PREPARATION AND CERTIFICATION OF A CERTIFIED REFERENCE MATERIAL FOR THE TOTAL AND METHYLMERCURY CONTENT IN FISH

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Mercury contamination of the marine environment has long been recognized as a serious environmental concern. Fish accumulate substantial concentrations of mercury in their tissues and thus can represent a major source of this element to humans. Among the different mercury species, methylmercury is of particular concern due to its extreme toxicity and its ability to bioaccumulate in fish tissues where it represents circa of 90% of total mercury. This is a matter of concern considering that fish tissues are an important part of the diet in several communities around the world and the presence of mercury can be a public health problem, particularly for women who are or may become pregnant, nursing mothers, and young children.

A significant effort has been made to develop methods for its determination in environmental and biological samples [1,2,3].

The demand for new certified reference materials for the assessment of accuracy and reproducibility of experimental data is increasing in several areas. Thus, competent authorities emphasized the need to produce certified reference materials (CRMs), which can support metrological traceability of the results in the total and methylmercury determination. As a response, the Laboratório de Caracterização Química (LCQ), of the Instituto de Pesquisas Energéticas e Nucleares (Ipen) took on the task to produce Dourada-1, a certified reference material containing Hg and MeHg in fish matrix, following the principles of ISO Guides 34 [4] and 35 [5].

This communication describes the production of the Dourada-1 fish material, including the material processing, the results of tests performed to assess its homogeneity and stability, and the strategy to assign reference Hg and MeHg values in the material.

The candidate reference material was collected in the Pará State, north of Brazil. It was produced from Dourada fish (*Brachyplatystoma Flavicans*). Amount of 18 kg of Dourada fish was sliced and minced using a domestic miller. After mincing, the material obtained was stored frozen in glass Petri plate. The material was then freeze-dried, ground handmade and sieved using a polypropylene sieve.

The material was transferred to polyethylene container and sent to the Instituto Tecnológico de Alimentos (ITAL), in Campinas city, where it homogenized in a mixing drum type “V” and then bottled in brown borosilicate glass bottles. A total of 80 bottles each containing 15 g of material was produced for candidate reference material. The material was stored at room temperature.

The between-bottle homogeneity was verified by the determination of total and methylmercury on samples intakes of 0.4-0.5 g taken from 10 bottles. For the Hg determination, the samples were digested with acid mixture using sulfuric, nitric and percloric acid and the final determination was performed by FIA-CV-AAS. For the MeHg determination, a mass equal to 0.5 g sample was

weighed following leaching with 10 mL of 6 mol L^{-1} HCl solution, organic and inorganic Hg was separated by anion exchange resin Dowex 1x8 100-200 Mesh. MeHg was decomposed to inorganic mercury (II) either by UV irradiation and finally the determination was also performed by FIA-CV-AAS. The uncertainty of the homogeneity study was evaluated using ISO Guide 35. For determining the magnitude of the between-bottle homogeneity standard uncertainty, the experimental data obtained were inspected for trends.

The minimum sample intake was determined by carrying out a within-bottle homogeneity study for different test portions.

The stability of the total and methylmercury content was tested to determine the suitability of this material as reference material. Bottles were kept at respectively $+8^0$C, $+20^0$C and $+40^0$C over a period of 12 months and total mercury was determined at regular intervals during the storage period. Tests were made at the beginning of the storage period after 35, 70, 150 and 365 days. Samples were analyzed using the same procedure as for homogeneity study. The uncertainty of the stability study was evaluated using ISO Guide 35. The first step in the evaluation of data from a stability study is a check of whether any trend in the data can be observed. After that, no trend was detected and stability was demonstrated for the longest time studied. For stability studies the classical layout was used.

The technique used in the characterization was isotope dilution mass spectrometry (IDMS) using enriched spike isotope ^{202}Hg obtained from IRMM (IRMM 640, Retieseweg, B-2440, GELLO, Belgium). Isotope ratio measurements of reference/spike isotope were carried out by isotope ratio measurement mode in the HRICP-MS equipment (Element 2, Finnigan-MAT, Germany).

The uncertainty associated with a certified value of a CRM can be expressed as [5]:

$$u_{CRM} = \sqrt{u_{char}^2 + u_{bb}^2 + u_{lts}^2 + u_{sts}^2} \qquad (1)$$

In the Table 1, were given the certified values and their expanded uncertainty for total and methylmercury.

TABLE 1. CERTIFIED VALUE AND THEIR UNCERTAINTY FOR TOTAL MERCURY

Compound	**Certified Value ± expanded uncertainty μg g^{-1}**
Total mercury	0.271 ± 0.057
methylmercury	0.245 ± 0.038

A reference material was produced and certified for the total and methylmercury in fish matrix in compliance with ISO Guides 34 e 35. The standard operational procedure for the preparation of reference material was proposed in this study. The homogeneity and stability of the material were demonstrated. This material represents the first national certified reference material for mercury compounds in fish matrix.

References

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