

MICRO-HOMOGENEITY EVALUATION OF A BOVINE KIDNEY CANDIDATE REFERENCE MATERIAL

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ABSTRACT

The minimum sample intake for which a reference material remains homogeneous is one of the parameters that must be estimated in the homogeneity assessment study of reference materials. In this work, Instrumental Neutron Activation Analysis was used to evaluate this quantity in a bovine kidney candidate reference material. The mass fractions of 9 inorganic constituents were determined in subsamples between 1 and 2 mg in order to estimate the relative homogeneity factor (H_E) and the minimum sample mass to achieve 5% and 10% precision on a 95% confidence level. Results obtained for H_E in all the analyzed elements were satisfactory. The estimated minimum sample intake was between 2 mg and 40 mg, depending on the element.

1. INTRODUCTION

Assessing the homogeneity is one of the critical steps in the process of preparing and certifying a reference material (RM). One of the parameters that must be estimated is the minimum sample intake for which the material remains homogeneous, that is, that the concentration and uncertainty values present in the certificate continue to be valid.

In general, Certified Reference Materials (CRM) producers establish a minimum sample intake of 200 mg, estimated in a conservative way, and useful for classical analytical methods that use sample masses greater than this value. From the appearance of analytical techniques that use less sample quantities, such as Inductively Coupled Plasma, ICP, it became increasingly important to be able to clearly estimate which is the real minimum sample intake of the RMs.

The present work describes the micro-homogeneity study of a bovine kidney candidate reference material prepared as described in a previous work [1].

In order to obtain an estimate of the minimum sample intake amount, assays were performed to determine the relative homogeneity factor, or Kurfurst constant, (H_E), a parameter used for this purpose by several authors [2-4]. To quantify the homogeneity of an element in a given matrix, Kurfurst [2] defined a relative homogeneity factor (H_E) based on a modification of the Ingamells sampling constant [5]. The H_E factor was found to be an appropriated estimator of the homogeneity when small amounts of sample mass (up to 2 mg) were analyzed. Once the H_E value is calculated, it is possible to calculate the minimum quantity of sample to reach a certain precision value with a certain level of confidence (6).

Measurements should be carried out using a method that allows analyzing a low sample quantity, with good accuracy and low measurement uncertainty. In this work Instrumental Neutron Activation Analysis, INAA, was used, a reference technique for the evaluation of the degree of homogeneity of RM because its intrinsic characteristics such as high sensitivity, accuracy and precision [7]. On the other hand, INAA does not require the pre-treatment of the samples, and for this reason, contamination and sample losses are minimized and would not affect the total variability of the determinations.

2. EXPERIMENTAL

2.1. Experimental Design

One bottle of the bovine kidney candidate reference material was randomly chosen from the batch of 175 bottles. Ten sub-samples of approximately 1 mg were analyzed by Instrumental Neutron Activation Analysis to evaluate the H_E for the elements Cl, K, Mn and Na, and ten sub-samples of approximately 2 mg to evaluate the H_E for Co, Fe, Mg, Se and Zn. Samples were weighted using a Shimadzu Libror AEL 40SM analytical balance, calibrated by the Brazilian Calibration Network.

2.2. Preparation of Element Calibration Standards

Calibration standards were prepared from high purity standard solutions (SPEX Certiprep Inc., USA and LGC Standards, UK) or appropriate dilutions of these standard solutions using Milli-Q water (Millipore Corporation, USA). Appropriate aliquots of these solutions were pipetted onto Whatman 40 filter papers and dried inside a laminar flow hood. After drying, filter papers were transferred to polyethylene bags, previously cleaned with 10 % nitric acid and Milli-Q water.

2.3. Irradiation and Element Determination

All the measured test portions were weighed in polyethylene bags, previously cleaned with 10 % nitric acid and Milli-Q water. Test portions and element calibration standards were irradiated simultaneously under a thermal neutron flux of approximately $5 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$ at the nuclear research reactor IEA-R1 of the Nuclear and Energy Research Institute, IPEN - CNEN/SP, São Paulo, Brazil.

Studies were performed in order to optimize the irradiation and measurement conditions for such a low amount of sample. These conditions depended on the concentration of the element in the sample and how much it activates under the neutron flux. Table 1 summarizes the conditions that were found to be the best for the analysis. The decay time is the time between the end of the irradiation period and the beginning of the measurement.

Gamma ray measurements were performed using a GC2018 Canberra HPGe detector coupled to a Canberra DSA-1000 digital spectral analyser. Gamma ray spectra were collected and processed using a Canberra Genie 2000 version 3.1 spectroscopy software and all element content calculations were carried out using a Microsoft Excel spreadsheet.

Table 1: Conditions used in the micro-homogeneity study

Radionuclide	Irradiation time	Decay time	Measurement time
³⁸ Cl	60 s	0 and 1800 s	300, 1800 and 3600 s
⁶⁰ Co	16 h	7 to 17 d	50000 s
⁵⁹ Fe	16 h	7 to 17 d	50000 s
⁴² K	8 h	2 d	1800 s
²⁷ Mg	90 s	0 s	300 s
⁵⁶ Mn	60 s	1800 s	1800 and 3600 s
²⁴ Na	8 h	2 d	1800 s
⁷⁵ Se	16 h	7 to 17 d	50000 s
⁶⁵ Zn	16 h	7 to 17 d	50000 s

3. RESULTS AND DISCUSSION

In Table 2 are presented the mean values obtained for the concentration of each element in the analyzed subsamples, as well as the overall relative standard deviation, RSD, and the number of determinations performed. In some cases, as for Cl and Mn, it was possible to measure some subsamples more than once, and for this reason $n > 10$. The mass value shown in the table is an average from the 10 subsamples weighted.

Table 2: Mean concentration values, relative standard deviation and number of determinations in the micro-homogeneity study

Radionuclide	Mass (g)	Mean value ($\mu\text{g g}^{-1}$)	RSD (%)	n
³⁸ Cl	0.96	9739	7.8	25
⁶⁰ Co	1.78	2.03	11.3	10
⁵⁹ Fe	1.78	298	8.0	10
⁴² K	0.96	12352	4.5	10
²⁷ Mg	1.78	783	9.6	9
⁵⁶ Mn	0.96	6.71	9.8	15
²⁴ Na	0.96	8358	3.2	10
⁷⁵ Se	1.78	6.07	9.2	10
⁶⁵ Zn	1.78	88.9	4.9	10

To quantify the homogeneity of each element in the reference material, the relative homogeneity factor (H_E) was calculated as follows [5]:

$$H_E = RSE\sqrt{m} \quad (1)$$

where m is the mass used and RSE is the experimental relative standard deviation calculated as,

$$RSE^2 = RSD^2 - (\text{relative analytical uncertainty})^2 \quad (2)$$

where RSD is the relative standard deviation of the n determinations as stated in Table 2 and the relative analytical uncertainty is the combined standard uncertainty of the measurements. When a direct method as INAA is used the number of uncertainties that influence the measurement results are minimized and have been well studied [7-8]. In this work the analytical uncertainty was calculated combining the uncertainties due to the counting statistics, the uncertainty of the weighted mass and the uncertainties of the calibration standards.

Table 3 shows the results obtained for the analytical uncertainties, RSD , RSE and the calculated relative homogeneity factor (H_E).

Table 3: Standard uncertainties, RSE , RSD and relative homogeneity factors calculated for each analyzed element

Radionuclide	u_A (%)	RSD (%)	RSE(%)	H_E (%$m^{1/2}$)
^{38}Cl	4,0	7,8	6,7	6,5
^{60}Co	4,4	11,3	10,5	14,0
^{59}Fe	10,8	8,0	-	10,6
^{42}K	3,4	4,5	2,9	2,9
^{27}Mg	11,8	9,6	-	12,8
^{56}Mn	8,5	9,8	4,8	4,7
^{24}Na	0,9	3,2	3,1	3,0
^{75}Se	6,2	9,2	6,9	9,1
^{65}Zn	2,9	4,9	3,9	5,3

As can be seen in Table 3 the uncertainty obtained for Fe and Mg was higher than the RSD, giving a negative value for RSE. This fact occurs because for these elements the analyzed peak was very small and so the counting statistics were elevated. For these elements the H_E was estimated taking into account the overall RSD, to have an indicative value, but it has to be considered that the result is an overestimation of the real value.

When a value of $H_E \leq 10$ is obtained, the material is considered to be very homogeneous [3]. Once the H_E value was calculated, it was possible to calculate the minimum quantity of sample to reach a certain precision value with a certain level of confidence [6] using the following equation:

$$M_{x\%} = m \cdot \left(\frac{k_2 H_E}{x\%} \right)^2 \quad (3)$$

where $x\%$ is the level of precision to be achieved, k_2 is the factor for the bilateral tolerance limit of the normal distribution and m is the mass used in the study of homogeneity.

For macro components like Cl, K and Na the minimum mass was estimated considering a target precision of 5%. For the rest of the elements the target precision was stated in 10% due to their low concentration level. Results are presented in Table 4.

Table 4: Minimum sample mass to achieve a 5% and 10% precision at a 95% confidence level

Radionuclide	k_2^*	$M_{5\%}$ (g)	$M_{10\%}$ (g)
^{38}Cl	2.60	11.3	-
^{42}K	3.38	3.7	-
^{24}Na	3.38	4.0	-
^{60}Co	3.38	-	39.6
^{59}Fe	3.38	-	22.9
^{27}Mg	3.53	-	36.0
^{56}Mn	2.95	-	1.9
^{75}Se	3.38	-	17.0
^{65}Zn	3.38	-	5.6

* k_2 is the factor for the bilateral tolerance limit of the normal distribution, at a 95% confidence level.

From the results presented it can be said that for the analytes Cl, K, Mn, Na, Se and Zn, the material presented a very high homogeneity, with minimum samples masses ranging from 2 mg for Mn to 17 mg for Se. For the analytes Co, Fe and Mg the homogeneity was moderate.

As it was already pointed, for Fe and Mg the obtained H_E value is an overestimation from the real one and so is the minimum sample intake. The obtained variability for these two elements is more related with the analytical capability than to sample heterogeneity. For Co the higher levels were obtained, however the obtained minimum sample intake for this element was 40 mg, a value that is adequate for most of the analytical techniques.

4. CONCLUSIONS

The micro-homogeneity study for a bovine kidney candidate reference material was conducted satisfactorily.

Results obtained for the relative homogeneity factors and the minimum sample intakes were considered adequate. The suggested minimum sample intake for the reference material will depend on the intended use and the element to be determined. Results obtained were between 2 mg and 40 mg, showing that for most of the studied elements the reference material will be useful for micro-analytical techniques.

Future works in this reference material should improve the analysis for Fe and Mg, maybe determining better irradiation and measurement conditions to obtain more accurate results for these elements.

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