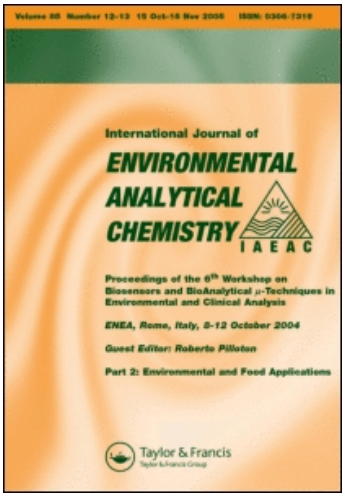

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INORGANIC AND METHYL ARSENICALS DETERMINATION BY IEC-HG-AAS IN MOLLUSCA

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Seven digestion methods for arsenic speciation in bivalve mollusca were investigated. A method using HNO_3:HCl (3:1) was found to be the best in order to provide the highest recovery. The method was used for As(III)), As(V), MMA and DMA determination in mollusca by IEC-HG-AAS.

Keywords: Speciation; organic arsenic; inorganic arsenic; IEC; HG-AAS; mollusca

INTRODUCTION

Arsenic is present naturally in foods but its presence can also be due to the contamination of the environment[1]. Arsenic concentrations in animal and vegetable foods are usually between 0.1 and 0.9 $\mu g.g^{-1}$[2]. In 1973 the FAO/WHO Mixed Comission of the Codex Alimentarius established arsenic as an element whose uncontrolled presence in foodstuffs could be potentially toxic[3]. In 1983[4], they lowered the maximum acceptable daily intake of arsenic from 50 to 2 μg per Kg^{-1} of body weight.

Seafood products generally have higher arsenic content, than other foods[5], but the determination of the total arsenic concentration is not sufficient to assess the toxicity of this element because it depends on the different chemical species involved.

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Some analytical methods that use different on/off line analytical techniques have been proposed in the last years. Liquid chromatography[6–12] is the most common separation technique used. Spectrophotometric techniques are used for the detection of the arsenic species, being the most commonly employed HG-AAS[6,7,13], ETAAS[14,15], ICP-AES[16,17] and ICP-MS[1,11,18].

Chemical speciation of arsenic in solid materials requires an extraction in which the reagent does not alter the oxidation state of arsenic. Classic wet ashing methods require some hours to ensure a complete digestion. In order to speed up these digestions, microwave energy has been applied to the analysis of arsenic species in marine samples[19–22].

EXPERIMENTAL

Reagents

Stock solutions of As(III), As(V), MMA and DMA (1000 $mg.L^{-1}$) were prepared by dissolving As_2O_3, $Na_2HAsO_4.7\ H_2O$, $CH_3AsO(ONa).6\ H_2O$ and $(CH_3)_2AsO(ONa).3\ H_2O$, respectively, in ultrapure water.

Exchange resins Dowex 50-X8 and Dowex 1-X8, 100–200 mesh, were used. All other chemicals were reagent grade.

Instrumentation

A Perkin-Elmer model 2380 Atomic Absorption Spectrophotometer equipped with a MHS-10 Perkin-Elmer Hydride Generator was used. An As hollow-cathode lamp (Perkin-Elmer) operated at 6W was used as the source. Absorption was measured at the 193.7 nm line with a spectral band pass of 0.7 nm.

For microwave digestions, a microwave oven (Model 1200 MLS, Milestone) coupled with an automatic closer dispositive (acm 100) was used.

Analytical Procedure

Sample Digestion

A new digestion method for total arsenic determination was optimized, verifying that it is adequate for arsenic speciation in bivalve mollusca. We have also checked that the digestion procedure proposed does not change the oxidation state of the arsenic species by studying its recoveries. Table I shows the digestion methods investigated.

TABLE I Digestion methods

Method	*Acid/S*	*Volume*	*Time*	*Energy*
1	HNO_3	7 mL	12 hours	—
2	HNO_3/HCl (3:1)	7 mL	12 hours	—
3	HCl	6 mL	3 hours	Boiling Water
	H_2O	2 mL		
4	HNO_3/HCl (1:1)	7 mL	12 hours	—
5	H_2SO_4/HNO_3 (1:4)	7 mL	2 hours	Heater (80–90°C)
6	HCl	2 mL	8 minutes	Microwave Oven
	HNO_3	3 mL		
7	HNO_3	4 mL	14 minutes	Microwave Oven

To compare the results obtained with the studied methods, the recovery of total arsenic was calculated. The highest recovery was obtained with method 2 (98%) followed by method 7 (81%), method 4 (78%), method 6 (53%), method 5 (47%), method 3 (31%), and method 1 (30%). Method 6 gives a worse recovery than classic wet digestions due to the high organic matter content in the samples.

Some modificatios were made to improve method 2. To this end, the proportion of the acid mixture (HNO_3/HCl) was varied obtaining the highest recovery (98%) with the HNO_3/HCl 3:1.5 mixture (see Table II).

We have also varied the volume of acid mixture, testing 5.5 and 8.5 mL. The recoveries obtained were smaller than those obtained with the original method 2 (75 and 82%, respectively). Therefore, it was concluded that method 2 is the best for sample digestion.

Chromatographic Separation of Arsenic Species

As(III), As(V), MMA and DMA were separated by the method proposed by González et al.[23]. The method consists on the separation of As(III) from As(V), MMA and DMA on the acetate-form of Dowex 1-X8 (100–200 mesh) ion-exchange resin in a 0.7 × 15 cm column glass. The resin in the column was converted into the acetate form by passing 5 mL of 1M sodium hydroxide

TABLE II Arsenic recoveries obtained varying the proportion of the acid mixture HNO_3/HCl

Acid Mixture (HNO_3/HCl)	*3:1.5*	*3.5:1*	*4:1*	*3:2*
Recovery (%)	98	87	68	75

through, followed by 10 mL of 4M acetic acid. The sample was then passed through the column, followed by 5 mL of 1.5M ammonia and 5 mL of hydrochloric acid, 10 mL of effluent being collected. As(III), when present, was found in this fraction. The DMA remained on the resin.

As(V) and MMA were separated on H^+-form Dowex 50-X8 ion-exchange resin (100–200 mesh) in a 0.7 × 15 cm column glass. The resin in the column was converted into the H^+ form by passing 30 mL of hydrochloric acid. The sample was then passed through the column, followed by 13 mL of hydrochloric acid and 10 mL of water. As(V), when present, was eluted in the first fraction. MMA, when present, was eluted in the second fraction.

In order to avoid a decrease in the signal, the samples must be measured in 4–6 hours time.

Mollusca are known to contain large amounts of arsenobetaine and other arsenosugars, being the predominant species in these matrices. In order to prove if arsenobetaine was released with the digestion procedure used, several samples were spiked with different amounts of this species. They were subjected to the digestion procedure described before, and analyzed by HG-AAS. It was proved that arsenobetaine was not transformed into hydride. So, we assume that the sum of As(III), As(V), MMA and DMA contents are equal to the total arsenic content.

Arsine Generation

As(III), As(V) and MMA were reduced quantitatively to their respective hydrides and the concentration of DMA was calculated by difference. 32% hydrochloric acid was used to give an acid medium, and 3% sodium borohydride (prepared in 1% sodium hydroxide) was used as reductant.

Linearity

The arsenic species can be determined in the concentration range 0–30 $\mu g.L^{-1}$ for As(V) and MMA, and 0–35 $\mu g.L^{-1}$ for As(III). The calibration graphs were similar to those normally found in atomic absorption spectroscopy, being linear over the ranges mentioned with curvature at higher levels.

RESULTS AND DISCUSSION

The method proposed has been applied to the analysis of ten bivalve mollusca purchased at local markets of La Coruña (NW Spain) in January 1994. Samples

were opened, washed with tap and ultrapure water, and the soft part triturated, frozen and lyophilized. Lyophilized samples were sieved through a 0.5 mm mesh to obtain a good homogenization.

Figure 1 shows total arsenic, As(III), As(V) and DMA concentrations in the studied samples. The samples studied were: S1 (*Cerastoderma edule*), S2 (*Venerupis rhomboideus*), S3 (*Venerupis semidecussata*), S4 (*Ostrea edulis*), S5 (*Spisula solida*), S6 (*Italian venerupis*), S7 (*Solen ensis*), S8 (*Pecten maximus*), S9 (*Pecten varius*) and S10 (*Mytilus galloprovincialis*).

The concentrations found ($\leq$4 μg As.g^{-1}) are lower than others found in the bibliography for fish analysis[2,24]. Luten et al.[24] found in fishes from different regions of North Sea, arsenic concentrations between 3 and 166 μg.g^{-1}. The UK Ministry of Agriculture, Fisheries and Food (MAFF)[2] reported results in the range 1–20 μg.g^{-1}. Besides Luten et al.[24] confirmed that the arsenic content for the same specie of fish varies with the location of the catch.

The low values found can be due to the fact that these samples were bought at local markets and came from a depuration process.

The results obtained agree with Branch et al.[8] who found very low DMA concentrations and MMA concentrations lower than the detection limit.

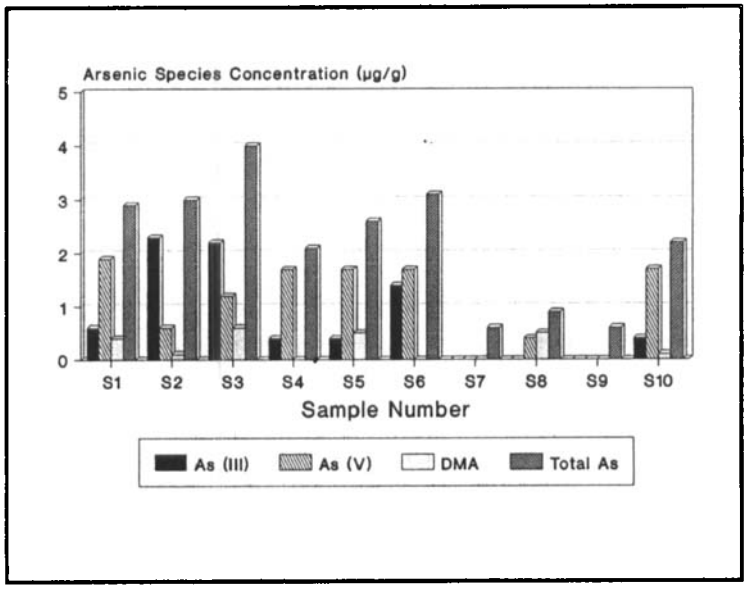

FIGURE 1 Total As and As species levels in the studied samples.

For As(III), the highest values were found for *Venerupis rhomboideus* and *Venerupis semidecussata* (2.3 and 2.2 respectively), being the As(III) levels lower than the detection limit for *Solen ensis*, *Pecten maximus* and *Pecten varius*. All samples showed very similar values of As(V), being the levels found lower than the detection limit for *Solen ensis* and *Pecten varius*.

DMA concentrations were calculated by difference between total arsenic and the sum of As(III) and As(V) contents, because MMA concentrations were lower than the detection limit in all samples. The highest concentrations were found for *Venerupis semidecussata* (0.6), *Spisula solida*, and *Pecten maximus* (0.5).

It was not possible the determination of DMA in *Solen ensis* and *Pecten varius* because all arsenic species had concentrations lower than the detection limit. For *Ostrea edulis* and *Italian clam*, the sum of As(III) and As(V) was equal to total As values, then the DMA level was not calculated for these samples.

MMA concentrations were lower than the detection limit in all the studied samples.

Quality Parameters

Sensitivity

This was evaluated using the detection and quantification limits. The obtained values are shown in Table III.

Precision of the Spectrophotometric Measurement

In order to estimate the precision of the spectrophotometric measurement, the coefficient of variation of ten measurements of a sample was calculated as 2.7%.

Precision of the Analytical Method

It was evaluated calculating the coefficients of variation of ten aliquots of a sample that were subjected to the procedure of analysis proposed. The %C.V.

TABLE III Limits of detection (L.O.D.) and quantification (L.O.Q.) for arsenic species

Species	*L.O.D.* ($\mu g.g^{-1}$)	*L.O.Q.* ($\mu g.g^{-1}$)
Total Arsenic	0.23	0.33
As(III)	0.31	0.51
As(V)	0.26	0.49
MMA	0.28	0.56

obtained were: 2.3% for Total As, 2.5% for As(III) and 4.5% for As(V). It was not possible to determine the coefficient of variation for MMA because none of the samples had a MMA content higher than the detection limit.

Accuracy

In order to investigate the accuracy of the analytical method for total arsenic determination, six replicate determinations for a BCR Reference Material (Mussel Tissue CRM 278) were made. This material has a certified arsenic content of 5.9 ± 0.2 $\mu g.g^{-1}$. The results obtained were submitted to a t-Student's test[25], obtaining a calculated t value of 1.63 lower than the critical t value$_{(2\ tailed,95\%,n=4)}$ 2.78. Therefore, it was concluded than the method for Total Arsenic is accurate.

High and low concentration addition recoveries were also studied for total arsenic (as As(V)) after the addition of 20 $\mu g.L^{-1}$ and 10 $\mu g.L^{-1}$, respectively. The results were 97% for high level additions and 101.4% for low concentrate additions.

In order to investigate the accuracy of the analytical method followed for the determination of each arsenic species, we have studied the recovery of the species. Recoveries of high and low additions of As(III), As(V) and MMA were investigated after the addition of 20 $\mu g.L^{-1}$ and 10 $\mu g.L^{-1}$. The results obtained were 103% for high addition and 97.2% for low addition for As(III), and 100% in the two cases for As(V). It was not possible the evaluation of the recovery for MMA because none of the samples had a MMA content higher than the detection limit.

Acknowledgements

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