Proficiency Tests
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Evaluation Report
proficiency test

DLA 29/2017

Coumarin in Pastry (Cookies)

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Allgemeine Informationen zur Eignungsprüfung (EP)
General Information on the proficiency test (PT)

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Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (21 February 2018) Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager) *- gezeichnet / signed G. Wichmann* Datum / Date: 21 February 2018
Unteraufträge *Subcontractors*	Falls im Rahmen der Eignungsprüfung eine Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern durchgeführt wurde, hat DLA diese im Unterauftrag vergeben. In case the analysis of the content, homogeneity and stability of PT-parameters was part of the proficiency test, the determinations were subcontracted by DLA.
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

Inhalt / Content

1. Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed, cosmetics and food contact materials. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the verification and/or validation of the particular testing method [1, 5].
The purpose of DLA is to offer proficiency tests for selected parameters in concentrations with practical relevance.
Realisation and evaluation of the present proficiency test follows the technical requirements of DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2009 / ISO 13528:2015 [2, 3].

2. Realisation

2.1 Test material

The basic test material are common in commerce speculaas biscuits of an European supplier. The material was crushed, sieved and homogenized. Subsequently a coumarin-solution was spiked to an aliquot of the basic matrix, mixed and homogenized again. Then aliquots of the basic matrix were added in 4 additional steps and in each case homogenized until the total amount had been reached.

Afterwards the samples were portioned to approximately 50 g into metallised PET film bags and chronologically numbered.

The composition of the PT samples is shown in table 1.

Table 1: Composition of DLA-Samples

Ingredients	**Content**
Spice-Speculaas Ingredients: wheat flour, sugar, palm fat, caramel sugar syrup, spice, baking agent: sodium, salt Nutrients per 100 g: protein 6,0 g, carbohydrates 70 g, fat 20 g	100 g/100 g
Coumarin Chemical for Analysis	52 mg/kg *

** Coumarin is also contained in the matrix of speculaas biscuits*

Note: *The metrological traceability of temperature, mass and volume during production of the PT samples is ensured by DAkkS calibrated reference materials.*

2.1.1 Homogeneity

The **mixture homogeneity of the bottled and numbered DLA-Samples** was examined 6-fold by HPLC-UV analysis. The repeatability standard deviation was determined to 3,8 %, which is in the range of usual relative repeatability standard deviations of comparable methods. The repeatability standard deviation of the German official method ASU § 64 for determination of coumarin in cinnamon biscuits with HPLC-DAD and external calibration is 4,1 % [17]. The results of the homogeneity test is given in the documentation.

The calculation of the **repeatability standard deviation S_r of the dublicate determination of the participants** was also used as an indicator of homogeneity. With 0,95% it was low for coumarin. Therefore the repeatability standard deviation is lower or rather comparable to precision data of the referring standardized methods (e.g. ASU §64, s. 3.6.2) (see Tab. 3) [17]. The repeatability standard deviation of the participants' results is given in the table of statistic data (see 4.1).

Furthermore, the homogeneity was characterized by the **trend line function of participants' results for chronological bottled single samples**. The maximum deviation from the mean value of the trend line was about 1,8% of the target standard deviation σ_{pt} (s. 5.2 homogeneity) and can therefore be regarded as low.

If the criteria for sufficient homogeneity of the test material are not fulfilled on a particular parameter, the impact on the target standard deviation is checked and optionally the evaluation of the results of the participants will be done using the z'-score considering the standard uncertainty of the assigned value (see 3.8 and 3.11) [3].

2.1.2 Stability

A water activity (a_W) of < 0,5 is an important factor to ensure the stability of dry or dried products during storage. Optimum conditions for storage is the a_W value range of 0,15 - 0,3. In this range the lowest possible degradation rate is to be expected [16].

The experience with various DLA reference materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameter coumarin for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the EP samples was 0,34 (23,3°C). The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

Two portions of test material were sent to every participating laboratory in the 46th week of 2017. The testing method was optional. The tests should be finished at 12th January 2018 the latest.

With the cover letter along with the sample shipment the following information was given to participants:

The two portions contain identical samples of crushed spiced shortcrust biscuits (speculaas) with added coumarin.

Please note the attached information on the proficiency test.
(see documentation, section 5.3 Information on the PT)

2.3 Submission of results

The participants submitted their results in standard forms, which have been handed out with the samples (by email).

The finally calculated concentrations of the parameter as average of duplicate determinations of both numbered samples were used for the statistical evaluation. For the calculation of the repeatability- and reproducibility standard deviation the single values of the double determination were used.

Queried and documented were single results, recovery and the used testing methods.

In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

22 out of 23 participants submitted results. One of them has not submitted any results.

3. Evaluation

3.1 Consensus value from participants (assigned value)

The robust mean of the submitted results was used as assigned value (X_{pt}) („consensus value from participants") providing a normal distribution. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3]. If there are < 12 quantitative results and an increased difference between robust mean and median, the median may be used as the assigned value (criterion: Δ median - rob. mean > 0,3 σ_{pt}) [3].

The condition is that the majority of the participants' results show a normal distribution or are distributed unimodal and symmetrically. To this end, an examination of the distribution is carried out, inter alia, using the kernel density estimate [3, 12].

In case there are indications for sources of higher variability such as a bimodal distribution of results, a cause analysis is performed. Frequently different analytical methods may cause an anomaly in results' distribution. If this is the case, separate evaluations with own assigned values (X_{pti}) are made whenever possible.

The statistical evaluation is carried out for all the parameters for a minimum of 7 values are present, in justified cases, an evaluation may also be carried out from 5 results onwards.

The actual measurement results will be drafted. Individual results, which are outside the specified measurement range of the participating laboratory (for example with the result > 25 mg/kg or < 2,5 mg/kg) or the indicating "0" will not be considered for the statistic evaluation [3].

3.2 Robust standard deviation

For comparison to the target standard deviation σ_{pt} (standard deviation for proficiency assessment) a robust standard deviation (S^x) was calculated. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3].

3.3 Repeatability standard deviation

The repeatability standard deviation S_r is based on the laboratory´s standard deviation of (outlier free) individual participant results, each under repeatability conditions, that means analyses was performed on the same sample by the same operator using the same equipment in the same laboratory within a short time. It characterizes the mean deviation of the results within the laboratories [3] and is used by DLA as an indication of the homogeneity of the sample material.

In case single results from participants are available the calculation of the repeatability standard deviation S_r, also known as standard deviation within laboratories S_w, is performed by: [3, 4].

The relative repeatability standard deviation as a percentage of the mean

value is indicated as coefficient of variation CV_r in the table of statistical characteristics in the results section in case single results from participants are available.

3.4 Reproducibility standard deviation

The reproducibility standard deviation S_R represents a inter-laboratory estimate of the standard deviation for the determination of each parameter on the bases of (outlier free) individual participant results. It takes into account both the repeatability standard deviation S_r and the within-laboratory standard deviation S_S. Reproducibility standard deviations of PT´s may differ from reproducibility standard deviations of ring trials, because the participating laboratories of a PT generally use different internal conditions and methods for determining the measured values.

In the present evaluation, the specification of the reproducibility standard deviation, therefore, does not refer to a specific method, but characterizes approximately the comparability of results between the laboratories, assumed the effect of homogeneity and stability of the sample are negligible.

In case single results from participants are available the calculation of the reproducibility standard deviation S_R is performed by: [3, 4].

The relative reproducibility standard deviation as a percentage of the mean value is indicated as coefficient of variation CV_R in the table of statistical characteristics in the results section in case single results from participants are available. Its meaning is explained in more detail in 3.9.

3.5 Exclusion of results and outliers

Before statistical evaluation obvious blunders, such as those with incorrect units, decimal point errors, and results for a another proficiency test item can be removed from the data set [2]. Even if a result e.g. with a factor> 10 deviates significantly from the mean and has an influence on the robust statistics, a result of the statistical evaluation can be excluded [3].
All results should be given at least with 2 significant digits. Specifying 3 significant digits is usually sufficient.

Results obtained by different analytical methods causing an increased variability and/or a bi- or multimodal distribution of results, are treated separately or could be excluded in case of too few numbers of results. For this results are checked by kernel density estimation [3, 12].

Results are identified as outliers by the use of robust statistics. If a value deviates from the robust mean by more than 3 times the robust standard deviation, it is classified as an outlier [3]. Detected outliers are stated for information only, when z-score are < -2 or > 2. Due to the use of robust statistics outliers are not excluded, provided that no other reasons are present [3].

3.6 Target standard deviation (for proficiency assessment)

The target standard deviation of the assigned value σ_{pt} (= standard deviation for proficiency assessment) can be determined according to the following methods.

If an acceptable quotient S^*/σ_{pt} is present, the target standard deviation of the general model by Horwitz is preferably used for the proficiency assessment. It is usually suitable for for evaluation of interlaboratory studies, where different analytical methods are applied by the participants. On the other hand the target standard deviation from the evaluation of precision data of an precision experiment is derived from collaborative studies with specified analytical methods.

In cases where both above-mentioned models are not suitable, the target standard deviation is determined based on values by perception, see under 3.6.3.

For information the z-scores of both models are given in the evaluation, if available.

For the valuation of coumarin the target standard deviation of the general model of Horwitz (see 3.6.1) was applied in the present PT.
In addition, the target standard deviation of a precision experiment (German official method ASU §64 L 00.00-134) was given for information (3.6.2).

3.6.1 General model (Horwitz)

Based on statistical characteristics obtained in numerous PTs for different parameters and methods Horwitz has derived a general model for estimating the reproducibility standard deviation σ_R [6]. Later the model was modified by Thompson for certain concentration ranges [10]. The reproducibility standard deviation σ_R can be applied as the relative target standard deviation σ_{pt} in % of the assigned values and calculated according to the following equations [3]. For this the assigned value X_{pt} is used for the concentration c.

Equations	**Range of concentrations**	**corresponds to**
$\sigma_R = 0{,}22c$	$c < 1{,}2 \times 10^{-7}$	< 120 µg/kg
$\sigma_R = 0{,}02c^{0{,}8495}$	$1{,}2 \times 10^{-7} \leq c \leq 0{,}138$	≥ 120 µg/kg
$\sigma_R = 0{,}01c^{0{,}5}$	$c > 0{,}138$	> 13,8 g/100g

with c = mass content of analyte (as relative size, e.g. 1 mg/kg = 1 ppm = 10^{-6} kg/kg)

3.6.2 Value by precision experiment

Using the reproducibility standard deviation σ_R and the repeatability standard deviation σ_r of a precision experiment (collaborative trial or proficiency test) the target standard deviation σ_{pt} can be derived considering the number of replicate measurements m of participants in the present PT [3]:

$$\sigma_{pt} = \sqrt{\sigma_R^2 - \sigma_r^2 \left(m - 1 / m \right)}$$

The values of relative repeatability standard deviation (RSD_r) and relative reproducibility standard deviation (RSD_R) given in Table 3 were determined in collaborative trials using the specified methods. The in the table indicated resulting target standard deviation σ_{pt} was applied for the evaluation of the present PT results.

Table 3: Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) from precision experiments and resulting target standard deviations σ_{pt} [17]

Parameter	**Matrix**	**Mean [g/100g]**	**RSD_r**	**RSD_R**	**σ_{pt}**	**Method / Literature**
Coumarin	cinnamon powder	2682,10 mg/kg	1,54%	12,8%	12,7%	HPLC-DAD external Calibration / ASU L00.00-134
Coumarin	cinnamon cookies	51,02 mg/kg	4,14%	8,57%	8,06%[1]	HPLC-DAD external Calibration / ASU L00.00-134
Coumarin	cinnamon powder	2561,4 mg/kg	1,25%	2,76%	2,62%	HPLC-DAD internal Standard / ASU L00.00-134
Coumarin	cinnamon cookies	45,60 mg/kg	2,12%	9,06%	8,94%	HPLC-DAD internal Standard/ ASU L00.00-134
Coumarin	cinnamon powder	6,09 mg/kg	3,39%	15,0%	14,8%	HPLC-MS/MS / ASU L00.00-134

[1] *used for evaluation (s. chapter 4)*

3.6.3 Value by perception

The target standard deviation for proficiency assessment can be set at a value that corresponds to the level of performance that the coordinator would wish laboratories to be able to achieve [3].

For the present evaluation the target standard deviation according to 3.6.1 were regarded suitable.
Table 4 shows selected characteristics of participants results of the present PT in comparison to the previous year.

3.7 z-Score

To assess the results of the participants the z-score is used. It indicates about which multiple of the target standard deviation (σ_{pt}) the result (x_i) of the participant is deviating from the assigned value (X_{pt}) [3].
Participants' z-scores are derived from:

$$z_i = \frac{\left(x_i - x_{pt}\right)}{\sigma_{pt}}$$

The requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z \leq 2 \ .$$

The z-score valid for the PT evaluation is designated z-score (σ_{pt}), while the value of z-score (Info) is for information only. The two z-scores are calculated using the different target standard deviations according to 3.6.

3.7.1 Warning and action signals

In accordance with the norm ISO 13528 it is recommended that a result that gives rise to a *z*-score above 3,0 or below −3,0, shall be considered to give an "action signal" [3]. Likewise, a *z*-score above 2,0 or below −2,0 shall be considered to give a "warning signal". A single "action signal", or "warning signal" in two successive PT-rounds, shall be taken as evidence that an anomaly has occurred which requires investigation. An error or cause analysis can be carried out by checking the analysis process including understanding and implementation of the measurement by the staff, details of the measurement process, calibration of equipment and composition of reagents, transmission or calculation errors, accuracy and precision and use of reference material. if necessary appropriate corrective measures should be applied [3].

In the figures of z-scores DLA gives the limits of warning and action signals as yellow and red lines respectively. According to ISO 13528 the signals are valid only in case of a number of ≥ 10 results [3].

Table 4: Characteristics of the present PT (on dark gray) in comparison to previous PTs since 2013 (SD = standard deviation, CV = coefficient of variation)

Parameter	**Matrix** (Powder)	**rob. Mean** [g/100g]	**rob. SD (S*)** [g/100g]	**rel. SD (VK_{S*})** [%]	**Quotient S^*/σ_{pt}**	**DLA Report**
Coumarin	Bakery product	166 mg/kg	12,3 mg/kg	7,41%	0,95	DLA 17/2013
Coumarin	Bakery product	88,6 mg/kg	6,43 mg/kg	7,26%	0,89	DLA 22/2015
Coumarin	Cinnamon powder	29,4 mg/kg	6,32 mg/kg	21,5%	1,45	DLA 28/2016
Coumarin	Bakery product	74,1 mg/kg	7,30 mg/kg	10,3%	1,18	DLA 29/2017

3.8 z'-Score

The z'-score can be used for the valuation of the results of the participants, in cases the standard uncertainty has to be considered (s. 3.11). The z'-score represents the relation of the deviation of the result (x) of the participant from the respective consensus value (X) to the square root of quadrat sum of the target standard deviation ($\hat{\sigma}$) and the standard uncertainty (Ux_{pt}) [3].

The calculation is performed by:

$$z_i' = \frac{x_i - x_{pt}}{\sqrt{\sigma_{pt}^2 + u_{(x_{pt})}^2}}$$

If carried out an evaluation of the results by means of z 'score, we have defined below the expression in the denominator as a target standard deviation σ_{pt}'.

The requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z' \leq 2 \ .$$

For warning and action signals see 3.7.1.

3.9 Reproducibility coefficient of variation (CV_R)

The coefficient of variation (CV_R) of the reproducibility (= relative reproducibility standard deviation) is calculated from the standard deviation and the mean as follows [4, 13]:

$$CV_R = \frac{S_R * 100}{X}$$

In contrast to the standard deviation as a measure of the absolute variability the CV_K gives the relative variability within a data region. While a low CV_R, e.g. < 5-10% can be taken as evidence for a homogeneous set of results, a CV_R of more than 50% indicates a "strong inhomogeneity of statistical mass", so that the suitability for certain applications such as the assessment of exceeded maximum values or the performance evaluation of the participants possibly can not be done [3].

3.10 Quotient S^*/σ_{pt}

Following the HorRat-value the results of a proficiency-test (PT) can be considered convincing, if the quotient of robust standard deviation S* and target standard deviation σ_{pt} does not exceed the value of 2.
A value > 2 means an insufficient precision, i.e. the analytical method is too variable, or the variation between the test participants is higher than estimated. Thus the comparability of the results is not given [3].

3.11 Standard uncertainty

The consensus value has a standard uncertainty $U(X_{pt})$ that depends on the analytical method, differences between the analytical methods used, the test material, the number of participant laboratories (P) and perhaps on other factors. The standard uncertainty of the assigned value ($U(x_{pt})$) for this PT is calculated as follows [3]:

$$u_{(x_{pt})} = 1{,}25 \times \frac{s^*}{\sqrt{p}}$$

If $U(x_{pt}) \leq 0{,}3\ \sigma_{pt}$ the standard uncertainty of the consensus value needs not to be included in the interpretation of the results of the PT [3]. A clear exceeded the value of 0,3 is an indication that the target standard deviation was possibly set too low for the standard uncertainty of the assigned value.

The quotient $U(X_{pt})/\sigma_{pt}$ is reported in the characteristics of the test.

4. Results

All following tables are anonymized. With the delivering of the evaluation-report the participants are informed about their individual evaluation-number.
In the first table the characteristics are listed:

Statistic Data
Number of results
Number of outliers
Mean
Median
Robust mean(X_{pt})
Robust standard deviation (S^*)
Number with m replicate measurements
Repeatability standard deviation (S_r)
Coefficient of Variation (CV_r)in %
Reproducibility standard deviation (S_R)
Coefficient of Variation (CV_R)in %
Target range:
Target standard deviation σ_{pt} or σ_{pt}'
Target standard deviation for information
lower limit of target range (X_{pt} – $2\sigma_{pt}$)*
upper limit of target range (X_{pt} + $2\sigma_{pt}$)*
Variation coefficient V_K in %
Quotient S/σ_{pt} or S*/σ_{pt}'*
Standard uncertainty U(X_{pt})
Quotient U(X_{pt})/σ_{pt} or U(X_{pt})/σ_{pt}'
Number of results in the target range
Percent in the target range

* Target range is calculated with z-score or z'-score

In the second table the individual results of the participating laboratories are listed formatted to 3 digits**:

Auswerte-nummer Evaluation number	Parameter [Einheit / Unit]	Abweichung Deviation	z-Score σpt	z-Score (Info)	Hinweis Remark

** In the documentation the results are given as submitted by the participants.

4.1 Coumarin in mg/kg

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	22
Number of outliers	2
Mean	75,3
Median	74,3
Robust Mean (X)	**74,1**
Robust standard deviation (S*)	**7,30**
Number with 2 replicates	20
Repeatability SD (S_r)	0,712
Repeatability (CV_r)	0,95%
Reproducibility SD (S_R)	7,66
Reproducibility (CV_R)	10,3%
Target range:	
Target standard deviation σ_{pt}	**6,20**
Target standard deviation (for Information)	5,97
lower limit of target range	**61,7**
upper limit of target range	**86,5**
Quotient S/σ_{pt}*	*1,2*
Standard uncertainty $U_{(Xpt)}$	*1,94*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,31*
Results in the target range	*17*
Percent in the target range	*77%*

Comments to the statistic data:

The target standard deviation was calculated according to the general model of Horwitz (s. 3.6.1). The target standard deviation for information was calculated according to precision experiments (s. 3.6.2) (German official ASU §64 method: L00.00-134).

The distribution of results showed a normal variability. The quotient S*/σ_{pt} was below 2,0. The robust standard deviation is in the range of prior PTs (s. 3.6.3). The comparability of results is given.
The repeatability and reproducibility standard deviations were in the range of established values for the applied methods (see 3.6.2).

The quotient $U_{(Xpt)}/\sigma_{pt}$ was slightly increased > 0,3 (0,31), and acceptable according to the other statistic data and usage of different analysis methods.

77% of results were in the target range.

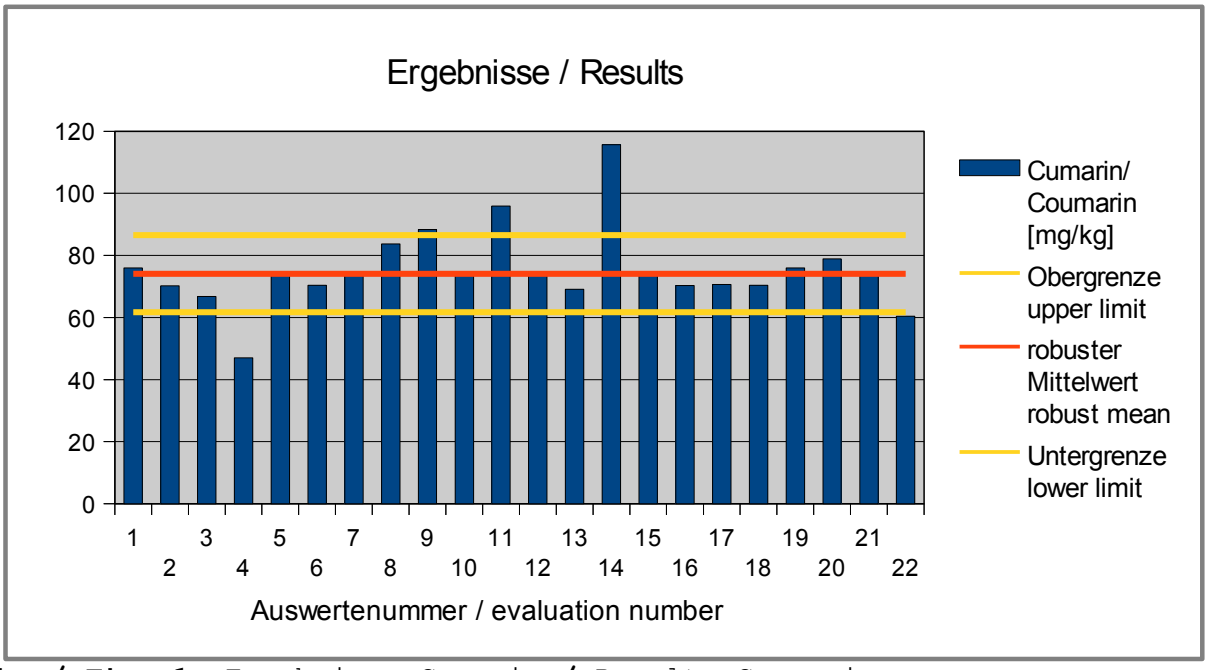

Abb. / Fig. 1: Ergebnisse Cumarin / Results Coumarin

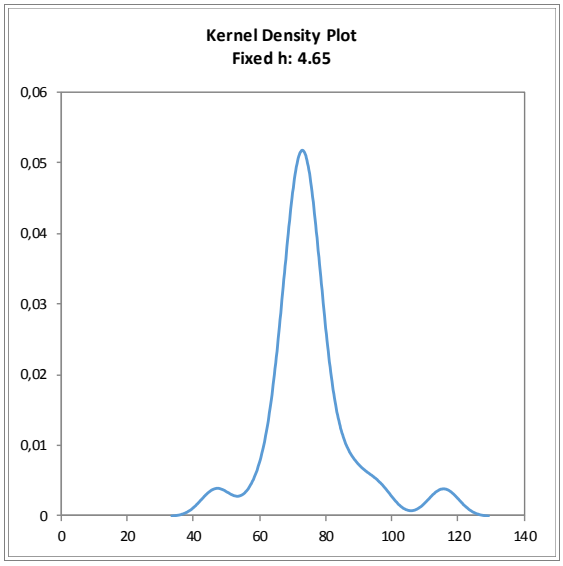

Abb. / Fig. 2:
Kerndichte-Schätzung der Ergebnisse (mit h = 0,75 x σ_{pt} von X_{pt})

Kernel density plot of results (with h = 0,75 x σ_{pt} of X_{pt})

Comment:
The kernel density plot shows almost a symmetrical distribution of results with two side peaks, which are due to the two outlier results. Moreover there is a slight shoulder, caused by two results above the target range.

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Cumarin/ Coumarin [mg/kg]	Abweichung [mg/kg] Deviation [mg/kg]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	76,0	1,91	0,31	0,32	
2	70,2	-3,89	-0,63	-0,65	
3	66,8 *	-7,29	-1,2	-1,2	
4	47,0	-27,1	-4,4	-4,5	Ausreisser / Outlier
5	74,5	0,407	0,07	0,07	
6	70,4	-3,72	-0,60	-0,62	
7	74,3	0,227	0,04	0,04	
8	83,7	9,61	1,5	1,6	
9	88,3	14,2	2,3	2,4	
10	74,2	0,107	0,02	0,02	
11	95,9	21,8	3,5	3,7	
12	74,6	0,507	0,08	0,09	
13	69,1	-5,01	-0,81	-0,84	
14	116	41,6	6,7	7,0	Ausreisser / Outlier
15	74,3	0,207	0,03	0,03	
16	70,3	-3,79	-0,61	-0,64	
17	70,6	-3,49	-0,56	-0,59	
18	70,4	-3,73	-0,60	-0,63	
19	76,0	1,86	0,30	0,31	
20	78,9	4,76	0,77	0,80	
21	74,5	0,407	0,07	0,07	
22	60,4	-13,7	-2,2	-2,3	

* Mean calculated by DLA

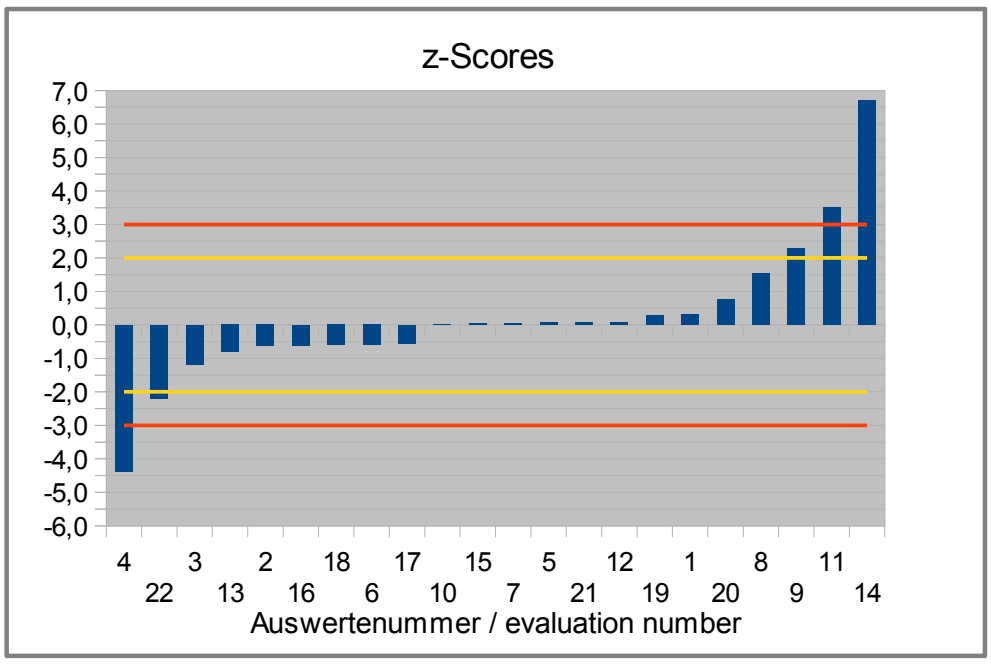

Abb. / Fig. 3: Z-Scores Cumarin / Coumarin

5. Documentation

Note: Information given in German was translated by DLA to the best of our knowledge (without guarantee of correctness).

5.1 Details by participants

5.1.1 Primary data

Parameter	Evaluation number	Unit	Sample A DLA No.	Sample B DLA No.	Date of analysis	Result (Mean)	Result sample A	Result sample B	Limit of quantitation	Incl. RR	Recovery rate [%]
Cumarin/ Coumarin	1	mg/kg	60	84	20.12.17	76	76	75	0,3	no	101
	2	mg/kg	51	93	18.12.18	70,2	70,4	70	1	yes	91,5
	3	mg/kg	63	81	28/11		66,8	69,0	2,0	yes	90,5
	4	mg/kg	12	132	27.11.17	47	44	49	0,1	no	
	5	mg/kg	22	122	16.11.17	74,5	74,6	74,3	2	no	96
	6	mg/kg	21	123	14.12.17	70,37	70,39	70,35	2	no	
	7	mg/kg	45	99	20.12.17	74,32	73,8	74,84	6,75	no	
	8	mg/kg	37	107	06.12.	83,7	83,96	83,43	0,9	no	106
	9	mg/kg	18	126	07.12.	88,3	88,4	88,1	5 mg/kg	no	
	10	mg/kg	55	89	20.11.17	74,2	74,5	73,8	0,5	no	
	11	mg/kg	54	90	21.11.17	95,9	97,5	94,3	2,5	no	104
	12	mg/kg	39	110	04.12.17	74,6	74,6	74,6	2	no	100
	13	mg/kg	43	101	28.11. /06.12.	69,08	68,92	69,24	0,6	no	103
	14	mg/kg	10	134	07.12.17	115,7	109,61	121,57	1,32	no	80
	15	mg/kg	11	133	01.12.17	74,3	74,4	74,1	1,04	no	
	16	mg/kg	14	130	27.11.	70,3	69,9	70,7	5	no	not detected
	17	mg/kg	106	38	01.12.17	70,6	70,8	70,3	2	no	
	18	mg/kg	64	80	22.11.17	70,36	70,17	70,55	2	no	103,2
	19	mg/kg	No. 41	No. 103	20.11.17	75,95	76,2	75,71	1	no	104
	20	mg/kg	53	91	18.12.17	78,85	79,2	78,5	0,2	no	-
	21	mg/kg	30	114	09.01.18	74,5	74,6	74,4	5	no	98,8
	22	mg/kg	66	78	09.01.18	60,43	60,36	60,5	2	no	99

5.1.2 Analytical methods

Evaluation number	Method description	Sample preparation	Analytical method	Calibration and reference material	Recovery with same matrix	Method accredited ISO/IEC 17025	Further remarks
1	In-house method	Coumarin is extracted after homogenization of the sample and slurried in the solvent mixture with stirring at room temperature.	The determination of coumarin in the sample extracts is carried out by liquid chromatographic separation (HPLC) by mass spectrometry (MS / MS).	The quantification is carried out according to the internal standard method.	yes	yes	Results are not recovery-corrected.
2	Extraction using 90% Methanol & HPLC-UV	Homogenisation using waring blender	N/A	Phytolab Ref Std	yes	yes	N/A
3	HPLC-UV			Sigma C4261-50g	yes	no	mean calculated by DLA
4	LC-MS/MS, in-house					yes	
5	HPLC-DAD (§64 L 00.00-134)	Extraction			yes	yes	
6	ASU L 00.00-134 mod.	Extraction methanol/water	HPLC/DAD	external calibration	no	yes / no	
7	PRM 0 51.2 0003 03 2015-06; Determination of coumarin and cinnamon components with RSLC and UV/VIS-Detection in backery wares, food precursors and flavors	Extraction from the sample matrix with ethanol, Carrez clarification	UHPLC-DAD at 279 nm, calculation via calibration standard with internal standard	internal standard: 6-Methylcumarin, e.g. Acros 12657-0050		yes	
8	ASU § 64 LFGB L 00.00-134, modified	20 g weighing/100 ml, dilution 1:2	HPLC-DAD	cinnemon	yes	yes	
9	In-house method	Extraction MeOH/water	HPLC-DAD	yes	no	yes	
10	In-house method HPLC				no	yes	
11	In-house method, LC-MS/MS	Extraction, Carrez-clarification	HPLC-MS/MS	Evaluation via internal standard	yes (starchy)	yes	
12	ASU L 00.00-134; Examination of food - Determination of coumarin in cinnamon-containing foods by HPLC / DAD or HPLC-MS / MS	Extraction with methanol/water 80/20 v/v for 30 min with magnetic stirrer	HPLC-DAD	int. Std. 6-Methylcoumarin	yes	yes	
13	§64 LFGB, L 00.00-134 mod, LCMSMS	Extraction with Methanol/water	LCMSMS	matrix calibration	yes	yes	
14	ASU L00.00-134 (2010-09)			yes	yes	yes	
15	ASU § 64 LFGB L 00.00-134	Extraction MeOH/water	HPLC-DAD	internal standard, 4-Hydroxy-2-Methylacetophenon		yes	
16	A5.52.0016 -HPLC/DAD-flavors	Extraction with ethanol, Carrez-clarification	HPLC-DAD	external calibration with 6 points	deleted	yes	
17	In-house method HPLC					yes	
18		Method Z1217: 10.0 g sample weight; Coumarin is exctracted with 80% methanol from the sample and determined after dilution with water using RP-8 UHPLC-UV.	wavelength 280 nm	Solved standards, calibration range 2 - 140 mg / kg	yes	yes	
19	in-house	extraction with Methanol-water 4-1	HPLC-DAD	internal standard (methylcoumarine); sigma-aldrich	yes	yes	-
20	HPLC-MS	Extraction with 80% Methanol	none	internal standard calibration with Coumarin D4	no	no	none
21	in-house method			Sigma	yes	yes	
22	in-house method	Extraction with methanol	HPLC-UV	external standard	yes	yes	

5.2 Homogeneity

5.2.1 Homogeneity of bottled PT samples

Homogeneity test with determination of coumarin by HPLC-UV:

Replicate measurements		mg/kg
Sample No.	34	64,8
Sample No.	44	60,4
Sample No.	59	63,5
Sample No.	85	58,6
Sample No.	100	63,8
Sample No.	110	62,8

General average	62,3	
Repeatability standard deviation	2,34	3,8%

5.2.2 Comparison of sample numbers / test results and trend line

By comparison of the increasing sample numbers and the measurement results of participants, the homogeneity of the chronological bottled PT item can be characterized with the help of the trend line function:

Coumarin				
Target standard deviation σ_{pt}	6,20			mg/kg
Sample numbers	11 – 133			
Total numbers of samples	40			
Slope	-0,0055			
Trend line range	74,8	–	74,6	mg/kg
Deviation trend line	74,7	±	0,11	mg/kg
Percent of σ_{pt}	1,8	%		

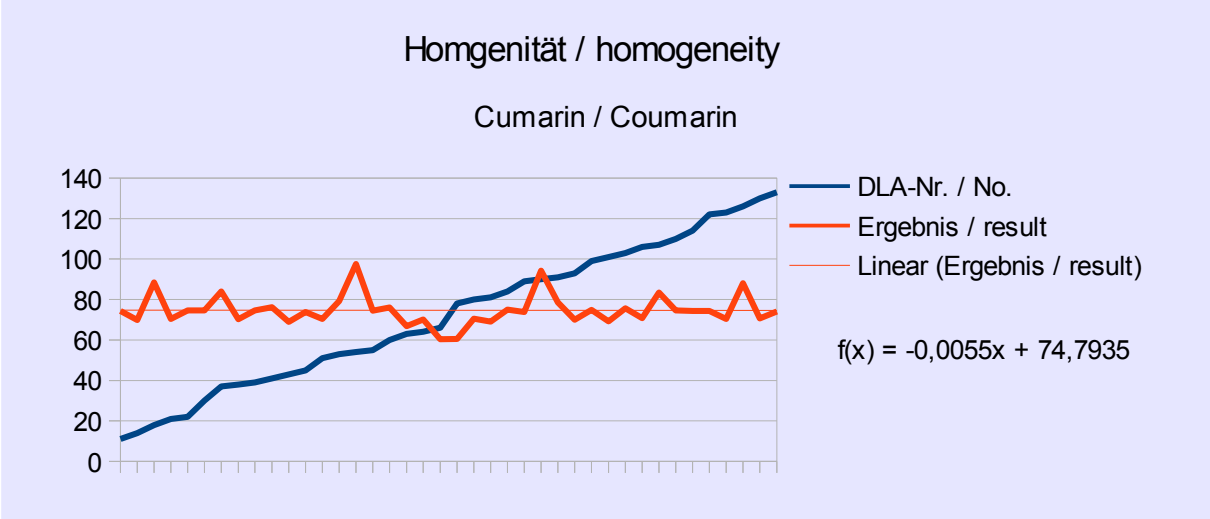

Abb./Fig. 4:
Trendfunktion Probennummern vs. Ergebnisse
trend line function sample number vs. results

5.3 Information on the Proficiency Test (PT)

Before the PT the participants received the following information in the sample cover letter:

Information on the Proficiency Test (PT)

PT number	***DLA 29-2017***
PT name	***Coumarin in Pastry (Cookies)***
*Sample matrix**	*Spice biscuits (speculaas), ground / Ingredients: Wheat flour, sugar, palm fat, caramel sugar syrup, spices, raising agent: sodium bicarbonate, salt and coumarin*
Number of samples and sample amount	*2 identical samples A + B: 50 g each.*
Storage	*Samples A + B: cooled 2 - 10°C (dark and dry)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*quantitative:* ***Coumarin***
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.*
Result sheet	*The results for sample A and B as well as the final results calculated as mean of the double determination (samples A and B) should be filled in the result submission file. The recovery rates, if carried out, has to be included in the calculation.*
Units	*mg/kg*
Number of significant digits	*at least 2*
Further information	*For information please specify:* – *Date of analysis* – *DLA-sample-numbers (for sample A and B)* – *Limit of detection* – *Assignment incl. Recovery* – *Recovery with the same matrix* – *Method is accredited*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest 12th January 2018***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler, PhD*

* Control of mixture homogeneity and qualitative testings are carried out by DLA. Testing of the content, homogeneity and stability of PT parameters is subcontracted by DLA.

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country

[Die Adressdaten der Teilnehmer wurden für die allgemeine Veröffentlichung des Auswerte-Berichts nicht angegeben.]

[The address data of the participants were deleted for publication of the evaluation report.]

7. Index of references

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