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Evaluation of high-performance liquid chromatography for the separation and determination of arsenic species by on-line high-performance liquid chromatographic–hydride generation–atomic absorption spectrometry

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Abstract

An on-line high-performance liquid chromatographic–microwave assisted oxidation-hydride generation-atomic absorption spectrometric (HG-AAS) system (using columns of different kinds) has been developed for the determination of arsenite, arsenate, dimethylarsinate (DMA), monomethylarsonate (MMA), arsenobetaine (AsB) and arsenocholine (AsC) in environmental samples. Ion-pair reversed-phase chromatography using tetrabutylammonium phosphate as the ion-pair reagent and anion-exchange chromatography were evaluated and the analytical performances of each are reported. The detection limits were 97–143 and 10–30 $\mu g\ l^{-1}$ for ion-pair reversed-phase and anion-exchange chromatography, respectively. The Hamilton PRP-X 100 anionic column was proposed for the determination of the six species; AsB can be quantitated independently of AsC by taking the difference between readings at pH 6 and pH 10.7. The proposed methods were applied to water samples and sediments and their potential for future application was demonstrated.

1. Introduction

One of the main goals of chemical speciation of arsenic is to extend the range of species separated beyond arsenite and arsenate, which are the two best known and most abundant forms of arsenic. Biomethylation by microorganisms and other higher organisms converts the inorganic species into charged forms, and the most common methylated varieties in environmental samples are monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB) and arsenocholine (AsC). Since the effect on the environment depends more on the chemical species than on the element, a sensitive and specific method must be developed for the individual determination of each arsenic species.

Arsenic toxicity decreases with increasing degree of methylation and hence arsenite and arsenate are the most toxic forms; MMA and DMA are less toxic and AsB and AsC seem to be innocuous for living organisms, including humans [1,2].

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Current speciation methodology must be improved not only by the development of fast efficient separation techniques, but also by coupling to detectors that can provide prompt and precise measurement of the eluate over a broad sensitivity range. The main analytical methods used for arsenic speciation are based on the coupling of chromatographic separation to atomic or mass detection. Gas chromatography coupled to atomic absorption spectrometry (GC–AAS) [3,4], to inductively coupled plasma atomic emission spectrometry (GC–ICP-AES) [5] or to mass spectrometry (GC–MS) [6] have been extensively employed. The main drawback of these coupled methods are the requirement for previous derivatization of the sample due to the low volatility of arsenic species. Also, AsB and AsC are difficult to derivatize and cannot be determined in this way. HPLC coupled to atomic or mass detection has been successfully used and allows speciation without any precolumn derivatization [7–9], although sensitivity and selectivity improve if post-column derivatization (usually to the hydride forms [10,11]) is employed. AsB and AsC have to be to destroyed before hydride formation.

A careful evaluation of the best HPLC columns for separating cationic and anionic arsenic species in a single run shows that the most suitable methods are anion-exchange chromatography or reversed-phase ion-pair chromatography. The organic cationic species of arsenic can be lightly retained on the anionic column by Van der Waals forces or by interaction with the counter ion in the resin. If there is no interaction, they elute in the dead volume. The retention of anionic species on the column depends on their effective charge at the pH used.

Ion-interaction chromatography using a neutral, non-polar stationary phase in conjunction with an ion-interaction reagent in the mobile phase is also potentially a good separation method. Quaternary ammonium compounds can be used as the counter ion for anionic arsenic species [12,13]. A new method based on a vesicle forming reagent such as didodecyldimethylammonium bromide (DDAB) has been recently proposed [14].

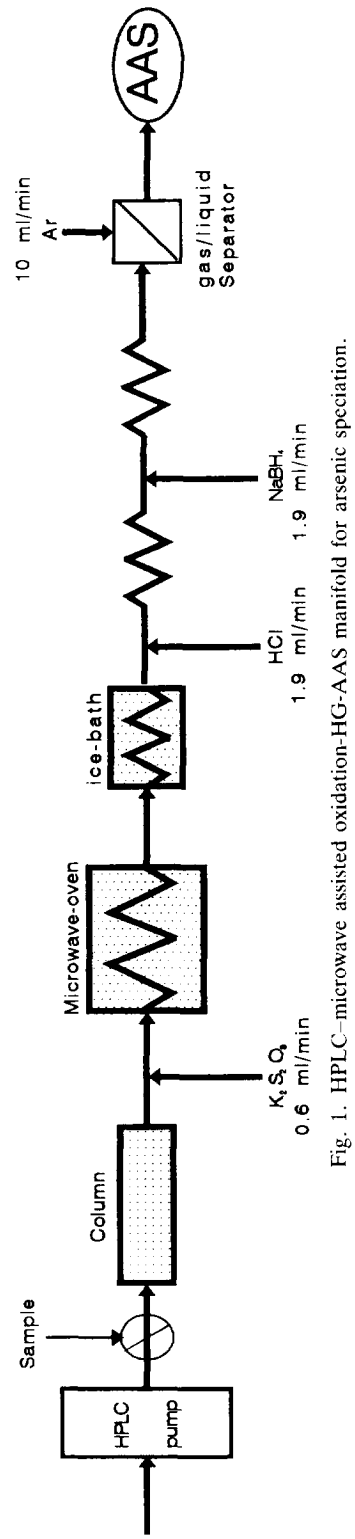

Fig. 1. HPLC–microwave assisted oxidation-HG-AAS manifold for arsenic speciation.

This work describes a systematic HPLC study of the ion-exchange and reversed-phase columns to identify the best chromatographic conditions for the separation of the six species studied, assuming that derivatization and final determination would be possible by HG-AAS.

2. Experimental

2.1. Chemicals

All reagents used were of analytical reagent grade. Deionized water from a Milli-Q system was used throughout. Stock solutions of arsenic compounds (1 g l^{-1} As) were prepared by dissolving appropriate amounts of $NaAsO_2$ (Carlo Erba, Milano, Italy), $Na_2HAsO_4 \cdot 7H_2O$ (Merck, Darmstadt, Germany), $CH_3AsO_3Na_2 \cdot 6H_2O$ (Carlo Erba) and $(CH_3)_2AsO_2Na \cdot 3H_2O$ (Sigma, St. Louis, MO, USA). The AsB and AsC were reference standards provided by the Bureau of Community Reference Material of the European Communities (BCR) (Brussels, Belgium). The stock standard solutions were stored in polyethylene bottles at 4°C in the dark. Working standards were prepared daily by dilution.

A 5% (w/v) $K_2S_2O_8$ (Merck) solution stabilized in 2.5% (w/v) NaOH (Merck) was used to oxidize the organic arsenical compounds. The reagents used to form arsine were 3% (w/v) $NaBH_4$ (Aldrich, Milwaukee, WI, USA) solution [stabilized in 1.0% (w/v)] NaOH and 3 *M* HCl (Carlo Erba).

The HPLC mobile phases for anion-exchange separation in the pH range 3–11 were prepared from 0.5–20 mmol l^{-1} H_3PO_4 (Carlo Erba) and 0.5–20 mmol l^{-1} Na_3PO_4 (Merck). When reversed-phase ion chromatography was used, 1.5–15 mmol l^{-1} tetrabutylammonium phosphate (TBAP) from Carlo Erba (Milano, Italy) was prepared by adjusting to pH 3–6.5 with H_3PO_4 or Na_3PO_4. The resulting solutions were filtered through a 0.45-μm membrane filter and degassed before use.

2.2. Apparatus

The hyphenated HPLC–microwave assisted oxidation-HG-AAS system is shown in Fig. 1. Alternatively, a UV-Vis detector was coupled to the HPLC system.

The chromatographic system consisted of a high-pressure solvent pump (Model 590, Waters, MA, USA) and a Rheodyne Model 7125 six-port sample injection valve fitted with a 100-μl loop (Cotati, CA, USA). Separations were performed on a Hamilton PRP-X 100 column (250 × 4.1 mm I.D., 10 μm) (Reno, NV, USA), on an IC-PAK A anionic column (50 × 4.5 mm I.D., 10 μm) from Waters (MA, USA) and on a Spherisorb ODS 1 (150 × 4.6 mm I.D., 5 μm) from Phenomenex (Torrance, CA, USA) for reversed-phase chromatography.

The UV-Vis detection module was a UV-Vis HP 8452 diode-array spectrophotometer from Hewlett-Packard (Avondale, PA, USA) interfaced with a integrator Milton Roy Model CI-4100 (FL, USA).

When HG-AAS detection was used, previous microwave assisted digestion (microwave oven, Balay Mode BAHM-III, Spain) was necessary. The microwave reaction coil was a loop of poly(tetrafluoroethylene) (PTFE) tubing (1.5 m × 0.5 mm I.D.) placed inside the microwave oven though the ventilation holes. The gas–liquid separator employed in the hydride generation module was a glass V-tube from Philips (Eindhoven, Netherlands), and mixing coils with dimensions 100 × 0.5 mm I.D. The coupling between the chromatographic system and the microwave oven-HG-AAS part of the system was optimized in earlier work [15].

A Model 2380 Perkin-Elmer atomic absorption spectrometer (CT, USA) with an electrodeless discharge lamp, 0.7-nm bandwidth and 193.7-nm wavelength was used. The signals were relayed to a Perkin-Elmer Model 56 printer (Avondale, PA, USA) and peak height was recorded.

2.3. Sample pretreatment

The samples collected were filtered through a

0.45-μm membrane filter and stored in acid-washed polyethylene bottles at 4°C in darkness until use.

3. Results and discussion

3.1. Preliminary observations

The chromatographic approach chosen for HPLC separation of the six arsenic species was based on a previous study using a Hamilton PRP-X 100 column at pH 6.0 [16], which allows separation of the six species into five chromatographic peaks in a single run.

In most instances where ion-exchange chromatography was applied, modified reversed-phase chromatography was examined as an alternative. A systematic study of the influence of pH, flow-rate and mobile-phase concentration on modified reverse-phase and anion-exchange chromatographic separation by the different columns in the isocratic mode was carried out in order to determine the best conditions for the separation and individual quantitation of the six arsenic species.

3.2. Reversed-phase ion chromatography

When aqueous tetrabutylammonium phosphate (TBAP) was used as the mobile phase, the retention times of the species were strongly affected by pH, as shown in Fig. 2. In the pH 4.5–5.5 range the toxic species As(V), MMA, DMA and As(III) could always be resolved and separated from the non-toxic highly methylated species AsB and AsC, which elute together.

Arsenious acid ($HAsO_2$, $pK_a = 9.2$) is not ionized in the pH range tested and elutes in the dead volume. AsB and AsC, which show a cationic behavior, are lightly retained on the column, probably by the non-polar C_{18} support, and elute just after As(III), but at a sufficient separation to allow quantitation of the toxic species individually and of the non-toxic species together. Arsenic acid (H_3AsO4, pK_a 2.3, 6.9 and 11.4), which is negatively charged over the total pH range studied, has the strongest electrostatic interaction with TBA^+ and the longest retention time. The order of elution of the two methylated arsenic species ($(CH_3)_2AsO_2H$ (pK_a 1.28 and 6.2) and $CH_3AsO_3H_2$ (pK_a 2.6 and 8.2) is explained by their effective charge and their interaction with the counter ion. Above pH 5.5, AsB separates from AsC and elutes in the dead volume together with As(III). In order to differentiate the toxic and non-toxic species, pH 5.2 was chosen as the optimum.

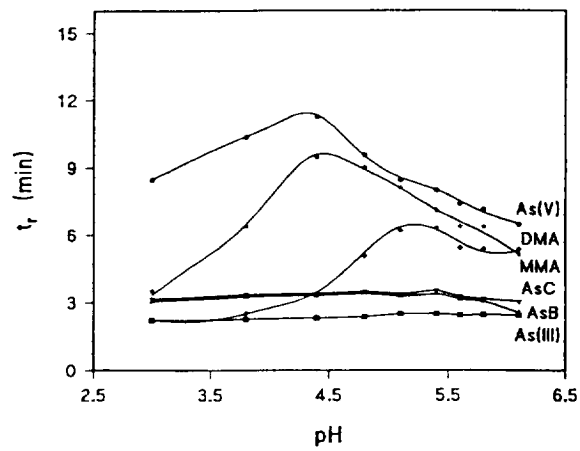

Fig. 2. Retention times of As(III), AsB, AsC, MMA, DMA and As(V) in a Spherisorb ODS 1 column as a function of pH of the mobile phase in the presence of 2.5 mmol l^{-1} TBAP at 1 ml min^{-1}.

TBAP concentrations from 1.5 to 15 mmol l^{-1} and flow-rates in the 0.5–2.0 ml min^{-1} range were tested and the values chosen as optimum were 2.5 mmol l^{-1} TBAP and a 1 ml min^{-1} flow-rate.

However, under the best chromatographic conditions achieved, five peaks were obtained for the six species (Fig. 3). The addition of methanol to modify the polarity of the mobile phase did not improve the chromatographic separation.

The dead volume of this interface is fairly high and, therefore, the likelihood of recombination of the As species increases. The feasibility of this column coupled to a UV-Vis detector, where the interface is shorter, was evaluated. However, the lower sensitivity of this technique made it necessary to use a more concentrated solution. Also, there was a one-to-one correspondence between

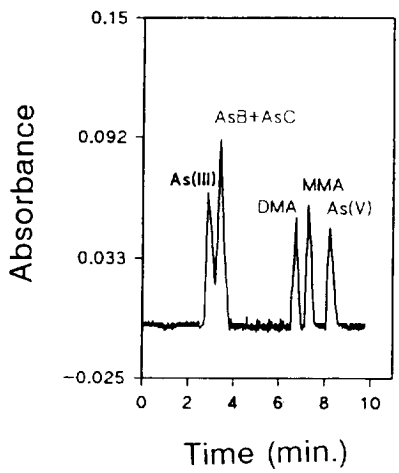

Fig. 3. Chromatographic separation of arsenic species (500 $\mu g\,l^{-1}$ As, 100 μl) by HPLC–microwave assisted oxidation-HG-AAS on a Spherisorb ODS 1 column (pH 5.2, 2.5 mmol l^{-1} TBAP and 1 ml min^{-1}).

the 6 peaks obtained and the As species tested, because AsC does not absorb at 196 nm. Some authors [17] have attributed the "unknown peak" to AsC, although we consider that it is due to the bromide which accompanied the AsC.

Hence, the experiments performed did not enable us to confirm whether the HPLC failed to separate the six species or whether the AAS was unable to determine them individually because of the high volume of the interface used.

3.3. Anion-exchange chromatography

Two different anion-exchange columns, i.e. Hamilton PRP-X 100 and a IC-PAK A, were tested for their ability to separate the six arsenic species. The mobile phase in both cases was a phosphate buffer. The effects of concentration, flow-rate and pH of the mobile phase were evaluated. These columns offer the possibility of eluting over wider pH ranges without any column deterioration. A pH range from 3 to 11, a phosphate concentration from 10 to 22 mmol l^{-1} and a flow-rate of 0.5–3.0 ml min^{-1} were used.

Fig. 4 shows the effect of pH on the retention time of the different species tested with the Hamilton PRP-X 100 column. The IC-PAK A column behaved similarly, but had different retention times because of its shortness. Better peak separation was achieved by working at a lower phosphate concentration than the optimum proposed for each column, but since As(V) has the longest retention time significant peak broadening was obtained. The best chromatographic conditions for the Hamilton PRP-X 100 column were pH 6.0 or 10.7, flow-rate 2 ml min^{-1} and 12 mmol l^{-1} phosphate buffer concentration, with one run taking 9 min. The optimum conditions for the IC-PAK A column were 1 mmol l^{-1} phosphate, pH 10.7 and a flow-rate of 1 ml min^{-1}, with one run taking 8.3 min.

The As(III) and AsB peaks always overlapped below pH 9.0; above pH 10 the AsB and AsC peaks overlapped and the arsenite peak was separated (see Fig. 5).

3.4. Analytical performance

Table 1 shows the analytical performance of the three columns tested. Calibrations were carried out by varying the amount of each arsenic species injected on the column and measuring the peak height obtained.

The detection limits for reversed-phase chromatography were about 50 times higher than those for anion-exchange chromatography. This fact and the high relative standard deviation might be due to the surface activity of the eluent, which interacts with the $NaBH_4$ to give a foam. The addition of a silicone antifoaming agent improved the reproducibility, but the signal decreased because the arsine generation was hindered. The low detection limit achieved with the anion-exchange columns makes them suitable for most environmental applications.

3.5. Analytical quantitation and application

Reversed-phase chromatography and anion-exchange chromatography with an IC-PAK A column allowed the sum of the non-toxic species (AsC and AsB) to be determined separately from the toxic forms, which run separately in the chromatogram. Using a Hamilton PRP-X 100 column and working at both pH 6 and pH 10.7, AsB can be determined by taking the difference

Table 1
Analytical performance of HPLC–HG-AAS for arsenic speciation

Column	Conditions	Species	Retention time (min)	Calibration ($\mu g\ l^{-1}$)	Slope ($\times 10^{-4}$)	Correlation coefficient	R.S.D.[a] (%)	D.L.[b] ($\mu g\ l^{-1}$)
Spherisorb ODS 1 (reversed phase)	TBAP: 2.5 mmol l^{-1} pH: 5.2 flow-rate: 1 ml min^{-1}	As(III)	2.83	400–1000	0.69 ± 0.01	0.9995	10	97
		AsB + AsC	3.70	800–2000	0.6 ± 0.1	0.994	5	100
		DMA	6.50	400–1000	0.49 ± 0.06	0.9992	7	122
		MMA	7.17	400–1000	0.47 ± 0.03	0.9998	7	128
		As(V)	8.00	400–1000	0.42 ± 0.04	0.9996	12	143
IC-PAK A (anion exchange)	Phosphate buffer: 1 mmol l^{-1} pH: 10.7 flow-rate: 1 ml min^{-1}	As(III)	2.25	50–200	3.8 ± 0.4	0.9993	5	24
		AsB + AsC	1.17	100–400	6.4 ± 0.2	0.99996	1	14.1
		DMA	1.63	50–200	7.3 ± 0.1	0.99998	2	12.3
		MMA	5.17	50–200	3.8 ± 0.4	0.9992	4	24
		As(V)	8.17	50–200	3.0 ± 0.4	0.9992	5	30
Hamilton PRP-X 100 (anion exchange)	Phosphate buffer: 12 mmol l^{-1} pH: 10.7 flow-rate: 2 ml min^{-1}	As(III)	2.65	50–250	4.2 ± 0.2	0.99990	3	14.3
		AsB + AsC	1.60	100–500	3.9 ± 0.2	0.9998	2	15.4
		DMA	2.00	50–250	4.1 ± 0.1	0.99995	5	14.6
		MMA	4.00	50–250	3.8 ± 0.3	0.9996	5	16
		As(V)	7.50	50–250	2.8 ± 0.2	0.9997	4	21
Hamilton PRP-X 100 (anion exchange)	Phosphate buffer: 12 mmol l^{-1} pH: 6.0 flow-rate: 2 ml min^{-1}	As(III) + AsB	1.60	100–500	4.9 ± 0.5	0.9995	1	12.1
		AsC	1.20	50–250	5.1 ± 0.4	0.9990	3	11.7
		DMA	2.40	50–250	3.8 ± 0.2	0.9993	3	15.9
		MMA	3.50	50–250	3.8 ± 0.2	0.9997	4	15.6
		As(V)	8.80	50–250	2.8 ± 0.1	0.9998	5	21.8

$n = 5$.
[a] R.S.D. (%) = $(S/C_M) \cdot 100$ (S = standard deviation of concentration, C_M = average concentration).
[b] D.L. = $3S_b/m$ (S_b = standard deviation blank, m = slope).

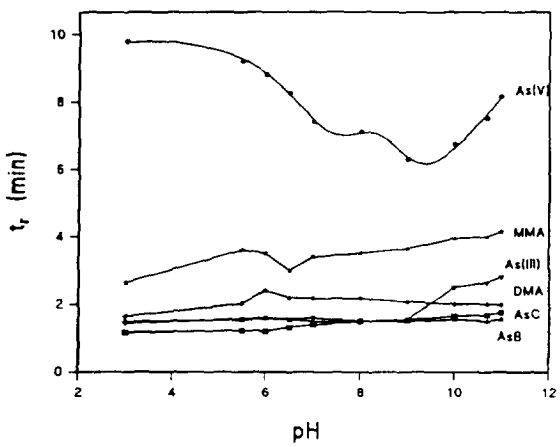

Fig. 4. Retention time of As(V), MMA, DMA, AsC and AsB in a Hamilton PRP-X 100 column as a function of pH of the mobile phase (12 mmol l^{-1} of NaH_2PO_4) (concentration: 200 $\mu g\ l^{-1}$ for each species)

between measurements at pH 6.0 [AsB and As(III) overlap] and pH 10.7 (AsC and AsB overlap). Each species can be quantitated.

Ground water, waste water and a sediment extract (provided by the measurement and testing programme of the European Community) were analyzed by this hydride technique. The species were quantitated on HPLC columns and the results are given in Table 2. The recoveries of 60 $\mu g\ l^{-1}$ of each arsenic species spiked in the sediment extract (Table 3) are acceptable and confirm the reliability of the proposed methods.

4. Conclusions

The proposed columns can be used for on-line HPLC–microwave assisted oxidation-HG-AAS and were successfully used to determine arsenite, arsenate, MMA, DMA and AsB + AsC in several types of waters and in a sediment extract. Toxic and non-toxic species can be determined and the proposed on-line system is efficient, reproducible, simple, fast, cheap and free from cross-contamination. The Hamilton PRP-X 100 column is the best one for determining the six arsenic species, since AsB and AsC can be independently determined by taking the difference between measurements at pH 6.0 and 10.7.

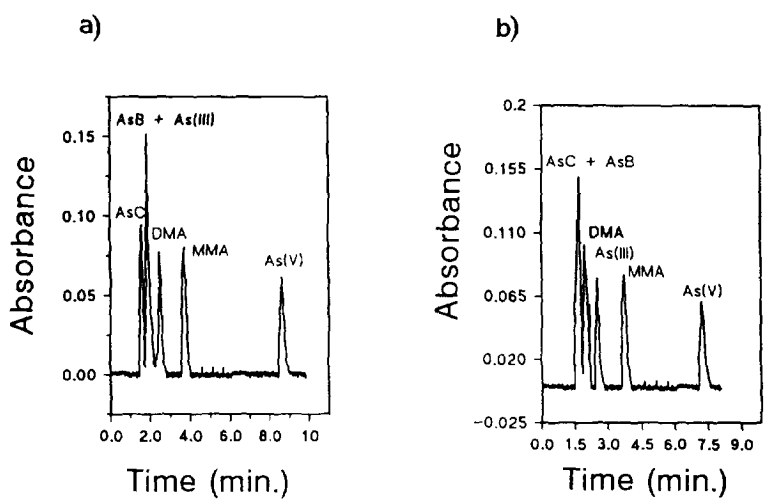

Fig. 5. Chromatographic separation of arsenic species (200 $\mu g\ l^{-1}$ As, 100 μl) using HPLC–microwave assisted oxidation-HG-AAS and Hamilton PRP-X 100 column: (a) pH 6.0 phosphate buffer (12 mmol l^{-1}, 2 ml min^{-1}); (b) pH 10.7 phosphate buffer (12 mmol l^{-1}, 2 ml min^{-1}).

Table 2
Speciation of arsenic in waters and synthetic sediment extract

Column	Ground water ($\mu g\ l^{-1}$)	Waste water[a] ($mg\ l^{-1}$)		Sediment extract[b] ($\mu g\ l^{-1}$)		
	As(V)	AsB + AsC	AsB	DMA	MMA	As(V)
Spherisorb	–	9.7 ± .06	–	–	–	–
IC-PAK A	86 ± 9	10.3 ± 0.5	–	51 ± 2	181 ± 6	113 ± 8
Hamilton PRP-X 100	86 ± 4	9.4 ± 0.4	9.3 ± 0.5	53 ± 7	179 ± 4	105 ± 9

Results are expressed as As: $\bar{x} \pm s$ ($n = 5$). The concentrations of As(III) and AsC were lower than the detection limit in all samples.
[a] Provided by CIEMAT (Spanish Research Center).
[b] Provided by the Measurement and Testing Programme (M&T) of the European Community.

Table 3
Recoveries of arsenic species in a synthetic sediment extract

Species	Total ($\mu g\ l^{-1}$)	Found ($\mu g\ l^{-1}$)		Recovery (%)	
		(a)	(b)	(a)	(b)
AsB + As(III)	60	59 ± 2	61 ± 3	98 ± 2	102 ± 3
DMA	114	113 ± 5	114 ± 2	99 ± 5	102 ± 2
AsC	60	63 ± 6	59 ± 2	105 ± 6	99 ± 2
MMA	243	238 ± 5	237 ± 2	98 ± 5	98 ± 2
As(V)	171	165 ± 8	179 ± 8	96 ± 8	103 ± 8

Anion-exchange column: (a) Hamilton PRP-X 100 at pH 6.0; (b) IC-PAK A.

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