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Certification of Trace Elements and Methyl Mercury Mass Fractions in IAEA-461 Clam (*Gafrarium tumidum*) Sample

IAEA

International Atomic Energy Agency

CERTIFICATION OF TRACE ELEMENTS AND METHYL MERCURY MASS FRACTIONS IN IAEA-461 CLAM (*Gafrarium tumidum*) SAMPLE

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CERTIFICATION OF TRACE ELEMENTS AND METHYL MERCURY MASS FRACTIONS IN IAEA-461 CLAM (*Gafrarium tumidum*) SAMPLE

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FOREWORD

The primary goal of the IAEA Environment Laboratories is to assist Member States in the use of both stable and radioisotope analytical techniques to understand, monitor and protect the environment. In this context, the major impact exerted by large coastal cities on marine ecosystems is an issue of primary concern for the IAEA and the IAEA Environment Laboratories. The marine pollution assessments required to understand such impacts depend on accurate knowledge of contaminant concentrations in various environmental compartments. The IAEA Environment Laboratories has been assisting national laboratories and regional laboratory networks since the early 1970s through the provision of reference material programme for the analysis of radionuclides, trace elements and organic compounds in marine samples.

Quality assurance, quality control and associated good laboratory practice are essential components of all marine environmental monitoring studies. Quality control procedures are commonly based on the analysis of certified reference materials and reference samples in order to validate analytical methods used in monitoring studies and to assess the reliability and comparability of measurement data. Data that are not based on adequate quality assurance and quality control can be erroneous, and their misuse can lead to poor environmental management decisions.

This publication describes the production of the IAEA-461 certified reference material, which was produced following ISO Guide 34:2009, General Requirements for the Competence of Reference Material Producers. A sample of approximately 60 kg of clams (*Gafrarium tumidum*) was collected in Noumea, New Caledonia, and processed at the IAEA Environment Laboratories to produce a certified reference material of marine biota. The sample contained certified mass fractions for arsenic, cadmium, calcium, chromium, cobalt, copper, iron, lead, mercury, methyl mercury, manganese, nickel, selenium, vanadium and zinc. The produced vials containing the processed biota sample were carefully capped and stored for further certification studies.

Between-unit homogeneity was quantified, as well as stability during dispatch and storage, in accordance with ISO Guide 35:2006, Reference Materials. Within-unit homogeneity was also quantified to determine the minimum sample intake. The material was characterized by laboratories with demonstrated competence and adhering to ISO/IEC 17025:2005, General Requirements for the Competence of Testing and Calibration Laboratories. No outlier was eliminated on statistical grounds only. Uncertainties of the certified values were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement, including uncertainty contribution related to possible heterogeneity and instability of the material, as well as to the characterization. The material was intended for the quality control and assessment of method performance. As with any reference material, it can also be used for control charts or validation studies.

The IAEA wishes to thank all the participants and laboratories who took part in this certification exercise and for the sampling of the raw material funded by the Mediterranean Science Commission (CIESM). The IAEA is also grateful to the Government of Monaco for its support. The IAEA officers responsible for this publication were E. Vasileva and S. Azemard of the IAEA Environment Laboratories.

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CONTENTS

1. INTRODUCTION

Many laboratories are involved in the production of local or regional environmental data leading, in many cases, to wider assessments. These laboratories may develop and validate new analytical methods, study the environmental impact of human activities, provide services to other organizations, etc. Because of the needs to base scientific conclusions on valid and internationally comparable data and to provide policy makers with correct information on the state of the environment, it is indispensable to ensure the quality of the data produced by each laboratory.

The Marine Environmental Studies Laboratory (MESL) of the IAEA’s Environment Laboratories (IAEA-EL) has the programmatic responsibility to provide assistance to Member States’ laboratories in maintaining and improving the reliability of analytical measurement results, both in trace elements and organic pollutants. This is accomplished through the provision of certified reference materials of marine origin, validated analytical procedures, training in the implementation of internal quality control, and through the evaluation of measurement performance by the organization of worldwide and regional interlaboratory. IAEA subprogram ‘Reference Products for Science and Trade’ represents an important benchmark in upgrading the quality of laboratory performances and assessing the validity of the analytical methods used for marine monitoring studies in the Member States.

Laboratories need to be able to check the performance of their methods for the determination of trace elements in difficult matrices such as marine biota. This is also true for standardized methods, the use of which does not guarantee accurate results. It is widely accepted that laboratories need to demonstrate their proficiency in the applicability of standard methods, for example, by using certified reference materials (CRMs).

While there are several CRMs certified for trace elements, there still is a noticeable lack of matrix CRMs.

The work presented in this report refers exclusively to the certification of the mass fractions of 15 trace elements (As, Ca, Cd, Co, Cr, Cu, Fe, Hg, Methyl Hg, Mn, Ni, Pb, Se, V and Zn) in clam (Gafrarium tumidum) biota sample.

The material is an IAEA certified reference material (CRM) released in December 2014. The CRM IAEA-461 has been developed to satisfy the demands of laboratories dealing with environmental and food safety analyses.

2. METHODOLOGY

2.1. COLLECTION AND PREPARATION OF THE MATERIAL

Sixty kg of clams (Gafrarium tumidum) were collected in Noumea, New Caledonia. Clams were opened, the soft-shell part was frozen and then freeze dried. The dry material was first ground and sieved at 250 µm. A portion of about 5 kg was micronized to achieve a final powder size <50 µm. The homogeneity test was performed by mixing the material in a stainless steel rotating homogenizer Moritz ERM-BB124 (Moritz, Chatou, France) for 14 days at a temperature of 20 (+/-2)°C, and relative humidity of 50%. After checking for the homogeneity of sample material, aliquots of about 8 g were packed into pre-cleaned brown borosilicate glass bottles with polyethylene caps and then sealed in plastic bags. The sample material was labeled as IAEA-461. The average moisture content of the sample after bottling was determined by oven drying of then subsamples (1 g each) to a constant mass at 85°C.

Particle size distribution was checked from the beginning to the end of the processing by using a particle analyser (Mastersizer, Malvern Instruments, Germany) on randomly selected units. The resulting material showed 100% particle size below 22 µm.

2.2. SELECTION OF LABORATORIES FOR THE CERTIFICATION CAMPAIGN

The selection of participants for this certification exercise was based on the measurement performances demonstrated by laboratories in the previous IAEA certification campaigns and interlaboratory comparisons on marine biota. Only results of laboratories having a quality system in place, using validated methods, applying uncertainty and traceability concepts and providing good results in the previous IAEA interlaboratory comparisons were accepted for the calculation of the assigned values and their uncertainties.

Each laboratory received one bottle of clam sample, accompanied by an information sheet and a report form. Participants were requested to analyse Al, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Ni, Pb, Se, Sn, V, Zn and Methyl Hg using a validated analytical method. They were asked to report the measurement results (three replicates and average value) along with the expanded uncertainty in addition to the information about the applied quality control procedure. The second request was to report results for the trace elements in a CRM with a similar matrix to the candidate reference material. Moisture determination method was also prescribed.

The list of laboratories participating in the certification exercise is presented in Appendix I.

2.3. HOMOGENEITY ASSESSMENT

A key requirement for any reference material is the equivalence between the various units. In this respect, it is not relevant whether the variation between units is significant compared to the analytical variation, but whether this variation is significant to the certified uncertainty.

Consequently, ISO Guide 34 requires RM producers to quantify the between-units variation. This aspect is covered in between-unit homogeneity studies. The within-unit homogeneity does not influence the uncertainty of the certified value when the minimum sample intake is respected, but determines the minimum size of an aliquot that is representative for the whole unit. Quantification of within-unit homogeneity is therefore necessary to determine the minimum sample intake. 'Unit' is defined as an individual glass bottle of IAEA-461.

Extensive homogeneity tests were carried out on this material in order to ensure its suitability as a certified reference material and to estimate the uncertainty associated with homogeneity of the sample.

The between-unit homogeneity was evaluated to ensure that the certified values of the CRM are valid for all produced units, within the stated uncertainty. The between-unit homogeneity was tested by the determination of the mass fractions of some typical elements (Cr, Cu, Hg, CH_3Hg, Mn, Ni, Pb and Zn) in the clam sample. In total, 11 bottles were selected using random stratified sampling of the whole batch. Care was taken to ensure that the order of measurements did not correspond to the filling sequence of the bottles, which enables the differentiation between potential trend in the filling sequence and analytical drift. Three sub-samples from each bottle were analysed for their total element mass fractions. For all elements except Hg and CH_3Hg, subsamples of 0.2 g were mineralized with 5 ml conc. HNO_3 in a microwave oven according to the protocol described in [1]. The final measurements were performed by flame and graphite furnace atomic absorption spectrometry under repeatability conditions, and in a randomized way, in order to be able to separate a potential analytical drift from a trend in the filling sequence. The determination of the total Hg was done in solid subsamples (50mg) with solid mercury analyser, and methyl mercury was determined by gas chromatography coupled with atomic fluorescence spectrometer (GC-AFS) after alkaline digestion and room temperature derivatization.

Results obtained by ICP-MS determination of As, Cd, Co, Se, Sr and V in 12 units, in the frame of long term stability studies, were also used for homogeneity assessment.

All methods used for homogeneity studies were previously validated in MESL, IAEA Inorganic Laboratory.

2.4. STABILITY STUDY

Stability testing is necessary to establish conditions for dispatching IAEA-461 to customers (short term stability) as well as conditions for storage (long term stability). Time, temperature and light were regarded as the most relevant influences on stability of the material. The influence of light was minimized by the choice of the container (i.e., amber bottles placed in aluminized sachet) which eliminates most of the incoming light. In addition, materials are stored and dispatched in the dark, thus practically eliminating the possibility of degradation by light. Therefore, only the influences of time and temperature needed to be investigated. During transport, especially in summer time, temperatures up to 60°C could be reached and stability under these conditions must be demonstrated if transport at ambient temperature will be applied.

The stability studies for IAEA-461 were carried out using an isochronous design. In this approach, selected units are stored for a certain time at different temperature conditions. Afterwards, the units are moved to conditions where further degradation can be assumed to be negligible (reference conditions), effectively 'freezing' the degradation status of the materials. At the end of the isochronous storage, the units are analyzed simultaneously under repeatability conditions, improving in this way the sensitivity of the stability tests.

2.4.1. Short term stability

One isochronous study over 4 weeks was applied in order to evaluate short term stability of the materials during the transportation. 8 bottles were stored in the dark at +20°C immediately after the bottling and 8 bottles at +60°C for 1, 2, 3 and 4 weeks respectively. After each of above mentioned time intervals two bottles kept at +20°C and another two kept at +60°C were transferred to -20C° temperature, considered as reference temperature for this study. Two independent measurements per bottle were performed under repeatability conditions. The obtained results were compared with the results from samples kept at -20°C during the entire period of the stability study.

The measurements were performed by flame atomic absorption for Cr, Cu and Zn; by graphite furnace for Cd and Pb and by Advance Mercury Analyser for total Hg. The measurements were performed under repeatability conditions, and in a randomized manner to be able to separate a potential analytical drift from a trend over storage time. The results were corrected for the water content determined in each unit by using the procedure in the section 2.1.

2.4.2. Long term stability

In the same way, an isochronous study over 24 months was performed to evaluate long term stability of the materials under prescribed storage conditions. Eight bottles were stored in the dark at +20°C for 6, 12, 18 and 24 months respectively. Reference temperature was kept at -20°C. Two independent measurements per bottle were performed under repeatability conditions. The obtained results were compared with the results from samples kept at -20°C during the entire period of the stability study.

The measurements were performed by ICP-MS for (Cd, Co, Cu, Mn, Ni, Pb, Sr and Zn); by Advance Mercury Analyser for total Hg and by gas chromatography coupled with atomic fluorescence spectrometry (GC-AFS) for CH_3Hg. The measurements were performed under repeatability conditions and in a randomized manner to be able to separate a potential analytical drift from a trend over storage time. The results were corrected for the water content determined in each unit by using the procedure in Section 2.1.

2.5. CHARACTERIZATION

Characterization refers to the process of determining the reference values. The material was initially analyzed in the IAEA-EL in Monaco. The final characterization was based on the results delivered by selected laboratories with demonstrated measurement capabilities, based on criteria that comprised both technical and quality management aspects. The characterization of the trace element mass fraction in the biota sample was based on the application of different analytical techniques as summarized in Figure 1; abbreviations used for instrumental techniques are given in Table 1.

All participating laboratories have used validated methods for determination of trace elements in marine samples. In addition, they provided results from the analyzed CRM with similar matrix composition, and the information on standard calibration solutions used for every trace metal. The results of laboratories which did not report any quality assurance information were excluded from the further evaluation.

Combined uncertainties were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [2], including uncertainties due to possible heterogeneity and instability.

All participating laboratories claimed traceability of provided results to the International System of Units (SI) via standard calibration solutions and CRM applied as a part of their analytical procedures.

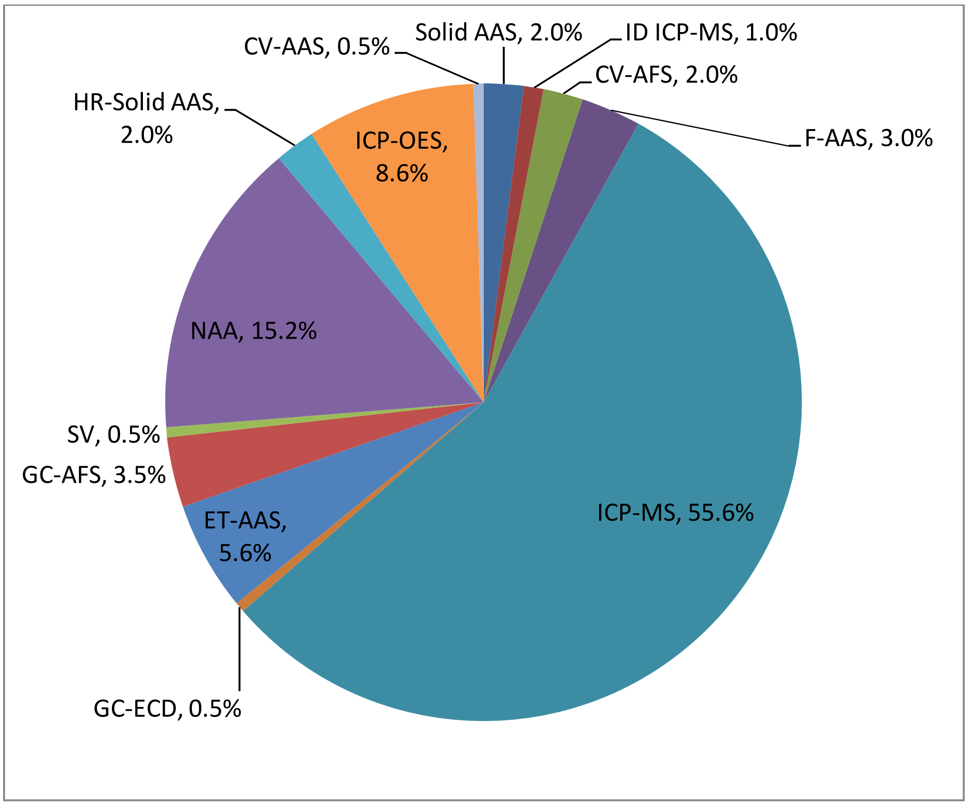

FIG. 1. Analytical methods used for the characterization of trace elements in the IAEA-461 clam sample.

TABLE 1. INSTRUMENTAL TECHNIQUES

Method code	Instrumental technique
AFS	Atomic Fluorescence Spectrometry
CV-AAS	Cold Vapour-Atomic Absorption Spectrometry
CV-AFS	Cold Vapour-Atomic Fluorescence Spectrometry
ET-AAS	Atomic Absorption Spectrometry-Graphite furnace
F-AAS	Atomic Absorption Spectrometry-Flame
GC-AFS	Gas Chromatography coupled with Atomic Fluorescence Spectrometry
GC-ECD	Gas Chromatography coupled with Electron Capture Detector
HR-Solid AAS	High Resolution Solid Sampling Atomic Absorption Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ID ICP-MS	Isotope Dilution Inductively Coupled Plasma Mass Spectrometry
NAA	Neutron Activation Analysis
Solid AAS	Solid Sampling Atomic Absorption Spectrometry
SV	Striping Voltammetry

2.6. MOISTURE DETERMINATION

The determination of the moisture content of the samples is to some extent ‘operationally defined’. In view of the comparability of results, the protocol for the correction of the moisture was developed at IAEA-EL and prescribed to other participants. The drying procedure at 85(±2)°C was established after experimental evaluation of sample stability. Correction for dry mass was obtained from separate portions of the material of minimum mass of 0.5 g (10 sub samples from 5 bottles). The weighing and repeated drying were performed until constant mass was attained. Moisture, determined at 85°C was found to be 6.5(±0.5)% for bottles kept at 20°C.

3. RESULTS AND DISCUSSION

3.1. RESULTS OF THE HOMOGENEITY STUDY

3.1.1. Between unit homogeneity

For the homogeneity study, 11 units (about 2% of the total batch) of biota sample were selected by using a random stratified sample picking scheme and analyzed for their trace elements contents in triplicate.

Regression analyses were performed to evaluate potential trends in the analytical sequence as well as trends in the filling sequence. No trends in the filling or analytical sequences were detectable for the majority of the measured elements at the 95% and 99% confidence levels. Grubbs-tests at 95% and 99% confidence levels were also performed to identify potentially outlying individual results as well as outlying bottles means.

Quantification of between-unit heterogeneity was done by analysis of variance (ANOVA), which can separate the between-unit variation (s_{bb}) from the within-unit variation (s_{wb}). The latter is equivalent to the method repeatability if the individual aliquots are representative for the whole unit [3].

Results for Al, Ni, Sr and Zn have shown significant trend in the analytical sequence. They were corrected as described in the Eq.(1).

$$\text{Corrected result}=\text{Measured result}-(b\times i) \tag{1}$$

Where: ‘b’ is the slope of the linear regression and ‘i’ is the position of the unit in the measurement sequence.

As presented in Table 3, few individual results were detected as outliers at 95% and 99% confidence levels. Additionally one outlying unit mean was found for Co. Since no technical reasons were identified for outlying results, all data were retained for statistical analysis. It was checked whether the retained individual results and unit means follow a normal distribution or are unimodally distributed. The series of results for investigated trace elements were normally distributed. One way analysis of variance ANOVA [3] was then applied to assess between-units and within-unit homogeneities. ANOVA allows the calculation of within-unit standard deviation s_{wb} and also between-units standard deviation s_{bb}:

$$s_{wb} = u_{wb} = \sqrt{MS_{wb}} \tag{2}$$

$$s_{bb} = u_{bb} = \sqrt{\frac{MS_{bb} - MS_{wb}}{n}} \tag{3}$$

s_{bb} and s_{wb} are estimates of the true standard deviations and are therefore subject to random fluctuations. Therefore, the mean square between groups (MS_{bb}) can be smaller than the mean squares within groups (MS_{wb}), resulting in negative arguments under the square root used for the estimation of the between-unit variation, whereas the true variation cannot be lower than zero. In this case, u^*_{bb}, the maximum heterogeneity that could be hidden by method repeatability, was calculated as described by Linsinger et al. [4]. u^*_{bb} is comparable to the limit of detection of an analytical method, yielding the maximum inhomogeneity that might be undetected by the given study setup.

For Hg, CH_3Hg, Pb and Se; ANOVA mean square between units was smaller than MS_{wb} (ANOVA mean square within units) and $s_{bb.}$ could not be calculated. Instead, u^*_{bb}, the heterogeneity was calculated, as described by Linsinger et al. [4]:

$$u^*_{bb} = \frac{s_{wb}}{\sqrt{n}} \sqrt[4]{\frac{2}{v_{MSwb}}} \tag{4}$$

Where: n is the number of replicate sub-samples per bottle; and v_{MSwb} is the degrees of freedom of MS_{wb}.

$$s_{bb} = u_{bb} = \sqrt{\frac{MS_{bb} - MS_{wb}}{n}} \tag{5}$$

However, a different approach was adopted for Co for which outlying unit average was detected. In this case, between-unit inhomogeneity was modelled as a rectangular distribution limited by the largest outlying unit average, and the rectangular standard uncertainty of homogeneity was estimated as given by:

$$u_{rec} = \frac{|outlier - y|}{\sqrt{3}} \qquad (6)$$

y : average of all results of the homogeneity study

It should be mentioned that the outlying unit averages are a result of presence of outlying individual values and do not necessarily reflect the real distribution of these elements in the material.

The between-units homogeneity could be quantified due to the good repeatability of the method used. The between-unit variations/heterogeneity were between 0.4 and 2%, small enough to ensure the homogeneity of the material. The uncertainty contributions due to the between-unit homogeneity were estimated according to ISO Guide 35 [2] as the maximum values obtained with Eq. (3), Eq.(4) or Eq. (5).

3.1.2. Within unit homogeneity

The within-unit homogeneity is closely correlated to the minimum sample intake. Due to the intrinsic heterogeneity, individual aliquots of a material will not contain the same amount of analyte. The minimum sample intake is the minimum amount of sample that is representative for the whole unit and thus can be used in an analysis. Sample sizes equal or above the minimum sample intake guarantee the certified value within its stated uncertainty.

In this study, the minimum sample intake was assessed by using the method information supplied by the laboratories participating in the characterization study. The smallest sample intake that still yielded results with acceptable trueness to be included in the respective studies was taken as minimum sample intake.

The minimum sample intake for all certified elements in this study was set to 200 mg.

TABLE 2. THE ESTIMATE OF INHOMOGENEITY CONTRIBUTIONS TO THE TOTAL UNCERTAINTY FOR THE CERTIFIED TRACE ELEMENTS

	Individual Outliers		Mean Outliers		s_{wb}	s_{bb}	u^*_{bb}	u_{rect}	u_{homo}
	95%	99%	95%	99%	%	%	%	%	%
Al	2	0	0	0	1.9	1.0	0.6		1.0
As	0	0	0	0	2.1	0.8	0.8		0.8
Cd	0	0	0	0	2.2	1.4	0.8		1.4
CH_3Hg	0	0	0	0	3.9	1)	1.4		1.4
Co	2	2	1	0	2.8	2.1	2.0	4.5	4.5
Cr	2	1	0	0	4.4	0.9	1.4		1.4
Cu	3	1	0	0	2.6	0.8	0.9		0.9
Hg	2	2	0	0	2.4	1)	0.8		0.8
Mn	1	0	0	0	2.8	0.2	0.9		0.9
Ni	1	0	0	0	1.7	1.3	0.5		1.3
Pb	3	3	0	0	3.5	1)	1.1		1.1
Se	0	0	0	0	4.4	1)	1.6		1.6
Sr	0	0	0	0	1.7	1.1	0.6		1.1
V	0	0	0	0	1.8	1.3	0.7		1.3
Zn	0	0	0	0	1.4	0.4	0.4		0.4

1) Not defined due to negative argument under the square root

The conclusion from the presented results for the tested trace elements was that the homogeneity of the candidate reference material complied with the provisions given by the ISO Guide 35, at the range of weights used. A minimum sample intake of 0.2 g was set, based on the smallest sample intake used in the characterization study.

3.2. RESULTS FOR STABILITY STUDY

3.2.1. Short term stability study

The samples selected for short term stability study were analyzed and each of the elements (Cd, Cr, Cu, Hg, Pb and Zn) was evaluated individually. The obtained data were evaluated individually for each temperature (20°C and 60°C).

Few outliers were found at 95% for Cr, Cd and Cu but none at 99%. Since no technical reasons were identified for outlying results, all data were retained for statistical analysis. A significant trend was detected for the analytical sequence of Cd, results were corrected following Eq. (1).

The evaluation of data was further carried out by performing a linear regression on the determined mass fractions versus time. The test material showed no significant trend of degradation over the timeframe at different temperatures: +20°C and +60°C. No significant slope at 95% level of confidence was detected for any of investigated trace elements in the short term study. As no degradation could be observed under any conditions it was concluded that no special precautions regarding temperature control during shipment were necessary. The uncertainty of the short term stability (u_{sts}) estimated as described by Linsinger et al. [5] for 2 weeks was less than 1% for all studied elements. It is considered to be negligible since no degradation was expected during this short time.

3.2.2. Long term stability study

The samples selected for short term stability study were analysed and each of the elements (As, Cd, Co, Cu, Cr, CH_3Hg, Hg, Mn, Ni, Pb, Se, Sr, V and Zn) was evaluated individually. Results were screened for trend and outliers. As presented in table 3 few individual outliers were found at 95% but also at 99%. Since no technical reasons were identified for outlying results, all data were retained for statistical analysis. A significant trend was detected for the analytical sequence of Se and Sr; results were corrected following Eq. (1).

The evaluation of data was further carried out by performing a linear regression on the determined mass fractions versus time. The test material showed no significant trend of degradation over the timeframe at prescribe storage conditions (+20C°C in the dark). No significant slope at 95% level of confidence was detected for any of the investigated analytes in the long term study.

Failure to detect degradation, however, does not prove stability. The uncertainty of stability u_{stab} describes the potential degradation which still can be reconciled with the data, even if the slope is not statistically and significantly different from zero. Although under these conditions an expansion of the total uncertainty of the certified values is generally not encouraged, in this case the approach of ISO Guide 35 [2] was followed, mainly due to the lack of sound alternatives. An uncertainty contribution related with the stability of the candidate reference material was estimated as uncertainty of the regression line with a slope of 0 multiplied with the chosen shelf life, as described by Linsinger et al. [5]. A factor of 36 was selected, which ensured the validity of the certificate for 3 years. Graphical representations of the long–term stability study are displayed in Appendix 2 (Figures 2 to 15). The estimated standard uncertainty of long term stability varies from 1.9 to 3.5%. The uncertainty from long term stability for elements not included in the study was set as 3.5%.

TABLE 3. THE ESTIMATE OF STABILITY CONTRIBUTIONS TO THE TOTAL UNCERTAINTY FOR THE CERTIFIED TRACE ELEMENTS

	Individual Outliers		Slope significance	u_{stab}
	95%	99%	(95%)	%
As	0	0	No	2.0
Cd	0	0	No	2.7
CH_3Hg	0	0	No	3.1
Co	2	2	No	3.4
Cr	0	0	No	2.2
Cu	2	1	No	3.1
Hg	0	0	No	3.5
Mn	0	0	No	2.1
Ni	0	0	No	2.3
Pb	1	0	No	2.1
Se	0	0	No	3.2
Sr	0	0	No	1.9
V	0	0	No	2.1
Zn	0	0	No	2.6

3.3. DETERMINATION OF CERTIFIED VALUES AND UNCERTAINTIES

The characterization campaign resulted in 4 to 16 results for the 18 elements of interest. The obtained data were first checked for compliance with the certification requirements, and then for their validity based on technical reasoning. All accepted set of results were submitted to the following statistical tests: Grubbs test to detect single and double outliers, Dixon’s test to detect outlying laboratory means and Kolmogorov-Smirnov’s test for normal distribution. Few individual outliers were found at 95% for As, Cd and CH3Hg but also at 99% for As and Cd. Since no technical reasons were identified for outlying results, all data were retained for statistical analysis. All datasets were normally distributed.

Robust statistics as described in ISO 13528 [6] were used for the determination of the assigned values. The robust mean and robust standard deviations were calculated as per Algorithm A, i.e., as described in ISO 13528, Annex C.21 [6].

Briefly, individual results were ranked in increasing order:

(x_1, x_2, x_i,...x_p)

Initial values of the robust average x^* and robust standard deviation s^* were calculated as:

x^* = median of x_i (i=1,2,3….p) (7)

$s^* = 1.483 \times$median $|x_i - x^*|$ (i=1,2,3…..p) (8)

The initial values x^* and s^* were updated by calculating:

$$\delta = 1.5 \times s^* \tag{9}$$

For each x_i $(i = 1,2,3 \ldots .p)$ calculate

$$x_i^* = \begin{Bmatrix} x_i^* = x^* - \delta, & \text{if } x_i < x^* - \delta \\ x_i^* = x^* + \delta, & \text{if } x_i > x^* + \delta \\ x_i^* = x^*, & \text{otherwise} \end{Bmatrix} \tag{10}$$

New values for x^* and s^* were calculated as:

$$x^* = \frac{\sum_1^p x_i^*}{p} \tag{11}$$

$$s^* = 1.134\sqrt{\frac{(x_i - x^*)^2}{(p-1)}} \tag{12}$$

The robust estimates of x^* and s^* were calculated by iteration by updating the values of x* and s* until they converged to the third significant Figure.

The medians and unweighted mean of the means were also calculated and compared with the respective robust mean. No significant differences were observed and the reference values obtained with the robust mean approach were further used. These values are considered to be the most reliable estimates of the property values.

The uncertainties associated with the reference values were calculated according to ISO Guide 35 [2]. The relative combined uncertainty of the certified value of the CRM consists of uncertainty related to characterization u_{char}, between-unit heterogeneity (u_{bb}) and long term stability (u_{stab}). These different contributions were combined to estimate the expanded relative uncertainty.

$$U = k \times \sqrt{u_{char}^2 + u_{stab}^2 + u_{hom}^2} \tag{13}$$

Where k: coverage factor equaling 2, representing a level of confidence of about 95%

u_{hom} was estimated as described in section 3.1.

u_{stab} was estimated as described in section 3.2.2.

u_{char} was estimated as described in ISO 13528 [6] using Eq.(14).

$$u_{char} = 1.25 \times \frac{s^*}{\sqrt{p}} \tag{14}$$

Where: s^* is the robust standard deviation calculated by Eq. (12); p is the number of laboratories.

Robust means and uncertainties are presented in Table 4.

TABLE 4. ROBUST MEANS AND UNCERTAINTIES

Element	Robust mean mg kg^{-1}	u_{char}, %	u_{hom}, %	u_{stab}, %	u_{CRM}, %
Al	870	8.6	1.0	3.5	9.3
As	30.2	1.2	0.8	2.0	2.5
Ca	9270	2.7	2.0	3.5	4.9
Cd	0.569	1.8	1.4	2.7	3.5
CH_3Hg^*	0.0623	4.4	1.4	3.1	5.6
Co	4.78	2.7	4.5	3.4	6.3
Cr	27.0	3.7	1.4	2.2	4.5
Cu	26.1	2.1	0.9	3.1	3.8
Fe	2600	1.5	2.0	3.5	4.3
Hg	0.390	3.6	0.8	3.5	5.1
Mg	6485	3.5	2.0	3.5	5.3
Mn	333	2.0	0.9	2.1	3.0
Ni	106	2.9	1.3	2.3	4.0
Pb	25.8	2.2	1.1	2.1	3.2
Se	2.78	4.3	1.6	3.2	5.6
Sr	112	2.5	1.1	1.9	3.3
V	6.86	4.3	1.3	2.1	4.9
Zn	156	2.1	0.4	2.6	3.4

* mg kg^{-1} as Hg

As shown previously in Figure 1, methods with different quantification steps (AAS, ET-AAS, AFS, ICP-MS) as well as methods without sample preparation step such as NAA, Solid Sampling AAS and X ray Fluorescence were used for characterization of the material. The agreement between results confirms the absence of any significant method bias and demonstrates the identity of the analyte.

The results provided by participants for trace element mass fractions grouped by methods are displayed in Figures 16–33 and in Tables 8−25 (Appendix III). In all figures, the reported results are plotted versus the robust mean denoted by a bold line, while the dashed lines represent the expanded uncertainty (k=2) associated with the robust mean (as calculated in Eq. (13)). The error bars represent the expanded uncertainty as reported by participants. The coverage factor used for calculation of expanded uncertainty for all data presented in Tables 8 to 24 was 2.

A good agreement within the stated uncertainty was observed for results obtained with different methods. Therefore, all of them were considered in deriving certified values.

A certified value was assigned when at least 5 independent results and 3 independent analytical methods were available and the relative expanded uncertainty was less than 15%. These criteria were fulfilled for As, Ca, Cd, CH_3Hg, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V and Zn. The certified values are presented in Table 5, together with their expanded uncertainty.

For Al, Mg and Sr criteria were not fulfilled; robust mean and uncertainties for those elements are given only as information values in Table 6.

4. METROLOGICAL TRACEABILITY AND COMMUTABILITY

If the results obtained by different laboratories are to be compared, it is essential that all results are based on reliable measurement standards the values of which are linked to the same reference.

Only validated methods applied within stated scope were used by participating laboratories in this certification exercise. Matrix CRMs with stated SI traceability purchased from NIST, EC JRC IRMM, NRC-CNRC and IAEA were used for validation of the methods applied in this study.

Pure metal standard solutions (CRMs) with stated purity were employed for calibration from all the laboratories participating in this certification campaign. As stated in the respective certificates of all CRM producers, the mass fractions of the trace element in the respective standard solutions were measured against another CRM (i.e. NIST, BAM or EMPA) with demonstrated SI traceability, followed by gravimetric preparation using balances calibrated with SI-traceable weights.

In addition, the agreement between the results confirms the absence of any significant method bias and demonstrates the identity of the measurand. Measurands are clearly defined as total element mass fractions and independent of the measurement method. The participants used different methods for the sample preparation as well as for the final determination, demonstrating absence of measurement bias.

The individual results are therefore traceable to the SI. This is also confirmed by the agreement among the technically accepted datasets. As the assigned values are combinations of agreeing results individually traceable to the SI, the assigned quantity values are also traceable to the SI system of units.

The degree of equivalence in the analytical behavior of real samples and a CRM with respect to various measurement procedures (methods) is summarized in a concept called 'commutability of a reference material'.

Commutability is a property of an RM, demonstrated by the closeness of agreement between the relation among the measurement results for a stated quantity in this material, obtained according to two given measurement procedures, and the relation obtained among the measurement results for other specified materials [7].

The appropriate characterization of CRMs, especially those materials intended to be used with routine measurement procedures, must carefully address fitness-for-use for all methods which the material is intended to be used for. Commutability is a critical requirement to avoid introducing unintended, and sometimes undetected, bias results when using a CRM.

Commutable CRMs should exhibit an analytical behavior for a given method similar to a real laboratory sample. However, CRMs might show behavior different from that of real samples, in particular during digestion, due to their small particle size in contrast to the possible larger particle size for real laboratory samples. IAEA-461 is a natural marine biota sample. The analytical behaviour should be the same as for a routine sample of dried biota samples. The agreement between results obtained with different analytical methods selected for the IAEA-461 characterization study confirms the absence of any significant method bias and demonstrates commutability of the material for all certified trace elements.

5. CONCLUSIONS

This exercise allows assignment of certified values for Al, As, Cd, Cr, Co, Cu, Fe, Hg, Li, Mn, Ni, Pb, Sn, Sr, V and Zn with associated uncertainties following ISO guidelines. The certified values are derived from measurement results provided by the laboratories participating in this certification campaign. Only validated methods were applied in the certification of the biota sample. As the certified values are combinations of SI traceable individual results, they are themselves traceable to SI. The produced clam (*Gafrarium tumidum*) sample is suitable for quality control, and as any certified reference material it can be used for validation studies.

TABLE 5. CERTIFED VALUES FOR TRACE ELEMENT MASS FRACTIONS AND THEIR EXPANDED UNCERTAINTY (*k=2*) IN THE IAEA-461 GAFRARIUM *TUMIDUM* SAMPLE

Element	Unit	Certified value[1]	Expanded uncertainty (*k*=2)[2]
As	mg kg^{-1}	30.2	1.5
Ca	mg kg^{-1}	$9.27\ .10^3$	$0.90.10^3$
Cd	mg kg^{-1}	0.569	0.040
CH_3Hg	µg kg^{-1} as Hg	62.3	6.9
Co	mg kg^{-1}	4.78	0.60
Cr	mg kg^{-1}	27.0	2.4
Cu	mg kg^{-1}	26.1	2.0
Fe	mg kg^{-1}	2600	220
Hg	mg kg^{-1}	0.390	0.040
Mn	mg kg^{-1}	333	20
Ni	mg kg^{-1}	106	8
Pb	mg kg^{-1}	25.8	1.7
Se	mg kg^{-1}	2.78	0.31
V	mg kg^{-1}	6.86	0.67
Zn	mg kg^{-1}	156	11

[1] The value is the robust mean of accepted sets of data, each set being obtained by different laboratory. The certified values are reported on dry mass basis and are traceable to the SI.

[2] Expanded uncertainty with a coverage factor *k*=2 estimated in accordance with the JCGM 100:2008 Evaluation of measurement data – Guide to the expression of uncertainty in measurement, corresponding to the level of confidence of about 95%.

TABLE 6. INFORMATION VALUES FOR TRACE ELEMENTS MASS FRACTIONS AND THEIR EXPANDED UNCERTAINTY (*k=2*) IN THE IAEA-461 GAFRARIUM *TUMIDUM* SAMPLE

Element	Unit	Information value[1]	Expended uncertainty (*k*=2)[2]
Al	mg kg^{-1}	870	160
Mg	mg kg^{-1}	$6.48\ .10^3$	$0.69.\ 10^3$
Sr	mg kg^{-1}	112	7

[1] The value is the robust mean of accepted sets of data, each set being obtained by different laboratory.

[2] Expanded uncertainty with a coverage factor *k=2* estimated in accordance with the JCGM 100:2008 Evaluation of measurement data – Guide to the expression of uncertainty in measurement, corresponding to the level of confidence of about 95%.

APPENDIX I

RESULTS OF THE LONG TERM STABILITY STUDY

Graphs 2 to 15 present individual mass fractions measured at each time-point relative to the mean at time zero, against the time that the samples were held at 20 ±2 ºC. Dashed lines represent expanded uncertainty of measurements.

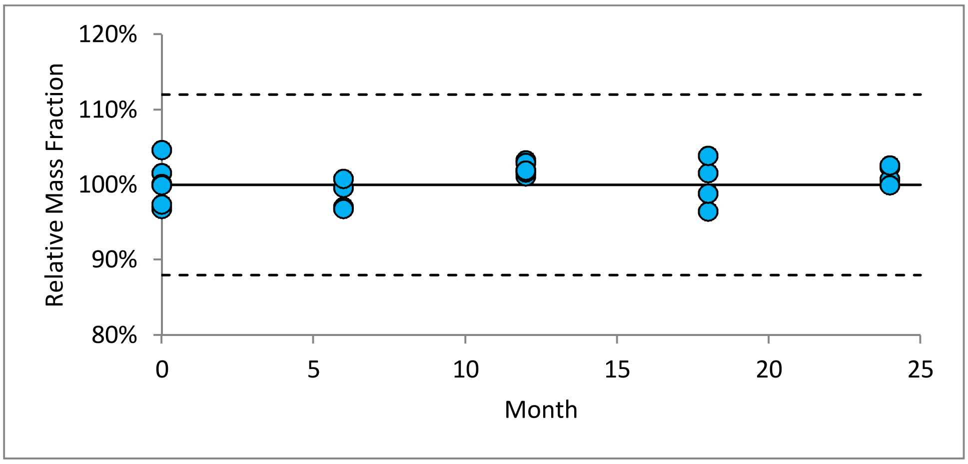

FIG. 2. Results of long term stability study for arsenic.

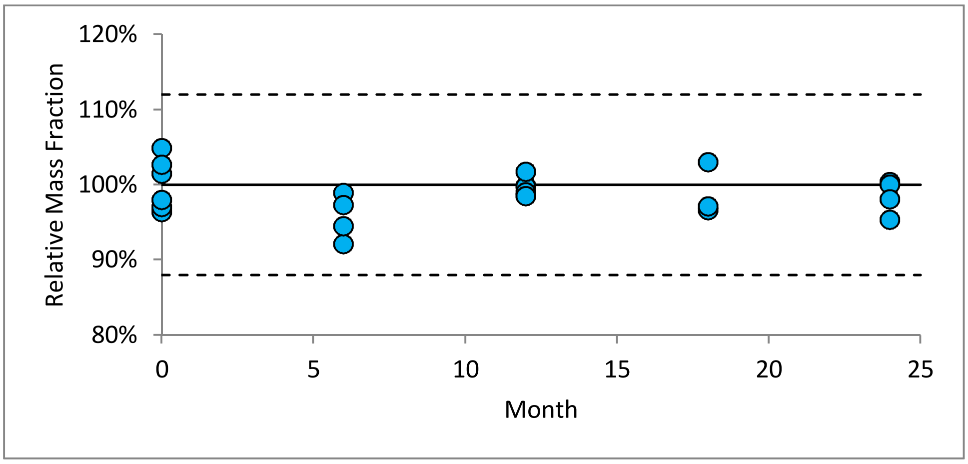

FIG. 3. Results of long term stability study for cadmium.

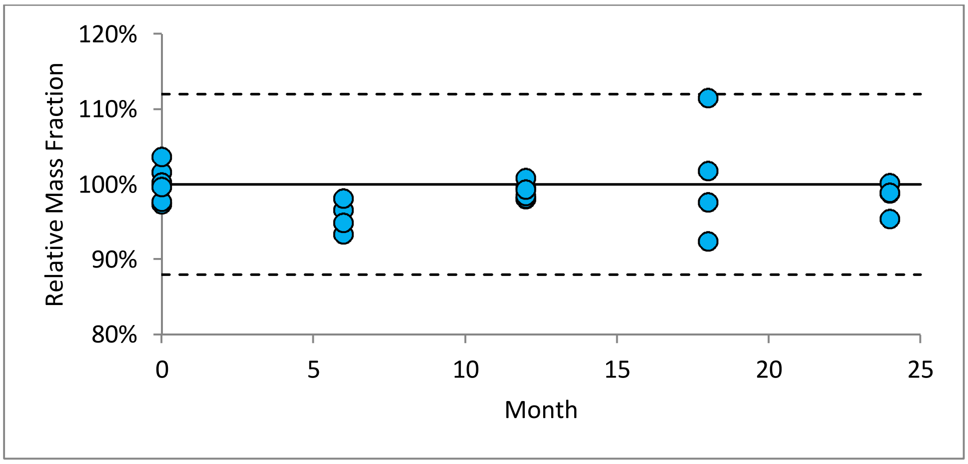

FIG. 4. Results of long term stability study for cobalt.

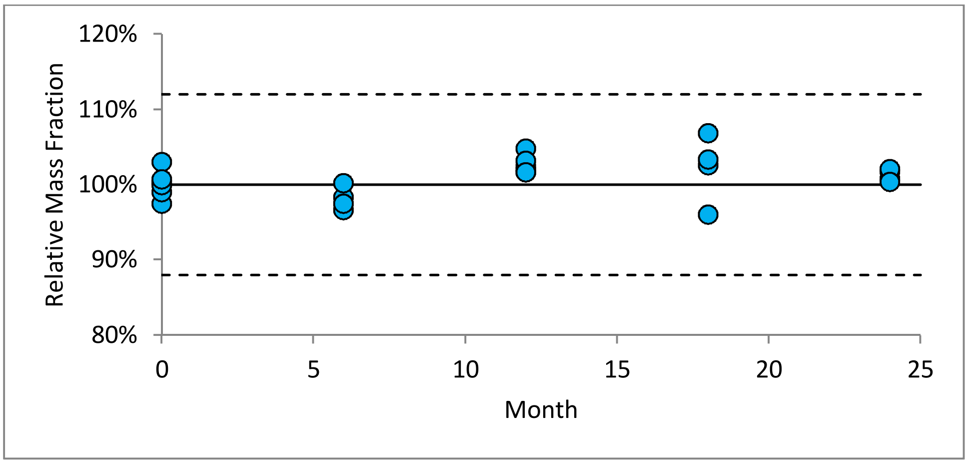

FIG. 5. Results of long term stability study for chromium.

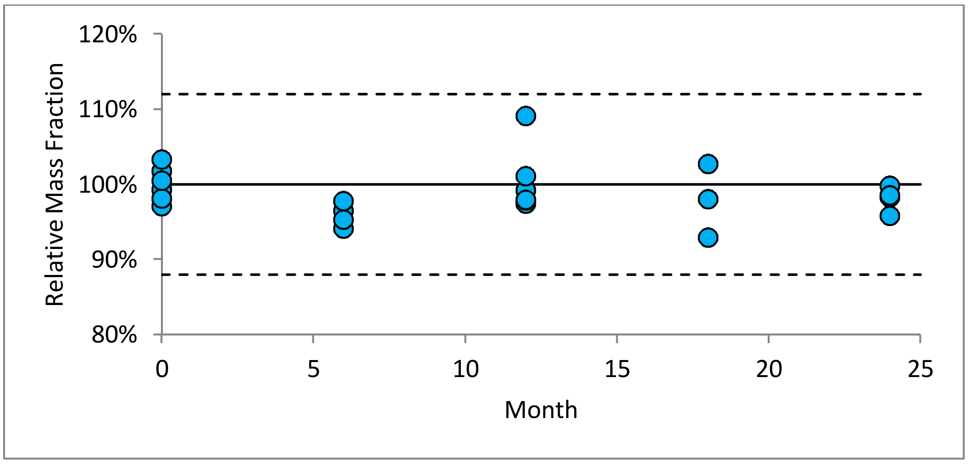

FIG. 6. Results of long term stability study for copper.

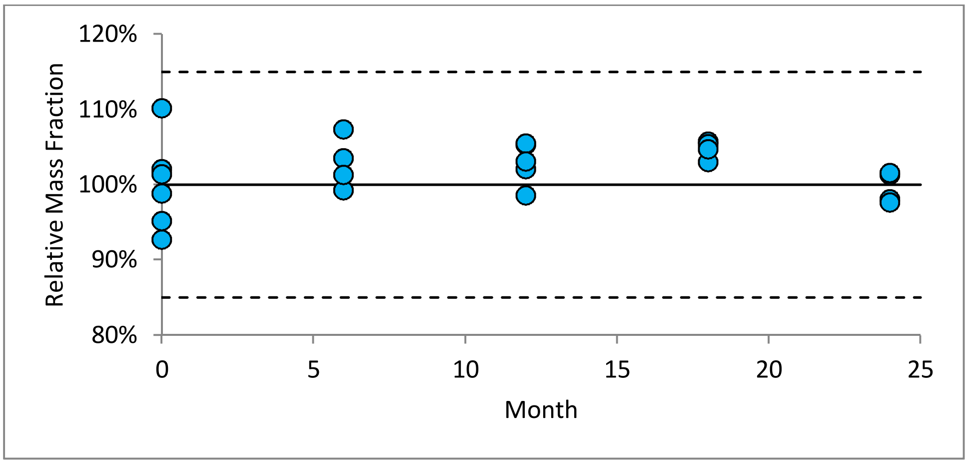

FIG. 7. Results of long term stability study for total mercury.

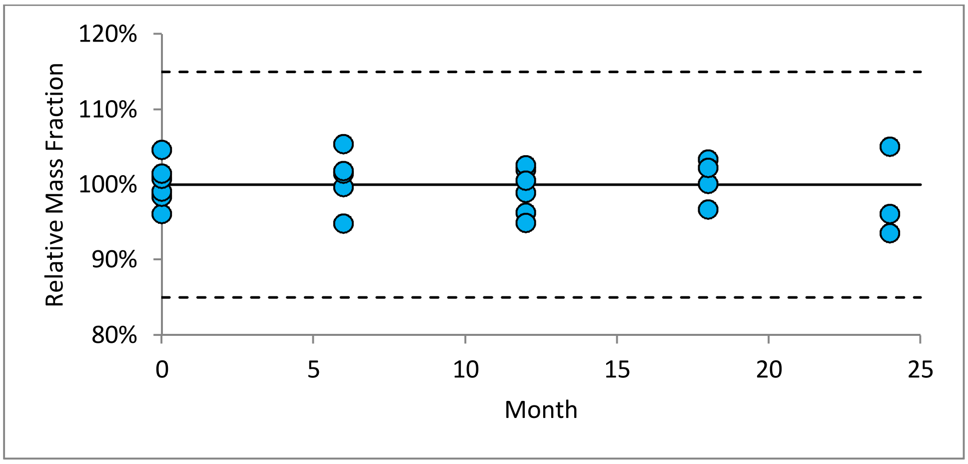

FIG. 8. Results of long term stability study for methyl mercury.

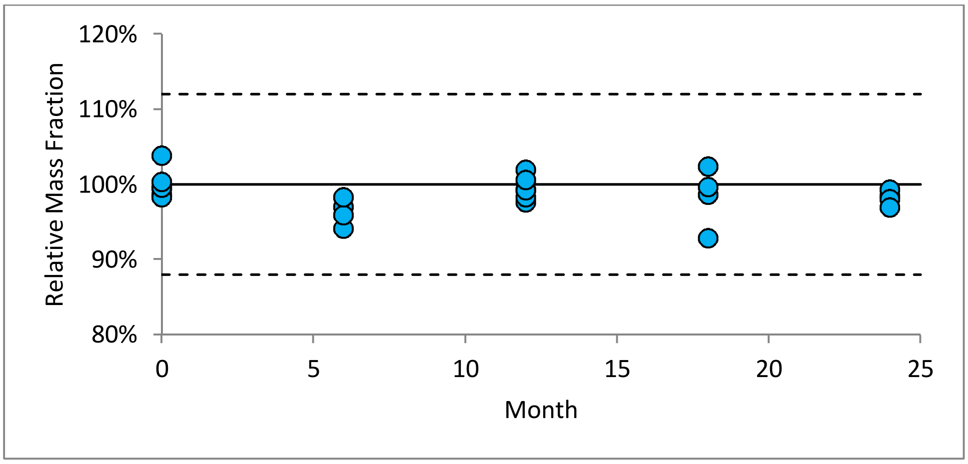

FIG. 9. Results of long term stability study for manganese.

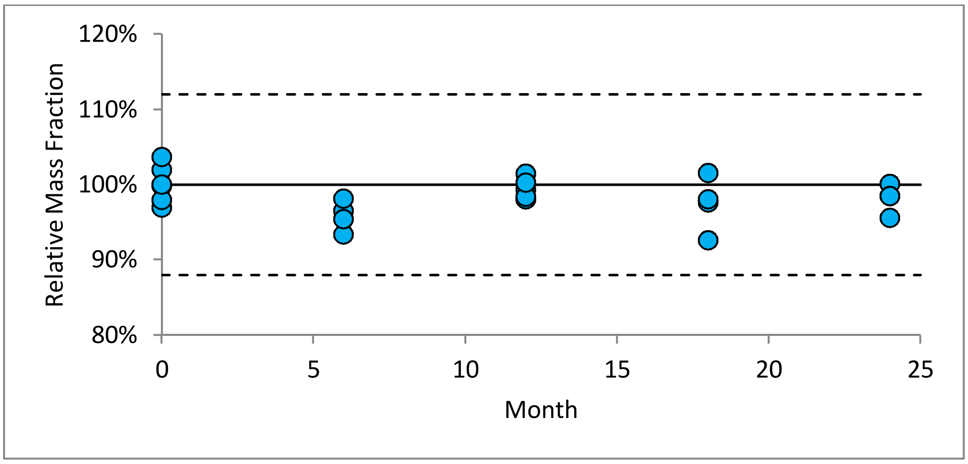

FIG. 10. Results of long term stability study for nickel.

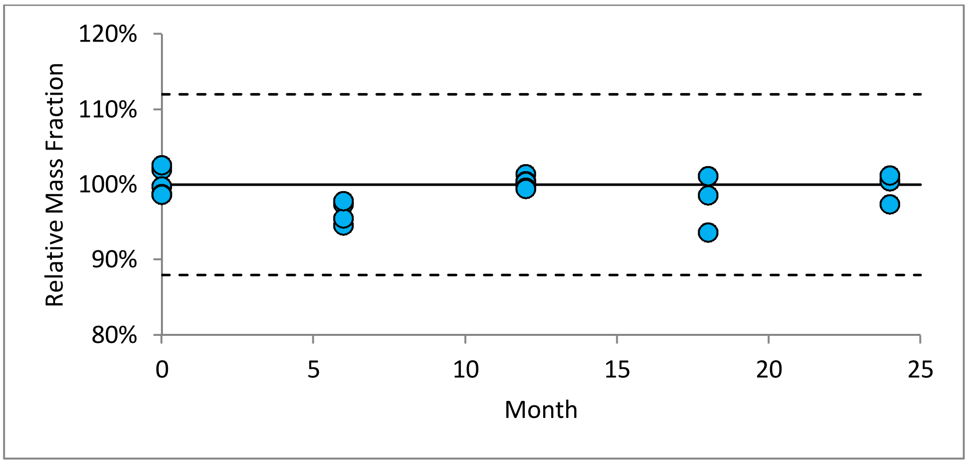

FIG. 11. Results of long term stability study for lead.

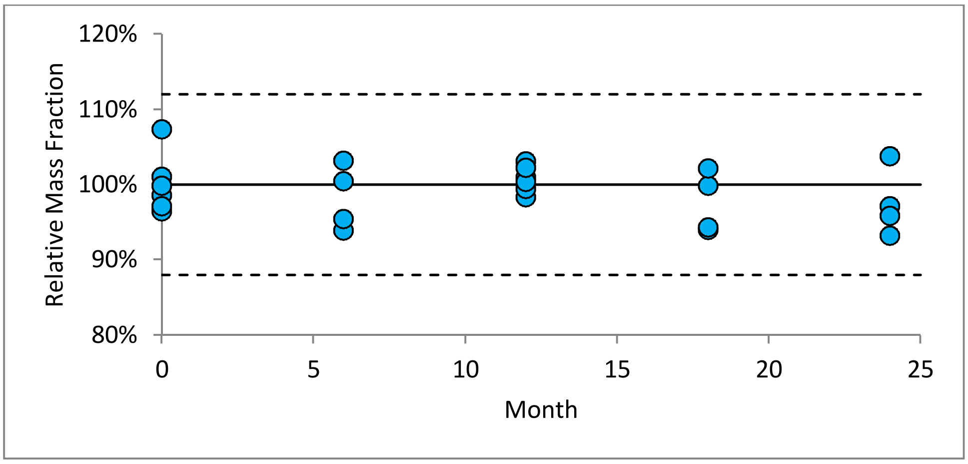

FIG. 12. Results of long term stability study for selenium.

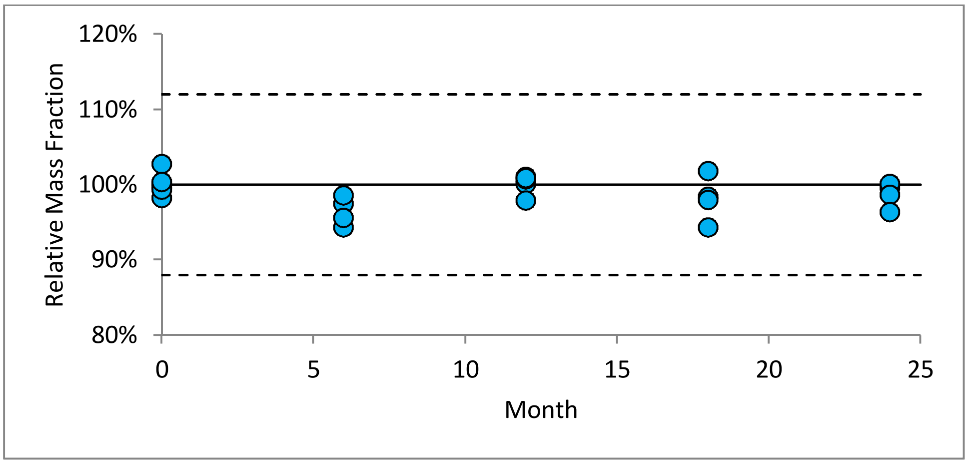

FIG. 13. Results of long term stability study for strontium.

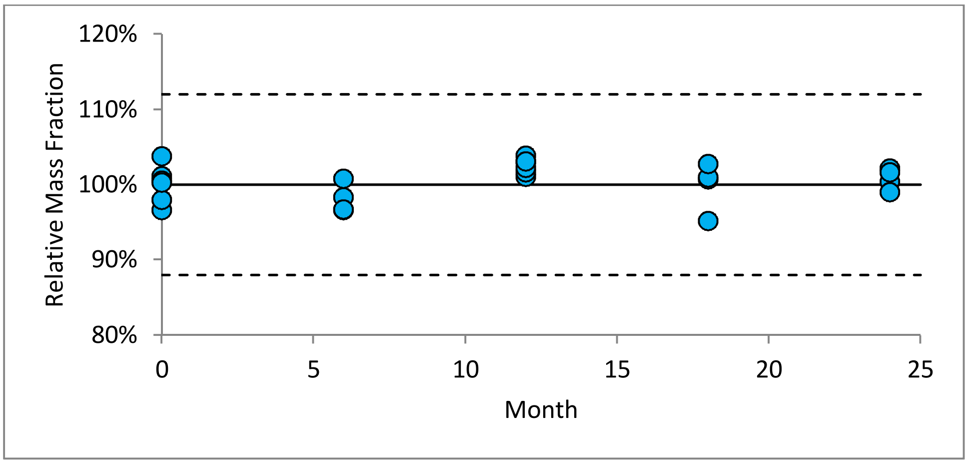

FIG. 14. Results of long term stability study for vanadium.

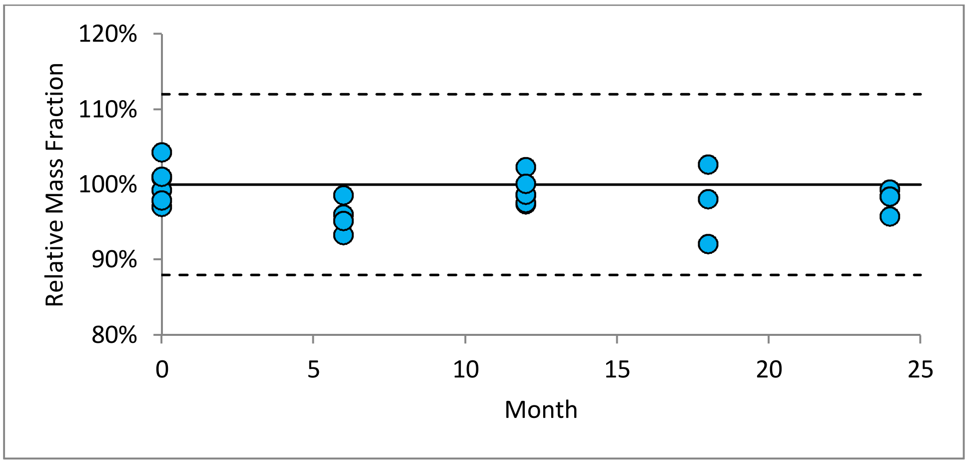

FIG. 15. Results of long term stability study for zinc.

APPENDIX II

RESULTS OF THE CHARACTERIZATION MEASUREMENTS

TABLE 7. ALUMINUM: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Method	Mean	Expanded uncertainty (U)
IAEA	F-AAS	1338	267
74	ICP-MS	627	11
33	ICP-MS	772	180
121	ICP-MS	812	497
32	ICP-MS	903	181
82	ICP-MS	942	65
49	ICP-OES	757	197
58	INAA	1145	31

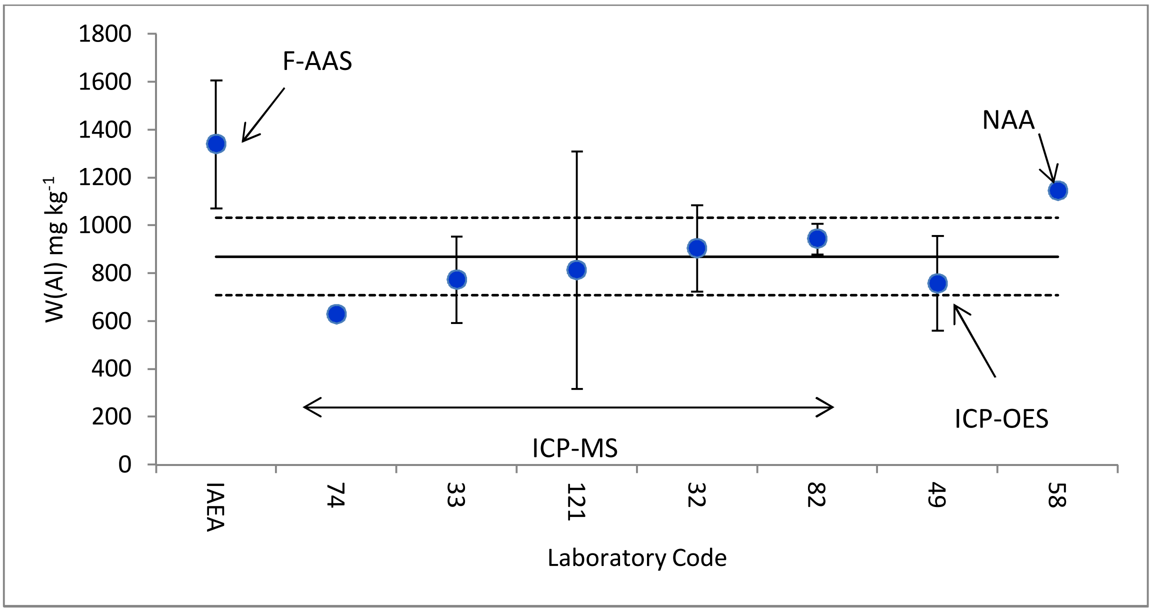

FIG. 16. Laboratory results for aluminum mass fraction (mg kg^{-1}) in the IAEA-461 Gafrarium tumidum sample.

TABLE 8. ARSENIC: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Method	Mean	Expanded uncertainty (U)
67	ET-AAS	29.3	4.4
IAEA	ET-AAS	30.6	6.1
IAEA	HR-Solid AAS	30.1	6.5
15B	ICP-MS	22.4	0.9
72	ICP-MS	29.2	1.4
IAEA	ICP-MS	29.6	3.5
49	ICP-MS	29.9	15.0
33	ICP-MS	29.9	18.6
82	ICP-MS	30.7	3.0
121	ICP-MS	31.5	11.5
32	ICP-MS	33.9	6.8
74	ICP-MS	36.3	0.5
65	NAA	29.1	1.4
58	NAA	30.0	1.7
47	NAA	32.0	2.4

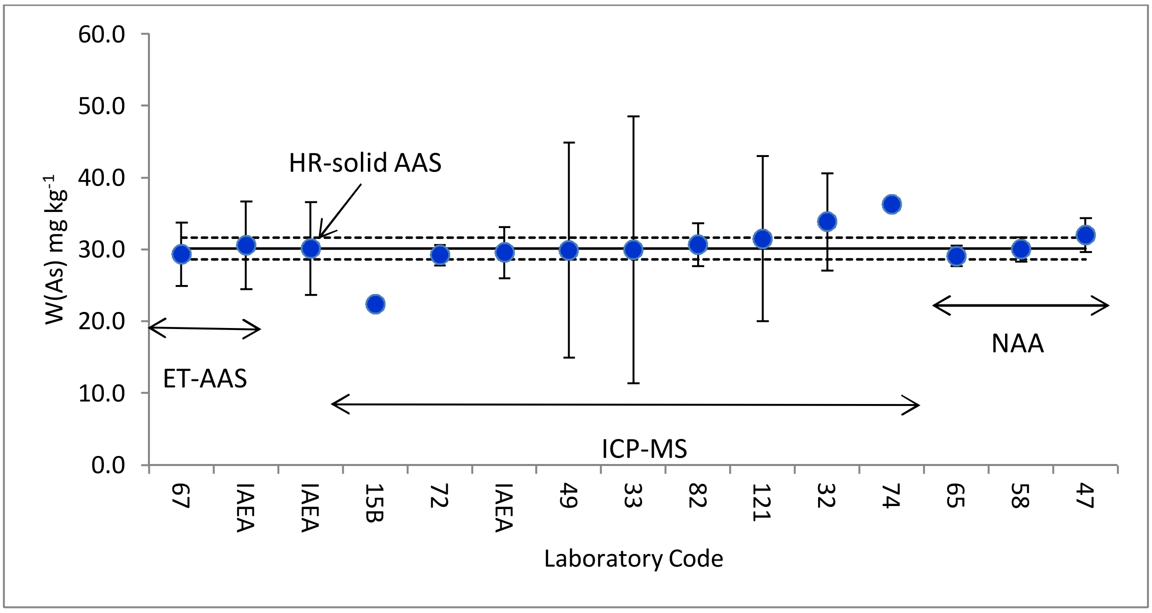

FIG. 17. Laboratory results for arsenic mass fraction (mg kg^{-1}) in the IAEA-461 Gafrarium tumidum sample.

TABLE 9. CALCIUM: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Method	Mean	Expanded uncertainty (U)
121	ICP-MS	8440	3376
74	ICP-MS	9550	80.8
82	ICP-MS	9707	1052
72	ICP-OES	8510	96
49	ICP-OES	9230	2770
47	NAA	9374	1126
65	NAA	9781	516

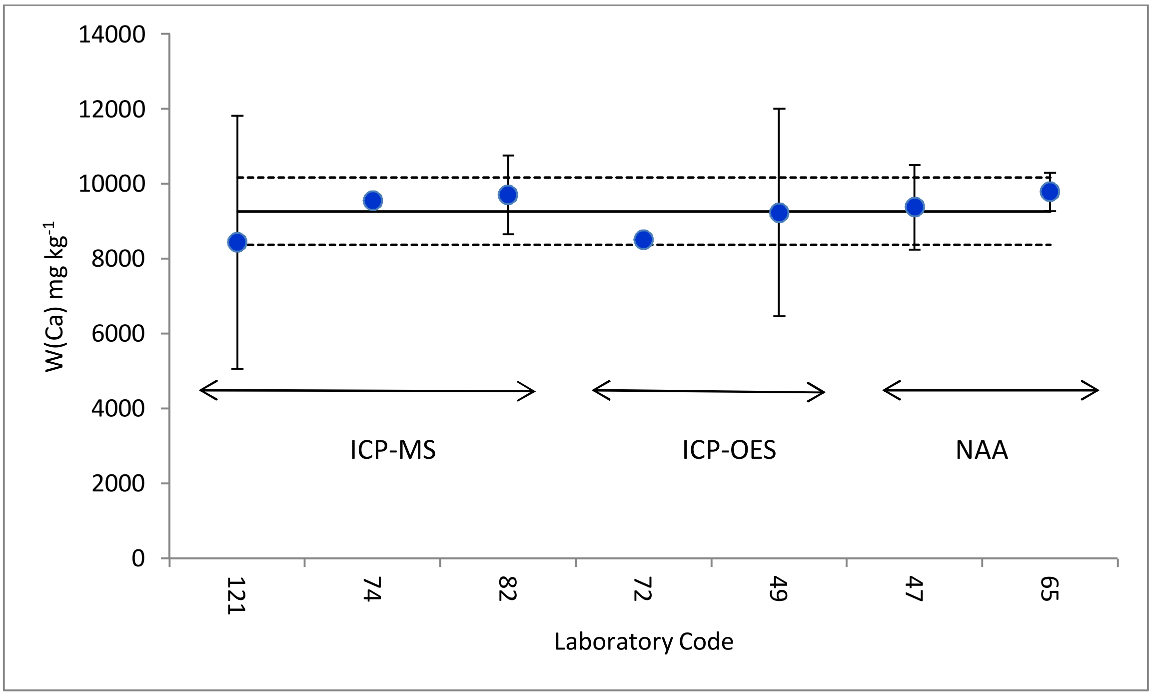

FIG. 18. Laboratory results for calcium mass fraction (mg kg^{-1}) in the IAEA-461 Gafrarium tumidum sample.

TABLE 10. CADMIUM: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Method	Mean	Expanded uncertainty (U)
IAEA	ET-AAS	0.567	0.113
67	ET-AAS	0.714	0.180
IAEA	HR-Solid AAS	0.594	0.127
49	ICP-MS	0.484	0.242
72	ICP-MS	0.500	0.022
33	ICP-MS	0.540	0.093
121	ICP-MS	0.546	0.184
15B	ICP-MS	0.551	0.039
74	ICP-MS	0.560	0.015
82	ICP-MS	0.582	0.029
IAEA	ICP-MS	0.584	0.070
75	ICP-MS	0.590	0.090
115	ICP-MS	0.590	0.059
32	ICP-MS	0.595	0.119

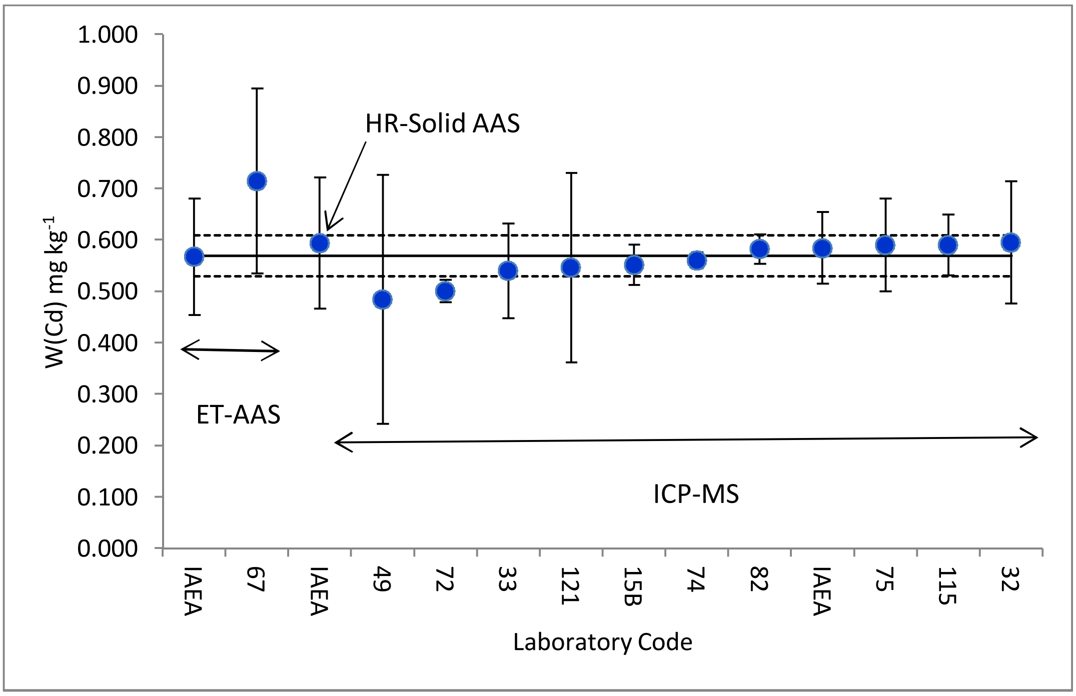

FIG. 19. Laboratory results for cadmium mass fraction (mg kg^{-1}) in the IAEA-461 Gafrarium tumidum sample.

TABLE 11. METHYL MERCURY: RESULTS AS REPORTED BY PARTICIPANTS (µg kg^{-1} as Hg)

Laboratory code	Method	Mean	Expanded uncertainty (U)
33	GC-AFS	47.9	9.6
74	GC-AFS	53.4	9.8
43	GC-AFS	56.8	11.4
IAEA	GC-AFS	58.1	7.6
IAEA	GC-AFS	65.7	15.9
47	GC-AFS	67.0	11.4
47B	GC-AFS	69.0	7.6
121	GC-AFS	84.4	54.2
38	GC-ECD	67.7	13.5
IAEA	HPLC ID ICP-MS	60.5	4.2
IAEA	ID ICP-MS	62.2	5.7
IAEA	Solid AAS	62.1	15.5

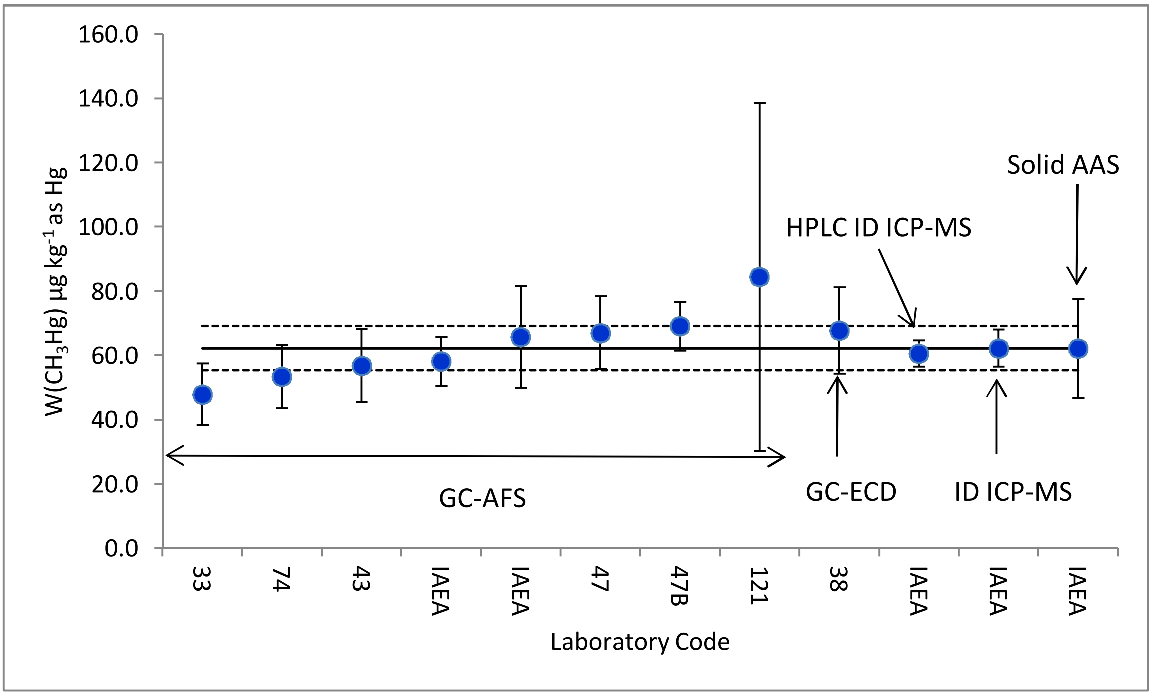

FIG. 20. Laboratory results for methyl mercury mass fraction (µg kg^{-1} as Hg) in the IAEA-461 Gafrarium tumidum sample.

TABLE 12. COBALT: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Method	Mean	Expanded uncertainty (U)
IAEA	ET-AAS	4.49	0.54
IAEA	HR-Solid AAS	4.56	1.59
121	ICP-MS	4.45	1.54
33	ICP-MS	4.51	0.90
IAEA	ICP-MS	5.02	0.60
74	ICP-MS	5.09	0.04
115	ICP-MS	5.10	0.51
32	ICP-MS	5.14	1.03
82	ICP-MS	5.47	0.29
49	ICP-OES	4.38	1.75
65	NAA	4.30	0.80
58	NAA	4.70	0.10
15A	NAA	4.70	0.87
47	NAA	5.09	0.36

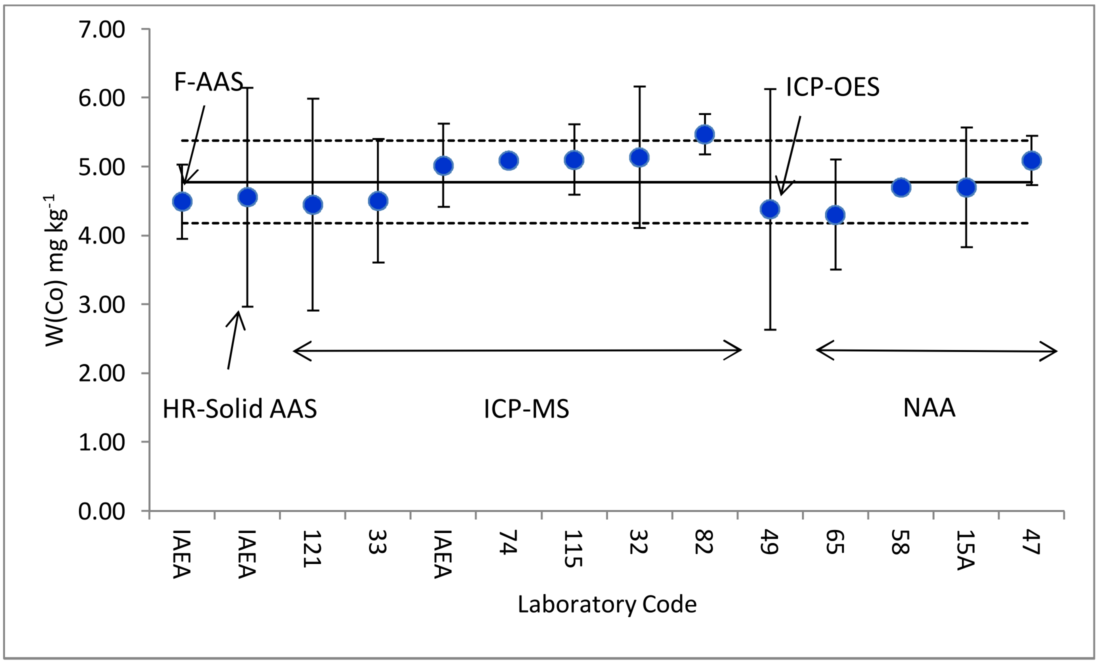

FIG. 21. Laboratory results for cobalt mass fraction (mg kg^{-1}) in the IAEA-461 Gafrarium tumidum sample.

TABLE 13. CHROMIUM: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Method	Mean	Expanded uncertainty (U)
IAEA	ET-AAS	27.0	4.3
33	ICP-MS	23.4	11.8
74	ICP-MS	24.7	0.5
121	ICP-MS	25.7	11.2
75	ICP-MS	25.8	7.7
32	ICP-MS	28.2	4.2
82	ICP-MS	31.3	1.7
49	ICP-OES	24.0	4.8
15A	NAA	26.7	4.3
58	NAA	27.0	1.3
47	NAA	29.5	2.0
65	NAA	31.4	2.8

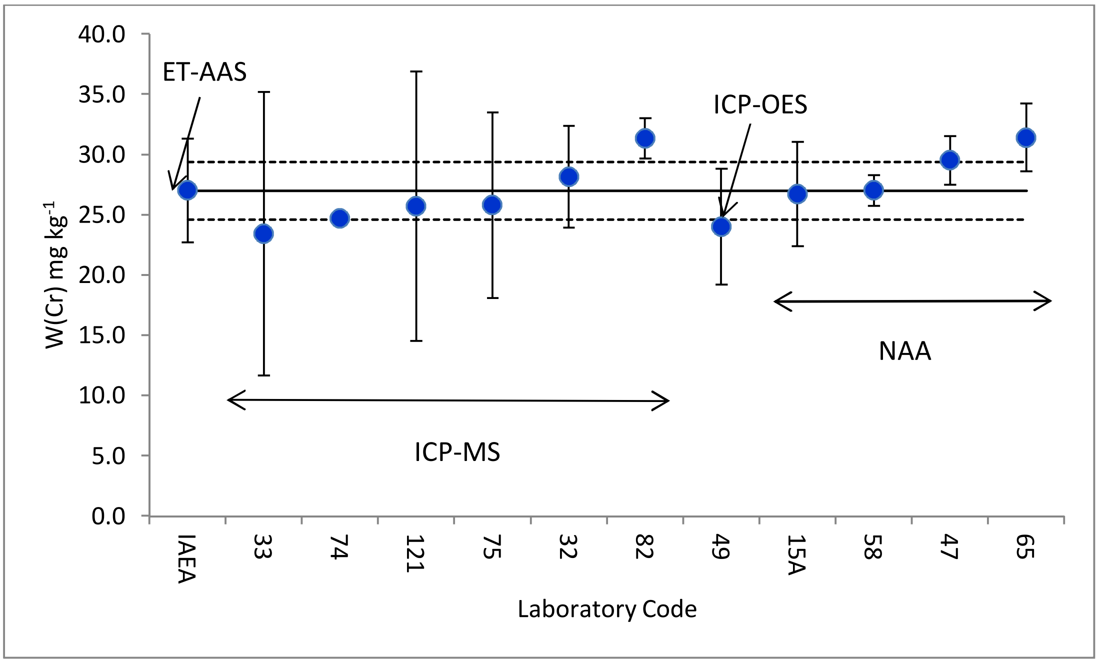

FIG. 22. Laboratory results for chromium mass fraction (mg kg^{-1}) in the IAEA-461 Gafrarium tumidum sample.

TABLE 14. COPPER: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Method	Mean	Expanded uncertainty (U)
IAEA	F-AAS	26.6	5.3
IAEA	HR-Solid AAS	27.7	2.5
33	ICP-MS	22.4	3.6
115	ICP-MS	23.7	1.9
75	ICP-MS	24.1	2.4
15B	ICP-MS	25.9	1.0
IAEA	ICP-MS	26.1	3.1
82	ICP-MS	26.8	0.7
32	ICP-MS	27.0	4.1
121	ICP-MS	27.4	10.0
74	ICP-MS	27.8	0.4
72	ICP-OES	25.1	1.2
49	ICP-OES	27.1	5.4

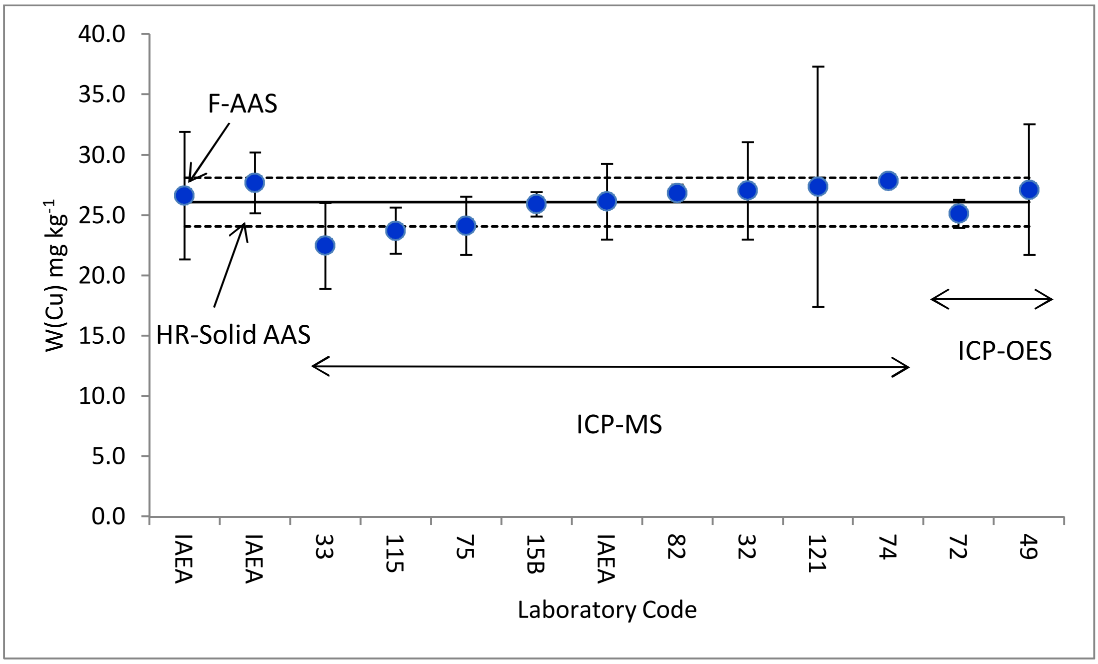

FIG. 23. Laboratory results for copper mass fraction (mg kg^{-1}) in the IAEA-461 Gafrarium tumidum sample.

TABLE 15. IRON: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Method	Mean	Expanded uncertainty (U)
IAEA	F-AAS	2503	500
115	ICP-MS	2525	379
32	ICP-MS	2645	529
74	ICP-MS	2650	60
82	ICP-MS	2685	96
121	ICP-MS	2770	665
72	ICP-OES	2361	126
49	ICP-OES	2510	703
15A	NAA	2544	140
65	NAA	2590	229
58	NAA	2650	130
47	NAA	2696	196

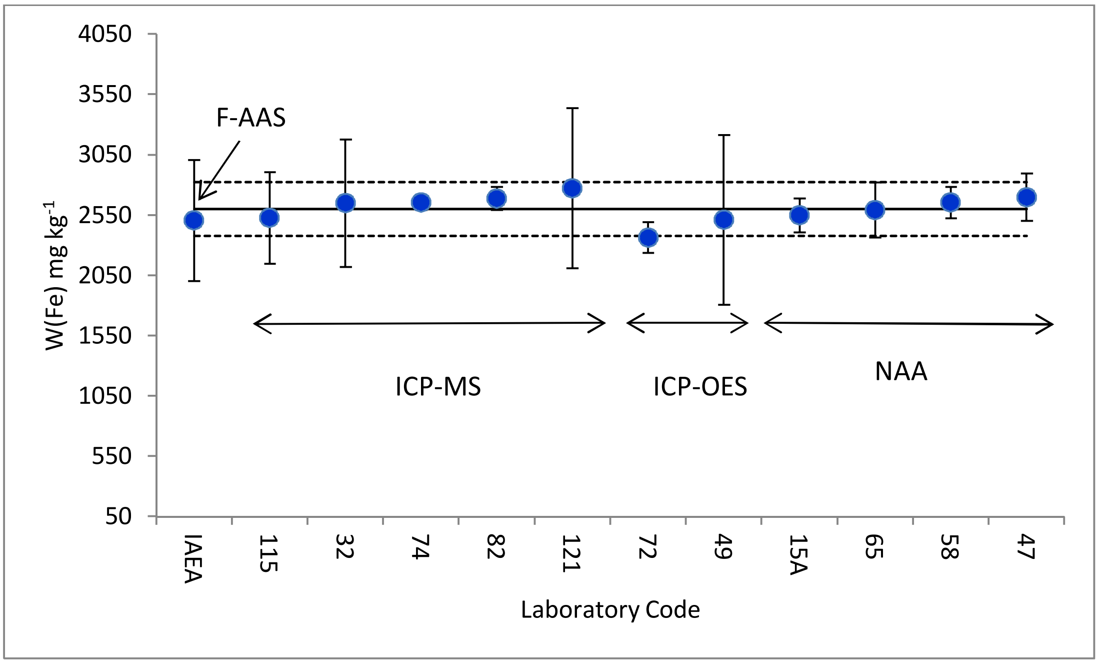

FIG. 24. Laboratory results for iron mass fraction (mg kg^{-1}) in the IAEA-461 Gafrarium tumidum sample.

TABLE 16. MERCURY: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Method	Mean	Expanded uncertainty (U)
49	AAS	0.377	0.123
33	CV-AFS	0.338	0.085
121	CV-AFS	0.340	0.123
43	CV-AFS	0.400	0.072
74	CV-AFS	0.405	0.028
IAEA	ID ICP-MS	0.390	0.009
15A	NAA	0.450	0.087
38	Solid AAS	0.350	0.070
IAEA	Solid AAS	0.383	0.042
67	Solid AAS	0.387	0.092
75	Solid AAS	0.417	0.063
47	Solid AAS	0.431	0.028

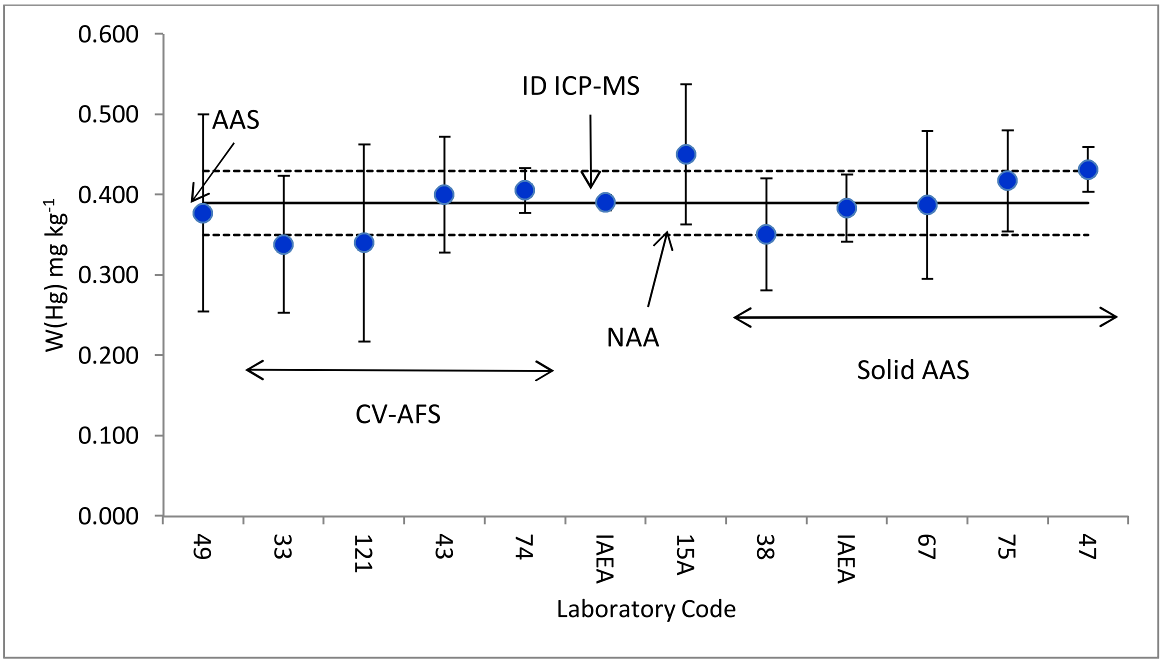

FIG. 25. Laboratory results for mercury mass fraction (mg kg^{-1}) in the IAEA-461 Gafrarium tumidum sample.

TABLE 17. MAGNESIUM: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Method	Mean	Expanded uncertainty (U)
33	ICP-MS	6160	1095
121	ICP-MS	6363	2609
74	ICP-MS	6790	61
82	ICP-MS	7018	222
72	ICP-OES	5979	236
49	ICP-OES	6600	1980

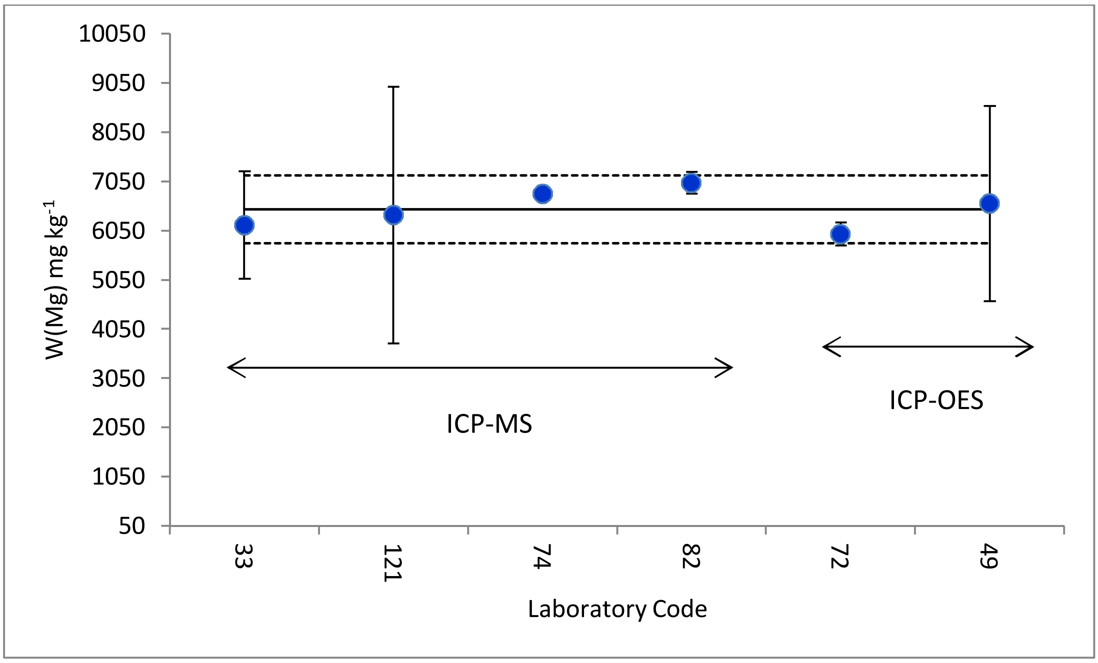

FIG. 26. Laboratory results for magnesium mass fraction (mg kg^{-1}) in the IAEA-461 Gafrarium tumidum sample.

TABLE 18. MANGANESE: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Method	Mean	Expanded uncertainty (U)
IAEA	F-AAS	336	67
33	ICP-MS	317	49
121	ICP-MS	335	148
IAEA	ICP-MS	339	41
82	ICP-MS	344	45
32	ICP-MS	351	35
74	ICP-MS	356	52
72	ICP-OES	316	13
49	ICP-OES	322	97
58	NAA	315	17

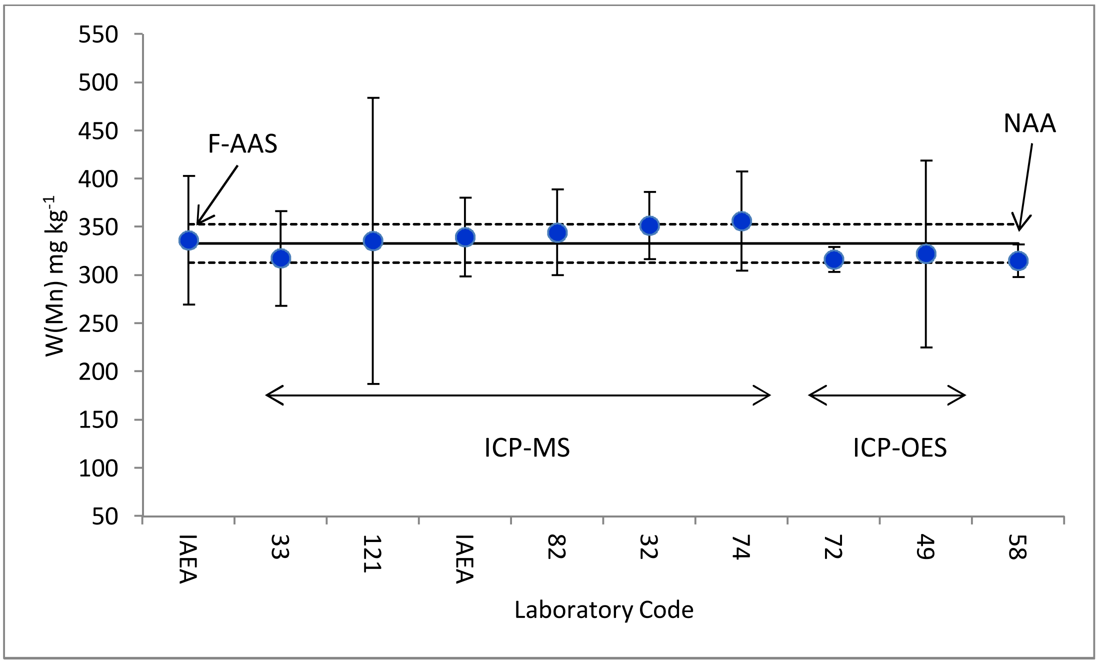

FIG. 27. Laboratory results for manganese mass fraction (mg kg^{-1}) in the IAEA-461 Gafrarium tumidum sample.

TABLE 19. NICKEL: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Method	Mean	Expanded uncertainty (U)
IAEA	F-AAS	122	24
33	ICP-MS	98	33
75	ICP-MS	100	20
IAEA	ICP-MS	101	12
115	ICP-MS	105	13
74	ICP-MS	106	1
121	ICP-MS	108	53
32	ICP-MS	109	22
82	ICP-MS	112	11
49	ICP-OES	91	32
15A	NAA	113	22

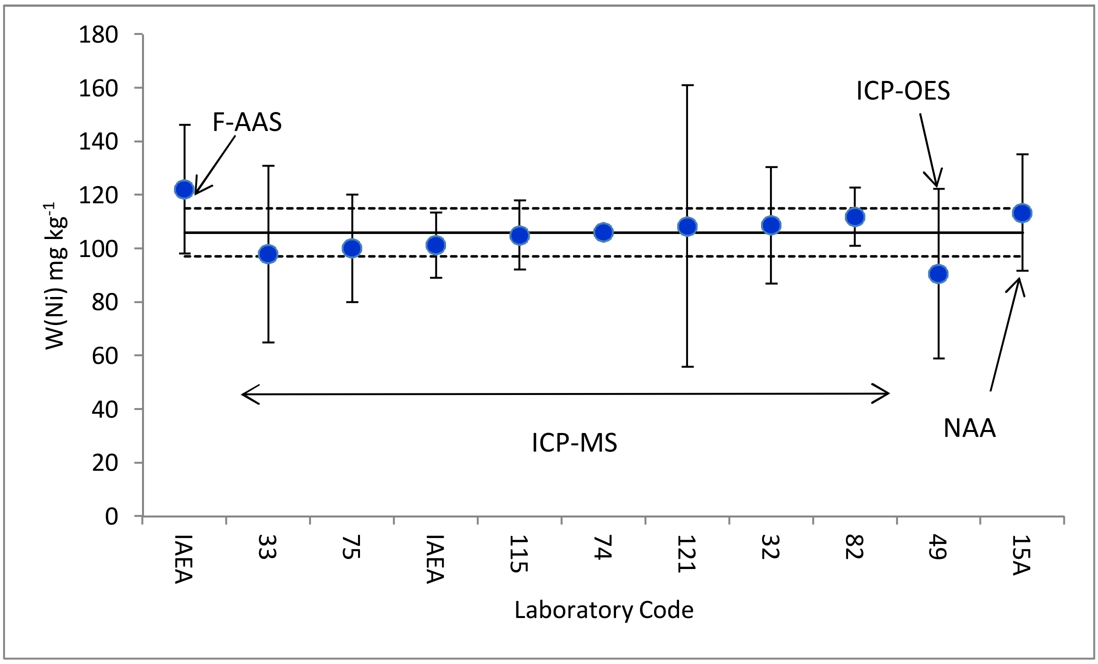

FIG. 28. Laboratory results for nickel mass fraction (mg kg^{-1}) in the IAEA-461 Gafrarium tumidum sample.

TABLE 20. LEAD: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Method	Mean	Expanded uncertainty (U)
IAEA	ET-AAS	24.1	4.4
33	ICP-MS	23.8	5.9
49	ICP-MS	24.9	6.2
IAEA	ICP-MS	25.1	3.0
72	ICP-MS	25.6	0.5
82	ICP-MS	25.7	2.0
115	ICP-MS	26.1	2.6
75	ICP-MS	26.6	4.0
74	ICP-MS	26.9	0.1
121	ICP-MS	27.6	17.4
32	ICP-MS	27.6	5.5
15B	ICP-MS	27.7	1.1
91	SV	23.8	0.9

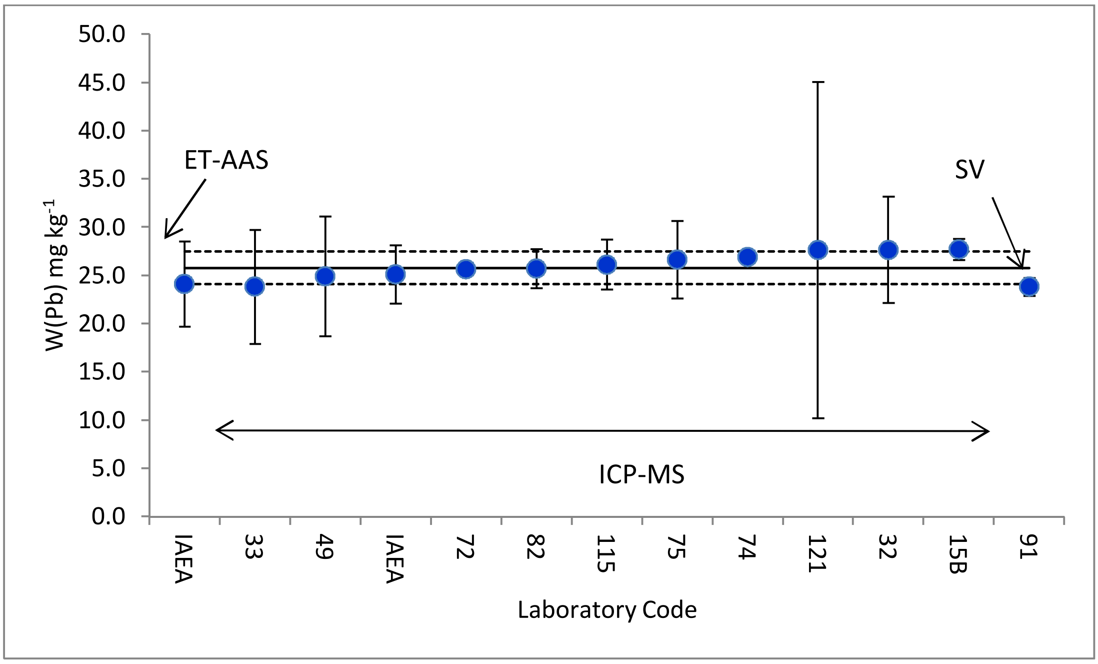

FIG. 29. Laboratory results for lead mass fraction (mg kg^{-1}) in the IAEA-461 Gafrarium tumidum sample.

TABLE 21. SELENIUM: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Method	Mean	Expanded uncertainty (U)
67	ET-AAS	2.15	0.22
IAEA	ET-AAS	2.43	0.73
121	ICP-MS	2.50	1.41
72	ICP-MS	2.70	0.18
15B	ICP-MS	2.72	0.13
33	ICP-MS	2.73	0.36
74	ICP-MS	2.77	0.07
IAEA	ICP-MS	2.86	0.34
49	ICP-MS	3.11	1.56
82	ICP-MS	3.12	0.53
32	ICP-MS	3.27	0.98
15A	NAA	2.37	0.41
47	NAA	3.00	0.22
65	NAA	3.10	0.40

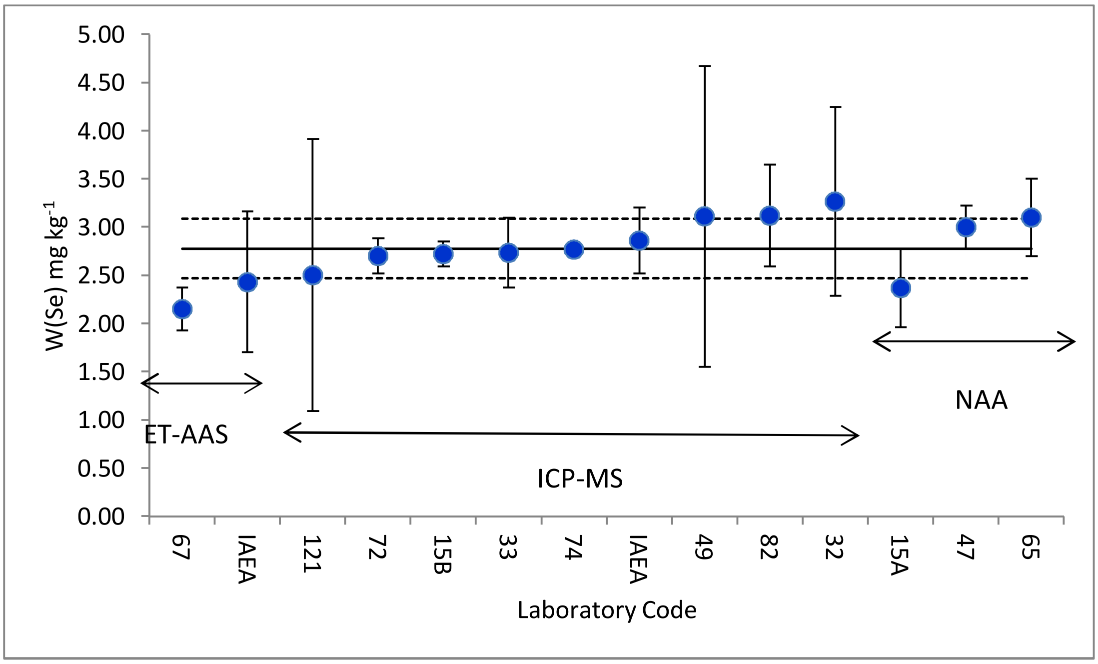

FIG. 30. Laboratory results for selenium mass fraction (mg kg^{-1}) in the IAEA-461 Gafrarium tumidum sample.

TABLE 22. STRONTIUM: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Method	Mean	Expanded uncertainty (U)
106	ET-AAS	115	24
33	ICP-MS	100	19
107	ICP-MS	111	13
47	NAA	116	12

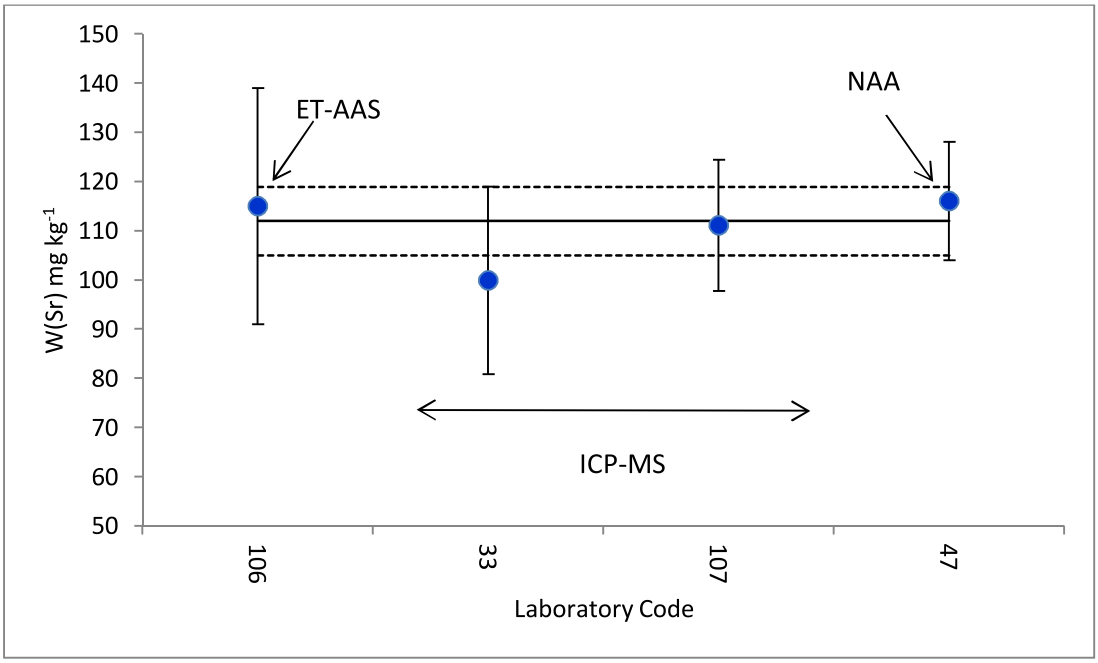

FIG. 31. Laboratory results for strontium mass fraction (mg kg^{-1}) in the IAEA-461 Gafrarium tumidum sample.

TABLE 23. VANADIUM: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Method	Mean	Expanded uncertainty (U)
IAEA	ET-AAS	7.34	0.88
33	ICP-MS	6.27	2.66
121	ICP-MS	6.62	2.00
74	ICP-MS	6.68	0.11
IAEA	ICP-MS	7.16	0.86
32	ICP-MS	7.51	1.50
82	ICP-MS	8.22	1.28
49	ICP-OES	5.90	1.48
58	NAA	6.50	0.70

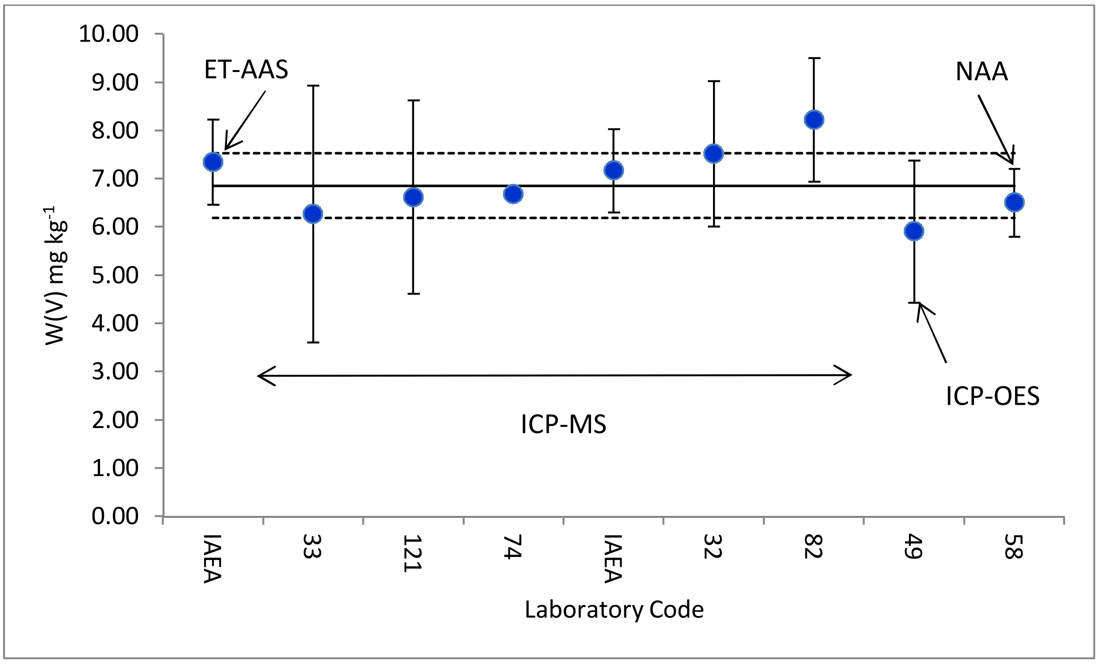

FIG. 32. Laboratory results for vanadium mass fraction (mg kg^{-1}) in the IAEA-461 Gafrarium tumidum sample.

TABLE 24. ZINC: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Method	Mean	Expanded uncertainty (U)
IAEA	Flame AAS	166	27
33	ICP-MS	141	21
15B	ICP-MS	142	6
74	ICP-MS	147	3
121	ICP-MS	150	67
115	ICP-MS	154	12
82	ICP-MS	160	6
IAEA	ICP-MS	162	19
32	ICP-MS	165	33
75	ICP-MS	169	17
49	ICP-OES	144	29
72	ICP-OES	151	4
58	NAA	157	22
65	NAA	160	24
15A	NAA	161	11
47	NAA	167	12

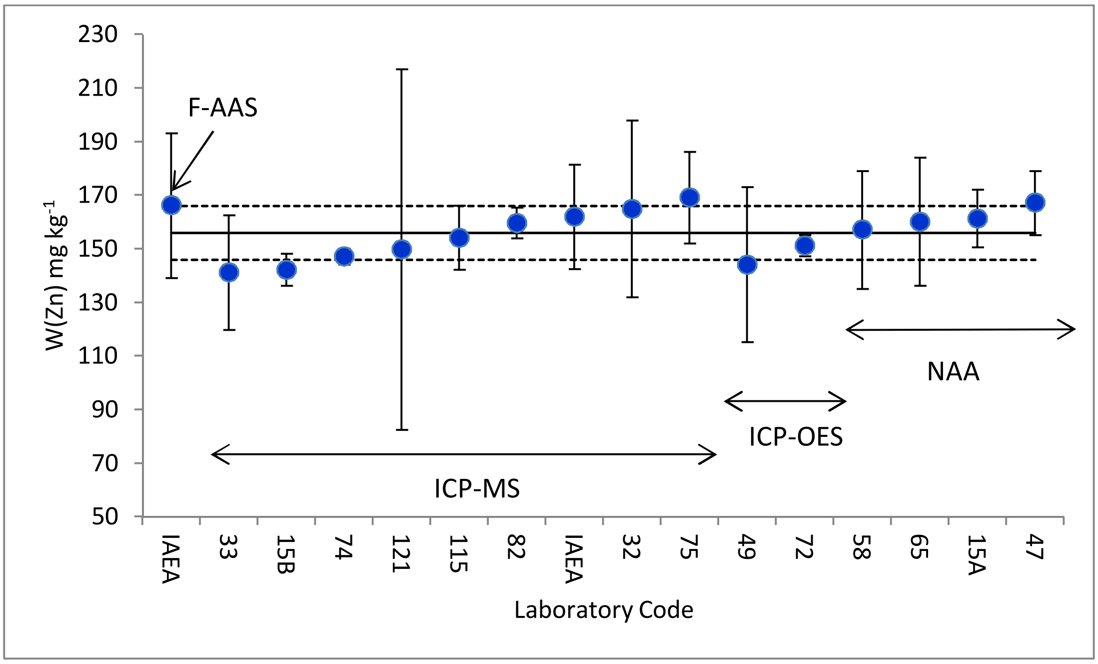

FIG. 33. Laboratory results for zinc mass fraction (mg kg^{-1}) in the IAEA-461 Gafrarium tumidum sample.

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