Analysis of Cd, Pb and Hg in human blood samples of the first Walloon biomonitoring program (BMW-1)

METHODOLOGICAL INFORMATION

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1. Introduction

This report describes the methodology used for the analysis of Cd, Pb and Hg in blood samples of the first Wallon 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). A multi-element standard solution of 1000 mg/kg used for quantification of Cd and Pb was purchased from Analytika (Prague, Czech Republic). A multi-elemental Varian tuning solution (10 mg L^{-1}) (Spectropure, Arlington, USA) was used to prepare tuning solutions for ICP-MS (5 µg L^{-1}, in 4% v/v nitric acid). Water used in this study was home produced doubly distilled water.

2.2. Determination of Cd and Pb by ICP-MS

After thawing, ± 1ml of blood was digested with HNO_3 and H_2O_2 in a DigiPREP heating block system (SCP SCIENCE, Quebec, Canada). Further sample preparation included centrifugation (15 min at 10 000 g) and dilution of the digested samples to a final dilution factor of 12.5. Sample solutions were stored at 4°C until ICP-MS analysis.
Total Cd and Pb concentrations of the blood samples were determined by ICP-MS (VARIAN 820; Varian, Melbourne, Australia), with H_2 as reaction gas. Cd was measured on mass 114 (and confirmed on mass 111), Pb was measured on mass 208 (and confirmed on mass 206). Quantification was carried out using an external calibration of the linear type. Calibration

standards were in the range 0.005 – 10 µg L^{-1} for Cd and 0.05-10 µg L^{-1} for Pb and prepared by making the appropriate dilutions of the multi element mother stock.

2.3. Determination of Hg by AMA

For determination of Hg in the blood samples an AMA 254 single purpose atomic absorption spectrometer (Altec, Czech republic) was used. 150 µl of blood sample was weight directly into a sampling boat, and quantification was done using the the first calibration range of the device (range 0.1- 40 ng Hg).

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 50 samples to allow verification of potential instrument drift and, a reference material (Seronom-level 1 or ERM-CE 195) to assess trueness and day to day variations (included on 1 out of 2 analysis days; see performance characteristics). 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 was calculated as 3.3 times the Limit of Detection (LOD = 3 times the standard deviation of 8 blanc samples). This resulted in LOQ values in the matrix (blood) of 0.16 µg L^{-1} for Hg, 0.07 µg L^{-1} for Cd and 0.75 µg L^{-1} for Pb (corresponding LOD of 0.05 µg L^{-1} for Hg, 0.02 µg L^{-1} for Cd and 0.23 µg L^{-1} for Pb), 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 pratice 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. Throughout the analytical series the Hg concentration in Seronorm-level1 was 1.56 ± 0.1 µg L^{-1}

(mean ± sd). (certified value 1.57 ± 0.32 µg L^{-1}, mean ± measurement uncertainty) resulting in an average trueness of 99.3%. Average Cd and Pb concentrations in ERM-CE 195 were respectively 5.36 ± 0.23 µg L^{-1} (mean ± sd) and 418± 0.15 µg L^{-1} (mean ± sd) corresponding to an average trueness of 106.7% and 100.5% respectively, compared to the value on the certificate (Cd: 5.06 ± 0.15 µg L^{-1}; Pb: 416 ± 0.15 µg L^{-1}; mean ± measurement uncertainty).

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-level I for Hg and ERM-CE 195 for Cd and Pb, that was analysed together with the samples on 10 different days in 2 independent replicates. The values were calculated via one-way analysis of variance using the equations below:

$$s_r = \sqrt{MSW} \tag{1}$$

$$s_d = \sqrt{\frac{MSB - MSW}{n}} \tag{2}$$

$$s_{ip} = \sqrt{s_r^2 + s_d^2} \tag{3}$$

MSW is the mean squares within days, MSB is the mean squares between days, and n is the number of measurements per day (2 replicates). Relative deviations (expressed in %) were obtained by expressing the corresponding standard deviations as a percentage of the mean measured values.

The repeatability standard deviation (s_r) for Hg, Cd and Pb was respectively 3.6%, 4.3% and 1.6%. The between-day standard deviation (s_d) respectively 6.1%, 3.1% and 3.5% This resulted in a within lab reproducibility (s_{ip}) of 7.1%, 5.3% and 3.8 % respectivily.