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A study of method robustness for arsenic speciation in drinking water samples by anion exchange HPLC-ICP-MS

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Abstract Regulating arsenic species in drinking waters is a reasonable objective, since the various species have different toxicological impacts. However, developing robust and sensitive speciation methods is mandatory prior to any such regulations. Numerous arsenic speciation publications exist, but the question of robustness or ruggedness for a regulatory method has not been fully explored. The present work illustrates the use of anion exchange chromatography coupled to ICP-MS with a commercially available "speciation kit" option. The mobile phase containing 2 mM NaH_2PO_4 and 0.2 mM EDTA at pH 6 allowed adequate separation of four As species (As(III), As(V), MMAA, DMAA) in less than 10 min. The analytical performance characteristics studied, including method detection limits (lower than 100 ng L^{-1} for all the species evaluated), proved the suitability of the method to fulfill the current regulation. Other parameters evaluated such as laboratory fortified blanks, spiked recoveries, and reproducibility over a certain period of time produced adequate results. The samples analyzed were taken from water utilities in different areas of the United States and were provided by the U.S. EPA. The data suggests the speciation setup performs to U.S. EPA specifications but sample treatment and chemistry are also important factors for achieving good recoveries for samples spiked with As(III) as arsenite and As(V) as arsenate.

Keywords As speciation · Drinking water · Anion-exchange chromatography · ICP-MS

Dedicated to Professor David M. Hercules on the occasion of his 70th birthday

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Introduction

Arsenic has been an element of some concern for many years because of its toxicological properties. Primary concern focuses on biological and environmental samples and their subsequent effect on human health. Arsenic occurs in agricultural chemicals, semiconductor materials, industrial gases, at mining sites, smelters, and other sources. Arsenic at ppm levels may be found in runoff from contaminated sites or volcanic hot springs. More recently, concern about arsenic is focusing on its particular forms or species [1, 2, 3] such as different oxidations states like As(III) or As(V). Inorganic arsenic undergoes biomethylation as a detoxification mechanism [4, 5]. Of the various forms (species) of arsenic, arsenobetaine and arsenocholine are essentially harmless to human life [6], while others, notably the inorganic forms or the methylated As(III), are not only specified as toxic, but are considered carcinogenic [7]. Millions of people globally are impacted by the toxic concerns of arsenic [8].

The World Health Organization (WHO) level of As in drinking water is 10 μg L^{-1}. The current level in the US is 50 μg L^{-1} and is based on the standard set by the Public Health Service in 1943. The U.S. EPA hoped to attain a new standard for arsenic in drinking water at 10 μg L^{-1} by 2006, but the new rule was withdrawn before it took effect. At the end of 2001, the U.S. EPA announced again a new standard for arsenic in 2006. However, these limits refer to total arsenic and there remains a compelling need for regulations for at least some of the individual arsenic species. Therefore, sensitive element analysis techniques such as ICP-MS are required to achieve the regulated detection limits for total arsenic [3, 9, 10] and will be even more necessary when regulations for individual species emerge. Separate identification and quantification of the individual forms of arsenic would be of much greater use in assessing overall risk. Also, speciation analysis is important for evaluating environmental impact [11].

The target species selected for this study were As(III) as arsenite (both toxic and a carcinogen), As(V) as arse-

Table 1 Instrumental operating conditions for HPLC and ICP-MS

Parameter	Setting
rf power	1300 W
ICP argon flows	Plasma (15 L min^{-1}), Aux 1.0 L min^{-1}, Carrier (1.0 L min^{-1})
Sample depth	5.5 mm
Nebulizer	Meinhard glass concentric
Spray chamber/temp	Scott double pass/2 °C
Acquired masses	35, 75
MS dwell time	0.5 s/mass
Oxide levels (CeO^+/Ce^+)	0.5%
Double charged (Ba^{++}/Ba^+)	1.1%
CPS (per ng ml^{-1} ^{89}Y)	2.5×10^4 (dwell time 0.1 s)
HPLC eluent	2.0 mM NaH_2PO_4, 0.2 mM EDTA pH=6.0
HPLC pump flow rate	1.0 mL min^{-1}
Injection volume	100 µL with autosampler and autostart
Column temperature	23 °C

nate, dimethylarsinic acid (DMA) (a carcinogen promoter), and monomethylarsonic acid (MMA). Arsenocholine and arsenobetaine are highly methyl-substituted species that are thought to be by-products, or possibly even end-products, of biological detoxification mechanisms, and are therefore considered innocuous. Also, arsenosugars have been shown to be present at significant levels in seaweed, algae, and kelp. Arsenic speciation in these samples is a current subject of research [12, 13, 14].

Anion exchange chromatography is one of the most widely used separation methods for arsenic speciation [3]. While a good option, some problems have been reported when applied to arsenic speciation in environmental water. Rapid, simultaneous analysis of different arsenic compounds is difficult because the retention mechanisms vary with each form. For example, As(III) as arsenite is weakly anionic, and is quickly eluted from an anion exchange column near where cationic or neutral arsenic compounds elute. Also, As(V) can react with other metallic species to form a precipitate. Some arsenic species may react with metals that may become deposited on the column during use.

This work illustrates the use of the anion chromatography coupled to ICP-MS as a robust and sensitive way to monitor arsenic species. The chromatography column and eluent are part of the Agilent Elemental Speciation System. The speciation system with remote start (HPLC and ICP-MS start their runs automatically and unattended) was evaluated in terms of the ruggedness that may be required for a potential regulatory method. Chromatographic and ICP-MS interferences (especially resulting from $^{40}Ar^{35}Cl^+$) were investigated as well as precision, method detection limits, spike recoveries, and application to waters from utility suppliers. Sample handling to preserve the original species is also discussed.

Experimental

Instrumentation

The chromatographic system was an Agilent 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a binary HPLC pump, autosampler, vacuum degasser system, and a thermostatted column compartment. The ICP-MS was also an Agilent 7500 s (Agilent Technologies, Tokyo, Japan). Together these were provided as a "metal speciation system" with a kit that included anion exchange pre- and analytical columns. The operating conditions for the LC and ICP-MS are shown in Table 1. The chromatographic stationary phase used in this work was an anion exchange marketed by Agilent Technologies and the column dimensions were 250×4.6 i.d. mm with 5 µm particle size; the pre-column was of the same characteristics and 50 mm long. The chromatographic run was isocratic at 1 ml min^{-1} with an injection volume of 100 µl. PEEK transfer tubing from the LC column to the ICP-MS nebulizer was (0.005 inches, Alltech, Deerfield, IL) inserted into a Tygon sleeve making an airtight seal inside the nebulizer. Data acquisition was automated by use of the remote start option in the ICP-MS software. When the LC autosampler injected a sample, a signal was sent to the ICP-MS computer via an APG cable to begin the ICP-MS data run.

Reagents

Commercial chemicals were analytical grade and were used without further purification. The mobile phase contained 2 mM NaH_2PO_4 (Fisher Scientific, Fair Lawn, NJ) and 0.2 mM EDTA (Fisher Scientific). The pH was adjusted to 6.0 using 1 M NaOH (Fisher Scientific). The standards for arsenic were arsenic (III) as arsenite, arsenic (V) as arsenate, dimethylarsinic (DMAA) acid and were obtained from Sigma (St. Louis, MO); monomethylarsonic acid (MMAA) was obtained from ChemService (West Chester, PA).

Procedures

The sampling was performed according to a simple sampling procedure sent to the water utilities in different areas of the United States with a sampling kit that included instructions. Opaque, clean, high-density polypropylene bottles (125 mL) (Fisher) were sent containing ethylenediamine (en) chelating agent. No rinsing of the sample bottle was specified and so when filled; the final sample would be approximately 50 mM en. This addition of the chelating agent to the samples helps to stabilize them for short-term storage, minimizing reactions with dissolved chlorine gas and metal ions, especially iron.

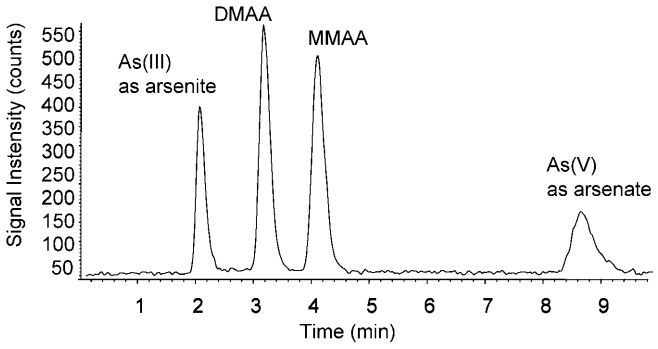

Fig. 1 Chromatogram showing separation of standard solution of 1 μg L^{-1} of each species As(III) as arsenite, DMAA, MMAA, As(V) as arsenate

Results and discussion

Analytical performance characteristics for regulatory requirements

The main objective of the present work was to evaluate the robustness, in terms of regulation requirements, of the speciation method [15] and its application to the analysis of drinking water samples. For this purpose, several parameters including method detection limits (MDL), interference rejection, laboratory fortified blanks (LFB), spiked recoveries, and reproducibility over a certain period of time were evaluated. Figure 1 shows the chromatogram of a standard solution containing 1.0 μg L^{-1} of each of the arsenic species (As(III) as arsenite, DMA, MMA, and As(V) as arsenate) and illustrates the complete separation of the four arsenic compounds in 10 min. The analytical figures of merit were calculated based on this separation and the instrumental detection limits (calculated as three times the standard deviation of the noise divided by the slope of the calibration curve) were in the ng L^{-1} range while the reproducibility (RSD%) for 1 μg L^{-1} injections (n=8) was less than 2%. Calibration curves for the concentration range from 0.5–10 μg L^{-1} for each of the four arsenic species showed correlation coefficients (R^2) better than 0.999.

Table 2 Method detection limits and recoveries calculated according to U.S. EPA guidelines. Each of the seven replicates represent DDI water fortified with 0.40 μg L^{-1} of each arsenic species

MDL #	As(III) as arsenite	DMAA	MMAA	As(V) as arsenate
1	0.39	0.37	0.40	0.71
2	0.40	0.43	0.39	0.78
3	0.38	0.39	0.41	0.79
4	0.41	0.41	0.39	0.75
5	0.36	0.39	0.40	0.77
6	0.35	0.36	0.41	0.76
7	0.39	0.40	0.38	0.79
Mean	0.383	0.393	0.397	0.764
% recovery (avg)	96	98	99	96
standard deviation	0.021	0.024	0.011	0.028
MDL (ng L^{-1})	67	74	35	89

Method detection limit

The method detection limit (MDL), calculated for method regulation requirements, is determined by injecting seven low concentration standards with a peak height not more than ten times above the magnitude of the baseline noise. The calculated determinations are then averaged and the standard deviation is calculated. The MDL is found by multiplying 3.14 (student t value for n=7 at 95% confidence level) by the standard deviation of the concentrations. The data shown in Table 2 illustrate the MDL values calculated. The average recovery for each arsenic species ranges from 96% to 99% and the MDL ranges from 35 to 89 ng L^{-1} depending on the species. These are well below the current U.S. EPA regulatory limit of 10 μg L^{-1} for total arsenic in drinking water indicating that detecting species at the low μg L^{-1} levels can be easily performed.

Laboratory fortified blanks

Another requirement, the laboratory fortified blank (LFB), was studied to determine recoveries in ideal solutions. According to U.S. EPA, the LFB solution is high purity water fortified with 1 μg L^{-1} of each species. The concentration is typically assigned as approximately ten times the MDL concentration, as per U.S. EPA procedures. The recoveries should be ±15% and the results for the four arsenic species investigated ranged from 95 to 99%.

System reproducibility

Another parameter evaluated was the system reproducibility over time, which is a concern in many speciation studies. Table 3 shows the results of reproducibility tests (n=8) of the four species under the same column and analytical conditions. The data were averaged from runs taken over several hours; the calibration standard and 1 μg L^{-1} standards were spaced every four injections between samples as a quality control check. This rigorous test of the

Table 3 Retention time (T_r) reproducibility for long-term analysis as %RSD (n=8). Data points taken over a 6-h period, 1 μg L^{-1} standard quality control injected every four samples analyzed

Species	Average T_r min	RSD %
As(III) as arsenite	1.99	0.36
DMAA	3.11	0.84
MMAA	4.03	0.69
As(V) as arsenate	8.61	1.15

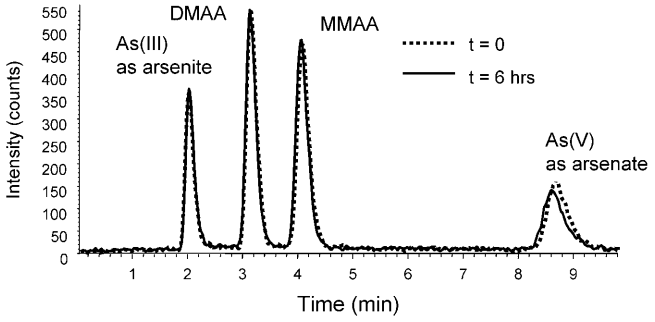

Fig. 2 Two chromatograms taken 6 hours apart where both are 1 μg L^{-1} quality control standards to illustrate long term stability

method performance suggests that the chromatography and the ICP-MS detection show very little observable drift. Two chromatograms are overlaid in Fig. 2 to illustrate the long-term stability of the speciation setup; the two chromatograms were taken 6 h apart with no tuning or mathematical corrections to the raw data. These results are an additional indication of the ruggedness of the speciation method. Since the system contains an LC autosampler, this precision suggests that unattended runs overnight could readily be accomplished, thereby increasing sample throughput. It is important to point out that U.S.EPA guidelines for other drinking water methods suggest sample set sizes of under 30. Taking into account that each run is only 10 min, the instrument stability is, therefore, excellent in the time period it takes to analyze a set of samples with accompanying quality control runs.

Investigation of potential interferences

The major interference for arsenic detection by ICP-MS is the polyatomic species $^{40}Ar^{35}Cl^+$ that is sometimes formed in the plasma at the same *m*/*z* of the only naturally occurring ^{75}As isotope. The drinking water samples possess a relatively high level of chlorine so the $^{35}Cl^+$ signal was monitored in addition to the $^{75}As^+$ signal during each run to check polyatomic interference formation. An example is shown in Fig.3 where both isotopes are monitored during the same chromatographic run. The top chromatogram illustrates the chloride ion eluting at nearly 7 min in a broad, tailing peak, chromatographically resolved from all the arsenic species except As(V). The count rates indicate the presence of Cl^- at high levels (~1 μg ml^{-1} or higher) but the polyatomic interference is not seen at the corresponding retention time on the *m*/*z* 75 chromatogram. This efficient decomposition of the matrix by the plasma is accomplished by optimization of the plasma and sample introduction. Thus, the polyatomic ion is not interfering under these conditions and the influence of this potential interference is negligible on the As(V) signal at *m*/*z*=75.

Application to drinking water samples

After evaluating the performance characteristics of the proposed speciation methodology, the application to the analysis of drinking water samples from different water utilities was performed. Samples B, C, and D were obtained from the U.S. EPA (Cincinnati) and had been previously stabilized with ethylenediamine. Sample A was obtained from an external laboratory and contained no preservation agent. Once the samples were received, each was analyzed twice: first the sample was analyzed diluted 1:2 in the mobile phase, and again diluted 1:2 in mobile phase fortified with 1 μg L^{-1} of each arsenic species (in samples B, C, and D) and with 20 μg L^{-1} in the case of sample A. Figure 4 illustrates the overlaid chromatograms of one of the water samples (spiked and non-spiked). The unretained peak prior to the As(III) retention time (only found in samples B, C, and D) illustrates the importance of spiking samples to avoid errors in assigning peaks due to slight differences in retention time. This unretained peak may be a neutral organoarsenic species, such as arsenobetaine or arsenocholine; identification of this peak would be interesting by use of ES-MS.

The arsenic determinations in four water samples (A, B, C, and D respectively) received from water utilities showed different levels of arsenic, as can be observed in

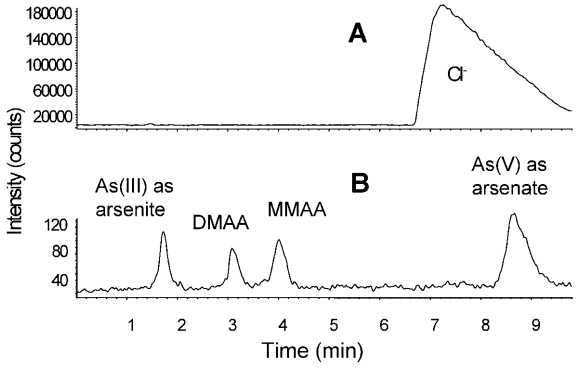

Fig. 3 ICP-MS chromatograms taken from the same injection of a water sample **A** *m*/*z*=35-signal monitoring $^{35}Cl^+$ signal **B** *m*/*z*=75-monitoring $^{75}As^+$ signal

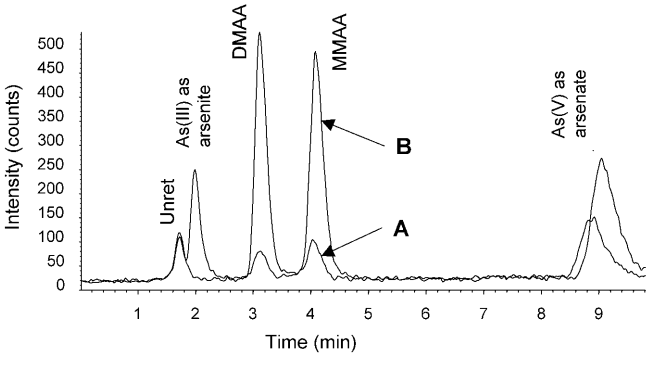

Fig. 4 Two overlaid chromatograms of the same water sample. **A** unspiked, **B** spiked with 20 μg L^{-1} each arsenic species (% recovery for each species spikes shown in Table 4)

Table 4 Arsenic species determination in four drinking water samples and % recovery for a 1 μg L^{-1} spike of each species in samples B, C, and D and for a 20 μg L^{-1} spike of each species in sample A

Compound	Sample							
	A		B		C		D	
	μg L^{-1}	% rec	μg L^{-1}	% rec	μg L^{-1}	% rec	μg L^{-1}	% rec
Unret	–	–	<0.1	–	0.2	–	0.2	–
As(III) as arsenite	16	96	–	75	–	94	–	61
DMAA	–	94	<0.1	92	<0.1	95	0.2	94
MMAA	–	94	–	91	–	91	0.2	101
As(V) as arsenate	32	95	<0.1	93	–	93	1.9	180

Table 4. Samples B and C were below 1 μg L^{-1} and samples A and D contained higher values. It is important to mention that a mass balance for total arsenic was carried out in sample A (by HG-AAS) and the sum of the two arsenic species found by HPLC-ICP-MS agreed with the total arsenic found by the other technique. In these samples, the species present were usually As(V) and small amounts of DMAA, though sample D contained MMAA and the unretained species (shown in Fig. 4) at levels of approximately 240 ng L^{-1} each. The response obtained by HPLC-ICP-MS for As(III) was used to quantify this peak due to its similarity in retention time.

The recoveries shown in Table 4 illustrate the challenges of arsenic speciation in water samples. The amount of As(III) as arsenite recovered from 1 μg L^{-1} spikes of the samples is notably low in sample D and somewhat better in sample B. The best recoveries (96%) were found in sample A, which was not treated prior to the analysis. This could be due to the sample matrix, suggesting a possible redox process (such as with iron and arsenic) or some other species conversion when the spike was added to the sample. Any conversion happens rapidly, as the samples were analyzed shortly after fortification with the standard arsenic mixture. Also, recoveries for As(V) as arsenate were significantly higher than 100% for sample D. This sample showed the lowest recoveries of As(III) suggesting that in these samples As(III) may have been oxidized to As(V). The methylated species exhibit good recoveries in all cases. Sample D requires more detailed study (perhaps by adding EDTA to complex any possible oxidizing cations prior to adding the spike). It appears that such a problem is more related to devising the appropriate sample treatment rather than any shortcoming in the chromatography/detection steps.

Conclusions

The method investigated to speciate arsenic in drinking water samples fulfills robustness requirements for potential regulations at the anticipated detection levels required (and even lower). Recoveries for fortified laboratory blank and reproducibility (short and long term) studies also show successful results. The chloride interference was studied by analyzing samples with high concentrations of chloride ion. At the retention time for chloride, where the potential for the formation of the $^{75}ArCl^+$ polyatomic interference was highest, the effect on the $^{75}As^+$ signal was negligible. Finally, a further study on species stability should be done relative to the fortified water samples. Although these reported well without fortification, the possible conversion of As(III) needs further investigation.

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References

1. Jain CK, Ali I (2000) Water Research 34:4304–4312
2. Pantsar-Kallio M, Manninen PKG (1997) J Chromatogr A 779: 139–146
3. Guerin T, Astruc A, Astruc M (1999) Talanta 50:1–24
4. Inoue Y, Date Y, Sakai T, Shimizu N, Yoshida K, Chen H, Kuroda K, Endo G (1999) Appl Organomet Chem 13:81
5. Heitkemper D, Creed J, Caruso JA, Fricke FL (1989) J Anal At Spectrom 4:279–284
6. Leonard A (1991) Metals and their compounds in the environment. VCH, Weinheim
7. Cullen WR, Dodd M (1989) Appl Organomet Chem 3:79–88
8. Cullen WR, Reimer KJ (1989) Chem Rev 89:713
9. Moldovan M, Gomez MM, Palacios MA, Camara C (1998) Microchem J 59:89–99
10. Killelea DR, Aldstadt JA (2001) J Chromatogr A 918:169–175
11. Helgesen H, Larsen EH (1998) Analyst 123:791–796
12. McSheehy S, Szpunar J (2000) J Anal At Spectrom 15:79–87
13. McSheehy S, Pohl P, Lobinski R, Szpunar J (2001) Anal Chim Acta 440:3–16.
14. Szpunar J (2000) Analyst 125:963–988
15. Sakai T, Kishi Y (2000) Agilent Technologies application note 5980–0262