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Speciation of five arsenic species (arsenite, arsenate, $MMAA^V$, $DMAA^V$ and AsBet) in different kind of water by HPLC-ICP-MS

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Abstract

A method using Ion Chromatography hyphenated to an Inductively Coupled Plasma-Mass Spectrometer has been developed to accurately determine arsenite (As^{III}), arsenate (As^V), mono-methylarsonic acid ($MMAA^V$), dimethylarsinic acid ($DMAA^V$) and arsenobetaine (AsBet) in different water matrices. The developed method showed a high sensitivity with detection limits for each arsenic species close to 0.4 pg injected. Arsenite and arsenate were the major species found in surface and well waters, but AsBet and $DMAA^V$ were found in some surface waters, which has never been reported before, while in some natural mineral waters located in volcanic region, the arsenic content exceeded the maximal admissible arsenic content by European legislation standards and the predominant form was As^V.

Keywords: Arsenic; IC-ICP-MS; Speciation; Trace analysis

1. Introduction

Arsenic exists under the form of various chemical species differing, not only by their physicochemical behaviour, but also in toxicity, bioavailability and biotransformation (Maeda, 1994). With more than 20 arsenic compounds present in the natural environment and biological systems, it is important to identify each individual chemical species of the element in the samples (Le et al., 2004). For this reason, the notion of speciation, defined as the determination of all the individual physicochemical forms of an element, has gained interest in recent years.

Arsenite (As^{III}) and arsenate (As^V) are the most toxic forms, considered and classified as human carcinogen substances and are the predominant forms found in water. Biologically-mediated methylation reactions, occurring in terrestrial and marine organisms, convert arsenite and arsenate to methylated compounds of moderate toxicity, such as methylarsonic acid ($MMAA^V$) and dimethylarsinic acid ($DMAA^V$). Arsenobetaine (AsBet), a more complex arsenic compound, was identified essentially in marine biota (Goessler et al., 1998a,b; Ackley et al., 1999; Kohlmeyer et al., 2002) or in marine sediments (Takeuchi et al., 2005) and is considered to be relatively non-toxic (Kaise et al., 1985).

The admissible level of arsenic in natural mineral water (Commission Directive 2003/40/CE of 16 May 2003) and in water intended for human consumption (Commission Directives 98/83/CE of 3 November 1998), has been lowered to 10 $\mu g\,l^{-1}$ of total arsenic. However, as reported previously in literature (Van Holderbeke et al., 1999), some

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commercially available natural mineral water exceeded this limit of 10 $\mu g\,l^{-1}$.

In order to assess the individual forms of arsenic, which may be present in water, and thus the potential toxicity, the analytical equipment must achieved very low detection limits.

Hyphenated analytical techniques are often necessary to achieve both selectivity and sensitivity for arsenic speciation at a low concentration level. Numerous instrumental methods for these five arsenic species speciation are reported in the literature. Most of them are based on chromatographic separation techniques such as High Performance Liquid Chromatography (HPLC) (Gailer and Irgolic, 1996; Teräsahde et al., 1996; Le and Ma, 1997; Dagnac et al., 1999; Kohlmeyer et al., 2002) or Capillary Zone Electrophoresis (Van Holderbeke et al., 1999), coupled with a selective and sensitive detector such as Atomic Absorption Spectrometry (AAS) (Gailer and Irgolic, 1996) or Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Teräsahde et al., 1996; Kohlmeyer et al., 2002). Post column derivatization of arsenic species with formation of the volatile arsine hydrides, has been coupled with AAS (Le and Ma, 1997), Atomic Fluorescence Spectrometry (Simon et al., 2004) or ICP-MS (Dagnac et al., 1999), but cannot be used for quantifying AsBet without prior chemical conversion (Slejkovec et al., 1999).

The hyphenated HPLC-ICP-MS technique is the most powerful method for arsenic speciation, allowing detection limits generally lower than 0.5 $\mu g\,l^{-1}$ (B'Hymer and Caruso, 2004, and references therein). The advantages associated with the HPLC-ICP-MS technique include high elemental specificity, the possibility to record real time chromatograms, the ability to separate the signals of interfering species from the peaks of interest, a high linear range, a multi-element capability and the possibility of isotopic information. The high sensitivity of ICP-MS allows the analysis of water samples without time-consuming preconcentration and derivatization steps. This consideration is crucial as species may be converted from one form to another or lost during the pre-treatment of the sample (Gong et al., 2002). However, the use of ICP-MS as a detector for HPLC gives rise to some constraints on the choice of chromatographic conditions concerning the nature and concentration of the buffer salts of the mobile phase and the presence of organic solvents. Moreover, because of its high sensibility, ICP-MS may suffer from many isobaric interferences caused mainly by phenomena occurring either in the plasma or in the ion extraction device. For example, presence of chlorine in the sample may give rise to the formation of $^{40}Ar^{35}Cl^{+}$ that interferes strongly with the mono-isotopic $^{75}As^{+}$ (Gray, 1986; Hywel Evans and Giglio, 1993).

For these reasons, the aim of this work was to develop a powerful speciation method suitable for trace analysis of all arsenic species found in drinking and surface water with appropriate performance characteristics in order to identify and quantify each arsenic species.

2. Experimental

2.1. Standards and reagents

Stock solutions of arsenic species (1000 $mg\,l^{-1}$) were prepared from $NaAsO_2$ (arsenite, As^{III}), $Na_2HAsO_4 \cdot 7H_2O$ (arsenate, As^{V}), $(CH_3)_2AsO(OH)$ (dimethylarsinic acid, $DMAA^{V}$), $CH_3AsO(OH)_2$ (mono-methylarsonic acid, $MMAA^{V}$) and $(CH_3)_3As^{+}CH_2COO^{-}$ (arsenobetain, AsBet). As^{III}, As^{V} and $DMAA^{V}$ were obtained from Sigma–Aldrich (Steinheim, Germany) while $MMAA^{V}$ and AsBet were obtained from Argus Chemicals (Florence, Italy). These individual standards were checked for purity by IC-ICP-MS, and the stock solutions were stored at 4 °C before analysis. In these conditions, the stock solutions were stable for at least one month. Analytical working solutions were prepared by diluting the stock solutions with ultrapure water (18.2 $M\Omega\,cm^{-1}$, Millipore, Molsheim, France) prior to analysis.

For the chromatographic mobile phase, $NH_4H_2PO_4$ (ammonium dihydrogen phosphate, >99%, Sigma–Aldrich, Steinheim, Germany), NH_4OH (ammoniac 25%, Suprapur®, Merck, Darmstadt, Germany) and methanol (Suprasolv®, Merck, Darmstadt, Germany) were used.

2.2. Instrumentation

The High Performance Liquid Chromatography (HPLC) module consisted of an Agilent 1100 Series (Agilent Technologies, Yokogawa Analytical System Inc., Tokyo, Japan) with a handheld control module. The separation of the arsenic species was performed on a Dionex AS7 anion-exchange column (250 × 4 mm; 10 μm) bearing alkyl quaternary ammonium exchange sites on a styrene-divinylbenzene copolymer. 20 μl of the sample was injected in the chromatographic column.

The Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) system consisted of an ICP-MS Hewlett-Packard 4500 Series 200 (Agilent Technologies, Yokogawa Analytical System Inc., Tokyo, Japan). A 40 cm PEEK® tubing (PolyEther Ether Ketone, 1/16″ OD X 0.010″ ID Blue-Stripe) with appropriate fittings was used to connect the outlet of the HPLC analytical column directly to the inlet of the ICP concentric nebulizer (Conikal® Concentric Nebulizer-STF, Glass Expansion, Switzerland). A double-pass spray chamber and a quartz torch were used in this study. The temperature of the spray chamber was maintained at 2 °C by cooling Peltier Effect. In accordance with B'Hymer et al. (1998), this concentric nebulizer/double pass spray chamber combination allows the best signal stability.

In addition to the ICP-MS Hewlett-Packard 4500 Series 200 (only used for the coupling with HPLC), an ICP-MS Hewlett-Packard 4500 Series 100 (Agilent Technologies, Yokogawa Analytical System Inc., Tokyo, Japan) was used for the total arsenic concentration in water. The ICP-MS Hewlett-Packard 4500 Series 100 was equipped with a Babington nebulizer.

2.3. Tuning and data acquisition

Before all analyses, the instrument was tuned using a 10 μg l^{-1} lithium (^{7}Li), yttrium (^{89}Y), thallium (^{205}Tl) and cerium (^{140}Ce) solution (all Certipur®, obtained from Merck, Darmstadt, Germany). Resolution and mass axis were optimized by monitoring m/z ^{7}Li, ^{89}Y and ^{205}Tl. Sensitivity was maximized at m/z ^{89}Y, allowing a very high sensitivity for ^{75}As, and thus very low detection limits, while maintaining the ratio of oxides ($^{140}Ce^{16}O/^{140}Ce$) and doubly charged ions ($^{140}Ce^{2+}/^{140}Ce^{+}$) at a low level to minimize the potential interferences.

For IC-ICP-MS data acquisition, the time resolved analysis mode was used. In addition of the arsenic signal at m/z 75, the interferences from chloride were also checked by monitoring m/z 35, 77, 82 and 83. The quantification of the chromatographic peaks was based on the peak area.

2.4. Speciation of arsenic in water

Surface, well and natural mineral water samples were analysed by IC-ICP-MS. These samples were collected from several locations in the Walloon Region (Belgium) and stored in polyethylene flasks at 4 °C without acidification to prevent changes in species distribution. The analyses were carried out within one week. Natural mineral waters were obtained from a local market, and covering different geological origins. The sum of arsenic species concentrations was compared to the total arsenic content obtained by ICP-MS analysis. All real samples were filtered through 5, 0.45 and 0.2 μm filter membranes (all Acrodisc® 25 mm Syringe filter with Versapor® membrane, Gelman Sciences, MI, USA) directly into the auto sampler vial and injected in the chromatographic system. All samples were analysed in triplicate.

3. Results and discussion

The optimized chromatographic conditions and the instrumental parameters used for IC-ICP-MS are summarized in Table 1.

3.1. Set up of the chromatographic elution

In HPLC, Ion Chromatography (IC) is an attractive technique for elemental speciation because it can separate charged species. Except AsBet, which is a zwitterion, all the other arsenic species of this study have a range of dissociation constants making them suitable for anion exchange column, as they exist in anionic form in alkaline mobile phase (Teräsahde et al., 1996). Na_2HPO_4 and NaH_2PO_4 are often used as mobile phase for the arsenic species separation, but deposition of salt on the sampling interface causes a rapid degradation and instability of the signal. For this reason, the selected mobile phase used in this study was ammonium dihydrogen phosphate ($NH_4H_2PO_4$), less deposit was observed together with a good stability of the signal. In the investigated $NH_4H_2PO_4$ concentration range (1–50 mM), the order of elution was AsBet, $DMAA^{V}$, As^{III}, $MMAA^{V}$ and As^{V} (Fig. 1). $NH_4H_2PO_4$ 5 mM gave the best compromise between a good AsBet/$DMAA^{V}$ resolution and minimum tailing for As^{III}. Furthermore, the effect of the pH of the mobile phase was tested (7.0, 8.0, 9.0 and 10.0) by adjusting with NH_4OH 1.35 M. Increasing the

Table 1
Instrumental settings

Total arsenic			
ICP-MS	HP 4500 Series 100		
Argon plasma gas flow rate (l min^{-1})	15		
Argon carrier gas flow rate (l min^{-1})	1		
Argon auxiliary gas flow rate (l min^{-1})	1		
Peristaltic pump flow rate (l min^{-1})	1		
Power (W)	1400		
Arsenic speciation			
HPLC	Agilent 1100 Series		
Analytical column	Dionex IonPak AS7 (250 × 4 mm; 10 μm)		
Mobile phase A	2.5 mM $NH_4H_2PO_4$ pH 10.0 (NH_4OH)		
Mobile phase B	50 mM $NH_4H_2PO_4$		
Gradient program	Time (min)	A (%)	B (%)
	0–4	100	0
	15–20	0	100
	24–30	100	0
ICP-MS	HP 4500 Series 200		
Argon plasma gas flow rate (l min^{-1})	15		
Argon carrier gas flow rate (l min^{-1})	0.7		
Argon auxiliary gas flow rate (l min^{-1})	1.0		
Argon blend gas flow rate (l min^{-1})	0.5		
Power (W)	1400		
Monitored signals (dwell times in ms)	^{75}As (500), ^{35}Cl (100), ^{82}Se (100) and ^{83}Kr (100)		

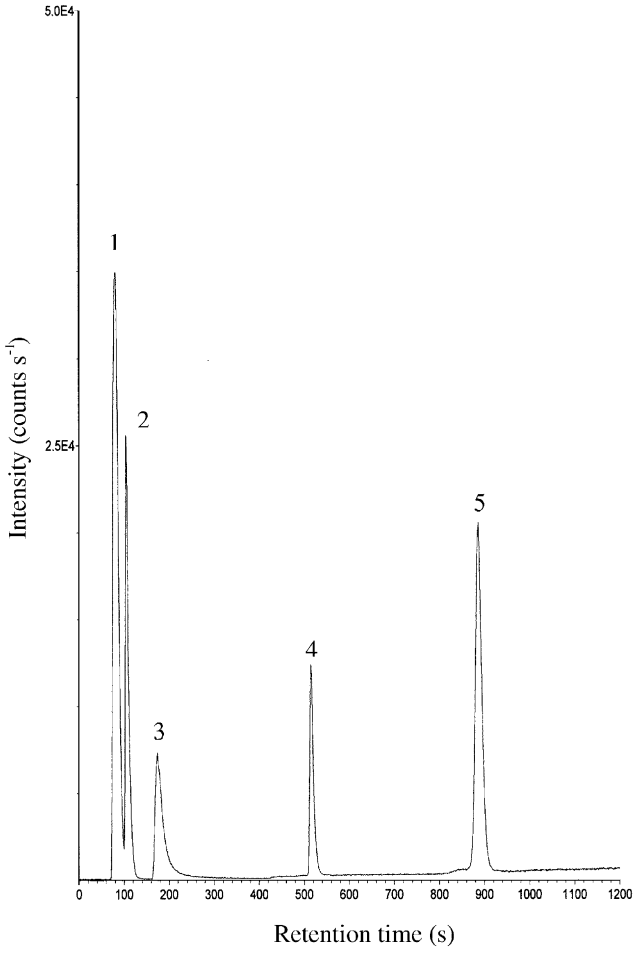

Fig. 1. Anion exchange HPLC-ICP-MS chromatogram of a 20 µl injected standard solution containing 5 µg l^{-1} of each arsenic species. Peak identification: (1) AsBet, (2) $DMAA^V$, (3) As^{III}, (4) $MMAA^V$ and (5) As^V. The analysis was performed according to the optimum conditions shown in Table 1.

pH of the mobile phase led to a higher ionisation degree of arsenic compounds according to their pK_a and should induced a higher affinity for the available exchange sites on the column and thus their retention time. But, the dissociation of the phosphate ions of the buffer, and thus the eluent power of the mobile phase, also increased with the pH. The retention time obtained as a function of the pH is the result of these antagonist phenomenons. For this reason, a 2.5 mM $NH_4H_2PO_4$ solution adjusted to pH 10.0 with NH_4OH allowing a good AsBet/$DMAA^V$ separation together with a minimum tailing for arsenite, was selected. This mobile phase was suitable for the separation of AsBet, $DMAA^V$ and As^{III}, but not for $MMAA^V$ and As^V due to the very long retention time. Consequently, an elution gradient with $NH_4H_2PO_4$ 50 mM was used. This gradient allowed a correct separation of the five molecules within 30 min including column re-equilibration time. In order to improve the ionisation of arsenic in the plasma, 3% (v/v) of methanol have been added in the mobile phase (Beauchemin et al., 1989; Larsen and Stürup, 1994). In spite of the fact that Brisbin et al. (2002) claimed that the use of carbonate as mobile phase brings less deposit than phosphate, no signal decrease was observed with ten repetitions. The relative standard deviations of the peak area were 1.5, 1.5, 2.0, 0.8 and 1.6% for AsBet, $DMAA^V$, As^{III}, $MMAA^V$, and As^V, respectively. The stability of the signal and the low standard deviation avoid the use of an internal standard.

3.2. Validation of the method

For the applicability of a method to real samples, a validation study is indispensable. Thus, the performance of the method was estimated by determining the limit of detection (LOD), the limit of quantification (LOQ), the linearity and the precision (within-assay and between-assay precision) of results following the XP T 90-210 (1994) guideline and calculations according to ISO 5725 (1996) (Table 2).

The linearity was checked in the concentration range 0.1–50 µg l^{-1} for each arsenic species by calculating the coefficient of determination (R^2) of the linear regression based on six different concentrations (three replicates) including the blank. The linear regression equations were $y = 17.237x - 0.8008$; $y = 18.223x - 0.5866$; $y = 20.172x - 2.6757$; $y = 19.09x + 1.4758$ and $y = 18.564x + 10.1$ for respectively AsBet, $DMAA^V$, As^{III}, $MMAA^V$ and As^V. R^2 obtained after linear regression were in all cases better than 0.9999, clearly illustrating adequate linearity for all the five arsenic species in the concentration range investigated.

Precision was expressed by within-assay precision (WAP) and between-assay precision (BAP) and were calculated according to Eqs. (1) and (2),

Table 2
Within-assay precision (WAP), between-assay precision (BAP), limit of detection (LOD) and limit of quantification (LOQ) of the method using conditions in Table 1

Arsenic species[b]	WAP[a]			BAP[a] (%)			LOD		LOQ	
	0.1	10	50	0.1	10	50	Relative (ng l^{-1})	Absolute (pg)	Relative (ng l^{-1})	Absolute (pg)
AsBet	2.4	1.0	3.0	3.4	1.0	3.7	24	0.5	80	1.6
$DMAA^V$	3.3	1.0	2.6	3.5	1.0	3.8	23	0.5	76	1.5
As^{III}	4.0	1.4	2.2	6.8	5.9	2.6	17	0.3	56	1.1
$MMAA^V$	4.3	1.4	3.7	4.3	2.2	3.8	26	0.5	88	1.8
As^V	5.7	1.4	4.0	6.2	1.7	4.6	26	0.5	85	1.7

[a] Concentrations used for calculating these parameters are expressed in µg l^{-1}.
[b] AsBet (arsenobetaine), $DMAA^V$ (dimethylarsinic acid), As^{III} (arsenite), $MMAA^V$ (methylarsonic acid), As^V (arsenate).

Table 3
Speciation of the standard material water and comparison with the total arsenic expecting concentration

Water samples	IC-ICP-MS[b]						Expecting value
	AsBet	$DMAA^V$	As^{III}	$MMAA^V$	As^V	Total	
Aquackeck 1	[a]	[a]	4.44 ± 0.36	[a]	1.98 ± 0.20	6.42 ± 0.41	6.50 ± 1.10
Aquackeck 2	[a]	[a]	1.88 ± 0.21	[a]	1.20 ± 0.13	3.10 ± 0.25	2.91 ± 0.49
Aquackeck 3	[a]	[a]	2.45 ± 0.26	[a]	1.25 ± 0.14	3.70 ± 0.29	3.53 ± 0.60
Aquackeck 4	[a]	[a]	4.73 ± 0.37	[a]	1.55 ± 0.16	6.28 ± 0.40	6.78 ± 1.15

The values are expressed in $\mu g\, l^{-1}$.
[a] <LOD.
[b] AsBet (arsenobetaine), $DMAA^V$ (dimethylarsinic acid), As^{III} (arsenite), $MMAA^V$ (methylarsonic acid), As^V (arsenate).

$$WAP = \frac{100}{m} \cdot \sqrt{\frac{\sum_{j=1}^{k}(n_j - 1) \cdot S_j^2}{\left(\sum_{j=1}^{k} n_j\right) - k}} \quad (1)$$

$$BAP = \frac{100}{m} \cdot n \cdot \frac{\sum_{j=1}^{k}[n_j \cdot (m_j - \bar{m})^2]}{k - 1} \quad (2)$$

where m is the used concentration; S_j^2 is the intergroup variance; n_j is the number of the group j repetition; k is the number of the group ($k = 4$); m_j is the average of the j group; $\bar{m}$ is the average m_j and n is the average number of values per group. WAP and BAP were determined at 0.1, 10 and 50 $\mu g\, l^{-1}$ with four analysis groups. The first group included 10 repetitions, while for the three others, only six replicates were carried out. At concentrations close to the LOQ, WAPs were <6%, showing good precision of the obtained results for trace arsenic analysis. Moreover, BAPs were similar to WAPs, proving low variation of the results in the course of time. Limit of detection and quantification (LOD and LOQ) were evaluated by analysing ten samples containing an arsenical concentration close to the expected LOD and LOQ in repeatability conditions and using Eqs. (3) and (4),

$$LOD = y_{signal} + 5 \cdot S_{signal} \quad (3)$$

$$LOQ = y_{signal} + 10 \cdot S_{signal} \quad (4)$$

where y_{signal} is the medium value and S_{signal} is the standard deviation.

LODs were closely the same for all the compounds and made the method particularly suitable for trace arsenic species determination in real water samples and were better than detection limits previously reported in the literature.

LOQ values were confirmed by analysing ten replicates of a solution containing all the arsenic species at the LOQ. Variation coefficient of 9.87, 8.38, 8.33, 12.3 and 8.84% were obtained for respectively AsBet, $DMAA^V$, As^{III}, $MMAA^V$ and As^V.

3.3. Arsenic analysis in reference materials

Certified reference materials for arsenic speciation were not available for drinking water. Therefore, interlaboratory solutions with well established concentrations for total arsenic were selected, namely Aquacheck® (Aquacheck Ltd, Bury Greater Manchester, England).

The certified total arsenic concentration agrees closely with the speciation results (Table 3). However, simultaneous presence of As^{III} and As^V was also noticed, probably

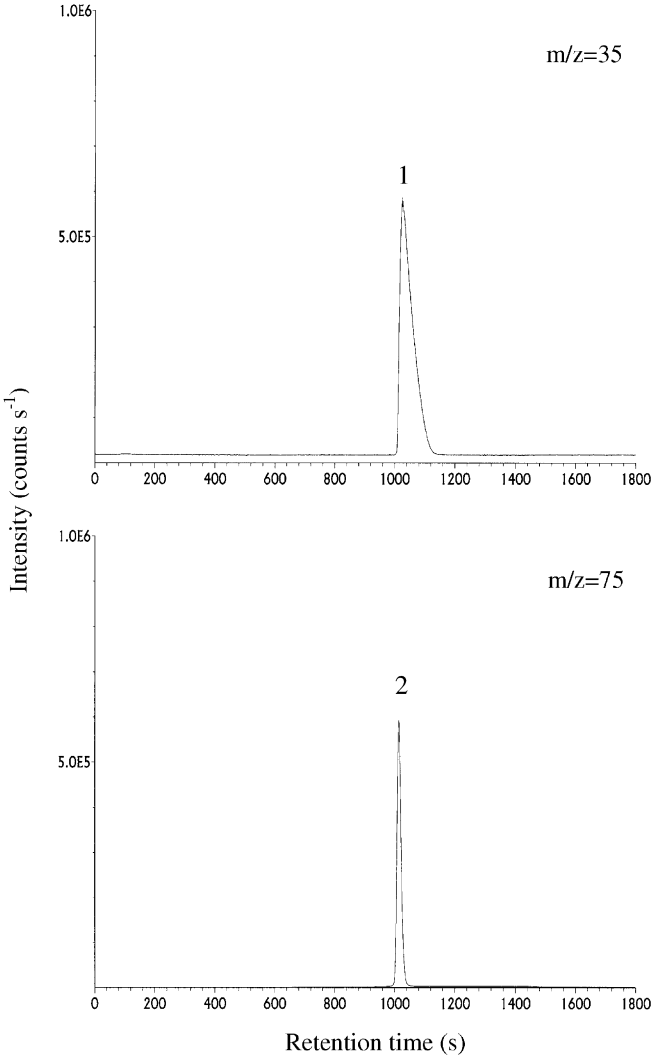

Fig. 2. Anion exchange HPLC-ICP-MS chromatogram of a 20 µl injected standard solution containing 10 $\mu g\, l^{-1}$ of As^V and 200 $mg\, l^{-1}$ of NaCl. Peak identification: (1) Chloride and (2) As^V. The analysis was performed according to the optimum conditions shown in Table 1.

resulting from the oxidation of the trivalent to the pentavalent form. Indeed, these solutions were prepared with an arsenite form and this conversion can be related to the 2% Aquacheck® HNO_3 acidification and prove the arsenic-form stabilisation problem in water. This hypothesis was confirmed by Bohari et al. (2001) who showed that 0.5% HNO_3 acidification changed arsenic speciation after ten days of storage. Thus, it will be important to consider this aspect for the preparation of certified water samples containing arsenic species, as this kind of sample does not exist yet.

3.4. Arsenic speciation in real samples

The possible interference of $^{40}Ar^{35}Cl^+$ with the monoisotopic $^{75}As^+$, has been studied by analysing solutions containing increased concentration (200, 500 and 1000 mg l^{-1}) of chloride and comparing the signal obtained at m/z 75 with the signal of a blank (Milli-Q water) using a t-test. This test was of particular importance since chloride eluted at the same retention time as arsenate (Fig. 2). We found that there is no noticeable $^{40}Ar^{35}Cl^+$ interference up to 500 mg l^{-1} chloride. These results are in good agreement with those published by Saverwyns et al. (1997) and Pantsar-Kallio and Manninen (1997).

Possible matrix effects on the calibration were estimated by spiking representative real water samples. These samples were collected at various locations in order to have different matrix contents, e.g. high suspended colloids. Samples from ground, well and surface water were spiked with a standard mixture of the five arsenic species giving an added arsenic concentration of 0.5 and 5 μg l^{-1} each. When the IC-ICP-MS procedure was applied to the analysis of three spiked real water samples, recoveries were satisfactory with values ranging from 95% to 108%. Then, surface, well and natural mineral waters were analysed for the native arsenic species quantification (Table 4).

As^{III} and As^{V} were the major arsenic species found in surface and well waters, but AsBet and $DMAA^{V}$ were present only in surface water. To our knowledge, it was the first time AsBet was found in surface waters, but the detection of this compound can be related to the fact that the proposed method was characterized by the lowest LOD in literature, permitting an AsBet ultra trace quantification. The presence of such methylated compounds probably results from a more intense biological activity in this kind of environment, as the bioaccumulation of the methylated forms has been largely discussed in terrestrial and marine organisms. Moreover, Hanaoka et al., 1997 found AsBet in suspended particles in marine biota (probably from degraded organisms), while Takeuchi et al. (2005) claimed an incorporation of this arsenic specie into the sediment. They quantified AsBet and $DMAA^{V}$ as the major compounds in the sediment, although the concentration of these organoarsenicals decreased with depth and is considered to be degraded within 60 years of deposition. In addition, Hasegawa (1997) found that the detritus on the surface of lake sediment was consumed by the anaerobic respiration of bacteria. The methylarsenic species were degradation products and/or the results of *in situ* bacterial methylation and were decomposed to inorganic arsenic under anoxic conditions by facultative and obligate anaerobes.

It was also reported that a portion of the $DMAA^{V}$ was transformed *in vitro* to AsBet by some organisms (Kaise

Table 4
Results of different natural sample analysis (concentration in μg l^{-1})

Water samples	IC-ICP-MS						ICP-MS
	AsBet	$DMAA^{V}$	As^{III}	$MMAA^{V}$	As^{V}	Total	
Drinking waters							
Well water 1	[a]	[a]	0.22 ± 0.03	[a]	1.40 ± 0.15	1.62 ± 0.15	1.76
Well water 2	[a]	[a]	0.44 ± 0.06	[a]	1.86 ± 0.19	2.30 ± 0.20	2.13
Well water 3	[a]	[a]	0.87 ± 0.11	[a]	1.03 ± 0.12	1.90 ± 0.16	1.98
Surface water 1	[b]	0.12 ± 0.01	0.23 ± 0.03	[a]	1.79 ± 0.21	2.12 ± 0.21	1.97
Surface water 2	[b]	[b]	0.11 ± 0.01	[a]	0.24 ± 0.03	0.35 ± 0.03	0.38
Natural mineral waters							
Sample 1[d]	[a]	[a]	[a]	[a]	8.52 ± 0.19	8.52 ± 0.19	7.30
Sample 2[d]	[a]	[a]	[a]	[a]	0.32 ± 0.04	0.32 ± 0.04	0.32
Sample 3[d]	[a]	[a]	0.07 ± 0.01	[a]	0.09 ± 0.01	0.16 ± 0.01	0.21
Sample 4[d]	[a]	[a]	[a]	[a]	26.20 ± 0.76	26.20 ± 0.76	26.54
Sample 5[e]	[a]	[a]	[a]	[a]	[a]	[a]	0.25
Sample 6[c]	[a]	[a]	[a]	[a]	0.08 ± 0.01	0.08 ± 0.01	[a]
Sample 7[c]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Sample 8[d]	[a]	[a]	[a]	[a]	7.59 ± 0.25	7.59 ± 0.25	7.40
Sample 9[d]	[a]	[a]	[a]	[a]	0.55 ± 0.06	0.55 ± 0.06	0.76
Sample 10[d]	[a]	[a]	[a]	[a]	3.57 ± 0.32	3.57 ± 0.32	3.39

[a] <LOD.
[b] <LOQ.
[c] Belgian mineral natural water.
[d] French natural mineral water.
[e] Italian natural mineral water.

et al., 1997). Indeed, organisms are transformed to particulate or dissolved species after their death, and experimental studies in vitro demonstrated that arsenobetaine contained in organisms degraded to inorganic forms via $DMAA^V$ intermediates (Hanaoka et al., 1993, 1997). This mechanism could be at the origin of the low levels of AsBet and $DMAA^V$ found in our study. This arsenic cycle shall explain why we detected both methylated and inorganic species in surface water.

Depending on the geological origin of the natural mineral water, the arsenic content was very variable from one type of water to the other (Table 4). According to Van Holderbeke et al. (1999), arsenic in such samples was exclusively in the arsenate form. Nevertheless, it was very interesting to notice that some natural mineral waters had a total arsenic content exceeding the 10 $\mu g\, l^{-1}$ limit of the European Commission Directive 2003/40/CE (2003). The presence of arsenic at this concentration level should be associated with the geochemical environment. Indeed, occurrence of arsenic in natural water depends on the local geology, hydrology and geochemical characteristics of the aquifer. In our study, the natural mineral waters with the higher arsenic content were located in a volcanic region in France. Thomas and Sniatechi (1995) reported that some mineral or thermal water were exceeding the maximum drinking water limit for arsenic values, e.g. spring water from Puy du Dôme in the volcanic region of Massif Central in France.

4. Conclusions

A method for arsenic speciation by IC-ICP-MS was developed which allowed the simultaneous separation of five arsenic species (AsBet, $DMAA^V$, As^{III}, $MMAA^V$ and As^V) in various types of water samples with LODs close to 20 $ng\, l^{-1}$ for each arsenic compounds. In each case the total arsenic concentration obtained by ICP-MS was in good agreement with the sum of the arsenic species obtained by IC-ICP-MS.

Based on arsenic speciation in well and surface waters, arsenite and arsenate were the major molecules, while in surface water, both $DMAA^V$ and AsBet were found. The occurrence of these methylated forms was probably due to micro-organisms as in well and natural mineral waters, no methylated products were detected.

The concentration of arsenic in some natural mineral waters analysed in this study exceeded the European Directive 2003/40/CE limit. The developed method is available to identify and quantify the arsenic species present in natural and drinking water at trace levels, which might be particularly important for the toxicity assessment in regions that may suffer of natural arsenic contamination.

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