APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2001; **15**: 306–314
DOI: 10.1002/aoc.142

Estimation of arsenobetaine in the NIES candidate certified reference material no. 18 human urine by HPLC–ICP-MS using different chromatographic conditions†

Amit Chatterjee,* Yasuyuki Shibata, Jun Yoshinaga and Masatoshi Morita
National Institute for Environmental Studies, Environmental Chemodynamics Section, Environmental Chemistry Division, 16-2 Onogawa, Tsukuba, Ibaraki-3050053, Japan

HPLC–ICP-MS, employing a silica-based LC-SCX cation-exchange column, styrene–divinylbenzene copolymer-based PRP-X100 anion exchange column, an ODS reversed-phase and gel-permeation (polyvinyl alcohol-based resin) GS-220 columns, has been used for the separation, identification, and quantification of arsenic compounds, particularly arsenobetaine (AB), present in NIES candidate certified reference material (CRM) no. 18 human urine. AB is the predominant arsenic species, followed by dimethylarsinic acid, methylarsonic acid and arsenic acid. The peak of each arsenic compound has been validated by spiking of the authentic standard solution to the urine sample and by using the above chromatographic systems. The high concentration of chloride that co-elutes with the arsenic acid from the LC-SCX and with the AB from the GS-220 columns has interfered with the ion signals of arsenic acid and AB, by forming the molecular ions $^{40}Ar^{35}Cl^{+}$ and $^{38}Ar^{37}Cl^{+}$ in the plasma. Thus, the concentration of AB has been carefully estimated on the GS-220 after extracting the chloride interference (^{37}Cl:^{35}Cl = 1:3.1271) by measuring the $^{40}Ar^{37}Cl^{+}$. The peak of AB overlapped with the peak of arsenous acid and hindered the estimation of AB on the ODS and PRP-X100 columns. But AB has been baseline separated from the other arsenic compounds and also from the chloride with 20 mM pyridine at pH 2.60 on the LC-SCX. So, the LC-SCX column has been proven and used for the determination of AB in NIES candidate CRM no. 18 human urine. The concentrations of AB, estimated by the standard addition method and found using the LC-SCX and GS-220 columns, are 70.5 ± 5.5 (n = 20) and 71.5 ± 4 μg l^{-1} (n = 9). The concentration of AB thus found has been applied as the baseline value for the collaborative study to certify the AB in the NIES candidate CRM no. 18 human urine.

Keywords: arsenobetaine; HPLC–ICP-MS; LC-SCX; Candidate Reference Material; urine; arsenic; arsenic compounds

Received 9 December 1999; accepted 3 October 2000

INTRODUCTION

Measurement of arsenic compounds in urine is important for clinical, occupational and environmental applications.[1] Most of the arsenic compounds that humans ingest come from food.[2] Ingested inorganic arsenic compounds are detoxified in the body by methylation[1] and excreted mostly through urine.[3] Urinary concentration of arsenic is increased by ingestion of seafood.[4,5] Exposure to inorganic toxic arsenic compounds has a greater risk compared with exposure to arsenobetaine (AB), which appears to be non-toxic.[6] However, the presence of AB does not influence the determination of arsenate (arsenic acid, arsenic(V)), arsenite (arsenous acid, arsenic(III)), methylarsonic acid (MA) and dimethylarsinic acid (DMA) in urine.[7] But arsenosugars, present in seaweed, are metabolized to DMA and other

* Correspondence to: Amit Chatterjee, National Institute for Environmental Studies, Environmental Chemodynamics Section, Environmental Chemistry Division, 16-2 Onogawa, Tsukuba, Ibaraki-3050053, Japan.
E-mail: amit@protect2.nire.go.jp
† Based on work presented at the Ninth Symposium of the Japanese Arsenic Scientists' Society (JASS-9), held 21–22 November 1999 at Hiroshima, Japan.

unknown arsenic compounds, and at least a part in the form of DMA is excreted through urine.[3,7] A realistic estimate of risk from ingested arsenic can be obtained only when the types and doses of the arsenic compounds that are ingested and subsequently excreted in the urine are known. As a result, speciation of arsenic compounds in urine is essential for biological monitoring of arsenic exposure.

Generally, arsenic speciation is performed by chromatographic separation followed by a suitable detection technique.[8–12] These conditions are met by using liquid chromatography, e.g. ion-exchange,[13] reversed-phase[14] and gel-permeation modes,[14] coupled to several detection systems.[14–20] Inductively coupled plasma mass spectrometry (ICP-MS),[14,19,20] inductively coupled plasma atomic emission spectrometry (ICP-AES),[13,21] hydride generation–atomic absorption spectrometry (HG–AAS),[15,16] and hydride generation–inductively coupled plasma mass spectrometry (HG–ICP-MS)[17] coupled to liquid chromatography, are generally used for arsenic speciation analysis. Moreover, HG–AAS is a sensitive technique for arsenic compound determination, but for the estimation of AB, arsenocholine bromide (AC) and trimethylarsonium iodide (TMI), a prior chemical conversion to hydride-generated arsenic compounds is essential.[3,22] High-performance liquid chromatography ICP-MS (HPLC–ICP-MS) is a widely recognized, reliable, sensitive, fast technique, and fulfils the ideal requirement of being an on-line real-time chromatographic detector for elemental speciation analysis.[23,24] It is highly sensitive for determination of arsenic compounds.[25,26] However, it suffers from spectroscopic and non-spectroscopic interferences.[23] The molecular ions, which are formed from the plasma gas (argon) and/or other matrix elements, complicate the sensitive analysis of elements from biological samples.[20,27] Moreover, in the case of arsenic (*m/z* 75) analysis, chloride within urine makes the polyatomic $ArCl^+$ ion ($^{38}Ar^{37}Cl^+$, $^{40}Ar^{35}Cl^+$ at *m/z* 75) in the plasma and interferes with the analysis of arsenic species co-eluted with chloride.

Development of certified reference materials (CRMs) for the speciation of arsenic is necessary in order to produce new speciation methods, as well as to provide quality assurance for the real analysis. In the present study, we report the determination of arsenic compounds, especially AB in NIES Candidate CRM no. 18 human urine by using anion-exchange, cation-exchange, reversed-phase and gel-permeation chromatographic systems in the presence of a high chloride matrix. Four chromatographic conditions have been used in order to confirm the identification of each arsenic compound. Finally, AB has been quantified using a standard addition technique to avoid non-spectroscopic interference.

EXPERIMENTAL

Reagents and solutions

The National Institute for Environmental Studies (NIES), Ibaraki, Japan, candidate freeze-dried human urine CRM no. 18 was used as a reference material from our institute. All solutions were prepared with Milli-Q (18.3 MΩ cm; Milli-QSP.TOC Reagent Water System, Nihon Millipore Ltd, Japan) water. The mobile phase for cation-exchange chromatography was prepared by dissolving 1.58 g pyridine (Merck, p.a.) in Milli-Q water and adjusting the pH of this solution to 2.6 by adding formic acid (~98%, Fluka puriss, p.a.) then made up to 1000 ml solution (20.0 mM) with Milli-Q water. The mobile phase for anion-exchange chromatography was prepared by dissolving 3.45 g $NH_4H_2PO_4$ (Merck, p.a.) to 1000 ml (30.0 mM) and adjusting the pH to 6.0 by addition of 2.0 M aqueous NH_3 solution (Merck, p.a.). The mobile phase for reversed-phase work was prepared by dissolving 14.7 g of tetraethylammonium hydroxide [(TEAH) Nacalai Tesque, Kyoto, Japan; 10% in water] in Milli-Q water/methanol (99.95:0.05, v/v). The pH was adjusted to 6.8 by adding 0.42 g of malonic acid (solid, Nacalai Tesque, Kyoto, Japan) and finally made up to 1000 ml solution by Milli-Q water (10 mM TEAH + 4 mM malonic acid). The mobile phase for the gel-permeation chromatography was prepared by dissolving 22.8 g of tetramethylammonium hydroxide [(TMAH) Nacalai Tesque, Kyoto, Japan G.R.; 10% in water] and 2.60 g malonic acid in Milli-Q water, and the pH adjusted to 6.8 by adding 2.0 M aqueous ammonia solution (Konto, Japan, p.a.). Finally, the solution was made up to 1000 ml solution by Milli-Q water (25 mM TMAH + 25 mM malonic acid). Standard solutions (1000 mg As l^{-1}) for the identification and quantification of arsenic compounds were prepared by dissolving 433.0 mg of $NaAsO_2$ (Merck, p.a.) [arsenic(III)], 1041 mg of $Na_2HAsO_4{\cdot}7H_2O$ (Merck, p.a.) [arsenic(V)], 460.5 mg of DMA, 466.6 mg of MA, 594.2 mg of AB, 817.5 mg of AC, 453.9 mg of trimethylarsine oxide (TMAO)

and 874.2 mg of TMI (Tri Chemical Laboratory Inc., Japan) to 250 ml of water. Mixture solutions of arsenic compounds (arsenous acid, arsenic acid, MA, DMA, AB, AC, TMAO and TMI) with concentrations 5.00, 10.0, 25.0, 50.0 or 100 μg As l^{-1} were prepared by appropriate dilution of the stock solutions with Milli-Q water. Calibration curves for the HPLC–ICP-MS measurements on the different (LC-SCX, PRP-X100, ODS and GS-220) columns were prepared by chromatographing aliquots (0.100 ml) of the above mixture solutions each containing 5.00, 10.0, 25.0, 50.0 or 100 μg As l^{-1} of arsenite, arsenate, MA, DMA, AB, AC, TMAO and TMI.

ICP-MS

For ICP-MS a PMS-2000 spectrometer (Yokogawa Electric, Tokyo, Japan) was used for this study. In Table 1 the operating conditions for ICP-MS are displayed. A Meinhard concentric-type nebulizer (PMS-2000, Yokogawa Electric Company, Japan; part number K9250YW) with 300 mm × 0.25 mm i.d. polyether ether ketone (PEEK) capillary tubing without a desolvation system was used for the sample introduction system. The exit of the HPLC column was connected directly to the nebulizer via the PEEK capillary tubing. More detailed information was described previously.[20,25]

Chromatography

The HPLC system consisted of a Perkin–Elmer Model Series 410 B10 solvent delivery unit (Perkin Elmer, Norwalk, CT 06856, USA) and a Rheodyne 9725 six-port injection valve (Rheodyne, Cotati, CA, USA) with a 100 μl injection loop. The separations were performed on a Supelcosil LC-SCX column (Supelco, Bellefonte, USA) cation-exchange (25 cm × 4.6 mm i.d. 5 μm silica-based particles with propylsulfonic acid exchange sites), connected with a guard cartridge (Supelcosil, 2 cm), an Asahipak GS-220 (Shodex, Asahi Kasei, Japan), gel-permeation column (7.6 mm i.d. × 50 cm 9 μm, polyvinyl alcohol-based resin), connected with a guard cartridge (GF IG, 7B, Shodex, Asahi Kasei, Japan), an Inertsil ODS (Gasukura Kogyo, Japan) reversed-phase column (4.6 mm i.d. × 250 mm, 5 μm ODS) and a PRP-X100 (Hamilton, Reno, NV, USA) anion-exchange (12.5 cm × 4.1 mm i.d.; spherical 10 μm particles of a styrene–divinylbenzene copolymer with trimethylammonium exchange-sites; stable between pH 1 and 13; exchange capacity 0.19 meq g^{-1}) column is connected with a guard cartridge (Hamilton, Reno, NV, USA). The guard cartridges were used to protect the analytical columns. The columns were equilibrated by passing at least 100 ml (flow rate 1.0 ml min^{-1}) of the mobile phase through the columns before injection of the arsenic compounds. The exit of the column was connected directly to the nebulizer with PEEK capillary tubing. The ion signals at *m/z* 35 (^{35}Cl), 51 ($^{35}Cl^{16}O$), 53 ($^{35}Cl^{18}O$), 75 (^{75}As), 77 ($^{40}Ar^{37}Cl$) and 82 (^{82}Se) were recorded with the time-resolved analysis software© version of Yokogawa, Japan. The chromatograms were exported, and peak areas and peak heights were determined. The concentrations were calculated with external calibration curves and with standard addition technique[20,28] considering peak areas. The details of chromatographic conditions used for the separation of arsenic compounds are displayed in Table 1.

Urine preparation

Freeze-dried human urine (NIES candidate CRM no. 18) served as the test material for the study. The tentative consensus value for the total arsenic is 134 μg l^{-1} when dissolved in 9.57 ml water. A detailed description about the preparation of the candidate CRM was described previously.[29] 9.57 ml of Milli-Q water was added to the bottle for the reconstitution. The bottle was swirled gently to dissolve the material completely. The reconstituted urine thus formed, was stored at 4 °C prior to analysis.

Standard addition method (SAM)

2.00 ml of urine was taken in each of five graduated (5.00 ml) conical flasks. 0.0, 0.14, 0.22, 0.31, and 0.41 ml of 1000 μg l^{-1} of AB was spiked to each of the flasks sequentially. Finally, every flask was diluted to 3.00 ml with Milli-Q water (final concentrations of AB were 0.00, 46.7, 73.3, 103, and 137 μg l^{-1}). Calibration curves for the HPLC–ICP-MS measurements on the different (LC-SCX, ODS and GS-220) columns were prepared by chromatographing the aliquots (0.100 ml) of the above spiked solutions. The concentration of AB in the urine was calculated after multiplying the volume correction. More details of the SAM are described elsewhere.[28]

Table 1 Operating conditions for the HPLC–ICP-MS system

ICP-MS	
Plasma	
rf power	Forward, 1.2 kW Reflected, <10 W
Argon gas flow	
plasma gas	14 l min^{-1}
nebulizer gas	0.8 l min^{-1}
auxiliary gas	1.0 l min^{-1}
Ion Sampling	
sample cone	Copper orifice, 1.00 mm diameter
skimmer cone	Copper orifice, 0.50 mm diameter
Measuring parameters	
monitored signal	*m/z* 35 ^{35}Cl; *m/z* 51 $^{16}O^{35}Cl$; *m/z* 53 $^{18}O^{37}Cl$; *m/z* 75 ^{75} As/^{40} $Ar^{35}Cl/^{38}$ $Ar^{37}Cl$; *m/z* 77 ^{40} $Ar^{37}Cl/^{77}Se$; *m/z* 82 ^{82}Se
total analysis time	400–1200 s
HPLC	
Columns:	
Inertsil ODS reversed-phase (**LC-1**)	Mobile phase: 10 mM $(C_2H_5)_4NOH$ + 4 mM malonic acid in 0.05% CH_3OH, pH 6.8; flowrate 0.75 ml min^{-1}
Asahipak GS-220 gel-permeation (**LC-2**)	Mobile phase: 25 mM $(CH_3)_4NOH$ + 25 mM malonic acid, pH 6.8; flow rate 1.0 ml min^{-1}
Supelcosil LC-SCX cation-exchange (**LC-3**)	Mobile phase: 20 mM pyridine, pH 2.6; flow rate 1.5 ml min^{-1}
Hamilton PRP-X100 anion-exchange (**LC-4**)	Mobile phase: 30 mM $NH_4H_2PO_4$, pH 6.0; flow rate 1.5 ml min^{-1}

RESULTS AND DISCUSSION

Chromatographic separation of arsenic compounds in NIES candidate CRM no. 18 human urine

Silica-based cation-exchange (LC-SCX), styrene–divinylbenzene copolymer-based PRP-X100 anion exchange, ODS reversed-phase and the polyvinyl alcohol-based resin gel-permeation GS-220 columns were used for the separation and identification of arsenic compounds, especially arsenobetaine (AB), present in NIES candidate CRM no. 18 human urine (Figs 1 and 2). Our approach was to separate, identify and revalidate each arsenic compound individually without any overlapping from the rest of the arsenic species, which are present in the NIES candidate CRM no. 18 human urine, for the certification study. Hence we have used different chromatographic systems (Table 1).

Of the many methods that have been applied to the chromatographic separation of arsenic compounds the most common one is ion-exchange chromatography.[20,26,27,30] The polarity of the organic and inorganic arsenic compounds makes them amenable to ion-exchange HPLC. Many arsenic compounds are acids with negative and positive charges on the species (Table 2); thus, their interaction with anion/cation-exchange resins is affected by the pH and the salt concentration of the mobile phase. The Supelcosil LC-SCX cation-exchange column is capable of separating AB, TMAO, TMI and AC (Fig. 2a) with an aqueous pyridine mobile phase solution (20 mM) at pH 2.6. The separation efficiency is governed due to dissimilar pK_a values of the arsenic compounds (Table 2) and their different hydrophobic interactions with the resin, which decrease in the order TMI > AC > TMAO > AB. Under these conditions (LC-3; Table 1) arsenous acid, arsenic acid and DMA are eluted before AB (Fig. 2a). Arsenic(V), MA and DMA are well separated (Fig. 2a). However, arsenous acid, which has the same retention time as that of MA, was co-eluted with MA under the LC-3 chromatographic conditions. So, separation and identification of both the compounds [arsenous acid and MA] under the LC-3 conditions are not possible. Consequently, identification of arsenic compounds, especially AB, arsenic(V) and DMA, in the NIES candidate CRM no. 18 human urine has been carried out using the LC-SCX column (Fig. 2b). On the PRP-X100 anion exchange column, DMA, MA and arsenic(V) are well separated with the 30 mM

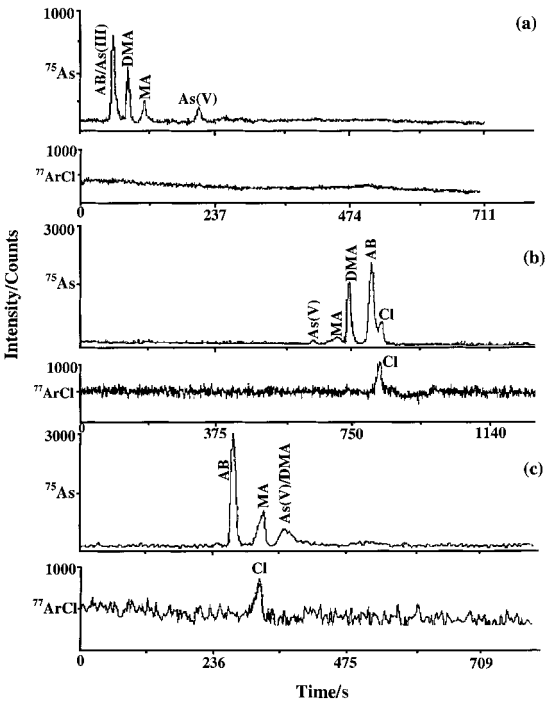

Figure 1 Chromatogram obtained with reconstituted NIES candidate CRM no. 18 freeze-dried human urine on a (**a**) PRP-X100 anion-exchange (10 µl urine injected), (**b**) GS-220 gel-permeating (25 µl urine injected), and (**c**) ODS reversed-phase (25 µl urine injected) columns using HPLC–ICP-MS (optimized conditions, see Table 1).

phosphate buffer[30] (LC-4; Table 1; Fig. 1a). AB is co-eluted with the solvent front along with the arsenous acid, complicating the identification of both compounds in the urine (Fig. 1a). Thus, separation and identification of both the compounds [arsenous acid and AB] under LC-4 conditions is not possible. Furthermore, on investigation, other cationic arsenic compounds (AC, TMAO, TMI) also co-elute with the solvent front before MA on this column.[19] So, the ion signals of AC, TMAO and TMI are overlapped with the ion signals of arsenous acid and AB. However, in our experience, LC-4 chromatographic conditions (phosphate buffer) are inappropriate for our PMS 2000 ICP-MS machine. Clogging of the cones has been observed, and the ion signals have decreased on increasing the phosphate mobile phase running time. So, we have used the LC-4 conditions only for the identification of arsenic compounds. The separation and identification of AB and arsenous acid has been reported[35] with a tartaric mobile phase at pH 2.91 on PRP-X100 column, but the peaks of AB and DMA, which are present in human urine, are not base-line separated under these chromatographic conditions. Hence, this chromatographic condition is also unsuitable for the quantification of AB and DMA in NIES candidate CRM no. 18 human urine. However, arsenous acid is retained in these separation conditions at pH 2.91 and even eluted after DMA and MA.[35] Therefore, using the PRP-X100 column under LC-4 chromatographic conditions, arsenic compounds, especially arsenic(V), MA and DMA, have been identified in NIES candidate CRM no. 18 human urine (Fig. 1a). Ion-pair reversed-phase chromatography[14,25,36] and micellar liquid chromatography[14,37] have also been applied for the arsenic separation study. Because of the wide range of pK_a values and different ionic characters of the arsenic species (Table 2), the separation of the arsenic compounds by ion-exchange chromatography is not easy to achieve. In particular, the separation of AB from arsenous acid in real matrices is difficult.[37] So, we have used the GS-220 and the ODS columns for further identification and validation of arsenic compounds (Fig.1b and c) in the urine.[14] The peak of AB is well separated from the other arsenic compounds (Fig. 1c) on the ODS column, but the peaks of DMA and arsenic(V) overlap and complicate their identification. On the GS-220, under LC-2 conditions, the peak of DMA has been separated (Fig. 1b) from the rest of the arsenic compounds and used to re-identify DMA. Hence, in NIES candidate CRM no. 18 human urine, AB, DMA, MA and arsenic(V) have been detected and confirmed by using these four different chromatographic conditions (Table 3). AB is the predominant arsenic species followed by DMA, MA and arsenic acid. We have further confirmed the peak identities of AB, DMA, MA and arsenic(V) by spiking arsenic standard to the urine. We have not detected peaks of arsenosugars in the study urine (Figs 1 and 2). The absence of arsenosugars is due to their very low concentration, which are below the detection limit,[20] or they are originally missing in the urine studied. The latter is most probable.[3,7,25] It is reported that ingested arsenosugars from the diet are metabolized in the human body to DMA and other unknown arsenic compounds.[3,7] Moreover, at least a portion is excreted through urine.[3,7] So, the possibility for detecting arsenosugars in human urine is uncertain. Ma and Le[7] did not detect arsenosugars in human urine when arsenosugars were ingested through the diet. Furthermore, our finding concerning the absence of arsenosugars in the NIES candidate CRM no. 18 human urine is in good agreement with

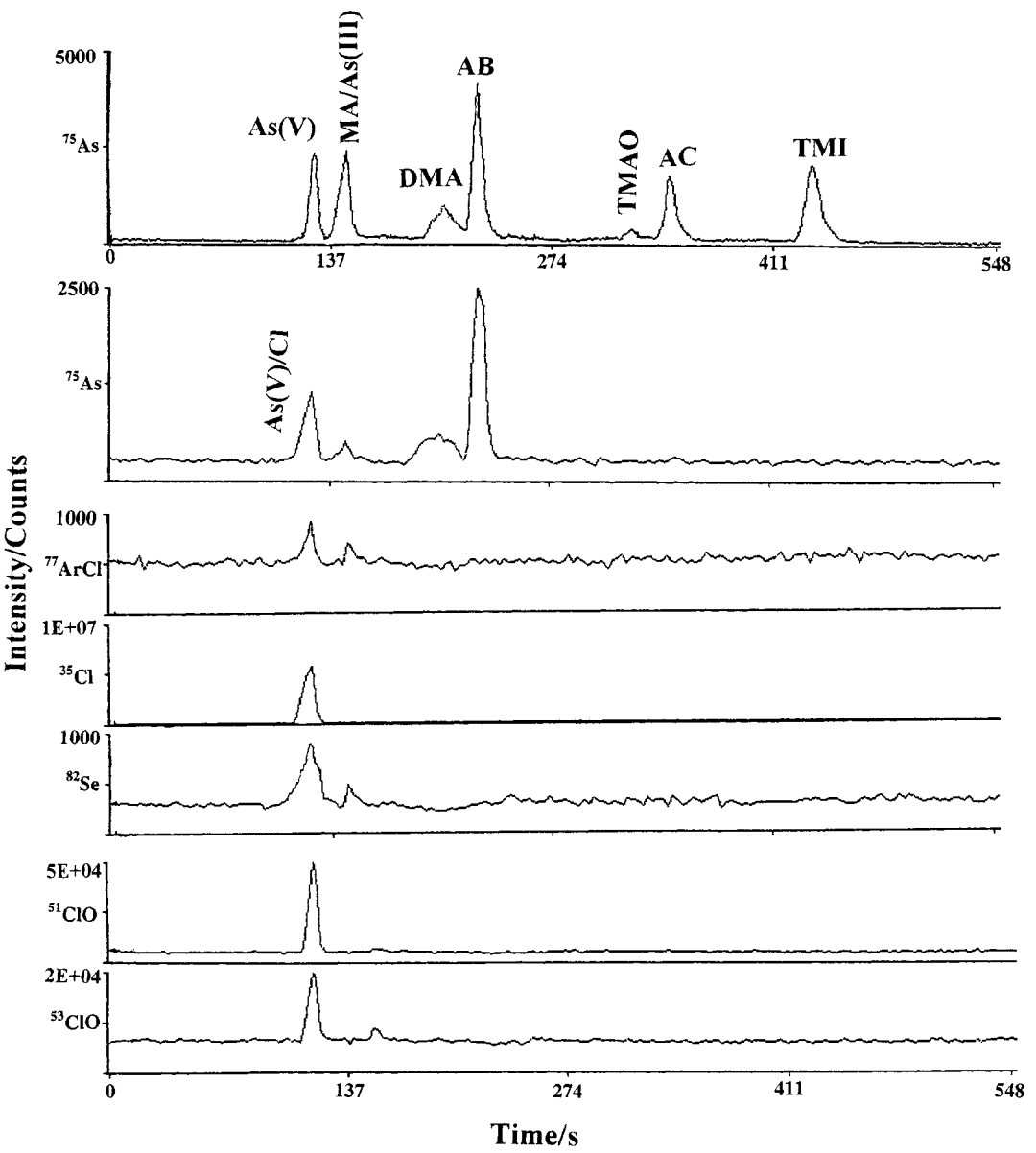

Figure 2 Chromatograms obtained with (**a**) a solution of DMA, MA, arsenate [arsenic(V)], AB, TMAO, AC and TMI (5 ng arsenic from each species) in distilled water and (**b**) reconstituted NIES candidate CRM no. 18 freeze-dried human urine (25 μl urine injected) on a Supelcosil LC-SCX cation-exchange column using HPLC–ICP-MS (optimized conditions, see Table 1).

previous reports, where typical Japanese human urine has been used for the arsenic speciation study.[14,25]

Argon chloride interference

High-resolution ICP-MS is used to separate the $^{40}Ar^{35}Cl^{+}$ (74.930 69 amu) from the parent $^{75}As^{+}$ (74.921 05 amu) but it requires an $M/\Delta M > 8000$ in order to achieve a full baseline separation of the peaks.[38] However, the sensitivity of the high-resolution ICP-MS at $M/\Delta M = 8000$ is very low compared with quadrupole ICP-MS instruments.[38] It is reported that, using liquid chromatography, chloride is separated chromatographically from arsenic.[26] To investigate argon chloride interfer-

Table 2 pK_a values of arsenic compounds

Compound	pK_a	Ref.
Arsenous acid [As(III)]	9.2	31
Arsenic acid [As(V)]	2.3, 6.8, 11.6	31
MA	3.6, 8.2	32
DMA	6.2, 1.28	31, 33
AB	2.18	33
TMAO	4	34
Dimethylarsinoyl group (sugar)	3.85	34

Table 3 Arsenic compounds identified in NIES candidate human urine by using four different chromatographic systems[a]

Column	Peaks identified	AB ($\mu g\ l^{-1}$)
ODS	AB, MA/Cl, As(V)/DMA	78.2 ± 4
GS-220	AB/Cl, MA, As(V), DMA	71.5 ± 4
LC-SCX	AB, MA, As(V)/Cl, DMA	70.5 ± 5.5
PRP-X100	AB/As(III), MA, As(V), DMA	79.5 ± 5

[a] Details of chromatographic conditions are given in Table 1.

ence with the detection of arsenic species in this study, 5000 $\mu g\ ml^{-1}$ of chloride (about the same concentration found in the NIES candidate CRM no. 18 human urine[20]) has been analysed using four chromatographic systems (LC-1 to LC-4; Table 1). Chloride is monitored as $^{35}Cl^{16}O^+$ ion at *m/z* 51; $^{37}Cl^{16}O^+$ ion at *m/z* 53; $^{37}Cl^{40}Ar^+$ ion at *m/z* 77 and $^{35}Cl^+$ at *m/z* 35 on the LC-SCX cation-exchange column with LC-3 chromatographic conditions. It co-eluted with the arsenic(V) (Fig. 2b). So, with LC-3 chromatographic conditions, chloride causes on interference problem in the speciation of arsenic(V) in urine and ultimately enhances the ion signals of arsenic(V) by forming $ArCl^+$. Moreover, chloride has not interfered with the peaks of arsenic compounds (Fig. 1a) on the PRP-X100 column with LC-4 chromatographic conditions. The $ArCl^+$ peak was not found within 711 s on the PRP-X100 column. Hence, the chloride has been separated on the PRP-X100 column chromatographically. However, it co-eluted with the MA on the ODS column with LC-1 chromatographic conditions. Moreover, AB and the other arsenic compounds has well separated from the chloride (Fig. 1c) on this column. On the GS-220 column with LC-2 conditions, chloride co-eluted with AB and enhanced the signal of AB by forming $ArCl^+$ (*m/z* 75; Fig. 1b). The ratio between the two intensities of ClO^+ (51 and 53) monitored in the chromatograms is approximately 3:1 (Fig. 2b) and corresponding to the natural abundance of the two chloride isotopes. The chromatographic peaks detected in different columns at *m/z* 51 and 53 (as ClO^+) have the same retention times as that of the peak detected at *m/z* 77 ($ArCl^+$, 77). Therefore, these peaks are assigned to be the chloride in the urine sample (for simplicity we only presented the peaks obtained from the LC-SCX column; Fig. 2b). However, a signal at *m/z* 77 can also be attributed to $^{77}Se^+$. We have detected selenium compounds in the urine by simultaneously measuring the *m/z* 82 ($^{82}Se^+$), having the same retention time as that of the chloride (Fig. 2b) on the LC-SCX cation-exchange column. Hence, it is recommended to measure both the *m/z* 51/53 and 77 to confirm the interference of chloride in urine. However, the signal at *m/z* 82 has not interfered with AB under any of the four chromatographic conditions (LC-1 to LC-4) used. Thus, the signal found at *m/z* 77, which interfered with the signal of AB on the GS-220 under the LC-2 conditions, is due to argon chloride, which is formed in the plasma by combination of argon (plasma gas) and the high chloride matrix of the urine.

Quantification of AB in the NIES candidate CRM no. 18 human urine

The AB peak obtained from the NIES candidate CRM no. 18 human urine on the LC-SCX column has been well separated from the rest of the arsenic compounds and also from the chloride peak (Fig. 2) with LC-3 conditions. So, LC-SCX is a suitable column (with LC-3 conditions) for the quantification of AB in urine. The concentration of AB, estimated using the standard additions technique (LC-SCX), is $70.5 \pm 5.5\ \mu g\ l^{-1}$ ($n = 20$; Table 3), and in agreement with the HPLC–MIP-MS[20] value ($76.2 \pm 3.2\ \mu g\ l^{-1}$). AB (53% of the total arsenic) is the major arsenic species in the urine, followed by DMA, MA and arsenic(V). The concentration of AB has also been calculated using an external aqueous calibration curve. However, the estimated value of AB ($82.2 \pm 8\ \mu g\ l^{-1}$) is higher with respect to the standard additions technique. So, the ion signals of AB have apparently been enhanced due to the urine matrix. It is necessary, therefore, to calibrate and estimate the AB by using the standard addition technique that we have applied. A value of *r* (calculated for SAM) better than 0.999 reveals a linear relationship between standards added to the

urine and the HPLC–ICP-MS signals. If a sufficient reconditioning step (about 4 min additional run of the mobile phase) is applied after each run of the urine sample, reproducibility and repeatability of the separation and quantification for AB on the LC-SCX column are good.[20] The mean concentration of AB, estimated by SAM with the four different columns conditions, is 74.9 ± 4.6 μg l^{-1} ($n = 7$; Table 3). This value of AB is slightly higher than that obtained using the LC-SCX column (70.5 ± 5.5 μg l^{-1}). Moreover, the AB peak overlaps with arsenous acid on the PRP-X100 and ODS columns with LC-4 and LC-1 chromatographic conditions. So, arsenous acid has confused the quantification of AB on these columns. Small amounts of arsenous acid (undetected with our present HPLC–ICP-MS conditions) in the urine will interfere. Hence, arsenous acid will elevate the concentration of AB in the NIES candidate CRM no. 18 human urine and ultimately may cause a positive error. Therefore, the mean value of AB on the four different columns is slightly higher than the value obtained using the LC-SCX column. As mentioned previously, AB is adequately separated from the rest of the arsenic compounds by using the GS-220 column, but it overlaps with the chloride peak. Therefore, this column is also unsuitable under the present chromatographic conditions (LC-2) for the quantification of AB in the NIES candidate CRM no. 18 human urine. However, the concentration of AB has been estimated carefully on the GS-220, after extracting the chloride interference (^{37}Cl:$^{35}Cl = 1{:}3.1271$) by measuring the $^{40}Ar^{37}Cl^{+}$ (where the peak of selenium at m/z 77 is not interfered).[20,26] This value has been used only for the reference value to revalidate and to confirm the quantified concentration of AB that was obtained using the LC-SCX chromatographic conditions. For the generation of CRMs, it is fundamental and essential that the value of AB should be rechecked. The value of AB generated should be reliable. So, we used different columns under different conditions for cross-examination of the value of AB obtained using LC-SCX (Table 2). The concentration of AB found, using the GS-220, is 71.5 ± 4 μg l^{-1} ($n = 9$; Table 3). This is in agreement with the LC-SCX value.

CONCLUSIONS

In NIES candidate CRM no. 18 human urine, the concentration of AB is 70.5 ± 5.5 μg l^{-1}. Chloride present in the urine matrix is eluted from the column and the formed argon chloride intensity is significant at m/z 75. Chloride interference has been removed by careful selection of the chromatographic systems. The LC-SCX cation-exchange column is the suitable one for the separation and determination of AB present in the NIES candidate CRM no. 18 human urine. The PRP-X100 and ODS columns are inappropriate with LC-1 and LC-4 conditions for the quantification of AB. This is due to the peak of arsenous acid overlapping with that of AB. However, the producer of AB in the food web has not yet been clearly identified.[39] AB is the major concern in understanding the biogeochemical cycling of arsenic in the marine ecosystem. Thus, estimation of AB is now essential in the understanding of its toxicity and environmental dynamics. Analytical quality assurance of arsenic compounds in urine is an important and ongoing demand nowadays. Accordingly, urine CRMs containing arsenic compounds are needed.[40] Taking this situation into account, NIES has prepared candidate CRM no. 18 human urine for the certification of arsenic species, particularly AB. The urine used here has been distributed to many organizations. A collaborative analysis of AB and other arsenic compounds is ongoing. The current result for AB thus found has been used as a baseline value for collaborative analysis, and will be used for the certification of AB in NIES candidate CRM no. 18 human urine.

REFERENCES

1. Vahter M. *Clin. Chem.* 1994; **40**(5): 679.
2. Lunde G. *Nature* 1969; **224**: 186.
3. Le X-C, Cullen WR, Reimer KJ. *Clin. Chem.* 1994; **40**: 617.
4. Chapman AC. *Analyst* 1923; **51**: 548.
5. Norin H, Vahter M. *Scand. J. Work Environ. Health* 1981; **7**: 38.
6. Kaise T, Watanabe S, Itoh K. *Chemosphere* 1985; **14**(9): 1327.
7. Ma M, Le X-C. *Clin. Chem.* 1998; **44**: 539.
8. Morita M, Edmonds JS. *Pure Appl. Chem.* 1992; **64**: 575.
9. Francesconi KA, Edmonds JS, Morita M. *Adv. Environ. Sci. Technol.* 1994; **26**: 189.
10. Sarzanini C. *J. Chromatogr. A* 1999; **850**: 213.
11. Benramdane L, Bressolle F, Vallon JJ. *J. Chromatogr. Sci.* 1999; **37**: 330.
12. Guerin T, Astruc A, Astrue M. *Talanta* 1999; **50**: 1.
13. Kurosawa S, Yasuda K, Taguchi M, Yamazaki S, Toda S, Morita M, Uehiro T, Fuwa K. *Agric. Biol. Chem.* 1980; **44**: 1993.

14. Shibata Y, Morita M. *Anal. Sci.* 1989; **5**: 107.
15. Zhang X, Cornelis R, De Kimpe J, Mees L. *Anal. Chim. Acta* 1996; **319**: 177.
16. Ng JC, Johnson D, Imray P, Chiswell B, Moore MR. *Analyst* 1998; **123**: 929.
17. Magnuson ML, Creed JT, Brockhoff CA. *J. Anal. At. Spectrom.* 1996; **11**: 893.
18. Murer AJL, Abildtrup A, Poulsen OM, Christensen JM. *Analyst* 1992; **117**: 677.
19. Chatterjee A. *Talanta* 2000; **51**: 303 and references cited therein.
20. Chatterjee A, Shibata Y, Yoshinaga J, Morita M. *Anal. Chem.* 2000; **18**: 4402, and references cited therein.
21. Morita M, Shibata Y. *Anal. Sci.* 1987; **3**: 575.
22. Le X-C, Cullen WR, Reimer KJ. *Appl. Organomet. Chem.* 1992; **6**: 161.
23. Jarvis KE, Gray AL, Houk RS. *Handbook of Inductively Coupled Plasma Mass Spectroscopy*. Blackie: Glasgow and London, 1992; 112–150.
24. Buchberger WW, Haddad PR. *J. Chromatogr. A* 1997; **789**: 67.
25. Shibata Y, Morita M, Fuka K. *Adv. Biophys.* 1992; **28**: 31.
26. Larsen EH, Pritzl G, Hansen SH. *J. Anal. At. Spectrom.* 1993; **8**: 557.
27. Tan SH, Horlick G. *Appl. Spectrosc.* 1986; **40**: 445.
28. Meier PC, Zund RE. *Statistical Methods in Analytical Chemistry*. Winefordner JD (ed.). John Wiley & Sons: New York, 2000; 120–122.
29. Yoshinaga J, Chatterjee A, Shibata Y, Morita M, Edmonds JS, *Clin. Chem.* 2000; **46**: 1781.
30. Gailer J, Irgolic KJ. *Appl. Organomet. Chem.* 1994; **8**: 129.
31. Weast RC. (ed.). *Handbook of Chemistry and Physics*, CRC Press: Cleveland, OH; 54th edn. 1973–1974.
32. Kortum G, Vogel W, Andrussov K. *Dissociation Constants of Organic Acids in Aqueous Solutions*. Butterworths: London, 1961; 492.
33. Hansen SH, Larsen EH, Pritzl G, Cornett C. *J. Anal. At. Spectrom.* 1992; **7**: 629.
34. Shibata Y, Morita M. *Biomed. Res. Trace Elements* 2000; **11**: 1.
35. Zheng J, Kosmus W, Pichler-Semmelrock F, Kock M. *J. Trace Elements Med. Biol.* 1999; **13**: 150.
36. Gailer J, Francesconi KA, Edmonds JS, Irgolic KJ. *Appl. Organomet. Chem.* 1995; **9**: 341.
37. Boucher P, Accominotti M, Vallon JJ. *J. Chromatogr. Sci.* 1996; **34**: 226.
38. Klaue B, Blum JD. *Anal. Chem.* 1999; **71**: 1408.
39. Francesconi KA, Edmonds JS. *Adv. Inorg. Chem.* 1997; **44**: 147.
40. Cornelis R, Zhang X, Mees L, Christensen JM, Byrialsen K, Dyrschel C. *Analyst* 1998; **123**: 2883.

