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Arsenic speciation in human urine by hyphenated ion chromatography (IC) and inductively coupled plasma mass spectrometry (ICP-MS)

Authors

Francesco Petrucci,
Beatrice Bocca,
Oreste Senofonte,
Giovanni Forte,
Istituto Superiore di Sanità,
Department of Environment
and Health, Viale Regina Elena,
299-00161 Roma, Italy

Keywords

Arsenic, inductively coupled plasma mass spectrometry, ion chromatography, urine, speciation

Goal

To validate a sensitive and high throughput research method for the determination of six species of As in urine using IC-ICP-MS.

Introduction

Arsenic (As) is a ubiquitous element in the environment and natural metabolic processes in the biosphere result in the existence of a large number of chemical forms, or so-called species, in which this element may be present. The different As species can be classified as inorganic arsenic (iAs) and organic arsenic compounds.[1,2] Exposure to iAs as the sum of arsenite As(III) and arsenate As(V) is a major concern for public health authorities worldwide. Exposure of the population to As predominantly occurs through drinking water and less frequently from other sources (pesticides, wood preservatives, dust emission and disposal of industrial waste). Dietary exposure to iAs through consumption of e.g. cereals, rice or fruit and vegetables may be an important source if drinking water levels are low. In contrast, exposure to organic species of As, such as arsenobetaine (AsBet), arsenocholine (AsChol) and arsenosugars, is primarily observed after consumption of fish or seafood.

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Exposure to iAs can result in a variety of adverse effects such as skin disorders, neuropathy, and lung, bladder and skin cancer. Following exposure, iAs is methylated in the body via the 1-carbon metabolism route and the main metabolites are dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA). This metabolic pathway is viewed as a detoxification process. Higher fractions of iAs and lower % of DMA in human urine have been associated with higher retention of As and higher risks for human health.[3] Organic As (AsBet and AsChol) is generally much less toxic, and is excreted in urine without any further metabolization. The need for As speciation is important because concentration of As may be interpreted in a risk-assessment context differently if As arises from exposure to iAs species (As(III) and As(V)), methylated As (MMA and DMA) or organic As (AsBet and AsChol). The measurement of total As concentration (which comprises both inorganic and organic As) cannot be used solely to explain the biological and toxic effects of As and judge potential hazards. Thus, the determination of the individual concentration of each As species, by using the speciation approach, becomes critical. The aim of this work is to develop and validate a method for the simultaneous separation and quantification of six different As species – namely AsBet, DMA, As(III), AsChol, MMA and As(V) – in human urine samples.

Instrumentation

A Thermo Scientific™ ICS-5000 Ion Chromatography (IC) System equipped with Dionex AG-7 (4 x 50 mm) and AS-7 (4 x 250 mm) anion exchange columns was used in order to separate the different As species. This system is perfectly suited for elemental speciation studies at trace levels because of its completely metal-free solvent pathway. A Thermo Scientific™ iCAP™ Q ICP-MS was used as a highly sensitive elemental detector for As species eluting from the ICS-5000 IC system. The instrument was equipped with a quartz torch, a PFA concentric nebulizer and a quartz, cyclonic spraychamber. The detection of As using ICP-MS is typically affected through polyatomic interferences such as $^{40}Ar^{35}Cl^{+}$, which can be completely removed using kinetic energy discrimination (KED), a generic approach for interference removal using the QCell Collision/Reaction Cell system. The instrument was tuned daily for highest detection sensitivity over the entire mass range. Furthermore, conditions for As were daily optimized using a blank solution containing the IC mobile phase and the same solution spiked with 1 µg·L^{-1} of As.

Using a flow rate of 1.0 mL·min^{-1} on the peristaltic pump, the system was optimized for the lowest background equivalent concentration (BEC) while maintaining suitable sensitivity for As. Operating conditions of the ICS-5000 IC System and the iCAP Q ICP-MS are reported in Tables 1 and 2 respectively. For the elution of the different As species, anion exchange chromatography was chosen using a linear gradient of water and a buffer solution containing 100 mM $(NH_4)_2CO_3$. Under these conditions, complete elution of all required species was achieved in less than 15 minutes, making for a very high throughput, routine chromatographic analysis. All calculations, including background subtraction, peak area integration and calibrations were performed using the Thermo Scientific™ Chromeleon Plug-in for Thermo Scientific™ Qtegra Intelligent Scientific Data Solution (ISDS) Software.

Table 1. Ion Chromatography operating conditions.

Parameter	Value			
Columns	Dionex IonPac AG7 (4x50 mm) and AS7 (4x250 mm)			
Injection Volume	100 µL			
Eluents	A: Ultrapure water B: 100 mM $(NH4)_2CO_3$ + 3% MeOH, pH 10.3			
Gradient	**Time min**	**Flow mL·min^{-1}**	**% A**	**% B**
	0-5	0.7	95	5
	5.1-10	1.5	70	30
	10.1-13	1.5	40	60
	13.1-14	0.7	95	5

Table 2. ICP-MS operating conditions.

Parameter	Value
Nebulizer	PFA-ST nebulizer, pumped at 40 rpm
Spraychamber	Quartz, cyclonic spraychamber cooled at 2.7 °C
Injector	2.5 mm i.d., Quartz
Interface	Ni cones with High Matrix (3.5 mm) insert
RF Power	1550 W
Nebulizer Gas Flow	1.17 L·min^{-1}
QCell settings	**KED**
Gas Flow	100% helium (99.999% Purity), 4.8 mL·min^{-1}
QCell Bias	-18 V
Quadrupole Bias	-21 V
Scan Settings	0.05 s dwell time per analyte, 14 minutes acquisition time

Sample preparation

A 2 mL aliquot of each urine sample was filtered through a 0.45 µm PTFE membrane filter (Sartorius, Goettingen, Germany) and diluted 1:5 with deionized ultrapure water (resistivity of 18 MΩ cm, EASY-pure UV). Standard solutions for the different arsenic compounds were prepared gravimetrically from the solid materials, and diluted freshly each day to the appropriate working concentration. AsBet, AsChol and MMA were purchased from Argus Chemicals srl (Vernio, Italy); As(III) and As(V) were obtained from CPA Chem (Stara Zagora, Bulgaria) and the DMA from Alfa Aesar (Thermo Fisher Scientific, Karlsruhe, Germany). For a recovery test within the validation protocol, a certified reference material NIST CRM 2669 - Arsenic Species in Frozen Human Urine, Level 1 and Level 2 (NIST, Gaithersburg, MD, USA) - with a certified content of As(III), As(V), AsBet, AsChol, DMA and MMA - was used. Finally, phenylarsonic acid (PAA) (Alfa Aesar - Thermo Fisher Scientific, Karlsruhe, Germany) was used as an internal standard (IS) to account for potentially occurring instrumental drift over longer analysis duration.

Method development and analysis

Urine samples were spiked with different amounts of As(III), As(V), AsBet, AsChol, DMA and MMA to optimize the chromatographic separation. Figure 1 shows the chromatographic profile in urine of the six As species investigated in this study (10 µg·L^{-1} for each species) including the internal standard PAA (added at a concentration of 1 µg·L^{-1}). The different As species were baseline separated in less than 15 minutes. Precision of retention times were typically less than 4%, and absolute peak widths were in the range of 10 - 20 s for each species. The reduced peak widths observed were due to the advanced stationary phase material of the AG7/AS7 column combination used. This contributed significantly to the demonstrated sensitivity of the method, with even low concentration species being easily detectable with such high signal-to-noise ratios. Quantification was performed by matrix-matched calibration in urine in the range 1–10 µg·L^{-1} for each As species. Figure 2 shows the typically observed calibration plots for AsBet, DMA, As(III), AsCol, MMA, PAA and As(V). All calibration curves showed an excellent linearity with correlation coefficients (R^2) being 0.999-1.000 for all species.

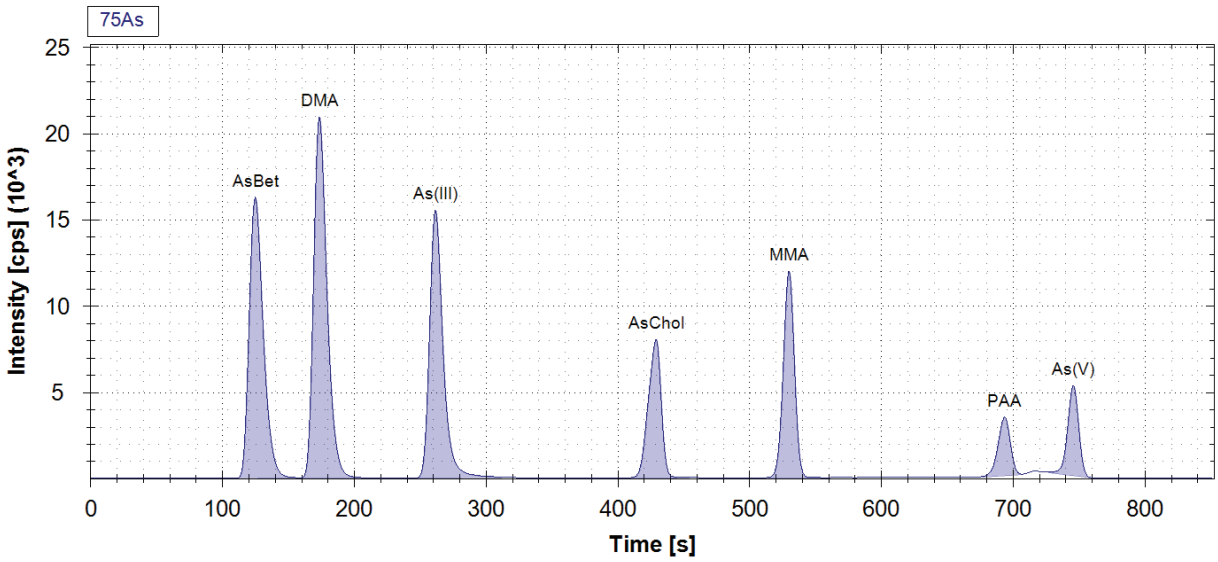

Figure 1. Chromatographic separation of all As species investigated in this study, including PAA used as internal standard.

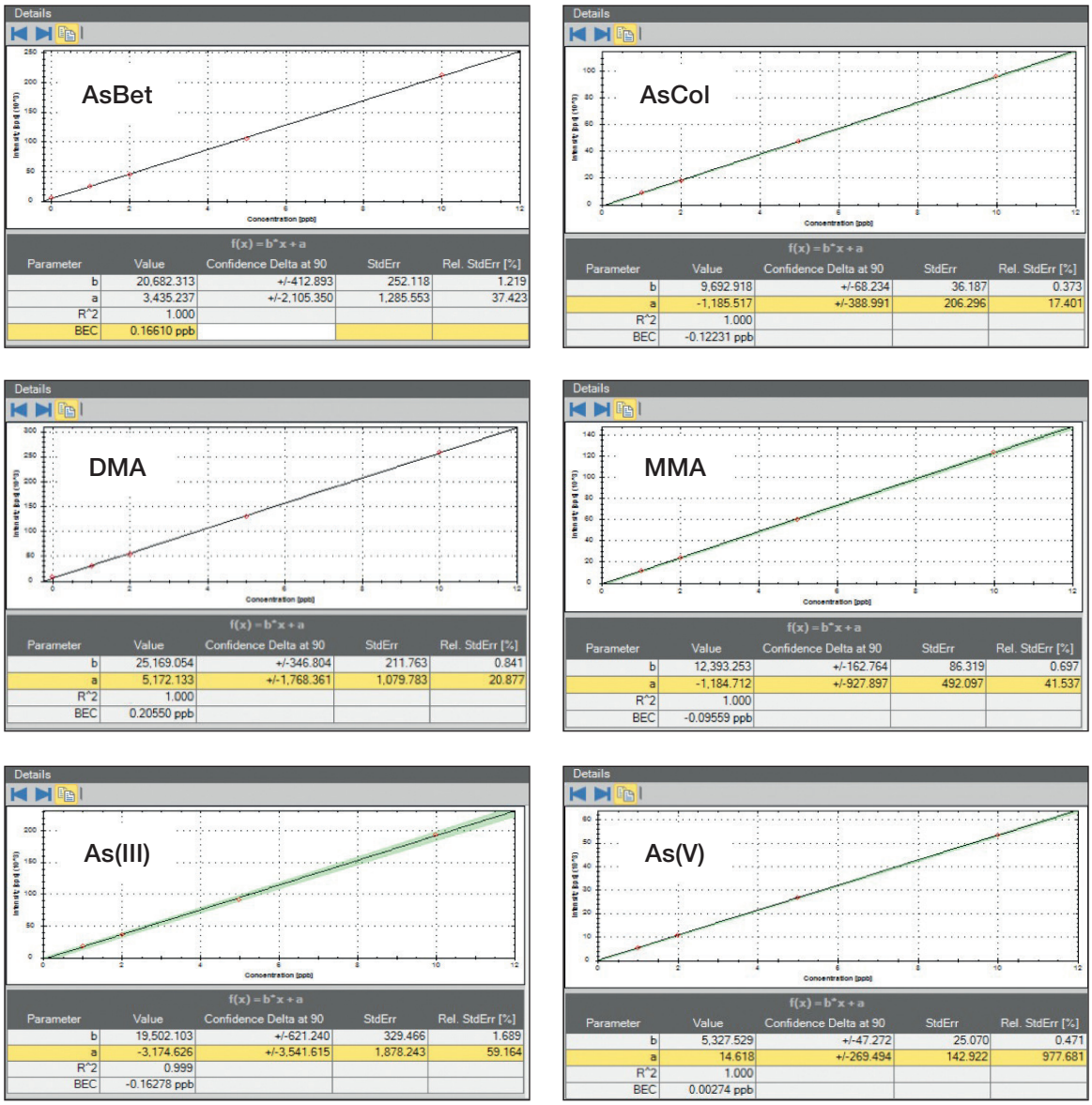

Figure 2. Quantitative IC-ICP-MS calibrations of six As species in urine samples diluted 1:5 with deionized ultrapure water, with calibration levels at 1, 2, 5, 10 µg·L^{-1}.

Results

The accuracy of the method was demonstrated using a certified reference material (NIST 2669 – Arsenic Species in Frozen Human Urine (Level I and Level II)) with certified concentration of different As species. The analytical precision was determined by intra-day and inter-day repeatability and expressed as the coefficient of variation in percent (CV%) of ten samples of urine spiked with the different As species at concentrations of 2 µg·L^{-1} each. The limit of detection (LoD) of the method (including all dilution steps carried out during preparation of the sample), describing the lowest concentration detectable with acceptable precision, was calculated following the 3 sigma criterion, analyzing ten samples of urine.

Table 2 reports the validation parameters for the As speciation analysis. The LOD was 0.25 µg·L^{-1} for all the species; the accuracy was in the ranges 86-107% for the Level I and 88-106% for the Level II materials. The intra-day and inter-day repeatability were on average 1.6% and 3.5%, respectively, for all the species. The sum of all As species (last column of Table 2) accounted for 98.6% of the total certified As for the Level I and 97.4% for the Level II, respectively, demonstrating that As was quantitatively recovered from the column.

Table 2. Results of method validation.

		AsBet	DMA	As(III)	AsChol	MMA	As(V)	Sum of species
LoD (µg·L^{-1})		0.25	0.25	0.25	0.25	0.25	0.25	
SRM 2669 Level I (µg·L^{-1})	Found	13.0±1.2	3.54±0.52	1.30±0.1	nc	2.00±0.42	2.07±0.32	21.9 ± 2.6
	Certified	12.4±1.9	3.47±0.41	1.47±0.1	nc	1.87±0.39	2.41±0.30	22.2±4.8*
SRM 2669 Level II (µg·L^{-1})	Found	1.38±0.10	26.4±1.05	4.45±0.40	3.94±0.32	7.58±0.52	5.65±0.41	49.4 ± 2.8
	Certified	1.43±0.08	25.3±0.7	5.03±0.31	3.74±0.35	7.18±0.56	6.16±0.95	50.7±6.3*
Intra-day repeatibiliy, N=10 (%)		2.23	1.63	1.10	1.93	1.34	1.49	
Inter-day repeatibiliy, N=10 (%)		3.50	3.25	2.98	3.52	3.20	4.45	

nc. not certified; *Certified value in SRM 2669 as total As.

Analysis of real samples

The fully validated method was applied to a human biomonitoring study with the objective to determine the concentrations of inorganic As (As(III) and As(V)), methylated As (MMA and DMA) and organic As (AsBet and AsChol) in the urine of children. Table 3 reports the observed levels of the different As species determined in a subset of all subjects investigated within the full study.

Table 3. Concentrations in µg·L^{-1} of the six As species after the chromatographic separation.

Subject	AsBet	DMA	As(III)	AsChol	MMA	As(V)
1	73.1	4.44	0.26	0.51	0.75	<LoD
2	27.3	1.75	<LoD	<LoD	0.53	<LoD
3	66.6	0.40	<LoD	<LoD	0.38	<LoD
4	22.4	5.50	0.47	<LoD	0.50	0.75
5	11.4	2.66	<LoD	<LoD	0.52	<LoD
6	27.2	3.33	0.90	0.32	0.79	0.37
7	243	3.65	<LoD	0.37	0.52	<LoD
8	769	4.30	0.41	0.31	1.71	<LoD
9	17.8	1.11	<LoD	0.34	0.41	<LoD
10	188	3.44	<LoD	1.20	0.39	<LoD

Conclusion

A research method for the analysis of As species in urine based on hyphenation of ion chromatography to ICP-MS has been developed and validated. The method combines an efficient separation of the different compounds with a highly sensitive detection obtained by ICP-MS.

The use of anion exchange chromatography in combination with a dedicated ion chromatography system enables narrow chromatographic peaks and reduces background signals, as there are no trace elements that can be washed out from the inert system. Using a generic interference removal approach such as kinetic energy discrimination removes all potentially occurring interferences on ^{75}As, so that lowest method detection limits can be achieved. Therefore, the method can be reliably applied to biomonitoring studies aiming at the determination of different inorganic and organic As species in human urine.

The results indicate that AsBet was the main arsenic species found in children's urine, representing about 90% of the total content of As found. Considering that AsBet is a non-toxic species of marine food origin, even though As levels were found to vary strongly between 11.4 and up to 769 µg·L^{-1}. Such variations may be explained through the subjects diet (AsBet is abundant e.g. in fish and seafood), and still the sum of As(III) and As(V) was around or far less than 1 µg·L^{-1}.

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