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Uncertainty assessment in instrumental neutron activation analysis of biological materials

E. G. Moreira,* M. B. A. Vasconcellos, M. Saiki

Instituto de Pesquisas Energéticas e Nucleares, IPEN/CNEN-SP, Research Reactor Center, Neutron Activation Analysis Laboratory, Caixa Postal 11049, 05422-970 São Paulo, Brazil

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The expression of measurement uncertainties in a standardized form is a requirement for result reliability as it imposes implications to the interpretation of analytical results. In this work, sample mass, elemental standard mass, element decay constant and sample and elemental standard activities were identified as the most important uncertainty sources for the relative method of instrumental neutron activation analysis. The contribution of these sources to the expanded standard uncertainty in the concentration of As, Co, Cr, Fe, K, Na, Se and Zn in biological materials of marine origin was assessed and sample activity was identified as the major contribution.

Introduction

The expression of measurement uncertainties in a standardized form allows the comparison of results from different laboratories. It is also important in reaching decisions about the result compliance to regulatory limits, as measurement uncertainties have implications on the interpretation of analytical results. Due to the growing demands on the quality assurance of analytical laboratories, the presentation of analytical results with their related uncertainties is a recent requirement in method validation and laboratory accreditation.

The Neutron Activation Analysis Laboratory (LAN) of Nuclear and Energy Research Institute (IPEN/CNEN-SP) has been using instrumental neutron activation analysis (INAA) in studies ranging from environment monitoring and reference material certification to food and diet analysis and archeology. In this work, the uncertainty sources for the relative method of INAA applied to biological materials were identified according to international accepted instructions,[1] as part of the Quality Assurance System implementation at LAN. The identified most important uncertainty sources were sample mass, elemental standard mass, element decay constant and sample and elemental standard activities.[2–5] Various uncertainty sources for the irradiation step and for the gamma-ray spectrometry measurement were considered. The contribution of the uncertainty sources to the expanded uncertainty in the concentration of As, Co, Cr, Fe, K, Na, Se and Zn in biological materials of marine origin were assessed. As biological matrix materials, four certified reference materials were used: DORM-1 (Dogfish Muscle), DOLT-1 (Dogfish Liver), NIST SRM 1566b (Oyster Tissue), and MR-CCHEN-002 ("Almejas").

The process of uncertainty assessment includes the specification of the measurand, the identification of uncertainty sources, the quantification of the individual standard uncertainties, the calculation of the combined standard uncertainty, and the presentation of the expanded uncertainty.[1]

Specification of the measurand

The measurand is the concentration of As, Co, Cr, Fe, K, Na, Se and Zn in the following biological matrix CRMs: DORM-1 (Dogfish Muscle), DOLT-1 (Dogfish Liver), NIST SRM 1566b (Oyster Tissue) and MR-CCHEN-002 ("Almejas"), by the relative method of INAA.

In the relative method of INAA, where the unknown sample is irradiated simultaneously with standards of the elements of interest, the concentration, C, is determined by means of the following equation:

$$C = \frac{m\, A_u\, e^{\lambda(t_u - t_s)}}{M\, A_s}$$

where m is the mass of the element to be determined in the standard; M is the mass of the unknown sample; A_u is the activity in the unknown sample; A_s is the activity in the elemental standard; t_u is the unknown sample decay time; t_s is the elemental standard decay time; λ is the decay constant, where $\lambda = \ln 2/T_{1/2}$ and $T_{1/2}$ is the half-life.

Traceability links

All the parameters used in the INAA element determination in biological materials are traceable to SI units. The traceability links were established by means of balance calibration by a laboratory accredited by the Brazilian National Institute for Metrology (INMETRO); use of calibrated radioactive sources for calibration of the gamma-ray spectrometer and use of certified reference solutions for the preparation of elemental standards.

* E-mail: emoreira@ipen.br

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Identification of uncertainty sources

The uncertainty sources considered in this work are shown in the cause and effect diagram in Fig. 1. The assessment of the various uncertainty sources is treated in the Discussion of this paper.

Experimental

Samples and elemental standard preparation

About 0.150 g of biological matrix CRM samples were weighed in a properly cleaned polyethylene vial using a Shimadzu AEM-5200 analytical balance. Elemental standards were prepared by pipetting standard element solutions (Spex) onto Whatman paper filters, using variable volume pipettors (Eppendorf or Jencons). For some elements, the original solution was diluted prior to pipetting and the contribution of pipettors and volumetric flasks was considered in the uncertainty assessment. After drying, paper filters were kept in polyethylene vials with the same geometry as for the samples. Four replicates of each CRM were used in this study.

Irradiation and element determination

CRM aliquots and elemental standards were irradiated simultaneously for 8 hours at 10^{12} n·cm^{-2}·s^{-1} thermal neutron flux of IEA-R1 Nuclear Research Reactor at IPEN. As, K and Na radionuclides were measured for 2 hours, after a 2-day period decay, while long-lived radionuclides were measured for 10 hours, after a 15-day decay period. Gamma-ray measurements were performed using a Canberra GX 2020 HPGe detector (coupled to a Canberra multi-channel system and electronics) with a 1.70 keV resolution for 1332 keV gamma-ray peak of ^{60}Co. The analysis of gamma-ray spectra and the calculation of element concentration were carried out using in-house software. Table 1 shows the radionuclides used as well as their gamma-ray energies and half-lives with uncertainties.[6,7]

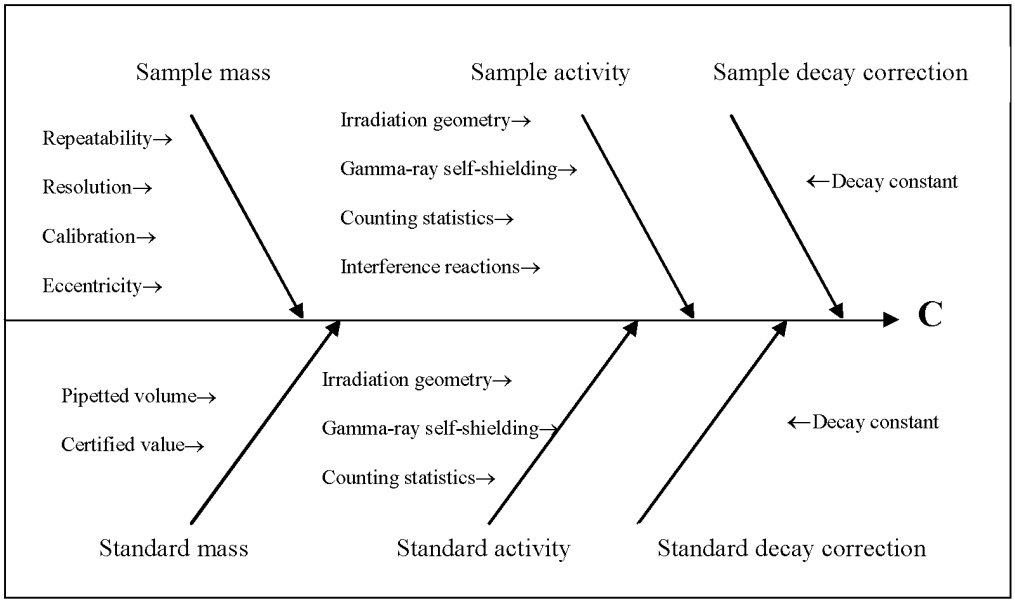

Fig. 1. Cause and effect diagram for INAA

Table 1. Radionuclides used for element determination in biological materials by INAA[6,7]

Element	Radionuclide	Energy, keV	Half-life
As	^{76}As	559.40	1.0778 ± 0.0020 d
Co	^{60}Co	1332.51	1925.1 ± 0.5 d
Cr	^{51}Cr	320.15	27.7025 ± 0.0024 d
Fe	^{59}Fe	1291.70	44.472 ± 0.008 d
K	^{42}K	1524.92	12.360 ± 0.003 h
Na	^{24}Na	1368.77	14.9512 ± 0.0032 h
Se	^{75}Se	264.74	119.779 ± 0.004 d
Zn	^{65}Zn	1115.62	244.26 ± 0.26 d

Results and discussion

Quantification of uncertainty components

Table 2 lists the contributions for sample mass combined standard uncertainty (u_M). The repeatability contribution, a Type A uncertainty, was taken from a control chart of 0.1 g measurements, with $n = 60$. The other contributions were taken from the balance calibration certificate.

The uncertainty in the elemental standard mass depends on the certified concentration values for the standard solutions (taken from the solution certificates) and on the volume of the solution pipetted onto paper filters. Three sources of uncertainty were considered: volume repeatability, evaluated from a series of weighings of pipettor dispensed water volume ($n = 10$); the statements by the pipettor producers about the imprecision of pipettors in dispensing liquids and the uncertainty from volume expansion due to differences in the temperature of the laboratory and the temperature at the time of the pipettor calibration, assumed as a 4 °C difference with the liquid expansion coefficient of $2.1 \cdot 10^{-4}$ °C^{-1}. The same approach was used in the estimation of the uncertainty of the 25-ml volumetric flask ($u = 0.022$ ml) used in the dilutions. In Table 3 are summarized the combined standard uncertainties for elemental standard masses (u_m) with the contributions of pipetted volume and concentration uncertainties.

The contribution of the decay constant (u_d) depends on the uncertainty of the half-lives of the radionuclides (Table 1). Half-lives were converted to minutes and uncertainties in decay constant were propagated as exponential uncertainties. Uncertainties due to the decay time of samples and elemental standards may be neglected for the radionuclides under consideration.

There are various sources of uncertainty in sample and elemental standard activities. In this work, the contribution due to geometry difference in the irradiation process and gamma-ray spectrometry measurement contributions (counting statistics and gamma-ray self-shielding) were considered.

The irradiation geometry difference contribution is due to neutron flux differences inside the irradiation capsule. From a flux density calibration certificate,[8] this contribution was estimated as 0.73% of samples and elemental standards activities, for the irradiation site and geometry used in this work.

Other sources or uncertainty in the irradiation as neutron self-shielding and scattering differences, neutron spectrum variations in time and space, nuclear reaction interferences, such as Fe in Cr determination, volatilization losses during irradiation and the duration of irradiation may be considered negligible for the elements under investigation, the matrix and irradiation scheme used.

The counting statistics component to uncertainty is available from the measurement result as the square root of the measured activity, as it follows the Poisson distribution. Usually, this is the most important contribution to activity uncertainty in INAA.

Gamma-ray self-shielding is negligible in most cases, but scattering differences may be important for low Z elements. From a gamma-ray self-shielding study, using europium as gamma-ray source, uncertainties in the activities were estimated as 0.4% for As, 0.6% for Na and K, 1.2% for Zn, 1.4% for Cr and Se and 2.3% for Co and Fe, for the samples under investigation.

Table 2. Contributions to sample mass combined standard uncertainty, u_M

Uncertainty source	Uncertainty, g	Probability distribution	Factor	Standard uncertainty, g
Repeatability	0.00002	Normal	1	$2.0 \cdot 10^{-5}$
Readability	0.00001	Rectangular	$1/2\sqrt{3}$	$2.89 \cdot 10^{-6}$
Calibration	0.00001*	Normal	1/2.05	$4.88 \cdot 10^{-6}$
Eccentricity	0.00002	Rectangular	$1/2\sqrt{3}$	$5.77 \cdot 10^{-6}$
Combined uncertainty:				$2.16 \cdot 10^{-5}$

* Expanded uncertainty, $k = 2.05$.

Table 3. Contributions to elemental standard mass combined standard uncertainties, u_m

Element	Concentration,* mg l^{-1}	Dilution volume, ml	Pippeted volume, µl	Element mass, µg
As	1000 ± 1.5	1.249 ± 0.001	99.06 ± 0.09	4.95 ± 0.02
Co	1000 ± 3	0.248 ± 0.002	99.06 ± 0.09	0.982 ± 0.005
Cr	1002.5 ± 3	1.243 ± 0.001	99.06 ± 0.09	4.96 ± 0.05
Fe	9991 ± 30	–	49.09 ± 0.09	490 ± 2
K	10027 ± 30	–	99.06 ± 0.09	993 ± 3
Na	9998 ± 30	–	99.06 ± 0.09	990 ± 3
Se	1000.5 ± 3	0.245 ± 0.002	99.06 ± 0.09	0.983 ± 0.008
Zn	1000 ± 3	5.018 ± 0.002	99.06 ± 0.09	19.90 ± 0.07

* Expanded uncertainty, $k = 2$.

Other sources of uncertainty in the gamma-ray spectrometry as counting geometry differences, gamma-ray interferences, pulse pile-up losses, duration of counting and dead time effects were kept to a minimum or are irrelevant for the elements under investigation and were neglected in this study.

Due to the large amount of data, the contributions to the combined standard uncertainties of samples and elemental standards activities (u_{A1} and u_{A2}, respectively) are not presented in this paper. Counting statistics was the major contribution for most elements. For Na and Zn, elements with high counting rates in the experimental design used, contributions due to irradiation geometry and gamma-ray self-shielding were comparably more important.

Combined standard uncertainty and expanded uncertainty

The standard uncertainties from the relevant sources of uncertainty were combined, using the relative method, yielding the combined standard uncertainty (u_c) for the concentration of As, Co, Cr, Fe, K, Na, Se, and Zn in the biological matrix materials. The expanded uncertainty (U) was determined from the combined standard uncertainties using the expression $U = k\,u_c$, with a coverage factor $k = 2$, which gives a level of confidence of approximately 95%. Figure 2 shows the contributions to the combined standard uncertainty obtained for DORM-1. Sample activity was the major contribution in the uncertainty assessment for all the determined elements. Similar contribution patterns were obtained for the other reference materials. Expanded uncertainties were lower than 5% of the concentration values for most CRMs and elements. The exceptions were Cr in DOLT-1 and SRM 1566b, due to its low concentration in these CRMs and K, an element that is not very favorable to be determined by INAA in the used conditions due to its relatively short half-life and low neutron capture cross section.

Element concentration in the CRMs by INAA

Table 4 presents the INAA concentration results obtained in this work for the various elements in the biological matrix CRMs, with associated expanded uncertainties. For comparison, certified values are also presented. A good agreement is observed between the obtained results and certified values. Most z score results are in the ±1 range, and the remaining results are in the ±2 range, confirming the suitability of the INAA method used. For most elements the reported uncertainties are in the same order of magnitude of the certified values uncertainties, showing the suitability of INAA to the analysis of biological matrix materials. The estimated expanded uncertainties for As are significantly lower in DORM-1 and DOLT-1 if compared to the uncertainties in the certificate. Direct comparison of these results is not an easy task since in this work uncertainties were calculated using ISO GUM principles, while in the reference material certificate confidence intervals for different analytical techniques are presented.

Table 4. Element concentration (in $\mu g \cdot g^{-1}$) in biological matrix CRMs obtained in this work by INAA[a]

Element	Certified reference material			
	DORM-1	DOLT-1	NIST SRM 1566b	MR-CCHEN-002
As	15.67 ± 0.43	9.18 ± 0.29	8.63 ± 0.55	6.26 ± 0.38
	(17.7 ± 2.1)	(10.1 ± 1.4)	(7.65 ± 0.65)	(6.1 ± 0.2)
Co	0.063 ± 0.008	0.203 ± 0.016	0.383 ± 0.023	0.753 ± 0.094
	(0.049 ± 0.014)	(0.157 ± 0.037)	(0.371 ± 0.009)	(0.68 ± 0.04)
Cr	3.38 ± 0.22	0.33 ± 0.12	0.51 ± 0.12	5.02 ± 0.22
	NC[c]	NC	NC	(4.35 ± 0.37)
Fe	72.0 ± 6.5	744 ± 44	205 ± 13	730 ± 43
	(63.6 ± 5.3)	(712 ± 48)	(205.8 ± 6.8)	(607 ± 109)
K[b]	1.45 ± 0.26	1.51 ± 0.28	NO[d]	0.87 ± 0.27
	(1.59 ± 0.10)	(1.01 ± 0.1)	(0.652 ± 0.009)	(1.066 ± 0.058)
Na[b]	0.75 ± 0.15	0.65 ± 0.13	0.305 ± 0.011	1.214 ± 0.026
	(0.8 ± 0.1)	(0.726 ± 0.073)	(0.33 ± 0.01)	(1.3016 ± 0.049)
Se	1.85 ± 0.23	8.15 ± 0.58	2.14 ± 0.20	1.17 ± 0.16
	(1.62 ± 0.12)	(7.34 ± 0.42)	(2.06 ± 0.15)	(1.07 ± 0.08)
Zn	23.2 ± 1.0	100.5 ± 4.8	1435 ± 51	36.6 ± 1.4
	(21.3 ± 1.0)	(92.5 ± 2.3)	(1424 ± 24)	(35.5 ± 0.93)

[a] Certified values in parentheses.
[b] Concentration in mass percentage.
[c] Not certified.
[d] Not obtained.
Reported uncertainties are expanded uncertainties calculated using a coverage factor of 2, which gives a level of confidence of approximately 95%.

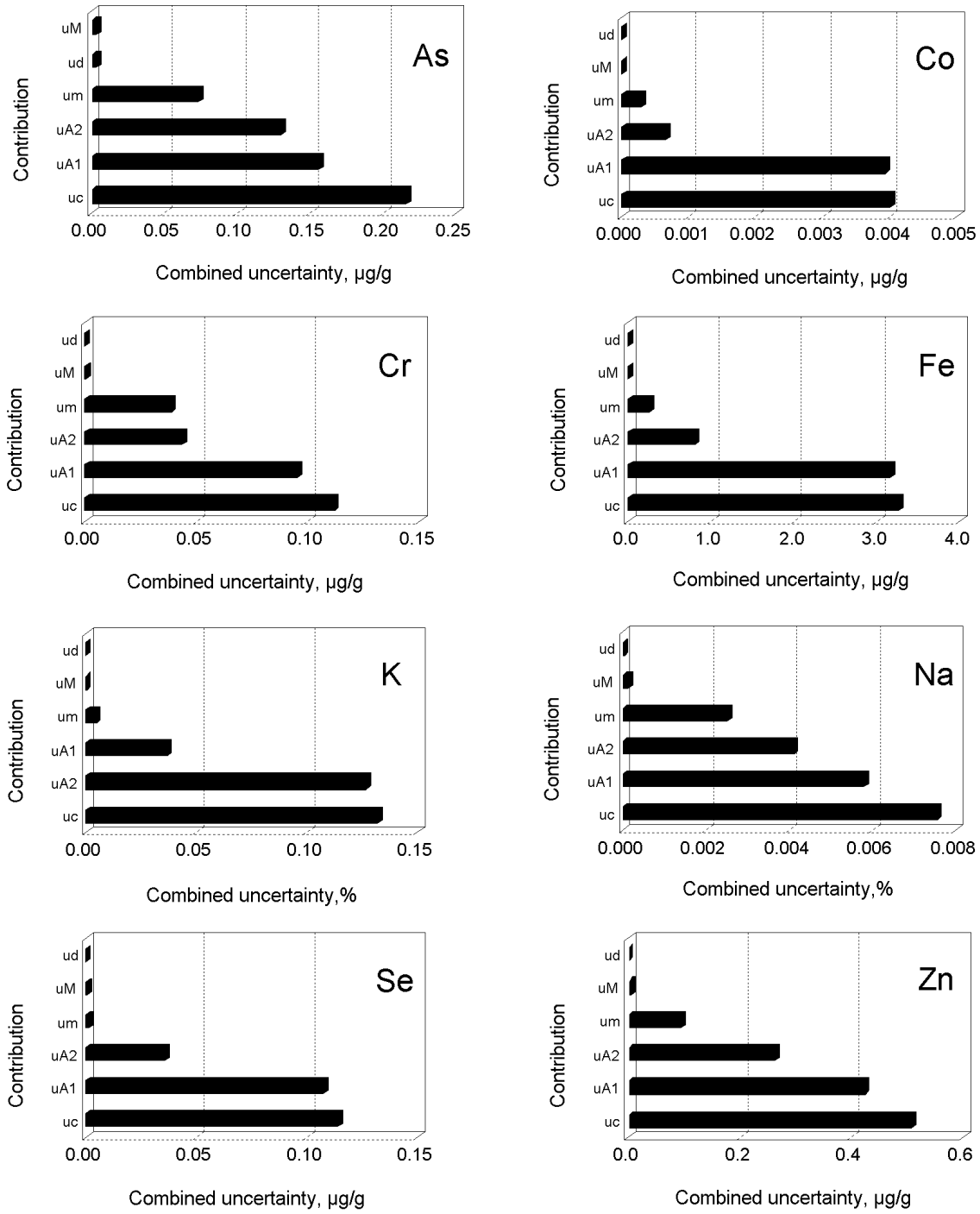

Fig. 2. Sample mass (u_M), elemental standard mass (u_m), decay constant (u_d), sample activity (u_{A1}) and elemental standard activity (u_{A2}) uncertainties contributions to the combined standard uncertainty (u_c) in DORM-1 element concentration by INAA

Conclusions

This paper shows the various steps involved in the expanded uncertainty assessment for the concentration of various elements in biological materials by INAA. Sample activity was the most important source of uncertainty. Gamma-ray counting statistics was the strongest contribution to activity uncertainty. It is possible that not all the sources of uncertainty were considered in this study and hence refinements of the uncertainty assessment are possible and are under investigation.

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