Evaluation Report
proficiency test

DLA ptAU06/2020

16-O-Methylcafestol

in three Coffee Blends

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

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EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: March 4, 2021
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. Realization

2.1 Test material

The test material were three ground coffee blends (samples A, B and C) with different ratios of Robusta coffee and arabica coffee contents:

Sample A (100 % Arabica):

Ingredient	**Amount**
Coffee blend 100% Arabica	100 %

Sample B (15 % Robusta):

Ingredients	**Amounts**
Sample A (100 % Arabica)	85,0 %
Coffee blend 100 % Robusta	15,0 %

Sample C (3 % Robusta):

Ingredients	**Amounts**
Sample A (100% Arabica)	97,0 %
Coffee Blend 100% Robusta	3,00 %

The raw materials were each sieved (*mesh size* 2,5 mm), added and homogenized

Afterwards the samples were portioned to approximately 20 g into metallised PET film bags and chronologically numbered.

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].
For the present PT, the microtracer analysis of samples B and C showed a probability of 85% and 59%, respectively. 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 HorRat value of 0,75 and 0,91, 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 activity of water (a_W value <0,5).

The a_W value of the PT samples was approx. 0,3 (23°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

The test material consisting of the three coffee blends (sample A, B and C) was sent to every participating laboratory in the 37th week of 2020. The testing method was optional. The tests should be finished at 6th November 2020 the latest.

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

The three portions contain different samples with the parameters 16-O-Methylcafestol (Methylcafestol), 1,2-Dihydrocafestol (Kahweol) and Cafestol in the matrix of roasted coffee blends with different ratios of arabica and robusta coffee contents. The analysis method is optional.

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).

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.
9 out of 12 participants submitted their results in time. Three participants have not submitted any results.

3. Evaluation

3.1 Consensus value from participants (assigned value)

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

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

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

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

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

3.2 robust standard deviation

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

3.3 Repeatability standard deviation

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

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

The relative repeatability standard deviation as a percentage of the mean value is indicated as coefficient of variation CV_r in the table of statistical characteristics in the results section in case single results from participants are available.

3.4 Reproducibility standard deviation

The reproducibility standard deviation S_R represents a inter-laboratory estimate of the standard deviation for the determination of each parameter on the bases of (outlier free) individual participant results. It takes into account both the repeatability standard deviation S_r and the within-laboratory standard deviation S_S. Reproducibility standard deviations of PT´s may differ from reproducibility standard deviations of ring trials, because the participating laboratories of a PT generally use different internal conditions and methods for determining the measured values.
In the present evaluation, the specification of the reproducibility standard deviation, therefore, does not refer to a specific method, but characterizes approximately the comparability of results between the laboratories, assumed the effect of homogeneity and stability of the sample are negligible.

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

The relative reproducibility standard deviation 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 16-O-Methylcaestol 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 46.02-4). Additionally, the standard uncertainty was considered by evaluation using the z'-score (see 3.6.8).
In addition, the target standard deviation according to the general model of Horwitz (see 3.6.1) was given for information.

Due to the number of <3, the results for cafestol and dihydrocafestol were not evaluated statistically.

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 experimnet

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

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

The values given in Table 1 relative repeatability standard deviation (RSD_r) and relative reproducibility standard deviation (RSD_R) were determined in collaborative trials using the specified methods. The in the table indicated resulting target standard deviation σ_{pt} is additionally given in the evaluation for information.

Table 1: Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) from precision experiments and resulting target standard deviations σ_{pt} [18]

Parameter	**Matrix**	**Mean (mg/kg)**	**RSD_r**	**RSD_R**	**σ_{pt}**	**Method / Literature**
16-O-Methyl-cafestol	Coffee blend (20% Robusta)	257,6	4,5%	11,6%	13,6%[1]	HPLC [18] ASU 46.07-4
16-O-Methyl-cafestol	Coffee blend (10% Robusta)	130,1	4,5%	9,8%	4,6%	HPLC [18] ASU 46.07-4

[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 2 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. For example a fault isolation or a root cause analysis through the examination of transmission error or an error in the calculation, in the trueness and precision must be performed and if necessary appropriate corrective measures should be applied [3].

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

Table 2: Characteristics of the present PT (on dark grey) in comparison to previous PTs since 2017 (SD = standard deviation, CV = coefficient of variation).

Parameter	**Matrix (powder)**	**robust mean [mg/kg]**	**robust SD (S*) [mg/kg]**	**rel. SD (CK_{S*}) [%]**	**Quotient S^*/σ_{pt}**	**DLA-Report**
16-O-Methyl-cafestol	Coffee blend Sample A (5% Robusta)	81,3	12,3	15,1	1,4	DLA 39/2017
16-O-Methy-lafestol	Coffee blend Sample B (10% Robusta)	116	40,6	35,0	1,9 [1]	DLA 39/2017
16-O-Methy-lafestol	Coffee blend Sample C (20% Robusta)	331	41,7	12,6	1,1	DLA 39/2017
16-O-Methy-lafestol	Coffee blend Sample A (3,5% Robusta)	53,5	23,7	44,3	1,9 [1]	DLA 41/2018
16-O-Methy-lafestol	Coffee blend Sample B (20% Robusta)	851	246	28,9	1,9 [1]	DLA 41/2018
16-O-Methy-lafestol	Coffee blend Sample C (25% Robusta)	274	146	53,3	2,2 [1]	DLA 41/2018
16-O-Methy-lafestol	Coffee blend Sample B (15% Robusta)	186	39,7	21,3	1,5 [1]	DLA ptAU06/2020
16-O-Methy-lafestol	Coffee blend Sample C (3,0% Robusta)	51,5	16,6	32,2	1,7 [1]	DLA ptAU06/2020

[1] ***with targed standard deviation σpt'***

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. 3.8). The z'-score represents the relation of the deviation of the result (x) of the participant from the respective consensus value (X) to the square root of quadrat sum of the target standard deviation (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.

3.9 Reproducibility cofficient of variation (CK_R)

The variation coefficient (CV) 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*)
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 16-O-Methylcafestol in sample A in mg/kg

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	2
Number of outliers	0
Mean	16,5
Median	16,5
Robust Mean (X)	**16,5**
Robust standard deviation (S*)	**10,4**
Target range:	
Target standard deviation σ_{pt}	
Target standard deviation (for Information)	
lower limit of target range	
upper limit of target range	
Quotient S/σ_{pt}*	
Standard uncertainty $U_{(Xpt)}$	
Quotient $U_{(Xpt)}/\sigma_{pt}$	
Results in the target range	
Percent in the target range	

Comments:

Due to <3 values, no statistical evaluation was conducted for this parameter in this sample.

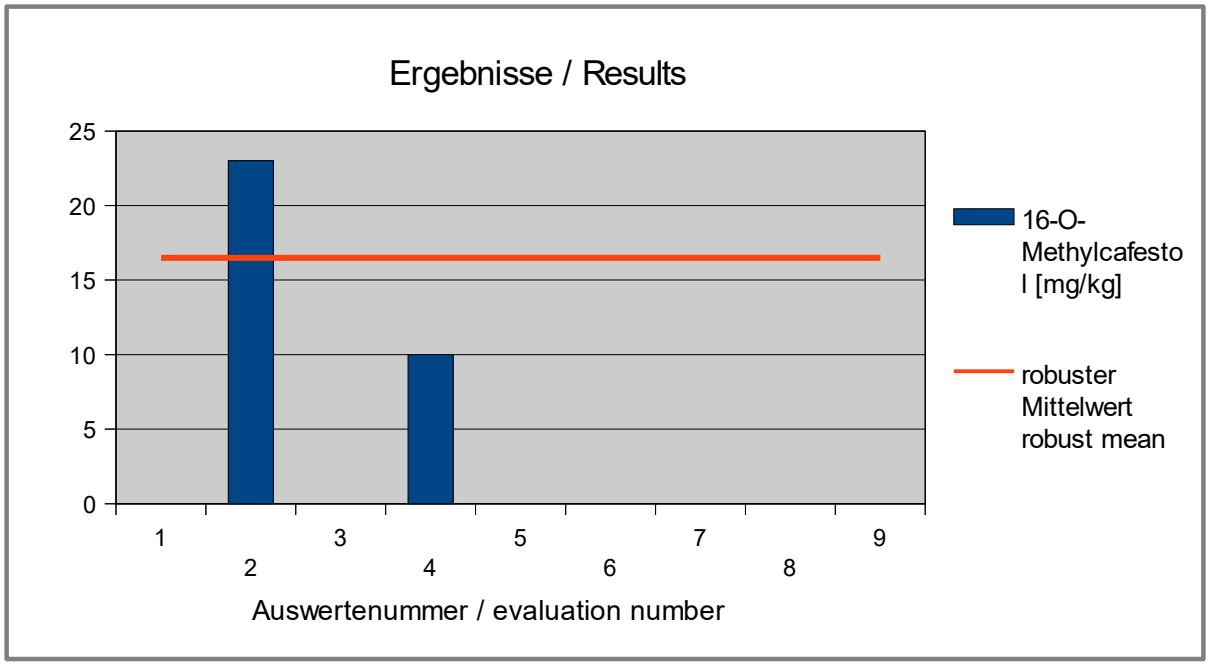

Abb. / Fig. 1: Ergebnisse 16-O-Methylcafestol in Probe A / Results 16-O-Methylcafestol in sample A

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	16-O-Methylcafestol [mg/kg]	Abweichung [mg/kg] Deviation [mg/kg]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1					
2	23,0	6,5			
3					
4	10,0	-6,5			
5					
6					
7					
8					
9					

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

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	9
Number of outliers	0
Mean	186
Median	184
Robust Mean (X)	**186**
Robust standard deviation (S*)	**39,7**
Target range:	
Target standard deviation σ_{pt}	**26,5**
Target standard deviation (for Information)	13,5
lower limit of target range	**133**
upper limit of target range	**239**
Quotient S/σ_{pt}'*	*1,5*
Standard uncertainty $U_{(Xpt)}$	*16,5*
Quotient $U_{(Xpt)}/\sigma_{pt}$'	*0,62*
Results in the target range	*8*
Percent in the target range	*89%*

Comments:

The target standard deviation was calculated using data from a precision experiment (ASU §64 L 46.02-4)(3.6.2). For information, the target standard deviation according to the model of Horwitz (s. 3.6.1) was given.

The distribution of results showed a slightly increased variability. Therefore the standard uncertainty was considered by evaluation using z'-scores. The quotient S/σ_{pt}' was below 2,0 then. The robust standard deviation was in the range of previous PTs (see 3.6.3). The comparability of results is given.*

89% of results were in the target range.

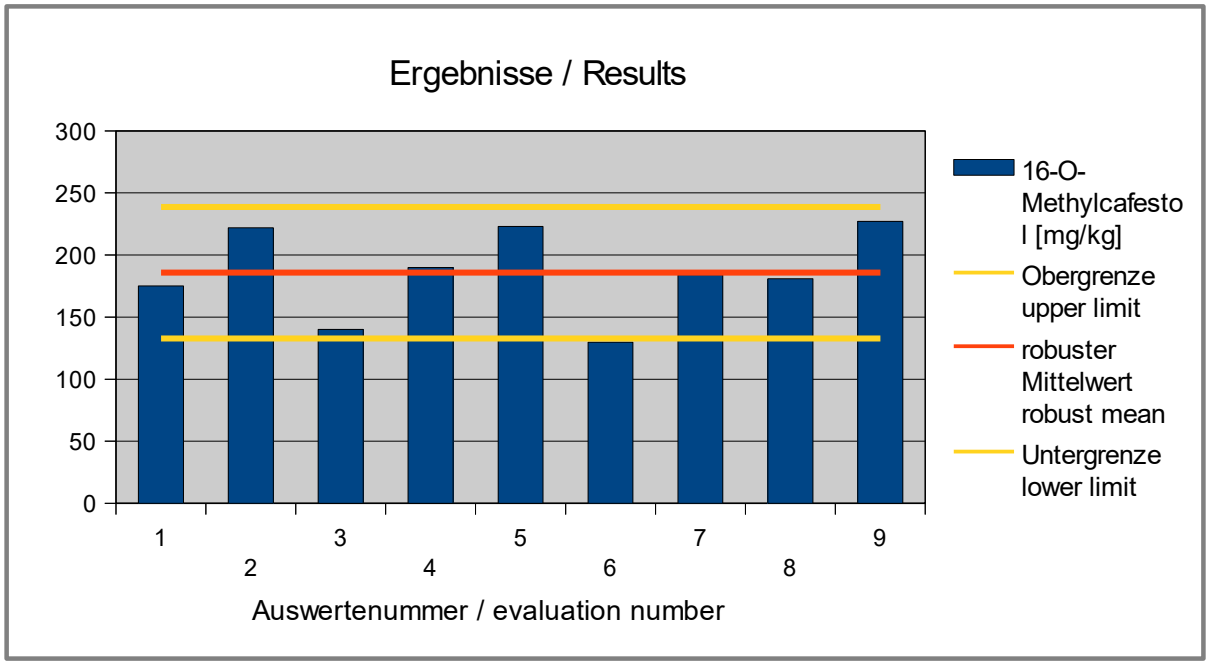

Abb. / Fig. 2: Ergebnisse 16-Methylcafestol in Probe B / Results 16-Methylcafestol in sample B.

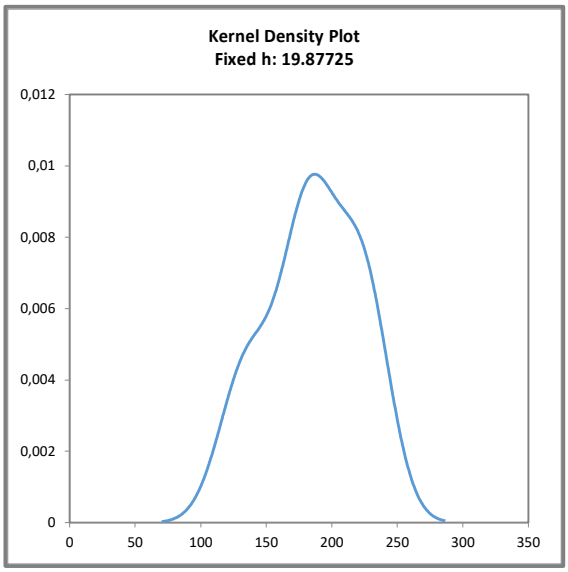

Abb. / Fig. 3:
Kerndichte-Schätzung der Ergebnisse (mit h = 26,503 x σ_{pt} von Xpt) / Kernel density plot of results (with h = 26,503 x σ_{pt} of Xpt)

Comment:
The kernel density shows almost a symmetrical distribution of results with two slight shoulders at approx. 40 and 220 mg/kg.

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	16-O-Methylcafestol [mg/kg]	Abweichung [mg/kg] Deviation [mg/kg]	z'-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	175	-11	-0,41	-0,79	
2	222	36	1,4	2,7	
3	140	-46	-1,7	-3,4	
4	190	4	0,16	0,31	
5	223	37	1,4	2,8	
6	130	-56	-2,1	-4,1	
7	184	-2	-0,07	-0,13	
8	181	-5	-0,18	-0,36	
9	227	41	1,6	3,0	

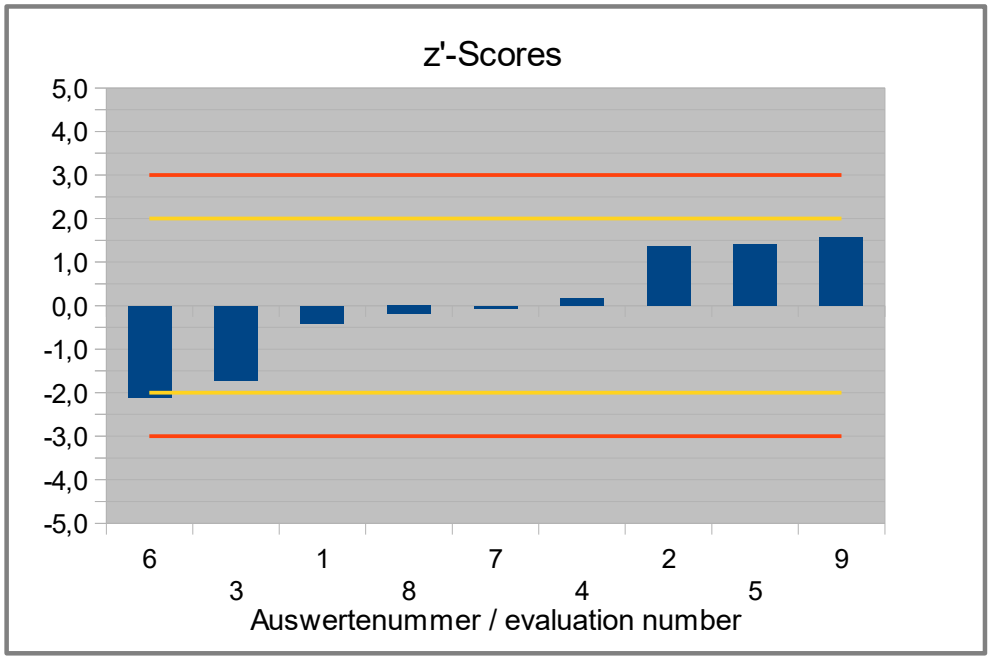

Abb. / Fig. 4: z'-Scores 16-O-Methylcafestol in Probe B / Results 16-O-Methylcafestol in sample B

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

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	7
Number of outliers	0
Mean	52,0
Median	51,0
Robust Mean (X)	**51,5**
Robust standard deviation (S*)	**16,6**
Target range:	
Target standard deviation $\sigma_{pt'}$	**9,72**
Target standard deviation (for Information)	4,55
lower limit of target range	**32,1**
upper limit of target range	**71,0**
Quotient S/$\sigma_{pt'}$*	*1,7*
Standard uncertainty $U_{(Xpt)}$	*7,84*
Quotient $U_{(Xpt)}/\sigma_{pt'}$	*0,81*
Results in the target range	*6*
Percent in the target range	*86%*

Comments:

The target standard deviation was calculated using data from a precision experiment (ASU §64 L 46.02-4)(3.6.2). For information, the target standard deviation according to the model of Horwitz (s. 3.6.1) was given.

The distribution of results showed a slightly increased variability. Therefore the standard uncertainty was considered by evaluation using z'-scores. The quotient S/$\sigma_{pt'}$ was below 2,0 then. The robust standard deviation was in the range of previous PTs (see 3.6.3). The comparability of results is given.*

86% of results were in the target range.

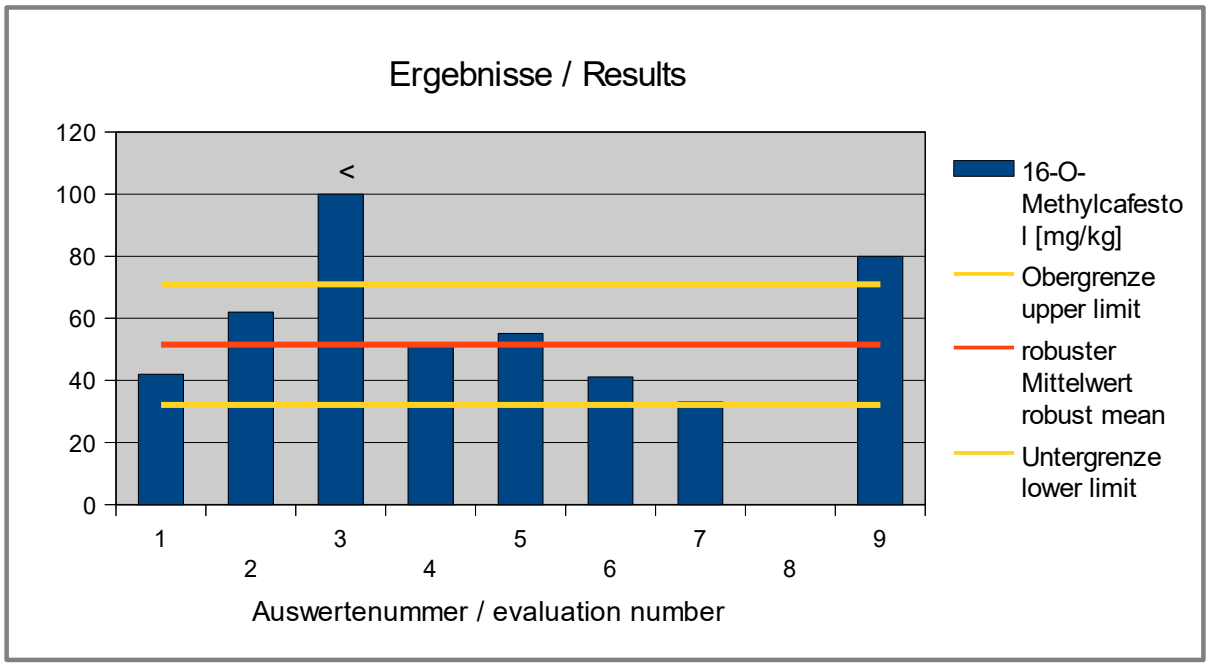

Abb. / Fig. 5: Ergebnisse 16-O-Methylcafestol in Probe C / Results 16-O-Methylcafestol in sample C

Comment: A kernel density was not calculated due to <8 results.

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	16-O-Methylcafestol [mg/kg]	Abweichung [mg/kg] Deviation [mg/kg]	z'-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	42,0	-9,5	-1,0	-2,1	
2	62,0	10,5	1,1	2,3	
3	< 100				
4	51,0	-0,5	-0,05	-0,11	
5	55,1	3,6	0,37	0,79	
6	41,1	-10,4	-1,1	-2,3	
7	33,0	-18,5	-1,9	-4,1	
8					
9	80,0	28,5	2,9	6,3	

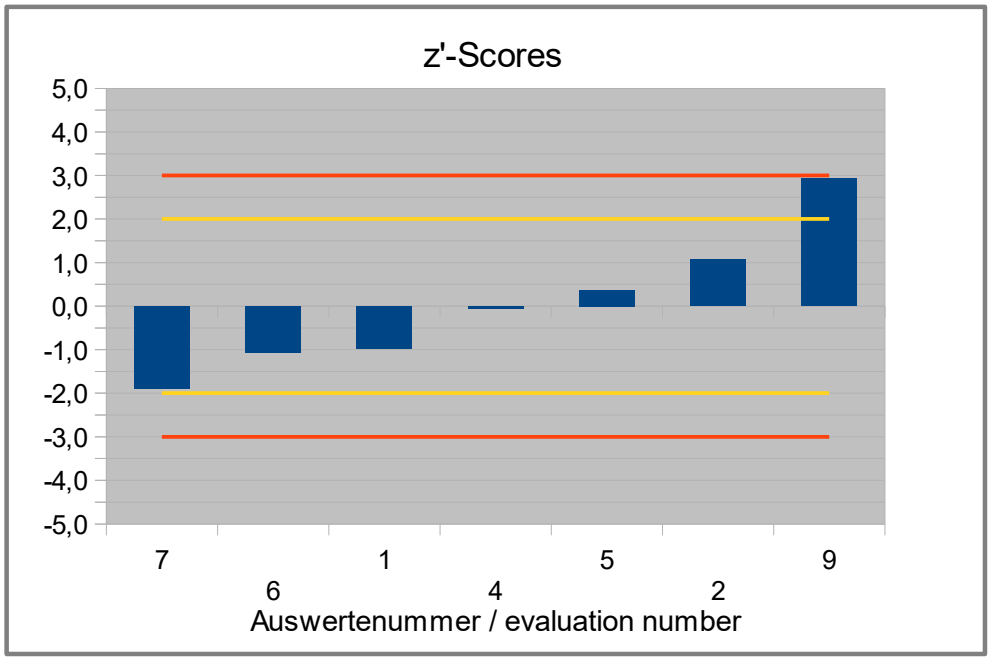

Abb. / Fig. 6: z'-Scores 16-O-Methylcafestol in Probe C / Results 16-O-Methylcafestol in sample C

4.4 Cafestol in sample A in mg/kg

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	2
Number of outliers	0
Mean	4740
Median	4740
Robust Mean (X)	**4740**
Robust standard deviation (S*)	**1171**
Target range:	
Target standard deviation σ_{pt}	
Target standard deviation (for Information)	
lower limit of target range	
upper limit of target range	
Quotient S/σ_{pt}*	
Standard uncertainty $U_{(Xpt)}$	
Quotient $U_{(Xpt)}/\sigma_{pt}$	
Results in the target range	
Percent in the target range	

Comments:

Due to <3 values, no statistical evaluation was conducted for this parameter in this sample.

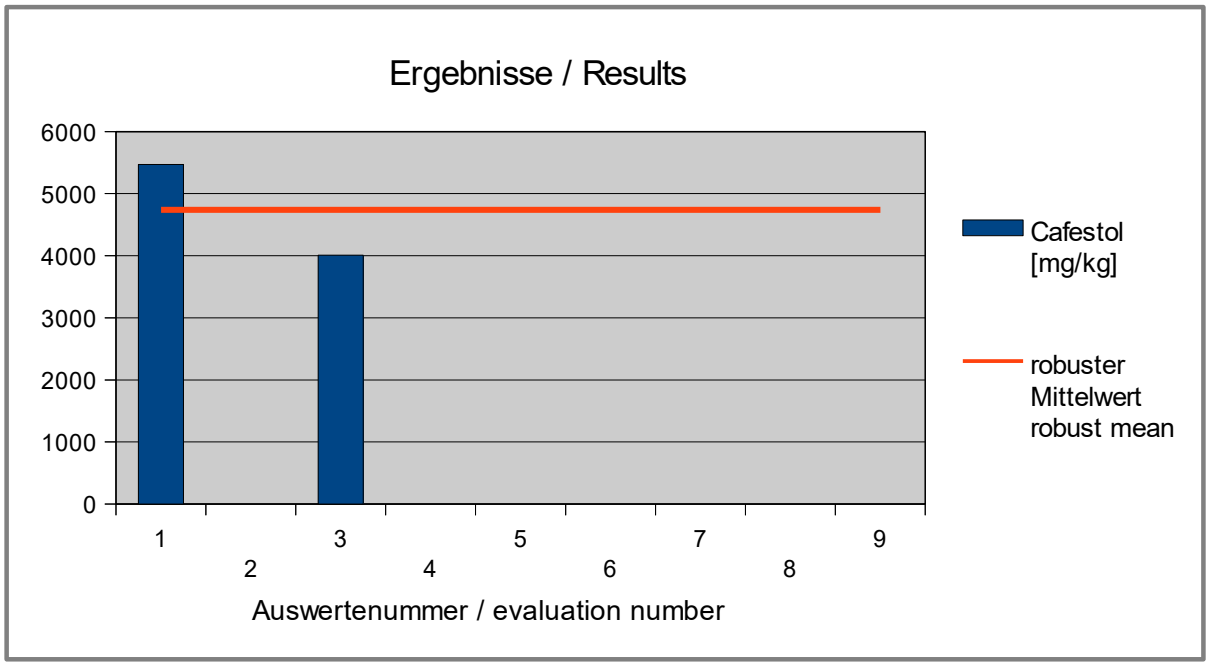

Abb. / Fig. 7: Ergebnisse Cafestol in Probe A / Results Cafestol in sample A

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Cafestol [mg/kg]	Abweichung [mg/kg] Deviation [mg/kg]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	5470	730			
2					
3	4010	-730			
4					
5					
6					
7					
8					
9					

4.5 Cafestol in sample B in mg/kg

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	2
Number of outliers	0
Mean	4318
Median	4318
Robust Mean (X)	**4318**
Robust standard deviation (S*)	**943**
Target range:	
Target standard deviation σ_{pt}	
Target standard deviation (for Information)	
lower limit of target range	
upper limit of target range	
Quotient S/σ_{pt}*	
Standard uncertainty $U_{(Xpt)}$	
Quotient $U_{(Xpt)}/\sigma_{pt}$	
Results in the target range	
Percent in the target range	

Comments:

Due to <3 values, no statistical evaluation was conducted for this parameter in this sample.

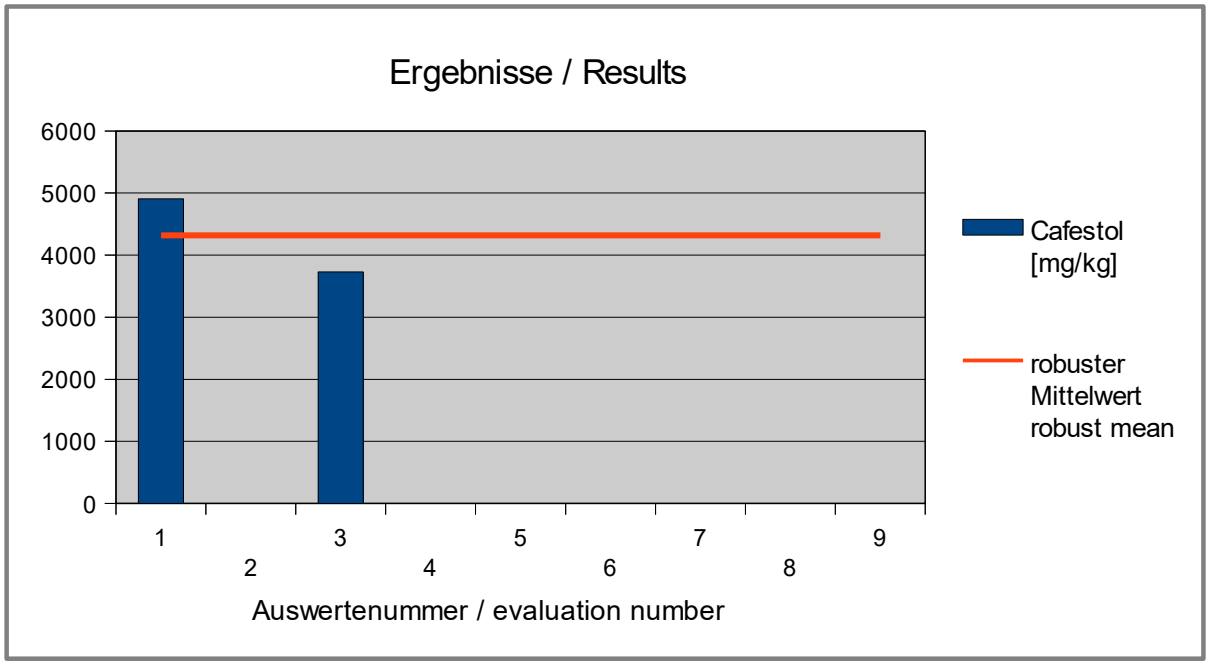

Abb. / Fig. 8: Ergebnisse Cafestol in Probe B / Results Cafestol in sample B

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Cafestol [mg/kg]	Abweichung [mg/kg] Deviation [mg/kg]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	4906	588			
2					
3	3730	-588			
4					
5					
6					
7					
8					
9					

4.6 Cafestol in sample C in mg/kg

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	2
Number of outliers	0
Mean	4618
Median	4618
Robust Mean (X)	**4618**
Robust standard deviation (S*)	**1006**
Target range:	
Target standard deviation σ_{pt}	
Target standard deviation (for Information)	
lower limit of target range	
upper limit of target range	
Quotient S/σ_{pt}*	
Standard uncertainty $U_{(Xpt)}$	
Quotient $U_{(Xpt)}/\sigma_{pt}$	
Results in the target range	
Percent in the target range	

Comments:

Due to <3 values, no statistical evaluation was conducted for this parameter in this sample.

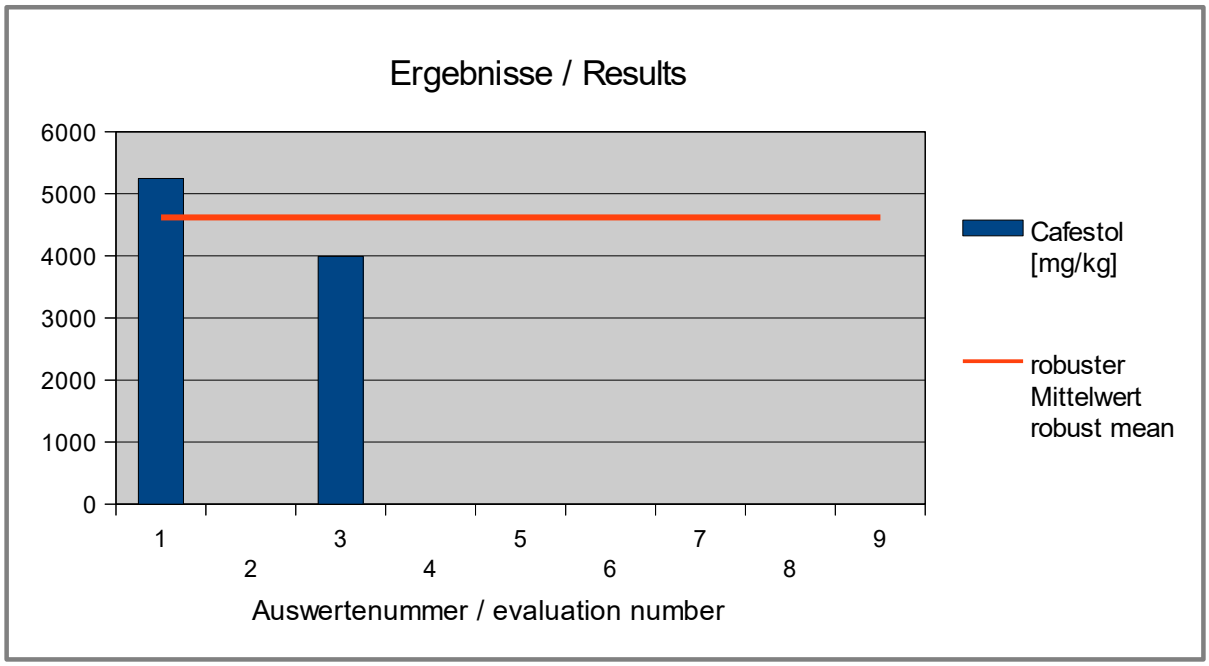

Abb. / Fig. 9: Ergebnisse Cafestol in Probe C / Results Cafestol in sample C

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Cafestol [mg/kg]	Abweichung [mg/kg] Deviation [mg/kg]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	5245	628			
2					
3	3990	-628			
4					
5					
6					
7					
8					
9					

4.7 1,2-Dihydrocafestol in sample A in mg/kg

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	2
Number of outliers	0
Mean	4251
Median	4251
Robust Mean (X)	**4251**
Robust standard deviation (S*)	**2038**
Target range:	
Target standard deviation σ_{pt}	
Target standard deviation (for Information)	
lower limit of target range	
upper limit of target range	
Quotient S/σ_{pt}*	
Standard uncertainty $U_{(Xpt)}$	
Quotient $U_{(Xpt)}/\sigma_{pt}$	
Results in the target range	
Percent in the target range	

Comments:

Due to <3 values, no statistical evaluation was conducted for this parameter in this sample.

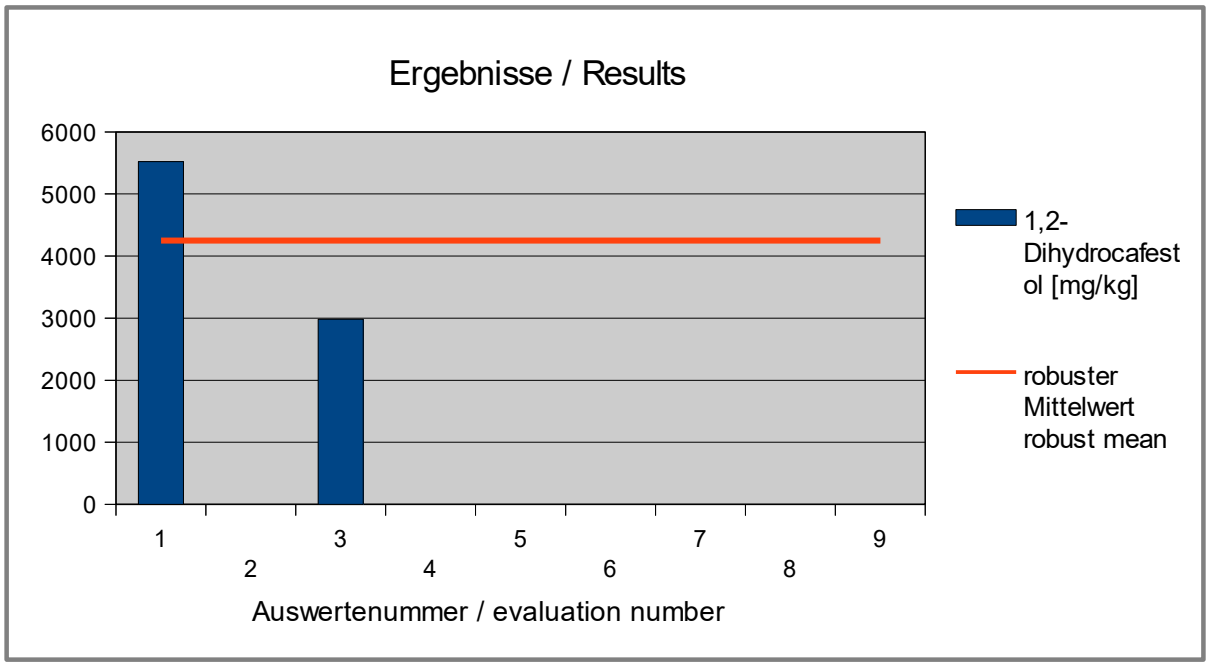

Abb. / Fig. 10: Ergebnisse 1,2-Dihydrocafestol in Probe A / Results 1,2-Dihydrocafestol in sample A

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	1,2-Dihydrocafestol [mg/kg]	Abweichung [mg/kg] Deviation [mg/kg]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	5521	1271			
2					
3	2980	-1271			
4					
5					
6					
7					
8					
9					

4.8 1,2-Dihydrocafestol in sample B in mg/kg

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	2
Number of outliers	0
Mean	3633
Median	3633
Robust Mean (X)	**3633**
Robust standard deviation (S*)	**1736**
Target range:	
Target standard deviation σ_{pt}	
Target standard deviation (for Information)	
lower limit of target range	
upper limit of target range	
Quotient S/σ_{pt}*	
Standard uncertainty $U_{(Xpt)}$	
Quotient $U_{(Xpt)}/\sigma_{pt}$	
Results in the target range	
Percent in the target range	

Comments:

Due to <3 values, no statistical evaluation was conducted for this parameter in this sample.

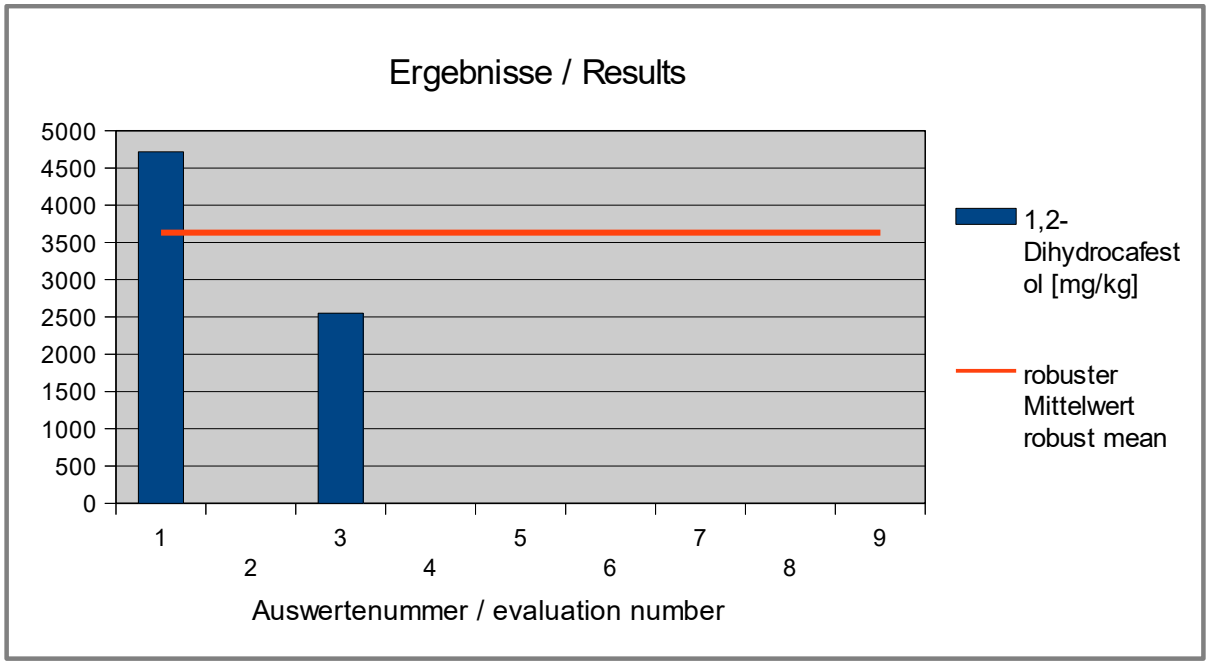

Abb. / Fig. 11: Ergebnisse 1,2-Dihydrocafestol in Probe B / Results 1,2-Dihydrocafestol in sample B

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	1,2-Dihydrocafestol [mg/kg]	Abweichung [mg/kg] Deviation [mg/kg]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	4715	1083			
2					
3	2550	-1083			
4					
5					
6					
7					
8					
9					

4.9 1,2-Dihydrocafestol in sample C in mg/kg

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	2
Number of outliers	0
Mean	4036
Median	4036
Robust Mean (X)	**4036**
Robust standard deviation (S*)	**1854**
Target range:	
Target standard deviation σ_{pt}	
Target standard deviation (for Information)	
lower limit of target range	
upper limit of target range	
Quotient S/σ_{pt}*	
Standard uncertainty $U_{(Xpt)}$	
Quotient $U_{(Xpt)}/\sigma_{pt}$	
Results in the target range	
Percent in the target range	

Comments:

Due to <3 values, no statistical evaluation was conducted for this parameter in this sample.

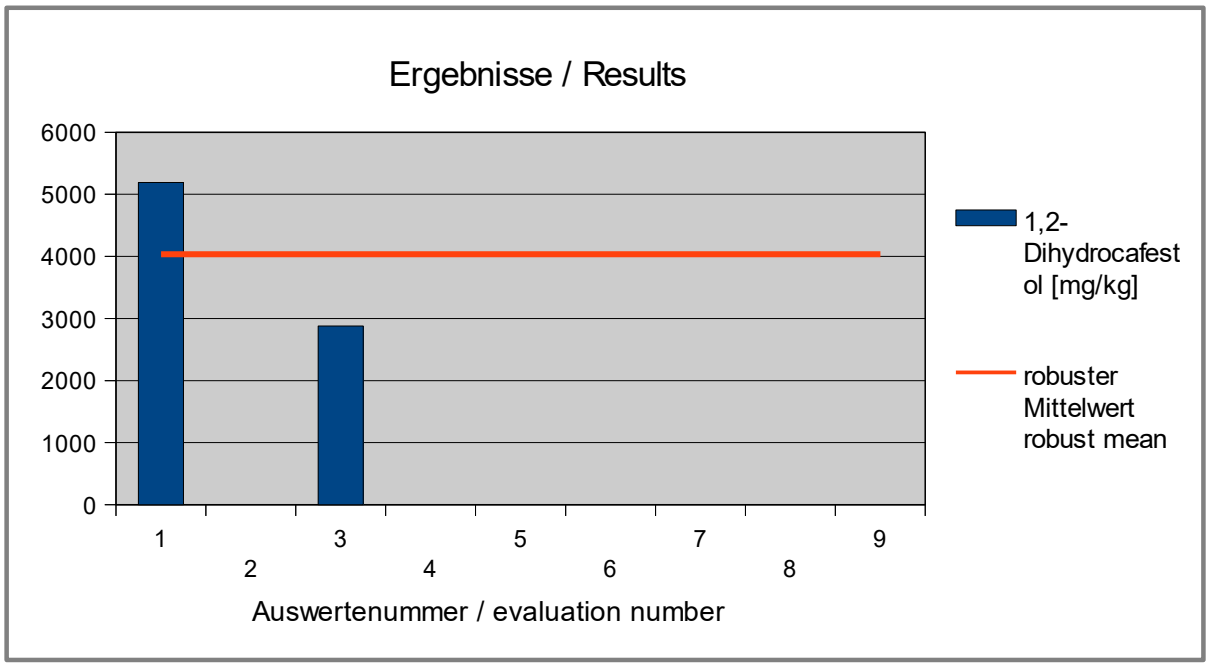

Abb. / Fig. 12: Ergebnisse 1,2-Dihydrocafestol in Probe C / Results 1,2-

Dihydrocafestol in sample C

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	1,2-Dihydrocafestol [mg/kg]	Abweichung [mg/kg] Deviation [mg/kg]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	5192	1156			
2					
3	2880	-1156			
4					
5					
6					
7					
8					
9					

4.10 z-Scores of the participants: tabular overwiev

Evaluation number	16-O-Methylcafestol			Cafestol			1,2-Dihydrocafestol		
Sample:	sample A	sample B	sample C	sample A	sample B	sample C	sample A	sample B	sample C
1		-0,41	-1,0						
2		1,4	1,1						
3		-1,7							
4		0,16	-0,05						
5		1,4	0,37						
6		-2,1	-1,1						
7		-0,07	-1,9						
8		-0,18							
9		1,6	2,9						

Bewertung des z-Scores / valuation of z-score (DIN ISO 13528:2009-01):
-2 ≤ z-score ≤ 2 erfolgreich / successful (in green)
-2 > z-score > 2 „Warnsignal“ / warning signal (in yellow)
-3 > z-score > 3 „Eingriffssignal“ / action signal (in red)

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	Date of analysis	Result sample A	Result sample B	Result sample C	Limit of determination	incl. RR	Recovery rate
			day/month					yes / no	in %
16-O-Methylcafestol	1	mg/kg	17.09.20	<20	175	42	20		100
16-O-Methylcafestol	2	mg/kg	09.10.20	23	222	62	0,15	no	101 - 110
16-O-Methylcafestol	3	mg/kg	15.09.20	<100	140	<100	100	no	
16-O-Methylcafestol	4	mg/kg	21.10.20	10	190	51	10	no	
16-O-Methylcafestol	5	mg/kg	30.10.20	< 30	223	55,1	30	no	95
16-O-Methylcafestol	6	mg/kg	13.10.20	0	129,8	41,1	5	no	101
16-O-Methylcafestol	7	mg/kg	05.11.20	<LOQ	184	33	30	yes	
16-O-Methylcafestol	8	mg/kg	30.10.	n.n.	180,85	n.b.	60	yes	73,5
16-O-Methylcafestol	9	mg/kg	30.10.20	0	227	80	10	no	

Analyte	Participant	Unit	Date of analysis	Result sample A	Result sample B	Result sample C	Limit of determination	incl. RR	Recovery rate
			day/month					yes / no	in %
1,2-Dihydrocafestol (Kahweol)	1	mg/kg	17.09.20	5521	4715	5192	300		100
1,2-Dihydrocafestol (Kahweol)	2	mg/kg							
1,2-Dihydrocafestol (Kahweol)	3	mg/kg	15.09.20	2980	2550	2880	100	no	
1,2-Dihydrocafestol (Kahweol)	4	mg/kg							
1,2-Dihydrocafestol (Kahweol)	5	mg/kg							
1,2-Dihydrocafestol (Kahweol)	6	mg/kg							
1,2-Dihydrocafestol (Kahweol)	7	mg/kg							
1,2-Dihydrocafestol (Kahweol)	8	mg/kg							
1,2-Dihydrocafestol (Kahweol)	9	mg/kg							

Analyte	Participant	Unit	Date of analysis	Result sample A	Result sample B	Result sample C	Limit of determination	incl. RR	Recovery rate
			day/month					yes / no	in %
Cafestol	1	mg/kg	17.09.20	5470	4906	5245	1200		100
Cafestol	2	mg/kg							
Cafestol	3	mg/kg	15.09.20	4010	3730	3990	2000	no	
Cafestol	4	mg/kg							
Cafestol	5	mg/kg							
Cafestol	6	mg/kg							
Cafestol	7	mg/kg							
Cafestol	8	mg/kg							
Cafestol	9	mg/kg							

5.1.2 Analytical methods

Analyte	Participant	Method description	Sample preparation	Measuring method	Calibration/ reference material	Recovery with same matrix	Method accredited	Further remarks
						yes / no	yes / no	
16-O-Methylcafestol	1	NMR, internal method	Extraction with CDCl3 after grinding	Analysis and Quantification via 1H-NMR	calibration by external standard	no correction via recovery rate	yes	
16-O-Methylcafestol	2	ASU L 46.02-4 (2012-01), modified	Extract filtration prior to analysis		DLA 39-2017 sample B & DLA 41-2018 sample B	yes	yes	
16-O-Methylcafestol	3	NMR					yes	
16-O-Methylcafestol	4	§64 L 46.02-04				no	yes	
16-O-Methylcafestol	5	DIN 10779(2011-03)			DLA 39/2017	yes	yes	
16-O-Methylcafestol	6	BVL L 46.02-4	extraction and alkaline hydrolysis	HPLC DAD			yes	
16-O-Methylcafestol	7	based on literature, in-house optimization	Homogenization and grinding, extraction with Chloroform-d	1H-NMR	Calibration via reference sample by the instrument manufacturer, analysis of a standard with known concentration	yes	yes	
16-O-Methylcafestol	8	ASU L 46.02-4				yes	yes	
16-O-Methylcafestol	9	In-house method	none	none	no	no	no	none

Analyte	Participant	Method description	Sample preparation	Measuring method	Calibration/ reference material	Recovery with same matrix	Method accredited	Further remarks
						yes / no	yes / no	
1,2-Dihydrocafestol (Kahweol)	1	NMR, internal method	Extraction with CDCl3 after grinding	Analysis and Quantification via 1H-NMR	calibration by external standard	no correction via recovery rate	yes	
1,2-Dihydrocafestol (Kahweol)	2							
1,2-Dihydrocafestol (Kahweol)	3	NMR					yes	
1,2-Dihydrocafestol (Kahweol)	4							
1,2-Dihydrocafestol (Kahweol)	5							
1,2-Dihydrocafestol (Kahweol)	6							not determined
1,2-Dihydrocafestol (Kahweol)	7							
1,2-Dihydrocafestol (Kahweol)	8							
1,2-Dihydrocafestol (Kahweol)	9							

Parameter	Participant	Method description	Sample preparation	Measuring method	Calibration/ reference material	Recovery with same matrix	Method accredited	Further remarks
						yes / no	yes / no	
Cafestol	1	NMR, internal method	Extraction with CDCl3 after grinding	Analysis and Quantification via 1H-NMR	calibration by external standard	no correction via recovery rate	yes	
Cafestol	2							
Cafestol	3	NMR					yes	
Cafestol	4							
Cafestol	5							
Cafestol	6							not determined
Cafestol	7							
Cafestol	8							
Cafestol	9							

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptAU06 Sample B

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	47,8	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,98	106	42,6
2	5,00	107	42,8
3	4,98	90	36,1
4	4,99	104	41,7
5	4,99	91	36,5
6	5,02	108	43,0
7	5,04	106	42,1
8	4,98	100	40,2

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	101,5	Particles
Standard deviation	7,00	Particles
χ^2 (CHI-Quadrat)	3,38	
Probability	**85**	%
Recovery Rate	85	%

Normal distribution		
Number of samples	8	
Mean	40,6	mg/kg
Standard deviation	2,80	mg/kg
rel. Stadard deviation	6,9	%
Horwitz Standard deviation	9,2	%
HorRat-value	**0,75**	
Recovery Rate	85	%

Microtracer Homogeneity Test
DLA ptAU06 Sample C

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	50,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,98	111	44,6
2	5,05	111	44,0
3	5,04	114	45,2
4	5,00	116	46,4
5	5,03	128	50,9
6	5,00	134	53,6
7	5,03	134	53,3
8	4,99	125	50,1

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	121,6	Particles
Standard deviation	9,86	Particles
χ^2 (CHI-Quadrat)	5,59	
Probability	**59**	%
Recovery Rate	97	%

Normal distribution		
Number of samples	8	
Mean	48,5	mg/kg
Standard deviation	3,93	mg/kg
rel. Stadard deviation	8,1	%
Horwitz Standard deviation	8,9	%
HorRat-value	**0,91**	
Recovery Rate	97	%

5.3 Informationen on the Proficiency Test (PT)

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

PT number	***DLA ptAU06 (2020)***
PT name	***Methylcafestol, Kahweol and Cafestol in 3 Coffee Blends***
*Sample matrix**	*Samples A, B + C: ground roasted coffee blends with different ratios of arabica and robusta contents*
Number of samples and sample amount	*3 different samples A, B + C, 20 g each.*
Storage	*Samples A, B + C: room temperature (PT period), cooled 2 - 10°C (long term)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*quantitative: 16-O-Methylcafestol (Methylcafestol), 1,2-Dihydrocafestol (Kahweol) and Cafestol*
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	*One result each should be determined for Samples A and B and the Spiking Level Sample. The results should be filled in the result submission file. The recovery rates, if determined, have 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***
Last Deadline	***the latest November 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
		Deutschland/Germany
		Deutschland/Germany
		FRANKREICH/FRANCE
		Deutschland/Germany
		Deutschland/Germany
		Deutschland/Germany
		Deutschland/Germany
		Deutschland/Germany
		Deutschland/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 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
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 funktion 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)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 – 196 (2006)
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