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Analysis of total As and As species in urine samples of the Walloon population (children (3-5y; 6-11y) and adults (20-39y; 40-59y) (BMH-Wal4)

Methodological information

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Trace elements and nanomaterials

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1. Introduction

This report describes the methodology used for the analysis of total arsenic (As) and As species in urine samples of children (3 – 11 y), and adults (20 – 59 y) in the context of BMH-Wal4 (2025 – 2026).
Urine samples were collected by ISSeP during BMHWal2 and 3 and transferred for analysis to the trace element laboratory of Sciensano. Samples were kept at -20°C until analysis.
Result data are summarised in the excel 'Sciensano BMHWAL4-Results'.

2. Materials and Methods

2.1. Standards and chemicals

Nitric acid (HNO_3, 67 – 69 %, Pico-Pure) was purchased from AnalytiChem (Belgium), and hydrogen peroxide (H_2O_2,30 %, Perhydrol, pro analyse) from Merck (Germany). A multi-element standard solution of 100 mg/kg used for quantification of total As was purchased from Analytika (Prague, Czech Republic). Arsenic standards to prepare a multi-species solution of 1000 mg/kg used for quantification of the As species (As3, As5, DMA, MA, AB) were purchased from Merck (Germany), Inorganic Ventures (Instrument Solutions, The Netherlands), and TRC (Toronto Research Chemicals, LGC Standards, Canada). A multi-elemental 5 µg L^{-1} tuning solution (Agilent Technologies, USA) or 1 µg L^{-1} tuning solution (Thermo Fisher Scientific, Belgium) was used for tuning the ICP-MS or IC-ICP-MS respectively. Water used in this study was home produced doubly distilled water (Aquatron, Cole-Parmer, UK) and ultrapure water (Millipore Milli-Q, Merck, Germany).

2.2. Determination of total arsenic by ICP-MS

After thawing, urine samples were centrifugated (10 min at 1500 g) and supernatant was diluted and acidified to a final dilution factor of 10 (final at 4 % HNO_3). Sample solutions were stored at 4 °C until ICP-MS analysis. Total element concentrations of the urine samples were determined by ICP-MS (Agilent 8800; Agilent Technologies, USA), with O_2 as reaction gas. Quantification was carried out using a matrix matched external calibration of the linear type (range 0.005 – 10 µg L^{-1}).

2.3. Determination of arsenic species by IC-ICP-MS

For determination of As species in the urine samples, the above mentioned supernatant was diluted to a final dilution factor of 5 in Milli-Q H_2O with phenylarsonic acid (PAA) added as an internal standard (final concentration 1 µg L^{-1}). Sample solutions were stored at 4 °C until IC-ICP-MS analysis. As species (As3, As5, DMA, MA, AB) concentrations of the urine samples were determined by IC-ICP-MS (Dionex ICS-6000 + iCAP RQ; Thermo Fisher Scientific, Belgium), with He as collision gas. For chromatographic separation of the As species, an anion exchange column was used (IonPac AG7 (4x50mm; PN 035394) + IonPac AS7 (4x250mm; PN 035393), Thermo Fisher Scientific). The mobile phase consisted of ammonium carbonate (200 mM + 3 % MeOH) in a gradient with Milli-Q water, for a run time of 20 minutes to obtain the maximum separation between

the different peaks. Quantification was carried out using a matrix matched external calibration of the linear type (range 0.2 – 5 µg L^{-1}), including 1.0 µg/L PAA.
Interspecies conversion between As3 and As5 might occur due to the long storage of the urine samples and aqueous dilution to minimize manipulation of the urine composition during sample preparation (Verdon et al., 2009). For this reason, further calculations/statistics were performed with the sum of both inorganic As species.

2.4. Quality control

Each analytical batch included internal quality control measures, specifically a reagent blank as a monitor for possible cross-contamination, a QC standard before and after a series of samples to allow verification of potential instrument drift and a reference material (Seronorm – Level 1 and Level 2 or NIST 2669 – Level 1 and Level 2) to assess trueness and day to day variations. A series of acceptance criteria were applied to each batch, including calibration blank value ≤ LOQ/2 and drift ≤ 20%.

2.5. Performance characteristics

Limit of Detection - Limit of Quantification – The LOQ of the method is the lowest level that can be determined with an acceptable performance. The LOQ was calculated as 3.3 times the Limit of Detection (LOD = 3 times the standard deviation of 10 blank samples or 10 pseudoblank (low spiked) samples for speciation). This resulted in LOQ and LOD values for urine in Table 1 (after taking into account the dilution factor of the samples).

Table 1: LOD and LOQ of elements determined by ICP-MS (Agilent 8800) and IC-ICP-MS (Thermo iCAP RQ)

Element	LOD (µg/L)	LOQ (µg/L)
total As	0.15	0.50
As species	0.15	0.50

Trueness – Trueness is a theoretical concept expressing how close the mean of an infinite number of results produced by the method is to a reference value. It can be assessed in practice by calculating the relative recovery compared to the reference value (in %). The reference values used in this study are the concentrations of the analyte cited on the certificate of the reference material used or the assigned values from the PT report of the PT material used. The results are given in Table 2, reference materials were measured on different (≥ 3) days and an average trueness was calculated.

Table 2: Trueness for measured elements

CRM or PT samples	Analyte	Reference value (± MU) [µg.L^{-1}]	Measured value (± SD) [µg.L^{-1}]	Trueness
Seronorm-L1 (urine)	total As	97 ± 19	98 ± 3	101 %
Seronorm-L2 (urine)	total As	198 ± 25	201 ± 10	102 %
EQUAS 67 2A (urine)	total As	210.6 ± 32.1	219 ± 3	104 %
EQUAS 67 2B (urine)	total As	83.3 ± 13.5	87.9 ± 1.9	105 %

CRM or PT samples	Analyte	Reference value (± MU) [µg.L^{-1}]	Measured value (± SD) [µg.L^{-1}]	Trueness
NIST 2669 L1 (urine)	AB	12.4 ± 1.9	16.78 ± 0.70	135 %
	DMA	3.47 ± 0.41	3.40 ± 0.68	98 %
	MA	1.87 ± 0.39	1.99 ± 0.14	106 %
	As3+As5	3.88 ± 0.32	3.92 ± 0.26	101 %
NIST 2669 L2 (urine)	AB	3.37 ± 0.28	4.17 ± 0.09	124 %
	DMA	25.3 ± 0.7	27.15 ± 1.13	107 %
	MA	7.18 ± 0.56	7.50 ± 0.33	104 %
	As3+As5	11.19 ± 1.00	11.11 ± 0.77	99 %
EQUAS 75 8A (urine)	AB	6.70 ± 1.56	7.91 ± 0.27	118 %
	DMA	1.62 ± 0.30	1.81 ± 0.07	112 %
	MA	0.52 ± 0.18	0.54 ± 0.08	104 %
	As3+As5	0.43 ± 0.17	< LOQ	
EQUAS 75 8B (urine)	AB	64.73 ± 8.16	76.70 ± 1.67	118 %
	DMA	9.47 ± 1.56	11.07 ± 0.52	117 %
	MA	1.47 ± 0.36	1.76 ± 0.20	120 %
	As3+As5	0.76 ± 0.22	< LOQ	

Precision – Repeatability standard deviation (s_r = within-day variation), between-day standard deviation (s_d) and intermediate precision standard deviation (s_{ip} = within-laboratory reproducibility) were determined based on results of the reference materials Seronorm-L1, Seronorm-L2, EQUAS-67 2A, and EQUAS-67 2B for total As; NIST 2669 L1, NIST 2669 L2, EQUAS-75 8A, and EQUAS-75 8B for As species, that were analysed on different days in independent replicates. The values were calculated via one-way analysis of variance using the equations below:

$$s_r = \sqrt{MSW} \quad (1)$$

$$s_d = \sqrt{\frac{MSB - MSW}{n}} \quad (2)$$

$$s_{ip} = \sqrt{s_r^2 + s_d^2} \quad (3)$$

Where MSW is the mean squares within days, MSB is the mean squares between days, and n is the number of measurements per day in routine (1 replicate).

Relative deviations (expressed in %) were obtained by expressing the corresponding standard deviations as a percentage of the mean measured values.

The repeatability relative standard deviation (RSD_r), the between-day standard deviation (RSD_d), and the resulting within-laboratory reproducibility (RSD_{ip}) for total As, AB, DMA, MA and As3+As5 in urine is given in Table 3.

Table 3: Repeatability relative standard deviation (RSD_r), between-day standard deviation (RSD_d) and resulting within lab reproducibility (RSD_{ip}) for measured elements

Analyte	RSD_r (%)	RSD_d (%)	RSD_{ip} (%)
As	3.1	1.4	3.4
AB	3.1	1.0	5.9
DMA	4.3	15	15
MA	3.8	9.0	9.2
As3+As5	5.5	3.7	4.6