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Simultaneous determination of twelve inorganic and organic arsenic compounds by liquid chromatography–ultraviolet irradiation–hydride generation atomic fluorescence spectrometry

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Abstract

A coupling between column liquid chromatography (LC) and atomic fluorescence spectrometry was developed for arsenic speciation. After separation, the compounds are oxidised on-line by UV irradiation, volatilised by hydride-generation and carried to the detector by a stream of argon. A combination of anion-exchange and hydrophobic interactions in a single column (Dionex AS7) was found suitable for the simultaneous separation of organic and inorganic species. Twelve compounds (arsenite, arsenate, monomethylarsonic acid, dimethylarsinic acid, arsenobetaine, arsenocholine, trimethylarsine oxide, tetramethylarsonium ion and four arsenosugars) were separated using an acetate buffer and a nitric acid solution as mobile phases. Limits of detection are 4–22 pg. The technique was applied to three marine samples. Arsenobetaine was detected as the main species in all samples, with concentrations varying from 59 to 1947 ng(As) g^{-1} of fresh mass.

Keywords: Marine samples; Speciation; Atomic fluorescence spectrometry; Arsenic

1. Introduction

Arsenic is widely known as a toxic element and is naturally present in all environmental compartments in various forms, depending on the nature of the sample. Inorganic species are predominant in water and soil, whereas organic compounds are mainly present in living organisms.

Many methods have been developed for the speciation of inorganic and methylated species, using different separation and detection processes. Ion-exchange chromatography coupled to atomic spectrometry is most frequently used. In the last decades, the interest for atomic fluorescence spectrometry (AFS) as an element-specific detector has increased, because of its low cost and ease of use. Recently, this detector has even been shown to be as efficient as inductively coupled plasma mass spectrometry (ICP-MS), as regards sensitivity and repeatability [1]. The interface between column liquid chromatography (LC) and AFS is hydride-generation, which allows a quantitative sample introduction and has the advantage of removing many matrix interferences. However, this approach is limited to hydride-forming species, namely for arsenic: arsenite (As^{III}), arsenate (As^{V}), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA); therefore, organoarsenic compounds cannot be detected.

Few authors have studied organoarsenic compounds detection with a hydride-generation-based interface. Almost always, species are first decomposed into hydride-forming compounds by reaction with an oxidant, using thermal [2,3], microwave [4–7] or UV irradiation [8–10] techniques. The last one is inexpensive and easy to use as no cooling system is required after decomposition.

As regards LC separations reported in the literature, the most common modes are cation-exchange [3,11–18] and anion-exchange chromatography [4,5,9,16,19–21], reversed-phase chromatography [22,23] or a combination of these techniques [20,24,25].

Anion-exchange chromatography can separate inorganic and mono- and di-methylated species, which have an

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anionic character. Other organic species, such as arsenocholine (AsC), trimethylarsine oxide (TMAO) and tetramethylarsonium ion ($TMAs^+$) are neutral or cationic, and may also have hydrophobic properties due to their alkyl groups. As a consequence, they should be separated by cation-exchange or reversed-phase chromatography.

For the simultaneous study of organic and inorganic species, a single chromatographic mechanism is not sufficient. Combinations of anion- and cation-exchange chromatography have therefore been applied, using columns in series [26,27] or column-switching systems [28]. Combining anion-exchange and hydrophobic mechanisms is an alternative approach [20]. The DIONEX AS7 column has such strong anion-exchange and hydrophobic characteristics. The LC conditions have been optimised by Londesborough et al. [24] and Kolhmeyer et al. [25] for arsenic speciation with an ICP-MS detector.

However, an AFS detector cannot be hyphenated directly with LC. The UV–HG interface increases the void volume between the column outlet and the detector which causes a loss of resolution. Moreover, the mobile phase should be compatible with the photooxidation and hydride-generation steps. Since the mobile phases commonly used for reversed-phase LC strongly affect the conversion steps, a mechanism based on the use of inorganic ions is to be preferred.

The aim of this work was to develop an anion-exchange separation for the simultaneous study of organic and inorganic As-containing species by coupling LC and AFS using a UV photooxidation and hydride-generation interface.

2. Experimental

2.1. Reagents

$NaAsO_2$ (Aldrich, 98%), $Na_2HAsO_4{\cdot}7H_2O$ (Prolabo, >98%), $CH_3AsO(ONa)_2{\cdot}6H_2O$ (Carlo Erba, >98%) and $(CH_3)_2AsO(ONa){\cdot}3H_2O$ (Fluka, >98%) were dissolved in high quality deionised water MilliQ gradient + A10 ($18\,M\Omega\,cm^{-1}$, TOC $< 4\,\mu g\,l^{-1}$) to prepare stock solutions of $1000\,mg(As)\,l^{-1}$. CRM 626 was used as arsenobetaine (AsB) standard with a certified value of $433 \pm 2\,mg(As)\,l^{-1}$. AsC, TMAO, $TMAs^+$ and arsenosugars A, B, C and D standards were kindly provided by Prof. Lobinski (LCABIE, Pau, France) and Prof. Francesconi (Karl Franzens University, Graz, Austria). All arsenic solutions were stored in the dark at +4 °C.

LC mobile phases were prepared using HNO_3 (Baker Instra-analysed, 69–70%), CH_3COOH (Baker Analysed, 99–100%) and $CH_3COONa{\cdot}3H_2O$ (Merck Normapur).

A 0.5% $K_2S_2O_8$ (Merck ACS, >99%) with 1% NaOH (Merck pro analysi) solution was used for photooxidation. Hydride-generation was performed using the following solutions: $3\,mol\,l^{-1}$ HCl (Merck pro analysi) and 2.5% (w/v) $NaBH_4$ (Sigma, 98%) stabilised by 1% (w/v) NaOH.

Methanol (Baker LC Analysed, >99.8%) was used for extraction.

2.2. Apparatus

A scheme of the coupled LC–UV–HG-AFS system is presented in Fig. 1.

The LC system consisted of a DIONEX P580 quaternary pump with an on-line degassing system, a Reodyne six-port injector equipped with a 100 μl loop. The chromatographic column was a DIONEX AS7 (250 mm × 4 mm) protected by a DIONEX AG7 guard column.

The oxidative solution was delivered by a peristaltic pump (Gilson Minipuls 2) at $0.5\,ml\,min^{-1}$ and was added using a T-joint at the outlet of the chromatographic column.

For photooxidation, a PTFE tube was wrapped around a Philips TUV-15 lamp (253.7 nm, 15 W, 44 cm long). Its length and inner diameter were chosen after optimisation because irradiation time directly depends on the geometry of the tube.

HCl and $NaBH_4$ solutions used for the hydride-generation were added with a flow rate of $0.3\,ml\,min^{-1}$ using a peristaltic pump (Labcraft) through a T-joint and a mixing valve, respectively. Another channel of this pump was required for removing waste from the gas–liquid separator developed in our laboratory, as described previously [29].

An Excalibur atomic fluorescence detector (PS Analytical 10033) equipped with a boosted discharge hollow cathode

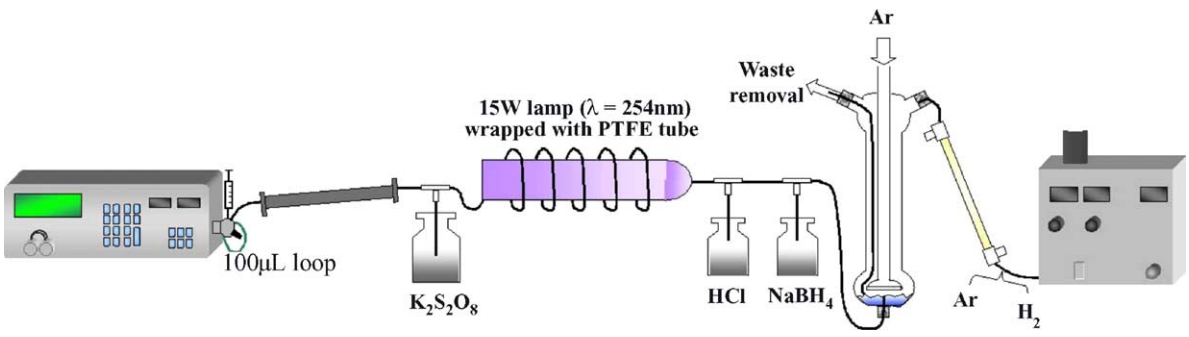


Fig. 1. Scheme of LC–photooxidation–HG-AFS coupling.

lamp (Photron) was used for detection. A computer recorded the output signal, using Borwin chromatographic software (JMBS, Grenoble, France).

2.3. Sample preparation and extraction

Surimi and shrimps were bought in a local supermarket. Oysters were collected in the Arcachon Bay in France. All samples were freeze-dried, powdered and sieved to 250 μm to obtain homogeneous materials. All samples were weighed before and after freeze-drying. The calculated moisture contents were 83.5, 74.4 and 79.4% for oyster, surimi and shrimp, respectively. These values were used to calculate the arsenic content in the fresh samples.

For extraction, 0.5 g of dried sample was mixed with 10 ml of methanol in a 50 ml polypropylene tube. The solution was magnetically stirred for 15 min, then diluted to 25 ml with pure water in order to prevent any solvent evaporation. The extract was filtrated through 0.2 μm porosity membranes and diluted with pure water before injection in the LC system.

To correct for possible matrix effects, quantitation of arsenic compounds was performed by standard additions using peak area measurement.

3. Results and discussion

Depending on the pH, arsenic species are neutral, anionic, cationic or zwitterionic. As a result, their retention will highly depend on the pH of the mobile phase. Cationic or neutral compounds such as AsC, TMAO and $TMAs^+$ cannot be retained by an anion-exchange mechanism. The hydrophobic nature of the DIONEX AS7 obviously causes the retention of such compounds as shown in the study of Londesborough et al. [24], where these organic species were the last to be eluted.

These authors separated eight arsenic compounds with a three-step gradient using nitric acid and benzene disulfonic acid (BDSA). The ion-pairing reagent was used to improve the separation of TMAO, AsC and $TMAs^+$.

When this program was used with UV–HG-AFS detection, many compounds overlapped partially or completely due to the increase of the void volume caused by the interface which led to significant broadening of all peaks. It was then necessary to modify the LC conditions to obtain sufficient overall resolution.

4. Optimisation of LC conditions

In our preliminary experiments BDSA did not significantly improve the retention of cationic compounds, consequently this ion-pairing reagent was not used in the following experiments.

To obtain a better retention of all compounds, the ionic strength of the eluents used by Londesborough et al. [24], was lowered by decreasing the HNO_3 concentration. This also led to an increase of the pH, and increased the anionic character of As^V, MMA, DMA and the carboxylic function of AsB. These compounds then have a higher affinity for the ion-exchange sites, leading to a stronger retention. The initial HNO_3 concentration (0.5 mmol l^{-1}) was lowered to 0.3 mmol l^{-1}, and this eluent was applied during 3.5 min to achieve a baseline separation of MMA and DMA. The second eluent (originally containing 50 mmol l^{-1}) was replaced by 25 mmol l^{-1} HNO_3. The gradient steps were modified accordingly.

The resulting program (No. 1 in Table 2) allowed a baseline separation for As^{III}, As^V, MMA, DMA and AsB. No improvement in resolution was obtained for TMAO, AsC and $TMAs^+$ which still partly overlapped.

An oyster extract, spiked with the eight arsenic species, was analysed to evaluate the effect of a complex matrix on retention times. Only MMA was highly affected, since its peak shifted towards As^{III}. Matrix effects did not significantly modify the retention of the other compounds. This phenomenon was also observed by Londesborough et al. [24].

To study the effects of pH and ionic strength of the extract on MMA retention, standards solutions of MMA containing increasing concentrations of phosphoric acid were injected. This acid was chosen because it was previously used by us to extract arsenic from vegetal samples [30]. Under these conditions, the phenomenon observed for MMA in the oyster extract also occurred. The MMA peak split into a large broad peak followed by a sharper one, even when the acid concentration was as low as 1 mmol l^{-1}. At higher acid concentrations the sharper peak tended to disappear, whereas the large peak eluted closer to the void volume.

This phenomenon can be explained by the pK_a value of MMA (pK_a = 3.6). A 0.3 mmol l^{-1} HNO_3 solution has a pH of 3.5, and for such a value, protonated and non-protonated MMA forms are equally abundant. At lower pH, i.e. total protonation of MMA, MMA is neutral and will not be retained by an anion-exchange mechanism. At higher pH, however, the anionic character will lead to a stronger retention. That is the large peak can be assigned to the protonated, and the sharp peak to the non-protonated form.

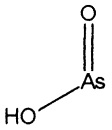
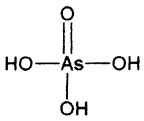
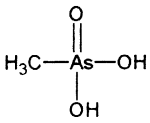
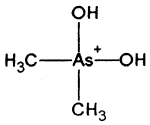
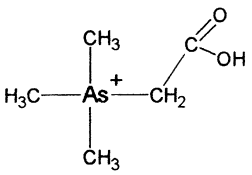
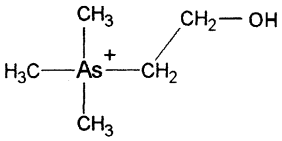
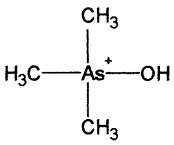
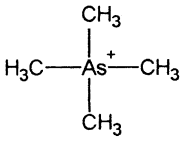
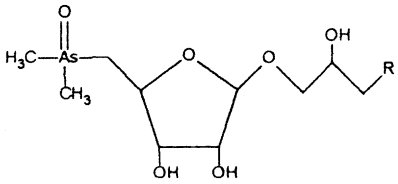
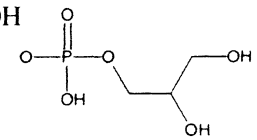
To solve the problem of MMA splitting, one should maintain the pH at a value at which MMA exists mostly in its deprotonated form. A buffered eluent was, therefore, used at the start of the LC run. The pH of the buffer solution should be higher than the pK_a value of MMA, i.e. 4–6.

A 0.5 mmol l^{-1} acetate buffer (pK_a = 4.8) was, therefore, used during the first minutes of elution.

However, the use of the buffered eluent, made it more difficult to decrease the pH in the following steps, and some modifications were then made as presented in No. 2 in Table 2, which allowed a good separation of the eight compounds.

To evaluate the matrix effects on the optimised LC conditions, a pure water solution and an oyster extract, both

Table 1
Formulae and pK_a values of studied arsenic compounds

Species	Formulae	pK_a [33]
Arsenite (As^{III})		9.29
Arsenate (As^{V})		2.24 6.96 11.5
Monomethylarsonic acid (MMA)		3.6 8.2 [34]
Dimethylarsinic acid (DMA)		1.78 6.14
Arsenobetaine (AsB)		2.18 [34]
Arsenocholine (AsC)		–
Trimethylarsine oxide (TMAO)		3.6 [11]
Tetramethylarsonium ion ($TMAs^{+}$)		–
Arsenosugars AsS(X)		–
A	R = OH 	–
B	R=	–
C	R: SO_3	–
D	R: OSO_3	–

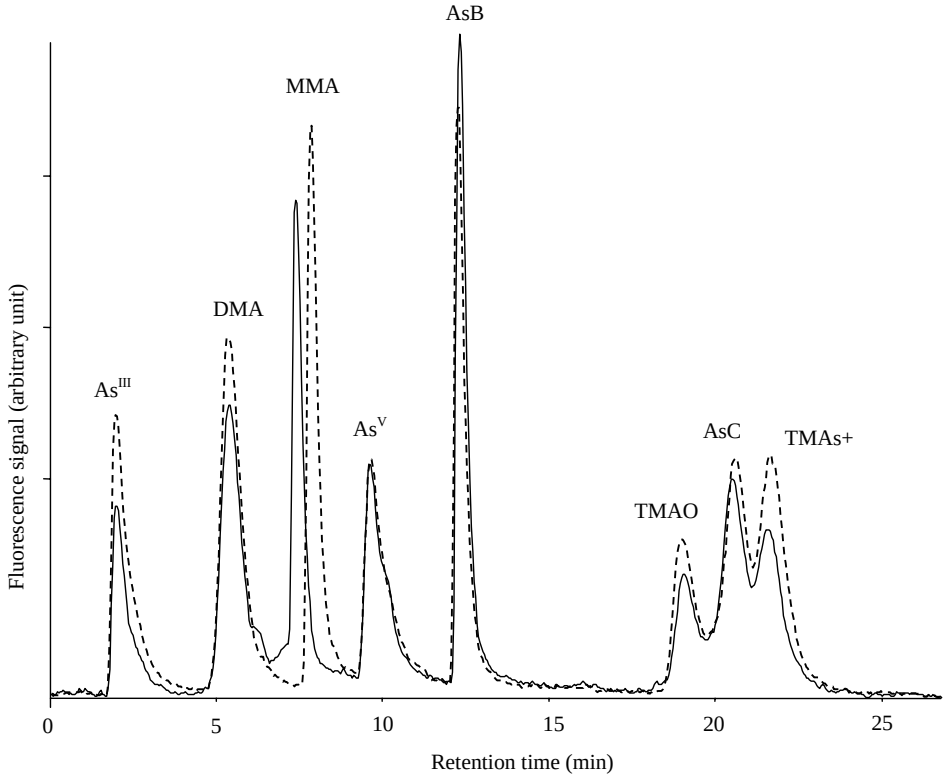


Fig. 2. Chromatogram of eight arsenic compounds in (- -) milliQ water or (-) in oyster extract. Conditions correspond to program 2 described in Table 2.

spiked with the eight arsenic compounds, were analysed. The chromatograms presented in Fig. 2 show that the oyster extract did not significantly change the retention times of the compounds. Furthermore, the MMA peak no longer split.

During analysis of other seafood samples, we detected peaks with retention times close to those of MMA and As^V. Standard addition revealed that these peaks did not correspond to MMA and As^V. It was supposed that these peaks could correspond to arsenosugars, since they were previously identified in such samples [31]. To test this hypothesis, the retention times of four arsenosugars were evaluated by injection of standard solutions. Three arsenosugars (B, C and D, see Table 1 for chemical formulae) eluted between DMA and MMA, with partial overlap, and arsenosugar A coeluted with AsB.

The LC conditions were then re-optimised to allow separation of the 12 species. This was achieved by increasing

Table 2
Elution programs based on nitric acid or ethanoate buffer and nitric acid

Program number					
1		2		3	
Time (min)	Composition[a] (%)	Time (min)	Composition[a] (%)	Time (min)	Composition[a] (%)
0–3.5	100 A	0–1	100 A	0–3	100 A
3.5–7	2 B	1–5	7 B	3–7	10 B
7–10	50 B	5–12	30 B	7–10	18 B
10–20	100 B	12–20	100 B	10–16	6 B
20–30	100 A	20–30	100 A	16–25	80 B
				25–35	100 A
A	0.3 mmol l^{-1} HNO_3	A	0.5 mmol l^{-1} CH_3COOH–CH_3COONa	A	0.5 mmol l^{-1} CH_3COOH–CH_3COONa
B	25 mmol l^{-1}	B	25 mmol l^{-1} HNO_3	B	25 mmol l^{-1} HNO_3

[a] All compositions are completed to 100% with pure water.

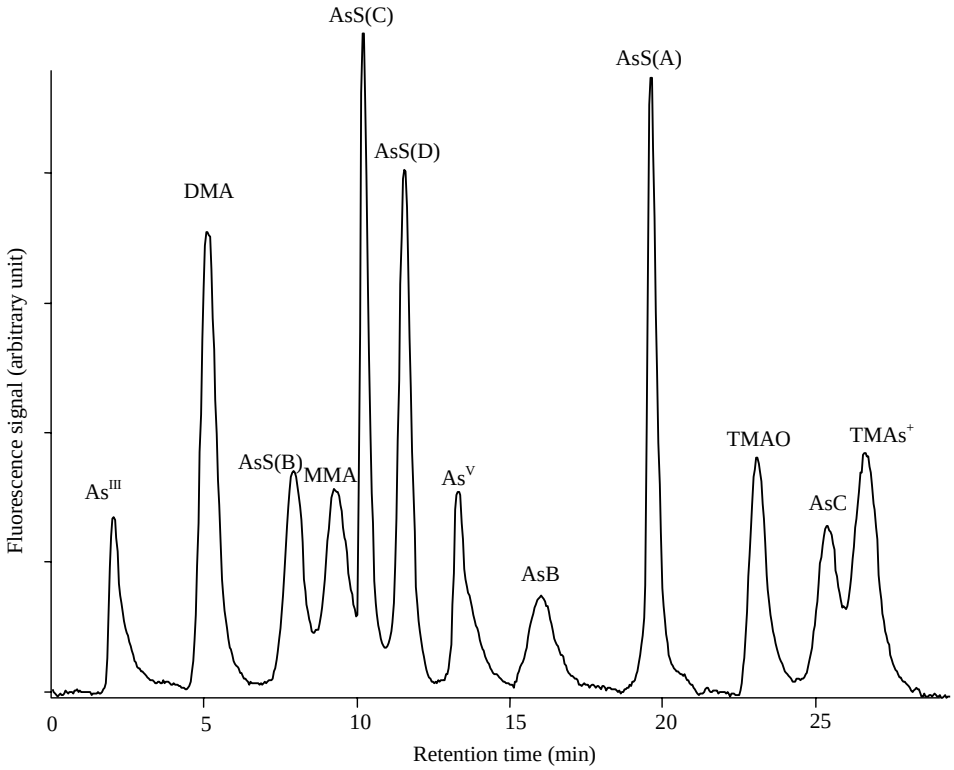


Fig. 3. Chromatogram of 12 arsenic standard, 5 ng g^{-1} each. Chromatographic conditions are presented in Table 2, column 3.

the length of the first step and decreasing the HNO_3 concentration in the following steps. The final program (No. 3 in Table 2) did not allow baseline separation of all compounds, but the resolution between all peaks was sufficient for identification of the 12 compounds. The increase of the HNO_3 concentration applied in step 3 was necessary to limit the broadening of the As^V and AsB peaks. In the next step, this concentration was lowered to 1.5 mmol l^{-1} in order to maintain a sufficient resolution between AsB and AsS(A). Finally, the HNO_3 concentration was set at 20 mmol l^{-1} to accelerate the elution of the last peaks. A chromatogram of 12 standards with concentrations of 5 ng(As) ml^{-1} each is presented in Fig. 3.

The relative standard deviations of retention times, evaluated by injecting five times a standard solution, were lower than 2% for all compounds. The repeatability of the retention for the arsenosugars was not evaluated due to insufficient amounts of standard solutions. Absolute limits of detection, determined as three times the standard deviation of noise level, were 14, 22, 14, 11, 15, 9, 17, 17, 10, 6, 4 and 6 pg(As) for As^{III}, As^V, MMA, DMA, AsB, AsC, TMAO, $TMAs^+$, AsS(A), AsS(B), AsS(C) and AsS(D), respectively. These values are in the same order of magnitude as those obtained with ICP-MS detection by Londesborough et al. (32–120 pg(As), calculated from published limits of detection) [24]. The high sensitivity obtained in our described method allows the determination of the 12 arsenic species at environmental relevant levels.

5. Application

The efficiency of the whole procedure for real samples was evaluated by analysing a dogfish muscle certified reference material, DORM-2 (certified for total arsenic, arsenobetaine and tetramethylarsonium ion concentrations). Furthermore, as this reference material has been frequently used to validate other techniques, many data are available in the literature. Table 3 presents results and standard deviation on five extractions with our technique and the certified values and the contents reported in the literature. Our results were in good agreement with the certified values, and in addition agreed well with the published contents for DMA (not certified). The extraction procedure was suitable for such matrices, and speciation and quantification analysis was reliable.

To evaluate the robustness of chromatographic separation, applications were performed on seafood products. Results obtained for each sample are presented in Fig. 4. In all cases, arsenobetaine was the major species, ranging from 69 to 100% of the quantified arsenic. The highest number of arsenic species was found in the oyster sample, with significant contents of inorganic in addition to several organic

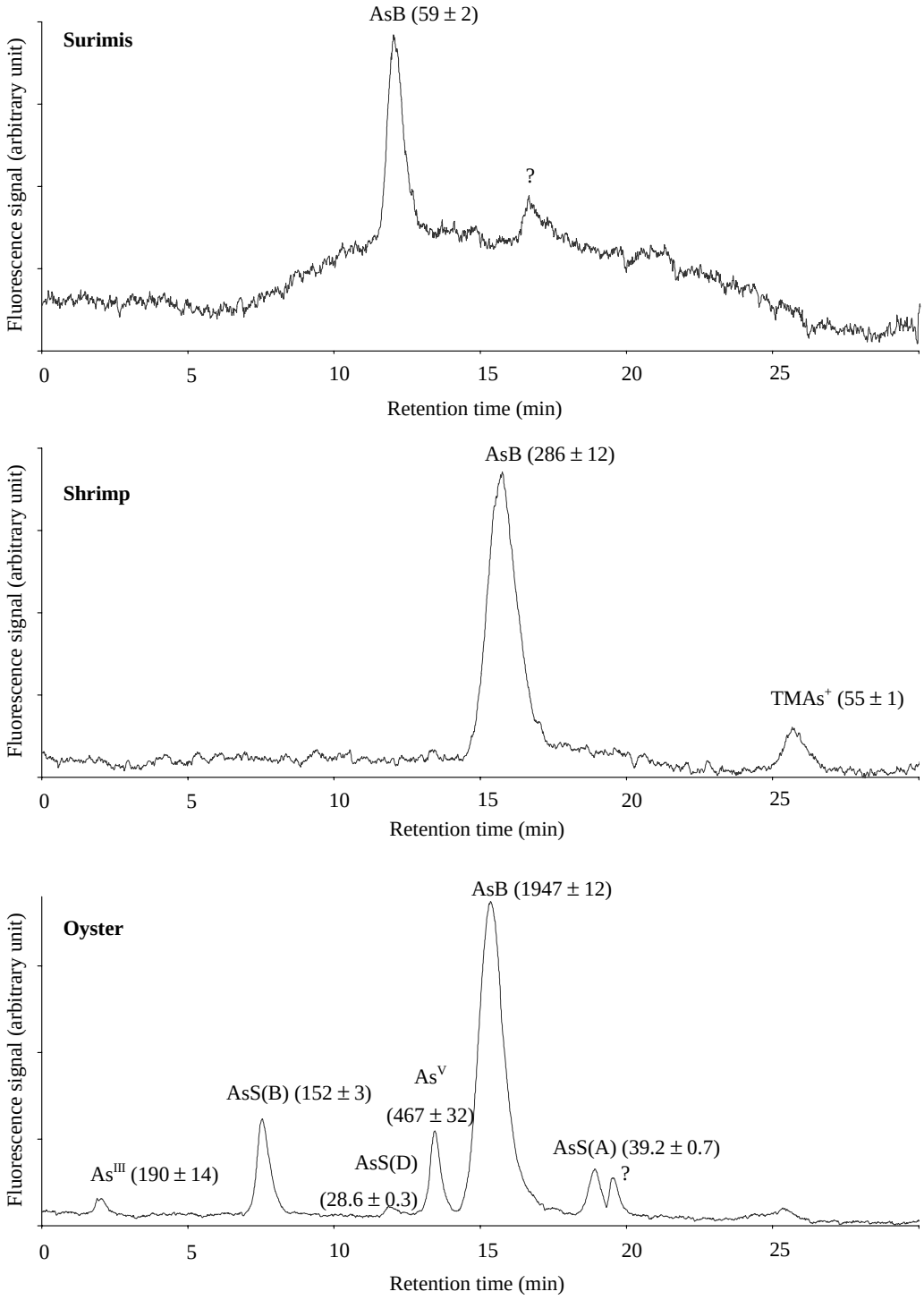


Fig. 4. Results of arsenic speciation in surimis, shrimp and oyster samples after methanol extraction (results expressed in ng(As) g^{-1} fresh mass; (?) unknown species).

compounds. The total inorganic arsenic content was close to 1 μg(As) g^{-1}, value fixed by Australia and New Zealand Food Authority as the maximum allowable level in edible molluscs [32].

As shown in the obtained chromatograms (Fig. 4), only low variations of the retention times for the As species were observed during these applications. The only exception was for the surimi extract which was not diluted prior to injection because of its very low arsenic concentration.

It appeared that a 10-fold dilution of the extract reduced the matrix effects, leading to similar retention times in standards solutions and various seafood matrices.

Table 3
Concentrations of arsenic species detected in DORM-2 expressed in μg(As) g^{-1}

As^{III}	As^{V}	MMA	DMA	AsB	TMAs+	AsC	TMAO	Unknown	Method	Ref.
nd	nd	nd	0.26 (±0.01)	15.9 (±0.6)	0.27 (±0.01)	nd	nd	nq	LC–UV–HG-AFS	This work
–	–	–	–	16.4 (±1.1)	0.248 (±0.054)	–	–	–	–	Certified values
<0.03	<0.03	<0.03	0.28 (±0.01)	16.0 (±0.7)	0.23 (±0.02)	0.02 (±0.01)	<0.03	0.16 (±0.01)	LC–HHPN–ICP-MS	[35]
nd	0.05 (±0.01)	nd	0.29 (±0.02)	16.1 (±0.1)	–	–	–	–	LC–ICP-MS	[36]
0.05 (±0.01)	0.05 (±0.02)	0.14 (±0.02)	0.49 (±0.03)	16.1 (±0.7)	0.30 (±0.02)	nd	0.30 (±0.03)	nd	LC–ICP-MS	[25]

Standard deviation on five extraction is given in brackets; nd: not detected; nq: not quantified; (–): not studied.

6. Conclusion

The LC conditions for both inorganic and organic arsenic species usually developed for ICP-MS detection were modified in view of the coupling to UV–HG-AFS. The optimised conditions allowed a satisfactory chromatographic resolution and were compatible with the UV–HG interface. Furthermore, due to the high sensitivity of MMA retention to pH, an acetate buffer solution was used in order to stabilise pH during the first minutes of elution and to avoid MMA peak splitting otherwise observed.

The repeatability of retention times was found satisfactory during the different applications to marine products evidencing the fact that the use of the buffered solution was sufficient to avoid strong matrix effects with a low dilution (5–10-fold) of the sample.

The LC–UV–HG coupled to AFS detection allowed a screening of species in biological matrices and their quantification at concentrations of a few ng g^{-1} of sample.

The good results obtained for sensitivity and reproducibility make this technique suitable for the determination of arsenic species in marine samples and constitutes an attractive alternative to LC–ICP-MS systems at significantly lower cost.

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