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Ion chromatography–inductively coupled plasma mass spectrometry determination of arsenic species in marine samples

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Arsenic speciation analysis in marine samples was performed using ion chromatography (IC) with inductively coupled plasma mass spectrometry (ICP-MS) detection. The separation of eight arsenic species, viz. arsenite, monomethyl arsonic acid, dimethylarsinic acid, arsenate, arsenobetaine, tetramethylarsine oxide, arsenocholine and tetramethylarsonium ion was achieved on a Dionex AS4A (weaker anion exchange column) by using a nitric acid pH gradient eluent (pH 3.3 to 1.3). The entire separation was accomplished in 12 min. The detection limits for the eight arsenic species by IC–ICP-MS were in the range 0.03–1.6 μg l^{-1}, based on 3σ of the blank response ($n = 6$). The repeatability and day-to-day reproducibility were calculated to be less than 10% (residual standard deviation) for all eight species. The method was validated by analyzing a certified reference material (DORM-2, dogfish muscle) and then successfully applied to several marine samples, e.g. oyster, fish muscle, shrimp and marine algae. The low power microwave digestion was employed for the extraction of arsenic from seafood products. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: ion chromatography; ICP-MS; arsenic; speciation; extraction

INTRODUCTION

Speciation of arsenic has received significant attention over the past 20 years in both mechanistic and exposure assessment research. This is due to species-dependent toxicity of arsenic. The inorganic arsenic species (arsenate (As^V) and arsenite (As^{III})) have been classified as carcinogenic,[1] and the methylated forms, i.e. monomethyl arsonic acid (MMA) and dimethylarsinic acid (DMA), have recently been identified as cancer promoters.[2] It is believed that arsenobetaine (AB) and arsenocholine (AC), which are highly methyl substituted, are considered to be nontoxic.[3] In marine organisms, more than 32 different inorganic and organic arsenic species have been reported.[4] Depending on the kind of organism, different kinds and concentrations of arsenic compounds are observed. In fish and crustaceans, AB is the dominant species, along with traces of MMA, DMA, tetramethylarsonium ion ($TeMAs^+$) and trimethylarsine oxide (TMAO).[5–8] In marine algae, arsenosugar compounds (derivatives of dimethylarsonylribosides and trimethylarsonioribosides) are most abundant.[3,5–12] Bivalves contain AB and arsenosugars as major compounds. Variations in the arsenic species in muscle and gonad of scallops were observed within the same organism.[9,13]

The analytical speciation of arsenic has been achieved with the use of coupled techniques, i.e. a combination of a separation process (chromatography) with a suitable detection techniques. Hydride-generation atomic absorption spectrometry (HG-AAS) has been used for arsenic speciation, in which online thermal or microwave oxidation is employed to convert many organoarsenicals into hydride-forming arsenicals.[14–16] Better sensitivity has been achieved with the use of HG-atomic fluorescence spectrometry (HG-AFS).[15,17] Nevertheless, inductively coupled plasma (ICP) mass spectrometry (MS)[7,12,18–33] is widely used for arsenic speciation analysis since it offers extremely high sensitivity, large dynamic range and easy coupling. In particular, electrospray tandem MS (ES-MS)[10,12,34–36] was used for the structural verification of arsenosugar compounds.

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Arsenicals have widely varying ionic characteristics, which are pH dependent. Therefore, two separation techniques are usually required to resolve the different arsenic species due their ionic nature. The anionic species As^{III}, As^{V}, DMA and MMA, and the cationic species AB, can usually be resolved using anion-exchange high-performance liquid chromatography (HPLC). However, problems arise when other cationic species are present; for instance, if AC present, it usually co-elutes with AB,[18–22] as does $TeMAs^{+}$ and TMAO.[20–22] Cation exchange can be used to separate the cationic species AB, AC, $TeMAs^{+}$ and TMAO,[22] in which As^{III}, As^{V}, MMA and DMA are expected to co-elute together.[22] Alternatively, ion pairing reversed-phase HPLC can be used to resolve inorganic arsenic and organoarsenicals in a single chromatographic run with help of ion-pairing agents. Tetrabutyl ammonium hydroxide is a common ion-pairing agent used for separating anionic species,[25,26] and pentanesulfonate[19,24] or hexanesulfonate[25,26] are used for separating cationic species. Recently, the use of a nitric acid pH gradient using a Dionex AS7 (strong anion-exchange column) has been proposed for the separation of eight arsenic species, viz. As^{III}, MMA, DMA, As^{V}, AB, TMAO, AC and $TeMAs^{+}$.[27,28] The separation of neutral, anionic and cationic species was successfully accomplished by the addition of an ion-pairing agent (benzene disulfonic acid) in the nitric acid mobile phase. Kohlmeyer *et al*.[29] have demonstrated a similar approach for the separation of 17 arsenic species from various seafood products. On the other hand, Dionex AS4A, a weak anion-exchange resin phase, has not been explored much for arsenic speciation studies except for a few reports on the separation of As^{III} and As^{V}.[28,30]

The present communication describes the use of the Dionex AS4A weak anion-exchange column to separate eight arsenic species, viz. As^{III}, MMA, DMA, As^{V}, AB, TMAO, AC and $TeMAs^{+}$ in a single chromatographic run using nitric acid pH gradient elution in combination with ICP-MS detection. The various chromatographic parameters were optimized for better separation and precision. The proposed method was validated by analyzing certified reference material DORM-2 (dogfish muscle) and applied to several marine samples, e.g. oyster, fish, shrimp and marine algae.

EXPERIMENTAL

Reagents and standards

For the preparation of reagents and standards, Milli-Q (Millipore, Milford, MA, USA) water of 18.3 MΩcm was used. For eluents, suprapure nitric acid was used. All reagents used were of analytical grade. The 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 of $NaAsO_2$ in 100 ml deionized water and As^{V} (1000 mg l^{-1}) was made by dissolving 0.416 g of Na_2HAsO_4 in 100 ml of water. The stock solutions ([As] = 1000 mg l^{-1}) for MMA, DMA, AC, AB, TMAO and $TeMAs^{+}$ were prepared separately by dissolving the corresponding amounts of salts, procured from Tri Chemical Laboratories Inc. (assay: >98% for all salts), Japan. All solutions were stored refrigerated and in the dark to prevent decomposition or oxidation. The final mixture was prepared daily from a 20 mg l^{-1} standard solution each time.

Reference material

Certified reference material DORM-2 (dogfish muscle), from the National Research Council, Canada, was used for validating the proposed procedure.

Instrumentation

The chromatography system consisted of a Dionex gradient pump (Dionex Corp, USA) and a Rheodyne Model 9125 six-port injection valve fitted with a 100 µl sample loop. An IonPac AG4 guard column and an IonPac AS4A analytical column (both from Dionex Corpn, USA) were used. This anion exchange resin (250 mm × 4 mm, 13 µm particles) has hydrophilic quaternary ammonium exchange sites on a divinyl benzene copolymer. 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 ICP-MS instrument was first 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. A Prolabo (MCS950) microwave instrument was used for arsenic extraction and wet digestion.

Chromatographic conditions

To separate anionic, neutral and cationic species, nitric acid gradient elution was employed on an IonPac AS4A anion-exchange column. During the chromatography run, the nitric acid concentration was changed from 0.4 to 50 mM (pH 3.3 to 1.3). The arsenic signals were monitored and their 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 for ion chromatography (IC)–ICP-MS are given in Table 1.

Samples

Oyster (*Crassostrea gigas*), fish (*Mylio macrocephalus*) and shrimp (*Metapenaeopsis barbata*) were purchased from a local market. These samples were directly freeze-dried continuously for 3 days to a constant weight and finely powdered then stored in desiccators. Two kinds of marine alga (*Sargassum piluliferum* and *Melanosiphen intestinalis*) were collected from Hiroshima Bay. The sample was cleaned thoroughly with tap water to remove salt and dirt and further rinsed with Milli-Q water. The algae were freeze-dried to a

Table 1. Instrumental operation conditions for IC–ICP-MS system

ICP-MS parameters	
R.f. power (W)	1200
Ar auxillary gas flow rate (l min^{-1})	0.90
Ar plasma gas flow rate (l min^{-1})	15
Ar nebulizer gas flow rate (l min^{-1})	0.90
Sample introduction	Concentric nebulizer
Dwell time (ms)	1000
Data acquisition	Graphic mode
Masses (m/z)	(^{75}As)
IC Parameters	
Guard column	IonPak AG4A (50 × 4 mm, Dionex)
Analytical column	IonPak AS4A (250 × 4 mm, Dionex)
Flow rate (ml min^{-1})	1.2
Mobile phase A	0.4 mM nitric acid
Mobile phase B	50 mM nitric acid
Gradient program	0–2 min, 100% A (pH 3.3) 2–3 min, linear gradient to 100% B 3–10 min, 100% B (pH 1.3) 10–11 min, linear gradient to 100% A 11–20 min, 100% A (reconditioning)

constant weight and then ground in a mixer. The resulting powder was used for extraction of arsenic.

Total arsenic concentration

The total arsenic concentrations in DORM-2 and natural samples were determined after microwave acid digestion using a closed-vessel system and the following procedure. Aliquots of ~200 mg powdered samples were weighed into the pre-cleaned, Teflon vessels. After addition of 5.0 ml of suprapure nitric acid and 1.0 ml of 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 the microwave oven (Prolabo MCS950), and the eight-step digestion program was started (time (min), power (W)): 2, 250; 0.5, 0; 5, 300; 0.5, 0; 5, 450; 0.5, 0; 5, 600; 10, 0 (ventilation).[31] After cooling, the digests were transferred into 50 ml calibrated flasks and diluted with Milli-Q water. The total arsenic contents in these sample solutions were determined by direct ICP-MS after a further 10 times dilution.

Extraction of arsenic compounds

DORM-2 and natural marine samples

Aliquots of ~100 mg dried powder of the various samples of DORM-2, oyster, fish muscle, shrimp muscle and shrimp shell were separately weighed into Teflon vessels in triplicate and 10 ml of 50% aqueous methanol was added into those vessels. These samples were digested using low-power microwaves (50 W, 10 min) in a closed system using the Prolabo microwave instrument. After cooling, these suspensions were centrifuged at 2500 rpm for 15 min. The supernatant extracts were filtered through a 0.45 μm Millipore filter and refrigerated at 4 °C until analysis. In the case of marine algae, ~100 mg of dried samples was weighed in triplicate into 50 ml glass vials. 10 ml of 50% aqueous methanol was then added to each vial and extraction was undertaken using ultrasonication (15 min) rather than microwaves. Subsequent procedures were the same as above. These extracts were further diluted 20 times with Milli-Q water prior to analysis. The speciation analysis of all sample extracts was carried out within 3 days after the sample preparation. Matrix match (2% methanol) standards were used separately for total extractable arsenic determination.

RESULTS AND DISCUSSION

The arsenic species have a wide range of ionic nature, and this ionic nature is highly pH dependent. The MMA and DMA exist either as neutral or anionic species. Arsenobetaine (pK_a 2.2) may be cationic or zwitterionic. The other species, such as AC, TMAO and $TeMAs^+$, exist mostly as cationic species.

Chromatographic separation

The separation of cationic/anionic arsenic species using a nitric acid gradient in the presence of an ion-pairing agent has been reported using a strong anion-exchange column.[28,29] Based on these reports, we carried out our preliminary experiments using a weaker anion-exchange column (Dionex IonPac AS4) to resolve eight arsenic species with nitric acid gradient elution in the presence of malonic acid at 1 ml min^{-1}. The resulting chromatogram (Fig. 1a) shows co-elution of five compounds (As^V, AC, AB, TMAO, $TeMAs^+$) under this condition. A change in malonic acid concentration in the range 0.25–2 mM did not show any improvement in separation. As an alternative, a mono-ionic ion-pairing agent, butane-sulfonic acid (0.25 mM), was also investigated in place of malonic acid. The results were the same as those for malonic acid. Then, the separation by nitric acid eluent in the absence of an ion-pairing modifier was studied. By using 0.4 mM HNO_3 under isocratic conditions, only three species, i.e. As^{III}, MMA and DMA, could be eluted (Fig. 1b), whereas the other five compounds were retained strongly. Subsequently, the pH gradient of nitric acid from pH 3.3 to 1.3 was examined. The resulting chromatogram is shown in Fig. 1c, which shows the possibility of the separation of eight species. However, a blank peak appeared at the r_T of As^V (Fig. 1d). This blank peak could not be eliminated, and may be appearing due to the drastic change in mobile-phase concentration during the gradient elution. Presumably, the separation of cationic

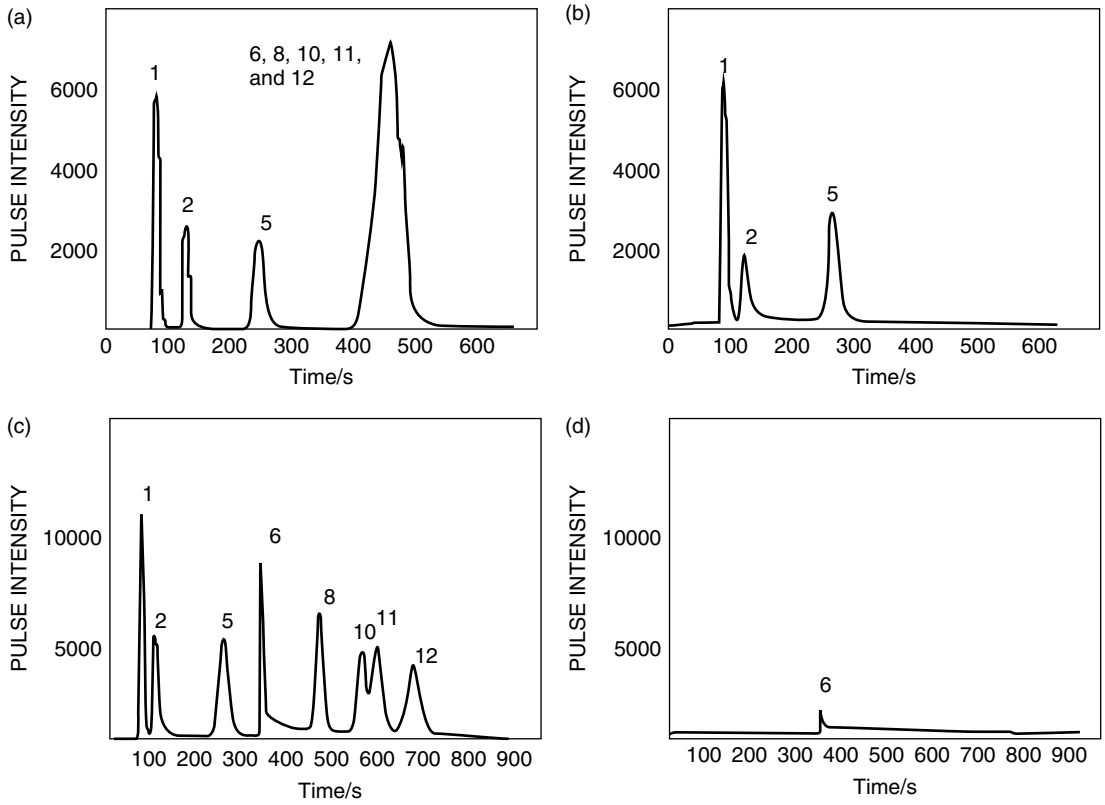

Figure 1. IC–ICP-MS chromatograms of mixtures of eight standards. (a) [As] = 5 μg l^{-1}, nitric acid pH gradient in presence of malonic acid (flow rate (f/r): 1.0 ml min^{-1}); (b) [As] = 5 μg l^{-1}, isocratic elution using 0.4 mM HNO_3 (f/r: 1.0 ml min^{-1}); (c) [As] = 10 μg l^{-1}, nitric acid pH gradient without ion-pairing agent (f/r: 1.2 ml min^{-1}); (d) nitric acid pH gradient of blank injection (f/r: 1.2 ml min^{-1}). Peaks are labeled as given in Table 3.

species was obtained based on hydrophobic interaction with the stationary phase. The overall separation is mainly based on ion exchange and reversed-phased mechanisms.[15,28] The TMAO and AC could not be resolved any better, in spite of varying the concentration of nitric acid between 30 and 75 mM, and the higher concentrations of nitric acid caused overlapping of the TMAO and AC peaks. Therefore, 50 mM nitric acid was used as the second mobile phase in further studies.

The influence of an organic modifier was investigated by using 0.2–1% methanol in the mobile phase. The addition of methanol caused broadening of the peak shape of MMA and As^{III}. Hence, methanol was not used in subsequent studies. The flow rate of the mobile phase was varied in the range 1–1.5 ml min^{-1}, and 1.2 ml min^{-1} was found to be suitable for shortening the analysis time without affecting separation factors. The use of dilute nitric acid as an eluent eliminates the clogging of the sampling cone in ICP mass spectrometer, which is frequently caused by other eluents in anion-exchange/reversed-phase chromatography.[19,33] Although chloride ions appeared between 100 and 200 s, less than 500 mg l^{-1} of chloride ions gave no peak and did not interfere with arsenic separation. The concentration of chloride ions in the injected samples was much less than 500 mg l^{-1}.

Detection limits and precision

The analytical characteristics of the method, i.e. detection limit (DL), precision, day-to-day reproducibility and linearity, were evaluated for each arsenic species. The characteristic performance of IC–ICP-MS is given in Table 2. The DLs were calculated based on 3σ of the baseline noise at the peaks' retention times ($n = 6$). The DLs obtained were from 0.03 μg l^{-1} (As^{III}) to 0.14 μg l^{-1} ($TeMAs^{+}$). These values are better than previously reported in the literature.[20,28] However, the detection for As^{V} is comparatively high (1.6 μg l^{-1}), which is high due to blank contamination. The absolute concentration of arsenate in the blank was calculated to be 1.0 μg l^{-1}. Similarly, the arsenic detection limits in the real samples were calculated to be in the range from 0.06 mg kg^{-1} for As^{III} to 0.28 mg kg^{-1} for AC. The exception is As^{V}, which is high at 3.2 mg kg^{-1}. The repeatability of IC–ICP-MS was examined by six consecutive runs at 10 μg l^{-1}, and the residual standard deviation (RSD) values were less than 5.3%, except for the 9.9% of As^{V}. The day-to-day reproducibility was also verified by running IC–ICP-MS on three different days. The

Table 2. Performance of IC–ICP-MS

Compound	Average t_R (min)	Detection limit[a] ($\mu g\ l^{-1}$)	Repeatability[b] at 10 $\mu g\ l^{-1}$ (%)	Linear equation[c]	Reproducibility[d] at 10 $\mu g\ l^{-1}$ (%)
As^{III}	1.23 ± 0.03	0.03	3.4	$y = 17\,284x$ ($r^2 = 0.9917$)	5.1
MMA	1.90 ± 0.06	0.05	2.2	$y = 15\,106x$ ($r^2 = 0.9983$)	12.2
DMA	4.45 ± 0.09	0.05	2.4	$y = 20\,834x$ ($r^2 = 0.9928$)	8.0
As^{V}	5.67 ± 0.07	1.6	9.9	$y = 12\,040x$ ($r^2 = 0.9914$)	9.7
AB	7.98 ± 0.13	0.08	2.6	$y = 19\,176x$ ($r^2 = 0.9906$)	6.4
TMAO	9.66 ± 0.16	0.13	4.3	$y = 9813x$ ($r^2 = 0.9989$)	10.4
AC	10.33 ± 0.19	0.14	5.3	$y = 12\,209x$ ($r^2 = 0.9936$)	11.7
$TeMAs^{+}$	11.81 ± 0.23	0.09	5.1	$y = 17\,849x$ ($r^2 = 0.9942$)	9.1

[a] Detection limits were determined as the elemental concentration giving a signal three times the standard deviation ($n = 6$) of the blank (Milli-Q water).
[b] Repeatability was determined from peak areas of six successive analyses at 10 $\mu g\ l^{-1}$.
[c] Linear equations have been calculated by best curve fitting for each species using Excel.
[d] Reproducibility was determined from peak area by calculating the RSD of three analyses on three different days with span of 2 days.

coefficients of variation were in the range 5.1–12.2%. The retention time of each arsenic compound was also shown to be reproducible. An eight-point calibration in the range [As] = 1–200 $\mu g\ l^{-1}$ was obtained for all eight species. Peak areas were used for the calibration data. The linearity was calculated by least-squares fit using Excel software, and the square of the correlation coefficient r^2 was 0.990 or better for all eight species.

Arsenic extraction

Low-power microwave digestion has been shown to be mild and fast for the extraction of arsenic species from seafood products.[16,19,22,37] In the preliminary studies, water and 50% methanol were investigated separately for arsenic extraction in marine algae. The aqueous methanol medium showed a better extractability (>75%) than the water medium (<65%). Hence, we used 50% aqueous methanol as the extract medium for all the samples. DORM-2 and other seafood samples were extracted as described in the Experimental Section. DORM-2 extract was initially analyzed for total arsenic and AB in order to assess the extraction efficiency, and satisfactory results were obtained in comparison with the certified values. In the case of marine algae, leaching with the help of sonication was used, since the microwave digestion method did not provide satisfactory results. The arsenic extraction from marine algae was carried out as described in the Experimental Section.

The extraction procedure was evaluated by determining the ratio between extractable arsenic (sum of arsenic from a single species) and the total arsenic after acid digestion. Extraction efficiencies were calculated for all sample types, and ranged from 45 to 105% (Table 3). Lower recoveries for marine algae (48–74%) are due to the difficulty in obtaining complete extraction in a marine algae matrix.

Validation

The accuracy of the proposed procedure was tested by analyzing DORM-2, which is certified for AB and $TeMAs^{+}$. The chromatogram of DORM-2 is shown in Fig. 2a. AB is the dominant species, with an arsenic concentration of $16.9 \pm 1.2\ \mu g\ g^{-1}$; the $TeMAs^{+}$ concentration, [As] = $0.36 \pm 0.11\ \mu g\ g^{-1}$, also compares closely with the certified value. These values were within the allowable certified values' errors (see Table 3). Smaller concentrations of TMAO, MMA, DMA and As^{III} were also detected, with [As] ranging from 0.11 to 0.34 $\mu g\ g^{-1}$ (Table 3). Though the certified values are not available for these species, they coincide with those reported in the literature.[15,28,29,38] In order to recognize possible interferences on signal intensity and retention time, spiking experiments were performed for DORM-2 extracts. Retention times were proved to be constant as long as the sample extracts were diluted, as mentioned in the Experimental Section. The recovery of standard solution spiked to DORM-2 was 90–102%.

Sample extracts

The various sample extracts were analyzed for arsenic species following the proposed IC–ICP-MS procedure. The analytical results are summarized in Table 3.

Seafood samples (microwave extraction)

The chromatogram of oyster extract is shown in Fig. 2b. Though AB is dominant here, reasonable concentrations of As^{III}, MMA and DMA in the range [As] = 0.51 to 2.00 $\mu g\ g^{-1}$ were detected (Table 3). A small peak appears between As^{V} and AB, which may be an arsenosugar compound with reference to an earlier report.[29]

The fish extract (Fig. 2c) was found mainly to contain AB, with [As] = 7.5 $\mu g\ g^{-1}$; minor concentrations of As^{III} and MMA were also present (Table 3). Similarly, extracts from shrimp muscle and shrimp shell mainly show the presence of AB, but at higher arsenic concentrations, i.e. 32.2–38.3 $\mu g\ g^{-1}$. An unknown peak appeared after AB. However, this peak did not match with TMAO standard addition. Hence, we could not label this peak specifically. The shell has a higher

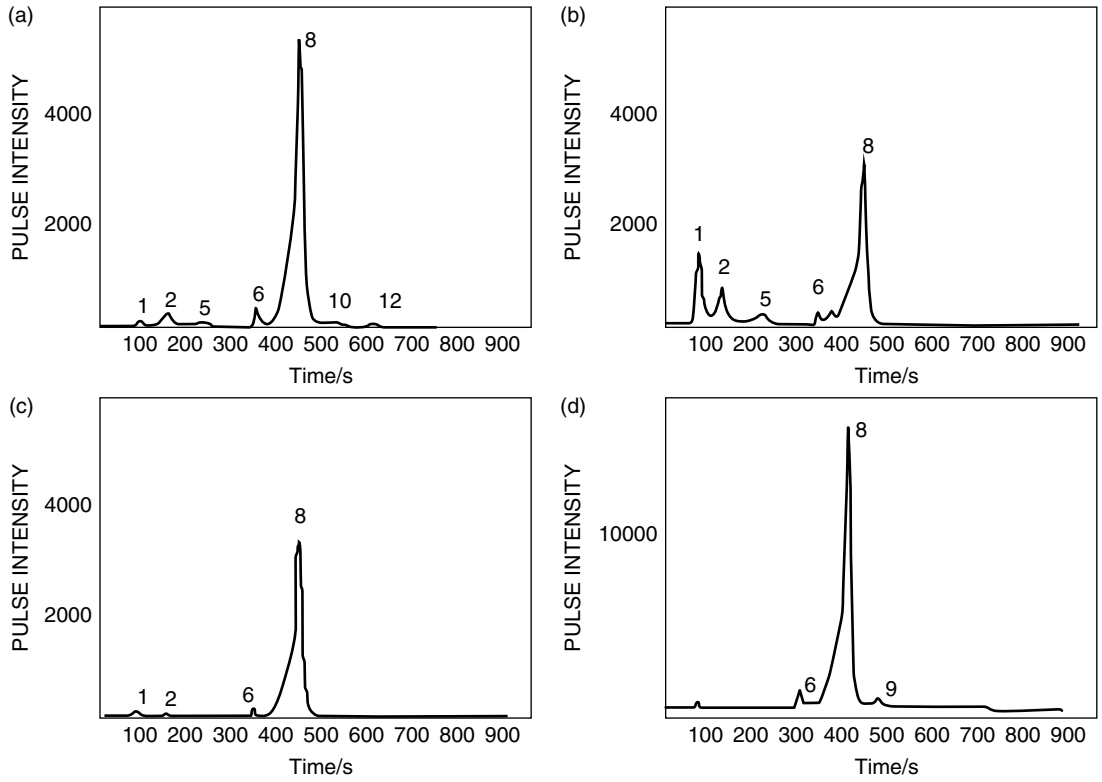

Figure 2. Nitric acid gradient anion-exchange ICP-MS chromatogram of methanol extract of seafood products: (a) DORM-2 (dogfish muscle); (b) oyster; (c) fish; (d) shrimp muscle (peaks are labeled as given in Table 3).

Table 3. Arsenic speciation analysis by IC–ICP-MS

		[As][a] ($\mu g\ g^{-1}$)						
					Shrimp		Marine algae	
Peak no.	Compound	DORM-2	Oyster	Fish	Muscle	Shell	*S. piluliferum*	*M. intestinalis*
1	As^{III}	0.11 ± 0.04	0.89 ± 0.08	0.09 ± 0.01	—	—	2.56 ± 0.53	3.91 ± 0.23
2	MMA	0.31 ± 0.12	2.00 ± 0.23	0.15 ± 0.09	—	—	2.00 ± 0.53	0.16 ± 0.03
3	Unknown 1	—	—		—	—	1.17 ± 0.08	—
4	Unknown 2	—	—	—	—	—	2.66 ± 0.35	—
5	DMA	0.16 ± 0.03	0.51 ± 0.03	—	—	—	12.2 ± 1.2	0.82 ± 0.14
6	As^{V}	—	—	—	—	—	113 ± 7	—
7	Unknown 3	—	—	—	—	—	0.082 ± 0.029	1.92 ± 0.22
8	AB	16.9 ± 1.2 [16.4 ± 1.1]	5.9 ± 0.2	7.5 ± 0.3	32.2 ± 0.7	38.2 ± 2.6	—	—
9	Unknown 4	—	—	—	0.07 ± 0.03	0.05 ± 0.02	—	—
10	TMAO	0.34 ± 0.06	—	—	—	—	—	—
11	AC	—	—	—	—	—	—	—
12	$TeMAs^{+}$	0.36 ± 0.11 [0.248 ± 0.054]	—	—	—	—	—	—
Sum of arsenic species		18.2 ± 1.2	9.3 ± 0.2	7.7 ± 0.3	32.3 ± 0.7	38.3 ± 2.6	133 ± 7	6.81 ± 0.23
Total arsenic (acid digestion)		17.4 ± 0.9 [17.1 ± 1.1]	11.1 ± 0.3	8.3 ± 0.3	36.7 ± 0.5	44.7 ± 1.8	181 ± 4	14.2 ± 1.6
Extraction efficiency (%)		105	84	93	88	86	74	48

[a] Average of triplicates. The values in the brackes are certified values.

concentration of AB than the muscle. The chromatogram of muscle extract is shown in Fig. 2d.

Marine algae (sonification)

The aqueous methanol extract of *S. piluliferum* was analyzed following the proposed IC–ICP-MS method. As^{V} was dominant, with [As] = 113 μg g^{-1}. Considerable concentrations of As^{III}, MMA and DMA, in the range [As] = 2.00–12.6 μg g^{-1}, were detected (Table 3). Two unknown arsenic compound peaks appeared between the MMA and DMA peaks. These may possibly be arsenosugar compounds (phosphate-arsenoriboside (Unknown 1) and sulfonate-arsenoriboside (Unknown 2)), by means of comparison with published literature.[29,36] A detailed investigation is necessary in order to label them specifically. Fig. 3a depicts the chromatogram of the marine algae extract. In contrast, the extract of *M. intestinalis* only shows the presence of As^{III}, MMA and DMA, along with one unknown compound (Unknown 3), at lower concentrations (Fig. 3b).

Verification of As^{V} values

The As^{V} concentrations of the various sample extracts were analyzed separately with another (carbonate eluent) chromatographic procedure[30] in order to verify the concentrations in the proposed procedure. This was carried out because of the As^{V} elution in the relatively high blank value. The estimated As^{V} concentrations in the marine algae extracts were in the range [As] = 100 ± 8.9 μg g^{-1}, which are comparable to

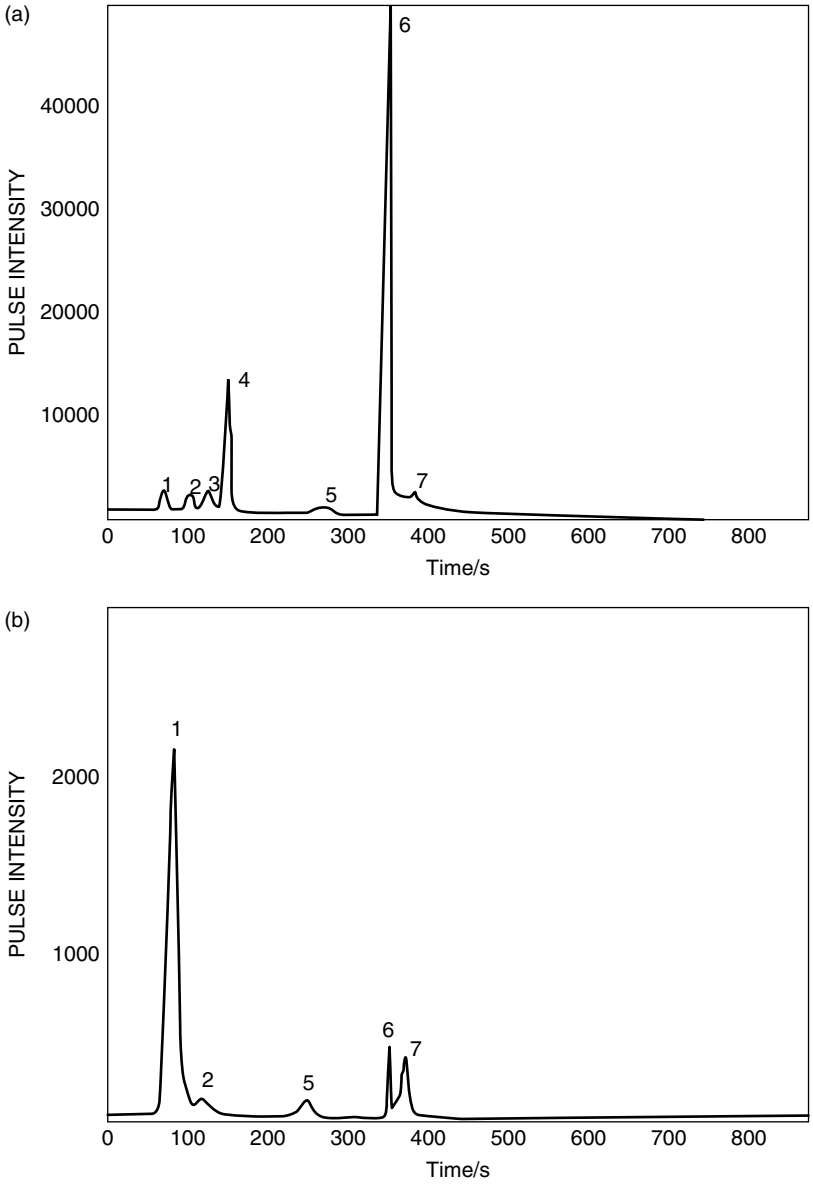

Figure 3. Nitric acid gradient anion-exchange ICP-MS chromatogram of marine algae. (a) Mamedawara *(Sargassum piluliferum)*; (b) Iwahige *(Myelophycus intestinalis)* (peaks are labeled as given in Table 3).

the values of our proposed procedure (Table 3). Similarly, the As^{V} peak was not detected in other extracts, such as DORM-2, oyster, fish or shrimp extracts. These results indicate that the proposed procedure is applicable for analyzing all arsenic species, including As^{V}.

CONCLUSIONS

The optimized proposed IC–ICP-MS analytical procedure offers sufficient detection limits and reproducibility for the investigation of species patterns of As^{III}, As^{V}, MMA, DMA, AB, TMAO, AC and $TeMAs^{+}$ in marine samples. This procedure was successfully validated by analyzing the certified reference material (DORM-2), and it was also applied to various natural samples, i.e. oyster, fish muscle, shrimp and marine algae. The results from the various sample analyses have demonstrated the capability of the method for the separation of 12 arsenic compounds. The low-power microwave extraction was shown to be simple, fast and efficient for seafood products extraction.

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