Certification Report

Certified Reference Materials

ERM®-BD513
ERM®-BD514
ERM®-BD515

Cocoa

May 2019

The certification of the mass fraction of Cd and acrylamide in cocoa powder

Certified Reference Materials
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ERM®-BD515

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Summary

This report describes preparation, analysis and certification of the cocoa reference materials ERM®-BD513, ERM®-BD514 and ERM®-BD515, certified for their cadmium mass fractions. The contents of acrylamide are given for information.
The certified reference materials (CRM) are available in the form of powder (ca. 8.3 g) in sealed aluminised plastic bags. They are intended for the verification of the accuracy of analytical measurements, for the measurement uncertainty estimation, to assess the traceability of the analytical results or for calibration of analytical instruments.

The following cadmium mass fractions and uncertainties have been certified:

CRM	**Mass fraction**[1] in mg/kg	**Uncertainty**[2] in mg/kg
ERM-BD513	0.181	0.009
ERM-BD514	0.541	0.024
ERM-BD515	0.690	0.029

1 Unweighted mean value of the means of accepted sets of data, each set being obtained by 19 laboratories with different methods of measurement. The values are traceable to the SI (Système International d'Unités) by the use of pure substances of known stoichiometry or certified standard solutions for calibration.

2 Estimated expanded uncertainty *U* with a coverage factor of $k = 2$ corresponding to a level of confidence of about 95%, as defined in the ISO/IEC Guide 98-3:2008 [Uncertainty of measurement -- Part 3: Guide to the expression of uncertainty in measurement (GUM:1995)].

This report contains detailed information on the preparation of the CRMs as well as on homogeneity investigations and on the analytical methods used for certification analysis.
The certified values are based on the results of 19 laboratories which participated in the certification inter-laboratory comparison.

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List of abbreviations
(if not explained elsewhere)

CRM	certified reference material
ERM	European reference material
ETAAS	electrothermal atomic absorption spectrometry
ICP-MS	inductively coupled plasma mass spectrometry
LC	liquid chromatography
MS	mass spectrometry
M	mean value
n	number of accepted data sets
s	standard deviation of an individual data set
s_M	standard deviation of laboratory means
s_{rel}	relative standard deviation
$\bar{s}_i$	square root of mean of variances of data sets under repeatability conditions
M_i	single result

1. Introduction

Food safety and quality are two of the most important factors determining the consumer acceptance and purchase of a product. Authenticity proof and detection of fraud or determination of residues and contaminants are therefore in the service of the consumer.
For implementation of food and feed legislation, there is a strong need for development and harmonisation of analytical methods. The official food control laboratories have to use validated methods whenever possible. For this reason, analytical methods must be subjected to validation procedures, in order to provide accurate, repeatable and reproducible results within and between laboratories. Method validation is done to check the performance of a method.
Food and feed reference materials and especially certified reference materials (CRM) are a very useful tool in the verification of the accuracy of analytical measurements. They can be used for the measurement uncertainty estimation, to assess the traceability of the analytical results or the calibration of analytical instruments.
In EU COMMISSION REGULATION (EU) No 488/2014 of 12 May 2014 a maximum level for Cadmium of 0.6 mg/kg in cocoa powder sold to the final consumer or as an ingredient in sweetened cocoa powder sold to the final consumer (drinking chocolate) is established from 1st January 2019. In some regions of cocoa producing countries, cadmium levels in soil can be naturally high. Therefore, it is necessary to check cocoa powder for Cd and therefore BAM decided to produce a series of CRM with increasing cadmium levels around the maximum levels established by the European Commission.
It was decided to characterise cocoa powder for its mass concentration of acrylamide as well, since acrylamide is also a contaminant of interest in food stuff since Swedish scientists in April 2002 reported the occurrence of acrylamide in many baked or fried food and a worldwide surveillance of this substance in food products started.
The candidate reference materials ERM-BD513, ERM-BD514 and ERM-BD515 to be certified were produced for the purpose of quality assurance and quality control for the determination of cadmium and acrylamide in food. The material has been prepared from cocoa powder from commercial sources intended for human consumption.
16 laboratories were selected based on documented experience and proficiency and invited to participate in the certification study of a candidate materials prepared at BAM.
Certification was carried out on the basis of ISO 17034 [1] and the relevant ISO-Guides [2, 3].

2. Laboratories involved

Preparation of the material:

Bundesanstalt für Materialforschung und -prüfung (BAM), Berlin, Germany

Test for homogeneity:

Bundesanstalt für Materialforschung und -prüfung (BAM), Berlin, Germany

Participants in the certification inter-laboratory comparison:

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, Berlin, Germany
Bundesanstalt für Materialforschung und -prüfung (BAM), Berlin, Germany
Cork Public Analyst's Laboratory St. Finbarr's Hospital, Cork, Ireland
CVUA Rheinland, Hürth, Germany
Estonian Agricultural Research Centre, Saku, Estonia
EXHM-GCSL/EIM Chemical Metrology Laboratory, Athens, Greece

Finnish Food Safety Authority, Helsinki, Finland
Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italia
Kreis Mettmann Amt für Verbraucherschutz, Mettmann, Germany
Laboratoire national de Santé, Dudelange, France
Landeslabor Schleswig-Holstein, Neumünster, Germany
Landesuntersuchungsamt für Chemie, Hygiene und Veterinärmedizin, Bremen, Germany
LUFA-ITL GmbH, Kiel, Germany
National Food and Veterinary Risk Assessment Institute, Vilnius, Lithuania
National Institute of Public Health - National Institute of Hygiene, Warsaw, Poland
National Laboratory for Health, Environment and Food, Maribor, Slovenia
Sanitary Veterinary and Food Safety Directorate, Bucharest, Rumania

Statistical evaluation of the data:

Bundesanstalt für Materialforschung und -prüfung (BAM), Berlin, Germany

3. Candidate material

In the run-up of the certification 9 different commercially available cocoa samples were bought in normal groceries. All samples were checked for their Cd-content immediately after purchase using solid sampling atomic absorption spectrometry. Table 1 shows the results of these determinations.

Table 1: Cd-content of candidate cocoa samples (mass fraction in mg/kg)

K1	K2	K3	K4	K5	K6	K7	K8	K9
0.488	0.573	0.570	0.575	0.268	0.432	0.836	0.223	0.640

It was decided to use batches K7 (ERM-BD515), K8 (ERM-BD513) and K9 (ERM-BD514) as candidate reference materials for certification, because K7 was well above, K8 was well below, and K9 near the maximum level of 0.6 mg/kg. The acrylamide contents were ca. 1.9 mg/kg in K7, 1.0 mg/kg in K8 and 2.3 mg/kg in K9.
Using Karl-Fischer-titration was used to determine the moisture of the three materials (see Table 2). 15 bags per CRM were used for moisture determination taking 3 sub-samples from each bag (single results see Annex 1).

Table 2: Moisture of ERM-BD513, ERM-BD514 and ERM-BD515 (n = 45)

CRM	M in %	s in %
BD513	5.04	0.08
BD514	3.70	0.19
BD515	3.58	0.08

In total 4.65 kg of K8, 7.43 kg of K9 and 8.25 kg of K7 were taken as starting material. Approximately 90 % of the powder had a particle size between 50 and 100 µm.
The three selected batches were homogenised in a drum-hoop mixer for 24 hours each using a stainless-steel drum. After homogenisation the candidate reference materials were filled in aluminised

plastic bags using a 10-dicision sample divider. Each plastic bag was filled with at least 8.3 g of cocoa powder.

In total, 500 bags of ERM-BD513, 760 bags of ERM-BD514 and 800 bags of ERM-BD515 were obtained.

4. Homogeneity testing

A total of 15 units of each candidate material were selected for homogeneity testing of cadmium and acrylamide using a random sample picking scheme (see Table 3).

Table 3: Units selected for homogeneity testing

BD513	022	072	093	130	153	203	230	257	305	355	371	407	437	473	499
BD514	045	071	090	168	196	253	314	373	439	544	571	608	662	710	765
BD515	006	063	095	148	217	301	347	405	451	513	573	610	705	732	788

Cadmium:
From each selected unit three sub-samples were analysed applying ICP-MS after digestion with 4 mL of HNO_3 and 1 mL of H_2O_2 in an UltraClave digestion unit using sample intakes of 0.5 g. All sample solutions from the respective batch were analysed together under repeatability conditions in one run with one calibration.

Acrylamide:
50 mg of the finely powdered food sample were placed in a 2 mL snaplock microtube and covered with 1.5 mL water containing the internal standard ($^{13}C3$- acrylamide, ~20 ng/g) and ninhydrin (10 mg/g). Samples were extracted at 85 °C for 2 hours at 1100 rpm in an MHR 13 thermomixer and subsequently centrifuged for 10 min by means of a micro-centrifuge at 14100×g. The aqueous phase was transferred to a second snaplock microtube by using a syringe fitted with a needle, avoiding the transfer of the upper lipid phase. Afterwards, an aliquot (1200 µL) was transferred to another snaplock microtube. Visual protein precipitation was achieved by addition of 150 µL of a 0.68 M potassium hexacyanoferrate(II) trihydrate solution and 150 µL of a 2 M zinc sulfate heptahydrate solution. After short shaking the mixture was centrifuged for 2 min at 14100×g and a 0.8 mL aliquot was transferred to a snaplock microtube. This Aliquot was extracted three times with ethyl acetate (1 mL, shaking) followed by a centrifugation step (0.5 min, 14500/min). The organic supernatants were combined in an 8 mL vial and 1 mL water was added as keeper. Subsequently, the ethyl acetate layer was evaporated under a gentle stream of nitrogen at 30 °C. The resulting aqueous solution was loaded onto a 100 mg Isolute® Multimode SPE cartridge, preconditioned with first acetonitrile (1 mL) and then water (3 mL). The aqueous solution was eluted and collected in a 4 ml vial. A 1 mL aliquot of water was loaded onto the cartridge, eluted, and collected in the same 4 mL vial of the previous fraction. Afterwards, the eluate was passed through a 0.2 µm membrane filter and collected in a 1.5 mL vial for LC-MS/MS analysis. All samples were stored at 4 °C before analysis.

All measurement results are given in Annex 2.

The estimates of analyte-specific inhomogeneity contributions u_{bb} to be included into the total uncertainty budgets were calculated according to ISO Guide 35 [3] using Eq. (1):

$$u_{\mathrm{bb}} = \sqrt{\frac{MS\mathrm{among} - MS\mathrm{within}}{n}} \qquad (1)$$

where

MS_{among}	mean of squared deviations between bottles (from 1-way ANOVA)
MS_{within}	mean of squared deviations within bottles (from 1-way ANOVA)
n	number of replicate sub-samples per bottle

Eq. (1) does not apply if MS_{within} is larger than MS_{among}. In this case the uncertainty contribution is set to zero (Cd in ERM-BD513 and acrylamide in ERM-BD513 and ERM-BD515).

Annex 2 shows the results of the calculations.

5. Stability testing

5.1 Initial stability study

From experience a temperature-driven deterioration especially of the acrylamide content was to be expected also for this material. Selected units of the candidate material were submitted to accelerated ageing at temperatures between - 20 °C and 60 °C over periods of 1 week to 4 weeks (short-term study) and 1 month to 12 months (long-term study) as shown in Table 4 to perform so-called isochronous stability studies [4]. After the respective periods of time individual units were stored at – 80 °C. All units were analysed for acrylamide as well as for Cd using the methods described above under homogeneity testing. Tables 5 and 6 show the results of the short- and long-term stability determinations for Cd, Table 7 the results of the short-term stability determinations for acrylamide and Annex 3 the single results.

Table 4: Accelerated ageing of exposed samples

Time [weeks]	Temperature [°C]				
	- 80	- 21	23	40	60
Short-term stability study					
1	X	X	X	X	X
2	X	X	X	X	X
3	X	X	X	X	X
4	X	X	X	X	X
Long-term stability study					
3	X	X	X	X	---
6	X	X	X	X	---
9	X	X	X	X	---
12	X	X	X	X	---
48	[1])	[1])	---	---	---

[1]) post-certification monitoring (due)

Table 5a: Results of short-term stability determination of Cd in ERM-BD513
(mass fraction in mg/kg)

Temperature in °C	Week 1	Week 2	Week 3	Week 4
- 80	0.22	0.22	0.22	0.22
- 20	0.22	0.22	0.22	0.23
23 (RT)	0.23	0.22	0.23	0.23
40	0.23	0.23	0.23	0.23
60	0.23	0.23	0.23	0.23

Table 5b: Results of short-term stability determination of Cd in ERM-BD514
(mass fraction in mg/kg)

Temperature in °C	Week 1	Week 2	Week 3	Week 4
- 80	0.64	0.64	0.64	0.64
- 20	0.64	0.65	0.64	0.64
23 (RT)	0.64	0.64	0.65	0.64
40	0.64	0.65	0.64	0.64
60	0.65	0.64	0.64	0.64

Table 5c: Results of short-term stability determination of Cd in ERM-BD515
(mass fraction in mg/kg)

Temperature in °C	Week 1	Week 2	Week 3	Week 4
- 80	0.89	0.88	0.88	0.88
- 20	0.89	0.89	0.87	0.88
23 (RT)	0.89	0.88	0.89	0.89
40	0.89	0.87	0.89	0.89
60	0.89	0.88	0.88	0.88

Table 6a: Results of long-term stability determination of Cd in ERM-BD513
(mass fraction in mg/kg)

Temperature in °C	3. month	6. month	9. month	12. month
- 80	0.23	0.23	0.22	0.22
- 20	0.23	0.22	0.22	0.22
23 (RT)	0.22	0.22	0.22	0.23
40	0.22	0.22	0.22	0.22

Table 6b: Results of long-term stability determination of Cd in ERM-BD514
(mass fraction in mg/kg)

Temperature in °C	3. month	6. month	9. month	12. month
- 80	0.65	0.65	0.64	0.64
- 20	0.65	0.65	0.64	0.64
23 (RT)	0.64	0.64	0.64	0.63
40	0.65	0.63	0.63	0.64

Table 6c: Results of long-term stability determination of Cd in ERM-BD515
(mass fraction in mg/kg)

Temperature in °C	3. month	6. month	9. month	12. month
- 80	0.80	0.79	0.79	0.79
- 20	0.80	0.79	0.79	0.79
23 (RT)	0.79	0.79	0.79	0.79
40	0.79	0.79	0.79	0.79

Table 7a: Results of short-term stability determination of acrylamide in ERM-BD513
(mass fraction in µg/kg)

Temperature in °C	Week 1	Week 2	Week 3	Week 4
- 80	166.76	166.67	167.66	165.54
- 20	165.38	170.35	167.63	162.01
23 (RT)	162.41	164.54	168.19	158.23
40	152.38	147.38	136.66	132.71
60	122.99	117.22	112.93	110.53

Table 7b: Results of short-term stability determination of acrylamide in ERM-BD514
(mass fraction in µg/kg)

Temperature in °C	Week 1	Week 2	Week 3	Week 4
- 80	213.33	217.00	213.29	205.34
- 20	211.19	211.74	218.88	207.26
23 (RT)	220.73	206.80	203.03	207.28
40	215.81	212.40	192.22	174.65
60	178.48	143.64	132.28	129.69

Table 7c: Results of short-term stability determination of acrylamide in ERM-BD515
(mass fraction in µg/kg)

Temperature in °C	Week 1	Week 2	Week 3	Week 4
- 80	199.06	189.46	183.52	197.39
- 20	191.48	184.04	192.73	185.57
23 (RT)	180.57	198.40	183.31	183.12
40	178.51	153.73	164.11	148.44
60	133.64	131.39	128.16	130.32

The statistical evaluation of the short-term stability data (ANOVA) showed no dependence of the Cd-content for all temperatures on exposure time. For acrylamide a non-negligible trend is obviously observed for all temperatures above RT. In order to obtain estimates for the thermal behaviour of the samples at the lower and especially at the storage temperature, a simple Arrhenius model is assumed for the dependence of the reaction rate k(T) on temperature. A plot of the reaction rate k(T) over the inverse temperature is given in Figure 1 (derived from the mean values measured at the corresponding sampling points in temperature and time).

Fig. 1a: Reaction rate for acrylamide in dependence on the inverse temperature (semi-logarithmic plot, ERM-BD513)

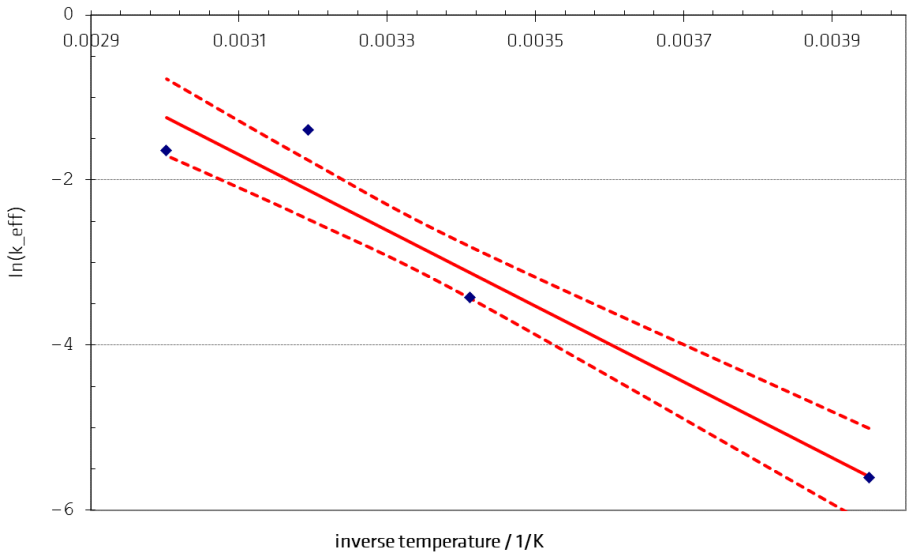


Fig. 1b: Reaction rate for acrylamide in dependence on the inverse temperature (semi-logarithmic plot, ERM-BD514)

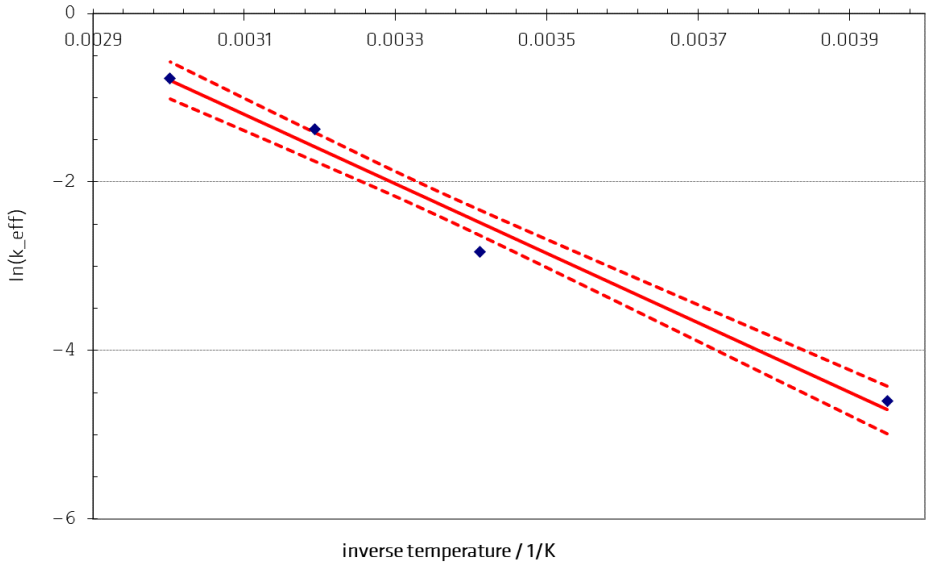
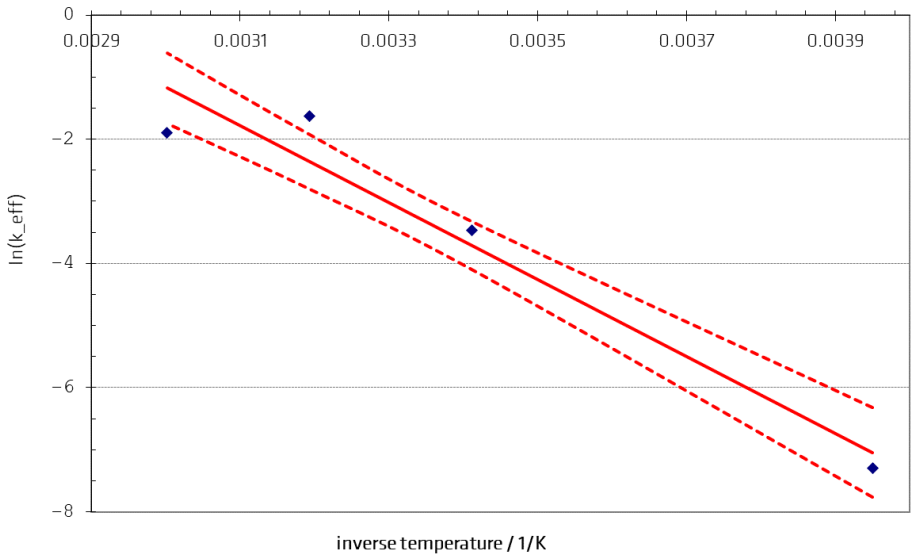


Fig. 1c: Reaction rate for acrylamide in dependence on the inverse temperature (semi-logarithmic plot, ERM-BD515)



Obviously the temperature dependence can be approximated by straight lines (as shown in the graphs). The corresponding confidence intervals for the lines are also given in the figures. By using these data and the assumed model, an estimate can be obtained when degradation will presumably force the acrylamide content to fall outside (i.e. below) the calculated lower expanded uncertainty limit. In the sense of a worst-case estimation, these calculations are carried out for the reaction rates at the upper confidence limit of the line as shown in Figures 1a-c. The results are given in Table 8.

Table 8a: Estimation of shelf life of ERM-BD513 (for acrylamide)

Value falls below lower limit after	Months	years
at – 20 °C	78.8	6.57
at + 20 °C	8.7	0.73
at + 40 °C	3.1	0.26
at + 60 °C	1.1	0.09

Table 8b: Estimation of shelf life of ERM-BD514 (for acrylamide)

Value falls below lower limit after	Months	years
at – 20 °C	35.0	2.92
at + 20 °C	4.3	0.36
at + 40 °C	1.7	0.14
at + 60 °C	0.7	0.06

Table 8c: Estimation of shelf life of ERM-BD515 (for acrylamide)

Value falls below lower limit after	Months	years
at – 20 °C	222.6	18.55
at + 20 °C	11.1	0.93
at + 40 °C	2.7	0.23
at + 60 °C	0.7	0.06

6. Characterisation study

6.1 Analytical methods

19 laboratories participated in the certification inter-laboratory comparison for Cd and 12 for acrylamide. For some elements part of the laboratories used more than one analytical method reporting more than one data set.
The laboratories were asked to analyse four subsamples. They were free to choose any suitable analytical method. Tables 9 and 10 show the analytical methods used by the participating laboratories.

Table 9: Analytical procedures for Cd-determination used by the participating laboratories

Lab-No.	Accreditation	Sample mass	Sample pretreatment	Analytical method
L01	yes	0.5 g	Dissolution with HNO_3/H_2O_2 microwave	ICP-MS, calibration with commercial mono-element solution (Inorganic Ventures)
L03	yes	0.5 g	Dissolution with HNO_3/H_2O_2 microwave	ETAAS, calibration with commercial mono-element solution (GUM)
L04	yes	0.5 g	Dissolution with HNO_3/H_2O_2 microwave	ICP-MS, calibration with commercial mono-element solution (Ultra Scientific)
L05	no	1 g	Dissolution with HNO_3	ICP-MS, calibration with pure metal (Sigma Aldrich)
L07	yes	0.4 g	Dissolution with HNO_3 under pressure acc. to DIN EN 13805:2014:2010	ICP-MS acc. to DIN EN 15763:2010, calibration with commercial mono-element solution (Labkings)
L09	yes	0.5 g	Dissolution with HNO_3/HCl	ICP-MS, calibration with commercial mono-element solution (Merck)
L10	yes	0.5 g	Dissolution with HNO_3/H_2O_2 microwave	ICP-MS, calibration with commercial mono-element solution (Romil)
L11	yes	0.35 g	Dissolution with HNO_3/H_2O_2 microwave acc. to EN 1404:2003	ETAAS, calibration with commercial mono-element solution (Roth)
L12	yes	0.5 g	Pressure digestion with HNO_3/H_2O_2	ICP-MS, calibration with commercial mono-element solution (Spex)
L14	yes	0.4 g	Dissolution with HNO_3 microwave	ICP-MS, calibration with commercial mono-element solution (Merck)
L15	yes	0.5 g	Dissolution with HNO_3/H_2O_2 microwave	ICP-MS, calibration with commercial mono-element solution (Merck)
L16	yes	0.5 g	Pressure digestion with HNO_3 acc. to DIN 13805:2010	ETAAS, calibration with commercial mono-element solution (Merck)
L17	yes	0.5 g	Dissolution with HNO_3/HCl/H_2O_2 microwave	ETAAS, calibration with commercial mono-element solution (Merck)
L18	yes	0.5 g	Dissolution with HNO_3/HCl/ microwave	ICP-MS, calibration with commercial mono-element solution (VWR)
L19	yes	0.3 g	Dissolution with HNO_3/H_2O_2 microwave	ICP-MS, calibration with commercial mono-element solution (Sigma-Aldrich)
L20	yes	0.5 g	Dissolution with HNO_3/H_2O_2 microwave	HR-ICP-MS, calibration with NIST-Std.
L21	no	0.5 g	Dissolution with HNO_3/H_2O_2 high-pressure microwave	ICP-MS, calibration with commercial mono-element solution (Merck)
L22	no	0.5 g	Dissolution with HNO_3/H_2O_2 high-pressure microwave	ETAAS, calibration with commercial mono-element solution (Merck)
L23	no	0.5 g	Dissolution with HNO_3/H_2O_2 high-pressure asher	ID-MS, spike with ^{113}Cd

Table 10: Analytical procedures for acrylamide-determination used by the participating laboratories

Lab-No.	Accreditation	Sample mass	Sample pretreatment	Analytical method
L02	yes	0.2 g	1. 40 mL extraction solvent: methanol/0,1 % acetic acid in water (60:40) (v/v) extraction for 60 min 2. centrifugation (15 min) 3. upper layer is filtered through PVDF syringe filter into HPLC vial	LC-MS/MS mobile phases (gradient elution): A: 0.2% formic acid in water B: ACN column: Kinetex® 2.6 µm C18 100 Å, LC column 150 x 4.6 calibration with AA (98%, Cambridge Isotope Lab.) no internal standard
L05	yes	2 g	1. extraction into water using ultra-turrax. 2. centrifugation and filtration, followed by SPE clean-up using MCX cartridges from Waters. 3. spiking with ISTD	LC-MS/MS mobile phases (gradient elution, 5 min): A: 99 % water with 0.1 % acetic acid B: 1 % isopropanol with 0.1 % acetic acid. Column: HSS T3 100 mm calibration with AA (Sigma-Aldrich) internal standard: AA-d5 (99 %)
L06	yes	2 g	according to EN 16618 (procedure for coffee)	LC-MS/MS mobile phase (isocratic elution): 1 % acetic acid in water calibration with AA (98%, Sigma-Aldrich, Tracecert) internal standard: AA-d3 (98%, Sigma-Aldrich)
L07	yes	1 g	extraction with water/ methanol, followed by centrifugation	LC-MS/MS mobile phase (isocratic elution): water/methanol internal standard: AA-d3 (99,9%)
L08	yes	1 g	according to EN 16618	LC-MS/MS mobile phase (isocratic elution): 0.1 % acetic acid in water column: Hypercarb 50 x 2.1 mm calibration with AA (> 99%, Sigma-Aldrich) internal standard: AA-d3 (> 99%, Sigma-Aldrich)
L10	yes	2 g	1. extraction into water (1 hour, 270 rounds/min). 2. centrifugation and filtration, followed by SPE clean-up (IST Isolute M-M) 3. centrifugation with cut off-filter (Amicon Ultra-4, 3 kDa) for 30 min (4000 rpm, 20°C)	LC-MS/MS mobile phase (isocratic elution): 0.1 % formic acid in water/2.5 % methanol column: Hypercarb 10 x 2.1mm calibration with AA (> 99%, Sigma-Aldrich) internal standard: AA-d3 (> 99%, Polymer Source Inc.)
L11	yes	1 g/2 g	according to EN 16618	LC-MS/MS mobile phase (isocratic elution): 0.1 % formic acid/1 % acetonitrile in water column: hypersil hypercarb 5 µm ODS 100x2,1 mm hichrome calibration with AA (99.9 %, Merck) internal standard: AA-d3 (98%)
L12	yes	2 g	Extraction with water in ultrasonic bath; clarification with Carezz I and II, Cleaning with Isolute MFC 18 cartouche	LC-MS/MS (isocratic elution) column: Luna C18, 3 µ with precolumn Phenomenex 150 mm x 3 mm calibration with AA (99.3 %, Ehrenstorfer) internal standard: AA-d3 (99.9%, HPC)

Table 10: Analytical procedures for acrylamide-determination (cont.)

Lab-No.	Accreditation	Sample mass	Sample pretreatment	Analytical method
L16	yes	2 g	Extraction with 5 ml hexane + 40 ml water, 1 hour shaking, 20 min. centrifuged (10°C, 3600U/min), Clean-up with 2 different SPE-columns	LC-MS/MS mobile phase (isocratic elution): 0.1 % formic acid column: Hypercarb (50mm x 2.1 mm) calibration with AA (>99 %, Sigma-Aldrich) internal standard: AA-d3 (99.9%, Sigma-Aldrich)
L18	yes	2 g	Extraction with water, clean up with SPR-cartridge Chromabond ABC 18, then extraction with ethylacetate	GC-MS, carrier gas: Helium column: DB Wax 60 m, 0,25 mm calibration with AA (99.9 %, Alfa-Aesar) internal standard: AA-d3 (98%)
L20	yes	2 g	Addition of 10 mL of water and 2 mL of hexane. Vortexing for 10 min. Separation by certifugation at 4500rpm. Clean-up withTwo SPEs (ISOLUTE multimode (MM) cartridge and ISOLUTE ENV). Elution with 60% MeOH/water.	ID-LC-MS/MS, mobile phase (isocratic elution): 0.1 % formic acid Column: C3 column 2.1 x 150 mm, 5µm calibration with AA (Tracecert Fluka) internal standard: AA-d3 (ACR)
L21	no	0.05 g	Addition of 1.5 mL water and ninhydrin (10 mg/g). Extraction at 85 °C for 2 hours at 1,100 rpm in an MHR 13 thermomixer centrifuging for 10 min at 14,100×g. Protein precipitation in the aqueous phase by addition of 150 µL of 0.68 M potassium hexacyanoferrate(II) trihydrate solution (Carrez I) and 150 µL of a 2 M zinc sulfate heptahydrate solution (Carrez II). Centrifuging for 2 min at 14,100×g. Extraction with ethyl followed by centrifugation (0.5 min, 14,500/min) and addition of water. Clean-up on Isolute Multimode cartridge.	LC-MS/MS Column: C3 column 2.1 x 150 mm, 5µm calibration with AA (Ultra Scientific) internal standard: AA-^{13}C3 (99 %, CIL)

6.2 Analytical results and statistical evaluation

The analytical results of the certification inter-laboratory comparison are listed in Tables 11 to 16. These tables show the single results (M_i) of each laboratory, the respective laboratories' mean values (M), absolute and relative intra-laboratory standard deviation (s and s_{rel}, respectively), the standard deviation of laboratory means (s_M), and in addition the square root of mean of variances of data sets under repeatability conditions ($\bar{s}_i$) where n is the number of accepted data sets. The continuous line marks the certified value (mean of the laboratories' means), the broken lines mark the standard deviation, calculated from the laboratories' means.
In the related figures for each laboratory its mean value and single standard deviation is given. Outliers which have been excluded are highlighted in yellow.

Table 11: Results for Cd in ERM-BD513

Lab./Meth.	L03	L19	L11	L18	L12	L05	L17	L20	L22	L07	L14	L16	L15	L09	L23	L04	L10	L21	L01		
M_i [µg/kg]	155.2	153.0	157.1	159.0	154.0	165.0	180.9	184.0	195.4	185.0	188.7	193.1	195.0	197.0	197.9	201.0	204.0	200.3	210.0		*n*
	152.2	147.0	163.7	159.0	177.0	171.0	179.5	175.0	179.0	184.0	184.0	182.1	190.0	198.0	197.8	195.0	199.0	208.8	211.0		19
	147.8	156.0	162.4	159.0	168.0		165.6	176.0	180.0	191.0	188.7	189.7	195.0	196.0	197.9	206.0	203.0	198.3	207.0		
	145.2	147.0	152.6	161.0	155.0		170.6	165.0	172.2	186.0	185.2	186.9	195.0	194.0	197.5	197.0	195.0	208.0	212.0		
***M* [µg/kg]**	**150.1**	**150.8**	**159.0**	**159.5**	**163.5**	**168.0**	**174.2**	**175.0**	**181.7**	**186.5**	**186.7**	**188.0**	**193.8**	**196.3**	**197.8**	**199.8**	**200.3**	**203.9**	**210.0**		**181.3**
s [µg/kg]	4.5	4.5	5.1	1.0	11.0	4.2	7.3	7.8	9.8	3.1	2.4	4.7	2.5	1.7	0.2	4.9	4.1	5.3	2.2	s_M [µg/kg]	18.631
																				$\bar{s}_i$ [µg/kg]	5.3147
s_{rel}	0.02972	0.02985	0.03212	0.00627	0.06746	0.02525	0.04192	0.04451	0.05395	0.01667	0.01282	0.02475	0.01290	0.00870	0.00102	0.02431	0.02054	0.02613	0.01029		0.10278

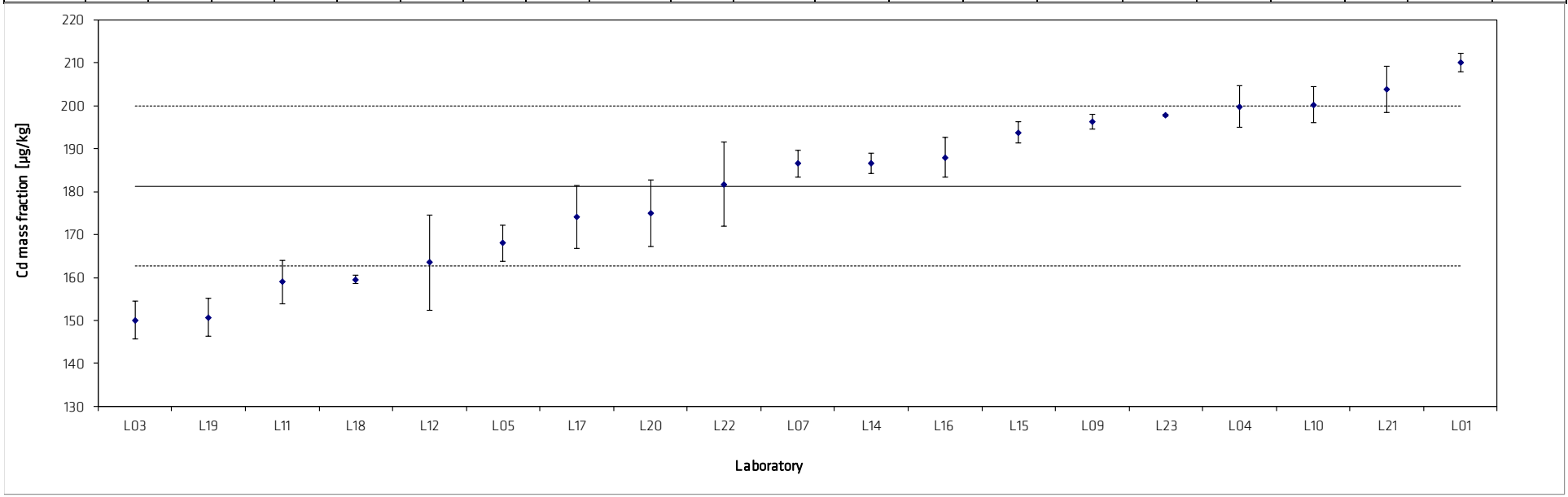


Table 12: Results for Cd in ERM-BD514

Laborcode	L19	L03	L18	L11	L17	L12	L07	L20	L15	L14	L22	L04	L05	L10	L23	L09	L16	L21	L01		
M_i [µg/kg]	452.0	508.1	470.0	472.8	521.1	508.0	554.0	570.0	552.0	554.0	588.2	564.0	555.0	571.0	573.6	573.0	582.6	593.0	607.0		*n*
	463.0	451.7	471.0	499.9	525.3	489.0	548.0	558.0	558.0	555.0	556.6	566.0	582.0	578.0	573.4	574.0	559.7	605.3	603.0		19
	454.0	418.0	471.0	483.3	489.4	513.0	542.0	552.0	557.0	556.3	558.2	574.0		585.0	574.7	577.0	587.6	589.1	605.0		
	455.0	481.2	472.0	476.9	462.8	516.0	541.0	537.0	553.0	555.7	534.4	564.0		552.0	570.0	579.0	579.2	601.8	593.0		
***M* [µg/kg]**	**456.0**	**464.8**	**471.0**	**483.2**	**499.7**	**506.5**	**546.3**	**554.3**	**555.0**	**555.2**	**559.3**	**567.0**	**568.5**	**571.5**	**572.9**	**575.8**	**577.3**	**597.3**	**602.0**		**541.2**
s [µg/kg]	4.8	38.8	0.8	11.9	29.3	12.1	6.0	13.7	2.9	1.0	22.1	4.8	19.1	14.2	2.0	2.8	12.2	7.5	6.2	s_M [µg/kg]	45.892
																				$\bar{s}_i$ [µg/kg]	14.9428
s_{rel}	0.01059	0.08339	0.00173	0.02468	0.05870	0.02394	0.01102	0.02475	0.00530	0.00178	0.03948	0.00840	0.03358	0.02485	0.00356	0.00478	0.02116	0.01260	0.01033		0.08479

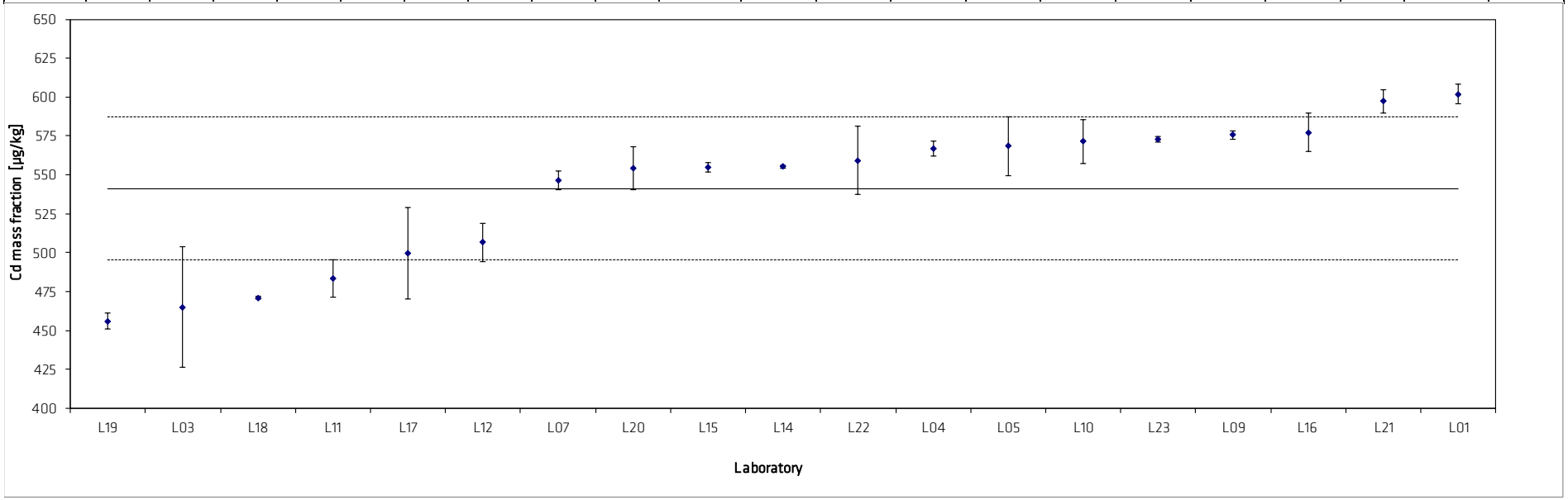


Table 13: Results for Cd in ERM-BD515

Laborcode	L19	L18	L11	L03	L12	L05	L20	L14	L17	L15	L07	L04	L23	L09	L22	L16	L10	L21	L01		
M_i [µg/kg]	582.0	597.0	594.4	611.9	670.0	663.0	690.0	706.7	681.5	692.0	718.0	720.0	726.6	725.0	728.7	736.9	756.0	762.9	773.0		*n*
	594.0	602.0	610.2	639.6	635.0	652.0	693.0	693.3	702.2	695.0	699.0	719.0	725.1	729.0	761.8	717.4	718.0	765.3	759.0		19
	595.0	597.0	607.6	599.5	662.0		692.0	674.5	707.5	696.0	709.0	715.0	725.5	723.0	721.0	733.5	767.0	763.2	778.0		
	606.0	604.0	598.1	601.9	660.0		695.0	695.9	687.9	699.0	720.0	710.0	723.8	726.0	696.5	732.9	740.0	763.3	770.0		
M **[µg/kg]**	**594.3**	**600.0**	**602.6**	**613.2**	**656.8**	**657.5**	**692.5**	**692.6**	**694.8**	**695.5**	**711.5**	**716.0**	**725.3**	**725.8**	**727.0**	**730.2**	**745.3**	**763.7**	**770.0**		**690.2**
s [µg/kg]	9.8	3.6	7.5	18.4	15.1	7.8	2.1	13.4	12.1	2.9	9.6	4.5	1.2	2.5	27.0	8.7	21.3	1.1	8.0	s_M [µg/kg]	55.053
																				$\bar{s}_i$ [µg/kg]	11.6389
s_{rel}	0.01651	0.00593	0.01250	0.02998	0.02304	0.01183	0.00301	0.01934	0.01744	0.00415	0.01351	0.00635	0.00159	0.00344	0.03708	0.01191	0.02856	0.00144	0.01044		0.07976

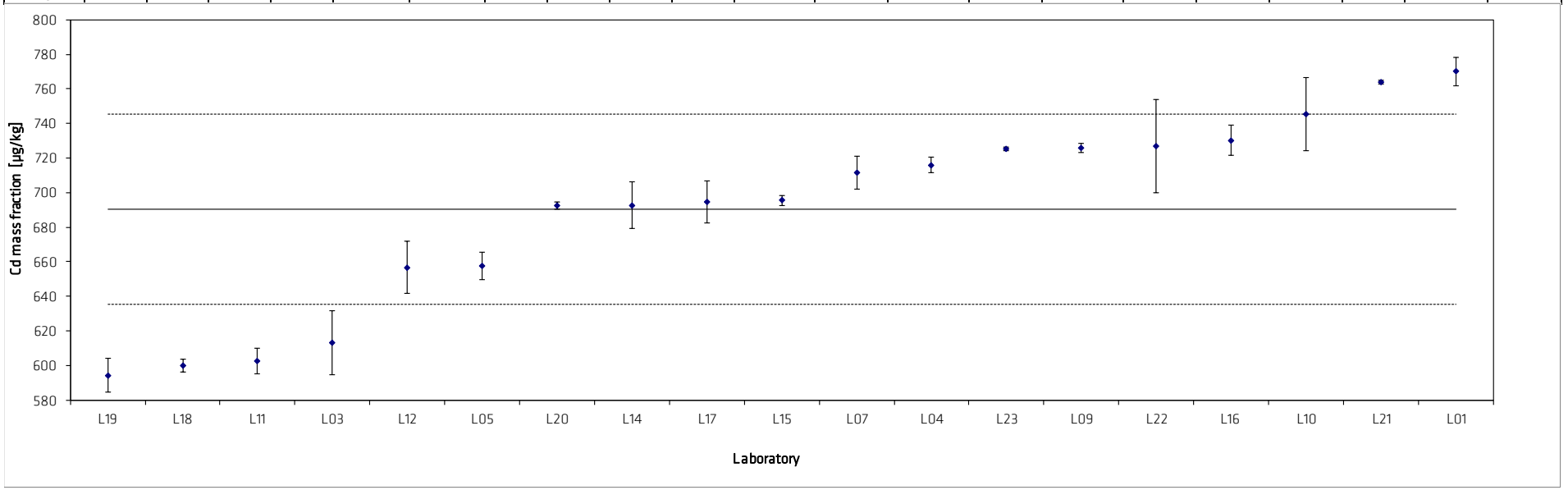


Table 14: Results for acrylamide in ERM-BD513

Lab./Meth.	L11	L07	L08	L16	L06	L18	L10	L21	L20	L05	L12	L02		
M_i [µg/kg]	30.5	29.8	32.3	33.6	31.8	61.0	47.8	78.9	109.0	95.5	168.0	426.0		*n*
	30.1	30.5	30.6	36.8	36.0	38.0	52.0	64.5	89.0	105.5	124.0	429.0		10
	19.5	32.0	32.0	38.6	33.5	35.0	44.1	46.9	105.0		132.0	398.2		
	19.7	31.1	30.9	27.1	35.1	37.0	44.7	60.0	98.0		79.8	420.1		
M **[µg/kg]**	**24.9**	**30.9**	**31.5**	**34.0**	**34.1**	**42.8**	**47.2**	**62.6**	**100.3**	**100.5**	**126.0**	**418.3**		**50.9**
s [µg/kg]	6.2	0.9	0.8	5.1	1.8	12.2	3.6	13.2	8.8	7.1	36.2	13.9	s_M [µg/kg]	28.155
													$\bar{s}_i$ [µg/kg]	7.3035
s_{rel}	0.24770	0.03023	0.02628	0.14928	0.05423	0.28609	0.07671	0.21128	0.08748	0.07036	0.28768	0.03327		0.55357

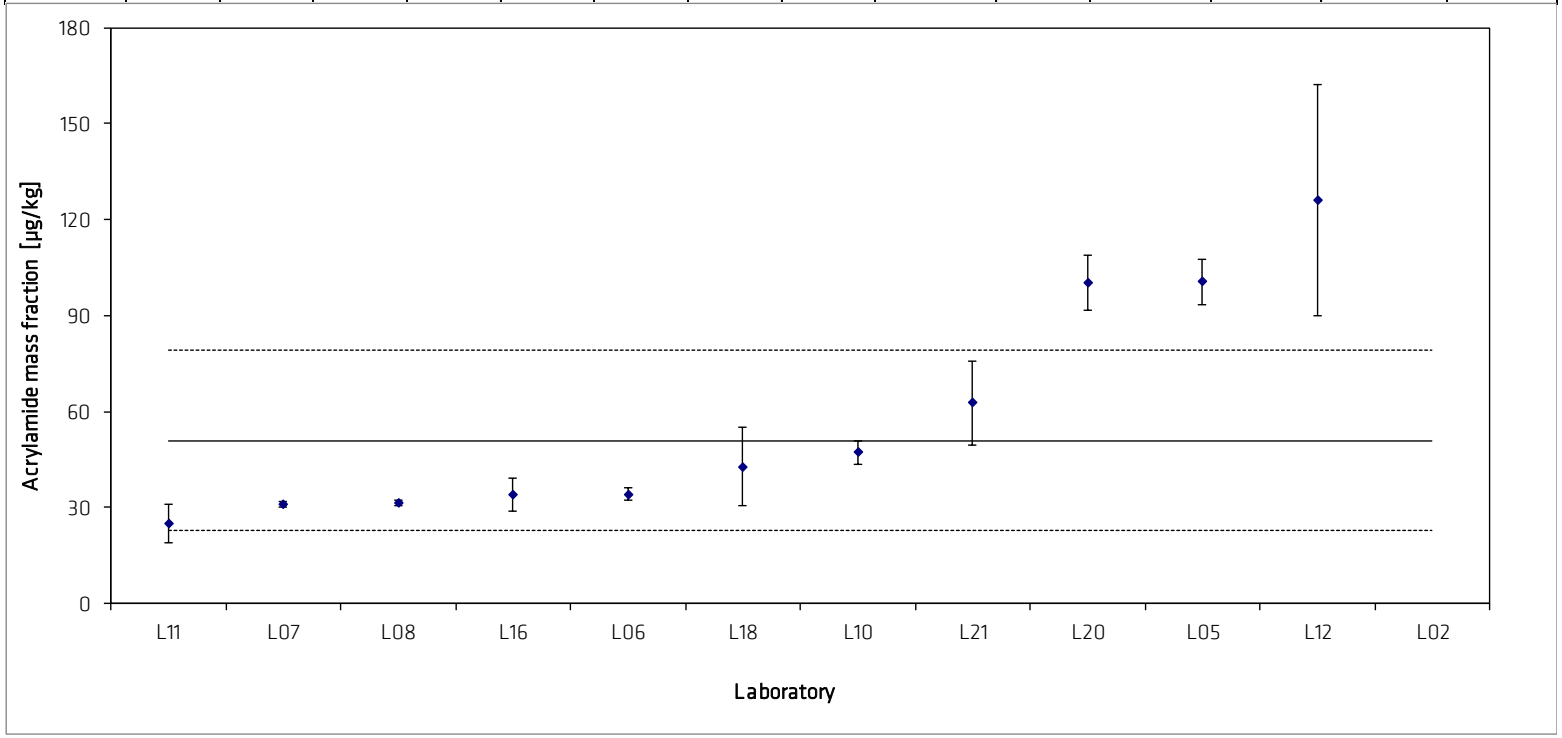


Table 15: Results for acrylamide in ERM-BD514

Lab./Meth.	L18	L08	L06	L16	L07	L10	L21	L11	L05	L12	L02		
M_i [µg/kg]		78.5	81.3	86.7	80.8	100.7	89.9	111.7	223.6	286.0	816.0		*n*
		75.5	82.2		90.8	89.3	95.7	108.5	160.0	203.0	673.0		9
	71.0	81.2	79.7	79.5	99.9	94.8	99.0	124.7		278.0	1005.0		
	79.0	79.3	76.9	74.4	84.8	86.1	116.7	120.9		181.0	1100.0		
***M* [µg/kg]**	**75.0**	**78.6**	**80.0**	**80.2**	**89.1**	**92.7**	**100.3**	**116.4**	**191.8**	**237.0**	**898.5**		**100.5**
s [µg/kg]	5.7	2.4	2.3	6.2	8.3	6.4	11.5	7.6	45.0	52.8	191.1	s_M [µg/kg]	36.642
												$\bar{s}_i$ [µg/kg]	16.3454
s_{rel}	0.07542	0.03016	0.02906	0.07730	0.09323	0.06920	0.11491	0.06518	0.23447	0.22292	0.21273		0.36472

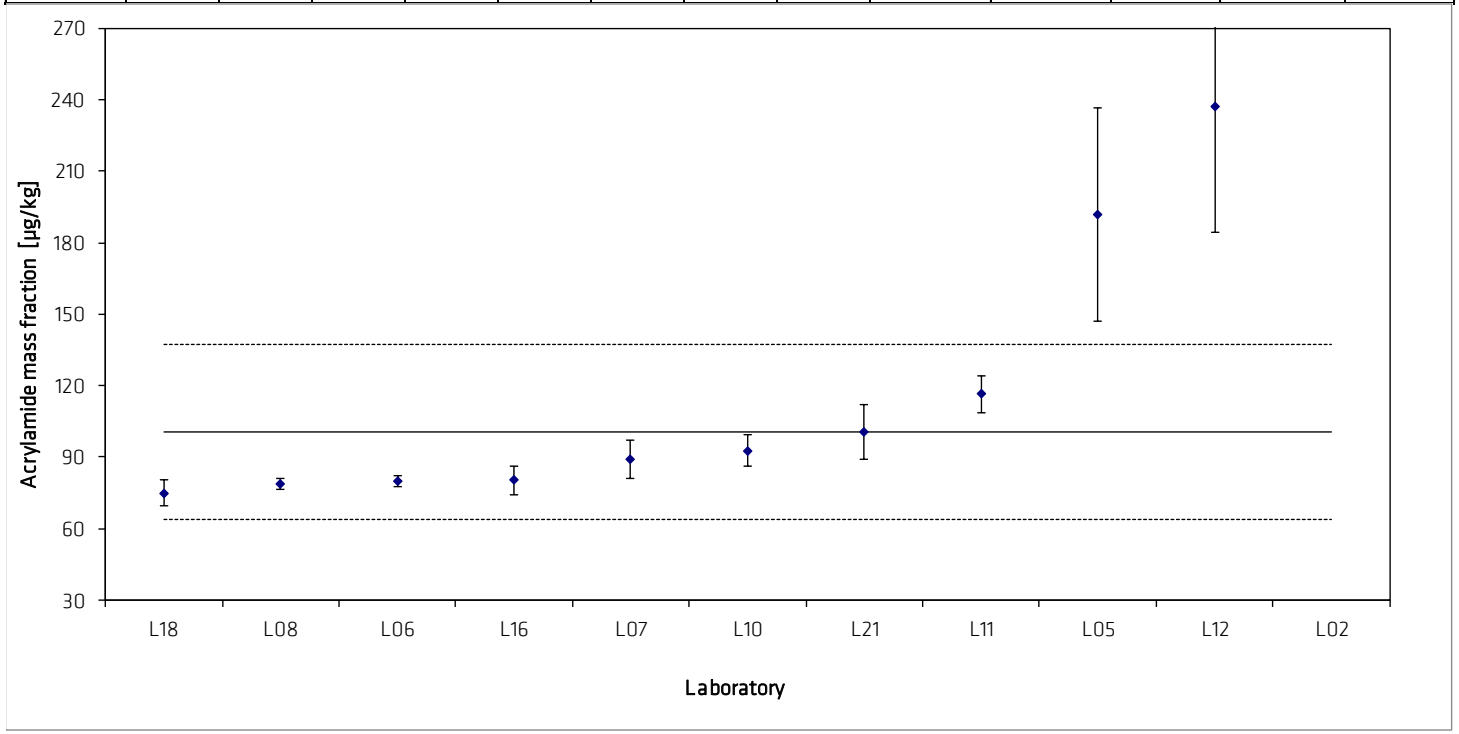
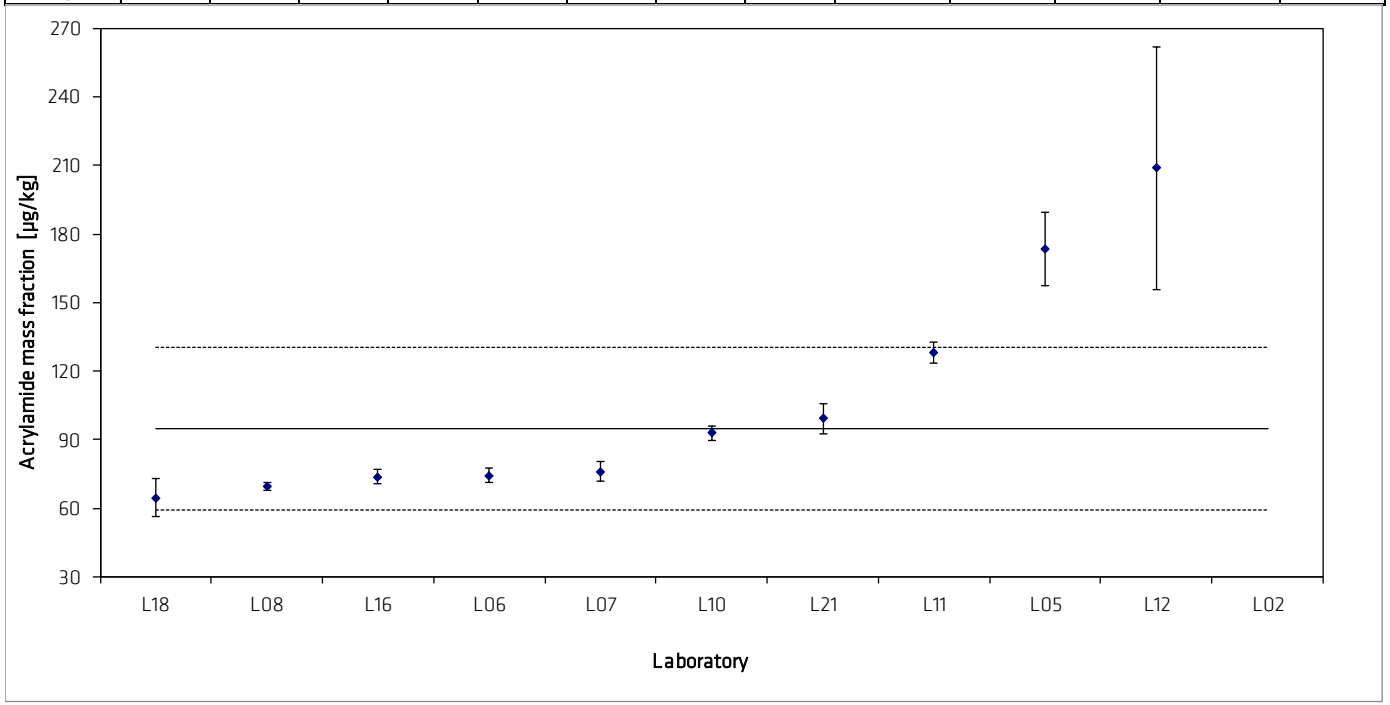


Table 16: Results for acrylamide in ERM-BD515

Lab./Meth.	L18	L08	L16	L06	L07	L10	L21	L11	L05	L12	L02		
M_i [µg/kg]	52.0	69.8	72.8	72.9	69.9	96.9	89.9	134.6	185.0	256.0	709.0		*n*
	69.0	72.1	78.5	74.9	78.5	91.6	103.5	128.9	162.3	154.0	660.2		9
	68.0	67.8	72.5	71.0	78.7	90.0	103.9	124.4		253.0	755.9		
	69.0	68.4	71.5	78.7	77.0	92.7	99.7	125.6		172.0	870.0		
***M* [µg/kg]**	**64.5**	**69.5**	**73.8**	**74.4**	**76.0**	**92.8**	**99.2**	**128.4**	**173.7**	**208.8**	**748.8**		**94.7**
s [µg/kg]	8.3	1.9	3.2	3.3	4.2	2.9	6.5	4.6	16.1	53.4	89.8	s_M [µg/kg]	35.591
												$\bar{s}_i$ [µg/kg]	7.0009
s_{rel}	0.12941	0.02748	0.04289	0.04429	0.05463	0.03178	0.06559	0.03567	0.09243	0.25557	0.11988		0.37583



The statistical evaluation of the data was performed using the software program SoftCRM 1.2.2. [5]. The following results were obtained:

Table 17: Outcome of statistical tests of results obtained for Cd in ERM-BD513

Number of data sets	19
Snedecor-F-Test and Bartlett-Test	Pooling not allowed
Dixon (α = 0.05)	---
Dixon (α = 0.01)	---
Nalimov (α = 0.05)	---
Nalimov (α = 0.01)	---
Grubbs (α = 0.05)	---
Grubbs (α = 0.01)	---
Grubbs Pair (α = 0.05)	---
Grubbs Pair (α = 0.01)	---
Cochran (α = 0.05)	---
Cochran (α = 0.01)	---
Kolmogorov-Smirnov-Lilliefors Test (α = 0.05)	Distribution: normal
Kolmogorov-Smirnov-Lilliefors Test (α = 0.01)	Distribution: normal
Skewness & Kurtosis Test (α = 0.05)	Distribution: normal
Skewness & Kurtosis Test (α = 0.01)	Distribution: normal

Table 18: Outcome of statistical tests of results obtained for Cd in ERM-BD514

Number of data sets	19
Snedecor-F-Test and Bartlett-Test	Pooling not allowed
Dixon (α = 0.05)	---
Dixon (α = 0.01)	---
Nalimov (α = 0.05)	---
Nalimov (α = 0.01)	---
Grubbs (α = 0.05)	---
Grubbs (α = 0.01)	---
Grubbs Pair (α = 0.05)	---
Grubbs Pair (α = 0.01)	---
Cochran (α = 0.05)	L03, L17, L22
Cochran (α = 0.01)	L03, L17
Kolmogorov-Smirnov-Lilliefors Test (α = 0.05)	Distribution: not normal
Kolmogorov-Smirnov-Lilliefors Test (α = 0.01)	Distribution: not normal
Skewness & Kurtosis Test (α = 0.05)	Distribution: normal
Skewness & Kurtosis Test (α = 0.01)	Distribution: normal

Table 19: Outcome of statistical tests of results obtained for Cd in ERM-BD515

Number of data sets	19
Snedecor-F-Test and Bartlett-Test	Pooling not allowed
Dixon (α = 0.05)	---
Dixon (α = 0.01)	---
Nalimov (α = 0.05)	---
Nalimov (α = 0.01)	---
Grubbs (α = 0.05)	---
Grubbs (α = 0.01)	---
Grubbs Pair (α = 0.05)	---
Grubbs Pair (α = 0.01)	---
Cochran (α = 0.05)	L22, L10
Cochran (α = 0.01)	L22
Kolmogorov-Smirnov-Lilliefors Test (α = 0.05)	Distribution: normal
Kolmogorov-Smirnov-Lilliefors Test (α = 0.01)	Distribution: normal
Skewness & Kurtosis Test (α = 0.05)	Distribution: normal
Skewness & Kurtosis Test (α = 0.01)	Distribution: normal

Table 20: Outcome of statistical tests of results obtained for acrylamide in ERM-BD513 (statistical evaluation without Lab. 02)

Number of data sets	10
Snedecor-F-Test and Bartlett-Test	Pooling not allowed
Dixon (α = 0.05)	---
Dixon (α = 0.01)	---
Nalimov (α = 0.05)	L12
Nalimov (α = 0.01)	---
Grubbs (α = 0.05)	---
Grubbs (α = 0.01)	---
Grubbs Pair (α = 0.05)	---
Grubbs Pair (α = 0.01)	---
Cochran (α = 0.05)	L12, L18
Cochran (α = 0.01)	L12
Kolmogorov-Smirnov-Lilliefors Test (α = 0.05)	Distribution: not normal
Kolmogorov-Smirnov-Lilliefors Test (α = 0.01)	Distribution: not normal
Skewness & Kurtosis Test (α = 0.05)	Distribution: normal
Skewness & Kurtosis Test (α = 0.01)	Distribution: normal

Lab. 02 was removed as obvious erroneous value. Lab. 12 was removed as technical outlier.

Table 21: Outcome of statistical tests of results obtained for acrylamide in ERM-BD514 (statistical evaluation without Lab. 02)

Number of data sets	10
Snedecor-F-Test and Bartlett-Test	Pooling not allowed
Dixon (α = 0.05)	---
Dixon (α = 0.01)	---
Nalimov (α = 0.05)	L12
Nalimov (α = 0.01)	---
Grubbs (α = 0.05)	L12
Grubbs (α = 0.01)	---
Grubbs Pair (α = 0.05)	---
Grubbs Pair (α = 0.01)	---
Cochran (α = 0.05)	L12, L05
Cochran (α = 0.01)	L12
Kolmogorov-Smirnov-Lilliefors Test (α = 0.05)	Distribution: not normal
Kolmogorov-Smirnov-Lilliefors Test (α = 0.01)	Distribution: not normal
Skewness & Kurtosis Test (α = 0.05)	Distribution: not normal
Skewness & Kurtosis Test (α = 0.01)	Distribution: not normal

Lab. 02 was removed as obvious erroneous value. Lab. 12 was removed as technical outlier.

Table 22: Outcome of statistical tests of results obtained for acrylamide in ERM-BD515 (statistical evaluation without Lab. 02)

Number of data sets	11
Snedecor-F-Test and Bartlett-Test	Pooling not allowed
Dixon (α = 0.05)	---
Dixon (α = 0.01)	---
Nalimov (α = 0.05)	L12
Nalimov (α = 0.01)	---
Grubbs (α = 0.05)	---
Grubbs (α = 0.01)	---
Grubbs Pair (α = 0.05)	---
Grubbs Pair (α = 0.01)	---
Cochran (α = 0.05)	L12, L05
Cochran (α = 0.01)	L12, L05, L18
Kolmogorov-Smirnov-Lilliefors Test (α = 0.05)	Distribution: not normal
Kolmogorov-Smirnov-Lilliefors Test (α = 0.01)	Distribution: not normal
Skewness & Kurtosis Test (α = 0.05)	Distribution: not normal
Skewness & Kurtosis Test (α = 0.01)	Distribution: not normal

Lab. 02 was removed as obvious erroneous value. Lab. 12 was removed as technical outlier.

The certified mass fractions of Cd as well as the informative mass fractions of acrylamide were calculated as mean of the accepted data sets.
The resp. combined uncertainties were calculated using Equation 2, taking into account contributions from the spread resulting from the certification inter-laboratory comparison (u_{ilc}), from possible

inhomogeneity (u_{bb}) and from long-term stability for Cd (u_{lts}) of the material and uncertainty terms for purity of calibrant (u_{pur}) and recovery (u_{rec}), see Table 23a/b.

$$u_{combined} = \sqrt{u_{ilc} + u_{bb} + u_{lts} + u_{pur} + u_{rec}} \quad (2)$$

with

$u_{ilc} = \sqrt{\frac{s_M^2}{n}}$: uncertainty contribution resulting from inter-laboratory comparison

n : number of data sets used for calculating the mean of means of each analyte

Table 23a: uncertainty contributions for Cd (all data in mg/kg)

CRM	M	u_{ilc}	u_{bb}	u_{lts}	u_{pur}	u_{rec}
BD513	0.181	0.0043	0.0	0	0.00092	0
BD514	0.541	0.0106	0.0029	0	0.00275	0
BD515	0.690	0.0127	0.0040	0	0.00350	0

Table 23b: uncertainty contributions for acrylamide (all data in mg/kg)

CRM	M	u_{ilc}	u_{lts}	u_{bb}	u_{pur}	u_{rec}
BD513	0.0509	0.00892	0	0.0	0.00030	0.00073
BD514	0.1005	0.01222	0	0.0023	0.00058	0.00144
BD515	0.0947	0.01187	0	0.0	0.00055	0.00135

The expanded uncertainties U are calculated by multiplication of $u_{combined}$ with a coverage factor of k = 2 using Equation 3.

$$U = k \cdot u_{combined} \quad (3)$$

Rounding of all values was performed following DIN 1333 [6]. The calculated and certified mass fractions and their resp. expanded uncertainties are given on Page 3 of this report.

7. Transport, storage and use

The certified reference materials ERM®-BD513, ERM®-BD514 and ERM®-BD515 are intended for the calibration and quality control of analytical methods used for the analysis of similar materials.
The minimum sample intake for Cd-determination is 0.5 g, for the determination of acrylamide at least 0.05 g of powder have to be taken.
The material is to dispatch in polystyrene boxes with cooling packs (stored at -20°C prior to use). On receiving, it is to be stored at a temperature equal to or lower than -20 °C. Before taking a sub-sample for analysis the bag has to have reached ambient temperature. Thereafter, the bag must be closed

tightly and stored at a temperature equal to or lower than -20 °C. The water content remains stable when the material is treated as described.

8. Information on and purchase of the CRM

Certified reference materials ERM-BD513, ERM-BD514 and ERM-BD515 are supplied by

Bundesanstalt für Materialforschung und -prüfung (BAM)
Division 1.6 „Inorganic Reference Materials“
Richard-Willstätter-Str. 11, D-12489 Berlin, Germany
Phone +49 (0)30 - 8104 2061
Fax: +49 (0)30 – 8104 72061
E-Mail: sales.crm@bam.de

Each bag of ERM-BD513, ERM-BD514 and ERM-BD515 will be distributed together with a detailed certificate containing the certified values and their uncertainties, the mean values and standard deviations of all accepted data sets and information on the analytical methods used and the names of the participating laboratories.
Information on certified reference materials can be obtained from BAM:
https://www.bam.de.
Tel. +49 30 8104 1111.

9. References

[1] ISO 17034, General requirements for the competence of reference material producers, 2016

[2] ISO Guide 31, Reference materials - Contents of certificates, labels and accompanying documentation, 2015

[3] ISO Guide 35, Reference materials - Guidance for characterization and assessment of homogeneity and stability, 2017

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Annex 1: Determination of moisture using Karl-Fischer-titration

ERM-BD513

Sample	sample intake [g]	Water, abs. [µg]	Water [mg/kg]	Water [%]	blank corrected [µg]	Water [mg/kg]	Water [%]	M water [%]	s water [%]	s(rel) water [%]
22	0.1038	5354.8	51587.7	5.16	5260.8	50682.4	5.07	**5.06**	**0.01**	**0.14**
	0.1028	5290.2	51461.1	5.15	5196.2	50547.0	5.05			
	0.1031	5310.4	51507.3	5.15	5216.4	50595.9	5.06			
72	0.1062	5422.3	51057.4	5.11	5328.3	50172.6	5.02	**5.06**	**0.10**	**1.89**
	0.1045	5305.5	50770.3	5.08	5211.5	49871.1	4.99			
	0.0994	5228.5	52600.6	5.26	5134.5	51655.3	5.17			
93	0.1028	5268.6	51251.0	5.13	5174.6	50336.9	5.03	**5.03**	**0.02**	**0.34**
	0.1037	5288.5	50998.1	5.10	5194.5	50091.9	5.01			
	0.1010	5186.4	51350.5	5.14	5092.4	50420.1	5.04			
130	0.1041	5295.2	50866.5	5.09	5201.2	49963.8	5.00	**5.01**	**0.02**	**0.31**
	0.0983	5031.3	51183.1	5.12	4937.3	50227.2	5.02			
	0.0963	4932.6	51221.2	5.12	4838.6	50245.4	5.02			
153	0.1028	5173.0	50321.0	5.03	5079.0	49406.9	4.94	**5.02**	**0.08**	**1.57**
	0.1033	5360.5	51892.5	5.19	5266.5	50982.9	5.10			
	0.0963	4932.6	51221.2	5.12	4838.6	50245.4	5.02			
203	0.0953	4806.2	50432.3	5.04	4712.2	49446.3	4.94	**4.91**	**0.04**	**0.83**
	0.0959	4801.0	50062.6	5.01	4707.0	49082.7	4.91			
	0.1038	5142.1	49538.5	4.95	5048.1	48633.3	4.86			
230	0.0953	4806.2	50432.3	5.04	4712.2	49446.3	4.94	**4.96**	**0.11**	**2.27**
	0.1001	5185.0	51798.2	5.18	5091.0	50859.5	5.09			
	0.1038	5142.1	49538.5	4.95	5048.1	48633.3	4.86			
257	0.0972	4936.7	50789.1	5.08	4842.7	49822.4	4.98	**5.01**	**0.03**	**0.54**
	0.0981	5034.6	51321.1	5.13	4940.6	50363.2	5.04			
	0.1055	5379.4	50989.6	5.10	5285.4	50098.9	5.01			
305	0.1014	5188.7	51170.6	5.12	5094.7	50243.9	5.02	**4.99**	**0.03**	**0.58**
	0.1018	5153.4	50622.8	5.06	5059.4	49699.7	4.97			
	0.1046	5303.5	50702.7	5.07	5209.5	49804.3	4.98			
355	0.0953	4887.4	51284.4	5.13	4793.4	50298.4	5.03	**5.05**	**0.02**	**0.38**
	0.1003	5157.7	51422.7	5.14	5063.7	50485.9	5.05			
	0.0992	5121.8	51631.0	5.16	5027.8	50683.8	5.07			
371	0.1000	5252.6	52526.0	5.25	5158.6	51586.3	5.16	**5.16**	**0.00**	**0.05**
	0.1045	5483.5	52473.7	5.25	5389.5	51574.5	5.16			
	0.1038	5443.3	52440.3	5.24	5349.3	51535.0	5.15			
407	0.1014	5286.0	52130.2	5.21	5192.0	51203.5	5.12	**5.14**	**0.02**	**0.41**
	0.1032	5406.8	52391.5	5.24	5312.8	51480.9	5.15			
	0.1040	5462.6	52525.0	5.25	5368.6	51621.5	5.16			
437	0.1017	5216.7	51295.0	5.13	5122.7	50371.0	5.04	**5.09**	**0.06**	**1.13**
	0.1042	5395.6	51781.2	5.18	5301.6	50879.4	5.09			
	0.1040	5452.1	52424.0	5.24	5358.1	51520.5	5.15			
473	0.0963	5032.3	52256.5	5.23	4938.3	51280.7	5.13	**5.14**	**0.06**	**1.09**
	0.1042	5507.5	52855.1	5.29	5413.5	51953.3	5.20			
	0.0957	4960.0	51828.6	5.18	4866.0	50846.7	5.08			
499	0.1004	5135.0	51145.4	5.11	5041.0	50209.5	5.02	**5.02**	**0.02**	**0.47**
	0.1000	5083.9	50839.0	5.08	4989.9	49899.3	4.99			
	0.0996	5109.7	51302.2	5.13	5015.7	50358.8	5.04			
M							**5.04**			
s							**0.08**			
s(rel)							**1.57**			

ERM-BD514

Sample	sample intake [g]	Water, abs. [µg]	Water [mg/kg]	Water [%]	blank corrected [µg]	Water [mg/kg]	Water [%]	M water [%]	s water [%]	s(rel) water [%]
45	0.1073	3867.2	36041.0	3.60	3750.0	34948.4	3.49	**3.50**	**0.04**	**1.19**
	0.1025	3756.5	36648.8	3.66	3639.3	35505.0	3.55			
	0.1031	3694.0	35829.3	3.58	3576.8	34692.2	3.47			
71	0.0965	3425.8	35500.5	3.55	3308.6	34285.7	3.43	**3.49**	**0.07**	**1.95**
	0.1017	3652.2	35911.5	3.59	3535.0	34758.8	3.48			
	0.1090	4000.4	36700.9	3.67	3883.2	35625.4	3.56			
90	0.0989	3506.9	35459.0	3.55	3389.7	34273.7	3.43	**3.52**	**0.10**	**2.78**
	0.0992	3711.5	37414.3	3.74	3594.3	36232.5	3.62			
	0.1021	3712.7	36363.4	3.64	3595.5	35215.1	3.52			
168	0.1083	3835.0	35410.9	3.54	3717.8	34328.4	3.43	**3.60**	**0.15**	**4.30**
	0.1049	3919.1	37360.3	3.74	3801.9	36242.8	3.62			
	0.0979	3777.5	38585.3	3.86	3660.3	37387.8	3.74			
196	0.1048	3846.8	36706.1	3.67	3729.6	35587.5	3.56	**3.54**	**0.11**	**3.16**
	0.1003	3541.9	35313.1	3.53	3424.7	34144.2	3.41			
	0.1045	3915.3	37467.0	3.75	3798.1	36345.1	3.63			
253	0.0999	3939.9	39438.4	3.94	3822.7	38264.9	3.83	**3.63**	**0.17**	**4.75**
	0.1053	3824.0	36315.3	3.63	3706.8	35202.0	3.52			
	0.1041	3798.7	36490.9	3.65	3681.5	35364.7	3.54			
314	0.0986	3707.6	37602.4	3.76	3590.4	36413.5	3.64	**3.66**	**0.03**	**0.76**
	0.1022	3844.4	37616.4	3.76	3727.2	36469.3	3.65			
	0.1064	4045.6	38022.6	3.80	3928.4	36920.7	3.69			
373	0.0978	3625.9	37074.6	3.71	3508.7	35875.9	3.59	**3.68**	**0.08**	**2.17**
	0.0942	3642.7	38669.9	3.87	3525.5	37425.3	3.74			
	0.0964	3681.4	38188.8	3.82	3564.2	36972.7	3.70			
439	0.0901	3538.4	39271.9	3.93	3421.2	37970.8	3.80	**3.70**	**0.09**	**2.35**
	0.1073	4006.7	37341.1	3.73	3889.5	36248.5	3.62			
	0.0930	3548.2	38152.7	3.82	3431.0	36892.1	3.69			
544	0.0960	3717.9	38728.1	3.87	3600.7	37506.9	3.75	**3.73**	**0.04**	**0.96**
	0.0948	3614.9	38131.9	3.81	3497.7	36895.2	3.69			
	0.0991	3835.9	38707.4	3.87	3718.7	37524.4	3.75			
571	0.1103	4310.1	39076.2	3.91	4192.9	38013.3	3.80	**3.83**	**0.14**	**3.59**
	0.1056	4319.6	40905.3	4.09	4202.4	39795.1	3.98			
	0.1062	4056.6	38197.7	3.82	3939.4	37093.8	3.71			
608	0.1036	4170.3	40253.9	4.03	4053.1	39122.3	3.91	**3.87**	**0.06**	**1.68**
	0.1086	4239.1	39034.1	3.90	4121.9	37954.6	3.80			
	0.0931	3750.6	40285.7	4.03	3633.4	39026.5	3.90			
662	0.1011	3837.6	37958.5	3.80	3720.4	36798.9	3.68	**3.70**	**0.02**	**0.52**
	0.1015	3885.9	38284.7	3.83	3768.7	37129.7	3.71			
	0.1015	3885.9	38284.7	3.83	3768.7	37129.7	3.71			
710	0.1109	4342.4	39156.0	3.92	4225.2	38098.9	3.81	**3.89**	**0.11**	**2.92**
	0.0948	3924.4	41396.6	4.14	3807.2	40160.0	4.02			
	0.1064	4193.4	39411.7	3.94	4076.2	38309.8	3.83			
765	0.1088	4529.2	41628.7	4.16	4412.0	40551.2	4.06	**4.13**	**0.07**	**1.69**
	0.0988	4261.2	43129.6	4.31	4144.0	41943.0	4.19			
	0.1022	4333.8	42405.1	4.24	4216.6	41258.0	4.13			
M							**3.70**			
s							**0.19**			
s(rel)							**5.06**			

ERM-BD515

Sample	sample intake [g]	Water, abs. [µg]	Water [mg/kg]	Water [%]	blank corrected [µg]	Water [mg/kg]	Water [%]	M water [%]	s water [%]	s(rel) water [%]
6	0.1044	3719.2	35624.5	3.56	3629.1	34761.0	3.48	**3.53**	**0.10**	**2.78**
	0.0993	3531.1	35559.9	3.56	3441.0	34652.1	3.47			
	0.0974	3635.6	37326.5	3.73	3545.5	36400.9	3.64			
63	0.1037	3635.8	35060.8	3.51	3545.7	34191.4	3.42	**3.47**	**0.05**	**1.42**
	0.1013	3616.5	35700.9	3.57	3526.4	34811.0	3.48			
	0.1016	3662.6	36049.2	3.60	3572.5	35161.9	3.52			
95	0.1005	3692.5	36741.3	3.67	3602.4	35844.3	3.58	**3.61**	**0.03**	**0.78**
	0.0982	3636.1	37027.5	3.70	3546.0	36109.5	3.61			
	0.1055	3931.5	37265.4	3.73	3841.4	36410.9	3.64			
148	0.0984	3773.1	38344.5	3.83	3683.0	37428.4	3.74	**3.74**	**0.03**	**0.89**
	0.1037	3931.7	37914.2	3.79	3841.6	37044.8	3.70			
	0.1030	3974.1	38583.5	3.86	3884.0	37708.3	3.77			
217	0.1022	3915.1	38308.2	3.83	3825.0	37426.1	3.74	**3.72**	**0.03**	**0.90**
	0.0957	3611.3	37735.6	3.77	3521.2	36793.6	3.68			
	0.1063	4054.9	38145.8	3.81	3964.8	37297.7	3.73			
301	0.1093	3988.4	36490.4	3.65	3898.3	35665.6	3.57	**3.59**	**0.03**	**0.78**
	0.1016	3728.8	36700.8	3.67	3638.7	35813.5	3.58			
	0.0986	3660.1	37120.7	3.71	3570.0	36206.4	3.62			
347	0.0968	3658.6	37795.5	3.78	3568.5	36864.2	3.69	**3.66**	**0.04**	**0.98**
	0.0991	3675.2	37085.8	3.71	3585.1	36176.1	3.62			
	0.1054	3958.9	37560.7	3.76	3868.8	36705.4	3.67			
405	0.0998	3688.1	36954.9	3.70	3598.0	36051.6	3.61	**3.62**	**0.02**	**0.55**
	0.1081	4003.5	37035.2	3.70	3913.4	36201.2	3.62			
	0.1017	3796.8	37333.3	3.73	3706.7	36446.9	3.64			
451	0.1015	3688.1	36336.0	3.63	3598.0	35447.8	3.54	**3.57**	**0.02**	**0.61**
	0.1049	3833.0	36539.6	3.65	3742.9	35680.2	3.57			
	0.1087	3990.4	36710.2	3.67	3900.3	35880.9	3.59			
513	0.1042	3797.6	36445.3	3.64	3707.5	35580.1	3.56	**3.57**	**0.02**	**0.49**
	0.1075	3918.8	36454.0	3.65	3828.7	35615.3	3.56			
	0.1031	3791.4	36774.0	3.68	3701.3	35899.6	3.59			
573	0.1004	3595.2	35808.8	3.58	3505.1	34910.9	3.49	**3.51**	**0.03**	**0.94**
	0.0987	3542.9	35895.6	3.59	3452.8	34982.3	3.50			
	0.1021	3716.2	36397.6	3.64	3626.1	35514.7	3.55			
610	0.1066	3808.9	35730.8	3.57	3718.8	34885.1	3.49	**3.51**	**0.03**	**0.77**
	0.1046	3755.0	35898.7	3.59	3664.9	35036.8	3.50			
	0.1076	3900.0	36245.4	3.62	3809.9	35407.5	3.54			
705	0.1087	3936.3	36212.5	3.62	3846.2	35383.2	3.54	**3.54**	**0.03**	**0.83**
	0.1023	3681.1	35983.4	3.60	3591.0	35102.2	3.51			
	0.0970	3552.3	36621.6	3.66	3462.2	35692.3	3.57			
732	0.0963	3474.8	36083.1	3.61	3384.7	35146.9	3.51	**3.54**	**0.03**	**0.84**
	0.1097	3967.8	36169.6	3.62	3877.7	35347.8	3.53			
	0.1043	3816.8	36594.4	3.66	3726.7	35730.1	3.57			
788	0.1031	3761.3	36482.1	3.65	3671.2	35607.7	3.56	**3.57**	**0.01**	**0.15**
	0.0996	3647.2	36618.5	3.66	3557.1	35713.4	3.57			
	0.0971	3553.7	36598.4	3.66	3463.6	35669.9	3.57			
M							**3.58**			
s							**0.08**			
s(rel)							**2.25**			

Annex 2: Homogeneity testing

Cd in ERM-BD513:

	Measurement results in mg/kg					
Sample #	**Sub-sample 1**	**Sub-sample 2**	**Sub-sample 3**	**Mean**	SD	RSD (%)
022	0.2176	0.2141	0.2153	0.2157	0.0018	0.82
072	0.2100	0.2152	0.2109	0.2120	0.0028	1.31
093	0.2139	0.2161	0.2126	0.2142	0.0018	0.83
130	0.2108	0.2167	0.2151	0.2142	0.0031	1.42
153	0.2120	0.2082	0.2131	0.2111	0.0026	1.22
203	0.2140	0.2170	0.2106	0.2139	0.0032	1.50
230	0.2097	0.2105	0.2156	0.2119	0.0032	1.51
257	0.2116	0.2129	0.2163	0.2136	0.0024	1.14
305	0.2074	0.2150	0.2136	0.2120	0.0040	1.91
355	0.2129	0.2119	0.2141	0.2130	0.0011	0.52
371	0.2112	0.2156	0.2133	0.2134	0.0022	1.03
407	0.2098	0.2137	0.2154	0.2130	0.0029	1.35
437	0.2085	0.2144	0.2132	0.2120	0.0031	1.47
473	0.2136	0.2140	0.2147	0.2141	0.0006	0.26
499	0.2107	0.2167	0.2097	0.2124	0.0038	1.78
Mean	0.2116	0.2141	0.2136	**0.2131**	(MM)	
SD	0.0026	0.0025	0.0020	0.0012		
RSD (%)	1.21	1.15	0.92	0.57		

Cd in ERM-BD514:

	Measurement results in mg/kg					
Sample #	**Sub-sample 1**	**Sub-sample 2**	**Sub-sample 3**	**Mean**	SD	RSD (%)
045	0.6473	0.6634	0.6681	0.6596	0.0109	1.65
071	0.6460	0.6443	0.6537	0.6480	0.0050	0.77
090	0.6525	0.6449	0.6572	0.6515	0.0062	0.95
168	0.6557	0.6511	0.6606	0.6558	0.0048	0.72
196	0.6621	0.6593	0.6550	0.6588	0.0036	0.54
253	0.6596	0.6539	0.6511	0.6549	0.0043	0.66
314	0.6613	0.6578	0.6602	0.6598	0.0018	0.27
373	0.6524	0.6579	0.6606	0.6570	0.0042	0.64
439	0.6542	0.6510	0.6567	0.6540	0.0029	0.44
544	0.6559	0.6562	0.6578	0.6566	0.0010	0.16
571	0.6562	0.6416	0.6543	0.6507	0.0079	1.22
608	0.6456	0.6555	0.6546	0.6519	0.0055	0.84
662	0.6489	0.6589	0.6568	0.6549	0.0053	0.81
710	0.6425	0.6517	0.6422	0.6455	0.0054	0.84
765	0.6526	0.6536	0.6456	0.6506	0.0044	0.67
Mean	0.6529	0.6534	0.6556	**0.6540**	(MM)	
SD	0.00589	0.00613	0.00623	0.00422		
RSD (%)	0.90	0.94	0.95	0.65		

Cd in ERM-BD515:

	Measurement results in mg/kg					
Sample #	**Sub-sample 1**	**Sub-sample 2**	**Sub-sample 3**	**Mean**	SD	RSD (%)
006	0.8523	0.8431	0.8457	0.8470	0.0047	0.56
063	0.8349	0.8579	0.8532	0.8487	0.0122	1.43
095	0.8602	0.8581	0.8577	0.8587	0.0013	0.16
148	0.8247	0.8234	0.8513	0.8331	0.0157	1.89
217	0.8563	0.8329	0.8412	0.8435	0.0119	1.41
301	0.8365	0.8274	0.8413	0.8351	0.0071	0.85
347	0.8395	0.8507	0.8451	0.8451	0.0056	0.66
405	0.8486	0.8435	0.8434	0.8452	0.0030	0.35
451	0.8296	0.8397	0.8453	0.8382	0.0080	0.95
513	0.8326	0.8394	0.8557	0.8426	0.0119	1.41
573	0.8456	0.8538	0.8390	0.8461	0.0074	0.88
610	0.8398	0.8402	0.8468	0.8423	0.0039	0.47
705	0.8407	0.8414	0.8411	0.8411	0.0004	0.04
732	0.8382	0.8394	0.8395	0.8390	0.0007	0.09
788	0.8364	0.8487	0.8294	0.8382	0.0098	1.17
Mean	0.8411	0.8426	0.8450	**0.8429**	(MM)	
SD	0.00988	0.01012	0.00728	0.00621		
RSD (%)	1.17	1.20	0.86	0.74		

Acrylamide in ERM-BD513:

	Measurement results in µg/kg					
Sample #	**Sub-sample 1**	**Sub-sample 2**	**Sub-sample 3**	**Mean**	SD	RSD (%)
6	191.6178	174.8953	180.6084	182.3738	8.4998	4.66
63	193.7010	180.4015	190.3636	188.1554	6.9193	3.68
95	197.3366	190.1584	183.2023	190.2324	7.0674	3.72
148	183.1212	196.7781	180.9402	186.9465	8.5839	4.59
217	179.0689	195.8398	198.4432	191.1173	10.5151	5.50
301	184.7962	190.6220	195.5511	190.3231	5.3837	2.83
347	176.2116	190.8839	194.9252	187.3402	9.8472	5.26
405	200.4943	199.1143	199.5419	199.7169	0.7064	0.35
451	174.4848	187.2993	196.8825	186.2222	11.2376	6.03
513	189.8151	185.0878	195.1060	190.0030	5.0118	2.64
573	184.7693	195.1917	186.3677	188.7762	5.6131	2.97
610	189.8175	182.9298	191.6742	188.1405	4.6071	2.45
705	191.9691	183.6703	183.8953	186.5116	4.7277	2.53
732	183.8661	179.6315	189.6396	184.3791	5.0237	2.72
788	190.7492	187.9028	196.0631	191.5717	4.1419	2.16
Mean	187.4546	188.0271	190.8803	**188.7873**	(MM)	
SD	7.4513	7.0028	6.4697	3.9387		
RSD (%)	3.97	3.72	3.39	2.09		

Acrylamide in ERM-BD514:

	Measurement results in µg/kg					
Sample #	**Sub-sample 1**	**Sub-sample 2**	**Sub-sample 3**	**Mean**	SD	RSD (%)
45	170.0357	188.8332	188.1502	182.3397	10.6611	5.85
71	187.8879	188.6980	183.1249	186.5703	3.0111	1.61
90	190.6086	177.6712	186.4712	184.9170	6.6072	3.57
168	177.5036	173.3345	181.6216	177.4866	4.1436	2.33
196	190.8518	188.9877	179.2322	186.3572	6.2404	3.35
253	180.5328	191.0361	171.3490	180.9727	9.8509	5.44
314	187.8398	190.5259	185.0342	187.8000	2.7461	1.46
373	178.8530	194.1844	198.3009	190.4461	10.2487	5.38
439	164.1996	171.4623	177.2947	170.9855	6.5606	3.84
544	197.0873	195.1969	178.6566	190.3136	10.1394	5.33
571	184.7518	194.0272	184.8696	187.8829	5.3215	2.83
608	191.8511	176.7024	178.5251	182.3595	8.2703	4.54
662	196.1883	188.6864	176.5730	187.1492	9.8976	5.29
710	162.1783	186.5955	183.7815	177.5184	13.3593	7.53
765	186.4460	193.2107	173.3433	184.3333	10.1008	5.48
Mean	183.1210	186.6102	181.7552	**183.8288**	(MM)	
SD	10.8261	7.8724	6.6308	5.3582		
RSD (%)	5.91	4.22	3.65	2.91		

Acrylamide in ERM-BD515:

	Measurement results in µg/kg					
Sample #	**Sub-sample 1**	**Sub-sample 2**	**Sub-sample 3**	**Mean**	SD	RSD (%)
6	191.6178	174.8953	180.6084	182.3738	8.4998	4.66
63	193.7010	180.4015	190.3636	188.1554	6.9193	3.68
95	197.3366	190.1584	183.2023	190.2324	7.0674	3.72
148	183.1212	196.7781	180.9402	186.9465	8.5839	4.59
217	179.0689	195.8398	198.4432	191.1173	10.5151	5.50
301	184.7962	190.6220	195.5511	190.3231	5.3837	2.83
347	176.2116	190.8839	194.9252	187.3402	9.8472	5.26
405	200.4943	199.1143	199.5419	199.7169	0.7064	0.35
451	174.4848	187.2993	196.8825	186.2222	11.2376	6.03
513	189.8151	185.0878	195.1060	190.0030	5.0118	2.64
573	184.7693	195.1917	186.3677	188.7762	5.6131	2.97
610	189.8175	182.9298	191.6742	188.1405	4.6071	2.45
705	191.9691	183.6703	183.8953	186.5116	4.7277	2.53
732	183.8661	179.6315	189.6396	184.3791	5.0237	2.72
788	190.7492	187.9028	196.0631	191.5717	4.1419	2.16
Mean	187.4546	188.0271	190.8803	**188.7873**	(MM)	
SD	7.4513	7.0028	6.4697	3.9387		
RSD (%)	3.97	3.72	3.39	2.09		

Results of ANOVA:

Cd	in mg/kg						
BD513	**property**	**factor**	**squares**	**dof**	**MS**	**F**	**p value**
	Cd content	between	0.000061008	14	4.35771E-06	0.587029316	0.853830989
		within	0.0002227	30	7.42333E-06		
		total	0.000283708	44			
inhom contribution:		0					
BD514	**property**	**factor**	**squares**	**dof**	**MS**	**F**	**p value**
	Cd content	between	0.00074963	14	5.3545E-05	1.844061322	0.078190049
		within	0.000871093	30	2.90364E-05		
		total	0.001620723	44			
inhom contribution:		0.002858236					
BD515	**property**	**factor**	**squares**	**dof**	**MS**	**F**	**p value**
	Cd content	between	0.001619232	14	0.000115659	1.698437769	0.109251384
		within	0.002042927	30	6.80976E-05		
		total	0.003662159	44			
inhom contribution:		0.003981704					

Acrylamide	in microgram/kilogram						
BD513	**property**	**factor**	**squares**	**dof**	**MS**	**F**	**p value**
	AA content	between	651.5599452	14	46.53999609	0.93123103	0.538731443
		within	1499.305584	30	49.9768528		
		total	2150.865529	44			
inhom contribution:		0					
BD514	**property**	**factor**	**squares**	**dof**	**MS**	**F**	**p value**
	AA content	between	1205.841894	14	86.13156383	1.22678889	0.307762298
		within	2106.268597	30	70.20895324		
		total	3312.110491	44			
inhom contribution:		2.303809208					
BD515	**property**	**factor**	**squares**	**dof**	**MS**	**F**	**p value**
	AA content	between	651.5599452	14	46.53999609	0.93123103	0.538731443
		within	1499.305584	30	49.9768528		
		total	2150.865529	44			
inhom contribution:		0					

Annex 3: Stability

Short-term stability Cd in ERM-BD513

Cadmium short-term stability ERM® - BD513 *blank corrected					
Sample-No.	Week	Temperature	Day of preparation	Cd mg/kg	Mean*
513-001	1	-80°C	1	0.21	
			2	0.22	
			3	0.23	
			4	0.23	0.222
513-158	2		1	0.21	
			2	0.22	
			3	0.23	
			4	0.24	0.224
513-277	3		1	0.21	
			2	0.22	
			3	0.23	
			4	0.23	0.224
513-406	4		1	0.21	
			2	0.23	
			3	0.24	
			4	0.23	0.225
513-044	1	-21°C	1	0.22	
			2	0.23	
			3	0.23	
			4	0.23	0.224
513-189	2		1	0.21	
			2	0.22	
			3	0.23	
			4	0.24	0.224
513-307	3		1	0.21	
			2	0.22	
			3	0.23	
			4	0.24	0.225
513-439	4		1	0.23	
			2	0.23	
			3	0.23	
			4	0.24	0.231

Cadmium short-term stability ERM® - BD513 *blank corrected					
Sample-No.	Week	Temperature	Day of preparation	Cd mg/kg	Mean*
513-069	1	RT (23°C)	1	0.21	
			2	0.23	
			3	0.23	
			4	0.23	0.226
513-217	2		1	0.21	
			2	0.22	
			3	0.23	
			4	0.24	0.225
513-346	3		1	0.20	
			2	0.23	
			3	0.23	
			4	0.24	0.225
513-468	4		1	0.23	
			2	0.23	
			3	0.23	
			4	0.24	0.231
513-096	1	40°C	1	0.22	
			2	0.22	
			3	0.23	
			4	0.23	0.225
513-249	2		1	0.21	
			2	0.22	
			3	0.23	
			4	0.24	0.225
513-367	3		1	0.20	
			2	0.24	
			3	0.23	
			4	0.23	0.226
513-474	4		1	0.23	
			2	0.23	
			3	0.23	
			4	0.24	0.229
513-133	1	60°C	1	0.21	
			2	0.22	
			3	0.23	
			4	0.24	0.227
513-276	2		1	0.21	
			2	0.22	
			3	0.23	
			4	0.25	0.229
513-379	3		1	0.21	
			2	0.23	
			3	0.23	
			4	0.24	0.228
513-496	4		1	0.23	
			2	0.23	
			3	0.22	
			4	0.24	0.229

Temperature	1 week	2 weeks	3 weeks	4 weeks
-80	0.22	0.22	0.22	0.22
-20	0.22	0.22	0.22	0.23
RT	0.23	0.22	0.23	0.23
40	0.23	0.23	0.23	0.23
60	0.23	0.23	0.23	0.23

Short-term stability Cd in ERM-BD514

Cadmium short-term stability ERM® - BD514 *blank corrected					
Sample-No.	Week	Temperature	Day of preparation	Cd mg/kg	Mean*
514-003	1	-80°C	1	0.65	
			2	0.63	
			3	0.63	
			4	0.67	0.645
514-187	2		1	0.63	
			2	0.63	
			3	0.63	
			4	0.68	0.644
514-430	3		1	0.64	
			2	0.63	
			3	0.63	
			4	0.68	0.642
514-643	4		1	0.64	
			2	0.63	
			3	0.63	
			4	0.67	0.640
514-040	1	-21°C	1	0.66	
			2	0.63	
			3	0.59	
			4	0.67	0.636
514-269	2		1	0.65	
			2	0.63	
			3	0.62	
			4	0.68	0.645
514-461	3		1	0.63	
			2	0.63	
			3	0.63	
			4	0.67	0.638
514-686	4		1	0.64	
			2	0.62	
			3	0.63	
			4	0.67	0.641

Cadmium short-term stability ERM® - BD514 *blank corrected					
Sample-No.	Week	Temperature	Day of preparation	Cd mg/kg	Mean*
514-072	1	RT (23°C)	1	0.64	
			2	0.63	
			3	0.63	
			4	0.68	0.642
514-280	2		1	0.64	
			2	0.63	
			3	0.62	
			4	0.68	0.638
514-496	3		1	0.64	
			2	0.64	
			3	0.63	
			4	0.68	0.648
514-725	4		1	0.64	
			2	0.63	
			3	0.62	
			4	0.66	0.637
514-089	1	40°C	1	0.64	
			2	0.63	
			3	0.62	
			4	0.68	0.645
514-357	2		1	0.65	
			2	0.63	
			3	0.64	
			4	0.68	0.650
514-515	3		1	0.64	
			2	0.62	
			3	0.63	
			4	0.67	0.640
514-771	4		1	0.64	
			2	0.63	
			3	0.63	
			4	0.67	0.643
514-164	1	60°C	1	0.65	
			2	0.62	
			3	0.64	
			4	0.68	0.647
514-371	2		1	0.64	
			2	0.63	
			3	0.63	
			4	0.68	0.644
514-605	3		1	0.64	
			2	0.62	
			3	0.63	
			4	0.67	0.638
514-792	4		1	0.64	
			2	0.63	
			3	0.62	
			4	0.66	0.638

Temperature	1 week	2 weeks	3 weeks	4 weeks
-80	0.64	0.64	0.64	0.64
-20	0.64	0.65	0.64	0.64
RT	0.64	0.64	0.65	0.64
40	0.64	0.65	0.64	0.64
60	0.65	0.64	0.64	0.64

Short-term stability Cd in ERM-BD515

Cadmium short-term stability ERM® - BD515 *blank corrected					
Sample-No.	Week	Temperature	Day of preparation	Cd mg/kg	Mean*
515-002	1	-80°C	1	0.83	
			2	0.87	
			3	0.95	
			4	0.91	0.889
515-202	2		1	0.85	
			2	0.84	
			3	0.92	
			4	0.93	0.882
515-426	3		1	0.84	
			2	0.83	
			3	0.94	
			4	0.92	0.884
515-644	4		1	0.86	
			2	0.84	
			3	0.92	
			4	0.90	0.879
515-050	1	-21°C	1	0.85	
			2	0.86	
			3	0.92	
			4	0.91	0.885
515-257	2		1	0.85	
			2	0.86	
			3	0.92	
			4	0.93	0.889
515-498	3		1	0.83	
			2	0.83	
			3	0.93	
			4	0.89	0.870
515-673	4		1	0.87	
			2	0.86	
			3	0.89	
			4	0.90	0.881

Cadmium short-term stability ERM® - BD515 *blank corrected					
Sample-No.	Week	Temperature	Day of preparation	Cd mg/kg	Mean*
515-085	1	RT (23°C)	1	0.86	
			2	0.86	
			3	0.93	
			4	0.92	0.893
515-277	2		1	0.84	
			2	0.85	
			3	0.90	
			4	0.93	0.878
515-522	3		1	0.85	
			2	0.86	
			3	0.94	
			4	0.93	0.894
515-726	4		1	0.85	
			2	0.85	
			3	0.92	
			4	0.91	0.886
515-113	1	40°C	1	0.87	
			2	0.85	
			3	0.91	
			4	0.92	0.889
515-365	2		1	0.83	
			2	0.83	
			3	0.94	
			4	0.89	0.873
515-553	3		1	0.86	
			2	0.85	
			3	0.93	
			4	0.92	0.890
515-769	4		1	0.86	
			2	0.84	
			3	0.93	
			4	0.92	0.888
515-167	1	60°C	1	0.86	
			2	0.86	
			3	0.93	
			4	0.92	0.891
515-391	2		1	0.85	
			2	0.85	
			3	0.92	
			4	0.92	0.885
515-589	3		1	0.85	
			2	0.85	
			3	0.92	
			4	0.91	0.885
515-795	4		1	0.86	
			2	0.85	
			3	0.91	
			4	0.90	0.879

Temperature	1 week	2 weeks	3 weeks	4 weeks
-80	0.89	0.88	0.88	0.88
-20	0.89	0.89	0.87	0.88
RT	0.89	0.88	0.89	0.89
40	0.89	0.87	0.89	0.89
60	0.89	0.88	0.88	0.88

Long-term stability Cd in ERM-BD513

Cadmium long term stability
ERM® - BD513
*blank corrected

Sample-No.	Month	Temperature	Day of preparation	Cd mg/kg	Mean*
513-010	3	-80°C	1	0.23	
			2	0.23	
			3	0.22	
			4	0.22	0.225
513-134	6		1	0.23	
			2	0.23	
			3	0.22	
			4	0.22	0.226
513-261	9		1	0.22	
			2	0.22	
			3	0.22	
			4	0.22	0.221
513-388	12		1	0.22	
			2	0.22	
			3	0.22	
			4	0.23	0.223
513-036	3	-21°C	1	0.23	
			2	0.23	
			3	0.22	
			4		0.225
513-152	6		1	0.23	
			2	0.23	
			3	0.22	
			4	0.22	0.223
513-289	9		1	0.22	
			2	0.23	
			3	0.22	
			4	0.22	0.224
513-435	12		1	0.22	
			2	0.23	
			3	0.22	
			4	0.23	0.224

Cadmium long term stability
ERM® - BD513
*blank corrected

Sample-No.	Month	Temperature	Day of preparation	Cd mg/kg	Mean*
513-079	3	RT (23°C)	1	0.23	
			2	0.23	
			3	0.22	
			4	0.22	0.224
513-210	6		1	0.23	
			2	0.23	
			3	0.22	
			4	0.22	0.224
513-318	9		1	0.22	
			2	0.23	
			3	0.22	
			4	0.23	0.222
513-463	12		1	0.23	
			2	0.22	
			3	0.22	
			4	0.23	0.226
513-102	3	40°C	1	0.23	
			2	0.23	
			3	0.22	
			4	0.22	0.224
513-240	6		1	0.22	
			2	0.23	
			3	0.22	
			4	0.22	0.221
513-350	9		1	0.22	
			2	0.23	
			3	0.22	
			4	0.23	0.223
513-486	12		1	0.22	
			2	0.22	
			3	0.22	
			4	0.23	0.223

ERM® - BD513

Temperature	3. Month	6. Month	9. Month	12. Month
-80	0.23	0.23	0.22	0.22
-20	0.23	0.22	0.22	0.22
RT	0.22	0.22	0.22	0.23
40	0.22	0.22	0.22	0.22

Long-term stability Cd in ERM-BD514

Cadmium long term stability ERM® - BD514 *blank corrected					
Sample-No.	Month	Temperature	Day of preparation	Cd mg/kg	Mean*
514-021	3	-80°C	1	0.65	
			2	0.64	
			3	0.65	
			4	0.66	0.651
514-230	6		1	0.64	
			2	0.64	
			3	0.65	
			4	0.65	0.648
514-408	9		1	0.63	
			2	0.64	
			3	0.64	
			4	0.64	0.639
514-617	12		1	0.63	
			2	0.63	
			3	0.64	
			4	0.65	0.640
514-078	3	-21°C	1	0.64	
			2	0.64	
			3	0.66	
			4	0.66	0.649
514-248	6		1	0.65	
			2	0.64	
			3	0.65	
			4	0.65	0.647
514-473	9		1	0.63	
			2	0.64	
			3	0.65	
			4	0.64	0.639
514-677	12		1	0.63	
			2	0.62	
			3	0.65	
			4	0.64	0.637

Cadmium long term stability ERM® - BD514 *blank corrected					
Sample-No.	Month	Temperature	Day of preparation	Cd mg/kg	Mean*
514-146	3	RT (23°C)	1	0.62	
			2	0.64	
			3	0.66	
			4	0.65	0.645
514-311	6		1	0.63	
			2	0.62	
			3	0.64	
			4	0.65	0.638
514-536	9		1	0.62	
			2	0.64	
			3	0.64	
			4	0.65	0.637
514-708	12		1	0.62	
			2	0.62	
			3	0.65	
			4	0.64	0.634
514-188	3	40°C	1	0.64	
			2	0.63	
			3	0.65	
			4	0.66	0.646
514-354	6		1	0.62	
			2	0.64	
			3	0.63	
			4	0.64	0.632
514-563	9		1	0.63	
			2	0.64	
			3	0.63	
			4	0.63	0.633
514-768	12		1	0.62	
			2	0.64	
			3	0.64	
			4	0.65	0.636

ERM® - BD514				
Temperature	3. Month	6. Month	9. Month	12. Month
-80	0.65	0.65	0.64	0.64
-20	0.65	0.65	0.64	0.64
RT	0.64	0.64	0.64	0.63
40	0.65	0.63	0.63	0.64

Long-term stability Cd in ERM-BD515

Cadmium long term stability
ERM® - BD515
*blank corrected

Sample-No.	Month	Temperature	Day of preparation	Cd mg/kg	Mean*
515-016	3	-80°C	1	0.80	
			2	0.79	
			3	0.81	
			4	0.80	0.801
515-225	6		1	0.78	
			2	0.78	
			3	0.80	
			4	0.80	0.791
515-438	9		1	0.79	
			2	0.78	
			3	0.81	
			4	0.80	0.794
515-604	12		1	0.79	
			2	0.78	
			3	0.79	
			4	0.79	0.790
515-069	3	-21°C	1	0.80	
			2	0.79	
			3	0.81	
			4	0.81	0.799
515-240	6		1	0.79	
			2	0.79	
			3	0.80	
			4	0.80	0.794
515-489	9		1	0.79	
			2	0.78	
			3	0.81	
			4	0.79	0.792
515-652	12		1	0.78	
			2	0.78	
			3	0.80	
			4	0.79	0.786

Cadmium long term stability
ERM® - BD515
*blank corrected

Sample-No.	Month	Temperature	Day of preparation	Cd mg/kg	Mean*
515-125	3	RT (23°C)	1	0.78	
			2	0.78	
			3	0.80	
			4	0.80	0.790
515-296	6		1	0.79	
			2	0.79	
			3	0.79	
			4	0.80	0.790
515-519	9		1	0.78	
			2	0.79	
			3	0.79	
			4	0.80	0.790
515-727	12		1	0.78	
			2	0.78	
			3	0.79	
			4	0.79	0.785
515-170	3	40°C	1	0.79	
			2	0.79	
			3	0.80	
			4	0.80	0.794
515-403	6		1	0.78	
			2	0.78	
			3	0.80	
			4	0.79	0.786
515-581	9		1	0.79	
			2	0.78	
			3	0.79	
			4	0.79	0.789
515-797	12		1	0.79	
			2	0.79	
			3	0.79	
			4	0.79	0.788

ERM® - BD515

Temperature	3. Month	6. Month	9. Month	12. Month
-80	0.80	0.79	0.79	0.79
-20	0.80	0.79	0.79	0.79
RT	0.79	0.79	0.79	0.79
40	0.79	0.79	0.79	0.79

Short-term stability acrylamide in ERM-BD513

Acrylamide short-term stability ERM® - BD513 *blank corrected					
Sample-No.	Week	Temperature	Day of preparation	Cd mg/kg	Mean*
1	1	-80°C	1	152.43	
			2	178.43	
			3	175.06	
			4	161.12	166.757
158	2		1	161.03	
			2	165.36	
			3	174.37	
			4	165.94	166.675
277	3		1	167.23	
			2	153.81	
			3	175.65	
			4	173.93	167.656
406	4		1	174.82	
			2	153.88	
			3	170.10	
			4	163.37	165.543
44	1	-21°C	1	173.83	
			2	164.88	
			3	156.33	
			4	166.49	165.382
189	2		1	172.28	
			2	160.09	
			3	171.13	
			4	177.88	170.347
307	3		1	168.08	
			2	156.35	
			3	179.98	
			4	166.10	167.627
439	4		1	156.02	
			2	158.10	
			3	164.94	
			4	168.99	162.011

Acrylamide short-term stability ERM® - BD513 *blank corrected					
Sample-No.	Week	Temperature	Day of preparation	Cd mg/kg	Mean*
69	1	RT (23°C)	1	170.14	
			2	155.93	
			3	155.94	
			4	167.63	162.409
217	2		1	161.74	
			2	167.88	
			3	172.27	
			4	156.29	164.544
346	3		1	170.57	
			2	170.52	
			3	159.70	
			4	171.98	168.191
468	4		1	156.56	
			2	158.67	
			3	155.24	
			4	162.45	158.232
96	1	40°C	1	163.02	
			2	136.88	
			3	157.44	
			4	152.16	152.376
249	2		1	160.34	
			2	145.64	
			3	139.33	
			4	144.21	147.379
367	3		1	131.89	
			2	134.39	
			3	138.76	
			4	141.60	136.661
474	4		1	133.83	
			2	134.14	
			3	124.81	
			4	138.06	132.709
133	1	60°C	1	121.45	
			2	120.44	
			3	131.46	
			4	118.62	122.994
276	2		1	116.92	
			2	124.75	
			3	119.65	
			4	107.54	117.217
379	3		1	112.47	
			2	109.81	
			3	117.19	
			4	112.23	112.927
496	4		1	111.05	
			2	99.11	
			3	115.62	
			4	116.35	110.534

Temperature	1 week	2 weeks	3 weeks	4 weeks
-80	166.76	166.67	167.66	165.54
-20	165.38	170.35	167.63	162.01
RT	162.41	164.54	168.19	158.23
40	152.38	147.38	136.66	132.71
60	122.99	117.22	112.93	110.53

Short-term stability acrylamide in ERM-BD514

Acrylamide short-term stability ERM® - BD514 *blank corrected					
Sample-No.	Week	Temperature	Day of preparation	Cd mg/kg	Mean*
3	1	-80°C	1	230.33	
			2	214.59	
			3	202.09	
			4	206.33	213.334
187	2		1	208.40	
			2	229.43	
			3	225.71	
			4	204.45	216.995
430	3		1	234.30	
			2	214.01	
			3	201.67	
			4	203.19	213.289
643	4		1	199.00	
			2	215.43	
			3	207.78	
			4	199.16	205.341
40	1	-21°C	1	221.83	
			2	210.60	
			3	203.47	
			4	208.86	211.189
269	2		1	231.20	
			2	213.37	
			3	183.72	
			4	218.68	211.744
461	3		1	201.92	
			2	233.26	
			3	210.90	
			4	229.45	218.882
686	4		1	203.18	
			2	227.23	
			3	185.05	
			4	213.59	207.261

Acrylamide short-term stability ERM® - BD514 *blank corrected					
Sample-No.	Week	Temperature	Day of preparation	Cd mg/kg	Mean*
72	1	RT (23°C)	1	216.99	
			2	216.33	
			3	217.24	
			4	232.34	220.725
280	2		1	203.27	
			2	222.84	
			3	202.38	
			4	198.71	206.798
496	3		1	194.98	
			2	207.47	
			3	206.95	
			4	202.72	203.029
725	4		1	193.86	
			2	210.53	
			3	206.98	
			4	217.75	207.279
89	1	40°C	1	213.31	
			2	203.04	
			3	217.24	
			4	229.63	215.808
357	2		1	219.01	
			2	217.31	
			3	197.04	
			4	216.22	212.395
515	3		1	199.87	
			2	208.68	
			3	182.91	
			4	177.40	192.215
771	4		1	179.06	
			2	163.57	
			3	171.86	
			4	184.13	174.654
164	1	60°C	1	177.24	
			2	192.68	
			3	159.02	
			4	185.00	178.485
371	2		1	150.57	
			2	141.83	
			3	142.83	
			4	139.32	143.638
605	3		1	120.69	
			2	136.08	
			3	134.62	
			4	137.74	132.284
392	4		1	121.02	
			2	141.05	
			3	118.74	
			4	137.97	129.691

Temperature	1 week	2 weeks	3 weeks	4 weeks
-80	213.33	217.00	213.29	205.34
-20	211.19	211.74	218.88	207.26
RT	220.73	206.80	203.03	207.28
40	215.81	212.40	192.22	174.65
60	178.48	143.64	132.28	129.69

Short-term stability acrylamide in ERM-BD515

Acrylamide short-term stability ERM® - BD515 *blank corrected					
Sample-No.	Week	Temperature	Day of preparation	Cd mg/kg	Mean*
2	1	-80°C	1	206.11	
			2	203.70	
			3	204.20	
			4	182.24	199.064
202	2		1	190.00	
			2	199.83	
			3	191.45	
			4	176.57	189.463
426	3		1	174.74	
			2	180.23	
			3	193.74	
			4	185.37	183.520
644	4		1	194.76	
			2	199.13	
			3	207.34	
			4	188.35	197.393
50	1	-21°C	1	190.57	
			2	203.59	
			3	192.30	
			4	179.47	191.484
257	2		1	180.48	
			2	179.12	
			3	186.72	
			4	189.87	184.044
498	3		1	199.21	
			2	191.96	
			3	197.24	
			4	182.53	192.733
673	4		1	176.98	
			2	196.67	
			3	187.74	
			4	180.90	185.572

Acrylamide short-term stability ERM® - BD515 *blank corrected					
Sample-No.	Week	Temperature	Day of preparation	Cd mg/kg	Mean*
85	1	RT (23°C)	1	186.53	
			2	174.12	
			3	183.99	
			4	177.64	180.572
277	2		1	204.45	
			2	192.67	
			3	202.25	
			4	194.23	198.402
522	3		1	188.37	
			2	168.47	
			3	187.87	
			4	188.55	183.313
726	4		1	179.51	
			2	199.37	
			3	183.05	
			4	170.53	183.117
113	1	40°C	1	179.14	
			2	172.92	
			3	181.17	
			4	180.82	178.512
365	2		1	145.97	
			2	151.97	
			3	161.76	
			4	155.22	153.731
553	3		1	163.96	
			2	160.43	
			3	176.29	
			4	155.76	164.110
769	4		1	142.11	
			2	157.98	
			3	149.34	
			4	144.33	148.440
167	1	60°C	1	132.32	
			2	137.30	
			3	139.68	
			4	125.24	133.636
391	2		1	130.88	
			2	138.30	
			3	134.72	
			4	121.65	131.387
589	3		1	131.54	
			2	129.59	
			3	118.10	
			4	133.42	128.164
795	4		1	123.40	
			2	133.23	
			3	132.94	
			4	131.70	130.315

Temperature	1 week	2 weeks	3 weeks	4 weeks
-80	199.06	189.46	183.52	197.39
-20	191.48	184.04	192.73	185.57
RT	180.57	198.40	183.31	183.12
40	178.51	153.73	164.11	148.44
60	133.64	131.39	128.16	130.32