Speciation of Five Arsenic Compounds in Urine by HPLC/ICP-MS

Introduction

High-performance liquid chromatography (HPLC) coupled to an ICP-MS has become an invaluable analytical tool for the determinations of trace levels of individual arsenic compounds (speciation). Speciation of arsenic is used to improve our knowledge of arsenic's biochemical interactions and to evaluate toxicity risks. The toxicity and bioavailability of arsenic is highly dependent on form or species. Five of the most commonly found arsenic compounds are listed in Table 1 with their LD_{50} values determined in mice.[1]

The World Health Organization has established that the total daily intake of inorganic As should not exceed 2 µg/kg body weight. Human exposure to the inorganic forms of arsenic has been linked to increased rates of cancer. Seafood can contain up to 25 µg As/g, but the major species is AsB which has a low toxicity; therefore the quantification of individual arsenic species is vital to accurate risk assessment. Analysis with speciation of urine can indicate the source of recent arsenic intake.[2]

In addition to arsenic's diverse toxicity, its chemical form determines arsenic's mobility in the environment. Therefore, new environmental regulations are being developed to specifically monitor for individual species of arsenic.[3]

While many different analytical techniques have been applied to analyses of arsenic species, high-performance liquid chromatography (HPLC) coupled to an ICP-MS has several significant advantages. These include the excellent separating power of HPLC, coupled with the high degree of elemental specificity and very low detection limits of the ICP-MS. ICP-MS can detect the inorganic and organic arsenic species without on-line digestion of organic forms.[4] Additionally, the system is totally integrated and easily automated giving excellent precision with ease of analysis.

Table 1. Toxicology of Arsenic Species.

Arsenic Species		LD_{50} in Mice (mg/kg)
Arsenious acid	(AsIII)	4.5
Arsenic acid	(AsV)	14-18
Monomethylarsonate	(MMA)	1,800
Dimethylarsinate	(DMA)	2,600
Arsenobetaine	(AsB)	10,000

Samples with a high chlorine component, such as urine, can potentially cause a polyatomic isobaric spectral overlap interference at the ICP-MS from the formation of $^{40}Ar^{35}Cl^{+}$, which has the same m/z as ^{75}As. Some investigators have resolved the $ArCl^{+}$ peak from the arsenic species by optimizing chromatographic parameters.[5-6] In this work we will use dynamic reaction cell (DRC) technology to "move" the arsenic ions away from the $ArCl^{+}$ by reacting the arsenic ions with oxygen to form the $^{75}As^{16}O^{+}$ ion, measurable at m/z 91.

This application note provides the analytical conditions, parameters, and procedures for the application of anion exchange HPLC/ICP-MS to the determination of the five most commonly found arsenic species in human urine. Two

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methods are investigated: one using a standard configuration ICP-MS with a gradient elution HPLC program, and a second method using the DRC ICP-MS technology with simpler and faster isocratic HPLC.[7]

Experimental

Instrumentation:

A schematic of the overall system is shown in Figure 1.

The HPLC system used for the gradient program consisted of a PerkinElmer Series 200 Quaternary Pump and a Series 200 Autosampler with a Hamilton PRP-X100 (4.1 mm x 250 mm, 10 µm) anion exchange column. A solid phase extraction (SPE) anion exchange cartridge (3 mL, from Alltech) was used for contamination reduction in the ammonium sulfate solvent reservoir. Operating parameters and the gradient program are given in Table 2.

For the isocratic method, a PerkinElmer Series 200 Micro Pump was used instead of the quaternary pump. The isocratic mobile phase was 10 mM ammonium nitrate, 10 mM ammonium phosphate adjusted to pH 9.4 with ammonium hydroxide. The flow rate was 1.5 mL/min with 100 µL sample injections. Details are given Table 3.

PerkinElmer SCIEX models 6100 ICP-MS and ELAN® DRC II ICP-MS were used for all arsenic measurements. The 6100 was equipped with nickel cones, and the DRC was equipped with platinum cones. Sample introduction system components were similar for both instruments: a cyclonic spray chamber (Glass Expansion, Inc., West Melbourne, Australia) and a Meinhard® type A nebulizer. The effluent from the LC column was directly connected to the nebulizer with PEEK tubing (1.59 mm o.d.) and a low dead volume PEEK connector (Part No.: WE024375). Operating parameters are given in Table 4.

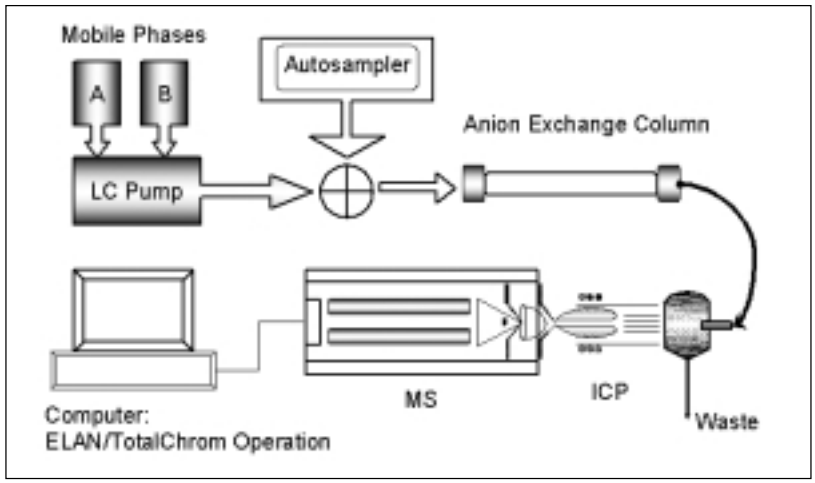

Fig. 1 Schematic of HPLC/ICP-MS instrumental setup.

Table 2. HPLC Gradient Method and Operating Parameters.

Parameter	Setting
Solvent A	20 mM Ammonium Bicarbonate @ pH 8.5
Solvent B	20 mM Ammonium Sulfate @ pH 7.0
Gradient Profile	6 min at 100% A; Step to 100% B for 12 min
Flow Rate	1.5 mL/min
Column	Anion Exchange, Hamilton PRP-X100, 4.1 mm i.d. x 250 mm, 10 µm
Column Temperature	Ambient
Autosampler Flush Solvent	5% Methanol / 95% DI Type I Water
Sample Injection Volume	100 µL
Re-equilbration Time	15 min
Urine Sample Prep	1:10 with DI Water
Solid Phase Extraction (SPE) Cartridge	3mL Supelclean LC-SAX SPE cartridge (Supelco™) in weak solvent reservoir
Detection	PerkinElmer/SCIEX ELAN 6100
Total Analysis Time	33 min

Table 3. HPLC Isocratic Method and Operating Parameters.

Parameter	Setting
Mobile Phase	10 mM Ammonium Nitrate and 10 mM Ammonium Phosphate (dibasic); pH 9.4
Flow Rate	1.5 mL/min
Run Time	10 min
Column	Anion Exchange, Hamilton PRP-X100, 4.1 mm i.d. x 250 mm, 10 µm
Column Temperature	Ambient
Autosampler Flush Solvent	5% Methanol / 95% DI Type I Water
Sample Injection Volume	100 µL
Urine Sample Prep	1:5 with Mobile Phase
Detection	PerkinElmer/SCIEX ELAN DRC II
Total Analysis Time	10 min

Table 4. ICP-MS Operating Conditions and Parameters.

Parameter	Setting/Type
Nebulizer	Meinhard Type A quartz *Part No.: WE02-4371*
Spray Chamber	ELAN 6100: Glass Cyclonic *Part No.: N812-2188*
	ELAN DRC II: Quartz Cyclonic *Part No.: WE02-5221*
RF Power	1500 w
Plasma Ar Flow	15 L/min
Nebulizer Ar Flow	0.95 L/min
Aux. Ar Flow	1.2 L/min
Injector	ELAN 6100: 2.0 mm i.d. Alumina *Part No.: N812-6041*
	ELAN DRC II: 2.0 mm i.d. Quartz *Part No.: WE02-3915*
Monitored Ion m/z	75 (^{75}As) and 91 ($^{75}As^{16}O$) for DRC
Dwell Time	500 ms
Total Acquisition Time	600 sec
CeO+/Ce+	<2%
Oxygen Flow for DRC	0.25 mL/min

Materials:

Laboratory pure 18 MΩ deionized water (ASTM Type I) was used throughout (US Filter). Arsenic (III) oxide, Sodium Arsenate (V) dibasic, Cacodylic acid (dimethylarsenic acid, DMA), and Arsenobetaine were obtained from Sigma-Aldrich (3300 South Second Street, St. Louis, MO, USA). Monosodium acid methane arsonate (MMA) was purchased from ChemService (660 Tower Lane, West Chester, PA USA). Second sources of As (III) and As (V) were obtained in 1000 mg/L solution form from Spex Certiprep (Metuchen, NJ USA). Reagents used for the HPLC mobile phases included ammonium bicarbonate and ammonium sulfate (Fisher Sci., Pittsburgh, PA USA), ammonium phosphate, nitric acid and ammonium hydroxide (hi-purity) (Baker Analyzed, Phillipsburg, NJ USA).

Mobile Phase Preparation for Isocratic Experiments:

Make up 550 mL solvent containing 10 mM Ammonium Nitrate and 10 mM Ammonium Phosphate, pH 9.4:

1. Add 500 mL HPLC-grade water to 500 mL solvent reservoir.
2. Using variable-volume pipette, pipette 345 µL (450 mg) conc. Nitric Acid into reservoir.
3. Using variable-volume pipette, pipette 660 µL (583 mg) conc. Ammonium Hydroxide into reservoir.
4. Weigh out 0.66 grams of Ammonium Phosphate (dibasic) and add to reservoir.
5. Adjust to pH 9.4 with Ammonium Hydroxide (~6-12 drops)

Solvent is stable for up to 3 days

Standard Preparation:

Stock standards of each species were prepared by serial dilutions of the starting compounds in laboratory pure water. Mixed standards of the following five concentrations were made: 1000, 500, 250, 100, 25 ng/L . These were prepared by mixing the individual stock species standards and prepared daily. All standards were verified for the proper total arsenic content by direct aspiration ICP-MS.

Sample Preparation:

Urine samples were obtained from various volunteers and stored at 4°C. For the gradient method, the samples were diluted 1:10 in 18 MΩ deionized water just prior to analysis. For the isocratic DRC method, the urine samples were diluted 1:5 with the buffered mobile phase.

Results

All evaluation of data for quantification and calibration was achieved via TotalChrom 6.2, after converting the ELAN .nsf data files to TotalChrom .raw files. TotalChrom offers powerful and flexible, yet intuitive, data review. The data conversion was performed either through ChromLink (SCIEX) or the Convert application under TotalChrom.

Gradient HPLC Using Conventional ICP-MS: $^{75}As^{+}$ Detection

The gradient method with the standard ELAN 6100 ICP-MS detection shows good separation of all arsenic species. The peaks were identified by the analyses of single component standards. The elution order is AsB, As (III), DMA, MMA, and As(V). Mixed standard chromatograms of varying concentrations are shown in Figure 2. The large peaks due to As-V result from contamination in the mobile phase. This problem was later corrected by adding a solid phase extraction cartridge to the weak solvent reservoir which scavenged most of the As-V contamination.

An example calibration curve (AsB) is shown in Figure 3. Except for As(V), all the curves had correlation coefficients ≥0.99; the problem with As(V) resulted from contamination in the mobile phase. With standard ICP-MS detection, an $ArCl^{+}$ peak appears in the urine sample between the MMA and As(V) peaks, as seen in Figure 4. Although not a concern for this application, the $ArCl^{+}$ peak could potentially overlap with the peaks of other organic arsenic species. (Note: For this gradient method, DRC ICP-MS can also be used with equal or better results.)

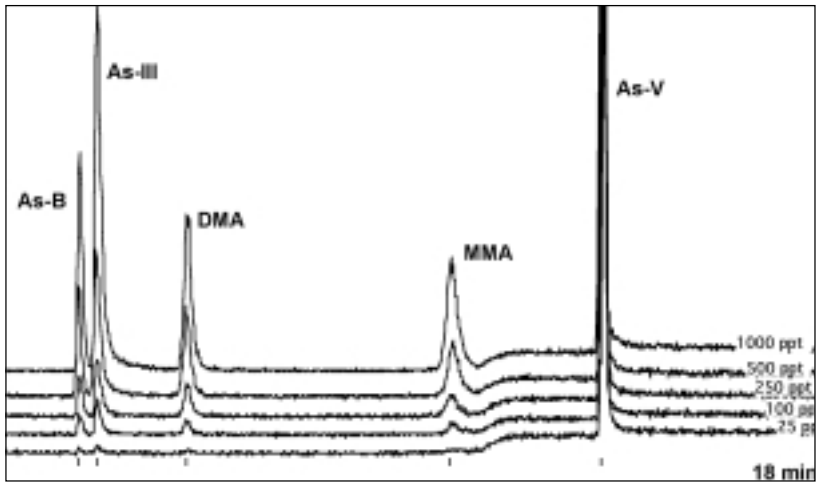

Fig. 2 Chromatograms of five arsenic species at five different concentrations using a gradient elution. The large peaks for As-V result from contamination of the mobile phase before the solid phase extraction column was used.

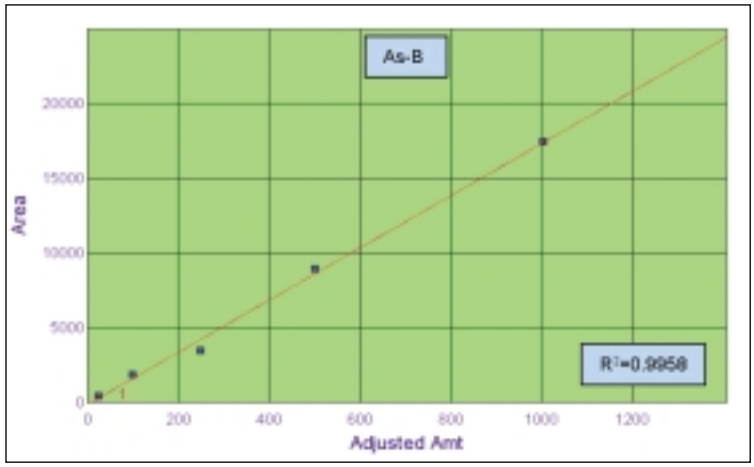

Fig. 3 A representative calibration curve (for arsenobetaine) generated in TotalChrom from the chromatograms displayed in Figure 2. The units on the x-axis are in ng/L.

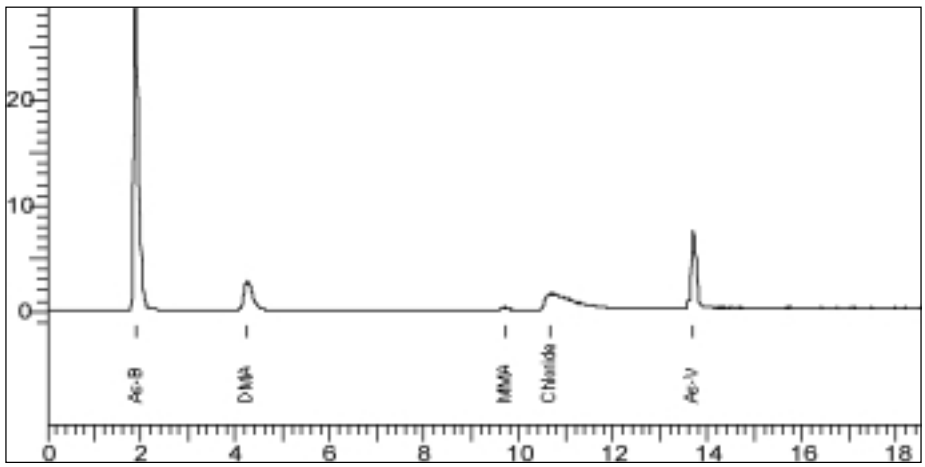

Fig. 4 Chromatogram of a urine sample (Urine A from Table 4) acquired on the ELAN 6100 monitoring $^{75}As^{+}$. The chloride peak which appears at about 11 minutes is $ArCl^{+}$, which results from the chloride present in urine.

Table 5 shows the speciation analysis of volunteers' urine samples. Volunteer A had recently consumed seafood, while volunteer B had not consumed seafood recently. As a result, urine A shows very high levels of arsenobetaine, the non-toxic form of arsenic common in deep water fish, while urine B has very low arsenic levels. By comparing arsenobetaine levels in urine samples, it is possible to determine if a person has recently consumed seafood.

Table 5. Concentrations of Arsenic Species in Volunteers' Urines (µg/L).

Peak	Component Name	Urine A	Urine B	Urine B%Recov.
1.	Arsenobetaine	123.5	1.0	88.6
2.	Arsenic III	< 0.25	0.8	93.8
3.	Dimethyl As	17.0	8.0	91.0
4.	Monomethyl As	28.5	1.8	66.4
5.	Arsenic V	N.D.	N.D.	N.D.

N.D. = Not Detected (due to contamination in the mobile phase)

Also displayed in Table 5 are the results of a spike recovery test on urine B. The urine was spiked with 5 µg/L of each arsenic species, and then diluted for analysis. The recoveries for arsenobetaine, As-III, and DMA are good, but the recovery for MMA is low, and As-V was not detected. The recovery of MMA may be affected by the chloride interference; from the chromatogram in Figure 4, the chloride peak occurs very close to the MMA peak, and this may affect the spike recovery. The problem with As-V results from contamination in the mobile phase (Solvent A in Table 2). This data was acquired before the solid phase extraction column was available; after it was installed on the intake line of the solvent reservoir, the As-V background was greatly reduced.

Isocratic HPLC with DRC ICP-MS: AsO^+ Detection (m/z 91)

An isocratic HPLC method was developed utilizing the same anion exchange column at pH 9.4, but with much faster analysis times. The HPLC parameters are shown in Table 3. In order to remove the effect of the $ArCl^+$ matrix interference, the ELAN DRC was used with oxygen as the reaction gas.[8] The analyte arsenic ions react with molecular oxygen inside the dynamic reaction cell of the ICP-MS to form AsO^+ (m/z=91), away from the $ArCl^+$ interference. Using this approach, the $ArCl^+$ peak is not recognized and, therefore, cannot interfere with the chromatography.

A chromatogram of the arsenic species, all at 1000 ng/L, is shown in Figure 5, along with the mobile phase. The resolution of the early eluting arsenic species is sufficient for this work but not as good as with the gradient method. The five arsenic species elute slightly earlier here than in the gradient method, and because it is an isocratic method, there is no need to for a column re-equilibration time. This reduces the total time of analysis to 10 minutes, about one third that of the gradient method.

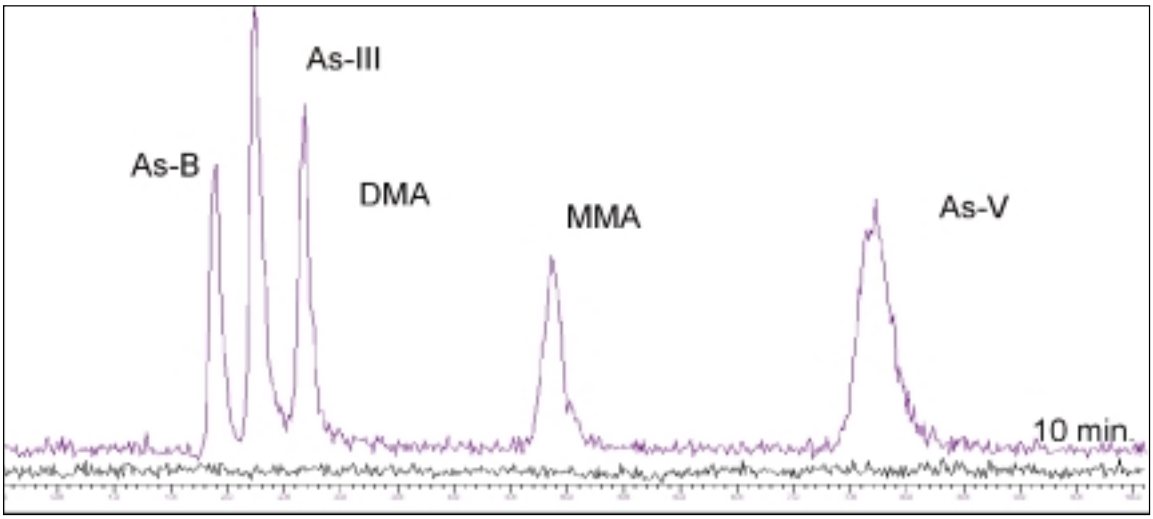

Fig. 5 Isocratic chromatograms of five arsenic species (1000 ng/L each) and a blank acquired with the ELAN DRC monitoring AsO^+ (m/z=91)

Figure 6 shows chromatograms of a urine sample obtained in both standard and DRC modes; the effect of eliminating the chloride peak is clearly seen and is important for MMA determination.

Figure 7 shows a chromatogram of Volunteer Urine C overlaid with the 1000 ng/L standard monitoring AsO^+ at m/z=91. Notice that no chloride peak is present in the chromatogram of the urine. It is important to note that the urine is diluted in the mobile phase buffer; when diluted in water, the retention times of the arsenic species shift from those of the standard. Presumably, the pH or high ionic strength of the urine is the cause of the retention time shift. Therefore, diluting in mobile phase reduces this shift.

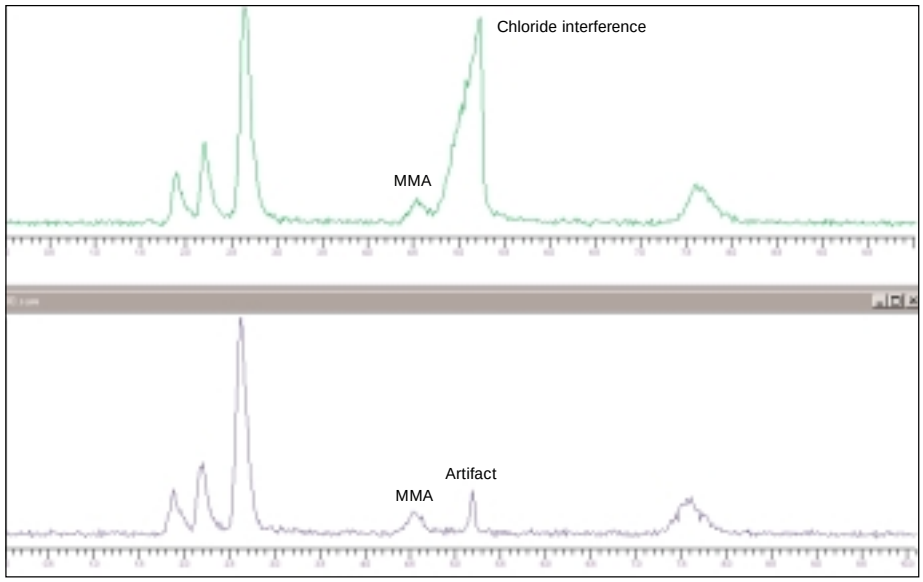

Fig. 6 Chromatograms of a urine sample obtained with the isocratic HPLC method. The top chromatogram was acquired monitoring $^{75}As^+$ in standard mode, without oxygen in the cell. A large chloride peak (due to $ArCl^+$) is present. The bottom chromatogram was the same sample acquired under the same chromatographic conditions, but monitoring AsO^+.

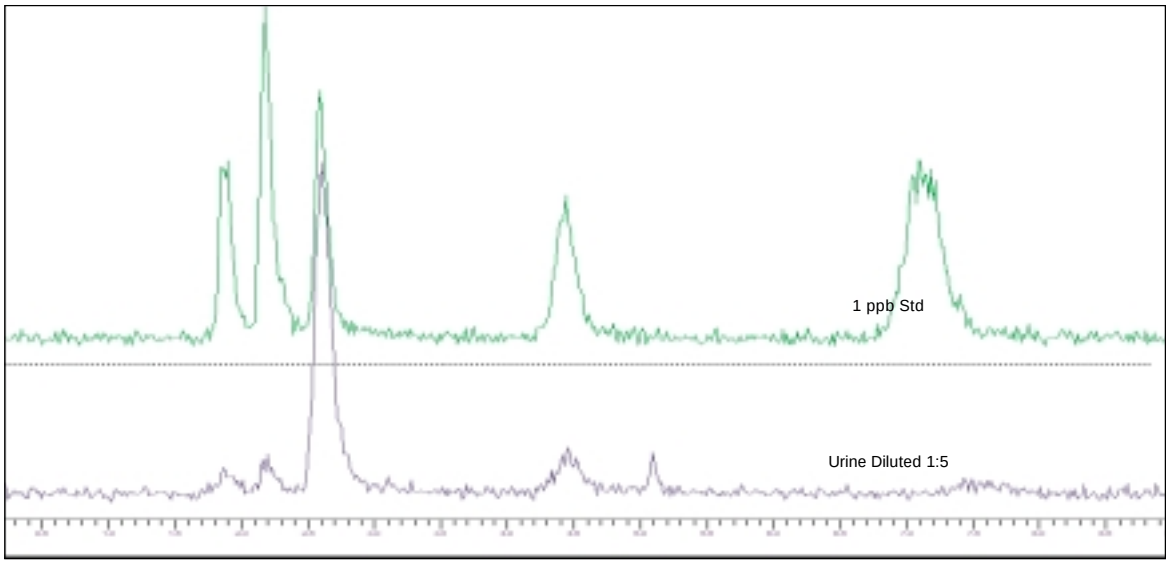

Fig. 7 Chromatograms of five arsenic species at 1000 ng/L each (top) and a urine sample diluted 5x (bottom). The data was acquired while monitoring AsO^+ (m/z 91).

An external standard calibration was performed by running 100, 250, 500, and 1000 ng/L standards of each species; the chromatograms for these calibration standards appear in Figure 8. The calibration curves have correlation coefficients ≥ 0.99 for each species, with the exception of As-V. The problem with this species is that the peak for the 100 ng/L standard is greater than that for the 250 ng/L standard. This problem most likely results from contamination. This calibration was applied to the urine sample displayed in Figure 6, and the corresponding quantitative results appear in Table 6.

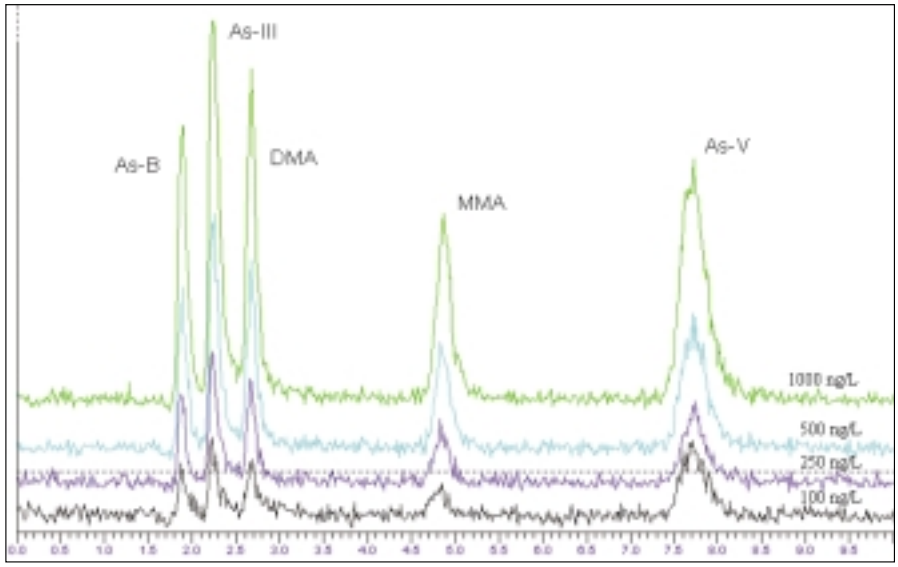

Fig. 8 Chromatograms of arsenic calibration standards (100, 250, 500, and 1000 ng/L of each species) obtained using isocratic conditions and monitoring AsO^+ (m/z 91).

Table 6. Quantitative Results for Volunteer's Urine Sample.

Peak	As Species	Concentration in Urine (µg/L)
1	As-B	0.2
2	As-III	0.3
3	DMA	5.3
4	MMA	1.1
5	As-V	< 0.1

Conclusions

Two anion exchange HPLC/ICP-MS methods for the determination of the five arsenic species most commonly found in human urine were presented. A method utilizing gradient mobile phases with standard ICP-MS detection was shown to separate the five arsenicals and the $ArCl^+$ matrix spectral interference. The gradient method requires the addition of an anion exchange SPE cartridge connected to the inlet of the weak solvent reservoir to control As(V) contamination. Urine samples can simply be diluted with water and analyzed. This method's major disadvantage is an analysis time greater than 30 minutes per sample.

An isocratic HPLC method was developed with the use of DRC ICP-MS technology for detection. This method uses a single buffered mobile phase with no column re-equilibration time needed. The DRC is used to react the analyte arsenic ions with oxygen to form AsO^+ and shift the analytical signal to m/z=91, away from the $ArCl^+$ spectral interference, as evidenced by the lack of a chloride peak in the chromatograms.

This method requires that the urine samples be diluted in the mobile phase, but the anaylsis time is about 10 minutes per sample, significantly less than the gradient method.

Both methods are highly automated, with total system integration and ease of analysis requiring no operator intervention other than sample preparation. By removing the effects of the $ArCl^+$ spectral interference, DRC ICP-MS allows the use of the more rapid chromatography and places the determination of arsenic species in urine within the capability of the routine clinical laboratory.

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