Proficiency Tests
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Evaluation Report
proficiency test

DLA 41/2018

16-O-Methylcefestol in 3 Coffee Blends

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

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EP-Nummer *PT-Number*	DLA 41/2018
EP-Koordinator *PT-Coordinator*	Dr. Gerhard Wichmann
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (24 November 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 (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler -Scharf* Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager) *- gezeichnet / signed G. Wichmann* Datum / Date: 24 November 2018
Unteraufträge *Subcontractors*	Die Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern wird von DLA im Unterauftrag vergeben. The analysis of the content, homogeneity and stability of PT-parameters are 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.

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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 materials are homogeneous blends with different rations of ground Arabica and Robusta beans:

Blend A (3,5% Robusta):

Ingredient	**percentage**
Roasted coffee 100% Robusta	3,5%
Roasted coffee 100% Arabica	96,3%
Premix Microtracer	0,2%

Blend B (20% Robusta)*:

Ingredient	**percentage**
Roasted coffee from the market, Ingredients: 20% Robusta/ 80% Arabica*	100 %

*** on the package**

Blend C (25% Robusta):

Ingredient	**percentage**
Roasted coffee 100% Robusta	25,0 %
Roasted coffee 100% Arabica	74,5 %
Premix Microtracer	0,2 %

Samples A and C were admixed with 0,2 % premix microtracer to determine the homogeneity.

Approximately 1 kg of the material was homogenized and then packaged lightproof in portions to approximately 20 g. The portions were numbered chronologically. The material was checked for homogeneity.

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 **micro-tracer 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 sample A and C showed a probability of 66% and 43%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. This gave HorRat values of 1,0 and 1,1 respectively. The results of microtracer analysis are given in the documentation.

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 PT samples was approx. 0,25 (24,5°C). The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Test

One portions of test samples A, B and C were sent to every participating laboratory in the 37th week of 2018. The testing method was optional. The tests should be finished at 26th march 2018 the latest.

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

In general, we recommend homogenizing a representative sample quantity according to good laboratory practice before analysis, especially for small analytical sample quantities.

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

2.3 Results

The participants submitted their results in standard forms, which have been handed out with the samples (by email). For statistical evaluation the final results for the numbered samples were used.

Queried and documented were single results for 16-O-Methylcafestol, recovery and the used testing method.

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.

9 participants have submitted their results in time.

3. Evaluation

3.1 Consensus values from participants (Assigned value)

For the evaluation as assigned value (Xpt) the robust mean value of the submitted results is usually used ("consensus value of the participants"). 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]. **In the present case, the median was used as the assigned value (Xpt) for samples A, B and C, since < 12 quantitative results were available and a relatively large difference between the median and the robust mean was present due to deviating individual results.**

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 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 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) and results for a another proficiency test item can be removed from the data set [2]. Even if a result clearly deviates from the robust mean (e.g. factor >10) and has an influence on the robust statistics, a result can be excluded from statistical evaluation [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 (algorithm A). If a value deviates from the robust mean by more than 3 times the robust standard deviation, it is classified as an outlier [3]. Due to the using of robust statistics, outliers are generally excluded from the evaluation, unless there are other reasons (see above) [3]. Determined outliers are only mentioned in the results section if they have been excluded from the statistical evaluation.

3.4 Target standard deviation

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.

In the present PT for evaluation the target standard deviation from evaluation of a precision experiment (see 3.6.2) was used. Additionally the standard uncertainty was considered and the results were evaluated by z´-score (see 3.6). The specified target standard deviation "for information" was calculated according to the Horwitz general model (see 3.6.1).

3.4.1 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)

3.4.2 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 statistical evaluation was realised with the target standard deviation according to ASU §64 LFGB L 46.02-4 (or DIN 10779/2011) because almost all of the participants have used this method.

The precision data of the ASU §64 LFGB L 46.02-4 (determination with HPLC-analysis) are: The repeatability standard deviation σ_r for the determination of 16-O-Methylcafestol is 4,5% and the reproducibility standard deviation σ_R is 11,6% for roasted coffee blends (portion Robusta = 20%).

The target standard deviation according to ASU § 64 LFGB L 46.02-4 (18) was used for the evaluation.

The target standard deviations according to Horwitz are listed for information additionally in this evaluation.

3.4.3 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).

3.5 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 is indicated as z-score (σ_{pt}) in the evaluation. The as z-score (info) designated value only obtains an informative character. The both z-scores were calculated with different target standard deviations described in 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, 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].

3.6 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. 3.8). The z'-score represents the relation of the deviation of the result (xi) of the participant from the respective consensus value to the square root of quadrat sum of the target standard deviation (σ_{pt}) 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.

3.7 Precision and coefficient of variation (V_K)

Precision describes the random deviation of values around the mean, given as standard deviation S^* or as coefficient of variation V_K (relative standard deviation).

The coefficient of variation (V_K) is calculated from the standard deviation S^* and the mean:

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

The V_K is used it to demonstrate the variability. The higher the V_K, the greater is the divergence. In contrast to the standard deviation as a measure of the absolute variability, the V_K shows the relative variability within a range of data.

A V_K of more than 50% suggest a "strong inhomogeneity of statistical mass".

3.8 Quotient $S^x/\hat{\sigma}$

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].

3.9 Standard uncertainty

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]:

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

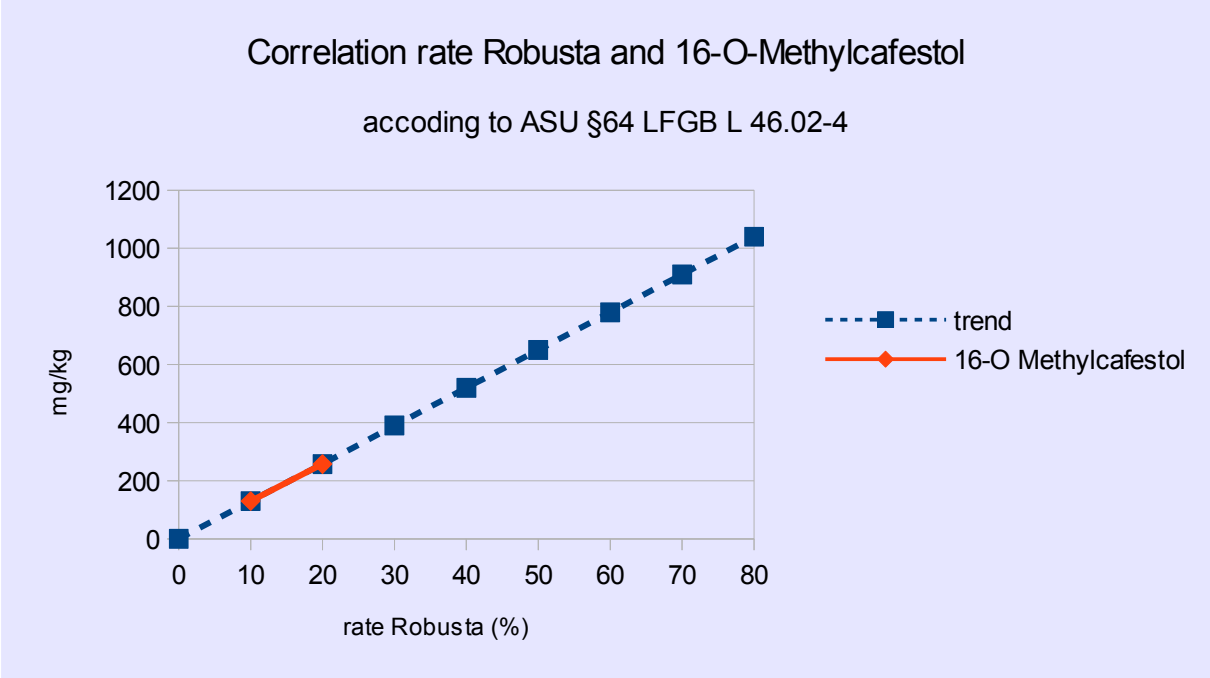
If $U_{(Xpt)} \leq 0{,}3\ \sigma_{pt}$ the standard uncertainty of the assigned value needs not to be included in the interpretation of the results of the PT [3]. Values exceeding 0,3 imply, that the target standard deviation could be too low with respect to the standard uncertainty of the assigned value.

The quotient $u_X/\hat{\sigma}$ 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.

From ASU §64 LFGB L 46.02-4 (13) and DIN 10779 (March 2011) resp. with 8 participating laboratories follows the correlation between the 16-O-Methylcafestol concentration and the Robusta rate in Arabica roasted coffee:



In the first table the characteristics are listed:

Statistic Data
Number of results
Number of outliers
Mean
Median (X_{pt})
Robust mean
Robust standard deviation (S^x)
Reproducibility (CV_{S^*})
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 ($X_{pt} + 2\sigma_{pt}$)
Quotient $S^x/\hat{\sigma}$
Standard uncertainty u_X
Quotient $u_X/\hat{\sigma}$
Number of results in the target range
Percent in the target range

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 σ_{pt}	z-Score (Info)	Hinweis Remark

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

4.1 16-O-Methylcafestol in sample A (mg/kg)

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	7
Number of outliers	0
Mean	53,5
Median (X_{pt})	**45,1**
Robust Mean	53,5
Robust standard deviation (S*)	**23,7**
Reproducibility (CV_{S*})	**52,6%**
Target range:	
Target standard deviation σ_{pt}´	**12,3**
Target standard deviation (for Information)	4,07
lower limit of target range	**20,5**
upper limit of target range	**69,7**
Quotient S/σ_{pt}´*	*1,9*
Standard uncertainty $U_{(Xpt)}$	*11,2*
Quotient $U_{(Xpt)}/\sigma_{pt}$´	*0,91*
Results in the target range	*5*
Percent in the target range	*71%*

Notes to the statistic data:

The target standard deviation was calculated according to precision data from ASU § LFGB L 46.02-4. Additionally the standard uncertainty was considered and the results were evaluated by z´-score (see 3.6). The specified target standard deviation "for information" was calculated according to the Horwitz general model (see 3.6.1).

The robust standard deviation shows an increased variability of the results and is relatively high compared to the reproducibility standard deviations of the ASU § 64 LMBG L 46.02-4. This is justified by the relatively low content of 16-O-methylcafestol. The ASU §64 LFGB L 46.02-4 has been tested for a mass fraction of 50 mg to 300 mg 16-O-methylcafestol per kg roasted coffee.

The quotient $U_{(Xpt)}/\sigma_{pt}$´ of 0,72 is above 0,3 and is acceptable due to the other characteristics and the use of different determination methods.

71% of results were in the target range.

For sample A, there was a roast coffee blend with Robusta content of 3,5%.

From the median (X_{PT}) and the values specified in the ASU § 64 LFGB L 46.02-4 a proportion of Robusta coffee of 3,5% can be calculated.

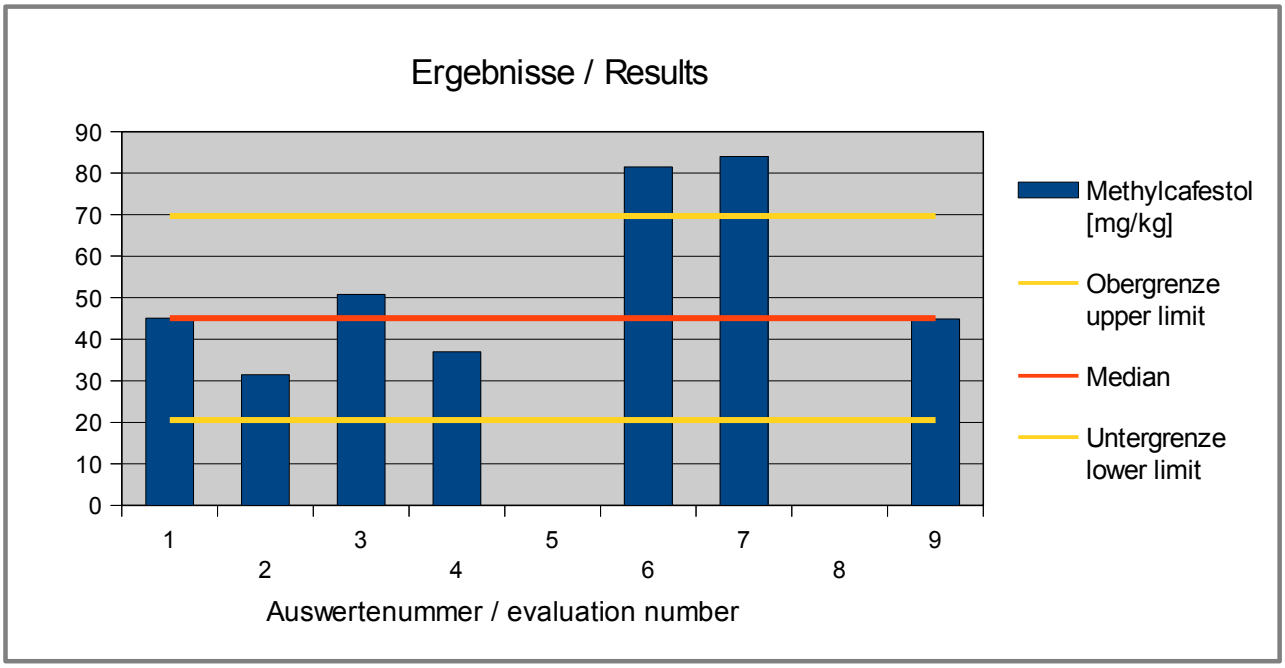


Abb. 1: Ergebnisse 16-O-MC, Probe A
Fig. 1: Results 16-O-MC, sample A

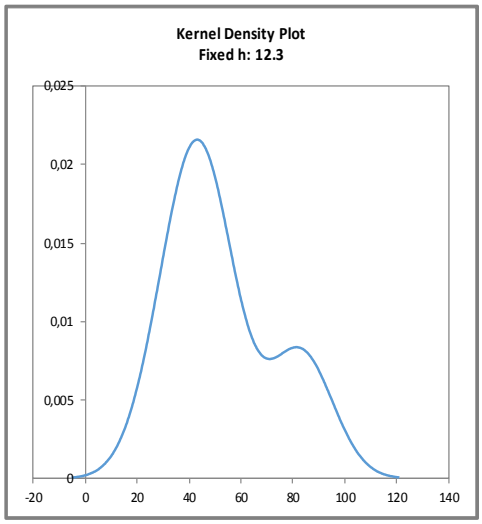


Abb. / Fig. 1:
Kerndichte-Schätzung der Ergebnisse (mit h = σ_{pt}´ von X_{pt})

Kernel density plot of results (with h = σ_{pt}´ of X_{pt})

Comment:
The kernel density shows almost a symmetrical distribution of results with a side peak at approx. 80 mg/kg, due to results outside the target range.

Ergebnisse der teilnehmenden Institute:
Results of Participants:

Auswerte-nummer Evaluation number	Methylcafestol [mg/kg]	Abweichung [mg/kg] Deviation [mg/kg]	z´-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	45,1	0,00	0,0	0,0	
2	31,5	-13,6	-1,1	-3,3	
3	50,8	5,70	0,46	1,40	
4	37,0	-8,10	-0,66	-2,0	
5	< LOQ				
6	81,5	36,4	3,0	8,9	
7	84,0	38,9	3,2	9,6	
8	< 100				
9	44,9	-0,200	-0,016	-0,049	

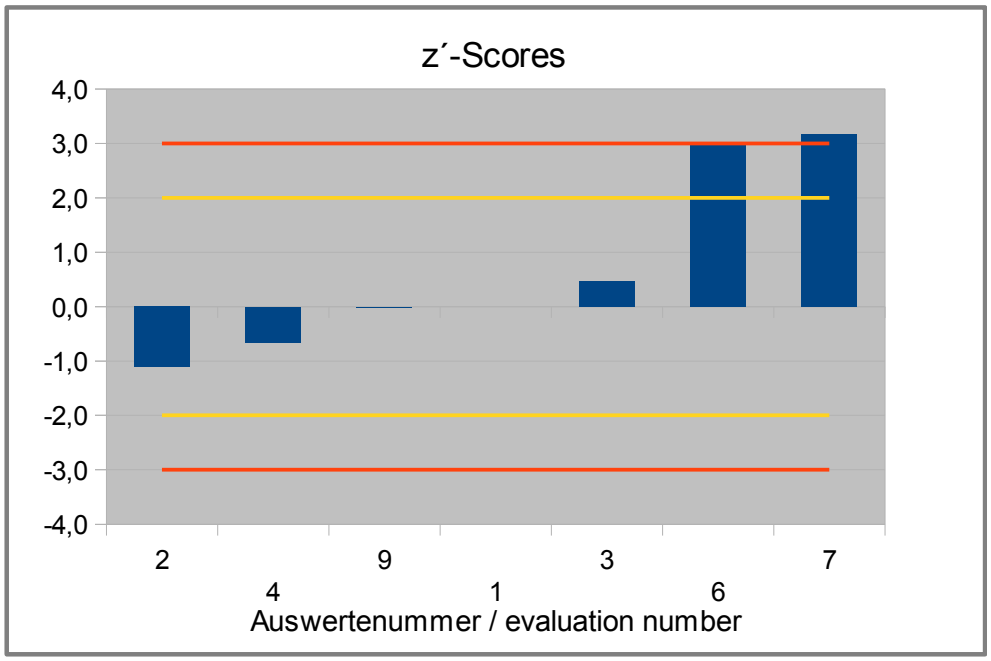


Abb. 2: Z-Scores 16-OMC Probe A
Fig. 2: Z-Scores 16-OMC sample A

4.2 16-O-Methylcafestol in sample B (mg/kg)

Statistic Data	
Number of results	9
Number of outliers	0
Mean	868
Median (X_{pt})	**730**
Robust Mean	851
Robust standard deviation (S*)	**246**
Reproducibility (CV_{s*})	**33,7%**
Target range:	
Target standard deviation σ_{pt}´	**131**
Target standard deviation (for Information)	43,3
lower limit of target range	**468**
upper limit of target range	**992**
Quotient S/σ_{pt}´*	*1,9*
Standard uncertainty $U_{(Xpt)}$	*102*
Quotient $U_{(Xpt)}/\sigma_{pt}$´	*0,78*
Results in the target range	*7*
Percent in the target range	*78%*

Notes to the statistic data:

The target standard deviation was calculated according to precision data from ASU § LFGB L 46.02-4. Additionally the standard uncertainty was considered and the results were evaluated by z´-score (see 3.6). The specified target standard deviation "for information" was calculated according to the Horwitz general model (see 3.6.1).

The robust standard deviation shows an increased variability of the results and is relatively high compared to the reproducibility standard deviations of the ASU § 64 LMBG L 46.02-4. This is acceptable due to the different characteristics and the use of different determination methods.

The quotient $U_{(Xpt)}/\sigma_{pt}$´ of 0,78 is above 0,3 and is acceptable due to the other characteristics and the use of different determination methods.

78% of results were in the target range.

Sample B was a roasted coffee blend with a declared Robusta content of 20%.

From the median (X_{PT}) and the values specified in the ASU § 64 LFGB L 46.02-4 a proportion of Robusta coffee of 57% can be calculated.

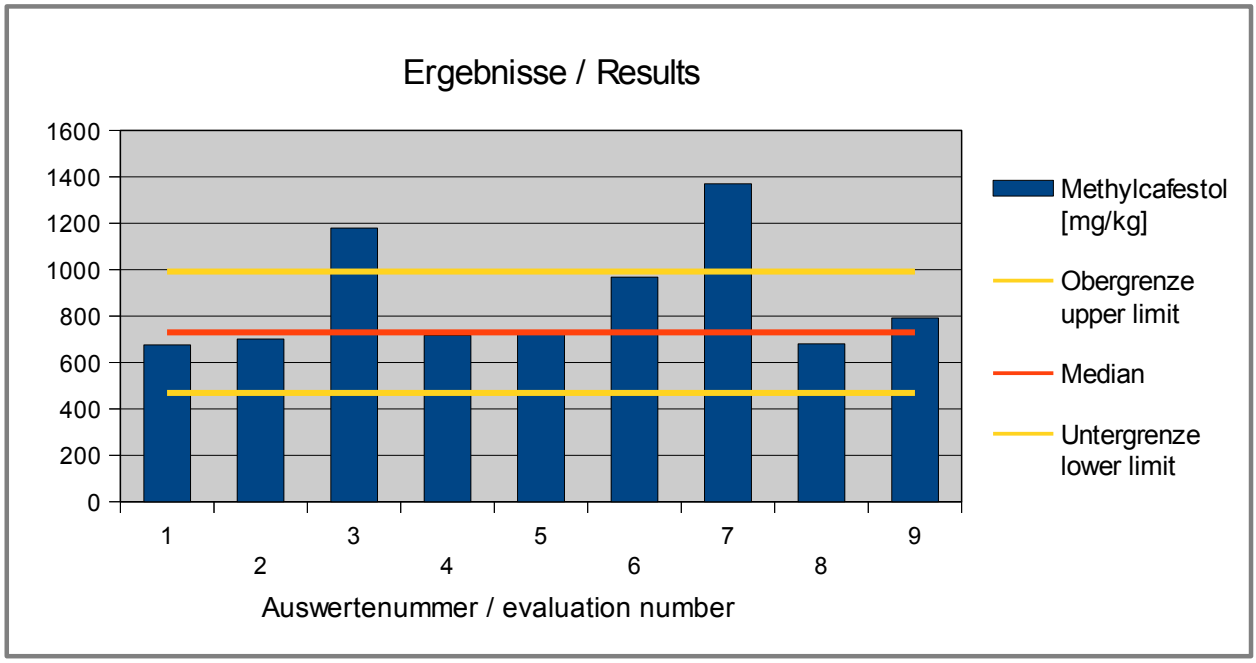


Abb. 4: Ergebnisse 16-OMC Probe B
Fig. 4: Results 16-OMC sample B

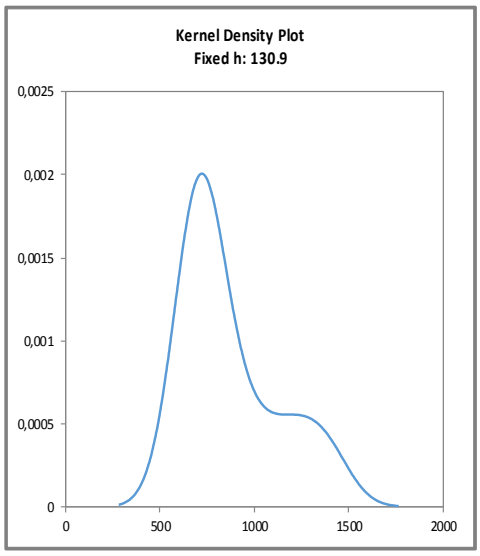


Abb. / Fig. 2:
Kerndichte-Schätzung der Ergebnisse (mit h = σ_{pt}´ von Xpt)

Kernel density plot of results (with h = σ_{pt}´ of Xpt)

Comment:
The kernel density shows almost a symmetrical distribution of results with a side peak at approx. 1300 mg/kg, due to results outside the target range.

Ergebnisse der teilnehmenden Institute:
Results of Participants:

Auswerte-nummer Evaluation number	Methylcafestol [mg/kg]	Abweichung [mg/kg] Deviation [mg/kg]	z´-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	676	-53,8	-0,41	-1,2	
2	701	-28,8	-0,22	-0,67	
3	1179	449	3,4	10	
4	717	-12,8	-0,10	-0,30	
5	730	0,0	0,0	-2,8	
6	968	238	1,8	5,5	
7	1370	640	4,9	15	
8	680	-49,8	-0,38	-4,0	
9	791	61,2	0,47	1,4	

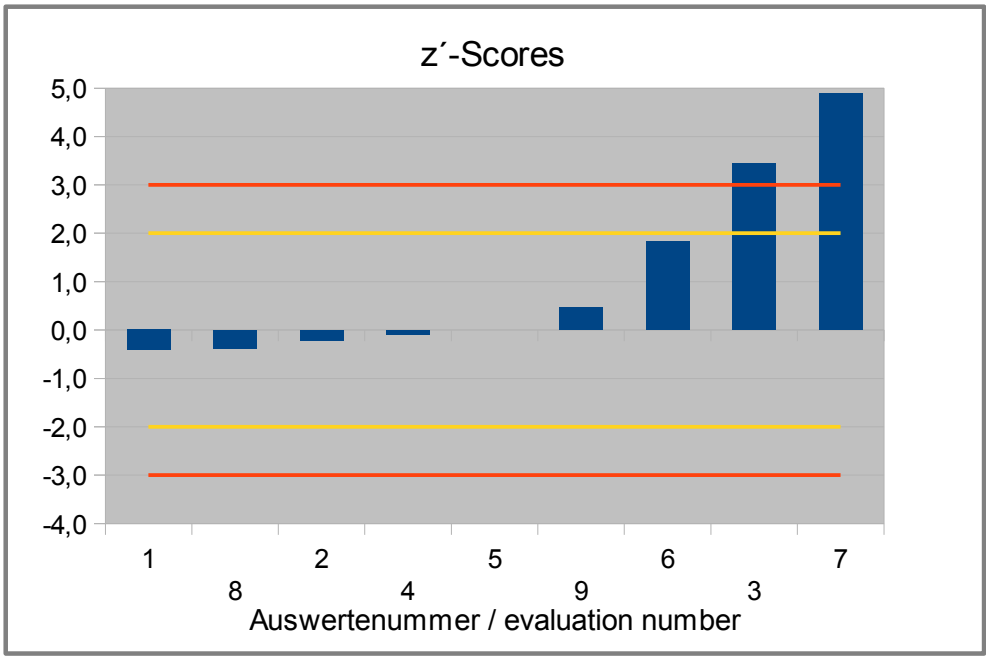


Abb. 5: Z-Scores 16-OMC Probe B
Fig. 5: Z-Scores 16-OMC sample B

4.3 16-O-Methylcafestol in sample C (mg/kg)

Statistic Data	
Number of results	9
Number of outliers	0
Mean	278
Median (x_{pt})	**215**
Robust Mean	274
Robust standard deviation (S*)	**146**
Reproducibility (CV_{s*})	**68,0%**
Target range:	
Target standard deviation σ_{pt}´	**65,5**
Target standard deviation (for Information)	15,3
lower limit of target range	**84,1**
upper limit of target range	**346**
Quotient S/σ_{pt}´*	*2,2*
Standard uncertainty $U_{(Xpt)}$	*60,9*
Quotient $U_{(Xpt)}/\sigma_{pt}$´	*0,93*
Results in the target range	7
Percent in the target range	*78%*

Notes to the statistic data:

The target standard deviation was calculated according to precision data from ASU § LFGB L 46.02-4. Additionally the standard uncertainty was considered and the results were evaluated by z´-score (see 3.6). The specified target standard deviation "for information" was calculated according to the Horwitz general model (see 3.6.1).

The robust standard deviation shows an increased variability of the results and is relatively high compared to the reproducibility standard deviations of the ASU § 64 LMBG L 46.02-4. This is acceptable due to the different characteristics and the use of different determination methods.

The quotient $U_{(Xpt)}/\sigma_{pt}$´ of 0,93 is above 0,3 and is acceptable due to the other characteristics and the use of different determination methods.

78% of results were in the target range.

Sample C was a roasted coffee blend with a Robusta content of 25%.

From the median (X_{PT}) and the values specified in the ASU § 64 LFGB L 46.02-4 a proportion of Robusta coffee of 17% can be calculated.

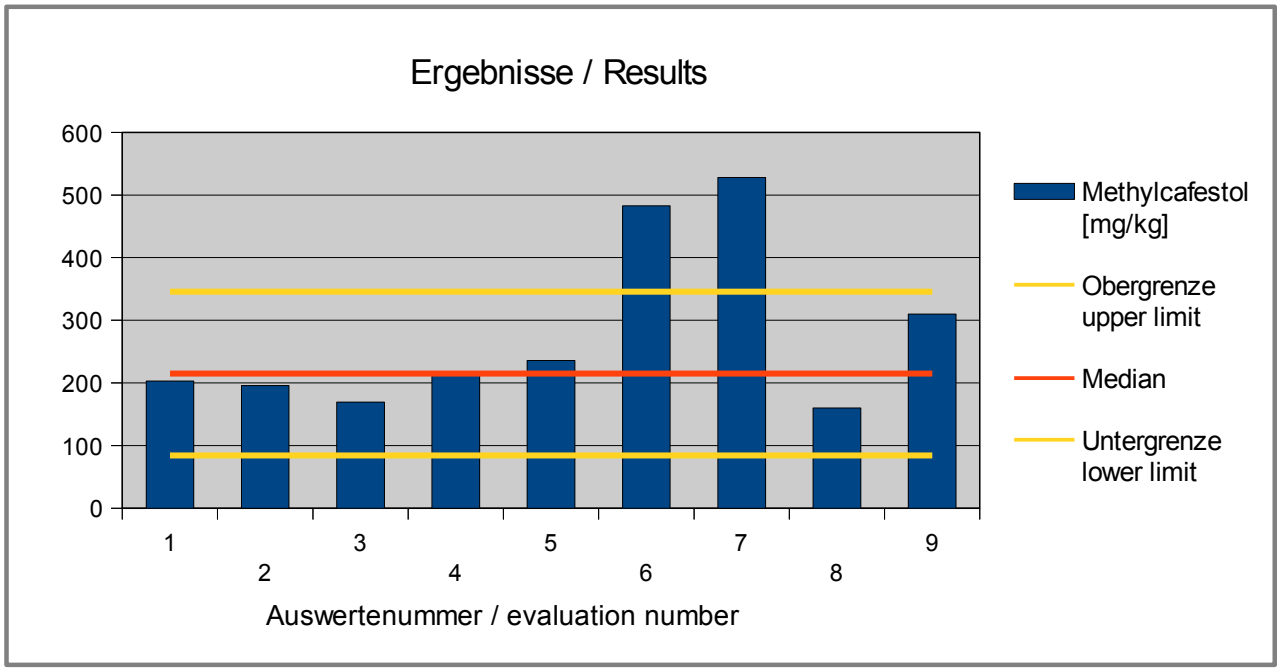


Abb. 7: Ergebnisse 16-OMC Probe c
Fig. 7: Results 16-OMC sample c

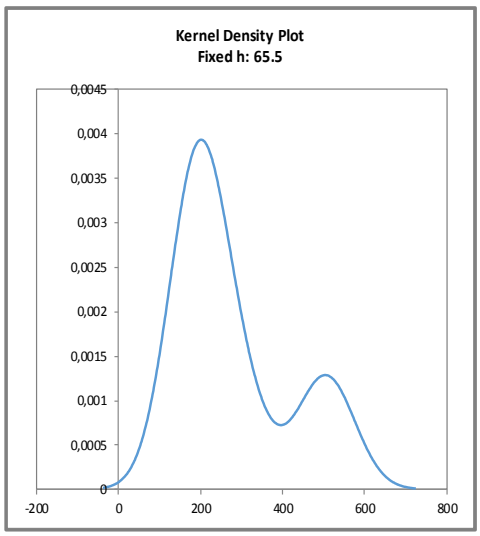


Abb. / Fig. 3:
Kerndichte-Schätzung der Ergebnisse (mit h = σ_{pt} von Xpt)

Kernel density plot of results (with h = σ_{pt} of Xpt)

Comment:
The kernel density shows almost a symmetrical distribution of results with a side peak at approx. 500 mg/kg, due to results outside the target range.

Ergebnisse der teilnehmenden Institute:
Results of Participants:

Auswerte-nummer Evaluation number	Methylcafestol [mg/kg]	Abweichung [mg/kg] Deviation [mg/kg]	z´-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	203	-12,0	-0,18	-0,78	
2	196	-19,0	-0,29	-1,2	
3	170	-45,5	-0,69	-3,0	
4	215	0,00	0,0	0,0	
5	236	20,6	0,31	-2,5	
6	483	268	4,1	17	
7	528	313	4,8	20	
8	160	-55,0	-0,84	-7,4	
9	310	95,0	1,5	6,2	

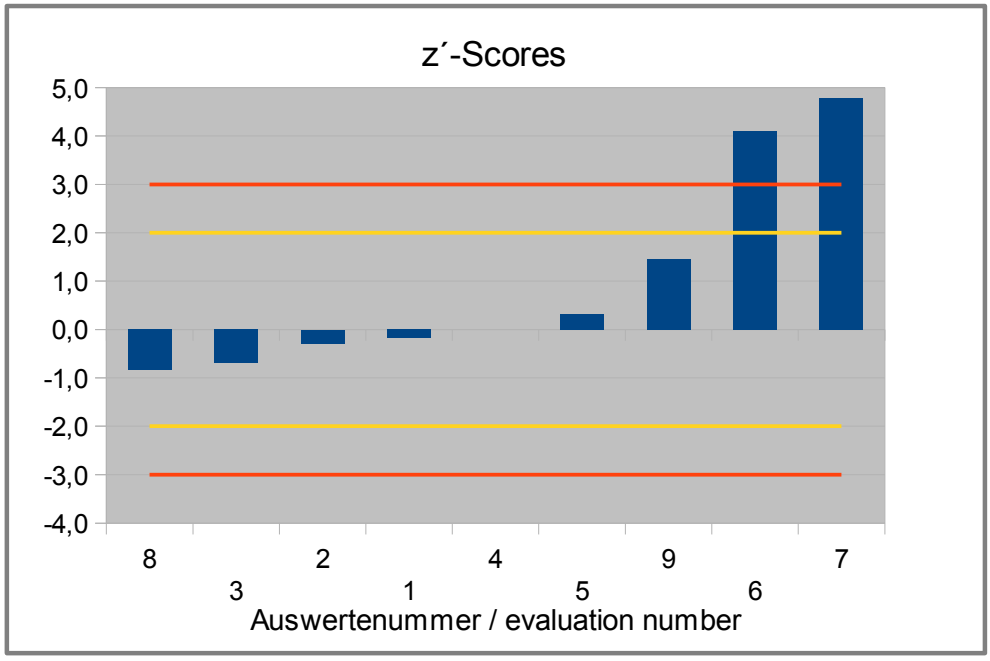


Abb. 8: Z-Scores 16-OMC Probe C
Fig. 8: Z-Scores 16-OMC sample C

5. Documentation

5.1 Details by the participants

5.1.1 Primary data

Parti-cipant	Date of analysis	Result sample A	Result sample B	Result sample C	Limit of quanti-ficatin	incl. RR	Recovery rate
Teil-nehmer	Datum der Analyse	Ergebnis Probe A	Ergebnis Probe B	Ergebnis Probe C	Bestim-mungs-grenze	Angabe inkl. Wiederfindung	Wieder-findungsrate
	day/month	mg/kg	mg/kg	mg/kg	mg/kg	yes/no	in %
1	23- 25.10	45,1	676	203	20	yes	99,5
2	08.10.2018 - 25.10.2018	31,5	701	196	24	yes	-
3	18.10.18	50,8	1179,2	169,5	5	no	101
4	18.10.	37	717	215	7	no	101
5	23.10.18	< LOQ	729,8	235,6	60		73,5
6	05.10.2018	81,5	968	483	15		
7	18.10.18	84	1370	528	15	yes	30
8	18.09.18	<100	680	160	100	no	
9	15.09.	44,9	791	310	30		

5.1.2 Analytical Methods

Teilnehmer	Methodenbeschreibung	Probenvorbereitung	Messmethode	Kalibrierung und Referenzmaterial	Wiederfindung mit gleicher Matrix	Methode akkreditiert	Sonstige Hinweise
Participant	Method description	Sample preparation	Measuring method	Calibration and reference material	Recovery with same matrix	Method accredited	Further remarks
					yes/no	yes/no	
1	§64 46.02-4 HPLC-method	unsifted	HPLC/DAD		yes	yes	
2	Literature-based, internal optimization	Homogenization and grinding of samples, extraction in chloroform-d	1H-NMR	Calibration via reference sample of the instrument manufacturer; carrying along of a standard of known concentration	yes	yes	-
3	BVL L 46.02-4				yes	yes	
4	Rapid determination by nuclear magnetic resonance spectroscopy (NMR)	Extraction with CDCl3	NMR		yes	yes	
5	ASU L 46.02-4				yes	yes	
6							
7	no	Direct saponification Column purification	HPLC/TOF	16-o-Methylcafestol	yes	no	
8	internal, RMN				no	yes	
9	LC-MS/MS					yes	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer homogeneity test
DLA 41-2018 sample A

Weight whole sample	1,000	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight of particle	2,0	µg
Addition of tracer	21,5	mg/kg

Result of analysis

Sample	Weight [g]	Paticle number	Particles [mg/kg]
1	8,07	62	15,4
2	10,06	76	15,1
3	7,89	51	12,9
4	8,41	49	11,7
5	8,74	62	14,2
6	9,03	54	12,0
7	8,65	53	12,3
8	8,83	51	11,6
9	9,55	72	15,1
10	9,19	59	12,8

Poisson distribution		
Number of samples	10	
Degree of freedom	9	
Mean	58,8	Particle
Standard deviation	6,67	Particle
χ^2 (CHI-Quadrat)	6,82	
Probability	**66**	%
Recoveryrate	62	%

Normal distribution		
Numver of samples	10	
Mean	13,3	mg/kg
Standard deviation	1,51	mg/kg
rel. Standard deviation	11,4	%
Horwitz Standard deviation	10,8	%
HorRat value	**1,0**	
Recovery rate	62	%

Microtracer Homogeneity test
DLA 41-2018 sample C

Weight whole sample	1,000	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	21,6	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	8,38	54	12,9
2	9,60	67	14,0
3	11,03	71	12,9
4	10,26	68	13,3
5	8,40	76	18,1
6	10,91	76	13,9
7	11,02	89	16,2
8	8,62	59	13,7
9	9,14	67	14,7
10	9,25	58	12,5

Poisson distribution		
Number of samples	10	
Degree of freedom	9	
Mean	68,6	Paticles
Standard deviation	8,32	Particles
χ^2 (CHI-Quadrat)	9,08	
Probability	**43**	%
Recovery rate	66	%

Normalverteilung		
Number of samples	10	
Mean	14,2	mg/kg
Standard deviation	1,72	mg/kg
rel. Standard deviation	12,1	%
Horwitz Standard deviation	10,7	%
HorRat value	**1,1**	
Recovery rate	66	%

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 41-2018***
PT name	***Methylcafestol in 3 Coffee Blends***
*Sample matrix**	*Samples A, B and C:* ***roasted coffee blends*** *(with different ratio of arabica : robusta)*
Number of samples and sample amount	*3 different samples, 15 g each.*
Storage	*Samples A, B and C: cooled 2 - 10°C*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*quantitative:* ***Methylcafestol***

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, B and C) 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, B and C)* – *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 26th October 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	*Dr. Gerhard Wichmann*

** 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

Teilnehmer/ participant	Ort/ location	Land/ country
		Switzerland
		Germany
		Germany
		France
		Germany
		Germany
		Germany
		Germany
		Germany

[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 literature

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)
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. A Horwitz-like function describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Analyst, 125, 385-386 (2000)
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13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messungen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
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.
15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
16. HORWITZ EQUATION AS QUALITY BENCHMARK IN ISO/IEC 17025 TESTING LABORATORY, C. Rivera, R. Rodriguez, Pimentel 4104 –B; Col. Las Granjas. Chihuahua Chihuahua Mexico. C.P. 31160
17. AOAC Guidlines for Standard Method Performance Requirements (2016)
18. ASU §64 LFGB L46.02-4; Bestimmung des Gehaltes an 16-O-Methylcafestol in Röstkaffee, HPLC-Verfahren (Januar 2012) (Übernahme der gleichnamigen Norm DIN 10779, Ausgabe März 2011)