Bundesanstalt für Materialforschung und -prüfung

CERTIFICATION REPORT

The certification of the mass content of acrylamide in crispbread

Certified Reference Material ERM®-BD272

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SUMMARY

This report describes the certification of one crispbread material intended for the use of acrylamide determination in food. The preparation of the material is described, the results of the homogeneity and stability study, the measurement technique used and the results of the certification round are given.

The certified value and the uncertainties are:

	Certified value	Uncertainty
Compound	Mass fraction in mg/kg	
Acrylamide	0.98	± 0.09

The value given represents the unweighted mean value of 15 laboratory means using various analytical methods. The uncertainty given for those value represents the estimated expanded uncertainty U_{CRM} with a coverage factor of $k = 2$, corresponding to a level of confidence of about 95% as defined in the Guide to the Expression of Uncertainty in Measurement, ISO (1993).

LIST OF ABBREVIATIONS AND SYMBOLS

AA	Acrylamide
ANOVA	Analysis of Variance
BAM	Federal Institute for Materials Research and Testing
CI	Chemical ionisation
CRM	Certified Reference Material
D_3-AA	Deuterated Acrylamide
EI	Electron impact
ERM	European Reference Material
ESI	Electro-Spray-Ionisation
GC	Gas chromatography
GUM	Guide to the Expression of Uncertainty in Measurement
ISO	International Organization of Standardization
ISTD	Internal Standard
LC	Liquid chromatography
MS	Mass spectrometry
SIM	Selected ion monitoring
W.E.J.	Dr. Wiertz – Dipl.-Chem. Eggert – Dr. Jörissen GmbH

CONTENTS

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.

As Swedish scientists in April 2002 reported the occurrence of acrylamide in many baked or fried food, a worldwide surveillance of this substance in food products started. Since 2002 many different analytical methods used for quantifying this substance in foodstuffs have been developed [JEZUSSEK, M., SCHIEBERLE, P. 2003; HOENICKE, K. ET AL. 2004; GUTSCHE, B. ET AL. 2002; BIEDERMANN, M. ET AL. 2002] but have not been validated [WENZL, T. ET AL. 2004].

The candidate reference material ERM BD 272 to be certified was produced for the purpose of quality assurance and quality control for the determination of acrylamide in food. The material has been prepared from crispbread sample 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 material prepared at Federal Institute for Materials Research and Testing (BAM). Following internationally accepted procedures the certified mass fraction of acrylamide, its uncertainty and the shelf life were evaluated.

2 Production of the candidate material

2.1 Preparation of the candidate material

The bulk material (34 kg) was procured from W.E.J. GmbH in Hamburg. It was grinded and sieved with a final particle size smaller than 0.5 mm. This material was homogenised with a cement mixer for 90 min. Further homogenisation and bottling was done using a version of the so-called “cross riffling” procedure [VEEN, A.M.H. VAN DER; NATER, D.A.G. 1993].

A total of 500 units were bottled in 125 mL amber glass bottles containing (68 ± 1) g sealed with screw caps with Teflon inserts and numbered in the order of leaving the bottling process. The whole batch has been stored at -20°C since bottling was finished.

Table 2.1: Matrix characterisation

Measurand	Value	Method
Particle size range	< 500 µm	Dry sieving
Dry mass	94.3 %	Coulometric Karl-Fischer-Titration
C,H,N-Analysis	w(C) = 43.2 %, w(H) = 6.5 %, w(N) = 1.7 %	
pH	6 - 7	ISO 10390

2.2 Analytical method

Many different methods are available for the determination of acrylamide (AA). The method we used is a GC-MS method after bromination with the deuterium-labelled [$^2H_3$] acrylamide (D_3-AA) as internal standard (ISTD).

Sample preparation

Approximately 2 g of homogenised sample was weighed into a brown glass vial and spiked with approx. 1 g of ISTD-solution (1 µg/g). The sample was suspended in 30 mL water/methanol (20:10; v/v) and extracted for 30 min by shaking. The suspension was centrifuged for 15 min and the clear supernatant was decanted in an other brown glass vial. Then, the extract was derivatised by using 5 g potassium bromide, hydrobromic acid (acidification to pH 1-3) and 7 mL of saturated bromine-water solution. The flask was transferred to an ice bath for at least one hour, and the excess bromine was decomposed by adding sodium thiosulfate (1 M) as drops until the yellow colour disappeared. The solution was extracted twice with 20 mL of ethyl acetate. The organic phases were dried and evaporated with a rotary evaporator to approx. 1 mL. The residue was transferred to a column filled with 2 g silica gel and eluted with 10 mL of ethyl acetate. The eluate was evaporated to dryness and the final residue was redissolved in 1 mL of ethyl acetate. Then 50 µL triethylamine was added to convert 2,3-dibromopropionamide to more stable 2-bromopropenamide. 2 µL of the final extract were analysed by GC-MS.

Measurement and calibration

Table 2.2: Parameters of the GC-MS system

	Measurement
Gas chromatography	
Instrument	Finnigan Ultra GC
Capillary column	60 m x 0.32 mm, DB 5-MS
	Film thickness 0.25 µm
Carrier gas	Helium 5.0
	1,0 mL / min
Oven program	65 °C for 1 min,
	10 °C / min to 280 °C for 10 min
Injection	Temperature : 240 °C
	Volume: 1 µL, autosampler
Detection	
Mass spectrometer	Finnigan Trace DSQ
Ionisation	Electron impact (EI)
Voltage	70 eV
Ion source temperature	280 °C
Quadrupol temperature	200 °C
Modus	Selected ion monitoring (SIM)

The ions monitored for identification and quantification were m/z 70, 106 (quantifier) and 149 for 2-bromopropenamide and 109 and 153 (quantifier) for 2-bromo(d_3)-propenamide (see fig. 2.1 and fig. 2.2).

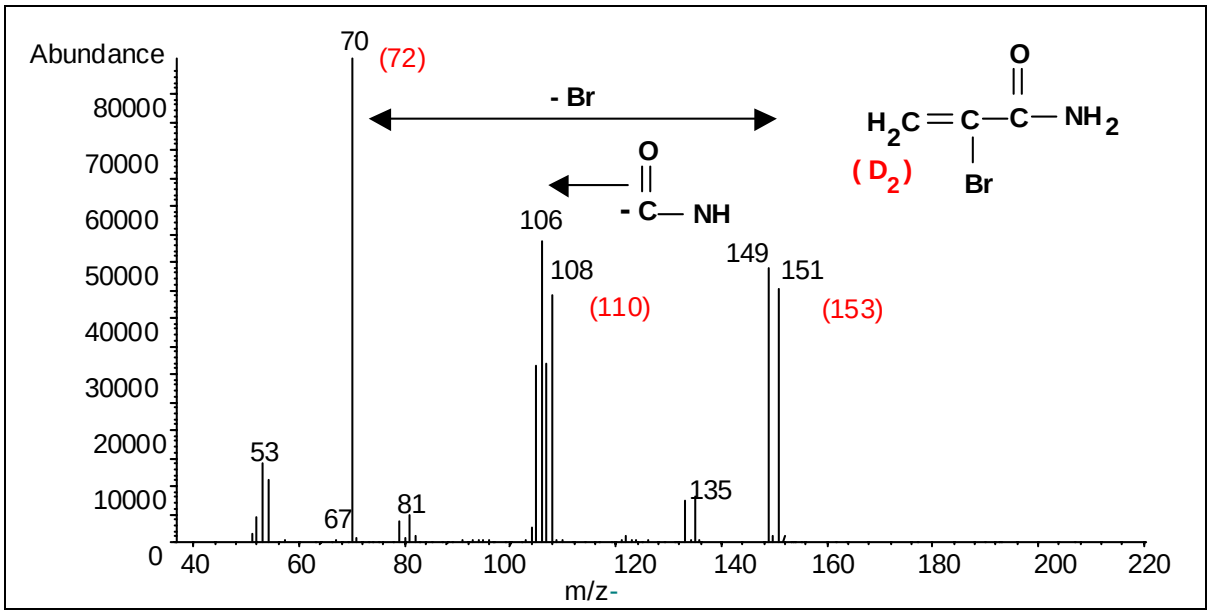

Fig. 2.1: EI mass spectrum of 2-bromopropenamide

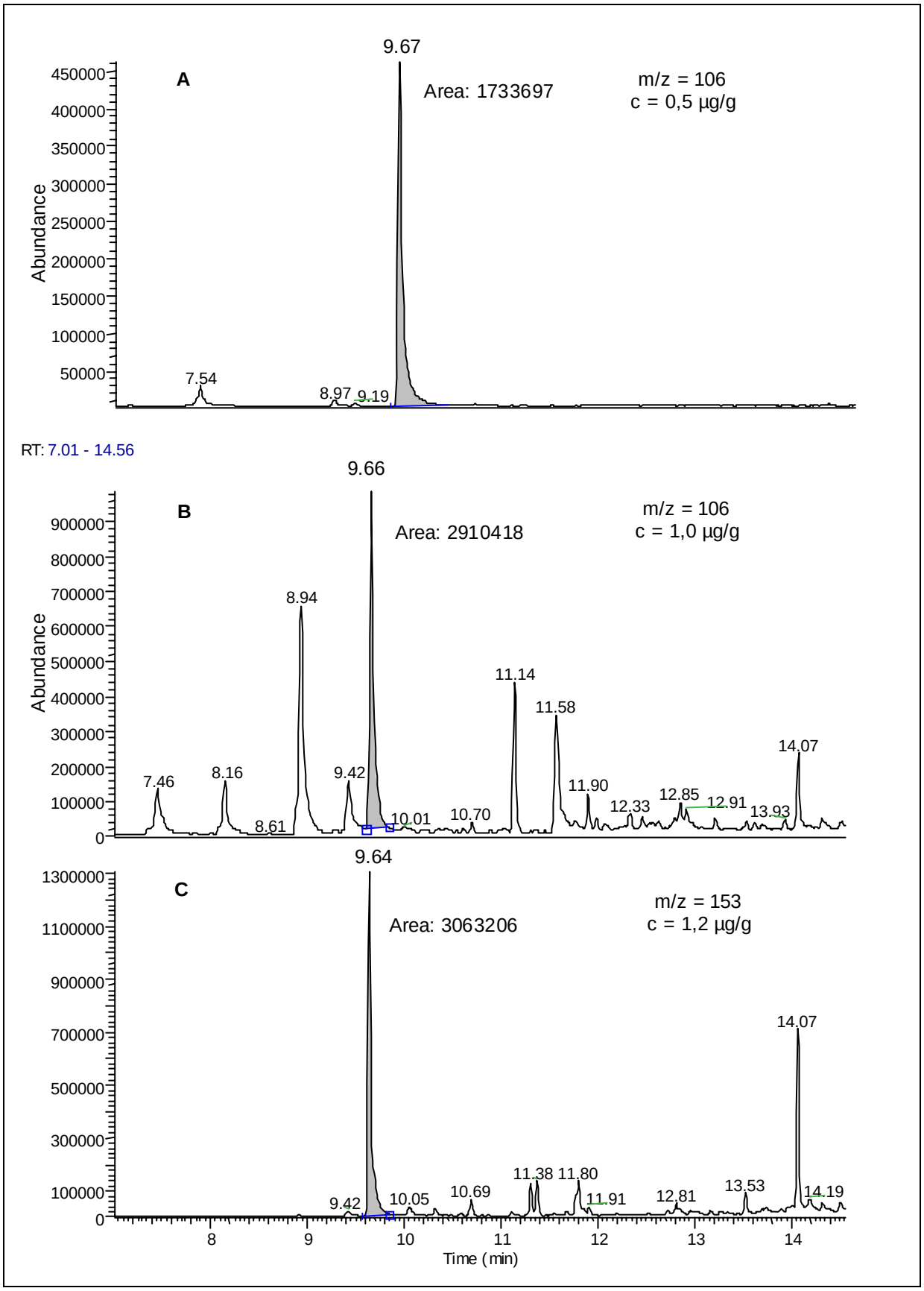

Fig. 2.2: Typical SIM-chromatograms of the monobromo-derivatives of: (**A**) acrylamide standard extract; (**B**) acrylamide and (**C**) D_3-acrylamide in crispbread extract

The calibration was done by linear regression analysis with six calibration points (see fig. 2.3). Each calibration solution was freshly prepared by weighing (range of mass fraction: 0.35-2.2 µg/g AA).

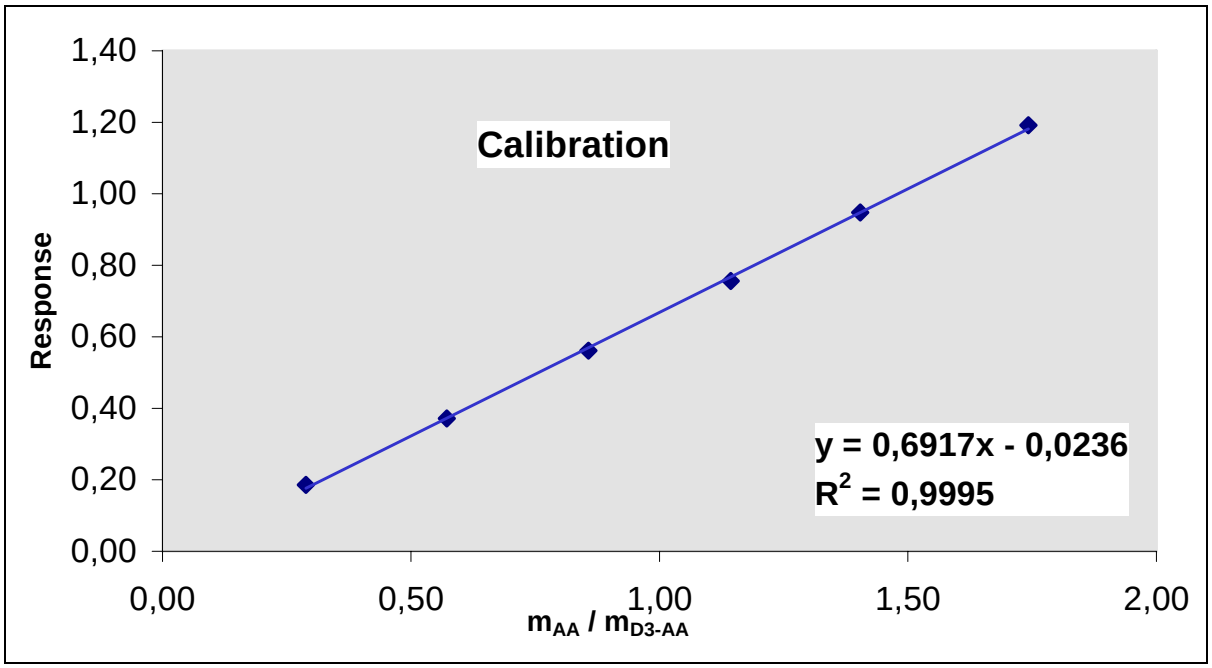

Fig. 2.3: Calibration by linear regression analysis

3 Homogeneity study

Based upon thorough batch homogenisation, and the results of preliminary studies, a satisfactory level of sample homogeneity was expected. For further quantitative demonstration, 10 units were selected randomly from the whole set of 500 bottles, and analysed four times each according to the analytical method we described before (chap. 2.2). All 10 units were extracted and processed once under repeatability conditions followed by the second set of extractions and processing in a randomised manner again under repeatability conditions and so on.

Processed extracts were analysed by GC-MS under repeatability conditions guaranteeing that all 40 extracts were quantified against one calibration after randomisation.

Results are given in figure 3.1 and the ANOVA table (table 3.1) below.

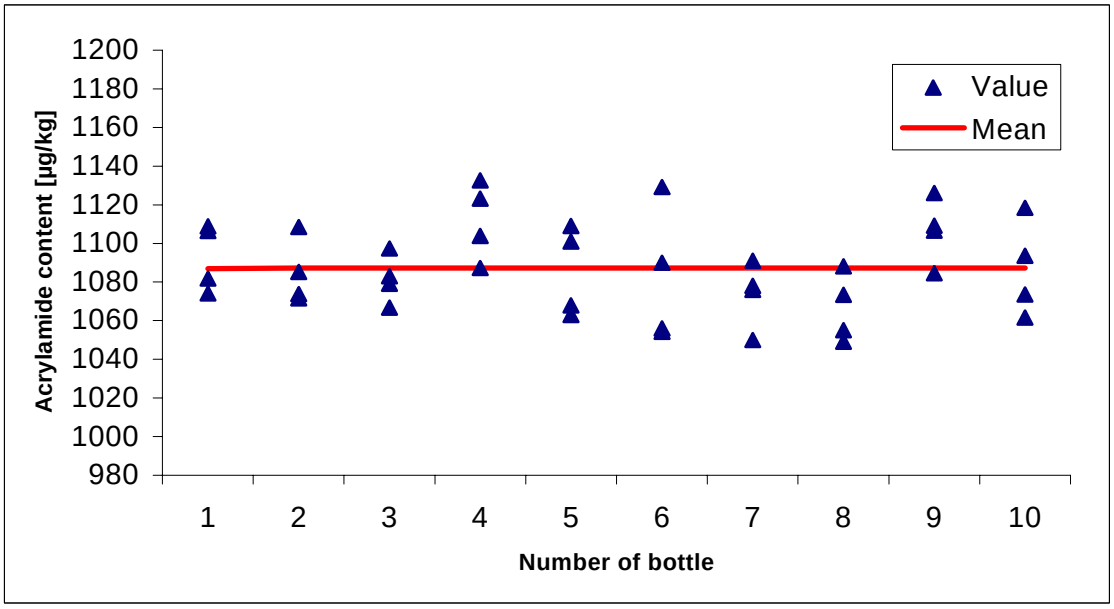

Fig. 3.1: Measurement results for the homogeneity test

Table 3.1 Analysis of Variance (ANOVA)

Variance		degree of freedom	Test criterion		Critical value	
between bottles	747.64	$f_1 = 9$ [1)]	$\frac{s^2_{between}}{s^2_{within}}$	1.68	$F(f_1, f_2, 5\%)$	2.21
within bottles	446.03	$f_2 = 30$ [2)]				

1) df = Z – 1 = 9
2) df = Z (M – 1) = 30

Variance between bottles

$$s^2_{between} = \frac{M \cdot \sum_{L=1}^{Z} \left(\bar{x}_L - \bar{\bar{x}}\right)^2}{Z - 1}$$

Variance within bottles

$$s^2_{within} = \frac{\sum_{L=1}^{Z} \sum_{k=1}^{M} \left(x_{L,k} - \bar{x}_L\right)^2}{Z \cdot (M - 1)}$$

$\bar{x}_L$: Mean value of bottle number L
$\bar{\bar{x}}$: Total mean of all bottles
$x_{L,k}$: Value of analysis k in bottle number L

L: Identifier of an individual bottle
Z: Total number of bottles
k: Identifier of an individual analysis
M: Total number of analyses per bottle

Table 3.2 Estimate for uncertainty contribution according to ISO Guide 35

	µg/kg	**relative in %**
u_{bb}	8.6834	0.7988
u_{bb}-min	10.7315	0.9873

$u_{bb...}$uncertainty between bottle

Because the test criterion is smaller than the critical value, no significant inhomogeneity of the batch was detected.

A contribution u_{bb} to the overall uncertainty of the certified reference material was nevertheless derived from the ANOVA results. The larger of the two estimates was used giving u_{bb} = 10.8 µg/kg.

4 Stability study

4.1 Initial stability study

From experience a temperature-driven deterioration 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 4 °C and 70 °C over periods of 1 week to 12 months as shown in Table 4.1 to perform a so-called isochronous stability study [LAMBERTY, A., SCHIMMEL, H., PAUWELS, J. (1998)]. After the respective periods of time individual units were stored at -20°C. All units were analysed for acrylamide using the method described above under repeatability conditions.

Table 4.1: Accelerated ageing of exposed samples

Time [months]	Temperature [°C]			
	4	20	40	70
0.25	X	X	X	X
0.5	X	X	X	X
0.75	X	X	X	X
1.5	X	X	X	X
3	X	X	X	--
6	X	X	X	--
12	X	X	X	--
24	[1])	[1])	--	--
48	[1])	[1])	--	--

[1]) post-certification monitoring (due)

The dependence on time of the thermal degradation is expected to be exponential. As a first step in the data analysis, the logarithmic plots of the data as obtained were tested for trends separately for each degradation temperature. Representive data for the initial values (at t = 0) for the samples stored at reference temperature -20 °C were taken from the homogeneity study. The results are illustrated in figure 4.1.

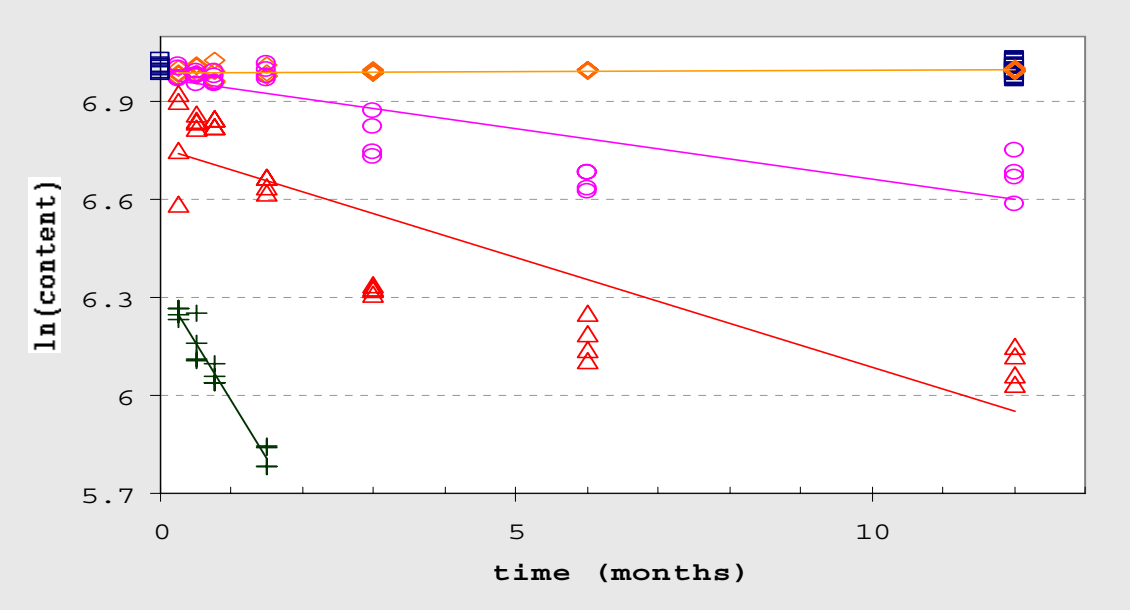

Fig. 4.1: Dependence on time of the mass content of acrylamide ratio of the measured value (degradation temperatures: -20°C - blue squares, 4°C - orange diamonds, 20°C - magenta circles, 40°C - red triangles, and 70°C - green crosses)

Given the relatively large intermediate precision of the method and a still moderate degradation at storage temperature 4 °C, initially an inverse trend was observed in the test on the single values as measured. Therefore, a data pre-treatment was applied for which the data were sorted with respect to a) the day of preparation and b) the sequence of measurement of the samples. Ratios were formed with respect to the value measured for the closest reference sample in the sequence.

For more detailed information on the approach, and the meaning of the statistical parameters used for assessing material stability see [BREMSER, W.; BECKER, R.; KIPPHARDT, H.; LEHNIK-HABRINK, P.; TÖPFER, A., ACQUAL (2006)].

A non-negligible trend is obviously observed for all temperatures above 4 °C. 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 4.2 (derived from the mean values measured at the corresponding sampling points in temperature and time).

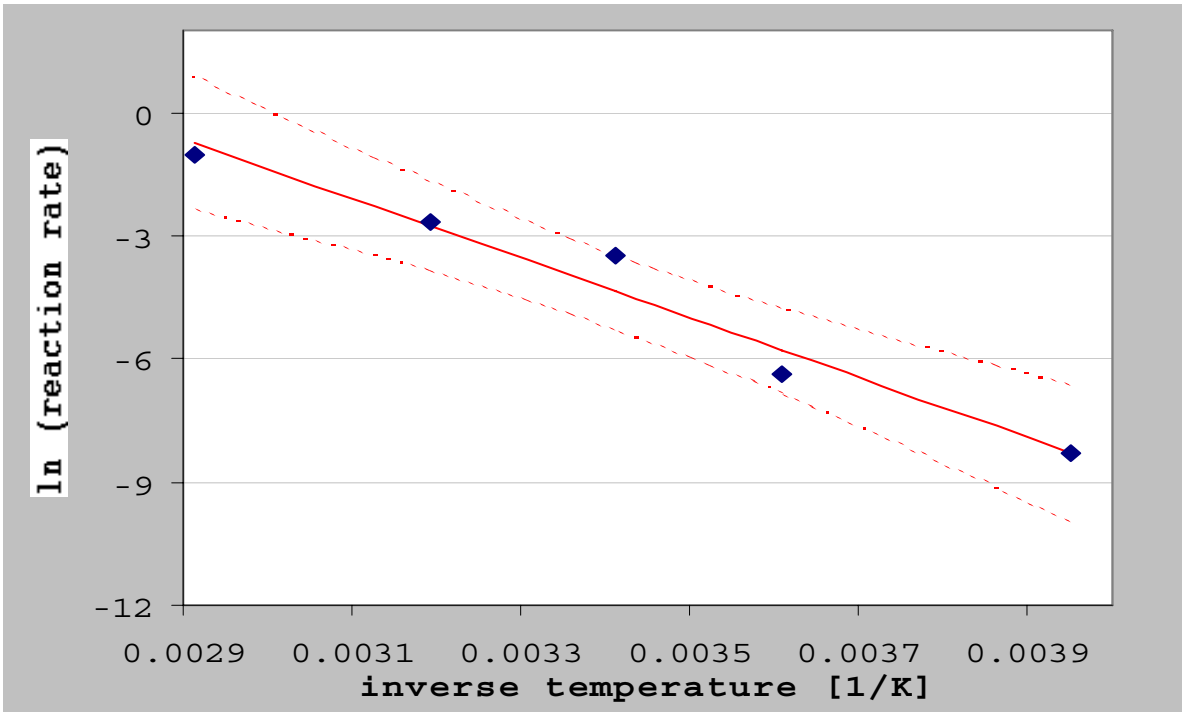

Fig. 4.2: Reaction rate for acrylamide in dependence on the inverse temperature (semi-logarithmic plot)

Obviously the temperature dependence can indeed be approximated by a straight line (as shown in the graph). The corresponding confidence interval for the line is also given in the figure. The estimated activation energy ΔE is 0.877 kJ/mol. 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 certified 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 figure 4.2. The results are given in table 4.2.

Table 4.2: Estimation of shelf life

Value falls below lower limit after		Months	years
at – 20 °C		38.91	3.24
	at 4 °C	5.97	0.50
	at 20 °C	1.61	0.13
	at 40 °C	0.28	0.02
	at 70 °C	0.02	

For further support of the estimated shelf life at the producer's side and its extension to a value which fits in the post-certification monitoring scheme, a contribution u_{lts} (long time stability) due to stability was taken into account in the CRM uncertainty calculation. This

contribution was calculated following the recommendations of the new edition of ISO GUIDE 35. A significant dependence of the mass content of acrylamide on the time at a temperature of -20 °C could not be observed (P value 0.74). A time dependence of the values measured for samples stored at a temperature of -20 °C was insignificant. The slope of the line was therefore neglected. From the uncertainty of the slope and the envisaged shelf life of 48 months, an u_{lts} of 36.73 µg/kg was obtained.

Although shelf life at a storage temperature of -20°C is still quite considerable, any exposure to room or even higher temperatures drastically reduces the time of validity of ERM-BD272. Therefore, a unique expiry date of **one year after delivery from storage** is established provided the sample is stored at –20 ± 4°C at the user's site. Transportation/delivery time must be kept at the possible minimum, and samples will be delivered in polystyrene boxes with two cooling packs to avoid any exposure to room temperature.

4.2 Post-certification stability monitoring

The first estimation of stability is updated by further measurements of units stored at 4°C and 20°C over the period of availability of the material. The first post-certification measurements will be done in due time before expiry of the initially estimated period of validity, namely at 24 and 48 months after initial storage.

5 Certification study

5.1 Participants

The certification study involved the following 15 laboratories using various analytical methods for the determination of acrylamide in food:

Table 5.1: Participants of the certification study

Laboratory	City/State
BAM, I.2	Berlin; Germany
Chemisches und Veterinäruntersuchungsamt Sigmaringen	Sigmaringen; Germany
Chemisches und Veterinäruntersuchungsamt Stuttgart	Fellbach; Germany
General Chemical State Laboratory, Food Division & Division of Environment	Athens; Greece
German Research Centre of Food Chemistry Lab (1)	Garching; Germany
German Research Centre of Food Chemistry Lab (2)	Garching; Germany
IRMM	Geel; Belgium
Kantonales Labor Zürich	Zürich; Switzerland
Lebensmittelchemisches Institut Köln	Köln; Germany
Lebensmittelversuchsanstalt	Vienna; Austria
National Food Administration Sweden	Uppsala; Sweden
Nestlé Research Centre	Lausanne; Switzerland
Public Analyst´s Laboratory Dublin	Dublin; Ireland
VWA/KvW Keuringsdienst van Waren Zuid Limburg, Noord Brabant	Eindhoven; The Netherlands
W.E.J. GmbH	Hamburg; Germany

The following analytical methods were employed by the participating laboratories:

Table 5.2: Analytical methods used for certification

Derivatisation	Analytical method
Bromination	GC-MS
Without	GC-MS
2-mercapto-benzoic acid	HPLC-ESI MS/MS
Without	HPLC-ESI-MS/MS

5.2 Design of the study

Three units of the candidate reference material were to be analysed by each laboratory in triple. The information on the level of acrylamide content to be expected between 200 and 2000 µg/kg was provided to ensure – as far as technically feasible – comparable analytical conditions.

In addition, each participant received one solution with unknown concentrations of acrylamide in water.

Results for the acrylamide content were to be reported on basis of total mass intake, no dry mass determinations. Results returned to BAM were scrutinised for consistency and a few obvious transcription errors were corrected after clarification with the respective laboratories.

5.3 Evaluation of results and certified values

The results of the certification study were evaluated in accordance with ISO GUIDE 35 and the specific requirements of the ERM agreement (For detailed information see: www.erm-crm.org/ermcrm). The computer software SoftCRM V1.2.2 was partially used for statistical tests and data treatment.

5.3.1 Technical evaluation

For each laboratory, the normalised mean value determined for the sample (normalised by the mean of laboratory means) was plotted against the recovery the laboratory attained for the control solution. Figure 5.1 shows the corresponding plot. As can be seen from the graph, 15 out of 16 laboratories group around the centre (formed by a recovery of unity and the normalised mean of unity). Most of the laboratories show positive correlation between the sample and the control value although obviously the inverse trend can also be observed. One single laboratory (lab number 08, highlighted) is distant from the main group, and therefore shows an underestimation concerning control and sample value and therefore in both cases a large correlation between sample and control value exists.

Although no further investigation into the reasons for this discrepancy have been conducted, it seems justifiable to exclude, for technical reasons, the sample value of this particular laboratory from further processing.

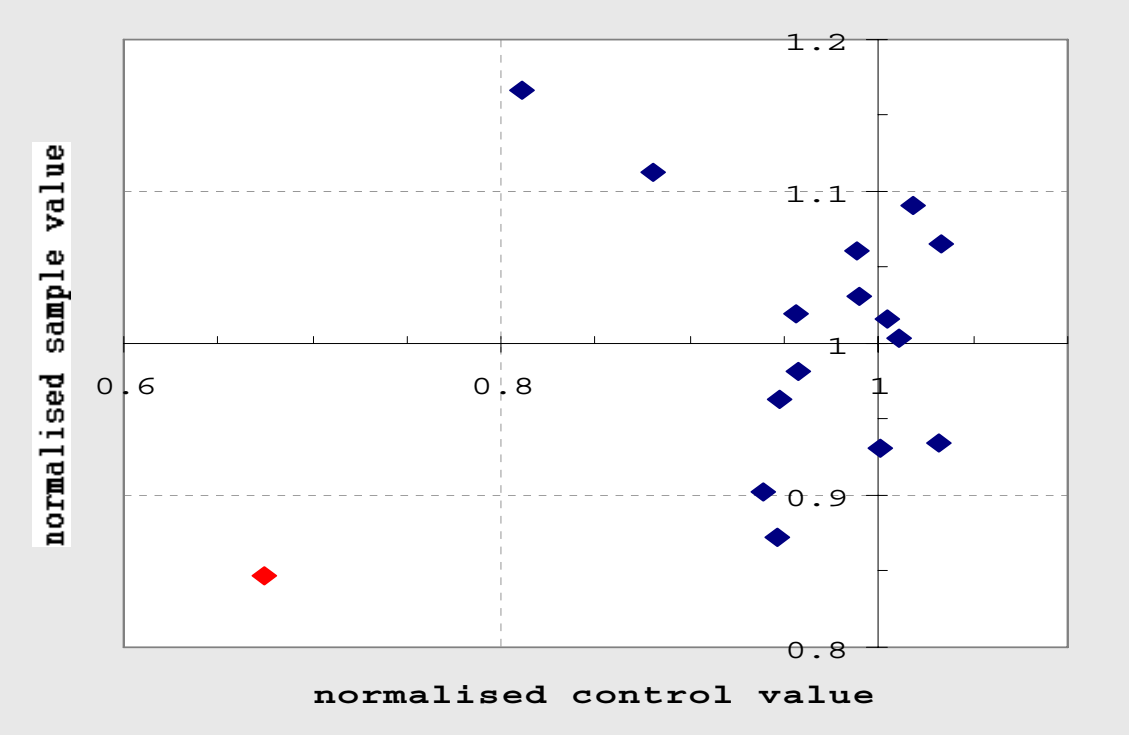

Fig. 5.1: Plot of the mean value found by the laboratories for the sample (normalised by the mean of laboratory means) against the recovery of the control solution attained by the laboratory

5.3.2 Statistical evaluation

After removal of laboratory 08 for technical reasons, the data set as shown in table 5.3 was used for further statistical processing.

Table 5.3: Accepted laboratory data sets

Lab	01	02	03	04	05	06	07	09	10	11	12	13	14	15	16
values	1090.0	1084.5	1070.0	947.0	1013.1	950.4	956.2	922.3	856.0	821.0	908.7	1034.9	1037.0	1030.9	977.0
	1095.0	1079.5	1081.5	975.5	1019.4	909.9	1086.5	935.0	855.0	808.5	937.2	1109.2	1112.5	1021.6	970.8
	1091.0	981.0	1029.5	974.0	1007.9	874.0	847.0	944.0	839.0	955.5	881.6	1063.3	1095.5	1026.8	979.1
	1082.0	1074.0	1071.5	1039.5	1004.5	948.9	705.6	965.3	977.5	871.0	909.1	1089.6	1141.0	994.5	990.3
	1090.0	1059.5	1049.6	1036.5	998.0	945.8	707.1	975.3	959.0	840.0	926.9	1099.6	1185.0	975.5	972.0
	1088.0	1077.0	1054.0	1008.5	968.6	950.6	735.1	960.7	931.5	913.5	901.4	1065.9	1059.5	1008.2	973.8
	1097.0	999.5	984.2	998.5	994.7	944.7	970.2	972.3	920.0	742.5	892.5	1035.7	1290.5	999.9	992.8
	1072.0	996.0	1031.7	1007.5	947.5	974.1	979.6	993.5	952.0	832.5	924.7	1069.3	1196.0	998.6	983.7
	1092.0	994.5	1012.7	996.5	998.9	984.7	958.1	979.2	934.5	895.5	919.7	1035.6	1158.5	1027.2	995.6
Inhom detect	No	No	No	Yes	No	No	Yes	Yes	Yes	No	No	No	No	No	No

As a first step, the accepted data set was investigated (using two-way ANOVA) for significant influence of factors, namely the laboratory and the unit investigated. As expected, laboratory is a significant factor (between-laboratory variation is significantly larger than within-lab variation), while the unit investigated is insignificant which is clearly in line with the results of the homogeneity tests carried out (compare chapter 3).

In a second step, it was tested (separately by one-way ANOVA on the data of each of the laboratories) whether significant differences between the units provided were detected by the participating laboratories. Although this was not expected to occur (compare chapter 3), 4 out of the 15 laboratories detected virtual differences between the units. This can most probably be attributed to a considerably large intermediate laboratory precision, in some cases comparable to the between-laboratory variability. As a consequence, individual laboratory standard deviations were taken into account as an additional contribution to the overall uncertainty by uncertainty propagation to the mean of means (see clause 5.3.4).

Participants in the intercomparison used different methods with different implementations in different laboratories. Obviously there was no good reason for assuming that the single values measured by the different laboratories would belong to a common mother distribution. Single measurement results cannot be pooled, and therefore the mean of laboratory means was considered an appropriate estimate for the certified value. This decision was justified by the outcome of the two-way ANOVA test as described above as well as by further statistical analysis within which the following statistical parameters were calculated:

- the mean of laboratory means
- the standard deviation of the distribution of laboratory means, and the standard deviation of the mean of laboratory means
- the confidence interval of the mean of laboratory means at the 0.05 significance level

and the following statistical tests were carried out (at significance levels of 0.05 and 0.01):

- Cochran test for the identification of outliers with respect to laboratory variance
- Grubbs test for the identification of outliers with respect to the mean
- Dixon and Nalimov test for the verification of possible outlier indications
- Kolmogorov-Smirnov Test (Lilliefors version) for the normality test
- Test for skewness and kurtosis

The results of the above calculations and tests for a data evaluation based upon the laboratory means are given in the table 5.4 below.

Table 5.4: Statistical parameters of the accepted data set

Acrylamide content [µg/kg]							
Value	**SD**	**u (x)**	**CI**	**TI**	**Data sets**		**Pooling**
988.462	79.97	20.6481652	44.286	236.231	15		No

Scheffé	Bartlett	Outlier α = 0.01 (0.05)				Gauss	Skew/Kurto
	α = 0.01	Cochran (0.01)	Grubbs E	Grubbs D	Nalimov	α = 0.01	
No	Inhom	- (-)	- (-)	- (-)	- (14)	Yes	Yes

CI - confidence interval of the mean at a 0.05 significance level
TI - tolerance interval of the mean at a 95/95 % confidence level

The main features are as follows:

- *Scheffé*- and *Snedecor*-F-Test: Data sets differ significantly.
- *Bartlett*-Test: Variances are inhomogeneous (at the significance level of 0.01).
- *Cochran*-Test: No outliers detected (significance levels 0.05 and 0.01).
- *Dixon*-, *Grubbs*- und *Nalimov*-Test: Laboratory means do not contain outliers (significance level 0.01). Laboratory 14 is a straggler in the Nalimov test and therefore retained.
- *Kolmogorov-Smirnov* and *Skewness/Kurtosis*-Test: Based on the available data, the hypothesis of normality cannot be rejected.

The mean of laboratory means of 988.46 µg/kg acrylamide was taken as the uncorrected (for purity) estimate for the value to be certified, and the standard deviation of the mean of laboratory means s_{char} of 20.65 µg/kg acrylamide as the uncertainty contribution from characterisation by intercomparison.

5.3.3 Traceability

The acrylamide mass fraction is, although not method-specific, clearly a parameter which is influenced by the method employed for its determination, namely the extraction and (if applicable) derivatisation procedure. The measurement step takes traceability from calibration using the pure substance (AA, 99+%; Merck Darmstadt), sample preparation steps from spiking using D_3-AA (99+%; Polymer Source), both having independently confirmed purities. Overall recovery was estimated to 95 - 105 %. Remaining systematic between-method biases are sufficiently covered by the allowance made for the intercomparison contribution to the total uncertainty budget. The certified value is traceable to the SI.

5.3.4 Certified value and combined uncertainty

The estimate of clause 5.3.2 for the certified value w_{cert} (acrylamide mass fraction) must be corrected for the purity of the calibration standard used in all of the experiments according to

$$w_{cert} = w_{char} * f_{pur}$$

The corresponding combined uncertainty must appropriately be composed from the uncertainty of characterisation u_{char}, the contribution from a possibly undetected inhomogeneity u_{bb}, the uncertainty of the purity correction u_{pur}, and a contribution u_{lts} due to stability according to

$$u_{c,\,r}^2 = u_{char,\,r}^2 + u_{pur,\,r}^2 + u_{bb,\,r}^2 + u_{lts,\,r}^2$$

where the index *r* refers to the corresponding relative uncertainties. The purity and its corresponding uncertainty were taken from the certificate of AA from Merck[1] as $f_{pur} = 0.995$ and $u_{pur} = 0.0029$ (assuming a rectangular distribution), u_{bb} is given in clause 3, and u_{lts} in clause 4. To the contribution s_{char} from the intercomparison (standard deviation of the mean of laboratory means) as given in clause 5.3.2, the standard deviations of the individual lab means $s_{lab,i}$ was added up by uncertainty propagation to give u_{char} as follows

$$u_{char}^2 = s_{char}^2 + \sum s_{lab,i}^2 / n^2$$

where n is the number of accepted laboratory data sets. The term $\sum s_{lab,i}^2 / n^2$ was estimated as 6.78 µg/kg (uncorrected for purity) and added due to the reasons explained under clause 5.3.2. The final certified values for the CRM are given in table 5.5 where the expansion factor for the expanded uncertainty is k = 2. The value and the expanded uncertainty are rounded according to the recommendations of [ISO, GUM, (1995)] and are given with respect to raw sample mass. The water content was seen to remain stable if the material is handled according to the instructions in the certificate (see also clause 6).

Table 5.5: Certified acrylamide content of ERM-BD272

CRM	Acrylamide content in mg/kg		
	Certified value, corrected for purity	Uncertainty of the certified value	Expanded uncertainty of the certified value
ERM-BD272	**0.98**	**0.045**	**0.09**

[1] The purity of AA was checked at BAM by means of quantitative ^{1}H-NMR. This analysis confirmed the purity of AA provided by the manufacturer (Merck).

6 Information on the proper use of ERM-BD272

6.1 Shelf life

From the initial stability study a preliminary shelf life of 48 month at a storage temperature of -20 °C was estimated (including a contribution to the total uncertainty). Since the dispatch to the end user may occur at any time during this period the certified properties will be valid for 12 months beginning with the dispatch of the material from BAM. The validity of this information will be maintained by the post-certification monitoring.

6.2 Transport, storage and use

The material is to dispatch in polystyrene boxes with two 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 bottle has to have reached ambient temperature. Thereafter, the bottle 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.

6.3 Safety instructions

The crispbread material was not sterilised, however, it is supposed to not exhibit any biological activity due to having been dried to constant weight. No hazardous effect is to be expected when the material is used under conditions usually adopted for the analysis of foodstuff matrices moderately contaminated with acryalmide.

6.4 Legal notice

Neither the Federal Institute for Materials Research and Testing (BAM) nor any person acting on their behalf make any warranty or representation, express or implied, that the use of any information, material, apparatus, method or process disclosed in this document may not infringe privately owned rights, or assume any liability with respect to the use of, or damages resulting from the use of any information, material, apparatus, method or process disclosed in this document.

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