Evaluation Report
proficiency test

DLA 42/2019

Nicotine

in E-Cigarette-Liquid

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

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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. Realisation

2.1 Test material

The test material is a common in commerce liquid-base solution for e-cigarretes with an addition of aroma and nicotine. The materials were mixed and homogenized.

Afterwards the samples were portioned to approximately 10 g into glass vials and chronologically numbered.

Table 1: Composition of the DLA-sample

Ingredients	**Content**
Liquid base solution (50% Glycerin / 50% Propylene glycol)	98,7 g / 100 g
Aroma „cream lemon“ (Ingredients: propylene glycol, natural and artificial flavouring agents)	0,50 g / 100 g
Nicotine	0,83 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 calculation of the **repeatability standard deviation S_r of the participants** was used as an indicator of homogeneity. It is 1,4% for nicotine. Thus it was lower to corresponding repeatability standard deviations of precision data of standardized methods for tobacco (e.g. ASU-Method §64 LFGB T 60.00-6, s. 3.6.2) (see Table 2) [18].
The repeatability standard deviation of the participants' results is given in the documentation in the statistic data (see 4.1).

Furthermore, the homogeneity was graphically characterized for information by the **trend line function of participants' results for chronological bottled single samples** (s. 5.2.1 Homogeneity).

In case the criterion for sufficient homogeneity of the test items is not fulfilled the impact on the target standard deviation will be verified. If necessary the evaluation of results will be done considering the standard uncertainty of the assigned value by z'-scores (s. 3.8 and 3.11) [3].

2.1.2 Stability

Experience has shown that commercially available e-cigarette liquids are stable for several years. For the product, the manufacturer gave a shelf life of 24 months. The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

Two portions of test material were sent to every participating laboratory in the 47th week of 2019. The testing method was optional. The tests should be finished at 10th January 2020 the latest.

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

The two portions contain identical samples of a liquid for E-cigarettes with added parameter nicotine to be determined. The methods of analysis are optional.

Note: please store the samples at 2-10 °C on arrival

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

2.3 Submission of results

The participants submitted their results in standard forms, which have been handed out with the samples (by email).

The finally calculated concentrations of the parameter as average of duplicate determinations of both numbered samples were used for the statistical evaluation. For the calculation of the repeatability– and reproducibility standard deviation the single values of the double determination were used.

Queried and documented were single results, recovery and the used testing methods. In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

All 10 participants submitted their results in time.

3. Evaluation

3.1 Consensus value from participants (assigned value)

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

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

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

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

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

3.2 Robust standard deviation

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

3.3 Repeatability standard deviation

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

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

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

3.4 Reproducibility standard deviation

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

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

The relative reproducibility standard deviation CV_R in percent of the mean is given as variation coefficient in the statistical data of participant for each parameter. The significance of CV_R is further explained in section 3.9.

3.5 Exclusion of results and outliers

Before statistical evaluation obvious blunders, such as those with incorrect units, decimal point errors, too few significant digits (valid digits) or results for another proficiency test item can be removed from the data set [2]. Even if a result e.g. with a factor >10 deviates significantly from the mean and has an influence on the robust statistics, a result of the statistical evaluation can be excluded [3].

All results should be given at least with 2 significant digits. Specifying 3 significant digits is usually sufficient.

Results obtained by different analytical methods causing an increased variability and/or a bi- or multimodal distribution of results, are treated separately or could be excluded in case of too few numbers of results. For this results are checked by kernel density estimation [3, 12].

Results are tested for outliers by the use of robust statistics (algorithm A): If a value deviates from the robust mean by more than 3 times the robust standard deviation, it can be classified as an outlier (see above) [3]. Due to the use of robust statistics outliers are not excluded, provided that no other reasons are present [3]. Detected outliers are only mentioned in the results section, if they have been excluded from the statistical evaluation.

3.6 Target standard deviation (for proficiency assessment)

The target standard deviation of the assigned value σ_{pt} (= standard deviation for proficiency assessment) can be determined according to the following methods.

If an acceptable quotient S^*/σ_{pt} is present, the target standard deviation of the general model by Horwitz is preferably used for the proficiency assessment. It is usually suitable for evaluation of interlaboratory studies, where different methods are applied by the participants. On the other hand the target standard deviation from the evaluation of precision data of an precision experiment is derived from collaborative studies with specified analytical methods.

In cases where both above-mentioned models are not suitable, the target standard deviation is determined based on values by perception, see under 3.6.3.

For information, the z-scores of both models are given in the evaluation, if available.

In the present PT for valuation of nicotine the target standard deviation according to the general model of Horwitz was applied (see 3.6.1).

Additionally the target standard deviation of the evaluation by a precision experiment (s. 3.6.2) was given for information (ASU §64 methods LFGB T 60.00-6).

3.6.1 General model (Horwitz)

Based on statistical characteristics obtained in numerous PTs for different parameters and methods Horwitz has derived a general model for estimating the reproducibility standard deviation σ_R [6]. Later the model was modified by Thompson for certain concentration ranges [10]. The reproducibility standard deviation σ_R can be applied as the relative target standard deviation σ_{pt} in % of the assigned values and calculated according to the following equations [3]. For this the assigned value X_{pt} is used for the concentration c.

Equations	**Range of concentrations**	**corresponds to**
$\sigma_R = 0{,}22c$	$c < 1{,}2 \times 10^{-7}$	< 120 µg/kg
$\sigma_R = 0{,}02c^{0{,}8495}$	$1{,}2 \times 10^{-7} \leq c \leq 0{,}138$	≥ 120 µg/kg
$\sigma_R = 0{,}01c^{0{,}5}$	$c > 0{,}138$	> 13,8 g/100g

with c = mass content of analyte (as relative size, e.g. 1 mg/kg = 1 ppm = 10^{-6} kg/kg)

3.6.2 Value by precision experiment

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

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

The relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviation (RSD_R) given in Table 2 were determined in ring tests using the indicated methods.
The resulting target standard deviations σ_{pt}, which were identified there, were used to evaluate the results and to provide additional information for the statistical data.

Table 2: Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) according to selected evaluations of tests for precision and the resulting target standard deviation σ_{pt} [18]

Parameter	**Matrix**	**Mean [g/100g]**	**RSD_r [%]**	**RSD_R [%]**	**σ_{pt} [%]**	**Method / Literature**
Nicotine	Tobacco	0,7	20,0	40,0	37,4	GC / [18]
Nicotine	Tobacco	1,0	25,2	44,8	41,1	GC / [18]
Nicotine	Tobacco	1,5	22,4	37,3	33,8	GC / [18]
Nicotine	Tobacco	3,5	11,2	28,8	27,7[1]	GC / [18]

[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].
For the present evaluation the target standard deviation according to 3.6.1 was regarded suitable.
Table 3 shows selected characteristics of participants results of the present PT in comparison to the previous year.

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

Parameter	**Matrix** (Powder)	**Rob. Mean**	**rob. SD (S*)**	**rel. SD (CV_{S*}) [%]**	**Quotient S^*/σ_{pt}**	**DLA-Report**
Nicotine	E-cigarette Liquid	1,01 g/100g	0,0866 g/100g	8,57%	1,6*	DLA 41/2017
Nicotine	E-cigarette Liquid	0,815 g/100g	0,0468 g/100g	5,73%	1,4	DLA 42/2019

** with target standard deviation σ_{pt}'*

3.7 z-Score

To assess the results of the participants the z-score is used. It indicates about which multiple of the target standard deviation (σ_{pt}) the result (x_i) of the participant is deviating from the assigned value (X_{pt}) [3].
Participants' z-scores are derived from:

$$z_i = \frac{\left(x_i - x_{pt}\right)}{\sigma_{pt}}$$

The requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z \leq 2 \ .$$

The valid z-Score for each parameter is indicated as z-Score (σ_{pt}). The value indicated as z-Score (Info) only obtains a informative character. The both z-Scores were calculated with the different target standard deviations in accordance with 3.6.

3.7.1 Warning and action signals

In accordance with the norm ISO 13528 it is recommended that a result that gives rise to a *z*-score above 3,0 or below −3,0, shall be considered to give an "action signal" [3]. Likewise, a *z*-score above 2,0 or below −2,0 shall be considered to give a "warning signal". A single "action signal", or "warning signal" in two successive PT-rounds, shall be taken as evidence that an anomaly has occurred which requires investigation.
An error or cause analysis can be carried out by checking the analysis process including understanding and implementation of the measurement by the staff, details of the measurement procedure, calibration of equipment and composition of reagents, transmission error or an error in the calculation, in the trueness and precision and use of reference material. If necessary, the problems must be addressed through appropriate corrective action [3].

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

3.8 z'-Score

The z'-score can be used for the valuation of the results of the participants, in cases the standard uncertainty has to be considered (s. 3.11). The z'-score represents the relation of the deviation of the result (x_i) of the participant from the respective consensus value (X) to the square root of quadrat sum of the target standard deviation (σ_{pt}) and the standard uncertainty ($U_{(Xpt)}$) [3].

The calculation is performed by:

$$z'_i = \frac{x_i - x_{pt}}{\sqrt{\sigma_{pt}^2 + u_{(x_{pt})}^2}}$$

If carried out an evaluation of the results by means of z 'score, we have defined below the expression in the denominator as a target standard deviation σ_{pt}'.

The requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z' \leq 2 \ .$$

For warning and action signals see 3.7.1.

3.9 Reproducibility cofficient of variation (CV_R)

The variation coefficient (CV_R) of the reproducibility (= relative reproducibility standard deviation) is calculated from the standard deviation and the mean as follows [4, 13]:

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

In contrast to the standard deviation as a measure of the absolute variability the CV_R gives the relative variability within a data region. While a low CV_R, e.g. <5-10% can be taken as evidence for a homogeneous set of results, a CV_R 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*)**
Number with m replicate measurements
Repeatability standard deviation (S_r)
Coefficient of Variation (CV_r)in %
Reproducibility standard deviation (S_R)
Coefficient of Variation (CV_R)in %
Target range:
Target standard deviation σ_{pt} or σ_{pt}'
Target standard deviation for information
lower limit of target range ($X_{pt} - 2\sigma_{pt}$) or ($X_{pt} - 2\sigma_{pt}$') *
upper limit of target range ($X_{pt} + 2\sigma_{pt}$) or ($X_{pt} + 2\sigma_{pt}$´) *
Quotient S/σ_{pt} or S*/σ_{pt}'*
Standard uncertainty U(X_{pt})
Number of results in the target range
Percent in the target range

* Target range is calculated with z-score or z'-score

In the table below, the results of the participating laboratories are formatted in 3 valid digits**:

Auswerte-nummer **Evaluation number**	**Parameter [Einheit / Unit]**	**Abweichung** **Deviation**	**z-Score σpt**	**z-Score** (Info)	**Hinweis** **Remark**

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

4.1 Nicotine in g/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	10
Number of outliers	0
Mean	0,814
Median	0,813
Robust Mean (X_{pt})	**0,815**
Robust standard deviation (S*)	**0,0468**
Number with 2 replicates	10
Repeatability SD (S_r)	0,0115
Repeatability (CV_r)	1,41%
Reproducibility SD (S_R)	0,0580
Reproducibility (CV_R)	7,13%
Target range:	
Target standard deviation σ_{pt}	**0,0336**
Target standard deviation (for Information)	0,226
lower limit of target range	**0,748**
upper limit of target range	**0,883**
Quotient S/σ_{pt}*	*1,4*
Standard uncertainty $U_{(Xpt)}$	*0,0185*
Results in the target range	*8*
Percent in the target range	*80%*

Comments:

The target standard deviation was calculated according to the general model of Horwitz (s. 3.6.1). Additionally the target standard deviation using data from precision experiments (ASU §64 LFGB T 60.00-6) is given for information.

The distribution of results showed a normal variability. The quotient S/σ_{pt} was below 2,0. The robust standard deviation is comparable to those of prior PTs (see 3.6.3). The comparability of results is given. The repeatability and reproducibility standard deviation were lower than established values for the used determination methods (s. 3.6.2).*

80% of results were in the target range.

The robust mean of the participant results was 98% of the gravimetric spiked value for nicotine in the EP sample (see Tab. 1, p.4)

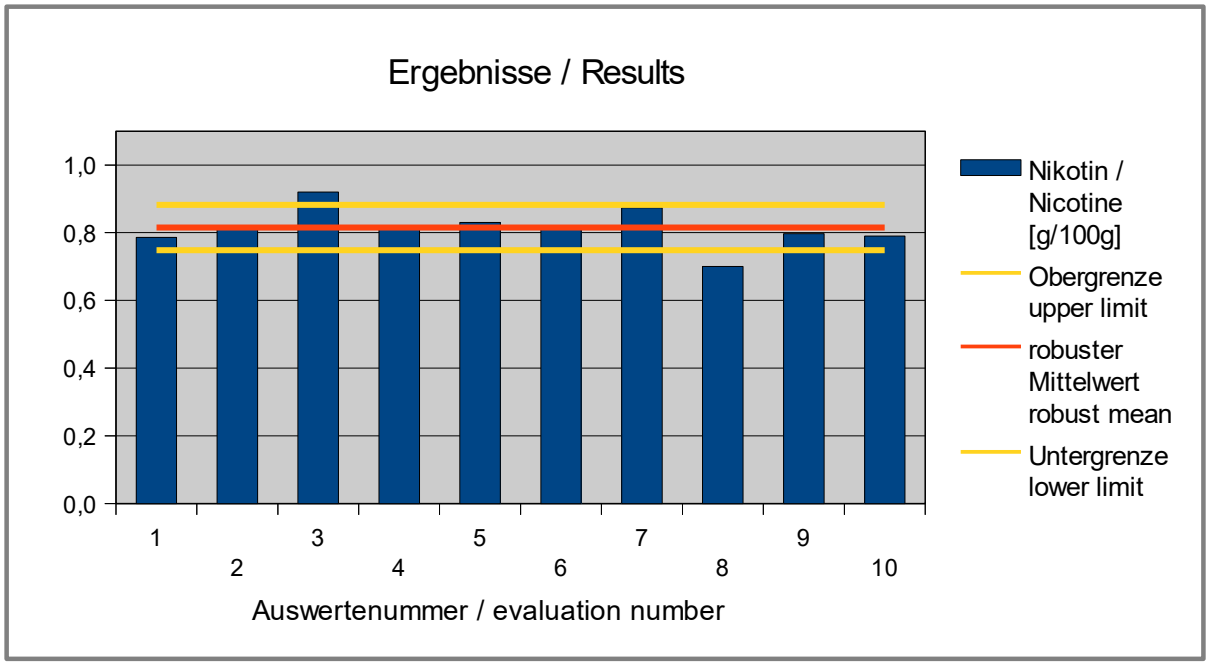

Abb. / Fig. 1: Ergebnisse Nikotin / Results Nicotine

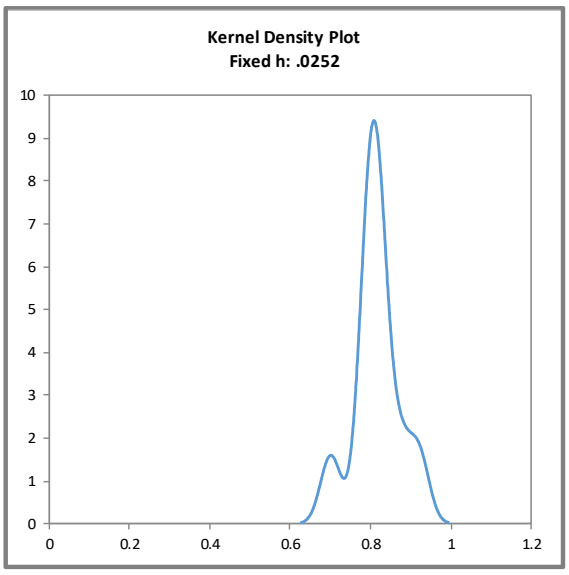

Abb. / Fig. 2:
Kerndichte-Schätzung der Ergebnisse (mit h = 0,75 x σ_{pt} von X_{pt})

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

Comment:
The kernel density shows almost a symmetrical distribution of results with a side peak at approx. 0,7 g/100g and a shoulder at approx. 0,9 g/100g, due to one and two results below and above the target range.

Ergebnisse der teilnehmenden Institute:
Results of Participants:

Auswerte-nummer Evaluation number	Nikotin / Nicotine [g/100g]	Abweichung [g/100g] Deviation [g/100g]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	0,786	-0,0294	-0,87	-0,13	
2	0,820 *	0,0046	0,14	0,02	
3	0,920	0,1046	3,1	0,46	
4	0,816	0,0006	0,02	0,00	
5	0,830	0,0146	0,43	0,06	
6	0,810	-0,0054	-0,16	-0,02	
7	0,874	0,0581	1,7	0,26	
8	0,700	-0,1154	-3,4	-0,51	
9	0,797 *	-0,0184	-0,55	-0,08	
10	0,791	-0,0249	-0,74	-0,11	

* Mean calculated by DLA

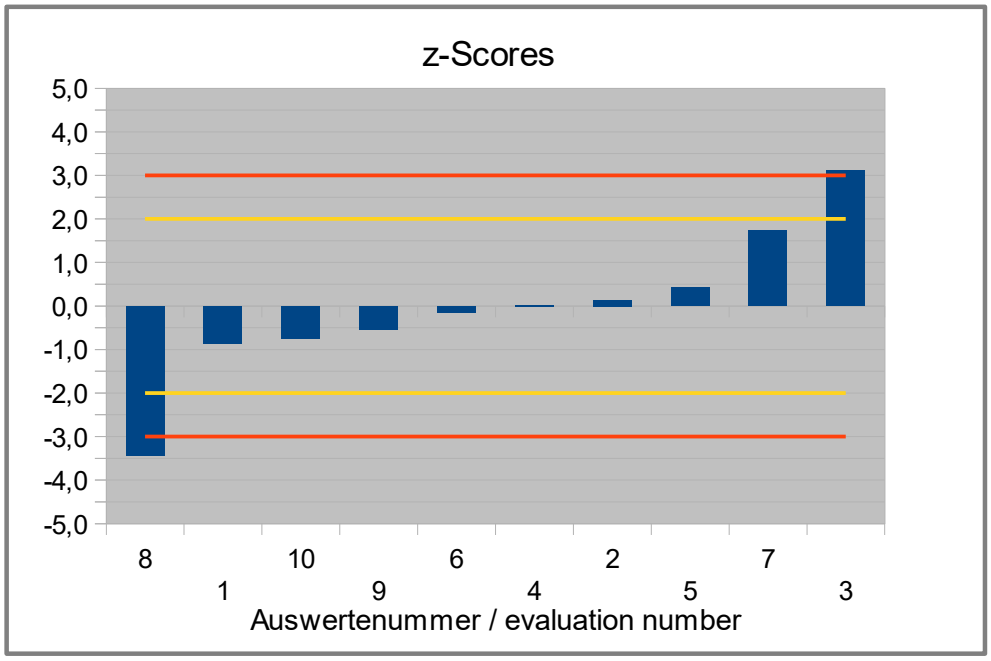

Abb. / Fig. 3: z-Scores Nikotin / Nicotine

5. Documentation

5.1 Details by the participants

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

5.1.1 Primary Data

Para-meter	Partici-pant	Unit	Sample I DLA No.	Sample II DLA No.	Date of analysis	Result (Mean)	Result 1	Result 2	Limit of quantification	Incl. RR	Recovery rate [%]
					Day/Month					yes / no	in %
Nikotin/ Nicotine	1	g/100g	38	2	28.11.19	0,786	0,7905	0,7815	0,0005	no	
	2	g/100g	13	27	09.12.19	0,82	0,82	0,82	0,0145	no	97,7
	2	g/100g	13	27	09.12.19	0,82	0,81	0,82	0,0145	no	97,7
	2	g/100g	13	27	09.12.19	0,82	0,82	0,82	0,0145	no	97,7
	3	g/100g	42/2019 Sample No 07	42/2019 Sample No 33	04.12.19	0,92	0,92	0,92	0,06	no	95 - 101
	4	g/100g	17	23	26.11.19	0,816	0,817	0,814	ca. 0,009	no	not determined
	5	g/100g	19	21	27.12.19	0,83	0,83	0,83	0,01	no	100
	6	g/100g	1	39	13.12.19	0,81	0,83	0,79	0,032	no	-
	7	g/100g	10	30	27.11.19	0,8735	0,8664	0,8806	0,02	no	95-100
	8	g/100g	15	25	09.01.20	0,7	0,7	0,7	0,05	No	88
	9	g/100g	8	32	08.01.20	43840	0,795	0,799	0,236	no	96,3
	10	g/100g			09.01.20	0,7905	0,777	0,804	10µg/L	no	nein

5.1.2 Analytical Methods

Para-meter	Partici-pant	Method description as in test report / norm / literature	Sample preparation and pro-cessing	Measuring method	Calibration / Reference material	Recovery rate with same matrix	Method Accredited ISO/IEC 17025	Further Remarks
						yes / no	yes / no	
Nikotin/ Nicotine	1	Determination of Alkaloids in Whole Tobacco, Health Canada- Official Method: T-301	extracted with alkaline methanol	LC-MS instead of GC	5-points with Nicotine (Alfa Aesar)	QC in every run.	yes	No recovery corrections are made. We use specific internal standards (Nicotine-D4 (LGC)).
	2	QSA-O-2102-02	Dil. w. 2-Propanol/GC-FID	HP5	internal standard series (heptadecane)	no	yes	
	2	QSA-O-2102-02	Dil. w. 2-Propanol/GC-FID	HP5	internal standard series (heptadecane)	no	yes	
	2	QSA-O-2102-02	Dil. w. 2-Propanol/GC-FID	HP5	internal standard series (heptadecane)	no	yes	
	3	PM 228-022 (house method)	0,2 g w. / 20 ml 2-propanol + int. Std.	GC- FID	cert. Nicotine-std.	yes	yes	
	4	qNMR Ph. Eur. 2.2.33	only dilution with D2O		maleic acid		yes	
	5	MP 2119 rev 1 - HPLC-UV/Vis			external calibration N3876-5ML (-)-Nicotine sigma	yes	no	
	6	in-house method	dilution	GC-MS	internal standard d4-nicotine	-	yes	-
	7	Determination of nicotine in liquid with HPLC-DAD 10045	50µl sample mixed with 950µl trifluoroacetic acid (1g/l water)	HPLC-DAD, isocratic	Calibration material: Sigma Aldrich 36733 Reference material CRM LGC Standards Promochem N-008 1,000mg/ml	yes	yes	
	8	In house method, GC-MS	Dissolution in methanol	GC-MS, SIM	In solvent, Sigma Aldrich	yes	no	
	9	House method HPLC-DAD	approx. 0.1 g are weighed to the nearest 0.1 mg and diluted to 20 ml with 10 mM NH4 formate buffer / ACN, syringe filter 0.4 µm	Phenomenex Lunar Omega polar C18 5 µm; 2,1 x 150 mm; gradient with 10 mM NH4-formiatpuffer/ACN, evalutaion at 260 nm	S(-)-nicotine, CAS Nr. 54-11-5, Sigma-Aldrich N-008, 1 mg/ml in methanol LGC/Ehrensdorfer 100 µg/ml in methanol certified reference solution	no	yes	
	10			LC-MS/MS	internal STD-calibration	no	no	

5.2 Homogeneity

5.2.1 Trend line function of the participants results

By comparison of the increasing sample numbers and the measurement results of participants, the homogeneity of the chronological bottled PT items can be shown by the trend line for information:

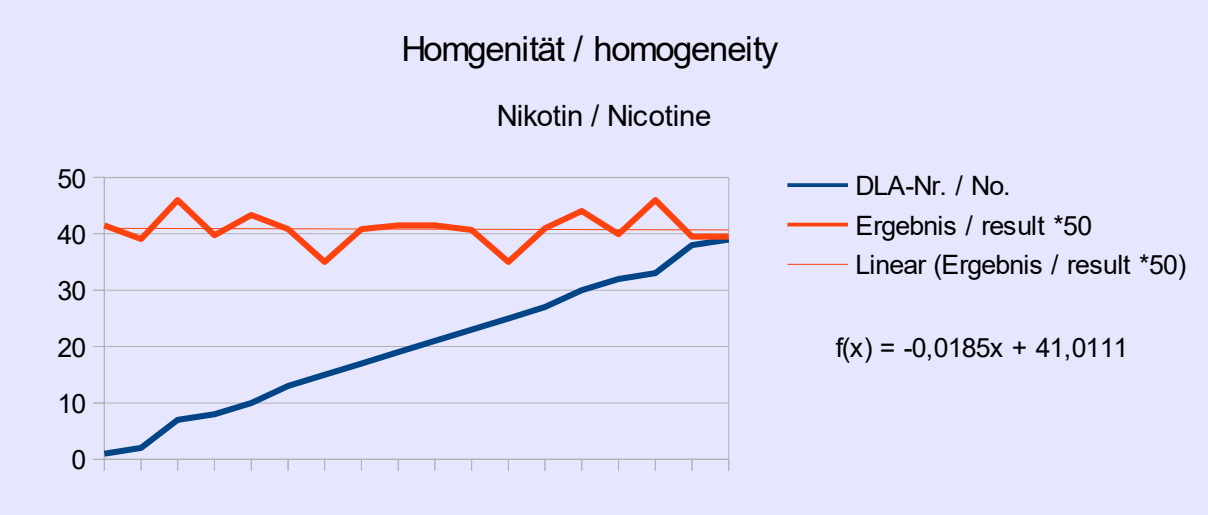

Abb./Fig. 4:
Trendfunktion Probennummern vs. Ergebnisse (1*50 dargestellt)
trend line function sample number vs. results (1*50 shown)

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 42-2019***
PT name	***Nicotine in E-Cigarette-Liquid***
*Sample matrix**	***Samples I + II:*** *E-Cigarette Liquid / Ingredients: Glycerin, propylene glycol, aroma, nicotine*
Number of samples and sample amount	*2 identical samples I + II, 10 g each.*
Storage	*Samples I + II: cooled 2 - 10°C*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*quantitative:* ***Nicotine***
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 January 10th 2020***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf PhD*

** Control of mixture homogeneity and qualitative testings are carried out by DLA. Any testing of the content, homogeneity and stability of PT parameters is subcontracted by DLA.*

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		AUSTRIA
		Germany
		Germany
		ITALY
		SWEDEN
		Germany
		SWITZERLAND
		Germany
		CROATIA
		CZECH REPUBLIC

[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

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17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Re-quirements, Appendix F, p. 2, AOAC Int (2016)
18. ASU §64 LFGB T 60.00-6, Bestimmung des Nikotingehaltes in Tabak und Tabakerzeugn-isse, GC-Verfahren (Juni 2012) / DIN 10373:2011-05 [Analysis of tobacco and to-bacco products - Determination of nicotine content - Gaschromatographic method]