EXPERIENCE OF THE NEUTRON ACTIVATION ANALYSIS LABORATORY OF IPEN – CNEN / SP IN REFERENCE MATERIAL PREPARATION

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Abstract

In this paper a description is made of the preparation of two reference materials in the Neutron Activation Analysis Laboratory of IPEN – CNEN / SP: Bovine Liver and *Perna perna* mussel. The Bovine Liver RM was designed as an in-house reference material, in the framework of a project supported by FAPESP, whose aim was to carry out nutritional studies of the diet of Brazilian industrial workers. About 15 kg of bovine liver acquired in a local market of the city of São Paulo were processed by homogenization and freeze drying and analyzed by several analytical techniques, after undergoing homogeneity test by INAA. Data evaluation was performed using the IAEA procedures for interlaboratory comparison exercises and using these criteria the results for 13 elements could be considered as reliable. The mussel reference material was prepared in the framework of a project on environmental biomonitoring with transplanted marine organisms. About 160 kg of the *Perna perna* mussel were purchased in a mussel farm in the Cocanha beach in the shore of São Paulo State and also submitted to several preparation steps and homogeneity tests by INAA, before being sent to various laboratories, in Brazil and abroad, to be analyzed by various analytical techniques. All the steps of the reference materials preparation and results obtained are presented.

***Keywords*:** reference material, neutron activation analysis, IPEN – CNEN / SP

1. INTRODUCTION

The demonstration of the quality of analytical results, i.e., their fitness for purpose, is becoming more and more an essential requirement in the quality assurance of products and processes, in industry, trade and in applied research, and it can affect economic, political and environmental protection related decisions [1].

In a chemical analysis, it is very important to demonstrate the quality of the results, that is, to show that the uncertainty sources are under control, which leads to reliable results. One of the best ways to attain this objective is the use of a reference material, RM, which presents values of properties that are sufficiently homogeneous and well established. RMs are also used for the validation of analytical methods, in interlaboratory studies and proficiency tests and quality assurance [2]. When the property values of a RM are certified by means of a procedure that establishes the traceability of these values to SI and when the property values are accompanied by an uncertainty for a given confidence level, the RM is designated as a certified reference material, CRM. In the case of CRMs for chemical composition, the property values are the concentrations of elements or of chemical substances [3].

On the other hand, despite the importance of the use of RMs for quality assurance of analytical results, their use in Brazil is not as disseminated as it should. Due to the complexity in the preparation and characterization of RMs, their price can become quite high and in some cases there are difficulties or restrictions to the purchase of these materials. Last but not least, in many instances, it is not possible to have available a material of a matrix similar to the samples in study, or with compatible concentration levels.

In Brazil, the production of CRMs can be considered still as incipient, due to the difficulties mentioned. The São Paulo State Institute for Technological Research, IPT, has given a very important contribution in the area of preparation and commercialization of CRMs for industrial applications, such as metal alloys and non-ferrous materials [4]. Other important initiatives to be mentioned are: the production of RMs of uranium and thorium by the Brazilian Nuclear Energy National Commission [5].

Recently uranium isotopic CRMs were prepared by Oliveira Junior, as part of a Ph. D. thesis at the Nuclear and Energy Research Institute, IPEN – CNEN / SP [6] and a CRM of bauxite was produced at the Center of Mineral Technology, CETEM, in Rio de Janeiro State [7]. The Institute of Geosciences of the University of Campinas, São Paulo State, also certified a reference material of basalt [8].

Other important Brazilian efforts in this area were the production of a fish RM for mercury at the Isotopic and Chemical Characterization Laboratory of IPEN – CNEN/SP and of a coffee RM for several trace elements at the Laboratory of Isotopes of the Center for Nuclear Energy in Agriculture, CENA, from the University of São Paulo [9].

The Neutron Activation Analysis Laboratory, LAN, of IPEN-CNEN/SP has been, for many years, developing research work in the environmental and nutritional fields and, as part of the efforts to comply with quality assurance and quality control requirements, has engaged in the preparation of two biological RMs: bovine liver and mussel tissue (*Perna perna*).

All the steps involved in the preparation and characterization of both RMs will be described in the present work.

2. OBJECTIVE

The objective of the present work is to describe all the steps involved in the preparation and characterization of the reference materials bovine liver and mussel tissue at the Neutron Activation Analysis Laboratory of IPEN-CNEN / SP.

3. MATERIALS AND METHODS

3.1. Bovine Liver Reference Material

About 15 kg of bovine liver, acquired in a local market in the city of São Paulo were homogenized in an industrial knife mill, previously washed and rinsed with deionized and distilled water.

After milling, the samples were frozen and then lyophilized, in an Atlas industrial lyophilizer, with 500 L capacity. Finally, the bovine liver was homogenized, in a domestic blender adapted with titanium knives, to avoid contamination with metals. The sample was then stored in demineralized polyethylene bags (Whirl-Paki) and kept in a freezer (-20 ^{o}C) before being distributed to the participating laboratories for analysis.

Homogeneity tests were made in 5 different lots of the sample, using instrumental neutron activation analysis, INAA, determining the elements Na, K, Fe e Zn. The results obtained allowed to conclude that the bovine liver material, prepared as described above, was homogeneous and adequate for further analysis.

The next step was the distribution of the candidate reference material to the laboratories that agreed to participate in the characterization of the material. A total of six laboratories of three research institutions participated of the intercomparison. The following analytical techniques were used by the laboratories: instrumental neutron activation analysis (INAA), atomic emission spectroscopy with argon plasma (ICP OES), energy dispersive X-ray spectroscopy (WD XRFS), differential pulse anodic stripping voltammetry (DP ASV), flame atomic absorption spectroscopy (FAAS), electrothermal atomic absorption spectroscopy (ET AAS) and high performance liquid chromatography (HPLC).

In this collaborative study, two NIST certified reference materials were distributed to the laboratories, together with the bovine liver candidate reference material: NIST SRM 1548a Typical Diet and NIST 1577b Bovine Liver, in order to conduct first a proficiency test.

The results sent by the laboratories were evaluated following the procedures used by the International Atomic Energy Agency (IAEA) for the intercomparison exercises that have been regularly conducted by IAEA for many years [11]. As for the analysis of the prepared bovine liver, the results of the laboratories were evaluated by the z-score criterion , in which the mean value of the final concentration , calculated for each element, was considered as the “target value” [12].

3.2. Mussel Tissue Reference Material

The marine organism chosen in the present study was the *Perna perna* mussel, which is the most abundant mussel species in the coast between the states of Rio de Janeiro and Santa Catarina. Nowadays the state of Santa Catarina is responsible for 93% of the Brazilian culture of this mussel, but in the state of São Paulo there are several farms too, mainly in the cities of Caraguatatuba, São Sebastião and Ubatuba.

In October 2005, 164 kg of the *Perna perna* mussel were acquired from a mussel farm situated in the Cocanha beach, in Caraguatatuba, where the mussel is cultivated by the longline system.

The mussels were submitted to a series of treatments for preparation of the candidate RM, including: washing with deionized water, homogenization in a blender with titanium blades, lyophilization, grinding and sieving. During all the preparation steps, care was taken to avoid contaminating the material with trace metals. It is also important to point out that, for the preparation of the mussel RM, the recommendations of the ISO Guide series 30 to 35 were followed. After the sieving step, 2.4 kg of the material, with particle size less than 105 μm was obtained.

The mussel tissue, prepared as described, was then homogenized in a Y stainless steel homogenizer, coated with polytetrafluorethylene, during 72 hours. After this step, the material was bottled in amber bottles, thoroughly , cleaned in nitric acid and Milli-Q water. The number of flasks of candidate reference material obtained was 171, with an average mass of 12.7 g each.

The next step was the sterilization of the flasks with gamma radiation, to enhance the stability of the matrix towards microbiological degradation. It was found that the irradiation with a dose of 5 kGy is enough for the sterilization of the material, at least for the microorganisms that grew in the culture conditions used in the present work.

The between bottles and within bottle homogeneity studies of the material were carried out by INAA, and the elements determined in the studies were : Ag, As, Br, Co, Cr, Cs, Eu, Fe, La, Na, Rb, Sc, Se, Th and Zn. The statistical tests applied revealed that the elemental concentrations can be considered as homogeneous along the bottles for the candidate reference material for the elements and test conditions used. As for the homogeneity study inside a flask, it was found that the material also can be considered as homogeneous, for the masses of 50, 150 and 250 mg.

Another important aspect of the preparation of a reference material is the short term and long term stability studies of the material. In the present work, the short term stability was studied by keeping vials of the material for various time periods, up to three months, at several temperatures (-20 ^{o}C , 20 ^{o}C, 40 ^{o}C and 60 ^{o}C). After the test periods, the elements : Ag, As, Br, Co, Cr, Fe, Na, Se and Zn were determined by INAA. It was concluded that the candidate mussel reference material is stable enough to be transported under normal transport conditions, without significant alterations in the composition of the elements studied.

As regards the long term stability , the tests made had a duration of one year, and consisted in the analyses of four aliquots of six bottles stored at room temperature and one bottle kept at – 20 ^{o} C. With the application also of the INAA method, considering the analysis of 15 elements, it was possible to confirm, by using statistical treatments, that there were no significant alterations in the concentrations of the elements considered in the study.

4. RESULTS

4.1. Results of the Bovine Liver intercomparison

For the bovine liver candidate reference material prepared and for the NIST CRMs, mean values were obtained for 17 elements, by 6 different analytical techniques. For each element, from 5 to 10 individual determinations were made.

The results obtained by the various laboratories , for CRM NIST 1577b Bovine Liver, allowed to conclude , using the z-score criterion, that all the elemental concentrations were in the range (+3, -3), with the exception of calcium determined by WD-XRFS, lead determined by DP ASV and potassium determined by all analytical methods.

As for the Typical Diet NIST CRM, practically all the elements analyzed were in the interval (+3, -3), with the exception of the elements potassium and manganese determined by INAA.

The results of the final concentrations of all the 17 elements determined are presented in Table 1, together with the respective values of the standard deviations and confidence intervals.

Element	Bovine Liver		
	Mean ± SD (target value)	RSD %	Confidence interval
Ca mg kg^{-1}	136 ± 22	16	82 -190
Cd mg kg^{-1}	0.09 ± 0.03	33	0.046 – 0.132
Cl %	0.2970 ± 0.0007	0.2	0.2911– 0.3029
Co mg kg^{-1}	0.34 ± 0.08	24	0.130 – 0.548
Cu mg kg^{-1}	209 ± 16	7.7	194 – 224
Fe mg kg^{-1}	227 ± 16	7.0	202 – 251
K %	1.11 ± 0.04	3.6	1.04 – 1.18
Mg mg kg^{-1}	612 ± 27	4.4	570 – 654
Mn mg kg^{-1}	9.1 ± 1.3	14	7.4 – 10.7
Mo mg kg^{-1}	3.6 ± 0.2	5.6	2.18 – 4.98
Na %	0.25 ± 0.02	8.0	0.211 – 0.283
P %	1.20 ± 0.04	3.3	1.09 – 1.31
Pb mg kg^{-1}	0.31 ± 0.05	16	-
Rb mg kg^{-1}	37.6 ± 0.5	1.3	33.1 – 42.0

S %	0.768 ± 0.009	1.2	-
Se mg kg^{-1}	0.311 ± 0.008	2.6	-
Zn mg kg^{-1}	139 ± 8	5.8	131 - 147

Table 1. Values of the final concentrations of the elements determined in the bovine liver candidate CRM

4.2. Results of the mussel tissue intercomparison

In order to start the certification process for the elements of interest in the mussel tissue, it was decided to use the approach of a collaborative study, in which the certified value can be obtained by means of application of statistical methods to the results of several laboratories, utilizing various analytical techniques. The organization of the program was made using international recommendations for the development and control of collaborative programs [13-14] .After evaluation of the results, it is possible, to assign the certified values for the elements studied in the program.

In this study, ten national and six international laboratories, with known analytical competence for analysis of environmental samples, agreed to participate of the project. It was suggested to the participants to determine all the elements possible, with the available analytical techniques in each laboratory. It was asked form each participant to send results, dry weight, for six determinations of each element, obtained independently in the two bottles of the candidate reference material that were sent. It was also asked that the laboratories send results of analysis of three aliquots of a quality control sample, a RM at choice of the laboratory.

Up to now, not all the participants have sent their results, nevertheless results for the analysis of 32 elements have been sent, by the following analytical techniques : ICP OES, AAS, WDXRF, INAA, k_o-INAA. Once all the results of the participant laboratories will be available, adequate statistical techniques will be applied, in order to obtain, as much as possible, certified values for the mussel candidate reference material.

5. CONCLUSIONS

As regards the preparation of the bovine liver RM, it can be concluded that it is suitable to be used as a secondary reference material. The relative standard deviations obtained for 13 elements were below 13 % and for calcium, below 16 %. The elements present at concentration levels of the order of μg kg^{-1} presented higher relative standard deviations, such as 17 % for lead, 25 % for cobalt and 30 % for cadmium.

In the case of the mussel tissue candidate RM, it can be considered that the preparation was very successful, since the material obtained was found to be homogeneous, stable at short and long term and free of microbiological degradation. The treatment of the elemental analytical data sent by the laboratories is underway and it will be decided later which elements can be certified.

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