Proficiency Tests

DLA

food
cosmetics
consumer goods
www.dla-lvu.de

Evaluation Report
proficiency test

DLA 41/2017

Nicotine in E-Cigarette-Liquid

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

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

Coordinator: Dr. Gerhard 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
EP-Nummer *PT-Number*	DLA 41/2017
EP-Koordinator *PT-Coordinator*	Dr. Gerhard Wichmann
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report : 13 February 2018
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) - *gezeichnet / signed M. Besler-Scharf* Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager) - *gezeichnet / signed G. Wichmann* Datum / Date: 13 February 2018
Unteraufträge *Subcontractors*	Die Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern wird von DLA im Unterauftrag vergeben. The analysis of the content, homogeneity and stability of PT-parameters are subcontracted by DLA.
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

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 mixture of a liquid-based solution, an aroma ("cream lemon") and nicotine. Approx. 0,5 kg of the material were homogenized. A homogeneous, clear liquid has been obtained.
The sample was filled into a 1000 ml glass container.
Subsequently, the samples were filled into glass screw tubes in portions of approx. 10 g and numbered chronologically.

The composition of the PT samples is shown in Table 1.

Table 1: Composition of the DLA-sample

Ingredients	**Content**
Liquid based solution (50% Glycerol/ 50% Propylene glycol)	94,0 g / 100 g
Aroma „cream lemon“ (Ingredients: propylene glycol, natural and artificial flavouring agents)	5,00 g / 100 g
Nicotine	1,00 g / 100 g

Note: *The metrological traceability of temperature, mass and volume during production of the PT samples is ensured by DAkkS calibrated reference materials.*

2.1.1 Homogeneity

The PT sample used is an apparently clear, homogeneous, light liquid solution. No analytical examination of the homogeneity prior to filling the samples was carried out.

The calculation of the **variation coefficient** (CV) of the repeatability standard deviation (CV_r) was used as an indicator of homogeneity. It is 0,85% for this PT. The repeatability standard deviation shall be considered to be very low, see also Table 2.

Furthermore, the homogeneity was characterized by the **trend line function of participants' results for chronological bottled single samples**. The maximum deviations for nicotine from the mean value of the trend line was in the range of 0,8% of the target standard deviation σ_{pt} (s. 5.2 homogeneity) and is to be judged as low.

If the criteria for sufficient homogeneity of the test material are not fulfilled on a particular parameter, the impact on the target standard deviation is checked and optionally the evaluation of the results of the participants will be done using the z´-score considering the standard uncertainty of the assigned value (see 3.8 and 3.11) [3].

2.1.2 Stability

The samples are a sufficiently preserved, commercially available e-cigarette smoke solution so that storage stability is ensured with regard to the shelf life of the sample (microbial spoilage) and nicotine content during the test period.

2.2 Sample shipment and information to the test

Two portions of test material were sent to every participating laboratory in the 48th week of 2017. The testing method was optional. The tests should be finished at January 26th 2018 the latest.

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

In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.

Further information see 5.3.

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.

Out of 11 participants, 10 participants submitted the results in time. 1 participant has not submitted any results.

3. Evaluation

3.1 Consensus values from participants (Assigned value)

The robust mean of the submitted results was used as assigned value (X) („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 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, 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. If a value deviates from the robust mean by more than 3 times the robust standard deviation, it is classified as an outlier [3]. Detected outliers are stated for information only, when z-score are < -2 or > 2. Due to the use of robust statistics outliers are not excluded, provided that no other reasons are present [3].

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 the evaluation the target standard deviation from the general model of Horwitz (s. 3.6.1) was applied. In addition, the standard uncertainty was taken into account and the results were evaluated using z'-score (see 3.6.8). For information, the target standard deviation of a precision experiment was given *(ASU §64 Method: [14]), see 3.6.2.*

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 standard deviation (RSD_R) given in Table 2 were determined in ring tests using the indicated methods.

The resulting target standard deviations σ_{pt}, which were identified there, were used to evaluate the results and to provide additional information for the statistical data.

Table 2: Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) according to selected evaluations of tests for precision and the resulting target standard deviation σ_{pt} [14]

Parameter	**Matrix**	**Mean (g/100)**	**RSD_r (%)**	**RSD_R (%)**	**σ_{pt} (%)**	**Method / Literature**
Nicotine	Tobacco	0,7	20,0	40,0	37,4	GC/14
Nicotine	Tobacco	1,0	25,2	44,8	41,1	GC/14
Nicotine	Tobacco	1,5	22,4	37,3	33,8	GC/14
Nicotine	Tobacco	3,5	11,2	28,8	27,7[1]	GC/14

[1] *Value used for information 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 z-score valid for the PT evaluation is designated z-score (σ_{pt}), while the value of z-score (Info) is for information only. The two z-scores are calculated using the different target standard deviations according to 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].

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 ($\hat{\sigma}$) 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^*/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

The consensus value has a standard uncertainty $U(X_{pt})$ that depends on the analytical method, differences between the analytical methods used, the test material, the number of participant laboratories (P) and perhaps on other factors. The standard uncertainty of the assigned value ($U_{(Xpt)}$) 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 consensus value needs not to be included in the interpretation of the results of the PT [3]. A clear exceeded the value of 0.3 is an indication that the target standard deviation was possibly set too low for the standard uncertainty of the assigned value.

The quotient $U(X_{pt})/\sigma_{pt}$ 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
Robust mean(X_{pt})
Robust standard deviation (S^*)
Coefficient of variation (CV_{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 Nicotine (g/100g)

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	10
Number of outliers	0
Mean	1,01
Median	1,01
Robust Mean (X)	**1,01**
Robust standard deviation (S*)	**0,0866**
Coefficient of variation (CV_{S*})	8,58%
Number with 2 replicates	10
Repeatability SD (S_r)	0,00856
Repeatability (CV_r)	0,846%
Reproducibility SD (S_R)	0,0933
Reproducibility (CV_R)	9,21%
Target range:	
Target standard deviation σ_{pt}´	**0,0529**
Target standard deviation (for Information)	0,279
lower limit of target range	**0,903**
upper limit of target range	**1,11**
Quotient S/σ_{pt}´*	*1,6*
Standard uncertainty $U_{(Xpt)}$	*0,0342*
Quotient $U_{(Xpt)}/\sigma_{pt}$´	*0,65*
Results in the target range	*8*
Percent in the target range	*80,0%*

Comments:

For the valuation the target standard deviation of the general model according to Horwitz was applied. In addition, the standard uncertainty was taken into account and the results were evaluated using z'-score (see 3.6.8). For information, the target standard deviation from a precision experiment (ASU § 64 LFGB T60.00-6) was given, see 3.6.2.

The distribution of the results showed a slightly increased variability. The quotient S*/σ_{pt}´ was well below 2,0. The coefficients of variation CV_r and CV_R are in the range of established values for the analytical methods used (see 3.6.2) and demonstrate an acceptable variability of the results.

The quotient $U_{(Xpt)}/\sigma_{pt}$´ is 0,65 over 0,3, but is acceptable due to the other characteristics and the use of different analytical methods.

80% of the results were in the target area.

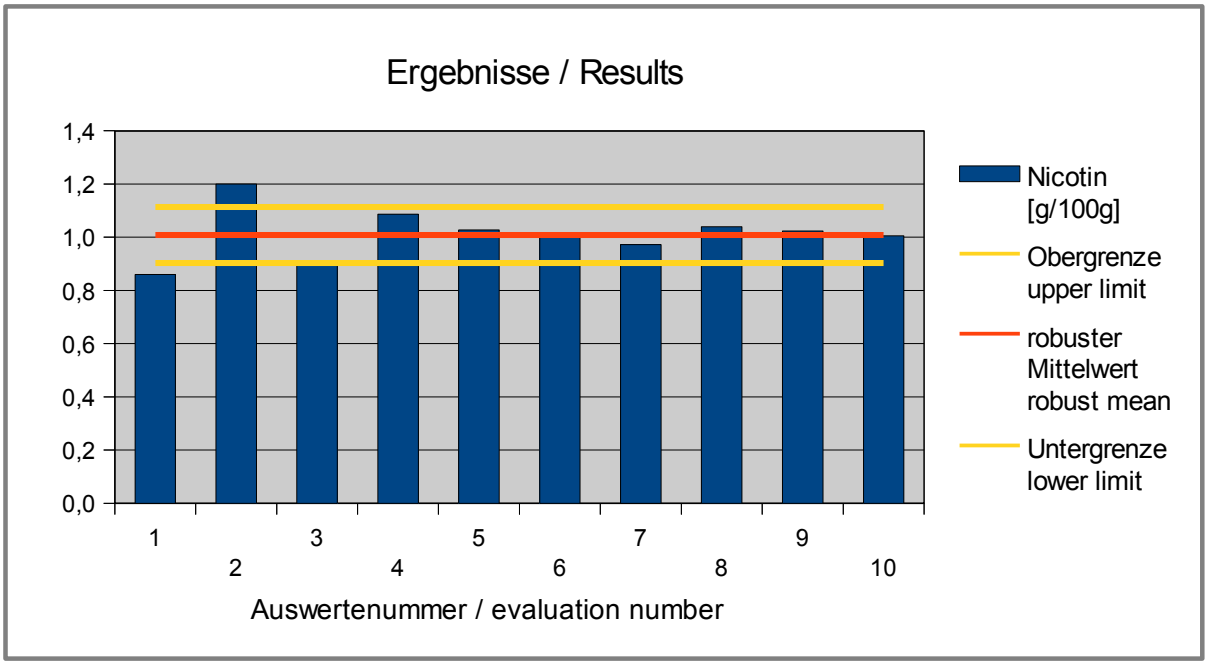

Abb. / Fig. 1: Ergebnisse/ Results Nicotine

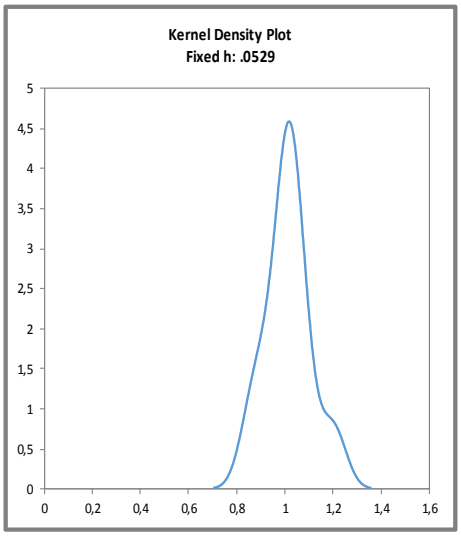

Abb. / Fig. 2:
Kerndichte-Schätzung der Ergebnisse (mit h = σ_{pt}´ von X_{pt})

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

Comment:
The kernel density shows a symmetrical distribution of results with two slight shoulders (at 0,8 g/100g and at 1,2 g/100g) due to the results outside of the target range.

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Nicotine [g/100g]	Abweichung [mg/kg] Deviation [mg/kg]	z´-Score ($\sigma_{pt'}$)	z-Score (Info)	Hinweis Remark
1	0,860	-0,149	-2,8	-0,53	
2	1,20	0,191	3,6	0,68	
3	0,910	-0,0987	-1,9	-0,35	
4	1,09	0,0778	1,5	0,28	
5	1,03	0,0193	0,36	0,069	
6	1,00	-0,00472	-0,089	-0,017	
7	0,972	-0,0367	-0,69	-0,13	
8	1,04	0,0313	0,59	0,11	
9	1,02	0,0145	0,27	0,052	
10	1,01	-0,00272	-0,051	-0,0097	

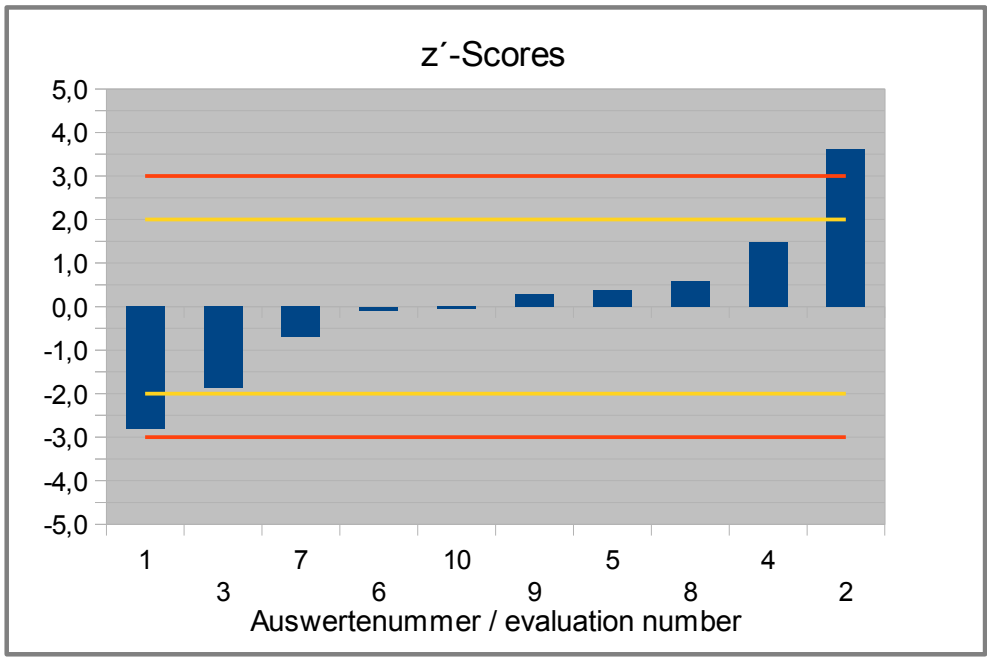

Abb. / Fig. 3: Z´-Scores Nicotine

5. Documentation

5.1 Details by 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).**

Teil-nehmer	Proben-Nr. I	Proben-Nr. II	Datum d. Analyse	Ergebnis (Mittel)	Ergebnis I	Ergebnis II	Bestim-mungs-grenze	Inkl. WF	Wiederfin-dungsrate [%]
Parti-cipant	Sample No. I	Sample No. II	Date of analysis	Result (Mean)	Result I	Result II	Limit of quanti-fication	Incl. RR	Recovery rate [%]
			day/month	g/100g	g/100g	g/100g	g/100g	yes/no	in %
1	2	46	18.01.18	0,86	0,855	0,867	0,0014	no	100
2	3	45	25/01	1,2	1,2	1,2		yes	105
3	11	37	12.01.18	0,91	0,91	0,9	0,14	no	97,17
4	28	20	24.01.18	1,0865	1,0863	1,08665	0,0002	no	98,1
5	14	34	27.12.17	1,028	1,027	1,029	0,02	no	-
6	18	30	04.01.18	1,00	1,01	1,00	0,2	no	98,5
7	19	29	10.01.18	0,972	0,979	0,965	0,08	no	100,61
8	9	39	18.12.2017 03.01.2018	1,04	1,05	1,03	0,0003	yes	105
9	8	40	05.12.17	1,02325	1,026	1,0205	5mg/kg	no	
10	13	35	21.12.17	1,01	0,99	1,02	0,010	no	100

5.1.2 Analytical Methods

Teilnehmer	Methodenbeschreibung	Probenvorbereitung	Messmethode	Kalibrierung und Referenzmaterial	Wiederfindung mit gleicher Matrix	Methode akkreditiert	Sonstige Hinweise
Participant	Method description	Sample preparation	Measuring method	Calibration ans reference Material	Recovery with same matrix	Method accredited	Further remarks
					yes/no	yes/no	
1	According to DGUV information 213-578, proceed 01	high dilution (Factor about 4.000)	GC/MSD in SIM	ext. Nicotine-standards	no	no	Test whether methods for the determination of nicotine in the air are suitable for material samples in principle
2	GC-MS analysis after dilution with organic solvent			Internal Standard		no	
3	B 04.006.02	0,2 g / 20 ml n-Heptan, 30 min. Ultrasonic bath		Nicotine 98,61 % Charge: S7222577615	yes	Range accreditation	
4	IPA added to sample and shaked for 30mins. The IPA is then analysed on the GC-FID based on the linearity plot of Nicotine standards.	IPA extraction for 30mins on orbital shaker, RPM 167	GC-FID using capillary column	Internal standard used in preparation and analysis	yes	Yes (for smoke condensate matrix)	
5	in house method	Weighing in of approx. 300 mg is diluted with 10 ml isopropanol, then measured and evaluated with GC.	GC-FID	Calibration with solubilized nicotine salicylate in isopropanol	no	yes	The result of sample 34 is not composed of 2 measured values because the second value has been deleted.
6	QSA-O-2102-01	Weighing 100 mg + 10 ml isopropanol with internal standard	GC-FID	internal standard: Heptadecan;Reference material Nicotine salicylate	yes	yes	
7	M318150501 in house method	Dilution with Eluent A/diaphragm filtration	HPLC/DAD	Nicotine(Sigma)>99%	yes	yes	LOD for sample dilution 1:100
8	internal procedure GC-MS	Weighing in depending on the expected concentration range (low/high); addition of NaOH solution and extraction solution; shake over a defined period of time; organic phase after filtration rapidly used for measurement.	GC-MS; according to ASU § 35 LMBG T60.00-6 (2012-06)	6 calibration points up to 500 mg/L; nicotine-containing product standard	yes	yes	Sample No. 09 was leaking during unpacking - product leakage. Sufficient material available for analytics; additional determination of density, since marking of nicotine-containing refills for e-cigarettes is carried out in[mg/mL]; density: 1.148 g/ml = 12.45 mg/ml nicotine
9	in house method	extracted with alkaline methanol	LC-MS		no	no	
10	MP 2119 rev 1 - HPLC-UV/Vis			External calibration N3876-5ML (-)-Nicotine sigma	yes	no	

5.2 Homogeneity

5.2.1 Comparison of sample number/test results and trend line

By comparison of the **increasing sample numbers** and the measurement results of chromium, the homogeneity of the chronological bottled PT item can be characterized with the help of the trend line function:

Nicotin				
Target standard deviation σ_{pt}´	0,0529			g/100g
Sample numbers	2 – 46			
Total numbers of samples	20			
Slope	0,0000450			
Trend line range	1,0130	-	1,0121	g/100g
Deviation trend line	1,0126	±	0,000450	g/100g
Percent of σpt´	0,851	%		

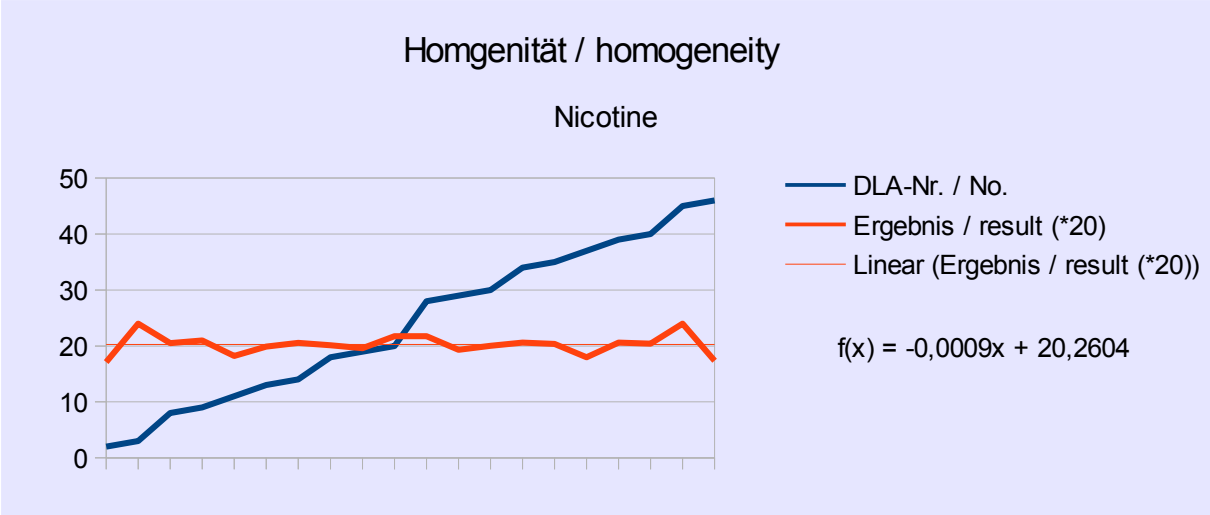

Abb./Fig. 4:
Trendfunktion Probennummern vs. Ergebnisse (*20 dargestellt)
trend line function sample number vs. results (*20 shown)

5.3 Sample cover letter: Information on the Proficiency Test (PT)

Before the PT, the participants are given the following information in the sample cover letter:

Information on the Proficiency Test (PT)

PT number	***DLA 41-2017***
PT name	***Nicotin in E-Cigarette-Liquid***
*Sample matrix**	*Samples I + II:* ***E-Cigarette-Liquid***
Number of samples and sample amount	*2 identical samples A + B, 10 g each.*
Storage	*Samples I + II: cooled 2 - 10°C*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*quantitative:* ***Nicotin***
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.*
Result sheet	*The results for sample I and II as well as the final results calculated as mean of the double determination (samples I and II) should be filled in the result submission file. The recovery rates, if carried out, has to be included in the calculation.*
Units	*g/100g*
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 26th January 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. Testing of the content, homogeneity and stability of PT parameters is subcontracted by DLA.

6. Index of participant laboratories

Teinehmer / Participant	Ort / Town	Land / country

[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. ASU §64 LFGB T 60.00-6, Bestimmung des Nikotingehaltes in Tabak und Tabakerzeugnisse, GC-Verfahren (Juni 2012)