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Reference measurements for total mercury and methyl mercury content in marine biota samples using direct or species-specific isotope dilution inductively coupled plasma mass spectrometry

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ABSTRACT

The analytical procedures for reference measurements of the total Hg and methyl mercury (MeHg) mass fractions at various concentration levels in marine biota samples, candidates for certified reference materials (oyster and clam *Gafrarium tumidum*), were evaluated. Two modes of application of isotope dilution inductively coupled plasma mass spectrometry method (ID ICP-MS), namely direct isotope dilution and species-specific isotope dilution analysis with the use of two different quantification mass spectrometry techniques were compared.

The entire ID ICP-MS measurement procedure was described by mathematical modelling and the combined uncertainty of measurement results was estimated. All factors influencing the final results as well as isotopic equilibrium were systematically investigated. This included the procedural blank, the moisture content in the biota samples and all factors affecting the blend ratio measurements (instrumental background, spectral interferences, dead time and mass discrimination effects as well as the repeatability of measured isotopic ratios). Modelling of the entire measurement procedures and the use of appropriate certified reference materials enable to assure the traceability of obtained values to the International System of Units (SI): the mole or the kilogram.

The total mass fraction of mercury in oyster and clam biota samples, after correction for moisture contents, was found to be: 21.1 (1.1) 10^{-9} kg kg^{-1} (U =5.1% relative, k=2) and 390.0 (9.4) 10^{-9} kg kg^{-1} (U=2.4% relative, k=2), respectively. For the determination of mercury being present as methyl mercury, the non-chromatographic separation on anion-exchange resin AG1-X8 of the blended samples was applied. The content of MeHg (as Hg) in oyster sample was found: 4.81 (24) 10^{-9} kg kg^{-1} (U=5.0%, k=2) and 4.84 (21) 10^{-9} kg kg^{-1} (U=4.3%, k=2) with the use of quadrupole (ICP QMS) or sector field (ICP SFMS) inductively coupled plasma mass spectrometers, respectively. In the case of clam sample, the concentration of MeHg (as Hg) was found to be: 61.0 (2.3) 10^{-9} kg kg^{-1} (U=3.8%, k=2) and 61.3 (2.2) 10^{-9} kg kg^{-1} (U=3.6%, k=2), respectively.

The mass fractions for total Hg and MeHg determined in this study were used as a contribution of the International Atomic Energy Agency (IAEA) Environment Laboratories in the characterisation of the IAEA 461 and IAEA 470 certified reference materials. The obtained good agreement with the reference values further validated the methods developed in this study.

1. Introduction

Mercury is a toxic element of widespread environmental, clinical and food safety importance. It is present in a trace amount in all compartments as a consequence of both natural and anthropogenic activities [1]. In October 2013, representatives of the governments of 140 member countries of the United Nations, agreed on the Mercury Convention, *i.e.* Minamata Convention on Mercury. This convention focuses on reducing the use of mercury compounds in mining and manufacturing, as well as its emission. The constitution of the Convention states that the Parties to the Convention have recognized that mercury is: "a chemical of global concern owing to its long-range atmospheric transport, its persistence in the environment once anthropogenically introduced its ability to bioaccumulate in ecosystems and its significant negative effects on human health and the environment" [2]. It is also well known that toxicity and ability of mercury to bioaccumulate in organisms depends on its chemical form. Adverse effects on

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human health associated with organic mercury compounds were brought to public attention in the late 1950's, when it was discovered that the widespread occurrence of neurological disease among residents in Minamata Bay and along the Agano River, Japan was linked to methyl mercury poisoning [3]. As a safeguard for human health, maximum permissible levels of Hg in fish <1 mg kg^{-1} and shellfish <0.50 mg kg^{-1} have been set by Regulation (EC) No 629/2008 to limit dietary exposure of consumers [4]. In 2004 the Joint Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) established a Provisional Tolerable Weekly Intake (PTWI) for methyl mercury of 1.6 μg kg^{-1} body weight (b.w.) and of 4 μg kg^{-1} b.w. for inorganic mercury. In 2012 in line with JECFA, the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM) established a Tolerable Weekly Intake (TWI) for methyl mercury of 1.3 μg kg^{-1} b.w., expressed as mercury [5]. Nowadays, it is well known that any mercury released into the environment undergoes biogeochemical transformation and can be converted into the most toxic methyl mercury derivative. The main source of human intake of mercury contaminants originates from methyl mercury in fish and fishery products. Establishment of the total concentration of element would not provide sufficient information in risk assessment regarding food or environmental safety. Therefore, it is essential to develop accurate and precise analytical method for the determination of individual mercury species.

The most widely analytical techniques used for the determination of total content of mercury in various samples are: a direct cold vapor atomic absorption spectrometry (CV AAS) [6] or with pre-concentration step [7], graphite furnace (GF) AAS [8], CV atomic fluorescence spectrometry (AFS) [9], inductively coupled plasma optical emission spectrometry (ICP-OES) [10] and ICP-MS [11,12]. Among listed techniques, ICP-MS is considered as the most powerful elemental detector at a trace level, due to its high sensitivity, low detection limit (often in the ng L^{-1} range) and good precision of measurement (<1% RSD). In general, speciation analysis involves the coupling of a separation technique with one of the detection method (*eg.* AAS, AFS, MS). Depending on the objective, different separation techniques for speciation of mercury could be applied, *e.g.* gas [13] or liquid [14] chromatography and capillary electrophoresis [15]. However, before final separation and detection of mercury in individual species, the sample preparation, *e.g.* pre-treatment (*eg.* extraction, derivatisation, distillation or complexation) or pre-concentration (*eg.* solid phase microextraction, amalgamation or trapping) are usually needed [16]. In this respect one should be aware of the commonly reported problems of non-quantitative recoveries and the formation of Hg artefacts during pre-treatment and pre-concentrations steps [17,18], which can be overcome by the use of isotopically enriched species (*i.e.* spikes) as tracers [19,20]. Thus, the analytical procedure involving isotope dilution inductively coupled plasma mass spectrometry (ID ICP-MS), regarded as a primary method of measurements, is recommended [21,22]. The main advantages of IDMS, in comparison with other calibration strategies, *e.g.* external calibration or standard additions, are: (i) once complete isotope equilibration between the sample and the spike has been achieved, there is no need to know the pre-concentration or dilution factor of the sample or to take into account any non-quantitative separation or evaporation process; (ii) any variation of the instrumental sensitivity such as signal drift has not influence on the final results; and (iii) the results are obtained with superior accuracy and precision, and with a small combined uncertainty [23,24].

The species-specific IDMS procedure requires the use of a spike solution containing the species to be analyzed in an isotopically labelled form. The spike solution is added to the sample at the very beginning of the analytical procedure, and once complete mixing between the added enriched species and the naturally occurring, the sample is ready for measurements, thus all traditional advantages of isotope dilution analysis can be fully exploited [20,24]. Every analytical measurement has an associated uncertainty, resulting from errors arising in the various stages of sampling and analysis. The estimation of the uncertainty budget being recognized as an essential part of the measurement process, because it facilitates improved inter-comparison of analytical results and is a requirement for the International Organization for Standardization (ISO) accredited methods [25,26].

Isotope dilution offers the possibility to determine major to trace mass fractions of elements in various matrices, and is often used for certified reference materials characterisation [22,27,28]. In this respect, a sector field mass spectrometer is preferable to quadrupole-based instruments, because the resulting flat-topped peak shape obtained at low resolution mode enables a precise isotope intensity measurement to be achieved [24]. It should be noted that, as in the case for other calibration strategies, isotope dilution cannot compensate for random contamination, which may occur during sample work up and an intensive blank monitoring is thus always necessary [21,29].

The development and validation of an ID ICP-MS reference procedure for the quantification of total Hg and mercury being present as MeHg in the marine biota samples with the objective of achieving an uncertainty target on final results of 1–5% ($k=2$) and SI traceable values are described in this study. Systematic assessment of all factors influencing the measurement results as sample-spike isotopic equilibrium, homogeneity study, factors affecting the blend ratio measurements, the efficiency of the sample digestion procedure, possible interferences, matrix effects *etc.* was done through the present study. Modelling of the entire measurement process and the use of reference materials links each of obtained results to the SI units: the mole and the kilogram [21,30].

2. Experimental

2.1. Instrumentation

Advanced mercury analyzer (AMA-254, Altech Czech Republic) and gas chromatograph coupled with atomic fluorescence spectrometer (GC-AFS, Brooks Rand Labs, Seattle, WA, USA) were used for homogeneity studies of total Hg and for MeHg, respectively.

All blends and working standard solutions were prepared gravimetrically by appropriate dilution of the stock standard solutions using an analytical balance (Mettler Toledo, Switzerland).

Digestion of the marine sediment samples was performed in a closed microwave system (Mars-X CEM) equipped with a carousel holding 12 digestion Teflon vessels.

High quality deionised water from Milli-Q system (Millipore, Bedford, MA, USA) was used throughout this work.

The isotope ratio measurements were carried out with either a quadrupole inductively coupled plasma mass spectrometer (XSeries 2 Thermo Scientific, Bremen, Germany), or a sector field inductively coupled plasma mass spectrometer (Attom, Nu Instruments Ltd., Wrexham, UK). Both instruments were equipped with a MicroMist nebulizer (0.2 mL min^{-1}, Glass Expansion, Australia) and a cyclonic spray chamber cooled by Peltier cooling system (Glass Expansion, Australia). The instrument conditions were checked daily and adjusted for optimum sensitivity and repeatability of the isotope ratio measurements. The ICP QMS was equipped with a collision cell for interferences reduction. Mixture of pure hydrogen and helium (Air Liquide, France) was used as the collision gas.

Servo operating mode and fast scan ion optics was used for all

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