APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2002; **16**: 437–445
Published online in Wiley InterScience (www.interscience.wiley.com). DOI:10.1002/aoc.316

Applications of liquid chromatography–electrospray ionization-single quadrupole mass spectrometry for determining arsenic compounds in biological samples†

Kevin A. Francesconi*

Institute of Biology, University of Southern Denmark, 5230 Odense M, Denmark

Received 18 January 2002; Revised 13 February 2002; Accepted 20 February 2002

Recent work on the determination of arsenic compounds in biological samples using liquid chromatography–electrospray ionization-single quadrupole mass spectrometry is reviewed. Specific examples include the quantification of arsenosugars in algae, confirmation of novel arsenic metabolites from marine microbes and in human urine, and the structural elucidation of two new arsenic compounds in marine animals. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: LC–electrospray ionization-MS; speciation; arsenic compounds; arsenosugars; arsenobetaine

INTRODUCTION

Molecular mass spectrometric techniques for determining environmental arsenic compounds were first carried out in 1983 by Luten *et al.*,[1] who used field desorption and fast atom bombardment ionization to identify arsenobetaine (Fig. 1) in fish and shrimp after its extraction and partial purification. In 1988, Siu *et al.*[2] reported the use of electrospray ionization for the mass spectrometric determination of organoarsenic compounds, and this ionization source seemed particularly suitable for the small polar arsenicals commonly encountered in environmental samples. The electrospray ionization source also had the advantage of being an excellent interface for coupling liquid chromatography (LC) with mass spectrometry (MS), because it can transfer ions in solution directly to the gas phase at low energy. Such a coupling, with a triple quadrupole MS, was first reported by Siu *et al.*[3] in 1991 to determine arsenobetaine in fish. This technique, LC–electrospray ionization-tandem MS, has subsequently been used for determining arsenic compounds in biological samples in several studies.[4–10] Among those, the investigation by Corr and Larsen[4] was particularly interesting: they used high orifice potentials in the ionization source to generate bare As^+ ions, and thus they were able to obtain elemental arsenic and molecular mass data on sequential chromatographic runs of the one sample.

The use of a single quadrupole mass analyser as an LC detector for arsenic compounds is restricted by sample matrix, and its application has been limited.[11] However, the approach of Corr and Larsen,[4] whereby arsenic ions are produced in the electrospray ionization source, greatly extends the capability of single quadrupole mass analysers as detectors for arsenic species. Over the last 2 years, LC–electrospray ionization-MS with a single quadrupole mass analyser has been used in several experiments to determine known arsenic metabolites, and in two investigative studies where novel arsenicals were identified. This paper summarizes those studies and aims to highlight the strengths and limitations of the method.

BRIEF DESCRIPTION OF THE METHOD AND THE INSTRUMENTATION

Electrospray ionization is able to transfer ionized analyte species in solution into the gas phase by non-energetic processes that efficiently deliver intact entities to the mass analyser.[12] By varying conditions in the electrospray interface, however, collision-induced dissociation may occur, resulting in fragmentation of the parent ion (or charged molecular species), which in turn can provide some structural information about the analyte.[12] These dissociation processes, which take place in the transport region of the ionization source as the potential difference between the exit capillary and the first skimmer plate is increased, are similar

*Correspondence to: K. A. Francesconi, Institute of Chemistry–Analytical Chemistry, Karl-Franzens University, 8010 Graz, Austria.
E-mail: kevin.francesconi@uni-graz.at
†This paper is based on work presented at the 10th International Symposium on Natural and Industrial Arsenic (JASS-10), Tokyo, 29–30 November 2001.
Contract/grant sponsor: Danish Research Council.

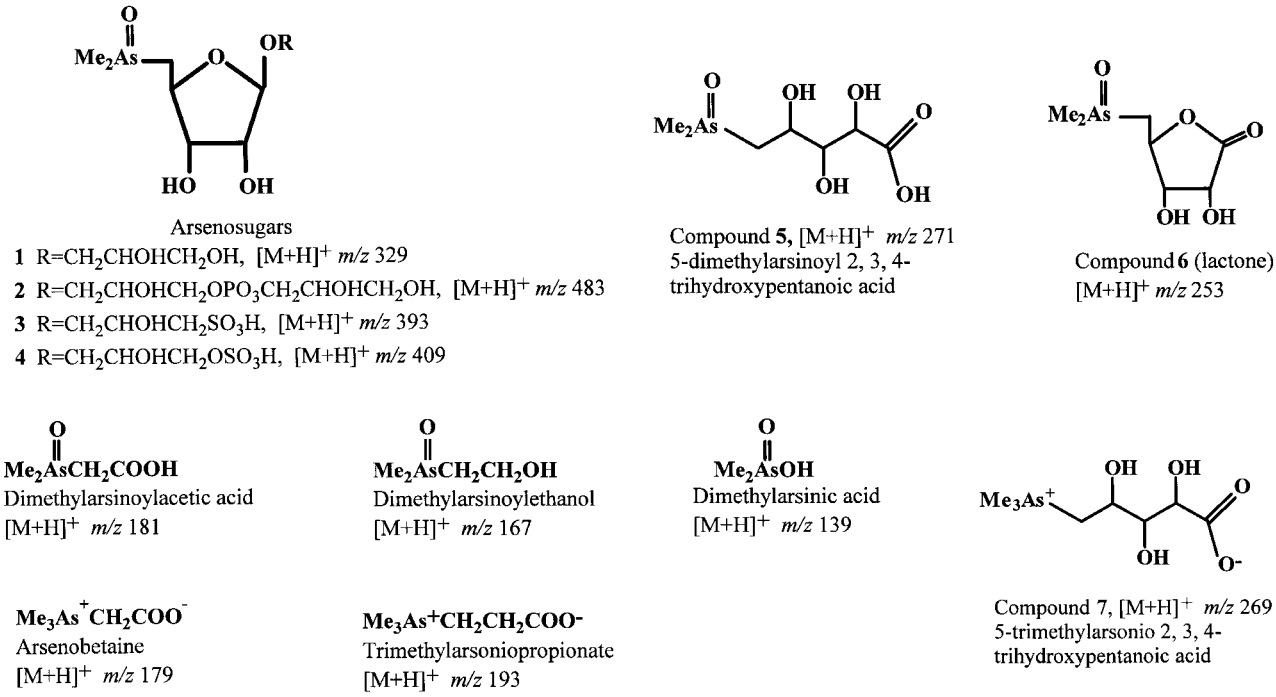

Figure 1. Arsenic compounds determined by LC–electrospray ionization-MS and the *m/z* values of their protonated molecular species.

to those taking place in the collision cell in tandem MS systems. The two techniques have been carefully compared by Voyksner and Pack[13] using pesticides and antibiotics as model compounds. Surprisingly, the single quadrupole MS operated in this manner had some advantages over the tandem MS system (a triple quadrupole instrument). One strength of LC–electrospray ionization-single quadrupole MS noted in that study was that the capillary–skimmer potential difference could be varied rapidly, permitting the acquisition of spectra of intact molecular species (no analyte dissociation) and spectra at increasing dissociation energies within the time frame of an LC peak. This capability has proven valuable when the technique has been applied to arsenic speciation analyses.

Voyksner and Pack also noted[13] that, with a single quadrupole mass spectrometer, a heavy burden is placed on the chromatography to deliver single components to the ionization source. This puts a severe limitation on the method when dealing with environmental samples. The comment of Voyksner and Pack,[13] however, referred to organic molecules (in their case pesticides and antibiotics), and the analysis of organoarsenic compounds by electrospray ionization-MS is less restricted by the chromatography. Exactly why this is so should become apparent in the following.

The arsenic speciation analyses reviewed here were performed on an LC–MS system supplied by Agilent (formerly Hewlett-Packard) comprising a Series 1100 liquid chromatograph and G1946A MSD single quadrupole mass spectrometer equipped with an electrospray ionization LC–MS interface. Full details of the operating conditions are provided in Pedersen and Francesconi.[14] The arsenic compounds were separated by either anion- or cation-exchange LC; conditions and mobile phases are recorded in the legends to the figures showing the separations. The compounds eluting from the column were introduced to the gas phase by electrospray ionization. The potential difference between the capillary exit and the first skimmer plate, termed fragmentor voltage, was varied to implement different degrees of dissociation in the analytes. At low voltages (70 V), dissociation is minimal and a high proportion of the analyte is transported to the mass spectrometer as the intact molecular species. As the fragmentor voltage is increased, the analyte begins to fragment and characteristic product ions are formed. At high fragmentor voltages (>200 V), the original molecular species is completely degraded and bare As^+ ions (*m/z* 75) become the dominant analyte species. This process, however, is not very efficient, and the intensity of the As^+ ions is only about 5–25% of that for the intact molecular species. The ions and molecular species were measured by selected ion monitoring in the positive ion mode.

The instrumentation is capable of rapidly changing the fragmentor voltage, thereby enabling the (essentially) simultaneous acquisition of molecular mass data and elemental arsenic data (and that of several characteristic fragments) on a single chromatographic run. This feature, and the information it can provide, are illustrated for the separation and identification of four standard arsenosugars

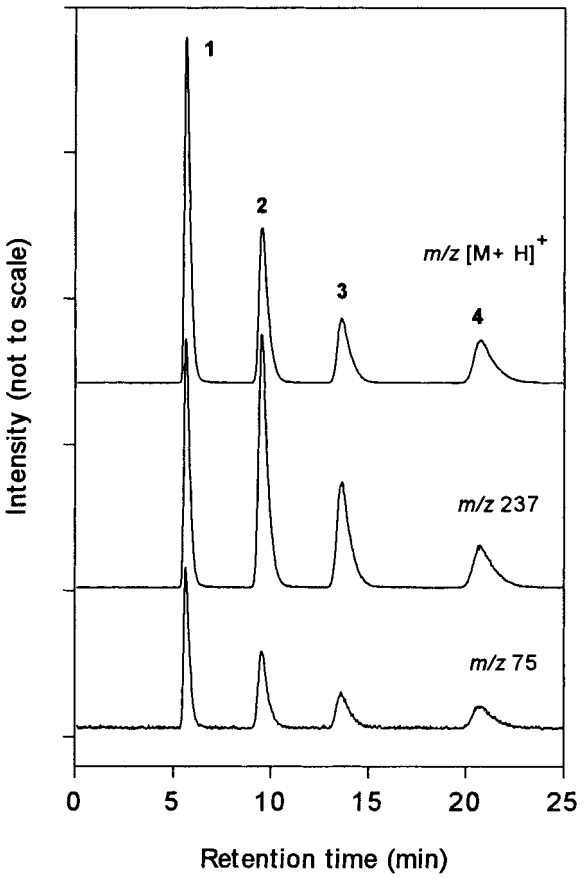

Figure 2. LC–electrospray ionization-MS chromatograms of four arsenosugar standards (each 27 pmol compound ≡ 2 ng arsenic) measured at *m/z* 75, *m/z* 237, and *m/z* $[M + H]^+$. Chromatography was performed using a PRP-X100 anion-exchange column (250 mm × 4.1 mm) and a mobile phase comprising a mixture of 20 mM aqueous NH_4HCO_3, pH 10.3 (adjusted with aqueous ammonia), and methanol (9 + 1 v/v) at 30 °C and a flow rate of 0.4 ml min^{-1}. Compounds were detected in variable fragmentor voltage mode (70 V for *m/z* $[M + H]^+$; 130 V for *m/z* 237; and 240 V for *m/z* 75). Signals for the three *m/z* values are presented to facilitate comparison of retention times and are not to scale; relative intensities are contained in the original report.[14]

1–4 (Fig. 2). The separation was performed on an anion-exchange column at pH 10.3, and it is of interest that the arsenosugars **2, 3**, and **4** readily produce protonated molecular species under such conditions, even though they enter the ionization source as anions. Similar cases of 'wrong-way-round' electrospray ionization, the mechanism for which is uncertain, have been reported for amino acids.[15] The ion at *m/z* 237 was first noted by Corr and Larsen[4] to be characteristic of arsenosugars, and it can assist in detecting this group of compounds. The generation of the *m/z* 75 signals deserves further comment. This has been assigned as bare As^+ ions, but non-arsenic fragments of *m/z* 75 might also be possible.[14] It was considered unlikely, however, that carbon-based fragments would remain intact under such strong ionization conditions. The fragmentation patterns for arsenosugars tended to support this view, because the *m/z* 75 signal began appearing at fragmentor voltage 160 V and was the dominant ion at 200 V with no other significant fragments present.[14] Although the assignment of the *m/z* 75 signal as bare As^+ must still be used cautiously, especially with crude samples, this signal enables LC–electrospray ionization-MS systems to serve as arsenic-specific detectors in many circumstances.

Another limitation of LC–electrospray ionization-single quadrupole MS is that the generation of As^+ ions is highly dependent on the type of arsenic compound, and some compounds may not produce this ion. For example, in the positive ion mode, arsenite and arsenate did not produce *m/z* 75 ions.[14,16] However, all organoarsenic compounds investigated so far have produced As^+ ions to a significant degree.

QUANTIFICATION OF ARSENOSUGARS IN ALGAE AND OYSTER

LC–electrospray ionization-MS was used to identify and quantify arsenosugars in aqueous extracts of two species of brown algae, *Laminaria digitata* and *Fucus vesiculosis*.[14] These algae were chosen because they represent two algal orders known to have appreciable concentrations of arsenic, and to show clear differences in the arsenic compounds that they contain. The LC–MS chromatograms (Fig. 3) were remarkably free from spectral interferences, and the compounds gave clear signals for *m/z* $[M + H]^+$, *m/z* 237 (characteristic product ion for arsenosugars) and *m/z* 75 (As^+), which matched those for standard compounds in all respects. Thus the identification of arsenosugars **2** and **3** (*Laminaria*) and **2, 3**, and **4** (*Fucus*) in these algae could be made with confidence. The absence of any other sizeable signals at *m/z* $[M + H]^+$ and *m/z* 237 was a surprising result; this probably reflects, at least in part, the high concentration of arsenosugars in these samples. The *m/z* 75 chromatograms were also clean, indicating that, for these sample matrices at least, the high fragmentor voltage was sufficient to decompose essentially all organic compounds. It was also considered likely that the intensity of the arsenosugar $[M + H]^+$ species would be reduced in the sample matrix when compared with standards. However, when concentrations of the arsenosugars in the algal extracts were determined by the method of standard additions, matrix effects were shown to be minimal.

The ability of LC–electrospray ionization-MS to serve as a quantitative detection system for arsenic species was then investigated in a study on arsenosugars in a standardized extract of the brown alga *Fucus serratus*.[17] The data from electrospray ionization-MS agreed well with those from inductively coupled plasma (ICP)-MS, an accepted quantitative method for arsenic speciation analyses (Table 1). The

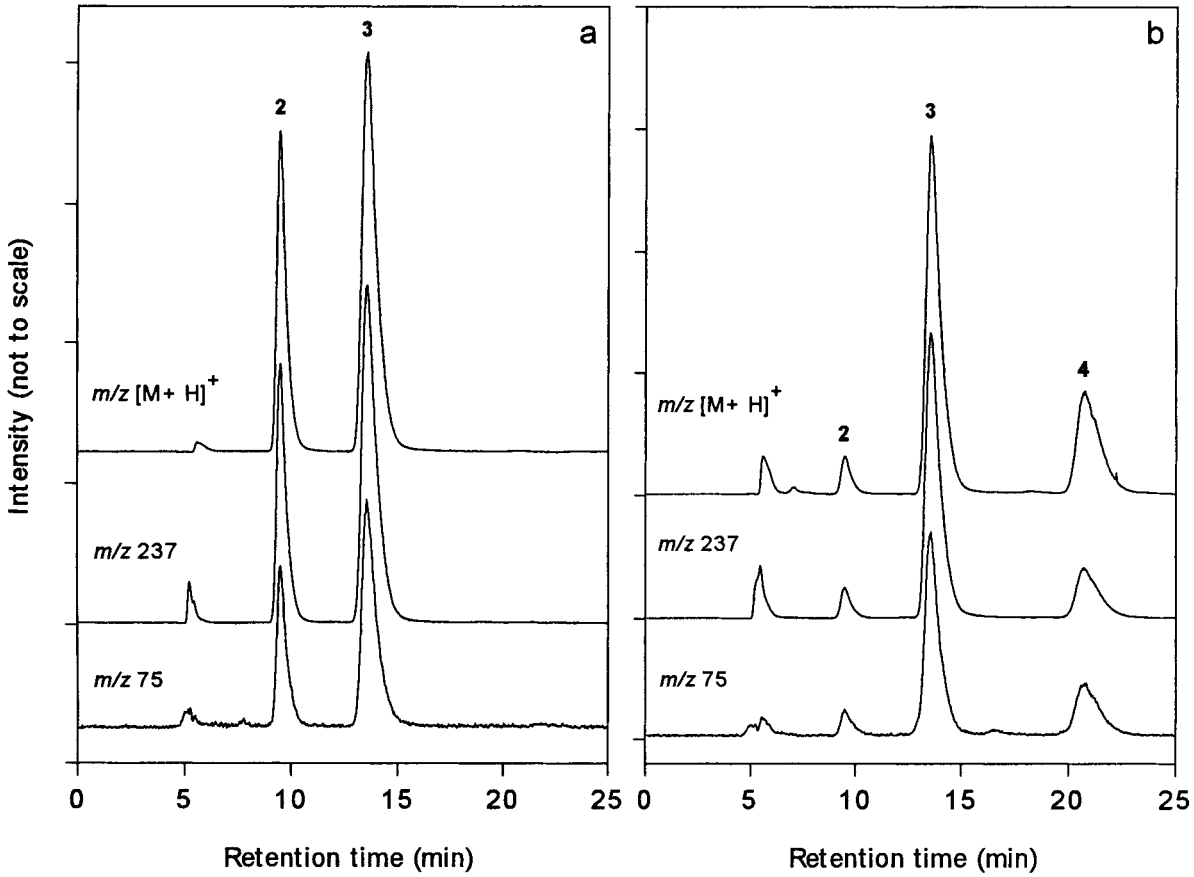

Figure 3. LC–electrospray ionization-MS chromatograms of crude extracts from two species of brown algae: (a) *L. digitata*; (b) *F. vesiculosis*. Conditions were identical with those described in Fig. 2 for standard arsenosugars. Arsenosugar **1** elutes at near the void volume and could not be determined reliably in these algal extracts.

method of standard additions, when applied to the electrospray ionization-MS data, again showed relatively small (<8%) matrix suppression of the signals relative to standard compounds.

Matrix suppression was, however, quite marked for the determination of arsenosugar **2** in an aqueous extract of oyster, *Crassostrea gigas*.[18] The concentration of arsenosugar **2** (as arsenic) in the oyster was 3.3 μg g^{-1} (dry mass), considerably lower than the arsenosugar concentrations in the algae examined previously. For the oyster sample, a clean chromatographic trace was obtained for the $[M + H]^+$ signal at *m/z* 483, but the *m/z* 237 signal could not be resolved from other (non-arsenic-containing) ions (Fig. 4). When the compound was quantified by the method of standard additions (using the peak area of the *m/z* 483 peak), a 50% matrix suppression was observed. To confirm the identification of arsenosugar **2** in the oyster, the compound was partially purified by preparative chromatography. LC–elecrospray ionization-MS on this partially purified sample produced clean chromatograms for *m/z* 483, 237 and 75, identical in all respects with chromatographic data for the standard arsenosugar. Furthermore, there was virtually no matrix suppression of the signals in this partially purified sample. The work with the oyster was of interest because it clearly showed the limitations of LC–electrospray ionization-MS for some sample matrices.

IDENTIFICATION OF ARSENIC METABOLITES IN BIOTRANSFORMATION STUDIES

LC–electrospray ionization-MS was found to be particularly suitable for monitoring the degradation of arsenobetaine by microbes in seawater.[19] A novel metabolite, dimethylarsinoylacetic acid, a transient species in the degradative pathway ending with dimethylarsinic acid, was unambiguously identified in the experiments. The two products could be quantified from their $[M + H]^+$ signals even though they were not fully resolved by the chromatography (retention times 9.48 min and 9.98 min for dimethylarsinic acid and dimethylarsinoylacetic acid respectively). There were early difficulties, however, because dimethlarsinoylacetic acid produces a small product ion with *m/z* 139. Presumably, this product ion is dimethylarsinic acid formed by loss of

Table 1. Comparison of quantitative determinations (LC–ICP-MS and LC–electrospray ionization-MS) of arsenosugars in a standardized extract of a brown alga (*F. serratus*)

Arsenic species	Mean As content (μg) [RSD(%)]	
	ICP-MS	Electrospray ionization-MS
Arsenosugar **1**	0.10 [4.8]	0.088 [21]
Arsenosugar **2**	0.086 [2.9]	0.075 [2.7]
Arsenosugar **3**	0.62 [3.8]	0.57 [2.2]
Arsenosugar **4**	0.40 [3.1]	0.41 [2.6]

CH_2CO and rearrangement in the ionization source; similar processes have been reported for related organoarsenic compounds.[20] Possible spectral overlap of molecular species with product ions is another limitation of LC–MS with a single quadrupole instrument.

The uptake and biotransformation of arsenate in seawater by the brown macroalga *F. serratus* was monitored by LC–MS using both ICP ionization and electrospray ionization.[21] The robustness and universality of ICP ionization was essential to detect reliably all the arsenic metabolites formed, a role that could not be filled by electrospray ionization. Nevertheless, the capability of electrospray ionization-MS to select molecular species of organoarsenic compounds for identification and quantification was valuable. In that study, arsenosugar **2** and methylarsonic acid were not well resolved, so arsenosugar **2** could not be quantified, based on the ICP-MS arsenic measurements, in the presence of the large quantities of methylarsonic acid that were produced by the algae in the early part of the experiments. This problem was overcome with electrospray ionization-MS by selecting *m/z* 483 (the $[M + H]^+$ for arsenosugar **2**), whereby a clean trace was obtained that could be easily quantified.

Recent work[22] examining arsenic metabolites in human

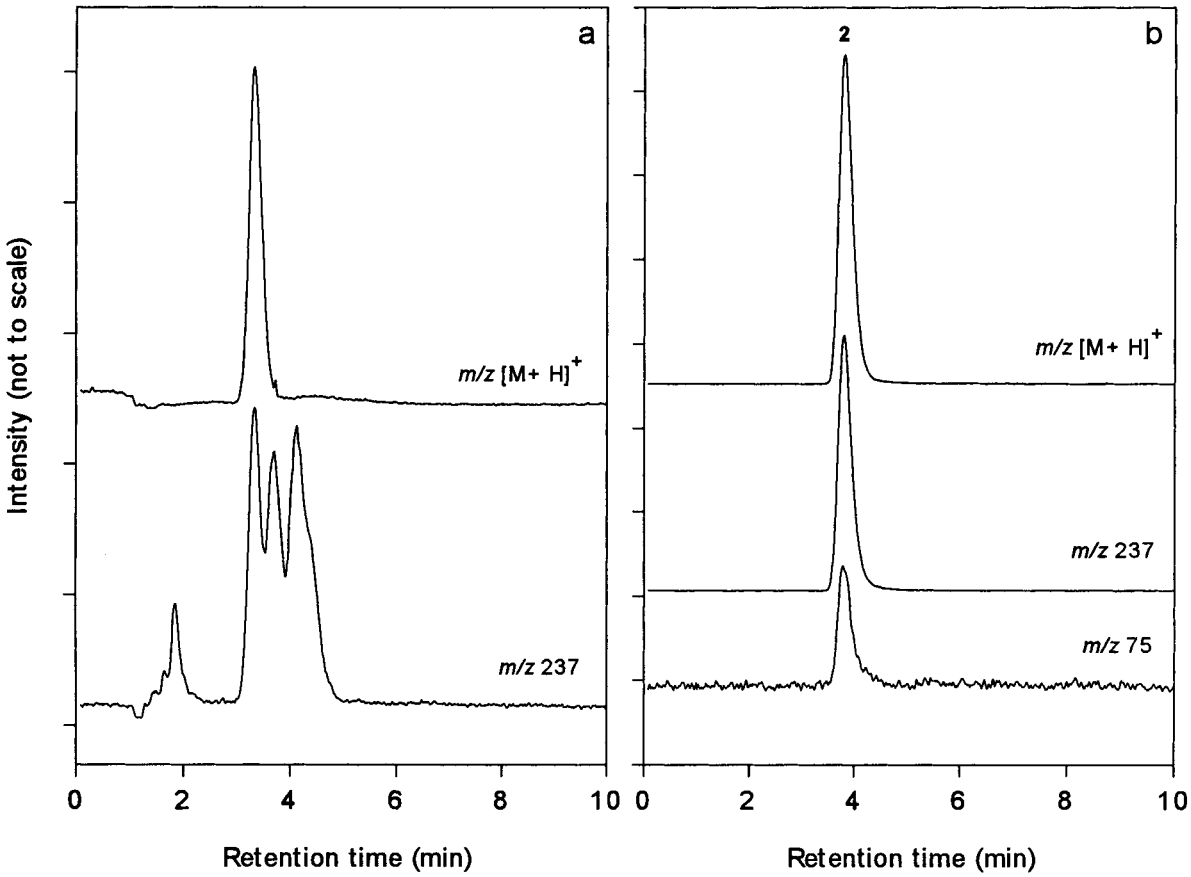

Figure 4. LC–electrospray ionization-MS chromatograms of extracts of the oyster *C. gigas*: (a) crude aqueous extract; (b) extract partially purified by preparative chromatography. Chromatography was performed using a PRP-X100 anion-exchange column (150 mm × 4.1 mm) and a mobile phase comprising a mixture of 20 mM aqueous NH_4HCO_3, pH 9.5 (adjusted with aqueous ammonia), and methanol (7 + 3 v/v) at 30 °C and a flow rate of 1.0 ml min^{-1}. Arsenosugar **2** was detected in variable fragmentor voltage mode: 70 V for *m/z* 483; 130 V for *m/z* 237; and 240 V for *m/z* 75 ((b) only). Signals for the three *m/z* values are presented to facilitate comparison of retention times and are not to scale; relative intensities are contained in the original report.[18]

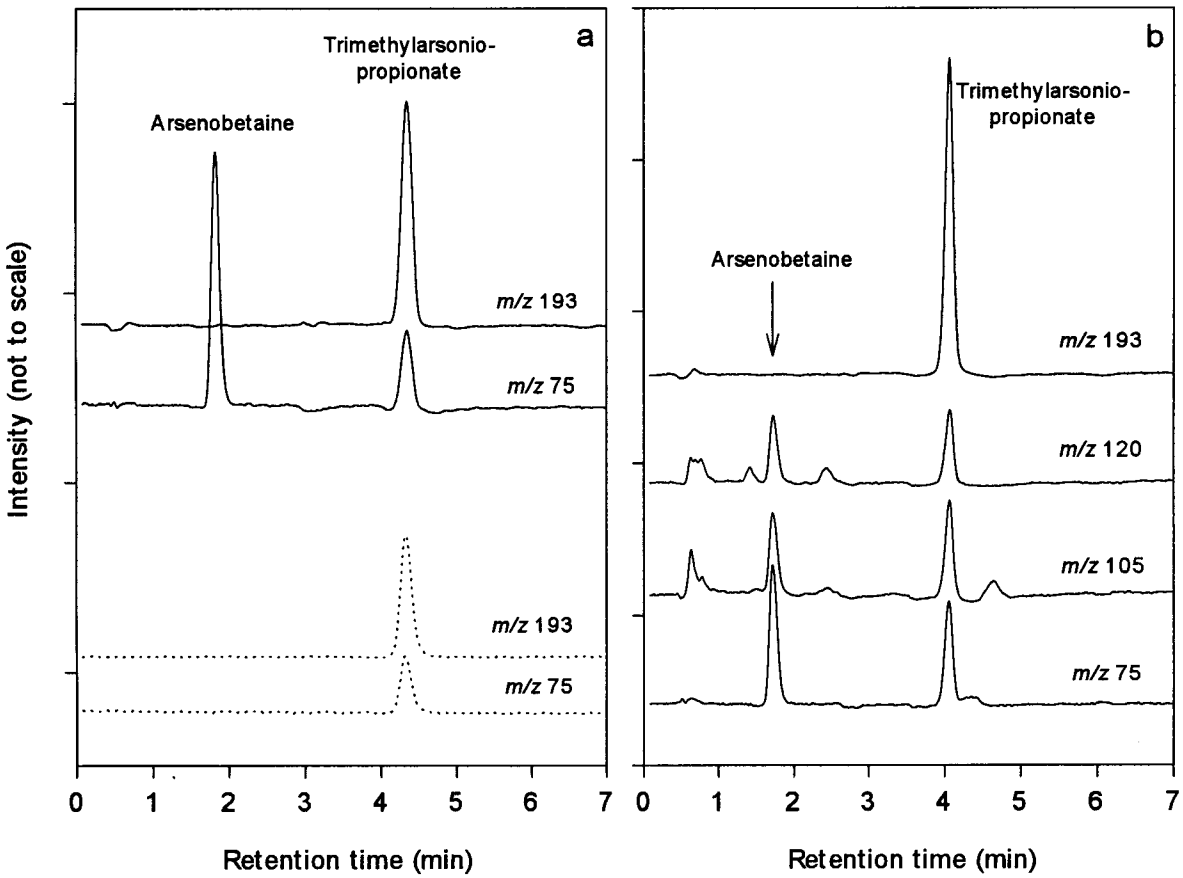

Figure 5. LC–electrospray ionization-MS chromatograms of partially purified (TLC) extract of the coral reef fish (*A. vaigiensis*) and authentic trimethylarsoniopropionate. (a) Fish extract (solid line) and authentic trimethylarsoniopropionate (dotted line) at *m/z* 75 and *m/z* 193; (b) mixture of fish extract and authentic trimethylarsoniopropionate (1 + 1 in terms of concentration of trimethylarsoniopropionate). Chromatography was performed with an Ionospher-C cation-exchange column (100 mm × 3 mm) and a mobile phase comprising a mixture of 20 mM aqueous pyridine pH 2.6 (adjusted with HCOOH) and methanol (9 + 1 v/v) at 30 °C and a flow rate 1.0 ml min^{-1}. Molecular species and ions were produced and detected simultaneously in variable fragmentor voltage mode: 70 V (*m/z* 193), 150 V (*m/z* 105 and 120) and 200 V (*m/z* 75). Signals for the *m/z* values are presented to facilitate comparison of retention times and are not to scale; relative intensities are contained in the original report.[24]

urine after ingestion of an arsenosugar also demonstrated the strength of parallel use of ICP-MS and electrospray ionization-MS detection. ICP-MS was able to detect at least 12 arsenic metabolites in the urine sample. The structure of one of these metabolites, dimethylarsinoylethanol, which had not previously been reported in urine, was positively identified with LC–electrospray ionization-MS by simultaneous detection of As^+ and the $[M + H]^+$ molecular species, and comparing the data with those from a synthetic specimen.

The above examples illustrate some applications of LC–electrospray ionization-MS to determine arsenic compounds in biological samples. However, in all cases the structures of the compounds were known, and standards were available so that the arsenicals in the samples could be positively identified and quantified. But can the method provide structural information about novel arsenic compounds? To obtain structural information on new organic compounds, the extra capabilities of tandem MS systems are normally required. For organoarsenic compounds, however, the ability of electrospray ionization-MS to detect arsenic ions and molecular species (and product ions), essentially simultaneously, provides an avenue for obtaining molecular information about novel compounds. Two recent examples will be used to illustrate this point.

STRUCTURAL ELUCIDATION OF NOVEL ARSENIC COMPOUNDS WITH LC–ELECTROSPRAY IONIZATION-SINGLE QUADRUPOLE MS

In 1993, Larsen *et al.*[23] reported the presence of an unknown

arsenic compound in several species of marine animals using LC–ICP-MS. What appeared to be the same compound was similarly detected[24] in an aqueous extract of a coral reef fish *Abudefduf vaigiensis*, where it constituted about 8% of the arsenic (the rest was present as arsenobetaine). By use of thin layer chromatography (TLC), a fraction was obtained in which the unknown arsenical represented 30% of the arsenic, and its arsenic concentration (175 ng cm^{-3}) was sufficient to measure the sample reliably for arsenic by LC–electrospray ionization-MS.

The sample was first analysed with a high fragmentor voltage in the electrospray ionization source, and with the mass spectrometer in selected ion monitoring mode measuring *m/z* 75 (As^+). A clean chromatographic trace was obtained, showing arsenobetaine and the unknown compound (Fig. 5a). The chromatography was then repeated at low fragmentor voltage (with the expectation that the $[M + H]^+$ molecular species of the unknown compound would be detected), and *m/z* values were scanned to see if any signal matched the retention time of the arsenic signal. To enhance the sensitivity of the method, *m/z* values were scanned in 'batches' of 20 mass units beginning with *m/z* 141–160 and ending at *m/z* 400. There was only one signal (*m/z* 193) of significant intensity with a retention time matching the arsenic signal for the unknown. As expected, there was also a strong signal at *m/z* 179 that matched the arsenic signal for arsenobetaine. The chromatography was then carried out under two sets of chromatographic conditions using the variable fragmentor voltage mode with simultaneous detection of *m/z* 75 and *m/z* 193. In both cases, the retention times of the two signals matched exactly, thereby providing good evidence that the unknown arsenical had a protonated molecular mass of 193. It may be worth pointing out that, in order for such an assumption to be made, the retention times for the two *m/z* values must match *exactly*, because they are just different ways of measuring the same chromatographic peak.

Further information about the unknown arsenical in coral reef fish was then obtained by looking for characteristic fragments at intermediate fragmentor voltages. The unknown arsenical produced clear signals at *m/z* 120 ($(CH)_3As^+$) and *m/z* 105 ($(CH_3)_2As^+$); this fragmentation closely matched that obtained for arsenobetaine, suggesting the presence of the trimethylarsonio moiety. Collectively, the LC–electrospray ionization-MS data obtained for the unknown arsenic compound indicated that it was homologous with arsenobetaine, and contained an additional methylene group in the functional group side chain. Thus, the structure proposed from the mass spectral data was trimethylarsoniopropionate; this assignment was then confirmed by comparison with a synthesized authentic specimen of trimethylarsoniopropionate (Fig. 5a), and by co-chromatography (Fig. 5b).

A second example of the structural elucidation of a new arsenic compound by LC–electrospray ionization-MS has recently been provided by a study on the arsenic constituents in the kidney of the giant clam *Tridacna derasa*.[25] Seven known arsenic compounds, including six arsenosugars, were identified by matching chromatographic retention times with standard compounds. However, the preliminary anion-exchange chromatogram, measured at high fragmentor voltage detecting *m/z* 75 (As^+), was dominated by a peak at retention time 3.5 min that did not match any of the available arsenic standards. So intense was this signal, relative to the other arsenic signals, that it was suspected to be an artefact of the matrix resulting from incomplete dissociation of a non-arsenic species. This possibility had

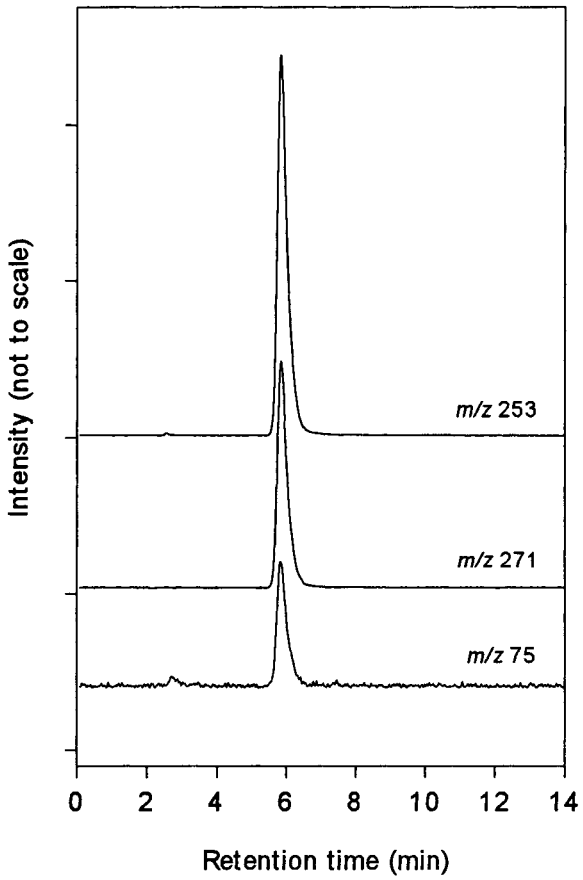

Figure 6. Anion-exchange LC–electrospray ionization-MS chromatogram of compound **5** from clam kidney detected at *m/z* 75, *m/z* 253, and *m/z* 271. Chromatography was performed with a PRP-X100 anion-exchange column (150 mm × 4.1 mm) and a mobile phase comprising a mixture of 20 mM aqueous NH_4HCO_3, pH 9.0 (adjusted with aqueous ammonia), and methanol (9 + 1 v/v) at 30 °C and a flow rate of 0.50 ml min^{-1}. Molecular species and product ions were produced and detected simultaneously in variable fragmentor voltage mode: *m/z* 253 and 271 at 70 V; and *m/z* 75 at 240 V. Signals for the *m/z* values are presented to facilitate comparison of retention times and are not to scale; relative intensities are contained in the original report.[25]

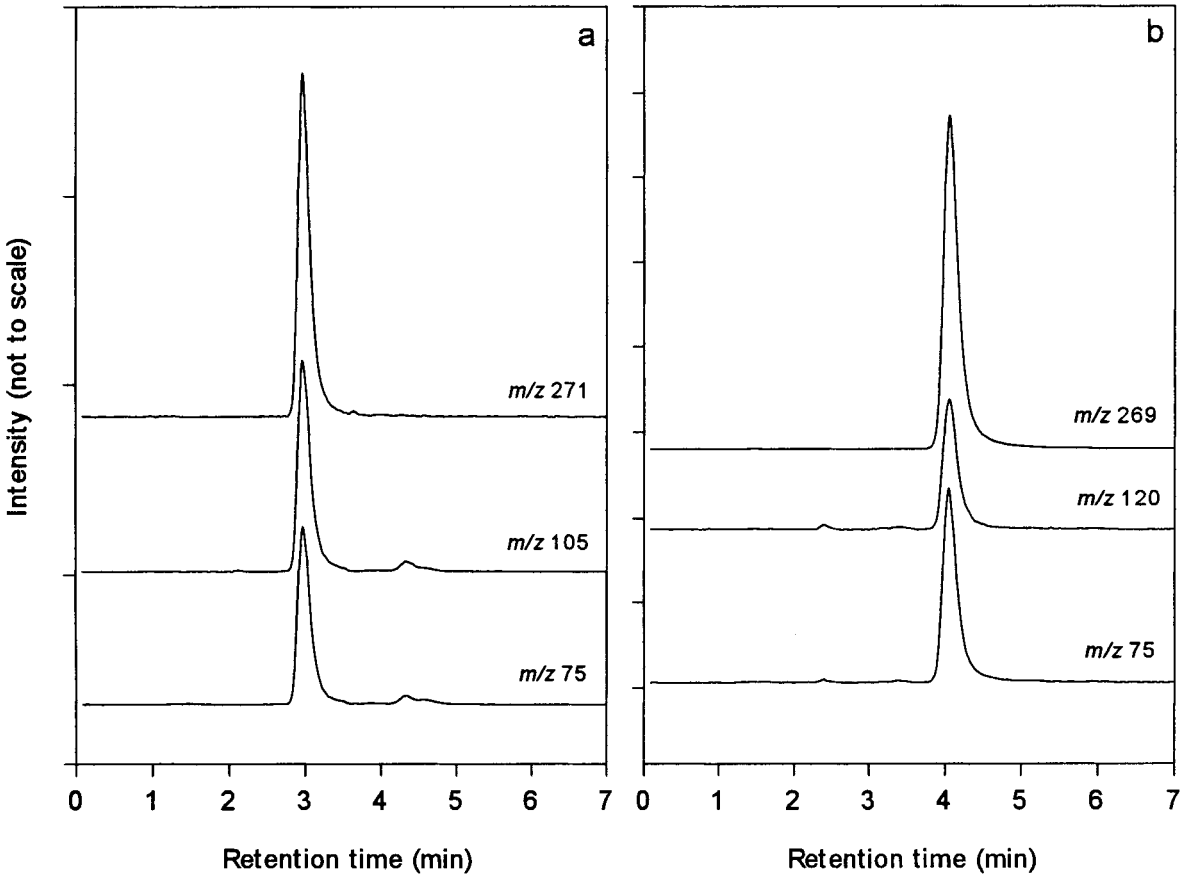

Figure 7. Cation-exchange LC–electrospray ionization-MS chromatograms of (a) compound **5** from clam kidney and (b) its trimethylated quaternary arsonio derivative **7**. Chromatography was performed with an Ionospher-C column (100 mm × 3 mm) and a mobile phase comprising a mixture of 20 mM aqueous pyridine, pH 2.6 (adjusted with HCOOH), and methanol (9 + 1 v/v) at 40 °C and 1.5 ml min^{-1} (a flow splitter directed 30% of the flow to the mass analyser). Molecular species and product ions were produced and detected simultaneously in variable fragmentor voltage mode: *m/z* 271 and 269 at 70 V; *m/z* 105 and 120 at 130 V; and *m/z* 75 at 240 V. Signals for the *m/z* values are presented to facilitate comparison of retention times and are not to scale; relative intensities are contained in the original report.[25]

been discussed previously (and considered unlikely) for algal samples.[14] To provide an independent check on the arsenic content of this peak, the chromatography was repeated with off-line detection of arsenic by graphite furnace atomic absorption spectrophotometry. The pattern of arsenic species was qualitatively the same as that recorded by electrospray ionization-MS detection, but the signal at 3.5 min was much less intense relative to the other arsenic signals. This was a reflection of the compound-dependent response for arsenic by electrospray ionization-MS. In contrast, graphite furnace atomic absorption spectrophotometry can quantify arsenic irrespective of the type of arsenic compound being determined.[26] This analysis demonstrated that the unknown compound constituted about 50% of the total water-soluble arsenic.

The molecular mass of this unknown compound was then sought in a manner similar to that used to identify the new arsenobetaine in coral reef fish. As in that case, the unknown arsenical was partially purified (this time by gel permeation chromatography) in order to simplify the LC–electrospray ionization-MS analysis. The data for the unknown arsenical from clam kidney were slightly more complicated than those for the new arsenobetaine because there were signals for two apparent $[M + H]^+$ values (*m/z* 271 and *m/z* 253) that exactly matched the retention time of the arsenic signal (*m/z* 75) (Fig. 6). Chromatography under anion-and cation-exchange conditions (the unknown was amphoteric) with several different mobile-phase conditions always produced identical retention times for these three signals. These data indicated that the *m/z* 253 ion was a product ion from dehydration of the *m/z* 271 $[M + H]^+$ species in the electrospray ionization source of the mass spectrometer. With due consideration to the chromatographic properties of the unknown (and their comparison with known compounds), and the likely

biogenetic origin of the unknown (from arsenosugars), the structure postulated was the trihydroxy carboxylic acid **5**, namely 5-dimethylarsinoyl-2,3,4-trihydroxypentanoic acid. The product ion at *m/z* 253 was postulated as the γ-lactone **6**. In-source dehydrations to γ-lactones, similar to that proposed for compound **5**, have been reported for other hydroxy carboxylic acids.[27]

Further evidence for the proposed structure of the clam kidney arsenical was obtained by LC–electrospray ionization-MS of the product obtained from derivatization of compound **5**. Thus, a small quantity ($\equiv$0.1 μg arsenic) of compound **5**, an arsine oxide, was reduced with dithiothreitol and the resultant arsine was quaternized with methyl iodide. The LC–electrospray ionization-MS data (Fig. 7) clearly showed the quantitative formation of the expected product, compound **7**, namely 5-trimethylarsonio-2,3,4-trihydroxypentanoic acid. It was interesting that this compound did not undergo in-source dehydration to a lactone.

CONCLUDING COMMENTS

Over the last 25 years, about 30 naturally occurring arsenic compounds have been discovered in environmental and biological samples.[28] Most of these compounds were isolated and identified by spectroscopic techniques such as NMR spectroscopy. The advent of LC–ICP-MS and its application to arsenic speciation analyses since the late 1980s has greatly increased our knowledge of the distribution of known arsenic compounds in the environment. The technique is robust and sensitive, and is increasingly becoming a common analytical method.

Structural elucidation of new compounds, however, cannot be achieved by LC–ICP-MS, and molecular MS with tandem mass spectrometric systems is usually required. The analytical power of such techniques has recently been impressively demonstrated by the structural elucidation of several new naturally occurring arsenic compounds.[29] The instrumentation, however, is still rather specialized, and is not common in analytical laboratories. In contrast, LC–single quadrupole MS is a relatively simple instrument routinely used in the analysis of a wide range of organic compounds. When used with an electrospray ionization source, it can determine the polar organoarsenic compounds found in environmental and biological samples. And when a high fragmentor voltage is used, the electrospray ionization source produces bare As^+ ions, and hence the system can also be used as an arsenic-specific detector.

LC–electrospray ionization-MS is still not widely used for arsenic speciation analyses, and its future use is likely to be restricted by problems associated with sample matrix. Nevertheless, the method has been employed successfully in the studies described here, and may soon be recognized as a useful adjunct to current methods for arsenic speciation analysis.

Acknowledgements

I thank collaborators on various aspects of the work, in particular Søren Pedersen for developing the electrospray ionization conditions for the analysis of arsenosugars. The Danish Research Council has provided consistent funding support.

REFERENCES

1. Luten JB, Riekwel-Booy G, van der Greef J and ten Noever de Brauw MC. *Chemosphere* 1983; **12**: 131.
2. Siu KWM, Gardner GJ and Berman SS. *Rapid Commun. Mass Spectrom.* 1988; **2**: 69.
3. Siu KWM, Guevremont R, Le Blanc JY, Gardner GJ and Berman SS. *J. Chromatogr.* 1991; **554**: 27.
4. Corr JJ and Larsen EH. *J. Anal. At. Spectrom.* 1996; **11**: 1215.
5. Pergantis SA, Winnik W and Betowski D. *J. Anal. At. Spectrom.* 1997; **12**: 531.
6. Gallagher PA, Wei XY, Shoemaker JA, Brockhoff CA and Creed JT. *J. Anal. At. Spectrom.* 1999; **14**: 1829.
7. McSheehy S and Szpunar J. *J. Anal. At. Spectrom.* 2000; **15**: 79.
8. McSheehy S, Marcinek M, Chassaigne H and Szpunar J. *Anal. Chim. Acta* 2000; **410**: 71.
9. McSheehy S, Pohl P, Lobinski R and Szpunar J. *Anal. Chim. Acta* 2001; **440**: 3.
10. McSheehy S, Pohl P, Lobinski R and Szpunar J. *Analyst* 2001; **126**: 1055.
11. Wu JC, Mester Z and Pawliszyn J. *Anal. Chim. Acta* 2000; **424**: 211.
12. Gaskell SJ. *J. Mass Spectrom.* 1997; **32**: 677.
13. Voyksner RD and Pack T. *Rapid Commun. Mass Spectrom.* 1991; **5**: 263.
14. Pedersen SN and Francesconi KA. *Rapid Commun. Mass Spectrom.* 2000; **14**: 641.
15. Mansoori BA, Volmer DA and Boyd RK. *Rapid Commun. Mass Spectrom.* 1997; **11**: 1120.
16. Florĕncio MH, Duarte MF, de Bettencourt AM, Gomes ML and Boas LF. *Rapid Commun. Mass Spectrom.* 1997; **11**: 469.
17. Madsen AD, Goessler W, Pedersen SN and Francesconi KA. *J. Anal. At. Spectrom.* 2000; **15**: 657.
18. Sánchez-Rodas D, Geiszinger A, Gómez-Ariza and Francesconi KA. *Analyst* 2002; 60.
19. Khokiattiwong S, Goessler W, Pedersen SN, Cox R and Francesconi KA. *Appl. Organomet. Chem.* 2001; **15**: 481.
20. Larsen BR, Astorga-Llorens C, Florĕncio MH and de Bettencourt AM. *J. Chromatogr. A* 2001; **926**: 167.
21. Geiszinger A, Goessler W, Pedersen SN and Francesconi KA. *Environ. Toxicol. Chem.* 2001; **20**: 2255.
22. Francesconi KA, Tangaard R, McKenzie CJ and Goessler W. *Clin. Chem.* 2002; **48**: 92.
23. Larsen EH, Pritzl G and Hansen SH. *J. Anal. At. Spectrom.* 1993; **8**: 1075.
24. Francesconi KA, Khokiattiwong S, Goessler W, Pedersen SN and Pavkov M. *Chem. Commun.* 2000; 1083.
25. Francesconi KA and Edmonds JE. *Rapid Commun. Mass Spectrom.* 2001; **15**: 1641.
26. Larsen EH. *J. Anal. At. Spectrom.* 1991; **6**: 375.
27. Michels JJ, Allain EJ, Borchardt SA, Hu P and McCoy WF. *J. Chromatogr. A* 2000; **898**: 153.
28. Francesconi KA and Kuehnelt D. Arsenic compounds in the environment. In *Environmental Chemistry of Arsenic*, Frankenberger WT (ed.). Marcel Dekker, Inc.: New York, 2001; 51–94.
29. McSheehy S, Szpunar J, Lobinski R, Haldys V, Tortajada J and Edmonds J. *Anal. Chem.* In press.

