Analysis of total As and As species in human urine samples of adolescents of the Walloon biomonitoring program (BMH-Wal2)

METHODOLOGICAL INFORMATION

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1. Introduction

This report describes the methodology used for the analysis of total As and As species in human urine samples of adolescents of the first Walloon Biomonitoring program (2019-2020).
Blood samples were collected by ISSeP and transfered for analysis to the trace element laboratory of Sciensano. Samples were kept at -20°C until analysis.

Statistical treatment of the data and calculation of reference values (RV_{95}) was done according to the document 'Méthodologie d'élaboration des valeurs de référence dans le cadre du projet BMH-Wal 1' issued by ISSeP. These results are summarized per element in separate reports. Raw data were transfered to ISSeP in excel files.

2. Materials and Methods

2.1. Standards and chemicals

Nitric acid (Suprapur, SpA 67-69%,) was purchased from Romil (UK), and H_2O_2 (30%, pergydrol, pro analyse) from Merck (Germany). Arsenic standards to prepare a multi species solution of 1000 mg/kg used for quantification of As and As species (As3, As5, DMA, MA, AB) was purchased from Analytika (Prague, Czech Republic), Sigma (Merck, Germany) and Inorganic Ventures (Instrument Solutions, The Netherlands). A multi-elemental 5 µg L^{-1} tuning solution (Spectropure, Arlington, 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).

2.2. Determination of total As 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 As concentrations of the urine samples were determined by ICP-MS (VARIAN 820; Varian, Melbourne, Australia), with H_2 as a reaction gas on mass 75. 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 As 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 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 (100 mM + 3%MeOH with pH of 10.3) in a gradient with Milli-Q water, for a run time of 14 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. For this reason, further calculations/statistics were performed with the sum of both inorganic As species.

Commenté [KC1]: Verdon, Carl P., Kathleen L. Caldwell, Mark R. Fresquez, and Robert L. Jones. "Determination of Seven Arsenic Compounds in Urine by HPLC-ICP-DRC-MS: A CDC Population Biomonitoring Method." *Analytical and Bioanalytical Chemistry* 393, no. 3 (February 1, 2009): 939–47. https://doi.org/10.1007/s00216-008-2537-3.

2.4. Quality control

Each analytical batch included internal quality control measures such two procedure blanks and a reagent blank as a monitor for possible cross-contamination, a QC standard check every 20 samples to allow verification of potential instrument drift and, a reference material (Seronom-level 1 and 2 or NIST 2996) to assess trueness and day to day variations. A series of acceptance criteria were applied to each batch, including calibration blank value ≤LOQ/2, procedure blank ≤ LOQ and drift ≤10%.

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 for total As was calculated as 3.3 times the Limit of Detection (LOD = 3 times the standard deviation of 10 blanc samples or 10 pseudoblanc (low spiked) samples for speciation). This resulted in LOQ values in the matrix

(urine) of 0.5 µg L^{-1} for both total As and As species (corresponding LOD of 0.15 µg L^{-1}), after taking into account the dilution factor of the samples.

Trueness –Trueness is a theoretical concept expressing how close the mean of 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 value used in this study is the concentrations of the analyte cited on the certificate of the reference material used. The results are given in Table 1, reference material was added in each measurement and an average trueness was calculated.

CRM or PT samples	Analyte	Reference value (± MU) µg L^{-1}	Measured value (± SD)	Trueness
Seronorm-L1	Total As	79 ± 16	84 ± 4 µg L^{-1}	107%
Seronorm-L2	Total As	184 ± 37	180 ± 4 µg L^{-1}	98%
	As3+As5			
NIST 2669 L1	AB	12.4 ± 1.9	15.9 ± 0.4	128%
	DMA	3.47 ± 0.41	4.37 ± 0.09	126%
	MA	1.87 ± 0.39	1.95 ± 0.10	104%
	As3+As5	3.88 ± 0.32	3.74 ± 0.40	96%
Equas 62A	AB	12.52 ± 3.63	13.53 ± 0.43	108%
	DMA	1.61 ± 0.48	1.65 ± 0.09	103%
	MA	1.24 ± 0.36	1.34 ± 0.09	108%
	As3+As5	0.65 ± 0.21	0.75 ± 0.16	115%

Precision –Repeatability standard deviation (s_r = within day variation), between-day standard deviation (s_d) and intermediate precision standard deviation (s_{ip}=within lab reproducibility) were determined based on results of the reference material Seronorm-L1 and L2 for total As and NIST 2669 L1 for As species, that were analyzed together with the samples on different days in independent replicates. The values were calculated via one-way analysis of variance using the equations below:

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

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

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

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) for As tot, AB, DMA, MA and As3+As5 was respectively 1.8%, 4.5%, 4.4%, 4.0% and 7.2%. The between-day standard deviation (RSD_d) respectively 2.1%, 2.0%, 1.5%, 2.6% and 5.5% This resulted in a within lab reproducibility (RSD_{ip}) of 2.8%, 5.0%, 4.6%, 4.8% and 9.1 % respectivily.