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CERTIFICATION REPORT

The Certification of the Mass Fractions of Elements in Fish Muscle

Certified Reference Material ERM®- BB422

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European Commission
Joint Research Centre
Institute for Reference Materials and Measurements

Contact information
Reference materials sales
Retieseweg 111
B-2440 Geel, Belgium
E-mail: jrc-irmm-rm-sales@ec.europa.eu
Tel.: +32 (0)14 571 705
Fax: +32 (0)14 590 406

http://irmm.jrc.ec.europa.eu/
http://www.jrc.ec.europa.eu/

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The Certification of the Mass Fractions of Elements in Fish Muscle

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J. Snell, H. Emteborg, H. Schimmel

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

Summary

This report describes the preparation of a fish muscle matrix reference material ERM®-BB422, and the certification of the contents (mass fractions) of eight 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 fish muscle (ERM®-BB422)

Element	Certified value, mg/kg [1,2)]	Uncertainty, mg/kg [3)]
As	12.7	0.7
Cd	0.0075	0.0018
Cu	1.67	0.16
Fe	9.4	1.4
Hg	0.601	0.030
I	1.4	0.4
Mn	0.368	0.028
Se	1.33	0.13
Zn	16.0	1.1

1) These values are related to dry mass and are based on the unweighted mean of accepted results
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) Where the standard combined uncertainty is multiplied by a coverage factor k = 2 for elements except I and k = 2.57 for I 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 and their uncertainties 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
CVAFS ..... Cold vapour atomic fluorescence spectrometry
d_f ..... degree of freedom (regression)
ERM® ..... European Reference Material®
GUM ..... Guide to the Expression of Uncertainty in Measurement
HG ..... Hydride generation
ICP-MS ..... inductively coupled plasma 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
n/a ..... not applicable
NAA. ..... Neutron activation analysis
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 standard deviation
s_{wb} ..... within-bottle heterogeneity standard deviation
se_b ..... standard error of slope b of linear regression
SI ..... International System of Units
u_{bb} ..... relative standard uncertainty due to between-bottle heterogeneity
u^*_{bb} ..... relative standard uncertainty due to heterogeneity that can be hidden by method repeatability
u_{char} ..... 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 development of a reference material to replace the CRM BCR-422 (Cod muscle).

The target parameters for certification were the mass fractions of As, Cd, Cu, Fe, Hg, I, Mn, Pb, Se, and Zn. The desired mass fractions were those at natural levels that are similar to BCR-422 and below regulatory limits for food contaminants as specified in EC Regulation no. 466/2001 (Cd < 0.05 µg/g, Hg < 0.5 µg/g, Pb < 0.3 µg/g "wet weight"). Values are to be traceable to the SI and expressed as dry mass fraction.

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

Throughout this report, results are expressed as a mass fraction on a dry mass basis. For practical purposes, the dry mass is established by determining the "loss of mass on drying" under defined conditions. It should be noted that determination of the dry mass correction factor under conditions other than specified in (section 10.2 of) 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 and stability measurements

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

Characterisation measurements

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

Ceinal, S.A. (Silliker), Área Análisis Físico-Químicos, Barcelona (ES)
(Measurements performed under ISO/IEC 17025 accreditation; ENAC 257/LE413)

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

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

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

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

The Food and Environment Research Agency, York (UK)
(Measurements performed under ISO/IEC 17025 accreditation; UKAS 1642)

Fødevareinstituttet, Danmarks Tekniske Universitet, Søborg (DK)
(Measurements performed under ISO/IEC 17025 accreditation; DANAK No 350)

Laboratoire National de Métrologie et d'Essais, Paris (FR)
(Measurements performed under ISO/IEC 17025 accreditation; COFRAC No 2-54)

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

Service Centrale d'Analyse (C.N.R.S.), Solaize (FR)

4 Processing of the material

4.1 Material selection

Since the preparation of BCR-422, which started with the fishing of the cod for the raw material in January 1989, concern has grown over the sustainability of European cod fisheries. Cod is now included on the IUCN "Red List" of endangered species. As the price of cod has risen, and the amount landed has reduced, food manufacturers and consumers have sought other white-fleshed seafish as alternatives to cod.

For the replacement of BCR-422, for which the stock is exhausted, an alternative fish was selected as the raw material for the preparation of the candidate CRM. Saithe (*pollachius virens*, NL: koolvis, FR: lieu noir, DE: Seelachs/Köhler) was chosen as the muscle is similar in matrix and trace element content to cod muscle. The taxonomic classification of saithe places it in the Gadidae family (cod-like fish), of which cod (*gadus moruha*) is also a member. Considering the sample preparation methodologies used as part of the measurements the CRM is designed to control, the matrix composition of saithe was expected to be similar to that of cod.

Fresh saithe fillets were purchased from a wholesaler in Belgium. The fish were caught in the European North-East Atlantic area (code FAO27) and were filleted by the wholesaler. Most fillets were in the mass range 0.7 – 1.2 kg. The filets were packed in plastic bags containing about 10 kg, on ice, in plastic boxes, and were transported to IRMM by refrigerated courier.

The material was supplied in three batches of about 40 kg that were freeze-dried on three occasions, each 1 week apart. The raw material was stored at 4 °C until processing, which took place less than 24 h after arrival at IRMM on all 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 Fish muscle processing

The raw material was manually cut into cubes of 1 to 2 cm length using ceramic knives and plastic cutting boards. The cutting took place under 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. About 29.5 kg of dried material was produced.

The dried cubes of fish muscle were then 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. The fraction > 250 µm, which was about 15 % of the starting mass, was transferred to the stainless steel drums used prior to the milling, again immersed into liquid nitrogen overnight and the Palla milling and sieving was repeated. The fraction that passed the sieve was added to the first fraction in the plastic drum and was kept at room temperature. The remainder (about 1.1 kg) was retained in a separate plastic drum and was not processed further.

About 27.5 kg of powder was produced, which was then 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 between 4.2 and 4.3 % (m/m), while no particles were larger in size than 435 µm and the particle-size distribution approximated log-normal. The material was transferred to plastic bags containing about 1 kg of powder, which were thermally sealed and stored at room temperature. While the particle size was considered suitable for the material to be bottled, the water contents indicated that an additional drying was necessary to ensure preservation of the material.

A batch of 50 ml brown glass bottles were acid washed with 2 % HNO_3, rinsed with Milli-Q purified water 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 placed in trays and loaded into the freeze drier in two batches. Vacuum drying was made during 4.5 h at 20 °C. The resulting water content was checked using Karl-Fischer titration (KFT) on two bottles in duplicate and was found to be < 3.0 % (m/m). At the end of the drying process, the vacuum chamber was filled with nitrogen to ambient pressure and the lyo-inserts were then hydraulically pushed by the shelves into the neck of the bottles to preserve the inert atmosphere over the material. The bottles were then capped with aluminium lids and labelled.

In total, 2520 units were produced, each containing about 10 g of material. Bottles for normal stock were stored at 18 °C.

4.3 Additional characterisation measurements

Five bottles were selected by random sampling for measurement of water content by 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 1.62 % (m/m) in the samples, with a standard deviation of 0.14 % (m/m) (and estimated measurement uncertainty of 0.2 % (m/m), $k = 2$). The material was therefore considered to be sufficiently dry to preserve the matrix for several years (in a sealed container), and the water content was the same between bottles, within measurement uncertainty.

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 146 and 192 µm with expanded uncertainty ($k = 2$) of 9.7 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 an optical microscope (Stemi 2000-C, Zeiss, DE) with < 10 µm resolution. The particles were irregularly shaped, with some agglomerates but no fibres apparent in any of the 10 images.

A selection of major elements was also measured in the samples, which were used for certification measurement, by SCK-CEN using *k*0-NAA. The element mass fractions, given in table 2, are given for addition information on the material that may be used in the optimisation of measurement methods.

Table 2: Major elements measured by NAA in ERM-BB422 (Fish muscle)

Element	*g/kg*	U_{rel} (k = 2)
Ca	0.342	9.8 %
Cl	3.5	5.2 %
K	21.4	5.4 %
Mg	1.37	5.1 %
Na	2.80	5.8 %

Where U_{rel} = Relative uncertainty for 6 replicate measurements, with 2 replicates made on each of 3 bottles.

Methylmercury (MeHg) was also measured in the samples that were used for certification measurement by Umweltbundesamt GmbH, Wien, using HPLC-ICP-MS (Double ultrasonic extraction with 50 mM pyridine, 0.5 % L-cysteine and 5 % methanol; measurement with a coupled HPLC (Perkin Elmer LC200) and ICP-MS (Perkin Elmer Elan DRC II)). The mass fraction of MeHg in the material, expressed as mass fraction of Hg present as MeHg, is given in Table 2.

Table 3: Methylmercury measured in ERM-BB422 (Fish muscle)

Element	*mg/kg as Hg*	s (n = 6)
MeHg	0.509	0.043

The value for MeHg is not given on the certificate as the material is intended to be stored at room temperature, and under this condition, MeHg was previously found to be unstable in similar materials (BCR-463 and BCR-464 tuna). Furthermore, as it was not a target analyte for certification, no assessment of MeHg stability was made in this study.

5 Homogeneity studies

5.1 Design of homogeneity studies

For the homogeneity study, 13 bottles of ERM®-BB422 (Fish 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, together with measurement of the water content. The measurements were made by methods using ICP-MS for As, Cd, Cr, Cu, I, Mn, Pb and Zn, ICP-OES for Fe and Se and CVAFS for Hg.

Samples were measured in a random order (predefined at IRMM and communicated to the laboratory) 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 reactions were corrected to the dry mass.

Grubbs tests the 99 % confidence level were performed to detect potentially outlying individual results as well as outlying bottle averages. Regression analyses were performed to detect possible trends regarding analytical or filling sequence. 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 4 for a summary of results, in which values were converted into relative uncertainties).

5.2 Results of the homogeneity study

The results are presented in Table 4, 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 Cr, Hg and Mn, and by the double Grubbs test for Cu. No significant slopes were found with either the filling sequences or the analytical sequences for any of the elements. All individual data and bottle averages showed normal or unimodal distributions, for all analytes. The consequences of these observations on the uncertainty estimations for between-bottle homogeneity are discussed below, by element:

For As, Cd, Cu, Fe, Hg, I, Se and Zn, no outliers, significant slopes or suspicious data were found and u_{bb} at an acceptable level were returned for each element.

For Cr, measurement RSD of over 100 % was found. Considering that the measurement uncertainties were typically 25 % (k = 2), this result indicates heterogeneity of Cr in the material.

For I, measurements were made by the conventional sample preparation technique of acid digestion in a heated pressurised vessel. While this method is suitable for most elements, there is a risk of loss of I through volatilisation, and the measurement laboratory declined to set an uncertainty for the measurements. However, measurements of a QC material with certified I mass fraction, measured in sequence with the sample returned a mean recovery of 98 % (s = 4.6 %, n = 8), which indicates that no significant losses took place. As the dataset otherwise showed no suspicious data, the estimate of u_{bb} was considered reliable.

For Mn, one outlier was found by the single Grubbs test at level α = 0.01, There was no technical reason to exclude the outlier, and it was included in the calculation.

For Pb, the mass fraction in the material was estimated to be below the method limit of quantitation (LOQ) of 0.002 mg/kg for almost all samples. Estimation of u_{bb} was therefore not possible with this study. However, for the long-term stability study, the sample intake mass was increased to 0.5 g, which lowered the method LOQ and allowed measurement of Pb. The data consisted of 5 replicate measurements on each of 8 bottles, and was used to estimate u_{bb} while ignoring the potential influence of the different storage conditions of the bottles. No outliers, trend or suspicious data was found. The u_{bb} returned of 5.3 % was higher than that of the other elements, and reflects the maximum heterogeneity hidden by the (relatively poor) measurement repeatability at this low mass fraction.

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

Table 4: Evaluation of homogeneity study for elements in ERM®-BB422 (Fish muscle)

	As	Cd	Cr	Cu	Fe	Hg	I	Mn	Pb[1]	Se	Zn
Mean [mg/kg]	11.75	0.0076	0.015	1.62	9.27	0.616	1.38	0.370	0.0030	1.19	16.4
RSD [%]	2.09	2.65	141	0.64	1.47	1.72	4.89	1.67	8.2	2.01	1.23
$MS_{between}$	0.361	2.4×10^{-7}	2.6×10^{-3}	0.7×10^{-3}	0.111	6.7×10^{-4}	0.027	2.3×10^{-4}	3.0×10^{-7}	3.4×10^{-3}	0.25
MS_{within}	0.421	2.8×10^{-7}	0.1×10^{-3}	1.7×10^{-3}	0.092	5.1×10^{-4}	0.026	1.9×10^{-4}	5.0×10^{-7}	7.9×10^{-3}	0.27
s_{bb} [%]	$MS_B<MS_W$	$MS_B<MS_W$	138	$MS_B<MS_W$	0.62	0.86	0.92	0.69	$MS_B<MS_W$	$MS_B<MS_W$	$MS_B<MS_W$
s_{wb} [%]	5.52	6.97	77	2.58	3.26	3.65	11.8	3.72	24	7.45	3.17
u^*_{bb} [%]	0.94	1.19	13.3	0.44	0.56	0.62	2.01	0.64	5.3	1.27	0.54
u_{bb} [%]	**0.94**	**1.19**	**138**	**0.44**	**0.62**	**0.86**	**2.01**	**0.69**	**5.3**	**1.27**	**0.54**
Significant slope in measured value/sample bottle number, at confidence level;											
95 %	No	No	No	No	No	No	No	No	No	No	No
99 %	No	No	No	No	No	No	No	No	No	No	No
Significant slope in measured value/measurement sequence number, at confidence level;											
95 %	No	No	No	No	No	No	No	No	No	No	No
99 %	No	No	No	No	No	No	No	No	No	No	No
Outliers by Grubbs tests, individual measurements											
Single	None	None	1	None	None	None	None	1	None	None	None
Double	None	None	n/a	None	None	None	None	n/a	None	None	None

1) Using data from the long-term stability study

5.3 Minimum sample intake

The minimum sample intake (MSI) is set to the mass used for the homogeneity study for all elements, which was 0.2 g with the exception of Pb that was estimated through measurements to assess long-term stability, made with a sample intake of 0.5 g. To verify the suitability of these masses, a study was made using solid sampling atomic absorption spectrometry, SSAAS, to measure the contents of selected elements in sub-milligram amounts of the material, without sample preparation.

The principle of the estimation of minimum sample intake by SSAAS is as follows: Between 3 and 50 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 residual standard deviation, 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 257 mg for As, 8 mg for Cu and 11 mg for Fe. While values for Cu and Fe lie well below the minimum sample intakes set for the material of 200 mg for all elements (except Pb at 500 mg), the value for As is higher. While real heterogeneity of the material can not be distinguished from the repeatability of measurements, the As content of the material lay close to the LOQ for the method, which was thus expected to provide poorer repeatability. It was therefore considered that the results of the SSAAS measurements support the MSI of 200 mg for this 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®-BB422 were chosen using a random stratified sample picking scheme. Element concentrations were measured with ICP-MS and ICP-OES method as used for the homogeneity study.

Samples were stored at 18 °C and 60 °C as well as at a reference temperature of -20 °C, at which the material is considered to be stable. Two bottles were stored at each temperature for 0, 1, 2 and 4 weeks. After the indicated storage periods, the samples were transferred to storage at -20 °C until analysis. Samples were analysed under intermediate precision conditions in an order specified by IRMM (randomised sample order). In each of the 10 bottles a determination of dry mass content was performed. All results per bottle were related to the mean of the respective duplicate dry mass determination.

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

For As, Cd, Hg, I and Se no outlying measurements, no significant slopes in mass fraction on storage at elevated temperature over time, and all data were accepted. The study returned u_{STS} for these elements significantly lower than the respective u_{CRM}. The results for the remaining elements are discussed below:

Cr: At 83 %, the RSD of measurement was significantly higher than that of the other elements, due to the heterogeneity also observed in the homogeneity study. Due to the RSD, the estimate of u_{STS} was considered to be unreliable.

Cu: One outlier was found by the single Grubbs test, for a replicate on a bottle stored at the reference temperature. As a consequence, significant slopes with time were found at the 95 % confidence level for both test temperatures. Although this was the same replicate also found to be an outlier for the Fe and Mn datasets, no technical reason for exclusion could be found. The outlier was retained for the calculation of u_{STS}, and the slopes for potential degradation were also included in the calculation.

Fe: For the 60 ºC data set, one outlier was found by the single Grubbs test. There was no technical reason to exclude the outlier and it was retained for the calculation of u_{STS}.

Mn: Two outliers were found by the double Grubbs test, in datasets for both test temperatures. There was no technical reason to exclude the outliers and they were retained for the calculation of u_{STS}.

Pb: Results for more than half the samples were reported as being below the method LOQ, 0.002 mg/kg. A reliable u_{STS} could therefore not be established.

Zn: While no outliers were found, a significant slope was apparent at the 95 % confidence level (but not at the 99 % level) for the study at 18 ºC. As no slope was found in samples stored at the higher temperature, and the measurement repeatability of the two datasets was similar, this is most likely to be a statistical artefact. However, as no technical reason could be found to ignore the slope, potential degradation was included in the calculation of u_{STS}.

In summary, reliable estimations of u_{STS} were obtained for all elements except Cr and Pb. The level of the u_{LTS} for 1 week, including potential degradation where found, varied between 0.4 and 1.1 %, with the exception of the dataset for Cd at 60 ºC at 1.7 %, which is most likely due to the Cd mass fraction lying close to the method LOQ. Each u_{STS} lay well below the respective u_{CRM} for the element is and thus negligible. ERM-BB422 may therefore be transported at room temperature, without cooling elements.

Table 5: Evaluation of short-term stability study for elements in ERM®-BB422 (Fish muscle)

Element	Test temperature	Grubbs test[1] single	Grubbs test[1] double	slope[2]	$u_{STS, rel}$ / %[3] stable	$u_{STS, rel}$ / %[3] degradation
As	18ºC	none	none	no	**0.6**	
	60 ºC	none	none	no	**0.8**	
Cd	18ºC	none	none	no	**0.6**	
	60 ºC	none	none	no	**1.7**	
Cr	18ºC	none	none	99 %	7.9	**17.4**
	60 ºC	none	none	99 %	6.2	**12.8**
Cu	18ºC	1	n/a	95 %	0.3	**0.4**
	60 ºC	1	n/a	95 %	0.3	**0.4**
Fe	18ºC	none	none	no	**0.4**	
	60 ºC	1	n/a	no	**0.3**	
Hg	18ºC	none	none	no	**0.4**	
	60 ºC	none	none	no	**0.6**	
I	18ºC	none	none	no	**0.9**	
	60 ºC	none	none	no	**1.1**	
Mn	18ºC	none	2	no	**0.4**	
	60 ºC	none	2	no	**0.4**	
Se	18ºC	none	none	no	**0.8**	
	60 ºC	none	none	no	**0.8**	
Zn	18ºC	none	none	95 %	0.2	**0.3**
	60 ºC	none	none	no	**0.3**	

[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, calculated assuming stability or including degradation, where observed

6.2 Long-term stability study

6.2.1 Design of the long-term stability study

A 12 month isochronous study [5] was performed to evaluate stability of the material during long-term storage. Eight 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 otherwise maintained, and after the study were returned, to storage at the reference temperature of -20 °C.

Water content was measured once in each bottle so that dry-mass correction of element concentrations could be performed, and five replicate measurements of each element were made on each bottle. Samples were analysed in a sequence defined by IRMM (with randomised sample order). The measurements were made using ICP-MS for As, Cd, Cu, I, Mn, Pb and Zn, ICP-OES for Fe and Se and CVAFS for Hg.

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 4 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 $\alpha = 0.05$ (95 % confidence interval) and for a confidence level $\alpha = 0.01$ (99 % confidence interval). The slope was considered as

statistically significant when $|b|/se_b > t_{\alpha,df}$. 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 40 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.

6.2.2 Results of the long-term stability study

For As, Cu, Hg, Mn, Se and Zn, no outlying measurements, no significant slopes in mass fraction on storage at elevated temperature over time, and all data were accepted. The results for the remaining elements are discussed below:

Cd: One outlier was found by the single Grubbs test. There was no technical reason to exclude the outlier and it was retained for the calculation of u_{LTS}. The concentration of Cd measured in the samples was close to the method LOQ, as evidenced by the measurement RSD of 13 %. The u_{LTS} was calculated to be 5.62 %, which is high relative to the other elements, however, the estimation can be considered reliable.

Fe: one outlier was found by the single Grubbs test. There was no technical reason to exclude the outlier and it was retained for the calculation of u_{LTS}.

I: one outlier was found by the single Grubbs test. There was no technical reason to exclude the outlier and it was retained for the calculation of u_{LTS}. As discussed for the homogeneity study, the sample preparation method used risks the loss of I by volatilisation. However, on this occasion, measurement on a QC material returned a recovery of 101 % (s = 2.2 %, n = 5), which indicated that the results were reliable.

Pb: One outlier was found by the single Grubbs test. There was no technical reason to exclude the outlier and it was retained for the calculation of u_{LTS}. The concentration of Pb measured in the samples was close to the method LOQ, as evidenced by the measurement RSD of 23 %. The u_{LTS} was calculated to be 9.73 %, which is high relative to the other elements, however, the estimation can be considered reliable.

In summary, reliable estimates of u_{LTS} were obtained for all elements and no significant slopes were found in mass fraction over storage time at elevated temperature. The material may therefore be stored at room temperature, + 18 ºC.

Table 6: Results of the 12 month stability study on element concentrations in ERM®-BB422 (Fish muscle)

	Grubbs test[1]				
Element	single	double	slope[2]	measurement RSD	$u_{LTS, rel}$ %[3]
As	none	none	none	2.47	2.10
Cd	1	n/a	none	13.2	11.3
Cu	none	none	none	2.13	1.81
Fe	1	n/a	none	7.43	6.3
Hg	none	none	none	1.66	1.41
I	1	n/a	none	5.59	4.8
Mn	none	none	none	3.76	3.2
Pb	1	n/a	none	22.9	19.5
Se	none	none	none	3.92	3.33
Zn	none	none	none	1.8	1.53

[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, extrapolated for a shelf-life of 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, laboratories 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 individual results for elements, where possible. Each laboratory was provided with two bottles of ERM®-BB422 (Fish muscle) and one bottle of BCR-422 (Cod 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, as required.

With the exception of measurements by 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 concentrations.

A table of methods employed by the participating laboratories 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 6:

$$\left| y_{mean} - c_{CRM} \right| < 2 \quad \sqrt{u_{CRM}{}^2 + u_y{}^2} \qquad (6)$$

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 the QC measurements.

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.

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.

For each element, variances between labs were significantly different (Snedecor *F*-test), therefore data could not be pooled and had to be grouped by labs. Unless an additional 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 results of Lab 4 were found to have differences in measured means between days of 19 % for the QC material and 29 % for the sample, respectively. As the Lab reported measurement uncertainty of 5 % and the certified As content of the QC material had associated uncertainty of about 2.5 % (k = 2), a measurement error was assumed and the results were rejected. For the remaining dataset, the result of Lab 6 was found to be an outlier by the Nalimov test at α = 0.05, and was found to have an outlying variance by the Cochran test at p = 0.05. While the result agreed with the mean of all labs, the estimated measurement uncertainty of 20 % was higher than all other labs. The results also showed an 11 % difference in sample means measured on different days. While this is lower than the measurement uncertainty, considering that their within day repeatability lay around 1 %, inconsistent performance between measurement days was suspected and the results were rejected. For the accepted dataset, the mean value of Lab 2 was found to be an outlier by Nalimov test at α = 0.05, but not α = 0.01. The result was therefore considered to be a straggler rather than a genuine outlier, and as there was no technical reason to exclude the result, it was retained.

Cd: Most participants reported higher than expected measurement uncertainties, of about 20 %, because the concentration of Cd in the material lay within an order of magnitude of the LOQs for most methods. Measurements of Lab 11 on the QC material showed a mean positive bias of 43 % which is greater than the combination of the maximum estimated measurement uncertainty and uncertainty of the certified value of 33 % (k=2), calculated according to equation 6. The result was therefore rejected.

Cu: Lab 6 reported QC measurements with a bias of 22 % compared to the maximum combined measurement and certificate uncertainty of 15.3 %, k = 2. The results were therefore excluded from calculation of the certified value. For the accepted dataset, the mean value of Lab 14 was found to be an outlier by Nalimov test at α = 0.05, but not α = 0.01. Their replicate measurements of Lab 14 were found to have a high variance; however, this was comparable to the variances of Labs 2 and 4, and their measurements on the QC material also showed no bias. The result was therefore considered to be a straggler rather than a genuine outlier, and as there was no sufficient technical reason to exclude the result, it was retained.

Fe: Measurements of the QC material by labs 12 and 14 returned biased results (of -17 % and 36 %) compared to the maximum combined measurement and certificate uncertainty (of 14.8 %, k = 2). Both results were therefore excluded from calculation of the certified value. For the remaining dataset, the result of Lab 2 was found to be an outlier by all tests at α = 0.05, and by Nalimov and Grubbs tests at α = 0.01: This resulted in a distribution of the dataset that was found not normal by skewness and kurtosis tests at α = 0.01. There was no technical reason to exclude the result as the lab's QC measurements showed no bias, the variance between replicates was low and the Fe concentration was more than 10 times the reported LOQ. However, as the result agreed with the dataset mean, within combined measurement uncertainty and u_{CRM}, it was retained for calculation of the certified value.

Hg: No outliers or suspicious data were found, and the results of all labs were accepted.

I: As only 5 labs submitted results, it was not possible to assess the normality of the results distribution. However, there were no outliers in either the means or variances, and all data was taken for calculation of the certified value.

Mn: Measurements of the QC material by labs 5 and 6 returned values biased high (at 32 % and 24 %) compared to the maximum combined measurement and certificate uncertainty (of 15.7 %, k = 2). Both results were therefore excluded from calculation of the certified value. No outliers or suspicious data were found in the remaining dataset.

Pb: 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. Labs 1, 4 and 10 reported uncertainties of greater than 100 %, and Labs 3, 5, 6 and 9 reported that the mass fractions measured were below their LOQ. While the two remaining Labs, 8 and 14, provided datasets with no suspicious data and estimated their measurement uncertainty to be at a suitable level for quantitative measurement, their mean values differed by a factor of 5. It was therefore not possible to establish a reliable mean for the dataset.

Se: For the QC measurements, values from 3 labs did not agree with those certified (with biases of 38 %, 19 % and 20 % compared to the sum of measurement and certified uncertainties of 18 %) and the results were excluded. For the remaining dataset, no outliers or suspicious data were found.

Zn: The mean value of Lab 2 was found to be an outlier by Nalimov test at α = 0.05, but not α = 0.01. However, as their measurements on the QC material showed no bias, the result was therefore considered to be a straggler rather than a genuine outlier, and it was retained for the dataset. Replicate measurements of Lab 14 were found to have a high variance of about 8 %. However, as this was comparable to the measurement uncertainties quoted by the labs their mean value was retained.

Table 7: Summary of the technical evaluation

Element	As	Cd	Cu	Fe	Hg	I	Mn	Se	Zn
Lab 0	OK			OK	OK	OK	OK	**Bias**	OK
Lab 1	OK	OK	OK	OK	OK	OK	OK	OK	OK
Lab 2	OK		OK	OK	OK		OK	OK	OK
Lab 3	OK	OK			OK	OK		OK	
Lab 4	**U/Rep'**	OK	OK	OK	OK		OK	OK	OK
Lab 5	OK		OK				**Bias**	**Bias**	OK
Lab 6	**U/Rep'**		**Bias**	OK			**Bias**		OK
Lab 7					OK				
Lab 8	OK		OK	OK		OK	OK	OK	OK
Lab 9	OK	OK	OK	OK	OK	OK	OK	**Bias**	OK
Lab 10		OK		OK	OK			OK	
Lab 11	OK	**Bias**	OK	OK	OK		OK	OK	OK
Lab 12	OK			**Bias**					OK
Lab 13	OK							OK	
Lab 14		OK	OK	**Bias**			OK		OK
Lab 15			OK				OK		OK
Lab 16	OK							OK	
Lab 17					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, showing poor repeatability or measurement uncertainty, 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	I	Mn	Se	Zn
Number of data sets	11	7	9	9	11	5	9	9	12
Number of replicate measurements	66	42	54	54	66	30	54	54	72
Mean of means [mg/kg]	12.65	0.0075	1.674	9.41	0.601	1.438	0.3680	1.332	16.02
Relative standard deviation of mean of means [%]	4.5	9.7	12.4	10.2	6.1	13.1	5.5	9.1	9.7
Relative standard error of mean of means (u_{char}) [%]	1.36	3.65	4.15	3.20	1.85	5.88	1.84	3.04	2.79
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	Lab 2	none	none	none	none	none
Outlying means? (Nalimov t-test; p = 0.05)	Lab 2	none	Lab 14	Lab 2	none	none	none	none	Lab 2
Outlying means? (Grubbs test; p = 0.05)	none	none	none	Lab 2	none	none	none	none	none
Outlying lab variances? (Cochran test; p = 0.05)	none	no	no	no	no	no	no	no	Lab 4
Lab variances homogeneous? (Bartlett test; p = 0.01)	yes	yes	yes	yes	yes	yes	yes	yes	yes
Variances between labs significantly different? (Snedecor F-test; p = 0.01)	yes	yes	yes	yes	yes	yes	yes	yes	yes
Distribution of means normal (p = 0.01)? (Skewness, kurtosis and normal probability plot)	yes	yes	yes	no	yes	< 6 sets	yes	yes	yes

8 Certified values and uncertainties

The certified values for ERM®-BB422 (Fish 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 7:

$$u_{CRM} = \sqrt{u_{bb}^2 + u_{lts}^2 + u_{char}^2} \qquad (7)$$

Table 9 summarises the individual standard uncertainty contributions and the resulting expanded uncertainties as well as the certified values and their uncertainties after rounding for ERM®-BB422 (Fish muscle).

Table 9: Certified values and uncertainties for ERM®-BB422 (Fish muscle)

Element	As	Cd	Cu	Fe	Hg	I	Mn	Se	Zn
u_{bb} [%]	0.94	1.19	0.44	0.62	0.86	2.01	0.69	1.27	0.54
u_{lts} [%] [1)]	2.10	11.3	1.81	6.3	1.41	4.8	3.2	3.3	1.53
u_{char} [%]	1.36	3.7	4.2	3.4	1.85	5.9	1.84	3.0	2.79
$u_{CRM, rel}$ [%]	2.67	11.9	4.6	7.2	2.48	7.9	3.8	4.7	3.2
n results	11	7	9	9	11	5	9	9	12
$U_{CRM, rel}$ (*95 % ci.*)[2)] [%]	5.3	24	9.1	14.4	5.0	22	7.5	9.4	6.5
Certified value [mg/kg]	12.7	0.0075	1.67	9.4	0.601	1.4	0.368	1.33	16.0
U_{CRM} (*95 % ci.*) [mg/kg]	0.7	0.0018	0.16	1.4	0.030	0.4	0.028	0.13	1.1

1) for a shelf life of 24 months

2) Where the standard combined uncertainty is multiplied by a coverage factor k = 2, or k = 2. 78 for I, 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

Reliable mean values were obtained for the mass fractions of nine elements. For Pb, the low number of accepted results, and the high deviation between them returned a U_{CRM} of over 100 % and no value could be assigned for the mass fraction.

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 [7]; 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 8:

$$u_\Delta = \sqrt{u_{meas}^2 + u_{CRM}^2} \qquad (8)$$

- 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 materials 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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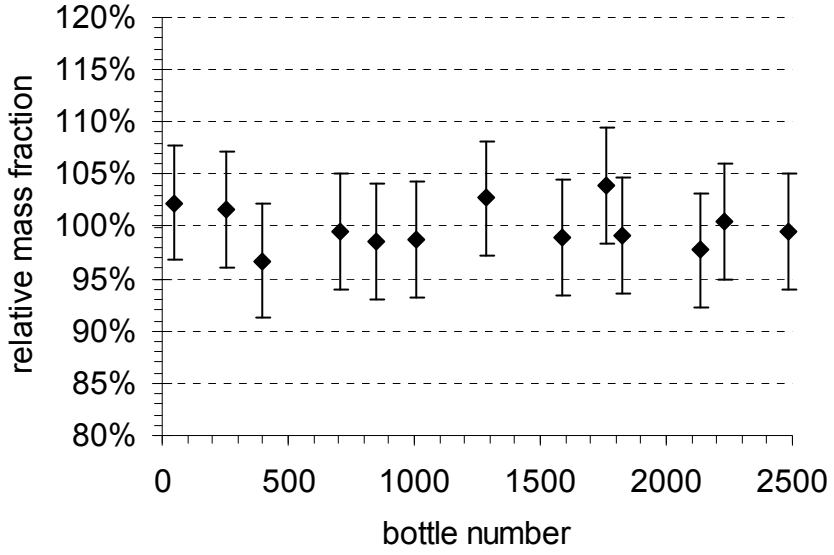
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Annex A ERM®-BB422 (Fish muscle) – Results of the homogeneity study

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.

Figure A1: As



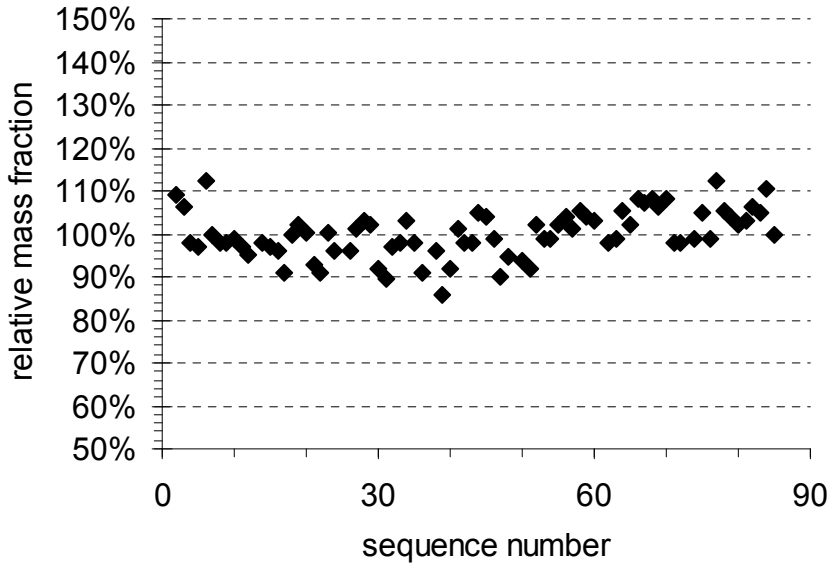
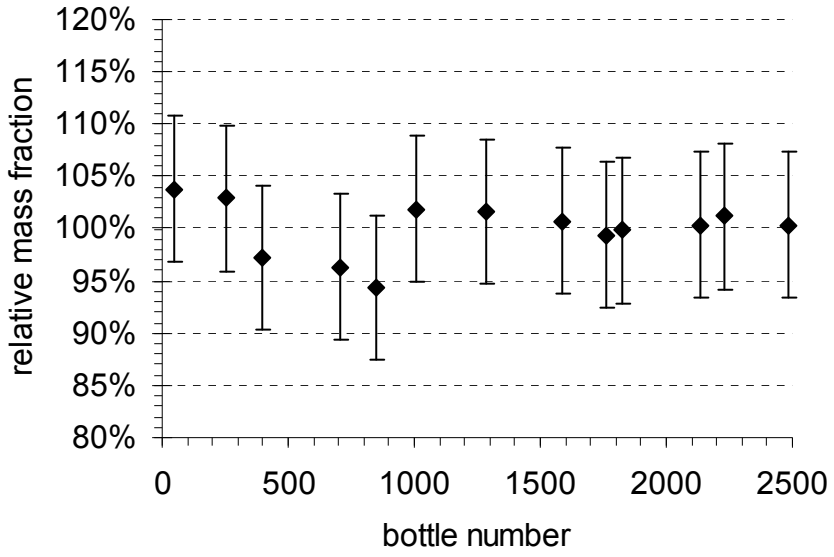


Figure A2: Cd


120%
115%
110%
105%
100%
95%
90%
85%
80%
relative mass fraction
0
500
1000
1500
2000
2500
bottle number

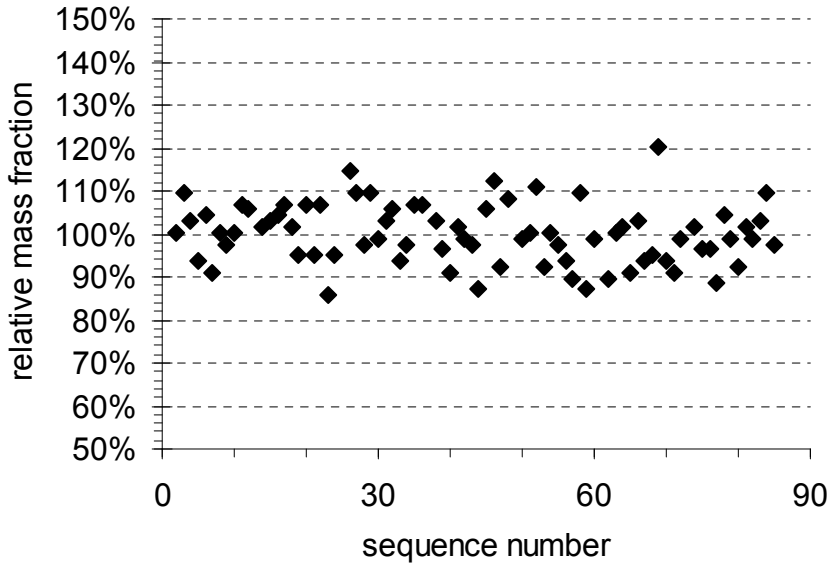
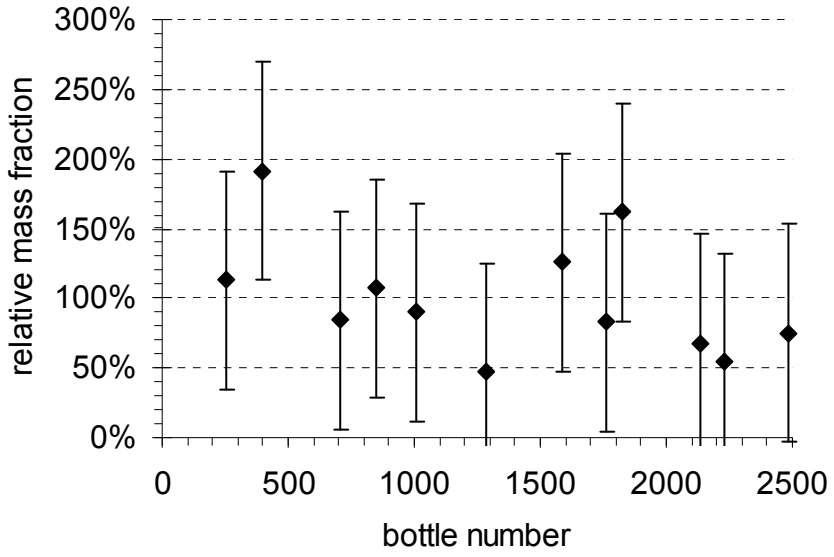

150%
140%
130%
120%
110%
100%
90%
80%
70%
60%
50%
relative mass fraction
0
30
60
90
sequence number

Figure A3: Cr



(one point not shown: Bottle 0047/936 %)

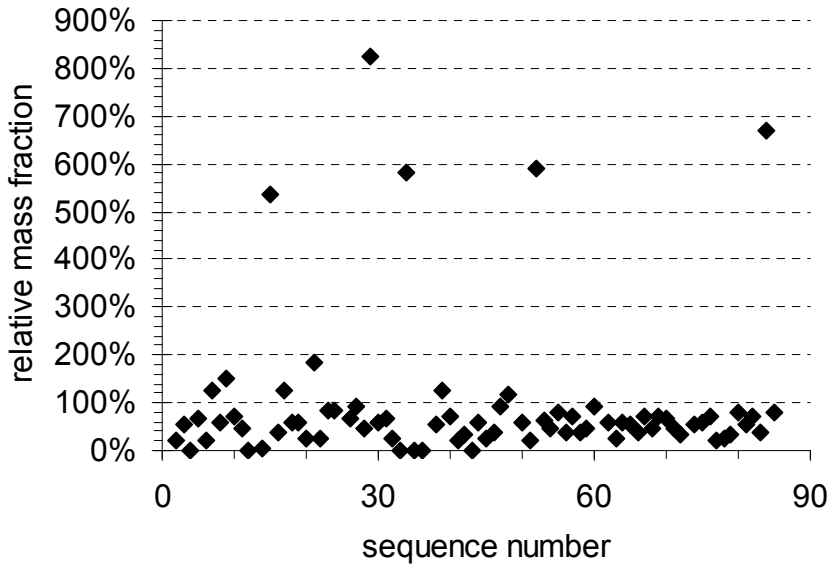
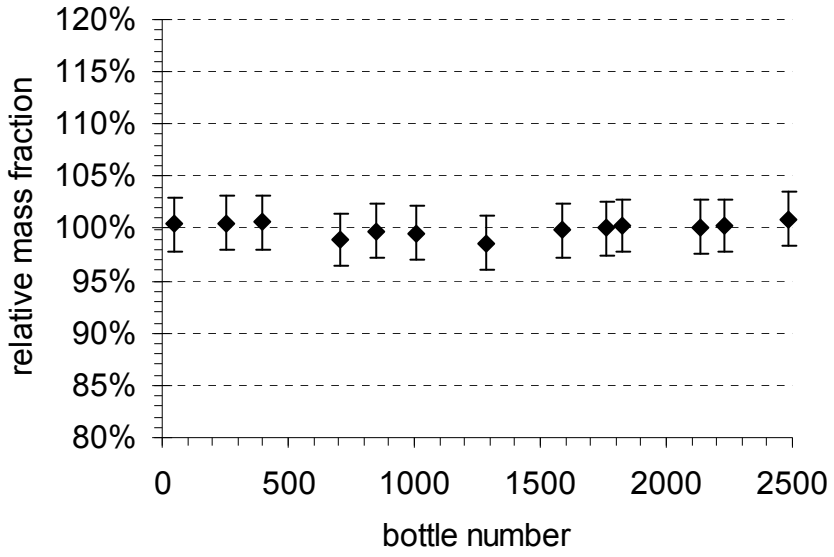


Figure A4: Cu


120%
115%
110%
105%
100%
95%
90%
85%
80%
relative mass fraction
0
500
1000
1500
2000
2500
bottle number

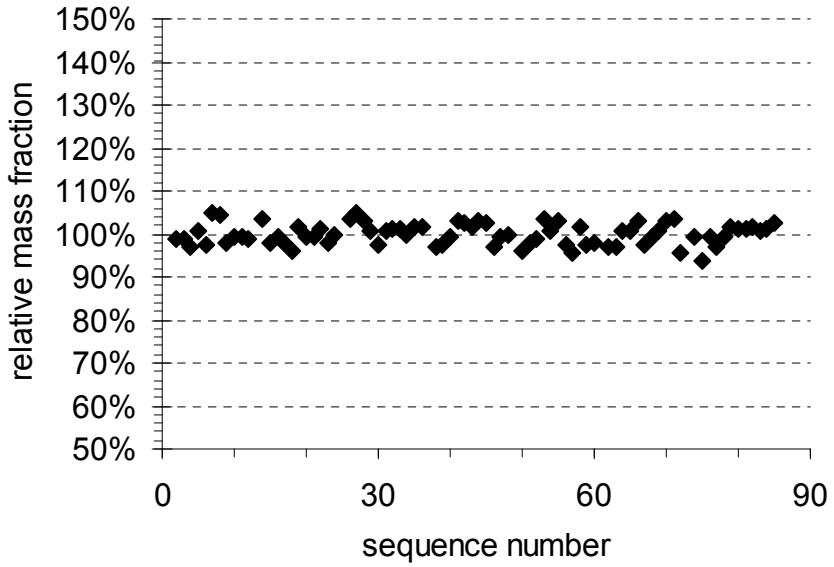

150%
140%
130%
120%
110%
100%
90%
80%
70%
60%
50%
relative mass fraction
0
30
60
90
sequence number

Figure A5: Fe

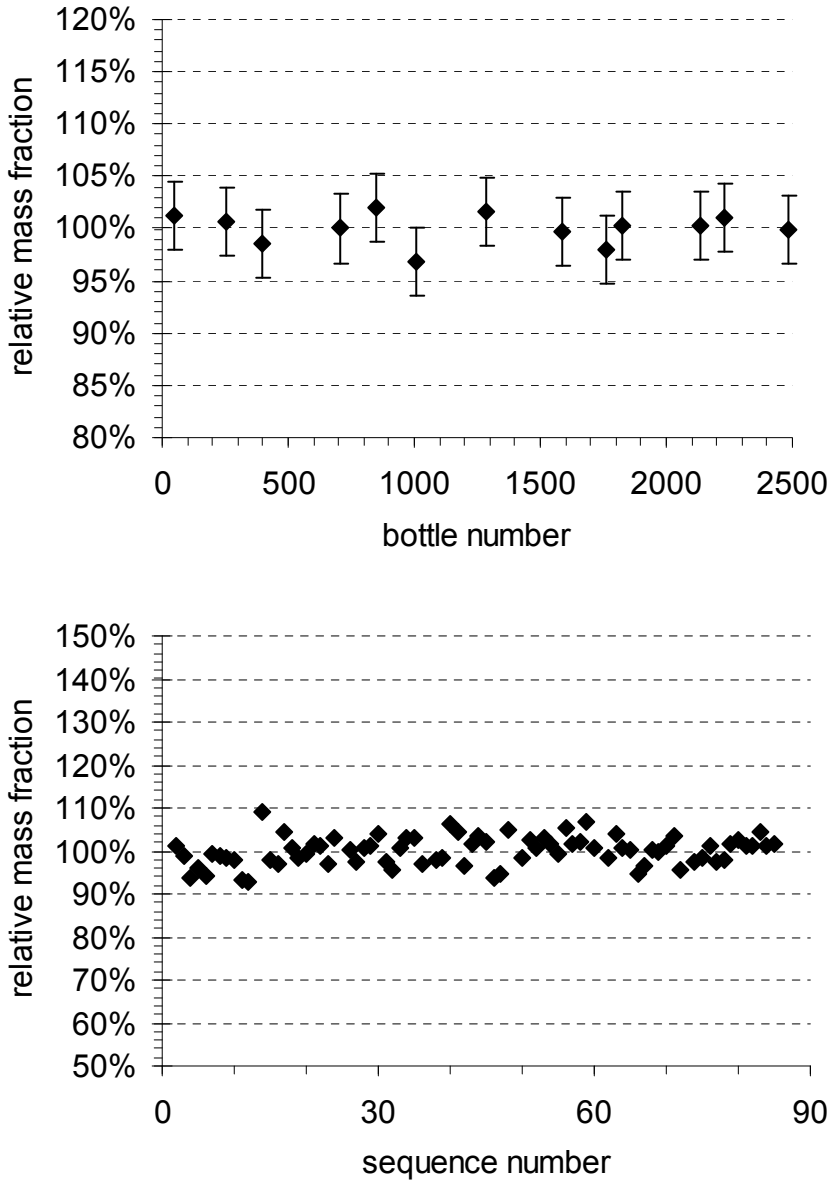

120%
115%
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105%
100%
95%
90%
85%
80%
relative mass fraction
0
500
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1500
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bottle number
150%
140%
130%
120%
110%
100%
90%
80%
70%
60%
50%
relative mass fraction
0
30
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90
sequence number

Figure A6: Hg

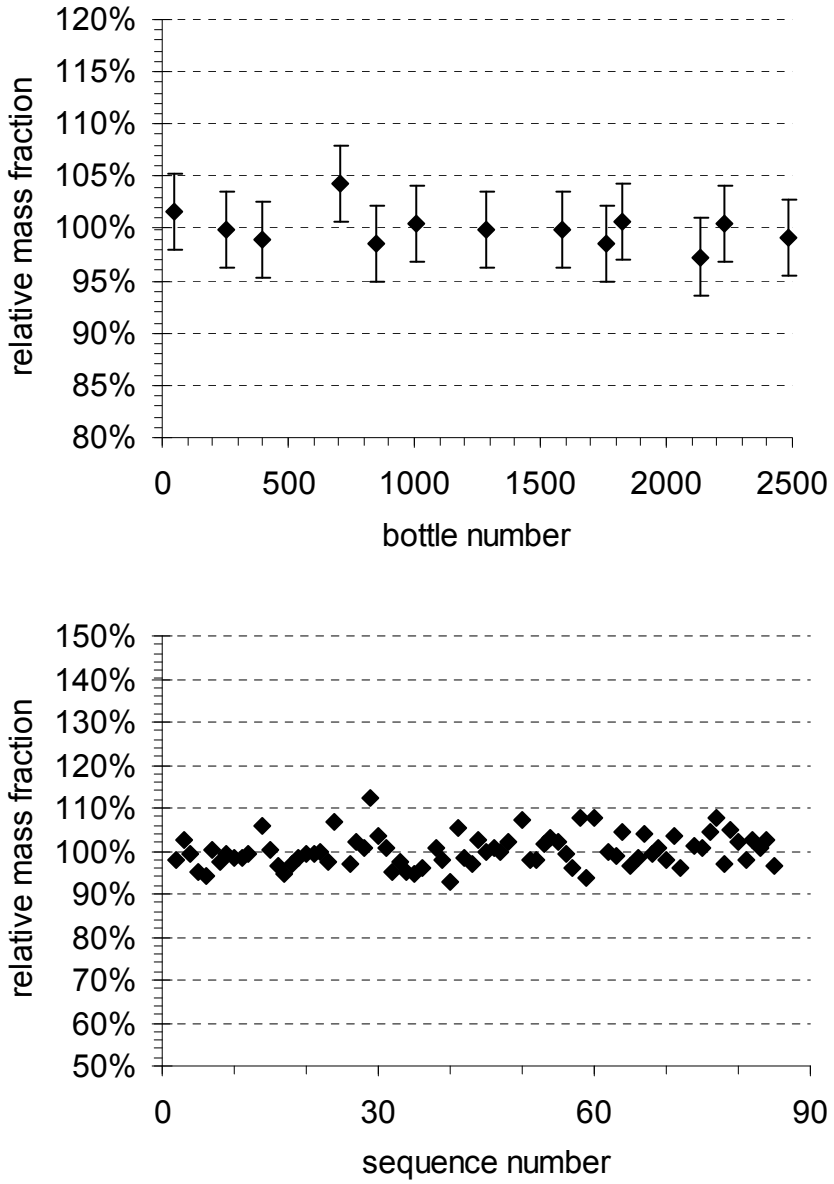
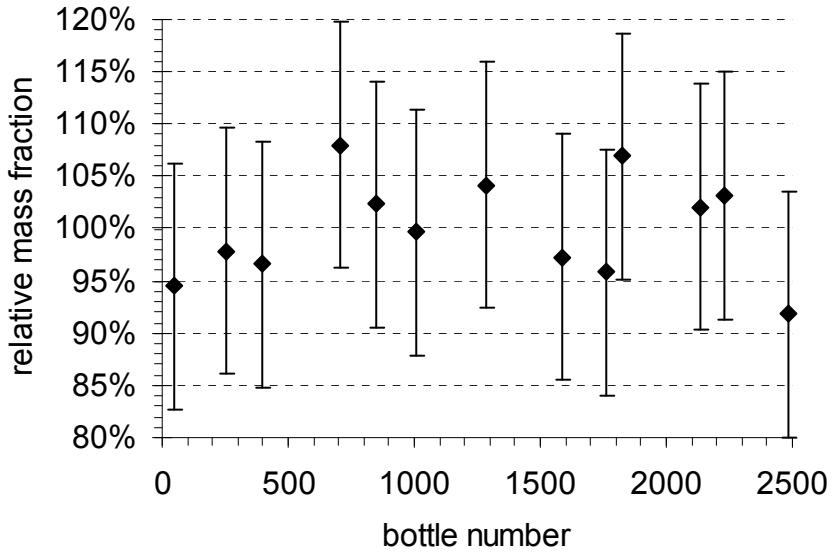

120%
115%
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105%
100%
95%
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85%
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relative mass fraction
0
500
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2000
2500
bottle number
150%
140%
130%
120%
110%
100%
90%
80%
70%
60%
50%
relative mass fraction
0
30
60
90
sequence number

Figure A7: I


120%
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105%
100%
95%
90%
85%
80%
relative mass fraction
0
500
1000
1500
2000
2500
bottle number

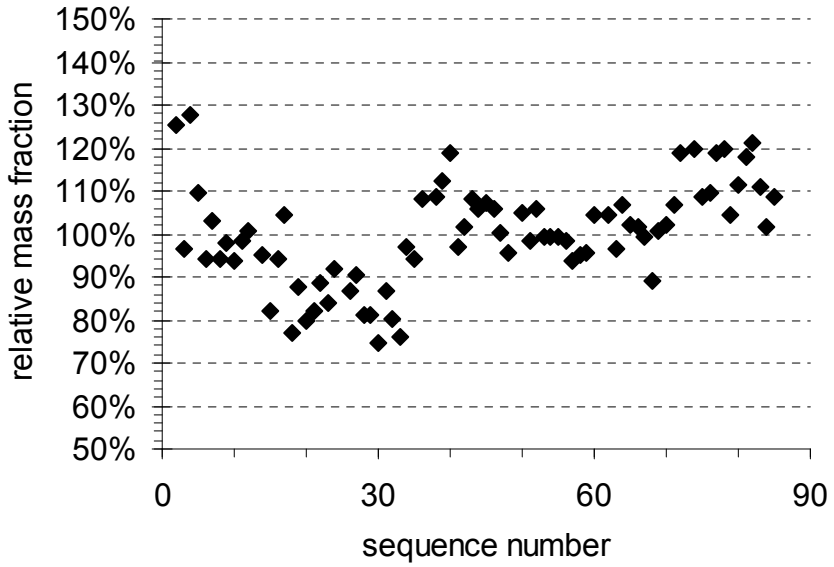
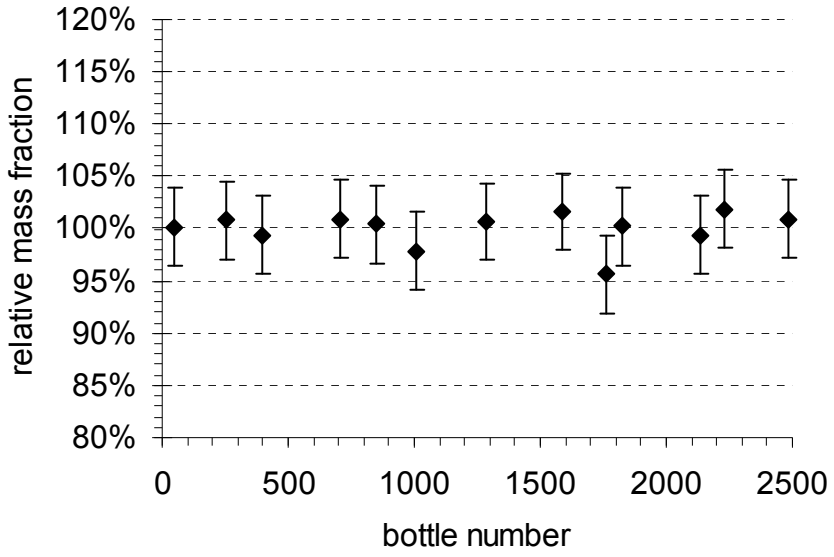

150%
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90%
80%
70%
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50%
relative mass fraction
0
30
60
90
sequence number

Figure A8: Mn


120%
115%
110%
105%
100%
95%
90%
85%
80%
relative mass fraction
0
500
1000
1500
2000
2500
bottle number

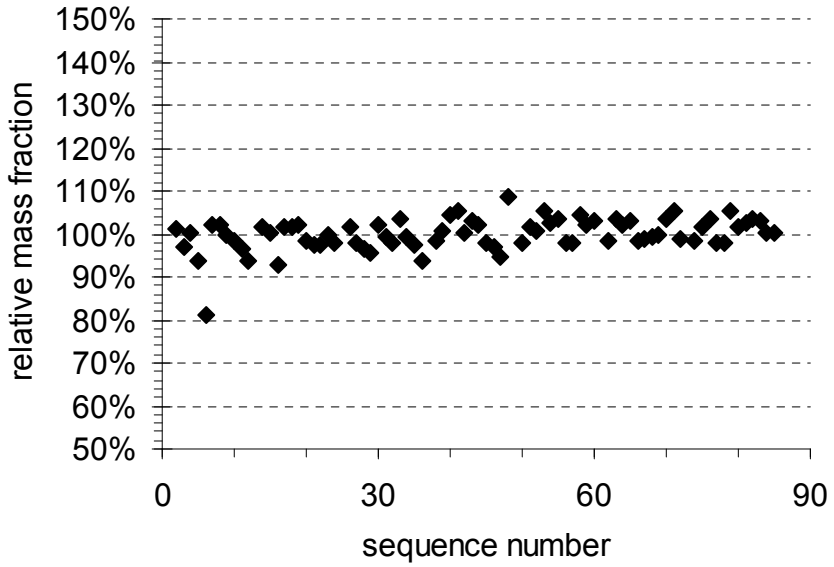
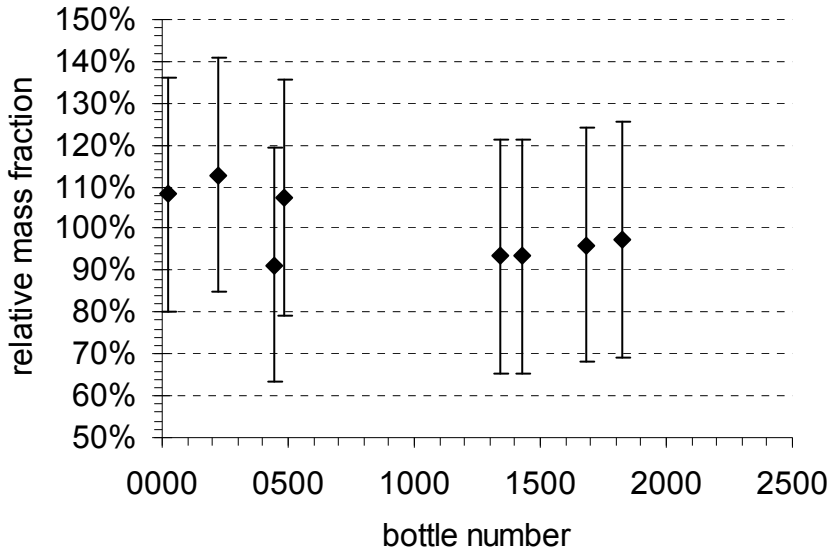
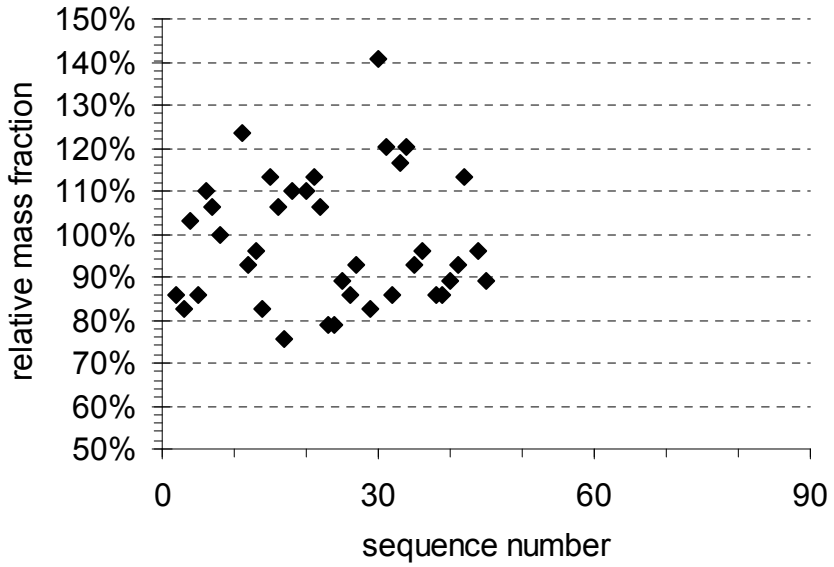

150%
140%
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100%
90%
80%
70%
60%
50%
relative mass fraction
0
30
60
90
sequence number

Figure A9: Pb





(values from long-term stability study)

Figure A10: Se

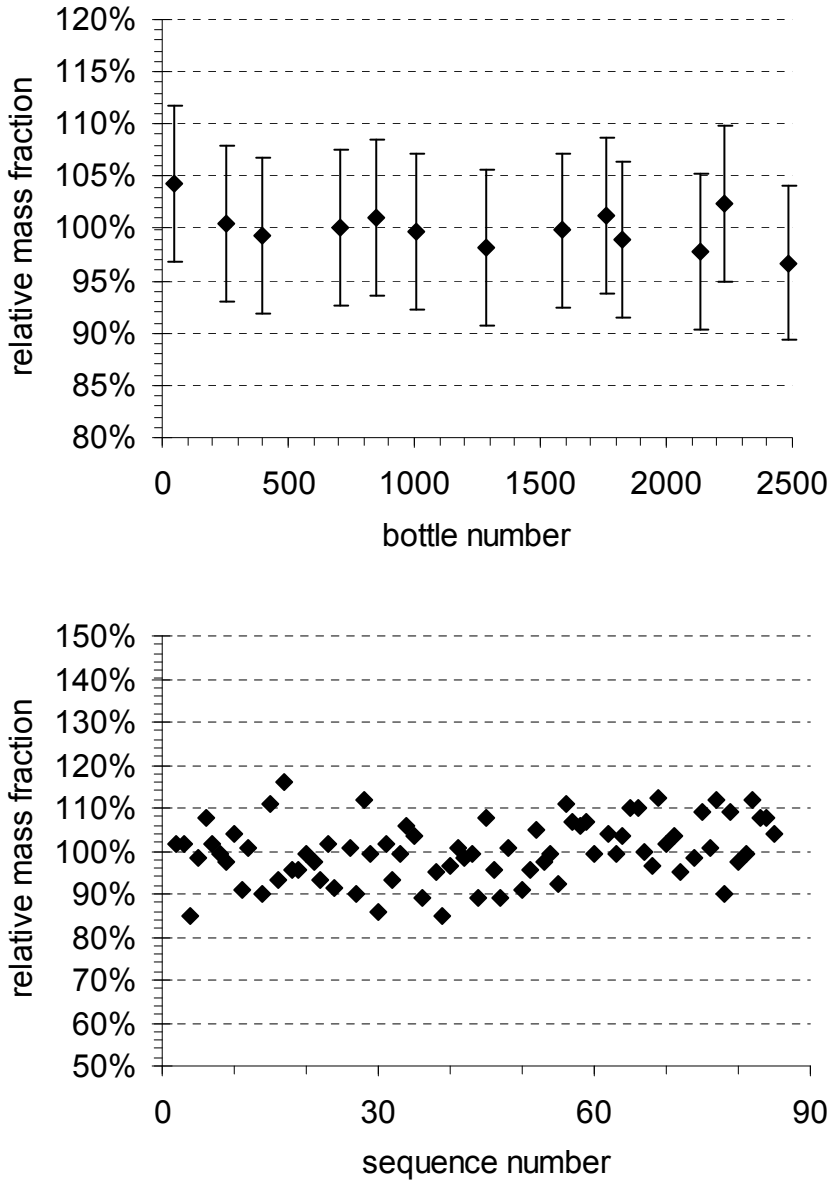
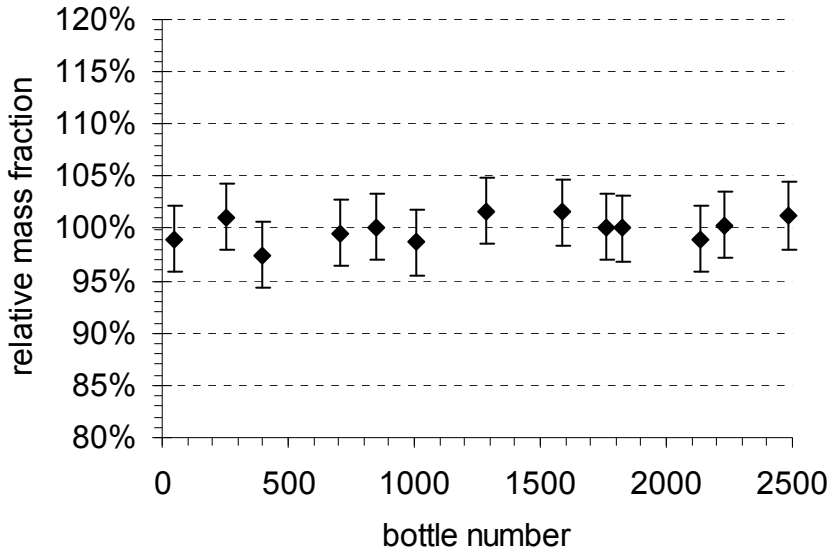
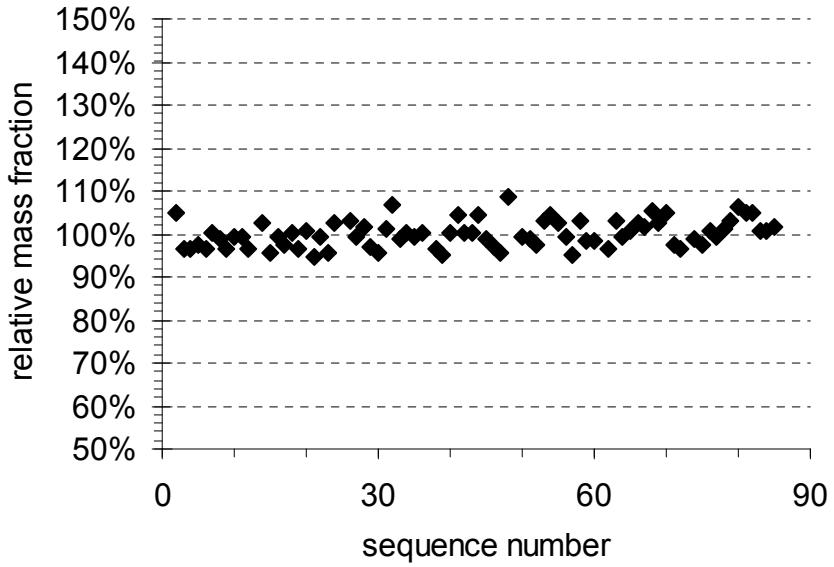


Figure A11: Zn





Annex B ERM®-BB422 (Fish muscle) – Results of the short-term stability study

Figure B1: Short-term stability of As mass fraction at 18 and 60 °C with associated u_{sts}

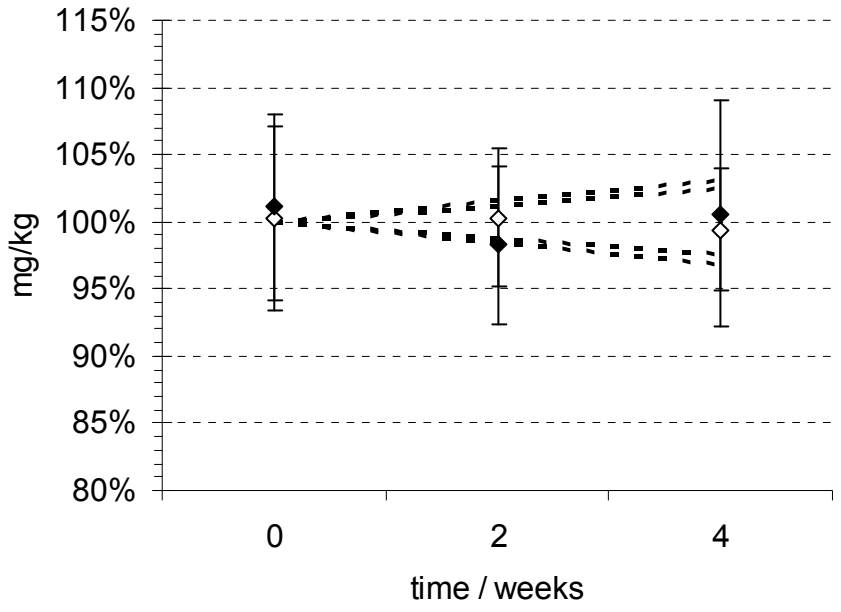


Figure B2: Short-term stability of Cd mass fraction at 18 and 60 °C with associated u_{sts}

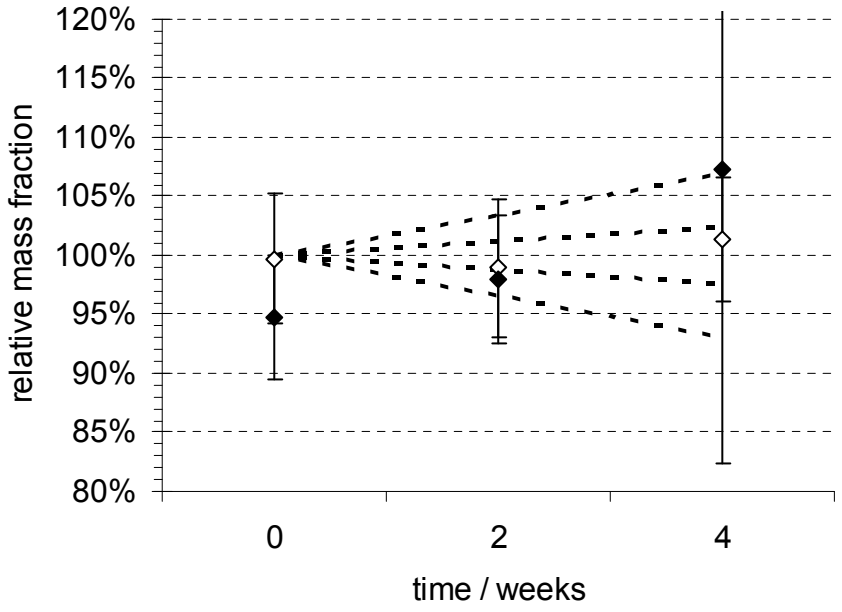


Figure B3: Short-term stability of Cu mass fraction at 18 and 60 °C with associated u_{sts}

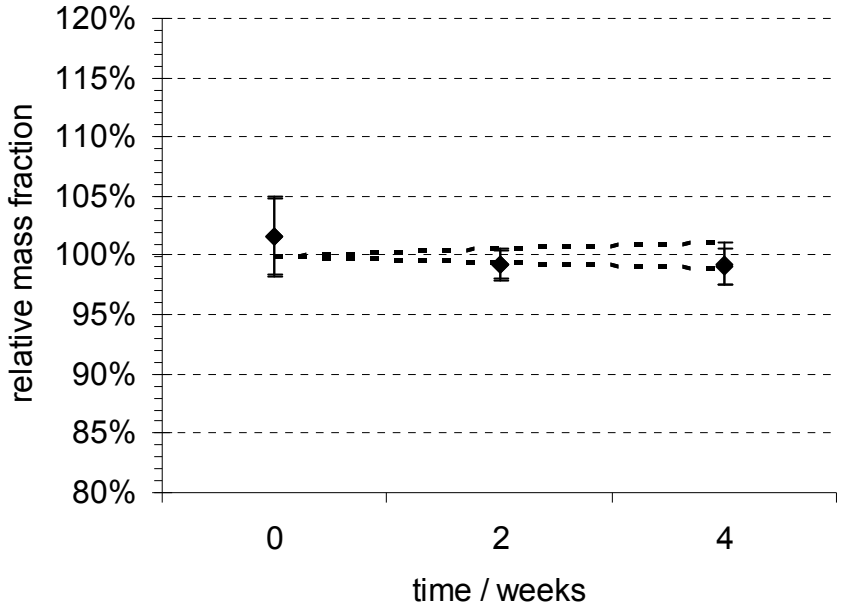


Figure B4: Short-term stability of Fe mass fraction at 18 and 60 °C with associated u_{sts}

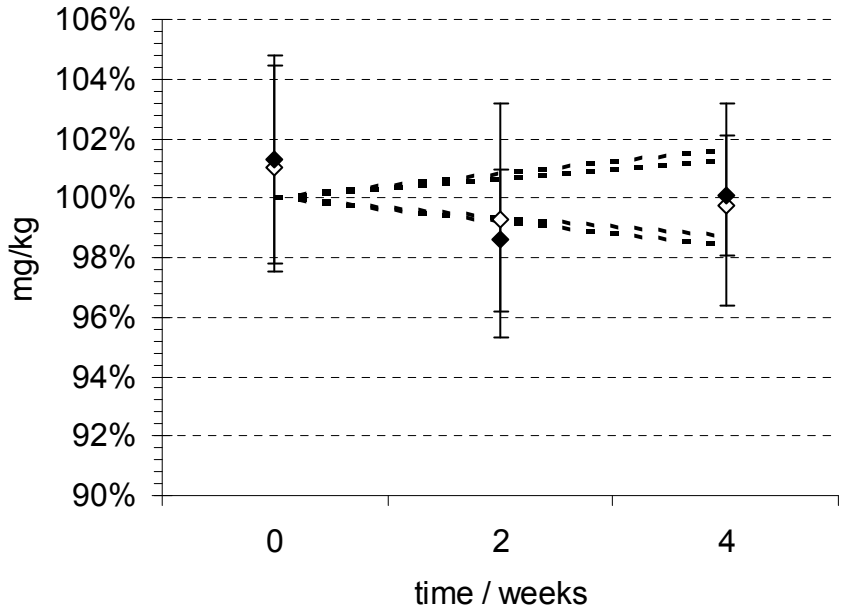


Figure B5: Short-term stability of Hg mass fraction at 18 and 60 °C with associated u_{sts}

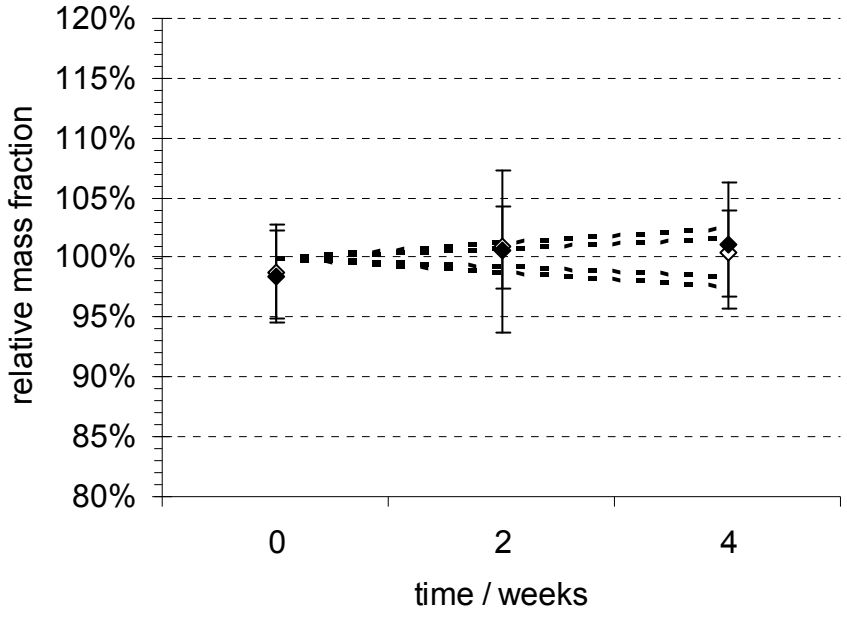


Figure B6: Short-term stability of I mass fraction at 18 and 60 °C with associated u_{sts}

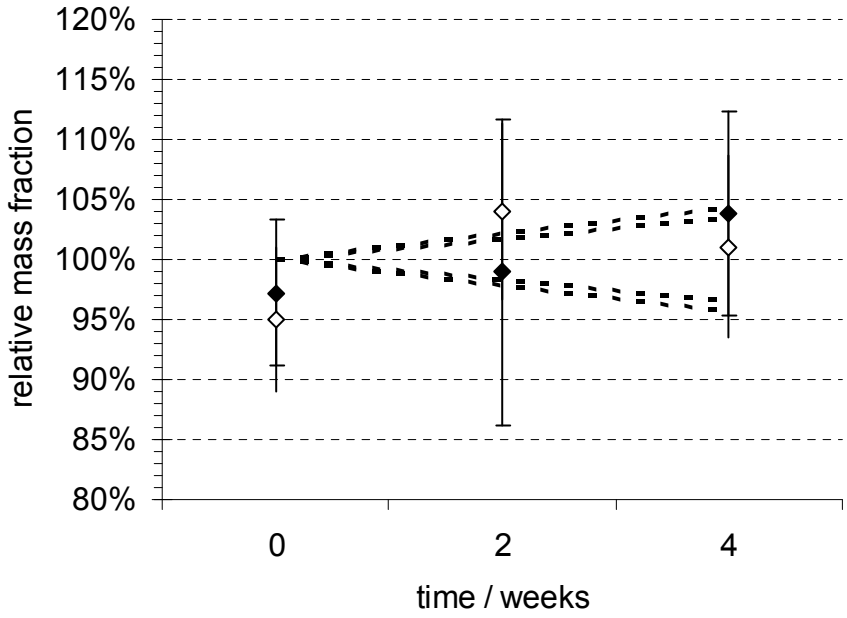


Figure B7: Short-term stability of Mn mass fraction at 18 and 60 °C with associated u_{sts}

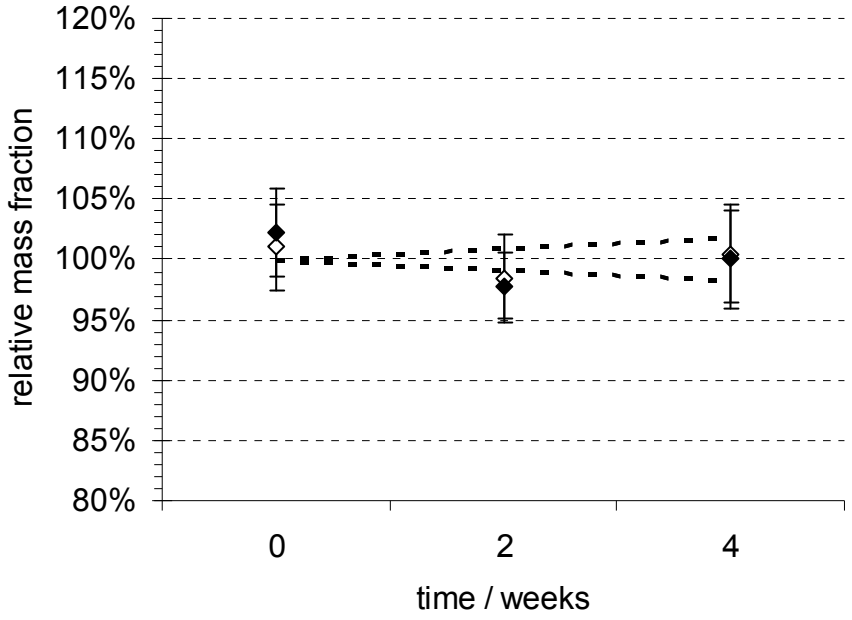


Figure B8: Short-term stability of Se mass fraction at 18 and 60 °C with associated u_{sts}

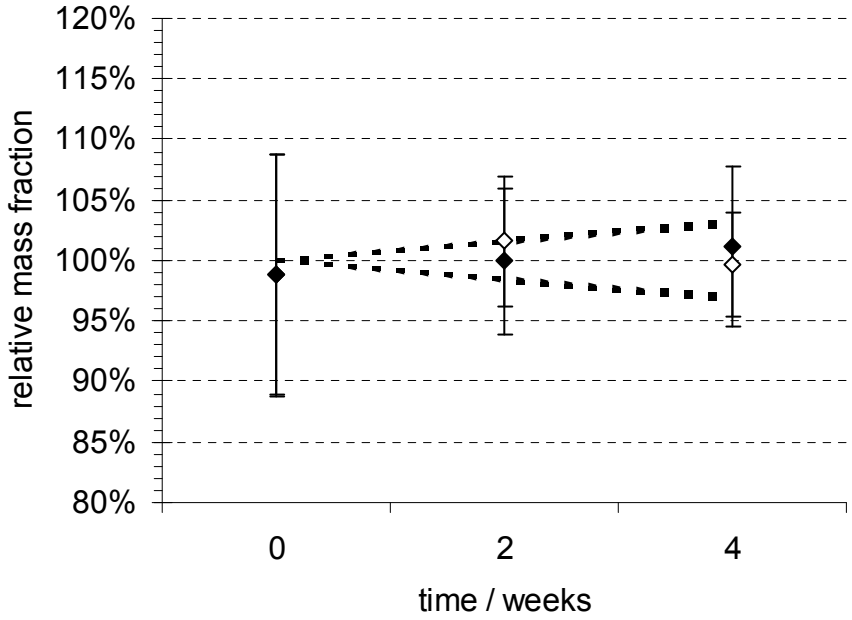
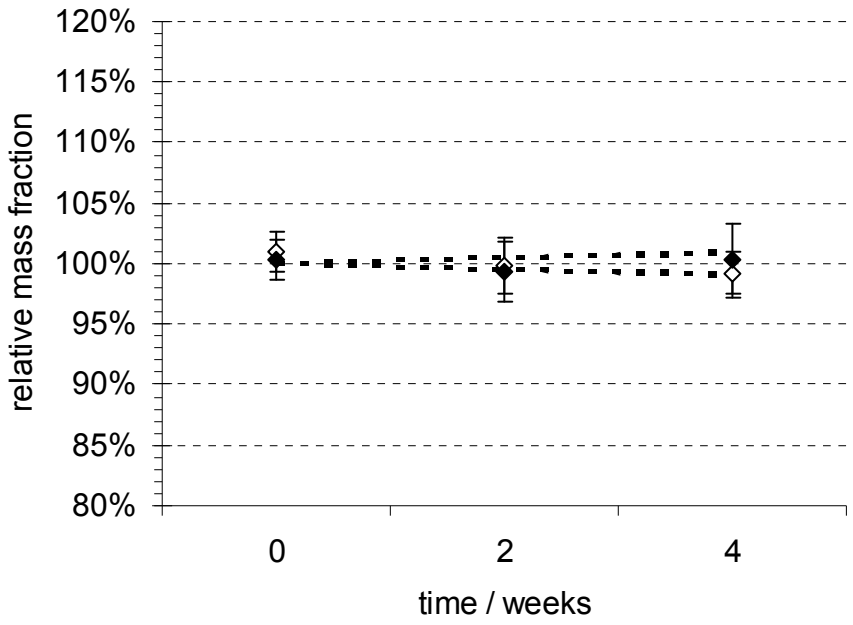


Figure B9: Short-term stability of Zn mass fraction at 18 and 60 °C with associated u_{sts}



Annex C ERM®-BB422 (Fish 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}

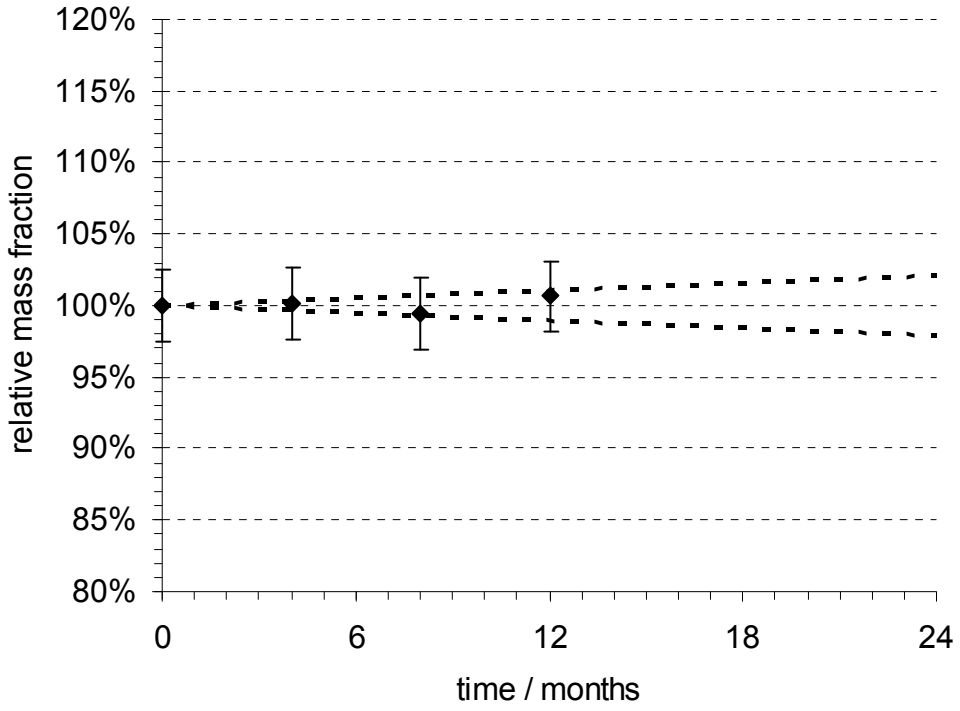


Figure C2: Long-term stability of Cd mass fraction at 18 °C with associated u_{lts}

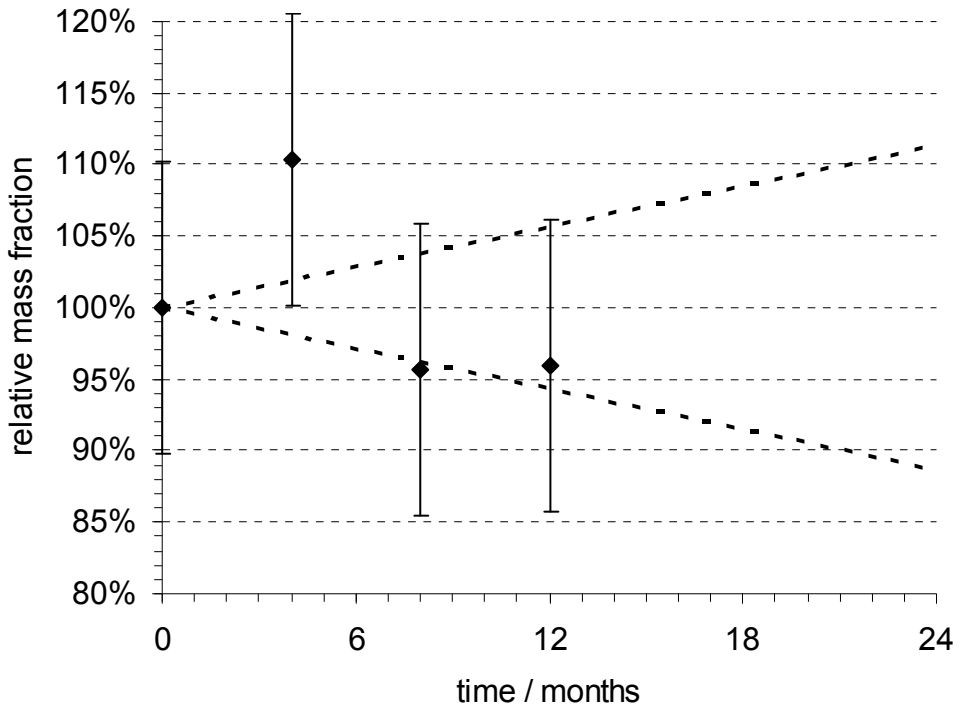


Figure C3: Long-term stability of Cu mass fraction at 18 °C with associated u_{lts}

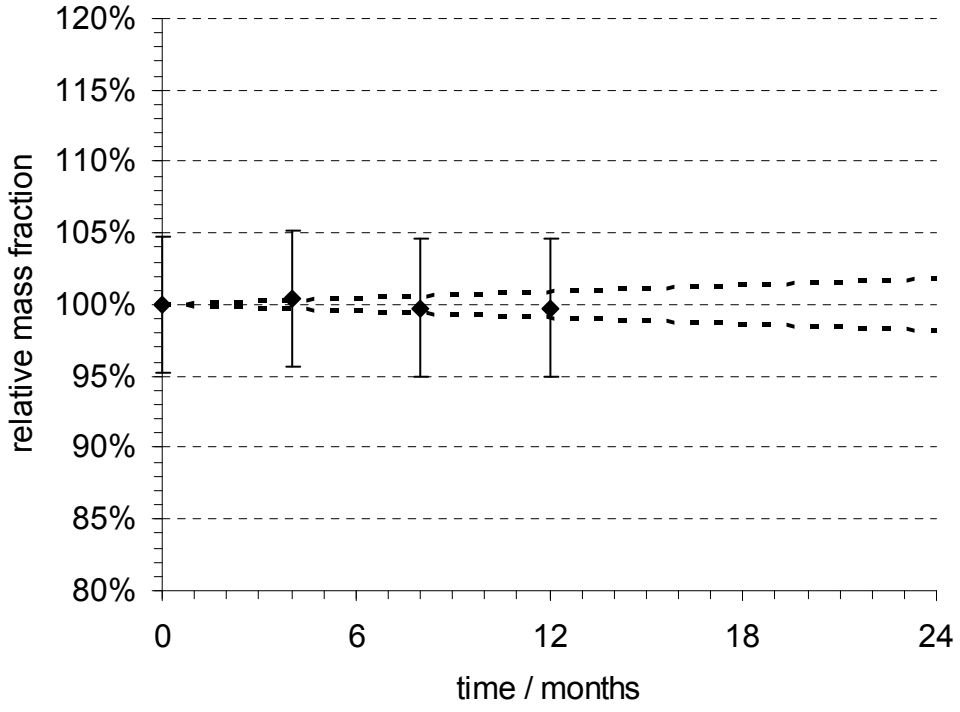


Figure C4: Long-term stability of Fe mass fraction at 18 °C with associated u_{lts}

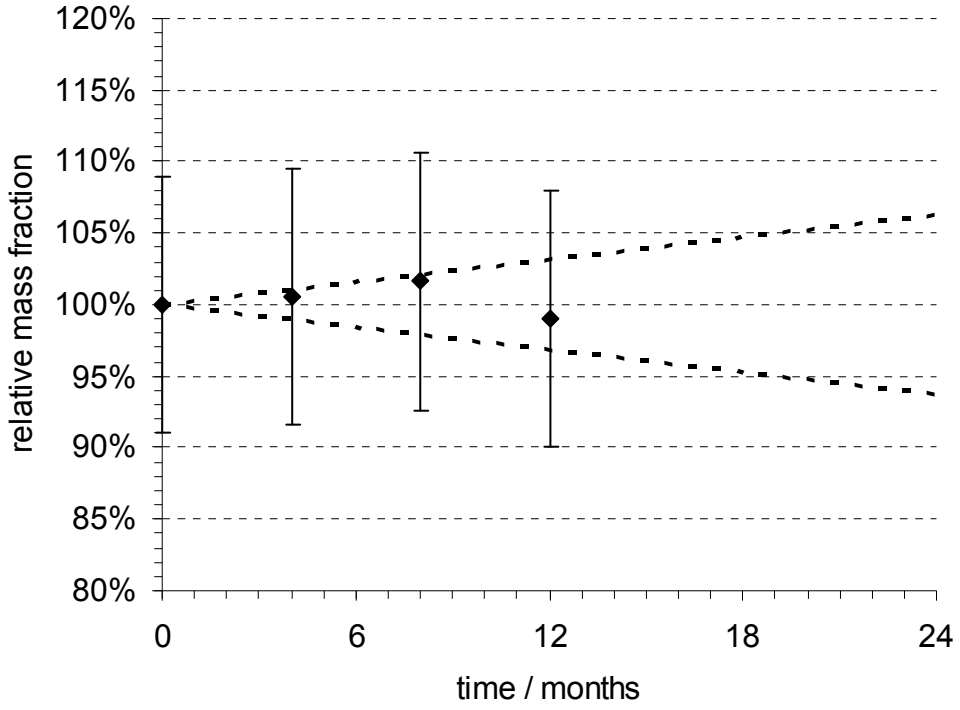


Figure C5: Long-term stability of Hg mass fraction at 18 °C with associated u_{lts}

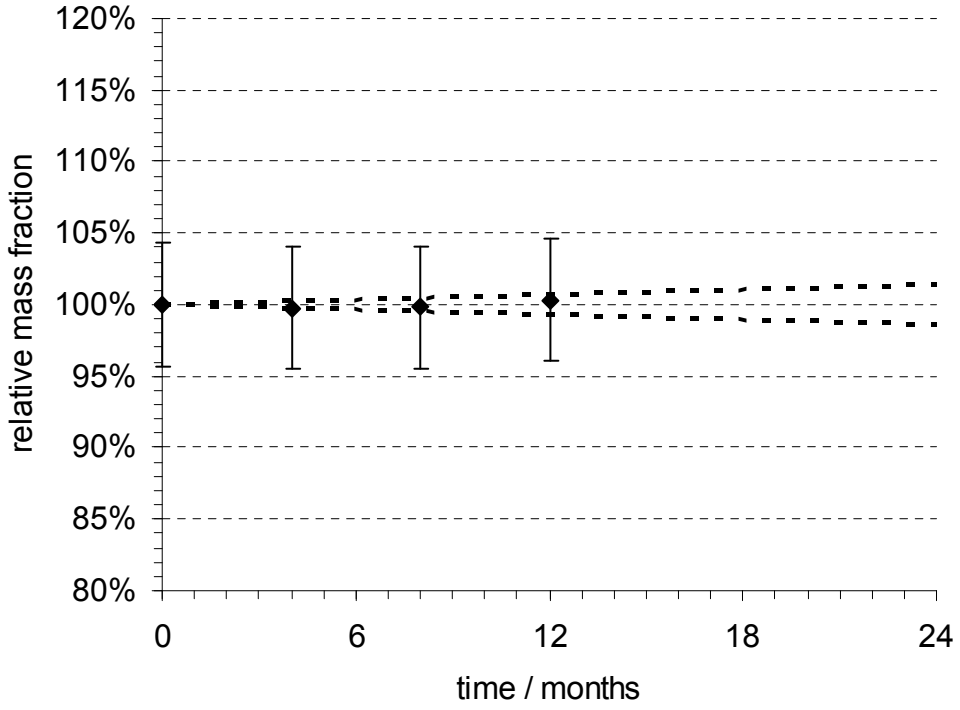


Figure C6: Long-term stability of I mass fraction at 18 °C with associated u_{lts}

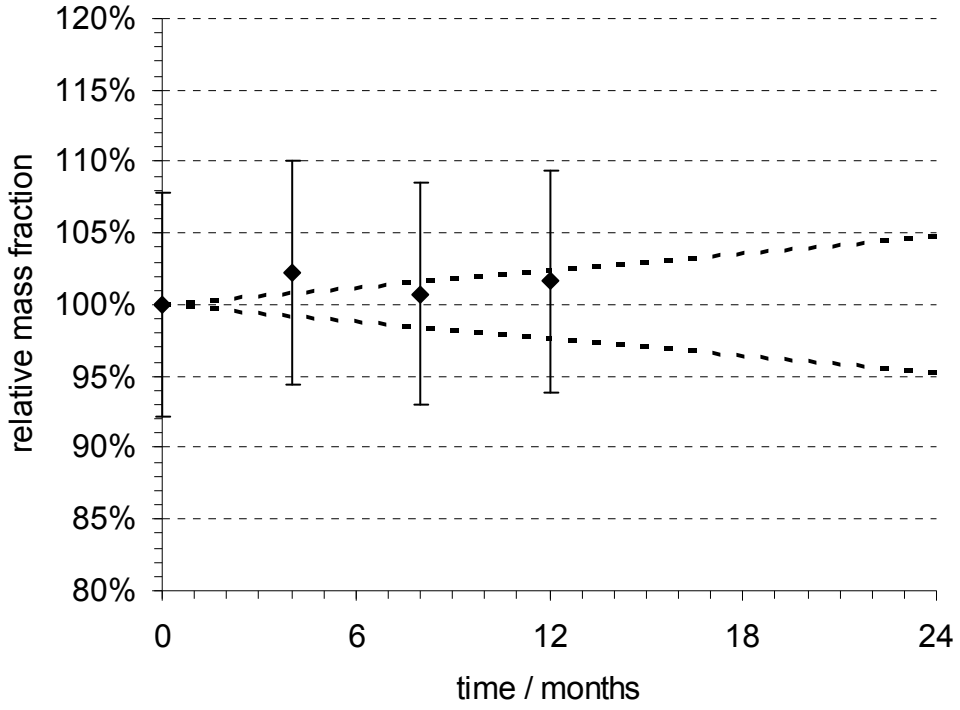


Figure C7: Long-term stability of Mn mass fraction at 18 °C with associated u_{lts}

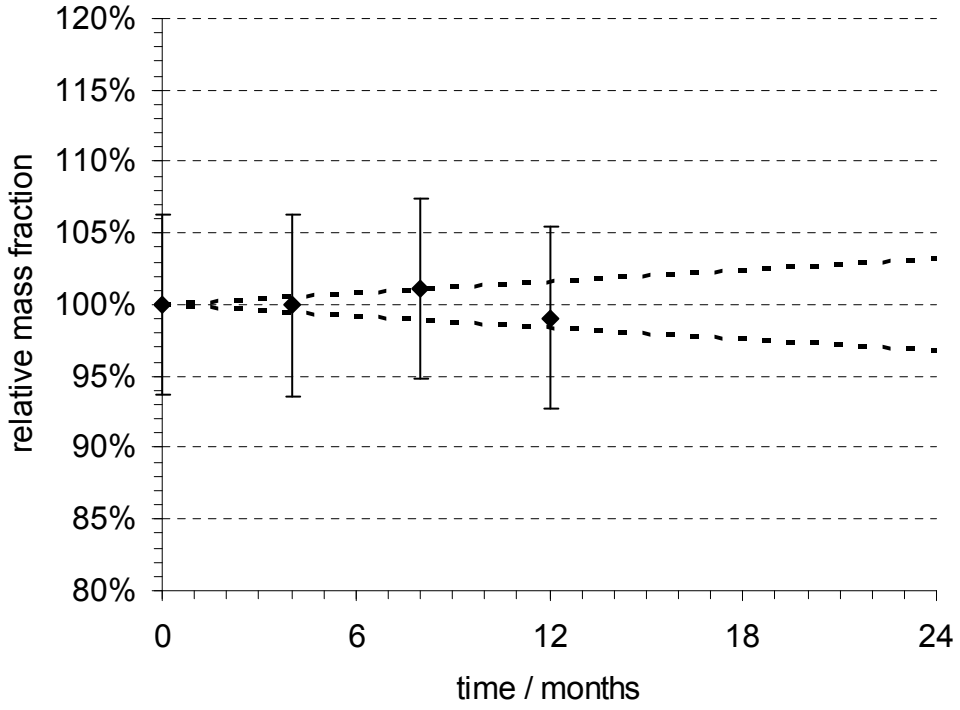


Figure C8: Long-term stability of Pb mass fraction at 18 °C with associated u_{lts}

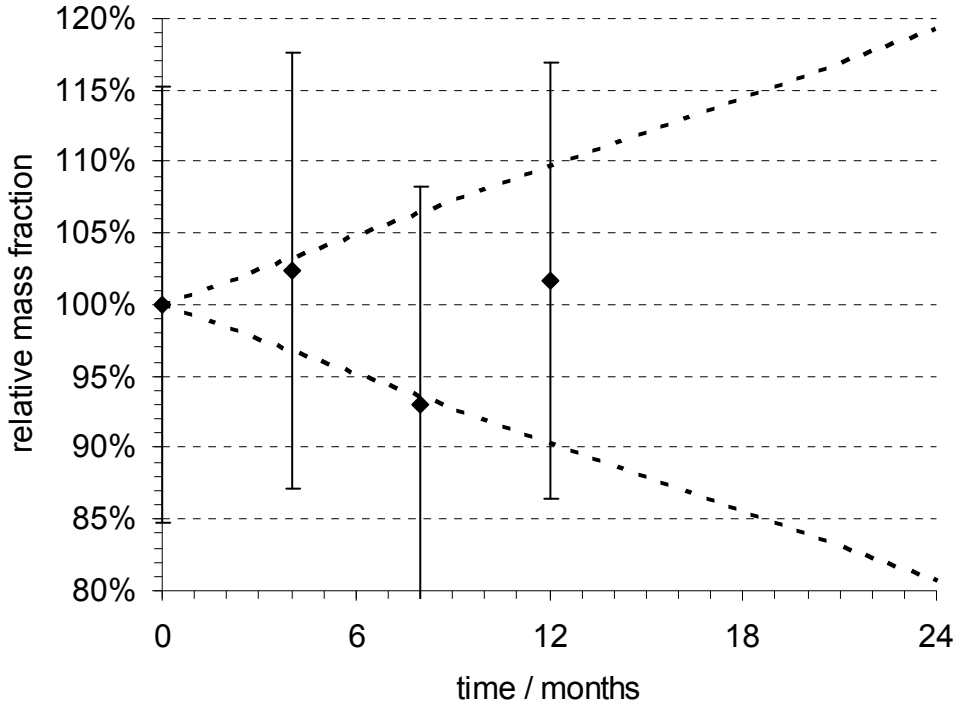


Figure C9: Long-term stability of Se mass fraction at 18 °C with associated u_{lts}

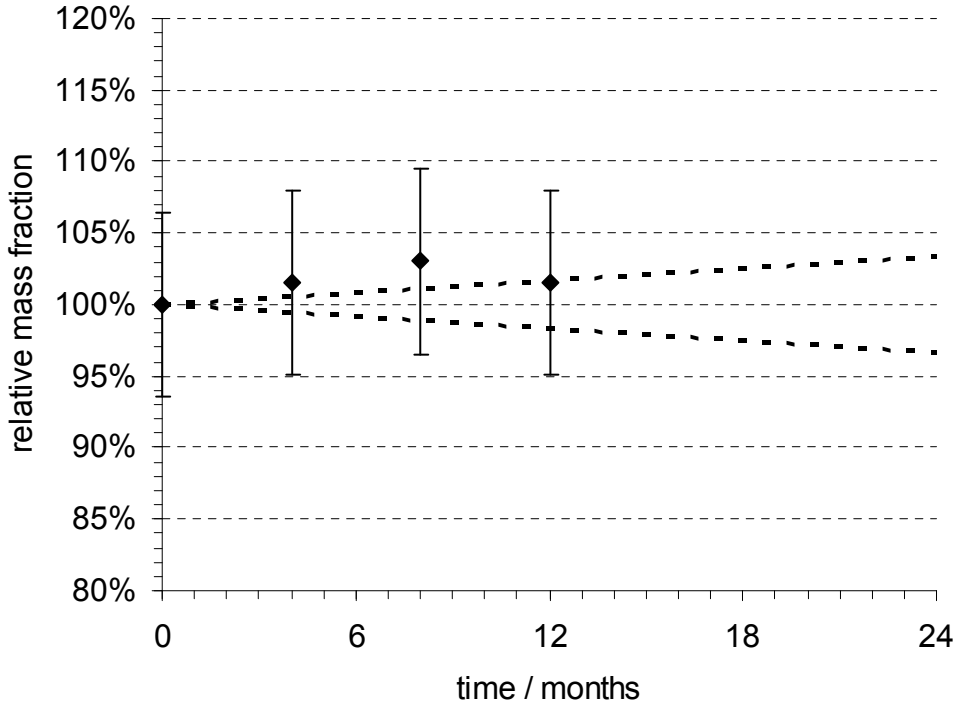
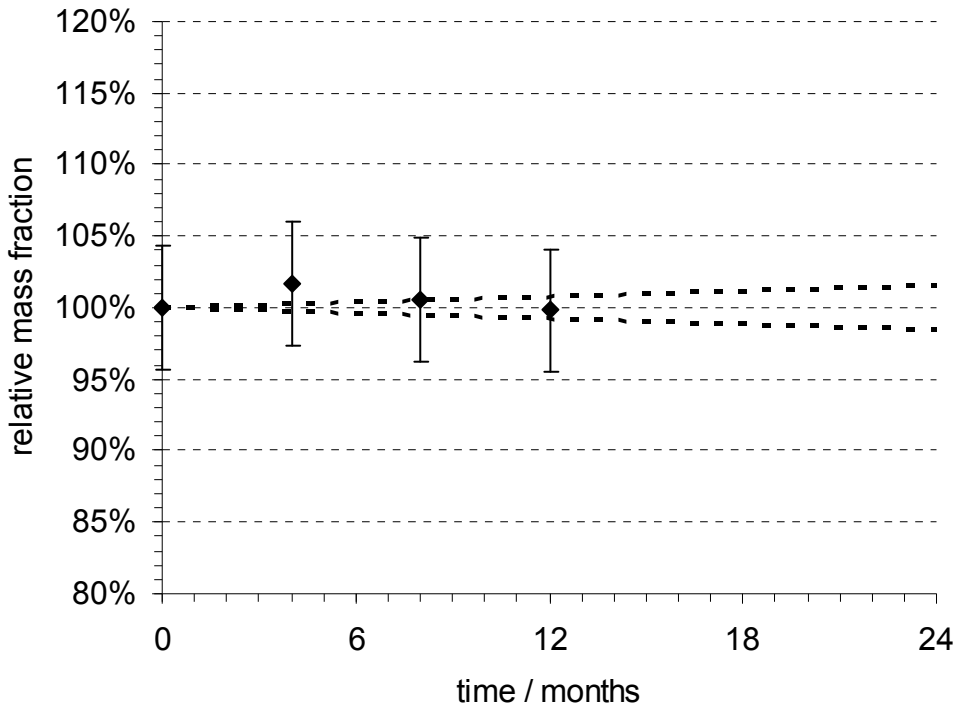


Figure C10: Long-term stability of Zn mass fraction at 18 °C with associated u_{lts}

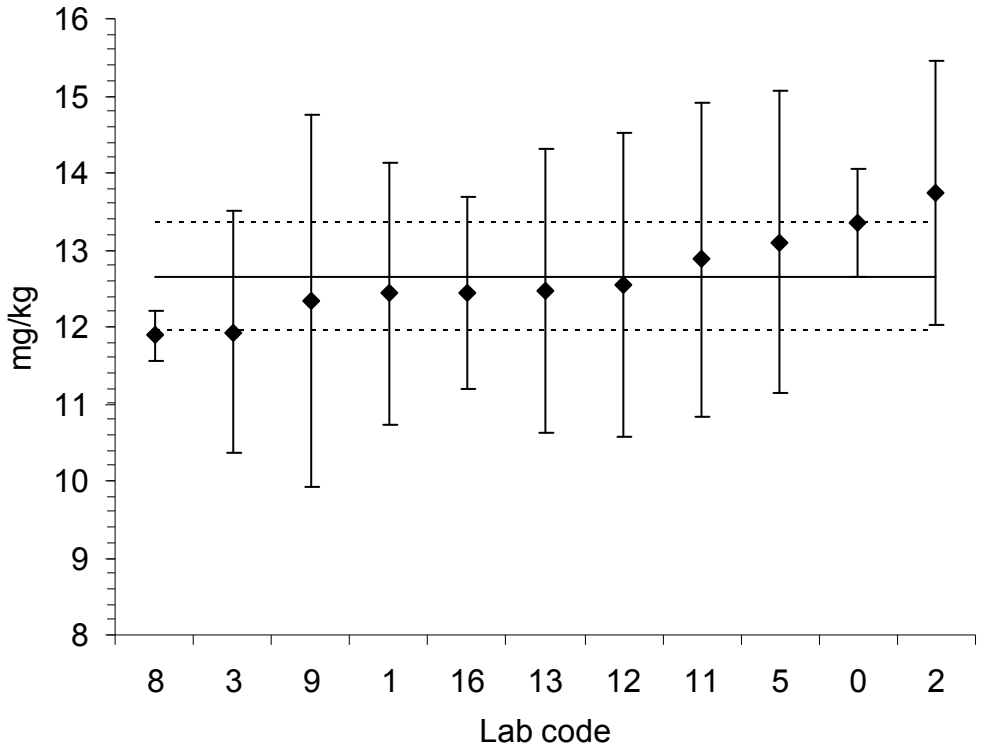


Annex D ERM®-BB422 (Fish muscle) – Characterisation data

Results of characterisation measurements (corrected to dry mass) as reported by participants.

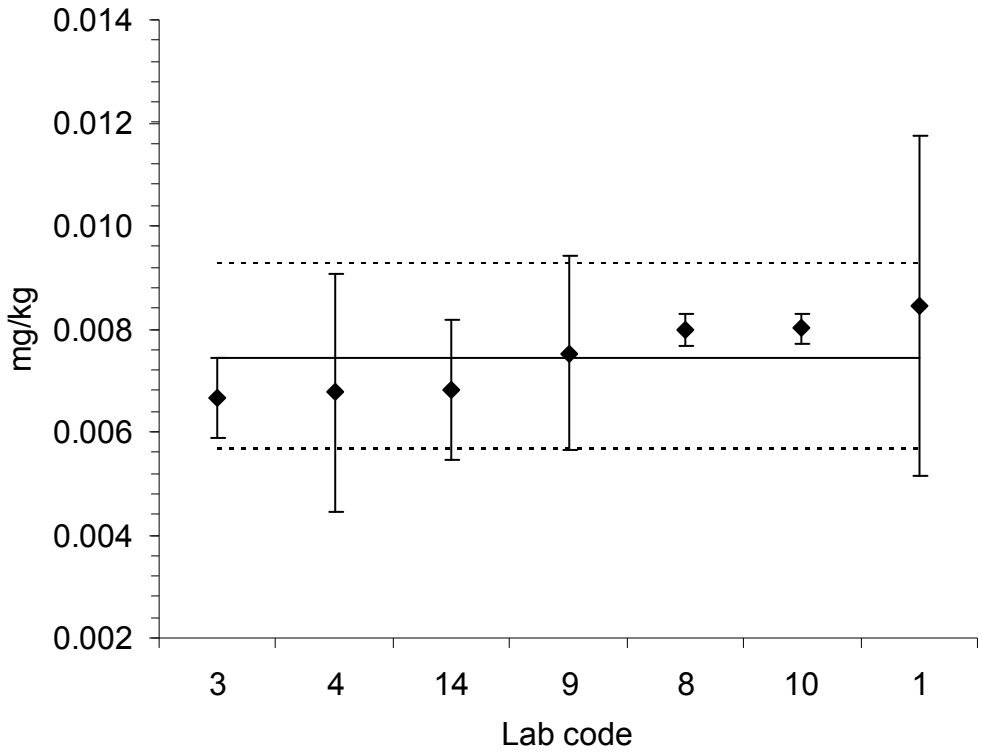
As mass fractions, mg/kg

Lab Code	#1	#2	#3	#4	#5	#6	Mean	U
0	13	13.3	13.9	13.4	13.4	13.1	13.35	0.70
1	12.6	13.1	12.3	12.4	12.2	12	12.43	1.70
2	14.23	13.84	14.13	13.5	13.52	13.2	13.74	1.71
3	12.1	11.5	11.6	12.8	11.7	11.9	11.93	1.56
4	~~10.35913~~	~~10.79413~~	~~10.59181~~	~~13.42875~~	~~13.6216~~	~~13.95656~~	~~12.13~~	~~0.61~~
5	13	13.2	13	13	13.1	13.3	13.10	1.97
6	~~15.3~~	~~15.2~~	~~15.2~~	~~13.7~~	~~13.5~~	~~13.4~~	~~14.38~~	~~2.88~~
7	-	-	-	-	-	-	-	-
8	11.7	11.8	11.8	12.1	12	11.9	11.88	0.33
9	11.94166	12.71344	12.56452	12.46696	12.42345	11.96893	12.35	2.42
10	-	-	-	-	-	-	-	-
11	12.886	12.761	12.764	13.62	12.853	12.363	12.87	2.04
12	12.05	12.448	13.092	12.688	12.762	12.25	12.55	1.96
13	12.792	13.182	11.782	12.972	11.953	12.065	12.46	1.85
14	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-
16	11.9	12.9	12.4	12.9	12.3	12.2	12.43	1.24
17	-	-	-	-	-	-	-	-



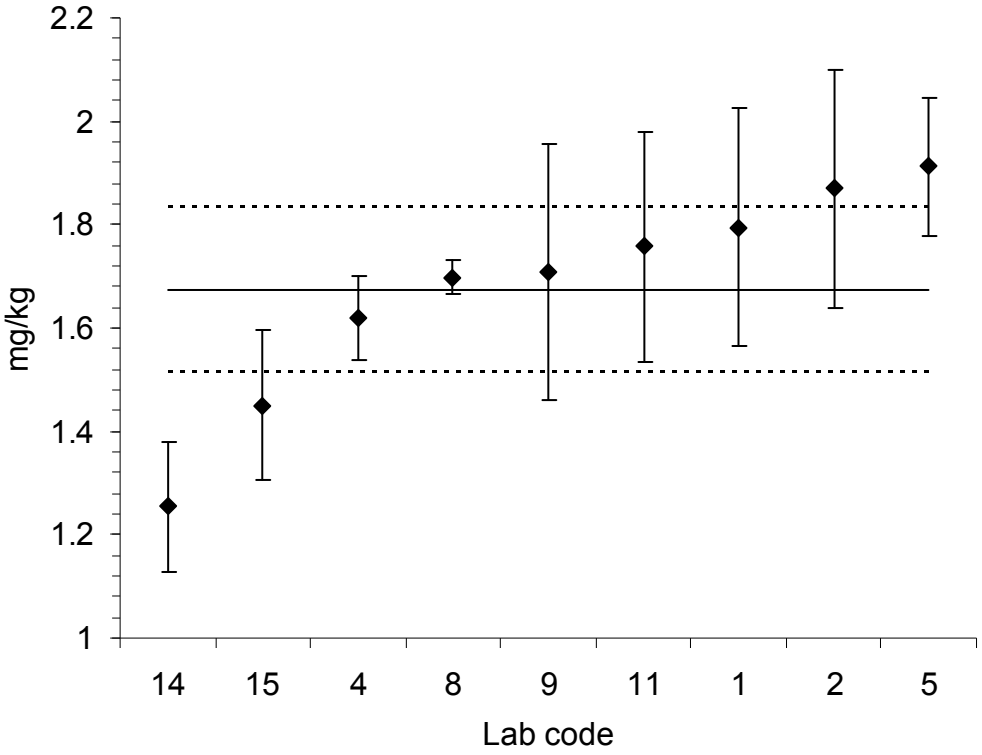
Cd mass fractions, mg/kg

Lab Code	#1	#2	#3	#4	#5	#6	Mean	U
0	-	-	-	-	-	-	-	-
1	0.0092	0.0086	0.0075	0.0085	0.0091	0.0078	0.0085	0.0033
2	-	-	-	-	-	-	-	-
3	0.0057	0.0072	0.005	0.0072	0.0075	0.0073	0.0067	0.0008
4	0.006813	0.006793	0.006325	0.006834	0.006751	0.007046	0.0068	0.0023
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	0.0003
9	0.009427	0.008576	0.005168	0.006447	0.007386	0.008096	0.0075	0.0019
10	0.0082	0.0082	0.0082	0.008	0.0078	0.0076	0.0080	0.0003
11	~~0.00831~~	~~0.0101~~	~~0.0103~~	~~0.0118~~	~~0.0121~~	~~0.013~~	~~0.0109~~	~~0.0026~~
12	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-
14	0.0075	0.0079	0.0059	0.0063	0.0072	0.0061	0.0068	0.0014
15	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-



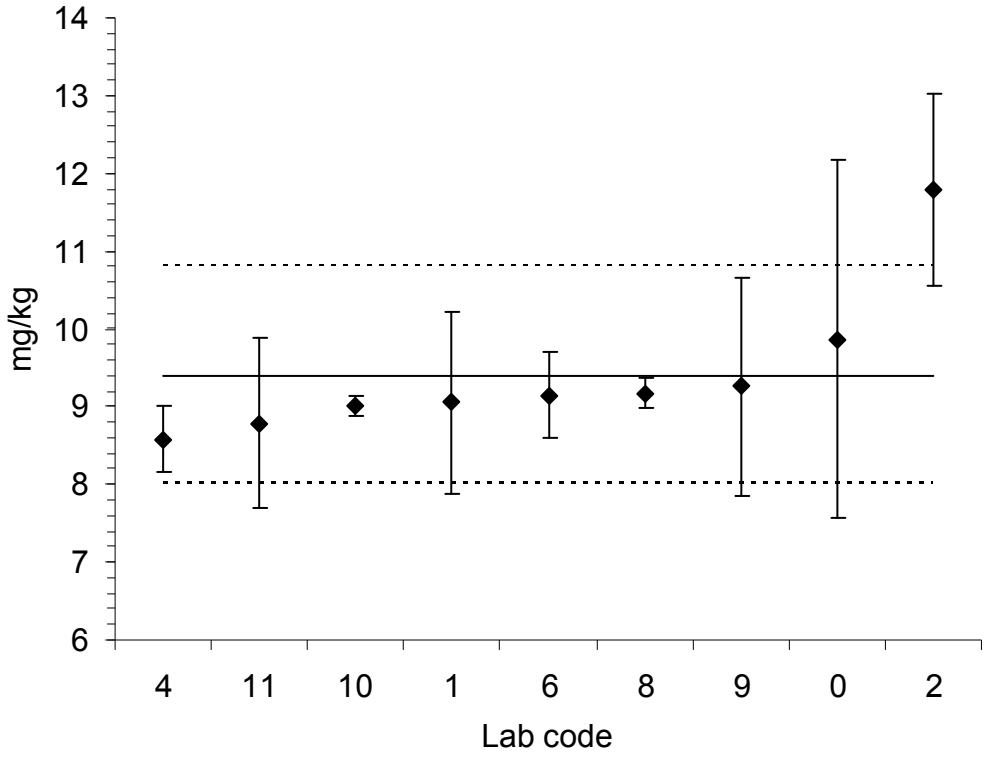
Cu mass fractions, mg/kg

Lab Code	#1	#2	#3	#4	#5	#6	Mean	U
0	-	-	-	-	-	-	-	-
1	1.8	1.8	1.84	1.82	1.77	1.74	1.795	0.230
2	1.84	1.83	2.26	1.7	1.81	1.78	1.870	0.230
3	-	-	-	-	-	-	-	-
4	1.511381	1.575114	1.518462	1.636216	1.727568	1.740763	1.618	0.081
5	1.83	1.92	1.9	1.87	1.93	2.02	1.912	0.134
6	~~1.92~~	~~1.87~~	~~1.92~~	~~1.8~~	~~1.78~~	~~1.79~~	~~1.847~~	~~0.185~~
7	-	-	-	-	-	-	-	-
8	1.69	1.7	1.67	1.7	1.71	1.72	1.698	0.034
9	1.661225	1.727852	1.771304	1.737817	1.691517	1.668555	1.710	0.248
10	-	-	-	-	-	-	-	-
11	1.752	1.726	1.755	1.866	1.775	1.669	1.757	0.224
12	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-
14	1.26	1.36	1.32	1.03	1.32	1.24	1.255	0.126
15	1.47	1.58	1.17	1.28	1.68	1.52	1.450	0.145
16	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-



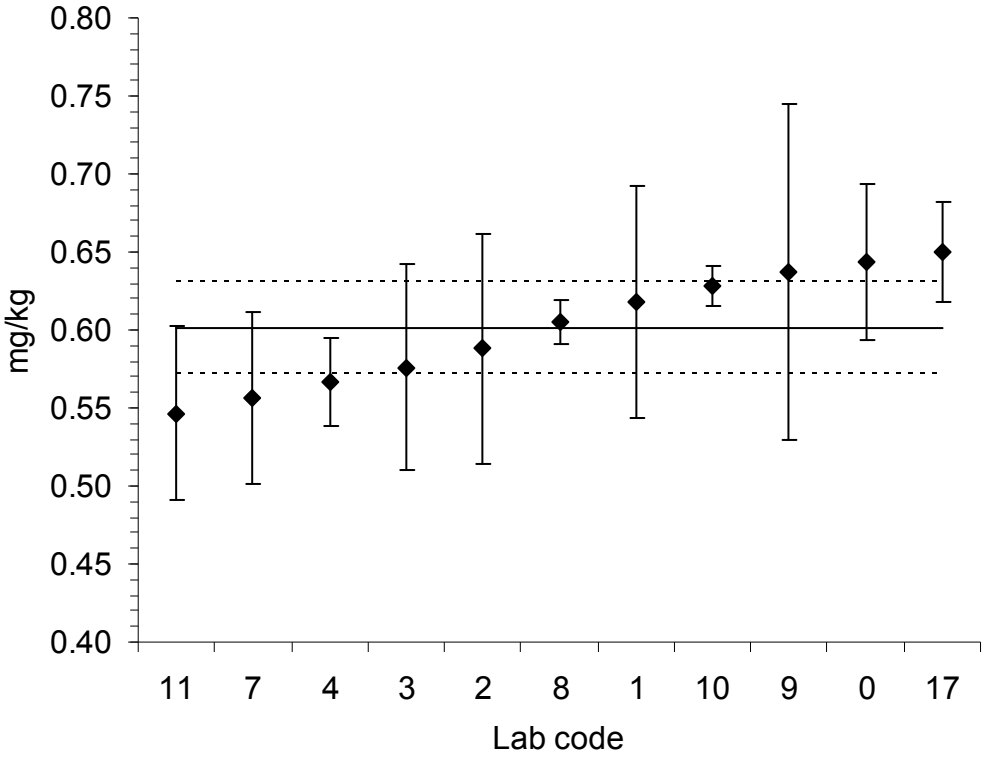
Fe mass fractions for indicative value, mg/kg

Lab Code	#1	#2	#3	#4	#5	#6	Mean	U
0	9.4	10.5	9.8	11.3	10.1	8.1	9.87	2.30
1	9.23	9.14	9.03	8.98	8.92	9	9.05	1.18
2	11.46	11.2	11.86	12.03	11.9	12.26	11.79	1.23
3	-	-	-	-	-	-	-	-
4	8.533131	8.208397	7.971674	9.003248	8.785018	9.002233	8.58	0.43
5	-	-	-	-	-	-	-	-
6	9	9.2	9.2	9.7	9.1	8.7	9.15	0.55
7	-	-	-	-	-	-	-	-
8	9.14	9.05	9.28	9.24	9.1	9.24	9.18	0.18
9	8.739973	9.012447	10.01838	9.304663	9.243023	9.263799	9.26	1.40
10	9	8.95	9.03	9	9	9.06	9.01	0.12
11	8.984	9.132	8.321	9.168	8.639	8.47	8.79	1.10
12	~~8.13~~	~~8.019~~	~~8.327~~	~~7.84~~	~~8.06~~	~~8.228~~	~~8.10~~	~~0.83~~
13	-	-	-	-	-	-	-	-
14	~~10.96~~	~~11~~	~~9.88~~	~~11.13~~	~~10.93~~	~~10.46~~	~~10.73~~	~~1.07~~
15	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-



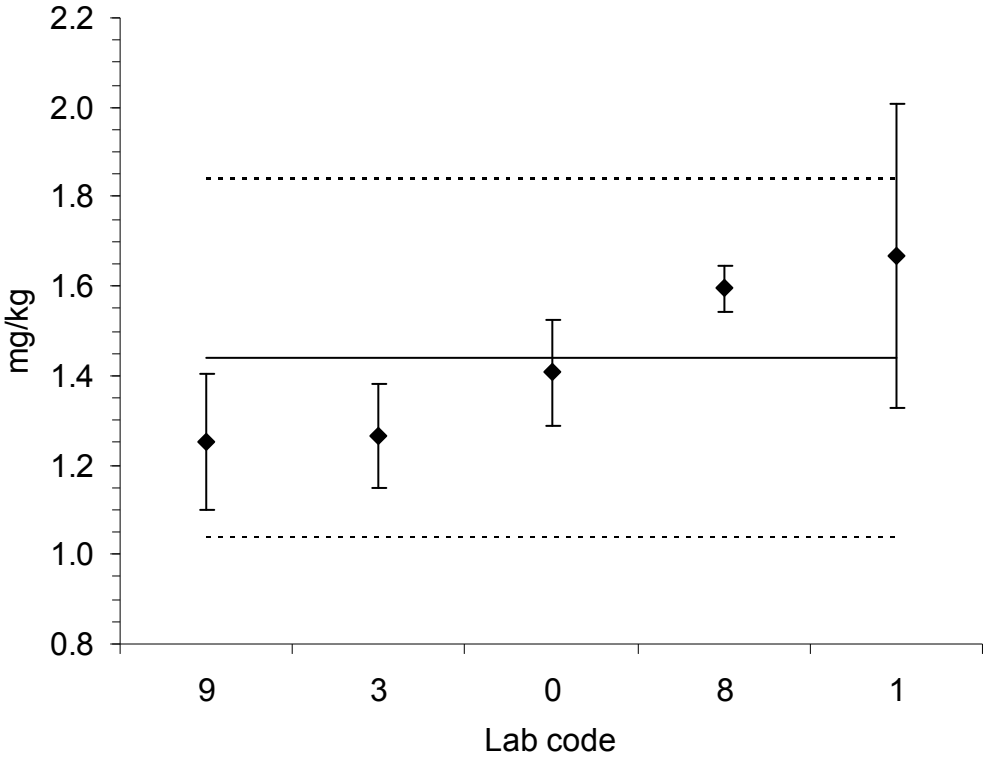
Hg mass fractions, mg/kg

Lab Code	#1	#2	#3	#4	#5	#6	Mean	U
0	0.642	0.63	0.633	0.657	0.646	0.655	0.6438	0.0500
1	0.624	0.624	0.63	0.632	0.602	0.597	0.6182	0.0740
2	0.571	0.565	0.571	0.598	0.616	0.607	0.5880	0.0740
3	0.59	0.535	0.548	0.623	0.575	0.583	0.5757	0.0660
4	0.56085	0.583915	0.592109	0.552253	0.551969	0.555928	0.5662	0.0283
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	0.589	0.575	0.565	0.528	0.544	0.538	0.5565	0.0557
8	-	-	-	-	-	-	-	0.0139
9	0.643773	0.61502	0.599936	0.673139	0.647799	0.643521	0.6372	0.1077
10	0.61	0.628	0.642	0.616	0.639	0.637	0.6287	0.0130
11	0.555	0.557	0.53	0.533	0.554	0.549	0.5463	0.0557
12	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-
17	0.65	0.655	0.653	0.639	0.647	0.656	0.6500	0.0325



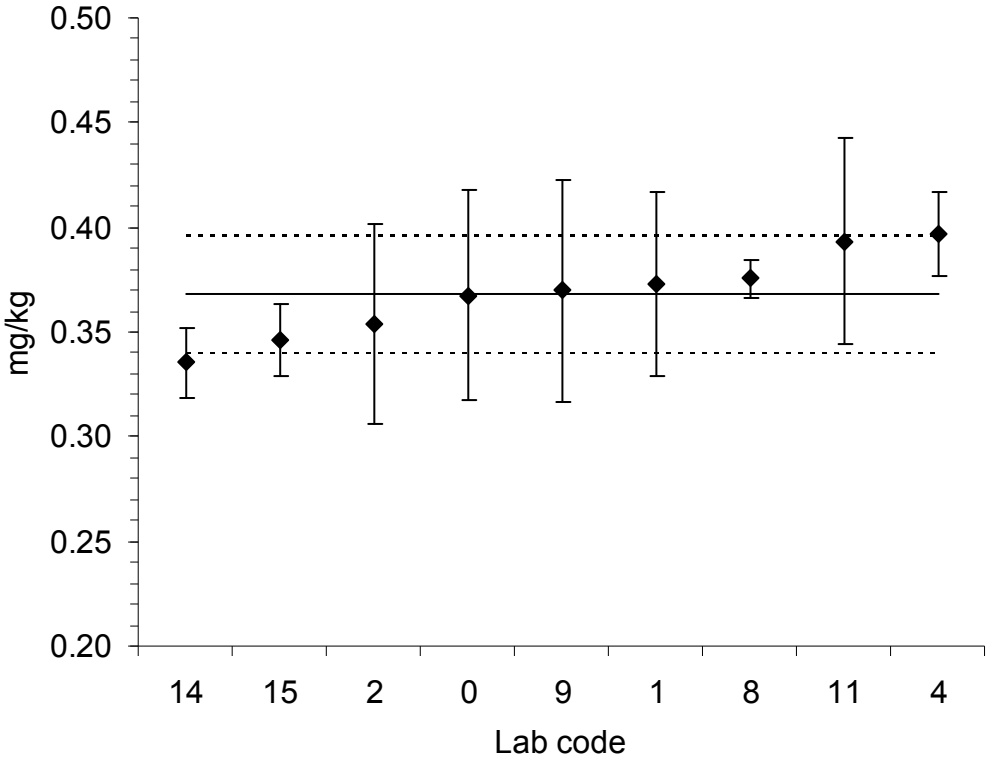
I mass fractions, mg/kg

Lab Code	#1	#2	#3	#4	#5	#6	Mean	U
0	1.48	1.37	1.39	1.32	1.42	1.46	1.407	0.120
1	1.66	1.6	1.56	1.72	1.78	1.69	1.668	0.340
2	-	-	-	-	-	-	-	-
3	1.28	1.29	1.29	1.23	1.23	1.28	1.267	0.116
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-
8	1.55	1.61	1.6	1.61	1.58	1.62	1.595	0.052
9	1.2663	1.275514	1.273711	1.239936	1.213469	1.23994	1.251	0.153
10	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-



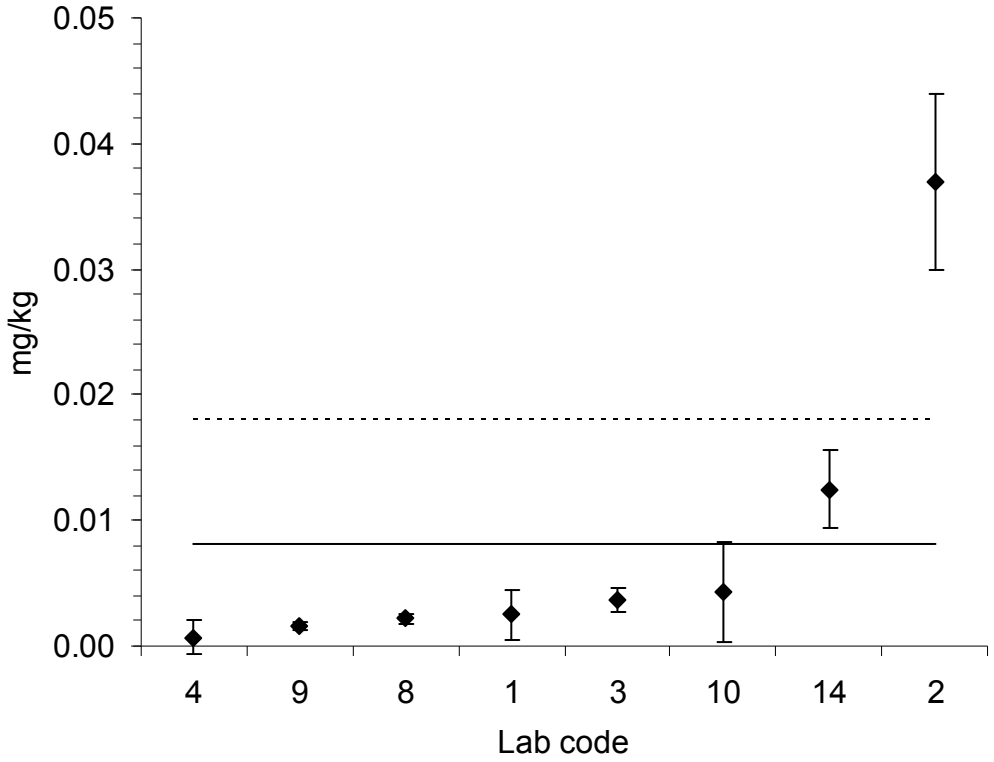
Mn mass fractions, mg/kg

Lab Code	#1	#2	#3	#4	#5	#6	Mean	U
0	0.393	0.367	0.369	0.365	0.341	0.37	0.3675	0.0500
1	0.382	0.364	0.38	0.372	0.377	0.365	0.3733	0.0440
2	0.343	0.351	0.344	0.396	0.349	0.341	0.3540	0.0480
3	-	-	-	-	-	-	-	-
4	0.426505	0.413859	0.402226	0.385607	0.371701	0.380227	0.3967	0.0198
5	~~0.605~~	~~0.602~~	~~0.576~~	~~0.545~~	~~0.508~~	~~0.5~~	~~0.5560~~	~~0.0834~~
6	~~0.55~~	~~0.51~~	~~0.46~~	~~0.47~~	~~0.54~~	~~0.57~~	~~0.5167~~	~~0.0258~~
7	-	-	-	-	-	-	-	-
8	0.37	0.376	0.371	0.377	0.378	0.382	0.3757	0.0090
9	0.345883	0.370215	0.381685	0.379928	0.365819	0.374134	0.3696	0.0534
10	-	-	-	-	-	-	-	-
11	0.408	0.381	0.398	0.4	0.395	0.378	0.3933	0.0489
12	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-
14	0.33	0.346	0.335	0.336	0.332	0.333	0.3353	0.0168
15	0.349	0.351	0.345	0.34	0.35	0.342	0.3462	0.0173
16	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-



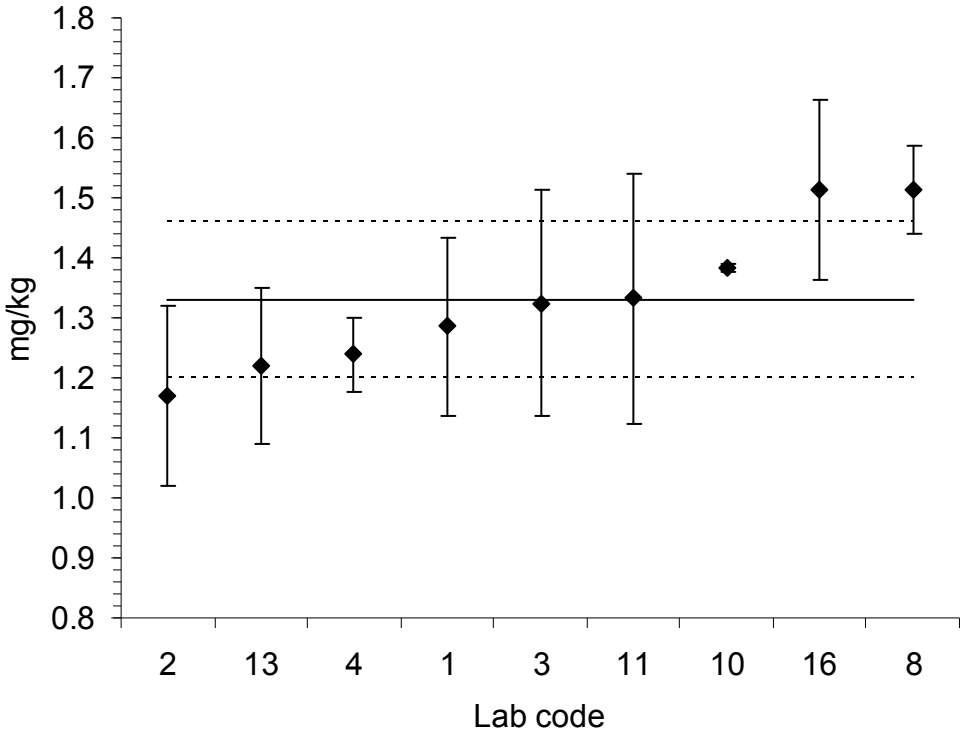
Pb mass fractions not certified, mg/kg

Lab Code	#1	#2	#3	#4	#5	#6	Mean	U
0	-	-	-	-	-	-	-	-
1	0.002	0.0027	0.0018	0.0031	0.0024	0.0029	0.0025	0.0020
2	0.033	0.037	0.037	0.041	0.036	0.038	0.0370	0.0070
3	0.0038	0.0042	-	0.0034	-	0.0031	0.0036	0.0010
4	0.001101	-	-	0.00136	0.000365	0.001248	0.0007	0.0014
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	0.0005
9	0.002223	0.00159	0.001426	0.002567	0.001426	0.000515	0.0016	0.0003
10	0.003	0.002	0.002	0.009	0.008	0.002	0.0043	0.0040
11	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-
14	0.013	0.019	0.007	0.008	0.013	0.015	0.0125	0.0031
15	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-



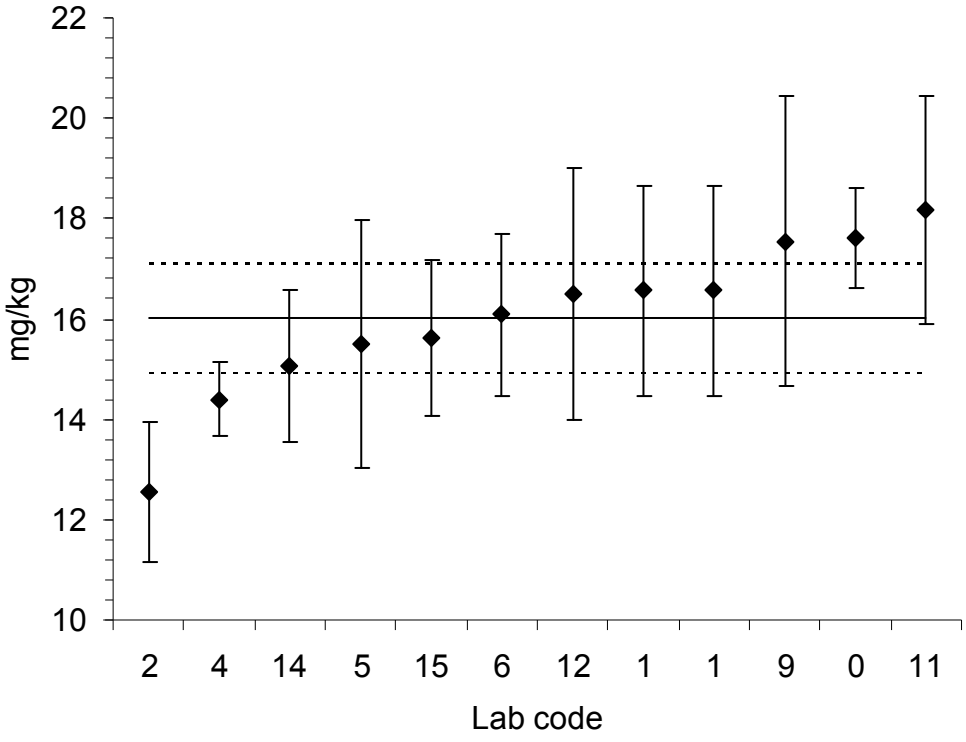
Se mass fractions, mg/kg

Lab Code	#1	#2	#3	#4	#5	#6	Mean	U
0	~~1.58~~	~~1.56~~	~~1.54~~	~~1.66~~	~~1.51~~	~~1.41~~	~~1.543~~	~~0.090~~
1	1.3	1.28	1.33	1.28	1.27	1.25	1.285	0.150
2	1.21	1.08	1.19	1.13	1.22	1.2	1.172	0.150
3	1.32	1.29	1.29	1.44	1.3	1.31	1.325	0.187
4	1.124937	1.161356	1.172484	1.278928	1.332724	1.365205	1.239	0.062
5	~~1.85~~	~~1.66~~	~~1.67~~	~~1.58~~	~~1.62~~	~~1.51~~	~~1.648~~	~~0.264~~
6	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-
8	1.48	1.553	1.487	1.48	1.547	1.535	1.514	0.073
9	~~1.467907~~	~~1.594321~~	~~1.547626~~	~~1.486256~~	~~1.545943~~	~~1.436269~~	~~1.513~~	~~0.287~~
10	1.386	1.403	1.375	1.418	1.38	1.337	1.383	0.007
11	1.253	1.343	1.364	1.377	1.376	1.279	1.332	0.207
12	-	-	-	-	-	-	-	-
13	1.178	1.162	1.239	1.213	1.313	1.215	1.220	0.131
14	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-
16	1.58	1.66	1.58	1.41	1.41	1.44	1.513	0.151
17	-	-	-	-	-	-	-	-



Zn mass fractions, mg/kg

Lab Code	#1	#2	#3	#4	#5	#6	Mean	U
0	17.5	18	18.1	17.7	17.3	17.1	17.62	1.00
1	16.9	16.6	16.5	16.5	16.5	16.4	16.57	2.10
2	12.13	13.7	12.45	12.85	12.2	11.99	12.55	1.40
3	-	-	-	-	-	-	-	-
4	13.05685	13.59636	13.4783	15.15428	15.33699	15.8039	14.40	0.72
5	15.3	15.6	15.5	15.5	15.4	15.7	15.50	2.48
6	16.2	16.1	16.2	16.2	16	15.8	16.08	1.61
7	-	-	-	-	-	-	-	-
8	16.6	16.5	16.5	16.4	16.7	16.7	16.57	0.25
9	16.72501	17.09337	17.66786	18.15451	18.00946	17.65193	17.55	2.90
10	-	-	-	-	-	-	-	-
11	18.223	19.024	17.238	19.071	17.93	17.563	18.17	2.29
12	16.14524	16.35324	16.48412	16.50384	16.73778	16.68105	16.48	2.51
13	-	-	-	-	-	-	-	-
14	14.7	14.8	14.2	16.1	14.5	16.1	15.07	1.51
15	16.2	15.8	15.9	15.2	15.9	14.7	15.62	1.56
16	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-



Annex E ERM®-BB422 (Fish muscle) – Participant methods

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

Lab code	*Element(s)*	*Sample preparation method*	*Measurement technique*	*Calibration method*	*Quality Assurance*	*Uncertainty estimation*
0	As, Cu, Fe, I, 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-422, BCR-184	Accoding to Robouch et al., Uncertainty budget for k0-NAA. J. Radioanal. Chem. 245, 195 (2000).
1	All	MW-assisted digestion (0.5 g sample + 5 ml HNO_3 + 1ml H_2O_2), 600 W, 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
2	As, Cd, 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	Control of digestion with one blank in each digestion batch, control of the response of the highest standard of the curve, the correlation coefficient (r^2) and residual analysis. One reference material is measured with each batch.	Considering the following contributions: - repeatability - reproducibility - Uncertainty related to exactitude (uncertainty of reference material)
	Hg	0.5 grams of sample. Microwave digestion with 6 ml HNO_3 (67%), 1 ml H_2O_2 (33%). Dilution to volume with HCl (37%) and H_2O	Cold vapour with Deuterium background correction	As above	As above	As above
	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
3	As, Cd, Hg, Pb, Se	Microwave assisted pressure digestion with conc. HNO_3	ICP-MS (quadrupole)	Addition calibration	1) Measurement of CRM 2) calibration standards were made from certified stock	According to the principles in "Guide to the expression of uncertainty in measurement - GUM:1995

					solution 3) addition of internal standards	BIPM/IEC/IFCC/ISO/IUPAC/IUPAP/OIML ". Software GUM Workbench used (www.gum.dk).
	I	Extraction with TMAH at 90 °C (3 h)	ICP-MS (quadrupole)	As above	As above	As above
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, Cu, Mn, Pb, Se, Zn	Acid digestion (1 g of sample, 10 ml HNO_3, 3 ml H_2O_2, final volume - 50 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, Cu, Fe, Mn, Se, 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.	As above
7	Hg	Acid digestion (1 g of sample, 10 ml HNO_3, 3 ml H_2O_2, final volume - 50 ml)	AMA - Atomic absorption spectrometry (on 0.1 ml aliquots)	11 point calibration using standard solutions - 0.01 and 0.05 mg Hg/L, range 0 - 25 ng Hg	Control of calibration for point 1 ng Hg prepared from another standard material in each series of measurement, measuring duplicate samples for each series of 20 samples	As above
8	Cd, Hg, Pb	Microwave assisted digestion: about 300 mg sample in 4 mL HNO_3, 2 mL H_2O_2, 1400 W (71 bar) for 10 min, cooling 20 min.	ICP-MS: Instrument: ELAN DRC II, Perkin-Elmer - LAS, Sample introduction using a peristaltic pump, Spetec, at a flow rate of 1.5 ml/min, Meinhard nebulizer and cyclone spray chamber.	External calibration with 6 standards and internal standards Ir and 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	Measurement of certified standards together with samples prepared at sample concentrations determined. Preparation and measurement of BCR-422 CRM together with the samples. Preparation and measurement of blank digestions together with the samples.	Uncertainty was estimated as twice the reproducibility standard deviation of BCR-422. Reproducibility standard deviation was derived from this sample, which was digested on 10 different days and analysed on 10 different days.

				matching the calibration range were appropriately diluted and re-measured.		
	Cu, Fe, Zn	As above	ICP-AES: Instrument: Optima 7300, Perkin-Elmer - LAS, Sample introduction using a peristaltic pump, Spetec, at a flow rate of 1.2 ml/min, Seaspray nebulizer and cyclone spray chamber.	External calibration with 9 standards; procedure and controls as above	As above	As above
	As, Se	As above	HG-ICP-AES: Instrument: Optima 7300, Perkin-Elmer - LAS, Sample introduction using a peristaltic pump, Spetec, at a flow rate of 2.5 ml/min, hydride generation introduction system.	External calibration with 9 standards; procedure and controls as above	As above	As above
	I	Complete dissolution with 5 % TMAH at 30 ºC in ultrasonic bath for 2 h.	As for Cd, Hg, Pb	As for Cd, Hg, Pb	As above	As above
9	As, Cd, Cu, Fe, Hg, Mn, Pb, Se, Zn	Aliquots (0.5 g) of test sample were digested in concentrated nitric acid using sealed quartz vessels and microwave heating.	ICP-MS with collision cell technology (Agilent 7500ce)	External 7-point calibration, plus internal standard correction using rhodium	Reagent blanks were included to correct for background contamination and to estimate the limit of detection and spiked blanks to estimate recovery of analyte. Certifed reference materials DOLT 2 dogfish liver, DORM 3 fish protein, NIST 1548a Typical diet were also included to measure accuracy.	Based on our performance in inter-laboratory comparisons.
	I	Aliquots (0.5 g) of test sample were digested in tetramethyl ammonium hydroxide solution using an open tube heater block set at 80 ºC.	ICP-MS (quadrupole)	External 7-point calibration, plus internal standard correction using antimony	Reagent blanks were included to correct for background contamination and to estimate the limit of detection and spiked blanks to estimate recovery of analyte. Certifed reference materials BCR 151 skimmed milk powder, NIST 1549 non-fat milk powder were also included to measure accuracy.	As above
10	Cd, Pb	Digestion of sample with 10 ml HNO_3 + 1 ml H_2O_2 on a heating block for 24 hours	Double IDMS on quadrupole ICP-MS	Digestion of high purity metal for reverse Isotope dilution; spike ^{111}Cd, ratio $^{112}Cd/^{111}Cd$; spike ^{206}Pb, ratio $^{208}Pb/^{206}Pb$	IDMS analysis accredited 2.54 Cofrac	An uncertainty budget had been established in compliance with the GUM comprising uncertainties for weighing, ratio measurements, fidelity, isotope abundances and corrections for blank. Our software calculate uncertainties

						propagation and the expanded final uncertainty (k=2)
	Fe, Hg, Se	Digestion of sample with 10 ml HNO_3 + 1 ml H_2O_2 in a closed vessel in a microwave oven.	As above, and using a collision cell for Fe, Se	As above, with isotopes: spike ^{54}Fe, ratio $^{54}Fe/^{56}Fe$; spike ^{202}Hg, ratio $^{202}Hg/^{200}Hg$, spike ^{78}Se, ratio $^{80}Se/^{78}Se$	As above	As above
11	As, Cd, Cu, Fe, Mn, Pb, Se, Zn	Closed microwave digestion (Multiwave 3000); 4 mL HNO_3 + 2 mL H_2O_2 + 2 mL H_2O	ICP-MS (Perkin Elmer Elan DRC II) with APEX sample introduction system for Cd and Pb	External calibration with standard solutions traceable to certified reference materials issued by NIST	The SRM 2976 Mussle Tissue (Trace Elements and Methylmercury) and digestion blanks were prepared and analysed in the same manner as samples.	Estimation of uncertainty was performed using CRM DORM-2, SRM 1577a, and SRM 2976. The uncertainty covers the whole analytical process (sample preparation, digestion, dilution, measurement procedure). For combined uncertainty (see average +/- uncertainty) bias, uncertainty of reference material and relative standard deviation of at least 6 independent measurements was taken into account.
12	As, Fe, Zn	As above	ICP-OES (Perkin Elmer Optima 7300 DV)	As above	As above	As above
13	Hg	As above	CV-AAS (Perkin Elmer FIMS 400)	As above	As above	As above
14	Cd, Cu, Mn, Pb, Zn	Dry ashing at 450°C for 6 hours. Residue dissolution with HNO_3.	ICP-MS (quadrupole, X7, Thermo Fisher Scientific)	External calibration; solutions are prepared with Spex certified solutions. Internal standard; In.	ISO 9001 Certification from AFAQ since October 2010. Metrology, apparatus maintenance, staff formation, chemical reagents are part of our quality manual.	Uncertainty expressed in % in the results table includes: - the uncertainty on the results given by the formula: $U = 2 \times (\sigma/\sqrt{n})$ (σ: standard deviation on measurements results, and n: number of measurement results) - the measurement uncertainty based on control standards solutions."
15	Cu, Fe, Mn, Zn	As above	ICP-AES (iCAP6500 DUO, Thermo Fisher Scientific)	As above	As above	As above
16	As, Se	Digestion in H_2SO_4/HNO_3 mixture with heating at 240°C.	ICP-MS (quadrupole, X7, Thermo Fisher Scientific)	As above	As above	As above
17	Hg	Solid-sampling (Treatment sample steps are integrated to the CVAAS device: combustion at 650°C after drying at 200°C. Mercury vapours are trapped on a gold wire.)	CVAAS (DMA80, Thermo Fisher Scientific)	Quadratic fit calibration curve. Calibration solutions are prepared with Spex certified solutions at 1g/L concentration. To check the adequacy of liquid calibration with solid samples, different weights of BCR-320R and ERM-464 were analysed on all the calibration range.	As above	As above

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European Commission

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Title: The Certification of the Mass Fractions of Elements in Fish Muscle
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Abstract
This report describes the preparation of a fish muscle matrix reference material ERM®-BB422, and the certification of the contents (mass fractions) of eight 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 fish muscle (ERM®-BB422)

Element	Certified value, mg/kg [1,2)]	Uncertainty, mg/kg [3)]
As	12.7	0.7
Cd	0.0075	0.0018
Cu	1.67	0.16
Fe	9.4	1.4
Hg	0.601	0.030
I	1.4	0.4
Mn	0.368	0.028
Se	1.33	0.13
Zn	16.0	1.1

1) These values are related to dry mass and are based on the unweighted mean of accepted results
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) Where the standard combined uncertainty is multiplied by a coverage factor $k = 2$ for elements except I and $k = 2.57$ for I 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 and their uncertainties are based on a minimum sample intake of 0.2 g.

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