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Selective arsenic speciation analysis of human urine reference materials using gradient elution ion-exchange HPLC-ICP-MS

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Arsenic speciation analysis was performed in two human urine certified reference materials (NIES No. 18 and NIST SRM2670a) and three human urine control materials (Seronorm, Medisafe and Lyphocheck). The samples were diluted 1 + 3 prior to analysis by gradient elution anion or cation exchange high-performance liquid chromatography (HPLC) coupled with inductively coupled plasma mass spectrometry (ICP-MS). Nine arsenic species, including arsenic acid, arsenous acid, monomethylarsonic acid, dimethylarsinic acid, arsenobetaine, trimethylarsine oxide, dimethylarsinoylacetic acid, trimethylarsoniopropionate and dimethylarsinoylethanol, were determined in the urines. Additionally, several unknown arsenicals were detected. This is the first time that dimethylarsinoylacetic acid and trimethylarsoniopropionate have been reported in human urine. The sums of the species concentrations determined by the chromatographic approaches were identical with the reference values given for total arsenic. The obtained values for arsenobetaine and dimethylarsinic acid were identical with the values certified for the NIES No. 18 urine CRM. The speciation data presented here may be valuable for the quality assurance of analytical method development and surveys of arsenic in urine samples.

Introduction

Arsenic is an element that is ubiquitous in the environment. Natural metabolic processes in the biosphere result in the existence of a large number of inorganic and organic arsenic compounds, and more than 25 arsenic compounds have been identified in the aquatic environment.[1] Due to the relatively high concentrations of arsenic found in samples of marine origin (usually in the mg kg^{-1} range), these sample types have been subjected to extensive research in the field of arsenic speciation. In seafood, arsenobetaine (AsB) and arsenosugars are the predominant species of arsenic found in fish *etc.* and algae, respectively, whereas other organoarsenic as well as inorganic compounds usually are found at low concentrations. Recent research has provided evidence for the existence of the same variability of arsenic compounds in the terrestrial environment although generally at much lower concentrations (usually in the μg kg^{-1} range).[2] A list of arsenic species often reported in marine biological samples is given in Table 1.

The human intake of arsenic mainly occurs *via* the diet and drinking water, whilst some individuals may be additionally exposed *via* the workplace environment.[3] Consumers highly exposed to arsenic are those with a high consumption of seafood (mainly as AsB) or people from areas of the world with a naturally high level of arsenic in their drinking water (mainly as inorganic arsenic).[4,5]

A dramatic variation of the environmental fate and behaviour, bioavailability, and toxicity between the species of arsenic is observed. Inorganic forms of arsenic are most toxic, whereas the methylated forms (*e.g.*, MMA, DMA) are low in acute toxicity and AsB is considered non-toxic.[3] Consequently, a toxicological evaluation of dietary arsenic intake and environmental exposure should be based on arsenic speciation data. In addition, knowledge about the occurrence of arsenic species in various foods and water (*e.g.*, AsB from seafood *versus* inorganic arsenic from drinking water) provides some information on the sources of the arsenic exposure. After absorption in the gastro-intestinal tract or in the lungs arsenic is eliminated from the body predominantly *via* the urine. Therefore, urinary arsenic is used as a biomarker of exposure in epidemiological and biological monitoring studies. The various arsenic compounds are metabolised in different ways in the human body: inorganic arsenic is methylated to MMA and DMA,[6] whereas AsB is excreted unchanged.[7] Several studies have reported DMA as the primary metabolite after ingestion of arsenosugars (from seaweed),[8,9] and several other (partly unknown) minor metabolites have also been found in addition to unmetabolised arsenosugar.[10]

For the determination of arsenic species in urine, hydride generation and detection by AAS has been widely used.[11,12] However, hydride generation methods are limited to the determination of hydride forming compounds, *i.e.*, As(III), As(V), MMA and DMA, unless a complete digestion of AsB and other organoarsenic compounds has been performed.[13–15] In recent years HPLC-ICP-MS appears to be the preferred methodology, allowing the simultaneous determination of both inorganic and organic (water-soluble) arsenic species.[16–19]

In the validation process of any analytical methodology certified reference materials (CRMs) are important tools for assessment of the accuracy of the method. To date only one urine CRM (NIES No. 18) has been certified for arsenic species, but the certification is limited to AsB and DMA. There is a lack of and a need for additional certified reference materials for arsenic species and especially reliable reference data for the species of greatest toxicological interest, *i.e.*, As(III) and As(V) are called for.

The purpose of this paper is to present arsenic species data for a range of human urine CRMs, including the NIES No. 18 Human Urine. The NIST SRM 2670 Human Urine has previously been used in several studies of arsenic speciation in human urine.[20–23] Recently, a new batch, NIST SRM 2670a Toxic Elements in Urine, was released,[24] and arsenic speciation data are for the first time presented for this new material. Finally, the development and performance of a new anion

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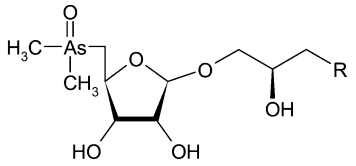
Table 1 List of arsenic species

	Acronym	Arsenic species	Formula
1	As(v)	Arsenic acid	$AsO(OH)_3$
2	As(III)	Arsenous acid	$As(OH)_3$
3	MMA	Monomethylarsonic acid	$CH_3AsO(OH)_2$
4	DMA	Dimethylarsinic acid	$(CH_3)_2AsO(OH)$
5	AsB	Arsenobetaine	$(CH_3)_3As^+CH_2COO^-$
6	TMAO	Trimethylarsine oxide	$(CH_3)_3AsO$
7	AsC	Arsenocholine ion	$(CH_3)_3As^+CH_2CH_2OH$
8	TMAs	Tetramethylarsonium ion	$(CH_3)_4As^+$
9	DMAA	Dimethylarsinoylacetic acid	$(CH_3)_2As(O)CH_2COOH$
10	TMAP	Trimethylarsoniopropionate	$(CH_3)_3As^+CH_2CH_2COOH$
11	DMAE	Dimethylarsinoylethanol	$(CH_3)_2As(O)CH_2CH_2OH$



12 –OH	Arsenosugar OH	R = OH
13 $-PO_4$	Arsenosugar PO_4	R = $OP(O)(OH)OCH_2$-$CH(OH)CH_2OH$
14 $-SO_3$	Arsenosugar SO_3	R = SO_3H
15 $-SO_4$	Arsenosugar SO_4	R = OSO_3H

exchange HPLC-ICP-MS method of analysis using gradient elution is described. In addition, data based on cation exchange HPLC-ICP-MS of the same set of samples supplement the anion exchange HPLC data.

Experimental

Instrumentation

An Agilent quadrupole ICP-MS 7500c instrument (Yokogawa Analytical Systems Inc., Tokyo, Japan), which was used as an arsenic specific detector, was run in the standard mode. For the liquid chromatographic separations an Agilent 1100 series quaternary HPLC pump, degasser and autosampler (Agilent Technologies, Waldbronn, Germany) were used. Polypropylene autosampler vials were used, as it was found that glass vials could contaminate the samples with arsenate. The same observation has been reported earlier.[10] The outlet of the HPLC column was connected to the nebuliser of the ICP-MS instrument by a short length of polyethylene tubing. For anion exchange chromatography two different polymer-based strong anion exchange HPLC columns, ICSep ION-120 (4.6 × 120 mm; 10 μm particles) (Transgenomics, San Jose, CA, USA) and Hamilton PRP X-100 (4.1 × 150 mm; 3 μm particles) (VWR, Oslo, Norway), were used. For cation exchange chromatography a silica-based strong cation exchange HPLC column, ChromPack Ionospher 5C (3.0 × 100 mm; 5 μm particles) (Varian, Middelburg, The Netherlands), was used. In Table 2 the instrumental settings for the HPLC and ICP-MS are given. Data was collected using the Agilent Chemstation ICP-MS chromatographic software.

Standard substances and chemicals

Deionised water (>17 MΩ cm^{-1}, Nanopure-system, Nanopure, Barnstead, UK) was used throughout the work. All chemicals were of *pro analysi* quality or better. Standard solutions of the following chemicals were prepared in water: arsenite standard (Fluka, Buchs, Switzerland), arsenate standard with an arsenic concentration of 1000 ± 3 mg l^{-1} (Spectrascan, TeknoLab, Drøbak, Norway), disodium methylarsenate (MMA) (Chem Service, West Chester, PA, USA), sodium dimethylarsinic acid (DMA) trihydrate (Merck, Hohenbrunn, Germany), trimethylarsine oxide (TMAO), arsenocholine (AsC) bromide and tetramethylarsonium (TMAs) iodide (Hot Chemicals, Tokyo, Japan), respectively. A certified standard solution was used for AsB (BCR CRM626, 1031 ± 6 mg kg^{-1} as AsB (Institute for Reference Materials and Measurements (IRMM), Geel, Belgium). Dimethylarsinoylacetic acid (DMAA), trimethylarsoniopropionate (TMAP), dimethylarsinoylethanol (DMAE) and the arsenosugar standards 3-[5′-deoxy-5′(dimethylarsinoyl)-β-ribofuranosyloxy]-2-hydroxypropylene glycol (arsenosugar OH), 3-[5′-deoxy-5′(dimethylarsinoyl)-β-ribofuranosyloxy]-2-hydroxypropyl 2,3-hydroxypropylphosphate (arsenosugar PO_4), 3-[5′-deoxy-5′-(dimethylarsinoyl)-β-ribofuranosyloxy]-2-hydroxypropanesulfonic acid (arsenosugar SO_3) and 3-[5′-deoxy-5′-(dimethylarsinoyl)-β-ribofuranosyloxy]-2-hydroxypropyl hydrogen sulfate (arsenosugar SO_4) were kindly donated by Professor K.A. Francesconi, Karl-Franzens University, Graz, Austria. Aqueous stock solutions of the arsenic species were prepared at a concentration of approximately 10 mg l^{-1} as As. The stock solutions were checked for impurities of other arsenic species using the HPLC-ICP-MS systems (no impurities found). The total arsenic concentration of the stock solutions was determined by ICP-MS against the certified arsenate standard. The stock solutions were stored in the dark at 4 °C. Multi-species calibration standard solutions in water were prepared from the stock solutions. Quantification was based on peak area evaluation using the method of standard addition for calibration.

The mobile phase solutions for anion exchange chromatography were prepared by dissolving an appropriate amount of ammonium carbonate (J.T. Baker, Philipsburg, NJ, USA) in an

Table 2 Instrumental settings used in this work

ICP-MS settings—		
Rf power/W	1600	
Carrier gas flow/l min^{-1}	1.15–1.25	
Plasma gas flow/l min^{-1}	15	
Auxilary gas flow/l min^{-1}	1	
Nebuliser	Meinhard concentric	
Spray chamber	Water cooled double pass	
Spray chamber temperature/°C	2	
Interface cones	Platinum	
Lens voltage/V	2–3	
Mass resolution/u	0.8	
Integration time/s	1000	
HPLC settings—		
Injection volume/μl	25	
Operating pressure/bar (at 1 ml min^{-1})	45–50	(ICSep ION120)
	150–160	(PRP X-100)
	85–90	(IonoSpher 5C)
Mobile phase (A) conc./mmol l^{-1} $(NH_4)_2CO_3$	0	
Mobile phase (B) conc./mmol l^{-1} $(NH_4)_2CO_3$	100	
Mobile phase (C) conc./mmol l^{-1} pyridinium ion)	0	
Mobile phase (D) conc./mmol l^{-1} pyridinium ion	50	

Mobile phase gradient elution programme (anion exchange)

Time/ min	Flow/ ml min^{-1}	Mobile phase (A) (%)	Mobile phase (B) (%)
0–5	0.7	95	5
5–10	1	70	30
10–14	1	50	50
14–15	1	95	5
15–17	1.5	95	5
17–18	0.7	95	5

Mobile phase gradient elution programme (cation exchange)

Time/ min	Flow/ ml min^{-1}	Mobile phase (C) (%)	Mobile phase (D) (%)
0–3	1	99	1
3–4	1	99 → 90	1 → 10
4–15	1	90	10
15–16	1	90 → 60	10 → 40
16–20	1	60	40
20–25	2	99	1

aqueous 3% (v/v) methanol solution (Merck, Darmstadt, Germany) followed by adjustment of the pH to 10.3 with 25% (v/v) aqueous ammonia (Merck, Darmstadt, Germany). The mobile phase solutions for cation exchange chromatography were prepared by dissolving an appropriate amount of pyridine (Merck, Darmstadt, Germany) in an aqueous 3% (v/v) methanol solution (Merck, Darmstadt, Germany) followed by adjustment of pH to 2.7 with formic acid (Merck, Darmstadt, Germany). Prior to use all mobile phase solutions were filtered through a 0.45 μm poly(vinyl difluoride) (PVDF) filter (Pall Gelman Sciences, Ann Arbor, USA).

Samples and sample preparation

The following certified reference materials have been used in this work: NIES no. 18 Human Urine (National Institute for Environmental Studies, Ibaraki, Japan), certified for AsB, DMA and total arsenic; together with NIST SRM2670a Toxic Elements in Urine (low and high level) (National Institute of Standards and Technology, Gaithersburg, MD, USA), certified for total arsenic concentration. In addition three commercially available control materials (all with two levels) with reference values for total arsenic content were used: Seronorm Trace Elements Urine (LOT NO2525) and Urine Blank (LOT NO2524) (Sero, Billingstad, Norway), Medisafe Metalle U (Level 1 (LOT 28351) and Level 2 (LOT 28352) (LGC Promochem, Borås, Sweden) and Lyphocheck Quantitative Urine Control (Level 1 and 2, LOT 62170) (Bio-Rad Laboratories, Oslo, Norway). All of the reference materials were supplied as lyophilised samples and were kept at 4 °C in the dark until use. The samples were reconstituted by adding water according to the supplier's instructions. Prior to analysis the samples were filtered using a 0.45 μm single use syringe and disposable filter (Sartorius MiniSart RC25, Sartorius, Göttingen, Germany) followed by a 1 + 3 fold dilution with water. This dilution prevented oxidation of As(III) to As(V) during analysis.[25]

Results and discussion

Method development

The ICP-MS instrument was tuned daily for optimum signal-to-noise ratio on *m/z* 75 for the ^{75}As isotope. Typically a sensitivity of approximately 8000 counts per second (cps) per ng As ml^{-1} in an aqueous 3% (v/v) methanol solution and a blank signal of approximately 30–50 cps were achieved when using the instrumental settings given in Table 2. An approximately 4-fold increase in signal intensity was achieved by the addition of 3% (v/v) methanol to the mobile phases.[26]

Anion exchange chromatography. The species retained on an anion exchange column include the inorganic species As(III) and As(V) in addition to MMA and DMA and other compounds, which become anionic under alkaline conditions (*e.g.*, several dimethylarsinoylribosides (arsenosugars)). The buffer concentration and pH of the mobile phases are important parameters, which influence the retention of the arsenic species. In order to ionise arsenous acid to arsenite (pK_a 9.2) to achieve a satisfactory retention of this species on the anion exchange column, an alkaline condition of the mobile phase is required. A pH value of 10.3 was earlier found to be optimum and was therefore chosen in the present study.[25,27] Carbonate was chosen as the buffer species of the mobile phase, serving both as an eluting agent and as a pH-buffer at the selected pH value.

Two different columns were tested, the Hamilton PRP X-100 and the ICSep ION120. Similar resolution capacity was obtained for the two columns: however, a different order of elution was observed; AsB, As(III), DMA, MMA, As(V) for PRP X-100 and AsB, DMA, As(III), MMA, As(V) for ION120, respectively. The latter order was preferred since in this system the As(III) peak (usually minor) was moved further away from the AsB peak (usually a major peak). This avoided the risk of co-elution of As(III) with AsB, which may impede the analysis of As(III). In order to investigate the often referred to $^{40}Ar^{35}Cl^{+}$ interference,[16,25,28] the $^{35}Cl^{+}$ signal was monitored. For the PRP X-100 column it was found that Cl^{-} eluted in the vicinity of MMA, potentially leading to overlap between $^{40}Ar^{35}Cl^{+}$ and MMA at high Cl^{-} concentrations. On the ION120 column Cl^{-} was eluted after As(V) and thus resolved chromatographically from the arsenic species. Consequently, the ION120 column was used throughout this work. It should, however, be noted that no $^{40}Ar^{35}Cl^{+}$ peak was observed in any of the urine samples analysed.

A gradient elution strategy with stepwise increase of mobile phase concentrations was chosen in order to gain the benefits of both a good resolution between the peaks eluting at the beginning of the chromatogram and an acceptable retention time ($k' < 12$) for the species, which are strongly retained on the column. A systematic investigation of the retention time variation as a function of mobile phase concentration using standard solution mixtures was conducted. A three step elution method as outlined in Fig. 1 and Table 2 was well suited for the anion exchange separation of the species investigated (AsB, DMA, As(III), MMA and As(V)). A large retention time difference between the arsenic species standards was deliberately pursued during optimisation of the gradient in order to allow for the separation and selective analysis of possible unknown species present in the urine samples. The retention times for the various arsenic species varied less than 4% ($n = 9$) between subsequent analysis of the different urine samples investigated.

This method does not allow for the separation of AsB and some other cationic organoarsenic compounds, which are not retained on the anion exchange column at pH 10.3. These include AsC, TMAs, TMAO and TMAP, which co-elute in the void volume on the anion exchange column. Consequently, the quantification of AsB (or other cationic species eluting in the void) using the void peak is usually not defendable. For this purpose alternative chromatographic methodologies which are able to separate these cationic species should be used (*e.g.*, cation exchange chromatography).[29,30] In many biological samples (including human urine) AsB is the predominant species while other cationic species are present only as minor constituents. As a consequence the uncertainty associated with the quantification of AsB using the void peak in anion exchange chromatography may in such cases be negligible. Various examples of the determination of AsB in biological samples using anion exchange chromatography can be found in the literature.[17,21,27,31] However, the analyst should be aware of

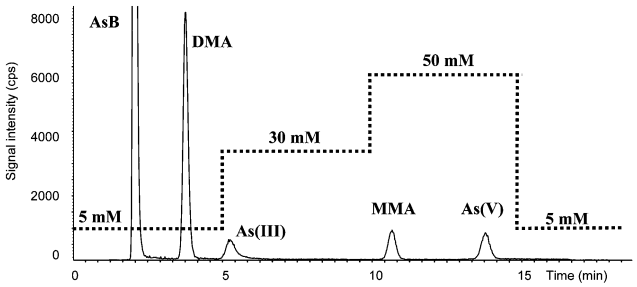


Fig. 1 Chromatogram of five arsenic standard compounds using gradient elution anion exchange HPLC (AsB 21 μg l^{-1} as As, DMA 7.1 μg l^{-1} as As, As(III) 0.8 μg l^{-1} as As, MMA 2.2 μg l^{-1} as As, As(V) 0.8 μg l^{-1} as As). The signal intensity of AsB is 42 000 cps. The broken line illustrates the gradient (concentration of $(NH_4)_2CO_3$) used for elution of the arsenic species (arbitrary scale). See Table 2 for information on the chromatographic conditions. For peak identification see Table 1.

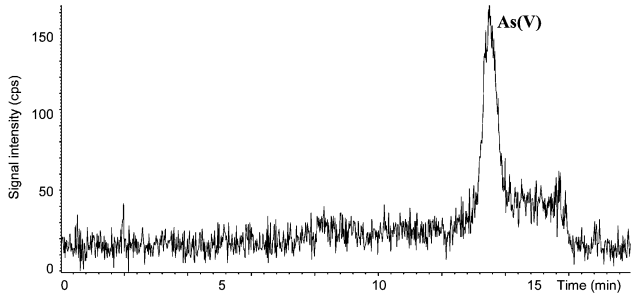


Fig. 2 Chromatogram of the baseline obtained when running the gradient elution programme for anion exchange chromatography. See Table 2 for information on the chromatographic conditions. For peak identification see Table 1.

the potential error that may be committed, especially when analysing samples of unknown arsenic species distribution.

An apparent drawback of the gradient elution procedure is that a blank peak occurs for As(v). This is apparent when detecting the baseline signal without any sample injected, as illustrated in Fig. 2. This phenomenon was previously reported by Falk and Emons[31] when using a similar gradient elution approach. The peak size varied depending on the column regeneration time, *i.e.*, a longer regeneration time led to larger peaks. The As(v) blank peak is caused by As(v) impurities in the mobile phase reagents, which are accumulated on the column during the column regeneration period. The low buffer concentration is not sufficient to elute As(v), which is therefore retained on the column until the subsequent higher buffer concentration in the gradient elution programme elutes As(v). Different commercial sources of mobile phase reagent (ammonium carbonate and ammonium hydrogen carbonate) were tested, and all contained impurities of As(v) to some degree. However, when running automated analysis with reproducible regeneration times between subsequent injections, repeated analysis of blank samples showed that the area of the blank peak was highly repeatable (RSD = 1.7%; $n = 12$). Hence, the As(v) blank concentration could be precisely quantified and subtracted from As(v) sample peaks.

In Table 3 the limit of detection (LOD) and the precision for the gradient anion exchange method are presented. LODs were calculated as three times the baseline noise (except for As(v)) and calibrated by extrapolation of the calibration curve in the low concentration range. LODs are in the range 0.04–0.2 $\mu g\,l^{-1}$ as As and are amongst the lowest reported so far for arsenic species in urine samples. The precision was determined by pooling of the RSD values from each of the duplicate determinations of the samples analysed in this study. Acceptable pooled RSD values from 2.0–5.3% for the different arsenic species were obtained, as shown in Table 3.

Cation exchange chromatography. The urine samples were also subjected to speciation analysis using a gradient elution cation exchange method for the separation of organoarsenic species recently developed by Sloth *et al.*[30] This method enables the separation of several organoarsenic compounds, including AsC, TMAs, TMAO and TMAP, all coeluting with AsB in the void volume in the anion exchange method. Additionally, this method allows for the determination of DMAA and DMAE. Both of these two compounds have recently been identified as arsenosugar metabolites in urine from humans (DMAE)[10,32] and sheep solely grazing on seaweed (DMAA and DMAE).[33,34]

Table 3 Figures of merit for the gradient elution anion exchange chromatography HPLC-ICP-MS method

	As(v)	As(III)	MMA	DMA
LOD ($3s$)/$\mu g\,l^{-1}$ as As[a]	0.2	0.08	0.05	0.04
Precision (RSD) (%)[b]	2.0	3.5	5.3	4.4

[a] LOD is expressed equivalent to a 4-fold dilution of the sample [b] RSD calculated from pooled s values ($n = 9$) from duplicate analysis of the samples analysed in this study.

Arsenic speciation analysis of human urine reference materials

For both chromatographic approaches identification of the arsenic species was based on matching retention times with available standard substances after spiking of the samples. The results from the arsenic speciation analysis of different human urine reference materials by the anion exchange and cation exchange methods are given in Tables 4 and 5, respectively. DMA was determined by both chromatographic approaches, and the concentration values obtained were in most cases not significantly different. The sums of species concentrations determined by anion and by cation exchange chromatography, respectively, were calculated. The results given in Tables 4 and 5 are in close agreement with each other. Furthermore, the data in the tables show that the sums of the arsenic species concentrations were identical with the reference values for total arsenic for all samples, except one. In contrast, the sums of species for the Lyphocheck materials did not confirm the reference values provided by the supplier. Interestingly, the sums of the concentrations of the hydride-generating arsenic species (*i.e.*, As(III), As(v), MMA and DMA) determined in this study are in agreement with the confidence range given by the supplier. This suggests that results obtained from hydride generation techniques may have contributed to the establishment of the reference values, thus excluding the analysis of AsB and other organoarsenic compounds, which do not form hydrides.

The AsB levels in the different materials reflect the dietary habits for the individuals or groups of individuals from whom the urines have been sampled. The materials NIST SRM2670a, Medisafe and Lyphocheck all have low AsB concentrations (<15 $\mu g\,l^{-1}$ as As), *i.e.*, low seafood intake is expected. In contrast, the materials NIES No. 18 and Seronorm Level 1 and 2, with a significantly higher concentration of AsB (68–77 $\mu g\,l^{-1}$ as As), may show a higher seafood intake.

Arsenic speciation analysis of NIES No. 18 Human Urine

The values for AsB and DMA determined in NIES No. 18 (Tables 4 and 5) are in good agreement with the certified values (mean $\pm$ 2s), 69 $\pm$ 12 $\mu g\,l^{-1}$ as As and 36 $\pm$ 9 $\mu g\,l^{-1}$ as As for AsB and DMA, respectively.[35] Furthermore, the values for MMA, As(III) and As(v) lie within the ranges of results reported by the participating laboratories in the certification round for this material (MMA: 2.2–5.4 $\mu g\,l^{-1}$ as As; As(III): 2.3–7.1 $\mu g\,l^{-1}$ as As; As(v): 0.6–11.4 $\mu g\,l^{-1}$ as As).[35] The results reported by the participating laboratories for the latter three species were too variable to establish any certified or reference values. Fig. 3 shows an anion exchange chromatogram of NIES No. 18. In addition to the species mentioned above three unknown peaks (U1, U2 and U3) were found. It was suspected that these unknowns might be arsenosugar compounds, since algae is known to be a major part of the diet of the Japanese.[32] However, spike experiments with the four main arsenosugars (–OH, $–PO_4$, $–SO_3$ and $–SO_4$) showed no coelution with any of these unknowns and the identity of these compounds remains to be identified by alternative techniques, *e.g.*, ESI-MS(MS). Fig. 4 shows a cation exchange chromatogram of NIES No. 18. Trace amounts of DMAA, DMAE, TMAP and three unknown species (X1, X2 and X3) were detected in addition to DMA, AsB as well as anionic compounds, which elute with the void volume. This is the first time that DMAA and TMAP have been reported in a sample of human urine. DMAA and DMAE may occur in the urine following metabolism of arsenosugars

Table 4 Arsenic speciation results from the analysis of human urine reference materials using anion exchange chromatography[e]

	Void[a]	As(v)	As(III)	MMA	DMA	U1[bf]	U2[b]	U3[b]	Sum species	Ref. value total As
NIES No. 18	68 ± 2	0.91 ± 0.02	2.2 ± 0.1	2.6 ± 0.1	39 ± 2	0.29 ± 0.02	16.2 ± 0.9	6.2 ± 0.3	140 ± 5	137 ± 11
NIST 2670a Level 1	0.79 ± 0.03	0.19 ± 0.01	0.18 ± 0.01	0.34 ± 0.02	1.2 ± 0.1	n.d.	0.21 ± 0.01	n.d.	3.0 ± 0.1	3[c]
NIST 2670a Level 2	1.1 ± 0.1	153 ± 3	59.7 ± 2.1	0.39 ± 0.02	2.0 ± 0.1	n.d.	n.d.	n.d.	221 ± 5	220 ± 10
Seronorm Level 1	69 ± 2	0.30 ± 0.01	0.32 ± 0.01	0.32 ± 0.02	9.1 ± 0.4	n.d.	0.40 ± 0.02	n.d.	82 ± 3	83 ± 7
Seronorm Level 2	77 ± 3	97 ± 2	0.10 ± 0.01	0.28 ± 0.02	9.7 ± 0.4	n.d.	0.33 ± 0.02	n.d.	190 ± 5	184 ± 17
Medisafe Metalle U Level 1	7.3 ± 0.2	47.0 ± 0.9	1.3 ± 0.1	n.d.	2.8 ± 0.1	n.d.	n.d.	n.d.	60 ± 1	50 (38.4 – 61.6)[d]
Medisafe Metalle U Level 2	7.8 ± 0.3	203 ± 4	0.30 ± 0.01	n.d.	3.8 ± 0.2	n.d.	n.d.	n.d.	220 ± 5	250 (190.0–310.0)[d]
Lyphocheck Level 1	12.1 ± 0.4	25.2 ± 0.5	0.28 ± 0.01	0.39 ± 0.02	6.1 ± 0.3	n.d.	n.d.	n.d.	45 ± 1	29 (23–34)[d]
Lyphocheck Level 2	14.4 ± 0.5	257 ± 5	0.25 ± 0.01	0.61 ± 0.03	9.2 ± 0.4	n.d.	n.d.	n.d.	288 ± 6	228 (183–274)[d]

[a] Void volume = cationic species not retained on the anion exchange column. The void was quantified against the AsB calibration curve. [b] Approximate retention times for unknowns; U1: 2.7 min; U2: 8.5 min; U3: 11.6 min (peaks are illustrated in Fig. 3). Unknowns were quantified against the As(v) calibration curve. [c] Information value. [d] Acceptance range. [e] All results in μg l^{-1} as As given as mean ± 2*s* (N = 2). Pooled standard deviations as presented in Table 3 were used. [f] n.d. = not detected.

Table 5 Arsenic speciation results from the analysis of human urine reference materials using cation exchange chromatography[e]

	Void[a]	DMA	AsB	TMAO[f]	DMAA	TMAP	DMAE	X1[b]	X2[b]	X3[b]	Sum species	Ref. value total As
NIES No. 18 Human Urine	32 ± 2	35 ± 1	68 ± 4	0.27 ± 0.02	0.79 ± 0.05	1.1 ± 0.1	1.2 ± 0.1	1.8 ± 0.1	0.60 ± 0.04	0.28 ± 0.02	140 ± 7	137 ± 11
NIST 2670a Level 1	1.8 ± 0.1	1.1 ± 0.1	0.84 ± 0.05	n.d.	n.d.	n.d.	n.d	n.d.	0.16 ± 0.01	n.d.	3.9 ± 0.2	3[c]
NIST 2670a Level 2	210 ± 10	2.1 ± 0.1	0.88 ± 0.05	n.d.	n.d.	n.d.	n.d.	n.d.	0.29 ± 0.02	n.d.	220 ± 10	220 ± 10
Seronorm Level 1	5.5 ± 0.3	8.2 ± 0.3	72 ± 4	0.24 ± 0.01	n.d.	0.36 ± 0.02	0.18 ± 0.01	0.82 ± 0.05	0.71 ± 0.04	n.d.	88 ± 5	83 ± 7
Seronorm Level 2	97 ± 5	8.0 ± 0.3	70 ± 4	0.26 ± 0.02	n.d.	0.41 ± 0.03	0.19 ± 0.01	0.87 ± 0.05	0.62 ± 0.04	n.d.	177 ± 9	184 ± 17
Medisafe Metalle U Level 1	47 ± 2	2.2 ± 0.1	7.0 ± 0.4	n.d.	n.d.	n.d.	n.d.	0.19 ± 0.01	0.22 ± 0.01	n.d.	57 ± 3	50 (38.4–61.6)[d]
Medisafe Metalle U Level 2	230 ± 10	2.0 ± 0.1	7.4 ± 0.4	n.d.	n.d.	n.d.	n.d.	0.32 ± 0.02	0.19 ± 0.01	n.d.	240 ± 10	250 (190.0–310.0)[d]
Lyphocheck Level 1	29 ± 2	5.1 ± 0.2	10.6 ± 0.6	n.d.	n.d.	0.46 ± 0.03	n.d.	0.24 ± 0.01	0.18 ± 0.01	n.d.	46 ± 2	29 (23–34)[d]
Lyphocheck Level 2	250 ± 10	8.7 ± 0.3	13.1 ± 0.8	n.d.	n.d.	0.35 ± 0.02	n.d.	0.26 ± 0.02	0.35 ± 0.02	n.d.	270 ± 10	228 (183–274)[d]

[a] Void volume = anionic species not retained on the cation exchange column. The void was quantified against the DMA calibration curve. [b] Approximate retention times for unknowns; X1: 3.5 min; X2: 9.1 min; X3: 9.5 min (peaks are illustrated in Fig. 4). X1 was quantified against the DMA calibration curve. X2 and X3 were quantified against the TMAP calibration curve. [c] Information value. [d] Acceptance range. [e] All results in μg l^{-1} as As given as mean ± 2*s*. Standard deviations as presented elsewhere[29] were used. [f] n.d. = not detected.

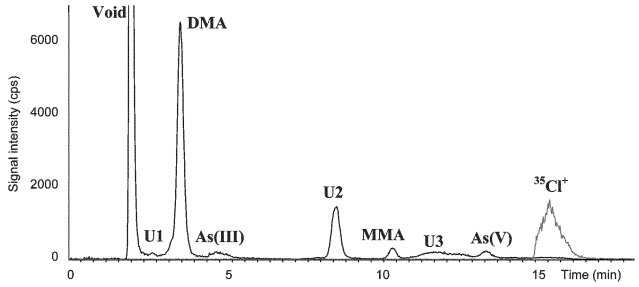


Fig. 3 Chromatogram from anion exchange HPLC-ICP-MS of NIES No. 18 CRM. See Table 2 for information on chromatographic conditions. For peak identification see Table 1. U1–U3 are unidentified arsenic species. Chloride was monitored as $^{35}Cl^+$ and was chromatographically resolved from the arsenic species.

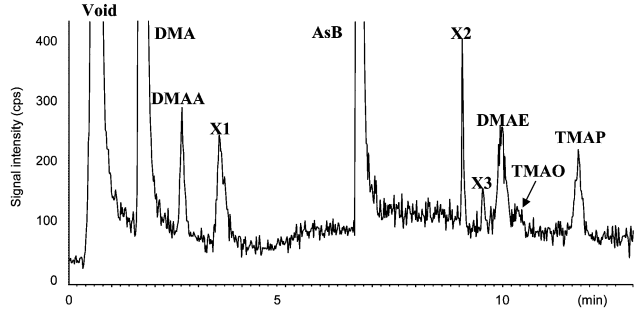


Fig. 4 Chromatogram from cation exchange HPLC-ICP-MS of NIES No. 18 CRM. No peaks were detected with retention time >12.5 min. See Table 2 for information on chromatographic conditions. For peak identification see Table 1. X1–X3 are unidentified arsenic species.

contained in ingested seaweed.[10,32–34] Additionally, DMAA may originate from the consumption of seafood, which may contain this species.[30,36,37]

Conclusions

Two human urine certified reference materials (NIES No. 18 (one level) and NIST SRM2670a (two levels)) and three human urine control materials (Seronorm, Medisafe and Lyphocheck, all two levels) were investigated using both anion and cation exchange HPLC-ICP-MS with respect to arsenic species. Speciation data for As(III), As(V), MMA, DMA, AsB, TMAO, DMAA, TMAP and DMAE were determined by the two different chromatographic approaches. Good agreement was obtained between certified and analysed values for AsB and DMA in NIES No. 18, the only CRM with certified values on the species level. The data reported here provide valuable speciation information and may be useful in the validation process of analytical methods for the speciation of arsenic in urine samples. However, new urine reference materials with certified values for more arsenic species are called for, and especially reference values for the species of greatest toxicological interest, *i.e.*, As(III) and As(V) are needed.

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