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Non-chromatographic speciation of toxic arsenic in fish

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Abstract

A rapid, sensitive and economic method has been developed for the direct determination of toxic species of arsenic present in fish and mussel samples. As(III), As(V), dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA) were determined by hydride generation–atomic fluorescence spectrometry using a series of proportional equations without the need of a chromatographic previous separation. The method is based on the extraction of arsenic species from fish through sonication with HNO_3 3 mol l^{-1} and 0.1% (m/v) Triton and washing of the solid phase with 0.1% (m/v) EDTA, followed by direct measurement of the corresponding hydrides in four different experimental conditions. The limit of detection of the method was 0.62 ng g^{-1} for As(III), 2.1 ng g^{-1} for As(V), 1.8 ng g^{-1} for MMA and 5.4 ng g^{-1} for DMA, in all cases expressed in terms of sample dry weight. The mean relative standard deviation values (R.S.D.) in actual sample analysis were: 6.8% for As(III), 10.3% for As(V), 8.5% for MMA and 7.4% for DMA at concentration levels from 0.08 mg kg^{-1} As(III) to 1.3 mg kg^{-1} DMA. Recovery studies provided percentages greater than 93% for all species in spiked samples. The analysis of SRM DORM-2 and CRM 627 certified materials evidenced that the method is suitable for the accurate determination of arsenic species in fish.

Keywords: Toxic arsenic; Speciation; Fish; MMA; DMA; As(V); As(III)

1. Introduction

Arsenic is an important element for speciation analysis, especially with regard to nutritional control. Human intake of arsenic occurs mainly via food chain, and it is closely related to the consumption of fish. Fortunately, arsenobetaine is the major component (typically more than 90% present arsenic) in fish and it is considered as a non-toxic compound. Therefore, the determination of total arsenic in a food sample does not reflect the level of hazard of this element and so it has become necessary to speciate arsenic forms in fish products to evaluate the potential toxicity [1].

The 50% lethal oral dose (LD_{50}) of As(III) is 4.5 mg kg^{-1} for mice, that of arsenate As(V) 14–18 mg kg^{-1}, monomethylarsonic acid (MMA) 1200 mg kg^{-1} and dimethylarsinic acid (DMA) 1800 mg kg^{-1} [2], being these the main toxic forms of As present in fish.

The provisional tolerable weekly intake (PTWI) for inorganic arsenic indicated by the Joint FAO/WHO Expert Committee on Food Additives is 0.015 mg kg^{-1} body mass/week [3] and Ysart et al. demonstrated that more than 90% of the total arsenic ingested comes from fish. However, less than 3% of the As in fish is present in the inorganic form (arsenite or arsenate) [4].

Speciation of arsenic usually involves several steps including derivatization, separation and detection [5]. Numerous analytical techniques have been applied involving separation by liquid chromatography and detection by inductively coupled plasma–atomic emission spectrometry (ICP–AES) [6,7], inductively coupled plasma–mass spectrometry (ICP–MS) [8,9], hydride generation–atomic absorption spectrometry (HG–AAS) [10–14] or hydride generation–atomic fluorescence spectrometry (HG–AFS) [15].

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The aim of this work has been to develop a selective extraction procedure of toxic arsenic for the direct speciation of the most commonly occurring chemical forms: As(III), As(V), MMA and DMA in fish without the need of a separation step. The developed methodology is based on the HG–AFS measurement of samples, previously sonicated at room temperature with HNO_3 and Triton X-114 and analyzed in four different experimental conditions, which provided a series of proportional equations from which the concentration of the four toxic As considered species can be determined.

2. Experimental

2.1. Apparatus and reagents

A PS Analytical Millennium Excalibur (Kent, UK) atomic fluorescence spectrometer was employed for arsenic detection. The system was equipped with an As boosted discharge hollow cathode lamp from Photron (Victoria, Australia), a specific filter, a solar blind and a Perma Pure® dryer.

Table 1 summarizes the experimental conditions employed for the HG–AFS determination of As in fish extracts, also indicating the four different measurement conditions selected for speciation purposes.

A microwave laboratory system Ethos SEL from Millestone (Sorisole, Italy) equipped with an optical fiber sensor for automatic temperature control, an automatic gas detector and ten high pressure vessels of 100 ml inner volume, operating at a maximum exit power of 1000 W was employed for microwave-assisted digestion of samples in order to determine total As and total toxic arsenic, as a function of the reagents employed.

An ultrasonic water bath from Selecta (Barcelona, Spain) of 350 ml volume with 50 W power and 50 Hz frequency was employed for sample sonication.

All reagents used were of analytical grade and all solutions were prepared in nanopure water with a maximum resistivity of 18.2 MΩ cm obtained from a Milli-Q Millipore system (Bedford, MA, USA).

A 1000 mg l^{-1} As(V) stock standard was supplied by Merck (Darmstadt, Germany). An As(III) stock solution of 1000 mg l^{-1} was prepared by dissolving As_2O_3 (Riedel de Häen, Hannover, Germany) in 20% (m/v) KOH solution from Panreac (Barcelona, Spain), neutralising with 20% (v/v) H_2SO_4 and diluting with 1% (v/v) H_2SO_4. A 1000 mg l^{-1} dimethylarsinic acid (DMA) stock solution was prepared from $(CH_3)_2AsO(ONa)\cdot 3H_2O$ (Fluka, Buchs, Switzerland). A 1000 mg l^{-1} monomethylarsonic acid (MMA) standard was prepared from $CH_3AsO(ONa)_2\cdot 6H_2O$ Carlo Erba (Milano, Italy).

A 1000 mg l^{-1} stock standard of arsenobetaine ($(CH_3)_3As^+CH_2COO^-$) was prepared from the Fluka salt (Buchs, Switzerland).

A 100 mg l^{-1} diluted standard solutions of As(III), As(V), MMA, DMA and arsenobetaine were prepared monthly and stored in a refrigerator at 4 °C to preserve the chemical species.

For the ultrasound-assisted extraction procedure HNO_3 3 mol l^{-1}, prepared from the 65% (m/v) concentrated acid J.T. Baker (Deventer, The Netherlands), a solution of the disodium salt of ethylenediaminotetraacetic acid EDTA 0.1% (m/v) (Panreac, Barcelona, Spain) and a surfactant agent, Triton XT114 from Feinbiochimica (Heidelberg, Germany) were also employed.

A reducing solution containing 50% (w/v) KI (Merck), and 10% (w/v) ascorbic acid (Guinama, Valencia, Spain) was employed to reduce As(V) and MMA to As(III) after the extraction step. For total arsenic determination a mixture of H_2SO_4 (Panreac, Barcelona, Spain) and HNO_3 (J.T. Baker) was used and L-cysteine (Sigma–Aldrich, Steinheim, Germany) was employed as reductant agent.

Sodium tetrahydroborate from Fluka dissolved in 0.1 mol l^{-1} NaOH, was used to form the arsine, previously to make the AFS measurements. This solution was prepared daily and filtered before use. Hydrochloric acid solution was prepared from the concentrated HCl (Scharlau).

Argon C-45 (purity > 99.995%) was employed as carrier gas and synthetic air was used to dry the formed hydride. Both were supplied by Carburos Metálicos (Barcelona, Spain).

2.2. Samples

Samples of different kinds of fish and mussels were purchased at the local market. They were cut into pieces with a plastic knife and frozen at −30 °C. Afterwards, they were freeze-dried in Cryodos system from Telstar (Barcelona,

Table 1
Experimental conditions employed for the determination of arsenic in fish extracts by HG–AFS

Parameter	Value
Wavelength (nm)	197.3
Measurement mode	Peak height
Measurement conditions HCl/mol l^{-1}	
A	2
B	4
C and D[a]	3.5
Measurement conditions $NaBH_4$/% (m/v)	
A	1.4
B	1.4
C and D[a]	1.2
Argon flow rate (ml min^{-1})	330
Air flow rate (l min^{-1})	2.5
Sample and HCl flow rate (ml min^{-1})	9
$NaBH_4$ flow rate (ml min^{-1})	4.5
Reaction coil length (cm)	150

Measurement conditions A, B and C correspond to different HCl and $NaBH_4$ concentrations for which different relative signals were found for As(III), As(V), MMA and DMA.

[a] In the D condition samples were previously treated with 1% (m/v) KI and 0.1% (m/v) ascorbic acid to produce a previous reduction of As(V) species.

Spain) for a minimum of 48 h at a chamber pressure of 0.05 mbar. The dried samples were crumbled and pulverized with a mill. The resulting fine powder was stored in a dessicator until analysis.

2.3. Reference material

A certified reference material CRM DORM-2 (dogfish muscle) was obtained from the National Research Council of Canada (CNRC), Institute of Environmental Chemistry, Ottawa (Canada).

A CRM 627 (tuna fish tissue) was obtained from the Institute for Reference Materials and Measurements (IRMM), Brussels (Belgium).

2.4. General procedures

2.4.1. Microwave-assisted digestion for total arsenic determination

0.5 g (±0.0001) powdered sample were weighted into a microwave high-pressure vessel, 5 ml of concentrated HNO_3 and 10 ml concentrated H_2SO_4 were added. The reactors' carousel was closed and placed into the microwave cavity.

The temperature of each sample was raised to 180 °C in 10 min and it was maintained at this value for a period of 10 min. Then, the temperature was raised to 200 °C in a short period of 5 min and it was maintained at 200 °C for 5 min. Afterwards, the reactor was cooled and opened, 0.2 g of L-cysteine suspended in 15 ml of water were added in order to reduce As(V) present in the digestion oxidant medium. The mixture was taken with 29.2 ml of concentrated HCl from the reactor and transferred to a volumetric flask, in which it was diluted to 100 ml. These solutions were heated at 100 °C for 1 min before total arsenic determination in the experimental conditions summarized in Table 1.

2.4.2. Reference microwave-assisted digestion for total toxic arsenic determination

1 g (±0.0001) powdered sample was weighted into a microwave high-pressure vessel and 10 ml of concentrated HNO_3 were added. The program temperature used was recommended by the EPA-3051 method [16]. The temperature was raised to 175 °C in 5 min and it remained at 175 °C for 10 min. The reactor was cooled and digested solutions treated as described in the previous section.

2.4.3. Extraction procedure for toxic arsenic speciation

1 g (±0.0001) portions of fish sample were weighted inside centrifuge tubes. 10 ml of a mixture of HNO_3 3 mol l^{-1} and Triton XT 114 0.1% (v/v) were added to each tube and the obtained slurries sonicated for 20 min. The extracts were separated by centrifugation at 3500 rpm for 20 min. The solid was washed with 10 ml of EDTA 0.1% (m/v). The final medium was fitted to 2, 3.5 or 4 mol l^{-1} HCl. One of the sub-samples was prepared in a medium containing HCl 3.5 mol l^{-1}, KI 1% (m/v) and ascorbic acid 0.1% (m/v) and it was left to react for 30 min before measurement in the experimental conditions described in Table 1.

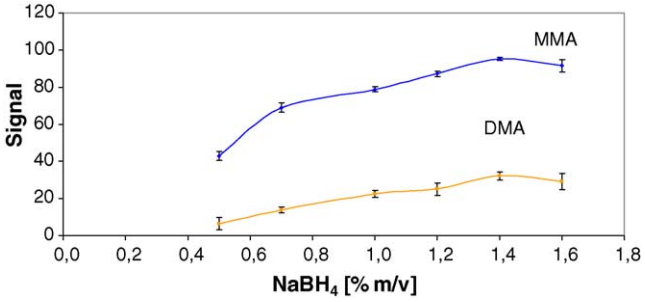

Fig. 1. Effect of $NaBH_4$ concentration on the fluorescence signals of solutions of 1 ng ml^{-1} MMA and 1 ng ml^{-1} DMA.

3. Results and discussion

3.1. Effect of $NaBH_4$ and HCl on the MMA and DMA fluorescence signals

The variation on the atomic fluorescence of MMA and DMA with the $NaBH_4$ concentration was evaluated in the range from 0.5% (m/v) to 1.6% (m/v) in 0.1 mol l^{-1} NaOH medium, using standard solutions of 1 ng ml^{-1} of MMA and DMA dissolved in 3.5 mol l^{-1} HCl. The remaining parameters (HCl, $NaBH_4$, argon and air flows) were maintained constant at the best values chosen in a previous work for As(III) and As(V) determination by HG–AFS [17].

The effect of borohydride concentration is clear (see Fig. 1). The signal rises significantly with the concentration of $NaBH_4$ reaching a maximum value for 1.4% (m/v) for both MMA and DMA, which corresponds to an improvement in the efficiency of the hydride generation from the methylated species. However, it is also evidenced from Fig. 1 that the hydride generation yield for MMA is of the order of 3.5 times higher than that obtained for DMA.

It was studied the effect of HCl on the arsine generation from MMA and DMA (see Fig. 2), in the range of 1–5 mol l^{-1}

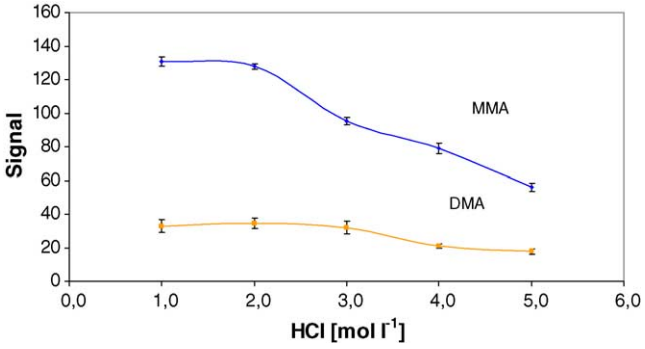

Fig. 2. Effect of HCl concentration on the fluorescence signals of MMA and DMA, note: in the same concentration as Fig. 1.

Table 2
Analytical characteristics of the developed method for non-chromatographic speciation of As by HG–AFS

Arsenic specie	Slope of calibration equation				LOD[a]	LOD[b]
	A	B	C	D		
As(III)	1736.4	1869.4	2070.6	2079.6	0.62	6.23
As(V)	790.9	872.1	1209.8	2036.7	2.1	20.58
MMA	1281.5	727.1	861.7	2052.0	1.8	18.26
DMA	360.1	194.5	292.5	285.7	5.4	54.15

Conditions A, B, C and D are indicated in Table 1.
[a] Limit of detection values, in ng g^{-1} of dry sample.
[b] Limit of detection, in ng l^{-1} for the measured solutions.

using $NaBH_4$ 1.4% (m/v). An increase of HCl concentration produces a decrease of the fluorescence signal of MMA and DMA and this change is especially dramatic for MMA. So to obtain the best sensitivity for HG–AFS determination of MMA and DMA a HCl concentration of 2 mol l^{-1} must be selected. On the other hand, the use of a 3.5 or 4 mol l^{-1} HCl concentration provides a different behaviour between MMA and DMA suitable to be employed for speciation.

3.2. Selection of the reductant agents

A preliminary study was made on the behaviour of As(III), As(V), MMA and DMA in the presence of KI and ascorbic acid in a 3.5 mol l^{-1} HCl medium. The aforementioned mixture proved to be highly efficient for the reduction of As(V) and MMA, having no effect on DMA. The slopes of the calibration equations obtained from As(V) and MMA were similar to those obtained for As(III) (see Table 2). In the case of DMA the slope does not change due to the addition of the reagents mixture.

However, the use of KI in a concentrated H_2SO_4 (>1.8 mol l^{-1}) and HNO_3 medium generates a considerable amount of I_3^-, a brownish compound which can not be reduced by the ascorbic acid and so, it can interfere in the subsequent HG–AFS determination.

In the strong acidic medium employed for microwave-assisted sample digestion, it is recommended to use L-cysteine as reducing agent. So it was evaluated the effect of L-cysteine from 0.1 to 0.5% (m/v) in a medium containing 10 ml H_2SO_4 and 5 ml HNO_3 for a 100 ml final volume, using 50 ng ml^{-1} As(V) standards in two different reaction conditions: (i) at room temperature for 30 min and (ii) at 100 °C for one minute. As can be seen in Fig. 3 the reaction at room temperature is incomplete, even for high concentrations of L-cysteine. However, the heating of solutions at 100 °C provides a quantitative reduction of As(V) to As(III) in a short period of time (only 1 min).

3.3. Selection of the extractant conditions for the toxic arsenic determination

The digestion with HNO_3 is not able to decompose the arsenobetaine, as it will be stated below; the measurement

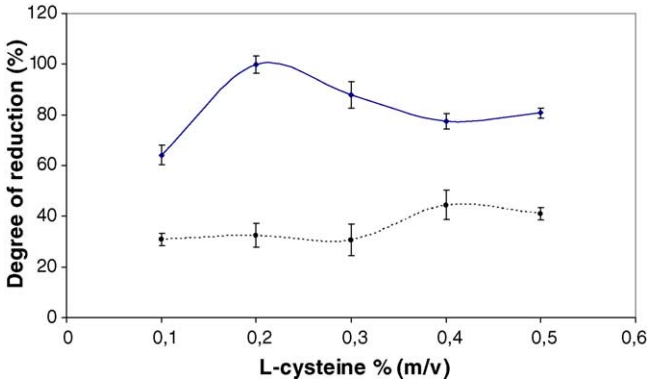

Fig. 3. Reduction of As(V) with L-cysteine in a medium HCl 3.5 mol l^{-1}, 10 ml H_2SO_4 and 5 ml HNO_3. Standards of As(V) 50 ng ml^{-1}. Dashed line (- - -) at room temperature for 30 min; continuous line (—) at 100 °C for 1 min.

by HG–AFS of the digested samples in this way only provides the content of the $NaBH_4$ reducible arsenic compounds, which includes inorganic arsenic [As(III), As(V)], monomethylarsonate (MMA), dimethylarsonate (DMA) and trimethylarsine oxide (TMAO) [18]. So, this procedure was employed as a reference method [16] for evaluation of toxic As recovery through sonication.

Six sub-samples of 1 g (salmon and mussel) were analyzed by HG–AFS after HNO_3 microwave-assisted digestion and alternatively after sonication in a water bath with 10 ml of different acids and Triton XT114 for 20 min. The extracts were separated by centrifugation and the solids were washed with a diluted solution of EDTA in order to prevent the retention of arsenic on the solid phase. The obtained recoveries are indicated in Table 3.

H_2SO_4 is able to degrade partially some organoarsenical compounds, such as arsenocoline or TMAO and, in a minor degree, arsenobetaine. So recoveries higher than 100% were obtained when this acid was used for sonication. On the other hand, HCl and HNO_3 provided quantitative recoveries of toxic arsenic, being HNO_3 the best extractant.

3.4. Strategy for speciation of As(III), As(V), MMA and DMA

The basis of the non-chromatographic speciation of As through HG–AFS is the measurement of standards and sam-

Table 3
Recoveries of As(III), As(V), MMA and DMA obtained through sonication with different acids respect to those found after microwave-assisted digestion for toxic arsenic determination

Acid	Recovery (%)
H_2SO_4 3 mol l^{-1}	153–176
HNO_3 3 mol l^{-1}	96–105
HCl 6 mol l^{-1}	87–95

Recovery values for 5 replicates.

ples at four different experimental conditions which allows us to establish a set of linear independent proportional equations, being the fluorescence intensity obtained in each case proportional to the concentration of the four species present in each solution:

$$I(A) = m_a[As(III)] + n_a[As(V)] + p_a[MMA] + q_a[DMA],$$

$$I(B) = m_b[As(III)] + n_b[As(V)] + p_b[MMA] + q_b[DMA],$$

$$I(C) = m_c[As(III)] + n_c[As(V)] + p_c[MMA] + q_c[DMA],$$

$$I(D) = m_d[As(III)] + n_d[As(V)] + p_d[MMA] + q_d[DMA]$$

The conditions chosen (A, B, C and D) are summarized in Table 1. Condition A represents a maximum signal for MMA and DMA, while in condition C the maximum sensitivity is achieved for As(III) and As(V). Condition B represents an intermediate behaviour of the species and finally, in condition D a mixture of KI/ascorbic acid was added in order to reduce As(V) and MMA to As(III).

The corresponding m_i, n_i, p_i and q_i coefficients correspond to the slope of the calibration lines obtained, for each compound in each experimental condition, from a series of pure standards and, as it can be seen in Table 2, these calibration lines involve different limit of detection values for each species.

3.5. Determination of total arsenic content

Arsenobetaine shows an extraordinary chemical stability and does not react at all with $NaBH_4$ to form the corresponding hydride. So it is not possible to determine this specie by HG-AFS without a previous degradation step.

The most usual techniques employed to break down arsenobetaine are UV-photooxidation [5,7,12], microwave-assisted digestion [14,15] and dry-ashing [19] and it has been reported in recent studies the problems found to assure the complete mineralization of samples [20,21].

Standard solutions containing from 5 to 10 mg l^{-1} arsenic as arsenobetaine were placed in the digestion vessel of the microwave oven and different digestion procedures, summarized in Table 4 were applied.

When only HNO_3 was used arsenobetaine does not decompose at relatively low temperatures (180 °C) and even at high temperatures (240 °C) poor recoveries were found. Schramel et al. [22] reported poor recoveries at the maximum attainable temperature (270 °C). This fact could be exploited in order to determine the partial content of arsenic, without consider arsenobetaine and other organoarsenic compounds.

The use of potassium peroxodisulfate, widely employed for the UV-photooxidation of organoarsenic compounds, is not effective in microwave-assisted digestion and the use of sodium peroxodisulfate, which has a higher solubility than $K_2S_2O_8$, and thus can be used at a concentration of 60% (m/v), only provided acceptable recoveries when it was combined with NaF.

The use of H_2SO_4 combined with HNO_3 in a 1:1 proportion and using a soft temperature program, provided similar recoveries than those obtained with $Na_2S_2O_8$. So, a method involving the use of HNO_3: H_2SO_4 in a 1:2 proportion was selected to obtain quantitative recoveries of the As from arsenobetaine (98 ± 2%).

3.6. Determination of toxic As species in actual fish samples

The concentration levels of As(III), As(V), MMA and DMA were evaluated in lyophilized samples of sardine, anchovy, salmon and mussel by using the developed procedure (see Table 5). Also, the total toxic arsenic and total arsenic were evaluated after microwave-assisted digestion with HNO_3 and HNO_3–H_2SO_4, respectively. The sum of potential toxic species of arsenic represents 9.6% in sardine, 12.7% in anchovy, 18.3% in salmon and 12.9% in mussel. Considering the toxic species, DMA is the predominant specie in fish samples (it supposes 5.4% in sardine, 6.9% in anchovy and 9.7% in salmon). This relatively high content of DMA could be due to the bioconversion of arsenite and arsenate to the methylated species (methylarsonic acid and mainly dimethylarsinic acid).

Table 4
Recovery of As from the microwave-assisted digestion of arsenobetaine using different reagents and irradiation programmes[a]

Method	Step	Reagents	Temperature (°C)	Time (min)	Recovery (%)
1	1.1	10 ml concentrated HNO_3	180	5	0
	1.2	5 ml concentrated HNO_3	240	5	<11
2		12.5 ml 4% (m/v) $K_2S_2O_8$	180	5	18–23
3	3.1	12.5 ml 4% (m/v) $K_2S_2O_8$ and 5 ml 5% (m/v) NaF	200	15	36–47
	3.2.	1 ml concentrated HNO_3 and 2.5 ml 5% (m/v) NaF	225	5	63–65
4	4.1	12 ml 60% (m/v) $Na_2S_2O_8$ and 4 ml 5% (m/v) NaF	200	15	77–81
	4.2	1 ml concentrated HNO_3 and 2.5 ml 5% (m/v) NaF	225	5	92–95
5		5 ml concentrated HNO_3 and 5 ml concentrated H_2SO_4	180	10	
			200	5	81–86
6		5 ml concentrated HNO_3 and 10 ml concentrated H_2SO_4	180	10	
			200	5	97–99

[a] The power (maximum 1000 W) was automatically controlled in order to achieve the programmed temperatures. Recovery values for five replicates.

Table 5
Analysis of As toxic species in fish samples by HG–AFS

Sample	DMA	MMA	As(V)	As(III)	Sum of toxic species	Total toxic arsenic[a]	Total arsenic[b]	Estimation of non-toxic species
Sardine	0.905 ± 0.05 (5.4%)	0.49 ± 0.04 (2.9%)	0.13 ± 0.02 (0.8%)	0.0803 ± 0.008 (0.5%)	1.81 ± 0.07 (10.8%)	1.75 ± 0.06 (10.5%)	16.7 ± 0.7	15.1 ± 0.7 (90.4%)
Anchovy	1.31 ± 0.13 (6.9%)	0.54 ± 0.05 (2.8%)	0.31 ± 0.04 (1.6%)	0.20 ± 0.01 (1.1%)	2.7 ± 0.1 (14.3%)	2.6 ± 0.2 (13.7%)	19 ± 1	16 ± 1 (87.3%)
Salmon	1.12 ± 0.07 (9.7%)	0.62 ± 0.03 (5.4%)	0.204 ± 0.01 (1.7%)	0.114 ± 0.009 (1.0%)	2.06 ± 0.08 (18.3%)	2.21 ± 0.09 (19.2%)	11.5 ± 0.5	9.4 ± 0.5 (81.7%)
Mussel	0.51 ± 0.04 (4.1%)	0.78 ± 0.09 (6.3%)	0.108 ± 0.009 (0.9%)	0.23 ± 0.01 (1.8%)	1.6 ± 0.1 (12.9%)	1.43 ± 0.03 (11.5%)	12.4 ± 0.4	10.8 ± 0.4 (87.1%)

Contents in mg kg^{-1} referred to dried sample.
[a] Total toxic arsenic determined by using the EPA method [16].
[b] Total content of As determined after microwave-assisted complete digestion of samples with HNO_3 and H_2SO_4. Values on parenthesis are the percentage of each specie respect to the total arsenic content.

3.7. Analytical figures of merit

The limit of detection values were calculated by dividing three times the standard deviation of the fluorescence signal of 10 reagent blanks by the slope of the calibration line, in the best experimental conditions, for each arsenic specie. The limit of detection was also established for the samples, taking into account the sample mass and dilution factor involved in the methodology (Table 2). The lowest LOD corresponds to As(III) (0.62 ng g^{-1}); As(V) and MMA have a similar LOD of 2.1 ng g^{-1} and 1.8 ng g^{-1}, respectively, whereas DMA gives the highest LOD, 5.4 ng g^{-1} due to its low sensitivity in comparison with the other arsenic species.

The precision of the method, expressed as the relative standard deviation evaluated from 4 replicate analysis of actual fish products like sardine, anchovy, salmon and mussel (see Table 5), corresponds to average RSD values from 7.1 and 7.4% for MMA and DMA to 8.9 % for As(III) and 11.4% for As(V).

3.8. Validation of the methodology

Six sub-samples of a lyophilized sardine tissue (1 g) were spiked before treatment with a similar content of each arsenic species than those values obtained: 0.1 mg kg^{-1} for As(III), 0.15 mg kg^{-1} for As(V), 0.5 mg kg^{-1} and 0.9 mg kg^{-1} for MMA and DMA, respectively and 10 mg kg^{-1} of arsenobetaine.

Quantitative recoveries were obtained for As(III) (97 ± 3%), As(V) (102 ± 5%), MMA (106 ± 4%) and DMA (93 ± 6%). These values evidence the excellent performance of the method for toxic arsenic species determination, while arsenobetaine was not detected and it did not interfere in the determination of the rest of species.

The comparison between the sum of toxic species determined by the proposed procedure and the toxic As determined by the EPA procedure [16] (see data in Table 5) evidences the good comparability of both procedures for the four samples analyzed, which provides a regression line $y = 0.2 \pm 0.3 + (0.9 \pm 0.2)x$, being y and x data found by the developed and the reference method respectively, which indicates a statistical comparability of a and b with 0 and 1, respectively and a regression coefficient of 0.96.

The total content of arsenic and the concentrations of As(III), As(V), MMA and DMA were determined by the proposed methodologies, after microwave-assisted digestion for total As or after sonication and measurement in four different experimental conditions for speciation, in two reference materials: a SRM DORM-2 and a CRM 627. Results obtained are shown in Table 6, with an estimation of the arsenobetaine content and, as it can be seen, the concentrations obtained are of the same order than some of data reported in the literature for the same sample and by using HPLC–atomic spectrometry hyphenated procedures.

Table 6
Concentrations of arsenic species ($\mu g\ g^{-1}$ As, referred to dry sample mass) found in reference materials DORM-2 and CRM 627

Total As	As(V)	As(III)	MMA	DMA	AsB	Technique	Reference
CRM 627							
4.8 ± 0.3	–	–	–	0.15 ± 0.02	3.9 ± 0.2		Certified
4.6 ± 0.2	0.07 ± 0.01	<LOD	<LOD	0.166 ± 0.008	4.3[a]	HG–AFS	This study
DORM-2							
	0.4	0.1	–	0.3	13.5	HPLC–ICP–MS	[23]
	0.05 ± 0.02	0.05 ± 0.01	0.14 ± 0.02	0.49 ± 0.03	16.1 ± 0.7	HPLC–ICP–MS	[24]
18.0 ± 0.6	–	–	<0.003	0.30 ± 0.01	15.9 ± 0.3	HPLC–HG–AFS	[19]
18.0 ± 0.6	–	–	–	–	16.3 ± 0.1	HPLC–HG–AFS	[25]
17.7 ± 0.6	–	–	–	0.28 ± 0.01	16.0 ± 0.7	HPLC–ICP–MS	[26]
	–	–	–		16.6 ± 0.6	HPLC–MS	[27]
	0.05 ± 0.01	–	–	0.29 ± 0.02	16.1 ± 0.6	HPLC–ICP–MS	[28]
18.0 ± 1.1	–	–	–	–	16.4 ± 1.1		Certified
17.8 ± 0.5	0.33 ± 0.01	<LOD	0.123 ± 0.009	0.61 ± 0.04	16.7[a]	HG–AFS	This study

[a] Arsenobetaine content estimated as the difference of total arsenic and the rest of arsenical species.

4. Conclusions

From the studies carried out it can be concluded that the developed methodology provides good recoveries and comparable results with those methods established for both, the total toxic As concentration and the chromatographic speciation.

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References

[1] C.D. Klaasen, L. Casarett, J. Doull, Caserett and Doull's Toxicology: The Basic Science of Poisons, Mc Graw-Hill, 2001.
[2] K. Shiomi, Arsenic in the environment. II part. Human health and ecosystem effects, in: Series in Advances in Environmental Science and Technology, Wiley, New York, 1994.
[3] FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series 759, WHO, Geneva, Switzerland, 1989.
[4] G. Ysart, P. Miller, M. Croasdale, H. Crews, P. Robb, M. Baxter, C. de L'Argy, N. Harrison, Food Add. Contam. 17 (2000) 775.
[5] C.M. Barra, R.R. Santelli, J.J. Abrão, M. de la Guardia, Quimica Nova 23 (2000) 58.
[6] M.C. Yebra-Biurrun, A. Gracia-Garrido, Food Chem. 72 (2001) 279.
[7] J. Albertí, R. Rubio, G. Rauert, Fresenius' J. Anal. Chem. 351 (1995) 415.
[8] I. Pizzarro, M. Gómez, C. Cámara, M. Palacios, Anal. Chim. Acta 495 (2003) 85.
[9] X.C. Le, W.R. Cullen, K.J. Reimer, Talanta 41 (1994) 495.
[10] A. Shraim, B. Chiswell, H. Olszowy, The Analyst 125 (2000) 949.
[11] S.P. Quináia, M.C. Ezequiel Rollemberg, J. Braz. Chem. Soc. 8 (1997) 349.
[12] D.L. Tsalev, M. Sperling, B. Welz, Spectrochim. Acta B 55 (2000) 339.
[13] S.J. Santosa, Anal. Sci. 17 (2001) i25.
[14] S. Ringmann, K. Bloch, W. Marquardt, M. Schuster, G. Schlemmer, P. Kainrath, Anal. Chim. Acta 452 (2002) 207.
[15] M. Vilanó, R. Rubio, J. AOAC Int. 84 (2001) 551.
[16] EPA Method 3051, http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3051.pdf, visited on 17th November 2004.
[17] P. Cava Montesinos, M.L. Cervera, A. Pastor, M. de la Guardia, Talanta 60 (2003) 787.
[18] M.O. Andreae, Anal. Chem. 49 (1977) 820.
[19] M.A. Suñer, V. Devesa, O. Muñoz, D. Vélez, R. Montoro, J. Anal. Atom. Spectrom. 16 (2001) 390.
[20] Z. Slejkovec, J.T. Van Elteren, U.D. Woroniecka, Anal. Chim. Acta 443 (2001) 277.
[21] W. Goessler, M. Pavkov, Analyst 128 (2003) 796.
[22] P. Schramel, S. Hasse, Fresenius' J. Anal. Chem. 346 (1993) 794.
[23] S. Londesborough, J. Mattusch, R. Wenrich, Fresenius J. Anal. Chem. 363 (1999) 577.
[24] U. Kohlmeyer, J. Kuballa, E. Jantzen, Rapid Commun. Mass Spectrom. 16 (2002) 965.
[25] M.A. Suñer, V. Devesa, I. Rivas, D. Vélez, R. Montoro, J. Anal. Atom. Spectrom. 15 (2000) 1501.
[26] W. Goessler, D. Kuehnelt, C. Schlagenchaufen, Z. Slejkovec, K.R. Irgolic, J. Anal. Atom. Spectrom 13 (1998) 183.
[27] J.J. Corr, J. Anal. Atom. Spectrom. 12 (1997) 537.
[28] K. Wrobel, K. Wrolbel, B. Parker, S.S. Kannamkumarath, J.A. Caruso, Talanta 58 (2002) 899.