Chromatographic Ion-Exchange Simultaneous Separation of Arsenic and Selenium Species with Inductively Coupled Plasma–Mass Spectrometry On-Line Detection

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Abstract

The high-performance liquid chromatographic (HPLC) simultaneous separation of arsenite, arsenate, monomethylarsonic acid, dimethylarsinic acid, selenite, and selenate is studied. The dependence of the retention times of these species on the pH of the mobile phase and on the concentration and chemical composition of buffer solutions (acetate, carbonate, and phosphate) is investigated using a Merck Polyspher IC AN2 and a Hamilton PRP-X100 anion exchange column. With a flame atomic absorption spectrometric detector (FAAS) and arsenic or selenium concentrations of at least 100 mg/L, the best simultaneous separation of these species is achieved on the PRP-X100 column using 0.015M ammonium phosphate buffer at pH 8.5, a 1-mL/min flow rate, and a 20-min analysis time. In a second study, these separation conditions are optimized by using an inductively coupled plasma–mass spectrometric (ICP–MS) detector. The use of a 12.5mM ammonium phosphate buffer adjusted to pH 8.5 with ammonia and a flow rate of 1.5 mL/min is found to be optimal for HPLC–ICP–MS studies with the PRP-X100 column. The analysis time is about 16 min; absolute detection limits are estimated in the range of 11–21 pg for arsenic species and 200 and 417 pg for Se^{IV} and Se^{VI}, respectively. Reproducibility ranges from 2.1 to 3.2%, and the linearity is verified in the 0–200-ng/mL range.

Introduction

Speciation analysis of metals and nonmetals in the various compartments of the environment is increasingly important due to the connection between toxicity and metal forms.

Arsenic toxicity and its ubiquity in the environment are well-known. Selenium presents a dual role as an essential element at low concentration levels and as a toxic substance at higher levels. In both cases, the most toxic forms in the aqueous phase are the less oxidated inorganic species, whereas methylated forms such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are much less toxic. In environmental matrices, the most frequently encountered inorganic species are arsenite (AsO_3^{2-}), arsenate (AsO_4^{2-}), selenite (SeO_3^{2-}), and selenate (SeO_4^{2-}).

Morita and Edmonds (1) presented an exhaustive technical report about the determination of arsenic species with references previous to 1989. They concluded that speciation studies have been carried out either to identify unknown compounds or to identify compounds by comparison with standards using chromatographic separation coupled with element-specific detectors.

Considering selenium species, Kölbl et al. (2) studied only high-performance liquid chromatographic (HPLC) separation. They reported several determinations of selenite and selenate using suppressed ion chromatography with conductivity detectors.

Dauchy et al. (3) reviewed the different analytical methods for selenium speciation; the most common separation methods used are ion-exchange chromatography with different detection methods or specific oxido-reduction reactions.

Finally, Pyrsynska (4) presented developments in the speciation analysis of selenium since 1992 and concluded that extensive investigations on total selenium or selenite and selenate have been done, but the studies were not systematic, and most of them were in standard solutions only.

In a review on inductively coupled plasma mass spectrometry (ICP–MS) used in speciation studies, Lespès et al. (5) noted six references on arsenic speciation, most of which used reversed-phase LC, and one on inorganic selenium speciation.

LC coupled to various element-specific detectors is increasingly used in speciation studies. ICP–MS (6–20) has been frequently chosen for chromatographic detection because of its ease in interfacing as an on-line detector and its ability to both separate interferences from significant peaks and simultaneously determine several elements with very high sensitivity. However, the use of ICP–MS as an HPLC detector leads to some constraints when choosing the nature and concentration of buffer salts in the chromatographic mobile phase. This technique can allow the simultaneous detection of species of various elements with a considerable decrease in analysis time.

When ICP–MS is chosen as an HPLC detector, the separation of inorganic and organic arsenic species is mainly made with ion-exchange (6–12) and ion-pairing reversed-phase (12–16)

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techniques. For the speciation of inorganic selenium, reversed-phase (16,17), ion-exchange (17,18), and size-exclusion (19) separation have been studied.

Only a few authors have reported on the simultaneous separation of some arsenic and selenium species (21,22). Schlegel et al. (21) and Colón and Barry (22) separated arsenous acid (As^{III}), arsenic acid (As^{V}), DMA, and selenous acid (Se^{IV}) with ICP–atomic emission spectroscopy (ICP–AES) and alternating current plasma (ACP) detection, respectively.

The aim of the present study was to develop a fast, sensitive, and accurate simultaneous separation of four arsenic and two selenium species considered to be possibly present in environmental sediment or soil samples using isocratic chromatographic conditions. Ion-exchange chromatography was used. Two anion-exchange columns were evaluated in which trimethyl ammonium groups were implanted on a polymeric solid. Several mobile phases compatible with on-line detection by ICP–MS were investigated and compared using the apparent charges method. The precision, the linear dynamic range, and the detection limits of the method were determined.

Table I. ICP–MS Operating Conditions

ICP–MS	VG Plasma Quad 3
Radio frequency power	1350 W
Sampler cone	platinum
Skimmer cone	nickel
Nebulizer	Meinhard type K
Spray chamber	doubled-pass Scott type, cooled at 3°C
Argon flow rates	
Outer	14.0 L/min
Intermediate	1.00 L/min
Aerosol carrier	0.92 L/min (variable)

Table II. pK_a Values* of Considered Species of Arsenic and Selenium

Species	Formula	pK_a†
Arsenous acid (As^{III})	$HAsO_2$	9.29
Arsenic acid (As^{V})	H_3AsO_4	2.24
		6.96
		11.5
MMA	$(CH_3)H_2AsO_3$	4.19
		8.77
DMA	$(CH_3)_2HAsO_2$	1.78
		6.14
Selenous acid (Se^{IV})	H_2SeO_3	2.35
		7.94‡
Selenic acid (Se^{VI})	H_2SeO_4	< 0
		1.7

* From reference 23.
† log *K* at 25°C (ionic strength = 0)
‡ log *K* at 25°C (ionic strength = 0.3)

Experimental

Reagents

Chemicals

Ammonium dihydrogen phosphate ($NH_4H_2PO_4$), ammonium hydrogen di-phosphate ($[NH_4]_2HPO_4$), ammonium hydroxide (NH_4OH) (all three of RPE purity), and MMA disodium salt (puro [> 98% pure]) were purchased from Carlo Erba (Nanterre, France). Sodium bicarbonate ($NaHCO_3$, p.a.), sodium hydroxide monohydrate ($NaOH–H_2O$, suprapur), sodium acetate trihydrate ($CH_3COONa–3H_2O$, p.a.), 100% glacial acetic acid (CH_3COOH), di-arsenium trioxide (As_2O_3, p.a.), and disodium selenite (Na_2SeO_3, suprapur) were purchased from Merck (Darmstadt, Germany). Cacodylic acid sodium salt trihydrate (DMA, purum), potassium hexahydroxyantimonate(V) (H_6KO_6Sb, analytical reagent), and a standard solution (1000 mg/L arsenic) of arsenate (H_3AsO_4, Spectrosol) were obtained from Fluka (Buchs, Switzerland), Prolabo (Fontenay sous Bois, France), and BDH (Dorset, England), respectively.

Solutions

All standards were used without further purification. Arsenite (As^{III}) standard (1000 mg/L arsenic) was prepared by dissolution of As_2O_3 in a 0.2% NaOH solution, which led to the formation of arsenous acid (salt). Other 1000-mg/L (as element) stock solutions were prepared in deionized water (Milliro-Milli Q system, Millipore, Saint Quentin, France). All standards (1000 mg/L) were stored at 4°C in the dark; stability over several months was confirmed, except for selenium compounds (one month). Working standards (10, 1, or 0.1 mg/L) were obtained daily by dilution in the chromatographic eluent; they were also stored in the dark and diluted further just before use.

Carbonate buffers (0.025–0.1M) were prepared by dissolving sodium bicarbonate in deionized water (Millipore), and the pH was adjusted by adding NaOH. Acetate buffers (0.03–0.06M) were prepared by mixing solutions of sodium acetate and acetic acid of the same molarity in the appropriate ratios to obtain buffer solutions in the pH range of 4–6. The same process was applied to phosphate buffers (0.010–0.045M) from ammonium dihydrogen and diammonium

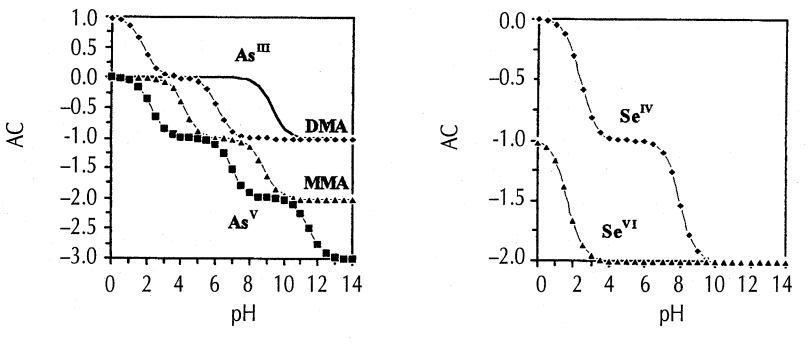

Figure 1. AC of arsenic and selenium species as a function of pH.

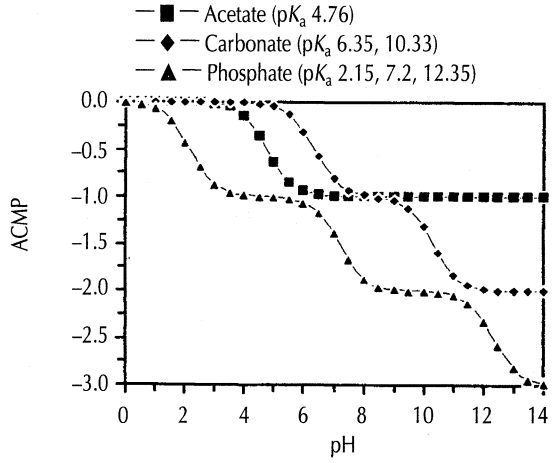

Figure 2. ACMP as a function of pH.

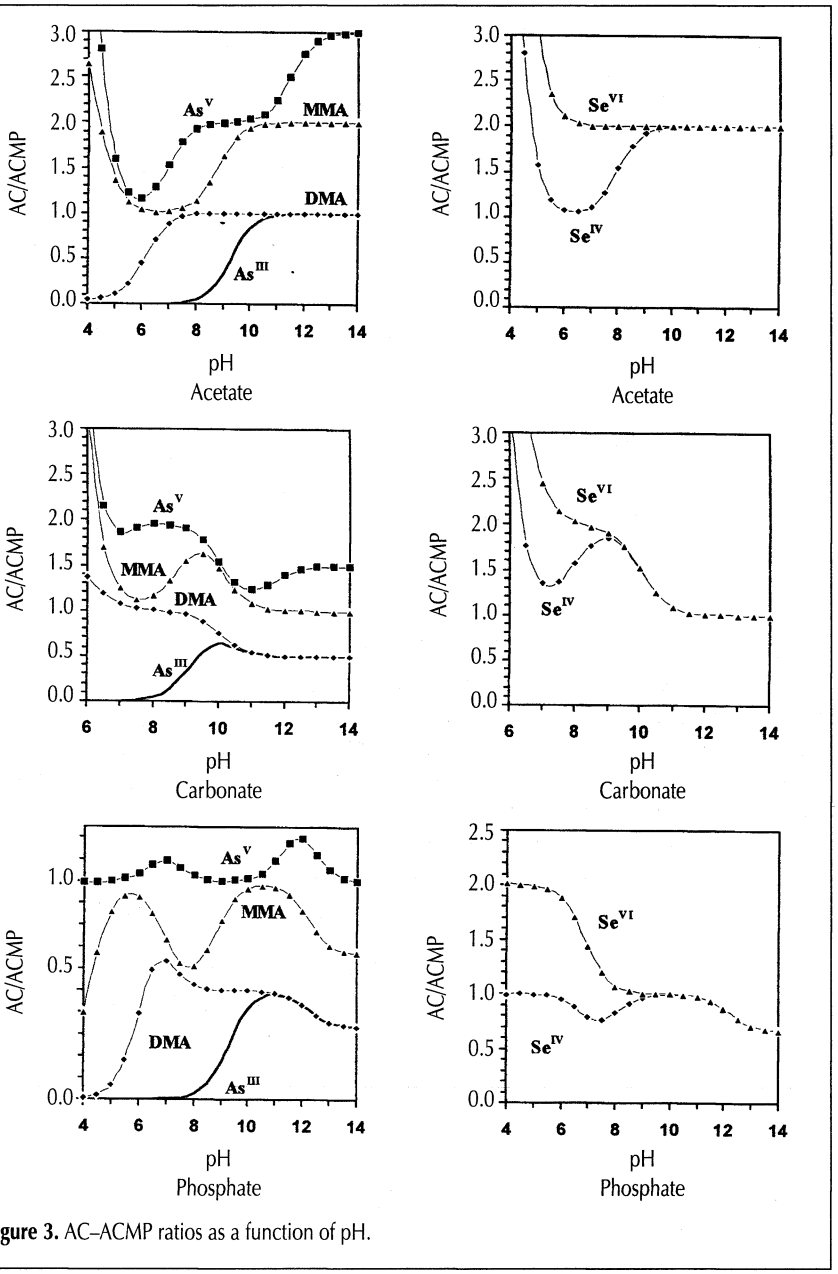

Figure 3. AC–ACMP ratios as a function of pH.

hydrogen phosphate solutions in the pH range of 5.75–8. For the pH range of 8–9, diammonium hydrogen phosphate was dissolved in deionized water, and the pH was adjusted by adding 30% ammonia. All mobile phases were filtered through a 0.45-µm membrane and degassed before use.

Apparatus

The HPLC system consisted of a Varian 9001 isocratic solvent delivery system (Varian, Les Ulis, France) and a Hamilton PRP-X100 anion exchange column (Hamilton, Reno, NV) (25 cm × 4.1-mm i.d., spherical 10-µm particles of a styrene–divinylbenzene copolymer with trimethylammonium exchange sites) or a Merck Polyspher IC AN2 resin-based anion exchanger (12 cm × 4.6-mm i.d., spherical 10-µm particles with quaternary ammonium functional groups).

A 100-µL injection loop (peek, Interchim, Asnières, France) was used in conjunction with a Rheodyne (Cotati, CA) six-port injection valve. An IL 551 (Instrumentation Laboratory, Wilmington, DE) flame atomic absorption spectrophotometer (FAAS) and a VG PQ3 ICP–MS (VG Elemental, Winsford, Cheshire, UK) were selected to detect arsenic and selenium compounds in the chromatographic effluent.

HPLC–FAAS

The HPLC system was coupled to the FAAS by connecting the column end to the FAAS nebulizer with a peek capillary (0.3 m × 0.5-mm i.d.) and a viton tube junction. The air–acetylene flame was adjusted to give a maximum signal-to-noise ratio. The arsenic (Photron, Victoria, Australia) and selenium (Varian, Victoria, Australia) hollow cathode lamps were operated at 8 and 10 mA, respectively. Arsenic absorption was measured at 193.7 nm, and selenium absorption was measured at 196.0 nm. Chromatographic peaks were recorded on a pen recorder (type PE, Sefram, Paris, France).

HPLC–ICP–MS

The MS was set to sample ion intensities (time-resolved acquisition) at the analyte mass-to-charge ratios of *m/z* 75 ($^{75}As^+$) and *m/z* 82 ($^{82}Se^+$). Additionally, the signal intensity was sampled at *m/z* 121 ($^{121}Sb^+$), and antimony was used for internal standardization.

Prior to the recording of signal intensities, instrument sensitivity was optimized by varying one instrumental setting at a time using standard solutions and a peristaltic pump for conventional sample introduction of the analyte solution at 1.5 mL/min. Instrument adjustments included the physical positioning of the MS relative to the plasma, ion lens voltages, aerosol carrier gas flow, and radio frequency power input to the argon

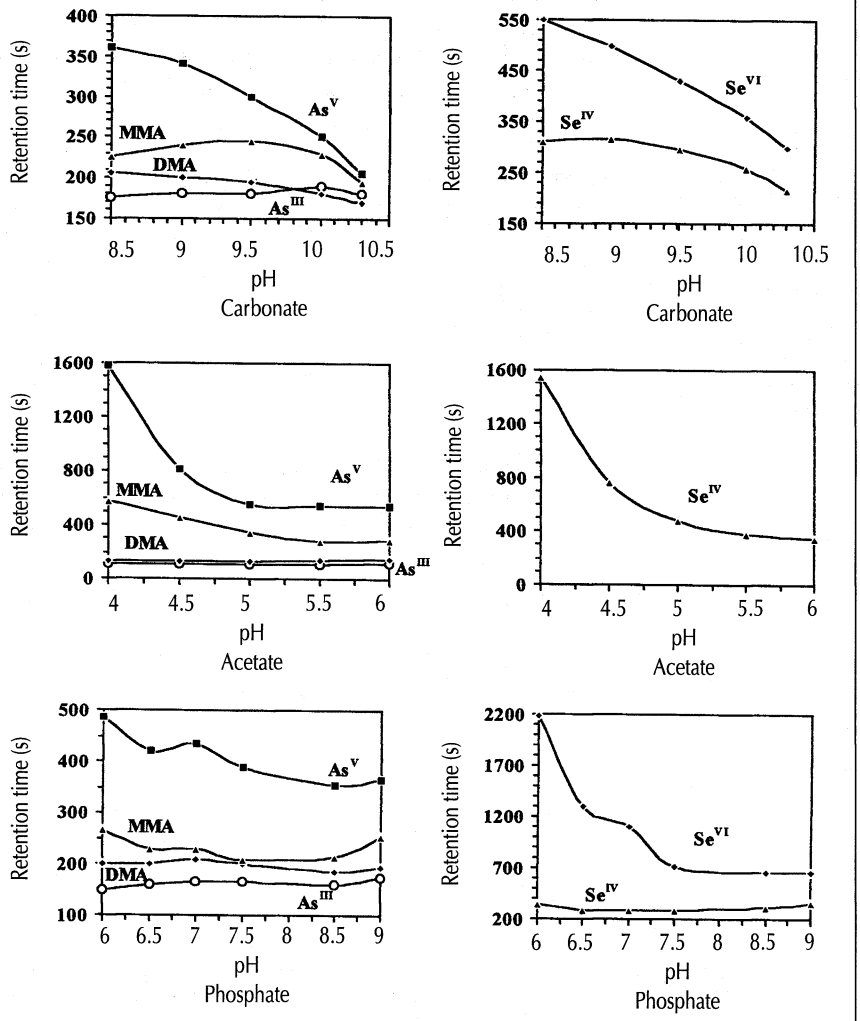

Figure 4. Dependence of the retention times of arsenic and selenium species on pH with the three different mobile phases: 0.1M carbonate buffer (1 mL/min flow rate), 0.03M acetate buffer (1.5 mL/min flow rate), 0.03M phosphate buffer (1 mL/min flow rate). A PRP-X100 column was used with an FAAS detector.

plasma. Optimal conditions found for the various elements differed somewhat. However, for multi-element speciation studies, one set of conditions must be chosen. Because arsenic was the main analyte of interest in this study, the settings optimized for this element were used for both arsenic and selenium.

The chromatographic system was interfaced with the ICP–MS instrument through a Teflon capillary tube (5-cm × 0.5-mm i.d.) that connected the HPLC column outlet to the inlet of the nebulizer. The chromatographic flow rate of 1.5 mL/min was compatible with the sample intake rate of the ICP–MS instrument. ICP–MS settings are given in Table I.

Results and Discussion

Preliminary studies were run using the FAAS for on-line HPLC detection. Then final optimization of chromatographic conditions was achieved by using HPLC–ICP–MS. Species of arsenic and selenium that are associated with polluted soils were considered. For arsenic, these included inorganic species (As^{III} arsenite ions and As^{V} arsenate ions) and organometallic species (MMA and DMA); cationic species such as arsenobetaïne or arsenocholine, found in biota, were not considered. Regarding selenium, this study was limited to Se^{IV} selenite ions and Se^{VI} selenate ions; organoselenium compounds such as selenoamino acids or methylated species were not considered. All arsenic and selenium retained species may be present as anionic forms if the pH is well-defined; this common property allows a single separation procedure to be designed for both elements. Two anion exchange columns were evaluated: a Merck Polyspher IC AN 2 and a Hamilton PRP-X100.

Several mobile phases were investigated. They should be compatible with on-line detection by both FAAS and ICP–MS and should allow a satisfying separation of all species in a reasonable time. Acetate, carbonate, and phosphate buffers were compared. To obtain optimal ICP–MS behavior, isocratic conditions were necessary, and ammonium salts were suggested.

All the species under study belong to weak acid–base systems (Table II). A strong influence of pH on their retention time by anion exchangers was foreseen. Gailer and Irgolic (24) clearly described the behavior of arsenic species in these conditions, but no equivalent exists for selenium species.

Apparent charges of species

The retention of arsenic and selenium species by anion exchangers relies mainly on their electrostatic interactions with the cationic sites of the solid surface, therefore on their "apparent charges" (AC), varying with pH. Other phenomena such as hydrophobic interactions of the various species with the polymeric solid may also influence the retention, especially for organometallic compounds such as MMA and DMA. Elution of analyte species electrostatically retained by the mobile phase at a given pH depends mainly on the AC of the mobile phase anion (ACMP). Buffer concentration also has a significant influence. Electrostatic interactions are examined here in some detail.

AC plots of these compounds as a function of pH are presented in Figure 1. AC values of a species were calculated from:

$$AC = \frac{\Sigma z \times (\text{concentration of the anion with charge } z-)}{\text{total concentration of this species}}$$

For example, if the considered species is As^{V} at a pH of 11.5:

$$AC = \frac{0 \times [H_3AsO_4] + (-1) \times [H_2AsO_4^-] + (-2) \times [HAsO_4^{2-}] + (-3) \times [AsO_4^{3-}]}{[H_3AsO_4] + [H_2AsO_4^-] + [HAsO_4^{2-}] + [AsO_4^{3-}]}$$

$$AC = \frac{0 - 0 - 2 \times (50\%) - 3 \times (50\%)}{0 + 0 + (50\%) + (50\%)} = -2.5$$

These diagrams may be considered for each individual element intended to define pH domains in which a good separation of all species may be achievable (i.e., domains in which AC values differ sufficiently).

One could expect separation of arsenic species to be possible in the pH range 4–11, whereas the pH range 0–10 would be suitable for selenium.

Simultaneous separation of selenium and arsenic species by anion exchange may therefore be attempted in the pH range 4–10. However, in the pH ranges 4–6 and 9–10, the differences between AC values of some arsenic species are quite low, and the optimal overall conditions should be found in the reduced pH domain 6–9.

Apparent charges of mobile phase anions

The mobile phases considered were acetate, carbonate, and phosphate buffers. To obtain a good separation of the various species of each element, the ACMP should be carefully chosen. An ACMP value that is too high will lead to a reduced resolution; one that is too low will considerably increase retention times and eventually lead to poor elution of the most strongly retained species. ACMP diagrams for acetate, carbonate, and phosphate solutions are presented in Figure 2.

It is clear from Figure 2 that, within the pH range in which separation of arsenic and selenium species is possible, acetate buffers covered the pH range 4–6, phosphate buffers covered the range 6–9, and carbonate buffers covered the range 8 to 10.

AC–ACMP ratios

The HPLC retention time of a given element species depends mainly on both the AC value at a given pH and the ACMP value at the same pH. Secondary effects (24) may somewhat alter this prediction, but major information can nevertheless be obtained from AC–ACMP plots as a function of pH (Figure 3).

Acetate buffers appear to be efficient for selenium species separation in the pH range 5–9; pH 6 is optimum. However, under these conditions, As^{III} is scarcely retained and should elute in the solvent front. Separation of As^{V}, MMA, and DMA appears possible at pH values from 6 to 7, a range in which the buffering capacity is very low.

The separation of arsenic species should be optimal at pH 8–9.5 in carbonate buffers. At pH values that are too low, As^{III} is not retained; MMA and DMA, which have similar AC–ACMP ratios, should not be significantly separated by electrostatic interactions; and As^{V} is strongly retained. At higher pH levels, DMA and As^{III} reach the same AC–ACMP ratio and should therefore not be separated. The separation of selenium species in carbonate solutions appears possible in the pH range 7–9. The buffering capacity of these solutions is again quite low due to the pK_a values of carbonic acid (6.35 and 10.33).

In phosphate buffers, AC–ACMP ratios indicate two possibilities for the separation of arsenic species: pH values less than 7 could be possible, but As^{III} is not retained at all. A pH range between 8 and 9.5 appears more satisfying; all arsenic species are retained with quite different AC–ACMP values. Selenium species separation is likely up to pH 9.5.

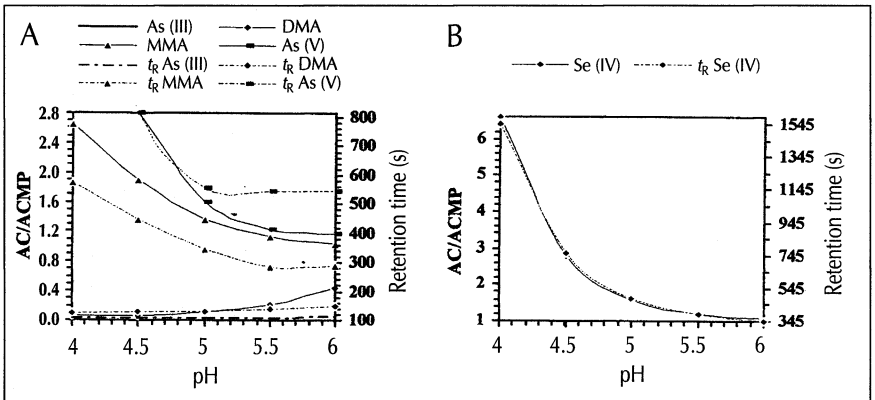

Figure 5. Comparison of experimental retention times and AC–ACMP ratios as a function of pH for (A) As^{III}, DMA, MMA, and As^{V} and (B) Se^{IV} and 0.03M acetate buffer. A PRP-X100 column was used.

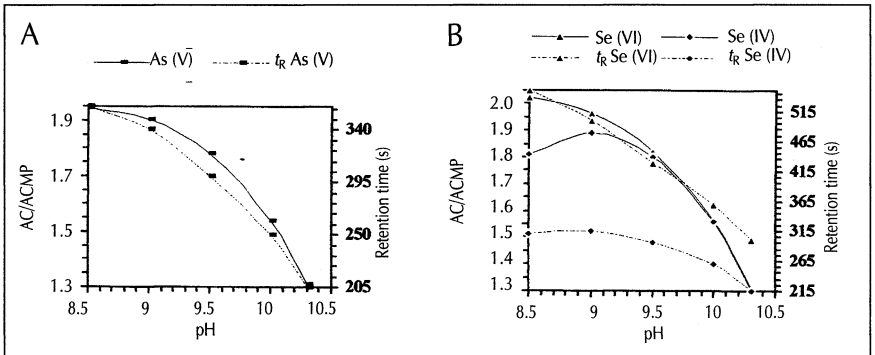

Figure 6. Comparison of experimental retention times and AC–ACMP ratios as a function of pH for (A) As^{V} and (B) Se^{IV} and Se^{VI} with 0.1M carbonate buffer. A PRP-X100 column was used.

HPLC–FAAS

Hamilton PRP-X100 column

The dependence of retention times on pH was studied experimentally by using HPLC–FAAS with the three mobile phases; the results are shown in Figure 4. It appears that ammonium carbonate buffer could be used at pH values close to 8.5; the separation of As^{III} and DMA peaks, however, was critical (difference in retention time, 25 s). Separation of arsenic species in acetate eluent occurred at pH 6, but As^{III} and DMA peaks were again very close. Moreover, Se^{VI} was very strongly retained. These results are in good agreement (for arsenic) with those of Gailer and Irgolic (24).

Optimal conditions were found in ammonium phosphate buffer at pH 8.5, where all arsenic and selenium species were separated and none were too strongly retained. Another possibility appeared to be at pH 6, where As^{III}–DMA separation was improved and Se^{VI} retention was strong.

The conclusion of this study was that ammonium phosphate buffers are the most efficient eluents for the simultaneous separation of the arsenic and selenium species studied.

Merck Polyspher IC AN2 column

The same type of study was conducted with an IC AN2 column by using carbonate and acetate buffers only. The separation of arsenic and selenium species with this column was not as efficient as with the PRP-X100.

Using carbonate buffer, for example, As^{III} and DMA could not be separated, even by varying pH or buffer concentration. Hansen et al. (11) obtained better results for these four arsenic species with the same buffer and the same column maintained in an oven set at 50°C. For the sake of experimental simplicity, this column was not retained for further studies.

Comparison of experimental and theoretical results

Experimental retention times obtained with the Hamilton PRP-X100 column were tentatively compared with theoretical AC–ACMP ratios as a function of pH for the three eluents tested. In these representations, an arbitrary adjustment of scales and origins was necessary because there is no simple theoretical relationship between retention times and AC–ACMP. This was done at both ends of the AC–ACMP range; as a result, only the curve shape is meaningful.

Figure 5 shows the results obtained in acetate buffer concerning arsenic species (Figure 5A) and Se^{IV} (Figure 5B). In acetate solutions in the pH range 4–6, Se^{VI} did not elute as a well-defined chromatographic peak. The behavior of arsenic species and Se^{IV} in acetate solutions appeared almost ideal because all AC/ACMP and retention time curves for all species had at least similar shapes, whereas only one arbitrary adjustment of scales and origins was made for the whole set of data. Inexplicably, the experimental retention of MMA was systematically and noticeably lower than expected from AC–ACMP ratios. Hydrophobic interaction of methyl groups with the polymer phase should, on the contrary, lead to increased retention. At low pH values (pH < 5), As^{III} and DMA are neutral species; they should not be retained at all. However, the retention time of DMA was slightly higher than that of As^{III}, probably due to hydrophobic interactions, as observed by Gailer and Irgolic (24).

Results in carbonate solutions (8.5 < pH < 10.5) were quite good, even for Se^{VI}. A few examples are presented in Figure 6 (As^{V}, Figure 6A; Se^{IV} and Se^{VI}, Figure 6B). Se^{IV} always eluted more rapidly than Se^{VI}, even between pH 9.5 and pH 10.3, where their AC–ACMP ratios merge into one single curve. The same manifestation appeared in phosphate buffer solutions at pH 9 (Figure 7B). This phenomenon may be explained by the larger size of Se^{VI} ions inducing its greater affinity towards the exchange sites of the stationary phase.

The correlations obtained for phosphate eluents (6 < pH < 9) were not as good. The general shapes of the retention time and AC/ACMP curves remained very similar (Figures 7A and 7B), but the agreement was not as good as that observed for acetate solutions. The pH range was quite wide, and at low pH values, the buffering capacity of the solution was low. This may lead to an insufficient control of local pH at the ion exchanger surface, which leads to unexpected retention time variations.

On the whole, AC–ACMP ratios appear to be a useful tool for qualitative prediction of the ion chromatographic retention of arsenic and selenium species. It is very efficient for acetate buffer solutions, remains useful for carbonate eluents, and provides some information for phosphate buffers.

The influence of buffer concentration on retention times was studied at a constant pH, differing with the mobile phase considered on the PRP-X100 column.

In the carbonate buffer, the best separation of arsenic and selenium species was obtained in 0.1M solution at the low pH of 8.5. However, arsenic species are not well-separated because the largest difference between As^{III} and DMA retention times was only 25 s (Figure 4). Under the same conditions, good separation of Se^{IV} and Se^{VI} was achieved after 10 min of analysis.

When a 0.03M acetate buffer was used at pH 6, the separation of arsenic species was quite good in a 10-min analysis (Figure 4),

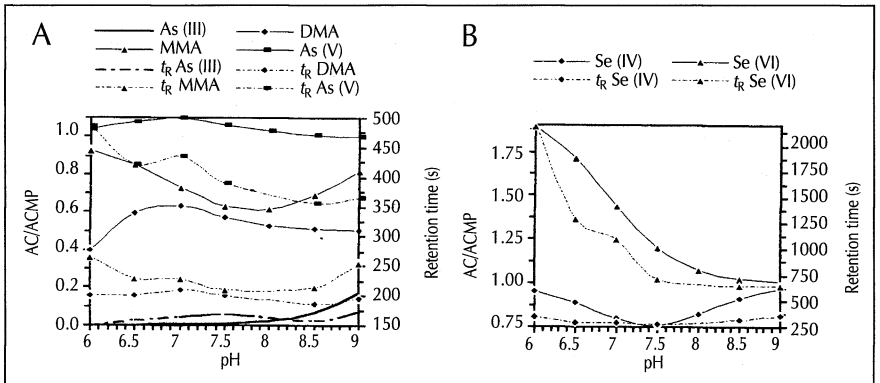

Figure 7. Comparison of experimental retention times and AC–ACMP ratios as a function of pH for (A) As^{III}, DMA, MMA, and As^{V} and (B) Se^{IV} and Se^{VI} with 0.03M phosphate buffer. A PRP-X100 column was used.

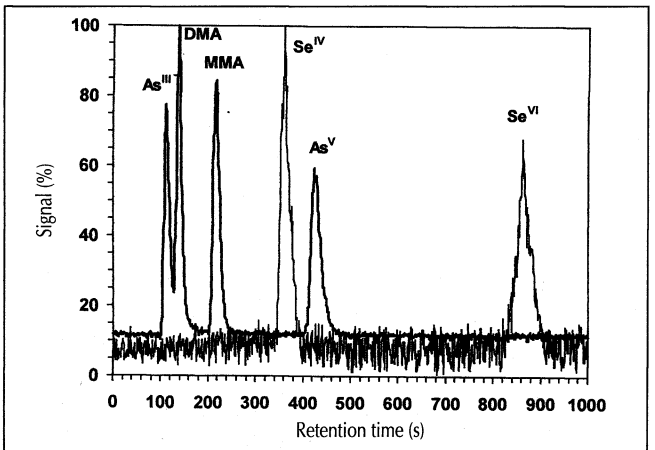

Figure 8. Typical HPLC–ICP–MS chromatogram of a mixture of 25 ng/mL (as element) of arsenic and selenium species in a 12.5mM ammonium phosphate buffer at pH 8.5. Signal intensities are given as a percentage of the highest species intensity (DMA). Flow rate: 1.5 mL/min.

Table III. Performance of the Anion-Exchange HPLC–ICP–MS Method

Species	RSD (%)*	Calibration curves: Slope (CPS/ng/mL)‡	Calibration curves: r^2	Detection limits†: Relative§ (ng/mL)	Detection limits†: Absolute (pg as element)
As^{III}	2.3	1929	0.9958	0.16	16
DMA	2.1	2886	0.9992	0.11	11
MMA	2.1	2278	0.9978	0.14	14
As^{V}	3.2	1519	0.9996	0.21	21
Se^{IV}	2.4	162	0.9978	2.0	200
Se^{VI}	2.1	77	0.9962	4.2	417

* Peak height reproducibility (6 replicates).
† Defined as 3 × standard deviation of 20 blank/slope of calibration curve (IUPAC, $k = 3$).
‡ Peak height mean for six replicate experiments.
§ Injection loop, 100 µL.

but only the Se^{IV} peak appeared. Se^{VI} was not detected as a definite peak but as a long delayed shift of the base line. These results are in good agreement with those of Gailer and Irgolic (24).

The best separation of all arsenic and selenium species was achieved with a 15mM ammonium phosphate buffer at pH 8.5, a 1-mL/min flow rate, and a 20-min analysis time (Figure 4). Separation of As^{III} and DMA could be improved at pH 6, but then Se^{VI} retention time would be too long. For a good simultaneous separation of arsenic and selenium species, a compromise between separation and analysis time was necessary.

HPLC–ICP–MS

The optimal separation conditions delimited in the HPLC–FAAS study were obtained at "high" analyte concentrations. Slight differences were observed using HPLC–ICP–MS, especially for As^{III} and DMA separation. This was probably due to a slight increase of peak width in the HPLC–ICP–MS method and also to the different concentration range used (about 10 ng/mL). A previous study (25) on the LC separation of organotin compounds demonstrated that, at trace levels, the chromatographic separation mechanisms of tin species differed from those evinced at higher concentrations.

The use of a phosphate buffer ($[NH_4]_2HPO_4$, 12.5mM adjusted to pH 8.5 with ammonia) and a flow rate of 1.5 mL/min gave an increased resolution between DMA and As^{III} peaks and was found to be optimal for HPLC–ICP–MS studies.

A flow rate change from 1 to 1.5 mL/min allowed a significant decrease in retention time while improving the peak shape and sensitivity. A typical chromatogram of a mixture of each arsenic and selenium species is shown in Figure 8. The total time required to obtain one chromatogram was lower than 16 min. The variability of retention times for any compound was less than 2 s.

Precision and linear dynamic range

In order to improve accuracy and overall analysis time, potassium hexahydroxyantimonate (Sb^{V}) was added as an internal reference standard. Reproducibility of peak heights was determined from six replicate injections of 5 ng arsenic and 20 ng selenium. The signal was normalized by 1 ng of Sb^{V} (Table III). Even without using an internal standard, the relative standard deviation (RSD) never exceeded 4%. Reproducibility was slightly better when peak heights were used rather than peak areas.

The linearity of calibration curves plotted with both peak heights and peak areas was found to be perfect for all species in the range of concentrations studied (0–200 ng/mL) (Table III). Correlation coefficients (r^2) obtained by using peak height and peak area measurements were excellent and almost identical. A more detailed study of the detection by ICP–MS is presented elsewhere (T. Guérin, M. Astruc, A. Batel, and M. Borsier. Multielemental speciation of As, Se, Sb, and Te by HPLC–ICP–MS. *Talanta,* in press.).

Detection limits, defined according to the International Union of Pure and Applied Chemistry (IUPAC) standard (26), are presented in Table III.

Conclusion

This work demonstrated that the coupling of the chromatographic anion-exchange separation (PRP-X100) with ICP–MS provided the resolution and sensitivity necessary to achieve simultaneous separation of arsenic and selenium species at ultratrace detection levels, which are required for environmental samples. The use of ACs and their ratios (ACMPs) in optimizing HPLC separation was of great help.

Acknowledgments

Thanks are due to A. Castetbon and M. Potin-Gautier from the University of PAU and R. Cottier at BRGM for their much appreciated technical assistance during the LC and ICP–MS studies, respectively.

References

1. M. Morita and J.S. Edmonds. Determination of arsenic species in environmental and biological samples. *Pure and Appl. Chem.* **64(4):** 575–90 (1992).

2. G. Kölbl, K. Kalcher, K.J. Irgolic, and R.J. Magee. Identification and quantification of inorganic and organic selenium compounds with high-performance liquid chromatography. *Appl. Organomet. Chem.* **7:** 443–66 (1993).
3. X. Dauchy, M. Potin-Gautier, A. Astruc, and M. Astruc. Analytical methods for the speciation of selenium compounds: A review. *Fresenius J. Anal. Chem.* **348:** 792–805 (1994).
4. K. Pyrsynska. Recent developments in speciation analysis of selenium. *Chem. Anal.* **40:** 667–86 (1995).
5. G. Lespès, M. Potin-Gautier, and A. Astruc. ICP–MS, a review: Applications to speciation of trace elements in aquatic and biological environments. *Environ. Technol.* **13:** 207–20 (1992).
6. Y. Inoue, K. Kawabata, H. Takahashi, and G. Endo. Determination of arsenic compounds using inductively coupled plasma mass spectrometry with ion chromatography. *J. Chromatogr.* **675:** 149–54 (1994).
7. S. Caroli, F. La Torre, F. Pettrucci, and N. Violante. On-line speciation of arsenical compounds in fish and mussel extracts by HPLC–ICP–MS. *Environ. Sci. & Pollut. Res.* **1(4):** 205–208 (1994).
8. M. Albert, C. Demesmay, M. Porthault, and J.L. Rocca. Séparation d'espèces arséniées organiques et minérales par chromatographie en phase liquide et électrophorèse capillaire. *Analusis* **20:** 383–90 (1992).
9. B.S. Sheppard, J.A. Caruso, D.T. Heitkemper, and K.A. Wolnik. Arsenic speciation by ion chromatography with inductively coupled plasma mass spectrometric detection. *Analyst* **117:** 971–75 (1992).
10. S. Branch, L. Ebdon, and P. O'Neill. Determination of arsenic species in fish by directly coupled high-performance liquid chromatography-inductively coupled plasma mass spectrometry. *J. Anal. Atom. Spectrom.* **9:** 33–37 (1994).
11. S.H. Hansen, E.H. Larsen, G. Pritzl, and C. Cornett. Separation of seven arsenic compounds by high-performance liquid chromatography with on-line detection by hydrogen–argon flame atomic absorption spectrometry and inductively coupled plasma mass spectrometry. *J. Anal. Atom. Spectrom.* **7:** 629–34 (1992).
12. X.C. Le, W.R. Cullen, and K.J. Reimer. Speciation of arsenic compounds by HPLC with hydride generation atomic absorption spectrometry and inductively coupled plasma mass spectrometry detection. *Talanta* **41(4):** 495–502 (1994).
13. S.C.K. Shum, R. Neddersen, and R.S. Houk. Elemental speciation by liquid chromatography–inductively coupled plasma mass spectrometry with direct injection nebulization. *Analyst* **117:** 577–82 (1992).
14. P. Thomas and K. Sniatecki. Determination of trace amounts of arsenic species in natural waters by high-performance liquid chromatography–inductively coupled plasma mass spectrometry. *J. Anal. Atom. Spectrom.* **10:** 615–18 (1995).
15. C. Hwang and S. Jiang. Determination of arsenic compounds in water samples by liquid chromatography–inductively coupled plasma mass spectrometry with an in situ nebulizer–hydride generator. *Anal. Chim. Acta* **289:** 205–13 (1994).
16. J.J. Thompson and R.S. Houk. Inductively coupled plasma mass spectrometric detection for multielement flow injection analysis and elemental speciation by reversed-phase liquid chromatography. *Anal. Chem.* **58:** 2541–48 (1986).
17. Y. Cai, M. Cabañas, J.L. Fernàndez-Turiel, M. Abalos, and J.M. Bayona. On-line preconcentration of selenium(IV) and selenium(VI) in aqueous matrices followed by liquid chromatography–inductively coupled plasma mass spectrometry determination. *Anal. Chim. Acta* **314:** 183–92 (1995).
18. S.C.K. Shum and R.S. Houk. Elemental speciation by anion exchange and size-exclusion chromatography with detection by inductively coupled plasma mass spectrometry with direct injection nebulization. *Anal. Chem.* **65:** 2972–76 (1993).
19. K.T. Suzuki, M. Itoh, and M. Ohmichi. Detection of selenium-containing biological constituents by high-performance liquid chromatography–plasma source mass spectrometry. *J. Chromatogr.* **666:** 13–19 (1995).
20. H. Ding, J. Wang, J.G. Dorsey, and J.A. Caruso. Arsenic speciation by micellar liquid chromatography with inductively coupled plasma mass spectrometric detection. *J. Chromatogr.* **694:** 425–31 (1995).
21. D. Schlegel, J. Mattush, and K. Dittrich. Speciation of arsenic and selenium compounds by ion chromatography with inductively coupled plasma atomic emission spectrometry detection using the hydride technique. *J. Chromatogr.* **683:** 261–67 (1994).
22. L.A. Colón and E.F. Barry. The determination of arsenic and selenium by HPLC combined with alternating current plasma detection and post-column hydride formation. *J. High Res. Chromatogr.* **14:** 608–12 (1991).
23. R.M. Smith and A.E. Martell. Critical Stability Constants, Vol. 3–5. Plenum Press, New York, NY, 1976–82.
24. J. Gailer and K.J. Irgolic. The ion-chromatographic behavior of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid on the Hamilton PRP-X100 anion-exchange column. *Appl. Organomet. Chem.* **8:** 129–40 (1994).
25. A. Astruc, M. Astruc, R. Pinel, and M. Potin-Gautier. Speciation of butyltin compounds by on-line HPLC–ETAA of tropolone complexes in environmental samples. *Appl. Organomet. Chem.* **6:** 39–47 (1992).
26. International Union of Pure and Applied Chemistry. Nomenclature, symbols, units and their usage in spectrochemical analysis-II. Data interpretation. *Spectrochim. Acta Part B* **33:** 241–45 (1975).

Manuscript accepted December 18, 1996.