Proficiency Tests
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Evaluation Report
proficiency test

DLA 29/2018

Estragole, Methyleugenol and Thujone in Tea Beverage Powder

Dienstleistung Lebensmittel Analytik GbR
Waldemar-Bonsels-Weg 170
22926 Ahrensburg, Germany

proficiency-testing@dla-lvu.de
www.dla-lvu.de

Coordinator: Dr. G. Wichmann

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

EP-Anbieter *PT-Provider*	**DLA - Dienstleistung Lebensmittel Analytik GbR** Gesellschafter: Dr. Gerhard Wichmann und Dr. Matthias Besler-Scharf Waldemar-Bonsels-Weg 170, 22926 Ahrensburg, Germany Tel. ++49(0)171-1954375 Fax. ++49(0)4102-9944976 eMail. proficiency-testing@dla-lvu.de
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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.

Inhalt / Content

1. Introduction

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

2. Realisation

2.1 Test material

The test material is a commercially available tea beverage powder of the components fennel (whole seeds), mugwort tea (cut) and bay leaves (cut) from European suppliers.

The raw materials were admixed and homogenized, a total of 1000 g. The samples were then filled into portions of approx. 30 g in vacuum bags and chronologically numbered.

In preliminary tests, our subcontracting laboratory determined the following contents of essential oils and ingredients (according to Ph.Eur. 9.0, 2.2.28 GC/FID):

Total essential oil content: 2,69 ml/100g

Estragole:	2,10 % in oil
Methyleugenol:	0,19 % in oil
Thujone:	< 0,05 % in oil

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 10-fold by **Tracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].

Before mixing, fennel seeds 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 and based on the normal distribution using the HorRat value. For the evaluation according to Poisson: A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15]. For the evaluation according to the normal distribution: According to [16, 17], the HorRat values between 0,3 and 1,3 are to be accepted under repeatability conditions (measurements within the laboratory).

The tracer analysis of the present PT sample showed a probability of 98%. 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 a HorRat value of 1,2. The results of tracer analysis are given in the documentation.

The calculation of the **variation coefficient** of the repeatability standard deviation (CV_r) was used as an indicator of homogeneity too. It is 9,2% for Estragole and 7,3% for Methyleugenol. The coefficient of variation CV_r is thus comparable to the precision data of the official method, see 3.6.2. The repeatability standard deviation of the participants is given at the characteristics (4.1 to 4.2).

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 < 0,6 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. 0,58 (22,6°C). The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Test

Two portions of test material were sent to every participating laboratory in the 41th week of 2018. The testing method was optional. The tests should be finished at November 23th 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).

The finally calculated concentrations as average of duplicate determinations of both numbered samples was 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 method, information on the limit of quantification, the date of the analysis and general points to the 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.

11 participants submitted at least one result.

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), 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 Robust Standard deviation

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

3.3 Repeatability standard deviation

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

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

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

3.4 Reproducibility standard deviation

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

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

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

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

3.5 Exclusion of results and outliers

Before statistical evaluation obvious blunders, such as those with incorrect units, decimal point errors, 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 (al-

gorithm 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.

3.6 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. The specified target standard deviation "for information" was calculated according to the Horwitz general model (see 3.6.1).

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 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 state deviations (RSD_R) given in Table 2 were determined in interlaboratory comparisons using the methods given.
The resulting target standard deviations identified in these tests (σ_{pt}) were used to evaluate the results or were additionally indicated in the key figures for information purposes.

Table 2: Relative repeatability standard deviation (RSD_r) and relative reproducibility state deviation (RSD_R) in fennel fruit tea according to precision tests and the resulting target standard deviation σ_{pt} [19].

Parameter	Matrix	Mean (mg/l)	RSD_r (%)	RSD_R (%)	σ_{pt} (%)	Method / Literature
Estragole	Fennel fruit tea	0,34	9,71	21,5	20,3[1]	19/ GC-MS-method

[1] *Values used in the evaluation (see section 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).

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 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].

3.8 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 (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.

3.9 Reproducibility coefficient of variation (CV)

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

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].

3.11 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.

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^*)
Number with 2 replicates
repeatability standard deviation (S_r)
Repeatability (Cv_r) in %
reproducibility standard deviation (S_R)
Reproducibility (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 $U(X_{pt})$
Quotient $U(X_{pt})/\sigma_{pt}$ or $U(X_{pt})/\sigma_{pt}$'
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	**Parameter [Einheit/ Unit]**	**Abweichung**	**Z´-Score** σ_{pt}´	**z-Score** (Info)	**Hinweis**
Evaluation number		**Deviation**			**Remark**

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

4.1 Estragole, Methyleugenol and Thujone in total oil

Since less than 5 results were available for the parameters estragole, methyleugenol and thujone, statistical evaluations and evaluations could not be carried out.

4.1.1 Estragole (% in oil)

Auswerte-nummer Evaluation number	Estragole [%in oil]	Abweichung [%in Öl] Deviation [%in Öl]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1					
2					
3					
4					
5	410*				* Indication in mg/kg
6	2,040				
7					
8					
9					
10					
11					

4.1.2 Methyleugenol (% in oil)

Auswerte-nummer Evaluation number	Methyleugenol [%in oil]	Abweichung [%in Öl] Deviation [%in Öl]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1					
2					
3					
4					
5	16*				* Indication in mg/kg
6	0,140				
7					
8					
9					
10					
11					

4.1.3 Thujone (% in oil)

Auswerte-nummer Evaluation number	Thujone [%in oil]	Abweichung [%in Öl] Deviation [%in Öl]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1					
2					
3					
4					
5	< 10*				* Indication in mg/kg
6	< 0,01				
7					
8					
9					
10					
11					

4.2 Estragole, Methyleugenol and Thujone in infusion

4.2.1 Estragole (mg/l)

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	9
Number of outliers	0
Mean	0,481
Median (X_{pt})	**0,519**
Robust Mean	0,482
Robust standard deviation (S*)	**0,188**
Number with 2 replicates	9
Repeatability SD (S_r)	0,0445
Repeatability (CV_r)	9,23%
Reproducibility SD (S_R)	0,169
Reproducibility (CV_R)	35,1%
Target range:	
Target standard deviation σ_{pt}	**0,105**
Target standard deviation (for Information)	0,0917
lower limit of target range	**0,309**
upper limit of target range	**0,729**
Quotient S/σ_{pt}*	*1,8*
Standard uncertainty $U_{(Xpt)}$	*0,0784*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,74*
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 47.08-3. The specified target standard deviation "for information" was calculated according to the Horwitz general model (see 3.6.1).

The quotient S^*/σ_{pt} was below 2.0. The results are comparable.

Repeatability- and reproducibility standard deviation are considered low or inconspicuous.

The quotient $U(X_{pt})/\sigma_{pt}$ of 0,74 is above 0,3, but is acceptable due to the other characteristics.

78% of results were in the target range.

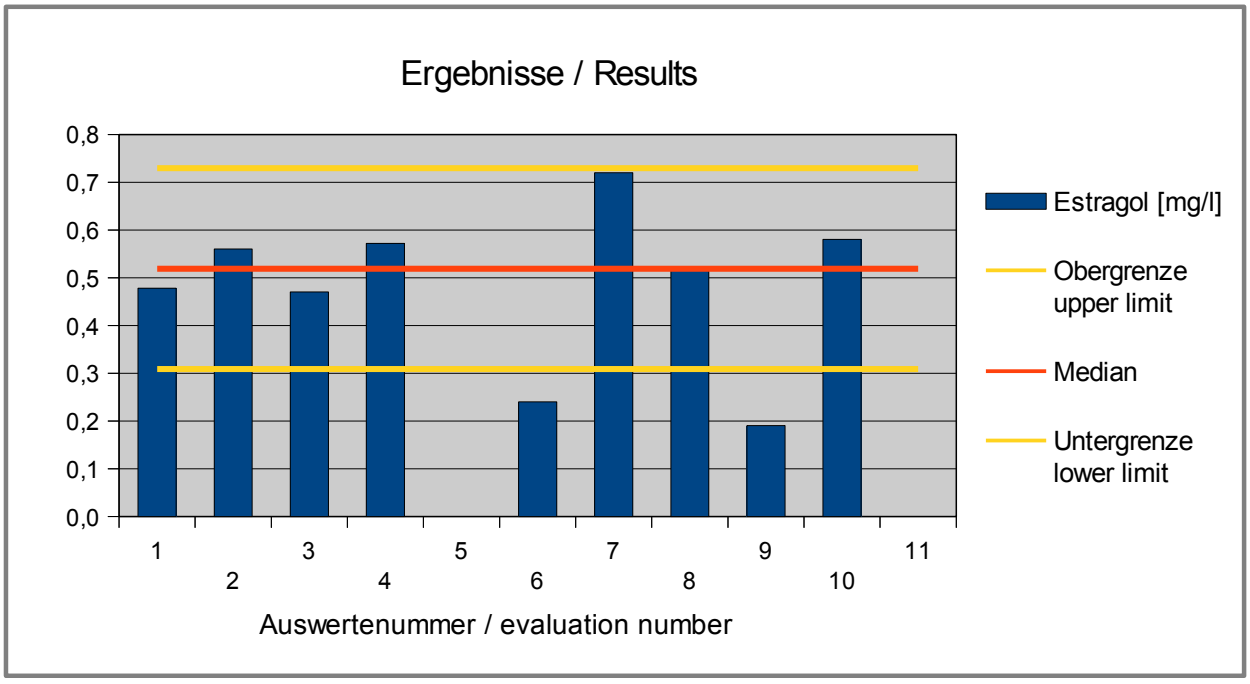

Abb. 1: Ergebnisse Estragol
Fig. 1: Results Estragole

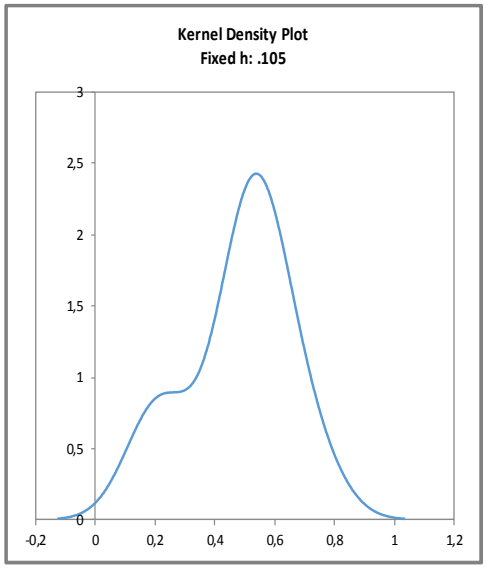

Abb. / Fig. 2:
Kerndichte-Schätzung der Ergebnisse (mit h = σ_{pt}´ von X_{pt})

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. 0,2 mg/l, due to results outside the target range.

Ergebnisse der teilnehmenden Institute:
Results of Participants:

Auswerte-nummer Evaluation number	Estragol [mg/l]	Abweichung [mg/l] Deviation [mg/l]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	0,478	-0,0410	-0,39	-0,45	
2	0,560	0,0410	0,39	0,45	
3	0,470	-0,0490	-0,47	-0,53	
4	0,572	0,0530	0,50	0,58	
5					
6	0,240	-0,279	-2,7	-3,0	
7	0,720	0,201	1,9	2,2	
8	0,519	0,00	0,00	0,40	
9	0,190	-0,329	-3,1	-3,6	
10	0,580	0,0610	0,58	1,1	
11					

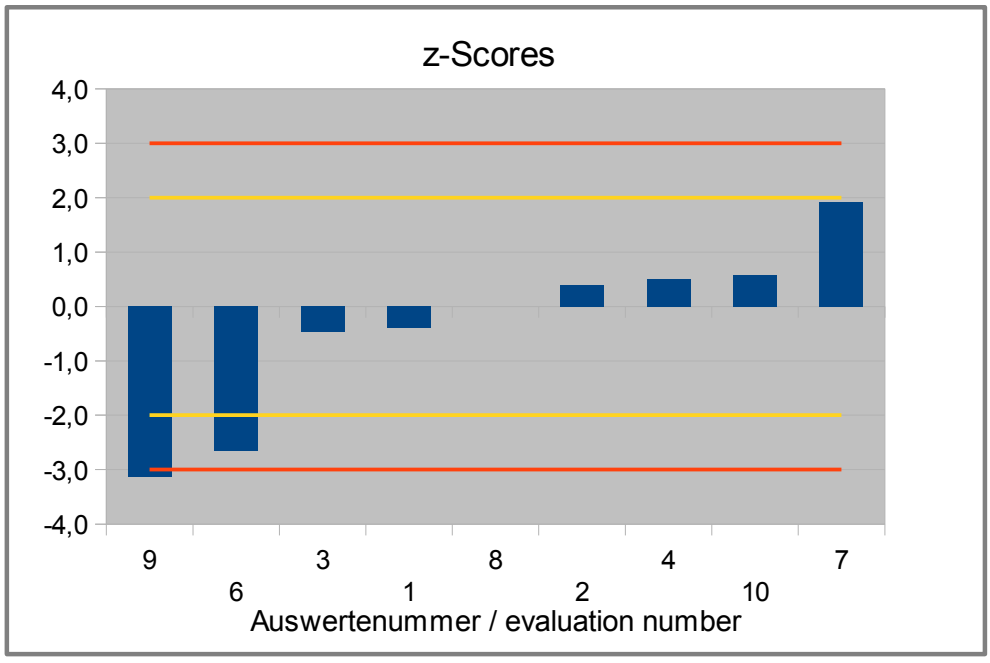

Abb. 3: Z-Scores Estragol
Fig. 3: Z-Scores Estragole

4.2.2 Methyleugenol (mg/l)

Statistic Data	
Number of results	9
Number of outliers	0
Mean	0,184
Median (X_{pt})	**0,200**
Robust Mean	0,189
Robust standard deviation (S*)	**0,067**
Number with 2 replicates	9
Repeatability SD (S_r)	0,0133
Repeatability (CV_r)	7,28%
Reproducibility SD (S_R)	0,0705
Reproducibility (CV_R)	38,6%
Target range:	
Target standard deviation σ_{pt}	**0,0405**
Target standard deviation (for Information)	0,0408
lower limit of target range	**0,119**
upper limit of target range	**0,281**
Quotient S/σ_{pt}*	*1,7*
Standard uncertainty $U_{(Xpt)}$	*0,0279*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,69*
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 47.08-3. The specified target standard deviation "for information" was calculated according to the Horwitz general model (see 3.6.1).

The quotient S*/σ_{pt} was below 2.0. The results are comparable.

Repeatability- and reproducibility standard deviation are considered low or inconspicuous.

The quotient $U(X_{pt})/\sigma_{pt}$ of 0,69 is above 0,3, but is acceptable due to the other characteristics.

78% of results were in the target range.

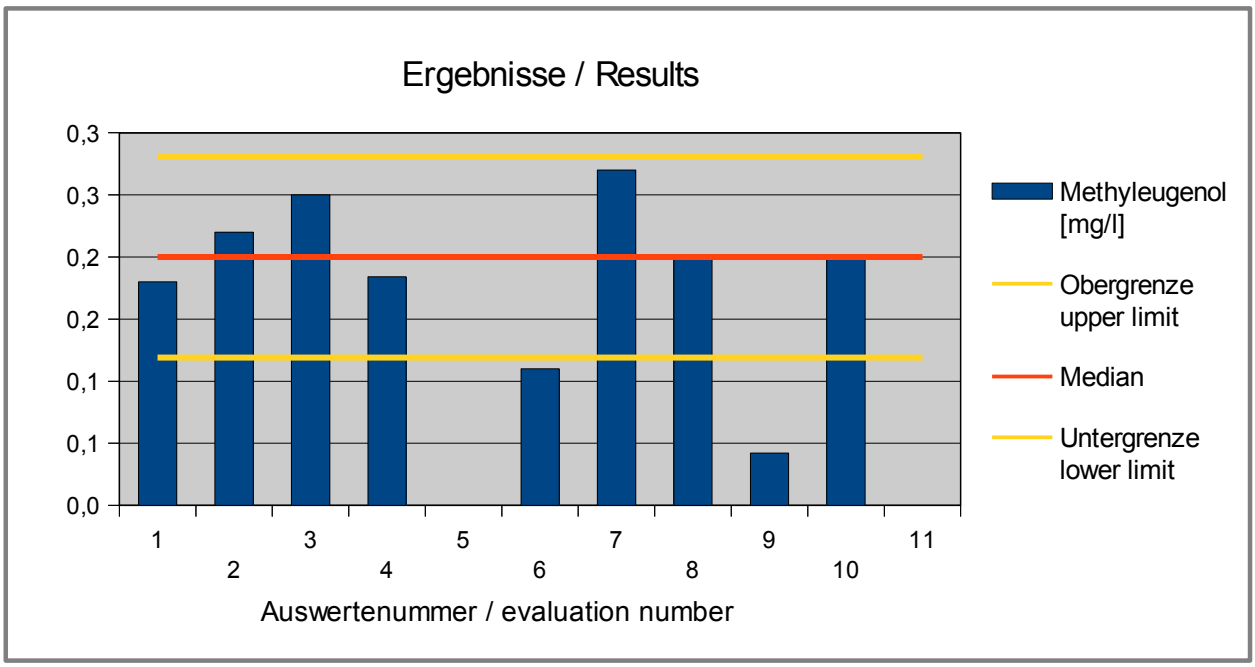

Abb. 4: Ergebnisse / results Methyleugonol

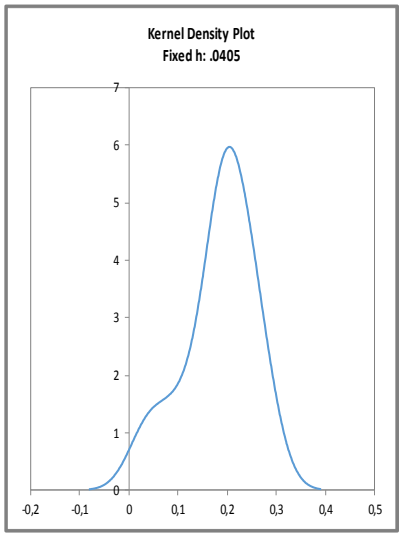

Abb. / Fig. 5:
Kerndichte-Schätzung der Ergebnisse (mit h = σ_{pt}´ von X_{pt})

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. 0,05 mg/l, due to result outside the target range.

Ergebnisse der teilnehmenden Institute:
Results of Participants:

Auswerte-nummer Evaluation number	Methyleugenol [mg/l]	Abweichung [mg/l] Deviation [mg/l]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	0,180	-0,0200	-0,49	-0,49	
2	0,220	0,0200	0,49	0,49	
3	0,250	0,0500	1,2	1,2	
4	0,184	-0,0160	-0,39	-0,39	
5					
6	0,110	-0,0900	-2,2	-2,2	
7	0,270	0,0700	1,7	1,7	
8	0,202	0,00	0,049	0,31	
9	0,042	-0,158	-3,9	-3,9	
10	0,200	0,00	0,00	0,26	
11					

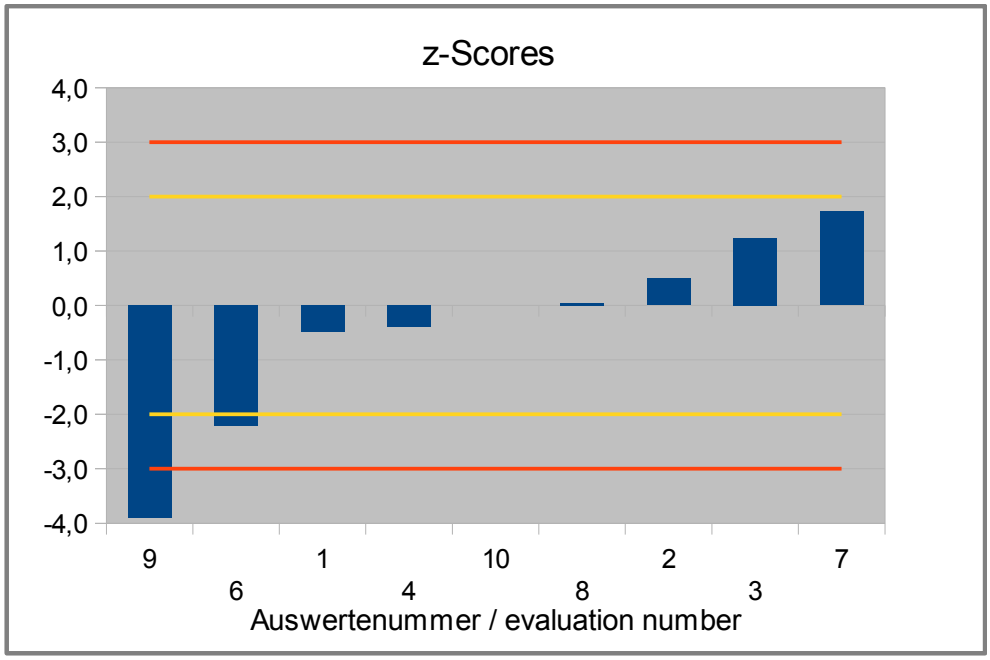

Abb. / Fig. 6: Z-Scores Methyleugenol

4.2.3 Thujone in mg/l

Vergleichsuntersuchung / Proficiency Test

8 results for Thujone were available, 6 (= 75%) of the results were below the respective detection limit or limit of quantification. For this reason a quantitative evaluation cannot be carried out:

Auswerte-nummer Evaluation number	Thujon [mg/l]	Abweichung [mg/l] Deviation [mg/l]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	<LOQ				LOQ < 2 mg/l
2	undetectable				LOQ < 0,2 mg/l
3	<0,05				
4	0,478				
5					
6	<0.01				
7					
8	< LOQ				LOQ < 0,05 mg/l
9	n.d.				LOQ < 0,01 mg/l
10	24,1				
11	<0,05				

Comment:
In 6 of 8 results (= 75%) the Thujone content was below the detection limit. This is in accordance with the preliminary examination by our reference laboratory, which was also unable to detect any Thujone in the essential oil of the tea mixture (<0,05 % Thujone in the oil).

5. Documentation

5.1 Details by the participants

5.1.1 Primary data

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

5.1.1.1 Estragole, Methyleugenol and Thujone in oil

Parameter	Teilnehmer Participant	Einheit Unit	Ergebnis Result	Ergebnis Probe I Result sample I	Ergebnis Probe II Result sample II	Bestim-mungs-grenze Limit of quantification	Incl. WF Incl. RR	Wieder-findungs-rate Recovery
							yes/no	in %
Estragole	5	mg/kg	410	420	390		no	108
Estragole	6	% in oil	2,04	2,06	2,02	0,01	no	n.a.
Methyl-eugenol	5	mg/kg	16	16	15		no	94
Methyl-eugenol	6	% in oil	0,14	0,14	0,14	0,01	no	n.a.
Thujone	5	mg/kg	<10	<10	<10		no	85
Thujone	6	% in oil	<0,01	<0,01	<0,01	0,01	no	n.a.

5.1.1.2.1 Estragole in infusion

Teil-nehmer	Probe Nr. I.	Probe Nr. II	Datum der Analyse	Ergebnis (Mittel)	Ergebnis Probe I	Ergebnis Probe II	Bestimmungs grenze	Incl. WF	Wiederfin-dungsrate
Partici-pant	Sample no.I	Sample no. II	Date of analysis	Result (mean)	Result sample I	Result sample II	Limit of quantification	Incl. RR	Recovery
			day month	mg/l	mg/l	mg/l	mg/l	yes/no	in %
1	3	29	21.11.2018	0,478	0,501	0,455	0,01	no	
2	8	24	22.11.18	0,56	0,53	0,58	0,2	no	100
3	4	28	23.11.18	0,47	0,42	0,53	0,05	yes	
4	11	21	21.11.18	0,572	0,579	0,565	0,02	no	95
5	10	22							
6	19	13	05.11.	0,24	0,27	0,22	0,01	no	n.a.
7	7	25	Sample I: 08.11.18, Sample II: 12.11.18	0,72	0,71	0,74	0,04	yes	93
8	6	26	17.10.18	0,519	0,547	0,491	0,05	no	65
9	12	20	25.10.	0,19	0,14	0,25	0,01	no	98
10	1	31	29.10.18	0,58	0,58	0,57	0,05 mg/l	no	
11	23	9							

5.1.1.2.2 Methyleugenol in infusion

Teilnehmer	Probe Nr. I.	Probe Nr. II	Datum der Analyse	Ergebnis (Mittel)	Ergebnis Probe I	Ergebnis Probe II	Bestimmungsgrenze	Incl. WF	Wiederfindungsrate
Partic-pant	Sample no.I	Sample no. II	Date of analysis	Result (mean)	Result sample I	Result sample II	Limit of quantification	Incl. RR	Recovery
			day/ month	mg/l	mg/l	mg/l	mg/l	yes/no	in %
1	3	29	21.11.2018	0,18	0,192	0,167	0,01	no	
2	8	24	22.11.18	0,22	0,22	0,22		no	96,3
3	4	28	23.11.18	0,25	0,25	0,25	0,05	yes	
4	11	21	21.11.18	0,184	0,179	0,188	0,02	no	90
5	10	22							
6	19	13	05.11.	0,11	0,12	0,09	0,01	no	n.a.
7	7	25	Sample I: 08.11.18, Sample II: 12.11.18	0,27	0,28	0,25	0,04	yes	102
8	6	26	17.10.18	0,202	0,208	0,195	0,05	no	91
9	12	20	25.10.	0,042	0,032	0,052	0,01	no	120
10	1	31	29.10.18	0,2	0,2	0,19			
11	23	9							

5.1.1.2.3 Thujone in infusion

Teilnehmer	Probe Nr. I.	Probe Nr. II	Datum der Analyse	Ergebnis (Mittel)	Ergebnis Probe I	Ergebnis Probe II	Bestimmungsgrenze	Incl. WF	Wiederfindungsrate
Participant	Sample no.I	Sample no. II	Date of analysis	Result (mean)	Result sample I	Result sample II	Limit of quantification	Incl. RR	Recovery
			day/month	mg/l	mg/l	mg/l	mg/l	yes/no	in %
1	3	29	21.11.2018	<LOQ	<LOQ	<LOQ	2	no	
2	8	24	22.11.18	n.d.	0	0	0,2	no	103,5
3	4	28	23.11.18	<0,05	<0,05	<0,05	0,05	yes	
4	11	21	21.11.18	0,478	0,459	0,496	0,02	no	86
5	10	22							
6	19	13	05.11.	<0,01	<0,01	<0,01	0,01	no	
7	7	25							
8	6	26	17.10.18	<LOQ	<LOQ	<LOQ	0,05	no	87
9	12	20	25.10.	n.d.	n.d.	n.d.	0,01	no	93
10	1	31		24,1	24,3	24			
11	23	9	16.11.18	<0,05	<0,05	<0,05	<0,05	no	96,9

5.1.1.2.3 Further in infusion: Eugenol

Teilnehmer	Probe Nr. I.	Probe Nr. II	Datum der Analyse	Ergebnis (Mittel)	Ergebnis Probe I	Ergebnis Probe II	Bestimmungsgrenze	Incl. WF	Wiederfindungsrate
Participant	Sample no.I	Sample no. II	Date of analysis	Result (mean)	Result sample I	Result sample II	Limit of quantification	Incl. RR	Recovery
			day/month	mg/l	mg/l	mg/l	mg/l	yes/no	in %
1	3	29							
2	8	24							
3	4	28							
4	11	21							
5	10	22							
6	19	13							
7	7	25							
8	6	26	17.10.18	0,547	0,535	0,558	0,05	no	112
9	12	20							
10	1	31							
11	23	9							

5.1.2 Analytical methods

5.1.2.1 Estragole, Methyleugenol and Thujone in oil

Teilnehmer	Methodenbeschrei-bung	Probenvorbereitung	Messmethode	Kalibrierung und Referenzmaterial	Wiederfindung mit gleicher Matrix	Methode ak-kreditiert	Sonstige Hinweise
Participant	Method description	Sample preparation	Measuring method	Calibration and reference matreial	Recovery with same matrix	Method ac-credited	Further remarks
					yes/no	yes/no	
1							
2							
3							
4							
5	LA-GC-604.05		GC-MS		yes	no	
6	Oil contents (GC-FID), acc. to the regulations, Ph. Eur Monographie Essential Oil (01/2008:2098)	Distillation with 15g sample material and inclusion in Xylene	GC-FID	n.a.	no	yes	n.a.
7							
8							
9							
10							
11							

5.1.2.2.1 Estragole in infusion

Teilnehmer	Methodenbeschrei-bung	Probenvorbereitung	Messmethode	Kalibrierung und Referenzmaterial	Wiederfindung mit gleicher Matrix	Methode ak-kreditiert	Sonstige Hinweise
Participant	Method description	Sample preparation	Measuring method	Calibration and reference matreial	Recovery with same matrix	Method ac-credited	Further remarks
					yes/no	yes/no	
1	Determination of Safrol, Methyleugenol and Estragole in food with GC/MS	Extract of NON-homogenized sample according to BVL L 47.08-3, liquid/liquid extraction.	GC/FID, Stabilwax-Da 50m x 0.25mm x 0.2µm	External calibration with intern standard methode	no	yes	
2	§ 64 L 47.08 -3 mod.	Infusion like standard, shaking with Kaltron	GC-MS mod. stand. Method.	Estragol OEKANAL, Sigma Aldrich, Article:34098, Lot.:SZBF335XV	no	yes	
3	ASU L47.08-3				yes	no	
4	ASU § 64 LFGB L00.00-145, mod.	Sample preparation after ASU § 64 LFGB L47.08-3	GC-MS	Standard doped synthetic juice	no (Synthetic juice)	yes	
5							
6	Estragol in tea infusion (GC-MS), ASU L 47.08-03	2*6.5g sample weight in 0.5 L water	GC-MS	Multi-point calibration	no	yes	n.a.
7	ASU L 47.08-3, 09-2006		GC-MS	Calibration with internal standard, Reference material certified	yes	yes	
8	ASU L 47.08-3	-	-	Solvent calibration	yes	yes	Experience has shown that recovery in the recovery matrix is poor
9	ASU L47.08-3: 2006-09	Grinding after <-18°C overnight	GC-MSD	5-point (selected from 9)	no	yes	Different appearence and smell of the samples when opening
10	ASU 47.08-3	12,5 g/l weighting quantity not further comminuted		Calibration 0 - 6,4 mg/l		yes	
11							

5.1.2.2.2 Methyleugenol in infusion

Teilnehmer	Methodenbeschrei-bung	Probenvorbereitung	Messmethode	Kalibrierung und Referenzmaterial	Wiederfindung mit gleicher Matrix	Methode ak-kreditiert	Sonstige Hinweise
Participant	Method description	Sample preparation	Measuring method	Calibration and reference matreial	Recovery with same matrix	Method ac-credited	Further remarks
					yes/no	yes/no	
1	Determination of Safrol, Methyleugenol and Estragol in food with GC/MS	Extract of NON-homogenized sample according to BVL L 47.08-3, liquid/liquid extraction.	GC/MS in Sim Mode with 30m x 0.25mm x 0.25µm HP-5MS column	External calibration with internal standard method.	no	yes	
2	§ 64 L 47.08 -3 mod.	Infusion like standard, shaking with Kaltron	GC-MS mod. Standard method.	Met4-Allyl-1,2-dimethoxybenzene, Sima Aldrich, Article: 284424, Lot.:MKBW1963V	no	yes	
3	ASU L47.08-3				yes	no	
4	ASU § 64 LFGB L00.00-145, mod.	Sample preparation according ASU § 64 LFGB L47.08-3	GC-MS	Standard doped synthetic juice	no (synthetic juice)	yes	
5							
6	Estragol in tea infusion (GC-MS), ASU L 47.08-03	2*6.5g sample weight in 0.5 L water	GC-MS	Muilt-point calibration	no	yes	n.a.
7	ASU L 47.08-3, 09-2006		GC-MS	Calibration with internal standard, Reference material certified	yes	yes	
8	ASU L 47.08-3	-	-	solvent calibratin	yes	yes	
9	ASU L47.08-3: 2006-09	Grinding after <-18°C overnight	GC-MSD	5-point (selected from 9)	no	yes	Different appearence and smell of the samples when opening
10				Calibration 0 - 6,4 mg/l			
11							

5.1.2.2.3 Thujone in infusion

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 matreial	Recovery with same matrix	Method accredited	Further remarks
					yes/no	yes/no	
1	Determination of the content of eucalyptol, thujone, menthone, camphor, menthol and methyl salicylate in spirits, cosmetics, liquids and tobacco products by GC/FID	Extract of NON-homogenized sample according to BVL L 47.08-3, liquid/liquid extraction	GC/FID, Stabilwax-Da 50m x 0.25mm x 0.2µm	External calibration with internal standard method	no	yes	
2	§ 64 L 47.08 -3 mod.	Infusion like standard, shaking with Kaltron	GC-MS modified standard procedure	Thujone pure, Roth, art.-Nr. 9540.1, Charge 277229062	no	yes	
3	ASU L47.08-3				yes	no	
4	ASU § 64 LFGB L00.00-145, mod.	Sample preparation according ASU § 64 LFGB L47.08-3	GC-MS	Standard doped synthethic juice	no (synthetic juice)	yes	
5							
6	Estragole in tea infusion (GC-MS), ASU L 47.08-03	2*6.5g sample weight in 0.5 L water	GC-MS	Multi-point calibration	no	yes	n.a.
7							
8	ASU L 47.08-3	-	-	Solvent calibration	yes	yes	
9	ASU L47.08-3: 2006-09	Grinding after <-18°C overnight	GC-MSD	-	no	yes	Different appearence and smell of the samples when opening
10				Calibration 0 - 70 mg/l		no	
11	liquid SPME & GC-MS	as prescribed by DLA		sigma aldrich	yes	no	

5.1.2.2.4 Eugenol in infusion

Teilnehmer	Methodenbeschrei-bung	Probenvorbereitung	Messmethode	Kalibrierung und Referenzmaterial	Wiederfindung mit gleicher Matrix	Methode ak-kreditiert	Sonstige Hinweise
Participant	Method description	Sample preparation	Measuring method	Calibration and reference matreial	Recovery with same matrix	Method ac-credited	Further remarks
					yes/no	yes/no	
8	ASU L 47.08-3			solvent calibration	yes	yes	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

DLA 29-2018

Weight whole sample 1,00 kg
Tracer fennel seed

Result of analysis

sample	Weight [g]	Particle number	Particle [mg/kg]
1	2,01	93	3483
2	2,04	104	3838
3	1,98	90	3422
4	1,82	94	3888
5	2,09	97	3494
6	1,89	90	3585
7	2,05	92	3379
8	1,95	102	3938
9	2,02	98	3652
10	1,90	92	3645

Poisson distribution		
Number of samples	10	
Degree of freedom	9	
Mean	95,3	Particle
Standard deviation	5,20	Particle
χ^2 (CHI-Quadrat)	2,56	
Prohability	**98**	%
Recovery rate	91	%

Normal distribution		
Mnumber of samples	10	
Mean	3632	mg/kg
Standard deviation	198	mg/kg
rel. Standard deviation	5,5	%
Horwitz Standard deviation	4,7	%
HorRat value	**1,2**	
Recovery rate	91	%

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 29-2018***
PT name	***Estragole, Methyleugenol and Thujone in Tea Beverage Powder***
*Sample matrix**	***Samples I + II: Tea Beverage Powder (Fennel seeds, bay leaves, mugwort)***
Number of samples and sample amount	*2 identical samples I + II, 30 g each.*
Storage	*Samples I + II: cooled 2 - 10°C*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*quantitative:* ***Estragole, Methyleugenol and Thujone***
Methods of analysis	*The content of estragole, methyleugenol and thujone is to be determined in the essential oil (%), additionally the content of estragole, methyleugenol and thujone in the tea infusion (for example according to ASU method, in mg / l) can be indicated.*
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	*% in the oil and additionally in mg/l*
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 23rd November 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/ Town	Land/ Country
		Austria
		Hungary
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		Ireland

[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 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)
12. AMC Kernel Density - Representing data distributions with kernel density estimates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
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 L 53.00-10 Bestimmung des ätherischen Ölgehaltes in Gewürzen, würzenden Zutaten und Kräutern, Wasserdampfdestillationsverfahren (September 2010) (DIN EN ISO 6571/ November 2008)
19. ASU § 64 LFGB L 47.08-3 Bestimmung von Estragol in Aufgüssen aus Fenchel und anderen teeähnlichen Erzeugnissen, GC-MS-Verfahren, September 2006
20. ASU § 64 LFGB L 47.08-2 Bestimmung von Estragol im Teeaufguss mittels GC-MS (Dezember 2004
21. Leitsätze für Gewürze und andere würzende Zutaten, vom 27 5. 1998 (BAnz. Nr. 183a vom 30. 9. 1998, GMBl. Nr. 30 S. 577 vom 30. 9. 1998)
22. Handbuch Aromen und Gewürze, Behr´s Verlag, www.behrs.de