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Speciation Analysis AOC

Speciation of arsenic in tube-well water samples collected from West Bengal, India, by high-performance liquid chromatography–inductively coupled plasma mass spectrometry†

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The objective of this study was to report on the arsenic species present in tube-well water samples collected from West Bengal, India, especially dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA), whose existence has not been reported in the literature. The water samples were collected from Jalangi Gram Panchayet (Murshidabad district, West Bengal, India). The samples were speciated for arsenic 11 days after collection. The samples were collected in duplicate. One part was acidified with nitric acid (final concentration 0.1%), whereas the other part was left unacidified. A quick and highly sensitive high-performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC–ICPMS) technique was employed for the separation and detection of the arsenic species. Four arsenic species, namely arsenite [arsenic(III)], DMA, MMA and arsenate [arsenic(V)] were separated and analysed in less than 5 min. Total arsenic concentration was determined by flow injection (FI)-ICPMS. Most of the samples were found to contain low concentrations of DMA and MMA (<2.1 ppb) and high concentrations of inorganic arsenic (>300 ppb). The existence of DMA and MMA in both acidified and unacidified water samples and in similar concentrations suggests that their presence is natural and not due to acidification. The detection limit of the four arsenic species was 0.06–0.10 ppb. The method was validated by spike recovery and analysis of two water standard reference materials (SRMs). The percentage recoveries of added spikes of all four species were 97–112%. The total arsenic concentration obtained by FI-ICPMS and the sum of the four arsenic species obtained by HPLC–ICPMS for the two water SRMs agreed with the certified values. Moreover, the difference between the total arsenic and the sum of the four arsenic species for most of the water samples was less than 10%. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: arsenic; speciation; tube-well water; HPLC–ICPMS; West Bengal; India; arsenite; dimethylarsinic acid (DMA); monomethylarsonic acid (MMA); arsenate

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INTRODUCTION

Arsenic poisoning in West Bengal, India, and Bangladesh is now one of the biggest pollution calamities in the world. Drinking water is naturally contaminated by arsenic in most of the districts of West Bengal and Bangladesh. Arsenic concentrations in tube-well water in the affected districts

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Table 1. HPLC–ICPMS operating conditions

HPLC	
Column	Reversed-phase C18 column (ODS-3, 150 mm × 4.6 mm, 3 μm particle size)
Temperature (°C)	50
Mobile phase	5 mM TBAH + 3 mM malonic acid + 5% methanol
Flow rate (ml min^{-1})	1.5
Injected volume (μl)	50
ICPMS	
Masses	35 (Cl), 75 (As), 77 (ArCl)
Integration time (s)	0.2
Sampling period (s)	0.31
RF power (w)	1400
Sample depth (mm)	7.0
SC temperature (°C)	2
Plasma argon flow rate (l min^{-1})	1

greatly exceed the World Health Organization (WHO) guidelines for drinking water (10 ppb). Percentages of water samples that contained arsenic in the ranges of 100–299 ppb, 300–499 ppb, 500–699 ppb and 700–1000 ppb in nine districts of West Bengal were 14%, 3%, 0.7% and 0.2% respectively.[1,2] The mean arsenic concentration in groundwater of three highly arsenic-affected districts in Bangladesh is 1955 ppb for Rajarampur, 996 ppb for Shamta and 253 ppb for Chaar Ruppur.[3] More than 300 000 people, including children, are suffering from arsenical skin lesions in West Bengal alone.[1,2,4] Cases of melanosis, keratosis, hyperkeratosis, cancer, gangrene, and early death cases related to arsenic have been reported in West Bengal and Bangladesh.[1,2]

Although the causes of arsenic contamination of tube-well water in West Bengal and Bangladesh could be geological,[2,3] exact explanation as to how this is happening is still lacking. However, there are conflicting reports on whether the release of arsenic is due to the oxidation of arsenic-bearing pyrite minerals,[2] due to the reductive dissolution of arsenic-rich iron oxyhydroxides[3] or due to both.

The species of arsenic that are commonly found in groundwater are arsenite [arsenic(III)], arsenate [arsenic(V)], monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA).[5,6] The former two species (inorganic) are much more toxic than the latter two (organic). Only arsenic(III) and arsenic(V) were reported to be present in tube-well water collected from the affected areas in West Bengal and Bangladesh; no MMA or DMA were reported.[1,7,8]

In the present study, tube-well water samples were collected from West Bengal, India, and analysed for arsenic species [arsenic(III), DMA, MMA and arsenic(V)] by high-performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC–ICPMS) and for total arsenic by flow injection (FI)-ICPMS.

EXPERIMENTAL

Equipment

Arsenic speciation was carried out using an HPLC–ICPMS system, whereas FI-ICPMS was used for total arsenic analysis. A Shimadzu (Kyoto, Japan) HPLC system consisting of an SCL-6A system controller, an LC-6A solvent delivery module, a CTO-6A column oven equipped with a mobile phase pre-heater and a GL Sciences (Tokyo, Japan) degasser (546B), and a Rheodyne (California, USA) 7125 six-port injection valve with 50 μl injection loop were used. A reversed-phase C18 column (Intersil ODS-3, 150 mm × 4.6 mm, 3 μm particle size, GL Sciences Inc., Tokyo, Japan) was used for arsenic species separation. A shield torch ICPMS system (HP 4500 series, Yokogawa Analytical Systems Inc., Tokyo, Japan) was used as a detector. The ICP mass spectrometer was equipped with an ASX500 autosampler (CETAC Technologies Inc., Nebraska, USA) and an integrated sample introduction system (ISIS, Yokogawa Analytical Systems Inc., Tokyo, Japan), which has two peristaltic pumps operated at a flow rate of 0.1 rps. The HPLC and ICPMS instruments were interfaced by a 50 cm PTTE tube (0.25 mm i.d.). Signals of masses *m/z* 35 (Cl), 75 (As) and 77 (ArCl) were monitored for arsenic analysis. Details of experimental parameters are summarized in Table 1. The pH values were measured using a 520A model pH meter (Orion Research Incorporated, Boston, MA, USA). The pH meter was calibrated before use (ranges: 1–4 and 4–7).

Reagents and solutions

All chemicals were of analytical-reagent grade unless stated otherwise. All glassware was soaked in 10% (v/v) nitric acid for a minimum of 12 h, washed with distilled water and finally rinsed with Milli-Q reagent water before use. All water used was obtained from a Milli-Q SP Reagent Water System (Yamato Millipore, Japan), and had a resistivity of

Table 2. Arsenic concentrations (ppb) and pH values of acidified tube-well water samples (values in parentheses are for unacidified samples; ND means not detected; $\sum$ means sum of the four species measured by HPLC–ICPMS; total arsenic was measured by FI-ICPMS)

Sample no.	pH	As(III)	DMA	MMA	As(V)	Σ	Total As
1	1.5 (6.7)	93.5 (4.5)	0.7 (0.3)	0.5 (0.7)	32.0 (88.1)	125.9 (93.3)	122.1
2	1.6 (6.8)	19.1 (14.8)	0.1 (0.2)	0.1 (0.3)	9.9 (9.3)	29.1 (25.5)	30.1
3	1.5 (6.7)	257.2 (54.0)	ND (0.1)	ND (0.1)	76.2 (272.3)	333.4 (326.5)	323.6
4	1.6 (6.7)	6.8 (8.8)	0.2 (ND)	0.2 (ND)	7.7 (6.9)	15.4 (15.7)	13.1
5[a]	1.3 (6.7)	501.7 (4.7)	0.1 (0.2)	0.1 (ND)	24.5 (57.7)	526.3 (62.4)	540.2
6[a]	1.4 (7.0)	418.4 (0.8)	ND (0.2)	0.1 (0.2)	184.5 (141.9)	602.9 (143.1)	546.5
7	1.4 (6.9)	23.6 (28.1)	ND (ND)	ND (ND)	9.9 (1.7)	33.5 (32.3)	33.1
8	1.3 (7.1)	461.9 (5.4)	ND (0.3)	ND (ND)	183.9 (285.6)	645.8 (291.3)	618.3
9	1.4 (6.9)	317.5 (1.1)	ND (ND)	2.1 (0.9)	184.5 (354.2)	504.1 (355.3)	469.7
JAC 0031[b]	~1	0.08	ND	ND	0.33	0.41	0.26
JAC 0032[b]	~1	0.19	0.15	0.27	4.91	5.5	5.8

[a] Brown precipitate was clearly seen at the bottom of the sample tube of the unacidified samples.
[b] The certified total arsenic for JAC 0031 is 0.28 ± 0.04 ppb and for JAC 0032 is 5.5 ± 0.3 ppb.

18.3 M Ω cm. Tetrabutylammonium hydroxide (TBAH, 0.5 M, HPLC grade), malonic acid (98%, 104.06 g mol^{-1}), methanol (99.7%, HPLC grade), sodium hydroxide (NaOH min. 96.0%), hydrochloric acid (HCl), nitric acid (for heavy metal analysis), arsenic(III) trioxide [arsenic(III), min. 98.0%], and DMA (min. 98.0%) were all obtained from Wako Pure Chemical (Osaka, Japan). Sodium arsenate dibasic heptahydrate [arsenic(V), min. 99.4%] was obtained from Sigma (St Louis, MO, USA), and MMA (98%) was obtained from Tori Chemicals Laboratory (Yamanashi, Japan). An atomic absorption spectrometry (AAS) arsenic standard solution (1013 ppm arsenic added as As_2O_3; Merck) was used for total arsenic analysis.

Stock arsenic species standard solutions (1000 ppm) were prepared by dissolving appropriate amounts of arsenic(V), DMA and MMA in water. Arsenic(III) was dissolved in a minimum volume of 4 M NaOH and neutralized with the same volume and concentration of HCl. Stock solutions were stored in glass containers in the dark and kept refrigerated at 4 °C. Working arsenic solutions were prepared daily as required by appropriate dilution of the stock solutions with Milli-Q water. For total arsenic analysis, the 1000 ppm stock solution was diluted as appropriate with 0.05 M nitric acid.

Two water standard reference materials (SRMs) (JAC 0031 and 0032) were purchased from the Japan Society for Analytical Chemistry (JAC). The SRMs were preserved with nitric acid (final concentration was 0.1 M, and the pH was approximately 1). The first SRM was natural river water (certified arsenic concentration 0.28 ± 0.04 ppb), whereas the second SRM was prepared by adding arsenic to the first SRM (certified arsenic concentration 5.5 ± 0.3 ppb). Both SRMs were certified only for total arsenic concentration, and no reports on speciation analysis were found in the literature.

Sample collection

Nine water samples were collected from Jalangi Gram Panchayet (GP) (Jalangi Block, Murshidabad district, West Bengal, India). Until now, no literature reference to arsenic analysis of water samples from GP was found (for more details, see the most recent report on the arsenic crisis in Murshidabad[9]). The samples were collected directly from tube-wells in duplicate and placed in 10 ml polypropylene plastic tubes. Tubes were first rinsed several times with the well-water and then completely filled with the same water and tightly closed to minimize any contact with air. The samples were then split into two groups. Samples in the first group were acidified with nitric acid (final concentration 0.1% v/v), whereas samples in the second group were left unacidified. The samples were air-shipped to Japan and arrived at the laboratory 48 h after collection. The samples were stored refrigerated at 4 °C until the time of analysis.

pH values

The pH measurements were done upon sample arrival at the laboratory (48 h after collection). The pH values of the acidified and unacidified samples ranged from 1.3 to 1.6 and 6.7 to 7.1 respectively (see Table 2).

Analytical procedure

For HPLC analysis, all calibration solutions, Milli-Q water and the mobile phase were filtered through 0.22 μm membrane filters before analysis. Acidified water samples were filtered through 0.45 μm membrane filters before analysis. No precipitate was observed in any of the acidified samples. On the other hand, a brown precipitate was clearly seen at the bottom of tubes of some unacidified samples (see Table 2). The samples were diluted 5 to 20 times with Milli-Q water; however, undiluted samples were also used when

necessary. The HPLC mobile phase was prepared by appropriate mixing of the required stock solutions to obtain a final solution containing 3 to 5 mM TBAH, 3 mM malonic acid and 2 to 5% (v/v) methanol. The pH of the mobile phase solution was approximately 5.6. The 0.05 M nitric acid used as a carrier solution for the total arsenic analysis was prepared by appropriate dilution of the concentrated solution with Milli-Q water. The mobile phase was delivered isocratically at a flow rate of 1.5 ml min^{-1}. For total arsenic analysis, the autosampler was interphaced with the ISIS system, which has a 100 µl injection loop and two peristaltic pumps operated at a flow rate of 0.1 rps. One of the pumps was used to deliver the arsenic standard or the sample solutions from the autosampler to the injection loop of the ISIS system, and the other pump was used to deliver the carrier solution. The injected solution, along with the carrier solution, was finally introduced to the ICP Mass Spectrometer.

Quantification was carried out using external calibration curves based on both peak area and peak height. No pronounced difference between the two methods were noticed; however, peak heights were used.

Calibration solutions ($n = 7$) were run in an increasing order of arsenic concentration. Water and acid blanks were run after switching from standards to samples and after each water sample to prevent contamination. Calibration curves, up to 100 ppb, for all four species were found to be linear ($R^2 > 0.995$) based on both peak area and peak height. Arsenic speciation was done 11 days after collection, whereas analysis of total arsenic was done after 2 months.

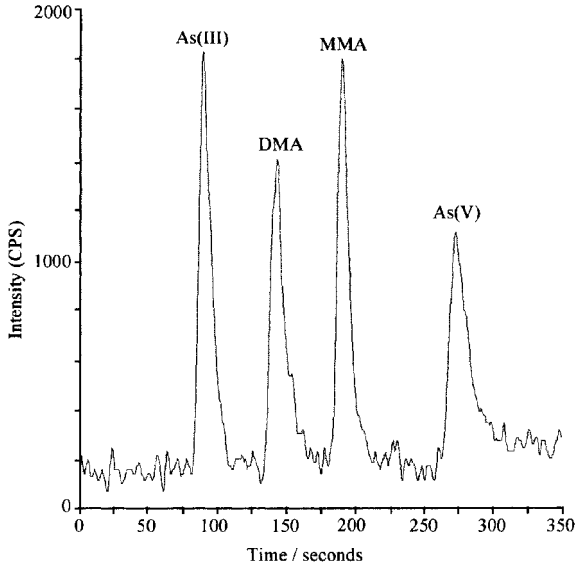

Figure 1. HPLC–ICPMS chromatogram of standard solution containing four arsenic species (1.0 ppb each). Experimental conditions: 5 mM TBAH + 3 mM malonic acid + 5% (v/v) methanol, flow rate 1.5 ml min^{-1}, temperature 50 °C, injected volume 50 µl. Reversed-phase C18 column (ODS-3, 150 mm × 4.6 mm, 3 µm particle size). Other experimental conditions are shown in Table 1.

Validation method

The method detection limit (DL) was calculated from three times the standard deviation of five readings of 50 µl injections of water blanks. The DLs for arsenic(III), DMA, MMA and arsenic(V) were 0.07 ppb, 0.10 ppb, 0.06 ppb and 0.09 ppb respectively, whereas the DL for total arsenic was 0.03 ppb. Recovery was calculated by spiking a standard solution containing a known concentration of arsenic species to the water samples. Accuracy was checked by analysis of the SRM. The method was also validated by comparing the sum of the four arsenic species concentrations obtained by HPLC–ICPMS with the total arsenic concentration obtained by FI-ICPMS.

RESULTS AND DISCUSSION

Optimization of the HPLC parameters

Concentrations of the mobile phase constituents, column temperature and mobile phase flow rate were optimized to achieve the best separation between the four arsenic species in the shortest practical possible time. The HPLC column used in the method of Le *et al.*,[10] where an HPLC–hydride generation (HG)-atomic fluorescence detection system was applied for the speciation of several arsenic species in urine samples, was used as a base for this investigation.

Effect of TBAH concentration

When a mobile phase containing 3 mM TBAH, 3 mM malonic acid and 2% (v/v) methanol at a flow rate of 1.5 ml min^{-1} and 50 °C was used, a partial peak overlap between arsenic(III) and DMA was observed. An increase in the TBAH concentration to 5 mM, under the above experimental conditions, eliminates this overlap and increases the difference in elution time of these two peaks from 10 s to more than 45 s. However, a decrease in the peak heights of 21%, 33% and 11% for arsenic(III), DMA and arsenic(V) respectively, and a 25% increase in the MMA peak height was also observed. Peak widths of arsenic(III), DMA and arsenic(V) were also increased, whereas the peak width for MMA was decreased, but the peak areas of the four species were not affected. This increase in the TBAH concentration also affected the elution times of all species except arsenic(III); the elution time for DMA was increased from 97 to 145 s, it decreased from 284 to 195 s for MMA, and it decreased from 387 to 319 s for arsenic(V).

Effect of methanol concentration

An increase in methanol concentration from 2 to 5% (v/v) (5 mM TBAH, 3 mM malonic acid, 1.5 ml min^{-1} flow rate and 50 °C) results in a 40% decrease in both the peak areas and

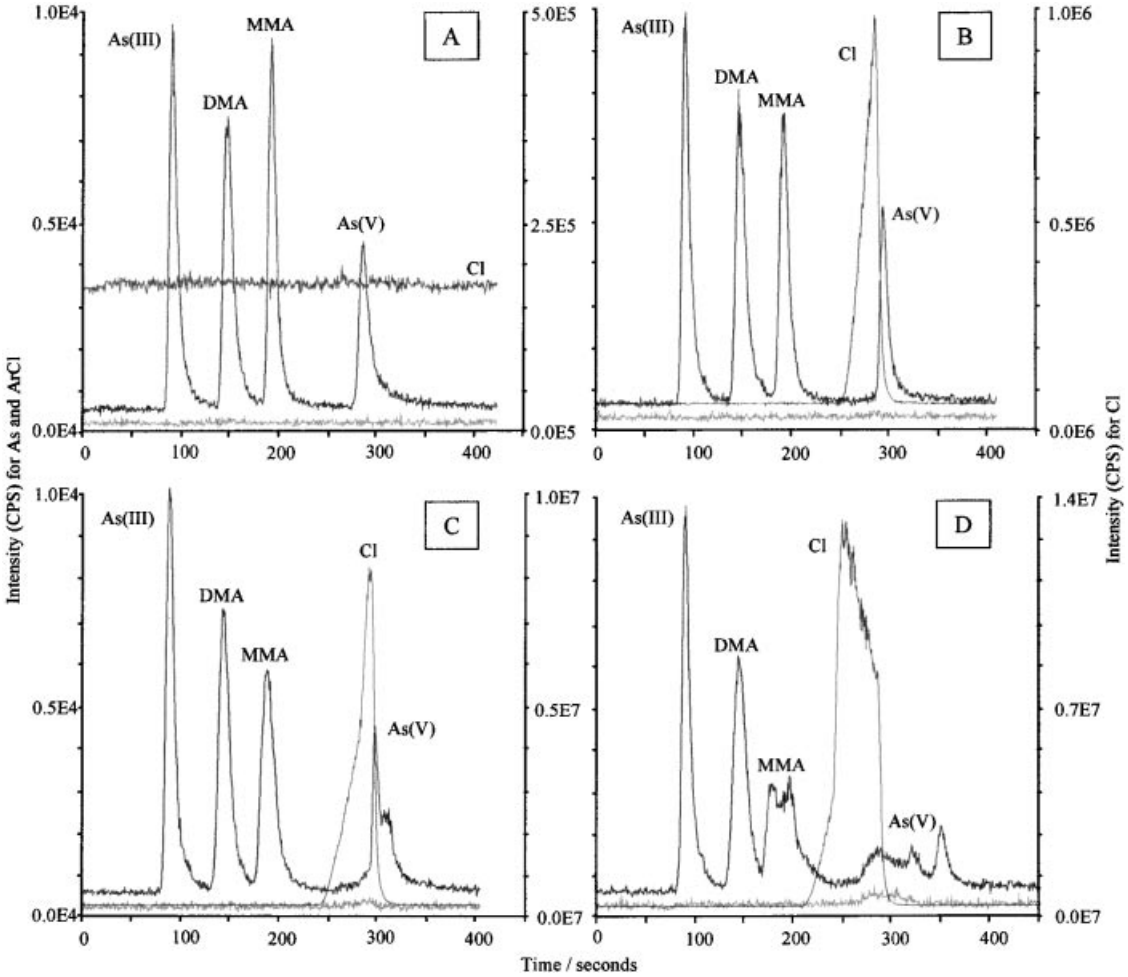

Figure 2. HPLC–ICPMS chromatograms of standard solution containing four arsenic species (10.0 ppb each): (A) 0 ppm NaCl; (B) 1000 ppm NaCl; (C) 3000 ppm NaCl; (D) 10000 ppm NaCl. The bottom trace, which has no clear peaks, represents the ^{77}ArCl response. The trace that has a very small peak in (A) and a very high signal just before 300 s in (B), (C) and (D) represents the ^{35}Cl response. The trace that has four distinctive peaks at 90, 144, 190 and 283 s represents the ^{75}As response. The left *X*-axis is identical in all chromatograms and represents the intensity of As and ArCl ions, whereas the right *X*-axis, which represents the intensity of chloride ion, is not identical and its range is indicated for each chromatogram. Experimental conditions are shown in Table 1.

heights. The peak widths were apparently not affected. There was also a decrease in the elution time for all the peaks except arsenic(III), which was not affected [40 s decrease for arsenic(V) and several seconds for the other two species]. Despite this effect on lowering the peak response, 5% (v/v) methanol was finally chosen because it provided a shorter analysis time.

Effect of flow rate

The effect of mobile phase flow rate (0.8–1.7 ml min^{-1}) was also studied. As expected, an increase in the flow rate decreases the elution time of each of the four arsenic species. The time difference between each two successive peaks also decreased. However, the effect was species dependent; e.g. an increase in the mobile phase from 1.0 to 1.5 ml min^{-1} resulted in 40 s, 65 s, 90 s and 160 s decreases in the retention times of arsenic(III), DMA, MMA and arsenic(V) respectively. However, resolution between peaks was good, and by using a flow rate of 1.5 ml min^{-1} a suitable chromatogram was obtained with a short analysis time.

Effect of column temperature

The column temperature was varied by adjusting the oven temperature, which is controlled by the temperature control unit. The mobile phase was also heated on-line to the same temperature as the column by the action of a mobile phase pre-heater installed just before the column oven. The pre-heater temperature was controlled by the temperature

control circuit of the column oven. The dead volume in the pre-heater is about 4 μl.

An increase in temperature, under the above-optimized conditions, results in a linear decrease in the peak response of the four arsenic species (R^2 = 0.880–0.997). An increase in temperature from 30 to 80 °C was accompanied by 70% and 30–40% decreases in both peak heights and areas for arsenic(V) and the other three species respectively. On the other hand, a change in temperature has little effect on peak widths. However, use of an HPLC column with small particle size (3 μm), such as the one used in this study, requires the application of temperatures higher than room temperature to reduce the column back-pressure and to allow the application of faster mobile-phase flow rates. As a compromise between the peak sensitivity and the flow rate, a column temperature of 50 °C was selected.

The separation chromatogram of the four arsenic species, under the optimized experimental conditions (Table 1), is shown in Fig. 1. The elution order of the four arsenic species is arsenic(III), DMA, MMA and arsenic(V) and their separation was complete in less than 5 min. This is consistent with the results reported by Le *et al.*[10]

Chloride interference

Under the optimized experimental conditions the chloride peak eluted just before the arsenic(V) peak and, therefore, it is important to make sure that this peak does not interfere with any of the arsenic peaks. Based on this, the effect of chloride concentrations of 0 to 10 000 ppm, added as NaCl to an arsenic standard solution containing 10 ppb of each of the four species, on the elution time and peak response of the four species was investigated; the results are shown in Fig. 2. Generally, no effect on either the peak shape or the elution time was observed until a chloride concentration of 3000 ppm was introduced, when a peak split and slight broadening only in the arsenic(V) peak was noticed in addition to the appearance of a small peak for ArCl just before 300 s (see Fig. 2c). However, 20% and 35% decreases in the peak height of MMA only were also observed when concentrations of 1000 ppm and 2000 ppm respectively were added (cf. Fig 2b and a). No effect on the peak area was noticed, which means that an increase in chloride concentration broadens the MMA peak. An increase in the chloride concentration to 5000 ppm results in a further peak broadening and split in the arsenic(V) peak, a slight broadening in the MMA peak and a slight increase in the ArCl peak. A further increase in chloride concentration to 10 000 ppm results in a spilt in the arsenic(V) peak to several broad peaks and further splitting and broadening in the MMA peak and an increase in the ArCl peak (Fig. 2d).

The chloride concentrations of all the samples (undiluted) analysed were found to be approximately 500 ppm. Figure 3 shows the chromatograms of chloride, arsenic and ArCl of sample no. 6, and it is clear from that the chloride peak is equivalent to approximately 500 ppm (cf. Fig. 2a) and it has

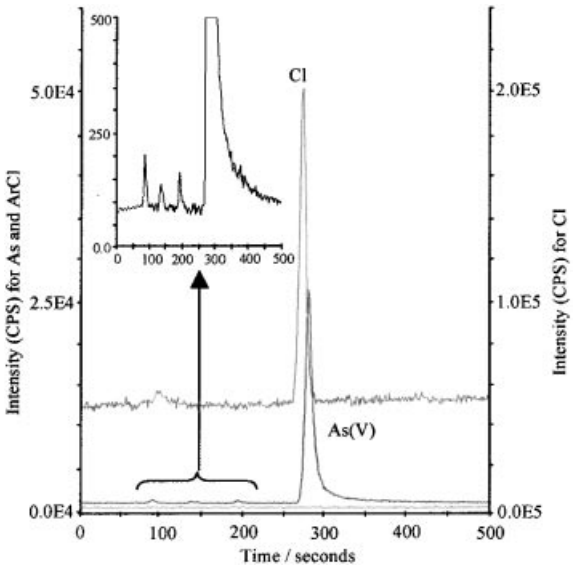

Figure 3. HPLC–ICPMS chromatogram of sample no. 6 (unacidified undiluted). The offset represents the ^{75}As response expanded to show the small peaks of arsenic(III) (first from left), DMA (second from left) and MMA (third from left), whereas the last large peak is for arsenic(V). Experimental conditions are shown in Table 1.

no effect on the arsenic peaks. Generally, all the samples were found to contain similar chloride concentrations, and sample no. 6 was selected to represent them. If the chloride concentration happens to be more than 3000 ppm in water samples, then two to three times sample-dilution will eliminate any chloride interference. Actually, a chloride concentration of 500 ppm is not likely to be present in groundwater samples as reported by Pantsar-Kallio and Manninen.[11] Therefore, it can be safely stated that, under our experimental conditions, no interference was observed from either chloride or ArCl ions on any of the arsenic peaks in any of the tube-well water samples analysed.

Recovery

Five tube-well water samples (three unacidified and two acidified) were spiked with 5 ppb of each of the four arsenic species and analysed by HPLC–ICPMS using the proposed method. Percentage recoveries for the arsenic species were found to be 112%, 112%, 108% and 97% for arsenic(III), DMA, MMA and arsenic(V) respectively.

Speciation of arsenic in water samples

Tube-well water samples

Our initial investigation (not published) on tube-well water samples collected from West Bengal showed that, in addition to the inorganic arsenic species [arsenic(III) and arsenic(V)], organic arsenicals (MMA and DMA) were detected, but in small quantities. The water samples in the initial investiga-

tion were analysed after being stored (for several months) acidified with nitric acid (0.1% v/v) and refrigerated at −20 °C. To check whether the existence of MMA and DMA in these samples was natural or due to storage and/or acidification, we collected more fresh water samples and analysed them 11 days after collection. Half of the freshly collected samples were acidified with nitric acid (0.1% v/v) and the other half were left unacidified. This method of acidification of tube-well water with nitric acid (0.1% v/v) was reported by several authors and shown to maintain the species distribution within 90 to 96% for approximately 3 weeks.[7,8]

Results of the acidified samples were considered for this discussion, a results of the unacidified samples were used for comparison purposes. The results of arsenic speciation are shown in Table 2. All the samples were found to contain total arsenic in concentrations greater than the WHO maximum permissible limit for arsenic in drinking water (10 ppb). Previous reports on tube-well water samples collected from West Bengal showed similar results to ours.[1,2,7–9]

As expected, the arsenic concentration in the acidified samples was greater than in the unacidified samples. The difference in arsenic concentration between the two types of sample is variable and is believed to be dependent on the composition of each sample, mainly the iron content. It was noticed that the difference was high when there was a brownish precipitate at the bottom of tubes of the unacidified samples (see sample nos 5 and 6 in Table 2), when arsenic species were believed to be co-precipitated or adsorbed on the iron hydroxide precipitate. On the other hand, this precipitation was not noticed in the acidified samples, due to lowering of the pH by acidification (compare the pH values for acidified and unacidified samples in Table 2). The phenomenon of precipitation of hydroxides of iron and other metals in natural water samples has been widely noticed and reported in the literature, and acid is usually added to prevent this phenomenon from occurring.[12,13]

It is clear from Table 2 that arsenic(III) concentrations in most of the unacidified samples are much less than those of the acidified samples, whereas arsenic(V) showed the opposite pattern. This pattern in arsenic(III) and arsenic(V) concentrations is more obvious in samples containing high arsenic concentrations. As an explanation for the difference in arsenic(III)/arsenic(V) concentrations between unacidified and acidified samples, arsenic(III) is reported to oxidize rapidly to arsenic(V) in unpreserved water samples.[12] The reverse process is also familiar, where under reducing conditions arsenic(V) is reduced to arsenic(III) by bacteria and marine phytoplankton.[6,13] This oxidation–reduction process is dependent on several factors, such as the arsenic concentration itself, chemical reactions between species, biological activity, light, temperature, pH and oxygen content.[12,13] Surprisingly, oxidation of arsenic(III) to arsenic(V) in the unacidified samples that contained low arsenic content was not obvious (see sample nos 2, 4 and 7 in Table 2), which seems to be in contradiction to what was reported by Ariza *et al.*[12] However, a mixed reduction–oxidation and oxidation–reduction phenomenon between arsenic(V) and arsenic(III) in water samples, especially at low concentrations, was noticed and thoroughly investigated by Hall *et al.*[13] In deionized water samples spiked with low concentrations of arsenic(III) and arsenic(V) (mixture and single species) and in river water samples, Hall *et al.*[13] noticed that arsenic(V) is rapidly reduced to arsenic(III) after 2 days. However, for natural water samples they reported that arsenic(III) and arsenic(V) concentrations were initially stable for 6 days. Then a decrease in the arsenic(III) concentration, accompanied by an increase in the arsenic(V) concentration, was observed. After that the arsenic(V) concentration started to decline gradually to a certain value after 14 days. Based on this, our results at low arsenic concentrations may be explained by a mixed reduction/oxidation process as reported by Hall *et al.*[13]

Table 2 also shows that the inorganic arsenic content [sum of arsenic(III) and arsenic(V)] is more than 97% of the total arsenic. The predominance of inorganic arsenic species in Indian and Bangladeshi tube-well water samples was also reported by other researchers[1,7,8] where only inorganic arsenicals were detected. Similar findings were also reported for Taiwanese well-water samples.[6,14]

When only acidified samples were taken into consideration, arsenic(III) was identified as the major species (60–90%) in most of our samples (eight out of nine). The predominance of arsenic(III) species in West Bengal tube-well water was reported for some samples collected from South-24-Parganas district.[7] Moreover, arsenic(III) was reported to be the major species in well-water samples collected from arsenic-endemic areas in Taiwan, where high arsenic concentrations were reported [462 ppb and 177 ppb for arsenic(III) and arsenic(V) respectively].[6,14]

In agreement with our findings, analysis of tube-well water samples collected from the same block as ours, but from different GPs,[9] showed that there is a wide variation in the total arsenic concentration between samples collected from the same area. The depth of the tube-well was a major factor in this variation. The authors classified seven different ranges of arsenic concentration for each interval of depth (usually 25 m), and the variation was wide for each depth interval.

Small quantities (0.1–2.1 ppb) of organic arsenic species (DMA and MMA) were found in most of our samples (see Table 2 and Fig. 3). The existence of such species, and in similar concentrations in both acidified and unacidified samples, ruled out the responsibility of acidification for their presence. It is also unlikely that methylation of inorganic arsenic species to MMA and DMA occurs in such a short time and, therefore, the presence of such species seems to be natural. This is the first time that organic arsenic species have been reported to be present in West Bengal tube-well water samples. In all the literature on tube-well water samples

from West Bengal and Bangladesh, neither DMA nor MMA was detected.[1,7,8] We believe that the inability to detect such species was mainly due to the use of methods that are not sensitive enough to detect small quantities of these species. The authors of these previous reports used combined cation- and anion-exchange resin columns and FI-HG-AAS to separate and analyse the arsenic species. The reported detection limits for arsenic(III), arsenic(V), MMA and DMA were 0.2 ng, 0.41 ng, 0.23 ng and 0.27 ng respectively when samples of 50 µl were injected.[1,7,8] These detection limits are equivalent to 4.0 ppb, 8.2 ppb, 4.6 ppb and 5.4 ppb respectively, which are more than 70–80 times higher than our detection limits.

It is not uncommon for organic arsenicals such as DMA and MMA to be present in well-water samples. Lin *et al.*[5] reported the presence of such species in Taiwanese well-water samples. Actually, before the appearance of that report in 1998, Lin's co-workers published two reports in 1995 and 1997[6,14] on water samples from the same wells and the organic arsenicals MMA and DMA were not detected. Chen and co-workers[6,14] used an ion pairing chromatography–HG-AAS technique for the speciation of arsenic, where the reported detection limits for arsenic(III), DMA, MMA and arsenic(V) were 3 ppb, 7 ppb, 3 ppb and 12 ppb respectively; they[6,14] stated 'since the two methylated species cannot be detected on the chromatograms, it may be concluded that the concentration of MMA and DMA in the real water samples should be lower than in the detection limit'. However, organic arsenicals in concentrations of 0.5–6.9 ppb were reported by the same group when improvements in the detection limits were achieved.[5]

Water SRMs

The two water SRMs were analysed for species and total arsenic concentrations and the results are shown in Table 2. The certified total arsenic concentration for JAC 0031 is 0.28 ± 0.04 ppb and the value obtained was 0.26 ppb (the sum of the four arsenic species was 0.41 ppb); the certified value for JAC 0032 is 5.5 ± 0.3 ppb and the value obtained was 5.8 ppb (the sum was 5.5 ppb).

Total arsenic

Total arsenic concentrations for the tube-well and SRM water samples were analysed by FI-ICPMS (Table 2). The sum of all four arsenic species concentrations measured by HPLC–ICPMS agrees very well with the total arsenic concentrations measured by FI-ICPMS. The percentage difference between the two types of result is less than 10% for most of the samples. This agreement in results adds more to the validity and accuracy of the proposed method.

CONCLUSIONS

The tube-well water samples collected from West Bengal were found to contain high arsenic concentrations, of which the inorganic species were the predominant ones, and this poses a real threat to humans drinking this water. Low concentrations of organic arsenic species were also detected for the first time in tube-well water samples collected from West Bengal. Our method is very sensitive, with detection limits of less than 0.1 ppb. Chloride concentrations of less 3000 ppm will not create any interference with any of the arsenic species analysed. The method is fast, reliable and accurate, as indicated by analysis of total arsenic and analysis of SRM water samples.

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