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Talanta 68 (2005) 406–415
Talanta
www.elsevier.com/locate/talanta

Speciation of arsenic in ground water samples: A comparative study of CE-UV, HG-AAS and LC-ICP-MS

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Available online 13 October 2005

Abstract

The performance of capillary electrophoresis-ultraviolet detector (CE-UV), hydride generation-atomic absorption spectrometry (HG-AAS) and liquid chromatography-inductively coupled plasma mass spectrometry (LC-ICP-MS) have been compared for the speciation of arsenic (As) in groundwater samples. Two inorganic As species, arsenite (As^{III}), arsenate (As^{V}) and one organo species dimethyl arsenic acid (DMA) were mainly considered for this study as these are known to be predominant in water. Under optimal analytical conditions, limits of detection (LD) ranging from 0.10 (As^{III}, AsT) to 0.19 (DMA) μg/l for HG-AAS, 100 (As^{III}, DMA) to 500 (As^{V}) μg/l for CE-UV and 0.1 (DMA, MMA) to 0.2 (As^{III}, As^{V}) μg/l for LC-ICP-MS, allowed the determination of the above three species present in these samples. Results obtained by all the three methods are well correlated ($r^2 = 0.996$*** for total As) with the precision of <5% R.S.D. except CE-UV. The effect of interfering ions (e.g. Fe^{2+}, Fe^{3+}, SO_4^{2-} and Cl^-) commonly found in ground water on separation and estimation of As species were studied and corrected for. Spike recovery was tested and found to be 80–110% at 0.5 μg/l As standard except CE-UV where only 50% of the analyte was recovered. Comparison of these results shows that LC-ICP-MS is the best choice for routine analysis of As species in ground water samples.

Keywords: Arsenic speciation; Ground water

1. Introduction

The toxic and carcinogenic properties of inorganic and organo arsenic (As) species make their speciation in natural water vitally important [1]. Hydride generation-atomic absorption spectrometry (HG-AAS) and liquid chromatography-inductively coupled plasma mass spectrometry (LC-ICP-MS) are currently being used for the estimation and speciation of As. Ideally methods commonly used for routine analyses would be rapid, reliable, cheap and easy to use [2]. Given that the toxicity of As varies with species, speciation and quantification of As is critical in assessing overall risk when regulations for individual as species will emerge. However, due to the different chemical and physical properties of As compounds, a reliable separation of the various species within one single run has been difficult. For this reason, a combination of various separation and detection procedures is commonly being employed. These techniques either separately or in combination are subject to significant errors due to interference arising from the presence of other chemicals, especially when the analyte is present at trace levels. Hydride generation (HG), liquid chromatography (LC), gas chromatography (GC) and capillary electrophoresis (CE) are commonly being utilized for the separation of As species.

The advantage of HG method is that it can easily be connected to various detection systems [AAS, electrothermal (ET)-AAS, ICP-atomic emission spectrometry (AES), atomic fluorescence spectrometry (AFS), and ICP-MS]. This improves the detection limits up to 100-fold over the commonly used liquid sample nebulization process [3]. It can eliminate spectral and chemical interferences encountered in the detection system as only gaseous hydrides are introduced into the detector. However, some drawbacks have been reported [4] for this method, including: (i) that the method is limited to the materials that form volatile arsines; (ii) reaction conditions have to be strictly controlled; (iii) the presence of certain interfering elements can reduce the efficiency of HG; (iv) method is laborious; and (v) single element detection technique.

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doi:10.1016/j.talanta.2005.09.011

CE offers high separation efficiency and rapid analysis. It requires only nano-litres of samples and the running costs are very low. Currently the majority of CE separations of As have been limited to pure standard solution or simple matrices due to its poor sensitivity and matrix interference [5,6]. However, sample stacking or on-column pre-concentration method has been introduced to increase the detection sensitivity and has been employed for As speciation [7].

The combination of LC with an element selective detector: ICP-MS is the most popular technique for As speciation in environmental samples [8]. Among the various LC techniques, ion exchange chromatography (IEC), ion interaction chromatography (IIC) has frequently been used. In IEC a mobile phase is used to transport the sample (and analyte) into the column where individual compounds (species) compete with oppositely charged functional-group ions on the stationary phase and the separation of analyte ions takes place by displacing mobile-phase ions. This IEC separation was often used in conjunction with ICP-MS detector owing to its multi-elemental capabilities, low detection levels (sub-nanogram) and greater linear range.

Following a review of the various speciation methods, three methods that are commonly used for the speciation of As were selected for a comparative study of their applicability and limitation to ground water samples of Bangladesh. There is no report so far comparing these three instrumental techniques for As speciation. In this study we compare these three contrasting speciation techniques for the speciation of As in ground water samples with a view to recommending a rapid and reliable technique. Despite of the lack of certified reference materials for the toxic As species [As^{III}, As^{V}], it is also of particular interest to compare As speciation determined by different methods on the same samples to establish their validity.

2. Experimental

2.1. Sample collection and preparation

Test samples were collected from tube-well of highly contaminated areas in Bangladesh. The samples were filtered with 0.45 μm syringe filter in the field and stored at 4 °C for analysis. No preservation was used as acid can change mobile phase pH and column condition. Samples were directly used for CE and LC-ICP-MS but L-cysteine, potassium iodide (KI), urea and acid were used for HG-AAS.

2.2. Instrumentation

2.2.1. HG-AAS

A vapour generation accessory (VGA-77, Varian, Australia Pty Ltd., Melbourne, Australia) connected to an atomic absorption spectrometer (GBC 906AA, GBC scientific, Melbourne, Australia) was used in this study. Arsenic hollow-cathode lamp was used as the radiation source. A deuterium background corrector was also used in the determination of As and operated according to manufacturer's instructions; instruments parameters used are summarized in Table 1. In this system, the solutions of sample, acid and reductant are pumped at the rate of 7, 1and 1 ml/min, respectively. Gaseous arsenic hydride generation was obtained by the continuous pumping of sample solution, acid solution and reducing agent ($NaBH_4$) through a reaction coil and into a gas–liquid separator. Nitrogen (N_2) was used as a carrier gas to transport the As-hydride to a heated quartz cell located in the optical path of an arsenic hollow-cathode lamp. The resulting absorption is proportional to the concentration of the sample. Samples were analyzed at a rate of 60 h^{-1} using a GBC FS3000 auto sampler (GBC Scientific, Melbourne, Australia).

Table 1
Operating conditions of the HG-AAS system (standard conditions as given by manufacturer)

Instrument mode	Absorbance
Calibration mode	Concentration
Measurement mode	Integration
Slit width (nm)	1.0
Slit height	Normal
Wavelength (nm)	193.7
Flame	Air–acetylene
Sample introduction	Auto
Delay time (s)	40
Time constant	0.05
Measurement time (s)	2
Replicates	3
Background correction	On
Sample flow rate (ml/min)	7
$NaBH_4$ flow rate (ml/min)	1
HCl flow rate (ml/min)	1

2.2.2. CE-UV

CE experiments were preformed using Quanta 4000 (Waters, Milford, USA). The system was controlled by Millennium (Waters, Milford, USA) software. Separation was carried out on fused-silica capillaries of 50 μm i.d. and 50 cm total length (42 cm effective length). The UV detector was set at 185 nm for direct UV detection and the voltage was applied at a positive polarity (+20 kV) for counter-EOF and at a negative polarity (−20 kV) for co-EOF. The temperature was varied between 28 and 30 °C, and the applied voltage was constant at +20 or −20 kV.

2.2.3. LC-ICP-MS

A simple isocratic separation using an Agilent 1100 LC (Agilent Technologies, Germany) equipped with LC pump, autosampler and a thermostatic column compartment was used in this study. The chromatographic stationary phase used in this work was an anion exchange column at 150 mm × 4.6 mm i.d. and 2 mM/0.2 mM phosphate buffer/EDTA eluent (pH 6.0) was used to detect all four toxic species in less than 10 min. One Guard column at 10 mm × 4.6 mm i.d. was also used to protect the main column. All of these column and mobile phase used in this work was marketed by Agilent technology. LC was connected with Agilent 7500c ICP-MS (Agilent Technologies, Tokyo, Japan) as an element selective detector and sample introduction was performed by Babington type nebulizer. The optimum operating condition for LC-ICP-MS is detailed in Table 2. Data acquisition

Table 2
Optimum working parameter for LC-ICP-MS (standard conditions as given by manufacturer)

LC parameter	LC setting	ICP-MS parameter	ICP-MS settings
Mobile phase flow rate	1 ml/min	Plasma RF power	1500 W
Injection volume	50 μl with autosampler	Sampling depth	7.5–8 mm
Run time	12 min	Carrier gas flow	1.14 l/min
Column temperature	Ambient	Spray chamber temperature	2 °C
Oxide levels (CeO+/Ce+)	1.33%	Interface	Nickel sample and skimmer cones
Double charged (Ba++/Ba+)	2.09%	Data acquisition mode	Time resolve analysis (TRA)
		Acquisition mass	75(As), 35(Cl^-), 57(Fe)
		Integration time	0.3 s for As, 0.1 s for Cl^-

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.

2.3. Reagents

All reagents obtained from Sigma and Aldrich (Sydney, Australia) was of analytical grade and were used without further purification. Standard solutions were prepared daily from 1000 mg/l stock solutions by dilution with Milli-Q water. The standard for As^{III} as $NaAsO_2$, As^V as $Na_2HAsO_4{\cdot}7H_2O$, DMA as $(CH_3)_2AsO(OH)$ were obtained from Sigma (Australia); MMA as $CH_3AsNa_2O_3{\cdot}6H2O$ was obtained from Chem Service (West Chester, PA, USA). Electrolytes required for CZE were prepared by dissolution of an appropriate amount of NaH_2PO_4 in Milli-Q water. The mobile phase of LC-ICP-MS was prepared from NaH_2PO_4 and EDTA in which pH was adjusted to 6.0 by using 1 M NaOH. All electrolytes were filtered through Millipore 0.45 μm membrane filters and degassed before use.

2.4. Analytical techniques

2.4.1. As speciation by HG-AAS

The HG-AAS technique is based on the atomic absorption measurement of As generated by thermal decomposition of As^{III} hydride. It is a single element specific technique with the advantage of being relatively inexpensive in terms of equipment. HG-AAS has been successfully used for the determination of As in water, urine and plant samples [9–11].

Speciation of arsenic (As^{III}, As^V) was carried out in two stages, estimation of total As (AsT) followed by As^{III}. As^V was determined by subtraction ($As^V = AsT - As^{III}$). A well-developed procedure was followed for the determination of total inorganic As where KI (10%) was used for the reduction of As^V to As^{III} and hydride was generated in 6N HCl in the presence of 0.6% $NaBH_4$ stabilized by 0.5% NaOH. For As^{III}, hydride was generated at pH 4–4.5 using an oxalate buffer. Detection limits was 0.1 μg/l for As^{III} and AsT following the same procedure described elsewhere [12].

For the determination of DMA, a published method was modified to satisfy the particular sample requirement [13]. HCl concentration was optimized with respect to arsine signal (absorbance) for 40 μg/l of DMA at 4% L-cystine and 0.6% $NaBH_4$, which was varied from 0.1 to 3 M and DMA signal reached steady state at around 1.5 M HCl (Fig. 1). In all our subsequent experiments 1.5 M HCl was used for estimating DMA.

DMA absorption was studied by using various concentration of L-cysteine. This study showed that L-cysteine had very limited effect on DMA absorption signal. Therefore, 2% L-cysteine was used as an optimum concentration. Since As^{III} is quite common in water samples, we investigated its interference on DMA signal in the presence of 2% L-cysteine. Our investigations showed that concentration of 500 μg/l As^{III} interfered with DMA absorbance at around 8%. Therefore, sample containing 500 μg/l As^{III} can be easily used for DMA speciation without As^{III} interference if we use a correction factor. Indeed this high level of As^{III} is rare in environmental samples.

Calibration curve for DMA was obtained by plotting concentration versus absorbance of the test solute under the optimum conditions using L-cysteine. The curve was linear in the concentration range of 0.19–40 μg/l with correlation coefficient (r^2) 0.999. The detection limit was 0.19 μg/l and calculated by the following equation:

$$\mathrm{DL} = 3 \times \mathrm{S.D.b} \times \frac{[\mathrm{St}]}{[\mathrm{Abs(St)} - \mathrm{Abs(blank)}]}$$

where S.D.b is standard deviation of the replicated blank ($n = 60$), St the highest standard concentration (40 μg/l), Abs(St) the mean of highest standard concentration ($n = 20$), and Abs(blank) the mean of blank.

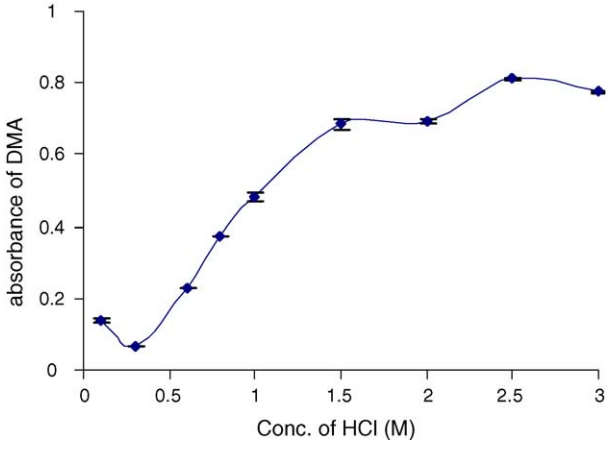

Fig. 1. Effect of HCl concentration on the absorption signal of DMA (40 μg/l) when using 4% L-cysteine and 0.6% $NaBH_4$ (m/v).

Table 3
Analytical performance of HG-AAS method

Species	Linear regression equation (a vs. c)	Linear range (μg/l)	Correlation coefficient (r^2)	Detection limit (μg/l)	% R.S.D.
AsT	$Y = -0.0004x^2 + 0.0429x + 0.0113$	0–50	0.999	0.1	<5
As^{III}	$Y = 0.0143x + 0.0139$	0–100	0.999	0.1	<5
DMA	$Y = 0.0227x + 0.0038$	0–40	0.999	0.19	<2

a vs. c means absorption vs. concentration.

The quantification limit was calculated using the following equation:

$$QL = 10 \times DL$$

where DL is the detection limit.

The above procedure were followed for the detection and quantification limit of AsT and As^{III}. The analytical performances of HG-AAS method for speciation of As^{III}, As^{V} and DMA are given in Table 3.

2.4.2. As speciation by CE-UV

CE is a new and attractive technique for the speciation of As. Despite its application difficulties, few researchers were able to apply the technique to natural waters, soil water extracts [14–16] and chicken feed samples [17] for As speciation. Using these published techniques, a CE method was developed to improve the detection limits of CE for the speciation of As (As^{III}, As^{V} and DMA) in ground water using sample stacking techniques. Separation of As species were achieved using an electrolyte solution containing 10 mM phosphate buffer and UV detection at 185 nm with both counter and co-electroosmotic (EOF) modes. In the co-EOF mode, cationic surfactants such as tetradecyltrimethylammonium bromide (TTAB) and cetyltrimethylammonium bromide (CTAB) were added to the electrolyte to modify the EOF. The concentration of such surfactants in the electrolyte affects the EOF, and in turn also influences the sample stacking efficiency. Other parameters such as electrolyte pH, run voltage, and injection modes and time were also varied in order to establish optimum analytical conditions. The best separation of the selected As species was achieved using 0.35 mM TTAB, a buffer pH of 9, and a run voltage of 20 kV (Fig. 2). Field amplified sample injection (FASI) with 10 kV for 10 s had decided advantages over large volume sample stacking (LVSS) because of short analysis time and sharp separation.

Calibration curves, obtained by plotting peak area versus concentration of the test solutes under the preferred conditions described above, found linear relationship with correlation coefficient greater than 0.99. The reproducibility of migration time and peak area were less than 2% and 9%, respectively. The analytical performances are given in Table 4.

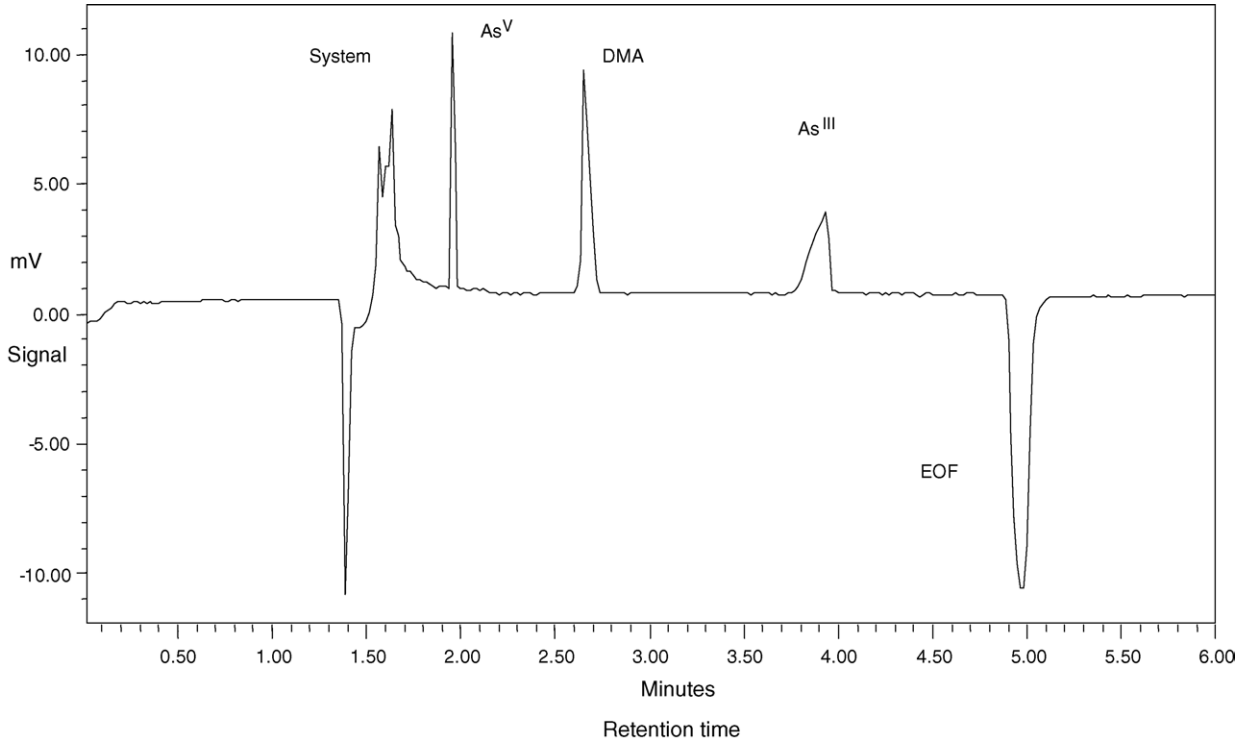

Fig. 2. Electropherogram of the three target As species (2 mg/l As mix) separated in the co-EOF mode. Conditions: fused silica capillary, 50 μm i.d × 50 cm (effective length, 42 cm); 10 mM phosphate buffer, pH 9; 0.35 mM TTAB, temperature 28 °C; −20 kV applied voltage; EI (−10 kV, 10 s).

Table 4
Analytical method performances of co-CZE with direct UV detection for arsenic species

Species	Linear regression equation[a] (*A* vs. *c*)	S.E.[b] (slope)	Linear range (mg/l)	Correlation coefficient	Detection limit[c] (mg/l)	Reproducibility (R.S.D.) (%)[d]	
						Migration time	Peak area
As(V)	$A = 3925c$	51	0.5–4	0.9984	0.5	0.4	8.8
DMA	$A = 13334c$	171	0.1–4	0.9985	0.1	0.8	7.7
As(III)	$A = 28221c$	376	0.1–4	0.9984	0.1	1.7	8.0

[a] *A*, peak area (arbitrary units); *c*, concentration (mg/l).
[b] Mean standard error.
[c] S/N = 3.
[d] For 0.5 mg/l.

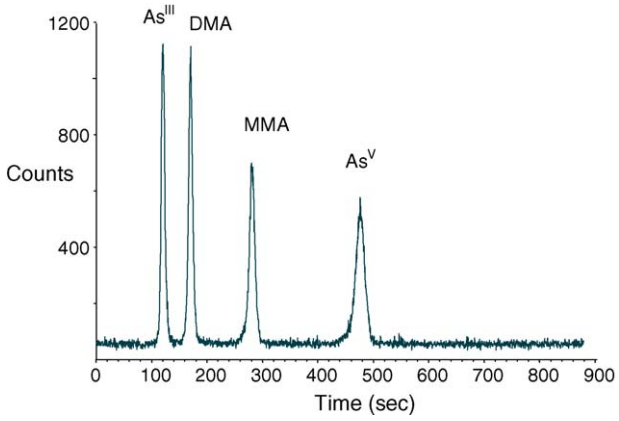

Fig. 3. Chromatogram showing separation of standard solution of 2 μg/l of each species As^{III}, DMA, MMA, As^{V}.

2.4.3. As speciation by LC-ICP-MS

Numerous investigators have successfully used LC-ICP-MS for the speciation of As in drinking water, river water, sea water, tissue extracts including oyster tissue and sea weeds containing a wider range of species [18]. LC-ICP-MS is a reliable technique for speciation of As in plant and aqueous samples with low detection limit and rapid analysis [19].

Using optimized LC and ICP-MS conditions described elsewhere [20] and summarized in Table 2, we were able to speciate As^{III}, DMA, MMA and As^{V} successfully within 10 min (Fig. 3). On-column loss of As was prevented by the addition of a complexing agent (0.2 mM EDTA) to the eluent. The mobile phase and column was used from a commercially available speciation kit marketed by Agilent Technology and verified by other researcher [20]. Peak area was considered for quantification purposes (integration time 0.5 s) and data were calculated by using normal Excel software. The analytical performance of the LC-ICP-MS method is shown in Table 5.

3. Result and discussion

3.1. Composition of ground water

The ground water pH is predominantly near neutral (pH 6.8–7.05). The composition of groundwater varied considerably among the samples examined. The concentration of aluminium (Al) and sulfur (S) is higher in samples which are extracted with disposable cartridge for As^{III} while filtered and acidified samples showed lower S and Al values. These might be due to the release of soluble Al and S from the cartridges. However, the concentration of iron, calcium and magnesium is high in all samples (Table 6). Presence of high concentrations of manganese (Mn) in these samples is likely to have enhanced As^{III} oxidation in the samples during transport and storage. As^{III} is oxidized by MnO_2 followed by the adsorption of the As^{V} reaction product on the MnO_2 solid phase [21] (Eq. (1)):

$$MnO_{2(s)} + 2H^+ + AsO_3^{3-} \leftrightarrow Mn^{2+} + AsO_4^{3-} + H_2O \quad (1)$$

3.2. Analytical instrument performance

3.2.1. HG-AAS

Prior to the analysis of real samples, the performance of the optimized method was tested and validated by estimating As in mineral water free of As and using spike recovery. Satisfactory result was obtained with 99–107% recovery. Sample matrix and ionic effect on absorption signal was evaluated and observed free of interferences. Finally, ground water samples of

Table 5
Retention times (RT) and analytical performance of anion-exchange LC-ICP-MS method

Species	Linear regression equation (*A* vs. *c*)	Linear range (μg/l)	Correlation coefficient (r^2)	Detection limit (μg/l)[a]	RT (min)
As^{III}	$Y = 681.27x + 573.28$	2–100	1.0	0.2	2.01 ± 0.01
As^{V}	$Y = 733.19x + 825.06$	2–100	1.0	0.2	6.93 ± 0.05
DMA	$Y = 772.77x + 76.758$	1–100	0.999	0.1	2.85 ± 0.03
MMA	$Y = 778.16x - 11.004$	1–100	1.0	0.1	3.64 ± 0.01

A, peak area; *c*, concentration.
[a] S/N = 3.

Table 6
Elemental information for water samples used in the present study

Lab ID	Samples ID	pH	EC (μS/cm)	Depth (ft)	Fe (mg/l)	Mn (mg/l)	Al (mg/l)	P (mg/l)	S (mg/l)	Ca (mg/l)	Mg (mg/l)
DHA-TW-17	W1	7.05	583	60	0.01	0.002	0.02	0.16	2.1	49.8	28.6
DHA-TW-19	W2 (3A)	7.05	583	60	2.6	0.31	8.4	0.04	72.0	96.1	26.7
DHA-TW-25	W3 (A)	6.87	615	95	7.02	0.42	0.07	1.03	<LD	66.9	20.5
DHA-TW-26	W4	6.87	615	95	0.02	0.23	<LD	0.12	<LD	67.2	21.9
DHA-TW-28	W5 (3A)	6.87	615	95	7.4	0.56	9.2	0.04	68.4	100.7	28.3
DHA-IW-44	W6 (3A)	6.8	555	85	13.6	0.21	11.7	0.12	88.8	107.7	32.4
KUN-TW-26	W7 (A)	6.86	825	75	19.1	0.65	0.01	1.3	1.1	86.4	31.2
KUN-TW-29	W8(3A)	6.86	825	75	19.9	0.89	20.2	0.05	101.1	109.9	35.4
NOY-TW-1	W9 (A)	6.76	664	85	13.0	0.53	0.005	1.5	0.1	62.1	22.1
NOY-TW-2	W10	6.76	664	85	15.2	0.78	0.04	2.5	1.7	72.9	25.8
NOY-TW-3	W11 (UA)	6.76	664	85	14.5	0.56	0.03	1.9	0.3	63.9	22.8
NOY-TW-4	W12 (3A)	6.76	664	85	12.8	0.73	3.8	0.03	107.8	85.1	25.5
BER-TW-4	W13 (3A)	6.92	638	35	12.0	0.55	0.8	0.03	83.5	92.8	32.9
BER-TW-9	W14 (3A)	7.08	600	80	8.15	0.59	14.02	0.09	124.6	108.7	36.9
BER-TW-20	W15 (A)	7.04	600	105	7.9	0.29	<LD	1.4	0.49	61.6	26.8

A means acidified sample; 3A means cartridge filtered sample; UA means unfiltered acidified sample.

Bangladesh was tested and results showed As^{III} and As^{V} are the major species with no organic As species being detected.

3.2.2. CE-UV

The optimized CE-UV method's performance was tested by spiking known concentration of As^{III}, As^{V} and DMA to mineral water. However, only 50% recovery was obtained. For this reason we investigated possible matrix effects arising from ions commonly found in water samples. Repeated investigation using ICP-MS revealed the presence of high concentration of Fe and S in the water samples. The ionic influence of these metals CE-UV speciation of As was evaluated using dilute ground water samples. Sample dilution by 20-fold improved spike recovery but this was found to limit speciation for samples low in As given CE's high detection limit.

3.2.3. Interference from Fe and S

In order to assess the effect of different inorganic ions on As speciation and detection by CE, we speciated As in the presence of Fe and S. These studies showed that As^{V} recovery was strongly dependent on the concentration of Fe^{3+}. Increasing Fe^{3+} led to a significant decline in the recovery of As^{V}. However, this decline was also dependent on the nature of the ligand ion accompanying Fe^{3+} which influences redox state of the aqueous phase. For instance, Fe^{3+}nitrate had a greater impact on As^{V} than Fe^{3+}sulfate. Increasing Fe^{3+}nitrate from 0 to 1.38 mg Fe^{3+}/l led to 15% decrease in recovery while at a concentration of 8.29 mg Fe^{3+}/l there was no recovery of As^{V}. Arsenate can co-precipitate with ferric hydroxides that can remove more than 90% of As^{V} from solution within 10 min [22]. The recovery of As^{V} decreased linearly ($r^2 = 0.98^{***}$) with ferrous (Fe^{2+}) concentration but was not as effective as Fe^{3+} ions. The recovery of As^{V} was quantitative when the concentration of Fe^{2+} was less than 6 mg Fe^{2+}/l, since at the higher pH, most of the Fe^{2+} remained in solution. The recovery of As^{V} initially decreased linearly with sulfate but it reached steady state after 44 mg SO_4^{2-}/l. This suggested that sulfate had little effect on As^{V} recovery but it depends on adsorbing sites and ligand ions.

Fe^{3+} had a marked impact on the recovery of As^{III}. Detailed work shows that there is a critical Fe^{3+} concentration above which the recovery of As^{III} dropped linearly ($r^2 = 0.97^{***}$). However, the effect of Fe^{+2} was logarithmic, i.e. recovery dropped by a factor of 10 with each unit increase in and then reached steady state. This suggests that there is a threshold Fe^{2+} concentration above which increasing Fe^{+2} has no effect on the recovery of As^{III} and this may be due to its transformation to Fe^{3+}. The latter may be possible due to the presence of oxidizing agents.

3.2.4. LC-ICP-MS

The analytical merit of optimized LC-ICP-MS method [18,20] was validated by testing spike recovery and investigating possible instrumental and matrix effects. The major interference for ^{75}As detection by ICP-MS is the polyatomic species $^{40}Ar^{35}Cl^{+}$ if the samples contain a high amount of chloride (Cl). Most of the drinking and ground water possess high concentration of Cl. Iron is also one of the major constituent of Bangladesh ground water sample under study (Table 6) and this can pose potential interference through adsorption or precipitation. Acidity of the sample needs consideration given that 0.5% HNO_3 was recommended for better stability of As species [23] which can create chromatographic problem.

3.2.5. Interferences of chloride (Cl)

To investigate $^{40}Ar^{35}Cl^{+}$ interference, the $^{35}Cl^{+}$ signal was monitored in addition to the $^{75}As^{+}$ signal during each run to check polyatomic interference formation. The result shows that there is no Cl interference when all of the As species were chromatographically resolved (Fig. 4). The most vulnerable As species which can be co-eluted with Cl was As^{V} but their expanded retention time (>1.31 min) suggested almost no effect. However, when the concentration of Cl was increased to 500 mg/l in a sample containing 50 μg/l As standard, there was significant tailing effect with no separation of Cl and As^{V} peak (Fig. 5). This suggests that water sample containing high level of Cl is likely to interference with As^{V} if Agilent column and mobile phase are used. Chloride concentration exceeding

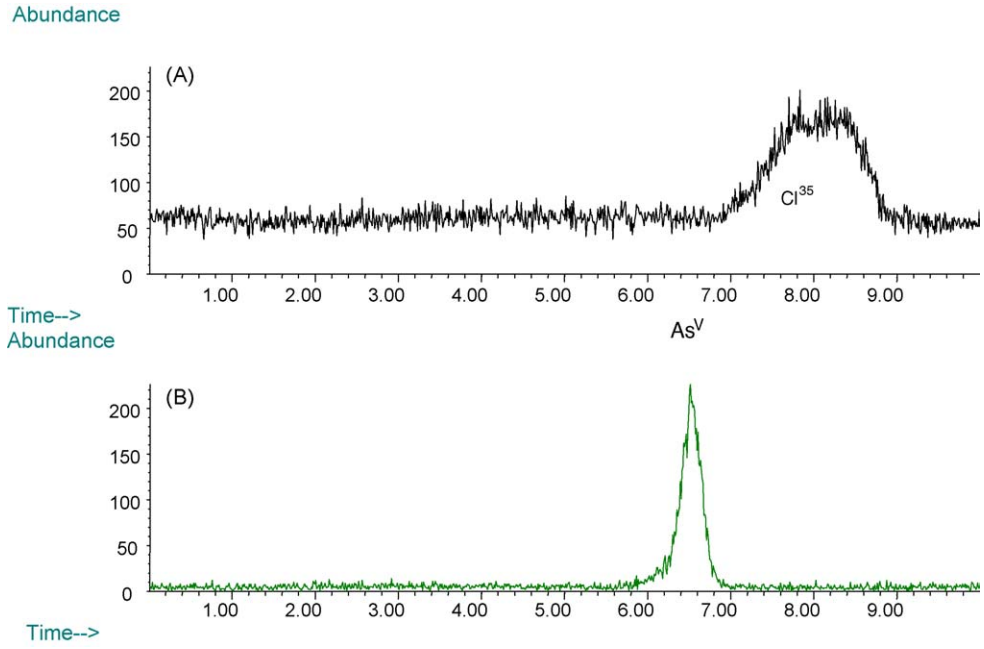

Fig. 4. Chromatogram taken from the same injection of a water sample A $m/z = 35$-signal monitoring Cl^{35}; B $m/z = 75$-monitoring ^{75}As signal.

500 mg/l has been reported in some soil solution and natural water samples of Australia [24].

3.2.6. Interferences of iron (Fe)

Iron (Fe) is an important co-existing element which is prevalent in most of ground water samples. Therefore, its effect on As speciation is crucial. Iron can interfere with As by adsorption or coprecipitation. Given the high concentration of Fe (often exceeding 20 mg/l), we investigated the effect of increasing Fe on the recovery of As. These investigations reveal that with the exception of As^V, all As species signal were successfully resolved. The effect of Fe on As^V was attributed to the presence

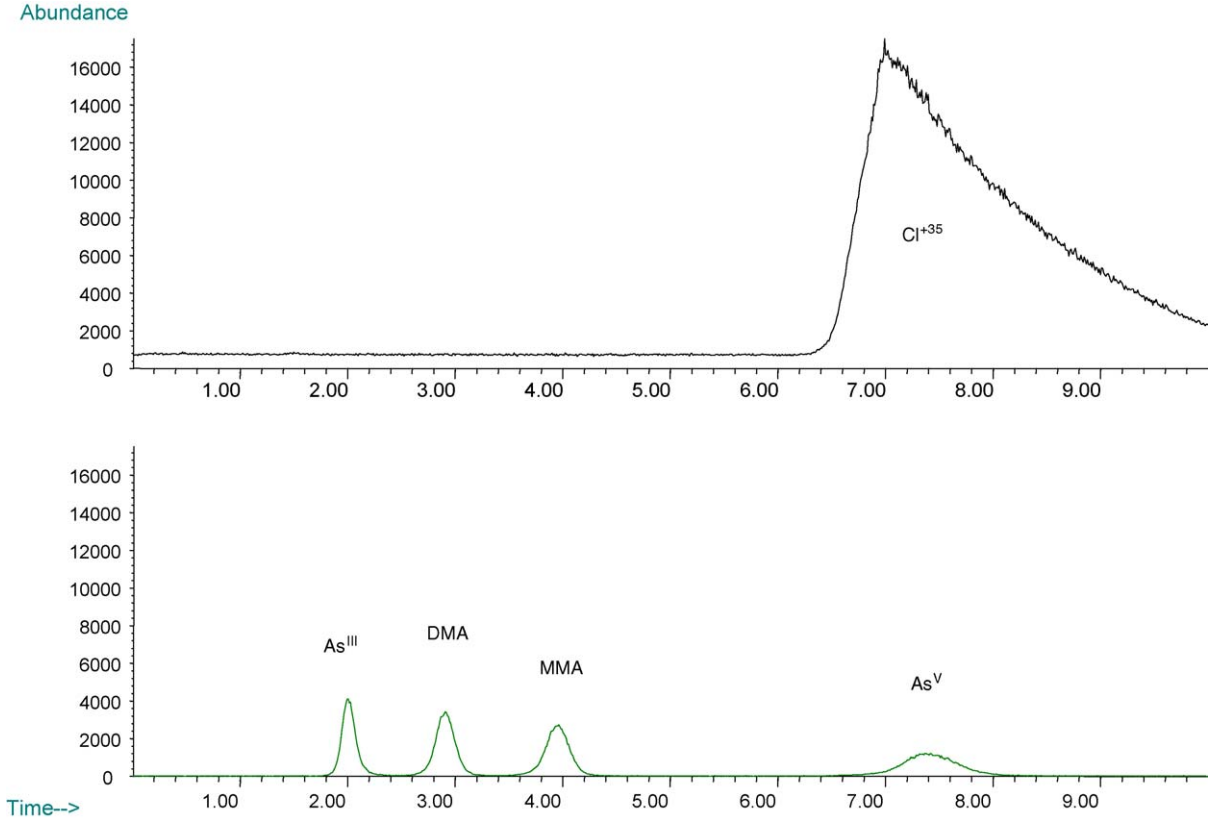

Fig. 5. Chromatogram showing overlapped Cl peak with As^V peak when spiked with 500 mg/l Cl in specific As standard.

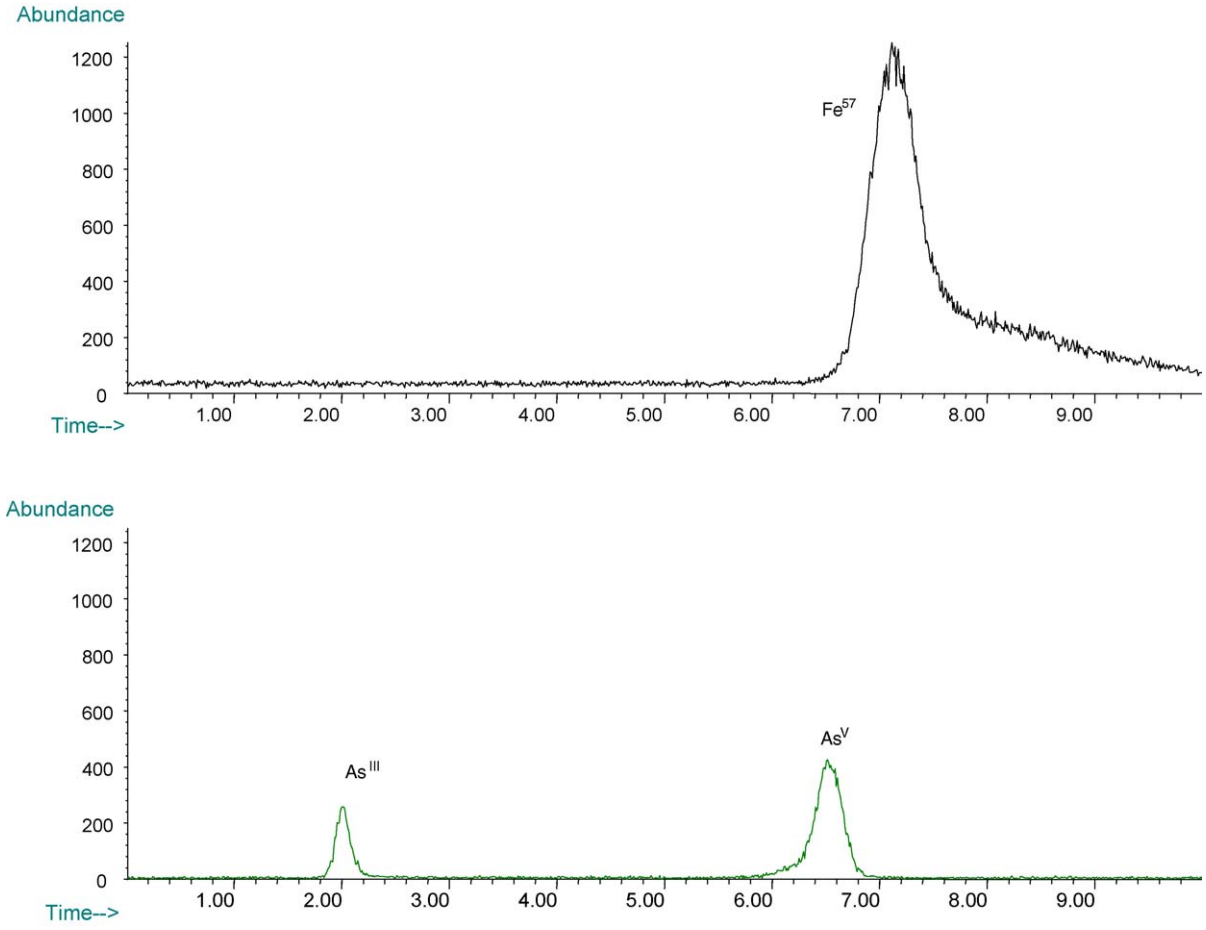

Fig. 6. Chromatogram showing Fe^{57} signal and As^{75} signal in the same tubewell water sample.

of Fe-EDTA complex that forms a broad tailing peak close to As^{V} (Fig. 6). However, single ion monitoring technique resolved this issue with little inference. Mobile phase without EDTA or different mobile phase might be a suitable solution for Fe interference.

3.2.7. *Interferences of acidified sample*

Acidified samples need special care before injecting to any anion or cation exchange column as acid can damage unique column characteristics which will shift retention time of species if we use weak mobile phase. To verify this issue, we studied selected acidified and non-acidified samples, and found negligible change of retention time (0.25 min) only in As^{V} which has highest mobility at our optimized pH. Acidified samples can be used if mobile phase has strong buffer capacity.

4. Application to ground water samples

The As species in ground water samples were evaluated following optimization of the three methods. Before speciation, samples mass balances (total As) were determined by ICP-MS and HG-AAS which showed a highly significant linear relationship ($r^2 = 0.996^{***}$) with each other.

These investigations revealed that 80–100% of As is present as As^{V} and the rest as As^{III} with no detectable level of DMA (Table 7). This contrasts strongly with investigations by Naidu et al [25] who found almost 80% of As in water samples of Bangladesh as As^{III}. However, their investigation was conducted on samples speciated with disposable cartridges. Thus, the presence of high concentration of As^{V} relative to As^{III} is not surprising given that the ground water samples had been transported from Bangladesh and stored at 4 °C for almost 4 months prior to analyses. During this period, it is likely that much original As^{III} has oxidized to As^{V}. There was a strong agreement between sums of different fractions of As and total As concentration in LC-ICP-MS and HG-AAS (Table 7). CE-UV was unable to detect any As species in these samples because of its high detection limit (500 μg/l for As^{V}).

5. Method comparison and concluding remarks

There is a significant difference in the ability of the three methods to measure As species. The CE method showed poor sensitivity in general to As in water samples while HG-AAS and LC-ICP-MS showed excellent sensitivity which can be expressed as CE < ICP-MS ∼HG-AAS (Table 7). In the presence of high concentration of As in standard solutions, resolution of different As species was high in CE-UV and LC-ICP-MS with CE-UV giving resolution of As species for less than 5 min compared to 10 min for the LC-ICP-MS. HG-AAS separates only

Table 7
Arsenic speciation in ground water samples (μg/l)

Sample ID	Mode	As^{III}	DMA	As^{V}	Sum of species	Total As
W1	I	5.0	<LD	62.0	67.0	–
	II	4.5	<LD	62.1	66.6	–
	III	<LD	<LD	<LD	–	–
	IV	–	–	–	–	81.2
W2	I	15.6	<LD	65.9	81.5	–
	II	15.6	<LD	65.1	80.7	–
	III	<LD	<LD	<LD	–	
	IV	–	–	–	–	80.0
W4	I	38.7	<LD	195.5	234.2	–
	II	38.2	<LD	201.2	239.4	–
	III	<LD	<LD	<LQ	–	–
	IV	–	–	–	–	286.7
W6	I	<LD	<LD	156.0	156.0	–
	II	<LD	<LD	155.5	155.5	–
	III	<LD	<LD	<LQ	–	–
	IV	–	–	–	–	160.0
W7	I	18.6	<LD	211.7	230.3	–
	II	18.5	<LD	211.6	230.1	–
	III	<LD	<LD	<LQ	–	–
	IV	–	–	–	–	212.0
W8	I	<LD	<LD	212.0	212.0	–
	II	<LD	<LD	211.0	211.0	–
	III	<LD	<LD	<LQ	–	–
	IV	–	–	–	–	210.0
W9	I	28.4	<LD	109.0	137.4	–
	II	27.9	<LD	108.4	136.3	–
	III	<LD	<LD	<LD	–	–
	IV	–	–	–	–	133.0
W10	I	38.5	<LD	109.0	147.5	–
	II	38.1	<LD	110.5	148.6	–
	III	<LD	<LD	<LD	–	–
	IV	–	–	–	–	163.0
W11	I	19.0	<LD	120.0	139.0	–
	II	18.6	<LD	117.2	135.8	–
	III	<LD	<LD	<LD	–	–
	IV	–	–	–	–	142.0
W12	I	36.8	<LD	100.7	137.5	–
	II	35.5	<LD	105.3	140.8	–
	III	<LD	<LD	<LD	–	–
	IV	–	–	–	–	138.0
W13	I	<LD	<LD	146.4	146.4	–
	II	<LD	<LD	137.1	137.1	–
	III	<LD	<LD	<LD	–	–
	IV	–	–	–	–	137.0
W14	I	<LD	<LD	155.7	155.7	–
	II	<LD	<LD	142.1	142.1	–
	III	<LD	<LD	<LD	–	–
	IV	–	–	–	–	142.0
W15	I	<LD	<LD	147.1	147.1	–
	II	<LD	<LD	135.1	135.1	–
	III	<LD	<LD	<LD	–	–
	IV	–	–	–	–	129.0

<LD, not detected; (–), not determined; <LQ, not quantified (defined as 10 × LD); I = LC-ICP-MS; II = HG-AAS; III = CE-UV; IV=ICP-MS.

Table 8
Comparison of the three methods

Analytical parameter	HG-AAS	CZE-UV	LC-ICP-MS
Separation capacity	Separate single species	Separate three species (As^{III}, DMA, As^{V})	Separate four species (As^{III}, DMA, MMA, As^{V})
Linearity range	0.13–40 μg/l	0.1–4 mg/l	2–100 μg/l
Method detection limit	0.19 μg/l (DMA); 0.1 μg/l (As^{III}, AsT)	100 μg/l (As^{III}, DMA); 500 μg/l (As^{V})	0.2 μg/l (As^{III}, As^{V}); 0.1 μg/l (DMA, MMA)
Interference	Free of inference	Fe and S interfere	500 mg/l Cl interfere with Agilent column
Sample preparation	Require complex sample preparation	No sample preparation except filtration	No sample preparation except filtration
Instrument availability	Easily available	Available	Rare
Running cost	Low	Low	High

single species at a time. Three or four analytical runs under different operating conditions are required for the speciation of toxic As species by HG-AAS which in turn increases the running time of the method. But the running cost and the instrument buying cost is much cheaper for HG-AAS and CE-UV, whereas LC-ICP-MS is highly expensive and requires significant investment and functioning cost. LC-ICP-MS can provide wide linear range while HG-AAS has limited linearity can hardly go up to 50 μg/l, and therefore recommended for low-level analytes. The matrix effects are more pronounced in CE while HG-AAS and LC-ICP-MS are largely free of interference. The most severe drawback observed in CE-UV because of its poor detection capability. This difficulty can be minimized with the addition of an appropriate detection system such as conductivity and amperometric detection [26,27].

The comparative study of the three methods showed that each method has its own merits and limitations (Table 8). The method of choice will depend on instrument availability, sensitivity and running cost. HG-AAS is an attractive method of As speciation because of its sensitivity and low costs but suffers with single species determination and laborious sample preparation. CE-UV is a simple, new and cheap technique but limited by its poor sensitivity and matrix effects. LC-ICP-MS is a real hope for As speciation at this moment despite its high running cost and restricted equipment. It was proved as a routine technique for speciation of As in drinking water. Further research is needed to develop method for other complex environmental matrices.

Acknowledgements

This work was supported by Australian Centre for International Agricultural Research (ACIAR) to Kazi Farzana Akter as a Ph.D. fellowship. We like to thanks CSIRO, Adelaide lab and CERAR, University of South Australia for instrumental support.

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