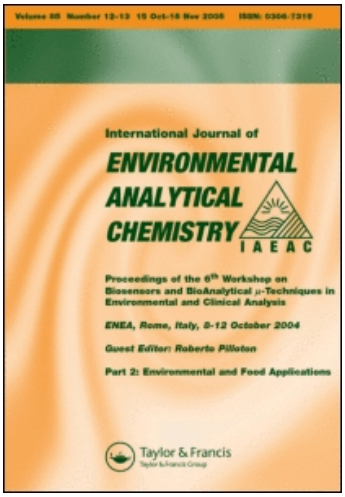

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Determination of arsenic species by microwave-assisted extraction followed by ion-pair chromatography-ICPMS: analysis of reference materials and fish tissues

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To cite this Article Karthikeyan, Sathrugnan , Hirata, Shizuko and Iyer, Chonattumadam S. P.(2004) 'Determination of arsenic species by microwave-assisted extraction followed by ion-pair chromatography-ICPMS: analysis of reference materials and fish tissues', International Journal of Environmental Analytical Chemistry, 84: 8, 573 — 582
To link to this Article: DOI: 10.1080/03067310310001658285
URL: http://dx.doi.org/10.1080/03067310310001658285

Intern. J. Environ. Anal. Chem.
Vol. 84, No. 8, pp. 573–582, 15 July 2004

Taylor & Francis
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DETERMINATION OF ARSENIC SPECIES BY MICROWAVE-ASSISTED EXTRACTION FOLLOWED BY ION-PAIR CHROMATOGRAPHY–ICPMS: ANALYSIS OF REFERENCE MATERIALS AND FISH TISSUES

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(Received 22 August 2003; In final form 18 November 2003)

A high-performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC–ICPMS) method for the determination of primary arsenic species in seafood products is described. A low-power microwave is used for efficient extraction of arsenic species from seafood products. The separation of arsenic species is accomplished on an ODS column by gradient elution at pH 9.3. Arsenobetaine (AB), arsenite (As^{III}), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA) and arsenate (As^{V}) were separated in less than 7 min. The detection limits are in the range of 44–66 ng As L^{-1} for all five arsenic species based on 3σ ($N = 6$) of the blank. The analysis of two reference materials (DORM-2 dogfish muscle and TORT-2 lobster tissue) showed good agreement with the certified values for total As. In the case of AB, the experimental results agreed well with those reported for DORM-2. For the other species mentioned above, for which there was no certification in the two reference materials, the values obtained by the proposed method were compared with those reported by other authors. In addition, samples of shrimp, fish and oyster were also analyzed by the proposed method and AB was found to be the dominant species in the range of 5.5–32.2 mg As kg^{-1} in these samples.

Keywords: Ion-pair chromatography; ICPMS; Arsenic speciation; Microwave-assisted extraction

INTRODUCTION

Arsenic species differ greatly in toxicity, depending on their ability to bind to thiol groups in proteins [1]. The median lethal dose (LD_{50}) values in rats for some As compounds are (in milligrams per kilogram) arsenite 14, arsenate 20, monomethylarsonic acid (MMA) 700–1800, dimethylarsinic acid (DMA) 700–2600 and arsenobetaine

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ISSN 0306-7319 print: ISSN 1029-0397 online
DOI: 10.1080/03067310310001658285

(AB) $>10\,000$ [2]. Methylation of inorganic As compounds is reported as the detoxifying mechanism in the human organism. Most seafoods contain milligram per kilogram concentrations of As, with AB being the dominant As species in crustaceans and arsenosugars in seaweed [3,4]. Other species such as arsenocholine (AC), tetramethylarsonium ion ($TeMA^+$), trimethylarsine oxide (TMAO) and trimethylarsine (TMA) have also been found in certain seafoods at much lower concentrations than AB and arsenosugars [5].

The different arsenic species can be separated by HPLC and detected by hydride generation atomic absorption spectrometry (HG-AAS) [7–9], hydride generation atomic fluorescence spectrometry (HG-AFS) [8,10] or inductively coupled plasma mass spectrometry (ICPMS) [10–19]. Of these ICPMS is preferred because of its easy coupling, wide dynamic range and extremely high sensitivity. For separation by anion-exchange HPLC, phosphate eluent is commonly used in the pH range 5 to 11. Generally, the elution order is AB, As^{III}, DMA, MMA and As^{V}. However, this order sometime changes depending on the pH, gradient condition, etc. [6–11]. Ion-pair chromatography, known for its potential to separate charged and neutral species in a single chromatographic run, has made rapid strides in recent times. The use of tetrabutyl ammonium ion (pH 6–11) and a number of alkylsulfonates (pH 2.7–4.5) in reversed-phase columns has resulted in efficient species separation of arsenic [12–17]. A dianionic ion-pairing agent, malonic acid, has also been successfully applied for arsenic speciation [18–20]. Woller *et al*. have used ammonium malonate at pH 8.5 on a microbore anion-exchange column for the separation of oxyanions of As and Se [18]. Le *et al*. have investigated mixed ion-pairing agents of tetrabutylammonium hydroxide, malonic acid and methanol on various reversed-phase columns for the speciation of organo arsenicals including arsenosugars [19,20]. Their results have demonstrated that the use of elevated temperatures shortens the separation time. Nevertheless, it is important to note that optimization of eluent composition, pH and flow rate are necessary, depending upon the choice of stationary phase.

Improvement in arsenic signals by the addition of methanol to the mobile phase has been proposed by several authors [19–21]. The enhancement in the intensity is due to a more efficient nebulization and transportation of aerosol into the ICP. However, higher methanol levels can lead to a decrease in the sensitivity of As determination, caused by the deterioration of the plasma properties for arsenic ionization [22].

The analysis of arsenic speciation in biological tissues generally involves critical steps owing to species decomposition during the sample preparation process. Many automated extraction techniques such as soxhlet, accelerated solvent extraction, supercritical fluid extraction and microwave-assisted extraction (MAE) are used for quantitative extraction of arsenic species [6,11,23–25,29]. MAE has been shown to be simple, fast and efficient. Dagnac *et al*. [11] have reported optimization of MAE for arsenic from the muscles of bivalves. Their results showed that the optimum power at 42 W for 3.6 min can yield 85% recovery using a methanol/water (55/45) mixture. Ackley *et al*. [24] have proposed MAE at 65°C for 4 min using 80% methanol for 100% extraction efficiency. However, they report a higher AB concentration in DORM-2 than other authors. Kirby and Maher [25] have also investigated the use of MAE for DORM-2 and TORT-2 materials. The authors reported the need for repeated extraction, and thrice at 70–75°C for 5 min using 50% v/v methanol for complete recovery. These studies indicate that the use of a microwave heating temperature near to the boiling point of the extractant mixture can yield better extraction efficiency.

Water has been tested as an extractant medium in MAE since it would be ideal for chromatographic introduction [23,24]. However, Ackley *et al*. [24] could not obtain satisfactory results by carrying out the extraction at either 80 or 100°C. The recoveries were lower in both cases and it was felt that the material was overheated at 100°C. Later, Brisbin *et al*. [23] from the same research group reported water as a suitable medium for MAE in the case of lobster tissues (90% extraction). In this case they used 75°C for 2 min. Although the extraction was greater with 2-min heating, it was nevertheless considered insufficient for uniform extraction since the mixing system was not as effective as with mechanical shaking or agitation techniques.

This study describes MAE of arsenic from seafood products followed by their speciation analysis using ion-pair chromatography with ICPMS detection. This experiment has been carried out with two objectives: namely (a) optimization of chromatographic parameters for the separation of the primary arsenic species As^{III}, As^{V}, MMA, DMA and AB using ion-pair chromatography; (b) evaluation of MAE for extraction of arsenic species from seafood using water as the extractant medium.

EXPERIMENTAL

Instrumentation

The chromatography system consisted of a Dionex gradient pump (Dionex Corp, USA), Rheodyne Model 9125 six-port injection valve fitted with a 100-μL sample loop and an Inertsil ODS column (GL Sciences Inc., Japan). The effluent from the column was connected to the concentric nebulizer in the cyclonic chamber of an Elan 6000 ICP mass spectrometer (Perkin-Elmer). The ICPMS instrument was optimized off-line for arsenic with an aqueous As^{V} standard each time. The intensity of the isotope at m/z 75 was monitored in all instances. The arsenic signal data were acquired using ELAN 6000 software. These raw data were later transferred into Total Chrom Work station Ver. 6.2 (Perkin-Elmer Inc, USA) to evaluate retention times and peak areas/peak heights. Peaks were identified according to retention time and confirmed by standard addition of arsenic compounds. The arsenic concentrations in real samples were quantified via peak areas on the basis of calibration curves of the known forms. The chromatographic gradient program and instrumental operating conditions of HPLC-ICPMS are given in Table I. A PROLABO (MCS950) closed microwave system was used for arsenic extraction as well as for acid wet digestion.

Reagents and Standards

For the preparation of reagents and standards, Milli-Q (Millipore, Milford, MA, USA) water of 18.3 $M\Omega\,cm^{-1}$ was used. Tetra-*n*-butyl ammonium hydroxide, 40% w/w (Lancaster, Morecambe, England) and malonic acid (Sigma Aldrich, Japan) were used for preparing the mobile phase. All reagents used were of analytical grade unless otherwise mentioned.

Mobile phase A (4 mM tetrabutylammonium hydroxide, TBA) was prepared by dissolving 2.600 g of TBA in 1 L MQ water and the pH was adjusted to 9.3 using dilute nitric acid.

TABLE I Instrumental operation conditions for HPLC–ICPMS system

HPLC Parameters	
Column	Inertsil ODS (150 mm × 4.6 mm)
Column temperature	Room temperature
Injection volume	100 µL
Flow rate	1.2 mL min^{-1}
Mobile phase A	4 mM TBA (pH 9.3), 0.5% methanol
Mobile phase B	4 mM TBA/10 mM malonic acid (pH 9.3), 0.5% methanol
Gradient program	0–1.5 min 100% A 1.5–3 min linear gradient to 100% B 3–8 min 100% B 8–9 min linear gradient to 100% A 9–15 min 100% A (reconditioning)
ICPMS Parameters	
Rf power	1200 W
Ar auxiliary gas flow rate	0.90 L min^{-1}
Ar plasma gas flow rate	15 L min^{-1}
Ar nebulizer gas flow rate	0.90 L min^{-1}
Sample introduction	Concentric nebulizer
Dwell time	1000 ms
Data acquisition	Graphic mode
Masses (m/z)	(^{75}As)

Mobile phase B (mixture of 4 mM TBA and 10 mM malonic acid) was prepared by dissolving 2.600 g TBA and 1.040 g malonic acid in 1 L MQ water and the pH was adjusted to 9.3 using an ammonium hydroxide solution.

Methanol was added to both mobile phases to maintain a concentration of 0.5% (v/v).

Standards

Concentration of arsenic species is always given as the concentration of elemental arsenic. As^{III} (1000 mg L^{-1}) was made by dissolving 0.1733 g $NaAsO_2$ in 100 mL water and As^{V} (1000 mg L^{-1}) was made by dissolving 0.416 g Na_2HAsO_4 in 100 mL water. The stock solutions (1000 mg As L^{-1}) for MMA, DMA and AB were prepared separately by dissolving corresponding amounts of salts, obtained from Tri Chemical Laboratories Inc, Yamanashi, Japan (assay: > 98% for all salts). All solutions were refrigerated and stored in the dark to prevent decomposition or oxidation. The final mixture was prepared daily from a 20 mg As L^{-1} standard solution each time.

Reference Material

The certified reference material, DORM-2 (dogfish mussel) and TORT-2 (lobster tissue) from National Research Council, Canada, were used.

Samples

Oyster, fish and shrimp were purchased from a local market. The edible parts were separated and freeze-dried continuously for three days to a constant weight, then finely powdered and stored in desiccators.

Total Arsenic Determination

The total arsenic concentrations in DORM-2 and natural samples were determined after microwave acid digestion using a closed vessel system. Aliquots (~200 mg) of powdered samples were weighed in triplicate into pre-cleaned, Teflon vessels. After addition of 5.0 mL suprapure nitric acid and 2.0 mL H_2O_2 (30%), the vessels were closed and kept at room temperature for 15 min. The vessels were then mounted in the rotor, which was placed in a microwave oven and an eight-step digestion program was started (time in min, power in W): 2, 250; 0.5, 0; 5, 300; 0.5, 0; 5, 450; 0.5, 0; 5, 600; 10, 0 (ventilation). After cooling, the digests were transferred into 50-mL calibrated flasks and diluted to volume. An aliquot of 5 mL was transferred into a 50 mL volumetric flask, diluted to the volume with MQ water and analyzed by direct ICPMS.

MAE of Arsenic

The effect of microwave power and time on arsenic extraction was determined by analyzing total arsenic and AB in certified reference material DORM-2. The temperature of water in the reactor vessel at 50 and 80 W was measured and found to be 88 ± 2 and $100 \pm 3^{\circ}C$, respectively.

Aliquots of ~100 mg dried powdered samples were separately weighed into Teflon vessels in triplicate, 10 mL water was added and the samples were extracted using low-power microwaves in the range 50 to 80 W for 5 to 10 min time in a closed system. After cooling, the suspensions were centrifuged at 2500 rpm for 15 min. The supernatant extracts were filtered through 0.45-μm Millipore filters and refrigerated at 4°C until analysis. One milliliter of these extracts was diluted to 20 mL with water prior to the analysis. Total arsenic was firstly determined by direct ICPMS. Speciation analysis of all sample extracts was carried out within three days of the sample preparation.

RESULTS AND DISCUSSION

Chromatographic Method Development

In the separation of arsenic species, both the electrical charge and the possible hydrophobic character of analytes have to be considered [26]. In ion-pairing, the tetrabutylammonium cation is used for separating As^{III}, DMA, MMA and As^{V}. The optimum pH range for separating the four arsenic species is between 5.0 and 7.0. In this pH range, As^{III} ($pK_a = 9.2$) is a neutral species, which is eluted in the void volume. AB is also eluted with As^{III} since it is considered to be a zwitterion. As^{III} becomes negatively charged when the pH of the mobile phase is increased over its pK_a value of 9.2. Therefore, As^{III} can be separated from AB using a resin-based column and a mobile phase pH of 9.0 [27].

In our preliminary studies, 4 mM TBA at pH 6.0 was initially investigated for the separation of the five arsenic compounds, AB, As^{III}, DMA, MMA and As^{V} on a reversed-phase ODS column. AB and As^{III} were eluted together under these conditions (Fig. 1a). When the TBA buffer at pH 9 was used, there was a separation between AB and As^{III} (cf. Fig. 1b). However, the other two arsenic species, MMA and As^{V} were eluted only after 20 min, indicating their stronger retention at higher pH. We investigated the effort of the pH of TBA buffer in the range 9.0–9.5 and found

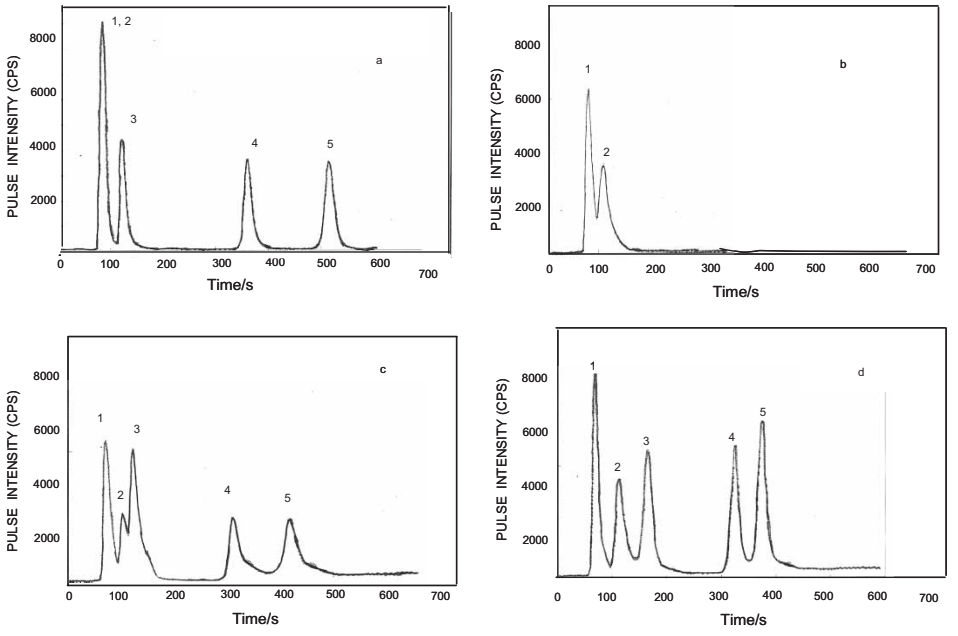

FIGURE 1 RP HPLC-ICPMS chromatograms of arsenic standards (a) 5 μg L^{-1} As; isocratic elution using 4 mM TBA, pH 6.0 (f/r = 1.2 mL min^{-1}). (b) 5 μg L^{-1} As (AB and As^{III}); isocratic elution using 4 mM TBA, pH 9.0 (f/r = 1.2 mL min^{-1}). (c) 5 μg L^{-1} As; isocratic elution using 4 mm TBA + 10 mM malonic acid mixture, pH 9.30 (f/r = 1.2 mL min^{-1}). (d) 5 μg L^{-1} As gradient elution using 4 mM TBA (pH 9.3) and 4 mM TBA/ 10 mM malonic acid mixture, pH 9.3 (f/r = 1.2 mL min^{-1}). Peaks are labeled as (1) AB, (2) As^{III}, (3) DMA, (4) MMA and (5) As^{V}.

the optimum at pH 9.2–9.4. Hence, the TBA buffer was adjusted to pH 9.3 for further studies.

For the elution of MMA and As^{V}, the addition of the anionic pairing agent malonic acid in the concentration range of 5–12 mM into 4 mM TBA at pH 9.3 was investigated. From these experiment results, it was observed that the lower concentrations of malonic acid (< 8 mM) delayed the elution of MMA and As^{V}, while they were coeluted at higher concentrations (> 10 mM). Therefore, 10 mM malonic acid was used with 4 mM TBA in subsequent studies. The resulted chromatogram is shown in Fig. 1(c) which shows the elution of MMA and As^{V}. However, the resolution between AB, As^{III} and DMA were not satisfactory under isocratic conditions using the mixture of 4 mM TBA and 10 mM malonic acid at pH 9.3.

Therefore, a gradient elution was proposed using two different mobile phase, i.e., mobile phase A (4 mM TBA at pH 9.3) and mobile phase B (4 mM TBA and 10 mM malonic acid mixture at pH 9.3). The gradient condition was chosen in such a way that AsB and As^{III} were allowed to separate using mobile phase A and mobile phase B was used to separate the remaining arsenic species. Accordingly, mobile phase A changes to mobile phase B from 1.5 to 3.0 min using a linear gradient. Mobile phase B was continued until 8 min for the elution of MMA and As^{V}. Under these conditions, the separation of these five arsenic compounds was found to be satisfactory (cf. Fig. 1d). It is known that the addition of carbon (as methanol) to the aqueous solution improves the ionization efficiency of arsenic in the plasma [23,24]. For that reason, 0.5% (v/v)

TABLE II Analytical figures of merit for the proposed RP HPLC–ICPMS

Parameter	*AB*	As^{III}	*DMA*	*MMA*	As^{V}
t_{ret} (min)$_{avg}$	1.17	1.87	2.76	5.30	6.13
Conc. range ($\mu g\,As\,L^{-1}$)	0.08–100	0.13–100	0.09–100	0.12–100	0.09–100
r^2	0.994	0.998	0.997	0.994	0.999
Slope [$\times 10^4$ L ($\mu g\,As$)$^{-1}$]	2.9	1.4	2.6	1.7	2.2
Intercept [$\times 10^4$]	2.0	1.4	2.9	1.4	3.2
DL ($ng\,As\,L^{-1}$)	42	66	44	59	45
R.S.D.% (2 μg As L^{-1})[a]	3.8	4.0	3.5	5.0	4.6
R.S.D.[a]% (5 μg As L^{-1})[a,b]	5.1	4.9	6.9	6.3	6.5

[a]$n = 6$; [b]R.S.D. for day-to-day reproducibility, calculated from three different days, analyses.

methanol was maintained in the eluent, which improved the peak intensities for all five arsenic species.

The analytical characteristics of the method, i.e. detection limit, precision, day-to-day reproducibility and linearity were evaluated for each arsenic species (Table II). A seven-point calibration is carried out in triplicate in the range 1–100 $\mu g\,As\,L^{-1}$ for which the r^2 values were better than 0.994 for all five species. The detection limits were calculated based on 3σ of the standard deviation of blank intensity at the respective retention times and found to be in the range 42–66 $ng\,As\,L^{-1}$. These are better than the earlier reports of B'Hymer and Caruso, 34–199 $ng\,As\,L^{-1}$ [14] and Londesborough *et al.* 160–600 $ng\,As\,L^{-1}$ [17]. The reproducibility and day-to-day repeatability were investigated and the relative standard deviations (RSDs) were less than 7% for all five species. The signals at m/z 75 ($ArCl^+$) due to chloride and m/z 53 (ArC^+) due to the sample matrix were monitored during As speciation analysis in order to control chloride and carbon concentrations. Under the selected conditions, chloride eluted after 6.0 min just after As^V. None of the matrixes analyzed caused a signal for m/z 75.

Microwave-assisted Extraction

Water has proven to be a good extraction medium for water-soluble arsenic species [23]. From previous reports, it was noted that microwave power in the range 50–75 W for 4–15 min was used for quantitative extraction of arsenic from various marine animal tissues. It was also mentioned that greater extraction efficiency can be achieved using an appropriate temperature near the boiling point of the extractant medium. Considering these two factors, three different digestion programs: (1) 50 W (5 min), 80 W (3 min); (2) 50 W (5 min), 80 W (5 min) and (3) 50 W (10 min), were investigated to obtain better quantification for total arsenic as well as AB concentration. The extraction was carried out as described above. These extracts were initially analyzed for total arsenic and then submitted to speciation analysis using the recommended HPLC-ICPMS procedure. The results are shown in Table III. It can be seen that 100% extraction was obtained using program (3), in which the temperature of the reactor vessel ($88 \pm 2^\circ C$) under slight pressure could come close to the boiling point of water. The longer time exposure, resulting in greater efficiency, is in good agreement with the previous report by Kirby and Maher [25]. In the case of programs (1) and (2), overheating was presumably responsible for low recovery as well as for low AB concentration. Dagnac *et al.* [11] reported on the instability of AB in microwave heating at

TABLE III Effect of microwave digestion program on DORM-2 sample

No.	*Program*	*Concentration found*[a] (μ As g^{-1})			
		Total As	*AB*	*DMA*	As^V
1	50 W (5 min); 80 W (3 min)	16.0 ± 1.2	13.6 ± 0.4	0.29 ± 0.05	0.77 ± 0.11
2	50 W (5 min); 80W (5 min)	15.5 ± 1.6	12.9 ± 0.6	0.31 ± 0.06	0.85 ± 0.12
3	50 W (10 min)	17.5 ± 1.4	16.0 ± 0.7	0.25 ± 0.02	0.36 ± 0.09
	Certified value	17.1 ± 1.1	16.4 ± 1.1		

[a]Average of triplicate determinations.

TABLE IV Arsenic extraction by microwave-assisted digestion

Sample	*Total As*[a] (μg g)		*Percentage extraction*
	Microwave-assisted	*Wet digestion*	
DORM-2	17.5 ± 1.4	17.4 ± 0.9	100.6
TORT-2	19.1 ± 0.7	20.7 ± 1.1	92.3
Shrimp	34.4 ± 0.6	34.3 ± 0.2	100.3
Oyster	9.4 ± 0.9	11.1 ± 0.3	85.0
Fish muscle	9.1 ± 0.6	8.3 ± 0.3	105.8

[a]Average of triplicate determinations.

80–90 W. To support this view, we have observed that the concentrations of AB were low in programs (1) and (2) while at the same time the concentrations of As^V were slightly higher. This may presumably be due to interconversion of AB caused by overheating. Therefore, it was decided to use low-power microwave heating of 50 W for 10 min as in program (3). Subsequently, all real samples were extracted using MAE, program (3). The resultant extracts were initially analyzed for arsenic (total extractable arsenic) by direct ICPMS. The total arsenic by acid wet digestion was also separately carried out as described above. The extraction efficiency was evaluated by determining the ratio between arsenic in the water extract and total arsenic after acid digestion. The results are given in Table IV showing extraction by water in the range 85–110% for all seafood samples.

Arsenic Speciation

The proposed HPLC-ICPMS procedure was tested using reference materials DORM-2 and TORT-2 and compared with certified values for total As in both, AB in DORM-2, and for the other species with those reported earlier. The assignment of chromatographic peaks was accomplished by matching retention times with those of standards and spiking experiments. From the spiking experiments, the recoveries were calculated to be in the range 90–105% which indicates the feasibility of speciation analysis for real-world samples. AB was the dominant species in both DORM-2 and TORT-2 extracts. The AB concentration (16.0 ± 0.7 μg As g^{-1}) in DORM-2 was in good agreement with the certified values (16.4 ± 1.1 μg As g^{-1}). The results obtained for other species are summarized in Table V, together with the values reported previously. Our results for DMA agreed well with the values reported by other authors for both DORM-2 and

TABLE V Quantitative results obtained in the analysis of reference materials, fish tissues ($\mu g\,As\,g^{-1}$)

Sample	*AB*	*As^{III}*	*DMA*	*MMA*	*As^{V}*	*Ref.*
DORM-2	16.0 ± 0.7	–	0.25 ± 0.02	–	0.36 ± 0.09	This work
DORM-2	15.9 ± 0.3	–	0.30 ± 0.01	< 0.003	–	[8]
DORM-2	16.1 ± 0.6	–	0.29 ± 0.02	–	0.05 ± 0.01	[12]
DORM-2	16.1 ± 0.7	$0.05 + 0.01$	0.49 ± 0.03	0.14 ± 0.02	0.05 ± 0.01	[30]
TORT-2	12.8 ± 0.3	–	1.06 ± 0.12	–	0.47 ± 0.13	This work
TORT-2	13.8 ± 0.2	–	$0.97 + 0.05$	0.03 ± 0.01	–	[8]
TORT-2	13.6 ± 3.9	–	1.33 ± 0.03	0.15 ± 0.11	0.68 ± 0.41	[12]
Shrimp	32.2 ± 1.7	–	–	–	0.37 ± 0.14	
Fish	8.1 ± 0.7	–	–	0.11 ± 0.05	0.19 ± 0.03	
Oyster	5.5 ± 0.5	–	0.19 ± 0.02	0.51 ± 0.08	0.08 ± 0.03	

Values shown are the average of triplicate determinations.

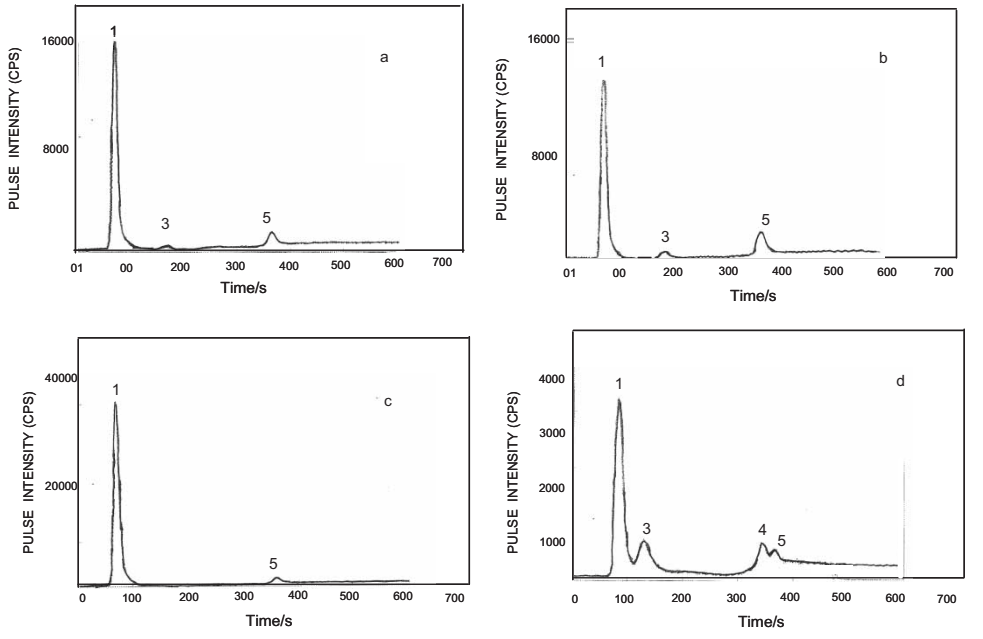

FIGURE 2 RP HPLC–ICPMS chromatograms of sample extracts using gradient elution with 4 mM TBA (pH 9.3) and 4 mM TBA/10 mM malonic acid mixture, pH 9.3 ($f/r = 1.2\,mL\,min^{-1}$). (a) DORM- 2 (dogfish muscle). (b) TORT-2 (lobster tissue) (c) shrimp (d) oyster. Peaks are labeled as (1) AB, (2) As^{III}, (3) DMA, (4) MMA and (5) As^{V}.

TORT-2 [8,12,30]. However, we found a higher concentration of As^{V} than the reported values for DORM-2. This may be due to the difference in the extraction procedures. The procedure was then applied to the analysis of arsenic speciation in the samples of tissues from shrimp, fish and oyster. From Table IV it can be seen that AB is the dominant species in all of them, with shrimp showing the highest value ($32.2 \pm 1.7\,\mu g\,As\,g^{-1}$). Lower concentrations of As^{V} were detected in all three species, and DMA and MMA were detected only in the oyster extract. Figure 2(a)–(d) depicts chromatograms of various sample extracts. It should be noted that DMA was eluted early in the case of oyster extract, showing the necessity for a further purification step.

CONCLUSION

A simple microwave-assisted aqueous extraction followed by an ion-pairing RP-HPLC-ICPMS procedure is described for arsenic speciation in seafood products. MAE employing water proved practical and efficient in the extraction of arsenic from seafood products. The proposed HPLC–ICPMS procedure is demonstrated to be useful for separation of AB, As^{III}, DMA, MMA and As^{V} in seafood samples. The analytical data on two reference materials, DORM-2 and TORM-2, were compared favourably with those reported in the literature. The analytical results of real fish tissue have shown AB to be the dominant arsenic species, which is considered to be non-toxic.

Acknowledgement

The authors thank the Japan Society for the Promotion of Science (JSPS) for their financial support.

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