Evaluation Report

proficiency test

DLA ptTX01 (2020)

Coumarin

in Pastry (Cookies)

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General Information on the proficiency test (PT)

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Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Keine As part of the present proficency test the following services were subcontracted: none
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.

Contents

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 test material consisted of ground spice cookies with the addition of Cassia cinnamon powder from European suppliers.

The cookies were crushed and sieved (mesh size 1,5 mm). The cinnamon powder was then added to an aliquot of the cookie matrix and the mixture was homogenized. Afterwards, cookie matrix was added again in three steps in portions and homogenized in each case until the total amount was reached.
Homogeneity was proofed by Microtracer analysis. The coumarin content was determined in preliminary analysis by LC-MS/MS.

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

The DLA-Samples' composition is shown in Table 1.

Table 1: Composition of DLA-Samples

Ingredients	**content**
Pastry (Cookies) Ingredients: wheat flour, sugar, palm fat, caramel sugar syrup, cinnamon, spices, baking agent: sodium hydrogen carbonate, salt nutritional values per 100 g: protein 6,0 g, carbohydrates 70 g, lipids 19 g	98,8 g / 100 g
Cassia cinnamon powder	1,2 g / 100 g

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 before bottling** was examined 8-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples A and the spiking level sample showed a probability of 83%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [16, 17]. This gave a HorRat value of 1,8. However, this value was accepted because of the more complicated microtracer analysis due to a lower recovery rate and the colouring cinnamom matrix. The results of microtracer analysis are given in the documentation.

The calculation of the **repeatability standard deviations S_r of the participants** was used as an indicator of homogeneity. It is 2,95% for coumarin. Thus, this value is comparable to corresponding repeatability standard deviations of precision data of the standardized methods (e.g. ASU § 64 LFGB L 00.00-134, s. 3.6.2) (see Table 2) [18].
The repeatability standard deviations of the participants' results are given in the documentation in the statistic data (see 4.1).

Furthermore, the homogeneity was graphically characterized for information by the **trend line function of participants' results for chronological bottled single samples** (s. 5.2.2 Homogeneity).

In case the criterion for sufficient homogeneity of the test items is not fulfilled the impact on the target standard deviation will be verified. If necessary the evaluation of results will be done considering the standard uncertainty of the assigned value by z'-scores (s. 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 test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the spiking level sample was approx. 0,27 (17°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 2020. The testing method was optional. The tests should be finished at 22th January 2021 the latest.

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

There are two identical samples with the parameter Coumarin to be determined. The matrix is Pastry (Cookies with spices). The analysis method is optional.
Note: please store the samples at 2-10 °C on arrival

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.

All 32 participants submitted their results in time.

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*) 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 CV_R in percent of the mean is given as variation coefficient in the statistical data of participant for each parameter. The significance of CV_R is further explained in section 3.9.

3.5 Exclusion of results and outliers

Before statistical evaluation obvious blunders, such as those with incorrect units, decimal point errors, too few significant digits (valid digits) or results for 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 tested for outliers by the use of robust statistics (algorithm A): If a value deviates from the robust mean by more than 3 times the robust standard deviation, it can be classified as an outlier (see above) [3]. Due to the use of robust statistics outliers are not excluded, provided that no other reasons are present [3]. Detected outliers are only mentioned in the results section, if they have been excluded from the statistical evaluation.

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 evaluation of interlaboratory studies, where different 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 valuation of coumarin, the target standard deviation according to the general model of Horwitz (see 3.6.1) was applied in the present PT.

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 relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviation (RSD_R) given in Table 2 were determined in ring tests using the indicated methods.
The resulting target standard deviations σ_{pt}, which were identified there, were used to evaluate the results and to provide additional information for the statistical data.

Table 2: Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) according to selected evaluations of tests for precision and the resulting target standard deviation σ_{pt} [18]

Parameter	**Matrix**	**Mean**	**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 (cf. 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 was regarded suitable.

Table 3 shows selected statistic data of participants results of present PT compared to PT results of previous years.

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 valid z-Score for each parameter is indicated as z-Score (σ_{pt}). The value indicated as z-Score (Info) only obtains a informative character. The both z-Scores were calculated with the different target standard deviations in accordance with 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 procedure, calibration of equipment and composition of reagents, transmission error or an error in the calculation, in the trueness and precision and use of reference material. If necessary, the problems must be addressed through appropriate corrective action [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 3: Characteristics of the present PT (on grey) in comparison to the previous PT since 2014 (SD = standard deviation, CV = coefficient of variation)

Parameter	Matrix	rob. Mean	rob. SD (S*)	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
Coumarin	Chocolate	36,0 mg/kg	1,67 mg/kg	4,62%	0,50	DLA 28/2018
Coumarin	Cinnamon powder	24,0 mg/kg	3,93 mg/kg	16,4%	1,3	DLA 29/2019
Coumarin	Bakery product	74,4 mg/kg	8,28 mg/kg	11,1%	1,3	DLA ptTX01/2020

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_i) of the participant from the respective consensus value (X) to the square root of quadrat sum of the target standard deviation (σ_{pt}) and the standard uncertainty ($U_{(Xpt)}$) [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 (VK_R)

The variation coefficient (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 gives the relative variability within a data region. While a low CV, e.g. <5-10% can be taken as evidence for a homogeneous set of results, a CV 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 levels or the performance evaluation of the participating laboratories 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 of the assigned value

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

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

If $U_{(Xpt)} \leq 0{,}3\ \sigma_{pt}$ the standard uncertainty of the assigned value needs not to be included in the interpretation of the results of the PT [3]. Values exceeding 0,3 imply, that the target standard deviation could be too low with respect to the standard uncertainty of the assigned value.

The traceability of the assigned value is ensured on the basis of the consensus value as a robust mean of the participant results.

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} – 2σ_{pt}) or (X_{pt} – 2σ_{pt}') *
upper limit of target range (X_{pt} + 2σ_{pt}) or (X_{pt} + 2σ_{pt}') *
Quotient S/σ_{pt} or S*/σ_{pt}'*
Standard uncertainty U(X_{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 table below, the results of the participating laboratories are formatted in 3 valid digits**:

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

*** In the documentation part, the results are given as they were transmitted by the participants.*

4.1 Coumarin in mg/kg

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	32
Number of outliers	-
Mean	74,2
Median	74,9
Robust Mean (X)	**74,4**
Robust standard deviation (S*)	**8,28**
Number with 2 replicates	32
Repeatability SD (S_r)	2,18
Repeatability (CV_r)	2,95%
Reproducibility SD (S_R)	10,4
Reproducibility (CV_R)	14,0%
Target range:	
Target standard deviation σ_{pt}	**6,22**
Target standard deviation (for Information)	5,99
lower limit of target range	**61,9**
upper limit of target range	**86,8**
Quotient S^*/σ_{pt}	*1,3*
Standard uncertainty $U_{(Xpt)}$	*1,83*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,29*
Results in the target range	*26*
Percent in the target range	*81%*

Comments:

The target standard deviation was calculated according to the general model of Horwitz (s. 3.6.1). Additionally, the target standard deviation using data from a precision experiment (official German method ASU §64 L 00.00-134)(3.6.2) was given for information.

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

81% of results were in the target range.

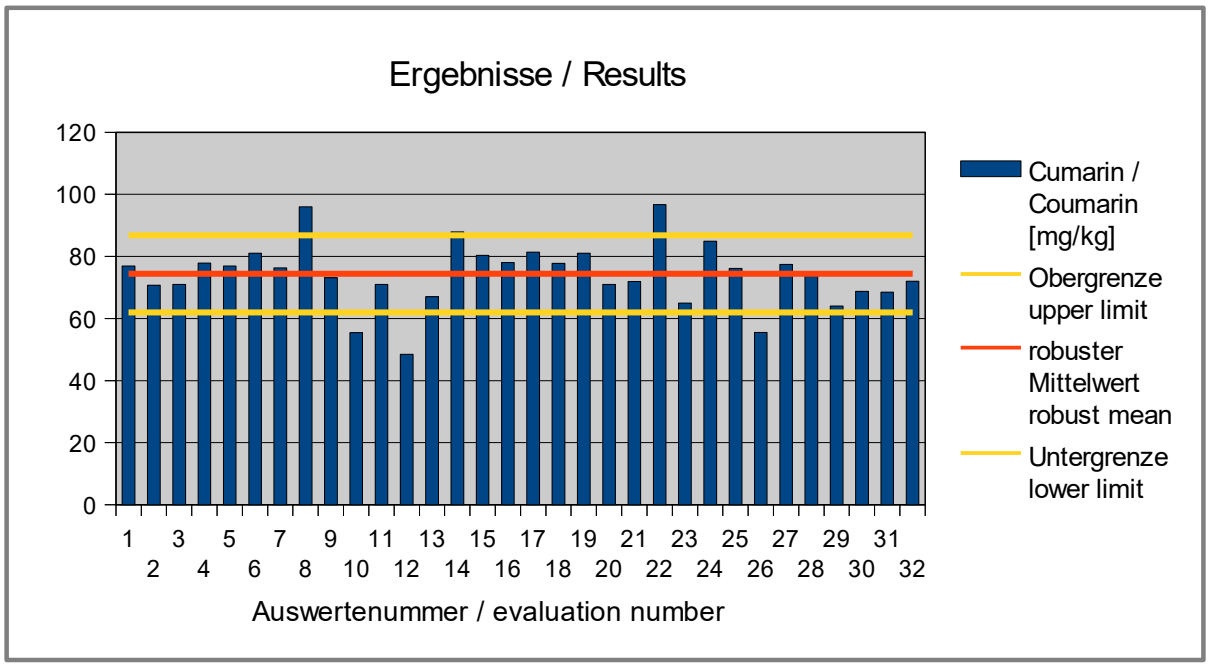

Abb. / Fig. 1: Ergebnisse Cumarin / Results Coumarin

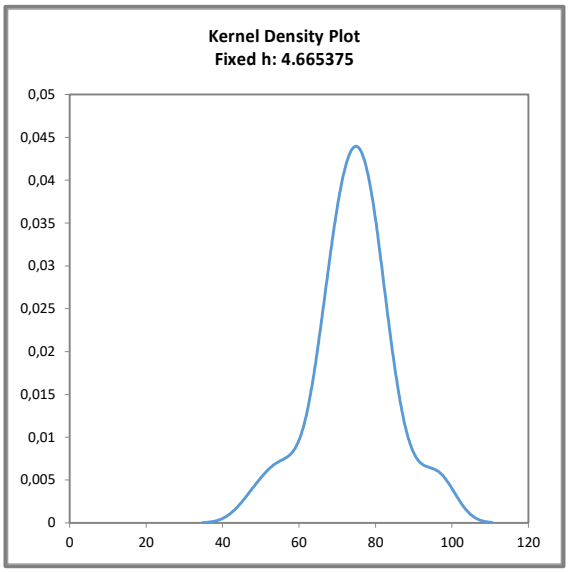

Abb. / Fig. 2:
Kerndichte-Schätzung der Ergebnisse
(mit h = 0,75 x σ_{pt} von X_{pt})

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

Comment:
The kernel density shows an almost symmetrical distribution of results with two shoulders due to participants' results outside 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	76,9	2,5	0,41	0,42	
2	70,7	-3,7	-0,59	-0,61	
3	71,0	-3,4	-0,54	-0,56	mean calculated by DLA
4	77,8	3,4	0,55	0,57	
5	76,9	2,5	0,41	0,42	
6	81,0	6,6	1,1	1,1	
7	76,3	1,9	0,31	0,32	
8	96,0	21,6	3,5	3,6	
9	73,1	-1,3	-0,20	-0,21	
10	55,4	-18,9	-3,0	-3,2	mean calculated by DLA
11	71,0	-3,4	-0,54	-0,56	
12	48,5	-25,9	-4,2	-4,3	
13	67,0	-7,4	-1,2	-1,2	
14	87,9	13,5	2,2	2,3	
15	80,3	5,9	0,95	1,0	
16	78,1	3,7	0,59	0,62	
17	81,4	7,0	1,1	1,2	
18	77,8	3,4	0,55	0,57	
19	81,0	6,6	1,1	1,1	
20	71,0	-3,4	-0,54	-0,56	
21	71,9	-2,5	-0,39	-0,41	
22	96,7	22,3	3,6	3,7	mean calculated by DLA
23	65,0	-9,4	-1,5	-1,6	
24	84,9	10,5	1,7	1,8	
25	76,2	1,8	0,29	0,30	
26	55,5	-18,9	-3,0	-3,1	
27	77,4	3,0	0,49	0,51	
28	73,7	-0,7	-0,11	-0,11	
29	64,0	-10,4	-1,7	-1,7	
30	68,7	-5,6	-0,90	-0,94	mean calculated by DLA
31	68,5	-5,9	-0,95	-1,0	
32	72,0	-2,4	-0,38	-0,40	

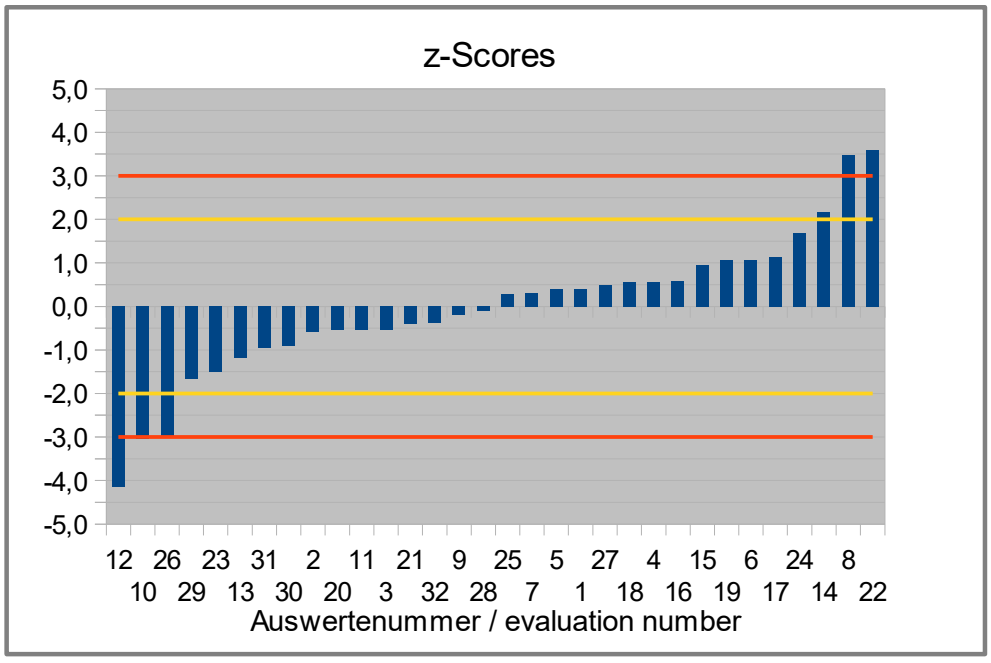

Abb. / Fig. 3: z-Scores Cumarin / Coumarin

5. Documentation

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

5.1 Details by the participants

5.1.1 Primary data

Analyte	Participant	Unit	Sample No. 1	Sample No. 2	Date of analysis	Result (Mean)	Result 1	Result 2	Limit of quantification	Incl. RR	Recovery rate [%]
										yes/no	
Coumarin	1	mg/kg	8	84	02.12.21	76,9	77	76,9	2,5	no	101,7
	2	mg/kg	24	68	18.11.	70,7	71,1	70,3	0,6	no	99,6
	3	mg/kg	2	90	19.11.20	71	70	72	1		
	4	mg/kg	14	78	23./26.11.2020	77,8	77,3	78,2	0,5	yes	85
	5	mg/kg	7	85	25./27.11. 2020, 07.12.2020	76,9	76,2	77,6		no	
	6	mg/kg	41	51	08.12.20	81	81	81	0,42	no	99
	7	mg/kg	27	65	09.12.20	76,3	76,8	75,7	1	no	
	8	mg/kg	22	70	11.01.21	96	89	103	5	no	
	9	mg/kg	42	50	22.12.20	73,1	69	77,2	0,5	yes	100
	10	mg/kg	21	71	05.01.21	55,44	55,73	55,15	0,01	yes	
	11	mg/kg	Nr. 35	Nr. 57	3.12.20-15.12.20	71	70	72	1,5	no	93-117
	12	mg/kg	64	28	18.01.21	48,5	48,3	48,6	0,5	no	
	13	mg/kg	5	87	02.12.20	67	67	67	0,1	no	
	14	mg/kg	25	67	10.01.21	87,89	88,35	87,43	0,2	no	
	15	mg/kg	1	91	22.12.20	80,3	81,53	79,07	1	no	99,43
	16	mg/kg	36	56	06.01.21	78,06	78,01	78,11	5	nein	94

Note: continued on next page.

Analyte	Participant	Unit	Sample No. 1	Sample No. 2	Date of analysis	Result (Mean)	Result 1	Result 2	Limit of quantification	Incl. RR	Recovery rate [%]
										yes/no	
Coumarin	17	mg/kg	20	72	11.01.21	81,4	81,6	81,2	0,2	yes	iStd
	18	mg/kg	11	81		77,78	76,86	78,69		no	
	19	mg/kg	23	69	16.12.20	81	82	80	< 1	yes	
	20	mg/kg	32	60	09.12.21	71	70,5	71,4	1	yes	98,5
	21	mg/kg	19	73	26.11.	71,91	71,66	72,15	0,05	no	
	22	mg/kg	13	79	12.01.21	96,65	97	96,3	0,2	no	
	23	mg/kg	29	63	09.12.21	65	65,1	64,9	0,5	no	
	24	mg/kg	03	89	16.11.20	84,9	83,5	86,3	3	no	91
	25	mg/kg	10	82	02.12.20	76,17	76,42	76,7	0,01	yes	111
	26	mg/kg	12	80	20.01.21	55,51	55,6	55,42	2,2	no	
	27	mg/kg	74 ptTX01 (2020)	18 ptTX01 (2020)	21.01.	77,4	77,8	76,9	3	no	100,9
	28	mg/kg	45	47	18.12.	73,7	73,5	73,9	7	no	
	29	mg/kg	15	77	03. Feb	64	65	63	3	no	90
	30	mg/kg	34	58	21.01.21	68,74	68,71	68,77	1	no	
	31	mg/kg	31	61	19.01.21	68,46	68,17	68,74	0,8	no	101
	32	mg/kg	88	4	28/01	72	72	72	2,7	no	

5.1.2 Analytical Methods

Parameter	Participant	Method description as in test report/ norm / literature	Sample reparation and processing	Measuring method	Calibration / Reference material	Recovery rate with same matrix	Method accredited ISO/IEC 17025	Further remarks
Cumarin / Coumarin	1	ASU L 00.00-134	4 g extracted with 100 ml MeOH/ H2O and filtered	HPLC-DAD mit ISTD		no	yes	
	2	§64 LFGB, L 00.00-134 mod., LCMSMS	Extraction with Methanol/Water	LCMSMS	matrix calibration	yes	yes	
	3	§ 64 LFGB L 00.00-134:2010-09	Sample was extracted with Ethanol/Water (70/30) after addition of d4-Coumarin as an internal standard	The extract was quantified by HPLC-MS/MS			yes	
	4	In-house method	After sample homogenization, Coumarin was extracted with by addition of solvent, stirring at room temperature.	Determination of Coumarin in the extracted samples was conducted after liquid chromatography (HPLC) via mass spectrometry (MS/MS).	Quantitation is conducted by internal standard method. Reference samples were analyzed in every batch.	yes	yes	Results are recovery-corrected
	5	ASU L 00.00-134 mod., LC-MS/MS	Homogenisierung, Extraktion im Methanol-Wasser-Lösungsmittelgemisch	LC-MS/MS	External calibration in food		yes	
	6	L 00.00-134	Extraction with MeOH/H2O, Carrez-clarification, filtration, analysis	HPLC-DAD	internal standard	no	yes	
	7	in-house method	estraction with MeOH/water	HPLC-UV/VIS	external calibration	no	yes	
	8	HPLC-DAD	Extraction with MeOH 80%		External calibration, no reference material	no	no	
	9	yes	none	none		yes	yes	none
	10	PV-SA-087		LC-MS/MS		yes	yes	
	11	ASU L00.00-134 modified	ASU L00.00-134. 80% EtOH was used instead of 80% MeOH, solution was diluted and extracted with TBME, analysis by GC/MS			yes	yes	
	12						no	
	13	SOP M3217, LC-MS/MS					yes	
	14	ASU L00.00-134	Extraction with methanol/water (80:20)	HPLC-DAD	calibration with internal standard		yes/no	
	15	in-house			internal standard	yes	yes	
	16	in-house method	extraction meoh/water	HPLC-DAD	sigma / ref cinamon	no	yes	

Note: continued on next page.

Parameter	Participant	Method description as in test report/ norm / literature	Sample reparation and processing	Measuring method	Calibration / Reference material	Recovery rate with same matrix	Method accredited ISO/IEC 17025	Further remarks
						ja/nein		
Cumarin / Coumarin	17	Cumarin (HPLC-MS)	extraction with MeOH 80%	RP-Separation, MS-Detection	Coumarin d4	yes	yes	
	18	ASU L00.00-134 (2010-09)		HPLC				
	19	FOOD PA 544, Determination via LC-MS/MS	Extraction mit ACN/H2O/Acetic acid	LC-MS/MS	IS (2-Hydroxy quinoline)	yes	yes	
	20	Extraction using 90% Methanol & HPLC-UV	Homogenisation using waring blender		Sigma Coumarin stock	yes	yes*	*accredited for bakery wares/ food supplements
	21	ASU L 00.00-134 mod.		LC-MS/MS	ISTD-method	no	yes	
	22	ASU L 00.00-134:2010-09	-	-		-	yes	-
	23	HPLC-DAD					yes	
	24	ASU L 00.00-134	Extraktion with 80 % Methanol	ESI(+)-LC-MSMS	solution calibration; internal standard for analysis; secondary standard available	Yes, via addition to a similar and non-containing sample	yes	non
	25	PV-24-Cumarin : 2013-03 (a)	Carrez-clarification		reference material	yes	yes	
	26	HPLC/UV					yes	
	27	in house method HPLC	Extraction, Carrez-clarification	HPLC-DAD	Usage of internal standards, reference material cookie	yes	yes	
	28	ASU L 00.00-134		HPLC-DAD			yes	
	29	Coumarin determination in food samples	extraction with methanol	HPLC-UV	spiked sample	no	yes	
	30	internal method HPLC-DAD P-330-230	Extraction MeOH/H2O 70/30 v/v for 30 Min. at 70 °C	HPLC-DAD at 279 nm	Three point calibration with standard, reference material: previous PT-sample (spice)	no	yes	
	31					yes	yes	
	32	HPLC/DAD						

5.2 Homogeneity

5.2.1 Mixture homogeneity before botteling

Microtracer Homogeneity Test
DLA-ptTX01

Weight whole sample	4,74	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	20,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	8	3,2
2	5,00	12	4,8
3	4,97	12	4,8
4	5,02	8	3,2
5	5,01	6	2,4
6	5,04	8	3,2
7	5,00	8	3,2
8	4,98	9	3,6

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	8,9	Particles
Standard deviation	2,13	Particles
χ^2 (CHI-Quadrat)	3,57	
Probability	**83**	%
Recovery rate	17	%

Normal distribution		
Number of samples	8	
Mean	3,5	mg/kg
Standard deviation	0,85	mg/kg
rel. Standard deviaton	24,0	%
Horwitz standard deviation	13,2	%
HorRat-value	**1,8**	
Recovery rate	17	%

5.2.2 Trend line function of the participants' results

By comparison of the increasing sample numbers and the measurement results of participants, the homogeneity of the chronological bottled PT items can be shown by the trend line for information:

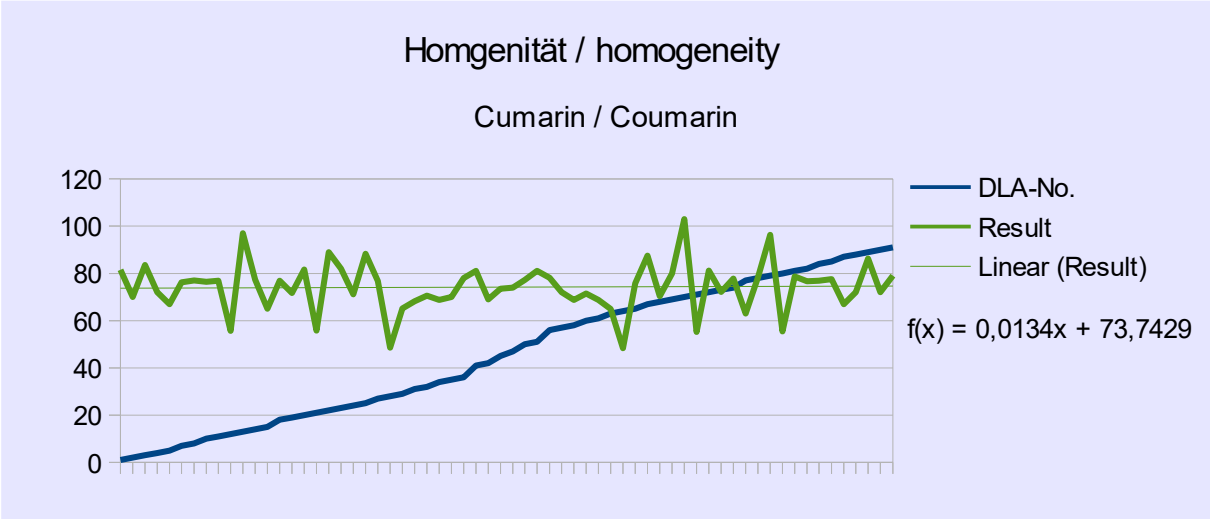

Abb./Fig. 4:
Trendfunktion Probennummern vs. Ergebnisse
trend line function sample number vs. results

5.3 Information on the Proficiency Test (PT)

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

PT number	***DLA ptTX01 (2020)***
PT name	***Coumarin in Pastry (Cookies)***
*Sample matrix**	***Samples I + II:*** *Spiced biscuits (speculoos), ground / Ingredients: wheat flour, sugar, palm fat, caramel syrup, cinnamon, spices, raising agent: sodium hydrogen carbonate, salt*
Number of samples and sample amount	*2 identical samples I + II, 50 g each.*
Storage	*Samples I + II: should be cooled 2 - 10°C on arrival (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 I and II as well as the final results calculated as mean of the double determination (samples I and II) 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 I and II)* – *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***
Last Deadline	***the latest January 22nd 2021***
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-Scharf, PhD*

* Control of mixture homogeneity and qualitative testings are carried out by DLA. Any 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
		ÖSTERREICH/AUSTRIA
		Deutschland/Germany
		Deutschland/Germany
		Deutschland/Germany
		Deutschland/Germany
		ITALIEN/ITALY
		Deutschland/Germany
		Deutschland/Germany
		Deutschland/Germany
		Deutschland/Germany
		Deutschland/Germany
		GRIECHENLAND/GREECE
		NIEDERLANDE/ NETHERLANDS
		Deutschland/Germany
		ZYPERN/CYPRUS
		GRIECHENLAND/GREECE
		Deutschland/Germany
		Deutschland/Germany
		Deutschland/Germany
		Deutschland/Germany
		LUXEMBURG/LUXEMBURG
		Deutschland/Germany
		Deutschland/Germany
		Deutschland/Germany
		Deutschland/Germany
		Deutschland/Germany
		Deutschland/Germany
		IRLAND/IRELAND
		Deutschland/Germany
		Deutschland/Germany
		Deutschland/Germany
		GRIECHENLAND/GREECE

[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

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by inter-laboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methoden-validierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and precision) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
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18. ASU § 64 LFGB L 00.00-134 (2010-09) Bestimmung von Cumarin in zimthaltigen Lebensmitteln mittels HPLC/DAD bzw. HPLC-MS/MS [Determination of coumarin in cinnamon containing foods by HPLC/DAD and HPLC-MS/MS