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Applying INAA to the homogeneity study of a *Perna perna* mussel reference material

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Abstract Instrumental neutron activation analysis (INAA) was used in the homogeneity study of a *Perna perna* (Linnaeus 1758) mussel reference material. Simultaneous determination of 15 elements in six bottles of the material, from a total of 171, was performed. The bottles were considered homogeneous for the analyzed elements, at the 95% confidence level, according to the analysis of variance test performed. Elements were also determined in one bottle with five different sample masses for minimum sample intake estimation. Results varied from 50 to 350 mg, depending on the element.

Keywords Reference material · Mussel · *Perna perna* · Homogeneity · INAA

Introduction

Several steps are involved in the characterization and certification of reference materials (RMs) of biological and environmental origin, including sampling site selection, sampling layout, material preparation and cleaning, freeze-drying, grinding and sieving, water content control, homogenization, bottling, sterilization, definition of storage conditions and chemical characterization [1].

In the homogeneity status characterization, the purpose of the between bottle homogeneity study is to assure that the property values of interest will not present variation among the bottles of the RM, more than allowed by its intended use. In other words, the homogenization of the bulk RM must be done in such a way that any residual inhomogeneity of the material will not be significant when the uncertainty of the analytical method in which the RM is used is concerned [2].

Good precision is the most important figure of merit of an analytical technique to allow it to be used in the homogeneity testing of candidate RMs. Due to its inherent characteristics, instrumental neutron activation analysis (INAA) is an analytical technique of choice for homogeneity testing [3]. With the use of appropriate INAA measurement schemes, it is possible to obtain precise measurement results simultaneously for a large number of elements. Another advantage is that solid samples are analyzed directly and, hence, problems in sample digestion and contamination from reagents are not an issue for the technique. Also, complementary gamma ray photopeaks, for the elements that present them, may be used in the homogeneity study of RMs, as presented in a previous communication [4].

The within bottle homogeneity study is another feature of the homogeneity characterization. In this case, the minimum sample intake for end users of the RM is estimated from property value determinations on one single bottle, using different sample masses [2].

In this study, INAA was used for the between bottle and within bottle homogeneity assessment of Ag, As, Br, Co, Cr, Cs, Eu, Fe, La, Na, Rb, Sc, Se, Th, and Zn in a *Perna perna* (Linnaeus 1758) mussel reference material prepared at. IPEN—CNEN/SP. The contribution of standard uncertainties due to the between bottle homogeneity (u_{bb}) to the standard uncertainties of property values (u_{MRC}) were estimated.

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Experimental

Homogeneity study layout

A random stratified scheme was applied for the selection of six bottles of the candidate RM for the between bottle homogeneity study (bottle numbers 19, 40, 75, 112, 143 and 156), from a total of 171 bottles. The HISTO software, provided by the International Atomic Energy Agency (IAEA) was used for bottle number selection [5]. The HISTO software was also used for randomization of all subsamples, prior to irradiation and gamma ray measurement. This procedure was necessary in order to avoid interferences from any possible trends that might arise in the results during the measurement campaign. For the within bottle homogeneity study, bottle number 90 was selected.

Sample and elemental standard preparation

For the between bottle homogeneity study, eight subsamples from each bottle, with approximately 150 mg, were weighed in properly cleaned polyethylene vials using a Shimadzu AEM-5200 analytical balance. For the within bottle homogeneity study, five subsamples of 50, 150, 250, 350 and 500 mg were used from the same bottle. Elemental standards were prepared by pipetting Spex standard element solutions onto Whatman paper filters, using variable volume pipettes (Eppendorf or Jencons). For some elements, the original solution was diluted in volumetric flasks prior to pipetting. After drying, paper filters were kept in polyethylene vials with the same geometry as for the samples.

Irradiation and element determination

Subsamples and elemental standards were irradiated simultaneously for 8 h at 10^{12} n cm^{-2} s^{-1} thermal neutron flux of the IEA-R1 Nuclear Research Reactor at IPEN—CNEN/SP. ^{76}As, ^{82}Br, ^{140}La and ^{24}Na radionuclides were measured for 1.5 h, after a 7-day decay period, while ^{110m}Ag, ^{60}Co, ^{51}Cr, ^{134}Cs, ^{152}Eu, ^{59}Fe, ^{233}Pa (for Th determination), ^{86}Rb, ^{46}Sc, ^{75}Se and ^{65}Zn radionuclides were measured for 10 h, after a 15-day decay period, using suitable gamma ray decay photopeaks[4]. Gamma ray measurements were performed using a GC2018 Canberra HPGe detector coupled to a Canberra DSA-1000 multichannel analyzer. Gamma ray spectra were collected and processed using a Canberra Genie 2000 version 3.1 spectroscopy software. Element content calculations were carried out using a Microsoft Excel spreadsheet.

Results and discussion

An analysis of variance (ANOVA) approach was performed to assess the between bottle homogeneity as well as the within bottle homogeneity of the mussel reference material [6].

Table 1 Mass fraction in mg kg^{-1} obtained by INAA for the between bottle homogeneity study of the mussel candidate reference material (wet mass basis)[a]

Element	Bottle					
	19	40	74	112	143	156
Ag	2.28 ± 0.12	2.30 ± 0.12	2.23 ± 0.12	2.26 ± 0.11	2.48 ± 0.18	2.27 ± 0.15
As	14.32 ± 0.59	13.89 ± 0.88	13.97 ± 0.46	14.01 ± 0.57	13.85 ± 0.49	14.03 ± 0.54
Br	203 ± 12	199 ± 6	213 ± 16	207 ± 10	204 ± 8	209 ± 6
Co	0.841 ± 0.010	0.829 ± 0.036	0.820 ± 0.023	0.840 ± 0.045	0.828 ± 0.006	0.834 ± 0.028
Cr	1.086 ± 0.089	1.183 ± 0.075	1.150 ± 0.028	1.137 ± 0.065	1.100 ± 0.049	1.155 ± 0.102
Cs	0.1033 ± 0.0088	0.1078 ± 0.0090	0.1028 ± 0.0063	0.1062 ± 0.0081	0.1070 ± 0.0076	0.1130 ± 0.0116
Eu	0.0293 ± 0.0016	0.0284 ± 0.0014	0.0285 ± 0.0023	0.0296 ± 0.0022	0.0291 ± 0.0013	0.0294 ± 0.0019
Fe	602 ± 22	609 ± 22	618 ± 26	614 ± 22	592 ± 4	613 ± 19
La	0.713 ± 0.058	0.718 ± 0.059	0.718 ± 0.083	0.679 ± 0.037	0.681 ± 0.030	0.700 ± 0.050
Na (%)	1.892 ± 0.081	1.872 ± 0.024	1.947 ± 0. 067	1.908 ± 0.050	1.912 ± 0.061	1.950 ± 0.044
Rb	4.59 ± 0.24	4.31 ± 0.19	4.51 ± 0.23	4.64 ± 0.18	4.50 ± 0.15	4.50 ± 0.33
Sc	0.1888 ± 0.0075	0.1868 ± 0.0078	0.1836 ± 0.0047	0.1865 ± 0.0061	0.1891 ± 0.0029	0.1865 ± 0.0088
Se	4.29 ± 0.17	4.29 ± 0.20	4.26 ± 0.10	4.37 ± 0.21	4.38 ± 0.11	4.23 ± 0.16
Th	0.257 ± 0.012	0.259 ± 0.010	0.254 ± 0.023	0.254 ± 0.016	0.261 ± 0.009	0.256 ± 0.018
Zn	115.3 ± 3.7	114.8 ± 3.5	118.3 ± 3.1	115.8 ± 2.4	113.7 ± 4.6	116.9 ± 2.2

[a] Mean result and confidence interval at 95% confidence level for $n = 8$

Between bottle homogeneity study

Table 1 presents the INAA mass fraction results obtained for the mussel candidate reference material bottles. Mean results are presented on a wet mass basis, as no correction for residual moisture was performed. Confidence intervals were calculated as $CI = t\,(s/\sqrt{n})$; where n is the number of determinations, s is the standard deviation and t is the upper critical value for the Student's t distribution for the $\alpha/2 = 0.025$ level of significance [7].

For each element, Table 2 summarizes the obtained ANOVA test output results. The test takes into account the variability between bottles (six bottles) and within each bottle (eight subsamples). The null hypothesis of the ANOVA test (H_0) is that there is no difference among the mean mass fraction of the bottles, or in other words, sample results come from populations with the same mean. If F, the calculated statistic of the test, is lower than the critical F_c value, there is no evidence to reject H_0. Hence, the reference material may be considered homogeneous for the test conditions.

Table 2 ANOVA output for the between bottle homogeneity study[a]

Element	Variation source	Mean square	F test statistic	p value	F_c
Ag	Between	0.0723	3.391	0.012	2.438
	Within	0.0213			
As	Between	0.247	0.723	0.610	2.443
	Within	0.342			
Br	Between	191	1.414	0.239	2.438
	Within	135			
Co	Between	0.000499	0.504	0.771	2.438
	Within	0.000989			
Cr	Between	0.00673	0.862	0.515	2.438
	Within	0.00781			
Cs	Between	0.000109	1.007	0.426	2.438
	Within	0.000108			
Eu	Between	0.00000194	0.416	0.835	2.438
	Within	0.00000467			
Fe	Between	719	1.210	0.321	2.438
	Within	595			
La	Between	0.00261	0.639	0.671	2.438
	Within	0.00409			
Na	Between	0.00748	1.883	0.118	2.438
	Within	0.00397			
Rb	Between	0.110	1.495	0.212	2.438
	Within	0.073			
Sc	Between	0.0000879	1.925	0.110	2.438
	Within	0.0000457			
Se	Between	0.0367	1.138	0.355	2.438
	Within	0.0323			
Th	Between	0.0000934	0.345	0.882	2.449
	Within	0.000271			
Zn	Between	20.51	1.263	0.298	2.438
	Within	16.24			

[a] F_c is the F distribution critical value for the level of significance $\alpha = 0.05$; and degrees of freedom $\nu_1 = 5$; $\nu_2 = 42$

It was observed from the ANOVA tests that, except for Ag, F is less than F_c for all the elements, and hence, there is no evidence to reject the null hypothesis. This indicates that the candidate reference material may be considered homogeneous for the investigated elements. In the case of Ag, it was observed that, for other ^{110m}Ag photopeaks, F is less than F_c, indicating that the candidate RM may also be considered homogeneous for this element, as discussed elsewhere [4].

Within bottle homogeneity study

For the estimation of the minimum sample intake, the relative standard deviation (*RSD*) obtained for the various sample masses were compared to the repeatability standard deviation of the INAA method (s_r) [8]. Figure 1 presents the results for selected elements.

For Ag, As, Co, Fe, Sc, Se, Th and Zn, *RSD* was higher than s_r for all masses and remained approximately constant, indicating that the minimum sample intake may be lower than 50 mg. For Cr, Cs, Eu and Rb, *RSD* varied inversely with mass and the minimum sample intake was indicated where *RSD* exceeded s_r. For Br, La and Na, results were anomalous, which is probably associated to the experimental limitations associated to these elements such as enhanced dead times and gamma ray self-absorption.

From data such as the ones on Fig. 1, minimum sample intakes were estimated as presented in Table 3. For some elements, it is likely that this value is an overestimation and, possibly much lower values are obtainable by INAA if lower masses are used in the within bottle homogeneity study.

Impact of residual homogeneity on assigned value uncertainties

The ANOVA approach was used not only to verify if there were significant differences in the mass fraction among the mussel RM bottles, but also to estimate the contribution of the variation among bottles to the combined uncertainty of the assigned values [2].

In the certified value assignment process, the first step is to assess the expanded uncertainty associated to the mass fraction values (U_{MRC}). Standard uncertainties due to element characterization (u_{char}); between bottle homogeneity (u_{bb}); and long term stability (u_{lts}) are combined in order to estimate the combined standard uncertainty (u_{MRC}) which

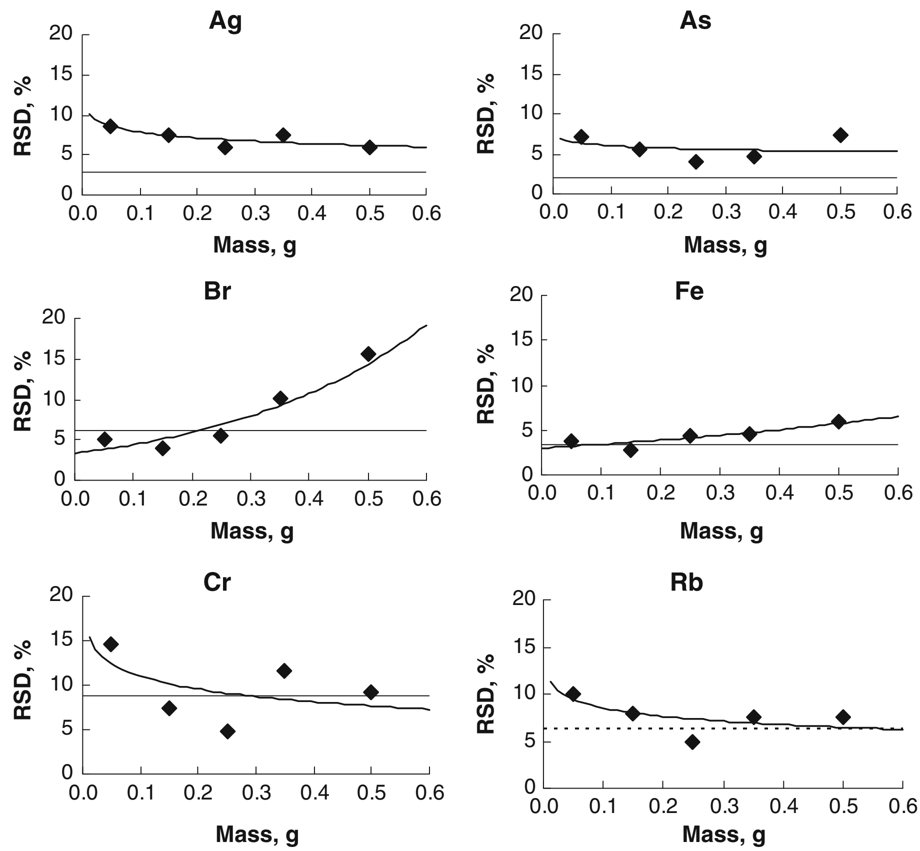

Fig. 1 Relative standard deviation (*RSD*) as a function of sample mass in the within bottle homogeneity study by INAA for selected elements. Method repeatability standard deviation (*dotted line*)

Table 3 Minimum sample intake estimates for the candidate mussel RM, as obtained by INAA

Minimum sample intake (mg)	Element
50	Ag, As, Br, Co, Fe, Na, Sc, Se, Th, Zn
150	Cs, Eu, La
250	Cr
350	Rb

multiplied by an appropriate coverage factor (k) will give U_{MRC}. The component u_{bb} is calculated from the quadratic means of the ANOVA test associated to the between bottle homogeneity study [2].

In relation to mass fractions, u_{bb} ranged from 0.4% for Co to 3.3% for Ag. This represents a small impact on the overall uncertainty, as would be expected from a between bottle homogeneity study on a well-homogenized material.

Conclusions

In this study, INAA was used in the homogeneity study of a *Perna perna* (Linnaeus 1758) mussel reference material. From the analysis of variance test performed on between bottle homogeneity study results, there was no evidence to reject the hypothesis that the bottles of the mussel reference material are homogeneous for the analyzed elements, within the 95% confidence interval. From the within bottle homogeneity study, the minimum sample intake was estimated as 50 mg for most of the elements. The impact of the residual non-homogeneity to the assigned values was considered small as it was lower than 1.5% for all but one element (Ag).

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References

1. Zschunke A (ed) (2000) Reference materials in analytical chemistry—a guide for selection and use. Springer, Berlin, p 222
2. ISO (International Organization of Standardization) (2006) Certification of reference materials-general and statistical principles, 3rd edn. ISO Guide 35. ISO, Geneva
3. Rossbach M, Groebecker KH (1999) Homogeneity studies of reference materials by solid sampling-AAS and INAA. Accredit Qual Assur 4:498–503
4. Moreira EG, Vasconcellos MBA, Catharino MGM, Maihara VA, Saiki M (2010) Application of INAA complementary gamma ray

photopeaks to the homogeneity study of a mussel candidate reference material. J Radioanal Nucl Chem 283:819–822
5. Radecki Z, Trinkl A, Burns KI (2004) Histo-a tool for evaluation of Analytical Quality Control Services (AQCS) interlaboratory studies. IAEA Interregional training course on organizational, reporting and certification aspects of proficiency tests, IAEA, Seibersdorf
6. van der Veen AMH, Linsinger T, Pauwels J (2001) Uncertainty calculations in the certification of reference materials. 2. Homogeneity study. Accredit Qual Assur 6:26–30
7. Farrant TJ (1997) Practical statistics for the analytical scientist. LGC, Teddington, p 98
8. ISO (International Organization of Standardization) (2005) Accuracy (trueness and precision) of measurement methods and results-practical guidance for the use of ISO 5725-2:1994 in designing, implementing and statistica lly analyzing interlaboratory repeatability and reproducibility results. ISO/TR 22971. ISO, Geneva