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Simultaneous determination of arsenic species by ion chromatography–inductively coupled plasma mass spectrometry

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Abstract

Six arsenic species, arsenite, arsenate, monomethylarsonic acid, dimethylarsinic acid, arsenobetaine and arsenocholine, were separated by coupled column ion chromatography using carbonate and nitric acid as eluents, and were detected by inductively coupled plasma mass spectrometry. Coupling of an anion column with a cation column made the simultaneous determination of both the cationic and the anionic arsenic species possible by ion chromatography. Extremely low detection limits, below 0.2 μg/l (as arsenic), were obtained for all the species studied.

Keywords: Coupled columns; Arsenic compounds

1. Introduction

Many trace elements, such as arsenic, mercury, selenium and tin, experience extensive chemical transformations in the environment. Once they have entered the environment, their physical and chemical properties, toxicity, mobility and biotransformation are controlled to a large extent by their physicochemical form. When assessing potential hazards of such elements, there is a need for analytical methods that are able to discriminate between the different chemical forms of a given element. Speciation of an element is defined as the determination of the individual physicochemical forms of an element which together make up its total concentration in a sample. Speciation of arsenic is of particular interest. Arsenic is widely distributed in the environment, and the different chemical forms of arsenic exhibit wide-ranging levels of toxicity. Humans may be exposed to arsenic compounds occupationally and through food, tobacco smoke, ambient air and water [1–3]. Arsenite (As^{III}) is the most toxic form of the water-soluble species of arsenic and it is a suspected human carcinogen. Arsenate (As^{V}) is also relatively toxic; while the methylated forms, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are much less toxic [4,5]. Arsenobetaine (AB) and arsenocholine, for example, are non-toxic [5].

Speciation studies of arsenic often employ high-performance liquid chromatography (HPLC) in various forms, in conjunction with inductively coupled plasma mass spectrometry (ICP-MS). The combination takes advantage of both the separation power offered by HPLC and the selectivity, extremely high sensitivity and large dynamic range offered by ICP-MS. Applications of ion-pair reversed-phase chromatography [6–13] and micellar liquid chromatography [14] have been reported, as well as ion chromatographic (IC) methods using anion- [15–26] or cation-exchange, [21–23,27] for the separation of either anionic or cationic arsenic species. However,

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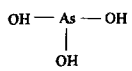
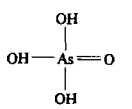
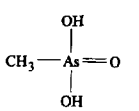
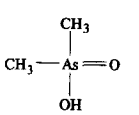
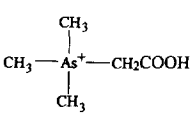
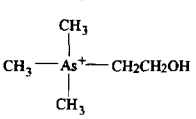
Table 1
Arsenic species studied

Compound	Formula	pK_a	ref
Arsenious acid (As III)		9.2	28
Arsenic acid (As V)		2.3 6.8 11.6	28
Monomethylarsonic acid		3.6 8.2	29
Dimethylarsinic acid		1.3 6.2	21 28
Arsenobetaine		2.2	21
Arsenocholine			

simultaneous determination of the arsenic species by ion chromatography is difficult, because of the wide-ranging pK_a values and different ionic characters of the species. Under any set of conditions, some of the arsenic species are in a cationic form and some are in an anionic or neutral form.

In this paper, we present a coupled column ion chromatographic method for the simultaneous determination of six commonly encountered arsenic species. The arsenic species studied and their pK_a values are shown in Table 1 [21,28,29]. The coupled column system was constructed by coupling a cation column with an anion column, in order to concentrate both the cationic and anionic species on the columns. A similar coupled column system has already been applied for speciation of chromium [30]. The performance of the speciation method and the possible spectral interference of the polyatomic $ArCl^+$ were studied.

2. Experimental

2.1. Ion chromatography

The chromatographic system consisted of a Pharmacia LKB dual piston reciprocating gradient pump (Model 2249, Pharmacia Biotech, Uppsala, Sweden), a syringe-loading sample injector with a 0.25-ml injection loop and a cation column coupled on-line with an anion column. The gradient pump was equipped with a ternary low pressure mixer unit. The anion column was a Waters IC-Pak A HC (150×4.6 mm, 10 μm particle size) (Waters, Milford, MA, USA) having trimethyl ammonium functionalized groups on polymethacrylate and the cation column used was a Waters Guard-Pak CM/D (5×3.9 mm, 5 μm particle size) containing sulphonic acid groups on polybutadiene maleic anhydride silica. A step gradient, with increasing ionic strength and decreasing pH, followed by additional restabilization steps was used. The gradient programme is presented in Table 2. The chromatographic system was interfaced with the ICP-MS instrument using 60 cm of 0.5 mm I.D. Teflon capillary tubing to connect the column outlet to the inlet hole of the nebulizer. The chromatographic flow-rate of 2.0 ml/min was found to be compatible with the nebulizer uptake rate of the ICP-MS instrument. A schematic diagram of the IC–ICP-MS system is shown in Fig. 1.

Table 2
IC gradient programme

	Elution steps			Restabilization steps	
Time (min)	0–1.0	1.5–6.0	7.0–11.0	12.0–18.0	19.0–24.0
$NaHCO_3/Na_2CO_3$ (mM)	0.3	2.5	0.0	10.0	0.3
HNO_3 (mM)	0.0	4.0–6.0	40.0	0.0	0.0

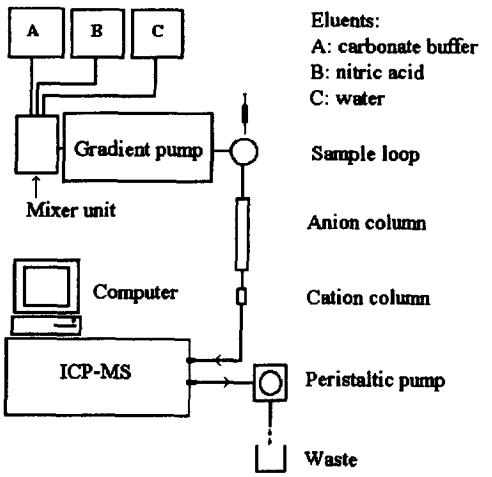


Fig. 1. Schematic diagram of the IC–ICP-MS system used in speciation studies.

2.2. Inductively coupled plasma mass spectrometry

A Fisons Plasma Quad PQ II+ ICP-MS instrument (VG Elemental, Winsford, UK) with a concentric nebulizer, a Scott-type quartz spray chamber and a Fassel-type quartz torch were used for detection. The peak heights and areas were obtained by PQ Vision integration software. For most of the work, a single-ion monitoring (SIM) mode, monitoring only the arsenic species at m/z 75, was used for data acquisition. When the argon chloride interference at m/z 75 was investigated, a time resolved analysis (TRA) software programme provided by the manufacturer was used to simultaneously monitor the ClO^+ ion at m/z 51 and 53, the $ArCl^-$ ion at m/z 75 and 77, and selenium at m/z 82.

Prior to the IC–ICP-MS runs, the instrument sensitivity was optimized by varying one instrumental setting at a time, using a solution of 10 μg/l of Indium in 2% nitric acid, fed by a peristaltic pump (Minipuls 3, Gilson Medical Electronics, Middleton, WI, USA). Adjustments included the ion-lens voltages, physical alignment of the ICP torch relative to the mass spectrometer and the optimization of the nebulizer gas flow. Optimization using an aqueous arsenic standard was also tested, but any enhancement in arsenic sensitivity was insignificant. The optimized ICP-MS operating conditions are summarized in Table 3. After optimization the peristaltic pump was manually replaced with the IC unit used in speciation studies.

Table 3
ICP-MS operating conditions

Parameter	Setting
RF Power, W	1350
Outer gas flow-rate, l min^{-1}	13.5
Intermediate gas flow-rate, l min^{-1}	0.90
Nebulizer gas flow-rate, l min^{-1}	0.865[a]
Spray chamber temperature, °C	4
Total acquisition time, s	720–900
SIM, m/z	75
TRA, m/z	51, 53, 75, 77, 82

[a]optimized each day

2.3. Reagents

The carbonate buffer was prepared by dissolving equal amounts of pro analysi grade sodium hydrogen carbonate and disodium carbonate (E. Merck, Darmstadt, Germany) in water. Supra pur grade nitric acid was from Baker (J.T. Baker, Phillipsburg, NJ, USA) and high purity water was obtained with a Millipore Milli-Q water purification system (Millipore, Milford, MA, USA). All the mobile phases were filtered through 0.45 μm filters and degassed before use. Standard aqueous solutions of As^{III}, As^{V} and DMA were prepared from arsenic trioxide (E. Merck) sodium arsenate and cacodylic acid sodium salt (Fluka, Buchs, Switzerland), respectively. MMA was obtained from Oulu Occupational Health Research Institute, Finland, and both AB and arsenocholine (AC) were obtained from the Pasteur Institute, France. The arsenic concentrations of the standards were verified by ICP-MS.

2.4. Procedures

After the last arsenic compound had eluted, the chromatographic system was disconnected and the columns were purged with 10 mM carbonate buffer for 6 min, according to the gradient programme presented in Table 2. At the same time, an alternative

flow of 2% nitric acid fed by a peristaltic pump was directed to the ICP-MS. When the columns were not in use, Milli-Q water was pumped through them.

Retention times, peak areas and peak heights of the arsenic species in a sample were evaluated by computer, and then standardized and identified against chromatograms resulting from similar IC–ICP-MS runs of the arsenic standard mixtures. Peak identification was confirmed, if necessary, by standard additions of the arsenic species.

3. Results and discussion

3.1. Separation of arsenic compounds

The anionic arsenic species As^{III}, As^{V}, MMA and DMA show large differences in their acid dissociation constants (Table 1), which makes them well suited for anion-exchange separation. However, arsenite has a high pK_a value (9.2) and it is not ionized over the pH range to which studies using silica-based columns are restricted. Consequently, arsenite tends to elute in the void volume together with arsenocholine, which is a cationic species, and arsenobetaine, which is present in a cationic or zwitterionic form, depending on the pH.

Because of the problems associated with the restricted pH range, a polymer-based anion column that was able to function over a wider pH range (1–12) was chosen. Carbonate buffer has the advantageous feature of high pH value and was chosen as the eluent. A gradient programme was used to give better resolution for the early eluting species and, at the same time, a reasonable analysis time. The gradient programme started with a dilute carbonate solution at a pH of 9.3. Under these conditions, As^{III}, As^{V}, DMA, MMA and, surprisingly, also AC were retained on the anion column, while AB eluted in the void volume. The species were eluted from the column by increasing the carbonate concentration and slightly decreasing the pH with nitric acid. However, AC co-eluted with DMA as a broad zone under all the conditions studied and it was necessary to add another separation mechanism to the system. Because AC has cationic character it was strongly retained on the cation guard column, which was coupled on-line with the anion column, as shown in Fig. 1. AC was eluted from the cation guard column with 40 m*M* nitric acid. The restabilization of the anion column was speeded up by using an additional step of 10 m*M* buffer solution in the gradient programme after the elution of AC. The final gradient, presented in Table 2, allowed good separation of all six arsenic species in less than 12 min. A chromatogram of a spiked river water sample, presented in Fig. 2, shows a typical separation. In the chromatogram of clean standard solutions and real water samples, the retention times, recoveries and responses of the species were the same, indicating that the method is robust.

3.2. Characteristics of the method

The coupled column IC–ICP-MS system was highly sensitive. Detection limits determined as

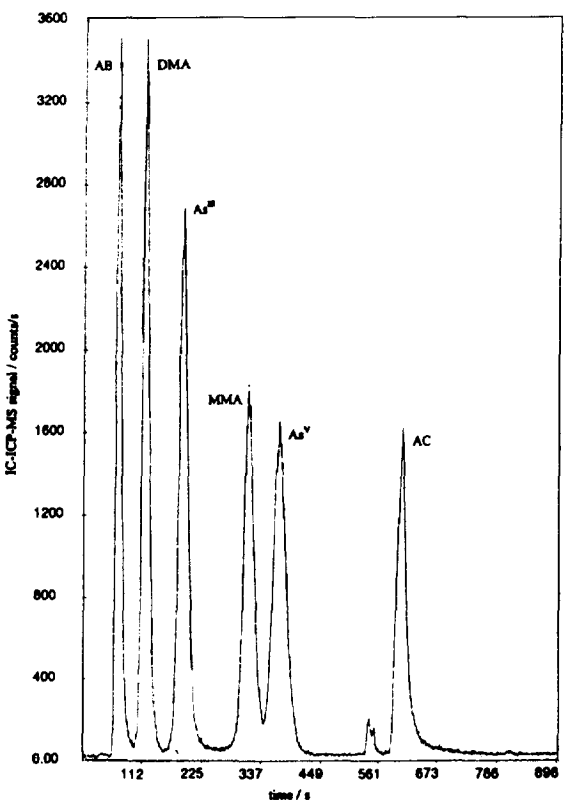


Fig. 2. Chromatogram of a river water sample spiked with 10 μg/l (as arsenic) of each arsenic species. The analysis conditions are described in Section 2. The peak near 561 s is an unidentified arsenic species.

arsenic or arsenic species concentration giving a signal three times higher than the standard deviation of the baseline were in a range from 0.04 μg/l (as arsenic) for DMA to 0.16 μg/l (as arsenic) for As^{V}. The detection limits are presented in Table 4. The relatively large differences in the detection limits arise from the changes in the baseline noise caused by the gradient elution steps and the different peak shapes of individual species.

The repeatability of the method, expressed as the R.S.D. values were calculated for both the peak heights and peak areas of each component from the results of three consecutive runs of a waste river water sample spiked with 10 μg/l (as arsenic) of each species. The R.S.D. values are presented in Table 4 and the chromatogram of this spiked river water sample can be seen in Fig. 2. The repeatability was better for peak areas of the components, with R.S.D. values of below 7%.

The linearity of the method was estimated by using the same river water sample and standard additions of 1, 10, 25, 50 and 100 μg/l (as arsenic) of each species except that 1, 10, 25 and 50 μg/l of AC were used. The regressions of the calibration curves based on the peak areas are presented in Table 4. The sensitivity of the IC–ICP-MS system used is slightly species-dependent. Differences in the ICP-MS sensitivity of individual arsenic species have earlier been reported by Larsen and Sturup [27]. The mathematical expressions for linearity in the range 1–100 μg/l were $y=5903x+3447$ for AB, $y=7419x+218$ for As^{III}, $y=6809x+8181$ for As^{V}, $y=7407x+210$ for DMA and $y=6103x+3987$ for MMA and in the range 1–50 μg/l $y=5444x-3249$ for AC.

Table 4
Characteristics of the speciation method: detection limits (DL), relative standard deviations (R.S.D.)[a] and regressions of the standard addition curves (R^2)[d] for the arsenic species studied

Compound	DL (μg/l)[b]		R.S.D. (%)[c]		
	Arsenic	Species	Peak area	Peak height	R^{2} [d]
AB	0.04	0.10	1.7	4.7	0.9994
DMA	0.04	0.08	2.6	4.1	0.9992
As^{III}	0.06	0.10	2.7	4.9	0.9999
MMA	0.11	0.19	4.9	8.3	0.9995
As^{V}	0.16	0.30	4.5	8.2	0.9984
AC	0.11	0.24	6.2	7.6	0.9994

[a] A spiked river water sample was used.
[b] Determined as a concentration of arsenic or arsenic species giving a signal that is three times higher than the standard deviation of the baseline.
[c] A standard addition of 10 μg/l (as arsenic) of each species was used. ($n=3$)
[d] Standard additions of 1, 10, 25, 50 and 100 μg/l (as arsenic) of each species were used, except that 1, 10, 25 and 50 μg/l (as arsenic) was used for AC.

3.3. Interference of chloride

In ICP-MS, formation of the polyatomic $^{40}Ar^{35}Cl^{+}$ ion in the plasma may cause spectroscopic interferences in the analysis of arsenic [16,18,31].The possible interference of $ArCl^{+}$ was investigated by monitoring $ArCl^{+}$ at m/z 75 and 77 and ClO^{-} at m/z 51 and 53. Selenium at m/z 82 was also monitored, because a peak at m/z 77 could also be attributed to $^{77}Se^{+}$. A very small peak was detected at m/z 75 and 77 when injecting a sample containing 1000 mg/l Cl^{-}, which indicated that the formation of the polyatomic $ArCl^{+}$ was very limited and did not cause spectral interferences. However, high chloride concentrations caused column overloading and seriously affected the peak resolutions of the arsenic species. At a chloride concentration of 500 mg/l, AB and DMA co-eluted, while the other species were still separated. The maximum chloride concentration that still allowed the successful separation of all the species was 250 mg/l. At this concentration the $ArCl^{+}$ ion was not detected at all.

4. Conclusions

A coupled column IC–ICP-MS method is presented for speciation of arsenic. The method offers extremely low detection limits (below 0.3 μg/l for all the species studied), a large linear range and good repeatability. With the method developed, six arsenic species can be separated in 12 min, with an elution order AB, DMA, As^{III}, MMA, As^{V} and AC. Cl^{-} did not cause any interferences for the separation at concentrations below 250 mg/l. The method has been applied in the analysis of natural and waste water samples.

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