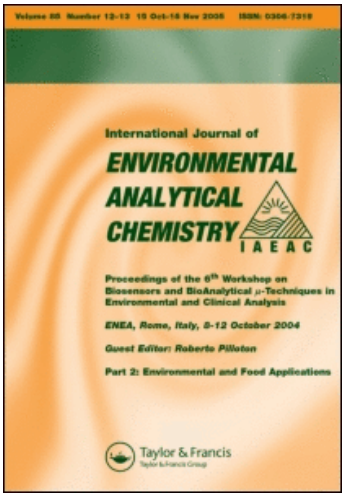

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Separation and Detection of Arsenic and Selenium Species in Environmental Samples by HPLC-ICP-MS

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SEPARATION AND DETECTION OF ARSENIC AND SELENIUM SPECIES IN ENVIRONMENTAL SAMPLES BY HPLC-ICP-MS

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A method is presented for arsenic speciation analysis of an oyster sample using ion chromatography coupled with an inductively coupled plasma mass spectrometry (ICP-MS) instrument. A strong anion exchange resin was employed with a step gradient elution of 0.1 mM/0.1 M K_2SO_4 at pH 10.2. Arsenobetaine and dimethylarsinic acid were determined following extraction based on trypsin enzymolysis with 95–100% extraction efficiency. Limits of detection in the range 0.1–0.3 mg kg^{-1} of arsenic were obtained for organic arsenic species. No inorganic arsenic was detected. Validation was performed using TORT-2 as a certified reference material.

Although high performance liquid chromatography (HPLC) coupled to ICP-MS is an effective method for speciation analysis it is not always necessary to obtain such a detailed picture. A simple liquid chromatographic separation technique based upon mini-column technology is presented. It was developed to obtain a fast, efficient and reliable separation of inorganic from organic, i.e. assumed toxic from non-toxic, arsenic and selenium species suitable for use as an initial screening method for environmental analysis. Two types of strong anion exchange resin were tested. Excellent separation was obtained for both min-column resins and analysis times were within 7 min. Limits of detection obtained for inorganic arsenic, organic arsenic, selenomethionine, Se^{IV} and Se^{VI} were 1.6, 1.8, 66, 32 and 22 μg kg^{-1}, respectively.

Keywords: Arsenic; Selenium; Speciation; High performance liquid chromatography; Mini-column; ICP-MS

INTRODUCTION

Trace metal or elemental speciation has become an area of increasing interest in analytical chemistry. The significance in understanding the toxicological, nutritional and biochemical impact of any element on a biological system depends on the chemical forms present. It is, therefore, essential to determine selectively individual species in a sample under investigation. Qualitative and quantitative speciation analysis together with total elemental determinations will provide an advanced analysis. Speciation-related activities reported in the literature can generally be regarded as demonstration of the ability to determine levels of an elemental species in a sample [1], exploratory investigations aimed at identifying unknown species in a sample [2] and the use of the combined information towards further understanding of biogeochemical cycling through the environment.

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Arsenic is ubiquitous element in the environment, having been introduced via natural and anthropogenic routes. It is an element that has been the subject of many studies as it is a known toxin. Inorganic arsenic is assimilated into many organisms where biotransformations give rise to the abundance of arsenical species seen. Inorganic forms of arsenic, As^{III} and As^{V}, are the most toxic. Organic species display decreasing toxicity with an increasing degree of substitution [3]. Seafood is known to be the most significant source of arsenic in the diet [4]. However, it is generally present as arsenobetaine, which is considered to be harmless [5]. However, exposure to certain arsenic species can give rise to mutagenic, teratogenic and carcinogenic effects [6]. Thus methods capable of separating and detecting specific chemical forms of arsenic are necessary for accurate risk assessment studies.

Selenium is widely recognised as both a toxic and an essential element depending on its concentration. Clinical studies by Clark *et al.* [7] found that dietary supplementation with selenium-enriched yeast significantly decreased the incidence of colorectal, lung and prostate cancers. This has given rise to the popularity of taking additional selenium in the diet. The absorption, metabolic fate and anti-carcinogenic activity of selenium are closely bound to the chemical form of the element. Selenium is easily absorbed in both inorganic and organic forms but their detailed metabolic pathways and toxicity in the human body are not yet fully elucidated. For example, it has been suggested that selenomethionine is less toxic than the inorganic forms of the element [8]. The uncertainty surrounding selenium, therefore, indicates the need for further research into the chemical speciation of this element.

Analytical data on the individual molecular species of an element can be aquired by ICP-MS when coupled to a suitable separation technique. Chromatography provides a powerful tool in this respect because of its inherent versatility. The main separation techniques based upon HPLC for arsenic and selenium species are ion exchange and reversed-phase. Both of these techniques are widely documented [9–11]. This paper describes the use of ion exchange media using both mini-columns and HPLC coupled with ICP-MS to separate various arsenic and selenium species. In many cases the full separation of species using HPLC means that only a few samples can be analysed in a given time frame. The development of a simple to operate, rapid yet efficient, system to detect and measure environmentally important arsenic and selenium species for use as an initial screening method is also presented. The advantages of the low pressure system are discussed.

EXPERIMENTAL

Instrumentation

Measurements were performed using a VG Plasmaquad 3 ICP-MS (T.J.A. Solutions, Winsford, Cheshire, UK) fitted with an Ebdon nebulizer (P.S. Analytical, Kent, UK), a double-pass Scott-type spray chamber cooled to 5°C and a Fassel torch with a 1.5 mm bore injector.

The chromatographic separation utilised a Waters 6000 A pump (Waters Associates, Milford, MA, USA) fitted with a Rheodyne 7125 (Rheodyne, Cotati, CA, USA) injection valve and 100 µl sample loop.

The pH of solutions was determined using a Hanna H19025 pH meter (Hanna Instrumentation, Singapore).

Materials and Methods

Samples of a CRM, TORT-2 (lobster hepatopancreas, supplied by the National Research Council, Canada), for method validation together with oyster tissue A and B supplied for a collaborative trial in a freeze dried and lyophilized form were prepared for total arsenic and arsenic species analysis. All commercial chemicals were of analytical grade and used without further purification. Sodium selenate, sodium selenite, selenomethionine, selenocystine, arsenous acid, arsenic acid, dimethylarsinic acid (Aldrich Chem. Co., Poole, Dorset), monomethylarsonic acid (kindly donated by Dr. A. Moreda-Pineiro, University of Santiago de Compostela) and arsenobetaine (BCR) were obtained. Stock solutions of $100\,\mu g\,ml^{-1}$ were prepared using Milli-Q water (Milli-pore, Bedford, MA, USA) and stored in the dark at 4°C. Solutions of the compounds for daily use were prepared by appropriate dilution from the stock solutions. Hydrogen peroxide 30% v/v (Sigma Aldrich) was stored in the dark at 4°C. K_2SO_4, K_2HPO_4 and potassium hydrogenphthlate (Fluka, Poole, UK) solutions were prepared daily using Milli-Q water and pH adjusted using a solution of NH_3 (Merck, Poole, Dorset, UK). Ammonium bicarbonate (Merck) was prepared as required.

Total arsenic was determined following microwave digestion. Samples (approximately 0.25 g, accurate to 0.1 mg) were weighed into poly(tetrafluoroethylene) bombs (Savillex Corporation, Minnetonka, MN, USA) and HNO_3 (69%, 4 ml) and H_2O_2 (30%, 1 ml) were added. The bombs were capped loosely and left overnight to allow easily oxidisable material to be destroyed. After predigestion, the bombs were microwaved on medium power for 1–2 min, or until the sample was a clear colour (indicating a complete digest). The digests were transferred quantitatively into 25 ml volumetric flasks made up to the mark with doubly deionised water. The samples and appropriate standards were spiked with a solution of indium (final concentration, $100\,\mu g\,l^{-1}$) as an internal standard. Analysis by ICP-MS was performed under the operating conditions shown in Table I with the addition of 4% nitrogen to the nebulizer gas flow to remove the spectroscopic interference from $^{40}Ar^{35}Cl^+$ [12,13].

Determination of arsenic species was achieved following an enzymolysis extraction technique. Samples (approximately 0.25 g, accurate to 0.1 mg) were weighed together with bovine trypsin, type III (approximately 0.1 g, accurate to 0.1 mg, Sigma, Dorset, UK) in ammonium bicarbonate solution (10 ml, 0.1 M, pH 8) and mixed in a 'Potter' homogenizer. The solutions were transferred to polyethylene centrifuge tubes and placed in a shaking water bath at 37°C for a minimum of 4 h. The samples were then

TABLE I Instrumental parameters for HPLC-ICP-MS

HPLC System:	
Column-1 guard column (50 × 4.6 mm), 2 columns – 150 × 4.6 mm	
Benson AX10 anion-exchange resin, 7–10 μm particle size.	
Mobile phase – 0.1 mM K_2SO_4 (4.5 min) with a step gradient to 0.1 M K_2SO_4 (10.5 min) @ pH 10.2	
Flow rate – $1\,ml\,min^{-1}$	
Injection volume – 100 μl loop	
ICP-MS Parameters:	
Nebuliser flow rate	$0.811\,min^{-1}$
Coolant gas flow rate	$13.11\,min^{-1}$
Auxiliary gas flow rate	$0.81\,min^{-1}$
Forward power	1350 W
Dwell time	500 ms (HPLC only)

centrifuged twice, with washings, at 2500 rpm for 20 min and transferred quantitatively to 50 ml volumetric flasks made up in ammonium bicarbonate buffer. The extracts were analyzed by HPLC-ICP-MS using a method described by Branch *et al.* [14]. Experimental conditions are also given in Table I. As part of the mass balance, the total arsenic in the extracts was determined by ICP-MS with 4% nitrogen addition to the nebulizer gas. The samples and appropriate standards were spiked with a solution of cesium (final concentration, $100\,\mu g\,l^{-1}$) as an internal standard. Cesium is more suitable than indium for the extracts as it remains in solution at pH 8 whereas indium is only soluble at a low pH.

RESULTS AND DISCUSSION

Total arsenic and arsenic species found in oyster tissues A and B together with TORT-2 are shown in Table III. All data reported have been corrected for moisture content and blank values. TORT-2 was chosen as the CRM as it most closely matched the samples under investigation. This gave a total arsenic value following trypsin digestion of $22.64 \pm 3.61\,mg\,kg^{-1}$ and following acid digestion of $21.08 \pm 2.06\,mg\,kg^{-1}$ which is comparable to the certified value of $21.6 \pm 1.8\,mg\,kg^{-1}$. This suggests that the trypsin and acid digestion methods were effectively 100% efficient. The predominant arsenic compound in sample A was found to be DMA although this species was undetected in sample B. For TORT-2 and oyster tissue B, AsBet was found to be the predominant arsenic compound. AsBet would be expected to be the most abundant arsenic compound present in marine organisms such as fish or shellfish in keeping with current understanding of biogeochemical cycling of arsenic through the environment [15]. The presence of elevated levels of DMA was, in this case, due to spiking of the sample by the trial co-ordinators. Inorganic arsenic was not detected in any of the samples. When examining the data on a mass balance basis some of the total arsenic found in the extract is unaccounted for. One suggestion is that unknown arsenic species may be present. Another suggestion is that some inorganic arsenic species are present but remain undetected due to losses on the chromatographic column.

There are many certified reference materials available for 'total' element concentrations but few are certified for their elemental species. In this analysis, experimental values for total arsenic were in good agreement with CRM values. This gives confidence in the results obtained for the total arsenic in oyster tissues.

Further to arsenic speciation by HPLC-ICP-MS, a technique based upon ion exchange liquid chromatography with low-pressure mini-columns was developed. Preliminary findings suggest that separation of inorganic from organic or toxic from non-toxic arsenic and selenium species can be achieved. To arrive at the separation of inorganic from organic arsenic and the 4 selenium species, Se^{IV}, Se^{VI}, selenomethionine (SeMet) and selenocystine (SeCys). Using mini-column technology, a range of experimental conditions were considered. Three eluent counter ions, phthalate, sulphate and phosphate, each in the concentration range 5 mM–0.1 M were examined. Conditions of pH ranging from 4–10 and eluent flow rates in the range of $1–2\,ml\,min^{-1}$ were tested. Two resins, Benson AX10 (Benson, Reno, Nevada, USA) and Hamilton PRP X100 (Phenomenex, Cheshire, UK), were assessed for suitability in micro-columns of size ranging from $25 \times 3 - 100 \times 3$ mm. Both resins are strong anion exchangers based upon styrene-divinylbenzene polymers. Hydrogen peroxide

(30% v/v, 0.25 ml in 25 ml) was added to arsenic standards in order to oxidise As^{III} to As^{V} allowing the inorganic components to elute together. The presence of the H_2O_2 did not appear to alter any of the other standards tested although further tests involving real samples are necessary to fully assess the impact of the H_2O_2. Simultaneous separation of As and Se was achieved using Benson AX10 anion exchange resin using a gradient elution of Milli-Q water followed by 0.1 M potassium hydrogen phthalate (see Table II). A typical chromatogram of the separation can be seen in Fig. 1. Excellent separation was obtained within 4.5 min for arsenic and 6 min for selenium. It is seen that the organic (AsBet, DMA) and inorganic arsenic (As^{III}, As^{V}) species are well separated within their groups. It is noteworthy that the column not only separated organic from inorganic selenium but that the selenium species shown give individual peaks. Resolution to within 10% of the baseline was obtained under the conditions employed (Fig. 2).

Using the Hamilton mini-column, separation of organic from inorganic arsenic species was achieved using an isocratic elution with 10 mM K_2SO_4 at pH 10.2 (see Table II). Again, all the selenium species under investigation were resolved under the same conditions for the separation of organic from inorganic arsenic species (Fig. 3). Limits of detection obtained for total inorganic arsenic, total organic arsenic, selenomethionine, Se^{IV} and Se^{VI} were 1.6, 1.8, 66, 32 and 22 $\mu g\,kg^{-1}$, respectively.

The Hamilton PRPX100 column gave a shorter analysis time (approximately 3 min) for the separation of the arsenic compounds compared with the Benson AX10.

TABLE II Chromatographic parameters for mini-column separation of arsenic and selenium species

Mini-column System for arsenic and selenium separation
Resin: Benson AX10, 7–10 μm particle size,
Column diameters: 25 mm × 3 mm
Mobile phase: Milli-Q water (2 min) followed by 0.1 M potassium hydrogen phthalate (5 min) pH 10.2
Flow rate: 1.4 ml min^{-1}
Resin: Hamilton PRPX100, 12–20 μm particle size,
Column diameters: 100 mm × 3 mm
Mobile phase: 10 mM potassium sulphate, pH 10.2
Flow rate: 1.0 ml min^{-1}

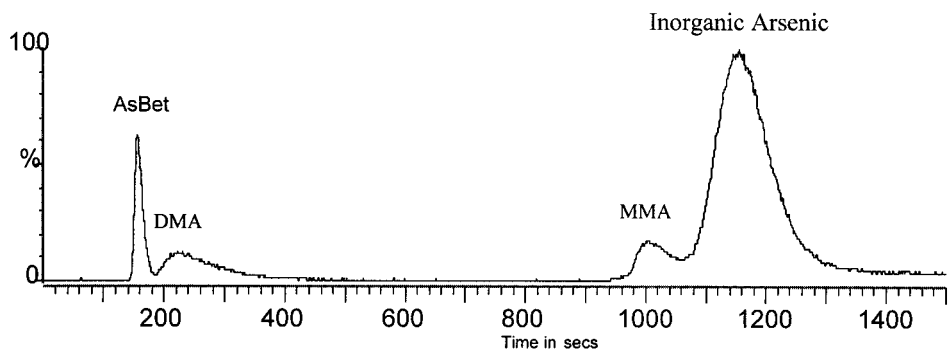

FIGURE 1 Typical chromatogram demonstrating the separation of 4 arsenic standards at 100 $\mu g\,l^{-1}$ under the given experimental conditions (Table I) for HPLC.

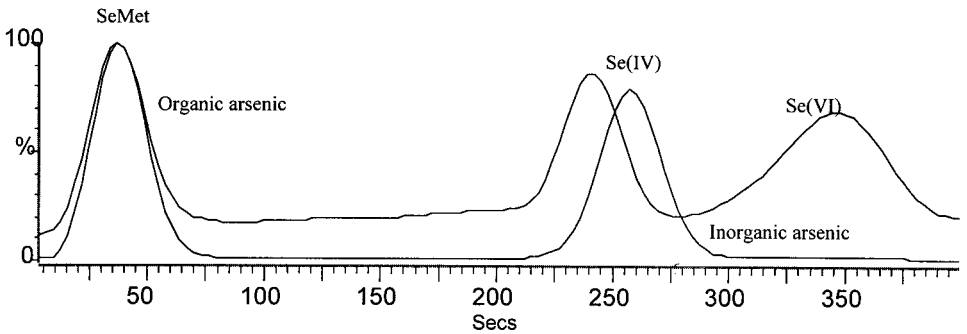

FIGURE 2 Mini-column separation using Benson AX10 resin for the separation of organic from inorganic arsenic species and selenomethionine, Se (IV) and Se (VI) (standards at $200\,\mu g\,l^{-1}$).

TABLE III Total arsenic by HNO_3 microwave extraction and arsenic species extracted by trypsin digest. All results in $mg\,kg^{-1}$ in terms of arsenic

Sample	*Total arsenic (HNO_3)*	*AsBet*	*DMA*	*Total arsenic (Trypsin)*
TORT-2	21.08 ± 2.06	17.5	0.95	22.64 ± 3.61
Oyster tissue A	NP	6.14 ± 0.52	41.51 ± 3.61	63.78 ± 1.57
Oyster tissue B	9.14 ± 0.24	7.6 ± 1.13	ND	NP
LOD's	0.11	0.17	0.29	0.21

Note: Certified value for TORT-2: Total arsenic $21.6 \pm 1.8\,mg\,kg^{-1}$; ND = Not detected; NP = Not performed.

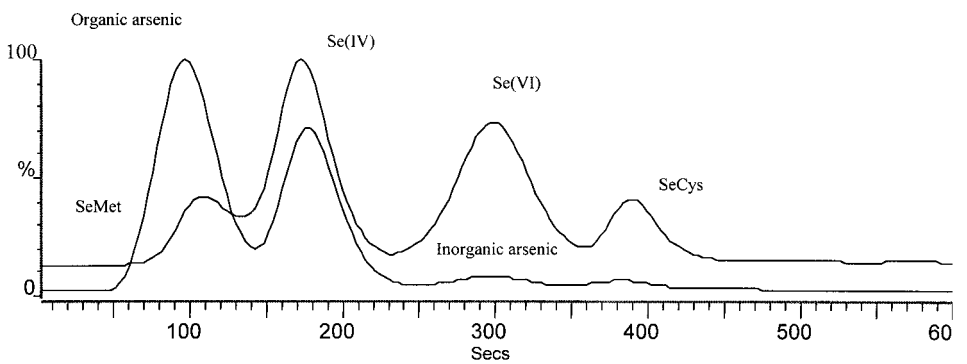

FIGURE 3 Mini-column separation Hamilton PRPX100 resin for the separation of organic from inorganic arsenic species and selenomethionine, Se (IV) and Se (VI) (standards at $200\,\mu g\,l^{-1}$).

The elution times for the detection of the selenium compounds are similar for both column types. The use of isocratic conditions with the Hamilton PRPX100 resin enable more rapid analysis as no column equilibration needs to be employed between injections. The use of the mini-column systems for the rapid screening of arsenic and selenium compounds upon the basis of their effect-related species, e.g. inorganic, organic-toxic, non-toxic species, is noted.

The optimum experimental conditions derived for both column systems under investigation provided the best separation whilst keeping the total solids in the mobile phase

to a minimum thereby reducing interferences caused by salt deposition in the ICP-MS torch injector or sampling cone orifice.

CONCLUSIONS

HPLC-ICP-MS is a successful and widely implemented technique used in speciation analysis. The technique is specific and sensitive and has been demonstrated that it is suitable for arsenic speciation determination.

The work involving micro-column technology has demonstrated its ability to separate inorganic from organic forms of arsenic and selenium, i.e. assumed toxic from non-toxic species, in a fast, simple and efficient way. This provides a cost-effective screening protocol for regulatory agencies when assessing environmental pollutants as it can eliminate a further speciation analysis step or identify certain samples for further analysis. Work will continue in refining the technique and extending the method for application in real environmental samples.

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