Evaluation Report

proficiency test

DLA 29/2019

Coumarin:

in Ceylon Cinnamon Powder

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General Information on the proficiency test (PT)

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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.

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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 is a common in commerce cinnamon powder „Ceylon Cinnamon" from an EU supplier from one production batch (see Table 1). The raw material packs were mixed and homogenized. 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 metal-lised PET film bags and chronologically numbered.

Table 1: Composition of DLA-Samples

PT-Sample
Ceylon Cinnamon Ingredients: cinnamon powder

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 66%. 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 0,8. 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 4,6% for coumarin in ceylon cinnamon. Thus is a little bit higher 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. 0,38 (18,2°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 2019. The testing method was optional. The tests should be finished at 6th January 2020 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 ceylon cinnamon powder with a natural content of the parameter coumarin to be determined. The methods of analysis are 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.

25 Participants of 26 submitted their results in time. One participant submitted no 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*) 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 of the evaluation by a precision experiment (s. 3.6.2) was applied in the present PT (German official method ASU §64 L 00.00-134).

In addition, the target standard deviation according to the general model of Horwitz (see 3.6.1) was given for information.

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%[1]	HPLC-DAD external Calibration / ASU L00.00-134
Coumarin	cinnamon cookies	51,02 mg/kg	4,14%	8,57%	8,06%	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 or given for information (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 3.6.2 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].

Tabelle 3: Characteristics of the present PT (on grey) in comparison to the previous PT from 2017 (SD = standard deviation, CV = coefficient of variation)

Parameter	Matrix	rob. Mean	rob. SD (S*)	rel. SD (VK_{S*}) [%]	Quotient $S*/\sigma_{pt}$	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	Chokolate	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

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 cofficient of variation (CV_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*/σpt

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 ($X_{pt} - 2\sigma_{pt}$) or ($X_{pt} - 2\sigma_{pt}$') *
upper limit of target range ($X_{pt} + 2\sigma_{pt}$) or ($X_{pt} + 2\sigma_{pt}$´) *
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°	18
Number of outliers	0
Mean	24,0
Median	24,3
Robust Mean (X_{pt})	**24,0**
Robust standard deviation (S*)	**3,93**
Number with 2 replicates	18
Repeatability SD (S_r)	1,10
Repeatability (CV_r)	4,58%
Reproducibility SD (S_R)	3,62
Reproducibility (CV_R)	15,1%
Target range:	
Target standard deviation σ_{pt}	**3,06**
Target standard deviation (for Information)	2,38
lower limit of target range	**17,9**
upper limit of target range	**30,1**
Quotient S/σ_{pt}*	*1,3*
Standard uncertainty $U_{(Xpt)}$	*1,16*
Results in the target range	*17*
Percent in the target range	*94%*

°results without outliers (results no. 2, 3, 7, 8, 13 and 14)

Comments:

Based on the kernel density estimation (Fig. 2) and the outlier test (see 3.5), 6 results were excluded in advance.

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

94% of results were in the target range.

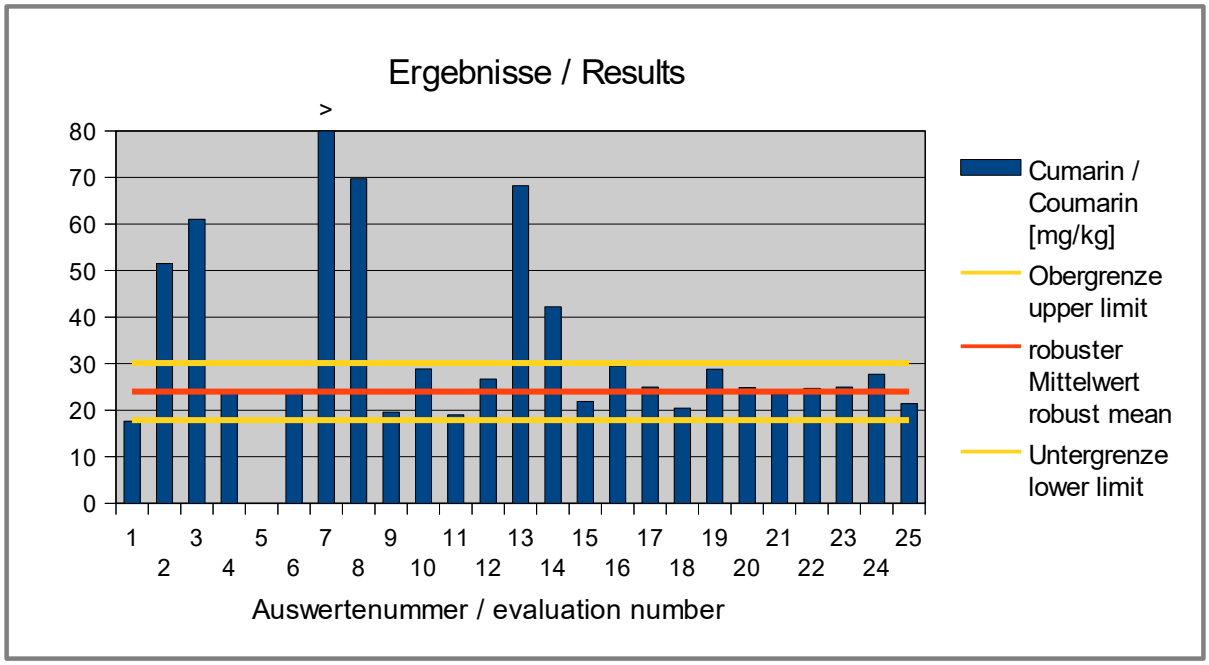

Abb. / Fig. 1: Ergebnisse Cumarin / Results coumarin

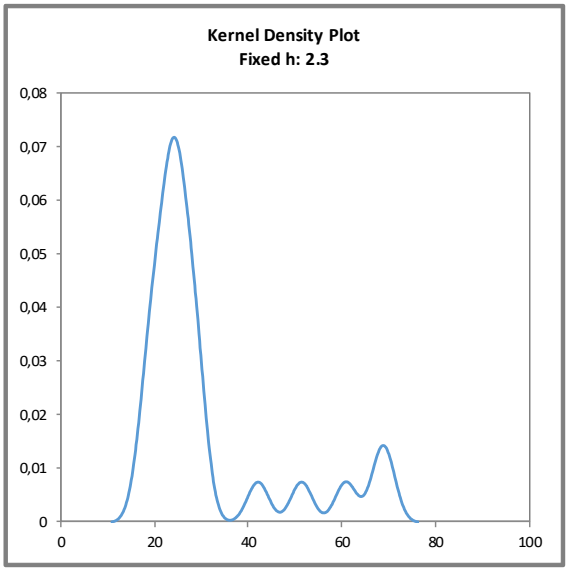

Abb. / Fig. 2:
Kerndichte-Schätzung der Ergebnisse (mit h = 0,75 x σ_{pt} von X_{pt})

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

Comment:
The kernel density shows a symmetrical distribution of results with several secondary peaks at > 35 mg/kg, due to participants' results outside the target range. The result at 524 mg/kg is not shown.

Ergebnisse der teilnehmenden Institute:
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	17,6	-6,39	-2,1	-2,7	
2	51,5		(9,0)		Ausreißer ausgeschlossen/ Outlier excluded
3	61,0		(12)		Ausreißer ausgeschlossen/ Outlier excluded
4	23,9	-0,12	-0,04	-0,05	
5	<BG				
6	23,6 *	-0,42	-0,14	-0,18	
7	524		(163)		Ausreißer ausgeschlossen/ Outlier excluded
8	69,7		(15)		Ausreißer ausgeschlossen/ Outlier excluded
9	19,6	-4,42	-1,4	-1,9	
10	28,9 *	4,83	1,6	2,0	
11	19,0	-5,02	-1,6	-2,1	
12	26,7	2,65	0,86	1,1	
13	68,2 *		(14)		Ausreißer ausgeschlossen/ Outlier excluded
14	42,2 *		(6,0)		Ausreißer ausgeschlossen/ Outlier excluded
15	21,9	-2,12	-0,69	-0,89	
16	29,5	5,48	1,8	2,3	
17	25,0	0,98	0,32	0,41	
18	20,4 *	-3,61	-1,2	-1,5	
19	28,8	4,78	1,6	2,0	
20	24,8	0,81	0,26	0,34	
21	23,4	-0,62	-0,20	-0,26	
22	24,7 *	0,67	0,22	0,28	
23	25,0	0,98	0,32	0,41	
24	27,7	3,68	1,2	1,5	
25	21,4	-2,62	-0,86	-1,1	

* Mean calculated by DLA

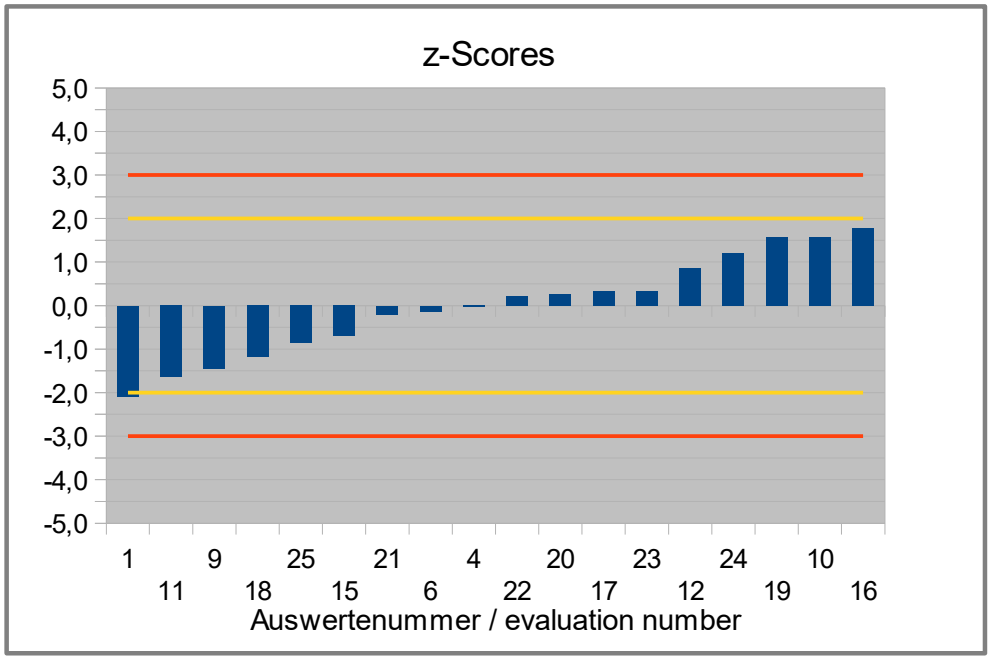

Abb. / Fig. 3: z-Scores Cumarin / Coumarin

5. Documentation

5.1 Details by the participants

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

5.1.1 Primary Data

Partici-pant	Unit	Sample I DLA No.	Sample II DLA No.	Date of analysis	Result (Mean)	Result I	Result II	Limit of quantification	Incl. Recove-ry rate	Recovery rate
				Day/Month					yes / no	in %
1	mg/kg	13	63	16.12.	17,63	17,52	17,74	0,5 mg/kg	no	DLA2019
2	mg/kg	7	67	28.11.19	51,5	48	55	0,5	ja	99
3	mg/kg	18	58	09.12.19	60,97	60,5	61,44	0,02	no	99
4	mg/kg	8	69	12.12.19	23,9	23,8	24	1	yes	82,8
5	mg/kg	23	53	18.12.19	<BG	<BG	<BG	35	no	deleted
6	mg/kg	No. 25	No. 51	19.12.19	03.01.20	23,3	23,9	5	yes	
7	mg/kg	9	68	13/12	524	539	508	5 mg/kg	no	
8	mg/kg	28	48	03.12.19	69,69	71,95	67,42	5	no	
9	mg/kg	10	66	03.01.	19,6	21,8	17,4	5	no	100
10	mg/kg	14	62	02.01.20		28,7	29	1	no	98
11	mg/kg	5	71	17.12.19	19	18	20	1	no	95,1
12	mg/kg	No. 16	No.60	09.12.19	26,67	26,89	26,45	20	no	80 - 110
13	mg/kg	6	70	17.12.19	30.12.19	69,43	67,03	1	no	103,2
14	mg/kg	3	73	9.12.		42,3	42,1	1	no	
15	mg/kg	52	24	05.12.19	21,9	21,782	21,921	3,4	no	
16	mg/kg	4	72	06.12.19	29,5	29	30,1	1	no	
17	mg/kg	17	61	12.12.19	25	26	25	12	no	99
18	mg/kg	No.29	No.50	11.12.19	12.12.19	20,735	20,093	25	no	98,53
19	mg/kg	32	44	11.12.19	28,8	28,7	29	0,2	yes	101,5
20	mg/kg	29/2019 Sample No. 12	29/2019 Sample No. 64	10.12.	24,83	24,68	24,98	0,005	no	none
21	mg/kg	33	43	21.11./26.11.	23,4	25,1	21,7	0,1	no	-
22	mg/kg	30	40	06.12.19	09.12.19	24,71	24,67	0,5	no	90
23	mg/kg	19	57	29.11.	25	26	24	1		
24	mg/kg	15	56	25.11.19	27,7	27,74	27,56	0,6 mg/kg	no	103,5
25	mg/kg	29/19 54	29/19 21	27.11.19	21,4 mg/kg	21,9 mg/kg	20,9 mg/kg	15,5 mg/kg	no	-

5.1.2 Analytical Methods

Partici-pant	Method description As in test report / norm / literature	Sample preparation and processing	Measuring method	Calibration / Reference material	Recovery rate w ith same matrix	Method Accredited ISO/IEC 17025	Further Remarks
					yes / no	yes / no	
1	internal procedure HPLC-DAD based on ASU L 00.00-134			Reference material: cinnamon from old PT		yes	
2	HPLC-UV	MeOH/ H2O, ultrasonic, centrifugation, membrane filtration	MeOH/ H2O, ultraso-nic, centrifugation, membrane filtration	External standard and carrying a reference material along: plum crumble cake	yes	yes	
3	ASU L.00.00-134	no	no	external calibration , RM-material ok	yes	yes	Sample was left uncooled for 3 days after arrival
4	Extraction using 90% Methanol & HPLC-UV	Homogenisation using waring blender		Sigma Coumarin stock	yes	yes*	*accredited for bakery wares/ food supplements
5	HPLC-DAD (§64 L 00.00-134)	Extraction			yes	yes	LOQ for matrix cinnamon
6	§64LFGB L 00.00-134 (2010-09)		LCMSMS			no	RR orrection using ISTD
7	HPLC-DAD	liquid extraction, Carrez purification	λ=275nm	external calibration, no reference material	no	no	
8	HPLC-UV	Methanol extraction	UV-Det. 280 nm			yes	
9	AHM 618 (HPLC-DAD)	Solvent methanol	HPLC-DAD	Calibration curve with coumarin	yes	yes	
10	ASU 00.00 134	Extraction with MeOH/water	internal Std.		yes	yes	
11	in-house method - MP-02111-NL	extraction water/methanol	HPLC-DAD (270nm)	external cal.	yes	yes	
12	HPLC-DAD	Extraction with MeOH:H2O/70:30 (v/v)		External calibration	yes	yes	
13	Yes	Sample weight 1g	HPLC-DAD	Sigma-Aldrich	yes	yes	result in mg/kg
14					no	yes	
15	ASU §64 LFGB L53.03/01 modified	Extraction with ethanol (94%)/water 75/25	HPLC-DAD	Quantification according to the method of the external standard		yes	
16	in-house method	estraction with MeOH/water	HPLC-UV/VIS	external calibration	no	yes	
17	in-house method	Methanol extraction, centrifugal filter 0,45 µm	HPLC-UV	external standard calibration	yes	yes	
18	Modified standard method based on official method L 00.00-134	Dilution of the sample solution with water for solvent composition such as eluent	other HPLC column; different solvent gradient		no	yes	
19	Coumarin, (UHPLC-MSD)	none	MS	Coumarin d4	yes	yes	none
20	ASU L 00.00-106 / 2006-12	Extraction with diethyl ether, SAFE distillation	GC-MS	internal standard d4-5-Coumarin	no	yes	
21	SOP M 3217, LC-MS/MS	Sample crushing with knife mill	LC-MS/MS			yes	
22	Determination of coumarin in food by LC/MS/MS		LC/MS/MS	present	no	yes	
23	§ 64 LFGB L 00.00-134:2010-09	The sample is extracted with ethanol/water (70/30) after adding d4-coumarin as an internal standard.	The extract is quantified using HPLC-MS/MS.			yes	
24	§64 LFGB, L 00.00-134 mod, LCMSMS	Extraction with methanol/water	LCMSMS	Matrix calibration	yes	yes	
25	HPLC-DAD, ASU L 00.00-134 2010/09 modified	-	-	external calibration, cinnamon powder	no	yes	-

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 29-2019

Weight whole sample	4,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	62,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,19	166	64,0
2	5,27	165	62,6
3	5,12	172	67,2
4	5,03	177	70,4
5	5,10	164	64,3
6	5,05	168	66,5
7	5,24	147	56,1
8	4,95	166	67,1

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	165,8	Partikel
Standard deviation	10,87	Partikel
χ^2 (CHI-Quadrat)	4,99	
Probability	**66**	%
Recovery rate	104	%

Normal distribution		
Number of samples	8	
Mean	64,8	mg/kg
Standard deviation	4,25	mg/kg
rel. Standard deviaton	6,6	%
Horwitz standard deviation	8,5	%
HorRat-value	**0,8**	
Recovery rate	104	%

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:

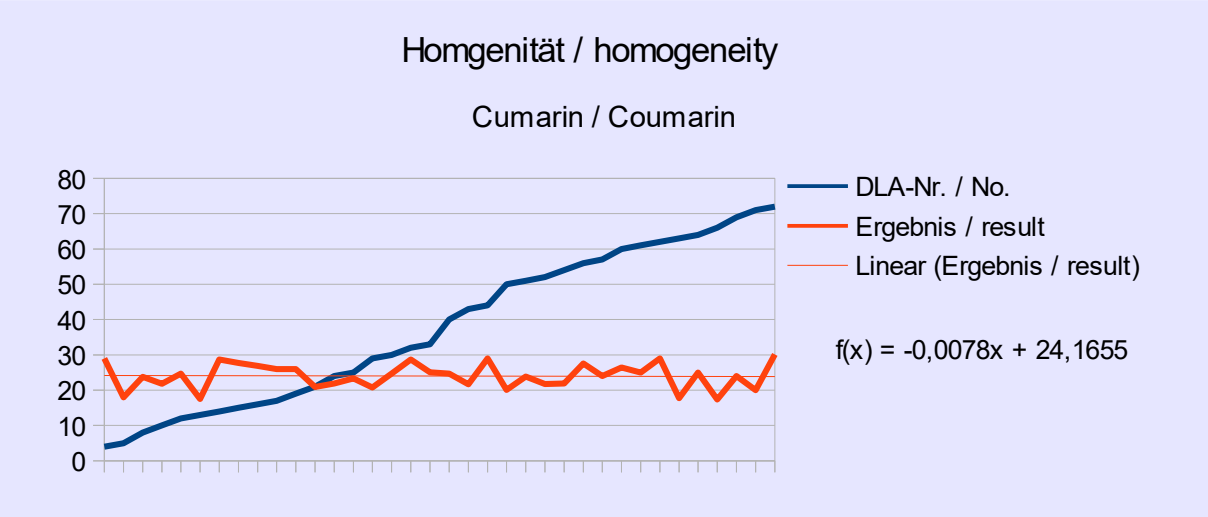

Abb./Fig. 4:
Trendfunktion Probennummern vs. Ergebnisse (ohne Ausreißer)
trend line function sample number vs. results (without outliers)

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 29-2019***
PT name	***Coumarin in Cinnamon Powder***
*Sample matrix**	*Samples I + II:* ***Cinnamon Powder*** *(Ceylon Cinnamon)*
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***
Deadline	***the latest January 06th 2020.***
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
		AUSTRIA
		Germany
		Germany
		Germany
		Germany
		Germany
		ITALY
		Germany
		NETHERLANDS
		Germany
		Germany
		LUXEMBOURG
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		IRELAND
		Germany
		VIETNAM
		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 interlaboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung / 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
6. Evaluation of analytical methods used for regulation of food and drugs; W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
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14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Checking procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
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16. Homogeneity and stability of reference materials; Linsinger et al.; Accred Qual Assur, 6, 20-25 (2001)
17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Requirements, Appendix F, p. 2, AOAC Int (2016)
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/M