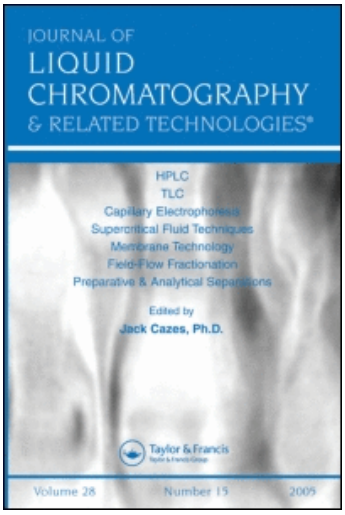

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Rubio, Roser , Peralta, Isabel , Albertí, Joan and Rauret, Gemma(1993) 'Arsenic Species Separation by IELC-ICP/OES: Arsenochoune Behavior', Journal of Liquid Chromatography & Related Technologies, 16: 16, 3531 — 3542
To link to this Article: DOI: 10.1080/10826079308019706
URL: http://dx.doi.org/10.1080/10826079308019706

JOURNAL OF LIQUID CHROMATOGRAPHY, 16(16), 3531-3542 (1993)

ARSENIC SPECIES SEPARATION BY IELC-ICP/OES: ARSENOCHOLINE BEHAVIOR

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ABSTRACT

The separation conditions and quantification of As(III), As(V), monomethylarsenate (MMA), dimethylarsinate (DMA), arsenobetaine (AsBet) and arsenocholine (AsChol) are studied by Liquid Chromatography (LC) coupled directly to an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP/OES) system. The separation conditions are optimized to improve the resolution of the six arsenic species. Arsenocholine shows a particular pattern of behaviour when phosphate is used as eluent: two peaks are observed in the chromatogram, thus a systematic study assaying different pH and concentration of phosphate is carried out to improve resolution and analysis time when the six arsenic compounds are analyzed in a mixture. Boric acid as mobile phase avoids the splitting of the arsenocholine peak and leads to a good separation of the six arsenic compounds. Detection limits are stablished for the six arsenic species.

INTRODUCTION

In the literature an increasing interest is observed in developing methods to determine arsenobetaine, arsenocholine and related compounds in sea food, and in reference materials in which the speciation of these compounds together with other organic and inorganic arsenic species is being carried out (1-7). It has been assumed that arsenic occurs mainly as arsenobetaine and, to a lesser extent, arsenocholine in marine invertebrates and fish (8,9,10), although arsenocholine is present only in some marine organisms like shrimps (5).

The analytical methods developed for arsenic speciation use coupled techniques, such as on-line hydride generation (cold-trap) coupled to AAS (11-13), LC-ICP/OES (14,15), LC-HG-AAS (16,17) and LC-HG-ICP/OES (18,19), LC-UV-HG-ICP/OES (20,21) and recently LC-ICP/MS (1,22,23). The last two coupled techniques also permit the determination at low concentrations of AsChol and AsBet, which cannot directly form their respective hydrides (as AsChol and AsBet). Recently we have established the conditions for the determination of five arsenic compounds using LC-UV-HG-ICP/OES (24,25) at low concentrations.

One of the main difficulties in the determination of AsBet, AsChol, DMA, MMA, As(V) and As(III) by coupled techniques (where liquid chromatography is used) is to achieve chromatographic separation with good resolution when the coupled system includes hydride generation step or photo-oxidation process before detection of arsenic species, and when sample volumes of more than 100 µl have to be injected to obtain low detection limits if an ICP/MS detector is not available.

In this study, the separation of the six arsenic compounds is achieved using a silica-based anionic exchange column. A particular pattern of behaviour was observed in the separation of arsenocholine using phosphate solution as eluent, namely the splitting of the chromatographic peak. To study this behaviour in more detail, the arsenocholine elution was carried out using not only phosphate solutions at different concentrations, but also other elution systems.

MATERIALS AND METHODS

Apparatus

LC system: a LKB pump and Perkin Elmer 250 LC Binary pump, an anion exchange Supelcosil LC-SAX column with particle size 5 µm (250 mm x 4.6 mm i.d.) and Reodyne 7125 with injection loop of 100 µL.

ICP-OES: "Plasmatherm" source, inductively coupled to a high frequency (27.12 MHz) magnetic field, operating at 1 kw. A Jobin-Ivon thermoregulated monochromator with a holographic grating, 3600 grooves mm^{-1}, focal length 1m, 0.1 A resolution. Argon was used as coolant (18 l min^{-1}) and carrier gas (0.5 l min^{-1}). Wavelength: 193,696 nm. An IBM PS/2 was used for data acquisition.

A PTFE tube, 0.25 mm internal diameter was used to connect the outlet of the HPLC column to the entry tube of a Cross-flow nebulizer.

Reagents

Stock solution of arsenic compounds were prepared of 1000 mg L^{-1} each of Arsenite (MercK), Arsenate (Carlo Erba), MMA (Carlo Erba) and DMA (Fluka). AsChol. and AsBet. solutions were supplied by Service Central d'Analyse from CNRS, Vernaison (France).

All the stock solutions were stored in polyethylene bottles, except AsChol. and AsBet. which were stored in glass bottles, and maintained at 4°C. Dilute solutions for analysis were prepared weekly.

Mobile phases:

a) Phosphate solutions: Prepared by mixing aqueous solution of Na_2HPO_4 and aqueous solution of H_3PO_4 or NaH_2PO_4 to reach the desired pH. The resulting solution was filtered through a 0.22 μm membrane.

b) Boric acid at different concentrations prepared from H_3BO_3 Merck pro analysi, dissolved in water. These solutions were filtered through a 0.22 μm membrane.

Procedures

A) Isocratic elution:

Phosphate solution at the selected pH value was used as mobile phase at 1 $ml.min^{-1}$, 100 μl of a solution containing the arsenic compounds was injected into the column and the elution was performed isocratically.

B) Gradient elution:

Solution 1: H_3BO_3 0.05 $mol.l^{-1}$, pH 5.0. Solution 2: 0.01 $mol.l^{-1}$ Na_2HPO_4/NaH_2PO_4 at pH 5.0. Elution conditions: 100% sol.1 for 1 min., changing in 0.1 min. to 100% sol. 2 for 8 min., then 100% sol. 1 is reached again in 0.1 min. and maintained for 8 min. at a flow rate of 1 $ml.min^{-1}$.

RESULTS AND DISCUSSION

Isocratic elution with phosphate solution as mobile phase:

Effect of pH and phosphate concentration on arsenic species separation.

The separation of arsenic compounds was studied using mobile phase solutions at two phosphate concentration (0.005 and 0.02 $mol.l^{-1}$) and different pH. The retention

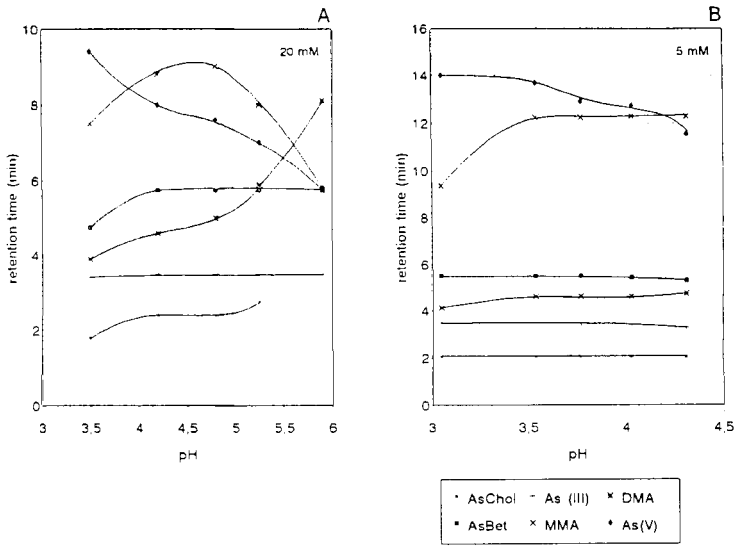

Figure 1. Retention time of arsenic species versus pH using an anion exchange column (Supelcosil LC-SAX) and isocratic elution using phosphate solution as mobile phase, a) 0.02 mol.l^{-1} PO_4^{3-}, b) 0.005 mol.l^{-1} PO_4^{3-}.

times values versus pH are plotted in Figure 1. The best separation for AsChol, AsBet, DMA, MMA, As(III) and As(V) using phosphate 0.005 mol.l^{-1} was achieved at pH 3.75 as is shown in the chromatogram in Figure 2.a. To reduce the retention time of MMA and As(V), we assayed a more concentrated mobile phase, 0.02 mol.l^{-1} phosphate; the chromatogram is shown in Figure 2.b.

Using these conditions we performed the separation of the six arsenic species with a short run time and good resolution.

As can be observed in the chromatograms, a shoulder appears in the arsenocholine peak, which becomes a single peak at higher phosphate concentration. This behaviour is observed at all pH values using phosphate as mobile phase.

Effect of phosphate concentration on arsenocholine peakshape.

The chromatograms in Figure 3 show the effect of phosphate concentration (from 0.005 to 0.05 mol.l^{-1}) of the mobile phase on arsenocholine retention. For low phosphate

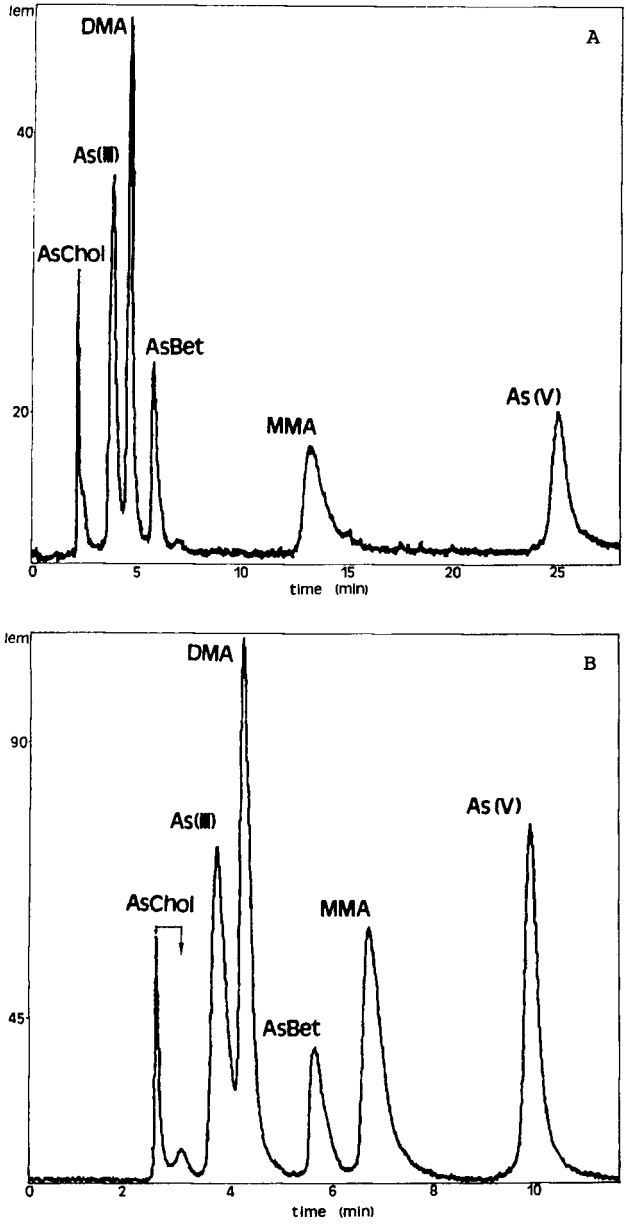

Figure 2. Chromatograms obtained with procedure A.

a) Mixture of 5 $mg.l^{-1}$ as As of AsChol, AsBet, DMA, MMA, As(III) and As(V). Mobile phase: 0.005 $mol.l^{-1}$ PO_4^{3-}.

b) Mixture of 10 $mg.l^{-1}$ as As of AsChol, AsBet, DMA, MMA, As(III) and As(V). Mobile phase: 0.02 $mol.l^{-1}$ PO_4^{3-}.

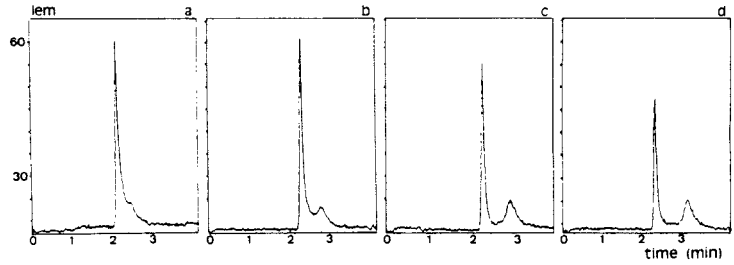

Figure 3. Arsenocholine (10 mg.l^{-1}) peak splitting when eluted using different phosphate solutions as mobile phase and isocratic elution (Procedure A).
a) 0.005 mol.l^{-1} PO_4^{3-}.
b) 0.01 mol.l^{-1} PO_4^{3-}.
c) 0.03 mol.l^{-1} PO_4^{3-}.
d) 0.05 mol.l^{-1} PO_4^{3-}.

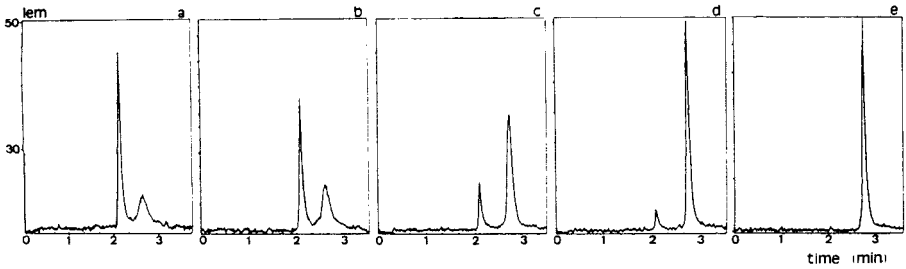

Figure 4. Arsenocholine behaviour after treatment with H_3PO_4 solutions at different concentrations. Conditions: 10 mg.l^{-1} AsChol, mobile phase: 0.02 mol.l^{-1} PO_4^{3-}.
a) 0.015 mol.l^{-1} H_3PO_4.
b) 0.03 mol.l^{-1} H_3PO_4.
c) 0.06 mol.l^{-1} H_3PO_4.
d) 0.09 mol.l^{-1} H_3PO_4.
e) 0.15 mol.l^{-1} H_3PO_4.

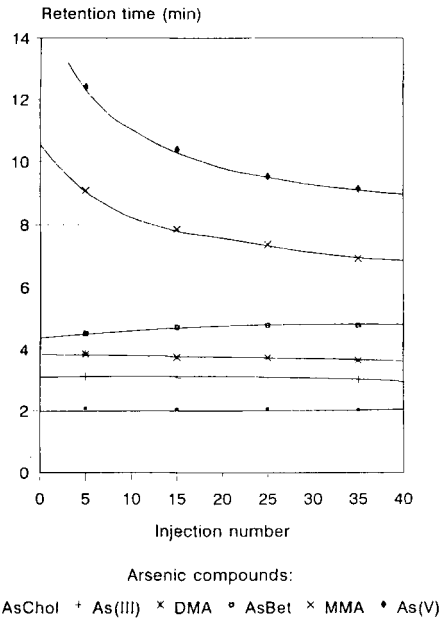

Figure 5. Variation of retention times of arsenic species versus injection number performed using isocratic elution (0.02 mol.l^{-1} PO_4^{3-} described in Procedure A.

concentration (0.005 mol.l^{-1}), only one peak is observed together with a shoulder. For higher phosphate concentrations two separate peaks are observed. This behaviour could be attributed to an interaction between arsenocholine and phosphate. This hypothesis lead us to study the effect of the addition of phosphoric acid to arsenocholine solutions before the separation process.

Figure 4 shows a different behaviour patterns of arsenocholine depending on phosphoric acid concentration. Each chromatogram corresponds to a solution of AsChol previously mixed with phosphoric acid at the corresponding concentration. Two peaks are observed in all the chromatograms. A revelant increase is observed in the peak height of the most retained species when phosphoric acid concentration increases. Finally, for 0.15 mol.l^{-1} phosphoric acid only the second peak is recorded.

Table 1: Detection limits obtained using Procedure A, and 0.02 mol.l^{-1} PO_4^{3-} as mobile phase. Mean value of three different days.

Detection limits (mg.l^{-1} as arsenic specie)					
As(III)	As(V)	MMA	DMA	AsChol	AsBet
0.22	0.87	1.52	0.41	0.34	0.41

Column longevity.

The major drawback in the use of this silica-based anionic exchange column in the separation conditions described above is that the column lasts for only a few days before splitting appears in the arsenocholine peak. Moreover, a gradual decrease in the retention time of MMA and As(V) is observed. Retention time of arsenic species depends on the number of injections performed using Procedure A as can be observed in Figure 5.

Detection limits.

Once the separation conditions were established (mobile phase: phosphate solution 0.02 mol.l^{-1}, pH 3.75. Injection volume: 100 µl), we determined the detection limits for each arsenic species. For this a calibration graph was calculated from three standard solutions containing the six species at three concentrations ranging from 1 to 10 mg L^{-1} of As(III), AsChol, AsBet and DMA and from 3 to 30 mg L^{-1} of MMA and As(V). The background signals were calculated from the baseline of the chromatograms obtained for each standard solution of the calibration curve. Detection limits were calculated as twice the standard deviation of the background signals. The values obtained are reported in Table 1.

Gradient elution with boric acid-phosphate as mobile phase.

The use of boric acid as an alternative to phosphate was considered. In this case, the splitting of arsenocholine peak is not observed. Thus, different boric acid

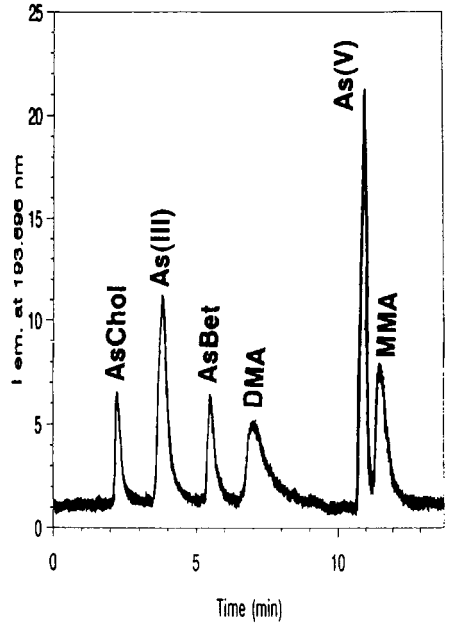

Figure 6. Chromatogram obtained using gradient elution described in Procedure B. Mixture of 5 $mg.l^{-1}$ as As of AsChol, AsBet, DMA, MMA, As(III) and As(V).

Table 2: Variation of retention times of arsenic species as a function of the injections performed using gradient elution described in Procedure B.

	Retention time (min)					
$N_{injection}$	AsChol	As(III)	DMA	AsBet	MMA	As(V)
15	2.1	3.3	8.6	4.8	13.1	14.6
25	2.1	3.3	5.8	4.8	9.4	10.2

concentrations at different pH were assayed for arsenic compounds separation. The best separation for AsChol, As(III), DMA and AsBet was obtained at concentration of 0.05 mol.l^{-1} boric acid (pH 5.0), but MMA and As(V) show a very high retention time (more than 25 min.). In order to shorten the retention time of the last two compounds, we decided to carry out their elution using 0.01 mol.l^{-1} phosphate at pH 5.0. Figure 6 shows the chromatogram obtained in the conditions described in Procedure B. Excellent resolution for the six species can be observed and only one peak appears for arsenocholine.

In these conditions there was a more noticeable decrease of the retention times of DMA, MMA and As(V) than when isocratic elution with phosphate is performed, and only about 30 injections can be performed with an acceptable resolution. Table 2 represents the values of the retention times according to the number of injections, when gradient elution with boric acid is used.

CONCLUSIONS

From this study it can be concluded that arsenic speciation in samples in which arsenocholine is present, special attentiom must be paid to the elution conditions in order to avoid the spliting of the arsenocholine peak, which can lead to some systematic errors in the identification and quantification. Moreover in samples containing phosphate, as in fish extracts, significant differences may appear when chromatograms of the samples and the standard solutions used for calibration are compared.

The arsenocholine behaviour might be attributed to the afinity between arsenocholine and phosphate. It has to be pointed out that in other related studies on arsenic speciation we did not observed the splitting of the arsenocholine peak when using a polymeric-based anionic exchange column (25), probably due to the lower resolution of these columns compared with the silica-based column used in this study.

It should be emphasized that good resolution is obtained for the six arsenic species with the anionic exchange column used in this study, although its life is short.

ACKNOWLEDGEMENTS

The authors thank Service Central d'Analyse from CNRS, Vernaison (France) for AsChol and AsBet supply.

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Received: March 14, 1993
Accepted: April 9, 1993