Acrylamide
Interlaboratory Study 2002

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Table of contents

1 Introduction

In addition to the different steps of internal quality control, the participation in external quality control measures like interlaboratory studies and especially proficiency tests is of utmost importance. Proficiency testing schemes are particularly suited to assess the analytical performance of laboratories. This particular interlaboratory study is divided in two parts. The sunshine test and the proficiency test.

2 Pre-Trial (Sunshine Test)

A so called “Sunshine” sample was distributed to interested laboratories before the main proficiency test was initiated. The aim of the “sunshine” sample was to pre-evaluate the laboratory efficiency and the method performance by the participants themselves. Since the approximate content of acrylamide was known and the results have been published quickly after closing date the laboratories were enabled to estimate their own performance. This “sunshine” test was designed to be an exercise for the laboratories exclusively.

The test material was already dispatched in July 2002. Each participant received 75 g sample of crisp bread test material in a plastic bag. With the sample the laboratories received also the information that the sample contains between 400-500 µg/kg acrylamide. The submission of results to the BgVV was voluntary.

47 sets of test materials were shipped to laboratories in eight different countries (35 Germany, 5 Swiss, 2 USA, 1 Austria, 1 India, 1 Oman, 1 Dubai, 1 Botswana). 34 laboratories sent back results to the BgVV. The results are given in Table 1.

Table 1: Results of the sunshine test. Concentration of acrylamide in the sunshine sample

Lab		µg/kg	Lab		µg/kg
1	*	989	24		407
2		474	25		462
4		446	26		517
5		469	27		484
6		546	28		525
7		490	29		479
8		493	31		421
11		439	32		430
12	*	631	33		436
14		490	34	*	200
15	*	350	36		426
16		490	37		470
17		447	38	*	81
19		496	40	*	4785
20		484	41	*	1162
21		501	45		468
23		463	46		490

* Extreme deviations according to Pearson [1]

Each laboratory was requested to estimate very critically its own performance and for modification of the analysis in case of questionable results.

After removing extreme values by applying the tolerance limits of Pearson (95%) [1] basic statistics were calculated on the remaining results.

Table 2: Statistics of the sunshine sample

Total number of results	„Outlier“	Number of accepted values	Mean in µg/kg	Standard deviation in µg/kg	Relative standard deviation in %
34	7	27	472	32,4	6,9

The results of the “sunshine test” were found to be encouraging for the conduction of the main proficiency test. Almost all laboratories which participated in the sunshine test took part in the main PT. Since some laboratories changed their methods of analysis and in the meanwhile some new laboratories wanted to participate and others did not submit results for the main trial, no pre-selection of laboratories for the main PT was done based on the results of the sunshine sample.

3 Proficiency Test

In September 2002, sample material was distributed to **47** laboratories, **34** of which sent back their results before the15th of November.

The organisation of the interlaboratory study and the statistical evaluation of the results were performed according to internationally recognised guidelines [2]. For that purpose a statistical software package obtained from quo data GmbH [3, 4] was used.

4 Participants

The order of the laboratories is random and not identical with the lab-code numbers.

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5 Objective

The Interlaboratory Study was designed in accordance with the *International Harmonised Protocol for the Proficiency Testing of Chemical Analytical Laboratories* (ISO/REMCO N 280) [2], jointly elaborated by ISO, IUPAC and AOAC.

The laboratories had to analyse the samples for their possible contents of acrylamide by applying their internal routine methods, irrespective to sample amounts, sample preparation procedures, detection techniques and the number of replicates.

The participants were provided with forms to state the analytical parameters and to indicate the results. The results had to be returned using exclusively the provided forms, which had to be filled in completely. The results had to be supported by meaningful raw data, e.g. chromatograms. The participants were requested to report the results of all parallel analyses without calculating the mean value.

Furthermore, the participants were asked to provide information by compiling a provided form on their methods including sample pre-treatment, sample preparation and detection, in order to assess the test results in relation to the respective analytical methods. Comments on the applied way of identification of the detected acrylamide and on the applied quality measures had to be added.

6 Sample Material

6.1 Production

Sample material was provided by a private German laboratory. The material was split into portions of approximately 50 g in plastic bags which were stored at –18 °C in a fridge. Table 3 provides an overview of the samples for the Proficiency Test.

Table 3: Samples

Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
Mashed Potato	Cocoa	Crisp bread	Butter biscuit	Mashed Potato	Crisp bread
highly contaminated		identical with sample F		Blank	identical with sample C

Note: Sample C and sample F are identical. **Sample A has been obtained "in house" by treating material of sample E under extreme laboratory conditions in order to produce artificially high acrylamide contents.**

6.2 Homogeneity

Homogeneity was tested by analysing five or ten randomly selected bags of each sample in duplicate. The obtained means and standard deviations for each sample and analyte are given in Table 4.

Table 4: Results of the homogeneity study

	n	Concentration [µg/kg]	STD analytical (S_a) [µg/kg]	STD between (S_s) [µg/kg]	Horwitz STD [µg/kg]	Quotient S_S/Horwitz STD (< 0.3)
Sample A	20	10679	428	328	1196	0.274
Sample B	10	629	47	31	108	0.289
Sample C/F	20	206	18.2	9.0	42	0.215
Sample D	10	630	19	23	108	0.213
Sample E	Blank	-	-	-	-	-

S_a is the analytical standard deviation calculated from the two parallel determinations. S_s is the standard deviation occurring between the samples (bags) [2].
The test for sufficient homogeneity was effected by comparing S_S with the Horwitz standard deviation [5, 6] in accordance with the *International Harmonised Protocol for the Proficiency Testing of (Chemical) Analytical Laboratories* [2]. The standard deviation between the samples (S_S) should be at least 3 times smaller than the Horwitz standard deviation from the mean (S_S/Horwitz S < 0.3). Recently a slightly higher quotient of < 0.4 was proposed and would be accepted for sufficient homogeneity [7]. However, the quotient (S_S/Horwitz S) was smaller than 0.3 for all samples.

Additionally the homogeneity of the sample material was checked by the Analysis of Variance (ANOVA) using the same set of data. It was confirmed that for **all** samples the variance within and between the samples is statistically not distinguishable.

6.3 Stability

In order to ensure the stability of the samples and to prove that the analyte content did not change for the leading time of the proficiency test, samples of the single test portions were taken from the predefined storage (cold or frozen ≤ 4°C) in defined intervals. From each sample bag two parallel determinations were carried out.
The results, which are shown in Table 5, indicate that the stability of the samples and analyte was sufficient for the duration of the proficiency test. Taking into account the analytical variance, no significant change at all could be observed during the storage.

	Sample A	Sample B	Sample C and Sample F	Sample D	Sample E
	Mashed Potatoes	Cocoa	Crisp Bread	Biscuits made with butter	Mashed Potatoes Blank
Storage time after shipment	Acrylamide [µg/kg]	Acrylamide [µg/kg]	Acrylamide [µg/kg]	Acrylamide [µg/kg]	Acrylamide [µg/kg]
7 days	11622	594	206	663	-
14 days	11973	646	279	710	-
30 days	10162	562	320	704	-
60 days	8645	743	201	556	-
90 days	13798	1138	304	665	-
mean	11240	737	270	660	-

Table 5: Stability study (the results are the means of two parallel determinations)

6.4 Shipment

Approximately 50 g of each sample were filled into plastic bags and stored at –18 °C until dispatch. The samples were shipped to the laboratories by express mail taking a maximum of two days. The addressed laboratories had to check the condition the samples were in. It was confirmed that all the samples arrived.

7 Statistical Evaluation

7.1 z-score

The interlaboratory study was evaluated according to the *International Harmonised Protocol for the Proficiency Testing of (chemical) Analytical Laboratories* [2] jointly elaborated by ISO, IUPAC and AOAC. This protocol determines that for the quantitative results of the laboratories, the z-scores must be calculated according to the following equation:

$$\text{z-score} = \frac{x - X}{\sigma}$$

x : laboratory result
X : assigned value
σ : target value for standard deviation

The advantage of the z-score determination is that it provides a standardised value allowing to compare the results both within one interlaboratory study and between different interlaboratory studies irrespective of the concentration of the analyte [3, 8].

In the case of a normal distribution, the probability of the absolute value of z not exceeding the value 2 is approximately 95 %. It is therefore sensible to establish the value 2 as a "quality limit" for the underlying measurements [2, 3]. Assuming a "well-behaved analytical system", the ISO protocol offers the following classification:

$\lvert z \rvert \leq 2$	satisfactory
$2 < \lvert z \rvert \leq 3$	questionable
$\lvert z \rvert > 3$	unsatisfactory

For the calculation of the z-scores two pivotal values must be determined: the target standard deviation and the assigned mean value.

- The assigned mean value is normally calculated as the mean of the laboratory results, from which outliers were eliminated previously. Alternatively, if robust statistics are applied, all values are considered, weighed by a certain factor. If certified reference material is used, the assigned value can really be an assigned value, employing the value that was certified in a study carried out previously. The classical elimination of outliers by statistical test procedures, as described in the DIN 38402 A 42 and ISO 5725 protocols for the calculation of z-scores, requires normally distributed data. If the data of a proficiency test are not normally distributed, a so-called robust calculation of the mean is recommended by [2] and was performed here. The model used was the calculation according to Huber (Q-Method) [3, 4].

- The target standard deviation strongly affects the sharpness of the evaluation. In proficiency testing, the target standard deviation was often determined in ring tests carried out previously, which were specially designed for the validation of methods. The concept of the free choice of method applied in this particular study, however, requires different target standard deviations. The protocol for the proficiency testing of analytical laboratories [2] describes the possibility of deriving the target standard deviation from general models of precision, such as the "Horwitz curve" [5, 6]. This is only a recommendation and in fact for example within the analysis of pesticides in the EU the use of the real standard deviation is established in proficiency testing. This seems to be in particular justified if a

reasonable number of laboratories participate, which have also a known expertise in a certain field of analysis. **For the calculation of z-scores of this proficiency test the observed standard deviation calculated by robust estimates has been taken.** However the z-scores calculated with the Horwitz-standard deviation as target were calculated also and are given in the ANNEX.

7.2 Laboratory results (x)

The final results of the participants having performed a quantitative examination, or – if parallel analyses were performed – the outlier-free mean value of these parallel determinations, were used as laboratory results (x).

7.3 Assigned value (X)

The so-called 'assigned value' (X) was obtained by calculating the Q-Huber estimator (see 7.5) of the results of all laboratories meeting the following criteria:

1. a quantitative examination had been performed
2. the result lay above the method's limit of determination as indicated by the laboratory,
3. the results were accepted although the method was not validated or, respectively, validation data were not provided.

The data and measurement results of the laboratories used to determine the assigned values are given in the ANNEX for each sample.

7.4 General model of prediction: Target value for the standard deviation (σ)

The target value for the standard deviation (σ) was determined according to the Horwitz Function [5, 6]:

$$\sigma = 0{,}02\ c^{\,0{,}8495},$$

where c is the mean value of the proficiency test samples expressed as a power of ten (e.g. 1 µg/kg = 10^{-9}).

7.5 Statistical parameters

The ISO protocol recommends the use of robust statistical methods since in the case of interlaboratory studies, normally distributed data cannot be expected and outliers which cannot be reasonably eliminated anymore with the help of classical outlier tests may occur [9]. It is an advantage of robust estimators that outliers do not need to be eliminated because they only play a minor or no role at all in the calculation of the parameters. Furthermore, robust procedures can be applied to data which are not normally distributed.

In the case of normally distributed data, the arithmetic mean is used as an estimate for the true value, whereas the standard deviation is used as an estimate for the scatter. Since an χ^2-test on normal distribution demonstrated that the results of more than one sample were not normally distributed, ***robust estimates*** like the Q-Huber estimation as an estimate for the real value and Q_n [8, 10] as a robust precision parameter ***were used for the assessment of the data of this interlaboratory study***.

7.5.1 Calculation of the assigned value

The calculation of the target value was done according to the Q-Huber estimation. In fact this is a kind of mix of the median and the arithmetic mean [3, 4].

7.5.2 Calculation of s_R

S_R = Reproducibility standard deviation

$$s_R = Q_n \cdot (x_1, ...,x_J) = c_J \cdot 2.22194 \cdot \{|x_r - x_s|; r \neq s\}_{(k)}$$

where $k = \binom{h}{2} \approx \binom{J}{2} \Big/ 4$ and $h = [J / 2] + 1$

c_J is a correction factor for small amounts of samples, J is the number of laboratories and [J/2] denotes the integer part of J/2 [4, 8, 10].
Thus Q_n corresponds to the lower quartile of the absolute differences of all the pairs of measured values. With regard to the relative coefficient of variation, an equivalent robust parameter CV_R was used to compare the scatter of the measurement values.

7.5.3 Calculation of CV_R

$$CV = \frac{s}{X}$$

CV_R = Relative standard deviation (coefficient of variance)

8 Summary of the methods

Table 6 summarises the analytical methods and their characteristics.

Table 6: Methods of analysis (LOD = limit of detection, LOQ = limit of quantification, Br = bromination, IS = internal standard)

Lab Code	Sample	LOD [µg/kg]	LOQ [µg/kg]	Method	Technique
1	A B C D E F	70 30 30 20 20 20	200 90 90 60 60 60	Extraction ASE: DCM/ethanol Clean Up: Water extraction IS: D_3-Acrylamide	LC-MS/MS
2	A B C D E F	20 50 20 20 20 20	50 100 20 50 50 50	Extraction: Water Clean Up: De-fatting/Carrez IS: D_3-Acrylamide	LC-MS/MS
5	A B C D E F	15 20 15 15 15 15	30 50 30 30 30 30	Extraction: Water/enzyme Clean Up: Carrez Derivatisation: Br IS: D_3-Acrylamide	GC-MS
6	A B C D E F			Extraction: Water Clean Up: De-fatting Derivatisation IS: D_3-Acrylamide	GC-MS
7	A B C D E F		5 5 5 5 5 5	Extraction: Water/i-propanol Clean Up: De-fatting IS: D_3-Acrylamide, methacrylamide, butyramid	GC-MS (CI)
8	A B C D E F		100 100 100 100 100 100	Extraction: Water Clean Up: De-fatting, SPE, Carrez IS: D_3-Acrylamide	LC-MS/MS
11	A B C D E F	10 10 10 10 10 10	30 30 30 30 30 30	Extraction: Water Clean Up: De-fatting/Carrez IS: D_3-Acrylamide	LC-MS/MS
12	A B C D E F	10 10 10 10 10 10	30 30 30 30 30 30	Extraction: Water Clean Up: De-fatting/Carrez Derivatisation: Br IS: D_3-Acrylamide	GC-MS
14	A B C D E F	25 25 25 25 25 25	75 75 75 75 75 75	Extraction: Water/1-propanol Clean Up: De-fatting IS: D_3-Acrylamide	GC-MS (CI)

15	A B C D E F			Extraction: Methanol/water IS: methacrylamide	GC-MS
16	A B C D E F	70 70 70 70	100 100 100 100	Extraction: methanol Clean Up: De-fatting IS: methacrylamide	GC-MS
17	A B C D E F	20 20 20 20 20 20	30 30 30 30 30 30	Extraction: Water/aceton Clean Up: De-fatting Derivatisation: Br IS: D_3-Acrylamide, methacrylamide,	GC-MS
18	A B C D E F	10 10 10 10 10 10	30 30 30 30 30 30	Clean Up: De-fatting Extraction: Water/acetonitrile Clean Up: Carrez IS: D_3-Acrylamide	LC-MS/MS
19	A B C D E F	30 30 30 30 50 30	60 60 60 60 100 60	Extraction: Water Clean Up: De-fatting, Carrez, re-extraction with ethyl acetate IS: D_3-Acrylamide	GC-MS
20	A B C D E F	154 300 40 30 180 40	550 1000 130 100 550 130	Extraction: Water Clean Up: SPE IS: $^{13}C_3$-Acrylamide	LC-MS/MS
21	A B C D E F			Extraction: Methanol/water Clean Up: Carrez Derivatisation: Br IS: Dimethylacryamid	GC-MS
22	A B C D E F	20 20 10 10 20 10	40 40 20 20 40 20	Extraction: Water Clean Up: De-fatting, Carrez SPE: Si with water IS: D_3-Acrylamide	LC-MS/MS
24	A B C D E F	10 10 10 10 10 10	40 40 40 40 40 40	Extraction: Water Clean Up: De-fatting, Carrez IS: methacrylamide	GC-MS (CI)
25	A B C D E F	2 2 2 2 2 2 for LC-MS/MS	5 5 5 5 5 5 for LC-MS/MS	Extraction: ASE: ACN/water Clean Up: De-fatting, SPE with ethyl acetate IS: D_3-Acrylamide	LC-MS/MS GC-MS GC-MS (HR)

26	A B C D E F	60 60 60 60 60 60	90 90 90 90 90 90	Extraction: Water Clean Up: Enzyme treatment amylase, Carrez External standard	LC-LC-DAD
27	A B C D E F		30 30 30 30 30 30	Extraction: Water/propanol Clean Up: freezing IS: D_3-Acrylamide, methacrylamide	LC-MS/MS
28	A B C D E F	15 15 15 15 15 15	50 50 50 50 50 50	Extraction: Water Clean Up: De-fatting, extraction with ethyl acetate IS: ^{13}C-Acrylamide	LC-MS
29	A B C D E F	10 30 10 10 10 10	30 100 30 30 30 30	Extraction: Water Clean Up: De-fatting, Carrez, SPE: MF18 with water IS: D_3-Acrylamide	LC-MS/MS
30	A B C D E F			Extraction: Water Clean Up: De-fatting IS: D_3-Acrylamide	LC-MS/MS
31	A B C D E F			Extraction: 1-propanol Clean Up: De-fatting IS: D_3-Acrylamide	GC-MS (CI)
32	A B C D E F	25 25 25 25 25 25	50 100 100 50 50 100	Extraction: Water Clean Up: Carrez, de-fatting, extraction with ethyl acetate IS: D_3-Acrylamide	GC-MS
33	A B C D E F	20 20 20 20 20	50 30 30 30 30	Extraction: Water/n-propanol Clean Up: De-fatting IS: D_3-Acrylamide	LC-MS/MS
36	A B C D E F	10 10 10 10	35 35 35 35	Extraction: Water Clean Up: De-fatting, SPE with ethyl acetate IS: $^{13}C_3$-Acrylamide	GC-MS (CI)
37	A B C D E F		30 40 30 30 30 30	Extraction: Water/1-propanol Clean Up: Carrez IS: D_3-Acrylamide, methacrylamide	LC-MS/MS

40	A B C D E F	10 10 25 25 10 25	50 50 60 60 50 60	Extraction: Water Clean Up: De-fatting Derivatisation: Br External standard	GC-ECD
41	A B C D E F	 250 250	 750 750	Extraction: Water Derivatisation: Br External standard	GC-MS
44	A B C D E F	5 5 5 5 5 5	10 10 10 10 10 10	Extraction: ASE: DCM/ethanol Re-extraction with water External standard	LC-MS/MS
45	A B C D E F	10 10 10 10 10 10	20 20 20 20 20 20	Extraction: Water Clean Up: De-fatting, Carrez Derivatisation: Br IS: D_3-Acrylamide	GC-MS
46	A B C D E F	25 25 25 25 25 25	50 50 50 50 50 50	Extraction: Water Clean Up: SPE with ethyl acetate IS: D_3-Acrylamide	GC-MS

Extraction

- Water (19 Labs, 56 %)
- Water/Alcohol (8 Labs, 24 %)
- Organic solvents (8 Labs, 24 %)
- SPE (7 x) and ASE (3 x)

Clean Up

- De-fatting (23 Labs, 68 %)
- Carrez-clearing (15 Labs, 44 %)
- Freezing (1 Lab, 3 %)
- Re-extraction (1 Lab)

Chromatography/detection

LC (47 % LC)

- 14 laboratories LC-MS/MS
- 1 laboratories LC-MS
- 1 laboratories LC-LC-DAD

GC (53 % GC)

- 17 laboratories GC-MS of which
  - 5 applied CI
  - 8 laboratories used derivatisations (7x Br)
- 1 laboratory GC-ECD

9 Results

Laboratory 15 and 40 have been excluded completely for the evaluation of this proficiency test, because both found very high concentrations of acrylamide in sample E (blank) **and** their results of the other samples differed unacceptable from the target values. Implementation of these two labs led to a significant alteration of the precision parameters even with robust evaluation, falsifying the outcome of this proficiency test. It must be assumed that these laboratories have not quantified acrylamide with their respective methods of analysis.

For a detailed overview of the results of the analyses of the participating laboratories please see **ANNEX**. Laboratory 25 submitted as results means obtained by different techniques of analysis.

9.1 Mean and standard deviation

The individual laboratory results are given in the **ANNEX**.

Table 7: Summary of the results

Sample	Assigned val. (Q-Huber) [µg/kg]	S_R [µg/kg]	CV_R %	Target CV_R %	Min. tolerance limit* [µg/kg]	Max. tolerance limit* [µg/kg]	Horwitz %	Horrat ratio <2
Sample A	7286	1866,3	25,6	25,6	3553,4	11018,4	11,9	2,2
Sample B	215,2	124,1	57,7	57,7	-32,9	463,3	20,2	2,8
Sample C	183,9	51,5	28,0	28,0	81,0	286,0	20,6	1,4
Sample D	531,8	102,4	19,3	19,3	327,0	736,6	17,6	1,1
Sample E								
Sample F	181,8	49,4	27,2	27,2	83,1	280,6	20,7	1,3

* = $|Z| \leq 2$

The ranges of tolerance are calculated for a z-score between –2 and 2. The Horrat ratio [11], which is the quotient of the obtained S_R and the predicted standard deviation (Horwitz), is one criterion for the acceptability of an analytical method during its assessment [12]. A value of < 2 indicates an acceptable method. For the evaluation of proficiency tests with free choice of method, this ratio gives a certain indication of the performance levels of the laboratories. If the Horrat ratio exceeds 2, the scatter of the quantitative results is unacceptably high, especially when considering that robust methods were applied for the mathematical analysis. The observed Horrat ratio is smaller than 2, almost in all cases, indicating a reproducibility standard deviation around the Horwitz prediction, so that in can be concluded that the quantification of the analyte was performed in agreement with the Horrat criterion.

Most of the laboratories found sample E to be free of analyte (22 of 34 labs below their LOD or LOQ). Some labs found quite high concentrations (3 of 31 labs > 100 µg/kg) and should therefore check their system of analysis for cross contamination.

9.2 Z-scores

The individual results of the laboratories are given in the **ANNEX**. Z-scores calculated with the Horwitz-standard deviation as target were calculated also and are given also in the ANNEX.

9.2.1 Differentiated evaluations of the results

It is **not** the aim of a proficiency test to figure out the “most suitable” method of analysis but because here information is available on the applied methods (see Table 6) a dedicated evaluation was performed in order to find indications for differences between the methods. Essentially for such a comparison are a reasonable number of results in each selected group to be distinguished. Since the applied methods are very different in terms of the path of analysis e.g. extraction, clean-up, chromatography, detection, only two comparisons were made.

- An evaluation was made by distinguishing the results by the measurement method applied, either GC or HPLC.
- Another approach was to test the influence of the solvent of the basic extraction, which was divided in aqueous and non-aqeous. Mixtures of water and alcohol or other organic solvents (acetone, acetonitrile) were assigned to the non-aqueous group.

The statistical approach for such a comparison is the Analysis of Variance (ANOVA) that has been applied on the appropriate set of data. Null hypothesis was always that the variance within the two groups and the variance between the two groups is not distinguishable statistically. The outcome of the ANOVA is the test value F that has to be compared with the critical value F (table value) for the chosen probability of 95 %. If the test value < critical value than the null-hypothesis is confirmed. The probability P gives the probability factor in the scale from 0-1 for the confirmation of the null-hypothesis. Here if it is < 0.05 than the test-value exceeds also the table-value (critical F-value).

Table 8: Summary of results of the laboratories for all samples indicating the chromatography/detection and the mode of the basic extraction.

Lab	Method	Basic extraction	Lab Mean [µg/kg] A	Lab Mean [µg/kg] B	Lab Mean [µg/kg] C	Lab Mean [µg/kg] D	Lab Mean [µg/kg] E	Lab Mean [µg/kg] F
1	LC-MS/MS	non-aqueous	6380	320,0	115,0	580,0	23,0	175,0
2	LC-MS/MS	aqueous	8028	*<100*	136,0	519,0	<20	128,5
5	GC-MS	aqueous	5912	151,0	165,5	615,5	<30	162,0
6	GC-MS	aqueous	11500 E	2225,0 E	395,0 E	35,0 E	151,5	229,0
7	GC-MS (CI)	non-aqueous	7500	250,0	190,0	580,0	20,0	165,0
8	LC-MS/MS	aqueous	8295	<100	216,7	654,0	<100	216,3
11	LC-MS/MS	aqueous	7540	206,0	124,0	458,0	<30	142,0
12	GC-MS	aqueous	8430	53,0	271,0	620,3	62,0	181,7
14	GC-MS (CI)	non-aqueous	7618	129,9	174,7	464,5	<25	178,1
15*	GC-MS	non-aqueous	41200	8500,0	5850,0	9850,0	45200,0	5900,0
16	GC-MS	non-aqueous	7220	456,0	150,0	495,0	*<LOD*	165,0
17	GC-MS	non-aqueous	5407	315,0	157,0	328,5	<20	158,5
18	LC-MS/MS	non-aqueous	8100	143,3	156,7	546,7	20	156,7
19	GC-MS	aqueous	8327	158,0	277,0	579,0	87,0	338,3 E
20	LC-MS/MS	aqueous	8773	*<300*	156,0	469,0	<180	152,0
21	GC-MS	non-aqueous	8860		194,0	522,0	<40	188,0
22	LC-MS/MS	aqueous	3513 E	69,0	148,7	437,7	<20	83,7
24	GC-MS (CI)	aqueous	4796	92,2	96,0	355,0	<10	138,6
25*	LC-MS/MS	non-aqueous	6891	127,0	129,0	411,0	7,0	131,0
	GC-MS							
	GC-MS (HR)							
26	LC-LC-DAD	aqueous	8210	128,0	181,5	643,0	<60	170,5
27	LC-MS/MS	non-aqueous	2252 E	225,0	208,0	512,5	<30	226,0
28	LC-MS	aqueous	8991	159,5	247,0	642,5	<50	179,5
29	LC-MS/MS	aqueous	8493	*<150*	180,0	614,0	<20	188,5
30	LC-MS/MS	aqueous	8826	106,0	213,7	612,3	72,7	238,0
31	GC-MS (CI)	non-aqueous	11316 E	299,0	196,0	561,5	916,0	201,0
32	GC-MS	aqueous	8615	248,3	231,7	585,0	72,7	260,3
33	LC-MS/MS	non-aqueous	1965 E	*n.a.*	38,0	142,5 E	40,0	34,0 E
36	GC-MS (CI)	aqueous	6478	172,5	170,5	581,5	<40	246,5
37	LC-MS/MS	non-aqueous	2464 E	220,7	213,0	508,0	<30	225,7
40*	GC-ECD	aqueous	614	4644,0	2319,0	408,0	1866,0	6475,0
41	GC-MS	aqueous	*n.a.*	*n.a.*	620,5	*n.a.*	*n.a.*	394,0
44	LC-MS/MS	non-aqueous	3770	580,0 E	155,0	605,0	215,0	140,0
45	GC-MS	aqueous	7376	3227,5 E	247,7	637,3	<20	206,0
46	GC-MS	aqueous	8410	200,0	170,0	500,0	*<50*	230,0

** = Lab excluded; n.a. = not analysed; E = IzI > 2*

In Table 8 the relevant information is given, whether a laboratory applied GC or HPLC and how the basic extraction was performed. Apart from Lab 15 and 40 also Lab 25 was excluded from this evaluation because its results are means obtained by different methods.

9.2.2 GC/HPLC

Table 9: ANOVA GC/HPLC with all valid data (Lab 25 was excluded for this calculation).

Sample		n Labs	Mean (µg/kg)	Variance (µg/kg)	CV %	Test value (F)	Probability	Critical F- value
A	GC	15	7851	3539195	24,0			
	HPLC	15	6373	7423064	42,7	2,987	0,095	4,196
B	GC	14	569,8	883707	165,0			
	HPLC	10	215,8	21477	67,9	1,377	0,253	4,301
C	GC	15	205,7	5050	34,5			
	HPLC	14	175,1	1579	22,7	2,014	0,167	4,210
D	GC	15	497,3	24664	31,6			
	HPLC	15	529,6	16538	24,3	0,379	0,543	4,196
F	GC	15	203,2	2635	25,3			
	HPLC	15	163,8	3053	33,7	4,103	0,052	4,196

Table 10: ANOVA GC/HPLC with reduced number of laboratories, only if IZI score ≤ 2 (S_R found). (Lab 25 was excluded for this calculation).

Sample		n Labs	Mean (µg/kg)	Variance (µg/kg)	CV %	Test value (F)	Probability	Critical F- value
A	GC	13	7304	1694647	17,8			
	HPLC	11	7764	2276290	19,4	0,645	0,431	4,301
B	GC	12	210,4	12288	52,7			
	HPLC	9	175,3	5739	43,2	0,664	0,425	4,381
C	GC	14	192,2	2486	25,9			
	HPLC	14	175,1	1579	22,7	1,011	0,324	4,225
D	GC	14	530,4	8944	17,8			
	HPLC	14	557,3	5460	13,3	0,703	0,409	4,225
F	GC	14	193,6	1334	18,9			
	HPLC	15	163,8	3053	33,7	2,889	0,101	4,210

All test values are smaller than the table values (critical F-value), what implies that **there is no statistically significant difference of the results obtained applying GC or HPLC**.

9.2.3 Basic extraction Aqueous/ non-aqueous

Table 11: ANOVA: Basic extraction aqueous/non-aqueous, with all valid data. (Lab 25 was excluded for this calcuation).

Sample		n Labs	Mean (µg/kg)	Variance (µg/kg)	CV %	Test value (F)	Probability	Critical F- value
A	aqueous	18	7806,2	3101253	22,6			
	non-aqueous	13	6134,0	8005265	46,1	4,114	0,052	4,183
B	aqueous	14	514,0	919917	186,6			
	non-aqueous	11	278,7	19622	50,3	0,645	0,430	4,279
C	aqueous	18	201,5	4933	34,8			
	non-aqueous	12	169,9	968	18,3	2,142	0,154	4,196
D	aqueous	18	531,0	22576	28,3			
	non-aqueous	13	481,3	16032	26,3	0,938	0,341	4,183
F	aqueous	18	194,0	3473	30,4			
	non-aqueous	13	164,9	2379	29,6	2,108	0,157	4,183

Table 12: ANOVA: Basic extraction aqueous/non-aqueous, with reduced number of laboratories only if IZI score ≤ 2 (S_R found). (Lab 25 was excluded for this calculation).

Sample		n Labs	Mean (µg/kg)	Variance (µg/kg)	CV %	Test value (F)	Probability	Critical F- value
A	aqueous	17	7589	2392158	20,4			
	non-aqueous	8	6857	2648866	23,7	1,180	0,289	4,279
B	aqueous	12	**145,3**	**3396**	40,1			
	non-aqueous	9	**262,1**	**9993**	38,1	**11,356**	**0,003**	**4,381**
C	aqueous	17	190,2	2765	27,7			
	non-aqueous	11	173,6	882	17,1	0,901	0,351	4,225
D	aqueous	17	560,2	7706	15,7			
	non-aqueous	11	518,5	5742	14,6	1,669	0,208	4,225
F	aqueous	17	185,5	2312	25,9			
	non-aqueous	12	167,7	2482	29,7	0,929	0,344	4,210

Sample B (cocoa powder) showed significant differences for the consideration of the laboratories with IZI score ≤ 2. In contradiction to the other samples the extraction with non-aqueous solvent as first step led to almost the double amount of acrylamide (262 µg/kg and 145 µg/kg). **This clearly demonstrates that the basic extraction significantly effects the final result found for some matrices, here cocoa**.

Since almost all laboratories used isotopically labelled internal standards it is obvious that the distribution of the analyte in the sample is different from the distribution of the internal standard that is achieved by spiking and mixing. So particularly the extraction procedure seems to be a crucial step for the analysis of acrylamide for some matrices. In addition it can be concluded that the fat/water distribution of the matrix affects the extraction and analysis.

10 Discussion and Conclusions

Apart from providing a laboratory assessment, this proficiency test has also shown that the quality of the analysis of acryamide is depending on the matrix. Good results were found for crisp bread and butter biscuits, still acceptable results for mashed potatoes.

Analysis of cocoa powder showed some problems. The robust calculated reproducibility standard deviation exceeds 2.8 times the Horwitz standard deviation, so the Horrat criterion has not been fulfilled for this matrix. In addition the mode of the basic extraction, aqueous or non-aqueous was found to be affecting significantly the results of analysis.

11 References

1 . Sachs L, (1996) Angewandte Statistik, 8th Edition, Springer Verlag Berlin

2. Thompson M, Wood R, International Harmonised Protocol for the Proficiency Testing of (Chemical) Analytical Laboratories, J AOAC Int, 76: 926-940 (**1993**)

3. Uhlig S, Entwicklung eines Programms zur Auswertung von anal. Laborvergleichsuntersuchungen gemäß int. Protokollen: Mathematisch-statistische Konzeption, Bericht im Rahmen eines BgVV-Projekts (**1995**)

4. Prolab 99 (Version 2.0), Software designed for the evaluation of ring test, Quo data GmbH, Dresden, (**1999**)

5. Horwitz W, Kamps L R, Boyer K W, J. Assoc. Off. Anal. Chem., 6, 1344-1354 (**1980**)

6. Horwitz W, Anal Chem, 54: 67A-76A (**1982**)

7. Thompson M, Lowthian P J, Analyst 121: 1593-1596 (**1996**)

8. Lischer P, (1994) Robust Statistics, Data Analysis and Computer Intensive Methods, Ed. Rieder H. Springer Verlag, Berlin.

9. Analytical Methods Committee Analyst 114: 1693-1697, (**1989**)

10. Rousseeuw P J, Croux C, J. Am. stat. Ass., 1273-1283 (**1993**)

11. Peeler J T, Horwitz W, Albert R, JAOAC 72: 784-806 (**1989**)

12. Pocklington W D, Pure and Appl. Chem. 62: 149-162 (**1990**)

ANNEX

Sample A (Mashed potatoes)

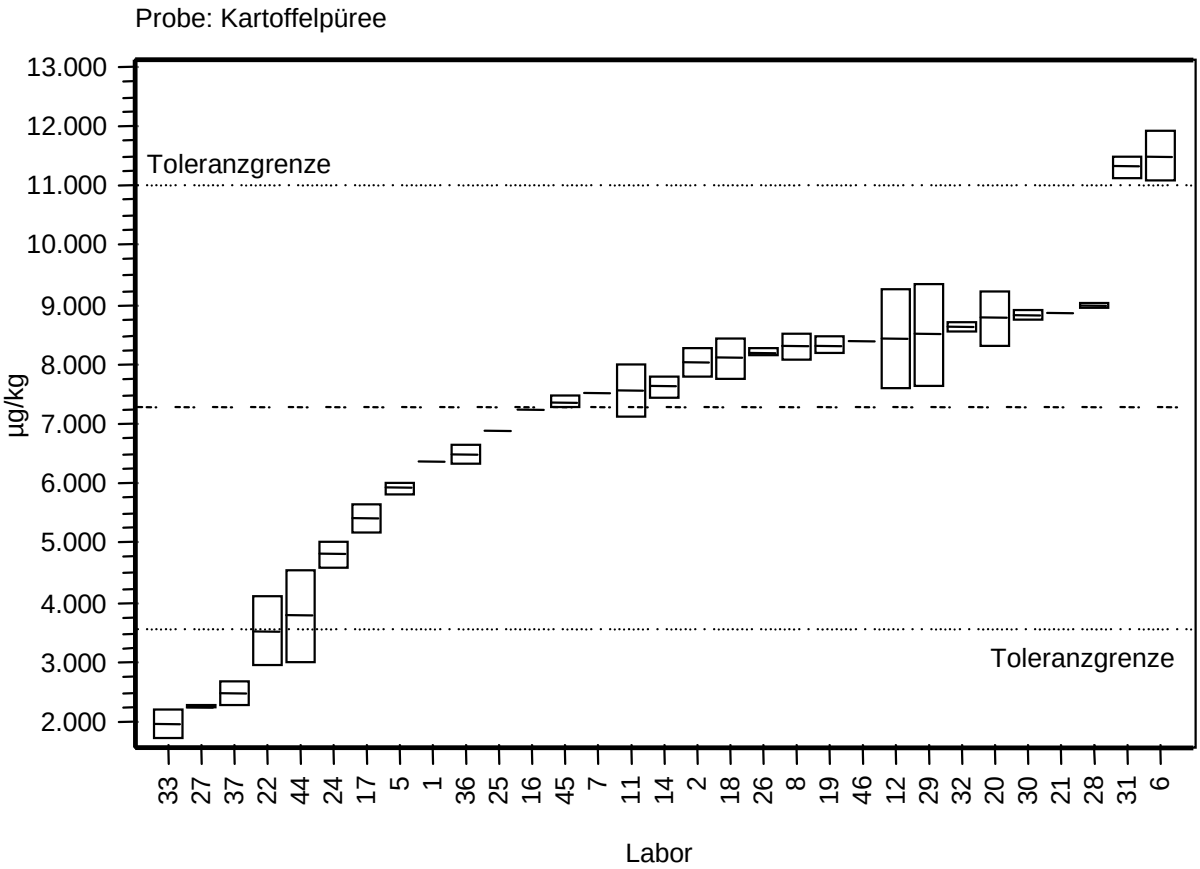
Lab	Method	Basic extraction	Lab Mean [µg/kg]		Lab SD [µg/kg]	n	Z-score S_R found	S_R Horwitz
1	LC-MS/MS	non-aqueous	**6380**				**-0,485**	-1,048
2	LC-MS/MS	aqueous	**8028**		252	4	**0,397**	0,858
5	GC-MS	aqueous	**5912**		106	2	**-0,736**	-1,589
6	GC-MS	aqueous	**11500**		424	2	**2,258 E**	4,875 E
7	GC-MS (CI)	non-aqueous	**7500**				**0,115**	0,248
8	LC-MS/MS	aqueous	**8295**		207	3	**0,541**	1,168
11	LC-MS/MS	aqueous	**7540**		439	5	**0,136**	0,294
12	GC-MS	aqueous	**8430**		817	3	**0,613**	1,323
14	GC-MS (CI)	non-aqueous	**7618**		168	4	**0,178**	0,384
15	GC-MS	non-aqueous	**41200**	*	19375	2		
16	GC-MS	non-aqueous	**7220**				**-0,035**	-0,076
17	GC-MS	non-aqueous	**5407**		245	2	**-1,007**	-2,174 E
18	LC-MS/MS	non-aqueous	**8100**		346	3	**0,436**	0,942
19	GC-MS	aqueous	**8327**		139	4	**0,558**	1,204
20	LC-MS/MS	aqueous	**8773**		458	2	**0,797**	1,720
21	GC-MS	non-aqueous	**8860**				**0,843**	1,821
22	LC-MS/MS	aqueous	**3513**		583	3	**-2,021 E**	-4,364 E
24	GC-MS (CI)	aqueous	**4796**		219	4	**-1,334**	-2,880 E
25	LC-MS/MS	non-aqueous	**6891**				**-0,212**	-0,457
	GC-MS							
	GC-MS (HR)							
26	LC-LC-DAD	aqueous	**8210**		57	2	**0,495**	1,069
27	LC-MS/MS	non-aqueous	**2252**		30	2	**-2,698 E**	-5,824 E
28	LC-MS	aqueous	**8991**		49	2	**0,913**	1,972
29	LC-MS/MS	aqueous	**8493**		852	4	**0,647**	1,396
30	LC-MS/MS	aqueous	**8826**		98	3	**0,825**	1,782
31	GC-MS (CI)	non-aqueous	**11316**		180	2	**2,159 E**	4,662 E
32	GC-MS	aqueous	**8615**		78	2	**0,712**	1,538
33	LC-MS/MS	non-aqueous	**1965**		233	2	**-2,851 E**	-6,156 E
36	GC-MS (CI)	aqueous	**6478**		154	2	**-0,433**	-0,935
37	LC-MS/MS	non-aqueous	**2464**		197	3	**-2,584 E**	-5,578 E
40	GC-ECD	aqueous	**614**	*				
41	GC-MS	aqueous	*n.a.*					
44	LC-MS/MS	non-aqueous	**3770**		778	2	**-1,884**	-4,067 E
45	GC-MS	aqueous	**7376**		84	2	**0,048**	0,104
46	GC-MS	aqueous	**8410**				**0,602**	1,300

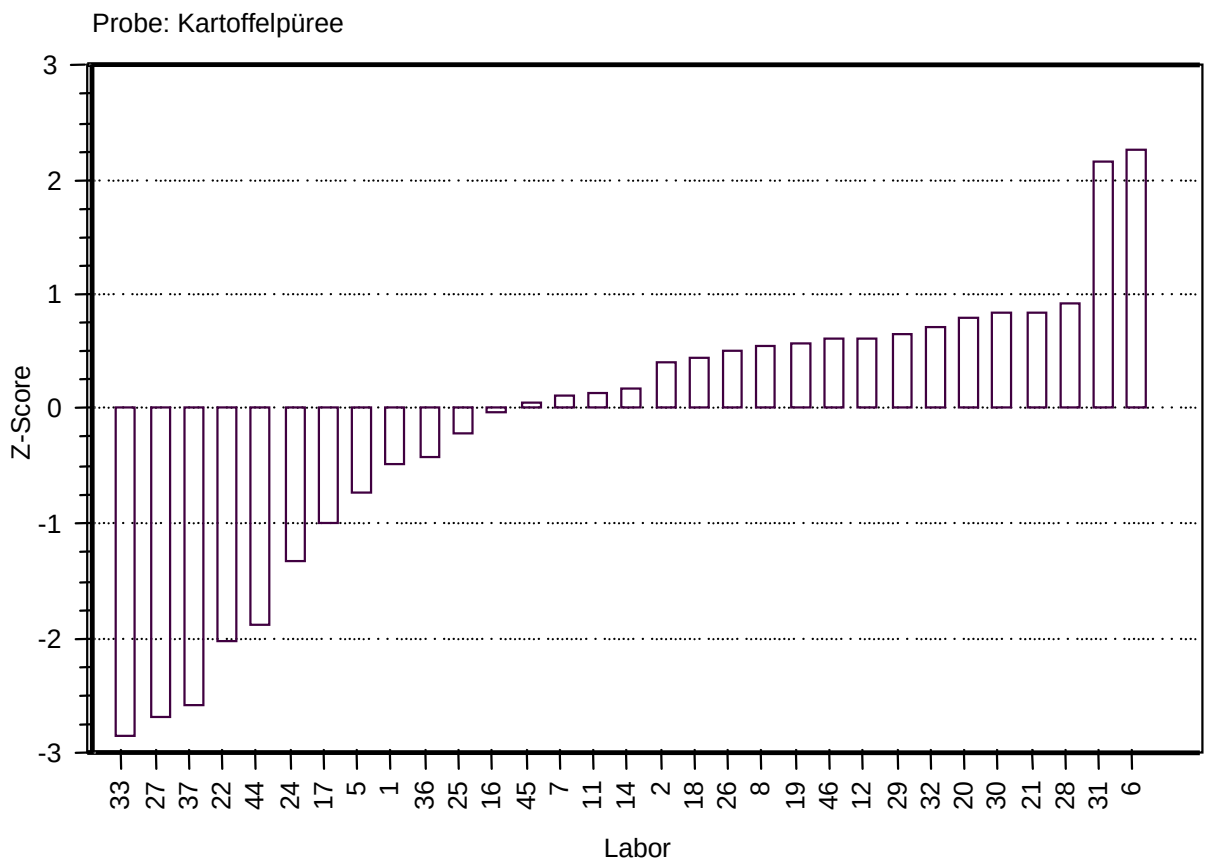
** = Lab excluded; n.a. = not analysed; E = IzI > 2*

Assigned value	**7285,9**	**µg/kg**
S_R	**1866,3**	**µg/kg**
CV_R	**25,6**	**%**
Number labs.	31	

	Found	Horwitz	
S_R Target	**1866,3**	864,4	µg/kg
CV_R Target	**25,6**	11,9	%
lower tolerance limit |Z|<2,000	**3553,4**	5557,1	µg/kg
upper tolerance limit |Z|<2,000	**11018,4**	9014,7	µg/kg

Sample A (mashed potatoes)





Sample B (Cocoa)

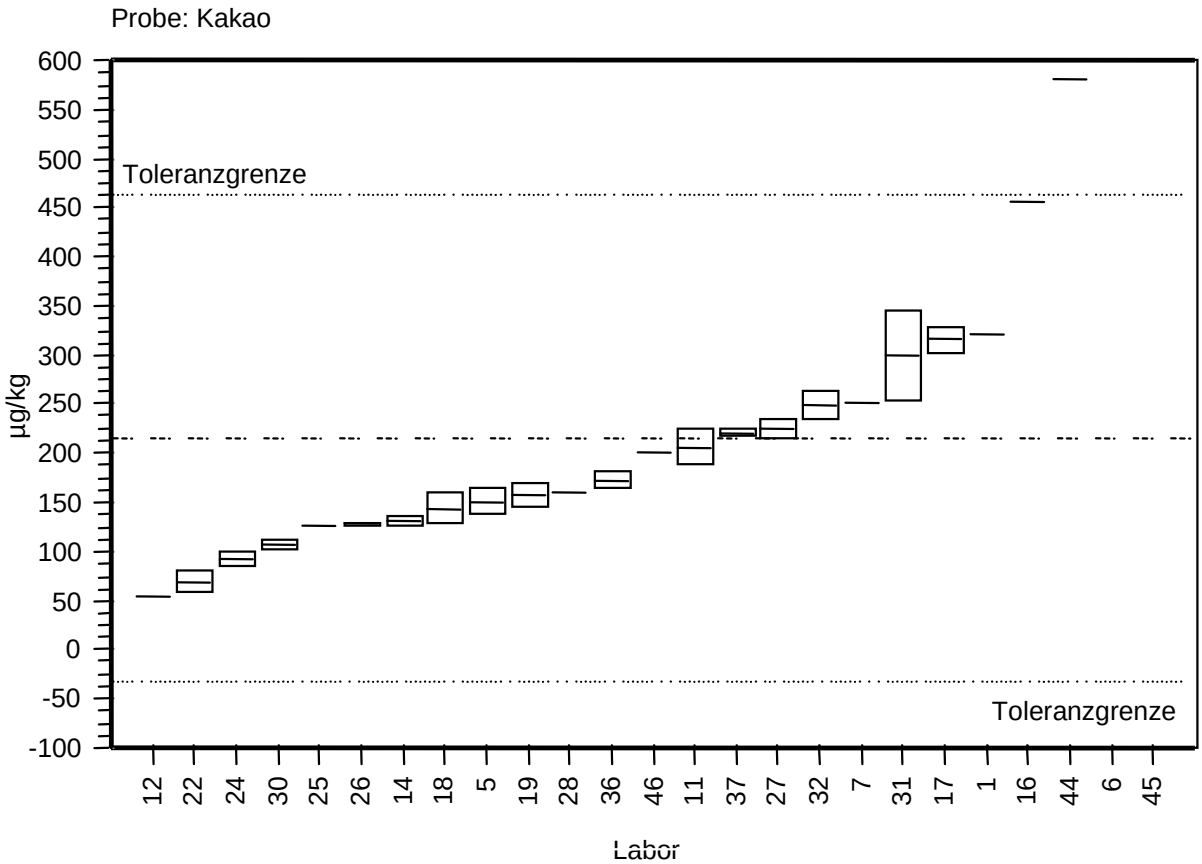
Lab	Method	Basic extraction	Lab Mean [µg/kg]		Lab SD [µg/kg]	n	Z-score S_R found	S_R Horwitz
1	LC-MS/MS	non-aqueous	**320,0**				**0,845**	2,416 E
2	LC-MS/MS	aqueous	*<100*	*<LOQ*				
5	GC-MS	aqueous	**151,0**		12,7	2	**-0,517**	-1,480
6	GC-MS	aqueous	**2225,0**		106,1	2	**16,201 E**	46,333 E
7	GC-MS (CI)	non-aqueous	**250,0**				**0,281**	0,803
8	LC-MS/MS	aqueous	<100	*<LOQ*				
11	LC-MS/MS	aqueous	**206,0**		18,2	5	**-0,074**	-0,212
12	GC-MS	aqueous	**53,0**				**-1,307**	-3,739 E
14	GC-MS (CI)	non-aqueous	**129,9**		4,8	4	**-0,688**	-1,967
15	GC-MS	non-aqueous	**8500,0**	*	3535,5	2		
16	GC-MS	non-aqueous	**456,0**				**1,941**	5,552 E
17	GC-MS	non-aqueous	**315,0**		14,1	2	**0,805**	2,301 E
18	LC-MS/MS	non-aqueous	**143,3**		15,3	3	**-0,579**	-1,656
19	GC-MS	aqueous	**158,0**		11,6	4	**-0,461**	-1,318
20	LC-MS/MS	aqueous	*<300*	*<LOD*				
21	GC-MS	non-aqueous		*n.a.*				
22	LC-MS/MS	aqueous	**69,0**		11,3	2	**-1,174**	-3,359 E
24	GC-MS (CI)	aqueous	**92,2**		7,4	4	**-0,991**	-2,835 E
25	LC-MS/MS	non-aqueous	**127,0**				**-0,711**	-2,033 E
	GC-MS							
	GC-MS (HR)							
26	LC-LC-DAD	aqueous	**128,0**		1,4	2	**-0,703**	-2,010 E
27	LC-MS/MS	non-aqueous	**225,0**		9,9	2	**0,079**	0,226
28	LC-MS	aqueous	**159,5**		0,7	2	**-0,449**	-1,284
29	LC-MS/MS	aqueous	*<150*	*<LOD*				
30	LC-MS/MS	aqueous	**106,0**		5,0	3	**-0,88**	-2,517 E
31	GC-MS (CI)	non-aqueous	**299,0**		45,3	2	**0,676**	1,932
32	GC-MS	aqueous	**248,3**		14,2	3	**0,267**	0,764
33	LC-MS/MS	non-aqueous		*n.a.*				
36	GC-MS (CI)	aqueous	**172,5**		7,8	2	**-0,344**	-0,984
37	LC-MS/MS	non-aqueous	**220,7**		3,8	3	**0,044**	0,126
40	GC-ECD	aqueous	**4644,0**	*				
41	GC-MS	aqueous		*n.a.*				
44	LC-MS/MS	non-aqueous	**580,0**		0,0	2	**2,941 E**	8,410 E
45	GC-MS	aqueous	**3227,5**		27,6	2	**24,282 E**	69,445 E
46	GC-MS	aqueous	**200,0**				**-0,122**	-0,350

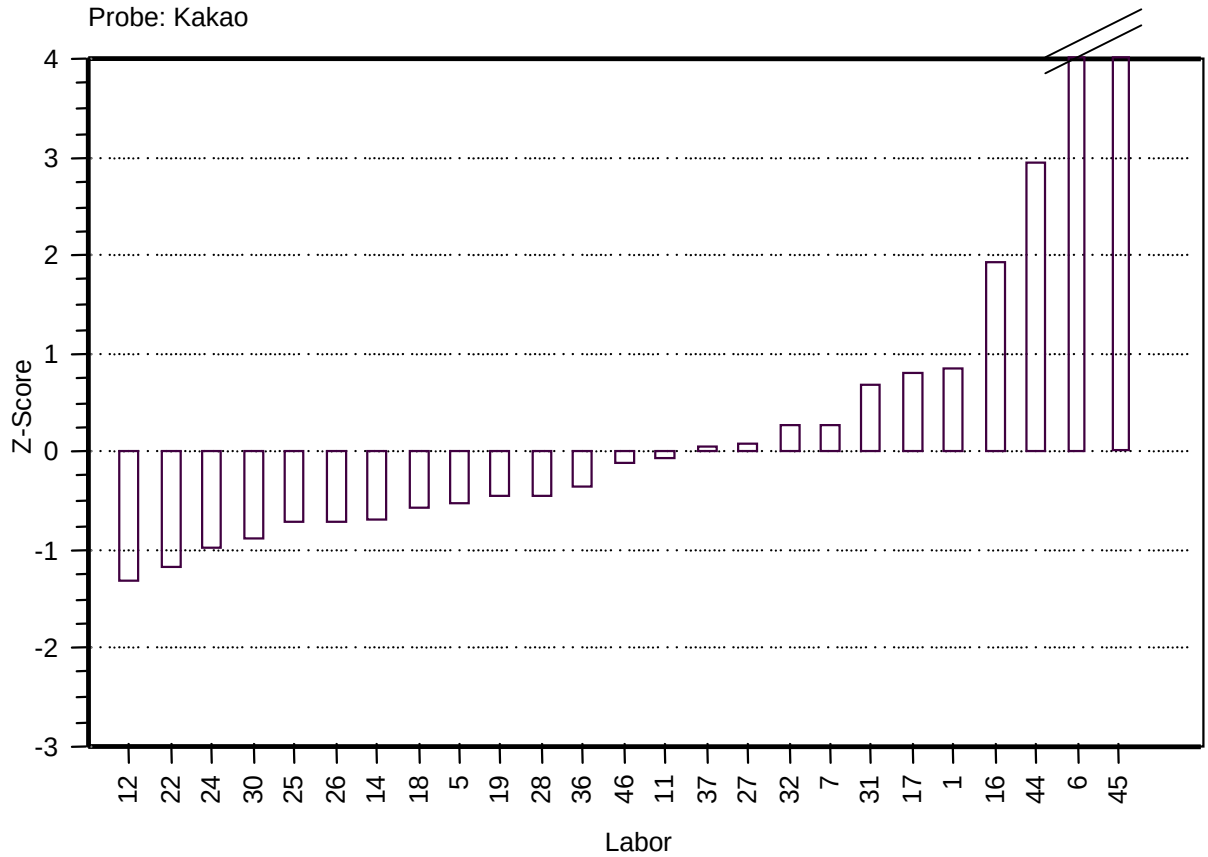
** = Lab excluded; n.a. = not analysed; E = |z| > 2*

Assigned value	**215,18**	**µg/kg**
S_R	**124,05**	**µg/kg**
CV_R	**57,7**	**%**
Number labs	25	

	Found	Horwitz	
S_R Target	**124,05**	43,37	µg/kg
CV_R Target	**57,7**	20,2	%
lower tolerance limit |Z|<2,000	**-32,92**	128,43	µg/kg
upper tolerance limit |Z|<2,000	**463,29**	301,94	µg/kg

Sample B (cocoa powder)





Sample C (Crisp bread)

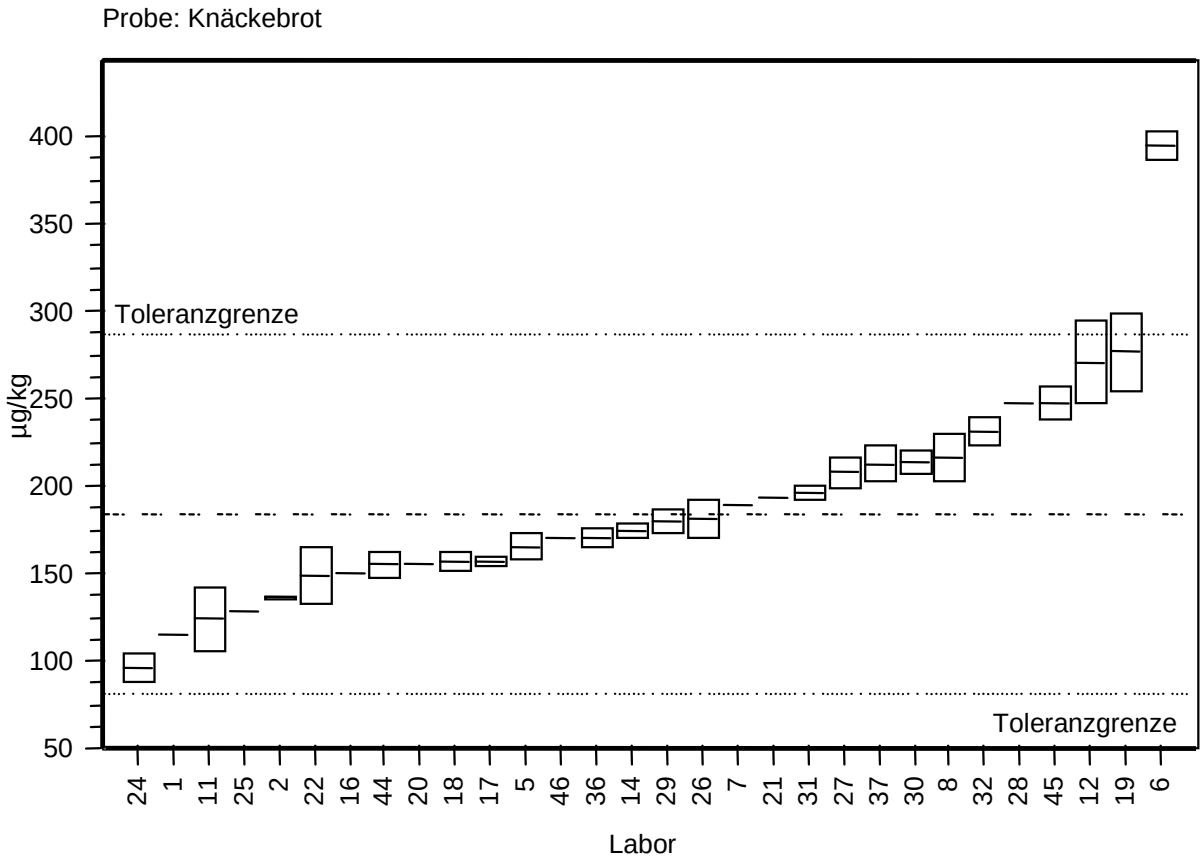
Lab	Method	Basic extraction	Lab Mean [µg/kg]		Lab SD [µg/kg]	n	Z-score S_R found	S_R Horwitz
1	LC-MS/MS	non-aqueous	**115,0**				**-1,339**	-1,815
2	LC-MS/MS	aqueous	**136,0**		0,8	4	**-0,930**	-1,262
5	GC-MS	aqueous	**165,5**		7,8	2	**-0,357**	-0,484
6	GC-MS	aqueous	**395,0**		8,5	2	**4,103 E**	5,562 E
7	GC-MS (CI)	non-aqueous	**190,0**				**0,119**	0,161
8	LC-MS/MS	aqueous	**216,7**		13,2	3	**0,637**	0,864
11	LC-MS/MS	aqueous	**124,0**		18,2	5	**-1,164**	-1,578
12	GC-MS	aqueous	**271,0**		24,0	2	**1,693**	2,295 E
14	GC-MS (CI)	non-aqueous	**174,7**		4,6	4	**-0,179**	-0,243
15	GC-MS	non-aqueous	**5850,0**	*	1202,1	2		
16	GC-MS	non-aqueous	**150,0**				**-0,658**	-0,893
17	GC-MS	non-aqueous	**157,0**		2,8	2	**-0,522**	-0,708
18	LC-MS/MS	non-aqueous	**156,7**		5,8	3	**-0,529**	-0,717
19	GC-MS	aqueous	**277,0**		22,6	2	**1,81**	2,453 E
20	LC-MS/MS	aqueous	**156,0**				**-0,542**	-0,735
21	GC-MS	non-aqueous	**194,0**				**0,197**	0,267
22	LC-MS/MS	aqueous	**148,7**		16,0	3	**-0,684**	-0,928
24	GC-MS (CI)	aqueous	**96,0**		7,5	4	**-1,708**	-2,315 E
25	LC-MS/MS	non-aqueous	**129,0**				**-1,066**	-1,446
	GC-MS							
	GC-MS (HR)							
26	LC-LC-DAD	aqueous	**181,5**		10,6	2	**-0,046**	-0,063
27	LC-MS/MS	non-aqueous	**208,0**		8,5	2	**0,469**	0,635
28	LC-MS	aqueous	**247,0**				**1,227**	1,663
29	LC-MS/MS	aqueous	**180,0**		7,1	2	**-0,075**	-0,102
30	LC-MS/MS	aqueous	**213,7**		6,5	3	**0,579**	0,785
31	GC-MS (CI)	non-aqueous	**196,0**		4,2	2	**0,236**	0,319
32	GC-MS	aqueous	**231,7**		8,1	3	**0,929**	1,259
33	LC-MS/MS	non-aqueous	**38,0**	**				
36	GC-MS (CI)	aqueous	**170,5**		4,9	2	**-0,26**	-0,353
37	LC-MS/MS	non-aqueous	**213,0**		10,5	3	**0,566**	0,767
40	GC-ECD	aqueous	**2319,0**	*				
41	GC-MS	aqueous	**620,5**	*<LOQ*	34,6	2		
44	LC-MS/MS	non-aqueous	**155,0**		7,1	2	**-0,561**	-0,761
45	GC-MS	aqueous	**247,7**		9,5	3	**1,240**	1,681
46	GC-MS	aqueous	**170,0**				**-0,270**	-0,366

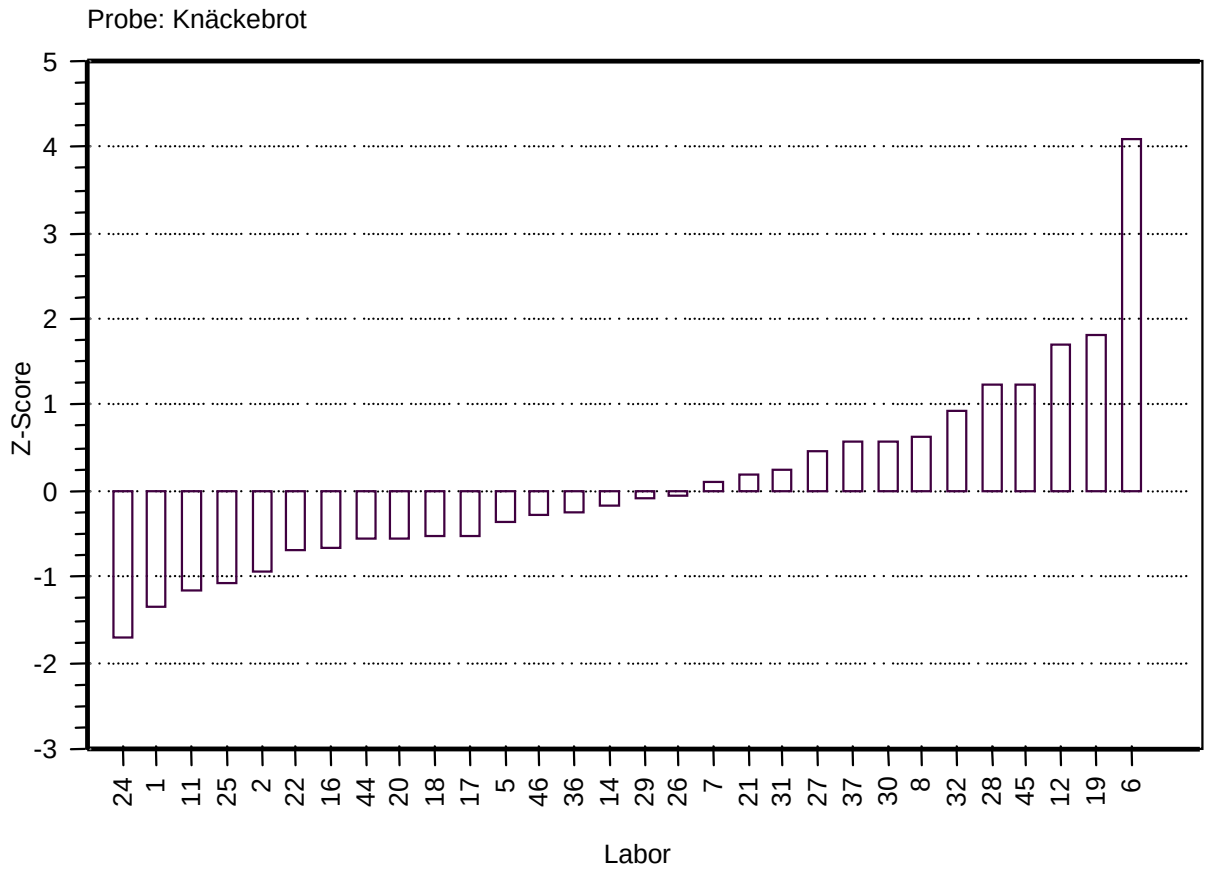
* = *Lab excluded;* ** another determination below the limit of quantification; *n.a. = not analysed; E = Izl > 2*

Assigned value	**183,88**	**µg/kg**
S_R	**51,46**	**µg/kg**
CV_R	**28,0**	**%**
Number labs	30	

	Found	Horwitz	
S_R Target	**51,46**	37,95	µg/kg
CV_R Target	**28,0**	20,6	%
lower tolerance limit |Z|<2,000	**80,96**	107,97	µg/kg
upper tolerance limit |Z|<2,000	**286,02**	259,79	µg/kg

Sample C (crisp bread)





Sample D (Butter biscuit)

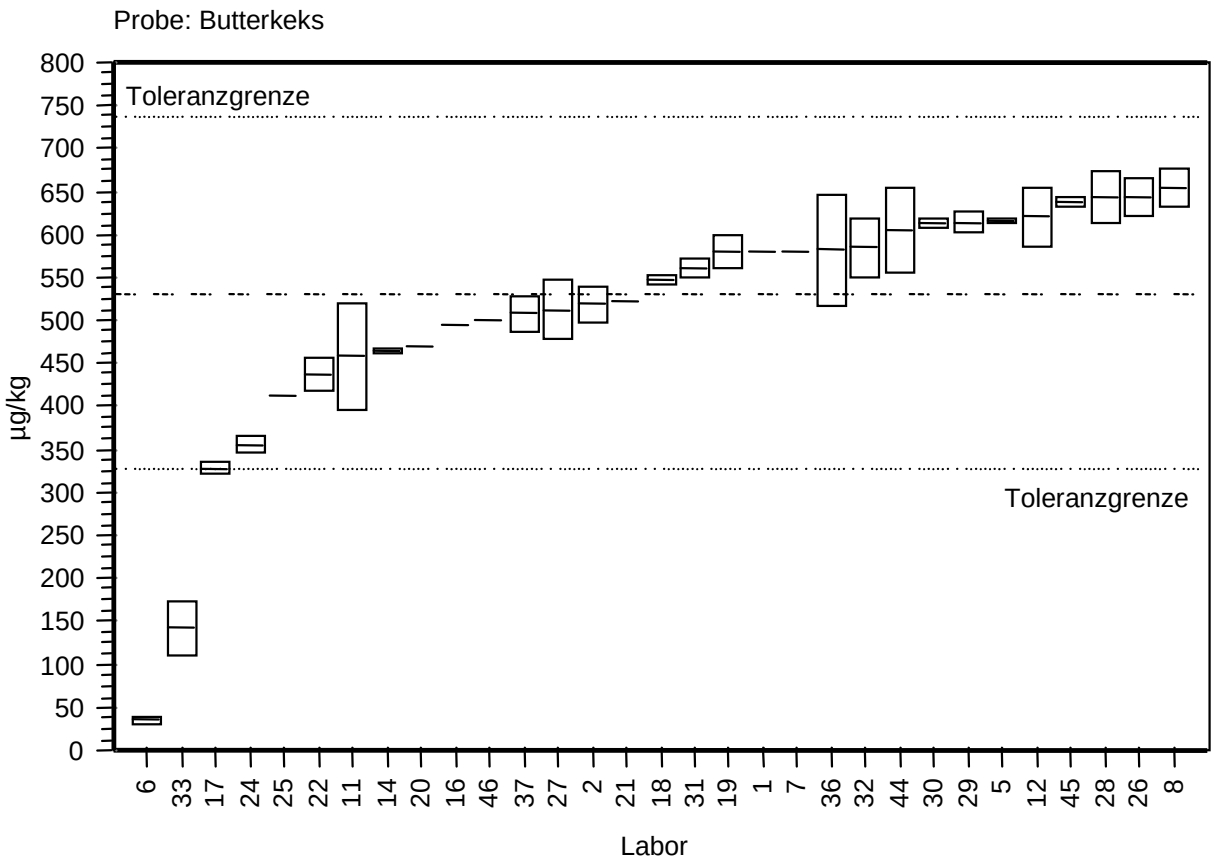
Lab	Method	Basic extraction	Lab Mean [µg/kg]		Lab SD [µg/kg]	n	Z-score S_R found	S_R Horwitz
1	LC-MS/MS	non-aqueous	**580,0**				**0,470**	0,515
2	LC-MS/MS	aqueous	**519,0**		20,8	4	**-0,125**	-0,137
5	GC-MS	aqueous	**615,5**		2,1	2	**0,817**	0,894
6	GC-MS	aqueous	**35,0**		4,2	2	**-4,851 E**	-5,310 E
7	GC-MS (CI)	non-aqueous	**580,0**				**0,470**	0,515
8	LC-MS/MS	aqueous	**654,0**		21,7	3	**1,193**	1,306
11	LC-MS/MS	aqueous	**458,0**		62,6	5	**-0,721**	-0,789
12	GC-MS	aqueous	**620,3**		34,5	3	**0,864**	0,946
14	GC-MS (CI)	non-aqueous	**464,5**		3,5	3	**-0,658**	-0,720
15	GC-MS	non-aqueous	**9850,0**	*	5161,9	2		
16	GC-MS	non-aqueous	**495,0**				**-0,360**	-0,394
17	GC-MS	non-aqueous	**328,5**		7,8	2	**-1,985**	-2,173 E
18	LC-MS/MS	non-aqueous	**546,7**		5,8	3	**0,145**	0,159
19	GC-MS	aqueous	**579,0**		19,4	4	**0,461**	0,504
20	LC-MS/MS	aqueous	**469,0**		0,0	2	**-0,613**	-0,672
21	GC-MS	non-aqueous	**522,0**				**-0,096**	-0,105
22	LC-MS/MS	aqueous	**437,7**		19,1	3	**-0,919**	-1,006
24	GC-MS (CI)	aqueous	**355,0**		10,0	4	**-1,727**	-1,890
25	LC-MS/MS GC-MS GC-MS (HR)	non-aqueous	**411,0**				**-1,180**	-1,291
26	LC-LC-DAD	aqueous	**643,0**		21,2	2	**1,086**	1,188
27	LC-MS/MS	non-aqueous	**512,5**		33,2	2	**-0,189**	-0,207
28	LC-MS	aqueous	**642,5**		30,4	2	**1,081**	1,183
29	LC-MS/MS	aqueous	**614,0**		12,7	2	**0,802**	0,878
30	LC-MS/MS	aqueous	**612,3**		5,1	3	**0,786**	0,861
31	GC-MS (CI)	non-aqueous	**561,5**		10,6	2	**0,290**	0,317
32	GC-MS	aqueous	**585,0**		34,0	3	**0,519**	0,568
33	LC-MS/MS	non-aqueous	**142,5**		31,8	2	**-3,802 E**	-4,161 E
36	GC-MS (CI)	aqueous	**581,5**		65,8	2	**0,485**	0,531
37	LC-MS/MS	non-aqueous	**508,0**		20,9	3	**-0,233**	-0,255
40	GC-ECD	aqueous	**408,0**	*				
41	GC-MS	aqueous		*n.a.*				
44	LC-MS/MS	non-aqueous	**605,0**		49,5	2	**0,715**	0,782
45	GC-MS	aqueous	**637,3**		5,5	3	**1,030**	1,128
46	GC-MS	aqueous	**500,0**				**-0,311**	-0,340

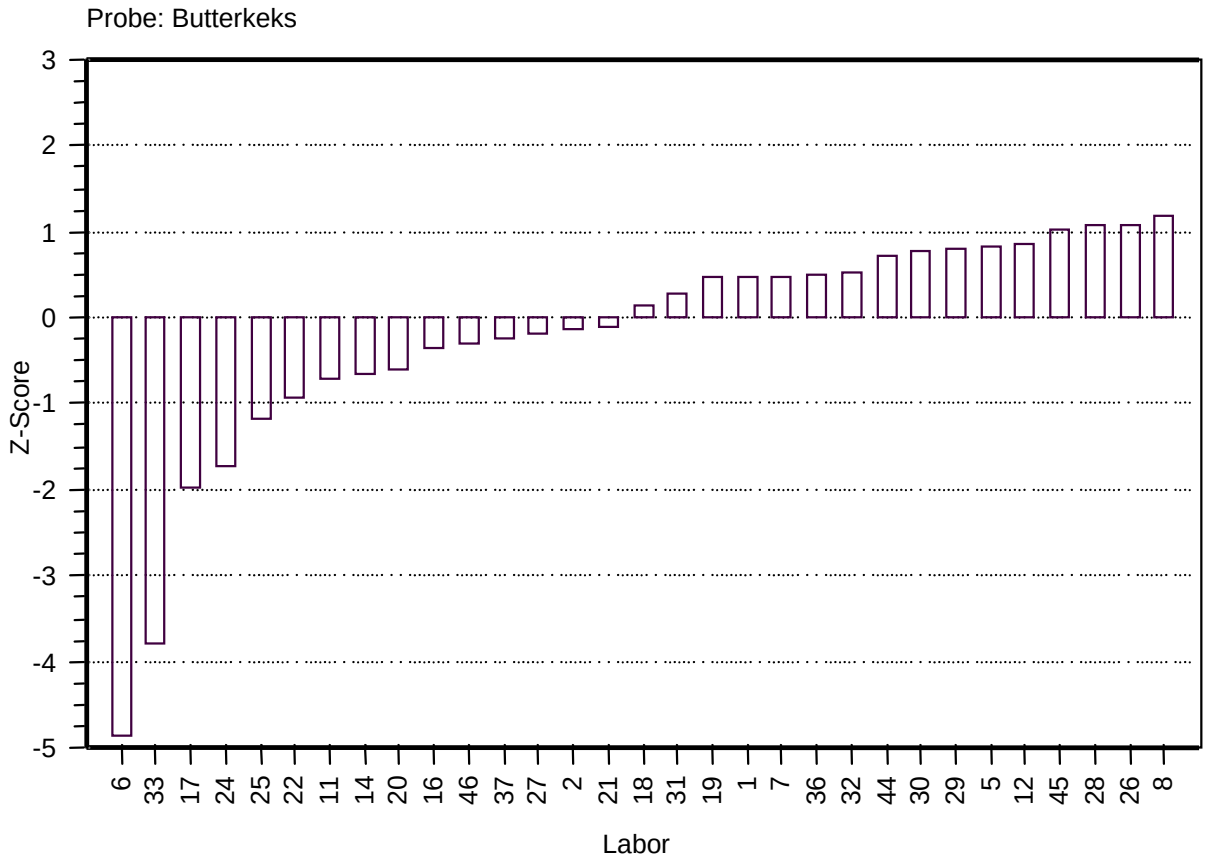
** = Lab excluded; n.a. = not analysed; E = IzI > 2*

Assigned value	**531,83**	**µg/kg**
S_R	**102,41**	**µg/kg**
CV_R	**19,3**	**%**
Number labs	31	

	Found	Horwitz	
S_R Target	**102,41**	93,56	µg/kg
CV_R Target	**19,3**	17,6	%
lower tolerance limit |Z|<2,000	**327,01**	344,71	µg/kg
upper tolerance limit |Z|<2,000	**736,64**	718,94	µg/kg

Sample D (butter biscuit)





Sample E (Mashed potatoes)

Lab	Method	Basic extraction	Lab Mean [µg/kg]		Lab SD [µg/kg]	n
1	LC-MS/MS	non-aqueous	**23,0**	*<LOQ*		
2	LC-MS/MS	aqueous	**<20**	*<LOD*		
5	GC-MS	aqueous	**<30**	*<LOQ*		
6	GC-MS	aqueous	**151,5**		9,2	2
7	GC-MS (CI)	non-aqueous	**20,0**			
8	LC-MS/MS	aqueous	**<100**	*<LOQ*		
11	LC-MS/MS	aqueous	**<30**	*<LOQ*		
12	GC-MS	aqueous	**62,0**	*<LOQ*		
14	GC-MS (CI)	non-aqueous	**<25**	*<LOD*		
15	GC-MS	non-aqueous	**45200,0**	*	19092	2
16	GC-MS	non-aqueous		*<LOD*		
17	GC-MS	non-aqueous	**<20**	*<LOD*		
18	LC-MS/MS	non-aqueous	**20**	*<LOQ*		
19	GC-MS	aqueous	**87,0**		14,1	4
20	LC-MS/MS	aqueous	**<180**	*<LOD*		
21	GC-MS	non-aqueous	**<40**	*<LOD*		
22	LC-MS/MS	aqueous	**<20**	*<LOD*		
24	GC-MS (CI)	aqueous	**<10**	*<LOD*		
25	LC-MS/MS	non-aqueous	**7,0**			
	GC-MS					
	GC-MS (HR)					
26	LC-LC-DAD	aqueous	**<60**	*<LOD*		
27	LC-MS/MS	non-aqueous	**<30**	*LOQ*		
28	LC-MS	aqueous	**<50**	*<LOQ*		
29	LC-MS/MS	aqueous	**<20**	*<LOQ*		
30	LC-MS/MS	aqueous	**72,7**		3,1	3
31	GC-MS (CI)	non-aqueous	**916,0**		39,6	2
32	GC-MS	aqueous	**72,7**		11,0	3
33	LC-MS/MS	non-aqueous	**40,0**			
36	GC-MS (CI)	aqueous	**<40**	*<LOQ*		
37	LC-MS/MS	non-aqueous	**<30**	*<LOQ*		
40	GC-ECD	aqueous	**1866,0**	*		
41	GC-MS	aqueous		*n.a.*		
44	LC-MS/MS	non-aqueous	**215,0**		21,2	2
45	GC-MS	aqueous	**<20**	*<LOQ*		
46	GC-MS	aqueous	***<50***	*<LOQ*		

** = Lab excluded; n.a. = not analysed*

Sample F (Crisp bread)

Lab	Method	Basic extraction	Lab Mean [µg/kg]		Lab SD [µg/kg]	n	Z-score S_R found	S_R Horwitz
1	LC-MS/MS	non-aqueous	**175,0**				**-0,138**	-0,181
2	LC-MS/MS	aqueous	**128,5**		1,3	4	**-1,079**	-1,418
5	GC-MS	aqueous	**162,0**		4,2	2	**-0,401**	-0,527
6	GC-MS	aqueous	**229,0**		11,3	2	**0,956**	1,255
7	GC-MS (CI)	non-aqueous	**165,0**				**-0,340**	-0,447
8	LC-MS/MS	aqueous	**216,3**		20,1	3	**0,699**	0,919
11	LC-MS/MS	aqueous	**142,0**		21,7	5	**-0,806**	-1,059
12	GC-MS	aqueous	**181,7**		31,5	3	**-0,003**	-0,004
14	GC-MS (CI)	non-aqueous	**178,1**		12,3	3	**-0,074**	-0,098
15	GC-MS	non-aqueous	**5900,0**	*	1272,8	2		
16	GC-MS	non-aqueous	**165,0**				**-0,340**	-0,447
17	GC-MS	non-aqueous	**158,5**		3,5	2	**-0,472**	-0,620
18	LC-MS/MS	non-aqueous	**156,7**		5,8	3	**-0,509**	-0,669
19	GC-MS	aqueous	**338,3**		38,7	4	**3,168 E**	4,162 E
20	LC-MS/MS	aqueous	**152,0**				**-0,604**	-0,793
21	GC-MS	non-aqueous	**188,0**				**0,125**	0,165
22	LC-MS/MS	aqueous	**83,7**		20,2	3	**-1,987**	-2,611 E
24	GC-MS (CI)	aqueous	**138,6**		12,5	4	**-0,875**	-1,149
25	LC-MS/MS	non-aqueous	**131,0**				**-1,029**	-1,352
	GC-MS							
	GC-MS (HR)							
26	LC-LC-DAD	aqueous	**170,5**		0,7	2	**-0,229**	-0,301
27	LC-MS/MS	non-aqueous	**226,0**		14,1	2	**0,895**	1,176
28	LC-MS	aqueous	**179,5**		3,5	2	**-0,047**	-0,061
29	LC-MS/MS	aqueous	**188,5**		6,4	2	**0,136**	0,178
30	LC-MS/MS	aqueous	**238,0**		13,1	3	**1,138**	1,495
31	GC-MS (CI)	non-aqueous	**201,0**		19,8	2	**0,389**	0,511
32	GC-MS	aqueous	**260,3**		19,9	3	**1,590**	2,089 E
33	LC-MS/MS	non-aqueous	**34,0**		5,7	2	**-2,993 E**	-3,932 E
36	GC-MS (CI)	aqueous	**246,5**		21,9	2	**1,310**	1,721
37	LC-MS/MS	non-aqueous	**225,7**		16,6	3	**0,888**	1,167
40	GC-ECD	aqueous	**6475,0**	*				
41	GC-MS	aqueous	**394,0**	*<LOQ*	46,7	2		
44	LC-MS/MS	non-aqueous	**140,0**		0,0	2	**-0,847**	-1,112
45	GC-MS	aqueous	**206,0**		1,4	2	**0,490**	0,644
46	GC-MS	aqueous	**230,0**				**0,976**	1,282

** = Lab excluded; n.a. = not analysed; E = IzI > 2*

Assigned value	**181,81**	**µg/kg**
S_R	**49,38**	**µg/kg**
CV_R	**27,2**	**%**
Number labs	31	

	Found	Horwitz	
S_R Target	**49,38**	37,59	µg/kg
CV_R Target	**27,2**	20,7	%
lower tolerance limit |Z|<2,000	**83,05**	106,63	µg/kg
upper tolerance limit |Z|<2,000	**280,57**	256,99	µg/kg

Sample F (crisp bread)