PRODUCTION OF A CERTIFIED REFERENCE MATERIAL FOR THE TOTAL MERCURY CONTENT IN FISH

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Abstract: Certified reference materials are widely applied to ensure the reliability of analytical data and contribution to establishing the accuracy and traceability of chemical measurements. Some types of reference materials may not be readily available, and the possibility of producing them in house has to be considered. This work describes the steps of production and certification, according to ISO Guides 34 e 35, of a new certified reference material. Homogeneity and stability study were carried out by flow injection analysis cold vapor-atomic absorption spectrometry (FIA-CV-AAS) and for characterization isotope dilution mass spectrometry (ID-ICPMS) was used. The certified value for total mercury is 0.271 ± 0.057 μg g^{-1}.

Key words: certified reference material, quality control, mercury, fish

1. INTRODUCTION

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.

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 various disciplines, among which environmental and food analysis is the prime importance. Thus, competent authorities emphasized the need to produce certified reference materials (CRMs), which can support metrological traceability of the results in the mercury determination. According to International Vocabulary of Metrology [4] and ISO Guide 30 [5], a CRM is "reference material, accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceabilities, using valid procedures".

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 in fish matrix, following the principles of ISO Guides 34 [6] and 35 [7].

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 value in the material.

2. MATERIALS AND METHODS

2.1. Collection of the candidate – CRM

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, as many as nine individuals, was frozen (- 20 ^{0}C) and transported to laboratory in Ipen. Then, the 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, resulting in 1.2 kg of material. All material used in the preparation, such as knives, plastic trays, cutting boards, container miller, plastic bottles and glass Petri plate were cleaned and decontaminated, and the last wash carried out with Milli-Q water (Academic, 18MΩ-cm, Millipore Corporation).

2.2. Homogenization and bottling

The freeze-dried material was 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.

To elimination of microorganisms and prevent their reproduction, the next step was sterilizing the material by irradiation at the Centro de Tecnologia das Radiações - CTR, in Ipen. The sample, consisting of approximately 1.2 kg of material, was irradiated with an activity of 4.9 kGy.

2.3. Homogeneity

The between-bottle homogeneity was verified by the determination of total mercury on samples intakes of 0.4 g taken from 10 bottles. Three portions of each bottle were digested and three replicate analysis in each portion was analysed, so a total of 90 concentration values were obtained.

For the determination, the samples were digested with acid mixture using sulfuric, nitric and percloric acid and the final determination was performed by FIA-CV-AAS. Calibrations were performed by standard solution of mercury.

One way analysis of variance (ANOVA) of the results was carried out, according described in ISO Guide 35. The uncertainty of the homogeneity study was evaluated using ISO Guide 35. Several approaches to obtain an uncertainty estimate that explain the insufficient repeatability of the measurement method has been proposed. The influence of the repeatability standard deviation on S_{bb} can be accounted for using:

$$u_{bb} = \sqrt{\frac{MS_{within}}{n}} \sqrt[4]{\frac{2}{\nu_{MS_{within}}}} \qquad (1)$$

where MS_{within} is equal to the repeatability variance of the measurements used in the between-bottle homogeneity study (mean square), n is number of observations and $\nu_{Mswithin}$ is degrees of freedom of MS_{within}.

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 within-bottle homogeneity was assessed by 10 replicate determinations on the content of one bottle.

It was concluded that the material is suitable for use as CRM.

2.4. Stability

The stability tests were carried out using ISO Guide 35 and protocol developed at the BCR [8].

The stability of the total mercury content was tested to determine the suitability of this material as reference material. In order to check the stability of the concentration of the element, 5 bottles were kept at different temperatures for different periods of time. 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. Total mercury was determined in five bottles. Two portions of each bottle were digested and three replicate analysis in each portion was analysed, so a total of 30 concentration values were obtained at each occasion of analysis.

The samples stored at room temperature were used as reference.

The uncertainty contribution due to long-term stability study was evaluated using ISO Guide 35:

$$u_{lts} = s_{b_1}.t \qquad (2)$$

where u_{lts} is the uncertainty contribution due to long-term stability, s_{b1} is the uncertainty associated with the slope (regression parameters: $y = b_0 + b_1.x$, where y = property value and x denotes time studied) and t is the time in months.

2.5. Certified values

The technique used in the characterization was isotope dilution mass spectrometry (IDMS) using enriched spike isotope ^{202}Hg obtained from Institute for Reference Materials and Measurements (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). A good accuracy on the isotope ratio determinations (^{200}Hg/^{202}Hg) is ensured by mass bias correction of

the observed isotope ratios. The mass discrimination correction factor ($f = R_{true}/R_{measured}$) was estimated daily from the analysis of mercury standard solutions (IRMM 639, Retieseweg, B-2440, GELL, Belgium). The factor obtained was equal to 1.0064.

The concentration of Hg was calculated by the following equation 1 [9]:

$$C_s = C_{sp} \cdot \frac{W_{sp}}{W_s} \cdot \frac{A_{r_s}}{A_{r_{sp}}} \cdot \frac{A_{sp}}{A_s} \cdot \frac{R_m - R_{sp}}{1 - R_m . R_s} \quad (3)$$

where Cs is the unknown concentration of the element in the sample (S) and C_{sp} the concentration of the element in the spike (Sp). W_s and W_{sp} are the weights taken from sample and spike respectively. Ar_s, and Ar_{sp} are the elemental atomic weights in the sample and spike, respectively. A_{sp} is the isotope abundance (At%) of the reference isotope in the spike (isotope a) and A_s is the isotope abundance of the reference isotope in the sample (isotope b). R_m and R_{sp} are the atomic ratios (isotope b/isotope a) in the mixture and the spike respectively and R_s is the atomic ratio (isotope a/isotope b) in the sample.

A mass equal to 0.4 g sample was weighed and added to 0.20 g of spike solution with concentration of mercury equal to 0.013 ug g^{-1}, directly into a Teflon® container. Then was added 2 mL of concentrated HNO_3 distillate and shaken manually. The container was sealed with a Teflon cap and Teflon tape was placed at the junction between the container and cap. The set (container and cap) was transferred to a hot plate and digestion proceeded for 100 minutes at a temperature of 100 ^{0}C. Finally, after cooling, the digested sample was transferred to a plastic tube, like Falcon, with a capacity of 15 mL and was diluted to a final weight of 10 g. Then, the determination of isotopic ratios in the mixture were performed at HRICP-MS.

For the determination of total mercury in homogeneity and stability study was used the flow injection analysis cold vapor-atomic absorption spectrometry (FIA-CV-AAS). This technique is traditionally used because of its high sensitivity, low cost and speed [10,11,12].

The uncertainty associated with a certified value of a CRM can be expressed as [7]:

$$u_{CRM} = \sqrt{u_{char}^2 + u_{bb}^2 + u_{lts}^2 + u_{sts}^2} \quad (4)$$

where: u_{char} is standard uncertainty due to characterization, u_{bb} is standard uncertainty due to between-bottle homogeneity, u_{lts} is standard uncertainty due to long-term stability and u_{sts} is standard uncertainty due to short-term stability.

The short-term stability is associated with any extra effects due to transport of the samples. Transport conditions have been chosen so that no additional uncertainty with respect to stability due to transport needs to be taken into account. Consequently, u_{sts} = 0.

3. RESULTS AND DISCUSSION

3.1. Homogeneity

In the homogeneity study was not detected any heterogeneity in the material.

Using the expression (1), an estimate of the between-bottle standard deviation can be obtained that reflects the relative value for the repeatability standard deviation:

$$u_{bb} = \sqrt{\frac{0{,}000917}{20}} \times \sqrt[4]{\frac{2}{80}} = 0{,}006771 \text{ x } 0{,}3976 = 0{,}002692 \text{ µg } g^{-1}$$

After within-bottle homogeneity study, was defined as the minimum mass 0.2 g. But, for convenience, we used a mass of the 0.4 g.

3.2. Stability

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.

Using the expression (2), an estimate of uncertainty contribution due to long-term stability study was obtained by:

$$u_{lts_{20C}} = 0{,}002356 \times 12 = 0{,}02827$$
$$u_{lts_{40C}} = 0{,}002875 \times 12 = 0{,}0345$$

On the basis of the results, it was concluded that no instability could be demonstrated. The material will be monitored at regular intervals.

3.3. Certified values

In the Table 1, is given the certified value and their expanded uncertainty (with k = 2 and 95% confidence level) for total mercury.

Table 1. Certified value and their uncertainty

Property	Certified Value ± expanded uncertainty $\mu g\ g^{-1}$
Total mercury	0.271 ± 0.057

4. CONCLUSIONS

A reference material was produced and certified for the total mercury in fish matrix in compliance with ISO Guides 34 e 35. The results of this work clearly showed that particular care has to be taken to achieve a good quality control of techniques used and the steps in preparation of material. The homogeneity and stability of the material were demonstrated. In-house fish reference material is homogeneous at 200 mg sample size. For a long-term stability study of material in a period of 12 months at temperature of 40 ^{0}C, no significant change was observed indicating the stability of the material. This material represents the first national certified reference material for mercury in fish matrix.

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