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Full validation and accreditation of a method to support human biomonitoring studies for trace and ultra-trace elements

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ABSTRACT

This work describes the full validation of a method, in a context of quality assurance (QA), for human biomonitoring (HBM) of known and emerging trace and ultra-trace elements by high-resolution-inductively coupled plasma mass spectrometry (HR-ICP-MS) in human urine, serum and blood. The validation procedure included distinct operational steps: i) definition of the fitness-for-purpose; ii) demonstration of method performances and systematic quality control (QC) measures (including the use of control charts); iii) evidence of compliance in proficiency testing (PT) exercises and accreditation to ISO/IEC 17025. The method can be applied to monitoring single elements or mixture of elements in a broad category of human samples and in populations differently exposed, as a tool for public surveillances, hot spot programs and health risk assessments. Hopefully, the protocol can be used as a guidance towards a greater harmonization of HBM procedures and comparability of HBM results on a European-wide level.

Contents

Abbreviations: BEI, biologic exposure index; BEs, biomonitoring equivalents; CCs, control charts; CFs, correction factors; CRMs, certified reference materials; EQC, external quality control; HBM, human biomonitoring; HR, high resolution; HR-ICP-MS, high-resolution-inductively coupled plasma mass spectrometry; IQC, internal quality control; ISs, internal standards; LoD, limit of detection; LoQ, limit of quantification; LR, low resolution; MR, medium resolution; MU, measurement uncertainty; PGEs, platinum group elements; PT, proficiency testing; QA, quality assurance; QC, quality control; r, correlation coefficient; rl, repeatability limit; Rec, recovery; RSD, relative standard deviation; RSD_R, relative standard deviation of intermediate precision; SOPs, standard operating procedures; Tru, trueness; *U*, expanded uncertainty; u_c, combined uncertainty; u_{Tru}, trueness uncertainty; u_{Rec}, recovery uncertainty; $u_{other\ terms}$, other terms uncertainty.

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1. Introduction

Human biomonitoring (HBM) is a series of procedures aiming to protect human health from exposure to contaminants, such as trace and ultra-trace elements, by controlling their amounts in the body [1]. Measuring the internal dose, HBM shows whether and to what extent elements are really intaked from all sources. It also enables to identify changes in exposure, populations at higher risk, and can also help to elucidate their metabolism *in vivo*. The determination of elements in HBM matrices is the first step in a top-down "exposome" approach; internal biomarkers of exposure can be associated and integrated with measurements or modelling of exposures in air, soil, water, food, endogenous processes (hormones, oxidative stress, ageing) and genetic susceptibility, and other non-chemical stressors (individual's social, economic and psychological environment as well as climate changes) in order to characterize how these exposures relate to the development of health outcomes in populations groups. One of the prerequisites for HBM is the availability of suitable and reliable analytical methods which are kept under control by quality assurance (QA) programs. At European scale, the validation and harmonization of common HBM measurement methods thought continuous exchange of capacities and experiences have been strictly encouraged to ensure comparability of data and a more effective use of resources [2–6]. Two European twin projects have been conducted – Consortium to Perform Human Biomonitoring on a European Scale (COPHES) and DEMOnstration of a study to Coordinate and Perform Human biomonitoring on an European Scale (DEMOCOPHES) – with the aim of developing standardized protocols for HBM in Europe, although a limited number of metals (Hg and Cd) have been already carried out [7,8]. In 2015, the European Food Safety Authority (EFSA) document recommended the development of European wide comparable study protocols, analytical methods, and the establishment of quality assurance/quality control (QA/QC) systems, providing in appendix a detailed overview of the analytical methods used for measuring different classes of substances (also metals) in various human biological matrices (i.e. blood, urine, hair, and milk) [9]. Also the World Health Organization (WHO) reported a number of sensitive biomonitoring-based analytical methods to measure low chemical concentrations, including mercury (Hg), cadmium (Cd), arsenic (As), lead (Pb) in urine and cord blood, as well as standard operating procedures (SOPs) for sampling, laboratory analysis and statistical analysis of data [10]. The US Centers for Disease Control and Prevention (CDC) in the fourth National Report on Human Exposure to Environmental Chemicals listed in appendix peer-reviewed HBM-based methods [11]. Analytical methods used for HBM within the program of the HBM Commission can also be found in "Biomonitoring methods" (part IV of the MAK collection) containing 150 methods covering about 350 chemicals including metals [12]. These resources provides well-described methods that may help to generate HBM reliable data but, as first step, it is the single laboratory involved in the sector who has the responsibility to validate its own method and give evidence of fitness. Every HBM study must have a QA system, which ensures the integrity and traceability of samples, analyses and HBM data produced [13]. Laboratory HBM methods must be validated demonstrating, minimally, appropriate specificity, limit of detection, trueness and precision [14]. Then, each method should have internal QC (IQC) samples analyzed concurrently with test samples, as well as external QC (EQC) samples by participating to proficiency testing (PT) schemes [15–18]. A forceful form of external assessment of laboratory performance is the accreditation, which is the physical inspection of the laboratory involved in HBM in order to ensure that it complies with externally imposed standards; accreditation to ISO/IEC 17025 of the laboratory is indispensable to demonstrate that it is applying the required QA principles [19,20].

Often, in the case of HBM study for trace and ultra-trace elements there is lack of information regarding the extent to which the methods have been validated and the data associated to their uncertainty. Moreover, method developers are often concentrated on the evaluation of performance parameters of a method and rarely set acceptance criteria. For emerging metals of medium and high priority, such as cobalt (Co), chromium (Cr), nickel (Ni), vanadium (V) and platinum group elements (PGEs), there is urgent need to develop and validate suitable HBM methods [9]. In addition, methods that give the elemental profile, i.e., the simultaneous determination of several elements even at very low level, while keeping specificity in mind, are very important to monitor combined or mixed chemical exposures which is an issue of increasing concern in risk assessment [21]. For example, the EU project Public Health Impact of long-term low-level Mixed Element Exposure in susceptible population strata (PHIME) addressed HBM of multi-elements and paid attention to interaction between elements and the complexity of mixed exposures [22]. In addition, methods of analysis which are applicable uniformly to various groups of matrices (as urine, serum and blood) are preferred over methods which apply to individual matrices.

Based on these considerations, a HBM method has been developed which simultaneously quantified 18 trace and ultra-trace elements in serum and urine and 4 in blood by a simple and uniform methodological approach covering main analytical performances and most of relevant random and systematic effects affecting measurements. Three procedural steps have been organized as follows: i) creation a fitness-for-purpose statement, by a general description of the method, and presentation of background information about the analyte, matrix, laboratory resources, and the nature of the analytical problems; ii) validation at the level of a single laboratory (in-house validation), by determining the minimum performance requirements and establishing the acceptance criteria, as well as identifying factors affecting the measurement uncertainty (MU), that it is, also, an explicit requirement of ISO/IEC 17025; iii) demonstration that the method possessed sufficient inter-laboratory performances and can be used at level of routine, by participating to PT schemes and obtaining the accreditation to ISO/IEC 17025 which demonstrated either the technical competence to perform the method as well as the capability of the QA system [19,23]. The method was consistent with the golden rules for method validation proposed by Massart et al. (1997) [24] namely: i) it was validated as a whole, including sample treatments prior to analysis; ii) it was validated covering the full range of analyte concentrations specified in the method scope; and, iii) it was validated for each kind of matrix where it will be applied. This method was developed and validated on the basis of past experience gained from the laboratory [25–27] and following several quality guides, published by Association of Official Analytical Chemists (AOAC), Eurachem, Cooperation on International Traceability in Analytical Chemistry (CITAC), European Accreditation (EA), European Federation of National Associations of Measurement, Testing and Analytical Laboratories (Eurolab), International Laboratory Accreditation Cooperation (ILAC), International Organization for Standardization (ISO) [28–38].

Because the validation protocol covered most relevant scenarios – operators, experimental conditions, concentration ranges – and demonstrated its strength under different conditions, the method may be extended, with reliable and reproducible findings, to similar matrices (i.e., plasma, saliva, cerebrospinal fluid, etc.) and different sample populations (unexposed and exposed).

The rationale of this method gives the basis to increase the knoweldge of the main aspects of designing and conducting a HBM study and can be used as a guideline for other field studies with the aim to achieve a reliable and harmonized assessment of the internal dose of trace and ultra-trace elements.