



## **CERTIFICATION REPORT**

**The certification of the mass fractions of elements in mussel tissue**

**Certified Reference Material ERM<sup>®</sup>-CE278k**

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Joint Research Centre  
Institute for Reference Materials and Measurements

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JRC 75296

EUR 25523 EN  
ISBN 978-92-79-26479-5 (pdf)  
ISSN 1831-9424 (online)  
doi:10.2787/6829

Luxembourg: Publications Office of the European Union

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## Summary

This report describes the production of ERM-CE278k, a mussel tissue material certified for the mass fractions of 13 elements. The material was produced following ISO Guide 34:2009 [1].

The starting material was wild mussels (*mytilus edulis*) harvested off the coast of the Netherlands. The mussels were collected in late spring, steam-cooked, shelled and frozen. About 150 kg of mussel flesh was freeze-dried, frozen in liquid nitrogen and milled before sieving to obtain a fine powder.

Between-unit heterogeneity was quantified and stability during dispatch and storage was assessed in accordance with ISO Guide 35:2006 [2]. Within-unit heterogeneity was quantified to determine the minimum sample intake.

The material was characterised by an intercomparison among laboratories of demonstrated competence and adhering to ISO/IEC 17025. Technically invalid results were removed but 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 (GUM) [3] and include uncertainties related to possible heterogeneity and instability and to characterisation.

The material is intended for quality control and assessment of method performance. As any reference material, it can also be used for control charts or validation studies. The CRM is available in glass vials containing 8 g of dry powder closed under a nitrogen atmosphere. The minimum amount of sample to be used is 200 mg.

The following values were assigned:

	Mass fraction	
	Certified value <sup>1,2)</sup> [mg/kg]	Uncertainty <sup>2,3)</sup> [mg/kg]
As	6.7	0.4
Cd	0.336	0.025
Cr	0.73	0.22
Cu	5.98	0.27
Fe	161	8
Hg	0.071	0.007
Mn	4.88	0.24
Ni	0.69	0.15
Pb	2.18	0.18
Rb	2.46	0.16
Se	1.62	0.12
Sr	19.0	1.2
Zn	71	4

1) Unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory and/or with a different method of determination. The certified value and its uncertainty are traceable to the International System of Units (SI).

2) Certified mass fractions are corrected for the water content of the material (and expressed as dry mass), determined as described in the section "Instructions for use".

3) The certified uncertainty is the expanded uncertainty with a coverage factor  $k = 2$  corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.



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## Glossary

AAS	Atomic absorption spectrometry
AFS	Atomic fluorescence spectrometry
ANOVA	Analysis of variance
AOAC	AOAC International (formerly Association of Official Analytical Chemists)
$b$	Slope in the equation of linear regression $y = a + bx$
BCR <sup>®</sup>	One of the trademarks of CRMs owned by the European Commission; formerly Community Bureau of Reference
BIPM	Bureau International des Poids et Mesures (International Bureau of Weights and Measures)
$c$	Mass concentration $c = m / V$ (mass / volume)
CC	Collision cell
CCT	Collision cell technology
CEN	European Committee for Standardization
CI	confidence interval
CIPM	CIPM Comité International des Poids et Mesures (International Committee of Weights and Measures)
C-KFT	Coulometric KFT
CRM	Certified reference material
CV-A-AFS	Cold vapour atomic fluorescence spectrometry with gold amalgamation
CV-AAS	Cold vapour atomic absorption spectrometry
CV-AFS	Cold vapour atomic fluorescence spectrometry
DRC	Dynamic reaction cell
EA	European co-operation for Accreditation
EC	European Commission
EN	European norm (standard)
ERM <sup>®</sup>	Trademark of European Reference Materials
EU	European Union
ET-AAS	Electrothermal atomic absorption spectrometry
FAAS	Flame atomic absorption spectrometry
GUM	Guide to the Expression of Uncertainty in Measurements <i>[ISO/IEC Guide 98-3:2008]</i>
HG-AFS	Hydride generation-atomic fluorescence spectrometry
IC	Ion chromatography
ICP	Inductively coupled plasma

ICP-MS	Inductively coupled plasma-mass spectrometry
ICP-QMS	ICP-Quadrupole mass spectrometry
ICP-SFMS	ICP-Sector field mass spectrometry
ID	Isotope dilution
IDMS	isotope dilution mass spectrometry
ILAC	International Laboratory Accreditation Cooperation
ILC	Interlaboratory comparison
(I)NAA	(Instrumental) neutron activation analysis
IRMM	Institute for Reference Materials and Measurements of the JRC
ISO	International Organization for Standardization
IU	International units
IUPAC	International Union of Pure and Applied Chemistry
JCGM	Joint Committee for Guides on Metrology
JRC	Joint Research Centre of the European Commission
<i>k</i>	Coverage factor
$k_0$ -NAA	$k_0$ -Neutron activation analysis
KFT	Karl Fischer titration
LOD	Limit of detection
LOQ	Limit of quantification
<i>M</i>	Molar mass
MRL	Maximum residue limit
MS	Mass spectrometry
$MS_{\text{between}}$	Mean of squares between-unit from an ANOVA
<i>MSDS</i>	Material safety data sheet
$MS_{\text{within}}$	Mean of squares within-unit from an ANOVA
<i>n</i>	Number of replicates per unit
<i>N</i>	Number of samples (units) analysed
n.a.	Not applicable
n.c.	Not calculated
n.d.	Not detectable
NIST	National Institute of Standards and Technology (USA)
OES	Optical emission spectrometry
PSA	Particle size analysis
PT	Proficiency testing
QA	Quality assurance

QC	Quality control
rel	Index denoting relative figures (uncertainties etc.)
RM	Reference material
RMP	Reference material producer
RM Unit	Reference Materials Unit of the IRMM
RNAA	Radiochemical neutron activation analysis
RSD	Relative standard deviation
RSE	Relative standard error (=RSD/ $\sqrt{n}$ )
$r^2$	Coefficient of determination of the linear regression
$s$	Standard deviation
$s_{bb}$	Between-unit standard deviation; an additional index "rel" is added when appropriate
$s_{between}$	Standard deviation between groups as obtained from ANOVA; an additional index "rel" is added as appropriate
SE	Standard error
SI	International System of Units
$s_{meas}$	Standard deviation of measurement data; an additional index "rel" is added as appropriate
$s_{ns}$	Standard deviation of results of normal stock samples
SPE	Solid phase extraction
$s_{within}$	Standard deviation within groups as obtained from ANOVA; an additional index "rel" is added as appropriate
$s_{wb}$	Within-unit standard deviation
T	Temperature
t	Time
$t_i$	Time point for each replicate
$t_{\alpha, df}$	Critical $t$ -value for a $t$ -test, with a level of confidence of $1-\alpha$ and $df$ degrees of freedom
$t_{sl}$	Proposed shelf life
$u$	standard uncertainty
$U$	expanded uncertainty
$u_{bb}^*$	Standard uncertainty related to a maximum between-unit heterogeneity that could be hidden by method repeatability; an additional index "rel" is added as appropriate
$u_{bb}$	Standard uncertainty related to a possible between-unit heterogeneity; an additional index "rel" is added as appropriate
$u_c$	combined standard uncertainty; an additional index "rel" is added as appropriate

$u_{\text{cal}}$	Standard uncertainty of calibration
$u_{\text{char}}$	Standard uncertainty of the material characterisation; an additional index "rel" is added as appropriate
$u_{\text{CRM}}$	Combined standard uncertainty of the certified value; an additional index "rel" is added as appropriate
$U_{\text{CRM}}$	Expanded uncertainty of the certified value; an additional index "rel" is added as appropriate
$u_{\Delta}$	Combined standard uncertainty of measurement result and certified value
$u_{\text{its}}$	Standard uncertainty of the long-term stability; an additional index "rel" is added as appropriate
$u_{\text{m}}$	Standard uncertainty of the measurement
$U_{\text{meas}}$	Expanded uncertainty of the measurement
$u_{\text{rec}}$	Standard uncertainty related to possible between-unit heterogeneity modelled as rectangular distribution; an additional index "rel" is added as appropriate
$u_{\text{sts}}$	Standard uncertainty of the short-term stability
$u_{\text{t}}$	Standard uncertainty of trueness
UV	Ultraviolet
V	Volume
Vap-C-KFT	Vaporisation coulometric KFT
VIM	International Vocabulary of Metrology – Basic and General Concepts and Associated Terms [ISO/IEC Guide 99:2007]
Vis	Visible
V-KFT	Volumetric Karl Fischer titration
WFD	Water Framework Directive
$\bar{x}$	Arithmetic mean
$\alpha$	significance level
$\Delta m$	Absolute difference between mean measured value and the certified value
$v_{\text{smeas}}$	Degrees of freedom for the determination of the standard deviation $s_{\text{meas}}$
$v_{\text{MSwithin}}$	Degrees of freedom of $MS_{\text{within}}$

# 1. Introduction

## Background: need for the CRM

This report describes the production of a candidate mussel tissue (*mytilus edulis*) reference material intended to replace ERM-CE278 (trace elements in mussel tissue). The project aimed to certify the mass fractions of trace elements with target contents at natural levels that are similar to ERM-CE278 and below regulatory limits for food contaminants (EC466/2001 and amendment 1881/2006 (Cd < 1.0 mg/kg, Hg < 0.50 mg/kg, Pb < 1.5 mg/kg, "wet weight")). Compared to ERM-CE278, a larger range of 15 trace elements was studied; Ag, As, Ba, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Rb, Sr, Se and Zn. The list of elements for which values are provided was update with the aim to provide a material useful for a wider variety of laboratory studies.

Like the material it replaces, the candidate reference material is intended to provide quality control for trace element measurements. In particular, the material may be of use for measurements made in support of upholding EC Directives such as quality of sources of drinking water (75/440/CEE, 79/869/CEE, 80/778/CEE), ground water (80/68/CEE, 91/676/CEE) or to protect fish life (78/659/CEE), as well as measurements on food safety (EC466/2001).

## Choice of the material

The mussels used as raw material were wild and were harvested in the Netherlands (fishery area FAO027). To ensure similar levels of trace elements to the previous reference material, the mussels were collected in late spring. The raw material was processed to give a dry powder that may be conveniently transported and stored.

## Design of the project

The stability and homogeneity of the material was evaluated through studies involving measurement of all certified elements. The certified mass fractions were established by an inter-comparison of different laboratories with different measurement methods and techniques.

# 2 Participants

## 2.1 Project management and evaluation

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE  
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No 268-RM)

## **2.2 Processing**

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE

(accredited to ISO Guide 34 for production of certified reference materials; BELAC No 268-RM)

## **2.3 Homogeneity study**

The Food and Environment Research Agency, York (UK)

(Measurements performed under ISO/IEC 17025 accreditation; UKAS 1642)

## **2.4 Stability study**

The Food and Environment Research Agency, York (UK)

(Measurements performed under ISO/IEC 17025 accreditation; UKAS 1642)

## **2.5 Characterisation**

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE

(Measurements performed under ISO/IEC 17025 accreditation; BELAC No 268-TEST)

Australian Nuclear Science and Technology Organisation, Kirawee (AU)

ALS Scandinavia AB, Luleå (SE)

(Measurements performed under ISO/IEC 17025 accreditation; SWEDAC 1087)

Ceinal, S.A. (Silliker), Área Análisis Físico-Químicos, Barcelona (ES)

(Measurements performed under ISO/IEC 17025 accreditation; ENAC 257/LE413)

Centre for Environment, Fisheries and Aquaculture Science, Lowestoft (UK)

(Measurements performed under ISO/IEC 17025 accreditation; UKAS 1875)

Institut "Jozef Stefan", Ljubljana, (SI)

(Measurements performed under ISO/IEC 17025 accreditation; Slovenska Akreditacija LP-090)

Laboratoire national de métrologie et d'Essais, Paris (FR)

(Measurements performed under ISO/IEC 17025 accreditation; COFRAC 22)

Solvias AG- Elemental and Microanalytical Services, Basel (CH)

Fødevareinstituttet, Danmarks Tekniske Universitet, Søborg (DK)

(Measurements performed under ISO/IEC 17025 accreditation; DANAK No 350)

Helmholtz Zentrum München - Deutsches Forschungszentrum für Gesundheit und Umwelt GmbH, München (DE)

SCK-CEN, Mol (BE)

(Measurements performed under ISO/IEC 17025 accreditation; BELAC 015-TEST)

Umweltbundesamt GmbH, Wien (AT)

(Measurements performed under ISO/IEC 17025 accreditation; Wirtschaftsministerium 92714/499-IV/9/01)

## **3 Material processing and process control**

### **3.1 Origin of the starting material**

The mussels used as raw material were wild and were harvested in the Netherlands (fishery area FAO027). The fresh mussels were steam-cooked and shelled by the supplier (Gastro-Fish, Zeebrugge, BE), before being frozen, packed into plastic bags and transported (by freezer-container) to IRMM. About 150 kg of mussel flesh was obtained.

### **3.2 Processing**

Upon arrival at the IRMM, the mussel flesh was immediately stored at -20 °C until further treatment. For vacuum-drying, four consecutive cycles were required to process all of the raw material. Firstly, large visible impurities (such as other shells, byssus and crabs) were removed manually. The batch was split into 1.1 kg portions and placed on 40 PE-foil covered trays, which were loaded into an Epsilon 2-85D freeze-dryer (Martin Christ, Osterode, Germany). The water contents were checked after each of the four drying cycles. Results were the same (within measurement uncertainty) for each batch, and were < 7 %. The material was therefore considered suitable for milling. In total, about 36 kg of vacuum dried mussel tissue was obtained.

Prior to milling, the mussels were placed in a stainless steel drum and crushed with a PTFE pestle. When the mussel pieces were sufficiently small to feed into the mill, they were transferred to another metallic drum for cooling over liquid nitrogen. The crushed mussels were subsequently immersed in liquid nitrogen over night. Mussel pieces were scooped up and fed slowly into a Palla vibrating mill (KHD Humboldt Wedag, Köln, Germany) which was previously cooled to -196 °C with liquid nitrogen. All machine parts in contact with the material were made of high purity titanium. The flow of liquid nitrogen was turned off just before milling was started, and the mill was stopped and new cooling sequences were started once the temperature at the outlet rose to -90 °C.

After milling, the material was passed through a 250 µm Nylon sieve (Russel Finex Model 17300, London, United Kingdom). The fraction < 250 µm represented about 99% of the milled material and was kept in an acid washed 200 L HDPE drum at room temperature. The coarse fraction > 250 µm (about 300 g) was transferred to the stainless steel drums used prior to the milling and immersed into liquid nitrogen again for 4 hours for pre-cooling. Palla milling and sieving were then repeated. Again, the fraction below 250 µm was transferred to the 200 L plastic drum and kept at room temperature. The fraction larger than 250 µm (estimated to be no more than a few grams) was not processed further. The total amount of powder <250 µm was 34.4 kg which was kept at room temperature prior to the next step.

The material was homogenised with a three-dimensional mixer( Dyna-MIX CM200 mixer, WAB, Basel, Switzerland) for 1 hour. The material was then checked for suitability for bottling by measuring water content and particle size distribution: The water content was found to be between 3 and 4 % (m/m), while in initial testing, the largest particle size was below 435 µm. The material was transferred to plastic bags with about 1 kg of powder each, thermo sealed and then stored at room temperature.

An automatic filling machine (All Fill, Sandy, United Kingdom) was used to transfer the mussel powder into 50 mL amber glass bottles. All bottles were acid washed with 2 % HNO<sub>3</sub> (m/m) and rinsed with Milli-Q water before drying in a drying cabinet. Each vial was filled with slightly more than 8.0 g. Once the vials were filled, lyo-inserts were automatically pressed down half-way in the bottle necks. The bottles were flushed with nitrogen before and after filling, and the hopper was also continuously flushed with nitrogen providing an inert atmosphere above the material. Just before collection at the end of the conveyor belt of the filling machine, the inserts were manually fully pressed down in the neck of the vials. Capping and labelling were done using automated systems (Bausch & Ströbel and BBK, respectively, Germany).

### **3.3 Process control**

After processing, 5 bottles were selected at random and 2 replicate micrographs, water measurements and particle size counts were made on each bottle.

Micrographs showed irregularly shaped particles with no fibres or agglomerations present in any of the images, and no discernable differences in particles of different sizes were detected.

The water content was measured using Karl Fischer titration. The mean result ( $n = 5$ ) was 3.3 % water (m/m) with 6 % RSD, which indicates that the material was homogeneously dried.

The particle size analysis was made using the Low Angle Laser Light Scattering technique with a Sympatec Helos instrument (Clausthal Zellerfeld, Germany). Five different samples of mussel powder were measured in duplicate. Results showed that the 90 % of particles were below a mean of 90.4  $\mu\text{m}$ , with measurement RSD of 2.57 %. This shows that the target particle size ( $< 250 \mu\text{m}$ ) was achieved and that the material is homogeneous and uniform over the batch with respect to particle size distribution.

## **4 Homogeneity**

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 unit variation. This aspect is covered in between-unit homogeneity studies.

The within-unit heterogeneity 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 heterogeneity is therefore necessary to determine the minimum sample intake.

In the case of ERM-CE278k, 'unit' is defined as an individual glass bottle of the material.

## 4.1 Between-unit homogeneity

The between-unit homogeneity was evaluated to ensure that the certified values of the CRM are valid for all bottles of the material, within the stated uncertainty.

Sixteen bottles were selected using a random stratified sampling scheme covering the whole batch for the between-unit homogeneity test. The batch was divided into a 16 groups (with a similar number of bottles) and one bottle was selected randomly from each group. The number of selected bottles corresponds to approximately the cubic root of the total number of produced bottles. Three independent samples were taken from each selected bottle, and analysed by ICP-MS. The measurements were performed under repeatability conditions, and in a randomised manner to be able to separate a potential analytical drift from a trend in the filling sequence. The results were corrected for the water content determined in each bottle (see Section 6.4). The results are shown as graphs in Annex A.

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 sequence were visible at the 99 % confidence level. Significant (99 % confidence level) trends in the analytical sequence were visible for Mn, Ni, Cu, Zn, As, Se and Pb pointing at instability of the analytical system. As the analytical sequence and the unit numbers were not correlated, correction for these trends can improve the sensitivity of the subsequent statistical analysis through a reduction in analytical variation without masking potential between-bottle heterogeneities. Therefore, trends in the analytical sequence were corrected if the trend was significant on at least a 99% confidence level as shown below.

$$\text{corrected result} = \text{measured result} - b \cdot i$$

$b$  = slope of the linear regression

$i$  = position of the result in the analytical sequence

The datasets were tested for consistency using Grubbs outlier tests on a confidence level of 99 % on the individual results and the unit means. Some outlying individual results and bottle means were detected. Since no technical reason for the outliers could be found, all the data were retained for statistical analysis.

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

Evaluation by ANOVA requires bottle means which follow at least a unimodal distribution and results for each unit that follow unimodal distributions with approximately the same standard deviations. Distribution of the bottle averages was tested using histograms and normal probability plots. Too few data are available for each unit to make a clear statement of the distribution of individual results. Therefore, it was visually checked whether all individual data follow a unimodal distribution using histograms and normal probability plots. Minor deviations from unimodality of the individual values do not significantly affect the estimate of between-unit standard deviations. The results of all statistical evaluations are given in Table 1.

Table 1: Results of the statistical evaluation;

Element mass fraction	Trends		Outliers		Distribution	
	Analytical sequence	Filling sequence	Individual results	Bottle means	Individual results	Bottle means
Ag*	NO	NO	none	none	unimodal	unimodal
As	YES	NO	none	none	unimodal	unimodal
Ba	NO	NO	none	none	unimodal	unimodal
Cd	NO	NO	none	none	unimodal	unimodal
Cr	NO	NO	1–statistical reason (retained)	none	unimodal	unimodal
Cu	YES	NO	none	none	unimodal	unimodal
Fe	NO	NO	none	none	unimodal	unimodal
Hg	NO	NO	none	none	unimodal	unimodal
Mn	YES	NO	none	none	unimodal	unimodal
Ni	YES	NO	1–statistical reason (retained)	none	unimodal	unimodal
Pb	NO	NO	none	1–statistical reason (retained)	unimodal	unimodal
Rb	NO	NO	none	none	unimodal	unimodal
Se	YES	NO	none	none	unimodal	unimodal
Sr	NO	NO	none	none	unimodal	unimodal
Zn	YES	NO	none	none	unimodal	unimodal

\*Ag results were given by evaluation of the samples measured for the long-term stability test (as described in the following section).

One has to bear in mind that  $s_{bb,rel}$  and  $s_{wb,rel}$  are estimates of the true standard deviations and therefore subject to random fluctuations. Therefore, the mean square between groups ( $MS_{between}$ ) can be smaller than the mean squares within groups ( $MS_{within}$ ), resulting in negative arguments under the square root used for the estimation of the between-bottle 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 heterogeneity that might be undetected by the given study setup.

Method repeatability ( $s_{wb,rel}$ ), between–bottle standard deviation ( $s_{bb,rel}$ ) and  $u_{bb,rel}^*$  were calculated as

$$s_{wb,rel} = \frac{\sqrt{MS_{within}}}{y}$$

$$s_{bb,rel} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}}$$

$$u_{bb,rel}^* = \frac{\sqrt{\frac{MS_{within}}{n}} \sqrt[4]{\frac{2}{v_{MS_{within}}}}}{\bar{y}}$$

- $MS_{within}$  mean square within a bottle from an ANOVA  
 $MS_{between}$ : mean squares between-bottle from an ANOVA  
 $\bar{y}$  average of all results of the homogeneity study  
 $n$ : average number of replicates per bottle  
 $v_{MS_{within}}$  : degrees of freedom of  $MS_{within}$

The results of the evaluation of the between-unit variation are summarised in Table 2. In almost half the cases, the uncertainty contribution for homogeneity was determined by the method repeatability.

Table 2: Results of the homogeneity study; n.c.: cannot be calculated as  $MS_{between} < MS_{within}$

Element mass fraction	$s_{wb,rel}$ [%]	$s_{bb,rel}$ [%]	$u_{bb,rel}^*$ [%]	$u_{bb,rel}$ [%]
Ag*	8.5	11.7	2.47	11.7
As	2.86	n.c.	0.83	0.83
Ba	7	1.2	2.02	2.02
Cd	9.6	n.c.	2.78	2.78
Cr	16.3	3.9	4.7	4.7
Cu	3.2	0.49	0.91	0.91
Fe	3.3	n.c.	0.94	0.94
Hg	5.8	1.88	1.67	1.88
Mn	2.6	n.c.	0.75	0.75
Ni	13.4	n.c.	3.9	3.9
Pb	2.78	n.c.	0.80	0.80
Rb	2.86	n.c.	0.83	0.83
Se	6.4	1.62	1.84	1.84
Sr	2.12	0.16	0.61	0.61
Zn	2.12	0.70	0.61	0.70

\*Ag results were given by evaluation of the samples measured for the long-term stability test (as described in the following section).

For Ag, a single outlying replicate about an order of magnitude higher than the mean was found in the homogeneity test. No technical reason could be found to explain the outlier, and it was unclear as to whether this represented contamination of the sample at the time of processing, or on the occasion of measurements for the study. Therefore, the homogeneity was assessed using the results of the long-term stability study, in which 5 replicate measurements were made on each of 8 bottles. For the homogeneity assessment, the potential influence of the different storage conditions (discussed in the following section) was ignored. The results are presented together with those for the other elements in tables 1 and 2.

The homogeneity study showed no outlying bottle means or trends in the filling sequence. Therefore the between-bottle standard deviation can be used as estimate of  $u_{bb}$ . As  $u_{bb}^*$  sets the limits of the study to detect inhomogeneity, the larger value of  $s_{bb}$  and  $u_{bb}^*$  is adopted as uncertainty contribution to account for potential heterogeneity.

## 4.2 Within-unit homogeneity and minimum sample intake

The within-bottle heterogeneity 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 bottle 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.

Homogeneity experiments were performed using a 200 mg sample intake. This sample intake gives acceptable repeatability, demonstrating that the within-bottle heterogeneity no longer contributes to analytical variation at this sample intake.

The minimum sample intake,  $m_{min}$ , is the minimal mass of sample that shall be used in an analysis, to ensure that the certified value is valid within its uncertainty. For ERM-C278k, it is set to the mass used for the homogeneity study for all elements, which was 0.2 g. To verify the suitability of this mass, a study was made using solid sampling atomic absorption spectrometry, SSAAS, to measure the contents of selected elements in sub-milligram amounts of the material, without sample preparation[5]. Sample intakes of about 0.02 – 0.46 mg were used and a minimum of 30 replicate measurements were carried out per element.

The data has been evaluated according to the following equation [6]:

$$m_{min} = \frac{k'_2 \cdot s_m}{u_{target}}^2 \cdot m$$

With  $m_{min}$  minimum sample mass,  $k'_2$  factor for the two-sided 95 % tolerance limits for a normal distribution,  $s_m$  relative standard deviation of the homogeneity experiment,  $u_{target}$  maximum relative uncertainty acceptable for sub sampling (in this case set to the uncertainties of the certified values) and  $m$  average mass used during the measurements.

The resulting minimum sample masses are summarised in Table 3.

Table 3: Minimum sample masses for a target uncertainty equal to  $u_{CRM,rel}$  as determined by solid sampling AAS

Element mass fraction	M (mg)	$s_m$	m (mg)
Cd	51	27 %	44
Cu	6	15 %	26
Pb	128	30 %	61

For each element measured by SSAAS, the estimated  $m_{min}$  was lower than the target of the homogeneity study of 200 mg. The overall minimum sample intake for this material, valid for all elements investigated in the homogeneity study, is therefore set to 200 mg. For Ag, the homogeneity was assessed through results of the long-term stability study, for which a mean sample intake of 500 mg was used. Therefore, for Ag only, the minimum sample intake is set to 500 mg.

## 5 Stability

Time, temperature and radiation were regarded as the most relevant influences on stability of the materials. The influence of ultraviolet or visible radiation was minimised by the choice of brown glass bottles for containment, which eliminates most of the incoming light. In addition, materials are stored and dispatched in the dark, thus eliminating practically the possibility of degradation by light. Therefore, only the influences of time and temperature needed to be investigated.

Stability testing is necessary to establish conditions for storage (long-term stability) as well as conditions for dispatch to the customers (short-term stability). 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 were carried out using an isochronous design [7]. In that approach, bottles are stored for a certain time at different temperature conditions. Afterwards, the bottles 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 bottles are analysed simultaneously under repeatability conditions. Analysis of the material (after various exposure times and temperatures) under repeatability conditions greatly improves the sensitivity of the stability tests.

### 5.1 Short-term stability study

For the short-term stability study, bottles were stored at 18 °C and 60 °C for 0, 1, 2 and 4 weeks (at each temperature). The reference temperature was set to -20 °C. Two bottles per storage time were selected using a random stratified sampling scheme. From each bottle, 3 samples were measured by ICP-MS. The measurements were

performed under repeatability conditions, and in a randomised sequence to be able to separate a potential analytical drift from a trend over storage time. The results were corrected for the water content determined once in each bottle.

The obtained data were evaluated individually for each temperature. The results were screened for outliers using the single and double Grubbs test. Some outlying individual results were found (see

Table 4). As no technical reason for the outliers could be found all data were retained for statistical analysis.

Furthermore, the data were evaluated against storage time and regression lines of mass fraction versus time were calculated. The slope of the regression lines were tested for statistical significance (loss/increase due to shipping conditions). For all elements, the slopes of the regression lines were not significantly different from 0 (on 99 % confidence level) at both 18 °C and 60 °C.

Table 4: Results of the short-term stability tests

Element mass fraction	Number of individual outlying results		Significance of the trend on a 99% confidence level	
	18 °C	60 °C	18 °C	60 °C
Ag	none	none	no	no
As	none	none	no	no
Ba	none	none	no	no
Cd	none	none	no	no
Cr	1	1	no	no
Cu	none	none	no	no
Fe	none	none	no	no
Hg	1	none	no	no
Mn	none	none	no	no
Ni	none	none	no	no
Pb	none	none	no	no
Rb	none	none	no	no
Se	none	none	no	no
Sr	none	none	no	no
Zn	none	none	no	no

The results of the short-term stability measurements are shown in Annex B. Individual outlying results were found in the studies for Cr at both test temperatures and for Hg in the study at 18 °C. There was no technical reason to exclude the outliers, and all were retained for the calculation of  $u_{STS}$ . There were no statistically significant trends at the 99 % confidence level for any of the elements at either temperature.

The material can be dispatched without further precautions under ambient conditions.

## 5.2 Long-term stability study

For the long-term stability study, bottles were stored at 18 °C for 0, 6, 12 and 18 months. The reference temperature was -20 °C. Two bottles per storage time were selected using a random stratified sampling scheme. From each bottle, 5 aliquots were measured by ICP-MS. The measurements were performed under repeatability conditions, and in a randomised 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 once in each bottle.

The results were screened for outliers using the single and double Grubbs test. One measurement result was removed from the dataset prior to statistical analysis, as the laboratory reported that an error was made in handling the sample. Some outlying individual results were found (see Table 5). The first (replicate) digest prepared of the series gave outlying high values for 5 elements. A handling error was assumed and the results were excluded from the study. For the remaining dataset, single outlying values were found for Ba and Cr. As no technical reason for these outliers could be found, they were retained for statistical analysis.

Furthermore, the data were plotted against storage time and linear regression lines of mass fraction versus time were calculated. The slope of the regression lines was tested for statistical significance (loss/increase due to storage conditions). Significant slopes on 99 % confidence level were found for Ni and Ag, and for Cr at the 95 % confidence level.

Table 5: Results of the long-term stability tests

Element mass fraction	Number of individual outlying results	Significance of the trend at a 99% confidence level
Ag	none	yes
As	none	no
Ba	1	no
Cd	none	no
Cr	1	no
Cu	none	no
Fe	none	no
Hg	none	no
Mn	none	no
Ni	none	yes
Pb	none	no
Rb	none	no
Se	none	no
Sr	none	no

Zn	none	no
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The results of the long-term stability measurements are shown in Annex C. Negative trends were observed for Ni and Ag at 18 °C. As neither element is expected to form volatile chemical forms in the sample, or react with the packaging material, the observed trends should most likely be regarded as statistical artefacts. For Ag, Cr and Ni, the standard deviation in measurements for the long-term stability study was significantly higher than that observed for the other elements, and was found at a similar level to that of the homogeneity study (as described in section 4.). However, without additional evidence for their stability, their mass fractions are given with combined uncertainties with  $u_{LTS}$  including potential degradation of the material. As additional information becomes available as part of the post-certification monitoring programme for this material, it may prove possible to revise the assigned uncertainties. The material can therefore be stored at 18 °C.

### 5.3 Estimation of uncertainties

Due to the intrinsic variation of measurement results no study can rule out degradation of materials completely, even in the absence of statistically significant trends. It is therefore necessary to quantify the potential degradation that could be hidden by the method repeatability, i.e. to estimate the uncertainty of stability. This means, even under ideal conditions, the outcome of a stability study can only be "degradation is  $0 \pm x$  % per time".

Uncertainties of stability during dispatch and storage were estimated as described in [8] for each analyte. For this approach, the uncertainty of the linear regression line with a slope of zero is calculated. The uncertainty contribution ( $u_{LTS}$ ) is then calculated as the product of the chosen shelf life and the uncertainty of the regression lines as

$$u_{LTS,rel} = \frac{RSD}{\sqrt{\sum (x_i - \bar{x})^2}} \cdot t_{sl}$$

*RSD* relative standard deviation of all results of the stability study

$t_i$ : time point for each replicate

$\bar{x}$ : mean results for all time points

$t_{sl}$ : proposed shelf life (12 months at 18 °C in this case)

The following uncertainties were estimated:

- $u_{sts,rel}$ , the uncertainty of degradation during dispatch. This was estimated from the 60 °C studies for a time of 0.25 months (1 week). The uncertainty therefore describes the possible change during a dispatch at 60 °C lasting for one week.
- $u_{LTS,rel}$ , the stability during storage. This uncertainty contribution was estimated from the 18 °C study. The uncertainty contribution therefore describes the possible degradation for 24 months at 18 °C.

The results of these evaluations are summarised in Table 6.

Table 6: Uncertainties of stability during storage and dispatch.  $u_{\text{sts,rel}}$  was calculated for a temperature of 60 °C and 1 week;  $u_{\text{lts,rel}}$  was calculated for a storage temperature of 18 °C and 2 years.

Element mass fraction	$u_{\text{sts,rel}}$ [%]	$u_{\text{lts,rel}}$ [%]
Ag	1.86	12.5
As	0.31	1.55
Ba	1.74	2.90
Cd	0.46	1.58
Cr	2.85	13.6
Cu	0.40	1.75
Fe	0.27	1.70
Hg	0.30	3.5
Mn	0.52	2.05
Ni	3.1	9.8
Pb	0.35	3.3
Rb	0.20	1.27
Se	0.59	2.17
Sr	0.18	0.88
Zn	0.49	1.53

The uncertainty of stability during dispatch is negligible compared to the overall uncertainty of the certified value even at 60 °C. Therefore the material can be transported at ambient conditions without special precautions.

After the certification campaign, the material will be subjected to IRMM's regular stability monitoring programme to control its further stability.

## 6 Characterisation

The material characterisation was based on an intercomparison of expert laboratories, i.e. the mass fractions of elements of the material were determined in different laboratories that applied different measurement procedures to demonstrate the absence of a measurement bias. This approach aims at randomisation of laboratory bias, which reduces the combined uncertainty.

## **6.1 Selection of participants**

Twelve laboratories were selected based on criteria that comprised both technical competence and quality management aspects. Each participant was required to operate a quality system and to deliver documented evidence of its laboratory proficiency in the field of element measurements in relevant matrices by submitting results for intercomparison exercises or method validation reports. Having a formal accreditation was not mandatory, but meeting the requirements of ISO/IEC 17025 was obligatory. Where measurements are covered by the scope of accreditation, the accreditation number is stated in the list of participants (Section 0).

## **6.2 Study setup**

Each laboratory received 2 bottles of ERM-CE278k and was requested to provide 6 independent results, 3 per bottle. The units for material characterisation were selected using a random stratified sampling scheme and covered the whole batch. The sample preparations and measurements had to be spread over at least two days to ensure intermediate precision conditions. An independent calibration was performed for each result. The water content had to be determined once in each unit and results are reported based on the dry mass.

Each participant received a sample of NIST SRM-2796 (Mussel tissue) as a blinded quality control (QC) sample. The results for this sample have been used to support the evaluation of the characterisation results.

Laboratories were also requested to give estimations of the expanded uncertainties of the mean value of the six results. No approach for the estimation was prescribed, i.e. top-down and bottom-up were regarded as equally valid procedures.

## **6.3 Methods used**

A variety of digestion methods with different quantification steps as well as methods without sample preparation were used to characterise the material. The combination of results from methods based on completely different principles mitigates undetected method bias.

All methods used during the characterisation study are summarised in Annex D. The laboratory code is a random number and does not correspond to the order of laboratories in Section 0. The lab-method code consists of a number assigned to each laboratory and abbreviation of the measurement method used.

## **6.4 Dry mass determination**

For all measurements carried out during certification (homogeneity, stability and characterisation studies) the following protocol for dry mass determination was applied:

An aliquot of approximately at least 0.2 g was accurately weighed on an analytical balance and dried in an oven at atmospheric pressure, at  $103\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ , until constant mass was attained.

The water content determined by the laboratories was in the range of 6 g/kg to 39 g/kg.

## **6.5 Evaluation of results**

The characterisation campaign resulted in between 5 and 13 datasets per element. All individual results of the participants, grouped by element are displayed in tabular and graphical form in Annex E.

### **6.5.1 Technical evaluation**

The obtained data were first checked for compliance with the requested analysis protocol and for their validity based on technical reasons. The following criteria were considered during the evaluation:

- compliance with the analysis protocol: sample preparations and measurements performed on two days, and the analytical sequence and water content determination.
- validity of the measurement procedure
- absence of values given as below limit of detection or below limit of quantification
- method performance demonstrated by agreement of the measurement results with the assigned value of the QC sample (only for the elements As, Cd, Cu, Fe, Hg, Pb, Se and Zn, for which mass fractions were certified in the QC sample)

Based on the above criteria, certain datasets were rejected as not technically valid (see Table 7).

Table 7: Datasets that showed non-compliances with the analysis protocol and technical specifications, and action taken

Element mass fraction	Lab-method code	Description of problem	Action taken
Ag	0, 13	some measurements reported as <LOQ	not used for evaluation
As	15, 16	QC measurements did not match the assigned value	not used for evaluation
Ba	6	outlying variance using the same technique as other labs; indicates relatively poor proficiency	not used for evaluation
Cr	2	outlying variance using the same technique as other labs; indicates relatively poor proficiency	not used for evaluation
Cu	2	measurement uncertainty reported as > 3 times that of other labs	not used for evaluation
Fe	14, 15, 16, 17	QC measurements did not match the assigned value	not used for evaluation
Hg	4, 6, 14, 16	QC measurements did not match the assigned value	not used for evaluation
Mn	4	outlying variance using the same technique as other labs; indicates relatively poor proficiency	not used for evaluation
Ni	2	outlying variance using the same technique as other labs; indicates relatively poor proficiency	not used for evaluation
Pb	2	outlying variance using the same technique as other labs; indicates relatively poor proficiency	not used for evaluation
Se	4, 14, 15, 16	QC measurements did not match the assigned value	not used for evaluation
Se	2	outlying variance using the same technique as other labs; indicates relatively poor proficiency	not used for evaluation
Zn	14, 15, 16	QC measurements did not match the assigned value	not used for evaluation
Zn	4	outlying variance using the same technique as other labs; indicates relatively poor proficiency	not used for evaluation

Datasets for Ba, Cr, Mn, Ni, Pb, Se and Zn contained at least one result with outlying variance. In each case, the laboratory/laboratories applied ICP-MS. As many other laboratories in the respective datasets also used ICP-MS, this demonstrates that the

laboratory/laboratories' proficiency in applying the method was significantly worse than that of the other laboratories. Datasets of laboratories using ICP-MS with outlying variance were therefore removed (as listed in Table 5).

### 6.5.2 Statistical evaluation

The datasets accepted based on technical reasons were tested for normality of dataset means using kurtosis/skewness tests and normal probability plots and were tested for outlying means using the Grubbs test and using the Cochran test for outlying standard deviations, (both at a 99 % confidence level). Standard deviations within ( $s_{\text{within}}$ ) and between ( $s_{\text{between}}$ ) laboratories were calculated using one-way ANOVA. The results of these evaluations are shown in Table 8.

*Table 8: Statistical evaluation of the technically accepted datasets for ERM-CE278k. p: number of technically valid datasets*

Element mass fraction	p	Outliers		Normally distributed	Statistical parameters			
		Means	Variances		Average [mg/kg]	s [mg/kg]	$s_{\text{between}}$ [mg/kg]	$s_{\text{within}}$ [mg/kg]
Ag	6	1	0	no	0.0442	0.0052	0.0085	0.0042
As	13	0	0	yes	6.72	0.45	0.75	0.27
Ba	5	0	0	yes	0.563	0.143	0.248	0.009
Cd	14	0	0	yes	0.336	0.023	0.039	0.012
Cr	10	0	0	yes	0.732	0.095	0.160	0.058
Cu	12	0	0	yes	5.98	0.23	0.36	0.23
Fe	10	0	0	yes	160.8	6.7	11.1	4.6
Hg	6	0	1	yes	0.0712	0.0036	0.0058	0.0033
Mn	13	0	0	yes	4.88	0.20	0.32	0.20
Ni	10	1	0	no	0.689	0.051	0.078	0.057
Pb	13	0	0	yes	2.184	0.167	0.285	0.072
Rb	9	0	0	yes	2.461	0.208	0.356	0.079
Se	11	0	0	yes	1.616	0.102	0.172	0.060
Sr	10	0	0	yes	18.98	1.77	3.06	0.38
Zn	12	0	0	yes	71.4	4.0	6.9	1.5

The laboratory averages follow normal distributions for all elements except Ag and Ni. None of the data contains statistically outlying means, except for Ag and Ni in which single statistically significant outliers are present; laboratory 6 outlier for Ag and 2 for Ni. However, the technical evaluation of results did not indicate any technical flaws with either of the results, and they were therefore retained for calculation of the values. As both results agreed with the respective dataset means within their  $u_{\text{CRM}}$ , the values and their uncertainties are considered to provide a

reliable estimate of the mass fractions measured by any of the techniques used for the characterisation.

The statistical evaluation flags laboratory 2 as outlying variance for Hg. However, as only one other laboratory applied the same method, the variance may merely reflect the fact that different methods have different intrinsic variability. As all measurement methods were found technically sound, all results were retained.

In addition to the statistical tests, the agreement of individual laboratories' results with the dataset means was tested according to ERM Application Note 1[9]. For 6 elements, one or more results did not agree with the dataset within the combined  $U_{CRM}$  and reported measurement uncertainty,  $U_m$ . As approaches to uncertainty estimation differ between laboratories, it is possible that not all sources of uncertainty are included in the budgets. For these labs,  $U_m$  were assigned based on the  $U_m$  reported by the other laboratories. For some of the disagreeing results, only one other result provided using the same technique was available, and it was therefore not possible to make a judgement on the expected  $U_m$  per technique. Therefore the 3<sup>rd</sup> quartile of all the reported  $U_m$  in the dataset was assigned to the disagreeing measurements, as this represented an acceptable level of  $U_m$  for inclusion in the dataset. The disagreeing results, together with their reported  $U_m$  and the 3<sup>rd</sup> quartile  $U_m$  of the complete dataset are given in table 9.

Table 9: Evaluation of results disagreeing with dataset means for ERM-CE278k.

Element mass fraction	Lab codes of disagreeing results		$U_{m,rel}$ [%]	
	with reported $U_m$	with 3 <sup>rd</sup> quartile $U_m$	Reported $U_m$	Dataset 3 <sup>rd</sup> quartile $U_m$
As	5	none	5	10
Ba	1	1	30	15
Cd	11, 12	none	8, 4	12
Pb	11, 12	none	8, 8	12
Rb	14	none	5	12
Sr	15, 16	none	3, 1	17

In all cases except that for the result of laboratory 1 for Ba,  $U_m$  reported for the results were significantly lower than the 3<sup>rd</sup> quartile of all reported  $U_m$ . On testing with the  $U_m$  assigned as the 3<sup>rd</sup> quartile of all results for the dataset, all results except that for Ba agreed with the dataset mean. For Ba, the result of laboratory 1 did not agree with the dataset mean.

The datasets for all elements except Ba are therefore consistent and the mean of laboratory means is a good estimate of the true value. For all elements, standard deviations between laboratories are considerably larger than the standard deviation within laboratories, showing that confidence intervals of replicate measurements are unsuitable as estimates of measurement uncertainty.

The methods used in the characterisation are methods routinely applied for measuring elements in shellfish tissue. The agreement of results from different methods demonstrates that the processing did not affect any properties relevant for these methods and that ERM-CE278k behaves like a real sample.

The uncertainty related to the characterisation is estimated as the standard error of the mean of laboratory means.

## 7 Value Assignment

Certified, indicative and informative values were assigned.

Certified values are values that fulfil the highest standards of accuracy. Procedures at IRMM require generally pooling of not less than 6 datasets to assign certified values. Full uncertainty budgets in accordance with the 'Guide to the Expression of Uncertainty in Measurement' [3] were established.

Indicative values are values where either the uncertainty is deemed too large or where too few independent datasets were available to allow certification. Uncertainties are evaluated according to the same rules as for certified values.

Additional material information refers to values that were obtained in the course of the study. For example, results reported from only one or two laboratories or in cases where individual measurement uncertainty is high, would fall under this category.

### 7.1 Certified values and their uncertainties

The unweighted mean of the means of the accepted datasets as shown in Table was assigned as certified value for each parameter.

The assigned uncertainty consists of uncertainties related to characterisation,  $u_{\text{char}}$  (see Section 0), potential between-unit heterogeneity,  $u_{\text{bb}}$  (see Section 4) and long-term storage,  $u_{\text{lts}}$  (see Section 0). These different contributions were combined to estimate the expanded, relative uncertainty of the certified value ( $U_{\text{CRM}}$ ) with a coverage factor  $k$  as

$$U_{\text{CRM}} = k \sqrt{u_{\text{char}}^2 + u_{\text{bb}}^2 + u_{\text{lts}}^2}.$$

- $u_{\text{char}}$  was estimated as described in Section 6
- $u_{\text{bb}}$  was estimated as described in Section 4.
- $u_{\text{lts}}$  was estimated as described in Section 0.

$u_{\text{sts}}$  was estimated as described in section 0. As can be seen in Table 6, the uncertainty of degradation during dispatch is negligible compared to the other uncertainty contributions.

Because of the sufficient numbers of the degrees of freedom of the different uncertainty contributions, a coverage factor  $k$  of 2 was applied for all elements to obtain the expanded uncertainties.

The certified values and their uncertainties are summarised in Table 10.

Table 10: Certified values and their uncertainties for ERM-CE278k

Element	Certified value <sup>a</sup> [mg/kg]	$u_{\text{char, rel}}$ [%]	$u_{\text{bb, rel}}$ [%]	$u_{\text{fts, rel}}$ [%]	$U_{\text{CRM, rel}}$ [%]	$U_{\text{CRM}}$ [mg/kg]
As	6.7	1.85	0.83	1.55	5.1	0.4
Cd	0.336	1.84	2.78	1.58	7.4	0.025
Cr	0.73	4.1	4.7	13.6	29.9	0.22
Cu	5.98	1.10	0.91	1.75	4.5	0.27
Fe	161	1.31	0.94	1.70	4.7	8
Hg	0.071	2.06	1.88	3.5	8.9	0.007
Mn	4.88	1.13	0.75	2.05	4.9	0.24
Ni	0.69	2.33	3.9	9.8	21.6	0.15
Pb	2.18	2.12	0.80	3.3	8.0	0.18
Rb	2.46	2.82	0.83	1.27	6.4	0.16
Se	1.62	1.91	1.84	2.17	6.9	0.12
Sr	19.0	2.95	0.61	0.88	6.3	1.2
Zn	71.4	1.62	0.70	1.53	4.7	4

<sup>a</sup>: reported on dry mass basis (see Section 6.4)

For Ni, the stability test under the specified storage conditions was inconclusive (as discussed in section 5.2). This led to a comparatively high uncertainty on the certified value. As additional information from long-term stability monitoring becomes available, it may become possible to revise the uncertainty on the certified value at a later date.

## 7.2 Indicative values and their uncertainties

An indicative value was assigned for Ag. Levels of uncertainty estimated for the between-bottle homogeneity and the long-term stability were high in comparison to the other elements certified, and led to an uncertainty on the mass fraction of 36 %. The uncertainty estimate was too high for the intended use of the material (checking method performance). However, the values found and the uncertainty estimates were reliable. Therefore, the value for Ag is given as indicative rather than certified. The uncertainty budgets were set up as for the certified values and are listed together with the assigned values in Table 11.

Because of the sufficient numbers of the degrees of freedom of the different uncertainty contributions, a coverage factor  $k$  of 2 was applied to obtain the expanded uncertainty.

Table 11: Indicative values and their uncertainties for ERM-CE278k

Element	Indicative value <sup>a</sup> [mg/kg]	$U_{\text{char, rel}}$ [%]	$U_{\text{bb, rel}}$ [%]	$U_{\text{lbs, rel}}$ [%]	$U_{\text{CRM, rel}}$ [%]	$U_{\text{CRM}}$ [mg/kg]
Ag	0.044	4.8	11.7	12.5	36	0.016

<sup>a</sup>: reported on dry mass basis (see Section 6.4)

### 7.3 Additional material information

Additional material information values are given for the elements listed in Table 12.

Table 12: Additional information values for ERM-CE278k

Element	Additional information value <sup>a</sup> [mg/kg]
Co	0.21

Element	Additional information value <sup>a</sup> [g/kg]
Ca	1.83
Cl	22.8
K	5.37
Mg	1.51
Na	13.9

<sup>a</sup>: reported on dry mass basis (see Section 6.4)

For Ca, Cl, K, Na and Mg, mass fractions were provided by a single technique, *k0*-NAA, and for Co, by ICP-MS. In each case, values are the mean of 6 replicate measurements on a set of 2 bottles provided for the characterisation study.

## 8 Metrological traceability and commutability

### 8.1 Metrological traceability

#### Identity

All certified properties are mass fractions of elements. The participants in the characterisation exercise used different methods for the sample preparation as well

as for the final determination, demonstrating absence of measurement bias. The measurands are therefore structurally defined and independent of the measurement method.

## **Quantity value**

Only validated methods were used for the determination of the assigned values. Different calibrants of known purity and with specified traceability of their assigned values were used and all relevant input parameters were calibrated. The individual results are therefore traceable to the SI, as it 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 themselves are thereby traceable to the SI.

## **9 Instructions for use**

### **9.1 Storage conditions**

The material shall be stored at  $18\text{ °C} \pm 5\text{ °C}$  in the dark. Care shall be taken to avoid change of the moisture content once the bottles are opened.

Please note that the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of opened bottles.

### **9.2 Safety and protection for the environment**

The usual laboratory safety measures apply.

### **9.3 Preparation and use of the material**

The units shall be shaken by turning upside down for at least 2 min before opening to ensure material re-homogenisation.

### **9.4 Minimum sample intake**

The minimum sample intake representative for all elements except Ag is 200 mg, and for Ag is 500 mg.

### **9.5 Dry mass correction**

Dry mass determination shall be carried out on a separate portion of at least 0.2 g, by drying in an oven at  $103\text{°C} \pm 2\text{°C}$  until constant mass is attained. Weighing of the samples for dry mass determination and weighing for the analysis shall be done at the same time to avoid differences due to possible take up of moisture by the material.

## Use of the certified value

The main purpose of the material is to assess method performance, i.e. for checking accuracy of analytical results. As any reference material, it can also be used for control charts or validation studies.

### Comparing an analytical result with the certified value

A result is unbiased if the combined standard uncertainty of measurement and certified value covers the difference between the certified value and the measurement result (see also ERM Application Note 1, [www.erm-crm.org](http://www.erm-crm.org) [9]).

For assessing the method performance, the measured values of the CRMs are compared with the certified values. The procedure is described here in brief:

- Calculate the absolute difference between mean measured value and the certified value ( $\Delta_m$ ).
- Combine measurement uncertainty ( $u_m$ ) with the uncertainty of the certified value ( $u_{CRM}$ ):  $u_{\Delta} = \sqrt{u_m^2 + u_{CRM}^2}$
- Calculate the expanded uncertainty ( $U_{\Delta}$ ) from the combined uncertainty ( $u_{\Delta}$ ) using an appropriate coverage factor, corresponding to a level of confidence of approximately 95 %
- If  $\Delta_m \leq U_{\Delta}$  no significant difference between the measurement result and the certified value, at a confidence level of about 95 % exists.

### Use as a calibrant

It is not recommended to use this matrix material as calibrant. If used nevertheless, the uncertainty of the certified value shall be taken into account in the estimation of the measurement uncertainty.

### Use in quality control charts

The materials can be used for quality control charts. Different CRM-units will give the same result as heterogeneity was included in the uncertainties of the certified values.

## **Acknowledgments**

The authors would like to acknowledge the support received from Katharina Teipel and Jean Charoud-Got from IRMM related to the processing of this CRM, from Maria Contreras concerning the set-up of the required isochronous studies, and Geert van Britsom for performing measurements for the characterisation.

Furthermore, the authors would like to thank Ringo Grombe and Eduardo Paredes (IRMM) for the reviewing of the certification report, as well as the experts of the Certification Advisory Panel "Elements", Steve Balsley (IAEA, AT), Thomas Prohaska (BOKU, AT) and Peter Vermaecke (SCK-CEN, BE) for their constructive comments.

## References

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- 2 ISO Guide 35, *Reference materials – General and statistical principles for certification*, International Organization for Standardization, Geneva, Switzerland, 2006
- 3 ISO/IEC Guide 98, *Guide to the Expression of Uncertainty in Measurement, (GUM 1995)*, International Organization for Standardization, Geneva, Switzerland, 2009
- 4 T.P.J. Linsinger, J. Pauwels, A.M.H. van der Veen, H. Schimmel, A. Lamberty (2001) *Homogeneity and stability of reference materials*, *Accred. Qual. Assur.* 6: 20-25
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## Annexes

Annex A: Results of the homogeneity measurements

Annex B: Results of the short-term stability measurements

Annex C: Results of the long-term stability measurements

Annex D: Summary of methods used in the characterisation

Annex E: Results of the characterisation measurements

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## Annex A: Results of the homogeneity measurements

Graphs present mass fractions of bottle means relative to the grand mean, against bottle number, and individual measurement replicates, against sequence number. Vertical bars are a confidence interval of 95 % derived from  $s_{wb}$  of the homogeneity study.

**Figure A1:** Ag

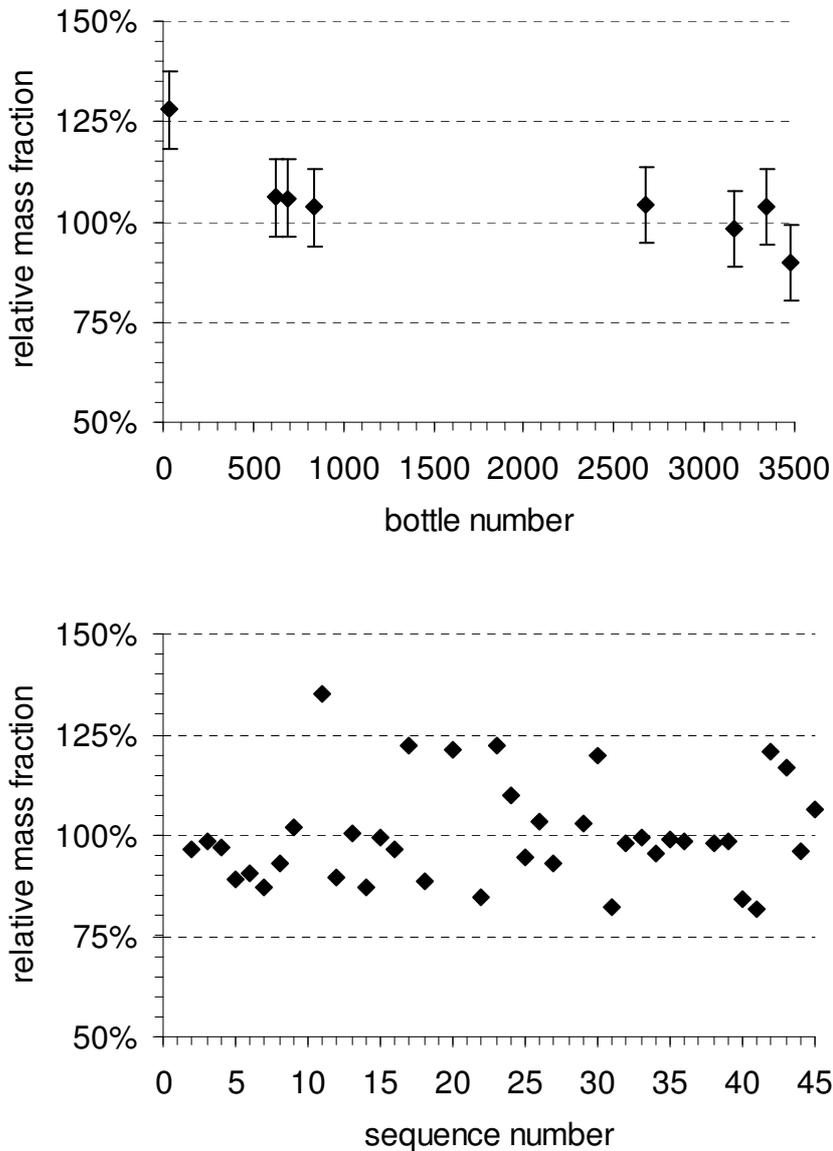


Figure A2: As

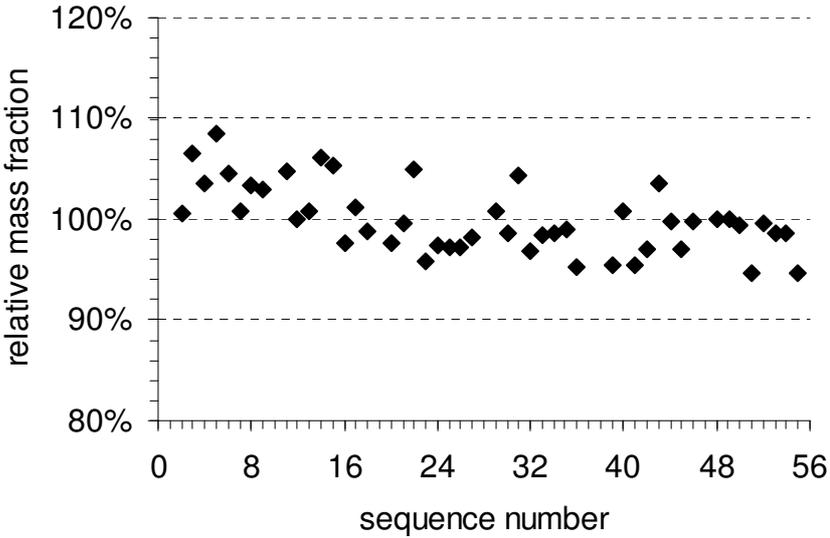
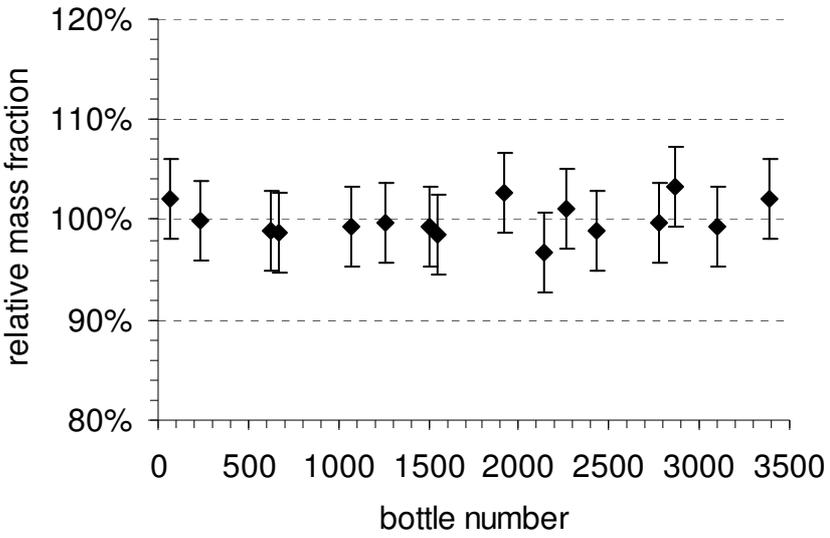


Figure A3: Ba

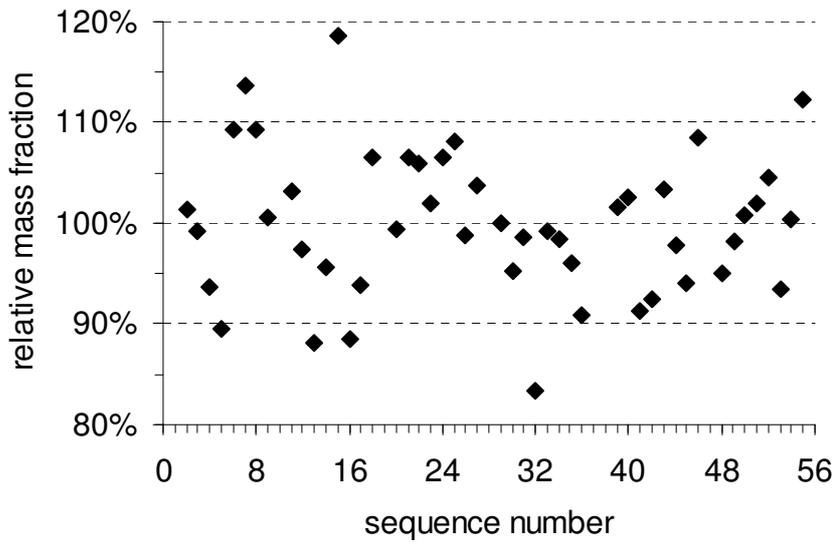
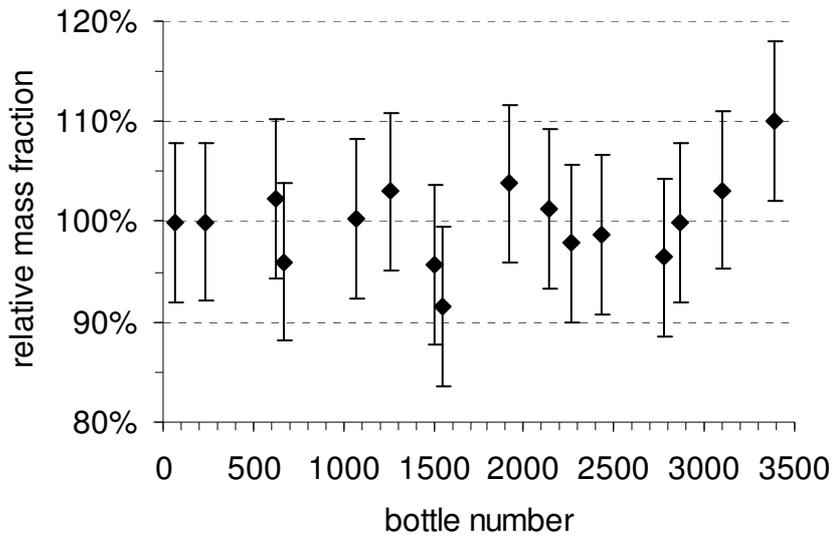


Figure A4: Cd

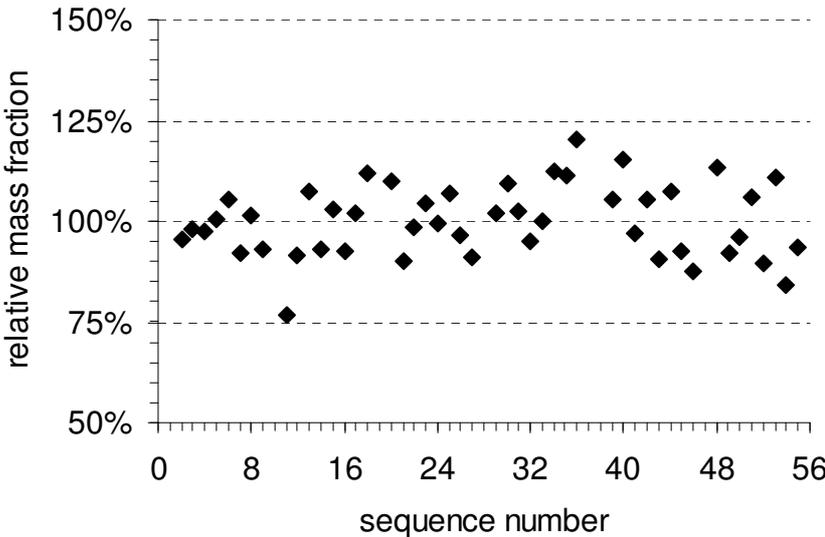
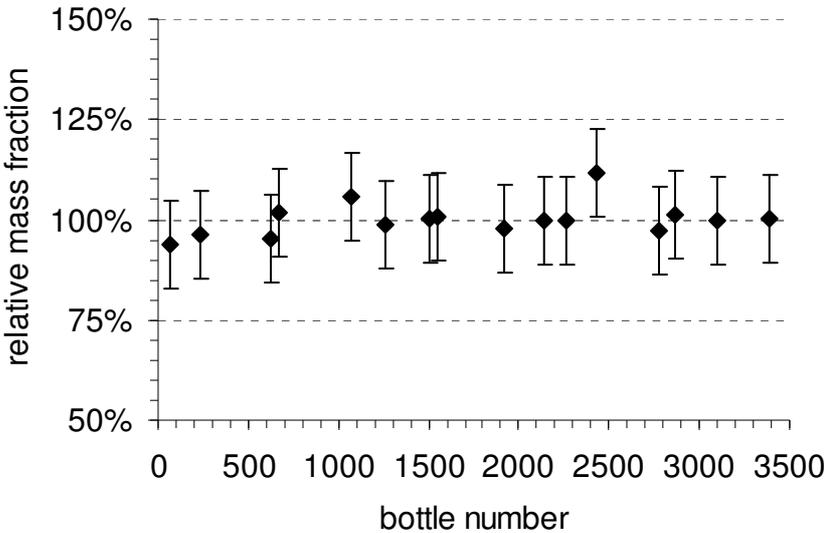


Figure A5: Cr

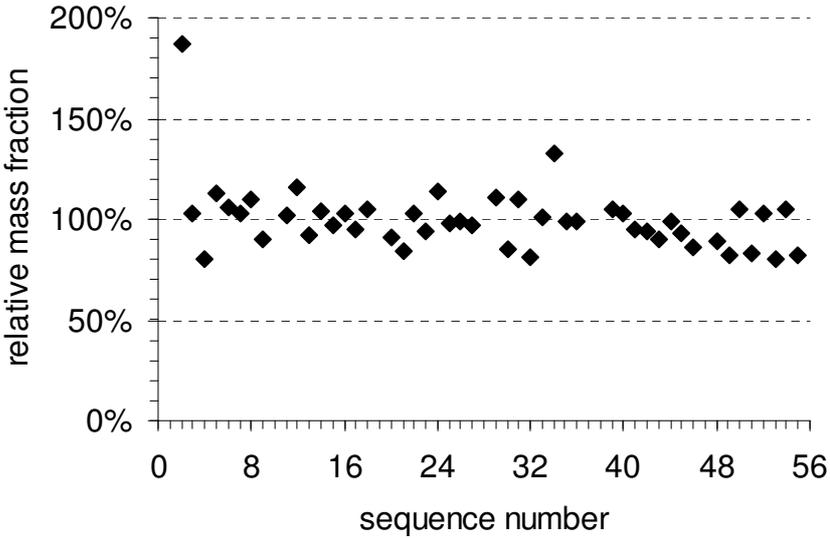
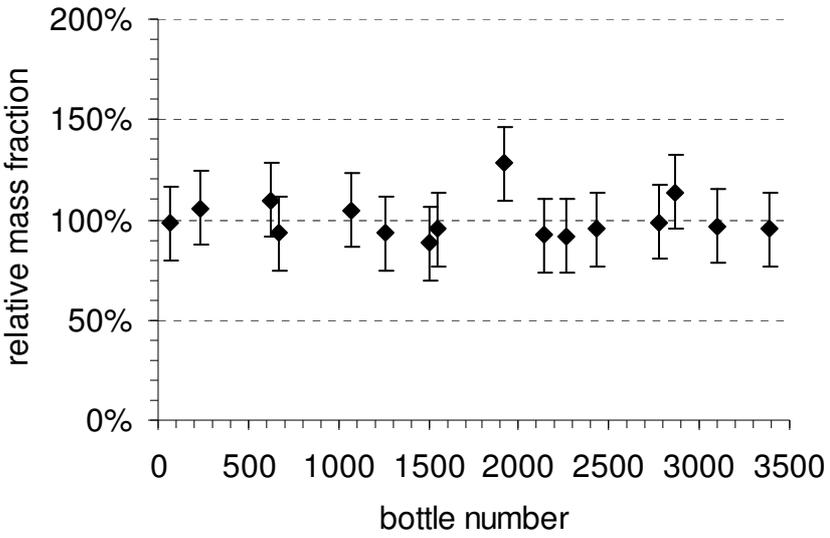


Figure A6: Cu

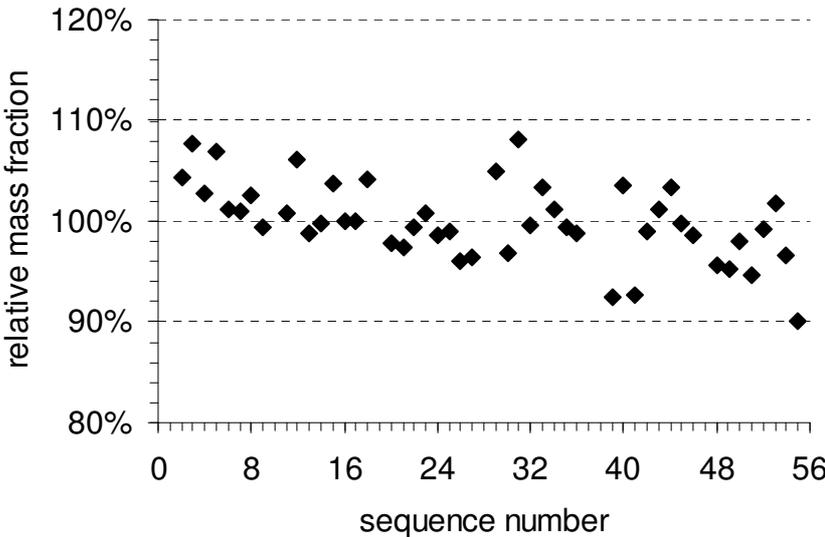
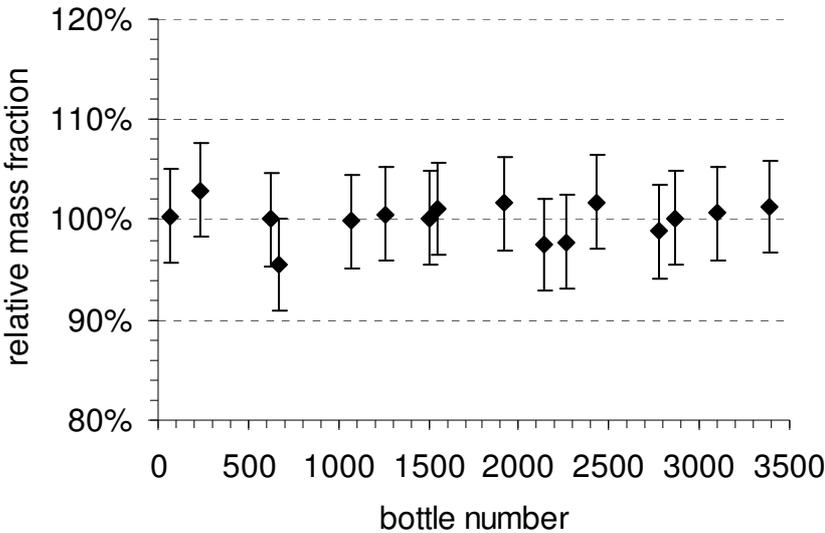


Figure A7: Fe

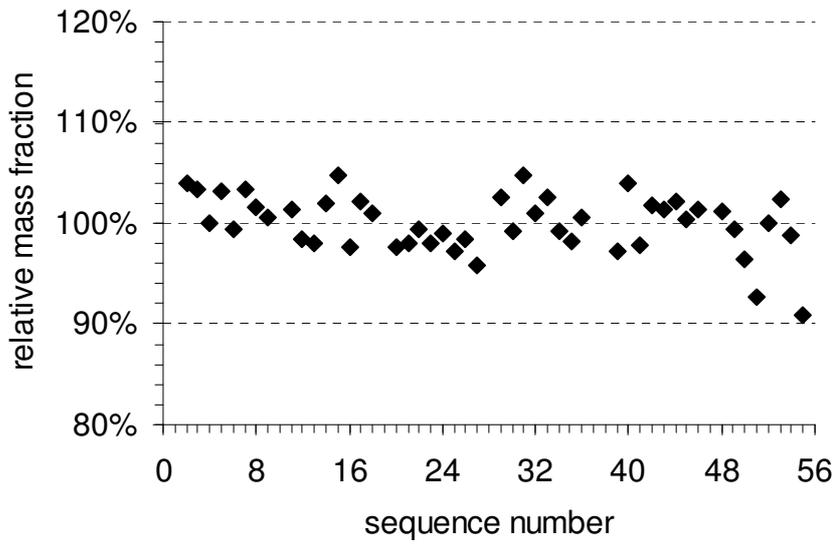
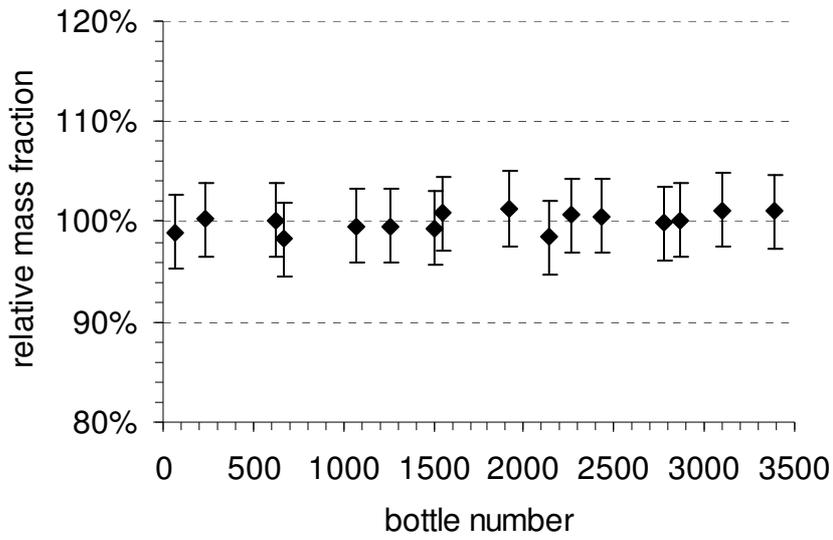


Figure A8: Hg

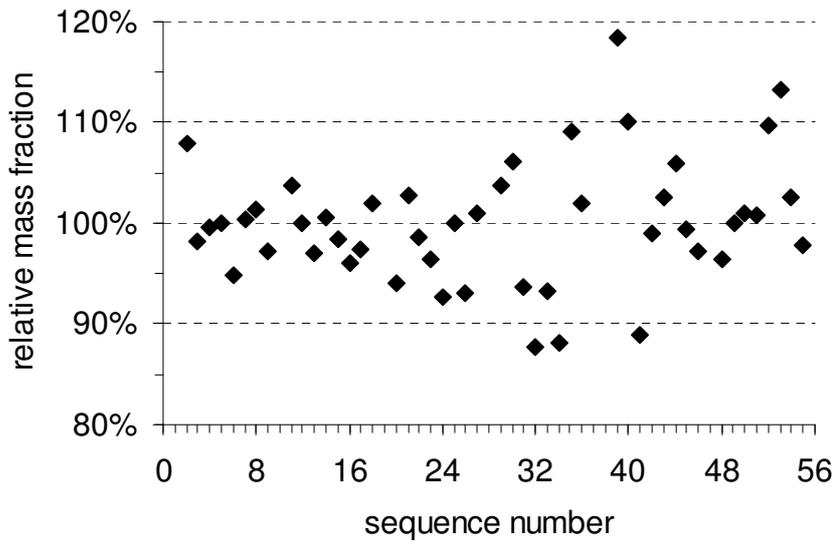
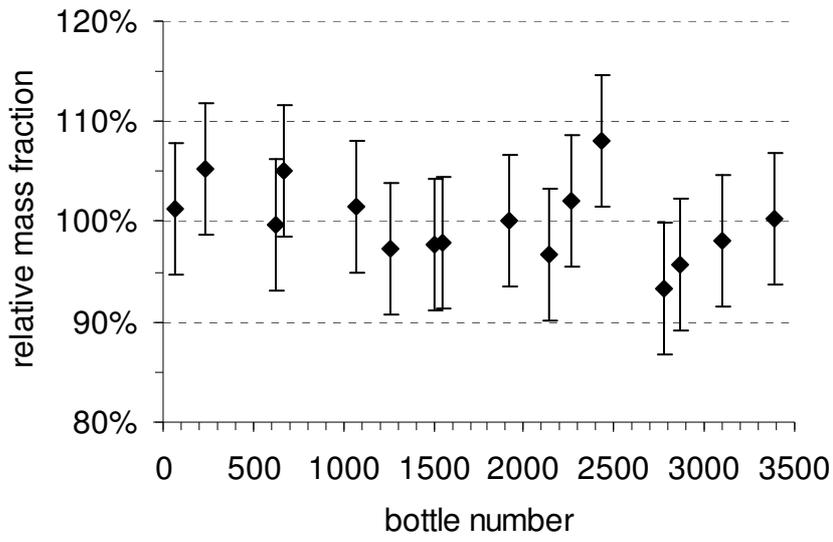


Figure A9: Mn

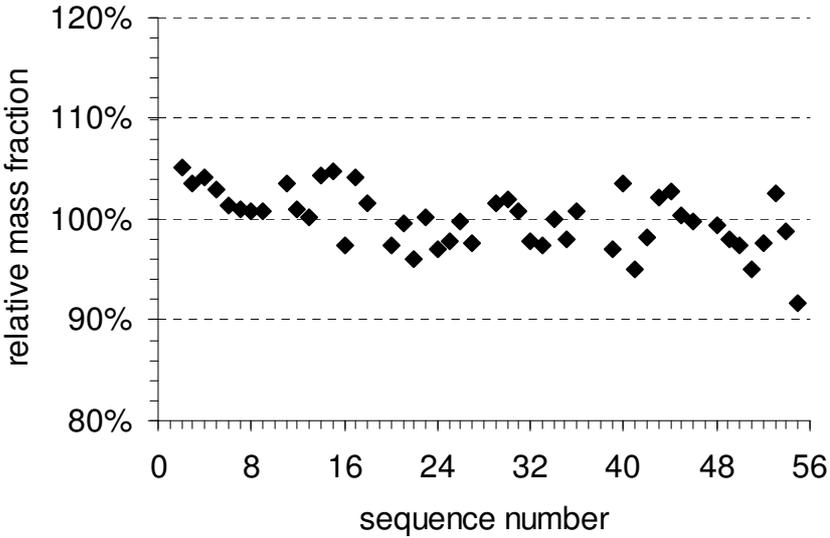
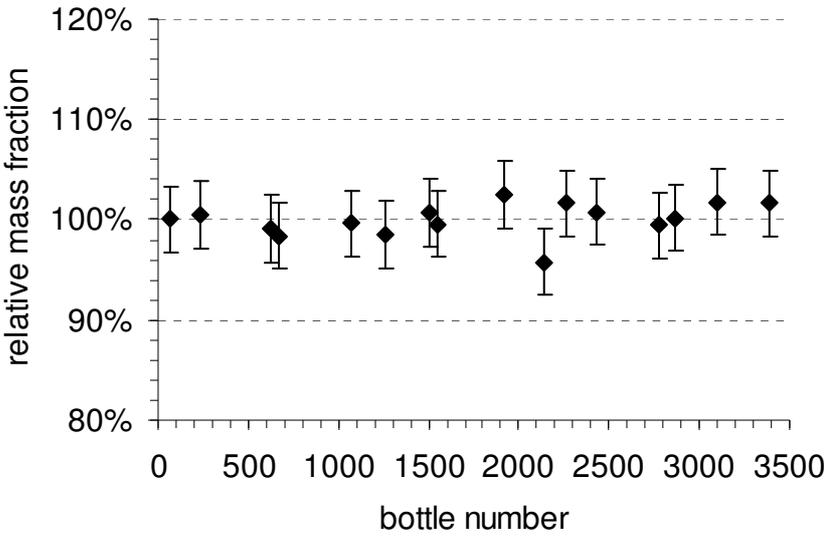


Figure A10: Ni

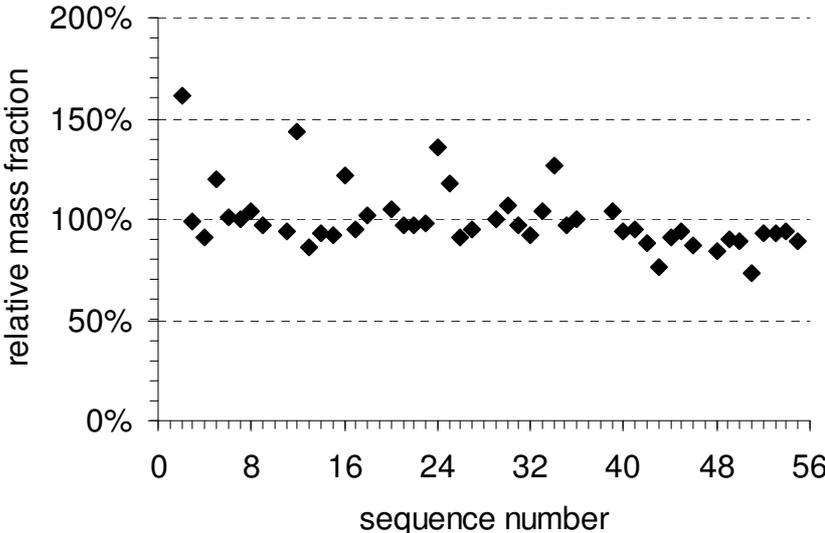
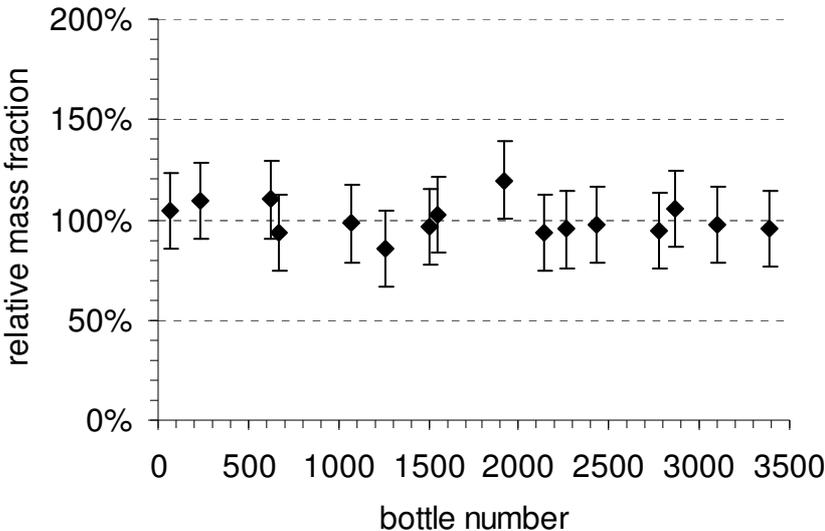


Figure A11: Pb

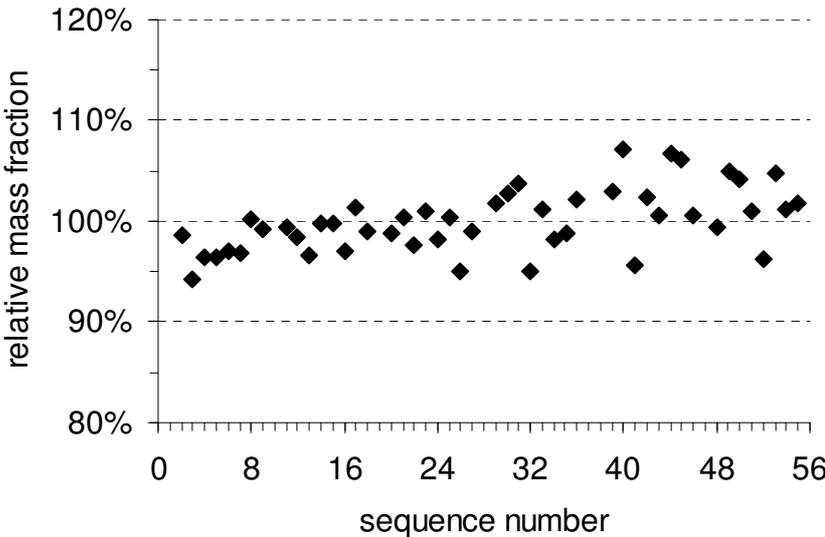
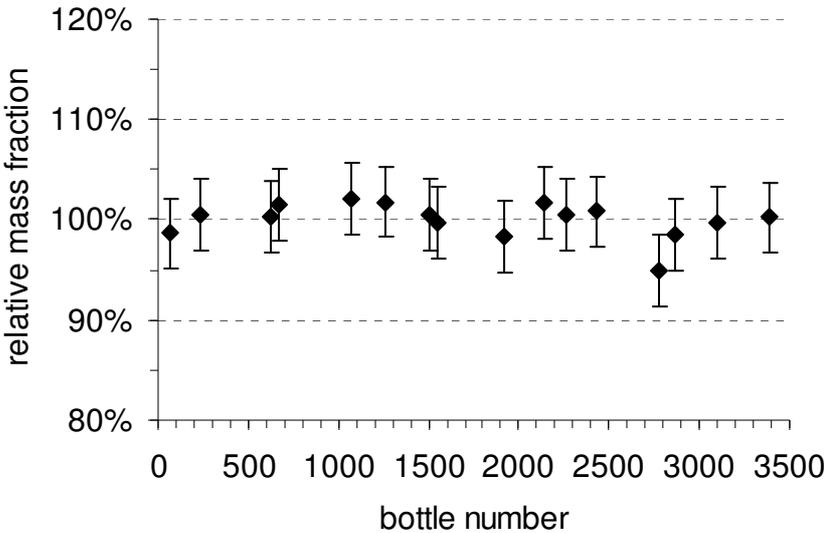


Figure A12: Rb

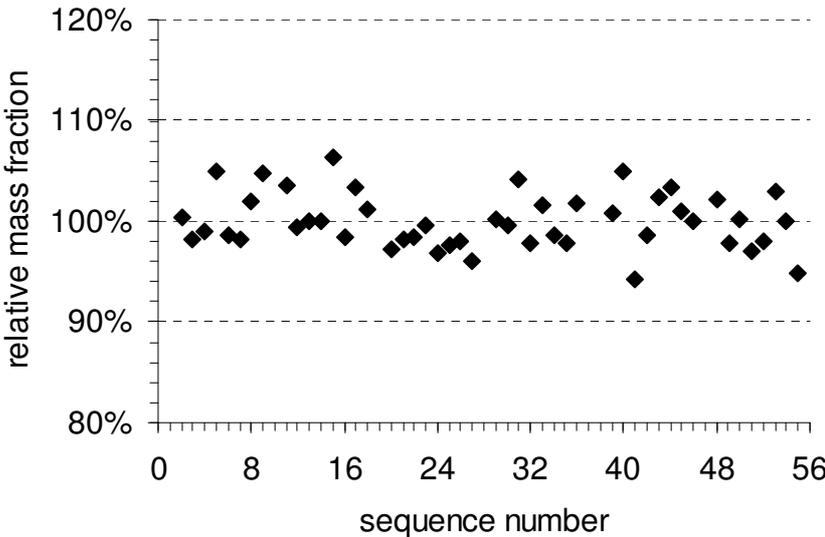
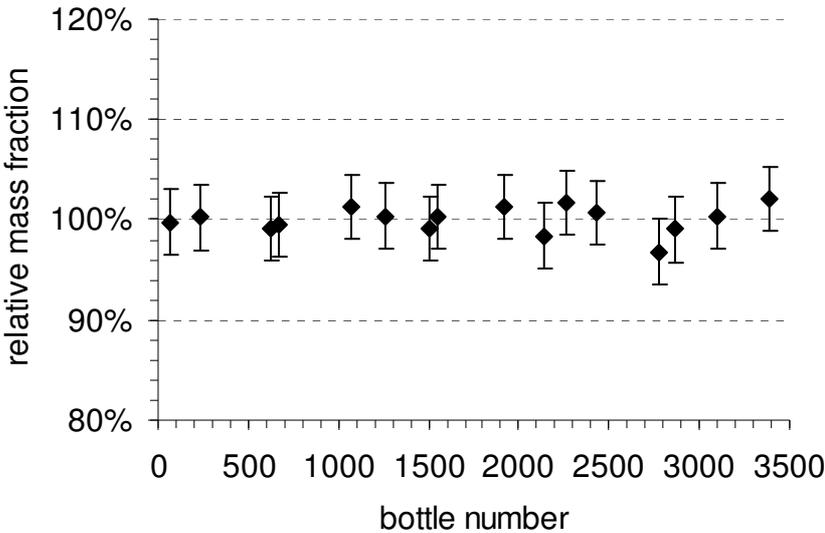


Figure A13: Se

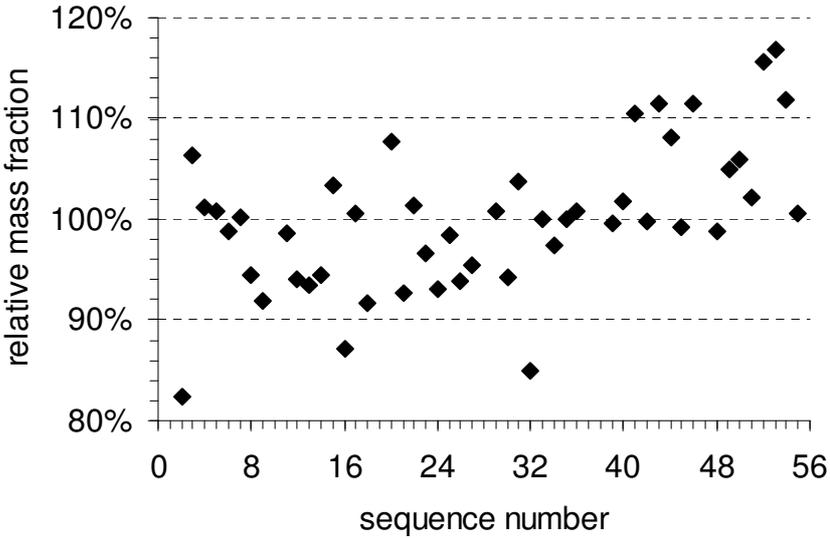
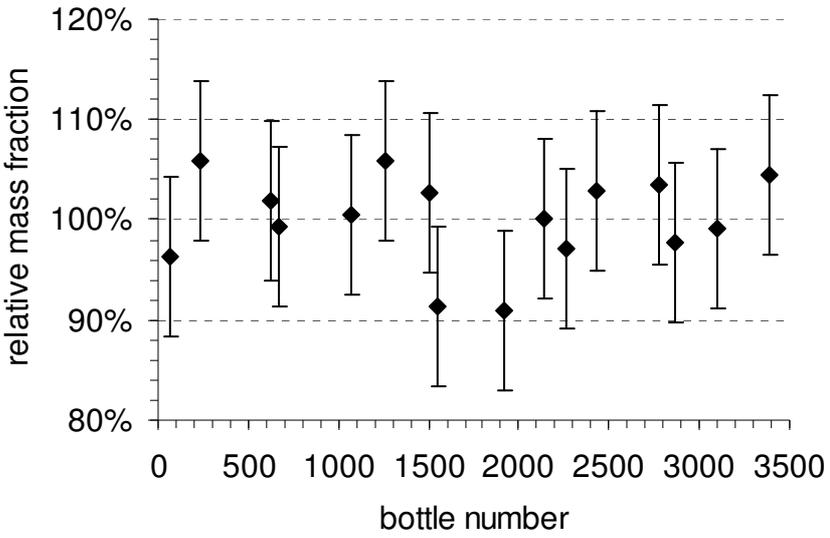


Figure A14: Sr

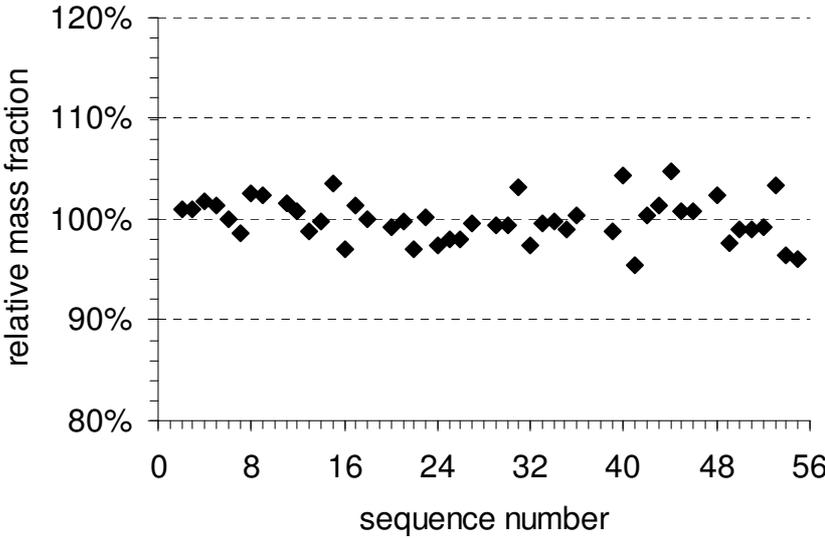
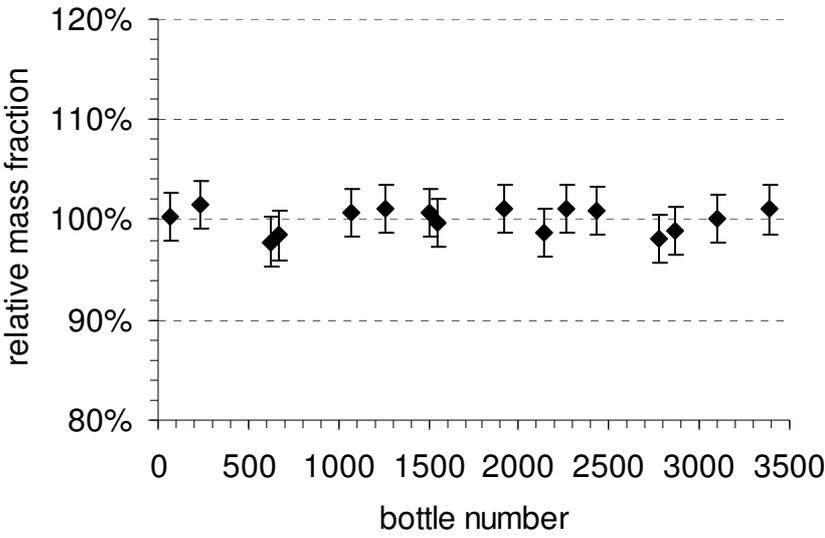
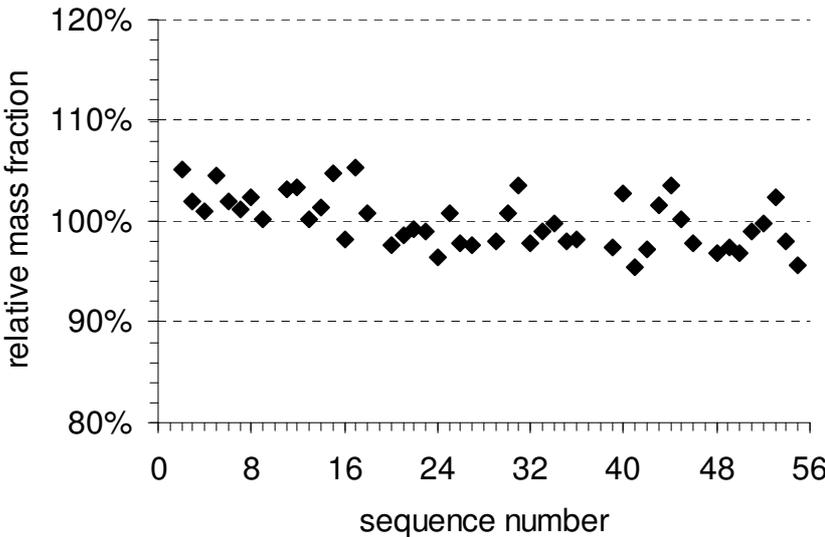
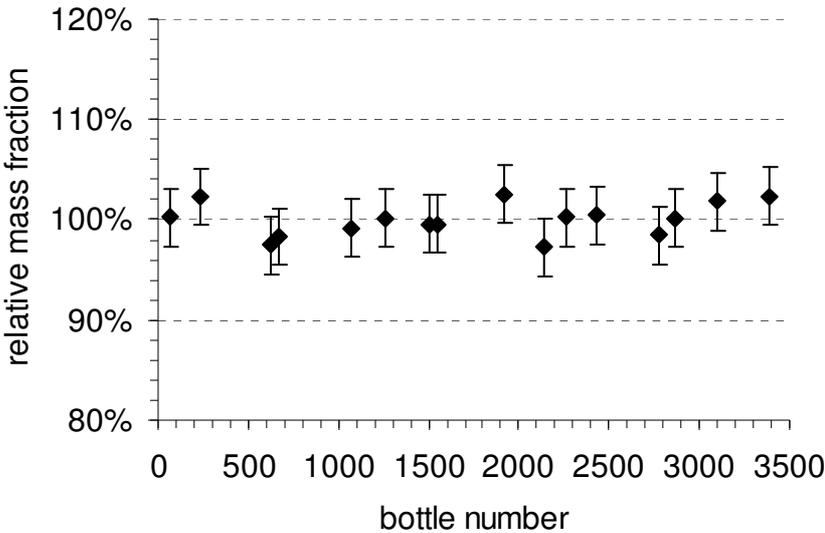


Figure A15: Zn



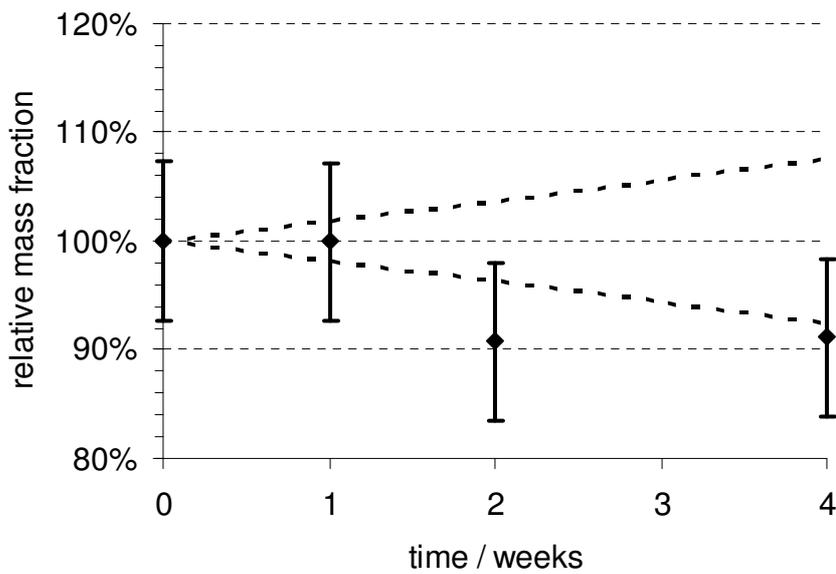
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## Annex B: Results of the short-term stability measurements

Graphs present the mean mass fractions measured at each time-point relative to the mean at time zero, against the time that the samples were held at 60 °C. Vertical bars represent the 95 % confidence interval of the measurements, based on the variance of measurements for each time-point calculated by ANOVA.

**Figure B1: Ag**



**Figure B2: As**

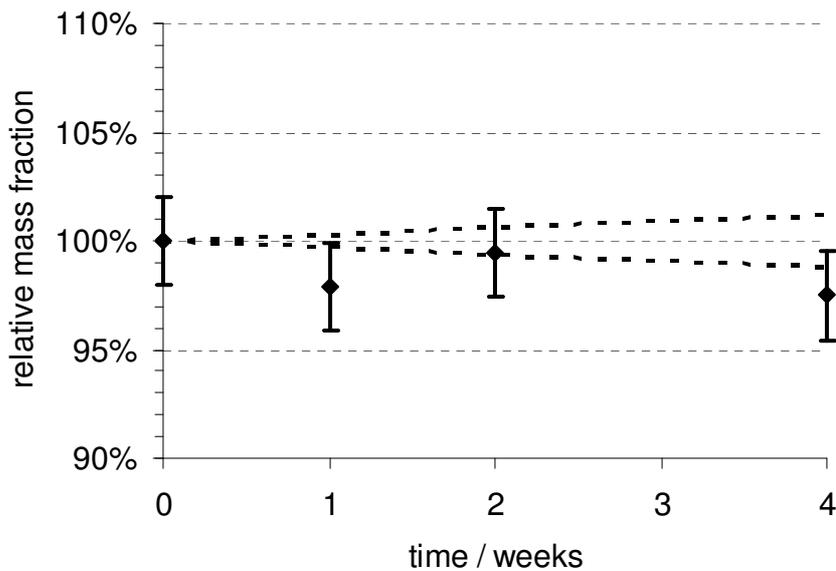


Figure B3: Ba

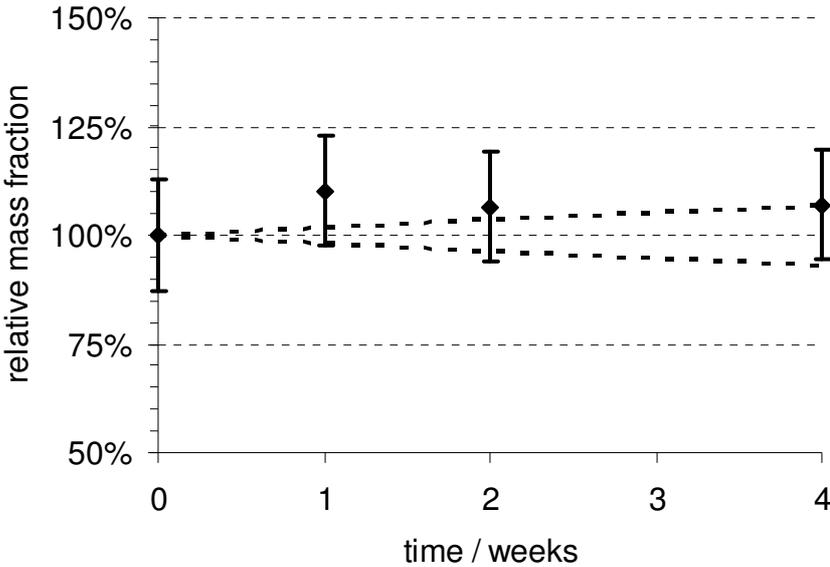


Figure B4: Cd

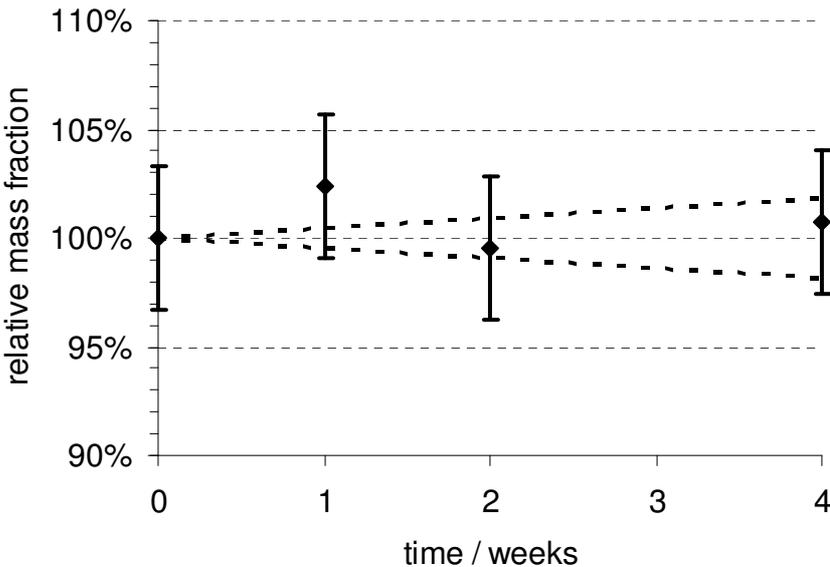


Figure B5: Cr

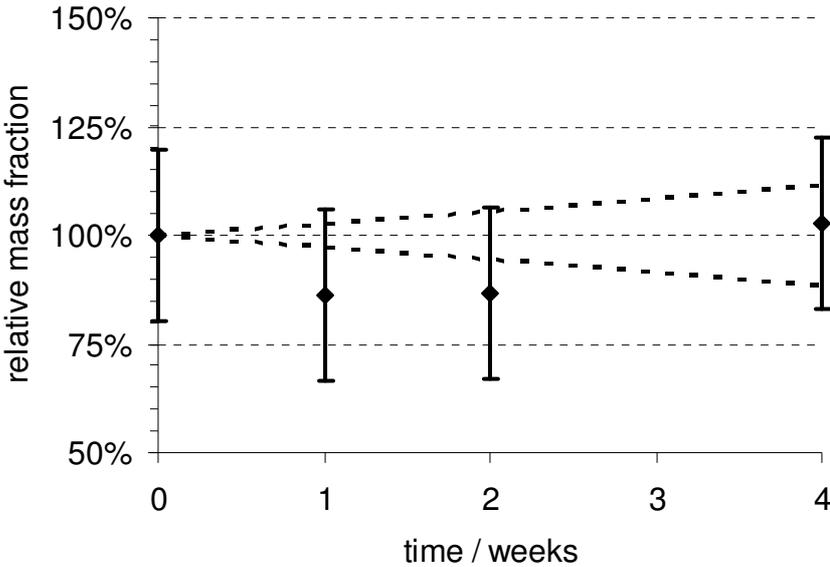


Figure B6: Cu

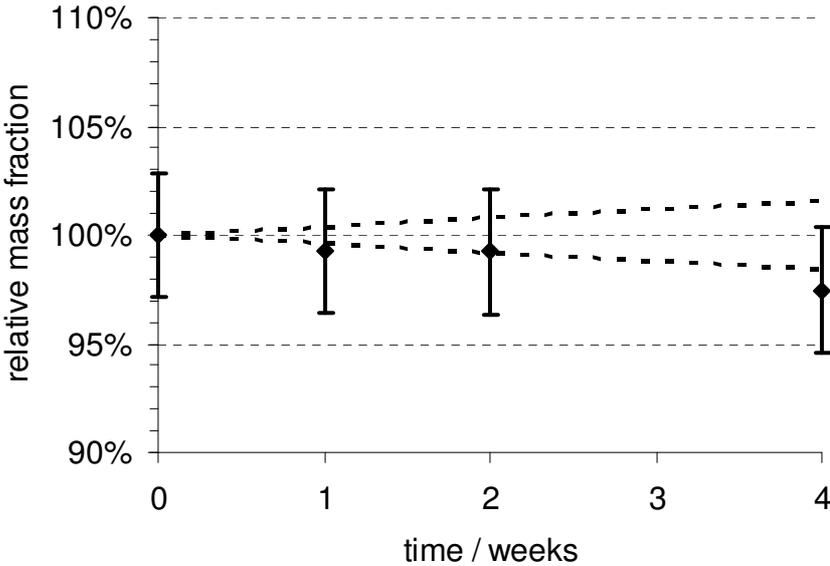


Figure B7: Fe

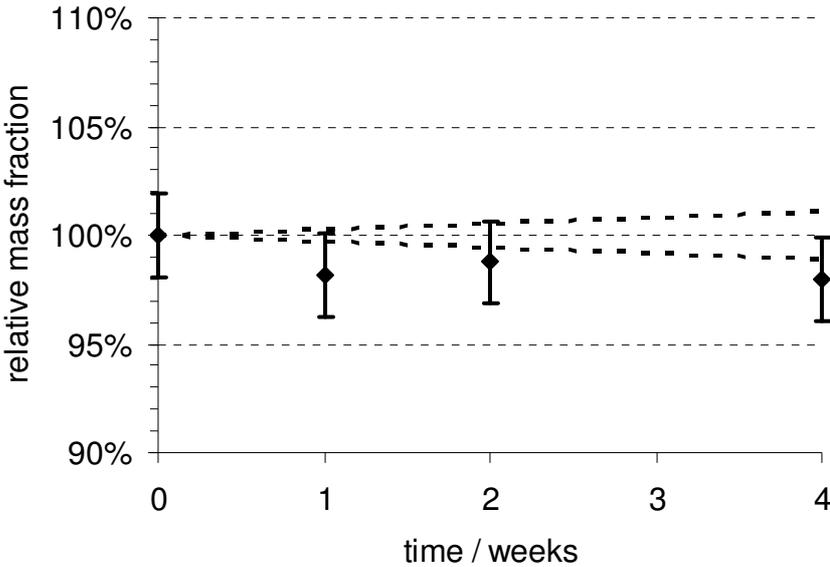


Figure B8: Hg

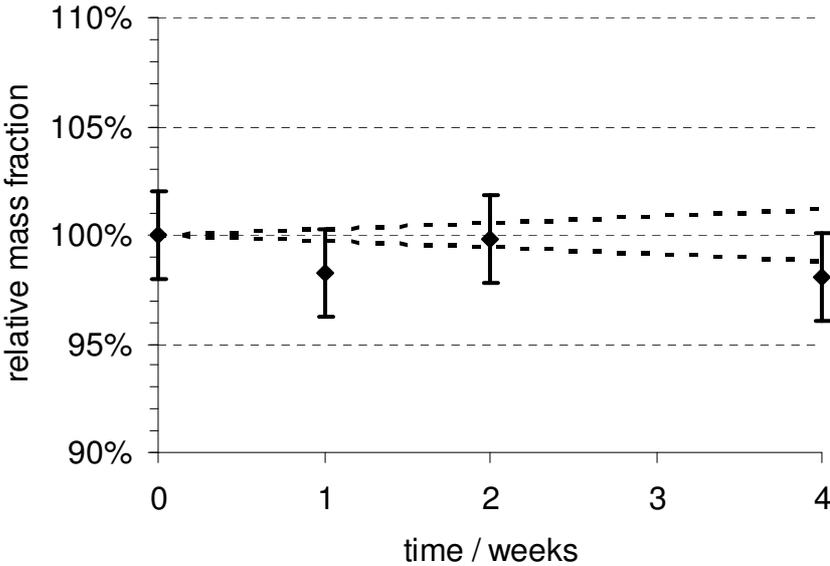


Figure B9: Mn

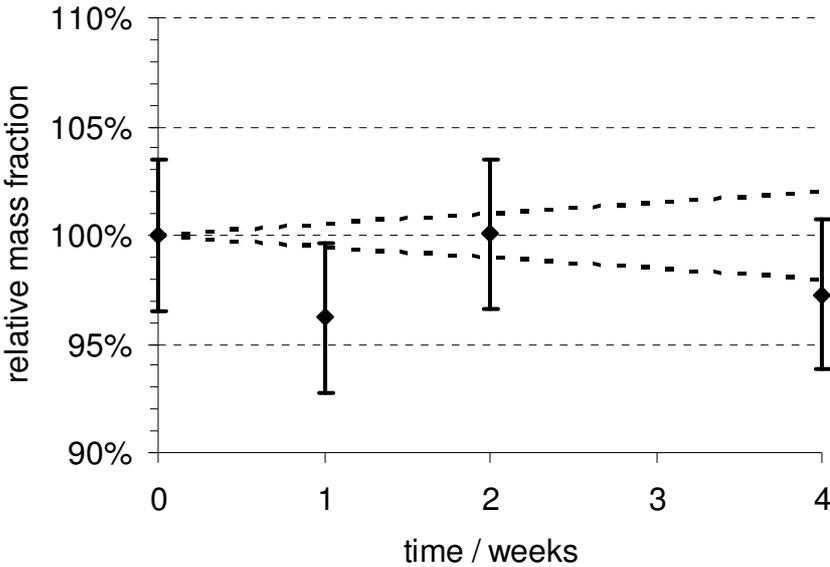


Figure B10: Ni

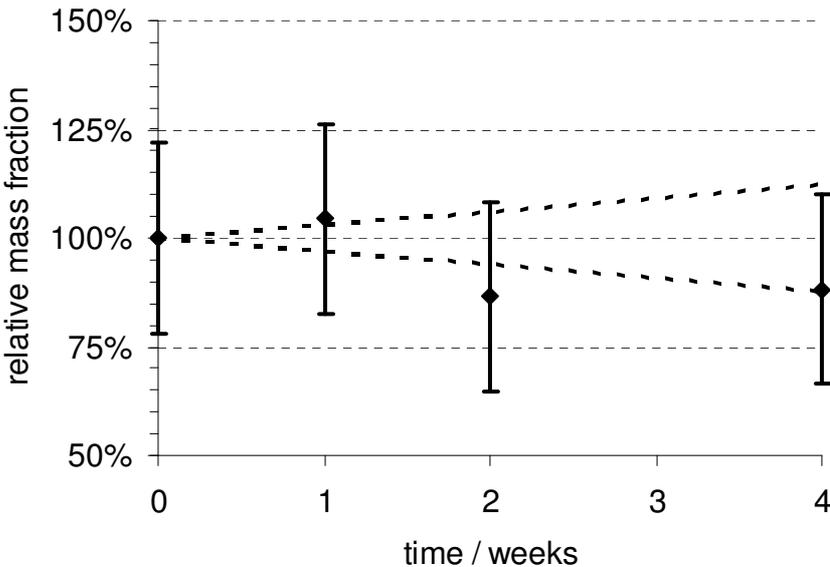


Figure B11: Pb

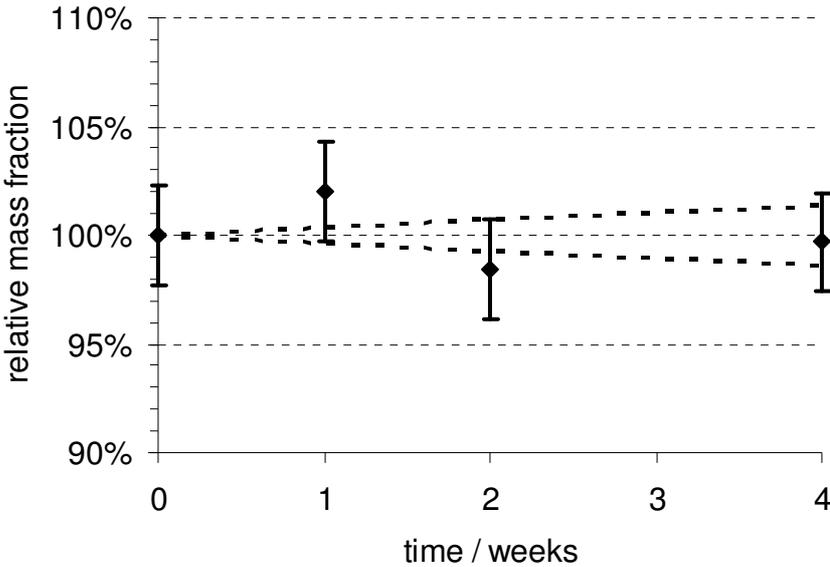


Figure B12: Rb

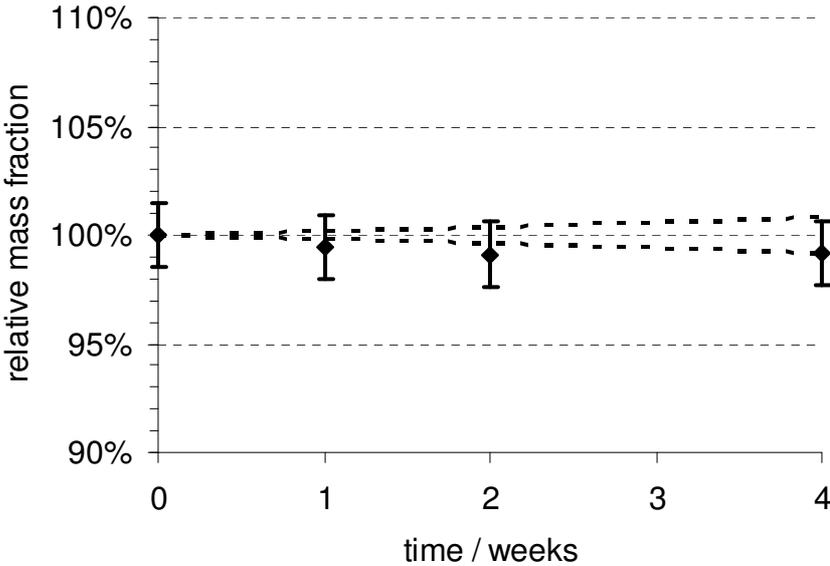


Figure B13: Se

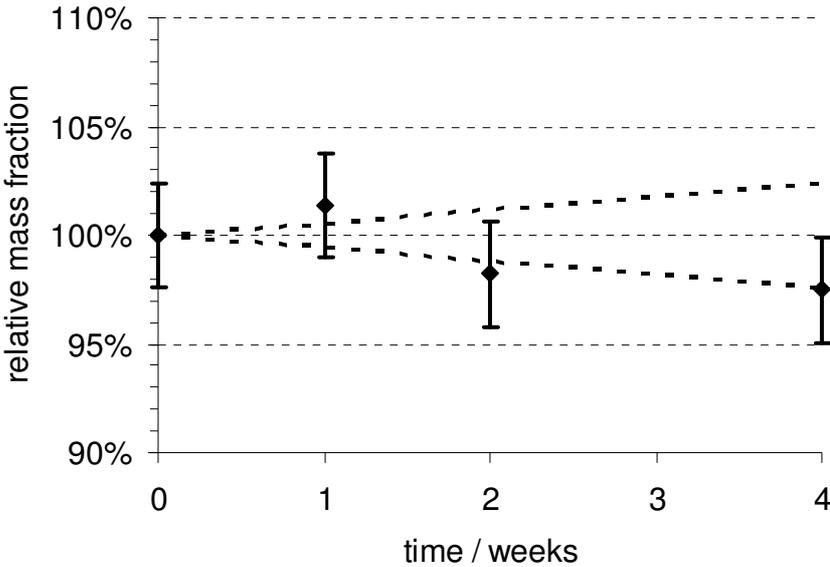


Figure B14: Sr

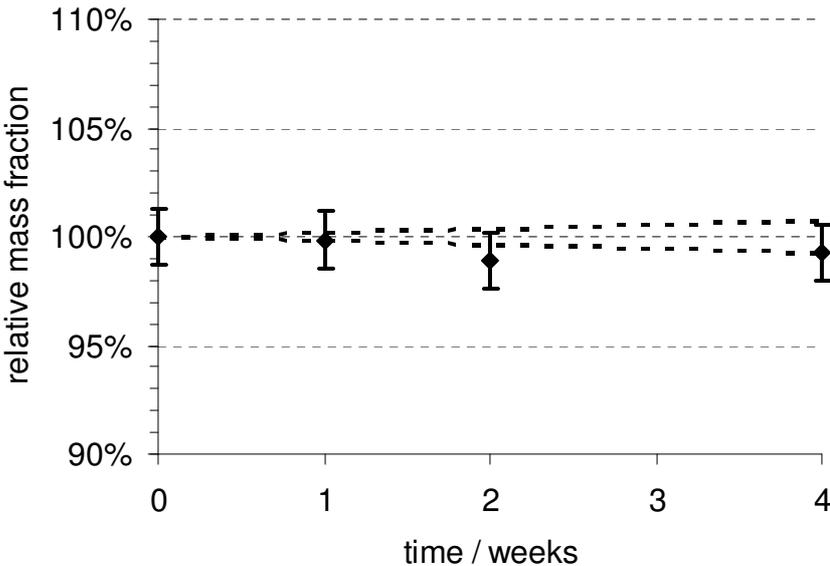
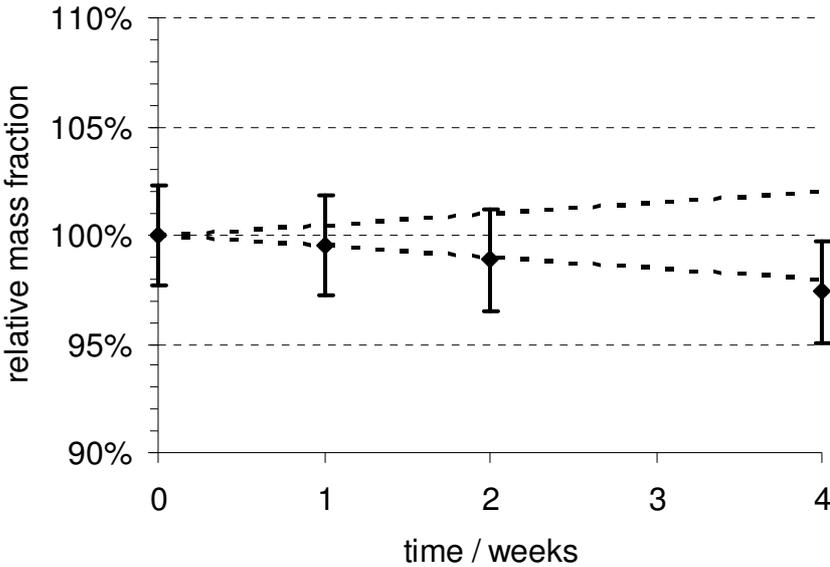


Figure B15: Zn



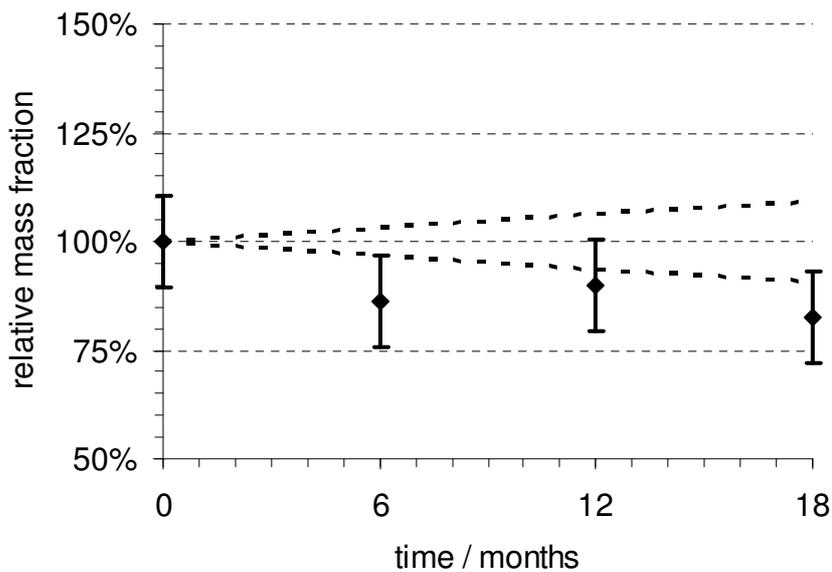
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## Annex C: Results of the long-term stability measurements

Graphs present the mean mass fractions measured at each time-point relative to the grand mean, against the time that the samples were held at 18 °C. Vertical bars represent the 95 % confidence interval of the measurements, based on the variance of measurements for each time-point calculated by ANOVA.

**Figure C1: Ag**



**Figure C2: As**

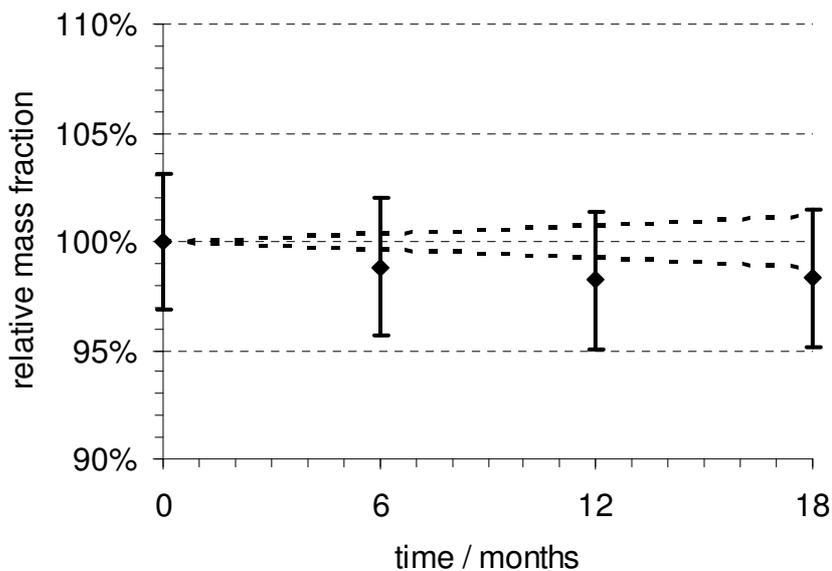


Figure C3: Ba

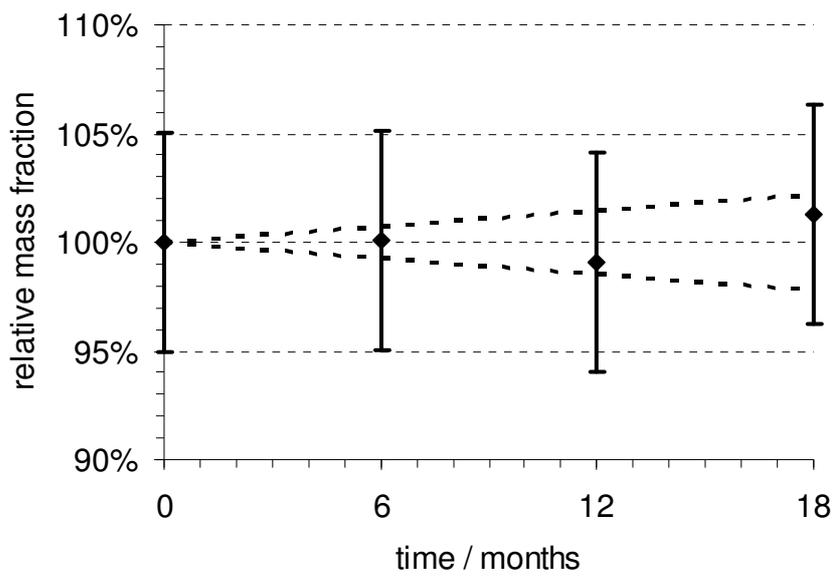


Figure C4: Cd

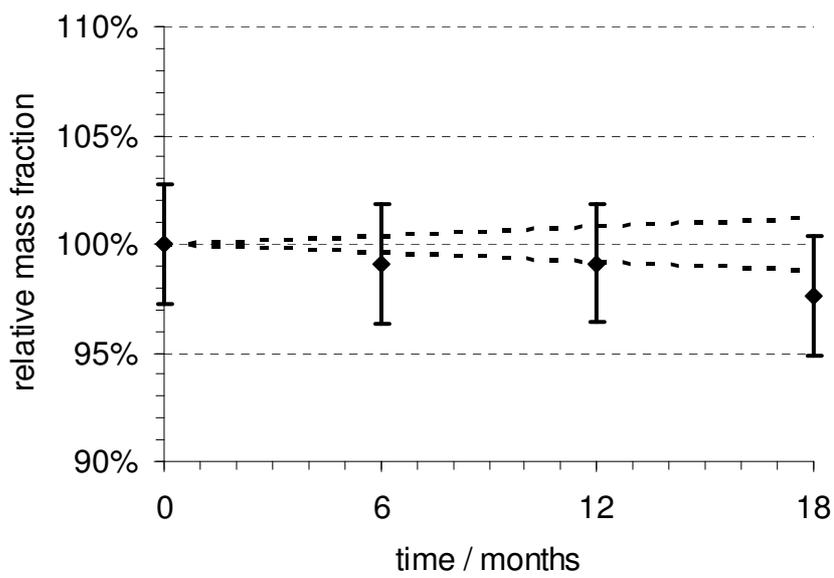


Figure C5: Cr

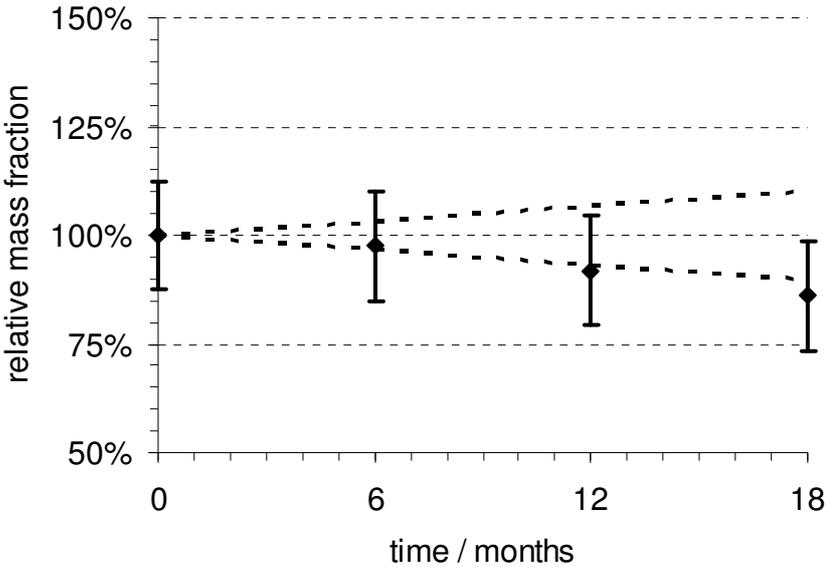


Figure C6: Cu

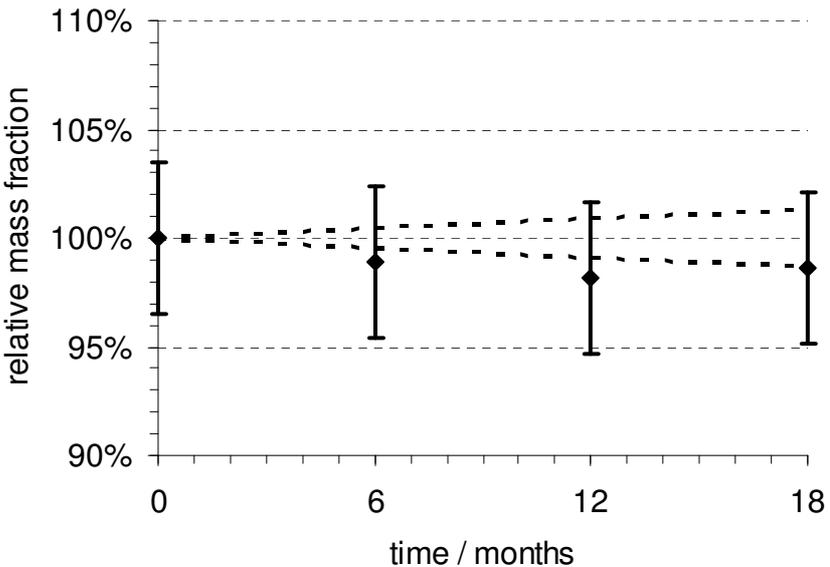


Figure C7: Fe

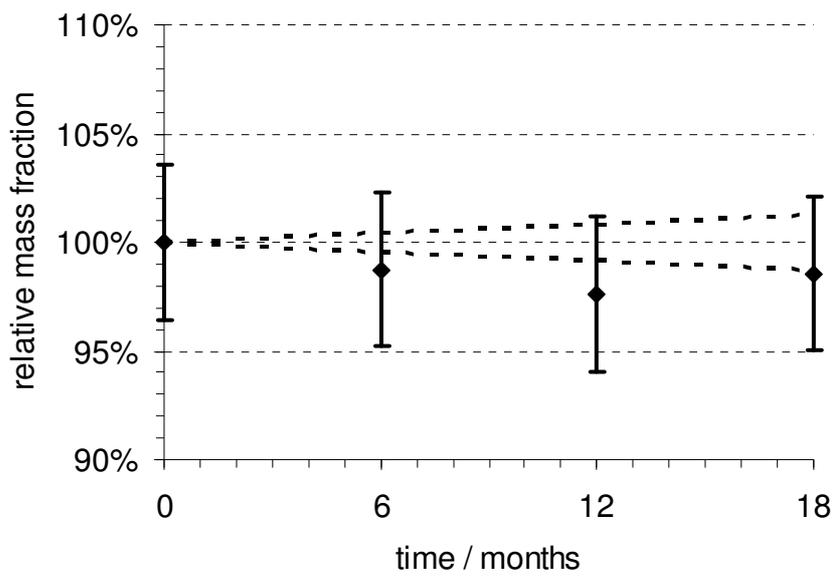


Figure C8: Hg

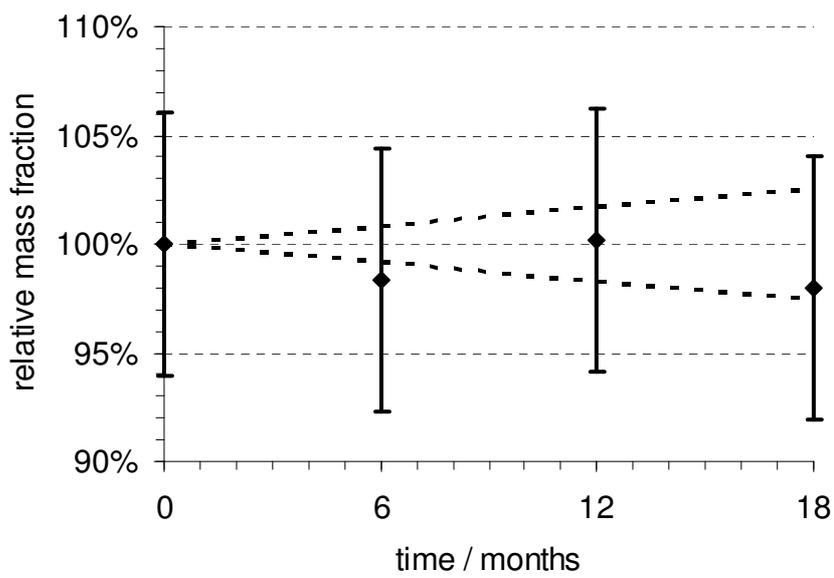


Figure C9: Mn

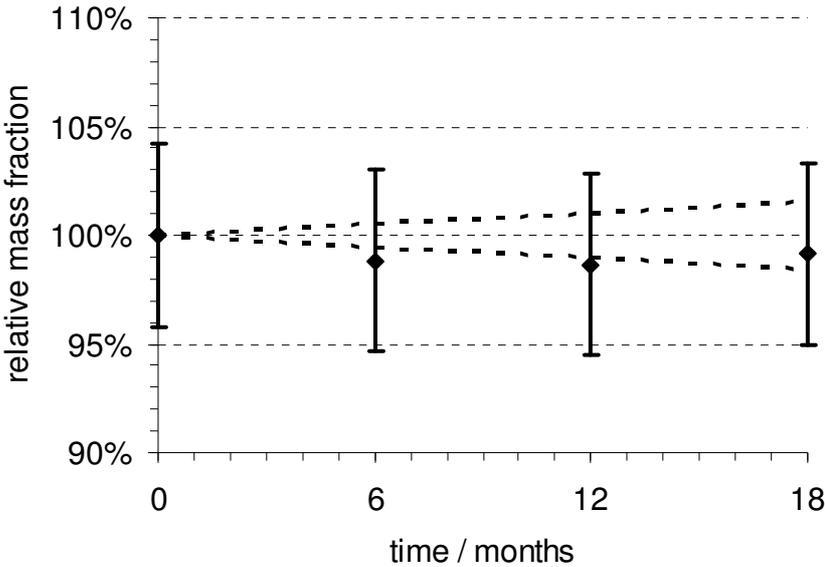


Figure C10: Ni

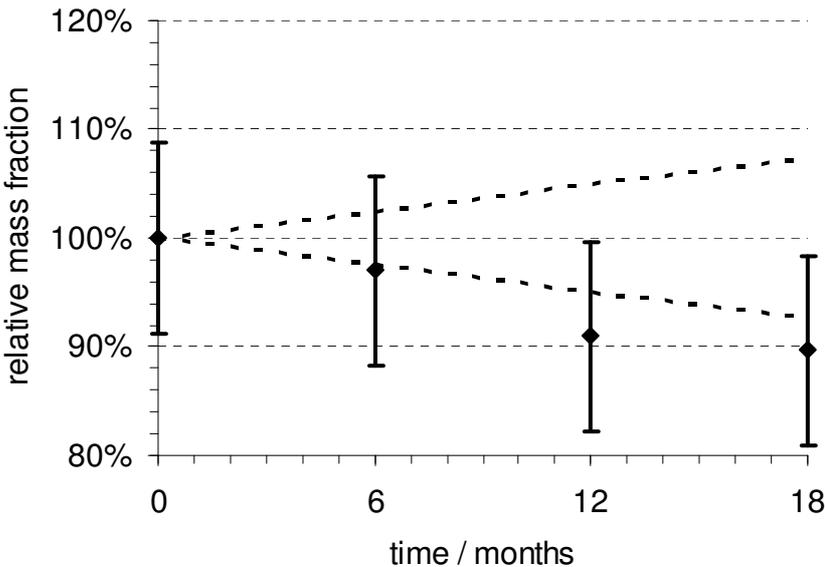


Figure C11: Pb

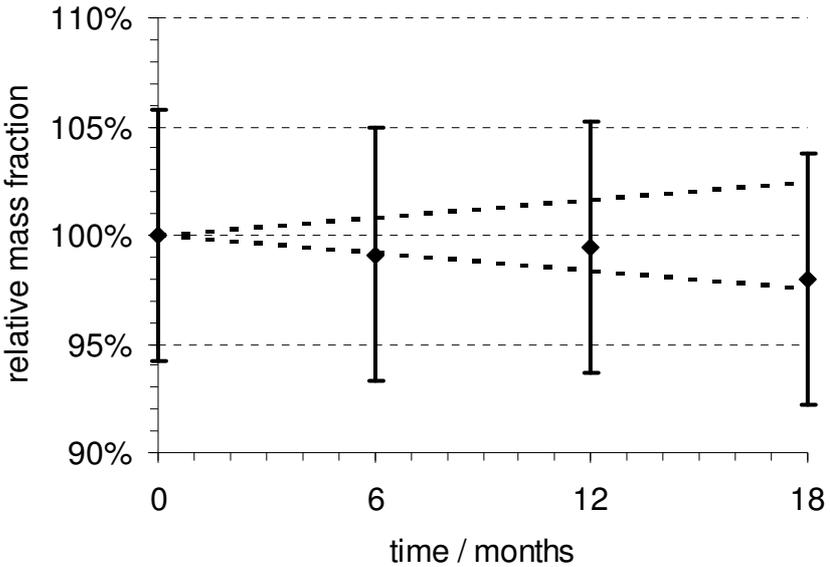


Figure C12: Rb

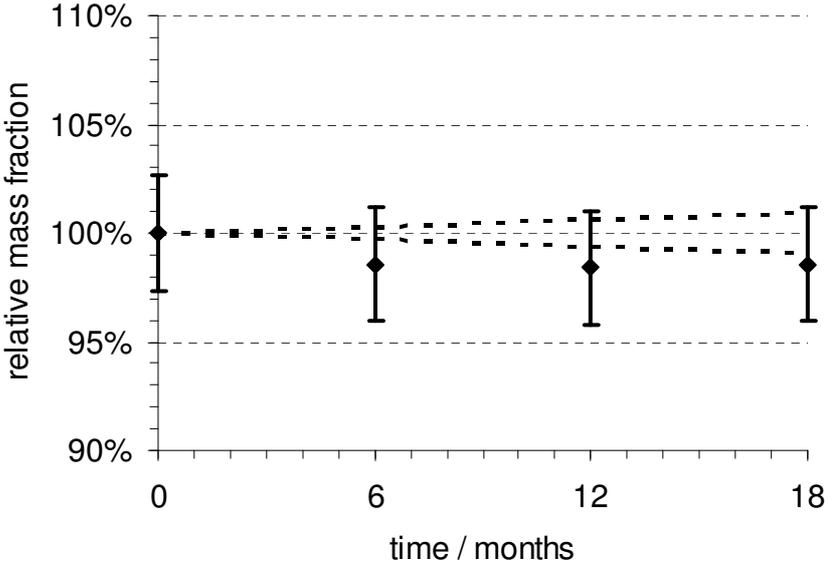


Figure C13: Se

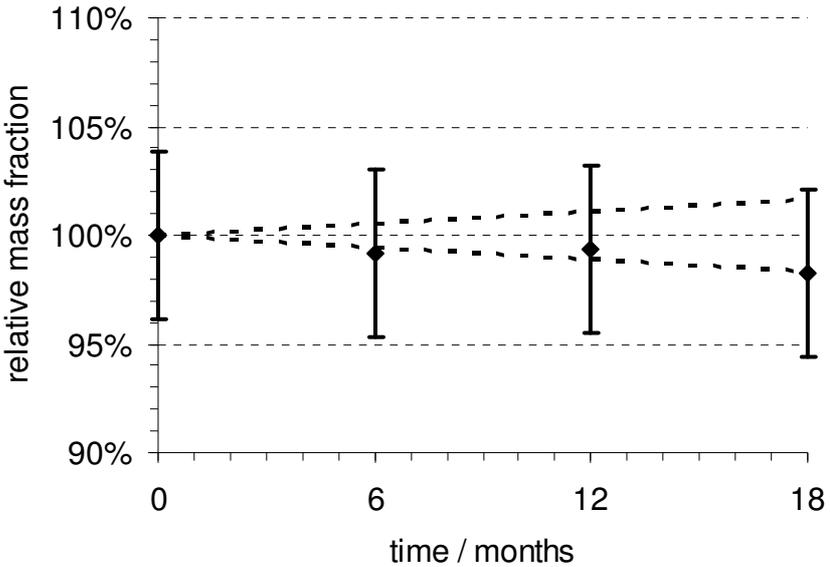


Figure C14: Sr

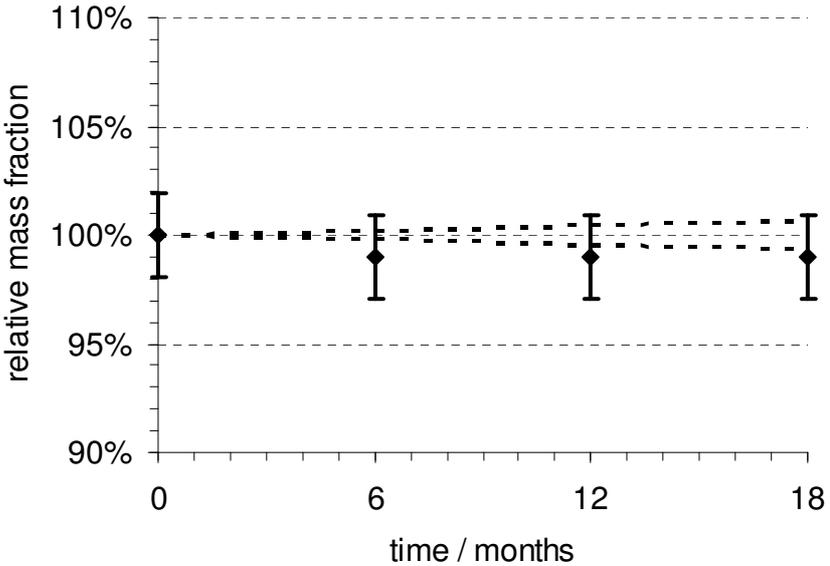
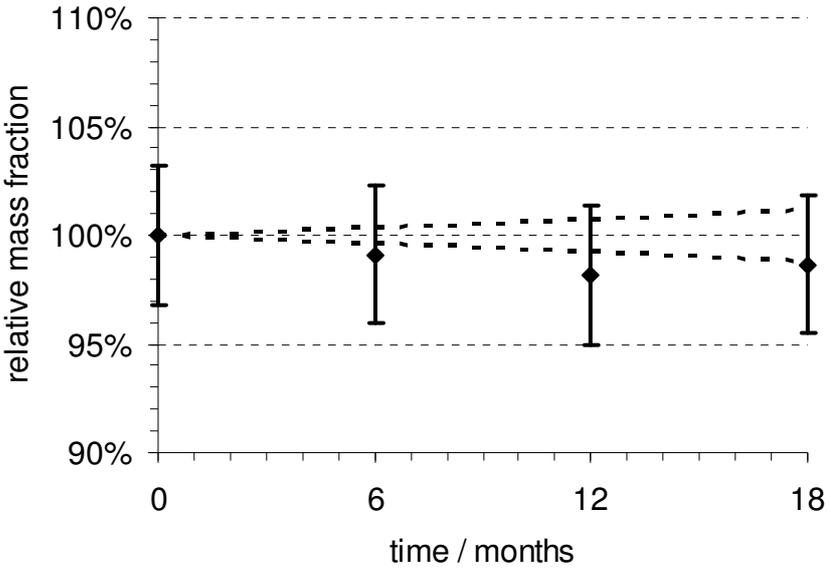


Figure C15: Zn



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### Annex D: Summary of methods used in the characterisation

Note that measurement methods are given as reported by the laboratories, and may not follow the terminology of the Guide to the Expression of Uncertainty in Measurements, [ISO/IEC Guide 98-3:2008], or the International Vocabulary of Metrology – Basic and General Concepts and Associated Terms, [ISO/IEC Guide 99:2007].

Lab Code	Element	Sample preparation method	Measurement technique	Calibration method	Standard or reference material used for calibration	Quality Assurance	Uncertainty estimation
0	Ag, As, Fe, Mn, Rb, Sr, Se, Zn	None	k0-NAA	k0 method	Traceability in k0-NAA is guaranteed through the use of IRMM-530R Al-Au fluence rate monitors		According to Robouch et al., Uncertainty budget for k0-NAA. J. Radioanal. Chem. 245, 195 (2000).

Lab Code	Element	Sample preparation method	Measurement technique	Calibration method	Standard or reference material used for calibration	Quality Assurance	Uncertainty estimation
1	ALL	MW digestion (HNO <sub>3</sub> +H <sub>2</sub> O <sub>2</sub> )	ICP-SFMS	External calibration	Single-element standard	QC, NIST1547, NIST1548, NIST1549, NIST1550, NIST1551, NIST1552, NIST1553, NIST1554, NIST1555, NIST1556, NIST1557, NIST1558, NIST1559, NIST1560, NIST1561	In accordance with T.Ruth 'A model for the evaluation of uncertainty i routine multi-element analysis', Accred Qual Assur (2004) 9:349-354

Lab Code	Element	Sample preparation method	Measurement technique	Calibration method	Standard or reference material used for calibration	Quality Assurance	Uncertainty estimation
2	As, Cd, Cr, Cu, Hg, Ni, Pb, Se, Zn  Fe, Mn, Zn	Microwave digestion using nitric acid	ICP-MS  ICP-OES	External Calibration (range 0.5-100ug/L), using linear regression line. Sample conc = (soln conc - blk conc) * dilution/weight	Stock mixed elements 1000mg/L calibration solution was purchased from SPEX Certiprep, Reference Material Producer CERT # 2495.01	Shewhart QC charts are built with results obtained from reference materials using the NWA Quality Analyst™ (Northwest Analytical Inc.). At least one reference material (TORT-2, DORM-3 or DOLT-4) must be run with every batch of analyses. Shewhart chart control limits are calculated from 30 points (whenever available) and these are recalculated at the start of each calendar year	U= 2xSD where SD is obtained from the QC charts

Lab Code	Element	Sample preparation method	Measurement technique	Calibration method	Standard or reference material used for calibration	Quality Assurance	Uncertainty estimation
3	As	Sample is digested in microwave (high pressure and temperature), with concentrated nitric acid and hydrogen peroxide. After digestion, sample is diluted to 10 ml with water	Graphite furnace, with 2 matrix modifiers: Pd (1000mg/l) and Magnesium nitrate (1000 mg/l). Wavelength: 193,7nm	external calibration curve with 4 standards (1 to 10 ug/l)	Arsenic (1000 mg/l): <i>Fluka 11082</i>	<ul style="list-style-type: none"> <li>- control of digestion with blank (one blank in each digestion batch)</li> <li>- control of the response of the highest standard of the curve</li> <li>- control of correlation coefficient (<math>r^2</math>) and residual analysis</li> <li>- one reference material in each batch</li> </ul>	we consider the following contributions: <ul style="list-style-type: none"> <li>- repeatability</li> <li>- reproducibility</li> <li>- Uncertainty related to exactitude (uncertainty of reference material)</li> </ul>
	Cd		Graphite furnace, with 2 matrix modifiers: DHPA (1%) and Magnesium nitrate (1000 mg/l). Wavelength: 228,8nm	external calibration curve with 4 standards (0,5 to 5 ug/l)	Cadmium (1000 mg/l): <i>Fluka 20895</i>		
	Cr		Graphite furnace, with one matrix modifier: Magnesium nitrate (5000 mg/l). Wavelength: 357,9nm	external calibration curve with 4 standards (1 to 10 ug/l)	Chromium (1000 mg/l): <i>Fluka 02733</i>		
	Cu		Graphite furnace, with 2 matrix modifiers: Pd (1000mg/l) and Magnesium nitrate (1000 mg/l). Wavelength: 327,4nm	external calibration curve with 4 standards (10 to 100 ug/l)	Copper (1000 mg/l): <i>Fluka 61147</i>		
	Fe		Graphite furnace, with one matrix modifier: Magnesium nitrate (5000 mg/l). Wavelength: 302,1nm	external calibration curve with 4 standards (10 to 100 ug/l)	Iron (1000 mg/l): <i>Fluka 44903</i>		
	Hg		Hydride generation/cold vapour. Wavelength: 253,7nm	external calibration curve with 4 standards (1 to 10 ug/l)	Mercury (1000 mg/l): <i>Fluka 16482</i>		
	Mn		ICP - Wavelength: 257,610nm	external calibration curve with 5 standards (1 to 50 ug/l) (Y as internal standard)	Manganese (1000 mg/l): <i>Fluka 63534</i> Yttrium (1000 mg/l): <i>Merck 1.70368.0100</i>		
	Ni		ICP - Wavelength: 231,604nm	external calibration curve with 5 standards (10 to 500 ug/l) (Y as internal standard)	Nickel (1000 mg/l): <i>Fluka 72223</i> Yttrium (1000 mg/l): <i>Merck 1.70368.0100</i>		

	Pb	Graphite furnace, with 2 matrix modifiers: DHPA (1%) and Magnesium nitrate (1000 mg/l). Wavelength: 283,3nm	external calibration curve with 4 standards (1 to 10 ug/l)	Lead (1000 mg/l): <i>Fluka 16595</i>		
	Se	Hydride generation Wavelength: 196,0nm	external calibration curve with 4 standards (1 to 10 ug/l)	Selenium (1000 mg/l): <i>Fluka 84896</i>		
	Zn	Flame atomic absorption. Wavelength: 213,9nm	external calibration curve with 4 standards (0,1 to 2,5 mg/l)	Zinc (1000 mg/l): <i>Fluka 6457</i>		

Lab Code	Element	Sample preparation method	Measurement technique	Calibration method	Standard or reference material used for calibration	Quality Assurance	Uncertainty estimation
4	As, Cd, Cu, Hg, Mn, Se, Zn  Pb	high-pressure $\mu$ -wave digestion with concentrated HNO <sub>3</sub> in quartz-vessels (Multiwave 3000, Anton Paar)	ICPMS	standard addition calibration at 4 concentration levels	Certified single element standard (SCP science)	Use of CRM (TORT-2)  Use of CRM (NIST1566a Oyster tissue)	according to GUM

Lab Code	Element	Sample preparation method	Measurement technique	Calibration method	Standard or reference material used for calibration	Quality Assurance	Uncertainty estimation
5	As	Digestion of sample with 7 ml HNO <sub>3</sub> + 1 ml H <sub>2</sub> O <sub>2</sub> + 0.1 ml HF in a microwave oven and closed vessel. Then ad hoc dilution	ICP/MS quadrupole mass spectrometer	Standard addition method (3 adding) and used of Selenium as internal standard. Ratio measurements of <sup>75</sup> As/ <sup>78</sup> Se	high purity standard of As <sub>2</sub> O <sub>5</sub> (99.9 % from Alfa Aesar)	Standard addition method not yet accredited Cofrac	An uncertainty budget had been established in compliance with the GUM comprising uncertainties for weighing, quantities of added arsenic, ratio measurements, fidelity, regression curve and corrections for blank. Our software calculates uncertainties propagation and the expanded final uncertainty (k=2)
	Cd	Digestion of sample spiked with <sup>111</sup> Cd in presence of 7 ml HNO <sub>3</sub> + 1 ml H <sub>2</sub> O <sub>2</sub> + 0.1 ml HF in a microwave oven and closed vessel. Then ad hoc dilution	ICP/MS quadrupole mass spectrometer	Double Isotope Dilution Mass Spectrometry IDMS	<sup>111</sup> Cd spike (96.5 % IRMM) characterised against high purity standard solution in house prepared from digestion of high purity solid (99.9999 % Prolabo). Used of this standard for mass bias correction	IDMS analysis accredited 2.54 Cofrac	An uncertainty budget had been established in compliance with the GUM comprising uncertainties for weighing, ratio measurements, fidelity, isotope abundances and corrections for blank. Our software calculates uncertainties propagation and the expanded final uncertainty (k=2)

Hg	<p>Digestion of sample spiked with <sup>202</sup>Hg in presence of 7 ml HNO<sub>3</sub> + 1 ml H<sub>2</sub>O<sub>2</sub> + 0.1 ml HF in a microwave oven and closed vessel. Then ad hoc dilution in presence of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (0.005 %)</p>	<p>ICP/MS quadrupole mass spectrometer</p>	<p>Double Isotope Dilution Mass Spectrometry IDMS</p>	<p><sup>202</sup>Hg spike (97.7% IRMM-ERM 640) characterised against high purity standard solution in house prepared from digestion of high purity solid (99.9999 % Merck). Used of CRM - ERM 639 for mass bias correction</p>	<p>IDMS analysis accredited 2.54 Cofrac</p>	<p>An uncertainty budget had been established in compliance with the GUM comprising uncertainties for weighing, ratio measurements, fidelity, isotope abundances and corrections for blank. Our software calculates uncertainties propagation and the expanded final uncertainty (k=2)</p>
Pb	<p>Digestion of sample spiked with <sup>206</sup>Pb in presence of 7 ml HNO<sub>3</sub> + 1 ml H<sub>2</sub>O<sub>2</sub> + 0.1 ml HF in a microwave oven and closed vessel. Then ad hoc dilution</p>	<p>ICP/MS quadrupole mass spectrometer</p>	<p>Double Isotope Dilution Mass Spectrometry IDMS</p>	<p><sup>206</sup>Pb spike (99.3 % Euriso-top) characterised against high purity standard solution in house prepared from digestion of high purity solid (99.99999 % Prolabo). Used of the SRM 981 and 982 for mass bias correction</p>	<p>IDMS analysis accredited 2.54 Cofrac</p>	<p>An uncertainty budget had been established in compliance with the GUM comprising uncertainties for weighing, ratio measurements, fidelity, isotope abundances and corrections for blank. Our software calculates uncertainties propagation and the expanded final uncertainty (k=2)</p>

	Se	Digestion of sample spiked with <sup>78</sup> Se in presence of 7 ml HNO <sub>3</sub> + 1 ml H <sub>2</sub> O <sub>2</sub> + 0.1 ml HF in a microwave oven and closed vessel. Then ad hoc dilution	ICP/MS quadrupole mass spectrometer with the collision/reaction cell	Double Isotope Dilution Mass Spectrometry IDMS	<sup>78</sup> Se spike (98.6 % Spectrascan) characterised against high purity standard solution in house prepared from digestion of high purity solid (99.999 % Alfa Aesar). Used of this standard for mass bias correction	IDMS analysis accredited 2.54 Cofrac	An uncertainty budget had been established in compliance with the GUM comprising uncertainties for weighing, ratio measurements, fidelity, isotope abundances and corrections for blank. Our software calculates uncertainties propagation and the expanded final uncertainty (k=2)
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Lab Code	Element	Sample preparation method	Measurement technique	Calibration method	Standard or reference material used for calibration	Quality Assurance	Uncertainty estimation
6	ALL	Oxidative decomposition of the test material using a high pressure autoclave with microwave heating	ICP-MS	external calibration (3 points)		calibration check using two independent standards (2-3 dilutions per standard), analysis of 3 standard reference materials	sample preparation: weighing (0.05%), filling of digestion solution to marker (1%), dilution of digestion solutions (0.3%), measurement itself (2.5%) --> in total 2.7% rounding error in case of low analyte content [%]: (0.001 mg/kg / analyte content)*100

Lab Code	Element	Sample preparation method	Measurement technique	Calibration method	Standard or reference material used for calibration	Quality Assurance	Uncertainty estimation
7	As	Neutron irradiation at flux of $5 \times 10^{12}$ cm <sup>2</sup> /s for 12 h, 6-7 day decay	Gamma spectrometry of <sup>76</sup> As at 559.1 keV, counting time 18-28 h	NAA k <sub>0</sub> standardisation method	IRMM-560RC		
	Fe	Neutron irradiation at flux of $5 \times 10^{12}$ cm <sup>2</sup> /s for 12 h, 12-17 day decay	Gamma spectrometry of <sup>59</sup> Fe at 1099.3 keV, counting time 22-28 h				
	Mn	Neutron irradiation at flux of $1.5 \times 10^{13}$ cm <sup>2</sup> /s for 30-60 s, 1-3 hour decay	Gamma spectrometry of <sup>56</sup> Mn at 846.8 keV, counting time ~2 h				
	Rb	Neutron irradiation at flux of $5 \times 10^{12}$ cm <sup>2</sup> /s for 12 h, 12-17 day decay	Gamma spectrometry of <sup>86</sup> Rb at 1077 keV, counting time 22-28 h				
	Sr	Neutron irradiation at flux of $5 \times 10^{12}$ cm <sup>2</sup> /s for 12 h, 12-17 day decay	Gamma spectrometry of <sup>85</sup> Sr at 514 keV, counting time 22-28 h				
	Se	Neutron irradiation at flux of $5 \times 10^{12}$ cm <sup>2</sup> /s for 12 h, 12-17 day decay	Gamma spectrometry of <sup>75</sup> Se at 264.7 keV, counting time 22-28 h				
	Zn	Neutron irradiation at flux of $5 \times 10^{12}$ cm <sup>2</sup> /s for 12 h, 12-17 day decay	Gamma spectrometry of <sup>65</sup> Zn at 1115.5 keV, counting time 22-28 h				

Lab Code	Element	Sample preparation method	Measurement technique	Calibration method	Standard or reference material used for calibration	Quality Assurance	Uncertainty estimation
8	As	microwave digestion	AAS	external	traceable to NIST SRM 3151	For quality assurance the standard reference material NIST 2976 Mussel Tissue and digestion blanks were prepared and analysed in the same manner as samples.	Estimation of uncertainty was performed using CRM DORM-2, SRM 1577a, and SRM 2976. The uncertainty covers the whole analytical process (sample preparation, digestion, dilution, measurement procedure). For combined uncertainty (see average +/- uncertainty) bias, uncertainty of reference material and relative standard deviation of at least 6 independent measurements was taken into account.
	Cd	microwave digestion	AAS	external	traceable to NIST SRM 3108		
	Cu	microwave digestion	AAS	external	traceable to NIST SRM 3114		
	Fe	microwave digestion	ICP-OES	external	traceable to NIST SRM 3126a		
	Pb	microwave digestion	AAS	external	traceable to NIST SRM 3128		
	Sr	microwave digestion	ICP-OES	external	traceable to NIST SRM 3153a		
	Se	microwave digestion	Hydride-AAS	external	traceable to NIST SRM 3149		
	Zn	microwave digestion	ICP-OES	external	traceable to NIST SRM 3168a		

Lab Code	Element	Sample preparation method	Measurement technique	Calibration method	Standard or reference material used for calibration	Quality Assurance	Uncertainty estimation
9	Ag	microwave digestion	ICP-MS	external	traceable to NIST SRM 3151	For quality assurance the standard reference material NIST 2976 Mussel Tissue and digestion blanks were prepared and analysed in the same manner as samples.	Estimation of uncertainty was performed using CRM DORM-2, SRM 1577a, and SRM 2976. The uncertainty covers the whole analytical process (sample preparation, digestion, dilution, measurement procedure). For combined uncertainty (see average +/- uncertainty) bias, uncertainty of reference material and relative standard deviation of at least 6 independent measurements was taken into account.
	As	microwave digestion	ICP-MS	external	traceable to NIST SRM 3103a		
	Ba	microwave digestion	ICP-MS	external	traceable to NIST SRM 3104a		
	Cd	microwave digestion	ICP-MS	external	traceable to NIST SRM 3108		
	Cr	microwave digestion	ICP-MS	external	traceable to NIST SRM 3112a		
	Cu	microwave digestion	ICP-MS	external	traceable to NIST SRM 3114		
	Fe	microwave digestion	ICP-MS	external	traceable to NIST SRM 3126a		
	Hg	microwave digestion	CV-AAS	external	traceable to NIST SRM 3133		
	Mn	microwave digestion	ICP-MS	external	traceable to NIST SRM 3132		
	Ni	microwave digestion	ICP-MS	external	traceable to NIST SRM 3136		
	Pb	microwave digestion	ICP-MS	external	traceable to NIST SRM 3128		

	Rb	microwave digestion	ICP-MS	external	traceable to NIST SRM 3145a		
	Sr	microwave digestion	ICP-MS	external	traceable to NIST SRM 3153a		
	Se	microwave digestion	ICP-MS	external	traceable to NIST SRM 3149		
	Zn	microwave digestion	ICP-MS	external	traceable to NIST SRM 3168a		

Lab Code	Element	Sample preparation method	Measurement technique	Calibration method	Standard or reference material used for calibration	Quality Assurance	Uncertainty estimation
10	Hg	none	Thermal decomposition (at 650°C), amalgamation of Hg vapour, atomic absorption detection (Direct Mercury Analyser DMA-80; Milestone Srl.)	calibration curve, linear regression	Hg aqueous solution prepared in-house from elemental Hg(0) (purity certificate available). Hg aqueous solution was verified against NIST SRM-3133.	Accuracy of Hg measurements was assured using reference material SRM 2976 (Mussel tissue), NIST. All results were in a very good agreement with the certified reference value and the measurement precision was 2 % (k=2).	EURACHEM/CITAC Guide (2000), Quantifying Uncertainty in Analytical Measurement and uncertainties of input parameters relevant for DMA technique. Uncertainties are given with a coverage factor k=2.

Lab Code	Element	Sample preparation method	Measurement technique	Calibration method	Standard or reference material used for calibration	Quality Assurance	Uncertainty estimation
11	Cd, Cr, Ni, Pb  Cu, Mn, Zn	To appx. 0.3 g of sample 4 mL of HNO <sub>3</sub> was added and microwave assisted digestion performed.	ETAAS  FAAS	external calibration	NIST	NIST SRM 2976	Measurement uncertainty was evaluated following EURACHEM/CITAC Guide (2000). For the expanded uncertainty the coverage factor was k=2.

Lab Code	Element	Sample preparation method	Measurement technique	Calibration method	Standard or reference material used for calibration	Quality Assurance	Uncertainty estimation
12	ALL	To appx. 0.3 g of sample 4 mL of HNO <sub>3</sub> was added and microwave assisted digestion performed.	ICP-MS	external calibration	NIST	NIST SRM 2976	Measurement uncertainty was evaluated following EURACHEM/CITAC Guide (2000). For the expanded uncertainty the coverage factor was k=2.

Lab Code	Element	Sample preparation method	Measurement technique	Calibration method	Standard or reference material used for calibration	Quality Assurance	Uncertainty estimation
13	Ag, As, Cr, Fe, Rb, Sr, Se, Zn	An aliquot of ERM-CE278k was pelletized in diameter 10 mm and 2.5 mm high. A sample and standard Al-0.1%Au (IRMM-530R) were stack together, fixed in the polyethylene vial in sandwich form and irradiated for about 15 h in the carousel facility of the TRIGA reactor with a thermal neutron flux of $1.1 \times 10^{12}$ n/(cm <sup>2</sup> s).	k <sub>0</sub> -INAA	k <sub>0</sub> -standardization method of instrumental neutron activation analysis (k <sub>0</sub> -INAA)	IRMM-530R (Al-0.1%Au alloy)	NIST SRM-1547 Peach Leaves	EURACHEM/CITAC Guide (2000), Quantifying Uncertainty in Analytical Measurement and uncertainties of input parameters relevant for k <sub>0</sub> -INAA technique. Uncertainties are given with a coverage factor k=2.

Lab Code	Element	Sample preparation method	Measurement technique	Calibration method	Standard or reference material used for calibration	Quality Assurance	Uncertainty estimation
14	Ag, Ba, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Rb, Se, Zn	microwave digestion in HNO <sub>3</sub>	ICP-MS: Instrument: Nexlon, Perkin Elmer, standard mode, sample introduction using a peristaltic pump at a flow rate of 0.3 ml/min, Meinhard nebulizer and cyclone spray chamber.	external, 6 point	CPI single standard, 1000 mg/L certified	Co-digestion and co-analysis of BCR 278. Each measurement series started with blanks, calibration standards, blanks, control standards <sup>1</sup> , followed from sample digests and control standards 2 + blanks (every tenth sample), and ended with control-calibration standards and blanks. Samples not matching the calibration range were appropriately diluted and measured again. Measurement of certified standards together with samples prepared at the sample concentrations determined. Preparation and measurement of blank digestions together with the samples. Preparation and measurement of a control standard at the measured sample concentration allowed fine-correction for instrumental drift.	Uncertainty was estimated as twice the reproducibility standard deviation of BCR-278. Reproducibility standard deviation was derived from 10 digests performed on 10 different days and analysed on 10 different days.

Lab Code	Element	Sample preparation method	Measurement technique	Calibration method	Standard or reference material used for calibration	Quality Assurance	Uncertainty estimation
15	As, Ba, Cd, Cu, Fe, Mn, Ni, Pb, Sr, Se, Zn	microwave digestion in HNO <sub>3</sub>	ICP-OES: Instrument: Optima 7300, Perkin-Elmer - LAS, Sample introduction using a peristaltic pump, low rate of 1.2 ml/min, Seaspray nebulizer and cyclone spray chamber. Analogous for all measured elements.	external, 6 point	CPI single standard, 1000 mg/L certified	Co-digestion and co-analysis of BCR 278. Each measurement series started with blanks, calibration standards, blanks, control standards <sup>1</sup> , followed from sample digests and control standards 2 + blanks (every tenth sample), and ended with control-calibration standards and blanks. Samples not matching the calibration range were appropriately diluted and measured again. Measurement of certified standards together with samples prepared at the sample concentrations determined. Preparation and measurement of blank digestions together with the samples. Preparation and measurement of a control standard at the measured sample concentration allowed fine-correction for instrumental drift.	Uncertainty was estimated as twice the reproducibility standard deviation of BCR-278. Reproducibility standard deviation was derived from 10 digests performed on 10 different days and analysed on 10 different days.

Lab Code	Element	Sample preparation method	Measurement technique	Calibration method	Standard or reference material used for calibration	Quality Assurance	Uncertainty estimation
16	ALL	microwave digestion in HNO <sub>3</sub>	ICP-MS: Instrument: Element 1, Thermo Fischer, sample introduction using a peristaltic pump at a flow rate of 1 ml/min, micromist nebulizer and cyclone spray chamber.	external, 6 point	CPI single standard, 1000 mg/L certified	Co-digestion and co-analysis of BCR 278. Each measurement series started with blanks, calibration standards, blanks, control standards <sup>1</sup> , followed from sample digests and control standards 2 + blanks (every tenth sample), and ended with control-calibration standards and blanks. Samples not matching the calibration range were appropriately diluted and measured again. Measurement of certified standards together with samples prepared at the sample concentrations determined. Preparation and measurement of blank digestions together with the samples. Preparation and measurement of a control standard at the measured sample concentration allowed fine-correction for instrumental drift.	Uncertainty was estimated as twice the reproducibility standard deviation of BCR-278. Reproducibility standard deviation was derived from 10 digestions performed on 10 different days and analysed on 10 different days.

Lab Code	Element	Sample preparation method	Measurement technique	Calibration method	Standard or reference material used for calibration	Quality Assurance	Uncertainty estimation
17	As, Cr, Cu, Mn, Ni, Pb, Rb, Sr, Se  Cd  Fe, Zn	microwave digestion in HNO <sub>3</sub>	ICP-MS (Agilent 7500ce) ETAAS (Analytik-Jena ZEENit 650) ICP-OES (Thermo Iris Intrepid II)	external, 6 point  external, 4 point  external, 6 point	Merck Certipur® 1000 mg/L solutions with certified concentration	Measurement of independently prepared control standards and digests of ERM-CE278 and NIST SRM2976	Uncertainties estimated through method validation, including components for repeatability, intermediate precision and trueness, and expressed with a coverage factor $k=2$ .

# CERTIFICATION REPORT

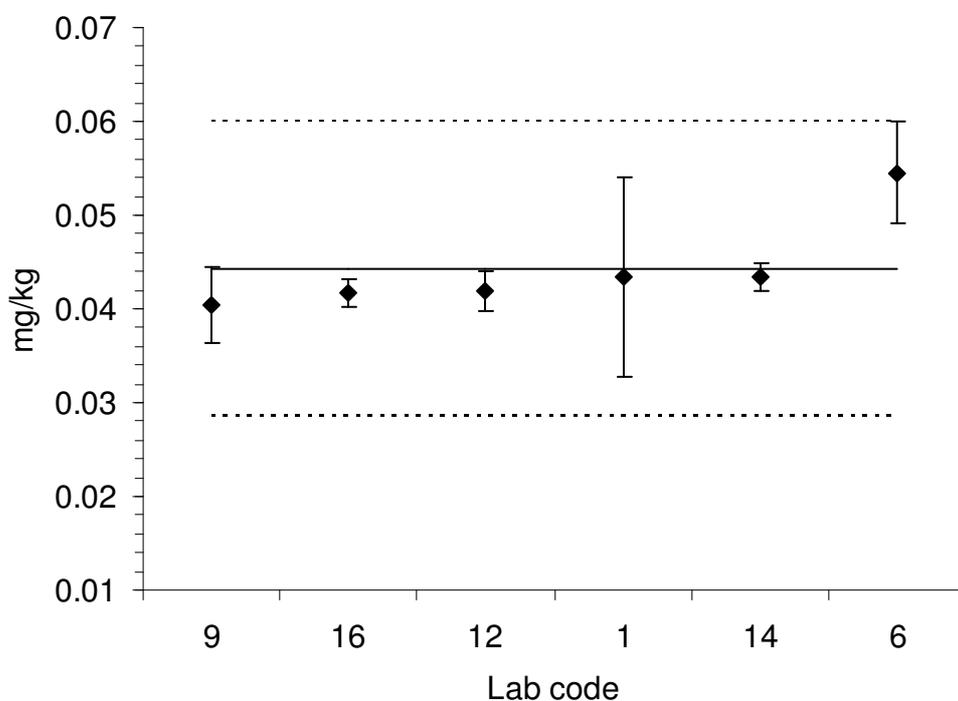
The certification of the mass fractions of elements in mussel tissue: ERM-CE278k

## Annex E: Results of the characterisation measurements

Tables present the results of characterisation measurements (corrected to dry mass) and the estimated uncertainties of the measurements, as reported by participants. Values are rounded for formatting reasons. Graphs show expanded uncertainties as stated by the laboratories and the certified range, and are given for the accepted datasets only.

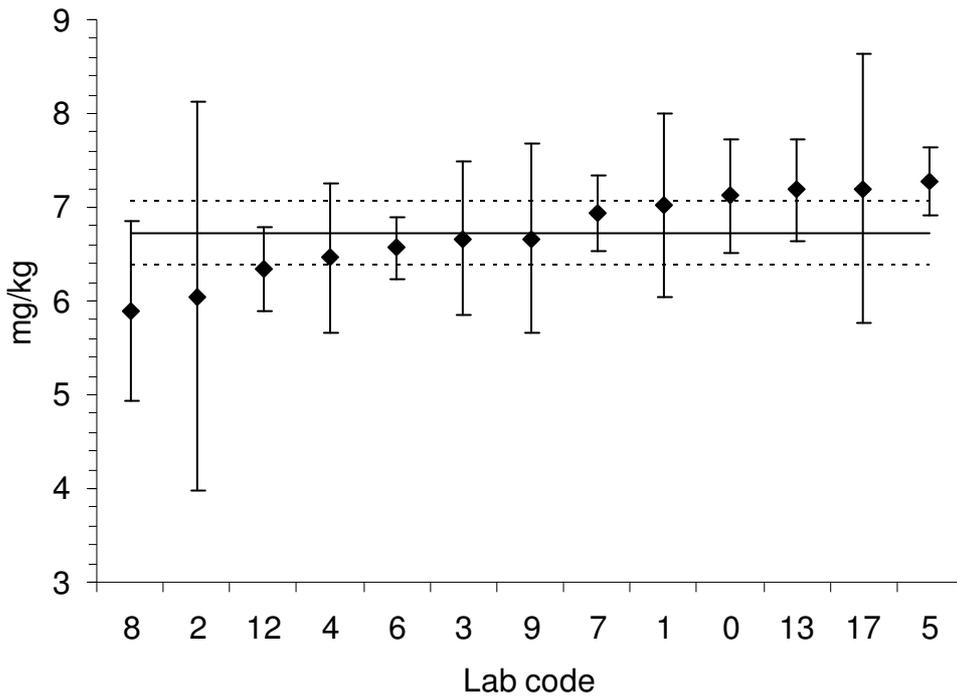
### Ag

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
1	ICP-SFMS	0.0466	0.0467	0.0455	0.0407	0.0409	0.0396	0.0433	0.0106
6	ICP-MS	0.044816	0.070116	0.045126	0.054690	0.054174	0.058508	0.0546	0.0055
9	ICP-MS	0.043	0.043	0.043	0.037	0.039	0.038	0.0405	0.0041
12	ICP-MS	0.0425	0.0429	0.0434	0.0409	0.0408	0.0409	0.0419	0.0022
14	ICP-MS	0.04255	0.04411	0.04359	0.04286	0.04349	0.04369	0.0434	0.0014
16	ICP-SFMS	0.04157	0.04416	0.04137	0.04032	0.04001	0.0428	0.0417	0.0015
<i>Results not used for certification</i>									
0	k0-NAA					0.072	0.052		
13	k0-INAA	0.05	0.067	0.042	< 0.049	< 0.048	0.053		



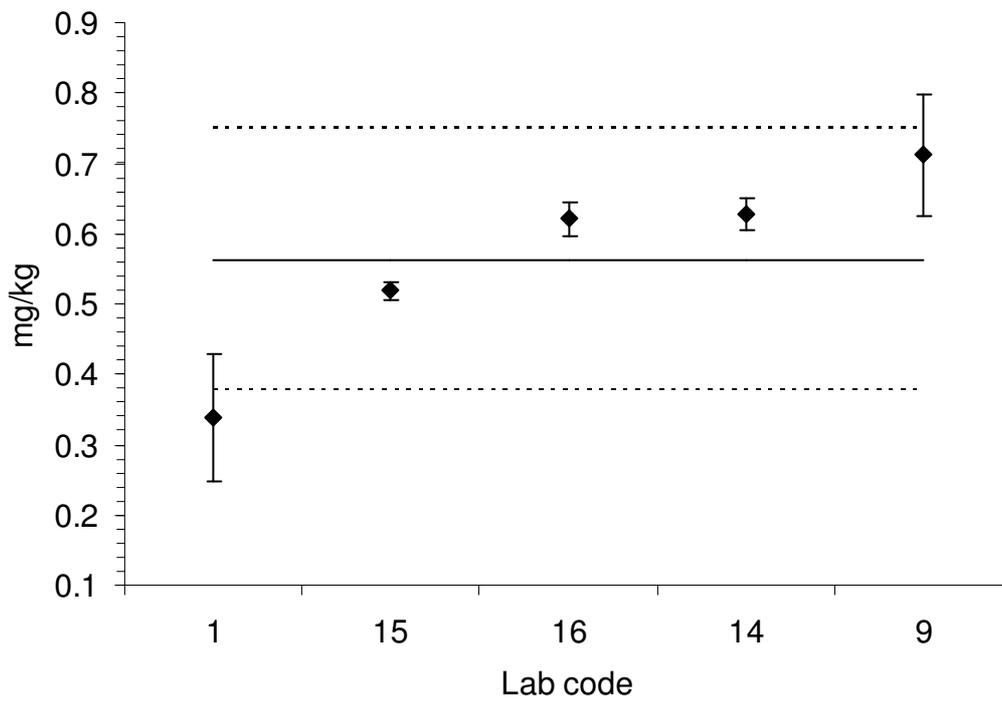
**As**

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
0	k0-NAA	7.18	7.19	6.9	7.1	7.17	7.17	7.12	0.60
1	ICP-SFMS	7.19	6.96	7.09	6.94	6.81	7.09	7.01	0.98
2	ICP-MS	6.21	6.59	5.876	5.816	5.701	6.093	6.05	2.07
3	ETAAS	6.7	6.7	6.6	6.9	6.5	6.6	6.67	0.82
4	ICP-MS	6.52	6.66	6.63	6.29	6.43	6.23	6.46	0.79
5	ICP-MS	7.31	7.51	7.48	7.28	6.95	7.14	7.28	0.37
6	ICP-MS	6.433292	6.713135	6.823627	6.340935	6.584460	6.489526	6.56	0.33
7	k0-NAA	6.894	6.927	6.739	6.978	7.025	7.032	6.93	0.41
8	HG-AAS	5.9	5.9	5.8	5.9	5.9	6	5.90	0.96
9	ICP-MS	6.8	6.8	6.6	6.6	6.6	6.6	6.67	1.01
12	ICP-MS	6.4	6.4	6.42	6.22	6.29	6.29	6.34	0.45
13	k0-INAA	7.38	7.06	7.2	7.32	7.13	7.01	7.18	0.55
17	ICP-MS	7.6	7.5	7.2	6.9	7	7	7.20	1.44
<i>Results not used for certification</i>									
15	ICP-OES	7.21	7.37	7.19	7.31	7.29	7.16		
16	ICP-SFMS	7.02	7.01	7.06	7.03	7.1	7.14		



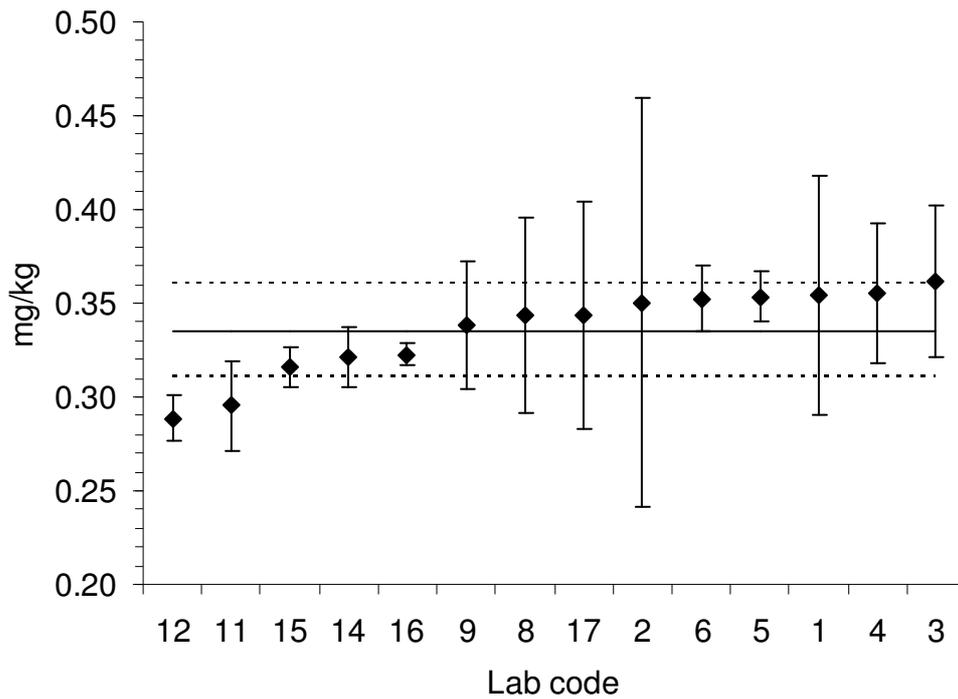
**Ba**

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
1	ICP-SFMS	0.336	0.346	0.321	0.334	0.346	0.346	0.3382	0.0919
9	ICP-MS	0.7	0.71	0.73	0.71	0.72	0.7	0.7117	0.0862
14	ICP-MS	0.63616	0.61852	0.61437	0.64451	0.62064	0.62998	0.6274	0.0227
15	ICP-OES	0.52508	0.51578	0.52616	0.5127	0.51063	0.52101	0.5186	0.0128
16	ICP-SFMS	0.6329	0.61325	0.60291	0.6203	0.62547	0.62961	0.6207	0.0230
<i>Results not used for certification</i>									
6	ICP-MS	0.551425	0.564436	0.581990	0.772778	0.778867	0.753689		



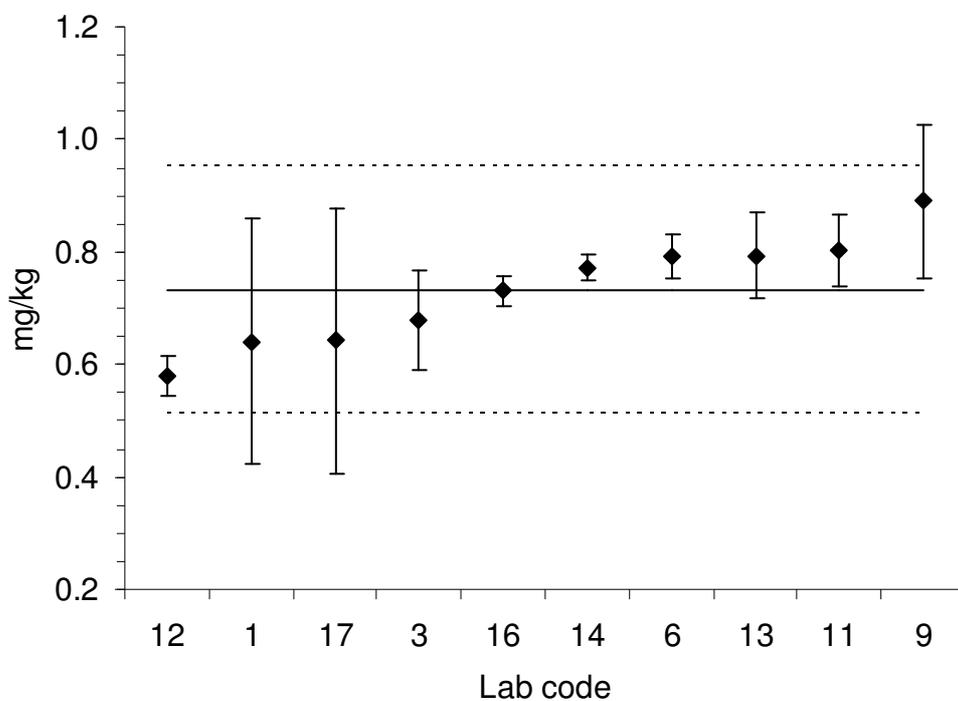
## Cd

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
1	ICP-SFMS	0.354	0.359	0.343	0.359	0.357	0.353	0.3542	0.0640
2	ICP-MS	0.349	0.338	0.334	0.37	0.356	0.356	0.3505	0.1091
3	ETAAS	0.37	0.36	0.36	0.36	0.36	0.36	0.3617	0.0402
4	ICP-MS	0.344	0.349	0.348	0.364	0.363	0.367	0.3558	0.0372
5	ID-ICP-MS	0.359	0.369	0.35	0.344	0.34	0.36	0.3537	0.0135
6	ICP-MS	0.357290	0.358943	0.349236	0.351666	0.342175	0.353937	0.3522	0.0176
8	ETAAS	0.35	0.35	0.34	0.34	0.34	0.34	0.3433	0.0520
9	ICP-MS	0.34	0.33	0.35	0.34	0.33	0.34	0.3383	0.0338
11	ETAAS	0.297	0.296	0.298	0.294	0.29	0.297	0.2953	0.0241
12	ICP-MS	0.302	0.298	0.285	0.287	0.274	0.285	0.2885	0.0121
14	ICP-MS	0.32275	0.3186	0.32068	0.31551	0.31966	0.33108	0.3214	0.0163
15	ICP-OES	0.31756	0.31652	0.3186	0.31862	0.3124	0.31343	0.3162	0.0105
16	ICP-SFMS	0.32679	0.31852	0.32265	0.32462	0.32049	0.32359	0.3228	0.0060
17	ETAAS	0.361	0.336	0.317	0.343	0.352	0.353	0.3437	0.0607



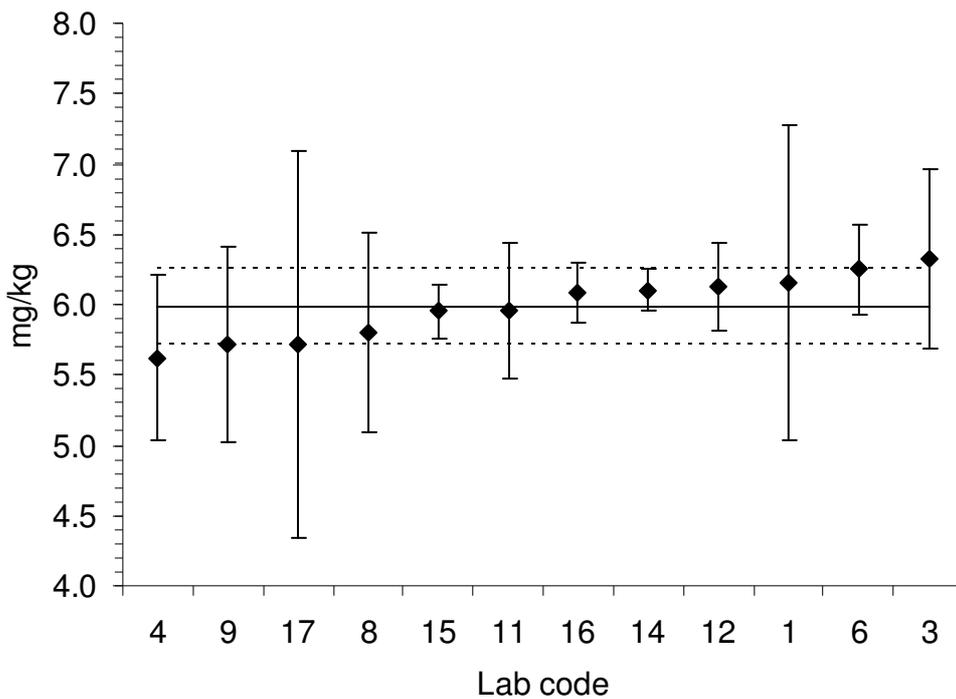
## Cr

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
1	ICP-SFMS	0.634	0.622	0.644	0.632	0.659	0.657	0.6413	0.2172
3	ETAAS	0.68	0.64	0.61	0.72	0.73	0.69	0.6783	0.0890
6	ICP-MS	0.737092	0.909851	0.717885	0.700856	0.844392	0.847075	0.7929	0.0396
9	ICP-MS	0.87	0.98	0.76	0.97	0.87	0.89	0.8900	0.1362
11	ETAAS	0.805	0.817	0.807	0.812	0.783	0.799	0.8038	0.0644
12	ICP-MS	0.634	0.644	0.638	0.52	0.523	0.523	0.5803	0.0351
13	ICP-MS	0.91	0.75	0.76	0.83	0.75	0.76	0.7933	0.0765
14	k0-INAA	0.75758	0.77315	0.76589	0.78566	0.76594	0.78462	0.7721	0.0228
16	ICP-SFMS	0.71563	0.74355	0.71977	0.7423	0.72058	0.74126	0.7305	0.0268
17	ICP-MS	0.64	0.62	0.62	0.67	0.65	0.65	0.6417	0.2369
<i>Results not used for certification</i>									
2	ICP-MS	0.549	0.489	0.506	0.835	0.853	0.87		



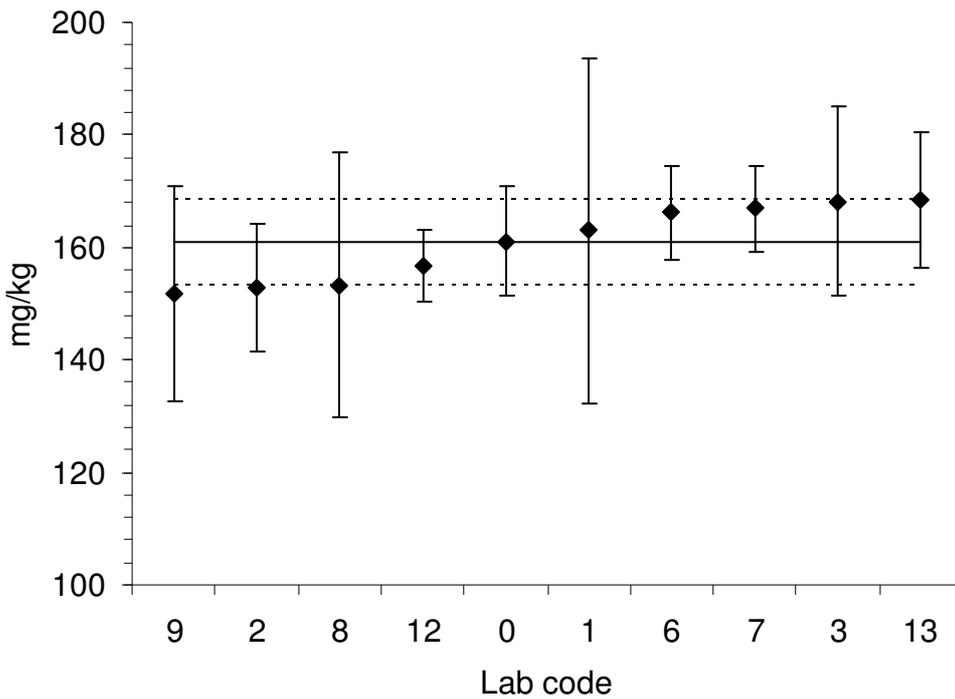
## Cu

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
1	ICP-SFMS	6.17	6.16	6.06	6.11	6.21	6.21	6.153	1.117
3	ETAAS	6.22	6.29	6.37	6.29	6.4	6.4	6.328	0.634
4	ICP-MS	5.94	6	5.17	5.49	5.62	5.5	5.620	0.587
6	ICP-MS	6.103883	6.246386	6.140025	6.350222	6.282117	6.368796	6.249	0.312
8	ETAAS	6.2	6.1	5.5	5.6	5.7	5.7	5.800	0.707
9	ICP-MS	5.7	5.8	5.9	5.8	5.6	5.5	5.717	0.694
11	FAAS	6.2	6.25	6.13	5.78	5.71	5.67	5.957	0.486
12	ICP-MS	6.08	6.09	6.07	6.17	6.2	6.19	6.133	0.312
14	ICP-MS	6.16	6.14	6.1	6.16	5.98	6.08	6.103	0.145
15	ICP-OES	6.02	5.98	5.92	5.9	6.02	5.88	5.953	0.188
16	ICP-SFMS	6.22	6.07	6.11	5.93	6.01	6.15	6.082	0.211
17	ICP-MS	5.8	5.7	5.6	5.6	5.8	5.8	5.717	1.380
<i>Results not used for certification</i>									
2	ICP-MS	5.542	5.619	4.873	5.609	5.88	5.757		



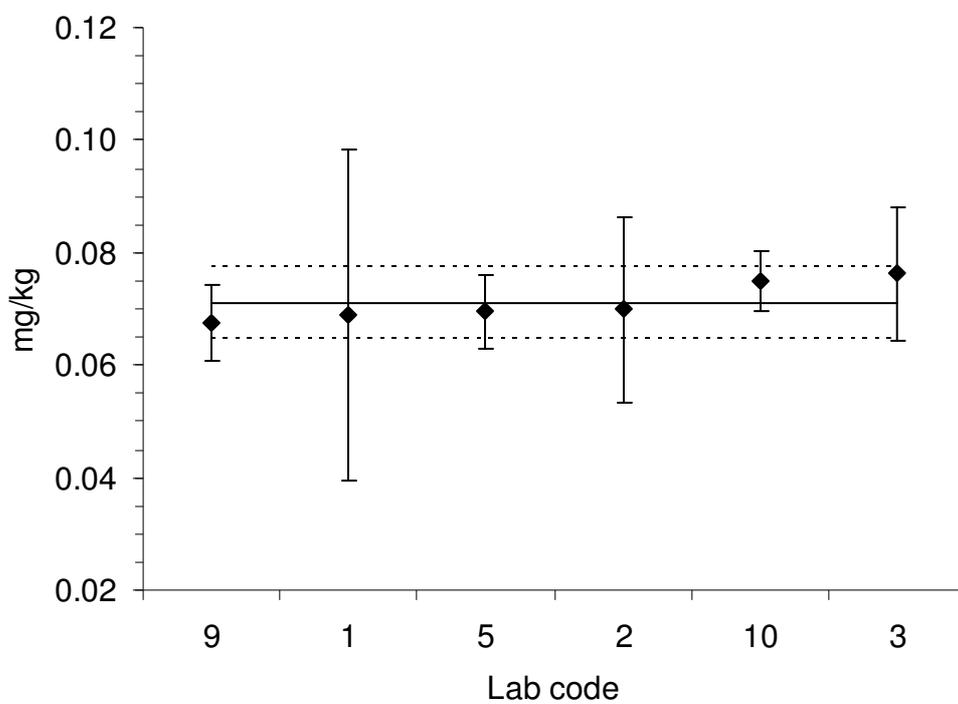
# Fe

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
0	k0-NAA	170	148	157	162	170	160	161.2	9.8
1	ICP-SFMS	162	165	156	167	170	158	163.0	30.6
2	ICP-OES	152.552	154.751	150.543	150.855	154.168	154.667	152.9	11.3
3	ETAAS	171.6	167.4	169.7	169.7	164.7	165.6	168.1	16.9
6	ICP-MS	166.8732	171.107	170.2809	160.2518	163.0379	165.5144	166.2	8.3
7	k0-NAA	176.8	166.3	164.9	165.7	166.3	162.1	167.0	7.6
8	ICP-OES	160	150	150	150	150	160	153.3	23.5
9	ICP-MS	150	160	150	150	150	150	151.7	19.2
12	ICP-MS	157.2	158.7	158.9	154.7	154.7	155.9	156.7	6.3
13	k0-INAA	169	169	168	168	168	168	168.3	12.0
<i>Results not used for certification</i>									
14	ICP-MS	176.42	196.14	175.39	200.31	176.44	185.78		
15	ICP-OES	181.61	178.5	176.42	175.4	183.7	181.63		
16	ICP-SFMS	177.87	183.04	173.74	186.09	196.43	172.65		
17	ICP-OES	180	181	181	178	180	180		



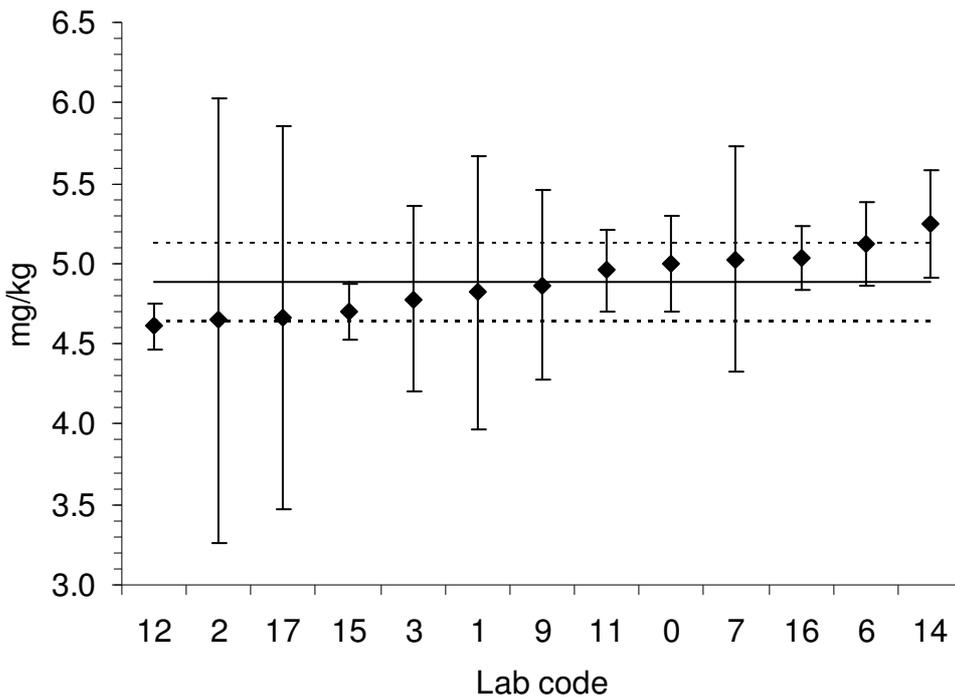
# Hg

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
1	ICP-SFMS	0.0697	0.0682	0.0692	0.0703	0.0663	0.0699	0.0689	0.0293
2	ICP-MS	0.084	0.063	0.072	0.071	0.066	0.063	0.0698	0.0166
3	CVAAS	0.077	0.08	0.073	0.07	0.077	0.081	0.0763	0.0119
5	ID-ICP-MS	0.065	0.067	0.074	0.075	0.07	0.066	0.0695	0.0064
9	ICP-MS	0.067	0.067	0.068	0.069	0.068	0.066	0.0675	0.0068
10	DMA	0.0737	0.0785	0.0742	0.0727	0.0735	0.0775	0.0750	0.0053
<i>Results not used for certification</i>									
4	ICP-MS	0.07	0.074	0.074	0.073	0.071	0.077		
6	ICP-MS	0.078893	0.078686	0.07786	0.042307	0.038799	0.05696		
14	ICP-MS	0.09454	0.09299	0.09247	0.09424	0.09465	0.09528		
16	ICP-SFMS	0.06836	0.07684	0.07508	0.06731	0.07795	0.07185		



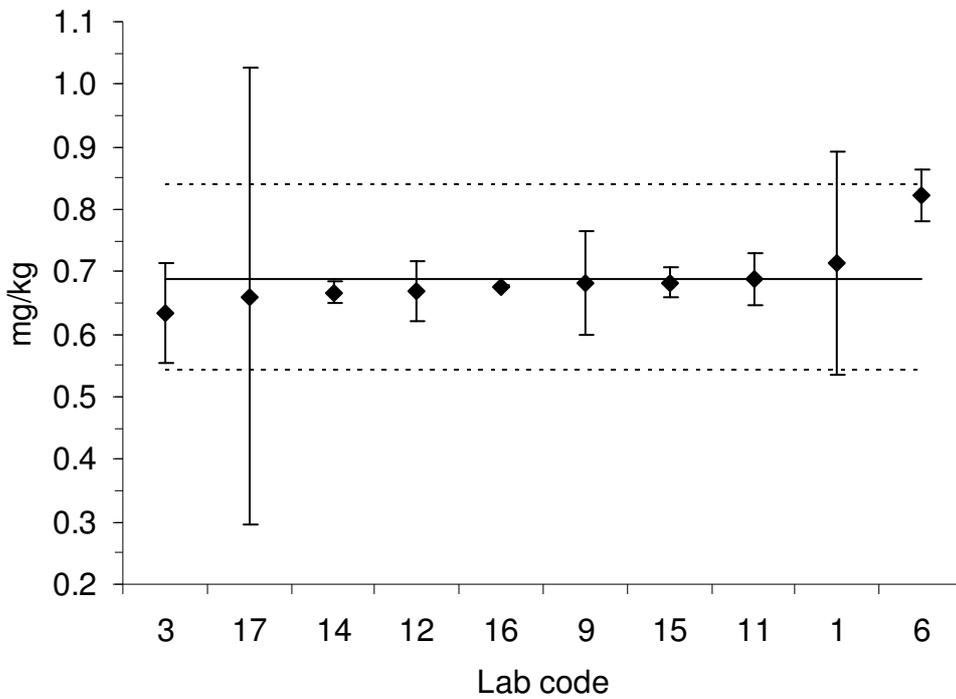
# Mn

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
0	k0-NAA	4.93	4.76	5.12	5.09	4.76	5.33	5.00	0.29
1	ICP-SFMS	4.78	4.95	4.78	4.72	4.96	4.72	4.82	0.85
2	ICP-OES	4.654	4.711	4.592	4.565	4.653	4.698	4.65	1.39
3	ICP-OES	4.83	4.77	4.71	4.86	4.83	4.68	4.78	0.58
6	ICP-MS	5.043371	5.092937	5.105328	5.153235	5.148076	5.19967	5.12	0.26
7	k0-NAA	5.12	5	5	5.07	5.03	4.95	5.03	0.59
9	ICP-MS	5	5	4.7	5	4.8	4.7	4.87	0.59
11	FAAS	4.74	4.73	4.77	5.06	5.3	5.14	4.96	0.25
12	ICP-MS	4.67	4.68	4.66	4.52	4.57	4.56	4.61	0.14
14	ICP-MS	5.3	5.1	5.44	5.39	5.13	5.12	5.25	0.33
15	ICP-OES	4.79	4.75	4.68	4.64	4.66	4.69	4.70	0.17
16	ICP-SFMS	4.96	5.1	4.94	5.05	4.97	5.19	5.04	0.19
17	ICP-MS	4.6	4.5	4.4	4.7	5	4.8	4.67	1.19
<i>Results not used for certification</i>									
4	ICP-MS	4.94	4.88	5.09	5.89	5.79	5.94		



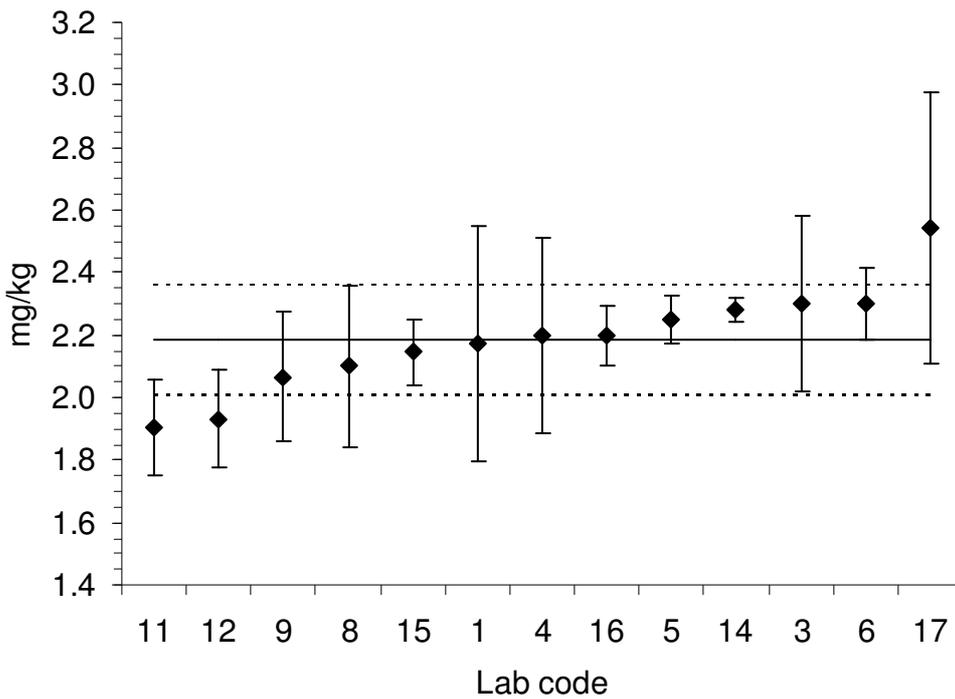
# Ni

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
1	ICP-SFMS	0.697	0.705	0.709	0.721	0.732	0.716	0.713	0.178
3	ICP-OES	0.56	0.6	0.6	0.7	0.67	0.67	0.713	0.178
6	ICP-MS	0.778088	0.888992	0.797398	0.808276	0.820555	0.836962	0.822	0.041
9	ICP-MS	0.7	0.66	0.68	0.69	0.69	0.67	0.682	0.082
11	ETAAS	0.708	0.735	0.748	0.637	0.715	0.584	0.688	0.041
12	ICP-MS	0.69	0.721	0.701	0.636	0.627	0.637	0.669	0.047
14	ICP-MS	0.66107	0.65381	0.66522	0.67357	0.67876	0.66942	0.667	0.018
15	ICP-OES	0.66626	0.68805	0.68494	0.70056	0.69122	0.66942	0.683	0.025
16	ICP-SFMS	0.67943	0.66185	0.68357	0.66843	0.68543	0.67613	0.676	0.002
17	ICP-MS	0.66	0.66	0.65	0.66	0.68	0.65	0.660	0.366
<i>Results not used for certification</i>									
2	ICP-MS	0.723	0.709	0.73	1.761	0.935	1.032		



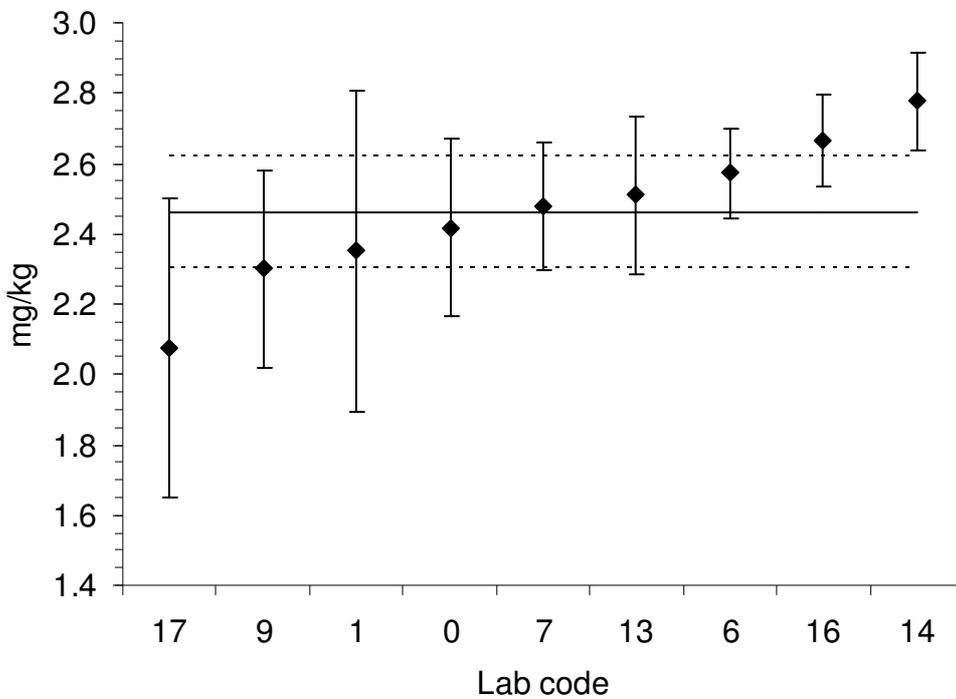
# Pb

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
1	ICP-SFMS	2.19	2.18	2.18	2.2	2.13	2.14	2.170	0.377
3	ICP-OES	2.3	2.37	2.32	2.35	2.24	2.21	2.298	0.281
4	ICP-MS	2.19	2.18	2.24	2.1	2.12	2.36	2.198	0.313
5	ID-ICP-MS	2.306	2.329	2.218	2.223	2.202	2.225	2.251	0.076
6	ICP-MS	2.337533	2.367479	2.359906	2.253981	2.241599	2.257077	2.303	0.115
8	ETAAS	2.1	2.1	2.1	2.1	2.1	2.1	2.100	0.260
9	ICP-MS	2.1	2.1	2.1	2.1	2	2	2.067	0.207
11	FAAS	1.914	1.86	1.904	1.921	1.947	1.87	1.903	0.153
12	ICP-MS	1.96	1.928	1.922	1.933	1.916	1.931	1.932	0.155
14	ICP-MS	2.3	2.27	2.25	2.3	2.28	2.29	2.282	0.041
15	ICP-OES	2.16	2.13	2.15	2.16	2.12	2.16	2.147	0.105
16	ICP-SFMS	2.18	2.22	2.24	2.25	2.16	2.14	2.198	0.097
17	ICP-MS	2.52	2.43	2.47	2.62	2.62	2.59	2.542	0.432
<i>Results not used for certification</i>									
2	ICP-MS	2.183	2.156	2.185	1.773	1.74	1.922		



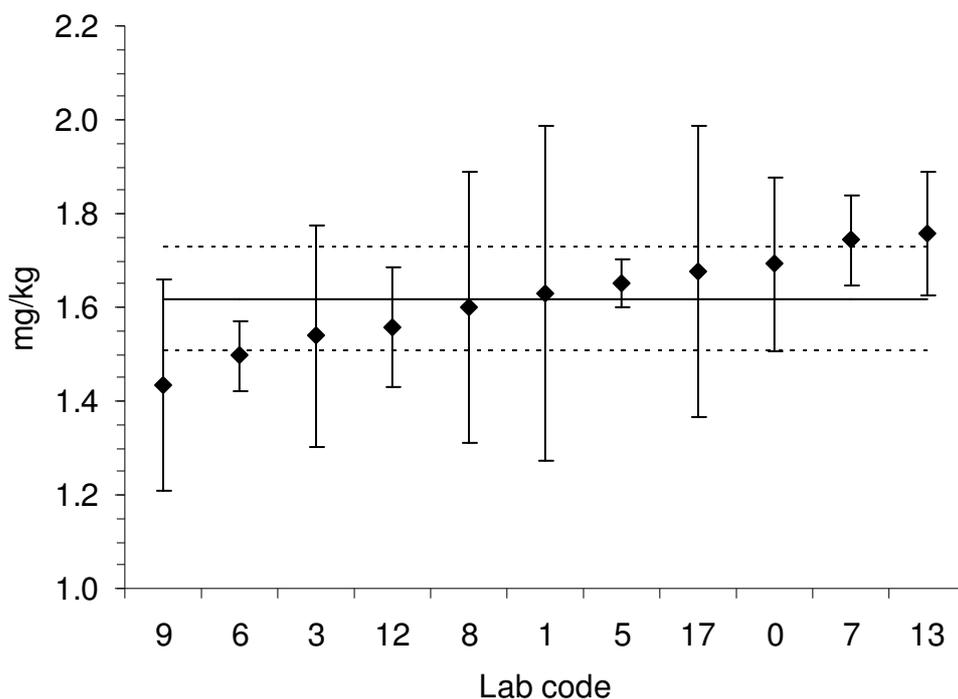
# Rb

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
0	k0-NAA	2.48	2.25	2.41	2.37	2.55	2.45	2.418	0.251
1	ICP-SFMS	2.32	2.33	2.34	2.32	2.39	2.41	2.352	0.456
6	ICP-MS	2.585708	2.565056	2.595002	2.541533	2.55598	2.589	2.572	0.129
7	k0-NAA	2.55	2.53	2.57	2.33	2.45	2.44	2.478	0.182
9	ICP-MS	2.3	2.3	2.3	2.3	2.3	2.3	2.300	0.280
13	K0-INAA	2.54	2.51	2.56	2.58	2.59	2.29	2.512	0.224
14	ICP-MS	2.81	2.78	2.82	2.85	2.68	2.72	2.777	0.139
16	ICP-SFMS	2.6	2.76	2.71	2.67	2.61	2.64	2.665	0.131
17	ICP-MS	2.06	2.05	2.06	2.11	2.06	2.1	2.073	0.425



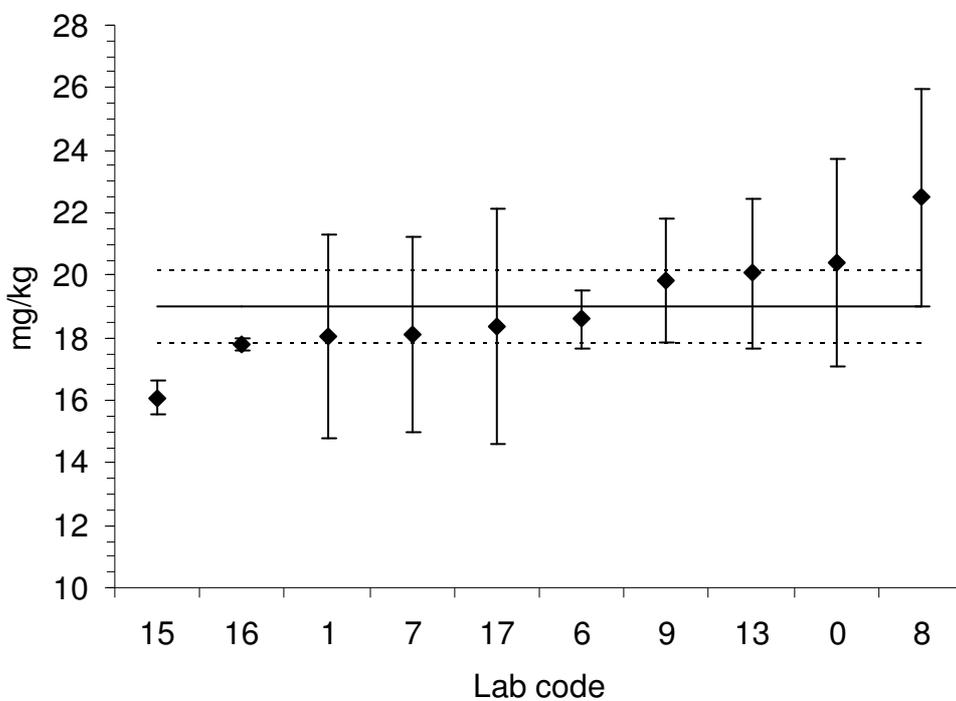
## Se

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
0	k0-NAA	1.84	1.47	1.63	1.67	1.71	1.83	1.692	0.184
1	ICP-SFMS	1.61	1.64	1.63	1.62	1.63	1.64	1.628	0.357
3	HG-AAS	1.55	1.59	1.6	1.49	1.5	1.5	1.538	0.236
5	ID-ICP-MS	1.678	1.712	1.653	1.626	1.613	1.623	1.651	0.053
6	ICP-MS	1.484924	1.435357	1.498348	1.610773	1.522031	1.431225	1.497	0.075
7	k0-NAA	1.79	1.77	1.73	1.65	1.79	1.73	1.743	0.095
8	HG-AAS	1.6	1.6	1.6	1.6	1.6	1.6	1.600	0.290
9	ICP-MS	1.5	1.5	1.3	1.5	1.4	1.4	1.433	0.225
12	ICP-MS	1.47	1.55	1.57	1.54	1.61	1.6	1.557	0.129
13	k0-INAA	1.73	1.77	1.81	1.75	1.73	1.76	1.758	0.132
17	ICP-MS				1.67	1.68	1.68	1.677	0.311
<i>Results not used for certification</i>									
2	ICP-MS	1.789	1.471	1.794	2.084	1.874	2.05		
4	ICP-MS	1.66	1.64	1.74	1.72	1.7	1.47		
14	ICP-MS	2.18	2.19	2.22	2.21	2.23	2.23		
15	ICP-OES	2.15	2.11	2.18	2.16	2.02	2.39		
16	ICP-SFMS	2.26	2.23	2.12	2.16	2.2	2.21		



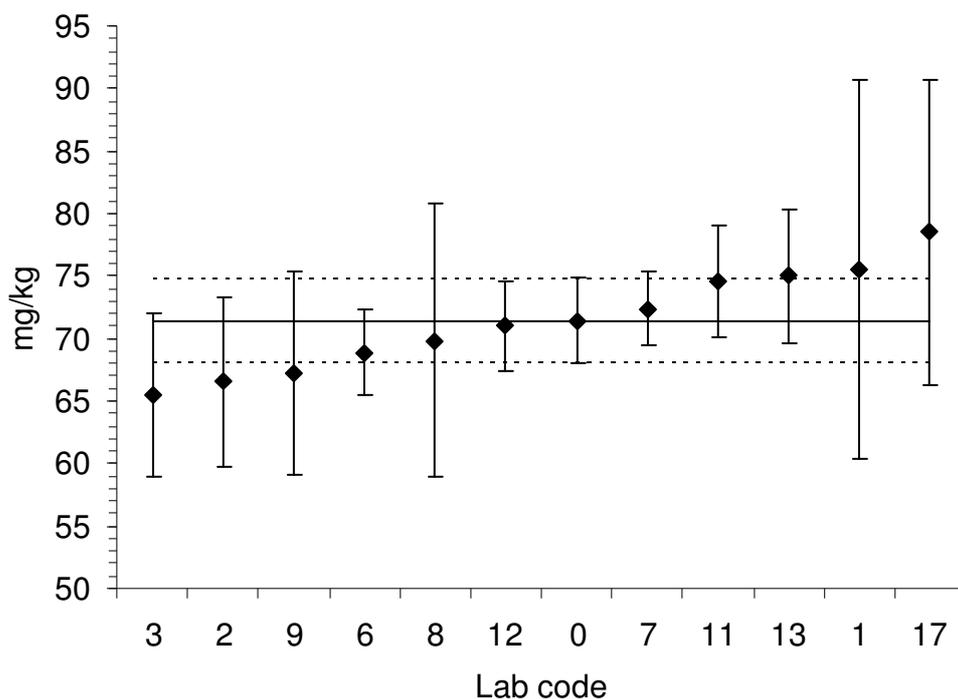
# Sr

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
0	k0-NAA	21.8	20	20.2	18.4	22	19.9	20.38	3.33
1	ICP-SFMS	18.3	18	18.1	17.9	17.9	18.2	18.07	3.26
6	ICP-MS	18.24659	18.29822	18.41181	18.81127	18.81127	18.97637	18.59	0.93
7	k0-NAA	17.6	18.1	18.8	18.3	17.9	18	18.12	0.93
8	ICP-OES	23	22	23	23	22	22	22.50	3.48
9	ICP-MS	19	20	20	20	20	20	19.83	1.98
13	k0-INAA	19.2	20.2	21.2	19.2	18.9	21.8	20.08	2.40
15	ICP-OES	16.09	16.19	16.09	15.98	16.19	15.98	16.09	0.52
16	ICP-SFMS	17.99	17.79	17.58	18.3	17.16	17.78	17.77	0.19
17	ICP-MS	18	18.2	18.6	18.5	18.4	18.3	18.33	3.77



## Zn

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
0	k0-NAA	75.8	66.3	69.6	71.5	74.2	71.2	71.4	3.5
1	ICP-SFMS	76.3	75.8	75.1	76.5	74.9	74.7	75.6	15.2
2	ICP-OES	59.148	69.772	67.366	66.78	68.046	68.17	66.5	6.8
3	FAAS	64.8	65.7	66.2	65.8	65.4	65.1	65.5	6.6
6	ICP-MS	73	73.1	73	71.4	72	72	72.4	3.0
7	k0-NAA	64.17	63.84	64.12	62.37	62.86	62.94	63.4	2.6
8	ICP-OES	70	69	70	70	70	70	69.8	11.0
9	ICP-MS	68	67	67	68	67	66	67.2	8.1
11	FAAS	73.44	74.81	74	75.3	74.46	75.66	74.6	4.5
12	ICP-MS	71.8	71.6	71.4	70.9	70.2	70.3	71.0	3.6
13	k0-INAA	74.7	75.5	76.3	75	74.5	74.2	75.0	5.4
17	ICP-OES	80	79	78	77	78	79	78.5	12.2
<i>Results not used for certification</i>									
4	ICP-MS	71.86	70.77	73.51	71.6	66.78	58.77		
14	ICP-MS	76.48	78.15	78.56	78.77	78.36	79.29		
15	ICP-OES	78.87	77	76.28	76.28	77.84	78.36		
16	ICP-SFMS	75.39	73.11	74.25	78.16	76.4	72.27		



**EUR 25523 EN – Joint Research Centre – Institute for Reference Materials and Measurements**Title: The certification of the mass fractions of elements in mussel tissue ERM<sup>®</sup>-CE278k

Authors: J. Snell, E. De Vos, H. Emteborg, H. Schimmel

Luxembourg: Publications Office of the European Union

2012 – 94 pp. – 21.0 x 29.7 cm

EUR – Scientific and Technical Research series – ISSN 1831-9424 (online)

ISBN 978-92-79-26479-5

doi:10.2787/6829

**Abstract**

This report describes the production of ERM<sup>®</sup>-CE278k, a mussel tissue material certified for the mass fractions of 13 elements. The material was produced following ISO Guide 34:2009.

The starting material was wild mussels (*mytilus edulis*) harvested off the coast of the Netherlands. The mussels were collected in late spring, steam-cooked, shelled and frozen. About 150 kg of mussel flesh was freeze-dried, frozen in liquid nitrogen and milled before sieving to obtain a fine powder.

Between-unit heterogeneity was quantified and stability during dispatch and storage was assessed in accordance with ISO Guide 35:2006. Within-unit heterogeneity was quantified to determine the minimum sample intake.

The material was characterised by an intercomparison among laboratories of demonstrated competence and adhering to ISO/IEC 17025. Technically invalid results were removed but 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 (GUM) and include uncertainties related to possible heterogeneity and instability and to characterisation.

The material is intended for quality control and assessment of method performance. As any reference material, it can also be used for control charts or validation studies. The CRM is available in glass vials containing 8 g of dry powder closed under a nitrogen atmosphere.

The minimum amount of sample to be used is 200 mg.

The following values were assigned:

	Mass fraction	
	Certified value <sup>1,2)</sup> [mg/kg]	Uncertainty <sup>2,3)</sup> [mg/kg]
As	6.7	0.4
Cd	0.336	0.025
Cr	0.73	0.22
Cu	5.98	0.27
Fe	161	8
Hg	0.071	0.007
Mn	4.88	0.24
Ni	0.69	0.15
Pb	2.18	0.18
Rb	2.46	0.16
Se	1.62	0.12
Sr	19.0	1.2
Zn	71	4

1) Unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory and/or with a different method of determination. The certified value and its uncertainty are traceable to the International System of Units (SI).

2) Certified mass fractions are corrected for the water content of the material (and expressed as dry mass), determined as described in the section "Instructions for use".

3) The certified uncertainty is the expanded uncertainty with a coverage factor  $k = 2$  corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.

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ISBN 978-92-79-26479-5



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