Speciation of Inorganic Arsenic with Mixed-Mode HPLC- Electrospray Ionization-Mass Spectrometry and Arsenite Oxidation

Tao Li

Center for Environmental Solutions and Emergency Response, Office of Research and Development, U.S. Environmental Protection Agency.

26 Martin Luther King Dr W., Cincinnati, Ohio, 45268, USA

Abstract

It has been challenging to analyze inorganic arsenic (iAs) with anion exchange HPLC-Electrospray Ionization-Mass spectrometry (HPLC-ESI-MS), because arsenite (As(III)) is difficult to retain on column and the salts in mobile phase causes ionization suppression of iAs. To address these issues, a method has been developed involving the determination of arsenate (As(V)) with mixed mode HPLC-ESI-MS and the conversion of As(III) to As(V) for total iAs. As(V) was separated from other chemicals on Newcrom B, a bi-modal HPLC column involving anion exchange and reverse phase interaction. The elution employed a two-dimensional gradient, including a formic acid gradient to elute As(V) and a concurrent alcohol gradient to elute organic anions used in sample preparations. As(V) was detected by Selected Ion Recording (SIR) in negative mode at $m/z = 141$ with a QDa (single quad) detector. As(III) was quantitatively converted to As(V) by mCPBA oxidation and measured for total iAs. By replacing salt with formic acid in elution, the ionization efficiency for As(V) was greatly enhanced in ESI interface. The limit of detection (LOD) for As(V) and As(III) were 0.0263 μM (1.97 ppb) and 0.0398 μM (2.99 ppb), respectively. The linear range was 0.05 – 1 μM.

The method has been used to characterize iAs speciation change in the solution and precipitation in a simulated iron-rich groundwater caused by air exposure.

Key words: *arsenic, speciation, mixed-mode HPLC, ESI-MS, groundwater*

1. Introduction

Groundwater contamination by arsenic impacts 94-200 million people worldwide.[1] Most of the natural arsenic exists as either As(V) or As(III). They have different mobilization and sequestration behaviors in a mineral-water-soil system. Their transportations are complicated by As(III) – As(V) interconversion through redox oscillation in different environmental settings.[2] Speciation of iAs has been a key issue in environmental characterization and management.

Speciation of iAs is also needed for drinking water production management. In the United States, groundwater is not only used for drinking through private wells, but also used as the main water source for community water systems (CWSs). For arsenic in drinking water, the Maximum Contaminant Level (MCL) is 10 ppb.[3] To meet the MCL, polluted water needs to be treated for human consumption. The treatment typically involves oxidation of As(III) to As(V), followed by chemical or physical removal.[4] However, spatiotemporal fluctuations of arsenic in various matrices present challenges to managing quality compliance of the potable water from CWSs. A recent report showed that high levels of arsenic was one of the major contributors to health-based violations based on the water quality data from 48,215 CWSs in the US. The arsenic MCL violations were significantly correlated to variations in climate factors such as precipitation and drought, and human consumption.[5] Since iAs speciation plays a fundamental role in its spatiotemporal fluctuation,[6] a comprehensive speciation analysis would help to customize infrastructure in CWS.

Although there is a tremendous need for iAs speciation, only a few options are available for lab analysis. The speciation methods are either based on the reactivity difference between As(III) and As(V), or carried out by HPLC separation.[7, 8] The most popular HPLC methods are anion exchange or ion-pair chromatography. [9] As(III) is generally eluted at close to void volume in these methods and the quantitation accuracy is strongly influenced by the matrix.[10, 11] To achieve ppb-level detection, atomic optical spectrometry and mass spectrometry are used, with the most popular being inductively coupled plasma mass spectrometry (ICP-MS).

ESI-MS has been typically used as a complimentary detector for As speciation identification in chromatography.[12, 13] It has not been commonly used for quantitation of iAs because it was strongly influenced by sample matrix when interfaced with the HPLC separations.[14] In ion-pair chromatography analysis, iAs gave very poor signal.[10, 11] In anion exchange chromatography with salt elution, the mobile phase depressed the signals and the co-eluted matrix chemicals had unpredictable impacts on ESI signals for As(III) and As(V).[15] In analyzing iAs with a Hamilton PRP-X100/Waters TQ MS, the groundwater matrix caused a 4-fold increase of the selected ion record signal for As(III), and 3- and 5-fold increases of multiple reaction monitor (MRM) signals for As(III) and As(V), respectively.[15]

The development of a standalone HPLC-ESI-MS method for iAs speciation is reported in this paper. The strategy focused on addressing three issues: eliminating salt from the mobile phase of HPLC to improve ESI-MS interfacing, separating matrix chemicals from As(V) for reliable detection, and converting As(III) to As(V) for total iAs determination. The first two issues were addressed by replacing anion exchange column with a mixed mode column.[16] Converting As(III) to As(V) eliminated problems associated with low retention, and improved its detectability.

The research was carried out with a HPLC separation system interfaced with an ESI- single quad MS detector (QDa). A method with HPLC-EIS-MS can be a valuable alternative to HPLC-ICP-MS for arsenic analysis. Apart from the difference in analysis capability, HPLC-EIS-MS has a different profile in terms of cost, operation support, and maintenance.[8, 17]

2. Experimental

2.1 *Chemicals*

Sodium arsenate heptahydrate was acquired from Spectrum Chemical (New Brunswick, NJ, USA). Sodium (meta)arsenite, Oxalic acid dihydrate, 3-Chloroperbenzoic acid (mCPBA), and formic acid (HCOOH) were from Sigma Aldrich (St. Louis, MO, USA). Reagent Alcohol (a mixture of 90 % EtOH, 5% MeOH, and 5% 2-propanol, Catalog # A962-4, Fisher Scientific), acetonitrile (MeCN), and methanol (MeOH) were from Fisher Scientific (Waltham, MA, USA). Ultrapure water was produced by a Milli-Q Advantage A10 Water Purification System from Millipore Sigma (Burlington, MA, USA). Sodium percarbonate, sodium perborate, hydrogen peroxide urea, sodium persulfate, and ammonia carbonate were purchased from Sigma Aldrich.

2.2 *LC-ESI-MS analysis*

All chromatography experiments were carried out with a Waters Alliance e2695 XC separation module equipped with a quaternary solvent system, autosampler, a 250 μl syringe, and a 100 μl sample loop (Waters Corp., Milford, MA, USA). Signal detection was carried out with a Waters 2998 Photodiode Array (PDA) and an Acuity QDa mass detector equipped with an electron spray ionization (ESI) interface. The nitrogen was supplied by a NM30A nitrogen generator (PEAK Scientific Inc., North Billerica, MA, USA). Empower 3 Software was used for chromatography data acquisition and processing.

The volume of sample injection was 10 μl. For 2-D elution gradient development, three mobile phase stocks were used, including water, Reagent Alcohol, and 10% v/v HCOOH in water. The column temperature was 25 ^{o}C.

Eluted chemicals were detected by QDa in negative mode with a probe temperature of 600 ^{o}C and a capillary voltage of 0.8 kV. The nitrogen flow of the nebulizer was 120 L/hour. Quantitative analysis of targeted analytes was carried out by Single Ion Recording (SIR) at the mass-to-charge (m/z) of the base peak. The matrix was monitored with total ion current (TIC) in the m/z range of 100 – 200, and UV absorption in the range of 200 – 300 nm. In the final method, total analysis time was 16 min.

2.3 Detection, chromatography, and sample preparation development

Prior to chromatography development, the detection was investigated by SIR of arsenic standards (2-1000 μM) eluted from a Xterra C18 column (4.6 x 150 mm, 3.5 μ, and 100 Å, Waters, Milford, MA, USA). The mobile phase was a gradient of 5-10% MeCN over 10 min in water containing 0.1% HCOOH and 15 mM $(NH_4)_2CO_3$. The sensitivity was characterized by the slope of the standard curve.

The candidate columns for iAs separation were Obelisc, Newcrom, and Primesep columns (3.2 x 100 mm, 5 μ, and 100Å, SIELS, Wheeling, IL, USA). The separation modes involved anion exchange plus hydrophobic interaction or cation exchange. Column evaluation was carried out with isocratic elution by $(NH_4)_2CO_3$ (2.5 – 12.5 mM) in 5% MeCN. The pH of the mobile phase was adjusted to 4.0 with formic acid.

For elution in anion exchange, $(NH_4)_2CO_3$ was replaced by formic acid (0.4 – 0.9 v/v%) in mobile phase. Methanol and ethanol (the Reagent Alcohol) were compared with MeCN in the second dimension of the elution. The composition and flow rate of mobile phase were systematically evaluated for their impacts on chromatographic separation as well as QDa detection. The experiment was designed and analyzed with JMP 15 software (JMP Statistical Discovery LLC, Cary, NC, USA). The chromatographic behavior was characterized in terms of retention time (t_R) and theoretical plate number (N). N was calculated by the equation below, where H is peak height and A is peak area.[18]

$$\text{Theoretical Plate Number: } N = 2\,\pi\,(\frac{H}{A}\,t_R)^2 \qquad \textbf{Eq. 1}$$

Oxalic acid was tested for its ability to preserve the speciation of 20 mM iAs stock solutions in long term (weeks) as well as low concentration samples in short term (days). To oxidize As(III), m-Chloroperbenzoic acid (mCPBA), percarbonate, perborate, hydrogen peroxide urea, and persulfate were compared. The oxidation was optimized for maximal efficiency, completeness, and minimal number of byproducts. After the protocols for sample modification with oxalic acid and As(III) oxidation were finalized, the elution was optimized to separate As(V) from the other chemicals, including oxalic acid, the oxidant, and the oxidation byproducts.

2.4 Method characterization

The HPLC method was characterized by linearity, limit of detection (LOD), and limit of quantitation (LOQ). The linearity of the standard curve was characterized by linear range, slope, and coefficient of determination. The LOD and LOQ were 3- and 10- fold of the quotient of the standard deviation divided by the slope of the calibration curve. The precision was evaluated by relative standard deviation (RSD) as a function of concentration. Recoveries of As(III) and As(V) were used to evaluate speciation accuracy.

2.5 *Arsenic stability and speciation change in simulated groundwater*

The method was applied to evaluate the change of arsenic speciation in a simulated groundwater matrix. The concentrations of chemicals were the median values of those in 53 groundwater samples collected between 2006 and 2007 at Fort Devens Superfund Site, Devens, MA (Table 1). [19] Air exposure was simulated by leaving vials open for 20 min, a typical time required for ground water sampling. The samples were allowed to stand for 16 h to complete ferric oxide precipitation. As(III) and As(V) were quantified in liquid and solid phases. Mass balance was evaluated in terms of total iAs recovery.

Table 1 Composition of the simulated groundwater

Chemical	Mol Wt	Cation (μM)	mg L^{-1}	[CO_3^{2-}] (μM)	[Cl^-] (μM)
$FeCl_2$ ($4H_2O$)	198.81	612	121.67		1224
$MnCl_2$	125.84	83	10.44		166
$CaCO_3$	100.1	1105	110.61	1105	
K_2CO_3	138.21	426	29.44	106	
$MgSO_4$	120.4	256	30.82		
$NaHCO_3$	84.1	847	71.23	847	
$(NH_4)_2CO_3$	96.09	286	13.74	72	
Sodium silicate (26.5% SiO2)	Na_2O 10.6% SiO_2 26.5%	310 Na (400 SiO_2)	90.72		
Na_2HPO_4 $2H_2O$	178	200 Na (100 PO_4^{3-})	17.8		
Total (μM)				2130	1390

3 **Results and discussion**

3.1 Arsenic detection and column selection

The detection of As(III) or As(V) with ESI-MS was evaluated by injecting standard solutions through a C18 column. The mobile phase was typical for the mixed mode columns to be screened, including a 5-10% MeCN gradient in 15 mM $(NH_4)_2CO_3$, and 0.1% HCOOH (pH 3.0). The two species were monitored with SIR in negative mode at the mass charge ratios of their base peaks.[14] With the cone voltage at 15 V, the base peaks were m/z = 141 and 107 for As(V) and As(III), respectively (Figure 1). Although both were eluted at void volume (3.89 min with flow rate = 0.4 ml/min), their detectability could be evaluated by the slopes of their standard curves. The slope for As (III) was 41.5 CPS μM^{-1} (50 -1000 μM). The slope for As(V) was 1839 CPS μM^{-1} (2-1000 μM), indicating As(V) detection was 44-times more sensitive. This trend was also reported in an anion exchange HPLC-ESI-MS method.[15] With an elution by a 20 – 47.5 mM $(NH_4)_2CO_3$ gradient in 5% MeOH, the SIR sensitivity for As(V) was about 10-fold higher than that for As(III).

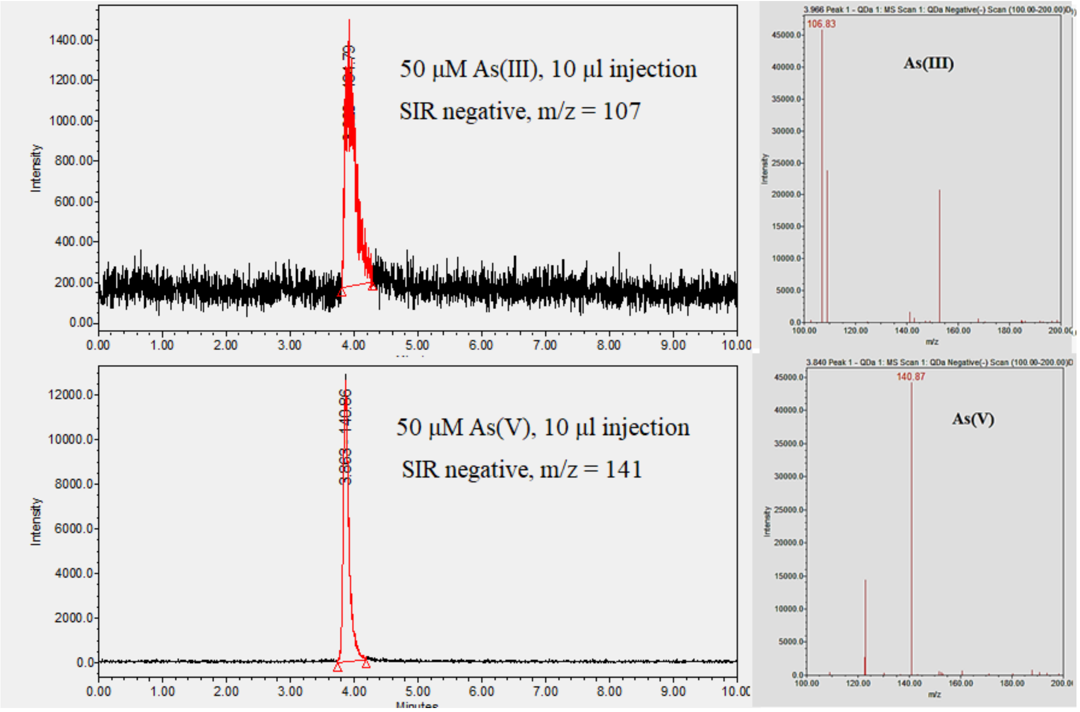

Figure 1 Chromatograms and Mass spectra and of As(III) and As(V) eluted by 5-10% MeCN, 15 mM $(NH_4)_2CO_3$ and 0.1 v/v % HCOOH in water from Xterra C18 (4.5 x 150 mm, 5 μ)

The screened columns included Obelisc R, Newcrom B, Primesep SB, and Primesep B2. All were in dimension of 3.2 x 100 mm, with particle size 5 μ and pore size 100 Å. Primesep SB, Primesep B2, Newcrom B employed reverse-phase and anion exchange interactions. Obelisc R was a tri-mode column that had cation exchange interaction in addition to the two modes. The mobile phases for isocratic elution were solutions of 2.5 – 12.5 mM $(NH_4)_2CO_3$ in 5% acetonitrile and the pH was adjusted to pH 4.0 with HCOOH. As(III) always eluted at void volume, suggesting no interaction in the employed modes. As(V) was retained in all four columns with retention factors ranging from 2.01 to 2.98. Newcrom B was selected for method development because As(V) was eluted as a sharp peak with 2.5 mM $(NH_4)_2CO_3$. The stationary phase of Newcrom B was a strong anion exchanger at the end of a 100 Å polymeric hydrophobic chain. Higher concentrations of $(NH_4)_2CO_3$ decreased As(V) retention and its peak area, indicating the interaction was anion exchange and $(NH_4)_2CO_3$ suppressed the ESI-MS signal.

The groundwater matrix presents additional challenges because the matrix chemicals can destabilize iAs speciation and interfere As(V) detection with ESI-MS. In a survey of As-polluted groundwater in Shepley's Hill Landfill (Fort Devens, MA, USA), the iAs in the sample was precipitated with newly formed iron oxide after exposure to air in less than 20 min. The groundwater also had carbonate, sulfate, and chloride in the range of 15-487 mg/L, 0.37-16.2 mg/L, and 1.31-35.4 mg/L, respectively. [19] The anions may impact ESI-MS detection if they are co-eluted with As(V). The objectives of HPLC would include separating As(V) from the anions in the matrix and the chemicals for sample preparation.

3.2. Impact of mobile phase on chromatographic behavior and detection of As(V)

Chemicals in mobile phase may impact ESI-MS detection in several stages, including formation of charged droplets, solvent evaporation, droplet fission, and gas-phase ion formation. Ammonium carbonate would increase the solution conductivity, change the surface tension of analyte droplets, and compete with As(V) in gas-phase ion formation.[20] These issues were addressed by replacing $(NH_4)_2CO_3$ with formic acid for elution. It was found that As(V) was eluted by 0.5 – 1.0 v/v% formic acid in 10- 40% of MeCN. By eliminating the salt in the elution, As(V) peak areas were increased by 1.9- to 7.5- fold. Organic solvent was evaluated by comparing MeCN with MeOH and EtOH (Reagent Alcohol, Fisher Scientific, catalog # A962-4). The peak area with Reagent Alcohol in mobile phase was twice as much comparing to those with MeCN or MeOH.

Formic acid, reagent alcohol, and flow rate influenced not only chromatography efficiency but also ESI-MS sensitivity for As(V). Their impacts were evaluated by systematically varying the mobile phases for isocratic elution by a design of experiment plan (Table 2).[21] The evaluation investigated formic acid in the range of 0.4 – 0.9 v/v%, Reagent Alcohol in the range of 20 – 60 v/v%, and flow rate in the range of 0.2-0.4 ml/min. The study employed a Surface Response Methodology based on Central Composite Design. Each factor was evaluated in three levels. There were 16 tests including 15 combinations of the three factors at different levels and 1 repeat at the center point (Table 2, Test 5, and 8). The sample was 10 μl of 1 μM As(V) in water. Retention time, peak area, and peak height were recorded. Theoretical plate numbers were calculated by Eq. 1 in Experimental Section.

Table 2. Impact of mobile phase and flow rate on the HPLC-ESI-MS analysis of As(V)

Test	Formic acid (v/v%)	Flow (ml/min)	Reagent Alcohol (v/v%)	t_R (min)	Area (CPS)	Height	Theoretical Plate Number
1	0.9	0.2	20	5.13	62528	4811	3530
2	0.65	0.2	40	5.34	80647	5261	2749
3	0.65	0.3	20	3.86	54151	5795	3869
4	0.4	0.2	20	7.53	56187	3191	4165
5	0.65	0.3	40	3.59	55650	5280	2644
6	0.4	0.2	60	6.70	88145	3587	1687
7	0.4	0.3	40	4.57	64686	4823	2627
8	0.65	0.3	40	3.57	73953	7063	2632
9	0.9	0.4	20	2.53	46404	7052	3353
10	0.4	0.4	20	3.75	37320	4120	3875
11	0.65	0.3	60	3.52	102164	8264	1837
12	0.9	0.3	40	3.12	75378	8141	2596
13	0.4	0.4	60	3.31	69381	5654	1644
14	0.9	0.4	60	2.31	103958	12467	1730
15	0.65	0.4	40	2.67	61360	7513	2422
16	0.9	0.2	60	4.59	143326	9554	2122

The effects of the three factors were evaluated with F-tests, and fitting to polynomial models that included terms for interactions and quadratic effects.[21] Retention times were in the range of 2.31 – 7.53 min. According to the F-test, all three factors significantly influenced retention time. The impacts were in the order of Flow > formic acid >> interaction between formic acid and flow ≈ Reagent Alcohol (Supplementary Material, Table 1). It was found formic acid was 11 times more efficient than Reagent Alcohol to elute As(V) in the experiment. Based on the peak area, variating elution conditions caused ESI-MS sensitivity to change by 3.84-fold (Supplementary Material, Table 2). Parameter estimates in modeling showed that Reagent Alcohol and formic acid increased the sensitivity. Their interaction was also significant. The impact from Reagent Alcohol was about 4.7-fold of that from formic acid. Signal enhancement by formic acid depends on alcohol concentration. In 60% alcohol, increasing formic acid from 0.4 – 0.9% caused peak area to increase by 64% (Test 6 vs 16, Table 2). It has been observed that carboxylic acids can form esters with arsenate at ESI interface. The detection limit of As(V) was reduced from 0.95 μM to 0.035 μM by forming a monoester with iminodiacetic acid.[22] Formic acid may form ester(s) with As(V) under acidic condition. It would be worthwhile to study if the esterification plays a role in the signal enhancement or can generate easier-to-detect anions in ESI. In terms of theoretical plate number, chromatography efficiency was reduced at higher concentrations of Reagent Alcohol or formic acid (Supplementary Material, Table 3). Apparently, Reagent Alcohol increased the peak width, while formic acid reduced the retention time. Flow rate changed both peak width and retention time. Overall, increasing flow rate caused little reduction in theoretical plate number.

3.3. As(III) oxidation and sample preparation

Since As(III) was difficult to analyze with HPLC-ESI-MS, converting As(III) to As(V) became the prioritized option. Many oxidants had been used to oxidize As(III) for water treatment. Examples of these oxidants are chlorine, oxygen, monochloramine, sodium hypochlorite, potassium permanganate.[23, 24] However, they are not suitable for As(III) oxidation in lab analysis. An ideal oxidant should be stable, efficient, and compatible with the HPLC-ESI-MS method. Hence, several stable peroxide or peracid oxidants were investigated. They included 3-chloroperbenzoic acid (mCPBA), hydrogen peroxide urea, sodium perborate, sodium percarbonate, and sodium persulfate. Although potassium permanganate is stable and efficient, it was not included in the evaluation due to the concern of forming refractory oxide byproducts.

The oxidants were compared both in water and in 5 mM oxalic acid because we had planned to use oxalic acid to solubilize ion oxides in samples.[25] The concentrations of oxidants were 0.2 – 1 mM and As(III) concentrations were 0.2 - 20 μM. The efficiency was compared by the oxidation yield in 1 hour. With 1 mM hydrogen peroxide urea, the yields were always < 3%. In water, oxidation yields with sodium percarbonate, sodium perborate, and sodium persulfate were between 77 – 92%, but in 5 mM oxalic acid their yields were reduced to 2- 7%. Of those tested, mCPBA was the most efficient oxidant. With 0.2 mM mCPBA in water, 0.2 to 20 μM As(III) was completely oxidized in < 1 h at ambient temperature. At 40 ^{o}C, the oxidation was complete in < 30 min. In 5 mM oxalic acid solution, only mCPBA oxidized As(III) completely. We also noted that in 5 mM oxalic acid, the oxidation gave 3-chlorobenzoic acid as the sole byproduct. This occurrence simplified the need for separation. Afterwards, the elution plan focused on separating As(V) from oxalic acid, mCPBA, and 3-chlorobenzoic acid.

The finalized condition for As(III) oxidation was with 1 mM mCPBA in 5 mM oxalic acid at 40 ^{o}C for 30 min. The solubility of mCPBA is around 1.1 g/L (6.4 mM) in water. The oxidation was carried out by adding mCPBA stock solution (100 mM in MeCN) to the sample.

The amount of inorganic arsenic (iAs) after mCPBA oxidation is the sum of As(III) and As(V). The concentration of As(III) is therefore calculated by the equation below.

$$[As(III)] = [iAs] - [As(V)] \qquad \textbf{Eq. } 2$$

3.4. Finalized method and characterization

In the finalized method, As(V) was eluted from Newcrom B by a 2-D gradient with 3.5-5 v/v% of 10% HCOOH and 35-50 v/v% of Reagent Alcohol. The flow rate was 0.35 ml/min (Table 3). As(V) was detected with SIR at m/z = 141. UV at 232 nm and TIC scan of m/z = 100 – 200 were used to monitor other chemicals in the elution. With this method, the As(V) was eluted at 4.2 min and the rest of the chemicals were eluted between 8.9 – 14.5 min (Figure 2). These chemicals include oxalic acid (m/z = 89, negative mode), and 3-chlorobenzoic acid (m/z = 155, negative mode) (Supplementary Material, Figure 1).

Table 3. Elution plan for As(V) from Newcrom B (3.2 x 100 mm, 3 μm)

Time (min)	Flow (ml/min)	10 v/v% HCOOH (%)	Reagent Alcohol (%)	Water (%)
0	0.35	3.5	35	61.5
5	0.35	3.5	35	61.5
6	0.35	5	40	55
9	0.35	5	40	55
10	0.35	3.5	35	61.5
16	0.35	3.5	35	61.5

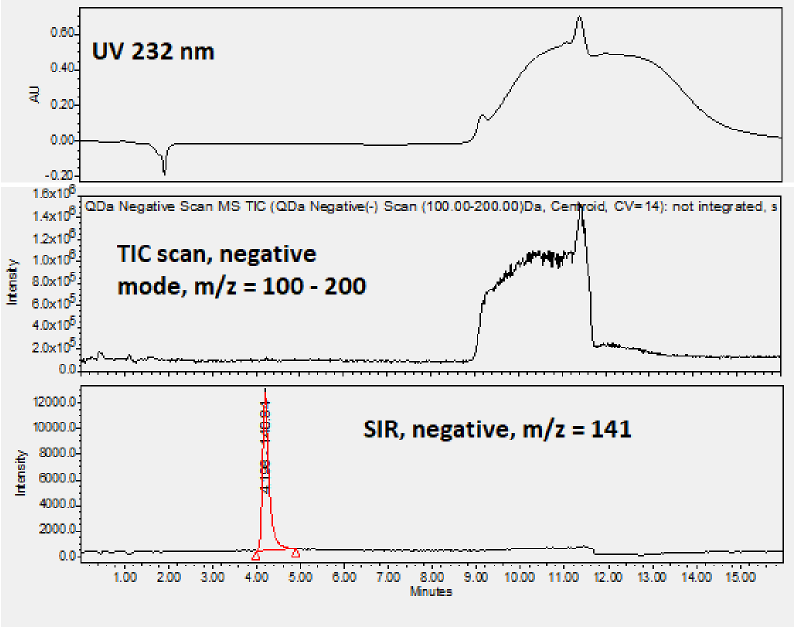

Figure 2. Chromatograms of a sample prepared by As(III) (1 μM) oxidation with 1 mM mCPBA in 5 mM oxalic acid. The sample was eluted from Newcrom B (3.2 x 100 mm, 3 μ) with the plan in Table 3.

3.4.1 Method characterization

The linearity, LOD, and LOQ were evaluated by the calibration curves for As(III) and As(V) with seven data points from 0.05 – 1.0 µM. The regression results (Table 4) showed that the peak areas were closely correlated to concentration over the concentration ranges ($r^2 > 0.99$). The slopes showed that the EIS-MS signals were greatly enhanced comparing to the method with ammonium carbonate gradient elution. For As(V), the increase was 15.7-fold. For As(III), the increase was 139-fold.[15] In the current method, the slope of the standard curve for As(III) was 98.1% of that for As(V), suggesting a complete recovery through mCPBA oxidation. With 10 µl injection volume, the LOD and LOQ for As(V) were 0.0263 µM (1.97 ppb) and 0.0876 µM (6.57 ppb), respectively. The precision for As(III) was slightly lower as indicated in r^2. The LOD and LOQ for As(III) were 0.0398 µM (2.99 ppb) and 0.133 µM (9.98 ppb), respectively.

Table 4. Method characterization for As(V) and As(III) analysis with HPLC-ESI-MS.

Arsenic	Regression Equation *y*, peak area (CPS), *x* arsenic concentration (µM)	r^2	Range (µM)	LOD (µM)	LOQ (µM)
As(III)	$y = 118103x$-278	0.9991	0.05-1.0	0.0398	0.133
As(V)	$y = 120378x - 462$	0.9996	0.05-1.0	0.0263	0.0876

The precision profile of As(V) showed that the RSD (n = 3) was dependent on the concentration. In the range of [As(V)] = 0.1 – 1 µM, the RSD was in the range of 0.39 – 3.87%. In lower range of [As(V)] = 0.01 – 0.07 µM, the RSD was in a higher range of 0.47 – 7.79%.

The accuracy of the method was evaluated by measuring the speciation of mixtures of As(V) and As(III) in 5 mM oxalic acid. In these samples, the concentration of added As(V) was fixed at 0.2 µM, while the concentration of added As(III) ranged from 0 – 0.6 µM. The measured concentrations ($As(V)_{est}$ and iAs_{est}) were compared to the expected values. The results showed that the recoveries of As(V) were in the range of 107 – 114%, while the recoveries for total iAs were in the range of 98 – 102%. (Table 5). These results indicated a good degree of accuracy for As(V) at low concentration. The accuracy was improved as the concentration increased. Overall, these results showed that the method was suitable for speciation analysis of iAs.

Table 5. Accuracy (% recovery) of the HPLC-ESI-MS method for iAs speciation

Sample	[As(V)] (µM)	[As(III)] (µM)	[iAs] (µM)	$[As (V)]_{est}$ (µM)	$[iAs]_{est}$ (µM)	Recovery of As(V) (%)	Recovery of iAs (%)
1	0.2	0	0.2	0.224	0.195	112	98
2	0.2	0.1	0.3	0.214	0.302	107	101
3	0.2	0.2	0.4	0.226	0.410	113	102
4	0.2	0.3	0.5	0.219	0.497	109	99
5	0.2	0.4	0.6	0.228	0.603	114	100
6	0.2	0.5	0.7	0.216	0.703	108	100
7	0.2	0.6	0.8	0.223	0.795	111	99

3.4.2 Sample stability and impact of air exposure on iAs in iron-rich groundwater

Sample stability has been a critical issue in iAs speciation in complex matrices. In Shepley's Hill Landfill Site, 0.02-81.5 mg/L of Fe and 0.01-7.56 mg/L Mn were found in the anoxic groundwater.[19] Short exposure to air during sampling had caused ferric oxide precipitation. It was expected that the reactions of Fe(II), As(III), and Mn(II) could be highly complicated and unpredictable.[26] The iron would not only catalyze As(III) oxidation but also cause iAs co-precipitation with ferric oxide. [27-30] Typically, sample preservation involved acid modification to slow down the oxidation and adding EDTA to prevent arsenic from co-precipitation with ferric oxide.[31]

The stability of iAs speciation was first evaluated in 5 mM oxalic acid as it was used to release iAs by dissolving ferric oxide. [25] Three samples made with different ratios of As(III) and As(V) in clear borosilicate glass vials were allowed to stand at ambient temperature (23-25 ºC) for 15 days. Samples were taken over the time from the stocks and measured for speciation. The recoveries of As(V) (0.2 – 0.8 μM) were from 92 – 106% with mean value of 97.8 ± 5.1% (n = 18), and the recoveries for total As (1 μM) were 88 – 106% with mean value of 99.6 ± 5.2% (n = 18) (Supplementary Material, Table 4). Neither time nor speciation had significant impact on the recoveries, indicating the speciation was stable in 5 mM oxalic acid.

The matrix of the simulated groundwater was based on the chemical composition of the groundwater in Shepley's Hill Landfill Site, Fort Devens, CT, USA.[19] The simulated groundwater had 612 μM of Fe(II) and 83 μM of Mn(II). The anions included carbonate (2130 μM), chloride (1390 μM), sulfate (256 μM), phosphate (100 μM), and silicate (400 μM) (Table 1).

Samples were prepared by adding 100 nmol of As(V) and/or As(III) to the synthetic groundwater to give 20 ml of 5 μM solutions of each species. After 20 min of air exposure, the vials were close capped and allowed to stand for 16 h at ambient temperature (21-25 ºC). For speciation determination, the solid and liquid were separated by centrifuge. Arsenic samples of for solid phase were prepared by dissolving ferric oxide in 2 ml of 50 mM oxalic acid. The arsenic solutions were diluted with 5 mM oxalic acid and centrifuged to remove calcium oxalate. Speciation was analyzed for the iAs in the solution and the iron oxide precipitate (Table 6).

The result showed that all As(V) was removed by the newly formed iron oxide from liquid phase (Test 1, Table 6). Most of the As(III) (>95%) was removed from liquid phase as co-precipitate. In the solid phase the ratio of As(V) vs As(III) was about 2:1 (Test 2, Table 6). The result suggested that although As(III) could be directly removed by iron oxide, most As(III) was actually oxidized to As(V) and then precipitated. [27] When As(V) and As(III) co-exist in groundwater, sample change was consistent with the two observations (Test 3, Table 6). The recoveries of total arsenic were 99.5 – 117%, indicating no arsenic loss to calcium oxalate solid.

Table 6. Arsenic speciation in solid and liquid phases of groundwater samples after 16 h

Test	Matrix	As Input (nmol)		As in liquid, 16 h (nmol)			As in solid, 16 h (nmol)			Recovery (%)
		As(V)	As(III)	As(V)	As(III)	iAs	As(V)	As(III)	iAs	
1	Groundwater	100	0	0	0	0	108.2	4.0	112.2	112
2	Groundwater	0	100	0	4.4	4.4	66.4	32.4	98.8	103.2
3	Groundwater	100	100	0	6.0	6.0	179.2	48.8	228	117
4	5 mM oxalic acid	100	100	102.4	96.6	199	n/a	n/a	n/a	99.5

4. Conclusion

This study demonstrated that As(V) can be separated from other anions on Newcrom B with a two dimensional elution strategy. The retention of As(V) was more sensitive to formic acid gradient, while the retention of organic anions could be fine-tuned by the organic solvent gradient. By replacing $(NH_4)_2CO_3$ with formic acid in mobile phase, this elution also enhanced ESI-MS detection of As(V) by 15.7-fold.[15] Interfacing the HPLC separation with a QDa detector, the LOD for As(V) reached 1.99 ppb when injection volume was 10 μl.

Direct determination of As(III) was difficult because it was not retained on the HPLC columns and the detection had low sensitivity. The problems have been solved by converting As(III) to As(V) with mCPBA oxidation. The conversion was complete and robust. The oxidant and its byproduct were separated from As(V) on Newcrom B. With this strategy, the sensitivity for As(III) was enhanced by 139-fold and the LOD reached 2.99 ppb.

The method had LOQ for As(III) and As(V) were 9.98 and 6.57 ppb, respectively. It can be a stand-alone method to monitor arsenic in groundwater and arsenic removal by community water system.

Air exposure caused several changes in arsenic speciation and solubility in iron rich groundwater samples, including As(III) oxidation, As(III) and As(V) precipitation with iron oxide. Oxalic acid was suitable to prepare As samples from the liquid and solid for speciation analysis.

Acknowledgements

The author would like to thank Dr. Matthew Small and Mr. Adam Ramos for discussions on community water systems in Navajo Nation, Dr. Wenhu Wang (University of Maine) for the evaluation of glass material of sample vials, Ms. Tri Richardson for manuscript proofreading, and Ms. Amy Zhao for support in lab management.

Disclaimer

The views expressed in this article are those of the authors and do not reflect the official policy or position of the United State Environmental Protection Agency (EPA). Mention of trade names, products, or services does not convey official EPA approval, endorsement, or recommendation. This manuscript has been subjected to the Agency’s review and has been approved for publication.

References

[1] J. Podgorski, M. Berg, Global threat of arsenic in groundwater, Science 368(6493) (2020) 845-850 https://doi.org/10.1126/science.aba1510.
[2] I. Herath, M. Vithanage, J. Bundschuh, J.P. Maity, P. Bhattacharya, Natural Arsenic in Global Groundwaters: Distribution and Geochemical Triggers for Mobilization, Current Pollution Reports 2(1) (2016) 68-89 https://doi.org/10.1007/s40726-016-0028-2.
[3] F.W. Pontius, K.G. Brown, C.-J. Chen, Health implications of arsenic in drinking water, Journal AWWA 86(9) (1994) 52-63 https://doi.org/10.1002/j.1551-8833.1994.tb06246.x.

[4] P. Mondal, S. Bhowmick, D. Chatterjee, A. Figoli, B. Van der Bruggen, Remediation of inorganic arsenic in groundwater for safe water supply: A critical assessment of technological solutions, Chemosphere 92(2) (2013) 157-170 https://doi.org/10.1016/j.chemosphere.2013.01.097.

[5] B.R. Scanlon, S. Fakhreddine, R.C. Reedy, Q. Yang, J.G. Malito, Drivers of Spatiotemporal Variability in Drinking Water Quality in the United States, Environ Sci Technol 56(18) (2022) 12965-12974 https://doi.org/10.1021/acs.est.1c08697.

[6] S. Fendorf, H.A. Michael, A. van Geen, Spatial and Temporal Variations of Groundwater Arsenic in South and Southeast Asia, Science (Washington, DC, U. S.) 328(5982) (2010) 1123-1127 https://doi.org/10.1126/science.1172974.

[7] D.Q. Hung, O. Nekrassova, R.G. Compton, Analytical methods for inorganic arsenic in water: a review, Talanta 64(2) (2004) 269-277 https://doi.org/10.1016/j.talanta.2004.01.027.

[8] J. Ma, M.K. Sengupta, D. Yuan, P.K. Dasgupta, Speciation and detection of arsenic in aqueous samples: A review of recent progress in non-atomic spectrometric methods, Analytica Chimica Acta 831 (2014) 1-23 https://doi.org/10.1016/j.aca.2014.04.029.

[9] M.S. Reid, K.S. Hoy, J.R.M. Schofield, J.S. Uppal, Y. Lin, X. Lu, H. Peng, X.C. Le, Arsenic speciation analysis: A review with an emphasis on chromatographic separations, TrAC Trends in Analytical Chemistry 123 (2020) 115770 https://doi.org/10.1016/j.trac.2019.115770.

[10] C. Yuan, X. Lu, N. Oro, Z. Wang, Y. Xia, T.J. Wade, J. Mumford, X.C. Le, Arsenic Speciation Analysis in Human Saliva, Clinical Chemistry 54(1) (2008) 163-171 https://doi.org/10.1373/clinchem.2007.092189.

[11] S. Afton, K. Kubachka, B. Catron, J.A. Caruso, Simultaneous characterization of selenium and arsenic analytes via ion-pairing reversed phase chromatography with inductively coupled plasma and electrospray ionization ion trap mass spectrometry for detection: Applications to river water, plant extract and urine matrices, Journal of Chromatography A 1208(1) (2008) 156-163 https://doi.org/10.1016/j.chroma.2008.08.077.

[12] K.A. Francesconi, D. Kuehnelt, Determination of arsenic species: A critical review of methods and applications, 2000–2003, Analyst 129(5) (2004) 373-395 https://doi.org/10.1039/B401321M.

[13] A. McKnight-Whitford, X.C. Le, Electrospray Mass Spectrometry of Arsenic Compounds and Thiol–Arsenic Complexes, Encyclopedia of Analytical Chemistry (2011) https://doi.org/10.1002/9780470027318.a9234.

[14] M.H. Florêncio, M.F. Duarte, A.M.M. de Bettencourt, M.L. Gomes, L.F. Vilas Boas, Electrospray Mass Spectra of Arsenic Compounds, Rapid Communications in Mass Spectrometry 11(5) (1997) 469-473 https://doi.org/10.1002/(SICI)1097-0231(199703)11:5<469::AID-RCM831>3.0.CO;2-7.

[15] G. Gilmartin, D. Gingrich, A comparison of the determination and speciation of inorganic arsenic using general HPLC methodology with UV, MS and MS/MS detection, Journal of Chromatography B 1083 (2018) 20-27 https://doi.org/10.1016/j.jchromb.2018.02.034.

[16] F.R. Mansour, N.D. Danielson, Multimodal liquid chromatography of small molecules, Analytical Methods 5(19) (2013) 4955-4972 https://doi.org/10.1039/C3AY40302E.

[17] R. Thomas, Money to Burn: Do you Know What is Costs to Run Your Atomic Spectroscopy Instrumentation? , International Labmate (2016) 18-19 https://doi.org/10.1201/b21952-24

[18] Shimadzu Service and Support, Formula for Calculating the Number of Theoretical Plates. https://www.shimadzu.com/an/service-support/technical-support/analysis-basics/basic/34/34tec.html.

[19] R.G. Ford, K.G. Schekel, S. Acree, R. Ross, B. Lien, T. Luxton, C. P, Final Report; Arsenic Fate, Transport and Stability Study; Groundwater, Surface Water, Soil And Sediment Investigation, Fort Devens Superfund Site, Devens, Massachusetts, in: U.E.P. Agency (Ed.) Washington, DC, 2008.

[20] F. Beaudry, P. Vachon, Electrospray ionization suppression, a physical or a chemical phenomenon?, Biomedical Chromatography 20(2) (2006) 200-205 https://doi.org/10.1002/bmc.553.

[21] J. Goupy, L. Creighton, Introduction to Design of Experiments with JMP Examples, 3rd ed., SAS Press, Cary, NC, United States, 2007.

[22] H. Kojima, S. Kurihara, Y. Watanabe, K. Iwamaru, K. Sato, K.-i. Tsunoda, H. Hotta, A novel method for determination of inorganic oxyanions by electrospray ionization mass spectrometry using dehydration reactions, Journal of Mass Spectrometry 51(2) (2016) 123-131 https://doi.org/10.1002/jms.3731.
[23] S. Sorlini, F. Gialdini, Conventional oxidation treatments for the removal of arsenic with chlorine dioxide, hypochlorite, potassium permanganate and monochloramine, Water Research 44(19) (2010) 5653-5659 https://doi.org/10.1016/j.watres.2010.06.032.
[24] G. Ghurye, D. Clifford, As(III) oxidation using chemical and solid-phase oxidants, Journal AWWA 96(1) (2004) 84-96 https://doi.org/10.1002/j.1551-8833.2004.tb10536.x.
[25] D. Panias, M. Taxiarchou, I. Paspaliaris, A. Kontopoulos, Mechanisms of dissolution of iron oxides in aqueous oxalic acid solutions, Hydrometallurgy 42(2) (1996) 257-265 https://doi.org/10.1016/0304-386X(95)00104-O.
[26] A.R. Kumar, P. Riyazuddin, Preservation of inorganic arsenic species in environmental water samples for reliable speciation analysis, TrAC Trends in Analytical Chemistry 29(10) (2010) 1212-1223 https://doi.org/10.1016/j.trac.2010.07.009.
[27] W. Ding, J. Xu, T. Chen, C. Liu, J. Li, F. Wu, Co-oxidation of As(III) and Fe(II) by oxygen through complexation between As(III) and Fe(II)/Fe(III) species, Water Research 143 (2018) 599-607 https://doi.org/10.1016/j.watres.2018.06.072.
[28] Z. Wen, Y. Zhang, X. Zhou, R. Chen, Effective As(III) and As(V) immobilization from aqueous solution by nascent ferrous hydroxide colloids (FHC), Separation and Purification Technology 176 (2017) 395-401 https://doi.org/10.1016/j.seppur.2016.12.022.
[29] S. Dixit, J.G. Hering, Comparison of Arsenic(V) and Arsenic(III) Sorption onto Iron Oxide Minerals: Implications for Arsenic Mobility, Environmental Science & Technology 37(18) (2003) 4182-4189 https://doi.org/10.1021/es030309t.
[30] K.P. Raven, A. Jain, R.H. Loeppert, Arsenite and Arsenate Adsorption on Ferrihydrite: Kinetics, Equilibrium, and Adsorption Envelopes, Environmental Science & Technology 32(3) (1998) 344-349 https://doi.org/10.1021/es970421p.
[31] G. Samanta, D.A. Clifford, Preservation of Inorganic Arsenic Species in Groundwater, Environmental Science & Technology 39(22) (2005) 8877-8882 https://doi.org/10.1021/es051185i.