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CERTIFICATION REPORT

The Certification of the Mass Fractions of Elements in Bovine Muscle

Certified Reference Material ERM®-BB184

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Joint Research Centre
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The Certification of the Mass Fractions of Elements in Bovine Muscle

Certified Reference Material ERM®-BB184

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Summary

This report describes the preparation of a bovine muscle matrix reference material ERM®-BB184, and the certification of the contents (mass fractions) of seven elements. All results are expressed as a mass fraction on a dry mass basis.

The preparation and processing of the material, homogeneity studies, stability studies and characterisation are described hereafter and the results are discussed. Uncertainties were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [1] and include uncertainties due to possible heterogeneity, instability and from characterisation. The certified values and their uncertainties are listed in Table 1:

Table 1: Certified mass fractions of elements and their uncertainties in Bovine muscle (ERM®-BB184)

Element	Certified value [1.2)] [mg/kg]	Uncertainty [3)] [mg/kg]
As	0.0234	0.0026
Cd	0.0022	0.0004
Cu	2.31	0.09
Fe	75	4
Mn	0.276	0.013
Se	0.45	0.04
Zn	146	7

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.

The assigned values are based on a minimum sample intake of 0.2 g.

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Glossary

α ..... confidence level
AAS ..... atomic absorption spectrometry
ANOVA ..... analysis of variance
b ..... slope of linear regression
CRM ..... certified reference material
CV ..... cold vapour
d_f ..... degree of freedom (regression)
DM ..... dry mass
ERM® ..... European Reference Material®
GUM ..... Guide to the Expression of Uncertainty in Measurement
HG ..... hydride generation
ICP-MS ..... inductively coupled plasma mass spectrometry
ICP-QMS ..... inductively coupled plasma quadrupole mass spectrometry
ICP-SFMS ..... inductively coupled plasma sector-field mass spectrometry
ICP-OES ..... inductively coupled plasma optical emission spectrometry
ID ..... isotope dilution
IRMM ..... Institute for Reference Materials and Measurements
ISO ..... International Organization for Standardization
JRC ..... European Commission's Joint Research Centre
KFT ..... Karl Fischer titration
LOQ ..... limit of quantification
$MS_{between}$ ..... mean of squares between groups (ANOVA)
MSI ..... minimum sample intake
MS_{within} ..... mean of squares within groups (ANOVA)
n ..... number of replicates
NAA. ..... neutron activation analysis
n/a ..... not applicable
n.c. ..... not calculable
n.d. ..... not determined
n.r. ..... not reported
p ..... level of significance
PSA ..... particle size analysis
RSD ..... relative standard deviation
RSD_{stab} ..... relative standard deviation of all results of stability study
s ..... standard deviation
s_{bb} ..... between-bottle heterogeneity based on standard deviation
s_{wb} ..... within-bottle heterogeneity based on standard deviation
se_b ..... standard error of slope b of linear regression
SI ..... International System of Units
SSAAS ..... solid-sampling (electro-thermal vaporisation) atomic absorption spectrometry
TIMS ..... Thermal ionisation mass spectrometry
$u_{bb,\,rel}$ ..... relative standard uncertainty due to between-bottle heterogeneity
$u^*_{bb,\,rel}$ ..... relative standard uncertainty due to heterogeneity that can be hidden by method repeatability
$u_{char,\,rel}$ ..... relative standard uncertainty of characterisation exercise
u_{CRM} ..... combined standard uncertainty of certified value
$u_{CRM,\,rel}$ ..... combined relative standard uncertainty of certified value
U_{CRM} ..... expanded uncertainty of certified value
$U_{CRM,\,rel}$ ..... expanded relative uncertainty of certified value
u_{lts} ..... relative standard uncertainty of long-term stability
u_{meas} ..... standard uncertainty of measurement result
u_{sts} ..... relative standard uncertainty of short-term stability
u_Δ ..... combined standard uncertainty of certified value and measured value
U_Δ ..... expanded uncertainty of certified value and measured value
t_{sl} ..... pre-defined shelf life
x_i ..... result at time point i in an isochronous stability study
$\overline{x}$ ..... average result of all time points in an isochronous stability study

$\overline{y}$ ..... average of all results of a homogeneity study
Δ ..... difference between two measurement results
Δ_m ..... difference between measured and certified value
$v_{MSwithin}$ ..... degrees of freedom (ANOVA)

1 Abstract

2 Introduction

2.1 Background

This report describes the preparation and certification of a reference material to replace the CRM BCR-184 (bovine muscle).

The target parameters for certification were the mass fractions of the total content of As, Cd, Cu, Cr, Fe, Hg, Mn, Pb, Se and Zn in bovine muscle. The desired mass fractions were those at natural levels that are similar to BCR-184 and below regulatory limits for food contaminants (EC466/2001 (Cd < 0.05 µg/g, Pb < 0.1 µg/g "wet weight"), with target uncertainties of 5 -10 % and values traceable to the SI, expressed as mass fraction of the dry mass.

The purpose of the reference material is for the validation and quality control of measurement methods for trace element mass fractions in meat.

Throughout this report, results are expressed as mass fraction on a dry mass basis. For practical purposes, the dry mass is established by determining the "loss of mass on drying" under conditions defined in Section 10.2. It should be noted that determination of the dry mass correction factor under conditions other than specified in this report might lead to results that differ from the certified values.

3 Participants

Project management and evaluation

European Commission (EC), Joint Research Centre (JRC)
Institute for Reference Materials and Measurements (IRMM), Reference Materials Unit, Geel (BE)
(Work performed under ISO Guide 34 accreditation; BELAC No. 268-RM)

Processing

European Commission (EC), Joint Research Centre (JRC)
Institute for Reference Materials and Measurements (IRMM), Reference Materials Unit, Geel (BE)
(Work performed under ISO Guide 34 accreditation; BELAC No. 268-RM)

Homogeneity measurements

ALS Scandinavia AB, Luleå (SE)
(Measurements performed under ISO/IEC 17025 accreditation; SWEDAC 1087)

Short-term stability measurements

Minton, Treharne & Davies Ltd., Llanelli (UK)
(Measurements performed under ISO/IEC 17025 accreditation; UKAS 1946)
ALS Scandinavia AB, Luleå (SE)
(Measurements performed under ISO/IEC 17025 accreditation; SWEDAC 1087)

Long-term stability measurements

ALS Scandinavia AB, Luleå (SE)
(Measurements performed under ISO/IEC 17025 accreditation; SWEDAC 1087)

Characterisation measurements

ALS Czech Republic s.r.o., Praha (CZ)
(Measurements performed under ISO/IEC 17025 accreditation; Czech Accreditation Institute 259/2006)

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(Measurements performed under ISO/IEC 17025 accreditation; FINAS T024)

SCK-CEN, Mol (BE)
(Measurements performed under ISO/IEC 17025 accreditation; BELAC 015-TEST)

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

Umweltbundesamt GmbH, Wien (AT)
(Measurements performed under ISO/IEC 17025 accreditation; Wirtschaftsministerium 92714/499-IV/9/01)

4 Processing of the material

4.1 Material selection

Trace element mass fractions were measured in samples of three types of bovine muscle; rump muscle, rib muscle of a fatty type, and leg muscle. For most elements, mass fractions were the same as those certified in BCR-184, within uncertainties that were not estimated, however expected to be broad as the samples were heterogeneous materials, and water contents were only estimated by freeze-drying experiments. Exceptions were noted for Se, which was about 2 times higher in all samples than in BCR-184 and for Cd and Pb, which were both present in concentrations below 10 µg/kg. In comparison, the leg muscle had the highest contents of Cd and Pb, and was therefore selected as the raw material.

The raw material was beef muscle from two healthy cattle slaughtered in Belgium: The first batch came from an animal of 39 months of age and the second batch from an animal of 47 months. All raw material was certified as "Fit for human consumption". The material was supplied in two batches of about 40 kg. It consisted of pieces of leg muscle trimmed of fat and sinew, of between 1 and 2 kg. It was sealed in plastic bags of about 10 kg for transport to IRMM.

The raw material was stored at 4 °C until processing, which took place less than 24 h after arrival at IRMM on both occasions. On arrival at IRMM, the material was weighed and sub-samples were taken and frozen at -20 ºC for later contamination checks on processing.

4.2 Bovine muscle processing

The raw material was cut into cubes of 1 to 2 cm length using ceramic knives and low-density polythene cutting boards. The cutting took place in an area that was curtained off and supplied from above with HEPA-filtered air. Cubes were placed on Teflon-coated stainless steel trays, weighed, and placed in a freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, DE). The material was dried under vacuum until a water content of about 3 % (m/m) was reached.

The dried cubes of bovine muscle were placed in stainless-steel drums that were immersed in liquid nitrogen overnight. Drums were removed from the liquid nitrogen one-by-one and the contents were transferred to a cryogenic Palla vibrating mill with titanium inner surface and milling rods (KDH Humboldt Wedag, Köln, DE) that had been pre-cooled with liquid nitrogen, and was maintained below -100 ºC. The resulting powder was sieved twice through a 250 µm Nylon sieve (Russel Finex, London, UK). The powder that passed the sieve was stored in acid washed 200 L HDPE drums, and the remaining fraction was discarded.

About 15 kg of powder was produced, which was further homogenised by 3-dimensional mixing in a Dyna-MIX CM200 mixer (WAB, Basel, CH). The material was tested for suitability of water content and particle size distribution: Sub-samples indicated water contents of less than half the target maximum content of 7 % (m/m) while no particles were larger in size than 435 µm and the particle-size distribution approximated log-normal. The material was therefore considered suitable to be bottled.

A batch of 50 ml brown glass bottles were acid washed with 6 % HNO_3 (p.a. grade, Merck, Darmstadt, DE), rinsed with Milli-Q purified water (18.2 MΩ/cm) and dried in a drying cabinet.

The powder was transferred to the bottles using an automatic filling machine (All Fill, Sandy, UK), fitted with PTFE coated hopper and auger. Lyo-inserts were automatically pressed into the bottles after filling, and the hopper and bottles were continually flushed with nitrogen gas. The bottles were then capped with aluminium lids and labelled.

In total, 1704 units were produced, each containing about 7 g of material. Bottles for general distribution were stored at 18 °C.

4.3 Additional characterisation measurements

Five bottles were selected by random sampling for measurement of water content by Karl Fischer titration (KFT) [2], particle size analysis, and for micrographs. All analyses were made in duplicate on each bottle.

KFT results showed a mean water content of 1.92 % (m/m) in the samples, with a standard deviation of 0.07 % (and estimated measurement uncertainty of 0.3 % (m/m), k=2). The material was therefore considered to be sufficiently dry to preserve the matrix over the desired shelf-life, and the water content was the same between bottles (within measurement uncertainty) at the time of their preparation.

Particles size analysis (PSA) was performed with laser diffraction spectrometry using a Helos laser light scattering instrument (Sympatec GmbH System-Partikel-Technik, Clausthal-Zellerfeld, DE) that measured in the range 0.5 to 875 µm. Within the 10 measurements, the x_{90} bands (size beneath which 90 % of particles are measured) were found between 156 and 191 µm with uncertainty (k=2) of 9.5 µm. All distributions approximated log-normal. These results indicate that the majority of particles are below the 250 µm target, and that the distribution appears to be homogenous between bottles.

Micrographs were made with a microscope (Stemi 2000-C, Zeiss, DE) with > 10 µm resolution. The particles were irregularly shaped, with no agglomerations or fibres apparent in any of the 10 images.

The mass fractions of major matrix components were estimated so that information values could be provided to assist in the optimisation of sample preparation methods. A selection of major elements were also measured, in the samples which were used for certification measurement by SCK-CEN, using NAA. The element mass fractions provided as additional information are given in Table 2.

Table 2: Major elements measured by NAA in ERM-BB184 (bovine muscle)

Element	*mg/kg*	U_{rel}
Ca	155	4.5 %
Cl	1538	1.2 %
K	15844	1.7 %
Mg	997	1.9 %
Na	1804	1.8 %

Where U_{rel} = Relative uncertainty for 6 replicate measurements.

5 Homogeneity study

5.1 Design of the homogeneity study

For the homogeneity study, 12 bottles of ERM®-BB184 (bovine muscle) were selected using a random stratified sample picking scheme. In each bottle, 6 replicate measurements of the mass fractions of each element for certification were made on sub-samples of about 0.2 g, together with measurement of the water content. The measurement methods used were ICP-MS for As, Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn, ICP-OES for Fe and Se and CVAFS for Hg.

Samples were measured in a sequence with replicates in ascending and descending order to allow distinction between an analytical trend and a trend in the filling sequence. All measurements were performed on one day, in a single analytical sequence. Water content was measured in each bottle by drying a sub-sample to constant mass at 105 ºC, and measured element mass fractions were corrected to the dry mass.

Grubbs tests on 95 and 99 % confidence levels were performed to detect potentially outlying individual results as well as outlying bottle averages. Regression analyses were performed to detect possible trends in the analytical or filling sequences. The uncertainty contribution from possible heterogeneity was estimated by a one-way analysis of variance (ANOVA) [3]. Method repeatability (s_{wb}) expressed as a relative standard deviation is given in equation 1:

$$s_{wb} = \frac{\sqrt{MS_{within}}}{\bar{y}} \qquad (1)$$

MS_{within} = mean square within a bottle from an ANOVA

$\bar{y}$ = average of all results of a homogeneity study

Between-unit variability (s_{bb}) expressed as a relative standard deviation is given by equation 2:

$$s_{bb} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}} \qquad (2)$$

$MS_{between}$ = mean square among bottles from an ANOVA

n = average number of replicates per bottle

Heterogeneity that can be hidden by method repeatability is defined in equation 3:

$$u^*_{bb} = \frac{s_{wb}}{\sqrt{n}} \sqrt[4]{\frac{2}{\nu_{MSwithin}}} \qquad (3)$$

$\nu_{MSwithin}$ = degrees of freedom of MS_{within}

The larger value of s_{bb} or u^*_{bb} was used as uncertainty contribution for (potentially hidden) heterogeneity, u_{bb} (see Table 2 for a summary of results, values were converted into relative uncertainties).

5.2 Results of the homogeneity study

The results are presented in Table 3, with graphs of sample means against bottle number, and replicate measurements against sequence, in Annex A. Outliers were detected by the single Grubbs tests for Mn and Pb, and by the double Grubbs test for Fe. Significant slopes were found for the filling sequences for Cd, Cu, Hg, Mn and Zn, and in the analytical sequences for Fe, Pb, Se and Zn. The consequences of these observations on the uncertainty estimations for between-bottle homogeneity are discussed below, by element:

For As and Co, no outlying data points, slopes or otherwise suspicious data were found, and reliable estimates of u_{bb} were obtained.

For Cd, a slope was found at the 95 % confidence level for the sample filling sequence. Caution should be taken in interpretation of this dataset as the mass fraction of Cd in the material was at a level close to the limit of detection for the method, 0.001 mg/kg. (In this range, the laboratory reported measurement uncertainty of between 25 and 37.5 % (k=2)). However, on calculation it can be seen that over the range of 1704 bottles, the slope equates to a potential change of 12 %. If a user is equally likely to receive any bottle from the sequence, the uncertainty is calculated as the potential difference divided by $\sqrt{3}$, to represent a rectangular distribution, which is 6.88 % in this case. As this value is higher than the measured s_{bb} of 4.62 %, the uncertainty introduced by the slope is taken as the u_{bb} to cover any potential trend.

For Cr, measurement RSD of almost 40 % was found. Considering that the measurement uncertainties were typically 25 % (k=2), which is a reliable estimate for the ICP sector-field MS technique applied, this result indicates heterogeneity of Cr in the material.

For Cu, a slope was found at the 99 % confidence level for the sample filling sequence. Over the range of bottles, the slope equates to a potential difference of 2.04 %. If a user is equally likely to receive any bottle from the sequence, the uncertainty is calculated as the potential difference divided by $\sqrt{3}$, to represent a rectangular distribution, which is 1.18 % in this case. As this value is higher than the measured s_{bb} of 0.63 %, the uncertainty introduced by the slope is taken as the u_{bb} to cover any potential trend.

For Fe, the first two measurements of the sequence lay about 5 % below the mean measured value, and were found to be outliers by the double Grubbs test at level a = 0.05. Their position in the analytical sequence and the relatively low RSD for all measurements (< 1.6 %), indicates that they were the result of an analytical error, and they were therefore excluded from the further evaluation. The analytical sequence itself shows a significant slope at the 99 % confidence level. However, as replicates of individual bottles were randomised in the measurement sequence, this could only lead to a conservative over-estimation of u_{bb}.

For Hg, slopes were found at the 99 % confidence level for the sample filling sequence. The measurement method did not have sufficiently low uncertainty at the level measured in the sample; measured values lay between 0.0011 and 0.0013 mg/kg, with uncertainty of 0.0018 (k=2). Using the same method to calculate the uncertainty introduced by the slope as described for Cd and Cu, an uncertainty of 0.70 % was found. This is the same as the u^*_{bb}, calculated by ANOVA, which is taken as the u_{bb}, however, given the method uncertainty, neither method can be considered to give a reliable estimate.

For Mn, one outlier was found by the single Grubbs test at level α = 0.01, which lay 15 % above the mean, and appears to be untrustworthy considering the measurement RSD of 3.5 %. However, there was no technical reason to exclude the outlier and it was retained for

the uncertainty calculation. A significant slope was found at the 95 % confidence level for the sample filling sequence, however, this is likely to be an artefact resulting from the aforementioned outlier; if it is removed from the data series, no slope is found. The s_{bb} calculated including the outlying data-point was therefore taken as the u_{bb}, and the slope ignored.

For Ni, measurement RSD of over 40 % was found. Considering that the measurement uncertainties were typically between 25 and 40 % (k=2, dependent on concentration), this result indicates heterogeneity of Ni in the material.

For Pb, one outlier was found by the single Grubbs test at level $\alpha = 0.05$. There was no technical reason to exclude the outlier and it was retained for the uncertainty calculation. A significant slope was found at the 95 % confidence level for the measurement sequence, however, this is likely to be an artefact resulting from the aforementioned outlier: If it is removed from the data series, no slope is found. The s_{bb} calculated including the outlying data-point was therefore taken as the u_{bb}, and the slope ignored.

For Se, no outliers were identified, however, a significant slope was found at the 95 % confidence level for the measurement sequence. As replicates of individual bottles were randomised in the measurement sequence, this could only lead to a slight over-estimation of u_{bb}.

For Zn no outlying measurements were identified by the single Grubbs test. A significant slope was found at the 95 % confidence level for the measurement sequence and a slope was observed at the 99 % confidence level for the sample filling sequence. While the double Grubbs test at level $\alpha = 0.05$ identified two outlying bottles, the slope with filling sequence was still observed on removing these bottles from the test. Over the range of bottles, the slope equates to a potential difference of 2.65 %. If a user is equally likely to receive any bottle from the sequence, the uncertainty is calculated as the potential difference divided by $\sqrt{3}$, to represent a rectangular distribution, which is 1.53 % in this case. As this value is lower than the measured s_{bb} of 0.80 %, the uncertainty introduced by the slope is taken as the u_{bb} to cover any potential trend.

All individual data and bottle averages showed normal or unimodal distributions, for all analytes.

In conclusion, the distribution of all elements except Cr and Ni can be considered as homogeneous, within acceptable uncertainties, in this material.

Table 3: Evaluation of homogeneity study for elements in ERM®-BB184 (bovine muscle)

	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Se	Zn
Mean [mg/kg]	0.0226	0.0014	0.0058	0.103	2.336	82.04	0.0011	0.291	0.0710	0.0014	0.478	158.4
RSD [%]	6.64	10.6	5.41	38.6	1.61	1.43	4.02	3.49	41.0	16.6	2.53	1.85
MS_{within}	2×10^{-6}	2×10^{-8}	5×10^{-8}	1.2×10^{-4}	1.2×10^{-3}	1.23	$< 1 \times 10^{-8}$	9.4×10^{-5}	5.1×10^{-3}	6×10^{-8}	1.4×10^{-4}	7.03
$MS_{between}$	1×10^{-6}	5×10^{-8}	3.9×10^{-7}	9.6×10^{-3}	2.5×10^{-3}	2.12	$< 1 \times 10^{-8}$	1.5×10^{-4}	7.2×10^{-5}	4×10^{-8}	1.7×10^{-4}	16.8
s_{bb} [%]	$MS_B<MS_W$	4.62	4.07	38.5	0.63	0.48	0.42	1.07	40.7	$MS_B<MS_W$	0.44	0.80
s_{wb} [%]	6.98	9.62	3.72	10.4	1.49	1.35	4.00	3.34	11.9	17.0	2.50	1.67
u^*_{bb} [%]	1.22	1.68	0.65	1.81	0.26	0.24	0.70	0.58	2.08	2.96	0.44	0.29
u_{bb} [%]	1.22	**6.88**	**4.07**	**38.5**	**1.18**	**0.48**	0.70	**1.07**	**40.7**	2.96	0.44	**1.53**
Significant slope in measured value/sample bottle number, at confidence level;												
95 %	No	**Yes**	No	No	**Yes**	No	**Yes**	**Yes**	No	No	No	**Yes**
99 %	No	No	No	No	**Yes**	No	**Yes**	No	No	No	No	**Yes**
Significant slope in measured value/measurement sequence number, at confidence level;												
95 %	No	No	No	No	No	**Yes**	No	No	No	**Yes**	**Yes**	**Yes**
99 %	No	No	No	No	No	**Yes**	No	No	No	No	No	No
Outliers by Grubbs tests, individual measurements												
Single; a=0.05	None	None	None	None	None	**1**	None	**1**	None	**1**	None	None
Double; a=0.05	None	None	None	None	None	n/a	None	n/a	None	n/a	None	None
Single; a=0.01	None	None	None	None	None	None	None	**1**	None	None	None	None
Double; a=0.01	None	None	None	None	None	None	None	n/a	None	None	None	None

5.3 Minimum sample intake

The minimum sample intake 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 at IRMM using solid sampling atomic absorption spectrometry, SSAAS, to measure the contents of selected elements in sub-milligram amounts of the material, without sample preparation. Initial tests showed that of all the target elements for certification, the mass fractions of Cu and Mn were in a suitable range for reliable measurement.

To estimate the minimum sample intake by SSAAS, about 30 absorbance peak area measurements are made on sub-samples weighed on a microbalance. These are blank subtracted by making an absorbance peak area measurement with an empty platform (or externally calculated mean of more than one measurement). A calibration slope (blank-subtracted absorbance peak area over sample mass) is calculated by the method of least-squares. For each measurement, the calibration predicted absorbance, residual absorbance (from the calibration) and relative residual are calculated, and thereby, the standard deviation of the relative residuals. This value is then used to calculate a minimum sample intake, based on the desired, or certified, standard uncertainty of the element mass fraction of the material, through the following equation:

$$MSI = \left(\frac{k'_2 \; s_m}{u} \right)^2 m \qquad (4)$$

Where k'_2 = the factor for two-sided tolerance limits for the number of measurements, s_m = the standard deviation of the relative residuals, u = the relative standard uncertainty of the element mass fraction in the material, m = the mean sub-sample mass.

The least-squares calibration and coefficient of determination are calculated according to common statistical convention. The estimation of minimum sample intake, based on this data, is made according to the calculations presented in reference [4].

The minimum sample intakes were estimated for the u_{CRM} given for the respective element mass fractions in Table 13. This returned values of 5 mg for Cu and 100 mg for Mn. Both values lie well below the minimum sample intake set for the material.

6 Stability studies

6.1 Short-term stability study

6.1.1 Design of the short-term stability study

A four weeks isochronous study [5] was performed to evaluate the stability of the material during transport. For the short-term stability study, 10 bottles of ERM®-BB184 (bovine muscle) were chosen using a random stratified sample picking scheme. Element concentrations were measured using ICP-MS and ICP-OES methods.

Samples were stored at 18 °C and 60 °C as well as at a reference temperature of -20 °C. Two bottles were stored at each temperature for 0, 2 and 4 weeks. After the indicated storage periods, the samples were transferred to storage at -20 °C until analysis. Samples were analysed under repeatability conditions in an order specified by IRMM (randomised sample order). In each of the 10 bottles a determination of dry mass content was performed and results for each element were corrected for the dry mass.

Grubbs tests at the 99 % confidence level were performed to detect potentially outlying results. Data points were plotted against time and the regression lines were calculated to check for significant trends (degradation, enrichment) due to shipping conditions (see Table 3). The observed slopes were tested for significance using a *t*-test, with $t_{\alpha,df}$ being the critical *t*-value (two-tailed) for a confidence level $\alpha = 0.05$ (95 % confidence interval) and for a confidence level $\alpha = 0.01$ (99 % confidence interval). The slope was considered as statistically significant when $|b|/se_b > t_{\alpha,df}$. Graphs can be found in Annexes B1 and B2.

6.1.2 Results of the short-term stability study

Unfortunately, the analytical performance for the first study was not sufficient to draw conclusions from the data for As, Cd, Hg and Pb. Therefore, another set of vials were held at 60 °C for 0, 2 and 4 weeks, and an additional study was made for As, Cd and Hg at a later date. For this second study, only the 60 °C temperature was tested as the long-term stability study described below concluded that all certified elements were stable in the material on storage at 18 ºC for 12 months. The results by element are discussed below:

As, Cd and Hg: Each of these elements was assessed by the second study, at 60 ºC only, and no outlying data points or significant slopes with time were found.

Cr: Results for Cr were not assessed because evidence of heterogeneity was found by the homogeneity tests described in the previous section.

Cu: For the 18 ºC data set, one outlier was found at the 99 % confidence level by the single Grubbs test, with a value almost 4 times the mean of the dataset, which otherwise showed an RSD of less than 10 %. A sample of the QC material was measured immediately before the outlier in the in the analytical sequence, and the result was also about 4 times the mean of the remaining values measured in the QC material. This outlier was therefore considered to be most likely caused by contamination, and was removed from the dataset. The remaining measurements on the QC material showed no bias (in comparison with the certified value). For the 18 ºC data set, one outlier was found at the 99 % confidence level, however, there was no technical reason to exclude it from the dataset. No significant slopes with time were found at either test temperature.

Fe: For the 18 ºC data set, one outlier was found by the single Grubbs test, at the beginning of the measurement sequence (position 3). However, there was no reason to exclude it from the dataset. No significant slopes with time were found at either test temperature.

Mn: For both datasets, no outliers were found and no significant slopes with time were found at either test temperature.

Pb: Results for more than half the samples were reported as being below the LOD of this method, 0.102 mg/kg. As results of the characterisation exercise were not suitable for the certification of Pb, the stability testing was not repeated.

Se: No outliers at the 99 % confidence level, no bias and no significant slopes with time were found at either test temperature.

Zn: No outlying measurements and no significant slopes with time were found for either test temperature.

In summary, reliable estimations of u_{STS} were obtained for all elements to be certified. In each case, no slope was found and u_{STS} for 1 week varied between 0.24 and 1.40 %, which lie well below the respective u_{CRM} and are thus negligible. The material is therefore considered to be sufficiently stable at elevated temperature to be transported without cooling elements.

Table 4: Evaluation of short-term stability study for elements in ERM®-BB184 (bovine muscle)

Element	Test temperature	single Grubbs[1]	double Grubbs[1]	slope[2]	$u_{STS, rel}$ %[3]
As	18ºC	-	-	-	-
	60 ºC	none	none	no	0.79
Cd	18ºC	-	-	-	-
	60 ºC	none	none	no	0.94
Cu	18ºC	none	none	no	1.09
	60 ºC	1	n/a	no	1.32
Cr	18ºC	none	none	no	4.62
	60 ºC	none	none	no	4.89
Fe	18ºC	1	n/a	no	0.61
	60 ºC	none	none	no	0.54
Hg	18ºC	-	-	-	-
	60 ºC	none	none	no	0.97
Mn	18ºC	none	none	no	1.27
	60 ºC	none	none	no	1.4
Se	18ºC	none	none	no	0.82
	60 ºC	none	none	no	0.73
Zn	18ºC	none	none	no	0.24
	60 ºC	none	none	no	0.27

[1] Number of outlying measurements by Grubbs tests at the 99 % confidence level
[2] Significant slope in mean measured value (bottle) over time at raised temperature at 95 or 99 % confidence level
[3] relative, for 1-week

6.2 Long-term stability study

6.2.1 Design of the long-term stability study

Data from two isochronous stability studies were combined to assess the stability of the CRM.

For the first isochronous study, 8 bottles were chosen using a random stratified sample picking scheme. Sets of two bottles were stored at 18 °C for 0, 4, 8 and 12 months and were maintained before and after the study at the reference temperature of -20 °C. Samples were analysed in a sequence with randomised sample order. The measurements were made methods using ICP-MS for As, Cd, Cu, Mn and Zn, ICP-OES for Fe and Se and CVAFS for Hg. The second isochronous study compared vials stored at -20°C as reference samples, with those from the normal stock stored at 18 °C. Two vials per storage time were selected using a random stratified sampling scheme, and element mass fractions were measured 39 months after the start of the first isochronous study. From each vial, 6 samples were measured by ICP-MS. For both studies, water content was measured once in each bottle so that dry-mass correction of element concentrations could be performed

Grubbs tests at the 99 % confidence level were performed to detect outlying results. Data points were plotted against time and the regression lines were calculated to check for significant trends of degradation or enrichment) due to storage conditions (see Table 5 for a summary). The observed slopes b were tested for significance using a t-test, with $t_{\alpha,df}$ being the critical t-value (two-tailed) for a confidence level α = 0.01 (99 % confidence interval). The slope was considered as statistically significant when $|b|/se_b > t_{\alpha,df}$. Finally, the uncertainty of stability u_{lts} [6] was calculated for a pre-defined shelf life of 12 months applying equation 5:

$$u_{lts} = \frac{RSD_{stab}}{\sqrt{\sum (x_i - \bar{x})^2}} \cdot t_{sl} \qquad (5)$$

with RSD_{stab} being the relative standard deviation of all individual results of the stability study, x_i being the time point for each replicate, $\bar{x}$ being the average of all time points and t_{sl} being the pre-defined shelf-life. Graphs can be found in Annex C.

A normalisation was applied to take into account differences between the two studies. This design allows separation of a potential analytical drift from a trend over storage time. 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. [6] t-tests were performed to evaluate potential significant difference between the measurements performed. In the case of a significant (95 % confidence level) difference between studies, results were normalised using the following equation:

$$d = \overline{x_2} / \overline{x_1} \qquad (6)$$

$\overline{x_1}$ mean measurement result of study 1

$\overline{x_2}$ mean measurement result of study 2

The relative uncertainty of this correction $u_{d,\,rel}$ was calculated as:

$$u_{d,rel} = \sqrt{\frac{1}{n_1} RSD_1^2 + \frac{1}{n_2} RSD_2^2} \qquad (7)$$

RSD_1: relative standard deviation of all results in study 1
RSD_2: relative standard deviation of all results in study 2
n_1: number of data points is study 1
n_2: number of data points in study 2

Whenever the bias correction is applied, the uncertainty on the correction is included in the calculation of RSD_{stab} according to equation 8:

$$RSD_{stab} = \sqrt{\frac{n_1 u_{d,rel}^2 + \sum_{j=1}^{n_1+n_2} \left(y_j - \hat{y}_j\right)^2}{n_1 + n_2 - 1}} \qquad (8)$$

The corrected RSD is then used for the estimation of u_{lts} according to equation 5.

The combined datasets were plotted against storage time and regression lines of concentration versus time were calculated. The slopes of the regression lines were tested for statistical significance.

6.2.2 Results of the long-term stability study

For Cd and Hg, significant differences in the means of all measurements were found between studies, and the results of the first studies were therefore normalised with correction factors, d = 0.81 ± 0.04 and d = 0.85 ± 0.03, respectively.

The obtained data were evaluated individually for each study, and in combination. Results of the statistical tests on the data from the first, second and combined studies are given in table 5.

Table 5: Statistical results of the long-term stability tests

Element	Number of individual outlying results			Significance of the trend on a 99% confidence level		
	first	second	combined	first	second	combined
As	none	none	none	no	no	no
Cd	none	none	none	no	no	no
Cu	none	none	none	no	no	no
Fe	none	none	none	no	no	no
Hg	1	none	none	yes	no	no
Mn	none	none	none	no	no	no
Pb	none	none	none	no	no	no
Se	none	none	none	no	no	no
Zn	none	none	none	no	no	no
As	none	none	none	no	no	no

Analytical performance of the long-term stability studies was similar to that of the homogeneity study, in that the mass fraction of Hg in the samples was close to the method LOQ of 0.001 mg/kg. However, as the sample intake mass was raised from 0.2 g to 0.5 g, measurement repeatability reached a suitable level to provide an estimate of u_{LTS}. One outlying measurement was found by the Grubbs test in the first stability study, which caused a statistically significant slope over time at the 99 % confidence level. There was no technical

reason to exclude the outlier and it was retained for statistical analysis. On combination of the two studies, the data point was no longer found to be an outlier, and no significant slope was detected.

For all other elements, no outliers or significant slopes with time were detected in either of the studies or the combined study.

In summary, reliable estimates of u_{LTS} were obtained for As, Cd, Cu, Fe, Mn, Se and Zn. The estimates are listed in table 6. No slopes were found in concentration over storage time at + 18 ºC. The material may therefore be stored at + 18 ºC.

Table 6: Results of the 12 month stability study on element concentrations in ERM®-BB184 (bovine muscle)

Element	$u_{LTS,\ rel}$ %[1]
As	1.98
Cd	4.27
Cu	1.10
Fe	0.70
Hg	2.00
Mn	1.21
Se	1.08
Zn	0.82

[1] relative, for 24-months

7 Characterisation

7.1 Design of the characterisation study

In providing measurements for the characterisation study, the laboratories applied validated methods. Measurements were either covered by the laboratory's scope of accreditation or measurement capabilities were proven through demonstration of previous experience and acceptable performance in the analysis of trace elements in comparable matrices.

In commissioning the study, collaborators were asked if they were able to provide measurements for the same element(s) by more than one analytical technique. This allowed the provision of a greater number of independent results for elements, where possible. Each laboratory was provided with two bottles of ERM®-BB184 (bovine muscle) and one bottle of BCR-184 (bovine muscle) that was to be measured with the samples, as a quality control. Because of the limited sample quantity per unit, laboratories that offered to measure one or more elements by more than one technique were supplied with extra bottles of the candidate reference material and quality control material, as required.

With the exception of measurements by neutron activation analysis (NAA), measurements on each sample bottle were spread over two days. NAA measurements were made under intermediate precision conditions. For each sample bottle, three replicate measurements were made and in each bottle of sample and QC material, the water content was determined to allow correction to dry mass for the element mass fractions.

A table of methods employed by the participants is given in Annex E.

7.2 Results and technical evaluation – Principles

After receipt of the data sets, the results were subjected to technical evaluation according to the following criteria.

Certain data sets from laboratories were excluded when their measured values for the QC material did not agree with that certified, when compared according to equation 9:

$$\left| y_{mean} - c_{CRM} \right| < 2\sqrt{u_{CRM}^{\,2} + u_y^{\,2}} \qquad (9)$$

Where y_{mean} = mean measured concentration in the QC, c_{CRM} = certified concentration in the CRM, u_{CRM} = standard uncertainty of the concentration in the CRM, u_y = standard uncertainty reported for measurements of the same element by the same (or similar) technique.

As approaches to uncertainty estimation differ between laboratories, decisions to exclude datasets on the basis of failed QC criteria were taken considering the level of uncertainty reported by other participants for measurements of the same element by the same or similar techniques: The maximum reported measurement uncertainty, u_y, of all labs reporting for the studied element was therefore applied in equation 6.

The evaluation of QC measurements for As and Mn was based on the results reported for the characterisation exercise for ERM-BB186 (Pig kidney), which was run concurrently with the exercise for ERM-BB184. For this study, the As mass fraction was not certified in the QC material, and the Mn mass fraction carried an uncertainty significantly higher that that of the other elements and above the target uncertainty of the new material.

Checks were made to see that results from the two different measurement days were consistent with the measurement uncertainty of the lab, and the level of between-bottle homogeneity established for the material.

The reported values for each element were checked against the method LOQ reported by the laboratory and values below the LOQ were excluded from the calculation of certified values.

Finally, comments and reports of anomalies in the measurements reported by the labs were considered in deciding whether to include values in the certification.

After passing the criteria above, the accepted sets of results were submitted to the following statistical tests:

- Scheffe multiple *t*-test to check if the means of two labs are significantly different
- Dixon test to detect outlying laboratory means
- Nalimov *t*-test to detect outlying laboratory means
- Grubbs test to detect single and double outliers
- Cochran test to check for outlying laboratory variances
- Bartlett test to check for homogeneity of laboratory variances
- ANOVA to assess between laboratory and within laboratory variances and test their significance employing the Snedecor *F*-test
- Skewness and kurtosis tests to assess the normality of the lab means distribution

7.3 Results and technical evaluation

The technical evaluation of results for inclusion in the accepted datasets is summarised in Table 7. The results of the accepted datasets, and statistical tests thereon are summarised in Table 8. Results from each participant and corresponding graphs can be found in Annex D.

With the exceptions of Cd and Pb, variances between labs were significantly different (Snedecor *F*-test), therefore data could not be pooled and had to be grouped by labs. Variances for Cd and Pb were high for some laboratories, as mass fractions lay close to their limits of quantification. Unless a technical reason for exclusion was found, data sets that had outlying laboratory variances (Cochran test) were kept. The results of the statistical and other tests are discussed for each element, below:

As: The value provided by Lab 1 was below their techniques LOQ, and was therefore excluded. Measurements of the QC material by Lab 5 and Lab 6 were about 2 times higher than those of the other participants. However, as the As mass fraction in the QC material was not certified, it remains possible that the values of Labs 5 and 6 were correct. The results of Labs 5 and 6 were provided by the same institute using different measurement techniques, and that institute participated in the characterisation exercise for ERM-BB186 pig muscle. In that case, the measurements made by (the same method used for) Lab 5 were also found to have a positive bias of about 2 times. The results of Labs 5 and 6 for this study were therefore considered unreliable, and were rejected.

Cd: Lab 4 reported that the results were below their LOQ, and the results were therefore excluded from calculation of the certified value. Measurement of the QC sample by Lab 5 returned a result of less than a quarter of the certified value, which lay outside their reported uncertainty of 15 %. Their result was therefore excluded.

Cu: Lab 14 reported that their measurements were unreliable due to an unexpectedly high measurement background, and their result was therefore excluded. Labs 8, 9 and 10

provided measurements of the QC samples with significant bias, lying 25, 22 and 25 % below the certified value, while the greatest measurement uncertainty reported by all participants (and applied in equation 9) was 15 %. The results, all of which were different measurement techniques used by the same institute, were therefore excluded. QC measurement results for the remaining labs lay within 7 % of the certified value. After exclusion of Labs 8, 9 and 10, the result of Lab 7 was found to be an outlier at α = 0.05 by the Nalimov test. However, as it was not an outlier at α = 0.01, it was considered to be a straggler and was retained for calculation of the certified value.

Fe: Labs 9 and 10 provided measurements of the QC samples with significant bias, lying 26 and 25 % below the certified value, while the greatest measurement uncertainty reported by all participants (and applied in equation 9) was 15 %. The results were therefore excluded. QC measurement results for the remaining labs lay within 11 % of the certified value. The mean of sample measurements from Lab 4 was found to be an outlier at α = 0.05 by the Nalimov test. However, as it was not an outlier at α = 0.01, it was considered to be a straggler and was retained for calculation of the certified value. Results of Lab 1 had an outlying (higher) variance by the Cochran test. However, no technical reason could be found for the higher variance and the result was included.

Hg: Lab 4 submitted results below their LOQ, and these were therefore rejected. Tests on the remaining 5 labs revealed no outlying means. While none of the Labs QC measurements showed evidence of bias, 3 labs reported measurement uncertainties of about 40 %. Given that the uncertainty reported by laboratories was much greater than the target for certification, and that the RSD between laboratories lay at a similar level, the results for Hg will only be used to calculate an indicative value for the mass fraction.

Mn: The QC material carried a relatively high uncertainty on the certified Mn mass fraction. This was reflected in the distribution in QC results from all participants, of 18 % RSD over all labs compared to 10 % for the sample, and the fact that more than half the labs reported results with bias greater than the maximum reported measurement uncertainty of 15 %. Therefore, for Mn analytical performance was assessed based on the results for the QC measurements made for the ERM-BB186 study (as described for As). Results of Labs 5 and 7 were provided by the same institute using the same measurement techniques that were applied in the characterisation exercise for ERM-BB186 pig muscle. For that exercise, their QC values were biased high (66 and 63 %), and sample measurements lay far from the mean. Therefore, measurements of Mn by this institute were not considered reliable, and were rejected. All other labs, except lab 2, participated in the ERM-BB186 study and provided QC measurements with no evidence of bias that lay within 7 % of the certified value. The results of lab 2 for both the QC material and the sample agreed with the respective intercomparison means, and their result was retained.

Pb: Labs 1, 5, 11 and 13 reported that the Pb mass fraction was below their method detection limits, and Lab 4 estimated their measurement uncertainty to be over 100 %. The results of Lab 2 were about 10 times higher than all other labs, the mean was found to be an outlier by all tests and the variance an outlier by the first pass of the Cochran test. However, no technical reason to exclude this result could be found. While measurements of the QC material demonstrated no bias for most labs, the Pb mass fraction in the material was about 100 times that of the sample and thus lay at a concentration suitable for measurement by the techniques applied. The dataset returned an estimate of u_{CRM} of about 50 % and was therefore not found to be of sufficient quality to provide a certified value for Pb.

Se: Lab 5 reported results for the QC material of more than twice the certified amount. Sample measurements were consistent between bottles and replicates, and were also about twice the mean of the study. Measurements therefore fell outside the maximum reported measurement uncertainty, of 25 % for Se, and the results were rejected. Lab 11 supplied

results for the 2 bottles (measured on 2 different days) with a 40 % difference in the mean (with a corresponding 30 % difference for the QC), which resulted in an outlying variance according to the Cochran test. However, as the mean value of the 2 QC measurements lay within the combined uncertainty of measurement and certificate, the result was retained.

Zn: Labs 8, 9 and 10 provided measurements of the QC samples with significant bias, lying 25, 25 and 24 % below the certified value, while the greatest measurement uncertainty reported by all participants (and applied in equation 8) was 16 %. The results were therefore excluded. QC measurement results for the remaining labs lay within 12 % of the certified value. For the accepted dataset, lab 5 was found to be an outlier by the Dixon test at $\alpha = 0.05$ and lab 11 by the Nalimov test at $\alpha = 0.05$. Lab 11 was also found to have an outlying variance by the Cochran Test. However, as both labs provided results that overlapped the intercomparison mean, both were retained.

Table 7: Summary of the technical evaluation

Element	As	Cd	Cu	Fe	Hg	Mn	Pb	Se	Zn
Lab 0	OK	OK	OK	OK	OK	OK	**<LOQ**	OK	OK
Lab 1	**<LOQ**		OK	OK		OK		OK	OK
Lab 2	OK	OK	OK	OK		OK	OK	OK	OK
Lab 3			OK	OK					OK
Lab 4	OK	**<LOQ**	OK	OK	**<LOQ**	OK	OK	OK	OK
Lab 5	**Bias**	**Bias**	OK			**Bias**	**<LOQ**	**Bias**	OK
Lab 6	**Bias**						OK		
Lab 7			OK	OK		**Bias**			OK
Lab 8		OK	**Bias**		OK		OK		**Bias**
Lab 9	OK	OK	**Bias**	**Bias**	OK	OK	OK	OK	**Bias**
Lab 10			**Bias**	**Bias**		OK		OK	**Bias**
Lab 11	OK	OK	OK	OK	OK	OK	**<LOQ**	OK	OK
Lab 12			OK	OK				OK	OK
Lab 13	OK	OK			OK		**<LOQ**	OK	
Lab 14			**Report**	OK		OK		OK	OK
Lab 15			OK						OK

Summary of results that were accepted for the characterisation datasets, and those that were excluded due to being either below the reported method LOQ, showing evidence of bias on measurement of a QC material or with other indications of measurement unreliability reported by the Lab.

Table 8: Results of the characterisation exercise for the accepted datasets

Element	As	Cd	Cu	Fe	Hg	Mn	Pb	Se	Zn
Number of accepted data sets	6	6	10	9	5	8	6	10	11
Number of replicate measurements	36	36	59	54	30	48	36	60	66
Mean of means [mg/kg]	0.0234	0.0022	2.307	75.0	0.0018	0.2756	0.0049	0.453	145.7
Relative standard deviation of mean of means [%]	12.3	7.18	3.35	6.21	41.2	4.78	114	12.0	4.71
Relative standard error of mean of means (u_{char}) [%]	5.02	2.93	1.06	2.07	18.44	1.69	46.4	3.79	1.42
All data sets compatible two by two? (Scheffe test)	no	no	no	no	no	no	no	no	no
Outlying means? (Dixon test; p = 0.05)	none	none	none	none	none	none	Lab 2	none	Lab 5
Outlying means? (Nalimov t-test; p = 0.05)	none	none	Lab 7	Lab 4	none	none	Lab 2	none	Lab 11
Outlying means? (Grubbs test; p = 0.05)	none	none	none	none	none	none	Lab 2	none	none
Outlying lab variances? (Cochran test; p = 0.05)	none	none	none	Lab 1	Lab 11	none	Lab 2, Lab 4	none	Lab 11
Lab variances homogeneous? (Bartlett test; p = 0.01)	yes	no	yes	yes	yes	yes	yes	yes	no
Variances between labs significantly different? (Snedecor F-test; p = 0.01)	yes	no	yes	yes	yes	yes	yes	yes	yes
Distribution of means normal (p = 0.01)? (Skewness, kurtosis and normal probability plot)	insufficient data	insufficient data	yes	yes	insufficient data	yes	insufficient data	yes	yes

8 Certified values and uncertainties

The certified values for ERM®-BB184 (bovine muscle) were calculated as the mean of means of the accepted data sets. The standard error of the mean of means was used as an estimation of the uncertainty contribution of the characterisation exercise. The standard error was calculated as the standard deviation divided by the square root of the number of accepted data sets.

The combined standard uncertainty of the certified value includes contributions from the between-bottle heterogeneity, long-term storage and the characterisation study. The relative combined standard uncertainty is calculated according to equation 10:

$$u_{CRM} = \sqrt{u_{bb}^2 + u_{lts}^2 + u_{char}^2} \qquad (10)$$

The combined expanded uncertainty, U_{CRM}, 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.

Table 9 summarises the individual uncertainty contributions and the resulting expanded uncertainties as well as the certified values and their uncertainties after rounding for ERM®-BB184 (bovine muscle).

Table 9: Certified values and uncertainties for ERM®-BB184 (bovine muscle)

Element	As	Cd	Cu	Fe	Mn	Se	Zn
u_{bb} [%]	1.22	6.88	1.18	0.48	1.07	0.44	1.53
u_{lts} [%] [1)]	1.98	4.3	1.10	0.70	1.21	1.08	0.82
u_{char} [%]	5.0	2.93	1.06	2.07	1.69	3.8	1.42
$u_{CRM, rel}$ [%]	5.5	8.6	1.93	2.23	2.34	4.0	2.24
n results	6	6	10	9	8	10	11
$U_{CRM, rel}$ (*95 % ci.*)[2)] [%]	11.1	17.2	3.9	4.5	4.7	7.9	4.5
Certified value [mg/kg] [3)]	0.0234	0.0022	2.31	75	0.276	0.45	146
$\boldsymbol{U_{CRM}}$ **(*95 % ci.*) [mg/kg]** [3)]	0.0026	0.0004	0.09	4	0.013	0.04	7

1) for a shelf life of 24 months

2) where the standard combined uncertainty is multiplied by 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

3) calculated from unrounded data

Reliable mean values were obtained for the mass fractions of seven elements. While a mean value was established for Hg, the variance inherent in the homogeneity and stability studies, resulting from the content being close to limits of quantification, do not allow the calculation of a reliable U_{CRM}. For Pb, the low number of accepted results, and the high deviation between them returned a U_{CRM} of over 100 %. Table 10 provides the summary for Hg, to which an indicative value was assigned.

Table 10: Summary of results for Hg in ERM®-BB184 (bovine muscle)

Element	Hg
u_{bb} [%]	0.70
u_{lts} [%] [1)]	2.85
u_{char} [%]	18.4
$u_{CRM, rel}$ [%]	18.7
n results	5
$U_{CRM, rel}$ (*95 % ci.*)[2)] [%]	52
Mean value [mg/kg]	0.0018
U_{CRM} (*95 % ci.*) [mg/kg]	0.0010

1) for a shelf life of 24 months

2) where the standard combined uncertainty is multiplied by a coverage factor k = 2.78 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

9 Metrological traceability

The measurement results for assigning mass fractions of the elements were obtained by different digestion and extraction, sample concentration, matrix separation and quantification procedures. Measurements were calibrated with either external calibrants or by isotope dilution using reference materials of known purity and concentration. The certified mass fractions are traceable to the International System of Units (SI).

10 Instructions for use and intended use

10.1 Safety precautions

Usual laboratory safety precautions apply.

10.2 Use of materials

- Allow the bottle to reach ambient temperature before opening.
- Shake the bottle before taking aliquots.
- Certified mass fractions are corrected for the water content of the material (dry-mass): To determine dry mass, accurately weigh an aliquot of approximately 1 g on an analytical balance and dry the sample in an oven at atmospheric pressure, at 103 °C ± 2 °C, until constant mass is attained. The weighing should be made at the same time as preparation of samples for element measurement.

10.3 Intended use

This material is intended to be used for method validation and performance control. To assess the method performance, the measured mass fractions are compared with the certified values following a procedure described by Linsinger [8]; described here in brief:

- Calculate the absolute difference between mean measured value and the certified value (Δ_m).
- Combine measurement uncertainty (u_{meas}) with the uncertainty of the certified value (u_{CRM}) according to equation 9:

$$u_\Delta = \sqrt{u_{meas}^2 + u_{CRM}^2} \qquad (9)$$

- Calculate the expanded uncertainty (U_Δ) from the combined uncertainty (u_Δ) using a coverage factor of two ($k = 2$), corresponding to a confidence interval of approximately 95 %.
- If $\Delta_m \leq U_\Delta$ then there is no significant difference between the measurement result and the certified value, at a confidence level of about 95 %.

10.4 Storage conditions

The material should be stored at a temperature of 18 °C ± 3 °C. However, the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially after opening of the bottles.

11 Acknowledgements

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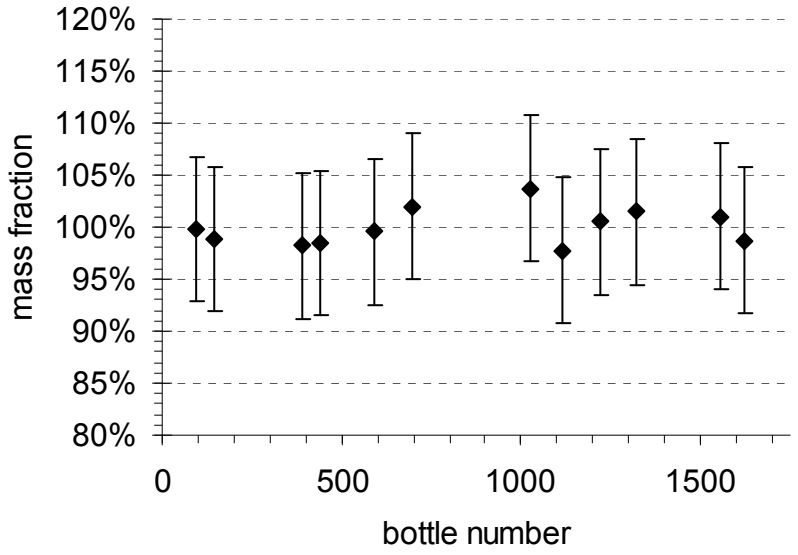
12 References

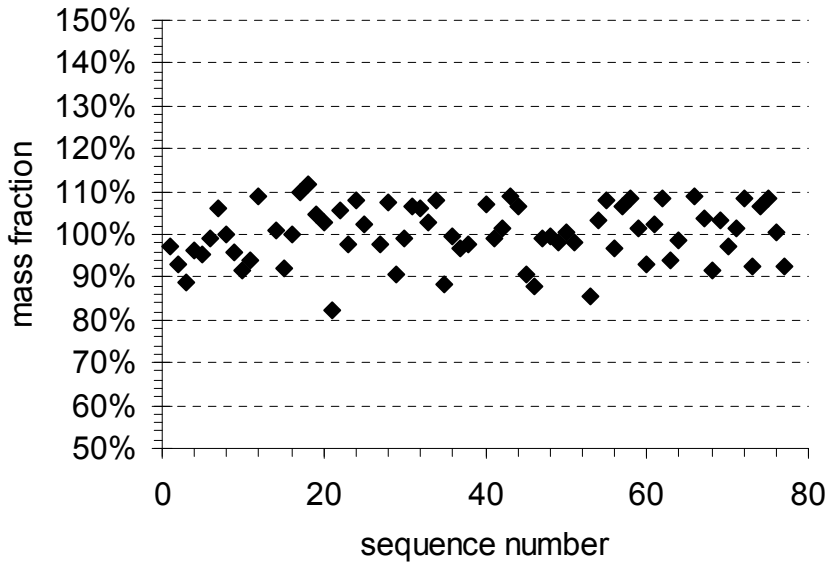
1. International Organisation for Standardization, ISO/IEC Guide 98-3: Uncertainty of measurement -- Part 3: Guide to the expression of uncertainty in measurement (GUM:1995) (2008), ISO, Geneva, CH.
2. Kestens, V., Conneely, P., Bernreuther, A. Vaporisation coulometric Karl Fischer titration: A perfect tool for water content determination of difficult matrix reference materials. Food Chem. 106 (2008) 1454.
3. Van der Veen, A.M.H., Linsinger, T.P., Pauwels, J. Uncertainty calculations in the certification of reference materials, 2. Homogeneity study. Accred. Qual. Assur. 6 (2001) 26.
4. Pauwels, J. and Vandecasteele, C., Fresenius J. Anal. Chem., 1993, 345, 121.
5. Lamberty, A., Schimmel, H., Pauwels, J. The study of the stability of reference materials by isochronous measurements, Fres. J. Anal. Chem. 360 (1998) 359.
6. T.P.J. Linsinger, A.M.H. van der Veen, B.M. Gawlik, J. Pauwels, A. Lamberty, Planning and combining of isochronous stabiliy studies of CRMs, Accred. Qual. Assur. 9 (2004) 464-472
7. Linsinger, T., Pauwels, J., Lamberty, A., Schimmel, H., van der Veen, A.M.H., Siekmann, L. Estimating the uncertainty of stability for matrix CRMs. Fres. J. Anal. Chem. 370 (2001) 183.
8. Linsinger, T.P.J. Comparison of measurement result with the certified value, ERM Application Note 1, July 2005, www.erm-crm.org.

Annex A ERM®-BB184 (Bovine muscle) – Results of the homogeneity study

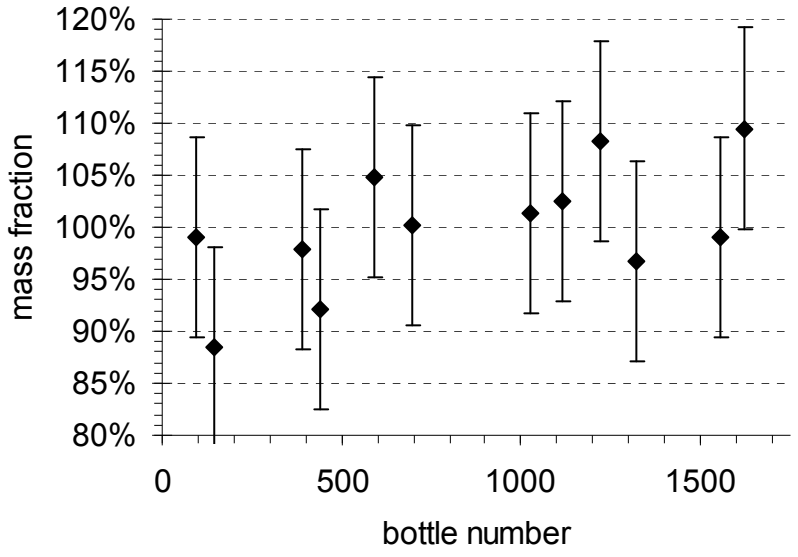
Graphs present mass fractions of bottle means relative to the grand mean, against bottle number, and individual measurement replicates, against sequence number. Vertical bars are a confidence interval of 95 % derived from s_{wb} of the homogeneity study.

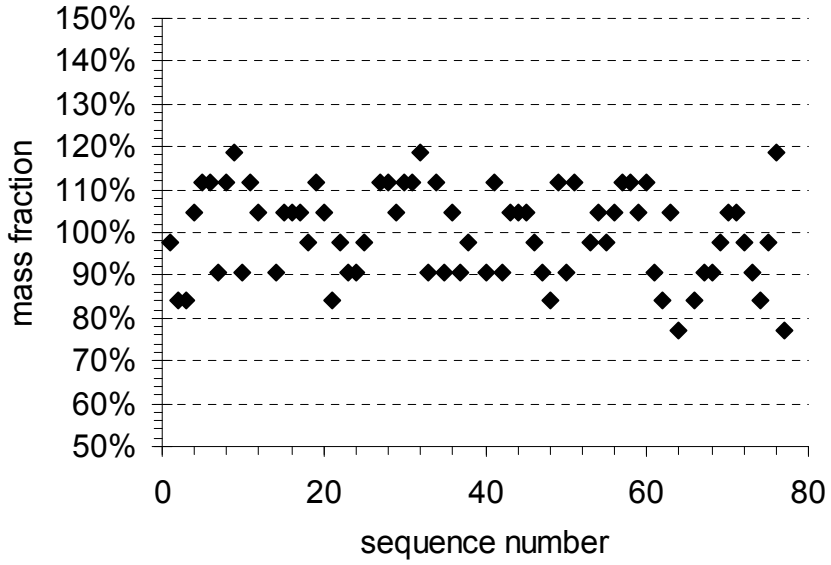
Graph A1: As



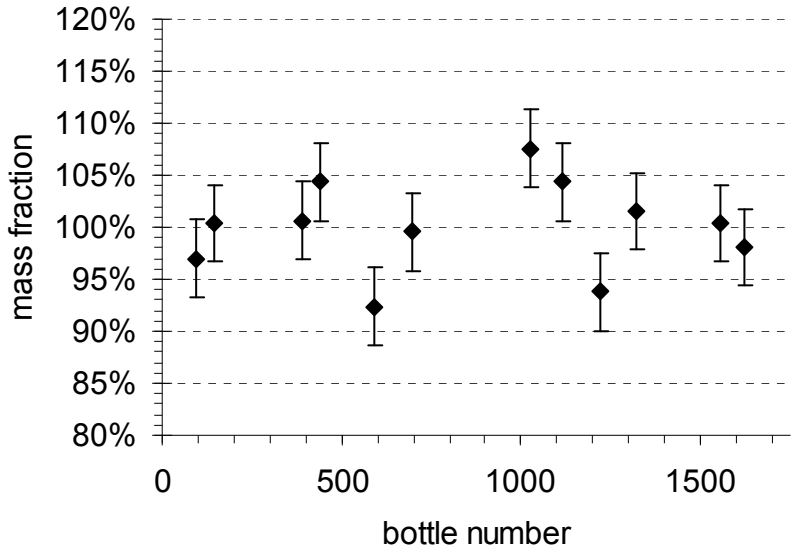


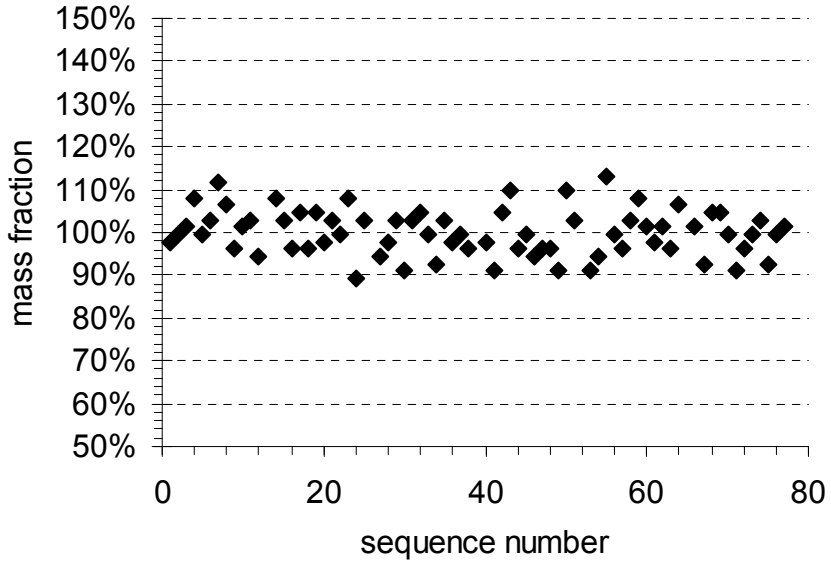
Graph A2: Cd


120%
115%
110%
105%
100%
95%
90%
85%
80%
mass fraction
0
500
1000
1500
bottle number

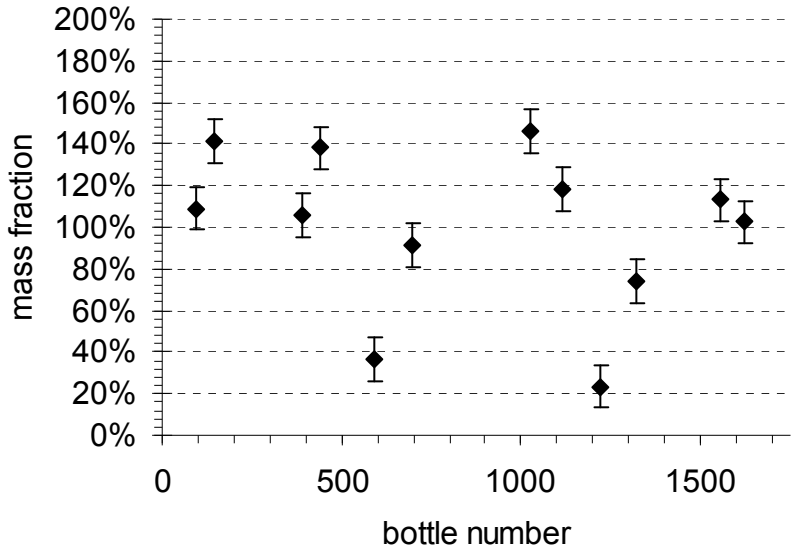

150%
140%
130%
120%
110%
100%
90%
80%
70%
60%
50%
mass fraction
0
20
40
60
80
sequence number

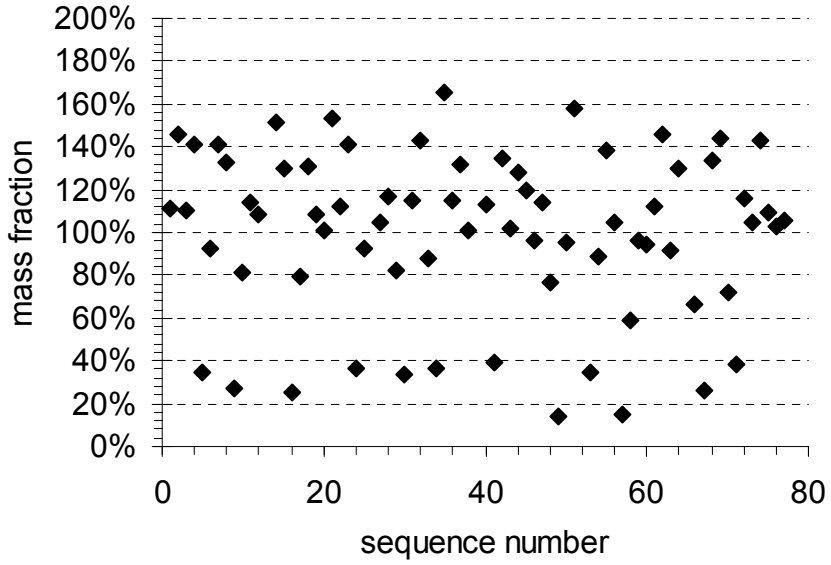
Graph A3: Co


120%
115%
110%
105%
100%
95%
90%
85%
80%
mass fraction
0
500
1000
1500
bottle number

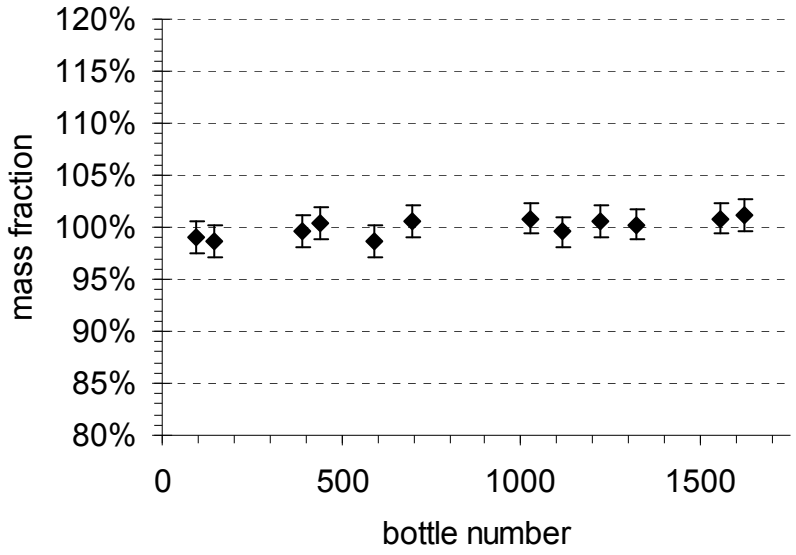

150%
140%
130%
120%
110%
100%
90%
80%
70%
60%
50%
mass fraction
0
20
40
60
80
sequence number

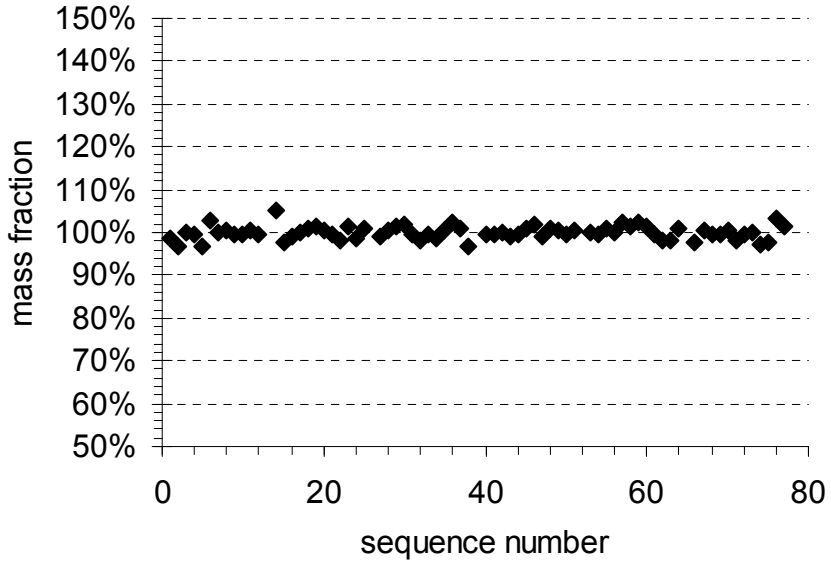
Graph A4: Cr


200%
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
mass fraction
0
500
1000
1500
bottle number

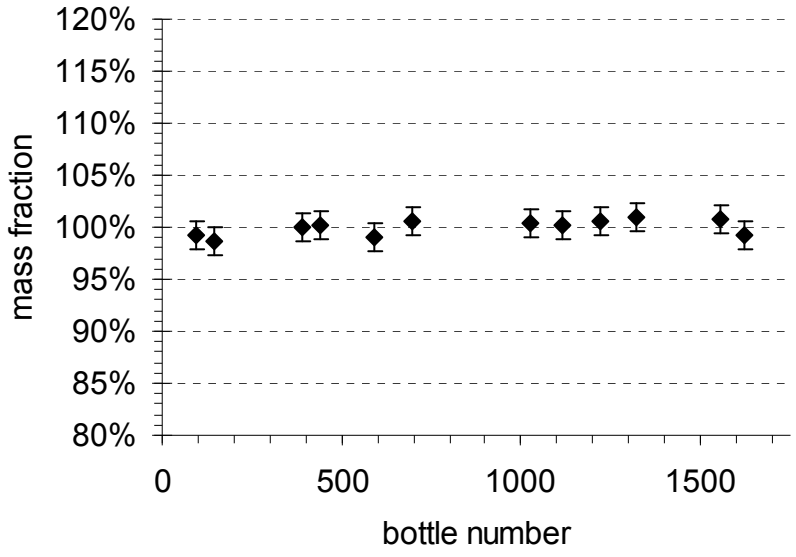

200%
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
mass fraction
0
20
40
60
80
sequence number

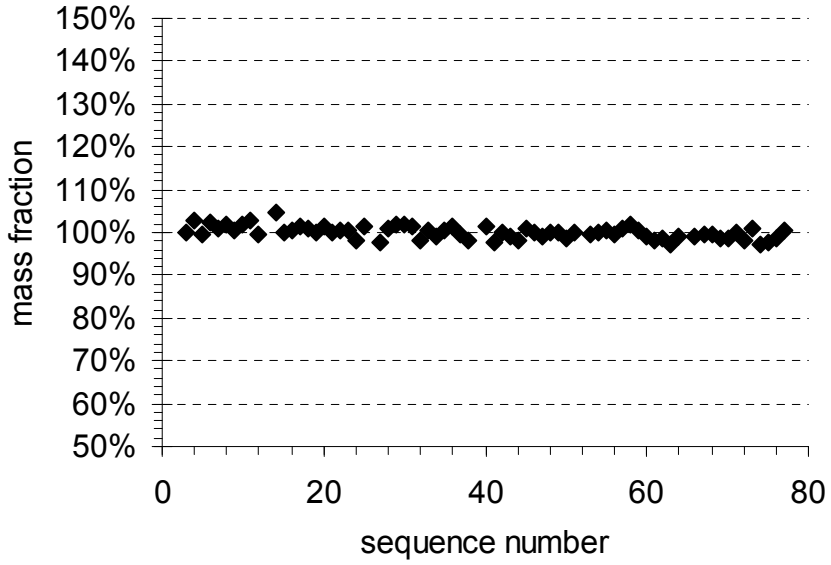
Graph A5: Cu


120%
115%
110%
105%
100%
95%
90%
85%
80%
mass fraction
0
500
1000
1500
bottle number

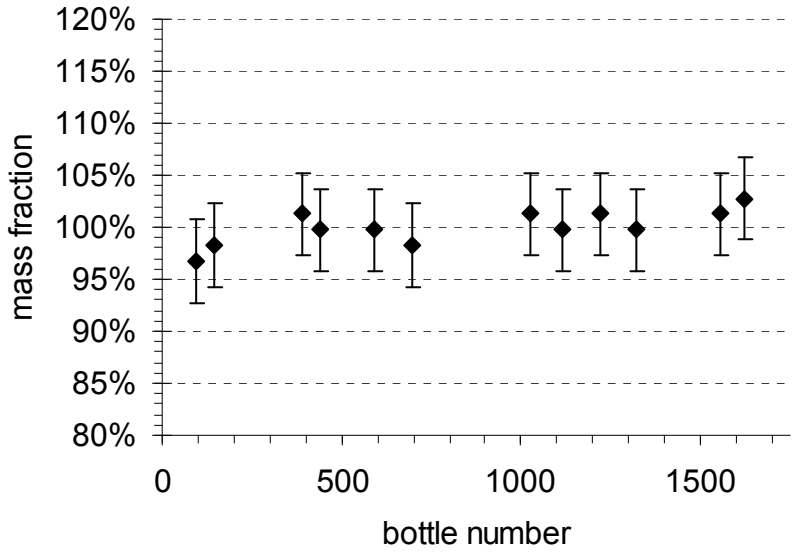

150%
140%
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120%
110%
100%
90%
80%
70%
60%
50%
mass fraction
0
20
40
60
80
sequence number

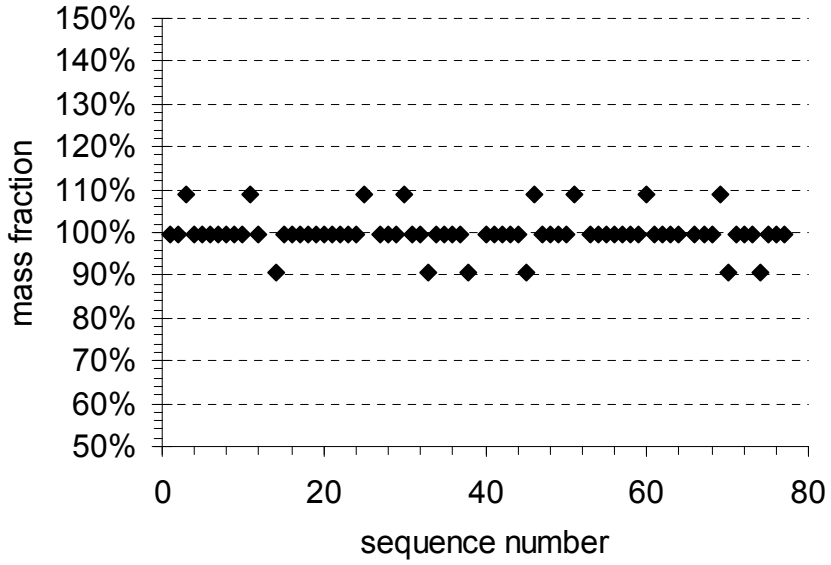
Graph A6: Fe


120%
115%
110%
105%
100%
95%
90%
85%
80%
mass fraction
0
500
1000
1500
bottle number

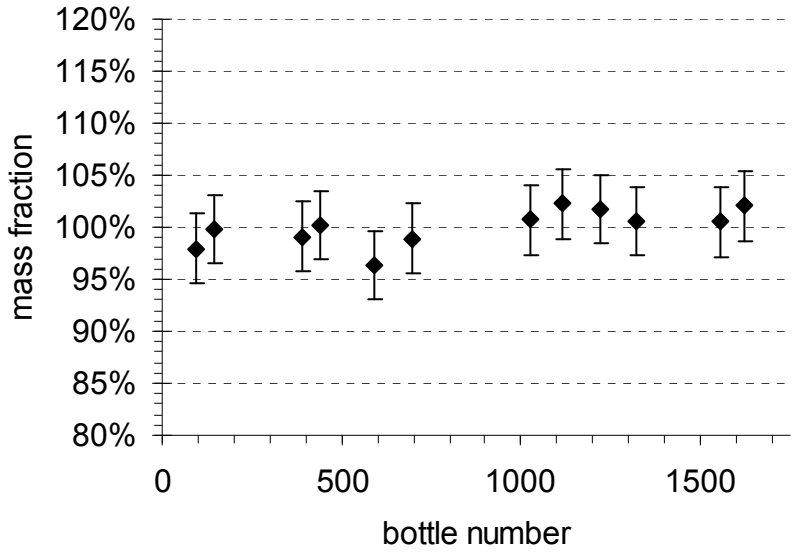

150%
140%
130%
120%
110%
100%
90%
80%
70%
60%
50%
mass fraction
0
20
40
60
80
sequence number

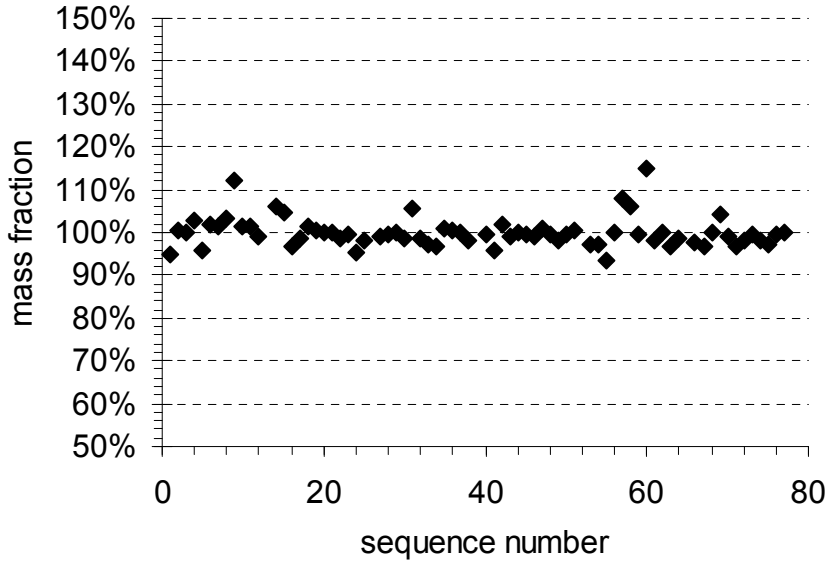
Graph A7: Hg


120%
115%
110%
105%
100%
95%
90%
85%
80%
mass fraction
0
500
1000
1500
bottle number

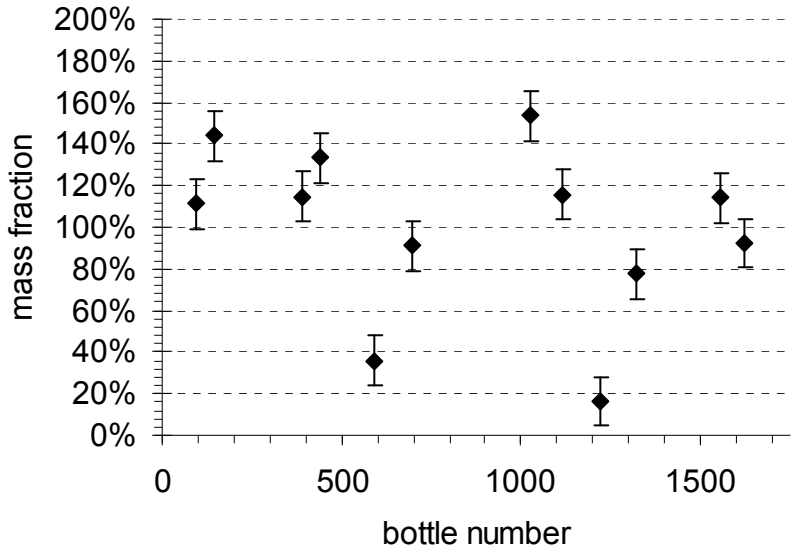

150%
140%
130%
120%
110%
100%
90%
80%
70%
60%
50%
mass fraction
0
20
40
60
80
sequence number

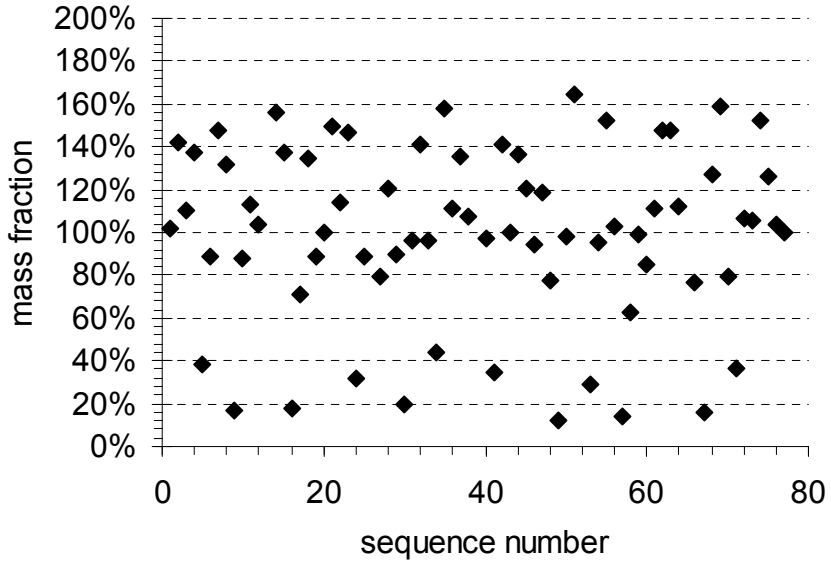
Graph A8: Mn


120%
115%
110%
105%
100%
95%
90%
85%
80%
mass fraction
0
500
1000
1500
bottle number

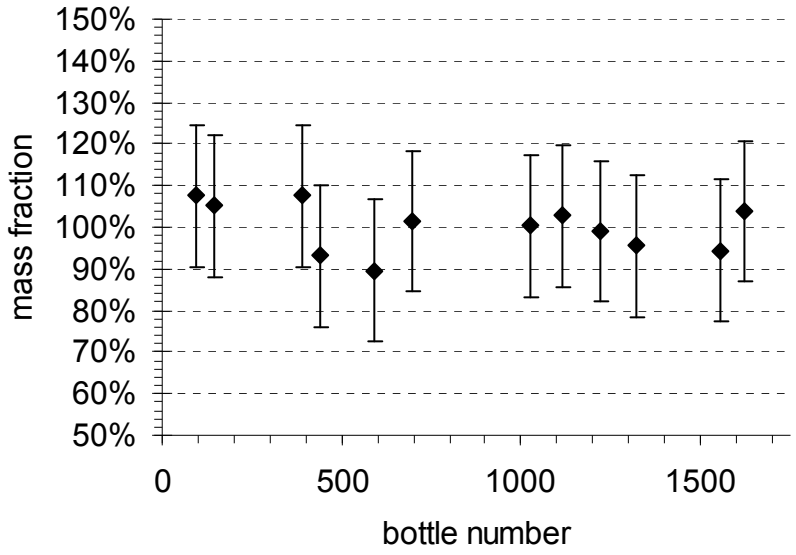

150%
140%
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90%
80%
70%
60%
50%
mass fraction
0
20
40
60
80
sequence number

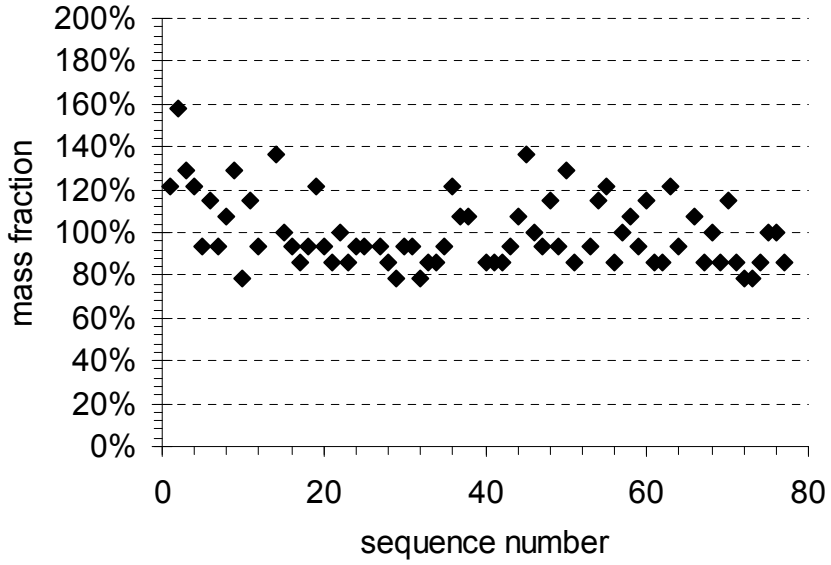
Graph A9: Ni


200%
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
mass fraction
0
500
1000
1500
bottle number

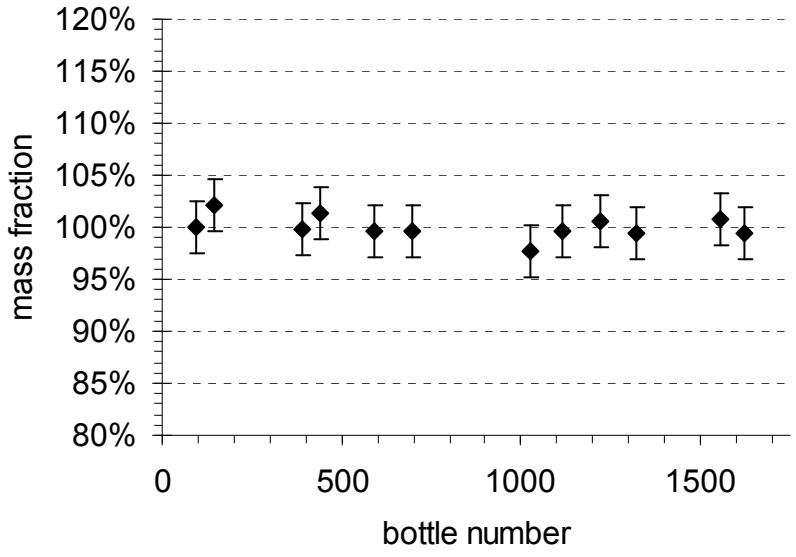

200%
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
mass fraction
0
20
40
60
80
sequence number

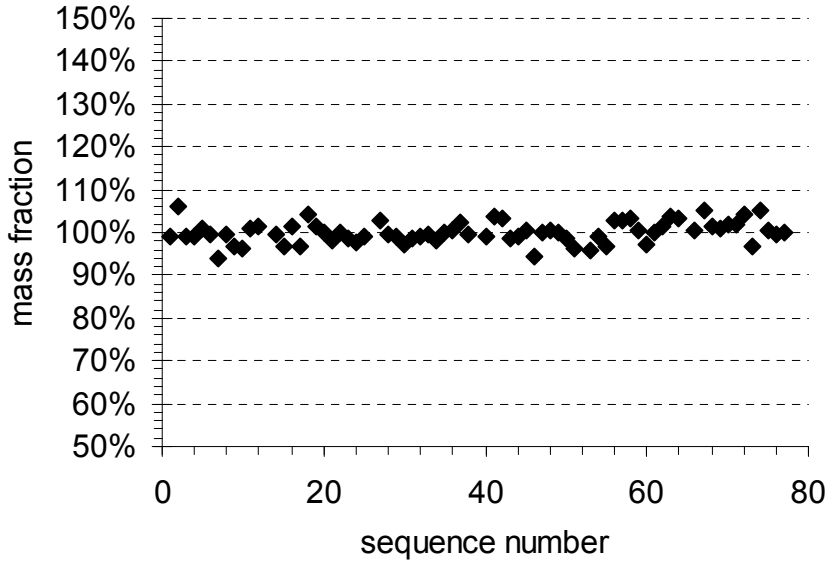
Graph A10: Pb


150%
140%
130%
120%
110%
100%
90%
80%
70%
60%
50%
mass fraction
0
500
1000
1500
bottle number

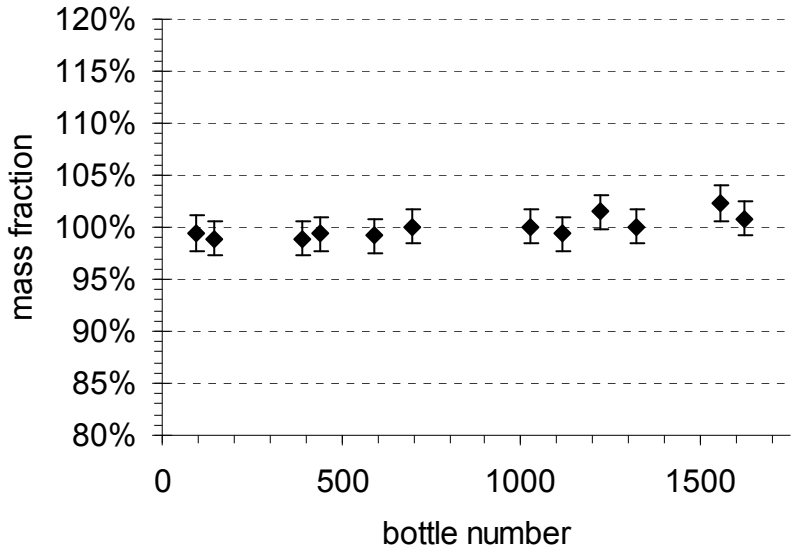

200%
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
mass fraction
0
20
40
60
80
sequence number

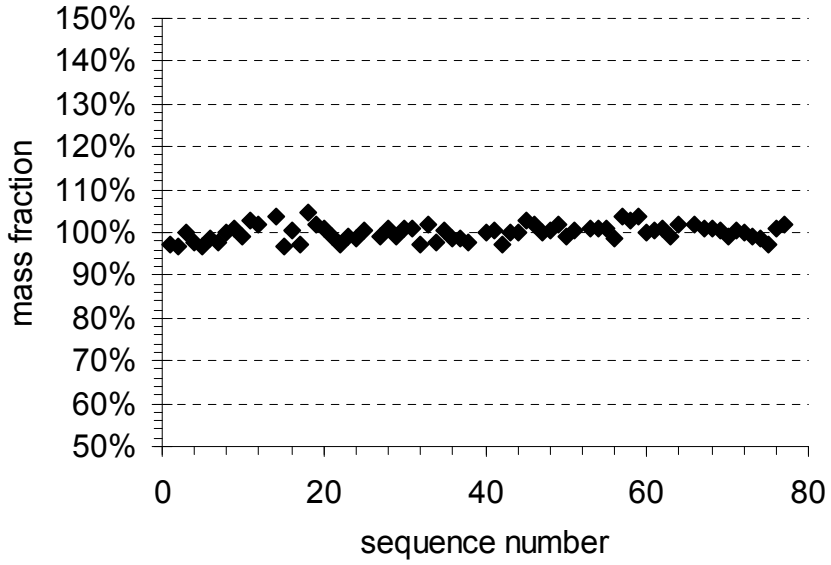
Graph A11: Se


120%
115%
110%
105%
100%
95%
90%
85%
80%
mass fraction
0
500
1000
1500
bottle number


150%
140%
130%
120%
110%
100%
90%
80%
70%
60%
50%
mass fraction
0
20
40
60
80
sequence number

Graph A12: Zn


120%
115%
110%
105%
100%
95%
90%
85%
80%
mass fraction
0
500
1000
1500
bottle number


150%
140%
130%
120%
110%
100%
90%
80%
70%
60%
50%
mass fraction
0
20
40
60
80
sequence number

Annex B ERM®-BB184 (Bovine muscle) – Results of the short-term stability study

Measured values are means at each time/temperature, relative to the global mean for samples at 18 ºC (◇) and 60 ºC (◆). Vertical bars are 2s of 10 replicate measurements.

Figure B1: Short-term stability of As mass fraction at 60 °C with associated u_{sts}

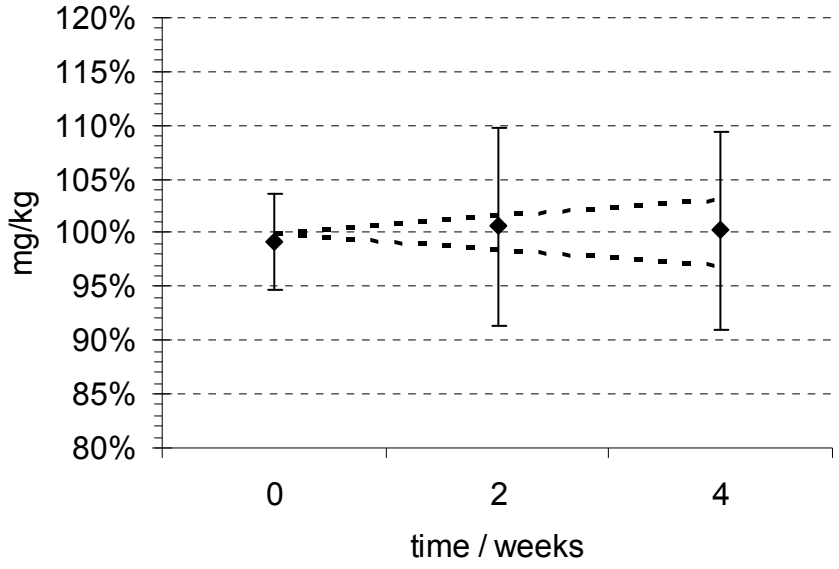


Figure B2: Short-term stability of Cd mass fraction at 60 °C with associated u_{sts}

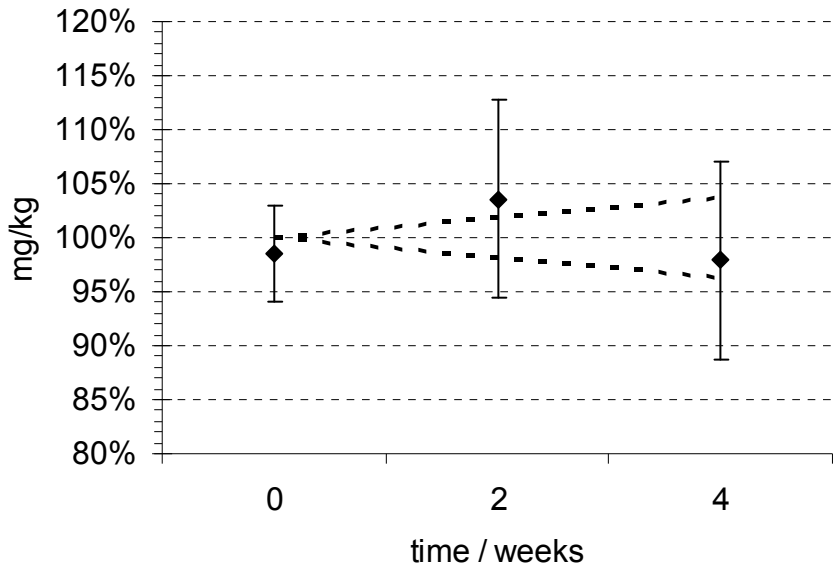


Figure B3: Short-term stability of Cr mass fraction at 18 and 60 °C with associated u_{sts}

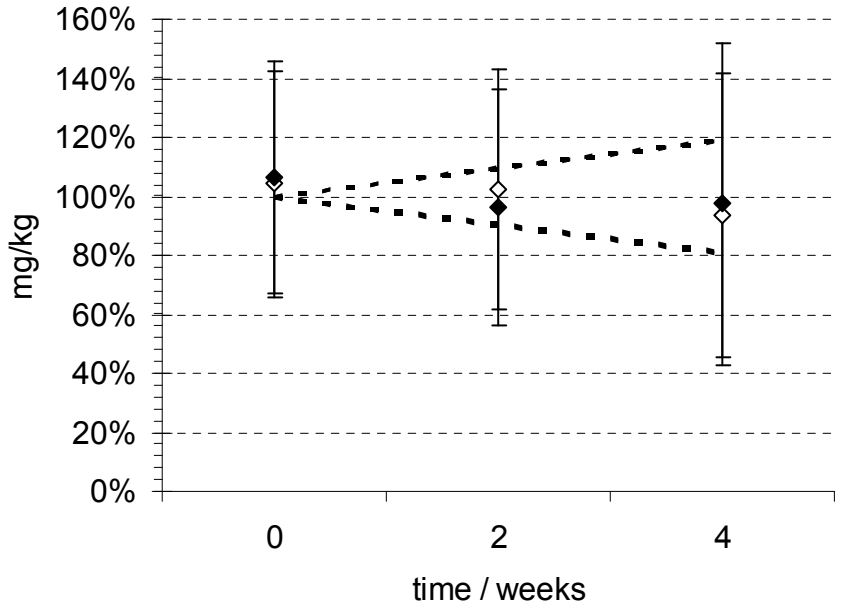


Figure B4: Short-term stability of Cu mass fraction at 18 and 60 °C with associated u_{sts}

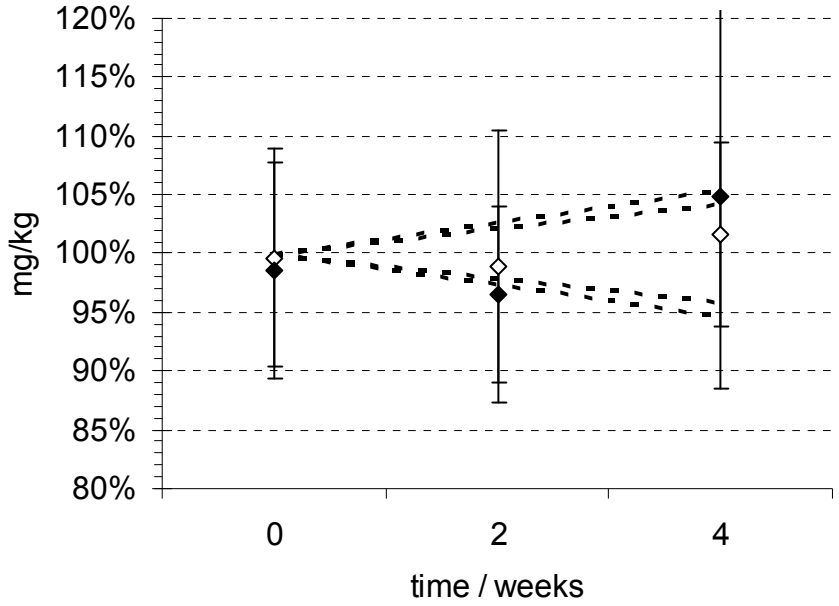


Figure B5: Short-term stability of Fe mass fraction at 18 and 60 °C with associated u_{sts}

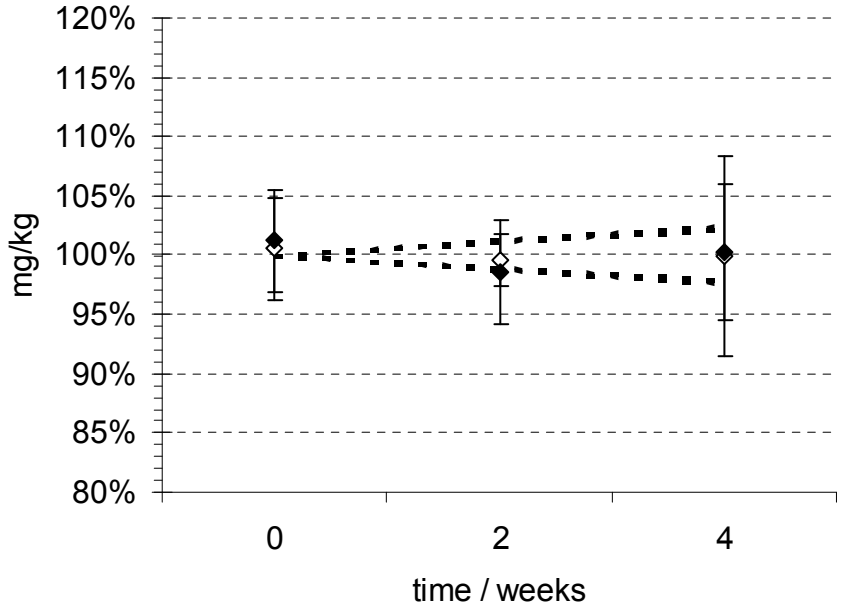


Figure B6: Short-term stability of Hg mass fraction at 60 °C with associated u_{sts}

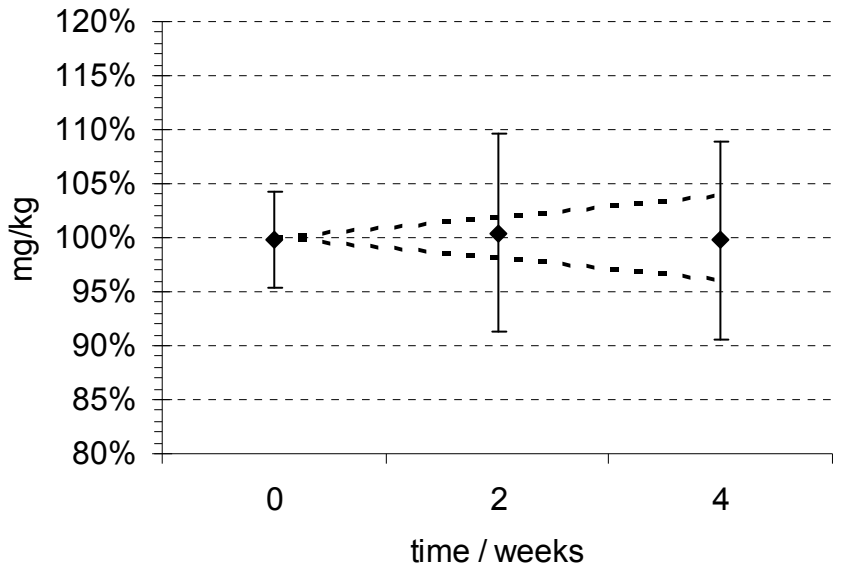


Figure B7: Short-term stability of Mn mass fraction at 18 and 60 °C with associated u_{sts}

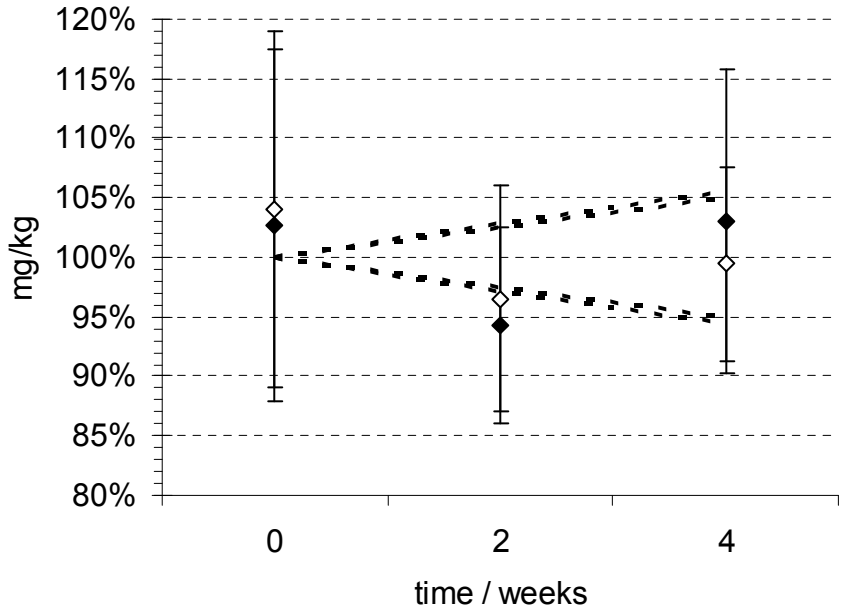


Figure B8: Short-term stability of Mn mass fraction at 18 and 60 °C with associated u_{sts}

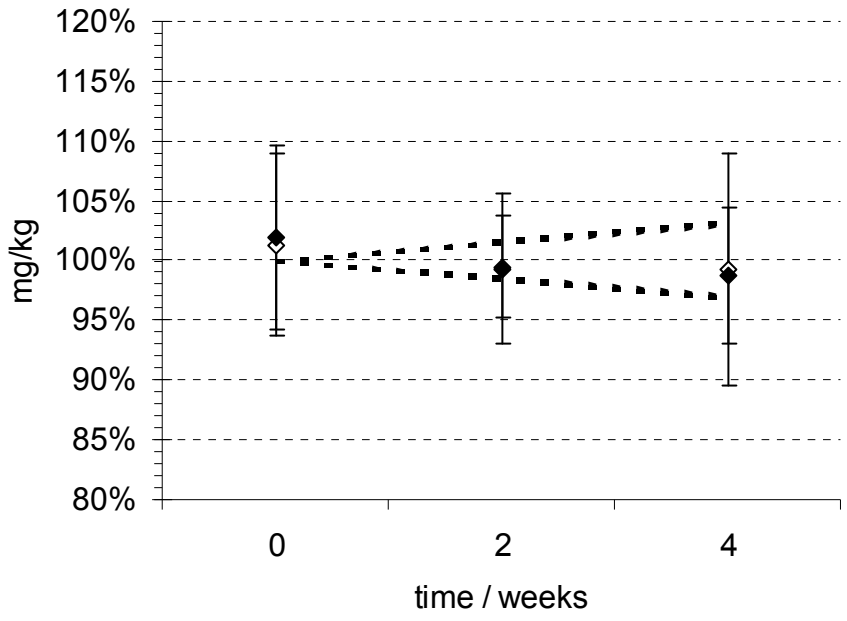
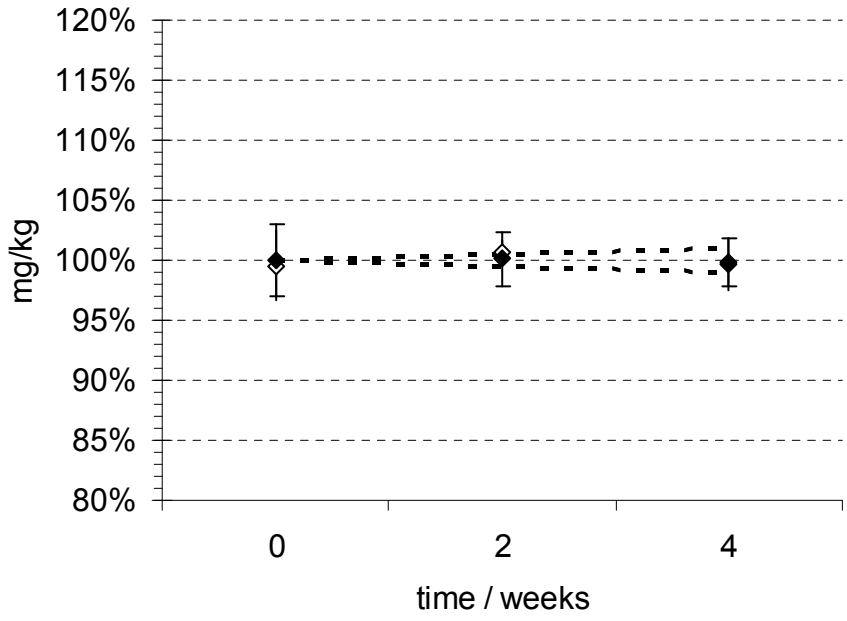


Figure B9: Short-term stability of Zn mass fraction at 18 and 60 °C with associated u_{sts}



Annex C ERM®-BB184 (Bovine muscle) – Results of the long-term stability study

Measured values are means at each time/temperature, relative to the mean of time-point zero. 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: Long-term stability of As mass fraction at 18 °C with associated u_{lts}

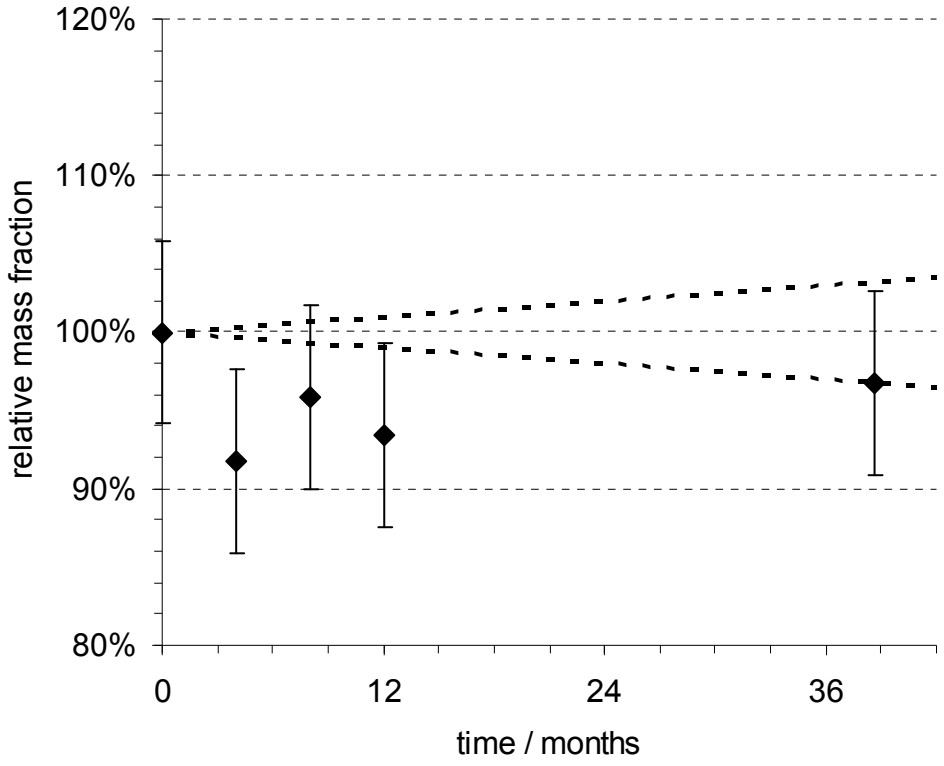


Figure C2: Long-term stability of Cd mass fraction at 18 °C with associated u_{lts}

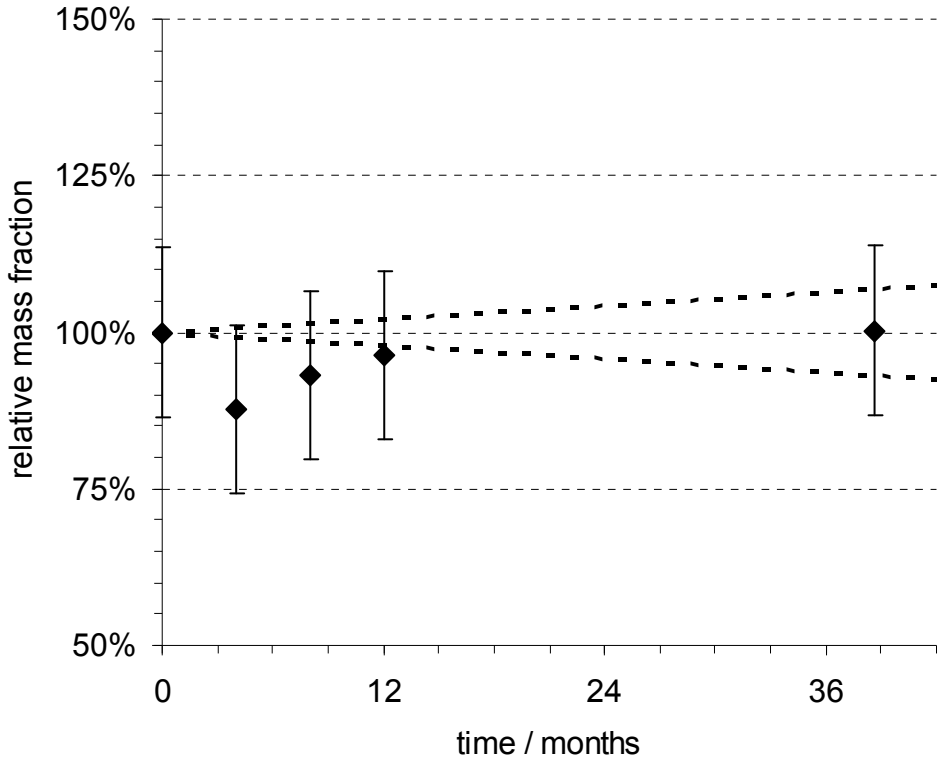


Figure C3: Long-term stability of Cu mass fraction at 18 °C with associated u_{lts}

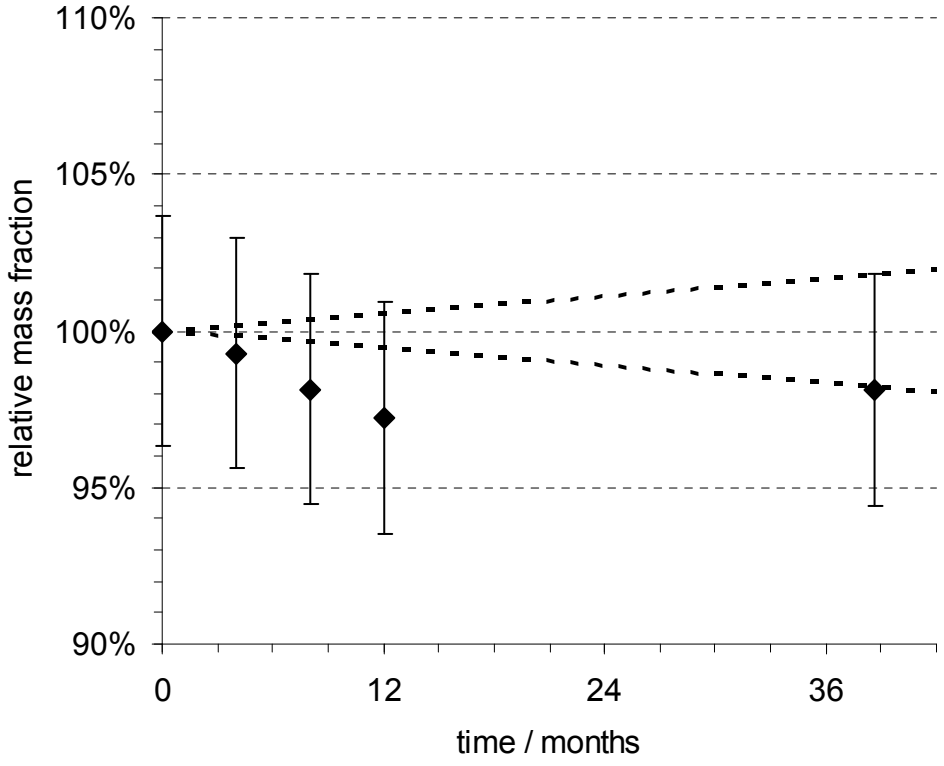


Figure C4: Long-term stability of Fe mass fraction at 18 °C with associated u_{lts}

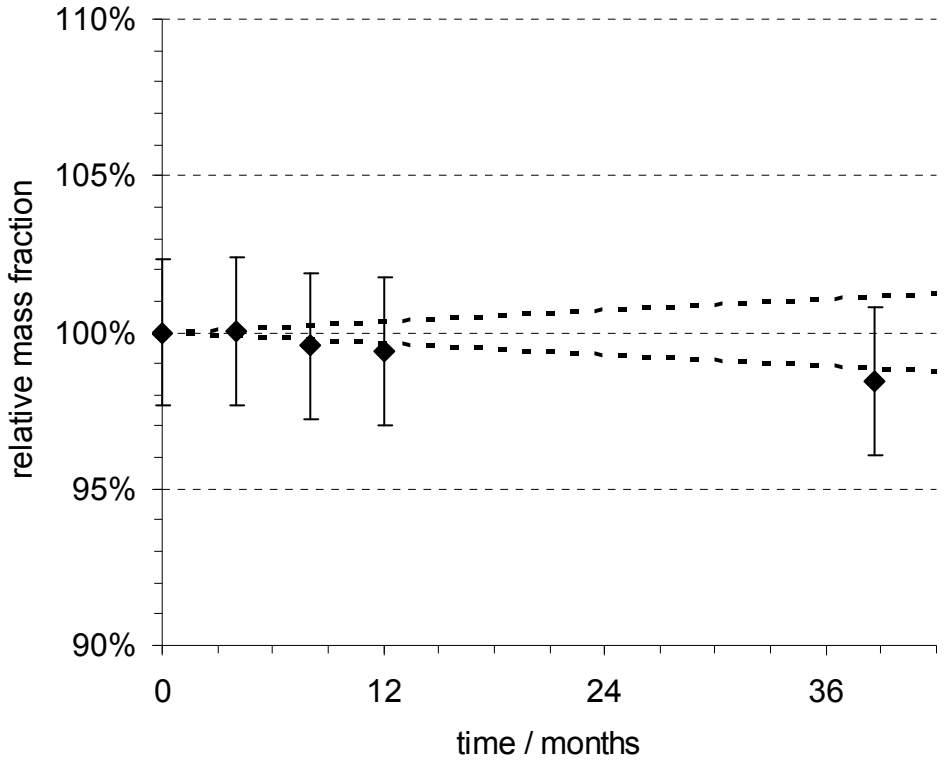


Figure C5: Long-term stability of Hg mass fraction at 18 °C with associated u_{lts}

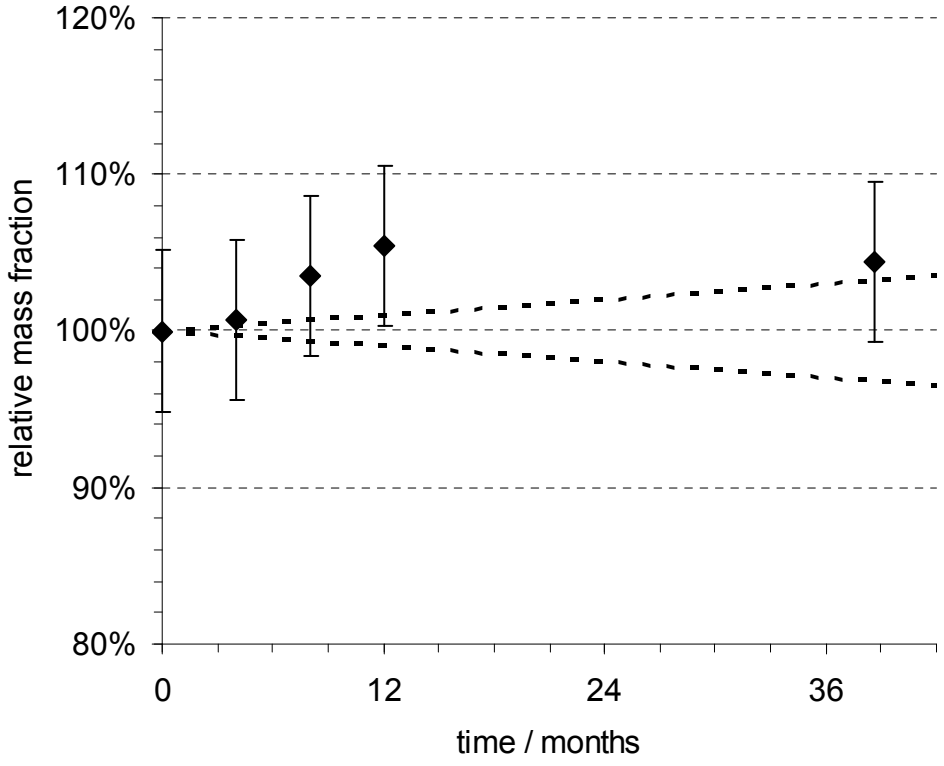


Figure C6: Long-term stability of Mn mass fraction at 18 °C with associated u_{lts}

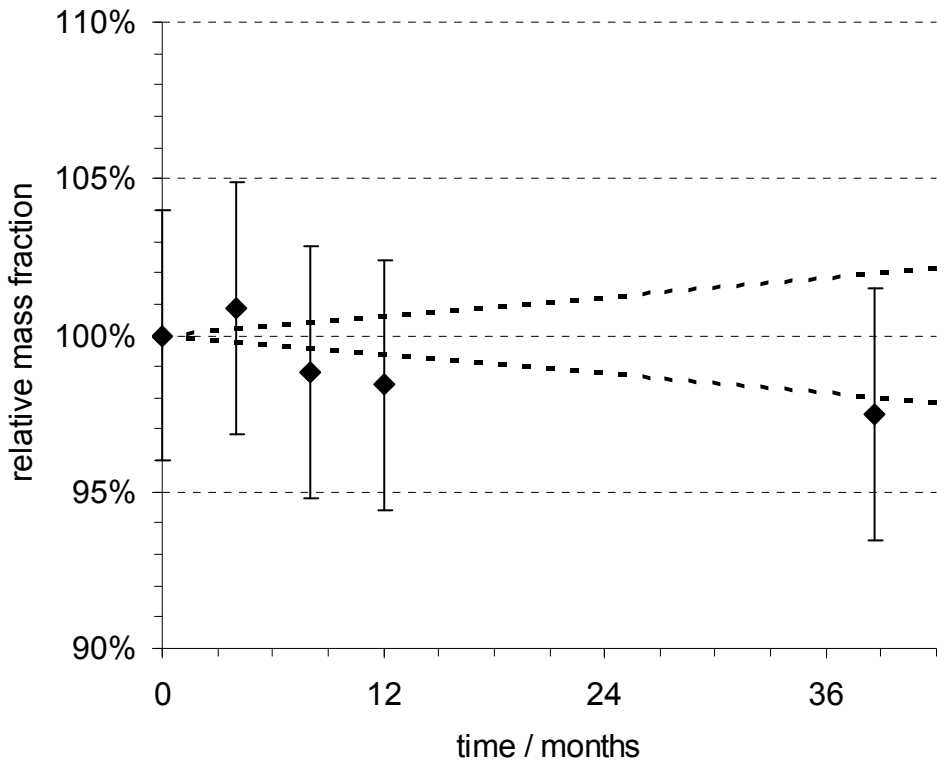


Figure C7: Long-term stability of Se mass fraction at 18 °C with associated u_{lts}

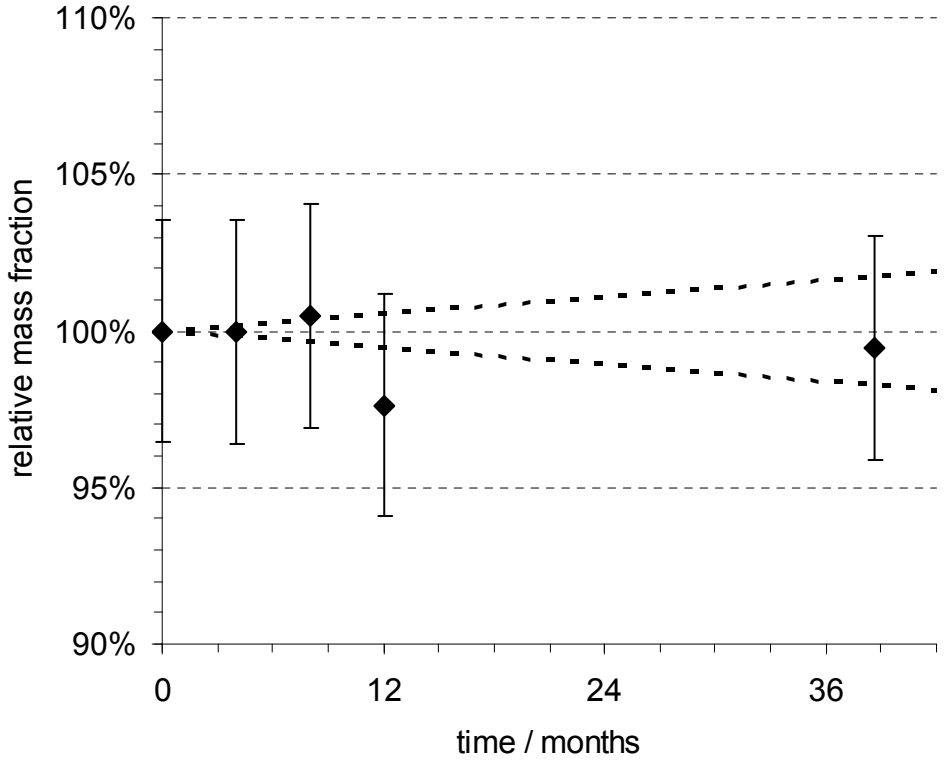
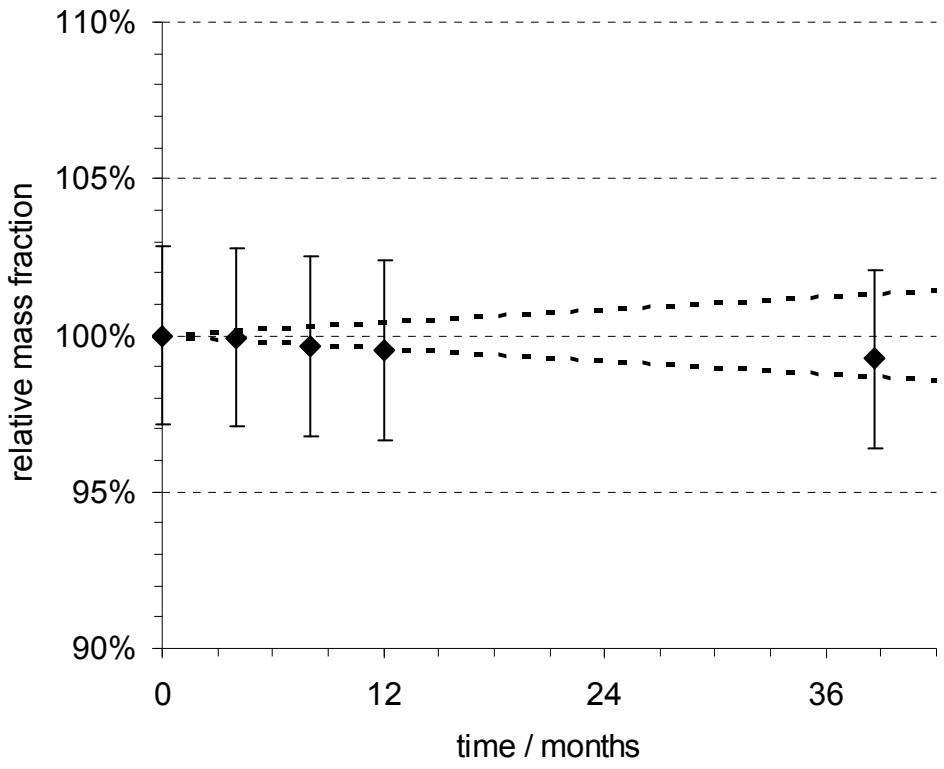


Figure C8: Long-term stability of Zn mass fraction at 18 °C with associated u_{lts}

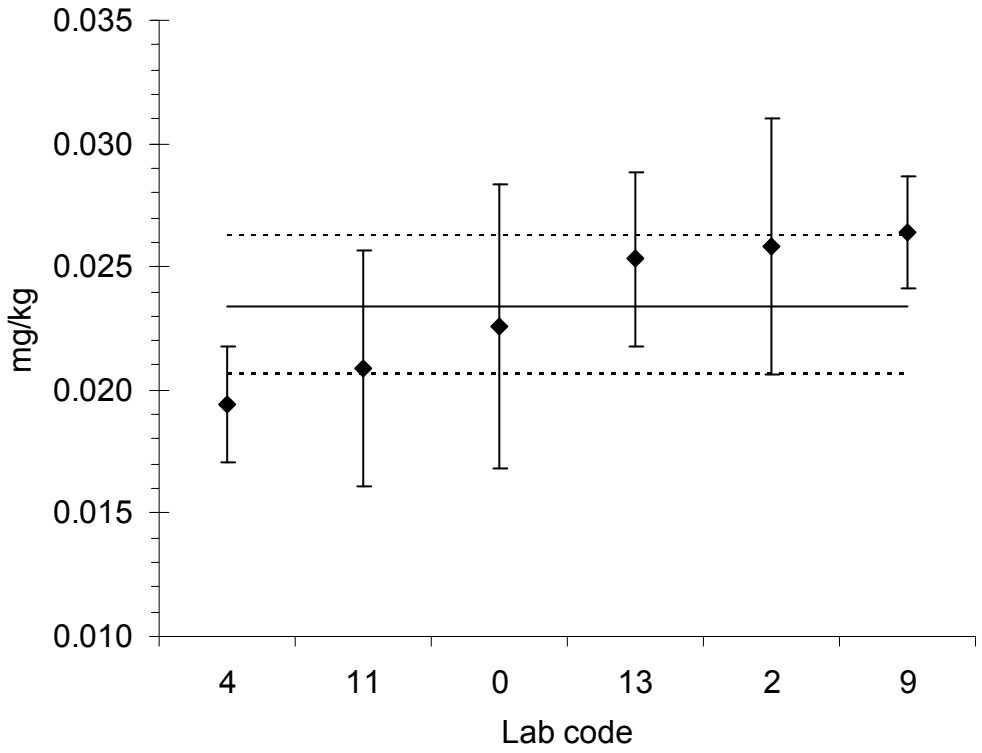


Annex D ERM®-BB184 (Bovine muscle) – Characterisation data

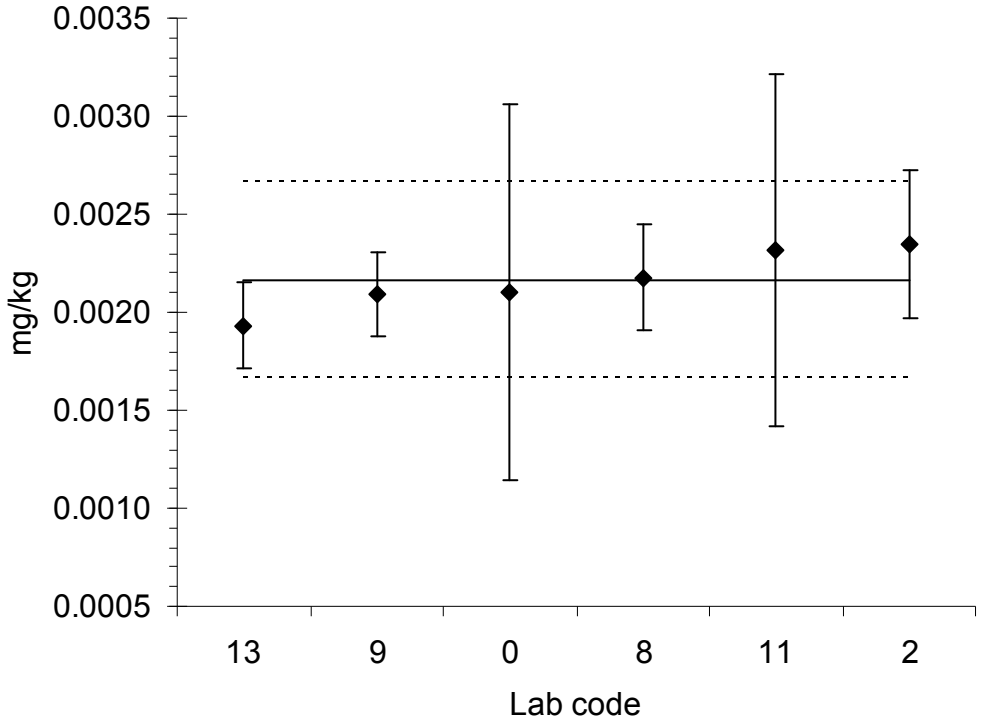
Results of characterisation measurements (corrected to dry mass), as reported by the participating laboratories. Values struck-through were excluded from calculation of the certified values. Individual replicate values are given with the mean and measurement uncertainty.

As mass fraction, mg/kg

Lab Code	#1	#2	#3	#4	#5	#6	Mean	U
0	0.0215	0.0225	0.0232	0.0226	0.0225	0.0232	0.0226	0.0058
1	~~0.081~~	~~0.073~~	~~0.063~~	~~0.075~~	~~0.072~~	~~0.07~~	~~0.0723~~	~~0.0130~~
2	0.026	0.026	0.027	0.024	0.027	0.025	0.0258	0.0052
3	-	-	-	-	-	-	-	-
4	0.021672	0.019608	0.019608	0.017797	0.020255	0.017539	0.0194	0.0023
5	~~0.06~~	~~0.069~~	~~0.058~~	~~0.059~~	~~0.063~~	~~0.056~~	~~0.0608~~	~~0.0091~~
6	~~0.06~~	~~0.06~~	~~0.064~~	~~0.053~~	~~0.067~~	~~0.065~~	~~0.0615~~	~~0.0062~~
7	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-
9	0.027	0.0261	0.0271	0.026	0.026	0.0261	0.0264	0.0023
10	-	-	-	-	-	-	-	-
11	0.0219	0.0235	0.024	0.0236	0.0142	0.0182	0.0209	0.0048
12	-	-	-	-	-	-	-	-
13	0.024	0.022	0.021	0.029	0.029	0.027	0.0253	0.0035
14	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-

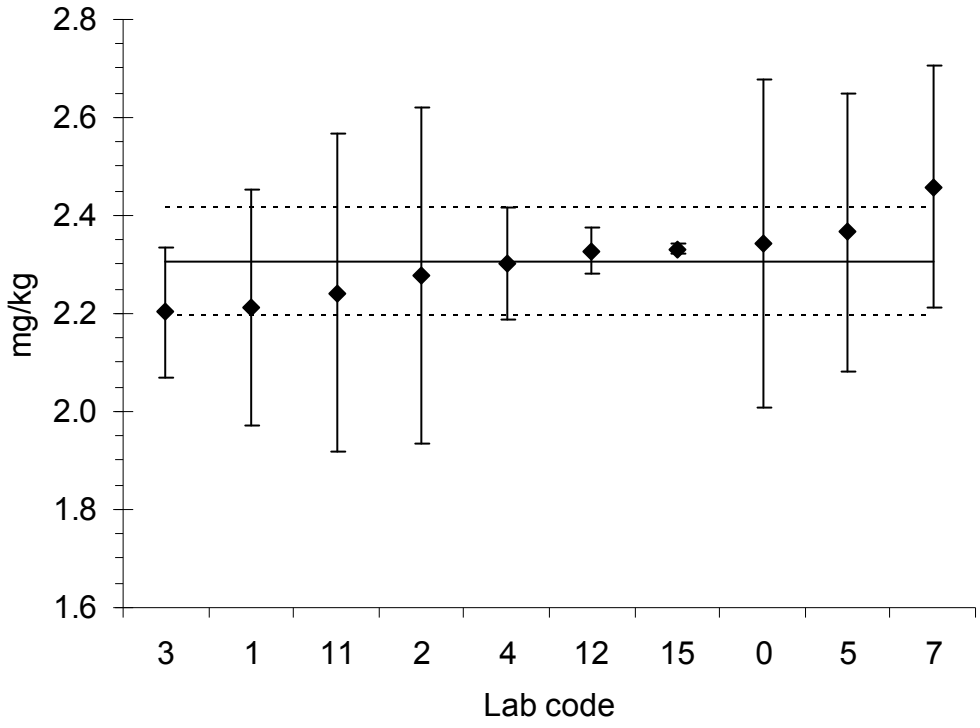


Lab Code	Cd mass fraction, mg/kg							
	#1	#2	#3	#4	#5	#6	Mean	U
0	0.0024	0.002	0.0022	0.0019	0.0021	0.002	0.0021	0.0010
1	-	-	-	-	-	-	-	-
2	0.003	0.0032	0.0027	0.0017	0.0012	0.0023	0.0024	0.0004
3	-	-	-	-	-	-	-	-
4	~~0.002064~~	~~0.002064~~	~~0.001032~~	~~0.001473~~	~~0.001441~~	~~0.001395~~	~~0.0016~~	~~0.0032~~
5	~~0.00092~~	~~0.00088~~	~~0.00072~~	~~0.00063~~	~~0.00069~~	~~0.00122~~	~~0.0008~~	~~0.0001~~
6	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-
8	0.0023	0.00212	0.00198	0.00209	0.00234	0.00222	0.0022	0.0003
9	0.00201	0.00221	0.00198	0.0022	0.002	0.00215	0.0021	0.0002
10	-	-	-	-	-	-	-	-
11	0.0019	0.0016	0.0012	0.0047	0.0023	0.0022	0.0023	0.0009
12	-	-	-	-	-	-	-	-
13	0.0019	0.0018	0.0021	0.002	0.0019	0.0019	0.0019	0.0002
14	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-



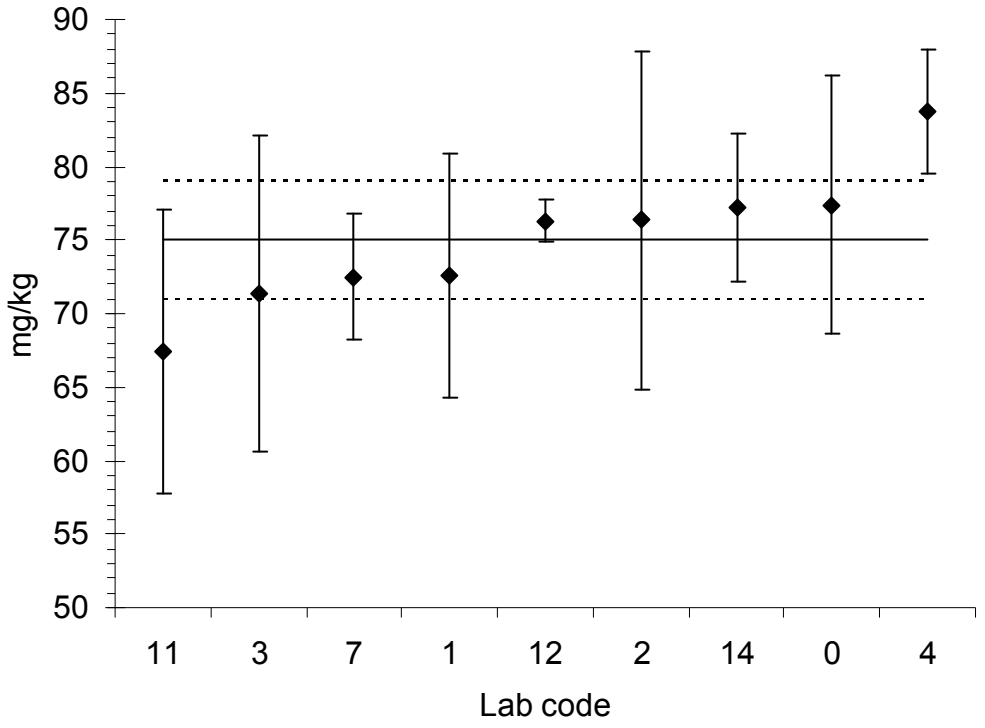
Cu mass fraction, mg/kg

Lab Code	#1	#2	#3	#4	#5	#6	Mean	U
0	2.39	2.34	2.36	2.33	2.35	2.29	2.343	0.335
1	2.25	2.34	2.27	2.14	2.1	2.17	2.212	0.242
2	2.3	2.25	2.22	2.28	2.23	2.39	2.278	0.342
3	2.208	2.131	2.231	2.19	2.221	2.234	2.203	0.134
4	2.208462	2.229102	2.208462	2.352639	2.407674	2.404074	2.302	0.115
5	2.39	2.35	2.33	2.34	2.43	2.36	2.367	0.284
6	-	-	-	-	-	-	-	-
7	2.57	2.42	2.43	2.39	2.47	2.47	2.458	0.246
8	~~2.07~~	~~2.12~~	~~2.09~~	~~2.12~~	~~2.12~~	~~2.03~~	~~2.092~~	~~0.070~~
9	~~2.01~~	~~2.06~~	~~2.1~~	~~2.11~~	~~2.14~~	~~2.01~~	~~2.072~~	~~0.110~~
10	~~2~~	~~2.06~~	~~2.07~~	~~2.11~~	~~2.05~~	~~2.05~~	~~2.057~~	~~0.050~~
11	2.229	2.207	2.193	2.312	2.209	2.306	2.243	0.324
12	2.39225	2.333719	2.283551	2.252365	2.277918	2.426925	2.328	0.047
13	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-
15	2.335	2.327	2.331	2.332	2.338	-	1.944	0.011



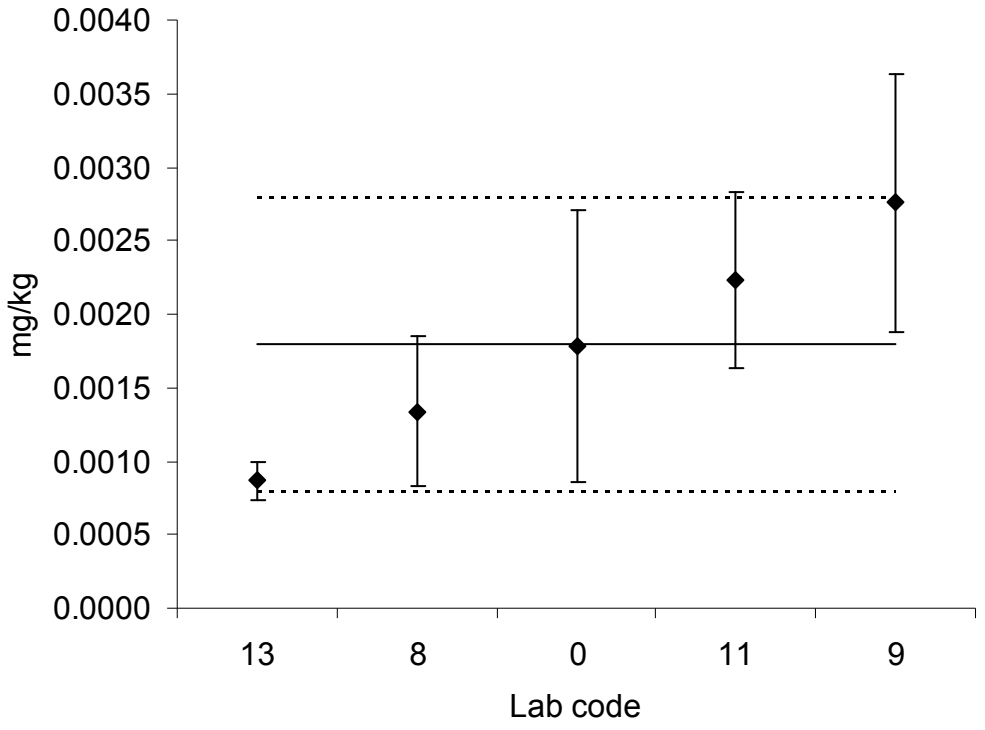
Fe mass fraction, mg/kg

Lab Code	#1	#2	#3	#4	#5	#6	Mean	U
0	79.8	76	76.8	77.9	78.1	75.7	77.38	8.78
1	78	73.7	74.9	69.4	69.7	69.6	72.55	8.27
2	74.9	76.3	74.1	78.9	76	77.8	76.33	11.45
3	70.85	70.93	71.73	71.74	71.57	71.2	71.34	10.76
4	85.44892	83.28173	83.90093	82.44008	84.14772	83.12931	83.72	4.19
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	72.5	74.2	72.4	71.7	69.3	75	72.52	4.35
8	-	-	-	-	-	-	-	-
9	~~67.1~~	~~68.5~~	~~69.8~~	~~67.5~~	~~69.2~~	~~68.5~~	~~68.43~~	~~2.01~~
10	~~71.1~~	~~69.3~~	~~71.7~~	~~69~~	~~69.8~~	~~68.1~~	~~69.83~~	~~2.68~~
11	67.69	68.8	67.5	66.92	65.34	68.21	67.41	9.63
12	75.25608	76.80665	79.09334	74.69631	75.39315	76.59036	76.31	1.45
13	-	-	-	-	-	-	-	-
14	77.3	74.2	76.1	78.4	79.2	77.9	77.18	5.00
15	-	-	-	-	-	-	-	-



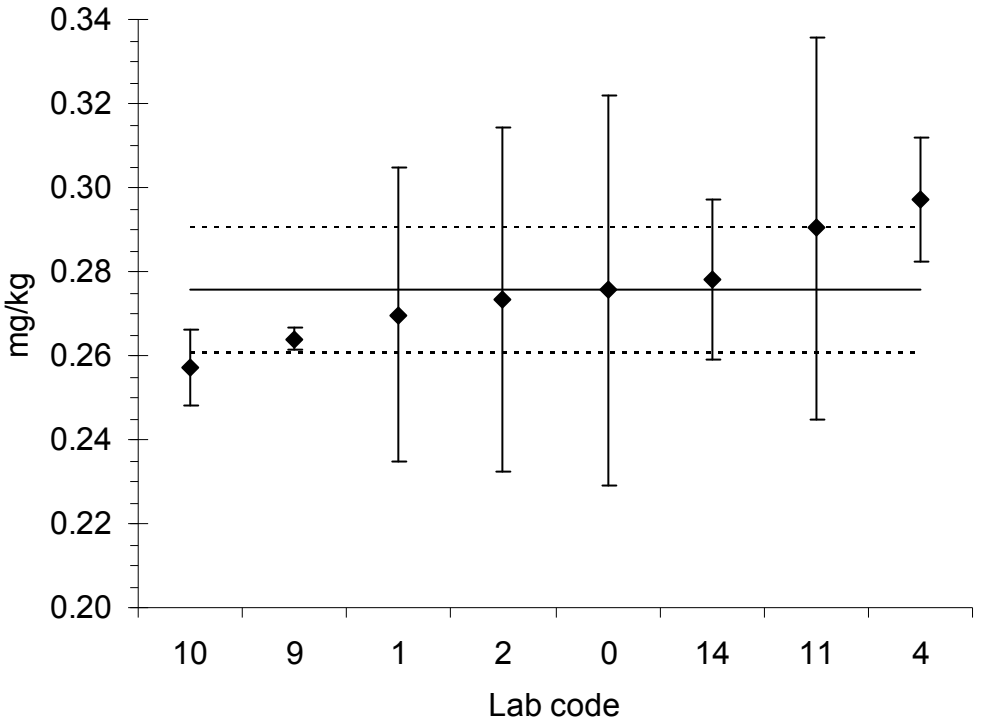
Hg mass fraction for indicative value, mg/kg

Lab Code	#1	#2	#3	#4	#5	#6	Mean	U
0	0.0023	0.0015	0.0015	0.0018	0.0017	0.0019	0.0018	0.0009
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	~~0.003096~~	~~0.007224~~	~~0.006192~~	~~0.001~~	~~0.001~~	~~0.001~~	~~0.0033~~	~~0.0065~~
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-
8	0.00143	0.00151	0.00144	0.00112	0.00148	0.00103	0.0013	0.0005
9	0.00235	0.00269	0.00234	0.00359	0.00259	0.00298	0.0028	0.0009
10	-	-	-	-	-	-	-	-
11	0.0006	0.0022	0.0013	0.0028	0.0028	0.0037	0.0022	0.0006
12	-	-	-	-	-	-	-	-
13	0.0009	0.0009	0.0006	0.0012	0.0008	0.0008	0.0009	0.0001
14	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-



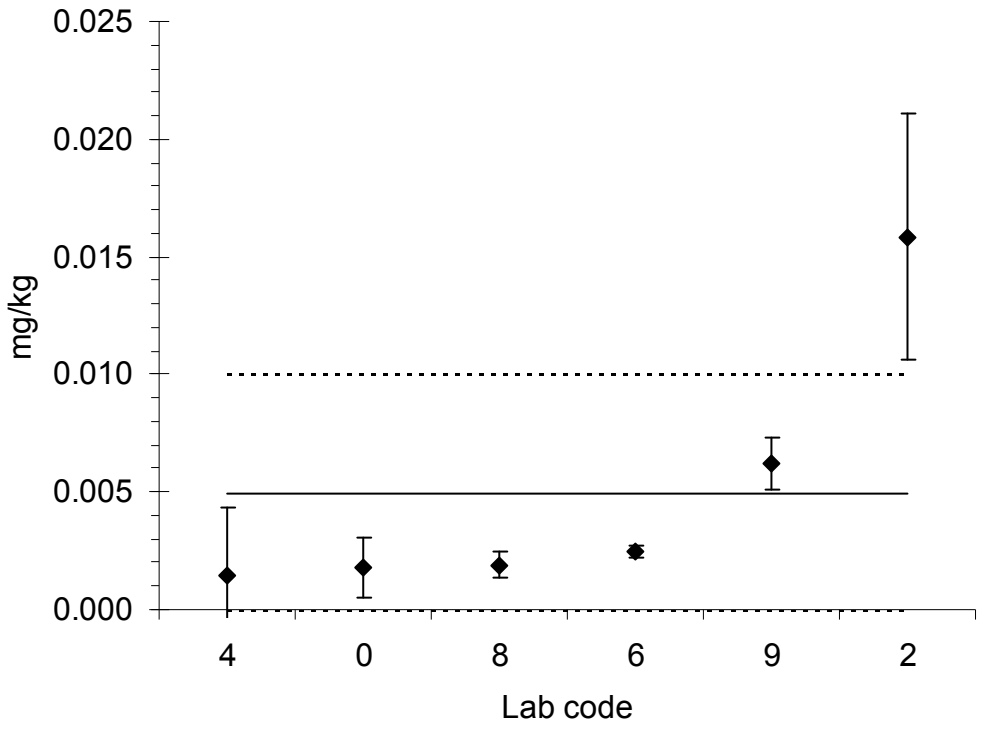
Mn mass fraction, mg/kg

Lab Code	#1	#2	#3	#4	#5	#6	Mean	U
0	0.277	0.266	0.271	0.272	0.278	0.289	0.2755	0.0462
1	0.274	0.27	0.278	0.268	0.259	0.269	0.2697	0.0350
2	0.28	0.32	0.23	0.26	0.25	0.3	0.2733	0.0410
3	-	-	-	-	-	-	-	-
4	0.283798	0.286894	0.283798	0.304701	0.315811	0.30861	0.2973	0.0149
5	~~0.33~~	~~0.32~~	~~0.34~~	~~0.34~~	~~0.33~~	~~0.33~~	~~0.3317~~	~~0.0498~~
6	-	-	-	-	-	-	-	-
7	~~0.35~~	~~0.36~~	~~0.35~~	~~0.35~~	~~0.33~~	~~0.32~~	~~0.3433~~	~~0.0172~~
8	-	-	-	-	-	-	-	-
9	0.265	0.266	0.263	0.261	0.264	0.265	0.2640	0.0026
10	0.264	0.263	0.257	0.253	0.252	0.253	0.2570	0.0090
11	0.2881	0.2986	0.2702	0.2656	0.324	0.2956	0.2904	0.0454
12	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-
14	0.272	0.291	0.28	0.272	0.277	0.276	0.2780	0.0190
15	-	-	-	-	-	-	-	-



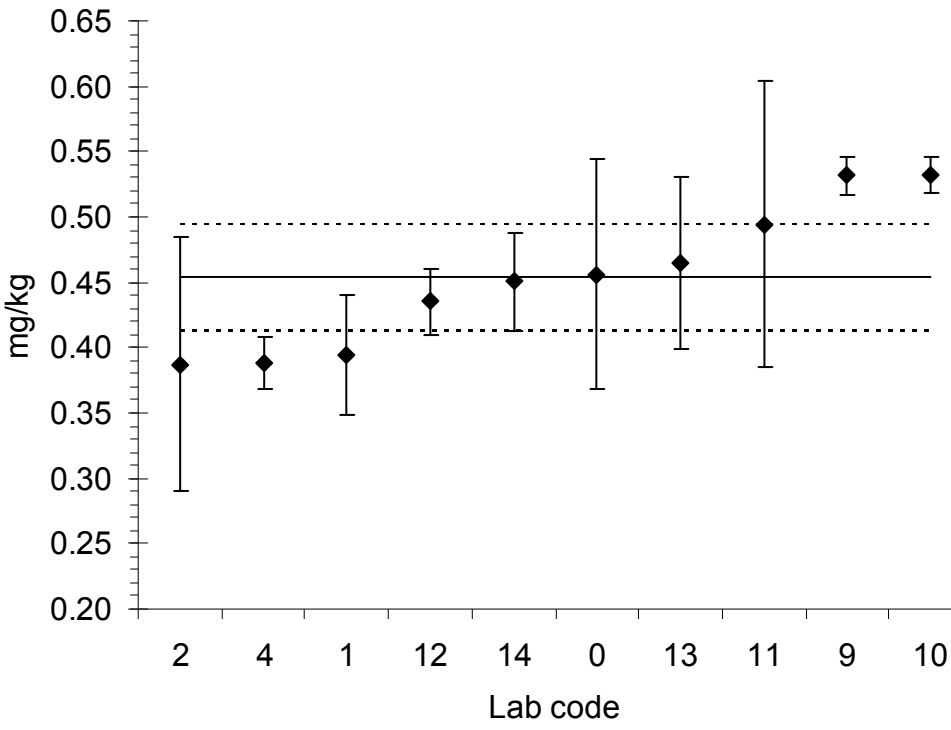
Pb mass fraction not for certification, mg/kg

Lab Code	#1	#2	#3	#4	#5	#6	Mean	U
0	0.0019	0.0016	0.0016	0.0017	0.0025	0.0015	0.0018	0.0013
1	-	-	-	-	-	-	-	-
2	0.027	0.011	0.012	0.015	0.014	0.016	0.0158	0.0052
3	-	-	-	-	-	-	-	-
4	0.001032	0.001032	0.001032	0.001447	0.001266	0.002872	0.0014	0.0029
5	-	-	-	-	-	-	-	-
6	0.0024	0.0023	0.0025	0.0026	0.0025	0.0024	0.0025	0.0002
7	-	-	-	-	-	-	-	-
8	0.00169	0.00226	0.00171	0.00224	0.00174	0.00175	0.0019	0.0006
9	0.00687	0.00638	0.00531	0.00661	0.00629	0.00578	0.0062	0.0011
10	-	-	-	-	-	-	-	-
11	-	-	~~0.1516~~	~~0.0283~~	-	-	~~0.0272~~	~~0.0197~~
12	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-



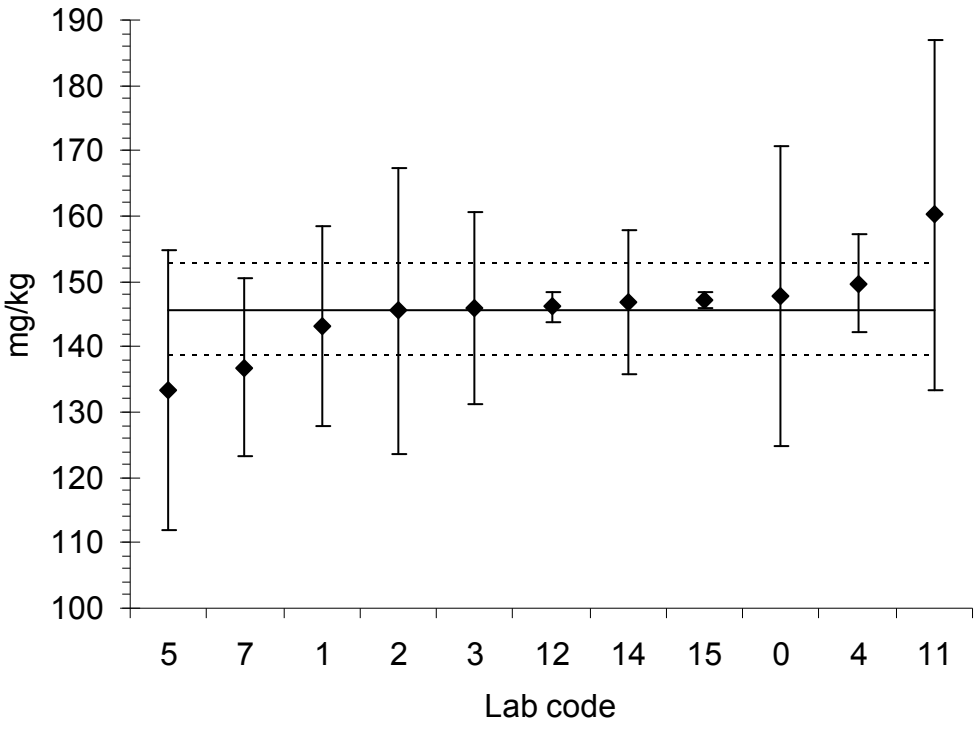
Se mass fraction, mg/kg

Lab Code	#1	#2	#3	#4	#5	#6	Mean	U
0	0.461	0.453	0.445	0.456	0.467	0.456	0.4563	0.0887
1	0.397	0.407	0.414	0.367	0.39	0.392	0.3945	0.0460
2	0.363	0.387	0.398	0.385	0.393	0.397	0.3872	0.0968
3	-	-	-	-	-	-	-	-
4	0.413829	0.423117	0.388029	0.36704	0.360354	0.376916	0.3882	0.0194
5	~~0.91~~	~~1.06~~	~~1.06~~	~~1.02~~	~~1.04~~	~~1.1~~	~~1.0317~~	~~0.1651~~
6	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-
9	0.522	0.525	0.536	0.541	0.535	0.53	0.5315	0.0140
10	0.528	0.532	0.542	0.535	0.52	0.538	0.5325	0.0140
11	0.6045	0.577	0.5543	0.4125	0.4156	0.4035	0.4946	0.1088
12	0.425802	0.424231	0.436887	0.439872	0.441529	0.445292	0.4356	0.0254
13	0.507	0.48	0.465	0.45	0.446	0.437	0.4642	0.0659
14	0.443	0.432	0.462	0.434	0.47	0.461	0.4503	0.0380
15	-	-	-	-	-	-	-	-



Zn mass fraction, mg/kg

Lab Code	#1	#2	#3	#4	#5	#6	Mean	U
0	145	147	148	149	149	148	147.67	23.00
1	145.9	144.7	145.8	140.8	142.1	140	143.22	15.30
2	144	147	143	145	145	149	145.50	21.83
3	144.5	146.3	145.5	147.5	145.6	146.4	145.97	14.75
4	152.9412	149.5356	150.1548	147.5157	149.8817	147.8243	149.64	7.48
5	130	136	134	134	135	131	133.33	21.33
6	-	-	-	-	-	-	-	-
7	136	139	133	132	140	141	136.83	13.68
8	~~129~~	~~130~~	~~129~~	~~131~~	~~130~~	~~130~~	~~129.83~~	~~1.50~~
9	~~125~~	~~133~~	~~128~~	~~130~~	~~126~~	~~128~~	~~128.33~~	~~5.80~~
10	~~131~~	~~130~~	~~130~~	~~131~~	~~133~~	~~135~~	~~131.67~~	~~4.00~~
11	166.3	167.3	162.6	155.9	152.2	156.7	160.17	26.80
12	145.9759	145.7029	147.0175	145.1496	146.6582	146.3436	146.14	2.30
13	-	-	-	-	-	-	-	-
14	148	142	149	142	152	148	146.83	11.00
15	147.35	147.29	147.23	147.26	147.11	147.23	147.25	1.20



Annex E ERM®-BB184 (Bovine muscle) – Participant methods

Methods applied by participants in the characterisation exercise, as reported by participants.

Participant code	*Element(s)*	*Sample preparation method*	*Measurement technique*	*Calibration method*	*Quality Assurance*	*Uncertainty estimation*
0	All	MW-assisted digestion (0.5 g sample + 5ml HNO3+1mlH2O2), 600W, 1 h	ICP-SFMS in high resolution mode	External	Preparation (method) blanks, DORM 2 CRM	In accordance with T.Ruth 'A model for the evaluation of uncertainty in routine multi-element analysis', Accred Qual Assur (2004) 9:349-354
1	As, Cr, Cu, Fe, Pb	0.5 grams of sample. Microwave digestion with 6 ml HNO_3 (67%), 1 ml H_2O_2 (33%). Dilution to volume with H_2O	AAS-Graphite furnace with Zeeman background correction	External calibration curve with 4 standards	Quality control sample in each batch; calibration curve linearity; blank control; repeatability of measurements	2 contributions: uncertainty related to intermediate precision and uncertainty related to trueness/bias. These contributions are established based on the validation data. For intermediate precision, 10 replicates obtained in 2-4 different days were used. For trueness, MRC, materials from PT schemes or spiked samples were used, depending on the availability.
	Se	0.5 grams of sample. Microwave digestion with 6 ml HNO_3 (67%), 1 ml H_2O_2 (33%). Ashing with $Mg(NO_3)_2$. Dilution of the ashes in HCl (37%). Reduction of Se at 90ºC, 10 min. Dilution to volume with H_2O	Hydride generation, with Deuterium background correction	As above	As above	As above
	Zn	0.5 grams of sample. Microwave digestion with 6 ml HNO_3 (67%), 1 ml H_2O_2 (33%). Dilution to volume with Li (2%) in HNO_3	AAS-Flame (Air-Acethylene)	As above	As above	As above
2	As, Cd, Pb	Wet digestion, concentrated nitric acid	ICP-MS	Standard addition for As, external calibration for	Reference materials used: ARC/CL Wheat flour, ARC/CL Animal muscle, NIST 156a Rice flour for As	The method uncertainty covers the whole analytical procedure, random error and systematic error
	Cr, Cu, Fe, Mn, Zn	As above	ICP-OES	External standards	Reference materials used: ARC/CL Wheat flour, ARC/CL Animal muscle	As above, with reservation: Method is not fully validated for Cr.
	Hg	Microwave digestion, concentrated nitric acid, and hydrochloric acid	Varian Mercury Analyzer	External standards	As above	As above
	Se	Wet digestion, mixture of concentrated	Graphite furnace	External	Reference materials used:	As above

		nitric, sulfuric and perchloric acid, extraction into MIBK	AAS	standards	ARC/CL Wheat flour, Total Daily Diet Pool -reference	
3	Cu, Fe, Zn	Microwave-assisted high-pressure digestion of 0.5g sample with 4 ml HNO_3 and 2 ml H_2O_2, filled up to 20ml.	ICP-OES: (With Cu 324.754 nm line), Meinhard nebulizer + cyclonic spray chamber (quartz)	External calibration with matrix matched standard solutions prepared from stock solutions traceable to (*certified reference materials issued by*) NIST	Analysis of CRM 1577a and digestion/reagents blanks. Check for comparability with alternative wavelengths. Check for stability by periodic measurement of quality control sample solutions. Calculation of recoveries of spiked sample solutions.	Uncertainty calculation based on statistics from CRM 1577a expressed as combined uncertainty and expanded with a coverage factor of 2.
4	All	Oxidative decomposition of the test material using a high pressure autoclave with microwave heating	ICP-MS	external calibration (2 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
5	As, Cd, Cr, Cu, Mn, Pb, Se, Zn	Acid digestion (2 g of sample, 5 ml HNO_3, 1.5 ml H_2O_2, final volume - 25.0 ml)	ICP-MS	2 point calibration (0 and 100 µg/L)	Control of calibration for point 100 µg/L prepared from another standard material in each series of measurement, measuring duplicate samples for each series of 20 samples, control of blank values, standard addition (50 µg/L) for each 20^{th} sample.	Repeated measurement of standard materials and calculation of RSD values.
6	As, Pb	Slow dry decompositon (24 hours at 450 °C), ash dissolved in HNO3 - (5 g of sample, 5 ml HNO_3, final volume - 50.0 ml)	ICP-MS, isotope ^{75}As with mathematical correction for $^{40}Ar^{35}Cl$ interference	2 point calibration (0 and 100 µg/L)	As above for elements measured by ICP-MS	Repeated measurement of standard materials and calculation of RSD values.
7	Cr, Cu, Fe, Mn, Zn	As above	ICP-OES, axial view	4 point calibration in range 0 - 20 mg/L	Control of calibration for point 2 mg/L prepared from another standard material in each series of measurement, measuring duplicate samples for each series of 20 samples, control of blank values, standard addition for each 20^{th} sample.	Repeated measurement of standard materials and calculation of RSD values.
8	Cd, Cu, Hg, Pb, Zn	Microwave assisted digestion Multiwave 3.000, Anton Paar with 8-XF-100	ICP-MS: Instrument: ELAN	External calibration with	Measurement of certified standards together with	Uncertainty was estimated as twice the reproducibility standard deviation of BCR-184.

		pressure vessels. Sample intake: exactly weighed. Acid addition: 4 ml HNO_3, 1 ml H_2O_2. When finished dilute the samples with H_2O exactly to a final volume of 30 ml. (HNO_3, subboiled distilled, H_2O_2 Suprapure, Merck)	DRC II, Perkin-Elmer - LAS, Sample introduction using a peristaltic pump, Spetec, at a flow rate of 1.3 ml/min, Meinhard nebulizer and cyclone spray chamber.	6 standards and internal standard Rh: Measurement series starts with blanks, calibration standards, blanks, control standard 1, followed by sample digests and control standard 2 + blanks (every tenth sample), ending with control-calibration standards and blanks. Samples not matching the calibration range were appropriately diluted and re-measured.	samples prepared at sample concentrations determined. Preparation and measurement of BCR-184 CRM together with the samples. Preparation and measurement of blank digestions together with the samples.	Reproducibility standard deviation was derived from this sample, which was digested on 10 different days and analysed on 10 different days.
9	All	As above	HR-ICP-MS: Instrument: Element 1 Thermo Fisher Scientific, Sample introduction using a peristaltic pump, Spetec, at a flow rate of 1.2 ml/min, Meinhard nebulizer and cyclone spray chamber.	External calibration with 6 standards and internal standards Ir and Rh; procedure and controls as above	As above	As above
10	Cr, Cu, Fe, Mn, Se, Zn	As above	ICP-AES: Instrument: Optima 7300, Perkin-Elmer - LAS, Sample introduction using a peristaltic pump, Spetec, at a flow rate of 0.6 ml/min, low-flow Micromist nebulizer and	External calibration with 7 standards; procedure and controls as above	As above	As above

			heated cyclone spray chamber (APEX).			
11	All	High pressure microwave assisted digestion using nitric and hydrochloric acid in quartz vessels	ICP-MS with collision cell technology (Agilent 7500ce)	External, acid matched, plus internal standard correction using rhodium	Reagent blanks, spiked samples, and their following CRM: BCR185R, ERM CE278, DORM3. Applied in each batch.	Based on our performance in inter-laboratory comparisons.
12	Cr, Cu, Fe, Se, Zn	Microwave acid digestion (HNO_3 and H_2O_2)	HR-ICP-MS Element 2, except Se; Agilent 7500ce ICP-MS.	IDMS, with procedures detailed in report of analysis	Independent Std used as quality check, NIST SRM1577c used as QC for recoveries/matrix effects. Analysis performed following LGCs SOP (UKAS accredited, ISO 17025) for the provision of characterisation values by ID-ICP-MS	The uncertainty of the moisture corrected value was calculated in accordance with ISO Guide to the Expression of Uncertainty in Measurement (GUM) guidelines. Combined uncertainties were calculated following the Kragten spreadsheet method taking into account instrumental performance, IDMS equation, blend to blend variation and uncertainty of moisture determination.
13	As, Cd, Hg, Pb, Se	Microwave assisted pressure digestion with conc. HNO_3	ICP-MS	Addition calibration (4 levels)	CRM (BCR185 Bovine Liver) was analysed	According to the principles in "Guide to the expression of uncertainty in measurement - GUM:1995 BIPM/IEC/IFCC/ISO/IUPAC/IUPAP/OIML ". Software GUM Workbench used (www.gum.dk).
14	Fe, Mn, Se, Zn	None	k0-NAA	*k*0 method - traceability in k0-NAA is guaranteed through the use of IRMM-530R Al-Au fluence rate monitors	BCR-184	Accoding to Robouch et al., Uncertainty budget for k0-NAA. J. Radioanal. Chem. 245, 195 (2000).
15	Cu, Zn	None	TIMS	Isotope dilution		

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Abstract

This report describes the preparation of a bovine muscle matrix reference material ERM®-BB184, and the certification of the contents (mass fractions) of seven elements. All results are expressed as a mass fraction on a dry mass basis.

The preparation and processing of the material, homogeneity studies, stability studies and characterisation are described hereafter and the results are discussed. Uncertainties were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [1] and include uncertainties due to possible heterogeneity, instability and from characterisation. The certified values and their uncertainties are listed in Table 1:

Table 1: Certified mass fractions of elements and their uncertainties in Bovine muscle (ERM®-BB184)

Element	Certified value [1.2)] [mg/kg]	Uncertainty [3)] [mg/kg]
As	0.0234	0.0026
Cd	0.0022	0.0004
Cu	2.31	0.09
Fe	75	4
Mn	0.276	0.013
Se	0.45	0.04
Zn	146	7

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.

The assigned values are based on a minimum sample intake of 0.2 g.

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