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Speciation of Arsenic Compounds in Urine by LC–ICP MS

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The method validation for the speciation of five arsenic species in urine samples by liquid chromatography hyphenated to inductively coupled plasma mass spectrometry is described. Arsenic species which are identified and quantified in urine samples are the anions of arsenic(III), arsenic(V), monomethylarsonic acid and dimethylarsinic acid, and the cationic arsenobetaine. Detection limits were obtained in the range 0.3–0.4 μg As l^{-1} while the repeatability was in the range 3–4% (RSD) for concentrations above five times the detection limit. Urine samples could be analysed directly after a tenfold dilution step. Arsenic compound concentrations were determined in urine samples from a volunteer who consumed a portion of tuna fish high in arsenobetaine. It was found that arsenobetaine was excreted rapidly via the urine with maximum concentrations after 12 h. Nearly complete arsenobetaine excretion was reached after 48 h. Background levels of arsenic compounds were determined in 61 urine samples from non-exposed inhabitants of The Netherlands. © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

Arsenic occurs naturally in foods at low concentrations. For foods from animal and vegetable origins the arsenic content varies from <0.1 and 1.0 μg g^{-1}.[1] However, seafood products generally have higher arsenic contents than other foods, with concentrations up to 25 μg g^{-1}.[1–3] According to the World Health Oganization (WHO), the total daily intake should not exceed 2 μg of inorganic arsenic per kg of body weight.[4]

In the human body about 80% of the inorganic arsenic species are detoxified by methylation, resulting in urinary excretions of 60% dimethylarsinic acid (DMA), 20% methylarsonic acid (MMA) and 20% inorganic acid under normal conditions.[5] Arsenobetaine (AsB), the major constituent in seafood products, is excreted unchanged in the urine.[6] Toxicities of the various arsenic species differ very widely. Inorganic arsenic is the most toxic form while arsenobetaine is almost non-toxic.[7,8] The methylated species MMA and DMA exhibit an intermediate toxicity.[7,8] Human exposure to arsenic can be estimated by determining its concentration in the urine. This is by far the predominant method used to estimate recent exposure. In Europe the total-As concentration in urine of non-exposed individuals is about 10–20 μg l^{-1}.[5,9]

In order to distinguish between the toxic inorganic arsenic compounds (and their metabolic products MMA and DMA) and the less toxic arsenobetaine, a speciation analysis is necessary. Generally arsenic speciation is performed by chromatographic separation and a suitable detection technique. However, recently a modified method for the determination of arsenic in urine using solvent extraction with toluene and graphite furnace atomic absorption spectrometry (AAS) was

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described.[10] With the described method, only inorganic and the mono- and di-methylated species are extracted and subsequently analysed as the sum of these arsenic species. The major drawback of this technique is that this arsenic fractionation technique will not give results for arsenic concentrations of individual species and furthermore it is very laborious. Better techniques are those which first separate the different arsenic species and finally detect these arsenic species, resulting in the concentrations of individual arsenic species. Inorganic arsenic and the methylated species MMA and DMA can be separated by gas chromatography after derivatization and determined with an element-specific detection system.[11–13] When arsenobetaine (AsB) and arsenocholine (AsC) also have to be determined, a liquid-chromatographic separation is the method of choice due to the non-volatility of AsB and AsC. These arsenic species can be determined at low concentrations after LC separation (both anionic and micellar chromatography) with ICP-based instrumentation such as ICP AES (atomic emission, spectrometry) or ICP MS (mass spectrometry).[11,12,14] Speciation analysis for arsenic by LC–ICP MS has been applied mainly to environmental samples.[11–13,15–18] Research papers dealing with arsenic speciation in biological fluids such as urine, blood and serum are rare, probably because legal provisions at present are almost exclusively concerned with the total amount of the element in foodstuffs and drinking water. Arsenic speciation in urine samples from exposed rats have been reported before, resulting in good detection limits (approx. 0.4 μg As l^{-1}) and reproducibility (3–5%).[19] However, detection limits were calculated as the square root of blanks and the reproducibility was calculated by analysing high-arsenic standard solutions (100 μg As l^{-1}), not indicating that similar results would be obtained in real-life samples. Using an LC–hydraulic high-pressure nebulizer system with ICP MS, good results were reported: detection limits of 0.5 μg As l^{-1} for several arsenic species and a good reproducibility of 2–4% at the 5 μg As l^{-1} level.[20]

In this paper the validation is described for the speciation analysis of arsenic in urine by LC–ICP MS using a normal cross-flow nebulizer. The species of interest are As (III), As (V), MMA, DMA and AsB. The method validated is compared with a total-arsenic determination by UV digestion–hydride AAS.[21] Finally, results from urine samples are presented. An demonstration experiment (fish consumption) is described, as well as a pilot study for the determination of background arsenic levels in urine samples from inhabitants of The Netherlands.

EXPERIMENTAL

Reagents and standards

The stock solution of As(V) (1000 mg l^{-1} in 5% nitric acid) was from Perkin-Elmer, Norwalk, CT, USA, and the stock solution of As (III) (1000 mg l^{-1} in 0.3 M hydrochloric acid) (Instra-analysed) was from J. T. Baker Deventer, The Netherlands, MMA $\{CH_3AsO(OH)_2\}$, DMA $\{(CH_3)_2AsO(OH)\}$ and AsB $\{(CH_3)_3As^+CH_2COO^-\}$ were obtained from Tri Chemical Laboratory, Japan. All arsenic standards used had a purity of 99.9% or more. The species purity of these individual standards was checked with LC–ICP MS in order to establish that the individual arsenic species standard did not contain other arsenic species. The concentration was checked against As(III) using inductively coupled plasma/optical emission spectroscopy (ICP/OES). No inadequacies were found in the purity of these standards. All other chemicals used were of *pro analysi* quality or equivalent and the water was produced by a Milli-Q system (Millipore, Milford, MA, USA).

Certified reference materials for arsenic species were not available, so a reference material with certified concentrations for total arsenic was selected, namely NIST-SRM-2670 (Standard Reference Materials, Gaithersburg, MD, USA), comprising: (1) elevated-level human urine, spiked with arsenate, certified total-As concentration 480 ± 100 μg l^{-1} (error expressed as 95% confidence interval of the mean value); and (2) normal-level human urine, indicative value 60 μg As l^{-1}.

Analytical instrumentation and instrumental settings

Arsenic speciation by LC–ICP MS

For the LC separations a Spectra System P2000 (Thermo Separation Products, The Netherlands) HPLC system and a Perkin-Elmer Advanced Sample Processor ISS 200 (Perkin-Elmer, Überlingen, Germany) with a 100 μl injection loop were used in conjunction with an anion-exchange column with a mobile phase, as indicated in Table 1.

The chromatographic system was interfaced to the Perkin-Elmer SCIEX ELAN 6000 (Perkin-

Table 1 Operating conditions for the ICP MS and the chromatographic system.

Inductively coupled plasma mass spectrometry	
RF power	1100 W
Spray chamber	Ryton™ Scott-type
Nebulizer	Cross-flow
Argon flow rates:	
Plasma/coolant	17 l min^{-1}
Auxiliary	1.2 l min^{-1}
Nebulizer	0.7 l min^{-1}
Dwell time	500 ms
Sweeps per reading	1
Readings per replicate	750
Number of replicates	1
Chromatography	
Anion-exchange column	ION 120 (125 mm × 3 mm i.d.), Interaction Chromatography (Mountain View, CA, USA)
Mobile phase	Ammonium carbonate buffer adjusted to pH 10.3 with ammonia. Gradient 0.0–3.1 min, 0.005 mol l^{-1} immediately followed by a 0.2 mol l^{-1} buffer solution
Flow rate	1 ml min^{-1}
Injected volume	100 µl
Chromatography software	Turbochrom Navigator, version 4.1

Elmer SCIEX, Thornhill, ON, Canada) ICP MS instrument by 30 cm of PEEK (polyetheretherketone) tubing (0.17 mm i.d.). The chromatographic effluent flow was compatible with the uptake requirements of the ICP MS sample introduction system, consisting of a Ryton™ Scott-type spray chamber and cross-flow nebulizer.

The mass spectrometer was set to monitor the ion intensity at *m*/*z* 75 ($^{75}As^{+}$). The ICP MS was tuned daily: the nebulizer flow rate, auto-lens voltage, radio-frequency power and MS resolution were optimized by continuously aspirating a standard solution of 20 µg l^{-1} of In, Co and Mg.

The raw ion-intensity data for *m*/*z* 75 were transported to PE Nelson Turbochrom Navigator software (version 4.1), peak areas were determined, and concentrations were calculated by external calibration curves.

Total-arsenic determination by UV digestion–hydride AAS

The decomposition of organic bound arsenic was performed by UV irradiation as described previously.[21] In short, a fivefold diluted urine sample was subjected to UV radiation (254 nm) at enhanced temperature (95 °C) for 8 h. During this period all organic bound arsenic compounds were quantitatively decomposed, resulting in inorganic arsenic. To 5 ml of the digested urine samples 0.5 ml of freshly prepared KI/ascorbic acid (both 5%, w/v) and 1.5 ml HCl (37%, w/v) were added in order to reduce arsenate to arsenite. A Perkin-Elmer FIAS-2100 (Norwalk, CT, USA) system was used to produce on-line arsines. Instrumental parameters were: wavelength 193.6 nm; slit width 0.7 nm; integration time 10 s, quartz-cell pathlength 180–190 mm. Samples were quantified by the external calibration method. The limit of detection (defined as LOD = 3 × SD, where SD is the standard deviation of the arsenic concentration in a urine sample near the LOD) for urine samples is 0.35 µg As l^{-1} while the precision is better than 5% at concentrations > 3 µg As l^{-1}. The linear range is 0.35–40 µg As l^{-1}.

Influence of methanol on the LC–ICP MS signal intensity

It is known that addition of carbon as methanol to the aqueous analyte solutions enhances ICP MS sensitivity for arsenic.[13,15,17] The amount of methanol needed for optimum sensitivity varies between 2 and 5%, depending on the instrumentation used.[13,17] It was proposed that an increased population of carbon or carbon-containing ions in the plasma facilitates a more complete ionization of elements lower in ionization energy than carbon itself. In order to obtain maximum signal intensities for the instrumentation used, various percentages of methanol were added to the LC buffers being used.

Validation of the LC–ICP MS method for the speciation of arsenic concentrations in urine

In order to obtain the performance characteristics of the method the following parameters were determined:

Limit of detection, defined as LOD = 3 × SD, where

SD is the standard deviation of the arsenic concentration in a 'real-life' sample near the LOD.

Repeatability, defined as $r = 2s_r\sqrt{2}$, where s_r is the repeatability standard deviation.

Accuracy, which can be checked with certified reference materials; the value obtained must fall within the 95% confidence interval.

Recovery, which can be checked by addition of standard solutions to the sample.

The method developed was checked by comparing the results obtained for several urine samples with a total-arsenic determination.

Urine samples

With the validated method two sets of experiments were performed: (1) a pilot study for the determination of background arsenic levels in urine samples from Dutch inhabitants, and (2) a study of the excretion of arsenic species after fish consumption.

To obtain an impression of background levels of arsenic species from healthy individuals, 61 urine samples were collected from all over the Netherlands. In a questionnaire participants were asked to give details of their food consumption in the past 48 h, especially in relation to the consumption of fish and fish products. Urine samples were collected between July 1996 and November 1996, and were stored in polyethylene sample containers at 4 °C. Samples were diluted 10-fold before analysis by LC–ICP MS. Total-arsenic concentrations were stable for at least three weeks.[21]

In order to study the excretion rate of arsenic species after eating one portion (250 g) of fresh tuna fish, urine samples were collected from a male volunteer during six consecutive days, during which he did not ingest any other fish product.

RESULTS AND DISCUSSION

LC–ICP MS

Optimization of the separation

The LC separation was optimized by applying various gradients and molarities of the ammonium carbonate/ammonia buffer. The main objective was to achieve a baseline–baseline separation of AS(III), As(V), MMA and DMA from each other and from cationic species such as AsB. Furthermore, chloride from the urine sample had to elute after all arsenic species of interest because the formation of the polyatomic $^{40}Ar^{35}Cl$ interferes with the detection of arsenic at *m*/*z* 75. A baseline–baseline separation of all five arsenic species of interest was achieved by applying the gradient as mentioned in the Experimental section. The chloride interference eluted 3 min after the elution of the last arsenic species [As(V)]. A chromatogram for the separation of the five arsenic species is shown in Fig. 1.

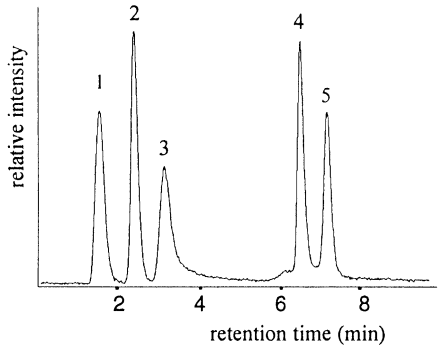

Figure 1 Chromatogram of anion-exchange ICP MS of five injected arsenic compounds. Peak identification: 1, AsB; 2, DMA; 3, As(III); 4, MMA; 5, As(V).

Influence of methanol on the column effluent

To the buffer solutions, 0–3% methanol was added in order to obtain maximum ICP MS intensities for the five arsenic species in a 'normal-level' urine sample. Results are given as signal intensity plotted against methanol percentage in Fig. 2 from which it is clear that maximum sensitivity is obtained with a

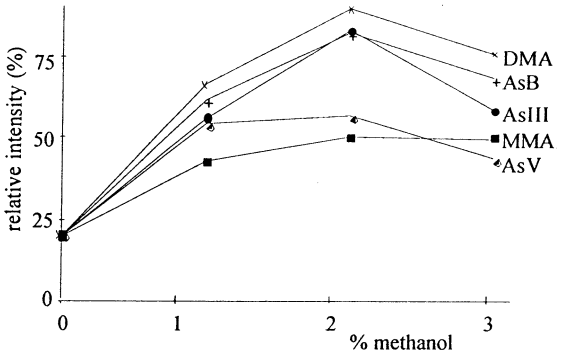

Figure 2 ICP MS response for five arsenic species as a function of the various methanol concentrations in the buffer solutions applied.

Table 2 Repeatability tested on a 'real-life' urine sample ($n = 10$)

Arsenic species	Concentration (μg As l^{-1})	Standard deviation (s_r) (μg As l^{-1})	RSD (%)	Repeatability ($2s_r \sqrt{2}$) (μg As l^{-1})
AsB	49.8	1.7	3.4	4.8
MMA	2.15	0.07	3.2	0.20
DMA	2.37	0.08	3.4	0.23
As(III)	< 0.40	—	—	—
As(V)	2.00	0.09	4.5	0.24

methanol addition of 2%. At this concentration the sensitivity for the different arsenic species increased by a factor of 2–4 compared with that obtained by applying buffer solutions without methanol. The sensitivity enhancement is in the same range as enhancements described earlier.[13,17]

Performance characteristics

Calibration mode and recovery

In order to check possible effects matrix, of the five different urine samples were analysed by both the standard addition technique and by an external calibration. The slopes of standard-addition calibrations were compared with the slopes obtained for normal calibrations, showing addition recoveries of 94% for AsB and 99–100% for the other arsenic species; this indicated minor matrix effects. For reasons of efficiency the external calibration procedure was used to quantify arsenic concentrations. The cationic arsenic species, e.g. AsB, arsenocholine (AsChol), tertramethylarsonium ion (TMAs) and trimethylarsine oxide (TMAO), are not retained on the anion-exchange column that was used, and consequently co-elute as one peak at the beginning of the chromatogram. In order to separate these cation arsenic species, cation exchange must be applied. However, from a toxicological point of view the main target in this study was to separate inorganic arsenic species and their metabolites from the other arsenic species. Furthermore, AsB is the most abundant cationic As species in human urine.[9,20] In order to quantify the cation species as AsB, the sensitivities of the cationic arsenic species AsChol, TMAs and TMAO were compared with the sensitivity of AsB. It was found that the sensitivities of these cations deviated less than 4% from the sensitivity of AsB. Therefore the sum of the cationic species can be quantified by an AsB calibration curve and the sum of the cationic arsenic species in urine samples will be expressed as AsB.

Detection limits

Detection limits, defined as $3 \times$ SD as before, were determined in real-life urine samples ($n = 10$) with arsenic species concentrations between 0.4 and 2.3 μg As l^{-1}. Detection limits were found to be 0.3 μg As l^{-1} for AsB and MMA, and 0.4 μg As l^{-1} for As (III), As (IV) and DMA in the non-diluted urine. These detection limits are very suitable for arsenic species determination in urine and are comparable with, or better than, detection limits reported in Refs 16–18.

Repeatability

The repeatability ($2S_r\sqrt{2}$) was tested on a real-urine sample, high in AsB. Each determination was performed 10 times and the results are shown in Table 2. At higher arsenic concentrations ($>10 \times$ LOD) the repeatability expressed as RSD, reaches a level of 3% or better, which in fact is almost the same as for normal ICP MS determinations. At lower concentrations near the detection limit the repeatability is of course influenced by the uncertainty of the analysis.

Validation of the method

Certified reference materials are not available for arsenic species in urine. Reference materials available for total arsenic in urine were found to be spiked with As (V) and to contain a maximum of 14% of organic arsenic species.[23] The NIST SRM-2670, both normal- and elevated-levels, were analysed for arsenic species and the results were compared with the certified (or indicative value in the case of the 'normal-level' urine) total concentrations. The results are presented in Table 3. The same material has been subjected to an As speciation determination recently by another group, whose results are also presented in Table 3.[20]

The concentration of total arsenic (430 μg l^{-1}) found in this study is in good agreement with the certified value of 480 ± 100 μg l^{-1} for the elevated

Table 3 Concentrations for arsenic species in SRM-2670[a] reference urine determined by LC–ICP MS, compared with literature data

	Concentration (μg As l^{-1})			
	This study		Data of Goessler *et al.*[b]	
Arsenic species	Normal level	Elevated level	Normal level	Elevated level
AsB	16.0 ± 1.1	16.2 ± 1.1	21.2 ± 3.7	24.7 ± 0.7
MMA	9.4 ± 0.9	9.2 ± 0.9	9.5 ± 3.0	10.9 ± 2.1
DMA	47.5 ± 1.6	50.7 ± 1.8	48.2 ± 2.4	51.6 ± 3.4
As(III)	< 0.4	< 0.4	15.0 ± 4.5	13.1 ± 4.5
As(V)	6.9 ± 0.8	354 ± 17	2.9 ± 0.7	386 ± 51

[a] Normal-level indicative value 60 μg l^{-1} total arsenic; elevated-level certified value 480 ± 100 μg l^{-1} total arsenic.
[b] Ref. 20.

level. Comparison of our results with speciation data published earlier by Goessler *et al.* for this SRM shows differences for the arsenic species AsB and arsenite.[20] In our study we could not detect any arsenite whereas Goessler *et al.* reported 13 μg l^{-1}.[20] This difference could appear from species transformation after reconstitution of the freeze-dried material. For the elevated level of SRM-2670 we found an AsB concentration of 16.2 ± 1.1 μg As l^{-1} whereas Goessler *et al.* reported 24.7 ± 0.7 μg As l^{-1}.[20] These differences in arsenic concentrations observed in the two studies indicate the need for certified reference materials for arsenic species in an urine matrix.

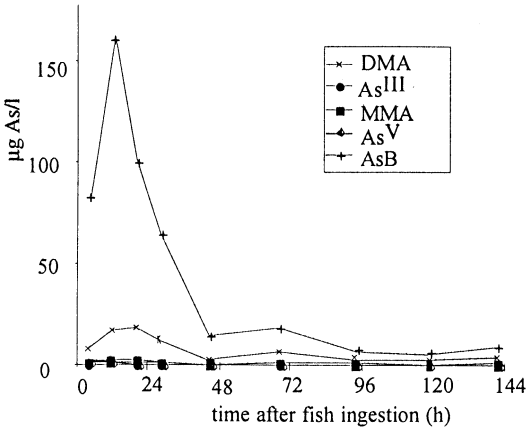

Figure 3 Dependence of concentrations of arsenic species in urine samples on time after ingestion of 250 g tuna fish (t = 0).

Comparison of speciation analysis with a total-arsenic determination

Verification of the method by another technique was performed by analysing seven urine samples both by LC–ICP MS and by UV–hydride AAS for a total-arsenic determination. A wide range of arsenic concentrations were chosen to check the speciation method. In Table 4 the individual and summed arsenic concentrations in the urine samples obtained by LC–ICP MS are given, together with the UV–hydride AAS results for total-arsenic determination. The results obtained were evaluated by applying the so-called Deming regression.[22] With this type of regression, two independent methods

Table 4 Comparison of two independent methods for the determination of total arsenic[a] in urine samples

	Speciation by LC–ICP MS						UV–hydride AAS
Sample	AsB	MMA	DMA	As^{III}	As^{V}	Sum	Total arsenic
A	370	1.51	32.5	2.89	0.71	408	435
B	27.5	2.67	8.83	3.09	1.38	43.5	42.0
C	0.34	0.56	1.84	0.60	0.40	3.74	2.66
D	18.1	0.40	8.07	0.40	0.64	27.6	25.3
E	70.6	0.98	1.86	1.24	0.40	75.1	96.2
F	183	0.30	19.7	0.84	0.63	204	200

[a] All concentrations are in μg As l^{-1}.

can be compared. It was calculated that the two methods yield slightly different results. The UV–hydride AAS gives 2–5% (95%, 5 degrees of freedom) lower results than the LC–ICP MS method. The difference in the results obtained is quite small compared with the repeatability of both methods, so when speciation analysis is requested in future the results obtained can always be compared with data obtained earlier for total arsenic in urine samples. This good comparison is of the utmost importance when analytical methods are changed during monitoring programmes.

Excretion of arsenic species

Excretion after fish consumption

Results of the excretion rate of arsenic species after eating one portion (250 g) of fresh tuna fish are depicted graphically in Fig. 3, which shows the arsenic species concentration versus the time after

Table 5 Arsenic species background concentrations[a] in urine samples from inhabitants of The Netherlands

No fish consumption				Fish consumption			
Sample	AsB	MMA	DMA	Sample	AsB	MMA	DMA
1	0.0	0.0	0.4	44	0.0	0.0	2.7
2	0.0	0.0	0.8	45	0.6	0.0	2.3
3	0.0	0.0	1.0	46	0.8	0.0	3.0
4	0.0	0.0	1.6	47	1.6	0.0	2.3
5	0.0	0.0	1.8	48	2.6	0.0	3.0
6	0.0	0.0	1.8	49	5.6	10.0	2.8
7	1.0	0.0	0.9	50	14.5	0.0	4.6
8	0.0	0.0	2.0	51	25.2	0.0	1.9
9	0.0	0.0	2.4	52	16.2	0.0	11.8
10	0.0	0.0	2.4	53	22.2	0.0	6.8
11	0.0	0.0	2.7	54	32.0	0.0	2.1
12	2.2	0.0	0.6	55	23.4	7.0	10.0
13	0.0	0.0	2.9	56	37.8	2.0	6.0
14	0.0	0.8	2.4	57	19.7	3.0	28.1
15	2.3	0.0	1.1	58	48.7	2.5	29.1
16	1.9	0.0	2.2	59	75.9	0.0	13.4
17	2.0	0.0	2.3	60	93.6	1.9	6.9
18	2.4	0.0	3.7	61	212	5.5	10.6
19	0.0	4.2	2.2				
20	3.7	0.9	2.6				
21	2.8	0.0	4.6				
22	0.0	5.5	2.0				
23	3.8	1.1	2.7				
24	5.6	0.0	2.4				
25	2.9	0.0	5.3				
26	0.0	0.0	9.3				
27	0.5	3.2	7.2				
28	9.2	0.0	6.2				
29	8.6	2.8	4.7				
30	8.2	0.0	8.0				
31	2.2	4.9	9.7				
32	6.6	2.1	9.7				
33	13.5	0.0	5.6				
34	15.5	0.0	3.8				
35	12.3	2.1	5.1				
36	5.9	6.7	8.1				
37	10.3	4.0	8.4				
38	0.0	17.0	9.4				
39	15.0	4.0	9.8				
40	25.0	7.7	5.5				
41	55.2	1.6	6.5				
42	67.0	2.0	7.0				
43	114	2.6	4.7				

[a] All concentration are in μg As l^{-1}.

fish ingestion. Total-arsenic concentration reaches a maximum (180 μg As l^{-1}) after 12 h, then decreases slowly to approximately 10 μg As l^{-1} after 100 h. AsB is the main constituent of the total arsenic present in the urine sample, ranging from 88% after 12 h to 58% after 94h. Excretion of AsB is almost complete within 48h. DMA follows the same pattern as AsB. Whether this DMA is a metabolic product of inorganic arsenic or was already present in the fish is unclear. Results of this study are in good agreement with results from Le *et al*. for total-arsenic determination in urine after consumption of fish products.[6] In their study they also observed a rapid excretion of total arsenic (70% after 37 h).

Background levels of arsenic species in non-exposed Dutch inhabitants

To obtain an impression of arsenic species background levels in urine samples from healthy individuals in the Netherlands, samples were collected ($n = 61$) and analysed by LC–ICP MS. The results are presented in Table 5. MMA concentrations ranged from below the detection limit to 17 μg As l^{-1}, DMA ranged from 0.4 to 29 μg As l^{-1} and AsB ranged from below the detection limit to 213 μg As l^{-1}. During these analyses we found much higher blank levels for As(III) and As(V) (standard deviation 1.7 μg As l^{-1}) than are normally observed, resulting in detection limits for arsenite and arsenate of at least 5 μg As l^{-1}. Due to these high detection limits we could not detect any positive concentration for either of the inorganic arsenic species in the samples analysed. These high blanks originated from the levels in the glass sample vials, as was found in later experiments. Polypropylene sample vials did not result in higher blank levels and will be used in future experiments.

People who ate fish during the preceding 48 h had four times higher AsB concentrations based on averages in their urine than people who had not ingested fish. Many volunteers said they did not consume fish or fish products in the previous 48 h, yet many of them had high concentrations of AsB in their urine. They had probably consumed 'hidden' fish products or products which also contained AsB. Poultry can contain arsenobetaine because they are normally fed with fish-containing products. Seaweed is also a well-known food product containing high levels of As species[6] and even some mushroom species contain AsB.[24]

Besides a speciation analysis, a total-arsenic determination was also performed. The mean arsenic concentration determined by UV–hydride AAS was 24.8 μg As l^{-1} while arsenic speciation yielded a summed (AsB, MMA and DMA) concentration of 24.1 μg As l^{-1}. Even without neglecting inorganic arsenic species, the sums of AsB, MMA and DMA obtained by LC–ICP MS were not significantly different from the UV–hydride AAS total-arsenic determination, according the Deming regression.[22]

Concentrations found in this study are in the same range as results described earlier by Goessler *et al*., who observed total-arsenic concentrations of between 9 and 315 μg As l^{-1}. However, the total-arsenic concentrations are much higher than the concentrations of 10–20 μg As l^{-1} mentioned previously in the literature.[5,9] It is most likely that these numbers reflect only the inorganic forms of arsenic, and probably just fractions of the MMA and DMA.

CONCLUSIONS

The validated method described here for the speciation of arsenic compounds in a urine matrix is very sensitive and has a good potential for obtaining more information about the toxic fraction of arsenic in urine. The method is fast, with a high sample throughput (approx. 50 samples per day). The comparability of the summed As species concentrations and the total-arsenic determination by UV–hydride AAS is good.

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