



Evaluation Report

proficiency test

DLA ptRE01 (2021)

Ethylene oxide/2-Chloroethanol

in Spice Mixture

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General Information on the proficiency test (PT)

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<i>Vertraulichkeit</i> <i>Confidentiality</i>	<p>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.</p>

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1. Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed, cosmetics and food contact materials. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the verification and/or validation of the particular testing method [1, 5].

The purpose of DLA is to offer proficiency tests for selected parameters in concentrations with practical relevance.

Realisation and evaluation of the present proficiency test follows the technical requirements of DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2009 / ISO 13528:2015 [2, 3].

2. Realisation

2.1 Test material

The test material is a mixture of ground spices (paprika, turmeric and cayenne), some of which contain residues of ethylene oxide / 2-chloroethanol. The raw materials were sieved, mixed and homogenized.

Afterwards the samples were portioned to approximately 50 g into metalised PET film bags and chronologically numbered.

The DLA-Samples' composition is shown in Table 1.

Table 1: Composition of DLA-Samples

Ingredients	content
Paprika powder, sweet	54,1 g/100 g
Turmeric powder	42,7 g/100 g
Cayenne powder	3,2 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 **mixture homogeneity before bottling** was examined 8-fold by **micro-tracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].

Before mixing dye coated iron particles of μm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of $\geq 5\%$ is equivalent to a good homogeneous mixture and of $\geq 25\%$ to an excellent mixture [14, 15].

The microtracer analysis of the present PT samples showed a probability of 93%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [16, 17]. This gave a HorRat value of 0,78. The results of microtracer analysis are given in the documentation.

The calculation of the **repeatability standard deviation S_r of the participants** was used as an indicator of homogeneity. It is 5,6% for the sum of ethylene oxide / 2-chloroethanol. Thus, this value is below the corresponding repeatability standard deviations of precision data of the standardized method (ASU §64 L 53.00-1, s. 3.6.2) (see Table 3) [18].

The repeatability standard deviations of the participants' results are 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. 5.2.2 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

A water activity (a_w) of $< 0,5$ is an important factor to ensure the stability of dry or dried products during storage. Optimum conditions for storage is the a_w value range of $0,15 - 0,3$. In this range the lowest possible degradation rate is to be expected [16].

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_w value $< 0,5$).

The a_w value of the PT samples was approx. $0,49$ ($18,5^\circ\text{C}$). The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

Two portions of test material were sent to every participating laboratory in the 13th week of 2021. The testing method was optional. The tests should be finished at 28th May 2021 the latest.

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

The two portions contain identical samples with the parameters Ethylene oxide / 2-Chloroethanol (to be reported in sum as Ethylene oxide) in the matrix of Spice Mixture. The analysis method is optional.

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

2.3 Submission of results

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

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.

18 participants submitted results. 3 registered participants submitted no results.

3. Evaluation

3.1 Consensus value from participants (assigned value)

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

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

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

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

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

3.2 Robust standard deviation

For comparison to the target standard deviation σ_{pt} (standard deviation for proficiency assessment) a robust standard deviation (S^*) 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 a 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 evaluation of the results in the present PT, the target standard deviation of the general model according to Horwitz/Thompson, suitable for levels $\leq 120 \mu\text{g}/\text{kg}$, was used for the parameter ethylene oxide / 2-chloroethanol (sum as ethylene oxide) (see 3.6.1).

For information, the target standard deviation of the evaluation of a precision experiment (see 3.6.2) was also given (ASU S64 L 53.00-1 [18]).

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
$\sigma_R = 0,22c$	$c < 1,2 \times 10^{-7}$	$< 120 \mu\text{g}/\text{kg}$
$\sigma_R = 0,02c^{0,8495}$	$1,2 \times 10^{-7} \leq c \leq 0,138$	$\geq 120 \mu\text{g}/\text{kg}$
$\sigma_R = 0,01c^{0,5}$	$c > 0,138$	$> 13,8 \text{ g}/100\text{g}$

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

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]:

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

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 [mg/kg]	RSD_r	RSD_R	σ_{pt}	Method / Literature
Ethylene oxide / 2-Chloroethanol	Paprika powder	0,0034	16,3%	58,7%	57,6%	GC / ASU \$64 L 53.00-1 [18]
Ethylene oxide / 2-Chloroethanol	Paprika powder	0,025	13,3%	32,7%	31,3%	GC / ASU \$64 L 53.00-1 [18]
Ethylene oxide / 2-Chloroethanol	Chillies powder	0,113	10,3%	27,8%	26,8% ¹	GC / ASU \$64 L 53.00-1 [18]

¹ used for information in the evaluation (cf. chapter 4)

3.6.3 Value by perception

The target standard deviation for proficiency assessment can be set at a value that corresponds to the level of performance that the coordinator would wish laboratories to be able to achieve [3].

For the present evaluation, the target standard deviation according to 3.6.1 was regarded suitable.

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:

$$z_i = \frac{(x_i - X_{pt})}{\sigma_{pt}}$$

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 an 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 ($U(x_{pt})$) [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 (VK_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 gives the relative variability within a data region. While a low CV, e.g. <5-10% can be taken as evidence for a homogeneous set of results, a CV of more than 50% indicates a "strong inhomogeneity of statistical mass", so that the suitability for certain applications such as the assessment of exceeded maximum levels or the performance evaluation of the participating laboratories possibly can not be done [3].

3.10 Quotient S^*/σ_{pt}

Following the HorRat-value the results of a proficiency-test (PT) can be considered convincing, if the quotient of robust standard deviation S^* and target standard deviation σ_{pt} does not exceed the value of 2.

A value > 2 means an insufficient precision, i.e. the analytical method is too variable, or the variation between the test participants is higher than estimated. Thus the comparability of the results is not given [3].

3.11 Standard uncertainty of the assigned value

Every assigned value has a standard uncertainty that depends on the analytical method, differences between the analytical methods used, the test material, the number of participating laboratories (P) and on other factors. The standard uncertainty ($U_{(x_{pt})}$) for this PT is calculated as follows [3]:

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

If $U_{(x_{pt})} \leq 0,3 \sigma_{pt}$ 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**:

Auswertenummer	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 Ethylene oxide/2-Chloroethanol in mg/kg (Sum as Ethylene oxide)

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	19
Number of outliers	0
Mean	0,108
Median	0,106
Robust Mean (X)	0,108
Robust standard deviation (S*)	0,0334
Number with 2 replicates	19
Repeatability SD (S_r)	0,00608
Repeatability (CV_r)	5,60%
Reproducibility SD (S_R)	0,0297
Reproducibility (CV_R)	27,3%
Target range:	
Target standard deviation σ_{pt}	0,0238
Target standard deviation (for Information)	0,0290
lower limit of target range	0,061
upper limit of target range	0,156
Quotient S^*/σ_{pt}	1,4
Standard uncertainty $U_{(X_{pt})}$	0,0096
Results in the target range	19
Percent in the target range	100%

Comments:

The target standard deviation was calculated according to the general model of Horwitz/Thompson (s. 3.6.1). Additionally, the target standard deviation using data from a precision experiment (official German method ASU §64 L 53.00-1) (3.6.2) was given for information.

The distribution of results showed a normal variability. The quotient S^*/σ_{pt} was below 2,0. The repeatability and reproducibility standard deviation were in the range of established values for the used determination methods and the present matrix (s. 3.6.2). The comparability of results is given.

All results were in the target range.

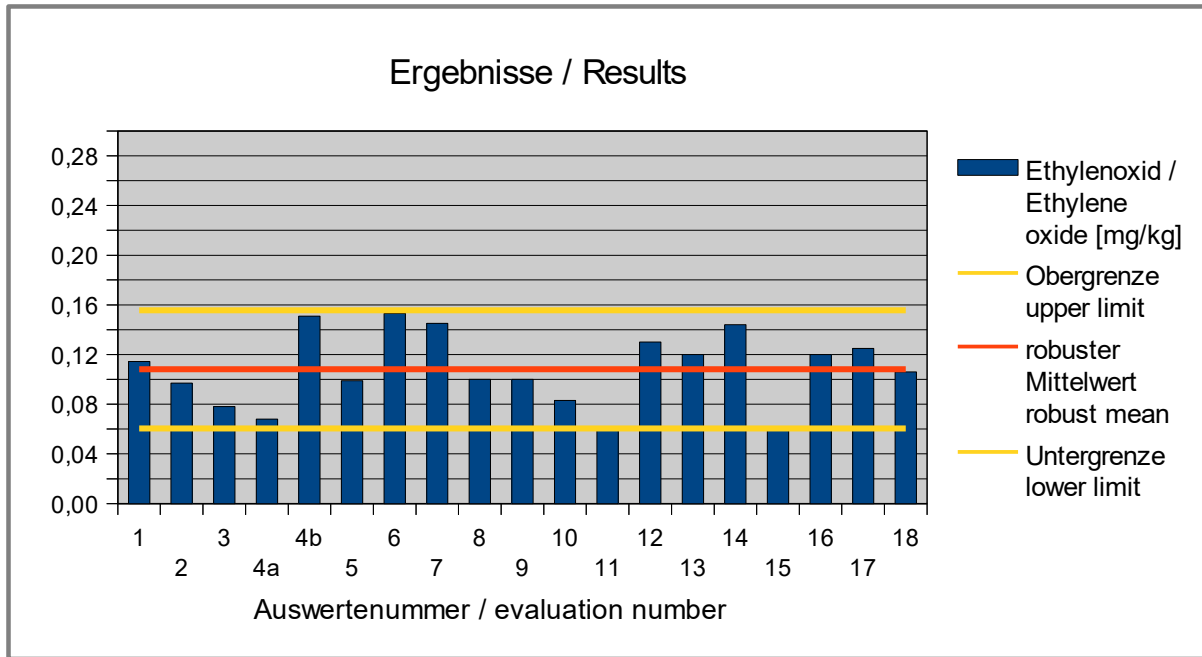


Abb. / Fig. 1: Ergebnisse Summe Ethylenoxid/2-Chlorethanol / Results sum ethylene oxide / 2-chloroethanol

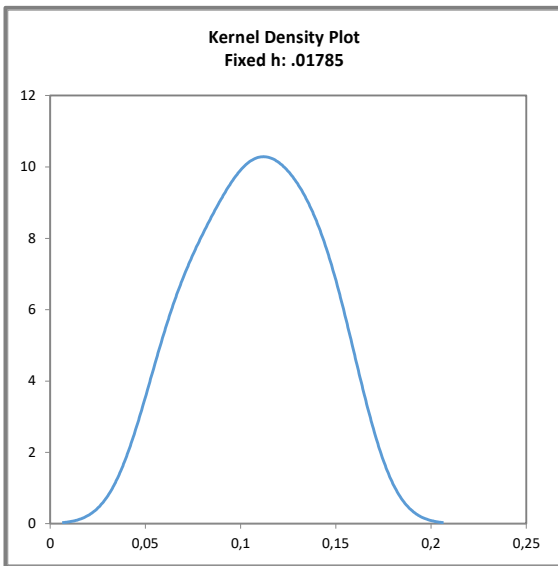


Abb. / Fig. 2:

Kerndichte-Schätzung der Ergebnisse (mit $h = 0,75 \times \sigma_{pt}$ von X_{pt})

Kernel density plot of results (with $h = 0,75 \times \sigma_{pt}$ of X_{pt})

Comment:

The kernel density shows a relatively broad almost symmetrical distribution of results.

**Ergebnisse der Teilnehmer:
Results of Participants:**

Auswertenummer Evaluation number	Ethylenoxid / Ethylene oxide [mg/kg]	Abweichung [mg/kg] Deviation [mg/kg]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	0,115 *	0,006	0,27	0,22	
2	0,097	-0,011	-0,47	-0,38	
3	0,078	-0,030	-1,3	-1,04	
4a	0,068	-0,040	-1,7	-1,38	
4b	0,151	0,043	1,8	1,48	
5	0,099	-0,009	-0,38	-0,31	
6	0,153	0,045	1,9	1,55	
7	0,145	0,037	1,5	1,27	
8	0,100	-0,008	-0,34	-0,28	
9	0,100	-0,008	-0,34	-0,28	
10	0,083	-0,025	-1,1	-0,87	
11	0,061	-0,047	-2,0	-1,62	
12	0,130 *	0,022	0,92	0,75	
13	0,120	0,012	0,50	0,41	
14	0,144	0,036	1,5	1,24	
15	0,060	-0,048	-2,0	-1,66	
16	0,120	0,012	0,50	0,41	
17	0,125	0,017	0,71	0,58	
18	0,106	-0,002	-0,09	-0,07	

* Mean calculated by DLA

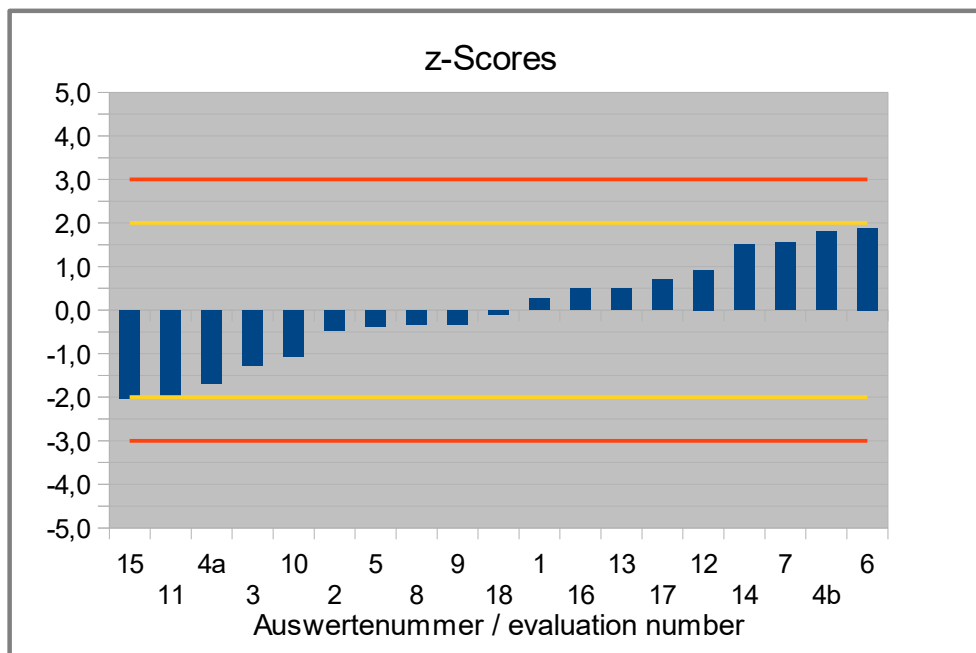


Abb. / Fig. 3: z-Scores Ethylenoxid / ethylene oxide

5. Documentation

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

5.1 Details by the participants

5.1.1 Primary data

Analyte	Participant	Unit	Sample 1 DLA No.	Sample 2 DLA No.	Date of analysis	Result (Mean)	Result 1	Result 2	Limit of quantification	Incl. RR	Recovery rate [%]
Ethylenoxid / 2-Chlorethanol (Summe als Ethylenoxid)	1	mg/kg	31	49	20.04.2021		0,112	0,117	0,03	no	102
	2	mg/kg	4	76	15.04.2021	0,097	0,097	0,097	0,01	no	88,4
	3	mg/kg	12	68	12.05.2021	0,078	0,078	0,079	0,01	no	-
	4a	mg/kg	20	60	13.04.2021	0,068	0,067	0,069	0,01	yes	1
	4b	mg/kg	20	60	14.04.2021	0,15	0,15	0,15	0,01	yes	1
	5	mg/kg	17	63	29.04.2021	0,099	0,1	0,098	0,01	no	65
	6	mg/kg	33	47	20.04.2021	0,153	0,162	0,143	0,1	no	103
	7	mg/kg	27	53	18.05.2021	0,145	0,139	0,150	0,02	no	101
	8	mg/kg	16	64	13.04.	0,1	0,099	0,110	0,05	no	72
	9	mg/kg	7	73	20.05.2021	0,1	0,110	0,099	0,01	no	—
	10	mg/kg	19	61	21.05.2021	0,083	0,086	0,080	0,01	no	72
	11	mg/kg	39	41	05.05./20.05.	0,061	0,061	0,061	< 0,010	no	88-103%
	12	mg/kg	14	66	14.04.2021	28.05.2021	0,13	0,13	0,01	yes	100
	13	mg/kg	25	55	21.05.2021	0,12	0,11	0,13	0,01	no	90
	14	mg/kg	No.05	No.75	04.05./21	0,144	0,144	0,145	0,01	yes	99
	15	mg/kg	35	45	11.05.	0,06	0,058	0,061	0,01	no	-
	16	mg/kg	37	43	19.05.	0,12	0,12	0,12	0,01	yes	112
	17	mg/kg	34	46	24.05.2021	0,125	0,131	0,12	0,01	No	70-120
18	µg/kg	9	71	08.06.2021	106	110	102	10	no	0,99	

5.1.2 Analytical Methods

Participant	Method description	Sample preparation	Measuring method	Calibration / Reference material	Recovery with same matrix	Method accredited	Further remarks
1	in house		GC-MS/MS	quadratic calibration, chloroethanol as pure substance	no	no	
2	ASU§54LFGB, L53.00-1	Sample weight: 5g	GC-MSD/SIM	calibration: 0,005 - 0,5 ng/µl 2-Iodoethanol / no reference material available	yes	yes	
3	Ethylene oxide and 2-Chloroethanol (GC) in plant products according to ASU §64 LFGB L 53.00-1 (mod.)	Separation of compounds by GC-ECD, Quantification of amount of Ethylene oxide and 2-Chloroethanol with internal Standard Bromopropanol, Identification by external Standard 2-Iodoethanol.	GC-ECD	6 Point Calibration, Reference material Iodoethanol	no	yes	-
4a	QuOil	-	-	-	yes	yes	-
4b	ASU L53.00-1 (1999-11)	-	-	-	yes	yes	-
5	ASU L 53.00-01, mod. 11/1999	5-fold Extraction	GC-ECD	Calibration 0,002 - 0,5 µg/ml 2-Iodoethanol	yes; Blank-Paprika powder	yes	Sample weight: 10 g
6	SOPC0208.03	Solvent extraction with Ethylacetate, d-SPE clean-up	GC-MS	Internal Standard Calibration with 2-Chloroethanol (Sigma-Aldrich) / d4-2-Chloroethanol (HPC Standards GmbH)	yes	yes	
7	SF-CS46520	Ultra sonic extraction after addition of acid/salt	SPME-HS-GC/MS	2-Chloroethanol/ 2-Chloroethanol-D4 (ISTD)	yes	yes	
8	Method L 53.00-1 for Determination of Ethylene oxide and 2-Chloroethanol of German Official Method according to ASU § 64 LFGB		GC-MS	Solvent calibration	yes (Paprika)	yes	Method for oil seeds validated
9	internal Method: LEI-SOP-00.65102.L	Reaction to Iodoethanol with NaI	GC-MSD	external calibration. Iodoethanol in ethylacetate	no	yes	---
10	ASU § 64 L 53.00-1 (1999-11)		GC-ECD	Lösemittelkalibr.	no	yes	

Note: continued on next page.

Participant	Method description	Sample preparation	Measuring method	Calibration / Reference material	Recovery with same matrix	Method accredited	Further remarks
11	IFP 003173 (HS-GC-MS) (a)	-	Headspace- GC/MSD	externe Kalibrierung, 2-CE-D4 als ISTD	yes	yes	
12	yes	Preparation : Analysis of Ethylene Oxide and its Metabolite 2-Chloroethanol by the QuOil or the QuEChERS Method and GC-MS/MS	none	control sample available	yes	yes	none
13	EURL-SRM- Analysis of Ethylene Oxide and its Metabolite 2-Chloroethanol by the QuOil or the QuEChERS Method and GC-MS/MS	5g of sample + 10ml AcN. Clean up C18/PSA/MgSO4	Injection mode: solvent vent. Column DB-WAX 30 * 0,25mm 0,25µm	External calibration with istd 2-chloroethanol 1000µg/l Dr. Ehrenstorfer Ethylene oxide solution 2000µg/ml SIGMA-ALDRICH	yes	no	Results obtained by mean value between two methods
14	PV-SA-E-399	ACN	GC-HE-MSD	external	yes, STA	yes	
15	§64 LFGB L53.00-1	-	GC-ECD	Iodoethanol	-	yes	-
16	P-14.194	-	GC-MSD	Iodoethanol, D4-2-Chloroethanol, 2-Chloroethanol	yes	yes	-
17	In-house method				Yes	Yes	
18	internal method			external	0,99	yes	GC/MS/MS

5.2 Homogeneity

5.2.1 Mixture homogeneity before botteling

Microtracer Homogeneity Test

DLA ptRE01

Weight whole sample	4,09	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	26,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,97	54	21,7
2	4,99	56	22,4
3	5,02	58	23,1
4	5,02	53	21,1
5	5,05	52	20,6
6	5,05	66	26,1
7	4,98	58	23,3
8	5,04	54	21,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	56,4	Particles
Standard deviation	4,40	Particles
χ ² (CHI-Quadrat)	2,40	
Probability	93	%
Recovery rate	86	%

Normal distribution		
Number of samples	8	
Mean	22,5	mg/kg
Standard deviation	1,75	mg/kg
rel. Standard deviaton	7,80	%
Horwitz standard deviation	10,0	%
HorRat-value	0,78	
Recovery rate	86	%

5.2.2 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:

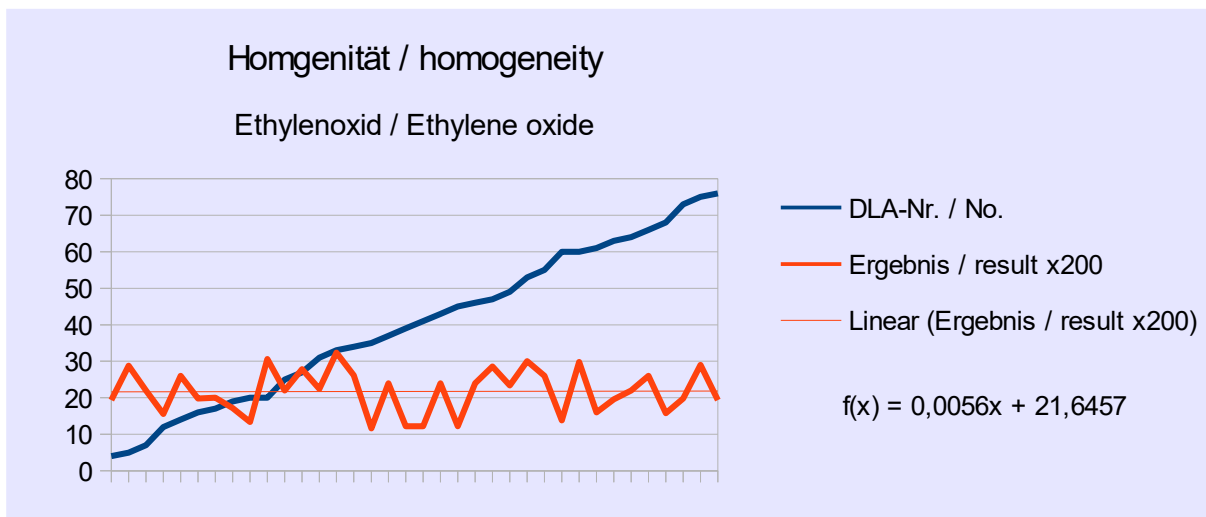


Abb./Fig. 4:

Trendfunktion Probennummern vs. Ergebnisse (1x200 dargestellt)
 trend line function sample number vs. results (1x200 shown)

5.3 Information on the Proficiency Test (PT)

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

<i>PT number</i>	DLA ptRE01-2021
<i>PT name</i>	Ethylene oxide /2-Chloroethanol in Spice Mixture
<i>Sample matrix*</i>	Samples I + II: Spice Mixture
<i>Number of samples and sample amount</i>	2 identical samples I + II, 50 g each.
<i>Storage</i>	Samples I + II: room temperature (PT period), cooled 2 - 10°C (long term)
<i>Intentional use</i>	Laboratory use only (quality control samples)
<i>Parameter</i>	quantitative: Ethylene oxide /2-Chloroethanol (in sum given as Ethylenoxid)
<i>Methods of analysis</i>	Analytical methods are optional
<i>Notes to analysis</i>	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.
<i>Result sheet</i>	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.
<i>Units</i>	µg/kg
<i>Number of significant digits</i>	at least 2
<i>Further information</i>	For information please specify: <ul style="list-style-type: none"> - 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
<i>Result submission</i>	The result submission file should be sent by e-mail to: pt@dla-lvu.de
<i>Last Deadline</i>	the latest May 28th 2021
<i>Evaluation report</i>	The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.
<i>Coordinator and contact person of PT</i>	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
		SWITZERLAND
		Germany
		ITALY
		ITALY
		Germany
		Germany
		SWITZERLAND
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		SPAIN
		SWITZERLAND
		Germany
		Germany
		Germany
		VIETNAM
		SWITZERLAND

[Die Adressdaten der Teilnehmer wurden für die allgemeine Veröffentlichung des Auswerte-Berichts nicht angegeben.]

[The address data of the participants were deleted for publication of the evaluation report.]

7. Index of references

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung - Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment - General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by inter-laboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and precision) of measurement methods and results
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16. Homogeneity and stability of reference materials; Linsinger et al.; Accred Qual Assur, 6, 20-25 (2001)
17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Requirements, Appendix F, p. 2, AOAC Int (2016)
18. ASU §64 L 53.00-1 (1999-11); Bestimmung von Lebensmitteln - Gaschromatographische Bestimmung von Ethylenoxid und 2-Chlorethanol in Gewürzen [Food analysis - Determination of ethylene oxide and 2-chloroethanol by gaschromatography]