European Commission

ERM
European Reference Materials

CERTIFICATION REPORT

The certification of the mass fractions of elements in Trout Muscle: ERM®- CE101

EUR 29355 EN

European Commission
Joint Research Centre
Directorate F – Health, Consumers and Reference Materials

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JRC113148

EUR 29355 EN

ISBN 978-92-79-94029-3 (PDF)
ISSN 1831-9424 (online)
doi: 10.2760/099555

Luxembourg: Publications Office of the European Union, 2019

Abstract

This report describes the production of ERM-CE101, which is a matrix material certified for the mass fractions of elements. This material was produced following ISO Guide 34:2009 and is certified in accordance with ISO Guide 35:2006.
The material was produced from fresh fish muscle from both trout and Nile perch. Batches of the fish muscle were frozen and cryogenically milled before being blended in mixing/cutting equipment. The resulting paste was pre-cooked and filled into glass jars. The jars were capped and autoclaved to ensure preservation of the sample.
Between unit-homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2006. Within-unit homogeneity was quantified to determine the minimum sample intake.
The material was characterised by an interlaboratory comparison of 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 accordance with the Guide to the Expression of Uncertainty in Measurement (GUM) and include uncertainties related to possible inhomogeneity, instability and characterisation.
The material is intended for quality control and assessment of method performance. As with any reference material, it can be used for establishing control charts or validation studies. The CRMs is available in glass jars containing at least 40 g of homogenised fish muscle, which were sealed with a pop-up safety lid. The minimum amount of sample to be used is 500 mg..

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The certification of the mass fractions of elements in Trout Muscle: ERM®- CE101

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Disclaimer

Certain commercial equipment, instruments, and materials are identified in this paper to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the European Commission, nor does it imply that the material or equipment is necessarily the best available for the purpose.

Summary

This report describes the production of ERM-CE101, which is a matrix material certified for the mass fractions of elements. This material was produced following ISO Guide 34:2009 [1] and is certified in accordance with ISO Guide 35:2006 [2].

The material was produced from fresh fish muscle from both trout and Nile perch. Batches of the fish muscle were frozen and cryogenically milled before being blended in mixing/cutting equipment. The resulting paste was pre-cooked and filled into glass jars. The jars were capped and autoclaved to ensure preservation of the sample.

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

The material was characterised by an interlaboratory comparison of 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 accordance with the Guide to the Expression of Uncertainty in Measurement (GUM) [3] and include uncertainties related to possible inhomogeneity, instability and characterisation.

The material is intended for quality control and assessment of method performance. As with any reference material, it can be used for establishing control charts or validation studies. The CRMs is available in glass jars containing at least 40 g of homogenised fish muscle, which were sealed with a pop-up safety lid. The minimum amount of sample to be used is 500 mg.

The following values were assigned:

	Mass Fraction	
	Certified value [1)] [mg/kg]	Uncertainty [2)] [mg/kg]
Hg	0.0219	0.0027
As	0.175	0.017
Fe	3.1	0.6
Mn	0.108	0.017
Se	0.113	0.011
Zn	4.5	0.6

[1)] Certified values are values that fulfil the highest standards of accuracy and represent the 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)] The uncertainty is the expanded uncertainty of the certified value 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
ASTM international	ASTM international (formerly American Society for Testing and Materials)
ANOVA	Analysis of variance
AOAC	AOAC International (formerly Association of Official Analytical Chemists)
BCR®	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
CEN	European Committee for Standardization
CI	Confidence interval
CIPM	CIPM Comité International des Poids et Mesures (International Committee of Weights and Measures)
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
DMA	Direct mercury analyser
EA	European co-operation for Accreditation
EC	European Commission
EN	European norm (standard)
EQS	Environmental Quality Standard
ERM®	Trademark of European Reference Materials
EU	European Union
ETAAS	Electrothermal atomic absorption spectrometry
FAAS	Flame atomic absorption spectrometry
GUM	Guide to the Expression of Uncertainty in Measurements *[ISO/IEC Guide 98-3:2008]*
HG-AFS	Hydride generation-atomic fluorescence spectrometry
IAEA	International Atomic Energy Agency
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
IEC	International Electrotechnical Commission
ILAC	International Laboratory Accreditation Cooperation
ILC	Interlaboratory comparison
(I)NAA	(Instrumental) neutron activation analysis
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JCGM	Joint Committee for Guides on Metrology
JRC	Joint Research Centre of the European Commission
k	Coverage factor
k_0-NAA	k_0-Neutron activation analysis
LOD	Limit of detection
LOQ	Limit of quantification
M	Molar mass
MS	Mass spectrometry
$MS_{between}$	Mean of squares between-unit from an ANOVA
MSDS	Material safety data sheet
MS_{within}	Mean of squares within-unit from an ANOVA
n	Number of replicates per unit
N	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)
PT	Proficiency testing
PTV	Programmable temperature vaporiser
QA	Quality assurance
QC	Quality control
rel	Index denoting relative figures (uncertainties etc.)
RM	Reference material
RMP	Reference material producer
RNAA	Radiochemical neutron activation analysis
RSD	Relative standard deviation
RSE	Relative standard error (=RSD/$\sqrt{n}$)
RT	Room temperature

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
RM Unit	Reference Materials Unit of Directorate F
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
TG	Thermogravimetry
$t_{\alpha, df}$	Critical t-value for a t-test, with a level of confidence of 1-α 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 inhomogeneity that could be hidden by method repeatability/intermediate precision; an additional index "rel" is added as appropriate
u_{bb}	Standard uncertainty related to a possible between-unit inhomogeneity; an additional index "rel" is added as appropriate
u_c	combined standard uncertainty; an additional index "rel" is added as appropriate
u_{cal}	Standard uncertainty of calibration
u_{char}	Standard uncertainty of the material characterisation; an additional index "rel" is added as appropriate
u_{CRM}	Combined standard uncertainty of the certified value; an additional index "rel" is added as appropriate
U_{CRM}	Expanded uncertainty of the certified value; an additional index "rel" is added as appropriate
u_Δ	Combined standard uncertainty of measurement result and certified value

u_{lts}	Standard uncertainty of the long-term stability; an additional index "rel" is added as appropriate
u_{meas}	Standard measurement uncertainty
U_{meas}	Expanded measurement uncertainty
u_{rec}	Standard uncertainty related to possible between-unit inhomogeneity modelled as rectangular distribution; an additional index "rel" is added as appropriate
u_{sts}	Standard uncertainty of the short-term stability; an additional index "rel" is added as appropriate
u_t	Standard uncertainty of trueness
V	Volume
VIM	International Vocabulary of Metrology – Basic and General Concepts and Associated Terms *[ISO/IEC Guide 99:2007]*
WFD	Water Framework Directive
$\overline{x}$	Arithmetic mean
$\overline{x}_{ns}$	Arithmetic mean of all results of normal stock samples
$\overline{x}_{ref}$	Arithmetic mean of results of reference samples
α	significance level
Δ_{meas}	Absolute difference between mean measured value and the certified value
$\nu_{s,meas}$	Degrees of freedom for the determination of the standard deviation s_{meas}
$\nu_{MSwithin}$	Degrees of freedom of MS_{within}

1 Introduction

1.1 Background

Chemical pollution of surface water presents a threat to the aquatic environment with effects such as acute and chronic toxicity to aquatic organisms, accumulation in the ecosystem and losses of habitats and biodiversity, as well as a threat to human health.

Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy lays down a strategy against pollution of water and requires further specific measures for pollution control and environmental quality standards (EQS). Directive 2008/105/EC lays down EQS in accordance with the provisions and objectives of Directive 2000/60/EC. In this, member states are obliged to apply, for mercury and its compounds, an EQS of 20 µg/kg Hg in fish (wet weight).

Commission Directive 2009/90/EC also lays down technical specifications for chemical analysis and monitoring of water status in support of Directive 2000/60/EC, and recommends methods be validated according to EN ISO/IEC 17025.

However, validation of methods to measure mercury at this EQS might present a challenge for laboratories. Most CRMs for fish muscle contain mercury at levels close to the legislative limits for food (Commission Regulation (EC) No 1881/2006). These limits are over an order of magnitude higher than the EQS. Hence, a new CRM with Hg content close to the EQS would be an important tool for validation of methods to measure Hg at this level.

1.2 Choice of the material

ERM-CE101 is homogenised wet fish muscle, prepared so as to resemble samples of fresh fish muscle as closely as possible. It was prepared by blending different amounts of fish muscle from two species of fish, brown trout (Salmo trutta) and Nile perch (Lates niloticus), so that the CRM would have a mass fraction of Hg close to 20 µg/kg.

1.3 Design of the CRM project

To determine the mass fractions of elements in the material, an inter-laboratory comparison was made, using results from independent laboratories selected for their expertise in measurement of elements in fish. Certified mass fractions are the unweighted mean values 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 Participants

2.1 Project management and evaluation

European Commission, Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, 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, Directorate F – Health, Consumers and Reference Materials, Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

2.3 Homogeneity study

European Commission, Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

ALS Laboratory Group, ALS Scandinavia AB, Luleå (SE)
(measurements under the scope of ISO/IEC 17025 accreditation SWEDAC; accreditation number 1087)

2.4 Stability study

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

ALS Laboratory Group, ALS Scandinavia AB, Luleå (SE)
(measurements under the scope of ISO/IEC 17025 accreditation SWEDAC; accreditation number 1087)

2.5 Characterisation

European Commission, Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Geel, BE
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ALS Laboratory Group, ALS Scandinavia AB, Luleå, SE

(measurements under the scope of ISO/IEC 17025 accreditation SWEDAC; accreditation number 1087)

The Food and Environment Research Agency, York, UK

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

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

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Havforskningsinstiuttet, Bergen, NO

(Measurements performed under ISO/IEC 17025 accreditation; Norsk Akkreditering; TEST 050)

Sciensano, Tervuren, BE

(Measurements performed under ISO/IEC 17025 accreditation; BELAC No. 172-TEST)

VITO NV, Mol, BE

3 Material processing and process control

3.1 Origin of the starting material

To obtain element contents close to the target values, the material was prepared from two fish species, trout (*Salmo trutta*) and Nile perch (*Lates niloticus*). The trout was supplied from two different sources. About 50 kg of fillets with skin was supplied from an aquaculture company near Malmedy, BE and the second batch of 40 kg originated from Italy and was delivered without skin. The 25 kg of Nile perch was bought at a local supermarket.

3.2 Processing

All batches of fish fillets were frozen at -20 °C after arrival. All fish was cut in cubes, any skin was removed and then shock-frozen in liquid nitrogen. The deep-frozen cubes were then stored in stainless steel drums over liquid nitrogen keeping the different species separated from each other.

About 80 kg of trout filet was cryogenically milled using a Palla VM-KT cryogenic vibrating mill (KHD, Köln, DE) and the resulting batches were kept at +4 °C until mixing. Thereafter 25 kg of Nile perch was cryo-milled and kept aside. In between these steps the cryogenic mill was cleaned with about 5 kg of crushed ice to prevent carry-over between the different fish species. The pooled trout materials and Nile perch were homogenised separately in a Stephan UM200 cutter/mixer (Hameln, DE) and then kept at -20 °C. Samples were taken in triplicate from each batch for measurement of the mercury content. Based on the results, different masses of the milled fish could be blended to achieve a desired mercury content.

The trout and Nile-perch pastes were thawed in a +4 °C cool cell. After all material had thawed, 60.03 kg of trout and 21.04 kg of nile perch were placed in a mixing bowl. After mixing for 5 minutes the double jacket of the mixing bowl was heated with steam to the pre-cook the paste, which is required before thermal sterilisation. The temperature was kept at 82 °C for 5 minutes under continuous mixing, thereafter the double jacked was cooled to about 40 °C and filling of jars was started.

The fish paste was transferred to a Unifiller (Lörrach, DE) and 40 g portions of paste were filled into 60 mL glass jars. The jars had been washed with 2 % nitric acid, rinsed with purified (18.2 MΩ.cm) water and dried in a dust-free environment before use. After about 3.5 h of the filling process the texture and consistency of the remaining paste had changed, so it was mixed again for about 15 minutes to slightly increase the temperature of the material. Twist-off lids of 66 mm diameter were placed on the jars using a Lenssen twist-off machine (Sevenum, NL). The lids are placed on the jars inside chamber filled with steam, which results in an under-pressure in the head space over the paste after cooling down. In this way the sensor of the lid will remain in place as long as the seal is not broken. Upon opening the lid will pop open with a click if the under-pressure has been maintained.

The filled jars were placed a metallic basket according to fill-order and were sterilised using a JBT autoclave with a program of about one hour (JBT, Sint-Niklaas, Belgium). Heating takes place by a steam-water spray process. When the program ended, the jars were removed, wiped dry with a paper cloth and stored at +4°C. Two batches were autoclaved. A few narrow-mouth 50 mL jars were also filled with fish paste and placed in the autoclave. These jars were deep enough to fit a probe that allowed real-time monitoring of the temperature inside the material during autoclaving. This confirmed that the core temperature had indeed reached 121 °C. The peak temperature was maintained for about 10 min. After cooling, labels were fixed to the lids that included a number corresponding to the filling sequence.

3.3 Process control

Average particle size distribution was measured in the paste just before filling, after pre-cooking at 82 °C. Measurements were made in triplicate, using a Helos laser light diffraction instrument (Sympatec, Clausthal Zellerfeld, DE). No particles larger than 420 µm were detected (X_{99}). The material in the jars after thermal sterilisation was found to contain larger granulates with a relatively large variability in size. This indicates that agglomerates were formed in an unpredictable way during autoclaving. Users are therefore recommended to mix the contents of the jar before taking a sample for analysis, to break agglomerates and re-mix the small amount of water that may surround the paste.

The certified element mass fractions were given for the material on a wet weight basis, as it is not intended to perform dry mass correction of analytical results. However, to control the production process, water content was measured in triplicate on 10 jars selected to cover the whole production sequence. Samples of between 6 and 8 g were dried in a ventilated oven at 102 °C, until they reached constant mass. A mean water content of 75 g/100g was found with a relative standard deviation of 1 g/100g over all measured samples. This showed that the water content corresponds to that normally found in fresh fish tissue and that the content is consistent between jars.

4 Homogeneity

A key requirement for any reference material aliquotted into units is equivalence between those units. In this respect, it is relevant whether the variation between units is significant compared to the uncertainty of the certified value, but it is not relevant if this variation between units is significant compared to the analytical variation. Consequently, ISO Guide 34 [1] requires RM producers to quantify the between unit variation. This aspect is covered in between-unit homogeneity studies.

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

4.1 Between-unit homogeneity

The between-jar homogeneity was evaluated to ensure that the certified values of the CRM are valid for all jars of the material, within the stated uncertainties.

The number of jars selected corresponds to approximately the cube root of the total number of jars produced. The 12 jars were selected using a random stratified sampling scheme covering the whole batch for the between-jar homogeneity test. For this, the batch was divided into 12 groups (with a similar number of jars) and one jar was selected randomly from each group. Three independent samples were taken from each selected jar, and analysed by ICP-MS after microwave-assisted acid digestion. The measurements were performed under repeatability conditions, and in a randomised manner to be able to separate a potential measurement drift from a trend in the filling sequence. The results are shown as graphs/Tables in Annex A.

Regression analyses were performed to evaluate potential trends in the analytical sequence as well as trends in the filling sequence. For some elements, trends in the filling sequence or the measurement sequence were observed at a 95 % confidence level. Filling trends were observed for Fe and Zn. A significant (95 % confidence level) trend in the analytical sequence for Ni was visible, pointing at a changing parameter, e.g. a signal drift in the analytical system. The correction of biases, even if they are statistically not significant, was found to combine the smallest uncertainty with the highest probability to cover the true value

[4]. The correction of the trend for Ni is therefore expected to improve the sensitivity of the subsequent statistical analysis through a reduction in analytical variation without masking potential between-jar heterogeneities. As the measurement sequence and the jar numbers were not correlated, trends significant on at least a 95 % confidence level were corrected as shown below:

$$x_{i\_corr} = x_i - b \cdot i \qquad \textbf{Equation 1}$$

b **=** slope of the linear regression

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

The datasets, trend-corrected where necessary, were assessed for consistency using Grubbs outlier tests at a confidence level of 99 % on the individual results and on the jar means. No outlying individual results or outlying jar means were detected.

Quantification of between-jar inhomogeneity was undertaken by analysis of variance (ANOVA), which separates the between-jar variation (s_{bb}) from the within-jar variation (s_{wb}). The latter is equivalent to the method repeatability if the individual samples were representative for the whole jar.

Evaluation by ANOVA requires mean values per jar, which follow at least a unimodal distribution and results for each jar that follow unimodal distributions with approximately the same standard deviations. The distribution of the mean values per jar was visually tested using histograms and normal probability plots. Too few data are available for the jar means to make a clear statement of the distribution. Therefore, it was checked visually 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-jar standard deviations. The results of all statistical evaluations are given in Table 1.

Table 1: Results of the statistical evaluation of the homogeneity studies

Method/Measurand	Trends (before correction)*		Outliers**		Distribution	
	Analytical sequence	Filling sequence	Individual results	Jar means	Individual results	Jar means
As	no	no	none	none	unimodal	unimodal
Cd	no	no	none	none	unimodal	unimodal
Fe	no	yes	none	none	unimodal	unimodal
Hg	no	no	none	none	unimodal	unimodal
Mn	no	no	none	none	unimodal	unimodal
Ni	yes	no	none	none	unimodal	unimodal
Pb	no	no	none	none	unimodal	unimodal
Se	no	no	none	none	unimodal	unimodal
Zn	no	yes	none	none	unimodal	unimodal

* 95 % confidence level

** 99 % confidence level

It should be noted that $s_{bb,rel}$ and $s_{wb,rel}$ are estimates of the true standard deviations and are 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-jar variation, whereas the true variation cannot be lower than zero. In this case, u^*_{bb}, the

maximum inhomogeneity that could be hidden by method repeatability, was calculated as described by Linsinger *et al.* [5]. u^*_{bb} is comparable to the LOD of an analytical method, yielding the maximum inhomogeneity that might be undetected by the given study setup.

Method repeatability ($s_{wb,rel}$), between–jar standard deviation ($s_{bb,rel}$) and $u^*_{bb,rel}$ were calculated as:

$$s_{wb,rel} = \frac{\sqrt{MS_{within}}}{\bar{y}} \qquad \textbf{Equation 2}$$

$$s_{bb,rel} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}} \qquad \textbf{Equation 3}$$

$$u^*_{bb,rel} = \frac{\sqrt{\frac{MS_{within}}{n}}\sqrt[4]{\frac{2}{v_{MSwithin}}}}{\bar{y}} \qquad \textbf{Equation 4}$$

MS_{within} mean of squares within-jar from an ANOVA

$MS_{between}$ mean of squares between-jar from an ANOVA

$\bar{y}$ mean of all results of the homogeneity study

n mean number of replicates per jar

$v_{MSwithin}$ degrees of freedom of MS_{within}

When a trend in the filling sequence was significant at least at 99 % confidence level, the uncertainty was assessed in a different way. This applies for Fe and Zn. Here, u_{rec} was estimated using a rectangular distribution between the highest and lowest jar mean. The corrected uncertainty in those cases where there was a significant trend in the filling sequence is given in:

$$u_{rec} = \frac{|highest\ result - lowest\ result|}{2 \cdot \sqrt{3} \cdot \bar{y}} \qquad \textbf{Equation 5}$$

Table 2: Results of the homogeneity studies

mass fractions in ERM-CE101	$s_{wb,rel}$ [%]	$s_{bb,rel}$ [%]	$u^*_{bb,rel}$ [%]	$u_{rec,rel}$ [%]	$u_{bb,rel}$ [%]
As	3.7	3.8	1.1	n.a.	3.8
Cd	26.0	n.c.	8.1	n.a.	8.1
Fe	11.0	4.9	3.4	7.2	7.2
Hg	8.2	3.8	2.6	n.a.	3.8
Mn	16.3	5.2	5.0	n.a.	5.2
Ni	12.5	7.7	3.8	n.a.	7.7
Pb	19.4	9.4	6.1	n.a.	9.4
Se	6.8	1.3	2.1	n.a.	2.1
Zn	4.5	3.9	1.4	4.6	4.6

[1)] n.c.: cannot be calculated as $MS_{between} < MS_{within}$

[2)] n.a.: not applicable

For most elements, the homogeneity study showed no outlying jar means or trends in the filling sequence. In these cases, the between-jar 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 inhomogeneity.

Trends in the filling sequence were found for Fe and Zn. However, taking the range of values for both elements into account, the inhomogeneity as quantified as u_{rec} is still sufficiently small to make the material useful. Therefore, u_{rec} was used as estimate of u_{bb}.

4.2 Within-jar homogeneity and minimum sample intake

The within-jar homogeneity is closely correlated to the minimum sample intake. Due to this correlation, 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 jar and thus should be used in an analysis. Using sample sizes equal or above the minimum sample intake guarantees the certified value within its stated uncertainty.

Homogeneity experiments were performed using a 500 mg sample intake. This sample intake gives acceptable repeatability/intermediate precision, demonstrating that the within-unit inhomogeneity no longer contributes to analytical variation at this sample intake.

5 Stability

Time, temperature, light (including ultraviolet radiation) and the presence of mould or bacteria were regarded as the most relevant influences on the stability of the material. The influence of ultraviolet or visible light was minimised materials being stored in the dark and dispatched in boxes, thus removing any possibility of degradation by light. Additionally the material was sterilized by heat treatment to eliminate microbial growth. Therefore, only the influences of time and temperature needed to be investigated.

Stability testing is necessary to establish the conditions for storage (long-term stability) as well as the conditions for dispatch of the materials to the customers (short-term stability). During transport, especially in summer time, temperatures up to 60 °C can be reached and stability under these conditions must be demonstrated, if the samples are to be transported without any additional cooling.

The stability studies were carried out using an isochronous design [6]. In this approach, samples were stored for a particular length of time at different temperature conditions. Afterwards, the samples were moved to conditions where further degradation can be assumed to be negligible (reference conditions). At the end of the isochronous storage, the samples were 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, samples were stored at 18 °C and 60 °C for 0, 1, 2 and 4 weeks (at each temperature). The reference temperature was set to 4 °C. Two jars per storage time were selected using a random stratified sampling scheme. From each jar, three samples were measured by ICP-MS after microwave assisted acid digestion. The measurements were performed under repeatability conditions, and a randomised sequence was used to differentiate any potential analytical drift from a trend over storage time.

The data were evaluated individually for each temperature. The results were screened for outliers using the single and double Grubbs test on a confidence level of 99 %. No outlying individual results were found (Table 3).

In addition, the data were evaluated against storage time, and regression lines of mass fraction versus time were calculated, to test for potential increases/decrease of the element mass fractions due to shipping conditions. The slopes of the regression lines were tested for statistical significance. Trends statistically significant at a 95 % confidence level were found for As in the 18 ºC study and for Hg and Pb in the 60 ºC study.

The results of the measurements are shown in Annex C. The results of the statistical evaluation of the short-term stability are summarised in Table 3.

Table 3: Results of the short-term stability tests

mass fractions in ERM-CE101	Number of individual outlying results*		Significance of the trend **	
	18 ºC	60 ºC	18 ºC	60 ºC
As	none	none	yes	no
Cd	none	none	no	no
Fe	none	none	no	no
Hg	none	none	no	yes
Mn	none	none	no	no
Ni	none	none	no	no
Pb	none	none	no	yes
Se	none	none	no	no
Zn	none	none	no	no

* 99 % confidence level

** 95 % confidence level

No technically unexplained outliers/Statistical outliers were detected for any of the elements, and all data was retained for the estimation of u_{STS}.

A significant increasing trend for As at 18 °C was found, but the material appeared to be stable at 60 °C. As it is unlikely that the material degrades faster at lower temperature than at higher one, this was regarded as statistical artefact. A positive trend also was observed for Pb at 60 °C. As the analyte cannot be created in the sample, a positive trend could only be due to degradation of the matrix. This, however, should be seen for all measurands, which is not the case. The observed trend was therefore regarded as statistical artefact.

A negative trend was observed for Hg at 60 °C. As the possibility of loss of volatile Hg compounds on storage cannot be excluded, the potential for change in the Hg value should be taken into account on transport.

The material shall be shipped under cooled conditions.

5.2 Long-term stability study

Data from two isochronous stability studies were combined to assess the stability of the CRM. An initial 12-month study was conducted as a precaution, as the stability of this CRM matrix is largely unknown, and was followed by a 24-month study.

For the first isochronous study, 8 jars were stored at 18 °C for 0, 4, 8 and 12 months. The reference temperature was set to -20 °C. Two jars per storage time were selected using a random stratified sampling scheme. For the second isochronous study, 8 jars were stored at 18 °C for 0, 8, 16 and 24 months. The reference temperature was set to -20 °C. Freezing of the reference samples is assumed to render the material degradation negligible. Two jars per storage time were selected using a random stratified sampling scheme. From each vial within the 12-month study, 3 samples were measured, and from each vial in the 24-month study 4 samples. These were analysed by ICP-MS after microwave assisted acid digestion. This design allows separation of a potential analytical drift from a trend over storage time. The measurements for each study were performed under repeatability conditions on separate occasions a year apart. A normalisation was applied to take into account differences between the two studies.

For Ni, an alternative stability test was made as the relative standard deviations of measurements for the previous (homogeneity and short-term stability) studies were relatively high. This indicated that the measurement performance would not be adequate to obtain a useful estimate of the uncertainty of stability during storage. Therefore, a study was made by comparative analysis of 3 jars of the CRM stored at the normal storage temperature (4 °C) and 3 reference samples (placed at -20 °C directly after the material processing). All the samples were analysed (with 3 replicates per each jar) by microwave-assisted acid digestion followed by ICP-MS. A measurement sequence alternating normal and reference samples was used to avoid potential analytical drift from causing any difference in results between sample types.

For the combination of the 12- and 24-month isochronous studies, ANOVA was performed to test for significant differences between the measurements performed for the two studies. Some significant (95 % confidence level) differences between the studies were detected, and results were normalised using the following equation:

$$corrected\ \ result\ =\ measured\ \ result\ \times\ d_i \qquad \textbf{Equation 6}$$

with $d_i = \overline{x_1} / \overline{x_i}$

d_i normalisation factor for study i

$\overline{x_1}$ mean value measured for all vials in study 1

$\overline{x_i}$ mean value measured for all vials in study i

The obtained data were evaluated individually for each study. The results were screened for outliers using the Grubbs test at a confidence level of 99%. One statistical outlier was detected in the 24-month study for Cd, and the result was retained for the estimation of u_{lts}.

Furthermore, the data were plotted against storage time and 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). While the slopes of the regression lines were not significantly different from zero (95 % confidence level) in the 12-month study, trends showing reductions in Hg, Pb and Zn mass fractions over time were observed in the 24-month study.

Afterwards the results of the two isochronous studies were combined as described in [7]. A measurement bias between the two studies was found and corrected using the normalisation factor *d* as calculated by equation 9.

The long-term stability data were evaluated individually. The combined results were screened for outliers using the single and double Grubbs test at a confidence level of 99 %. Again, one outlying individual result was found for Cd (Table 4). As no technical reason for the outlier could be found all data were retained for statistical analysis.

Again, the combined data were plotted against storage time and regression lines of mass fraction versus time were calculated. The slope of the regression lines was tested for statistical significance. The slopes of the regression lines were significantly different from zero (95 % confidence level) for Hg, Pb, Se and Zn.

For Ni, The results were screened for outliers using the single and double Grubbs test at a confidence level of 99 %. No outlying individual results were found. The combined data were plotted against a storage time at 4 ºC of 47 months for the Normal Stock samples, and a regression line of mass fraction versus time was calculated. The slope of the regression line was not found to be significantly different from zero (95 % confidence level).

The results of the long-term stability measurements are shown in Annex C. The results of the statistical evaluation of the long-term stability study are summarised in Table 4.

Table 4: Results of the long-term stability tests

Mass fractions in ERM-CE101	Number of individual outlying results*	Significance of the trend**
	18 ºC	18 ºC
As	none	no
Cd	1-statistical, retained	no
Fe	none	no
Hg	none	yes
Mn	none	no
Ni	none	no
Pb	none	yes
Se	none	yes
Zn	none	yes

* 99 % confidence level

** 95 % confidence level

Negative trends were observed for Hg, Se and Zn at 18 °C, while a positive trend was seen for Pb. The trend for Pb can be disregarded, as a positive trend could only be due to degradation of the matrix, which was not seen for any of the other analytes. The observed trend was therefore regarded as statistical artefact. In contrast, there is no apparent explanation for the negative trends observed for Hg, Se and Zn. Therefore, the potential for analyte loss must be taken into account in estimation of the uncertainty associated to long term-stability.

5.3 Estimation of uncertainties

Due to the intrinsic variation of measurement results, no study can entirely rule out degradation of materials, 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/intermediate precision, i.e. to estimate the uncertainty of stability. This means that, even under ideal conditions, the outcome of a stability study can only be that there is no detectable degradation within an uncertainty to be estimated.

The uncertainties of stability during dispatch and storage were estimated, as described in [8] for each analyte. In this approach, the uncertainty of the linear regression line with a slope of zero was calculated. The uncertainty contributions u_{sts} and u_{lts} were calculated as the product of the chosen transport time/shelf life and the uncertainty of the regression lines as shown in equations 7 and 8: For the LTS studies for Hg, Se and Zn, the uncertainty was based on the magnitude of the observed slope, and its standard error, as shown in equation 9.

$$u_{sts,rel} = \frac{s_{rel}}{\sqrt{\sum \left(t_i - \bar{t}\right)^2}} \cdot t_{tt} \qquad \textbf{Equation 7}$$

$$u_{lts,rel} = \frac{s_{rel}}{\sqrt{\sum \left(t_i - \bar{t}\right)^2}} \cdot t_{sl} \qquad \textbf{Equation 8}$$

$$u_{sts,rel} = \sqrt{\left(\frac{b}{\sqrt{3}}\right)^2 + se_b{}^2} \qquad \textbf{Equation 9}$$

b	observed slope; change of mass fraction with time
s_{rel}	relative standard deviation of all results of the stability study
se_b	standard error of the observed slope
t_i	time elapsed at time point *i*
$\bar{t}$	mean of all t_i
t_{tt}	chosen transport time (1 week at 18 ºC)
t_{sl}	chosen shelf life (24 months at 18 ºC)

The following uncertainties were estimated:

- $u_{sts,rel}$, the uncertainty of degradation during dispatch. This was estimated from the 18 °C studies. The uncertainty describes the possible change during a dispatch at 18 °C lasting for one week.
- $u_{lts,rel}$, the stability during storage. This uncertainty contribution was estimated from the 18 °C studies. The uncertainty contribution describes the possible degradation during 24 months storage at 18 °C.

The results of these evaluations are summarised in Table 5.

Table 5: Uncertainties of stability during dispatch and storage. $u_{sts,rel}$ was calculated for a temperature of 18 °C and 1 week; $u_{lts,rel}$ was calculated for a storage temperature of 18 °C and 24 months

Mass fractions in ERM-CE101	$u_{sts,rel}$ [%]	$u_{lts,rel}$ [%]
As	0.6	1.5
Cd	4.0	5.9
Fe	2.1	4.0
Hg	1.3	4.1
Mn	2.0	4.9
Ni	2.6	5.3
Pb	1.8	5.9
Se	1.4	3.4
Zn	1.6	4.3

The material showed significant degradation at 60 °C but no significant degradation was observed for transport below 18 °C. Cooled shipment is therefore necessary. During the studies it was also observed that samples that had been stored at -20 ºC often lost the vacuum applied on sealing the lids. To preserve sample integrity it is therefore recommended that users of the CRM do not freeze the jars before use.

After the certification study, the material will be included in the JRC's regular stability monitoring programme, to control its further stability.

6 Characterisation

The material characterisation is the process of determining the property values of a reference material.

This was based on an interlaboratory comparison of expert laboratories, i.e. the properties of the material was 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

Thirteen 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 2).

6.2 Study setup

Each laboratory received 2 jars of ERM-CE101 and was requested to provide 6 independent results, 3 per jar. The units for material characterisation were selected using a random stratified sampling scheme and covered the whole batch. The sample preparations (if

necessary) and measurements had to be performed on two separate days to ensure intermediate precision conditions. Independent calibrations were performed for each day. Three laboratories offered to measure the same element or elements by two different measurement techniques. The laboratories were sent additional samples in this case, and made separate sample preparations and calibrations for each technique used.

Each participant received a sample of DORM-4 (a fish protein CRM, National Research Council, Ottawa, Canada) that had been re-bottled for use as a blinded quality control (QC) sample. Participants measured 1 sub-sample of the QC with ERM-CE101 on each day. The results for this sample were 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.

Characterisation of Cd and Pb was not attempted, as the mass fractions lay close to the LOQ of techniques for characterisation, and high variance in replicate measurements was observed in both homogeneity and stability studies. The studies (by ICP-MS) indicated that the mass fraction of Pb was in the range 5 – 8 µg/kg, but they did not show good agreement for Cd.

6.3 Methods used

A variety of digestion methods (based on microwave-assisted acid digestion) with different quantification steps (for example, AAS, ICP-OES, ICP-MS) as well as methods without sample preparation (NAA, pyrolysis-AAS) were used to characterise the material. The combination of results from methods based on completely different measurement principles mitigates undetected method bias.

All methods used during the characterisation study are summarised in Annex D. The laboratory code (e.g. L01) is a random number and does not correspond to the order of laboratories in Section 2. The lab-method code consists of a number assigned to each laboratory (e.g. L01) and abbreviation of the measurement method used, (for example, ICP-MS).

The water content was not determined by the laboratories, as the mass fractions of elements in the sample are to be certified as wet-weight.

6.4 Evaluation of results

The characterisation study resulted in between 9 and 12 datasets per element. All individual results of the participants, grouped per element are displayed in tabular and graphical form in Annex E.

6.4.1 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:

- appropriate validation of the measurement procedure
- compliance with the analysis protocol: sample preparations and measurements performed on two separate days
- absence of values given as either below limit of detection or below limit of quantification

- method performance, i.e. agreement of the measurement results with the assigned value of the QC sample, between-measurement standard deviation and between-day difference

Based on the above criteria, the following datasets were rejected as not technically valid (Table 6). In addition to the specific exclusions listed in Table 6, no data from laboratories L01, L03 and L11 was included as they reported after the reporting deadline.

When considering QC results, it was found that a total of 13 laboratory means, from different participants and for different elements, did not agree within the combined uncertainties of the assigned value and that reported for measurement. Laboratories' approaches to uncertainty estimation differ, and in most cases in which QC results did not agree, that laboratory reported lower uncertainties than most other participants despite using a similar technique. Therefore, a measurement uncertainty of 20 %, representing the median reported, was applied for the QC test for all laboratories.

Table 6: Datasets that showed non-compliances with the analysis protocol and technical specifications, for which actions were taken

Element mass fraction	Lab-method code	Description of problem	Action taken
Fe	L02	Only 2 measurements were made on each jar	not used for evaluation
Fe	L06	Results were below the reported LOQ	not used for evaluation
Fe	L16	Results showed excessive variance (79 % RSD), which could not be explained by sample inhomogeneity.	not used for evaluation
Mn	L13	Results were below the reported LOQ	not used for evaluation
Mn	L16	Results for the sample showed excessive variance (133 % RSD), which could not be explained by sample inhomogeneity. Result for the QC did not agree with the assigned value.	not used for evaluation
Ni	L13	Results were below the reported LOQ	not used for evaluation
Se	L04	Result for the QC did not agree with the assigned value.	not used for evaluation

6.4.2 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_{within}) and between ($s_{between}$) laboratories were calculated using one-way ANOVA. The results of these evaluations are shown in Table 7.

Table 7: Statistical evaluation of the technically accepted datasets for ERM-CE101. *p*: number of technically valid datasets

Element mass fraction	*p*	Outliers		Normally distributed	Statistical parameters			
		Means	Variances		Mean [mg/kg]	*s* [mg/kg]	$s_{between}$ [mg/kg]	s_{within} [mg/kg]
Hg	10	0	2	yes	0.0219	0.0014	0.0013	0.0016
As	11	0	1	yes	0.175	0.013	0.013	0.006
Fe	9	0	0	yes	3.09	0.26	0.21	0.35
Mn	10	0	2	yes	0.108	0.009	0.002	0.023
Ni	9	0	2	yes	0.0514	0.0105	0.0101	0.0069
Se	10	0	2	yes	0.113	0.009	0.008	0.013
Zn	12	0	1	yes	4.50	0.31	0.28	0.33

The laboratory means follow normal distributions. None of the data contains outlying means. The statistical evaluation flags a number of datasets as having outlying variance. This merely reflects the fact that different methods have different intrinsic variability. As all measurement methods were found technically sound, all results were retained. The datasets are therefore consistent and the mean of laboratory means is a good estimate of the true value. Standard deviations between laboratories are not larger than the standard deviation within laboratories for 4 of the 7 elements. In such cases, confidence intervals of replicate measurements could be used to estimate measurement uncertainty. However, as the two deviations are similar in all cases, the standard error of results between laboratories was used to make reliable estimates for all elements.

The uncertainty related to the characterisation is estimated as the standard error of the mean of laboratory means. (Table 8).

Table 8: Uncertainty of characterisation for ERM-CE101

Element mass fraction	*p*	Mean [mg/kg]	*s* [mg/kg]	u_{char} [mg/kg]
Hg	10	0.02190	0.00141	0.0004
As	11	0.175	0.013	0.004
Fe	9	3.09	0.21	0.09
Mn	10	0.108	0.009	0.003
Ni	9	0.0514	0.0105	0.0035
Se	10	0.113	0.009	0.003
Zn	12	4.50	0.31	0.09

7 Value Assignment

Certified and indicative values were assigned.

Certified values are values that fulfil the highest standards of accuracy. Procedures at the JRC, Directorate F 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' [4] 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.

7.1 Certified values and their uncertainties

The unweighted mean of the means of the accepted datasets as shown in Table 9 was assigned as certified value for each parameter.

The assigned uncertainty consists of uncertainties relating to characterisation, u_{char} (Section 6), potential between-unit inhomogeneity, u_{bb} (Section 4.1), and potential degradation during transport, u_{sts}, and long-term storage, u_{lts} (Section 5). The uncertainty related to degradation during transport was found to be negligible. These different contributions were combined to estimate the relative expanded uncertainty of the certified value ($U_{CRM,\ rel}$) with a coverage factor k given as:

$$U_{CRM,rel} = k \cdot \sqrt{u_{bb,rel}^2 + u_{sts,rel}^2 + u_{lts,rel}^2 + u_{char,rel}^2} \qquad \textbf{Equation 18}$$

- u_{char} was estimated as described in Section 6
- u_{bb} was estimated as described in Section 3.4.
- u_{sts} and u_{lts} were estimated as described in section 4.3

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 uncertainties. The certified values and their uncertainties are summarised in Table 13.

Table 9: Certified values and their uncertainties for ERM-CE101

ERM-CE101	Certified value [mg/kg]	u_{char} [mg/kg]	u_{bb} [mg/kg]	$u_{lts,}$ [mg/kg]	U_{CRM} [1)] [mg/kg]
Hg	0.0219	0.0004	0.0008	0.0009	0.0027
As	0.175	0.0039	0.0067	0.0027	0.017
Fe	3.1	0.09	0.22	0.12	0.6
Mn	0.108	0.0030	0.0056	0.0052	0.017
Se	0.113	0.0030	0.0024	0.0038	0.011
Zn	4.5	0.09	0.21	0.19	0.6

[1)] Expanded (k = 2) and rounded uncertainty.

7.2 Indicative values and their uncertainties

An Indicative value was assigned for Ni. The mass fraction of Ni lay close to the LOQ of many of the measurement procedures used for testing the material, and the combined relative uncertainty of the certified value is significantly higher than those of the other elements. In addition, all results used in the characterisation were obtained by the use of a single principle, ICP-MS, and traceability to the SI for this indicative value can only be realised when using this measurement principle. However, results from all nine laboratories that were accepted for characterisation agreed well, and the results were regarded as sufficiently trustworthy to assign an indicative value. An indicative value may not be used as certified value. The uncertainty budget was set up as for the certified values and is listed together with the assigned value in Table .

Table 10: Indicative values and their uncertainties for ERM-CE101

ERM-CE101	Indicative value [mg/kg]	$u_{char, rel}$ [%]	$u_{bb, rel}$ [%]	$u_{lts, rel}$ [%]	U_{CRM} [mg/kg] [1)]
Ni	0.051	0.0035	0.0040	0.0027	0.012

[1)] Expanded (k = 2) and rounded uncertainty.

8 Metrological traceability and commutability

8.1 Metrological traceability

Identity

The mass fractions of Hg, As, Fe, Mn, Se and Zn are chemically clearly defined properties. The participants used different methods for the sample preparation as well as for the final determination, demonstrating absence of measurement bias. The measurand is therefore structurally defined and independent of the measurement method.

The mass fraction of Ni has been obtained by ICP-MS measurement. This measurand is therefore operationally defined by ICP-MS measurement.

Quantity value

For the element mass fractions, only validated methods were used for the determination of the assigned values. Different calibrants/calibrants of (known purity and) 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 International System of units (SI), the assigned quantity values themselves are traceable to the SI as well.

8.2 Commutability

Many measurement procedures include one or more steps which select specific (or specific groups of) analytes from the sample for the subsequent whole measurement process. Often the complete identity of these 'intermediate analytes' is not fully known or taken into account. Therefore, it is difficult to mimic all analytically relevant properties of real samples within a CRM. The degree of equivalence in the analytical behaviour of real samples and a CRM with respect to various measurement procedures (methods) is summarised in a concept called 'commutability of a reference material'. There are various definitions that define this concept.

For instance, the CLSI Guideline C53-A [9] recommends the use of the following definition for the term *commutability*:

> "The equivalence of the mathematical relationships among the results of different measurement procedures for an RM and for representative samples of the type intended to be measured."

The commutability of a CRM defines its fitness for use and is therefore a crucial characteristic when applying different measurement methods. When the commutability of a CRM is not established, the results from routinely used methods cannot be legitimately compared with the certified value to determine whether a bias does not exist in calibration, nor can the CRM be used as a calibrant.

ERM-CE101 was produced from fish muscle by freezing, milling, mixing and preserving by heat treatment. The analytical behaviour will be the same as for a routine sample of fish muscle. For samples other than fish muscle the commutability has to be assessed.

9 Instructions for use

9.1 Safety information

The usual laboratory safety measures apply.

9.2 Storage conditions

The material should be stored at 4 ± 3 °C in the dark. The material should not be frozen, as this may release the vacuum seal.

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 for opened jars.

9.3 Preparation and use of the material

The contents of the jar must be re-homogenised by mixing with a clean plastic implement before taking samples. For effective mixing the material may be transferred to a clean, dry container. Any water that has separated from the fish muscle must also be mixed back into the material.

9.4 Minimum sample intake

The minimum sample intake representative for all parameters is 500 mg.

9.5 Use of the certified value

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

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.

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 [10].

When assessing the method performance, the measured values of the CRMs are compared with the certified values. The procedure is summarised here:

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

Use in quality control charts

The materials can be used for quality control charts. Using CRMs for quality control charts has the added value that a trueness assessment is built into the chart.

10 Acknowledgments

The authors would like to acknowledge the support received from Håkan Emteborg and members of the RM Processing Group from the JRC, Directorate F relating to the processing of this CRM and from María Contreras López concerning the set-up of the required isochronous studies.

Furthermore, the authors would like to thank Marta Dabrio and Giovanni Emma (JRC, Directorates F and E, respectively) for reviewing the certification report, as well as the experts of the Reference Material Review Panel "Inorganic", Thomas Meisel (Montanuniversität Leoben, AT) and Jens Sloth (Technical University of Denmark, DK) for their constructive comments.

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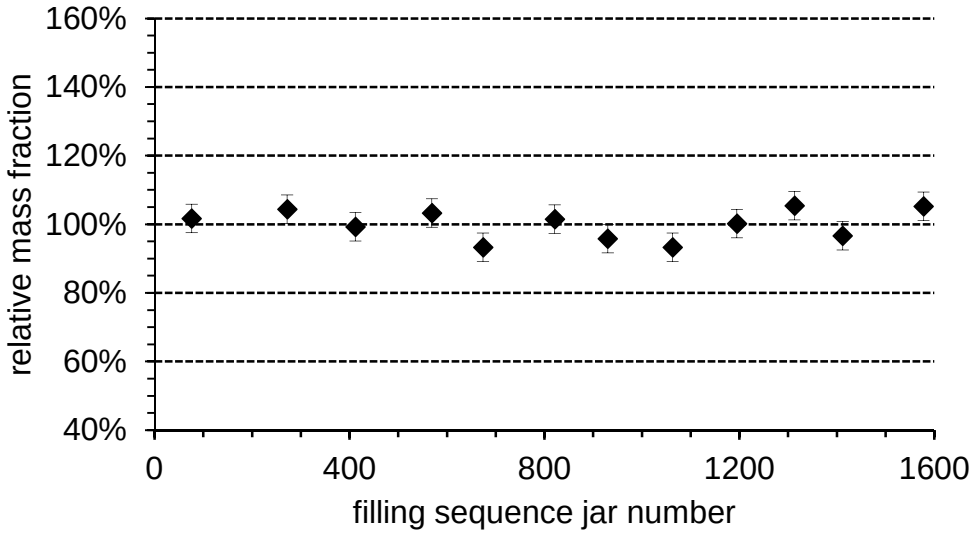
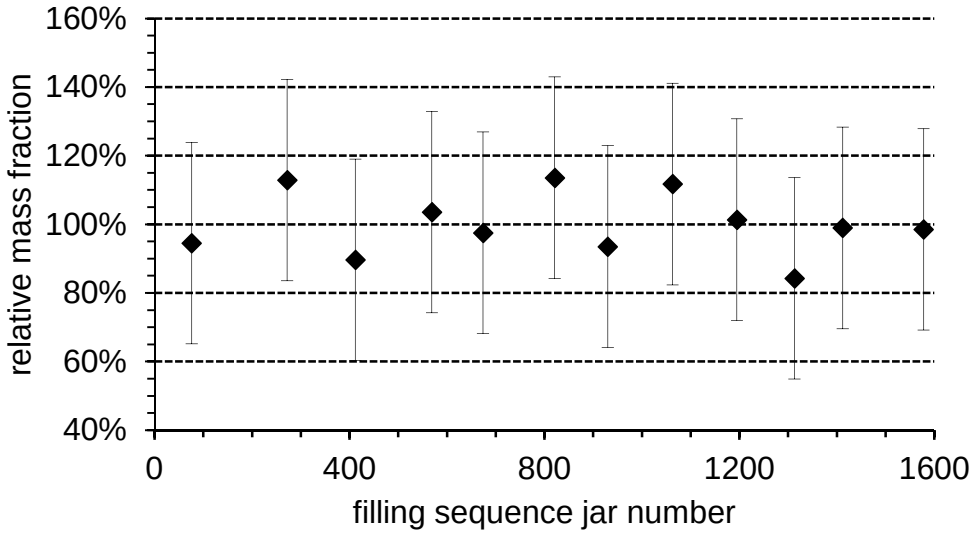
Annex A: ERM-CE101 Homogeneity Test Results

Graphs present the mean mass fractions measured in each jar relative to the grand mean, against the sequence in which jars were filled. Vertical bars represent the 95 % confidence interval of the measurements on each jar, based on the variance of measurements for each jar calculated by ANOVA.

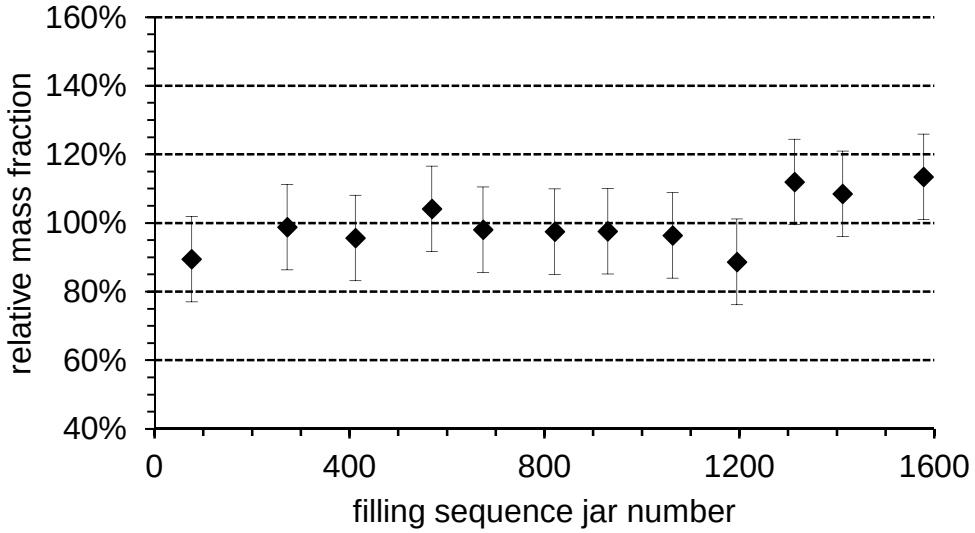
A1: As



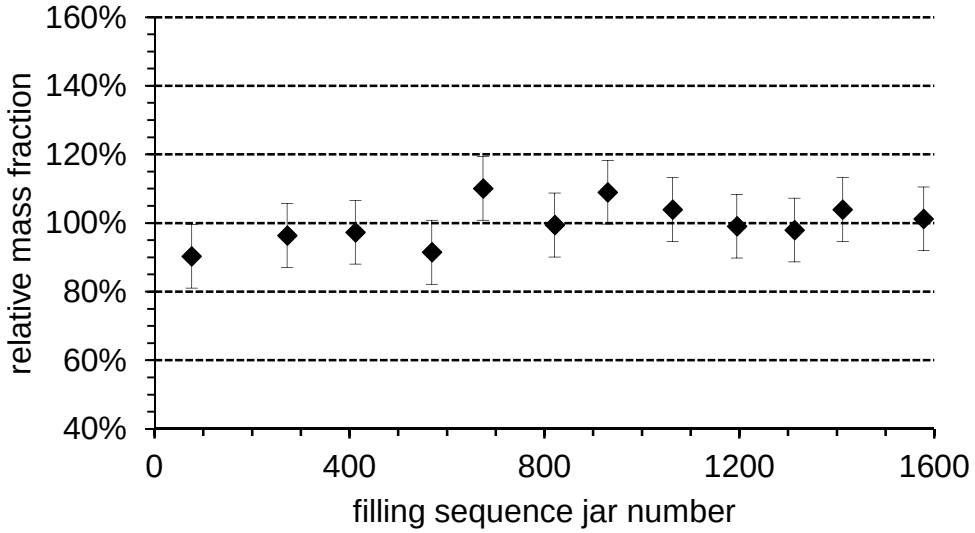
A2: Cd



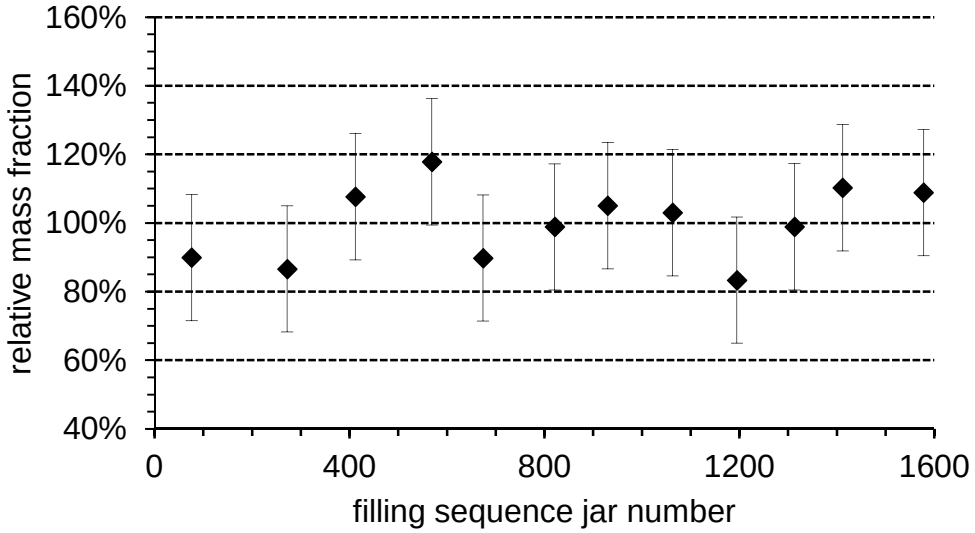
A3: Fe


160%
140%
120%
100%
80%
60%
40%
relative mass fraction
0
400
800
1200
1600
filling sequence jar number

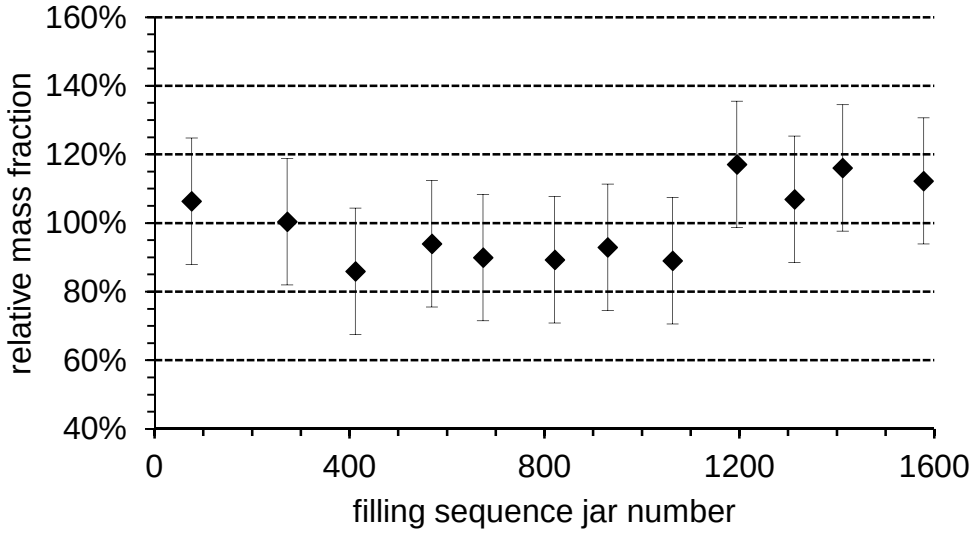
A4: Hg


160%
140%
120%
100%
80%
60%
40%
relative mass fraction
0
400
800
1200
1600
filling sequence jar number

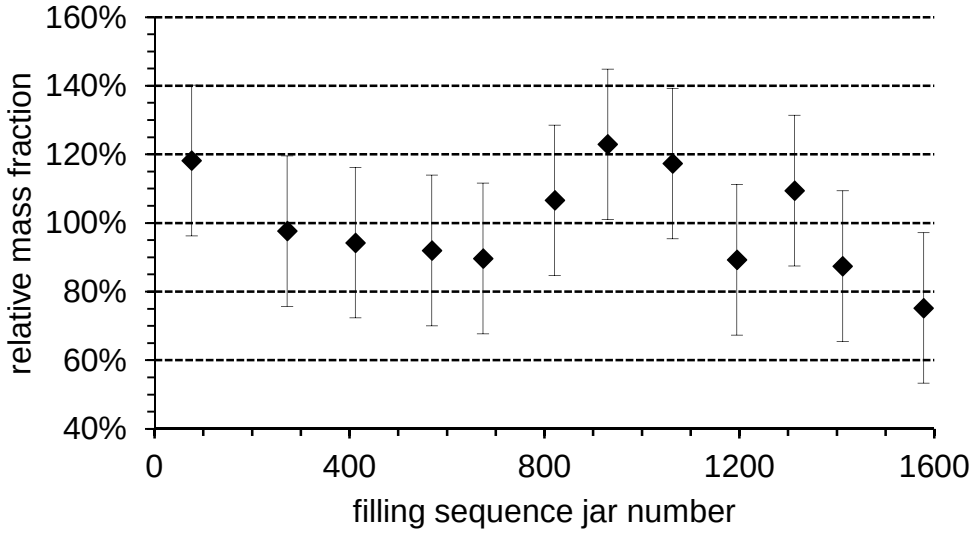
A5: Mn


160%
140%
120%
100%
80%
60%
40%
relative mass fraction
0
400
800
1200
1600
filling sequence jar number

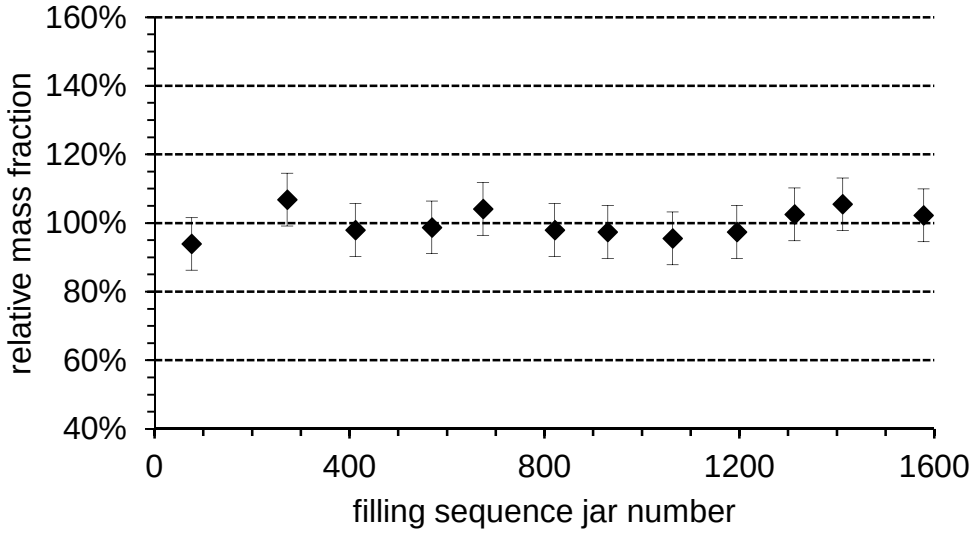
A6: Ni


160%
140%
120%
100%
80%
60%
40%
relative mass fraction
0
400
800
1200
1600
filling sequence jar number

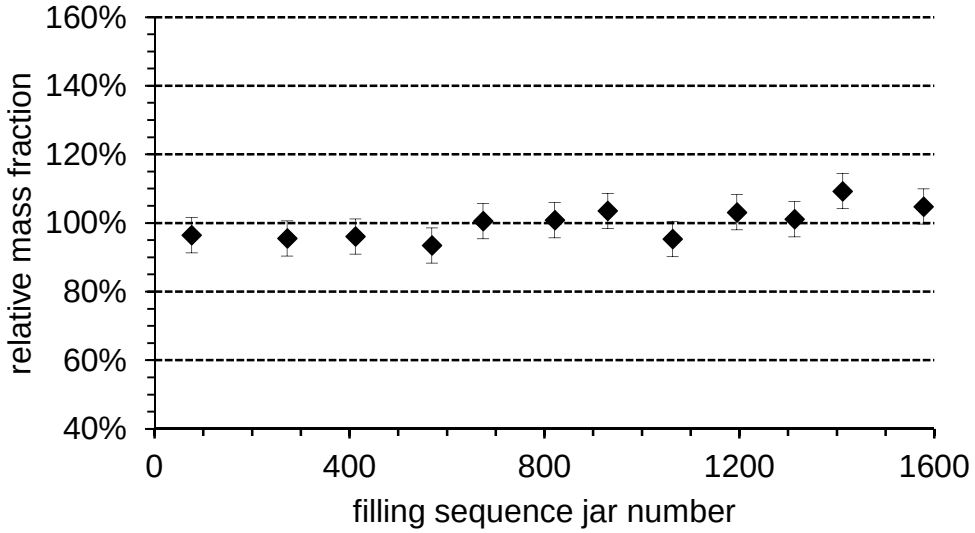
A7: Pb


160%
140%
120%
100%
80%
60%
40%
relative mass fraction
0
400
800
1200
1600
filling sequence jar number

A8: Se


160%
140%
120%
100%
80%
60%
40%
relative mass fraction
0
400
800
1200
1600
filling sequence jar number

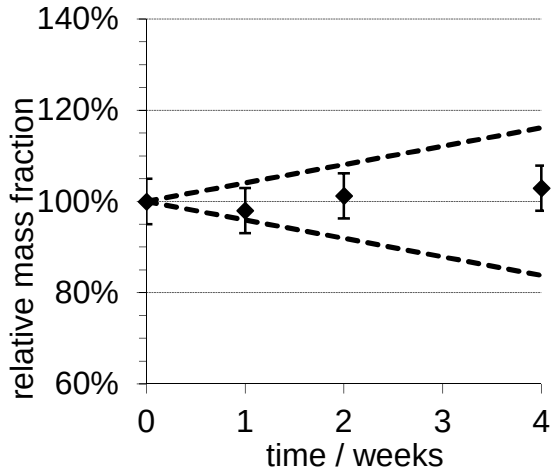
A9: Zn


160%
140%
120%
100%
80%
60%
40%
relative mass fraction
0
400
800
1200
1600
filling sequence jar number

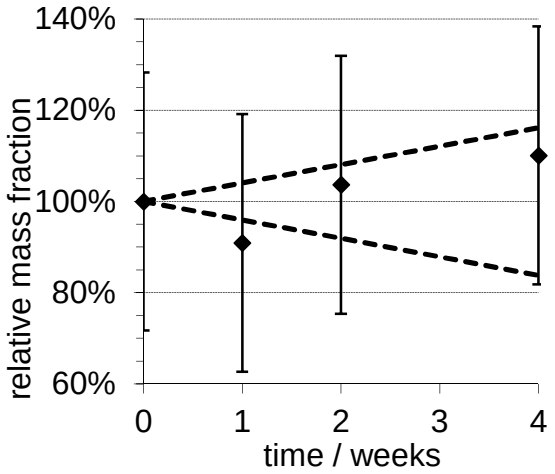
Annex B: ERM-CE101 Short-Term Stability Test Results

Graphs present the mean mass fractions measured at each time-point relative to mean at time point 0, against the time that the samples were held at 60 ºC. Vertical bars represent the 95 % confidence interval of the measurements, based on the variance of measurements for each time-point calculated by ANOVA. Dotted lines represent u_{STS}.

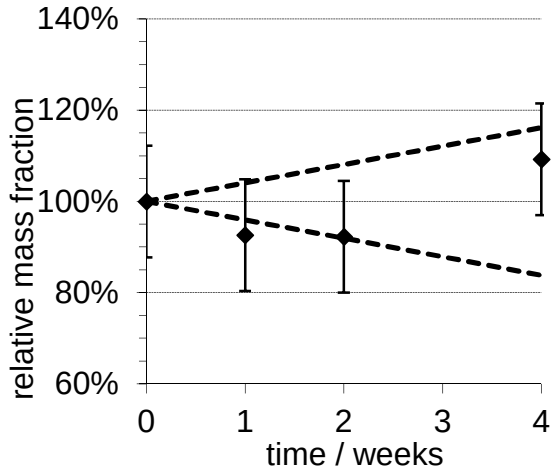
B1: As



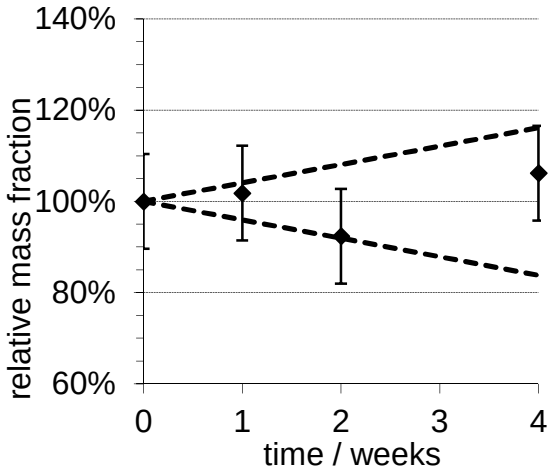
B2: Cd



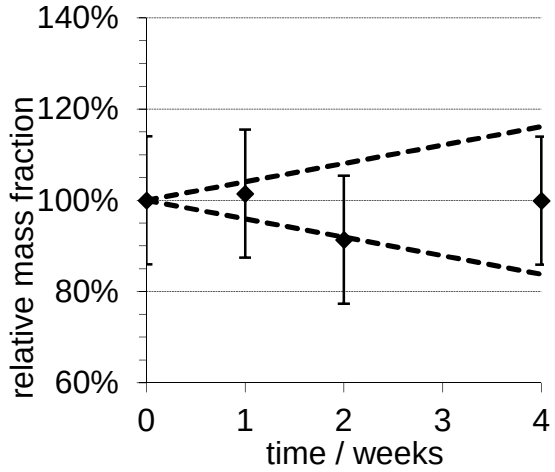
B3: Fe



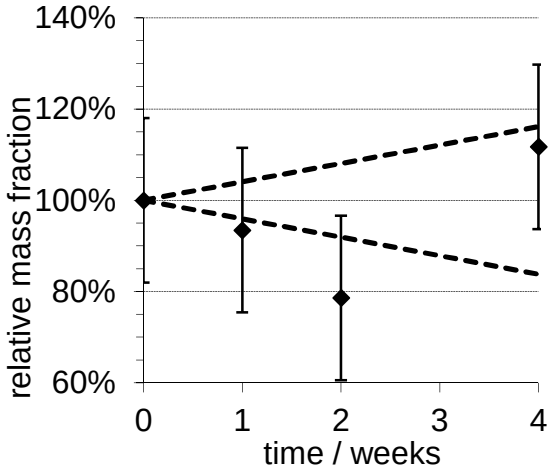
B4: Hg



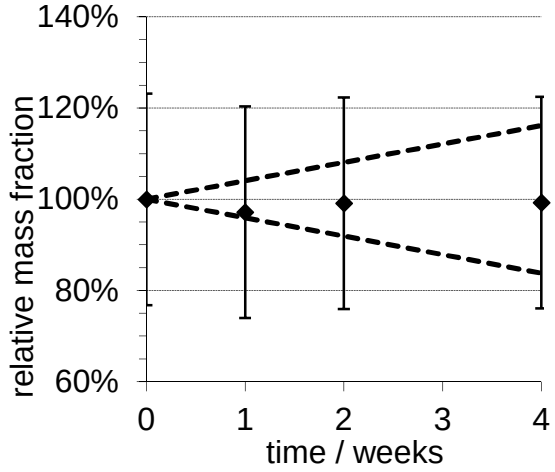
B5: Mn



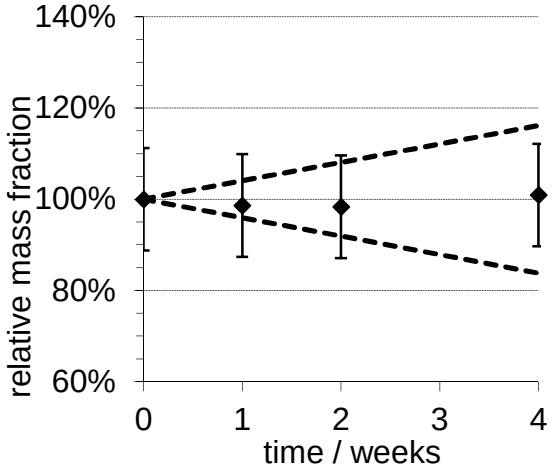
B6: Ni



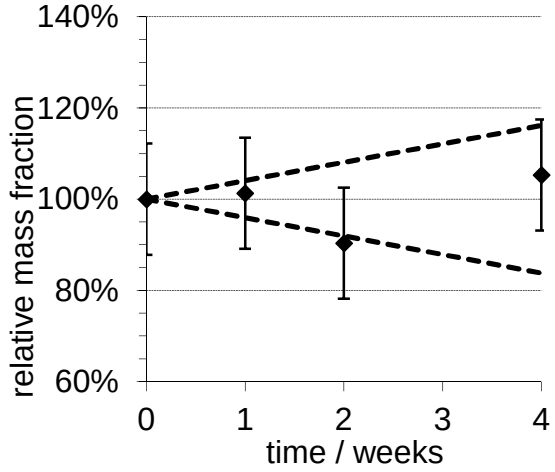
B7: Pb



B8: Se



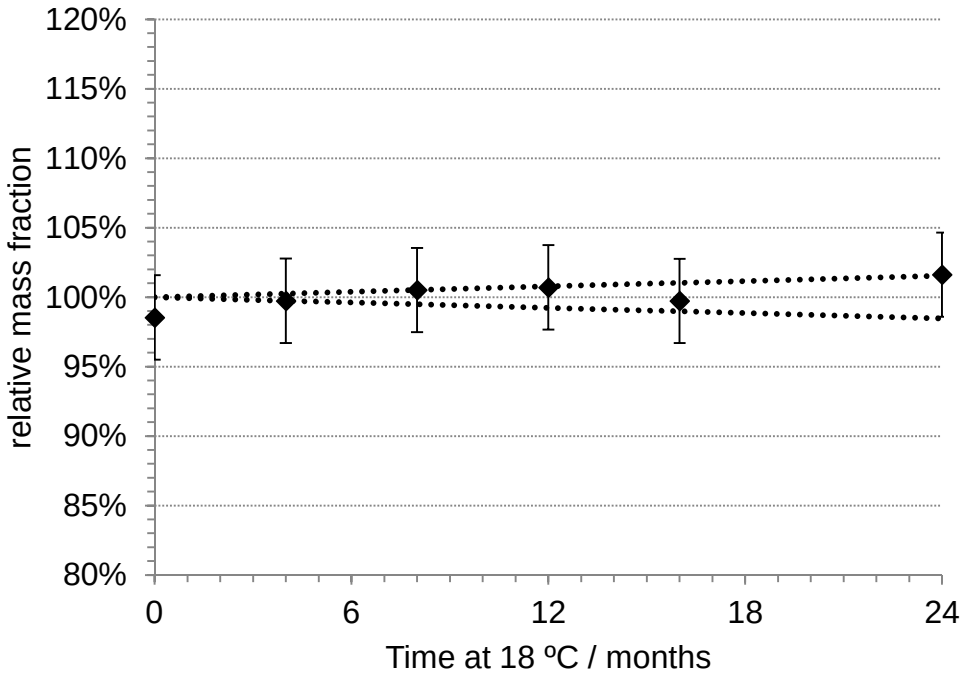
B7: Zn



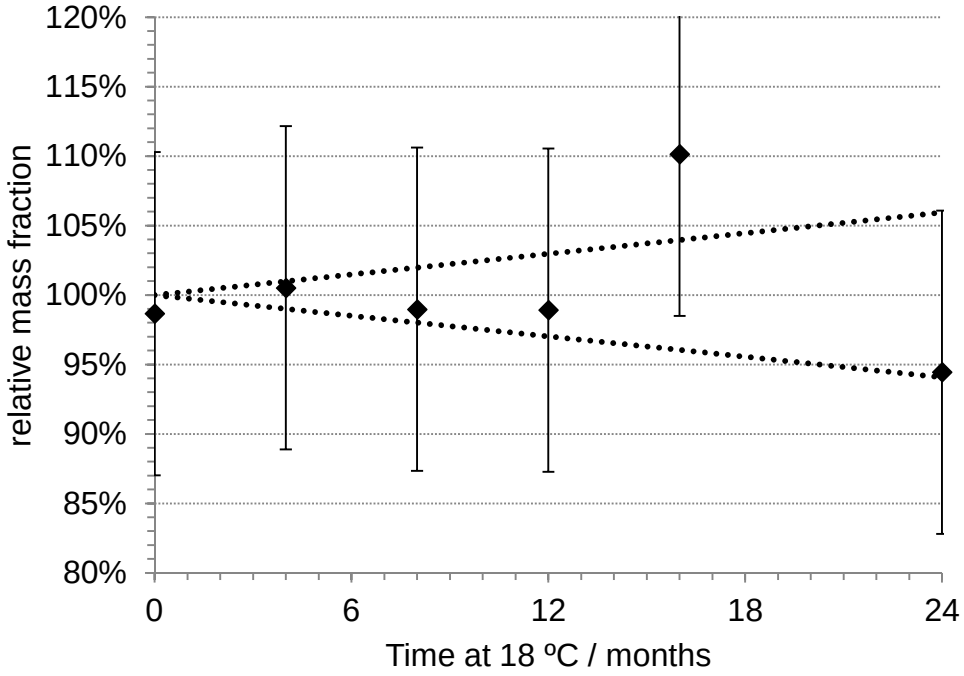
Annex C: ERM-CE101 Long-Term Stability Test Results

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 ºC. Vertical bars represent the 95 % confidence interval of the measurements, based on the variance of measurements for each time-point calculated by ANOVA. Dotted lines represent u_{LTS}.

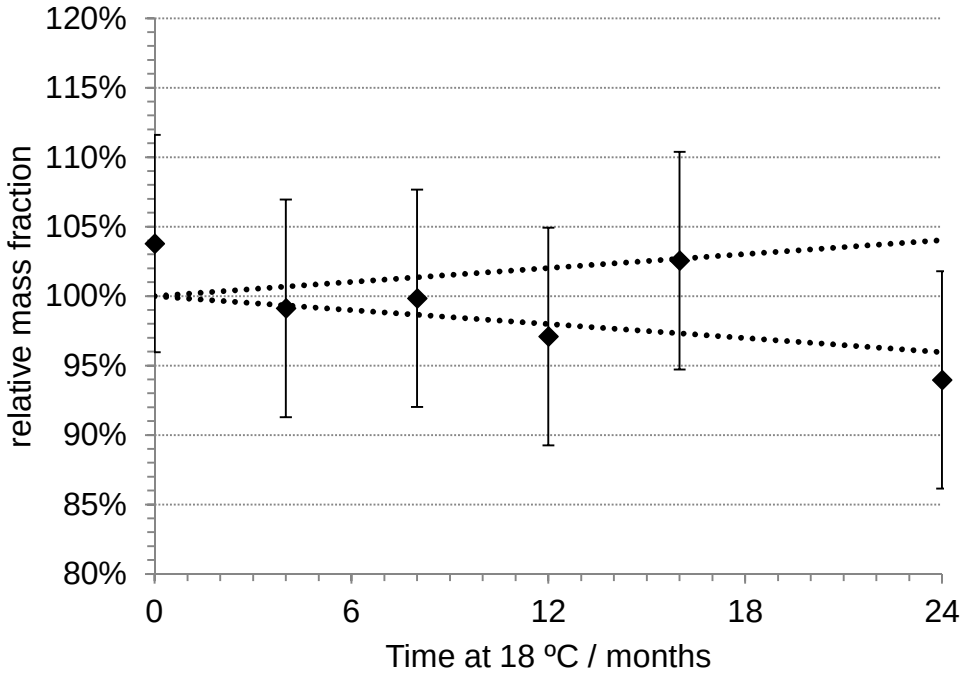
C1: As



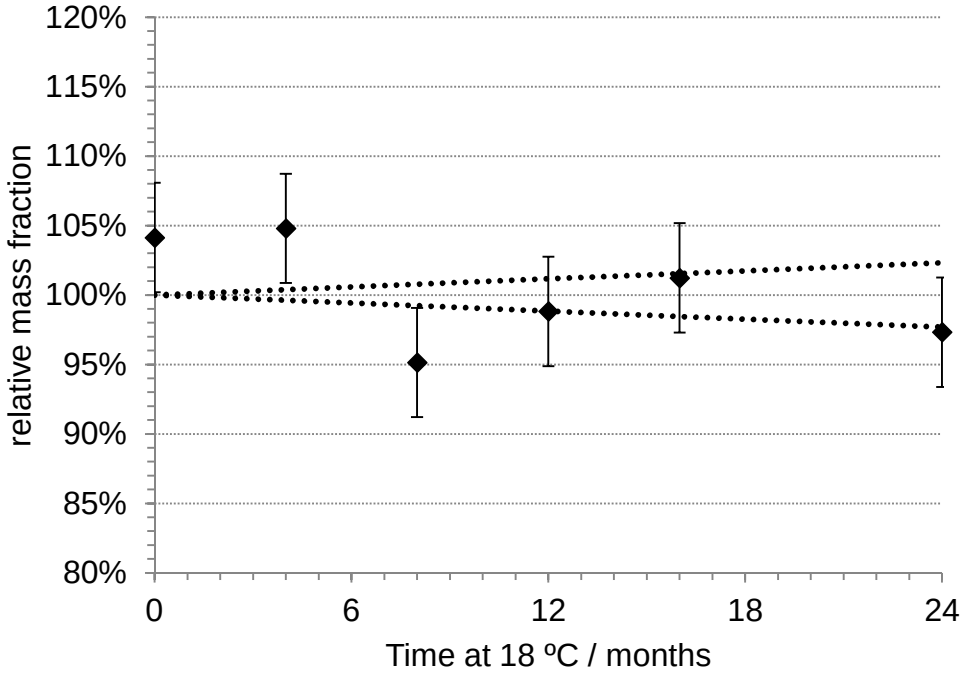
C2: Cd


120%
115%
110%
105%
100%
95%
90%
85%
80%
relative mass fraction
0
6
12
18
24
Time at 18 ºC / months

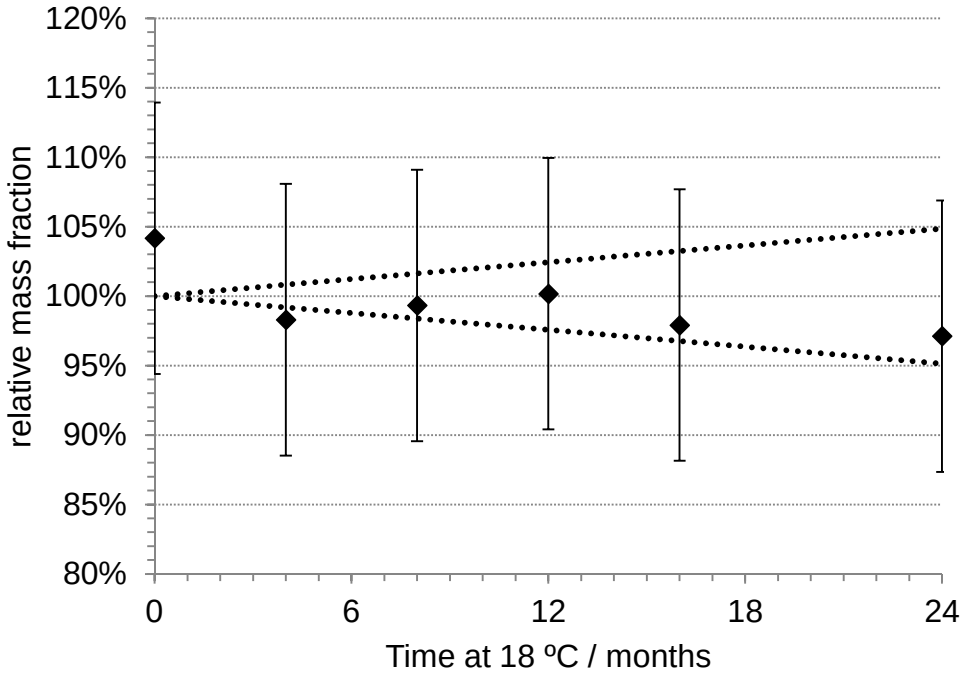
C3: Fe


120%
115%
110%
105%
100%
95%
90%
85%
80%
relative mass fraction
0
6
12
18
24
Time at 18 ºC / months

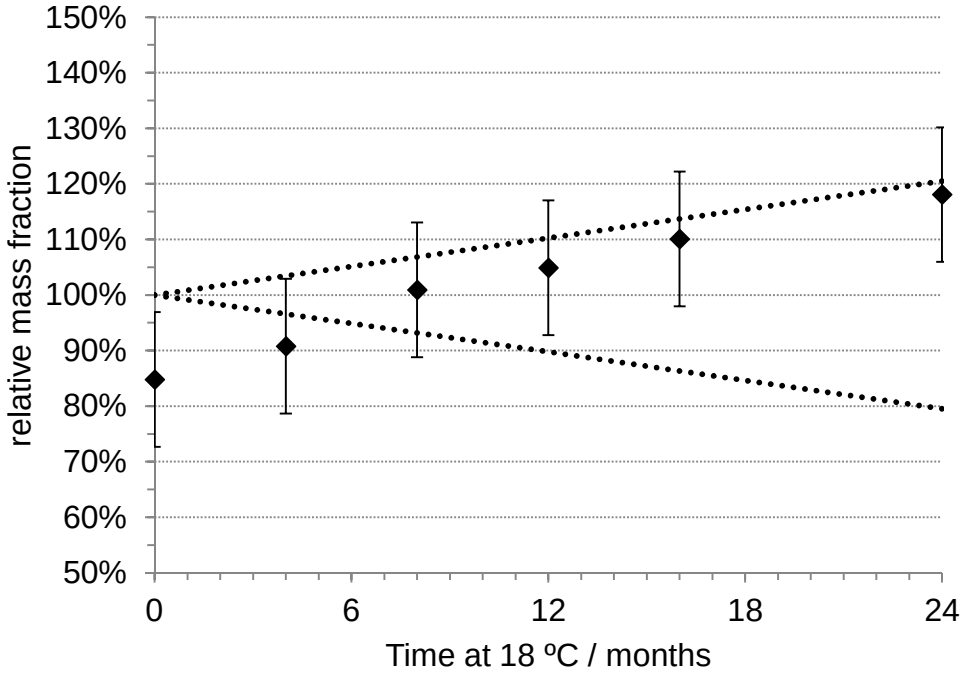
C4: Hg


120%
115%
110%
105%
100%
95%
90%
85%
80%
relative mass fraction
0
6
12
18
24
Time at 18 ºC / months

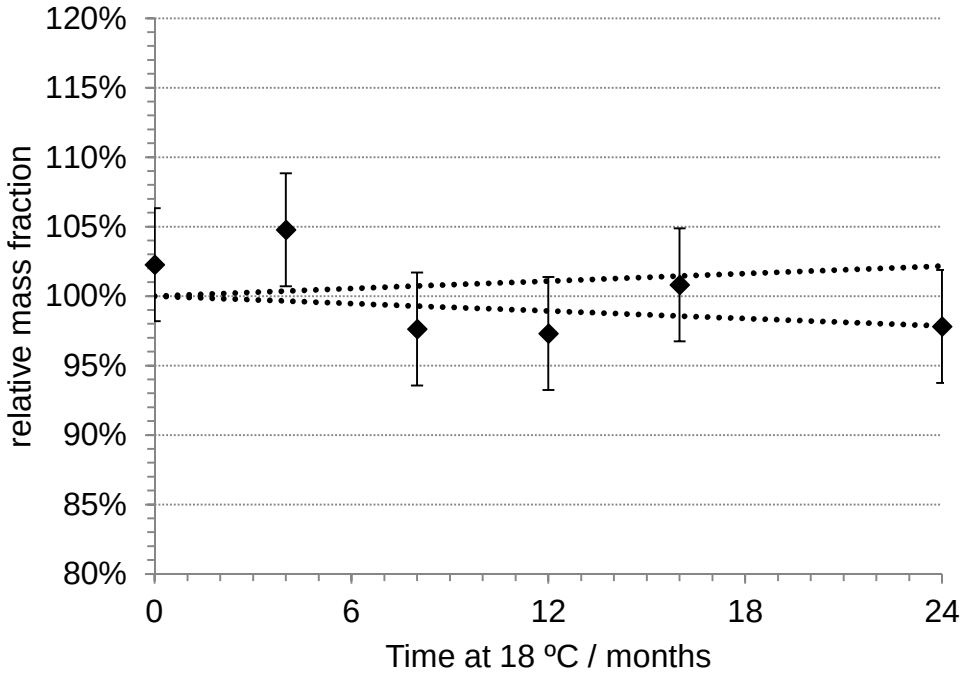
C5: Mn


120%
115%
110%
105%
100%
95%
90%
85%
80%
relative mass fraction
0
6
12
18
24
Time at 18 ºC / months

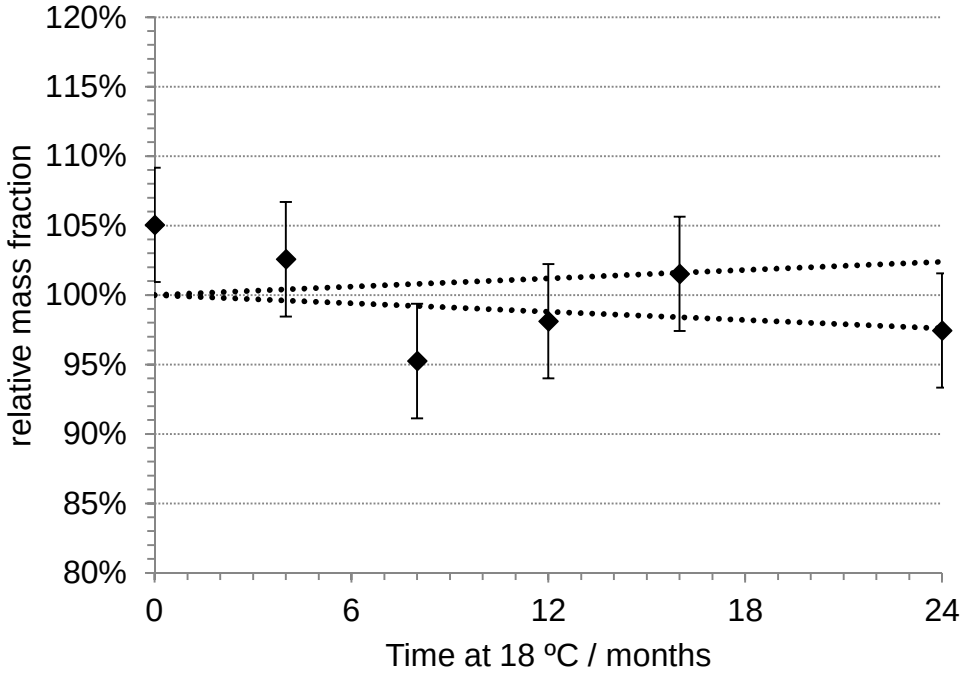
C6: Pb


150%
140%
130%
120%
110%
100%
90%
80%
70%
60%
50%
relative mass fraction
0
6
12
18
24
Time at 18 ºC / months

C7: Se


120%
115%
110%
105%
100%
95%
90%
85%
80%
relative mass fraction
0
6
12
18
24
Time at 18 ºC / months

C8: Zn


120%
115%
110%
105%
100%
95%
90%
85%
80%
relative mass fraction
0
6
12
18
24
Time at 18 ºC / months

Annex D: Summary of analytical techniques used in the characterisation of element mass fractions in ERM-CE101 as reported by the laboratories

Lab-method code	Sample pre-treatment	Analytical method	Calibrant	Instrument
L01	Microwave assisted digestion with nitric acid and hydrogen peroxide; sample weight: approx. 0.5 g, final volume 30 mL	Hg: CV-AAS according to ÖNORM EN ISO 12846 ($NaBH_4$). Other elements: ICP-MS, external calibration	Alfa Aesar, traceable to NIST SRM 3133, 3103a, 3126a, 3132, 3136, 3149, 3168a	Perkin Elmer Flow Injection Mercury System and Perkin Elmer Nexion 350D
L02	Microwave digestion (5 mL conc. HNO_3) in quartz vessels in a high-pressure microwave oven from Anton Paar (Multiwave 3000) following the principles in EN13805:2014.	ICP-QQQ-MS, external calibration	SPS Science	Agilent 8900 ICP-QQQ
L03	*Laboratory did not report results*			
L04	Acid digestion and dilution	ICP-MS, external calibration with In internal standard	Spex Certiprep	Agilent 7900ce
L05	Microwave digestion with HNO_3+HF+H_2O_2	ICP-SF-MS, external calibration	Ultra Scientific, traceable to NIST SRM 3133, 3126a, 3132, 3136, 3149, 3168a	ELEMENT 2, Thermo Fisher
L06	None (sample intake 0.3 g)	k_0-NAA	IRMM-530R	GA, TRIGA Mark II reactor; Canberra, HPGe detector

L07	Hg: Microwave digestion with HNO_3, Other elements: Microwave digestion wtih HNO_3 and H_2O_2	Hg: CV-AAS with external calibration, Other elements: ICP-MS with Collision-Reaction Cell in He Mode, with external calibration	Hg: NIST SRM 3133, As: NIST 3103a, Fe and Mn: Merck XVI multi-element solution, traceable to NIST SRMs, Ni: NIST 3136, Se: NIST3149, Zn: NIST3168a	Hg: SemiAutomatic Mercury Analyzer Model Hg-201, Sanso Seisakusho Co., LTD, Other elements: Agilent Technologies 7900x
L08	Microwave assisted digestion (CEM MarsXpress) with 6 ml of HNO_3 and 1 ml HCl at 220 ºC.	ICP-MS, external calibration with internal standard	Spectrascan, traceable to NIST SRMs	Agilent 7700X
L09	Hg: No sample preparation, Other elements: Closed microwave digestion with HNO_3:H_2O_2 (7ml:1ml) controlled temperature program (10 min. to 200 °C, 20 min at 200 °C).	Hg: Pyrolysis AAS, Fe, FAAS, Other elements: ICP-MS. All external calibration	Merck VI Multi-element standard	Hg: Milestone DMA 80, Fe: Analytik-Jena contrAA 300, Other elements: Perkin Elmer Elan DRC-e
L10	Closed microwave digestion with HNO_3:H_2O_2 (7ml:1ml) controlled temperature program (10 min. to 200 °C, 20 min at 200 °C).	AFS, external calibration	Inorganic Ventures	Analytik-Jena Automated Mercury Analyser
L11	*Laboratory did not report results*			
L12	Microwave digestion HNO_3/H_2O_2	ICP-SFMS, external calibration with Ge and Rh internal standards	LabKings, traceable to NIST SRM 3103a, 3126a, 3132, 3136, 3149, 3168a	Thermo Fisher Element 2
L13	Microwave digestion HNO_3/H_2O_2	ICP-OES, 2-point external calibration with Rh internal standard	SCP Science traceable to NIST SRM 3126a, 3132, 3136	Agilent 5100

L14	Hg: no preparation, Ni: Digestion in heating block with HNO_3/diluted HF, other elements: Closed microwave digestion with HNO_3	Hg: Solid-sampling pyrolysis AAS; As, Ni and Se: ICP-MS (triple quad with mass shift to 91 for As and 96 for Se), external calibration; Fe, Mn and Zn: ICP-OES, external calibration	Hg: factory calibration, all other elements: Analytika CZ calibrants	ALTEC AMA254, Agilent 8800, Varian 720
L15	Microwave assisted decomposition using HNO_3 and H_2O_2	ICP-MS with He collision cell, external calibration with Tm and Ge internal standards	Spectroscan	Thermo Fisher iCAP Qc
L16	Digestion with nitric and hydrochloric acids using a Milestone UltraWave digestion system	ICP-MS with He collision cell (except Hg), external calibration with In and Rh internal standards	VWR International Ltd standards, traceable to NIST SRMs	Agilent 7700x

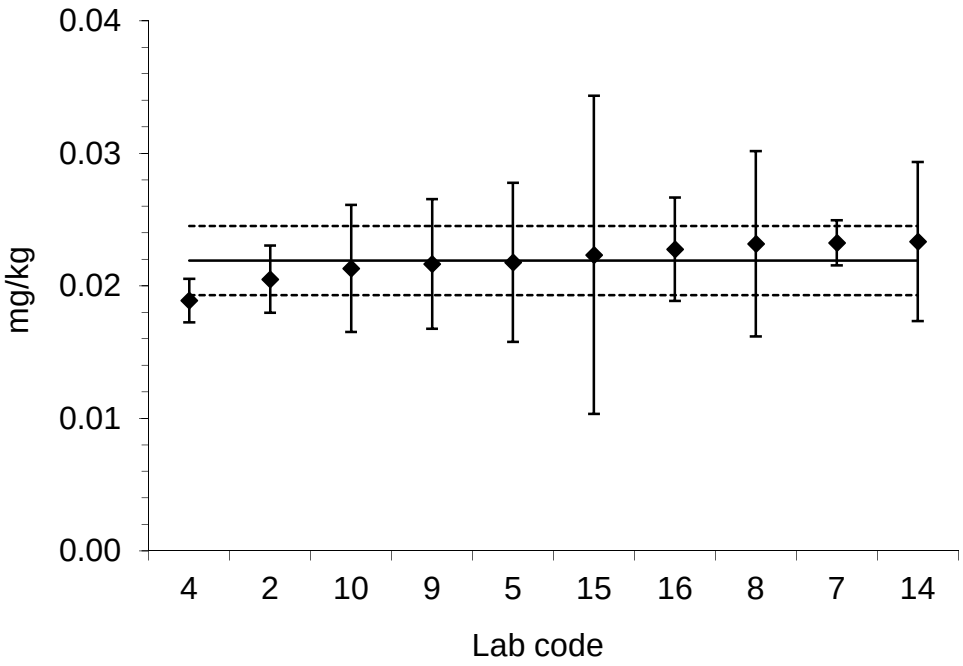
Annex E: Results of the characterisation study

Individual measurement results from each laboratory participating in the intercomparison. Vertical bars represent expanded uncertainties as reported by participating laboratories. The solid line represents the certified values (the mean of the laboratory means), while the broken lines represent the expanded uncertainty of the certified value.

Approaches to number rounding differed between participants. Values supplied without rounding were were rounded to assist document formatting.

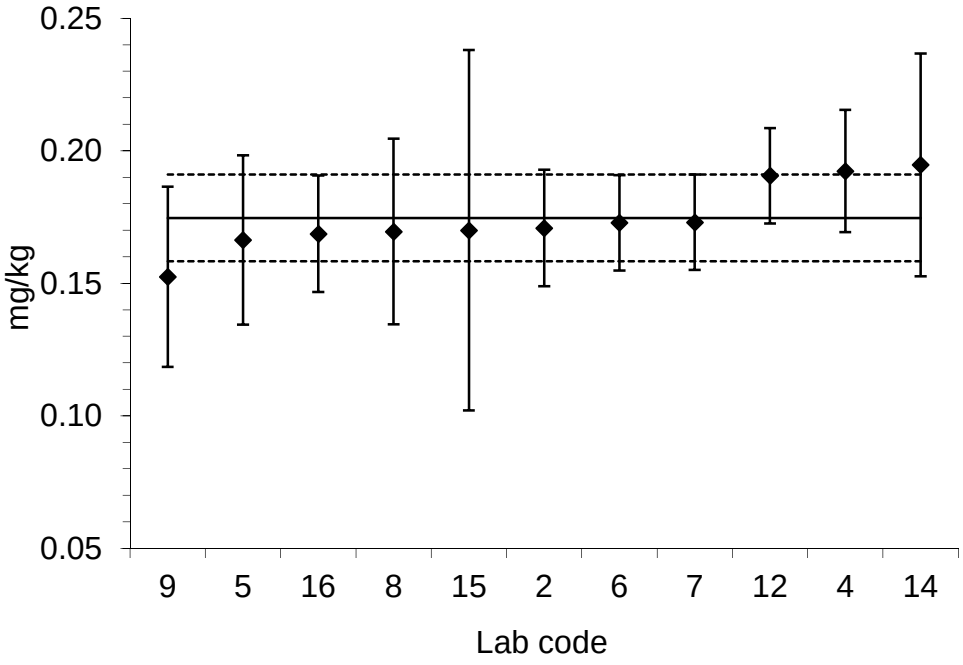
E.1 Hg mass fraction [mg/kg]

Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Mean	U
L02	0.0240	0.0200	0.0200	0.0210	0.0220	0.0160	0.0205	0.0025
L04	0.0161	0.0185	0.0182	0.0206	0.0195	0.0204	0.0189	0.0017
L05	0.0213	0.0212	0.0223	0.0215	0.0225	0.0218	0.0218	0.0060
L07	0.0226	0.0234	0.0221	0.0241	0.0236	0.0237	0.0233	0.0017
L08	0.023	0.021	0.022	0.025	0.024	0.024	0.023	0.007
L09	0.0217	0.0205	0.0199	0.0231	0.0232	0.0215	0.0217	0.0049
L10	0.0211	0.0214	0.0212	0.0214	0.0211	0.0217	0.0213	0.0048
L14	0.023	0.023	0.023	0.023	0.024	0.024	0.023	0.006
L15	0.022	0.024	0.022	0.023	0.022	0.021	0.022	0.012
L16	0.0206	0.0196	0.0200	0.0247	0.0263	0.0254	0.0228	0.0039



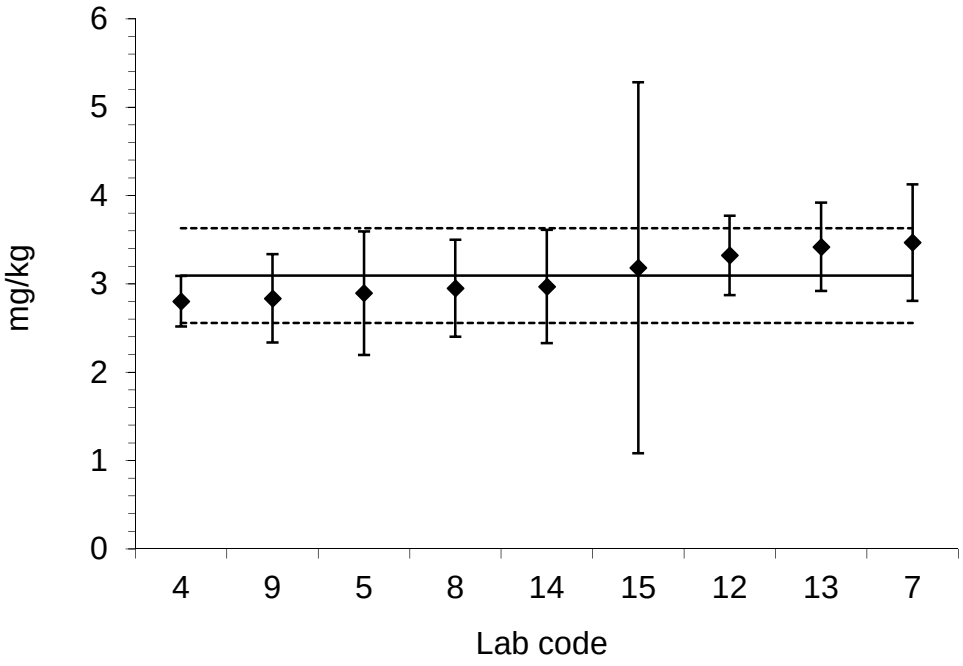
E.2 As mass fraction [mg/kg]

Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Mean	U
L02	0.177	0.180	0.178	0.164	0.164	0.162	0.171	0.022
L04	0.188	0.194	0.191	0.189	0.191	0.201	0.192	0.023
L05	0.162	0.167	0.167	0.175	0.157	0.170	0.166	0.032
L06	0.178	0.178	0.169	0.175	0.170	0.167	0.173	0.018
L07	0.165	0.176	0.178	0.178	0.164	0.177	0.173	0.018
L08	0.166	0.174	0.168	0.169	0.169	0.171	0.170	0.035
L09	0.156	0.135	0.172	0.141	0.157	0.154	0.153	0.034
L12	0.19567	0.19198	0.19426	0.18685	0.18971	0.18506	0.19059	0.018
L14	0.192	0.185	0.192	0.197	0.201	0.201	0.195	0.042
L15	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.068
L16	0.168	0.171	0.170	0.168	0.167	0.168	0.169	0.022



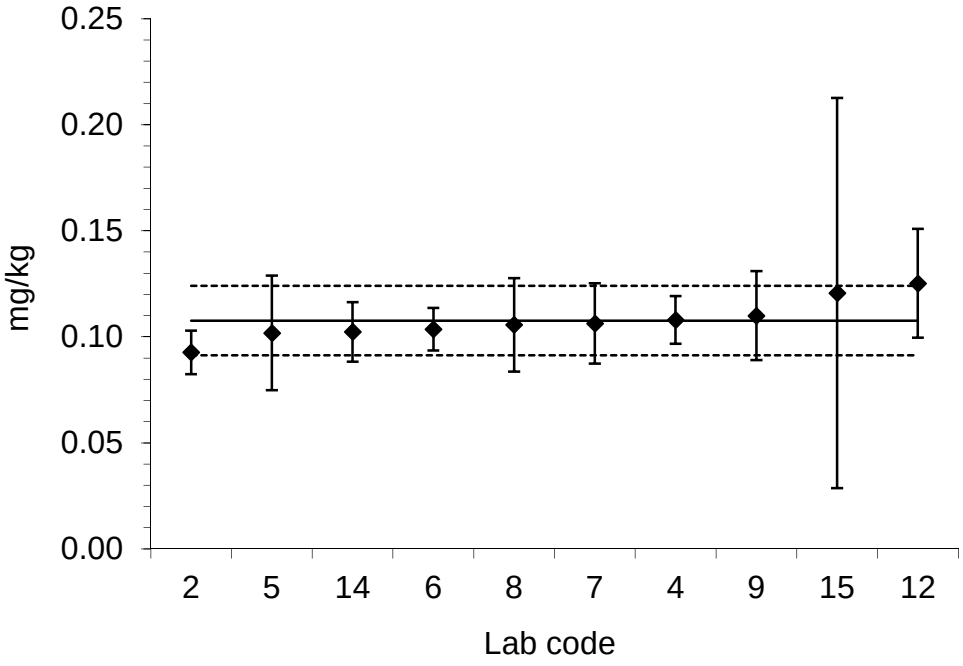
E.3 Fe mass fraction [mg/kg]

Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Mean	U
L04	2.80	2.30	2.34	2.98	3.15	3.25	2.80	0.29
L05	3.21	3.18	3.19	2.38	2.87	2.54	2.90	0.70
L07	3.65	3.09	3.41	3.48	3.40	3.77	3.47	0.66
L08	2.47	2.37	2.93	3.07	3.42	3.44	2.95	0.55
L09	2.38	2.84	2.62	3.35	2.85	2.97	2.835	0.5
L12	3.4975	3.3231	3.2212	3.0846	3.5400	3.2656	3.3220	0.45
L13	3.3004	3.5617	3.6495	3.2338	3.6052	3.1581	3.4181	0.5000
L14	2.98	2.99	2.77	3.22	2.91	2.95	2.97	0.64
L15	3.10	2.50	2.80	3.20	3.30	4.20	3.18	2.10
Results not used in calculation of the certified value								
L02	*2.23*	*2.55*	*-*	*2.57*	*2.65*	*-*	*2.50*	*0.27*
L06	*< 4*	*< 5*	*< 2*	*< 3*	*< 3*	*< 3*		
L16	*4.79*	*3.40*	*3.55*	*2.38*	*2.97*	*12.79*	*4.98*	*2.17*



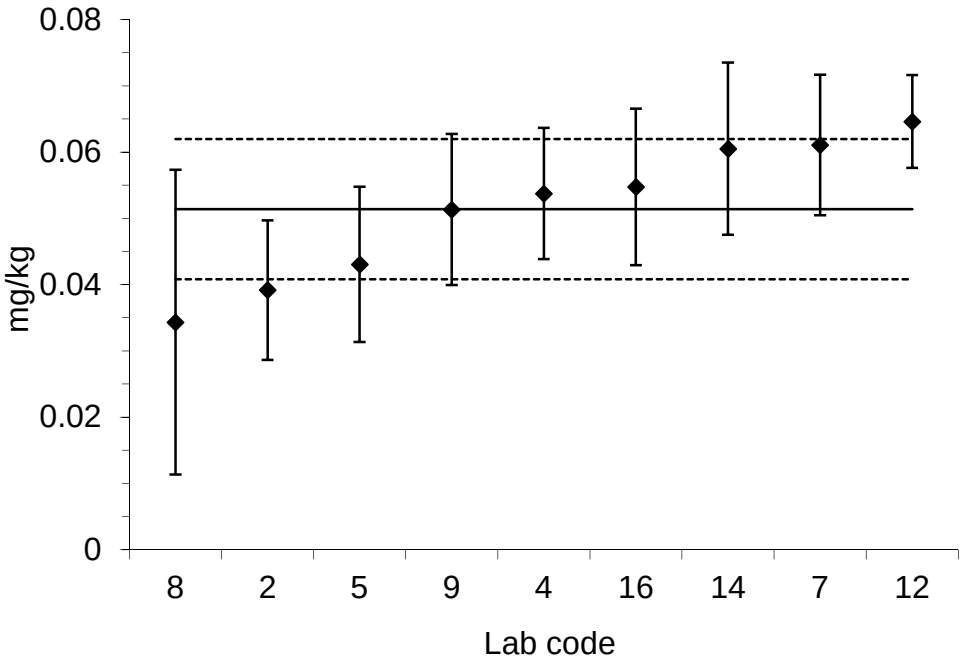
E.4 Mn mass fraction [mg/kg]

Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Mean	U
L02	0.103	0.101	0.103	0.080	0.095	0.074	0.093	0.010
L04	0.104	0.118	0.102	0.108	0.109	0.107	0.108	0.011
L05	0.1020	0.0994	0.0960	0.0902	0.1280	0.0953	0.1018	0.0270
L06	0.1100	0.1050	0.1040	0.0968	0.1060	0.0996	0.1036	0.0100
L07	0.101	0.110	0.102	0.118	0.104	0.103	0.106	0.019
L08	0.074	0.058	0.126	0.096	0.149	0.131	0.106	0.022
L09	0.119	0.100	0.106	0.119	0.111	0.105	0.110	0.021
L12	0.1295	0.1170	0.1339	0.1211	0.1158	0.1341	0.1252	0.0256
L14	0.092	0.091	0.104	0.107	0.106	0.114	0.102	0.014
L15	0.100	0.080	0.084	0.100	0.130	0.230	0.121	0.092
Results not used in calculation of the certified value								
L13	*0.12119*	*0.09555*	*0.08270*	*0.14583*	*0.11792*	*0.12399*	*0.1145*	*0.2500*
L16	*0.087*	*0.108*	*0.104*	*0.094*	*0.121*	*0.835*	*0.225*	*0.100*



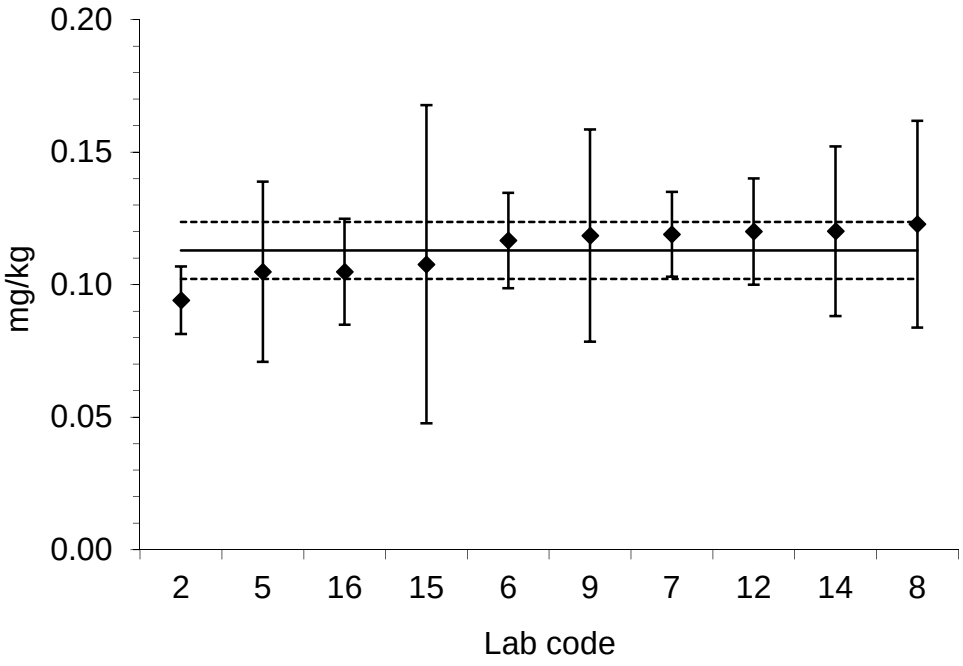
E.5 Ni, indicative mass fraction [mg/kg]

Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Mean	U
L02	0.042	0.038	0.035	0.047	0.036	0.037	0.039	0.011
L04	0.058	0.055	0.056	0.054	0.046	0.054	0.054	0.010
L05	0.0468	0.0417	0.0457	0.0409	0.0425	0.0407	0.0431	0.0117
L07	0.057	0.066	0.061	0.061	0.066	0.055	0.061	0.011
L08	0.022	0.024	0.058	0.031	0.036	0.035	0.034	0.023
L09	0.0505	0.0419	0.0450	0.0566	0.0568	0.0572	0.0513	0.0114
L12	0.06500	0.05516	0.06936	0.06721	0.06249	0.06843	0.06461	0.007
L14	0.063	0.059	0.063	0.057	0.060	0.061	0.061	0.013
L16	0.0474	0.0451	0.0438	0.0635	0.0695	0.0592	0.0548	0.0118
Results not used in calculation of the indicative value								
L13	*0.02624*	*0.07576*	*0.04383*	*0.00830*	*0.06956*	*0.02488*	*0.0414*	*0.2500*
L15	*<0.2*	*<0.2*	*<0.2*	*<0.2*	*<0.2*	*<0.2*		



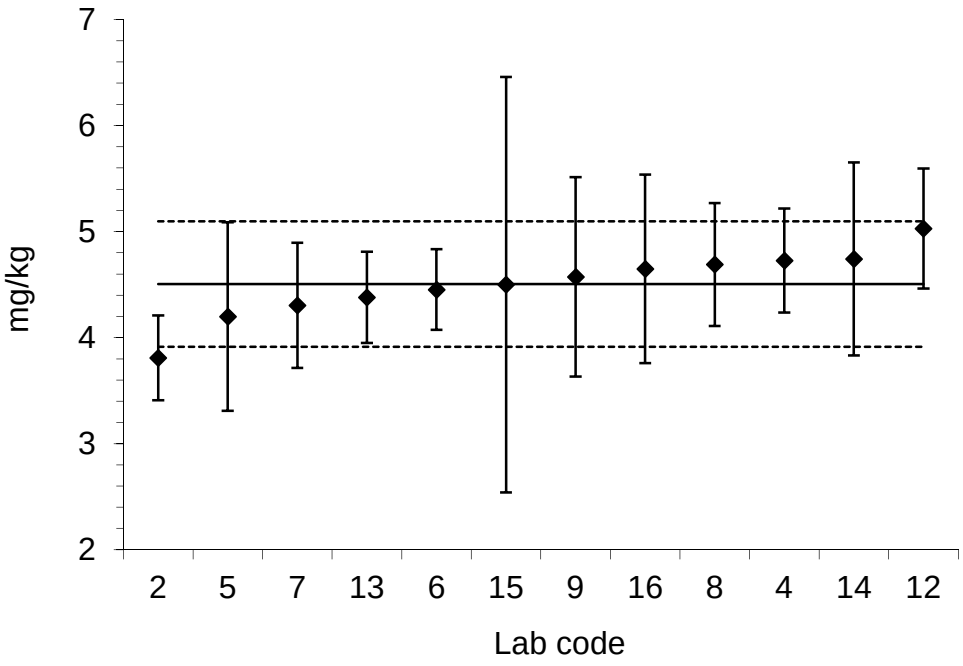
E.6 Se mass fraction [mg/kg]

Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Mean	U
L02	0.098	0.093	0.092	0.088	0.098	0.096	0.094	0.013
L05	0.111	0.108	0.109	0.100	0.108	0.093	0.105	0.034
L06	0.106	0.105	0.117	0.126	0.125	0.121	0.117	0.018
L07	0.108	0.114	0.110	0.134	0.127	0.121	0.119	0.016
L08	0.075	0.148	0.119	0.123	0.121	0.151	0.123	0.039
L09	0.138	0.101	0.115	0.108	0.101	0.148	0.119	0.040
L12	0.11276	0.12352	0.10833	0.12865	0.12572	0.12118	0.1200	0.0200
L14	0.120	0.126	0.116	0.123	0.120	0.116	0.120	0.032
L15	0.110	0.120	0.110	0.110	0.096	0.100	0.108	0.060
L16	0.096	0.090	0.093	0.117	0.118	0.115	0.105	0.020
Results not used in calculation of the certified value								
L04	*0.123*	*0.124*	*0.121*	*0.124*	*0.132*	*0.123*	*0.125*	*0.014*



E.7 Zn mass fraction [mg/kg]

Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Mean	U
L02	3.87	3.78	3.63	3.87	4.00	3.70	3.81	0.40
L04	4.27	4.39	5.09	5.26	4.61	4.74	4.73	0.49
L05	4.22	4.26	4.18	4.21	4.17	4.15	4.20	0.89
L06	3.99	4.18	4.18	4.74	4.91	4.72	4.45	0.38
L07	4.48	4.20	4.31	4.25	4.54	4.05	4.305	0.59
L08	4.69	4.47	4.72	4.63	4.79	4.84	4.69	0.58
L09	4.51	4.60	4.69	4.66	4.62	4.36	4.57	0.94
L12	5.0436	5.2827	4.7764	5.0604	5.0201	4.9952	5.0297	0.57
L13	4.2798	4.0558	3.9080	4.6729	4.8073	4.5619	4.3809	0.43
L14	4.36	4.58	4.44	5.03	5.02	5.03	4.74	0.91
L15	4.5	4.4	4.4	4.4	4.4	4.9	4.5	2.0
L16	3.92	4.08	3.91	4.87	5.19	5.92	4.65	0.89



European Commission

EUR 29355 EN – Joint Research Centre – Directorate F – Health, Consumers and Reference Materials
Title: **CERTIFICATION REPORT: The certification of the mass fractions of elements in Trout Muscle: ERM®- CE101**
Author(s): James P. Snell, Boryana Koleva, Marie-France Tumba-Tshilumba, Andrea Held
Luxembourg: Publications Office of the European Union
2019 – 52 pp. – 21.0 x 29.7 cm
EUR – Scientific and Technical Research series – ISSN 1831-9424
ISBN 978-92-79-94029-3
doi: 10.2760/099555

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KJ-NA- 29355-EN-N

Publications Office

doi: 10.2760/099555

ISBN: 978-92-79-94029-3