Arsenic Speciation by Ion-Pair Reversed-Phase Liquid Chromatography with Coupled Amperometric and Ultraviolet Detection

P. Boucher, M. Accominotti, and **J.J. Vallon***

Laboratoire de Biochimie, Pharmacotoxicologie et Analyse des traces, Hôpital Edouard Herriot, place d'Arsonval, 69437 Lyon 03, France

Laboratoire de Chimie Analytique III, Faculté de Pharmacie, Université Claude Bernard, 8, avenue Rockefeller, 69373, Lyon 08, France

Abstract

An ion-pair reversed-phase liquid chromatographic method is used to separate As(III), As(V), arsenobetaïn, arsenocholin, monomethylarsonic, and dimethylarsonic acids in a single run on a common C_{18} stationary phase with coupled amperometric and ultraviolet detection. As(III) is not separated from arsenobetaïn, but only As(III) is detected by amperometry. The calibration curves of the tested species are linear from 0.5 to 3 µmol/L. Coefficient of variation values for intra-assay precision range between 2.5% and 9.2%. The limit of quantitation ranges from 0.1–1 µmol/L, depending on the species concerned.

Introduction

Arsenic is naturally present in soil, air, and water, and its concentrations can be increased by pollution of industrial or agricultural origin. Epidemiological studies have demonstrated that exposure to high levels of arsenic is correlated to serious diseases such as skin and lung cancer (1,2). It is well known that arsenic toxicity is highly dependent on the chemical species concerned or on its oxydative degree (4); inorganic arsenical compounds are highly toxic (arsine more than arsenite and arseniate) (3,5,6). Organic arsenicals are nontoxic, as they are promptly excreted in urine. Examples include arsenobetaïn (Asbet), arsenocholin (Aschol), monomethylarsonic (MMA), and dimethylarsonic (DMA) acids, which originate from marine foods. For the most part, arsenic speciation has been studied in the environment or in marine animals (7–9), but a few studies were done on humans (8,10,11) and these studies generally did not take into account all the arsenic species concerned. Chromatographic separations are generally used for speciation; there are reports in the literature that use gas chromatography (14,15) and liquid chromatography (LC) with ion-exchange (7,9,10,12,13,16,17) or ion-pairing (16,17). A recent study (16) succeeded in separating As(III), MMA, DMA, and As(V) on an anionic-exchange column with a phosphate mobile phase (pH 5.3). The ultraviolet detector was set at 200 nm. The same authors tried to separate six different species (Asbet, As[III], DMA, MMA, Aschol and As[V]) by ion pairing with tetrabutylammonium hydroxyde (TBAH) (16), but the separation of As(III) from Asbet could not be achieved. The authors proposed using a stationary phase and an alkaline mobile phase (pH 9.0), thus allowing dissociation of arsenious acid (pK_a 9.3) and its separation from arsenobetaïn (17). Nevertheless the resolution of these two species was not satisfactory. Due to the relative failure of these studies, we decided to try differentiation of the various arsenical species by combining ultraviolet and amperometric detection after ion-pair chromatographic separation.

Experimental

Instrumentation

All separations were done using an LC6A liquid chromatograph (Shimadzu) equipped with a Rheodyne 7010 injection valve (Touzart and Matignon; LesUlis, France) and a 20-µL loop. A 250-mm C_{18} Lichrospher column with a 5-µm silica gel stationary phase was used for the separations (Helwett-Packard; Bron, France). Two detectors were used: a variable wavelength detector containing an 8-µL flow cell (Shimadzu) and an amperometric detector composed of a DL1 cell (Tacussel; France) with a vitrous carbon working electrode (DL1/CV) and platinum (DL1/PT) and Ag/AgCl electrodes (DL1/ER). The amperometric cell was connected at the output of the ultraviolet detector, and it worked on the wall-jet mode.

Reagents

As_2O_3 (Sigma), $Na_2HAsO_4 \cdot 7H_2O$ (Sigma), monomethylarsinic (Carlo Erba; Rueil-Malmaison, France), and dimethylarsinic acids (Sigma; Saint-Quentin Fallavier, France) were in 10^{-2}M solutions. Arsenobetaïn and arsenocholin were in 1 g/L solutions. The LC mobile phase was tetrabutylammonium hydroxyde (Fluka; Saint-Quentin Fallavier, France) 40% in bidistilled water. The pH value was set at 7.3. The column conditioning was done with water (pH 7.3) for 30 min followed by an acetonitrile–water (80:20) mixture for 24 h.

* Author to whom correspondence should be addressed.

Ultraviolet detection

Ultraviolet spectra were determined in the mobile phase medium (5mM TBAH, pH 7.3) at a concentration of 75 mg As per liter. Maximum absorbances were between 190 and 200 nm. The detector was set at 200 nm, which was the lowest detectable wavelength.

Amperometric detection

The voltammogram that showed the amperometric detection of arsenite ion in the mobile phase was obtained in the wall-jet mode in the potential range of –1.5 to +2.5 V.

Chromatographic separation

We used the ion-pair chromatographic conditions as previously described (16). TBAH was a good ion-pairing agent, and pH 7 was adequate for the separation of most compounds except arsenite and arsenobetaïn. Nevertheless, some compounds were found to elute in a different order from that found by Morin (16). This led us to study the influence of TBAH concentrations between 1.5 and 15 mmol/L.

Results

Influence of TBAH concentration

Various chromatographic parameters were measured as a function of TBAH concentration: capacity factor, selectivity, and resolution (Tables I and II). Table II shows that Asbet and As(III) cannot be separated regardless of the TBAH concentration. According to these data, it is evident that the retention of every solute in the column is dependent either on its pK_a value or on the apparent charge as calculated by Morin (16). Higher absolute values of the apparent charge result in easier ion-pairing and retention on the stationary phase. When the concentration of TBAH reaches 1mM, arsenical compounds are best separated, except for Asbet and As(III). Asbet is the first solute to be eluted. Arsenite is a very weak acid (pK_a 9.3) but it is more polar than Asbet (pH 7.3), and it is more retained on the column. This is in favor of an association with the counter ion. The capacity factor of Aschol is not influenced by TBAH concentration. The arseniate ion, which has a low pK_a value and a high apparent charge (–1.7) at pH 7.3, has the highest capacity factor (k') value (Table I), and therefore, ion-pairing is easy. A comparison between our results and those of Morin (16) should be done; increasing concentrations of TBAH leads to opposite effects on k' values: an increase in values observed by Morin and a decrease in our assays. This can be explained by a partition of ion-pairs between the stationary and mobile phases (Morin) or an ion-exchange phenomenon (our result). The conditioning mode for the stationary phase could be at the origin of this difference; when we achieved prolonged conditioning with TBAH, one could suppose that the counter-ion was fixed on the C_{18} phase by hydrophobic links, leaving the ammonium ionic group outside the phase and leading to an ion exchange stationary phase. On the contrary, conditioning over a short time period or the absence of conditioning only resulted in a partitioning of ion-pairs.

Table I. Capacity Factor for Each TBAH Concentration

TBAH conc. (mM)	Capacity factor					
	Asbet	AsIII	DMA	MMA	Aschol	AsV
1.5	0.4	0.4	2.5	3.2	5.9	20.1
3	0.4	0.4	1.8	2.4	5.4	13.9
4.5	0.16	0.4	1.8	2.2	6.1	13
7.5	0.15	0.14	1.4	1.7	5.7	8.8
10.5	0.15	0.32	0.92	0.92	4.7	5.6

Choice of conditions for chromatography

A mobile phase containing 6M (10^{-3}) TBAH at pH 7.3 and at a flow rate of 0.3 mL/min resulted in good resolution of the six arsenical species, except for arseniate, which was eluted only after 70 min. Various methods could lead to a shorter elution time. A pH or an ionic strength gradient was chosen by Albert and co-workers (18) to decrease the k' values. We decided to use coupled ultraviolet and amperometric detection. The total elution time was shortened to 30 min using a flow rate of 0.7 mL/min. Ultraviolet detection quantitates As(III) and Asbet, but the only electroactive species is arsenite.

Electrochemical detection of arsenite

We decided to use the electroreduction mode for arsenite (amperometric detector set at –1.4 V), which has the advantage of having a greater intensity than oxidation ($I_{ox,1}$ = 150; $I_{Red,1}$ = 220 in arbitrary units) (Figure 1).

Simultaneous ultraviolet–amperometric detection

The ultraviolet absorbance measured at the retention time common to As(III) and Asbet can quantitate both of these species. The amperometric intensity measured at the same re-

Table II. Ion-Pair Chromatographic Parameters for Each TBAH Concentration and for Each Pair of Solutes

Solute pair	1.5mM	3mM	4.5mM	7.5mM	10.5mM
Asbet–As(III)					
Selectivity	1	1	2.5	2.66	2.13
Resolution	–	–	0.74	0.74	0.74
As(III)–DMA					
Selectivity	6.25	4.5	4.5	3.5	2.87
Resolution	7.35	6.05	7.47	7.74	7.47
DMA–MMA					
Selectivity	1.28	1.33	1.22	1.2	1.0
Resolution	2.70	2.40	2.18	2.06	–
MMA–Aschol					
Selectivity	1.84	2.25	2.77	3.35	5.11
Resolution	8.13	13.2	18.2	22.35	23.5
Aschol–AsV					
Selectivity	3.41	2.57	2.13	1.54	1.20
Resolution	20.82	17.06	17.02	9.90	4.96

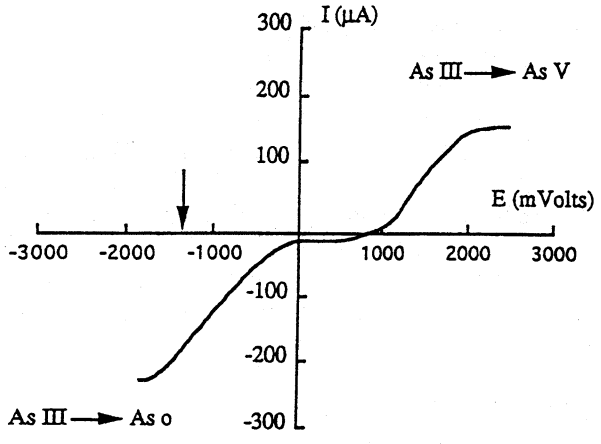

Figure 1: Voltammogram of arsenite in the mobile phase (6mM TBAH) at pH 7.3. The voltammogram shows two different redox systems. The first one (+ 1.50 V) corresponds to oxidation of As(III) to As(V); the half wave potential (–0.90 V) indicates electroreduction of As(III) to As(o). The arrow between –2000 and –1000 indicates the potential set by us.

tention time gives only the concentration of As(III). The absorbance at 200 nm of such a concentration of As(III), when substracted from the total absorbance, gives the Asbet concentration. Two calibration curves are used for this quantitation; one is for As(III) by amperometry. When ultraviolet detected is used, two curves are needed: one curve is used for As(III) in pure solution and one is used for Asbet.

Quality control

The calibration curve of the tested species was linear from 0.5 to 3 μmol/L. To test intra-assay precision, 10 assays were analyzed from a 0.5M (10^{-6}) mixture of each solute. Values for the coefficient of variation ranged between 2.46% and 9.24% depending on the species (Table III). The same mixture was tested once each day over the course of three days to test interassay precision. It ranged between 3.12% and 9.70% (Table III). The limit of quantitation (LOQ) was defined as the sample concentration giving a signal equal to 3 times the noise at the same retention time. It was determined by injection of an aliquot of mobile phase and measuring the noise. LOQ values are higher for species whose absorbance is low (Asbet, DMA, MMA, and As[V]) (Table III). Arseniate has the highest LOQ (56.2 μg/L) but its urinary concentration is always low because arnsenate is intensely metabolized. When one considers total arsenic concentrations in normal urine samples (20 to 50 μg/L) the LOQ observed seems to be sufficient to evaluate toxic concentrations.

Discussion

A recent study (16) shows that one way of separating Asbet and As(III) is to use an alkaline medium. This choice necessitates the use of a special and expensive stationary phase. This inconvenience is avoided through the use of coupled detection. The present LC method proved to be applicable for the quantitation of arsenical species in a single run without the use of an alkaline mobile phase at toxic levels in urine. Toxicological and metabolic studies have shown that speciation of arsenical compounds is necessary to ascertain intoxication. Organometallic species (MMA, DMA, Asbet, Aschol) are nontoxic or poorly toxic and originate from foodstuff. Inorganic arsenic (arsenite, arseniate, and arsine), on the other hand, is highly toxic. The lethal dose is considered to be around 100–200 mg of arsenious oxide. Arsin is volatile, absorbed via the lungs, hemolytic, and the most toxic of all species. Arsenite is the next most toxic species; it is rapidly absorbed in the intestinal tract where it combines rapidly to form thiols. It is responsible for the inhibition of pyruvate and glutathion decarboxylase. The arseniate ion is quickly reduced to arsenite in vivo, which is an inhibitor of phosphate in oxidative phosphorylations in the mitochondrial respiratory chain. Inorganic arsenic is quickly methylated to MMA and DMA, which undergo urinary elimination. If the ingested quantity of inorganic As is low, even when repeated chronic ingestions take place, DMA is mainly excreted (70% of ingested dose). In regard to MMA, the percentage is only 11%–19% and 3%–18% of total inorganic arsenic (7). In acute poisoning, these percentages are inversed; there is a high percentage of inorganic arsenic in urinary elimination and a low percentage of DMA (as compared with the level of MMA). It must be observed that seafood absorption leads to 90–100% of DMA and 0–10% of MMA (7). Urinary speciation of arsenic indicates an increase of the MMA–DMA ratio with the severity of intoxication. Arsenobetaïn and arsenocholin are indicators of normal food absorption. Consequently, speciation must be carried out after a 50-h interval during which no seafood should be ingested.

Table III. Interassay Precision, Intra-assay Precision, and Limit of Quantitation

Solute	Interassay coefficient of variation (%)	Intra-assay coefficient of variation (%)	Mean±SD (μmol/L)	Quantitation limit (μmol/L)
As(II)	4.27	3.76	0.46 ± 0.02	0.10
Asbet	4.95	4.60	0.45 ± 0.02	0.30
DMA	5.34	5.07	0.45 ± 0.02	0.30
MMA	8.21	8.14	0.42 ± 0.03	0.40
Aschol	3.12	2.46	0.47 ± 0.01	0.12
As(V)	9.70	9.24	0.41 ± 0.04	1.00

Conclusion

Coupled detection offers quantitative and selective separation in an aqueous medium. This method could be useful for the detection of arsenic compounds in urine. The total arsenic concentration in normal urine is near 50 μg/L; however, no indication of normal or toxic ranges for arsenical species can be found in the literature to our knowledge. The proposed LC method should allow

such a determination in humans in a single run without the use of an alkaline mobile phase.

Acknowledgment

We gratefully acknowledge CNRS Solaise (Lyon, France) for providing us with arsenobetaïn and arsenocholin.

References

1. W. Morton, G. Starr, J. Pohl, and S. Stoner. Skin cancer and water arsenic in Lane County Oregon. *Cancer* **37:** 2523–31 (1976).
2. S. Yesch. Skin cancer in chronic arsenicism. *Human Pathol.* **4:** 469–75 (1973).
3. J.T. Hindmarsh and R.F. Mc Curdy. Clinical and environmental aspects of arsenic toxicity. *Clin. Rev. Clin. Labor Sci.* **23:** 315–47 (1986).
4. W.R. Penrose. Arsenic in the marine and aquatic environments: analysis, occurence and significance. *Crit. Rev. Environ. Contr.* **4:** 465–69 (1974).
5. M. Vahter and H. Norin. Metabolism of ^{74}As labelled trivalent and pentavalent inorganic arsenic in mice. *Environ. Res.* **21:** 446–51 (1980).
6. B. Calesnick, A. Wase, and L.R. Overby. Availability during human consumption of the arsenic in tissues of chicks fed arsanilic ^{74}As acid. *Toxicol. Appl. Pharm.* **9:** 27–31 (1966).
7. M.W. Arbouine and H.K. Wilson. The effect of seafood consumption on the assessment of occupational exposure to arsenic. *J. Tr. Element Electrolytes Hlth. Diseases* **6:** 153–60 (1992).
8. E.A. Crecelius. Changes in chemical speciation of arsenic following ingestion by man. *Environ. Health. Persp.* **19:** 147–51 (1977).
9. C.T. Tye, S.J. Haswell, P. O'Neil, and K.C.C. Bancroft. High-performance liquid chromatography with hydride generation atomic absorption spectrometry for the determination of arsenic species with application to some water samples. *Anal. Chim. Acta* **169:** 195–200 (1985).
10. B.S. Chama and N.J. Smith. Urinary arsenic speciation by high performance liquid chromatography/atomic absorption spectrometry for monitoring occupational exposure to inorganic arsenic. *Anal. Chim. Acta* **197:** 177–86 (1987).
11. A. Colombi, M. Maroni, and V. Foa. Significance of arsenic metabolic forms in urine. Part I. chemical speciation. *Int. J. Environ. Anal. Chem.* **17:** 25–34 (1984).
12. J.G. Farmer and L.R. Johnson. Assessment of occupational exposure to inorganic arsenic based on urinary concentrations and speciation of arsenic. *Brit. J. Ind. Med.* **47:** 342–48 (1990).
13. G. Rauret, R. Rubio, and A. Padro. Arsenic speciation using HPLC–HG–ICP–AES with gas–liquid separator. *Fresenius Z. Anal. Chem.* **340:** 157–60 (1991).
14. F.T. Henry and T.M. Thorpe. Gas chromatography of the trimethylsilyl derivatives of arsenic, arsenious and dimethylarsinic acids. *J. Chromatogr.* **166:** 577–86 (1978).
15. K. Dix, C.J. Cappon, and T.Y. Toribara. Arsenic speciation by capillary gas–liquid chromatography. *J. Chromatogr. Sci.* **25:** 164–69 (1987).
16. P. Morin, M.B. Amran, S. Favier, R. Heimburger, and M. Leroy. Speciation of arsenical species by anion-exchange and ion-pair reversed-phase liquid chromatography. *Fresenius Z. Anal. Chem.* **339:** 504–509 (1991).
17. P. Morin, M.B. Amran, M.D. Lakkis, and M.J.F. Leroy. Ion pair reversed phase liquid chromatography of arsenic species on polymeric styrene-divinylbenzene packed columns with an alkaline aqueous mobile phase. *Chromatographia.* **33:** 581–85 (1992).
18. M. Albert, C. Demesmay, M. Porthault, and J.L. Rocca. Séparation d'espèces arseniées organiques et minérales par chromatographie en phase liquide et électrophorèse capillaire. *Analusis.* **20:** 383–90 (1992).

Manuscript accepted October 20, 1995.