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Arsenic speciation by micellar liquid chromatography with inductively coupled plasma mass spectrometric detection ☆

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Abstract

Four environmentally and biologically important arsenic species, dimethylarsenic acid (DMA), monomethylarsonic acid (MMA), As(III) and As(V) are separated by micellar liquid chromatography. Linear dynamic ranges for the four species are three orders of magnitude and detection limits are in the picogram range with inductively coupled plasma mass spectrometric (ICP-MS) detection. This paper discusses in detail the development of the chromatographic conditions. The micellar mobile phase, which consisted of 0.05 *M* cetyltrimethylammonium bromide, 10% propanol and 0.02 *M* borate buffer, showed good compatibility with ICP-MS. This method allowed direct injection of urine samples onto the chromatographic system without extensive pretreatment and presented no interference from chlorine in the matrix. Detection limits are comparable with other LC–ICP-MS studies. An SRM urine sample was used to demonstrate the applicability of this technique to "real-life" situations. Results indicated that DMA, MMA and As(V) were present in the urine sample.

1. Introduction

Micellar liquid chromatography (MLC) refers to the type of chromatography that uses surfactants in aqueous solutions well above their critical micelle concentrations (CMC), as alternative mobile phases for reversed-phase liquid chromatography (RPLC). Armstrong and Henry [1] were the first to demonstrate the usefulness of MLC and there have been excellent reviews of this unique technique [2,3]. In addition to the number of compounds with a wide range of polarities amenable to separations by RPLC, MLC extends the analyte candidates to almost all hydrophobic and many hydrophilic compounds providing they can partition to the micelles. Other advantages MLC offers over RPLC, such as simultaneous separation of both ionic and non-ionic compounds, faster analysis times and improved detection sensitivity and selectivity [4], stem from its unique three-way equilibrium mechanism [5] where micelles act as a pseudo phase in addition to the mobile and stationary phases.

Elemental speciation is becoming more and more important since the environmental toxicity and biological importance of many elements depend on their different chemical forms and oxidation states. Inductively coupled plasma

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mass spectrometry (ICP-MS) is the dominant detection method used in elemental analysis due to its elemental specificity and trace level detection limits. By combining the separation power of chromatography and the element-selective detection of ICP-MS, HPLC–ICP-MS becomes an excellent technique in trace level speciation [6,7].

Arsenic has found wide use in pesticides, herbicides, wood preservatives, desiccants, etc., and arsenic exposure occurs in many circumstances including occupations (coal mines, smelters), air and food [8]. The toxicity of arsenic varies widely with the different chemical forms. The inorganic arsenics are more toxic than the organoarsenicals, while trivalent arsenic compounds are more toxic than their pentavalent counterparts [8]. Monomethylarsonic acid (MMA) and dimethylarsenic acid (DMA) are the two main metabolites found to be toxic. The four arsenic compounds, As(III), As(V), DMA and MMA investigated in this work are the arsenic species studied most in the literature. The speciation of these four compounds by chromatography have mainly focused on ion-exchange [9–15] and ion-pairing techniques [10,16,17]. HPLC–ICP-atomic emission spectrometry (AES) for arsenic speciation was first discussed in 1981 [9]. Since then the detection limits have been greatly improved over the optical work with the use of ICP-MS [10–17].

The main focus of this work was to investigate the use of MLC for direct injection of "dirty samples" and better compatibility with ICP-MS. With conventional hydro–organic mobile phases, "dirty samples" such as body fluids, usually have to be deproteinized before chromatography to prevent protein precipitation in the column. However, the unique micelle aggregates in MLC should dissolve proteins in the sample and cause them to elute with the void volume without plugging the column. The possibility of direct sample introduction should greatly simplifies sample treatment and improves accuracy. In addition, MLC should give better compatibility to ICP-MS than RPLC. This is because MLC uses no or low concentrations of organic solvents, while the high organic content in RPLC usually leads to plasma instability, increased background and carbon deposition on the sampling cone of the ICP.

2. Experimental

A VG PlasmaQuad PQII + turbo (VG Elemental, Winsford, UK) ICP-MS system was used with a nickel sampler and skimmer, both of 1.0 mm diameter orifice. The sample introduction system consisted of a C-1 type concentric nebulizer (Precision Glassblowing, Parker, CO, USA) and a double-pass spray chamber cooled to 5°C with a Neslab (Portsmouth, NH, USA) Endocal refrigerated chiller.

The HPLC system was a Dionex AI-450 metal-free model (Dionex, Sunnyvale, CA, USA) with a Rheodyne Model 9125 injector (Cotati, CA, USA) and a laboratory-built 100-μl injection loop. A Hamilton (Reno, NV, USA) PRP-1 column (15 cm $\times$ 4.1 mm), with a guard column made of the same material was used.

LC was operated isocratically with a flow-rate of 1.0 ml/min. The outlet of the analytical column was connected to the nebulizer through a piece of polyether ether ketone (PEEK) (25 cm $\times$ 0.10 mm) and then PTFE capillary tubing (25 cm $\times$ 0.12 mm), with a total volume of approximately 4.8 μl which would have a minimum effect on extra-column peak broadening.

For preliminary work, an Alltech RP metal-free column (15 cm $\times$ 4.6 mm) (Deerfield, IL, USA) and a guard column of the same material were coupled to ICP-AES (PlasmaTherm, Kresson, NJ, USA). The early HPLC system consisted an ISCO Model 2350 pump and 2360 programmer (Lincoln, NE, USA), and a Rheodyne 7010 injector with a 100-μl sample loop.

Void volumes were measured by the injection of water.

2.1. Standards and reagents

Chemicals used were reagent grade, and obtained from Fisher Scientific (Fair Lawn, NJ, USA) except when otherwise indicated.

The mobile phase used was prepared from

cetyltrimethylammonium bromide (CTAB) and distilled deionized water, with a resistance of 18 MΩ (Barnstead, Boston, MA, USA). The buffer was made from boric acid and the pH of solutions was adjusted with sodium hydroxide. *n*-Propanol was added as the mobile phase modifier. Stock solutions (1000 ppm as arsenic) of DMA, As(III) and As(V) in mobile phase, were prepared from cacodylic acid, sodium arsenite and sodium arsenate, respectively. MMA stock solution (from Dr. John S. Thayer, Department of Chemistry, University of Cincinnati) was used as received.

Freeze-dried urine SRM 2670, elevated level, was obtained from National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). It was reconstituted according to instructions and filtered once with a 0.45-μm syringe filter (Alltech).

3. Results and discussion

3.1. Mobile phase compositions

The preliminary optimization of the chromatography was carried out with the ISCO HPLC system and ICP-AES.

In aqueous solvents, these arsenic species dissociate to give anions, thus CTAB became the surfactant of choice since the cationic CTAB would allow better partitioning of solutes into the micelles due to electrostatic interactions. Fig. 1 shows that the capacity factor, k', of arsenic compounds, decreased with increasing mobile phase concentrations, and resulted in reasonable retention times with CTAB concentrations ≥ 0.05 *M*. However, for ICP operations, it is important to note that salt (CTAB) content should be kept as low as possible to minimize clogging problems. With 0.1 *M* CTAB, deposition of salt at the nebulizer, spray chamber and torch injector tip occurred. Therefore 0.05 *M* is a practical compromise. For As(V), no data were plotted for CTAB concentrations below 0.1 *M*, because at mobile phase concentrations below 0.1 *M*, As(V) eluted at very long times ($k' > 15$) with very broad peaks.

In order to decrease the retention time of As(V), and improve the peak shape, the *n*-propanol concentration was varied. It has been reported that 3% propanol as the mobile phase additive is essential to enhance column efficiency in MLC [18] by improving slow mass transfer. Fig. 2 shows that with increased propanol concentration, k' of all four arsenic compounds decreased, giving shorter retention times (within 15 min). Signal-to-background ratios improved dramatically from 5 to 10% propanol in the mobile phase, as seen in Fig. 3. This is because increased propanol concentration improved the chromatography while having negligible degradation on plasma stability or analyte sensitivity. This improvement resulted in better peak shapes and thus better signal-to-background ratios as calculated from peak heights. A 10% propanol concentration was chosen because the signal-to-background ratios at 10% propanol were com-

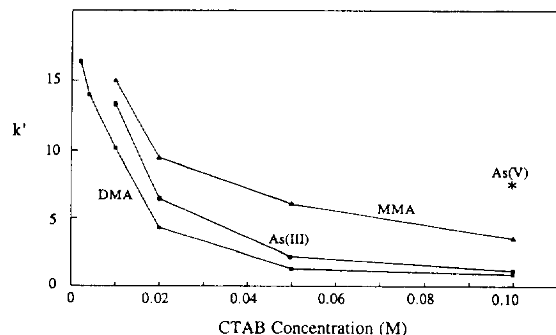

Fig. 1. Effect of CTAB concentration on k' with 5% propanol and pH 10.

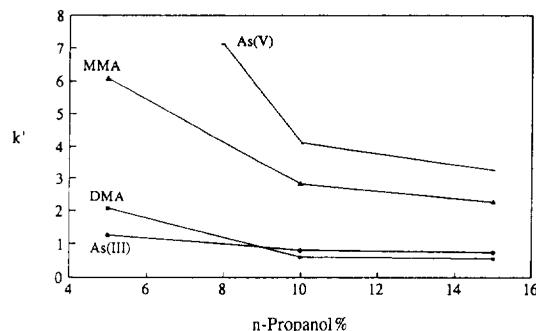

Fig. 2. Effect of percentage of propanol on k' at 0.05 *M* CTAB and pH 10.

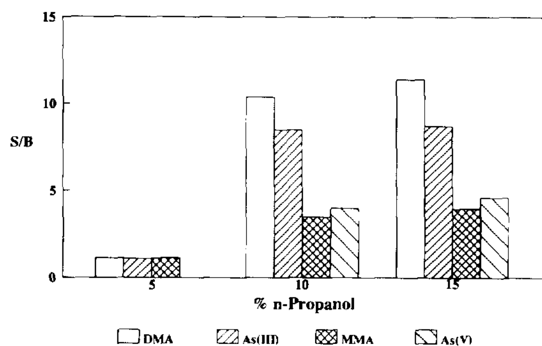

Fig. 3. Effect of propanol concentration on sensitivity at 0.05 *M* CTAB and pH 10.

parable to those obtained at 15% and the organic solvent should be kept as low as possible to favor micelle formation.

In order to improve the resolution between DMA and As(III), the pH of the mobile phase was studied. Although the chromatography used here was not an ion-exchange mode, the pH of the mobile phase had an important effect on retention. This is mainly because arsenic compounds are more anionic under more basic conditions, [for As(V), $pK_{a1} = 2.25$, $pK_{a2} = 6.77$ and $pK_{a3} = 11.6$; $pK_a = 9.23$ for As(III); $pK_a = 6.19$ for DMA; pK_a for MMA is not available), thus giving more interaction with the cationic micelles. As expected, longer retention of arsenic species was observed with increasing pH of the mobile phase. As can be seen from Fig. 4, a mobile phase of pH 12 would give the best separation between DMA and As(III). However, pH 12 was not practical because the large amount of NaOH needed to reach pH 12 caused severe salt deposition in the torch, and on the sampler and skimmer of the ICP. A pH value of 10.2 provided the best compromise, giving a baseline resolution ($R_s = 8$) between the two peaks while maintaining reasonable analysis times. The sudden change of k' (evident in Fig. 4), as a function of mobile phase pH values higher than pH 12, might be caused by changes in the stationary phase. The polystyrene–divinylbenzene beads were believed to swell and shrink with extreme mobile phase pH, causing changes in the active sites responsible for retention.

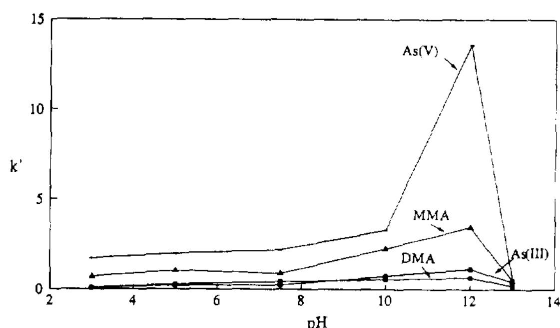

Fig. 4. pH effect on k' at 0.05 *M* CTAB and 15% propanol.

In summary, 0.05 *M* CTAB, 10% propanol, 0.02 *M* borate buffer and 40°C column temperature were used for mobile phase conditions.

3.2. *HPLC–ICP-MS*

After the preliminary study, the Dionex HPLC system was interfaced to ICP-MS to carry out calibration and sample analysis. Parameters for ICP-MS were optimized based on signal-to-background ratios and are summarized in Table 1. The spray chamber was cooled to minimize solvent vapor loading to the plasma. However, temperatures below 5°C were not advisable due to precipitation of CTAB. (Other surfactants such as cetyltrimethylammonium chloride, could be used should lower temperatures be needed). A separation of the four arsenic standards with good resolution is shown in Fig. 5 and figures of merit are illustrated in Table 2. For the arsenic standards, concentrations of 5 ppb, 10 ppb, 100 ppb and 1 ppm were used. The response (based on peak height) of this technique was linear over

Table 1
ICP-MS conditions

Forward power	1.35 kW
Reflected power	2 W
Nebulizer flow	0.74 l/min
Auxiliary flow	1.0 l/min
Coolant flow	12 l/min
Spray chamber temperature	5°C

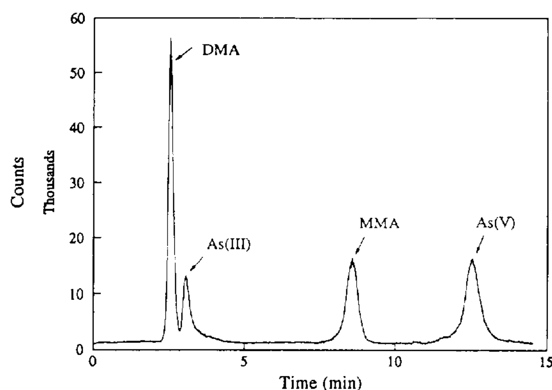

Fig. 5. Chromatogram of mixture of four arsenic standards. Hamilton PRP-1 column. Chromatographic conditions: 0.05 *M* CTAB, 10% propanol, pH 10.2, column temperature 40°C. $m/z = 75$.

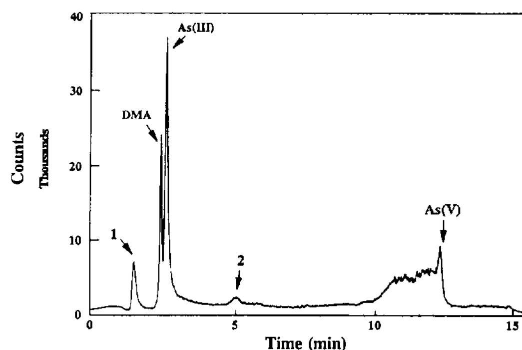

Fig. 6. Chromatogram arsenic speciation in urine. Hamilton PRP-1 column. Chromatographic conditions as in Fig. 5. $m/z = 75$. Peaks 1 and 2 are different forms of Cl^-.

three orders of magnitude. Detection limits were 90 pg for DMA, and 300 pg for As(III), MMA and As(V). Reproducibilities were determined by eight injections of a 100 ppb test mixture, and relative standard deviations were less than or equal to 5%. The better detection limit for DMA, compared to the other three compounds, is primarily due to the better peak shape.

Detection limits of this work compare favorably with those of ion-pairing chromatography (from 50 to ca. 300 pg) [10]. However, they are higher than the results obtained with an ion-exchange technique (from 20 to ca. 91 pg) [12]. This can be ascribed to the larger peak volumes [19] in this work, and possibly the complex mobile phase content, especially the Na^+ ions (coming from the buffer), which might have caused suppression of the ionization of arsenic, resulting in decreased signal sensitivity.

3.3. Analysis of urine

Urine samples were filtered prior to injection, and were then injected directly onto the column without any further treatment. Fig. 6 shows that DMA, As(III) and AS(V) were found with concentrations listed in Table 3. Total arsenic found was 0.52 ± 0.02 ppm, which is in agreement with the certified value of 0.48 ± 0.10 ppm. The last peak was, however, very broad and may represent more than one arsenic species. Spiking a 30 ppb standard in urine confirmed the presence of As(V), however, to this point we do not have a positive identification of the hump before the peak of As(V).

Table 2
Analytical figures of merit for arsenic speciation

	DMA	As(III)	MMA	As(V)
Linear dynamic range (orders of magnitude)	3	3	3	3
R^2	0.9990	0.9797	0.9880	0.9856
R.S.D. (%)[a]	3.4	4.7	4.9	5.0
Slope of log–log plot	0.99	0.93	0.92	0.91
Detection limit (pg)[b]	90	300	300	300

[a] R.S.D. of peak height for 8 replicate injections of 10 ng arsenic analyte.
[b] Detection limit defined by 3 × standard deviation of background/slope of calibration curves.

Table 3
Speciation of arsenic in urine

	Urine	Certified value
DMA	0.07 ± 0.01 ppm	
As(III)	0.40 ± 0.01 ppm	
As(V)	0.05 ± 0.01 ppm	
Total	0.52 ± 0.02 ppm	0.48 ± 0.10 ppm

3.4. Chlorine interference

A chloride interference ($^{40}Ar^{35}Cl^{+}$, the polyatomic ion formed in the plasma at $m/z = 75$) with arsenic can be severe in chloride-containing matrices, particularly in biological samples. In other studies, elimination of the $ArCl^{+}$ was investigated using hydride generation [17] and by chromatographically separating Cl^{-} from arsenics using an ion-exchange column with appropriate sample dilution [13]. In this method, however, the Cl^{-} interference did not pose a problem. Figs. 6 and 7 show two Cl^{-}-containing complexes eluting from the column (confirmed by monitoring at $m/z = 77$, $^{40}Ar^{37}Cl$ where monoisotopic arsenic is not present). One of the two complexes may be in the form of hyperchloride containing species (some Cl^{-} may be converted to hyperchloride under the very basic conditions of the mobile phase). Nevertheless, they did not co-elute with any of the four arsenic compounds. Some Cl^{-} was found to be retained on the column after 30 to 40 injections of pure 0.15 *M* NaCl solution (human urine is about 0.15 *M* in NaCl [20]), as observed by the high background at $m/z = 77$. This can be explained by the electrostatic attraction between Cl^{-} and some of the cationic CTAB adsorbed on the column. However, the retained Cl^{-} was readily removed by flushing the column with methanol–water.

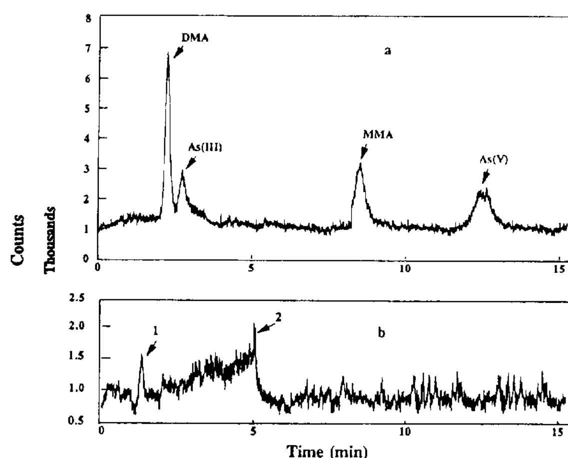

Fig. 7. Comparison of retention of arsenic standards and NaCl. $m/z = 75$. (a) Injection of 10 ppb standard, (b) injection of 0.15 *M* NaCl. Peaks 1 and 2 in chromatogram b are different forms of Cl^{-}.

4. Conclusions

MLC is compatible with ICP-MS and provides comparable figures of merit with other LC–ICP-MS methods. No O_2 addition to the nebulizer gas was necessary as is usually the case with methanol–or acetonitrile–water mobile phases. This reduces instrumental complexity, and prolongs the life-time of the sampling cone. No serious salt deposition was observed if periodically, a short rinse with 3% HNO_3 solution was carried out. Urine samples were filtered only before injection and no pressure build-up was observed, illustrating the capability for direct introduction of "dirty samples" onto the chromatographic system. The many advantages of MLC demonstrated by this work, clearly indicates that it is worthy of further investigation as an alternative chromatographic mode for ICP-MS detection.

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