DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

DLA 26/2016

Patulin in apple juice

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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 was clear and merchantable apple juice of a local supplier, mixed with apple juice with a natural content of patulin. For preservation we added potassium sorbate and vinegar essence. Approximately 3 kg of the material were homogenized and then packaged lightproof in portions to approximately 50 ml. The portions were numbered chronologically.

2.1.1 Homogeneity

To verify the homogeneity of the test material sorbic acid was added before homogenisation. The five-fold determination was made by HPLC/UV modified according to ASU §64 LFGB L 00.00-9. The standard deviation between the results was < 1,0 %. The result is comparable with the precision data of ASU § 64 LFGB L 48.03-2 [16]. Homogeneity is thus sufficiently assured. The results are given in the documentation.

The calculation of the repeatability standard deviation of the participants for patulin was used as an indicator of homogeneity. The result is similar to the repeatability standard deviation of the official method ASU § 64 LFGB 48.03-2 [16]. The repeatability standard deviation of the participants is given in the documentation and in the statistic data (see 4.1).

In the documentation the portion numbers are graphically assigned to the results of patulin. There is no trend recognizable in the results which could suggest inhomogeneity.

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].

2.2 Sample shipment and information to the test

Two portions of test material were sent to every participating laboratory in the 11th week of 2016. The testing method was optional. The tests should be finished at 29th April 2016 the latest.

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 of patulin 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 for patulin.

From the 12 participants one participant has not delivered a result and one participant had submitted the results late. All other participants submitted the result in time.

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].

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.

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.

The calculation of the repeatability standard deviation S_r is performed by: [3, 4].

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.

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. Its meaning is explained in more detial 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]. 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.

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)

The target standard deviation according to Horwitz/Thompson [6, 10] was used.

3.6.2 Precision experiment

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

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

The values given in Table 1 relative repeatability standard deviation (RSD_r) and relative reproducibility standard deviation (RSD_R) were determined in collaborative trials using the specified methods.

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Table 1: Relative repeatability standard deviation (RSD_r) and relative reproducibility standard deviation (RSD_R) for Patulin according to evaluations of experiments for precision [15, 16]

Parameter	**Matrix**	**Mean values**	**RSD_r**	**RSD_R**	**Method/ Literature**
Patulin	cloudy apple juice	26 µg/l	14%	33%	HPLC/15
Patulin	cloudy apple juice	69 µg/l	6%	14%	HPLC/15
Patulin	cloudy apple juice	106 µg/l	10%	12%	HPLC/15
Patulin	clear apple juice	26 µg/l	14%	33%	HPLC/15
Patulin	clear apple juice	54 µg/l	11%	25%	HPLC/15
Patulin	clear apple juice	128µg/l	8%	11%	HPLC/15
Patulin	apple juice	10,7µg/kg	12,1%	24,3%	HPLC/16

From the average precision data (RSD_r= 10%; RSD_R= 22%) the relative target standard deviation of 20.8% is obtained.

This target standard deviation is given for information in the evaluation.

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 of results the target standard deviation according to Horwitz/ Thompson was applied.

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

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

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

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

3.8.1 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].

3.9 Reproducibility coefficient (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^*)
repeatability standard deviation (S_r)
relative reproducibility standard deviation (S_R)
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 ($X_{pt} + 2\sigma_{pt}$´) *
Variation coefficient CV_R in %
Quotient S/σ_{pt} or S*/σ_{pt}'*
Standard uncertainty $U(X_{pt})$
Quotient $U(X_{pt})/\sigma_{pt}$ or $U(X_{pt})/\sigma_{pt}$'
Number of results in the target range
Percent in the target range

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

In the second table the individual results of the participating laboratories are listed:

Auswerte-nummer **Evaluation number**	**Parameter [Einheit / Unit]**	**Abweichung** **Deviation**	Z-Score σ_{pt}	**z-Score** (Info)	**Hinweis** **Remark**

4.1 Patulin in µg/kg

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	11
Number of outliers	0
Mean	90,9
Median	100
Robust Mean (X_{pt})	91,4
Robust standard deviation (S^x)	26,0
Repeatability standard deviation (S_r)	5,61
Reproducibility standard Deviation (S_R)	27,9
Target range:	
Target standard deviation Horwitz/ Thompson (σ_{pt})	20,1
Target standard deviation by ASU (for Information)	19,0
lower limit of target range	51,2
upper limit of target range	132
coefficient of variation (CV_R) in %	30,5
Quotient S^x/σ_{pt}	*1,3*
Standard uncertainty $u(X_{pt})$	*9,8*
Quotient $u(X_{pt})/\sigma_{pt}$	*0,49*
Results in the target range	*10*
Percent in the target range	*91*

Comments:

The target standard deviation was calculated by Horwitz/ Thompson.

The evaluation of the results shows an acceptable variability of results, in particular because the tests using different methods (HPLC, LC-MS). The quotient S^x/σ_{pt} was below 2,0. The quotient $U(X_{pt})/\sigma_{pt}$ is 0,49 above 0,3, but to accept because of the different methods.

The robust standard deviation is in the range of established values for the standard deviation of the methods used. The reproducibility of the results is given.

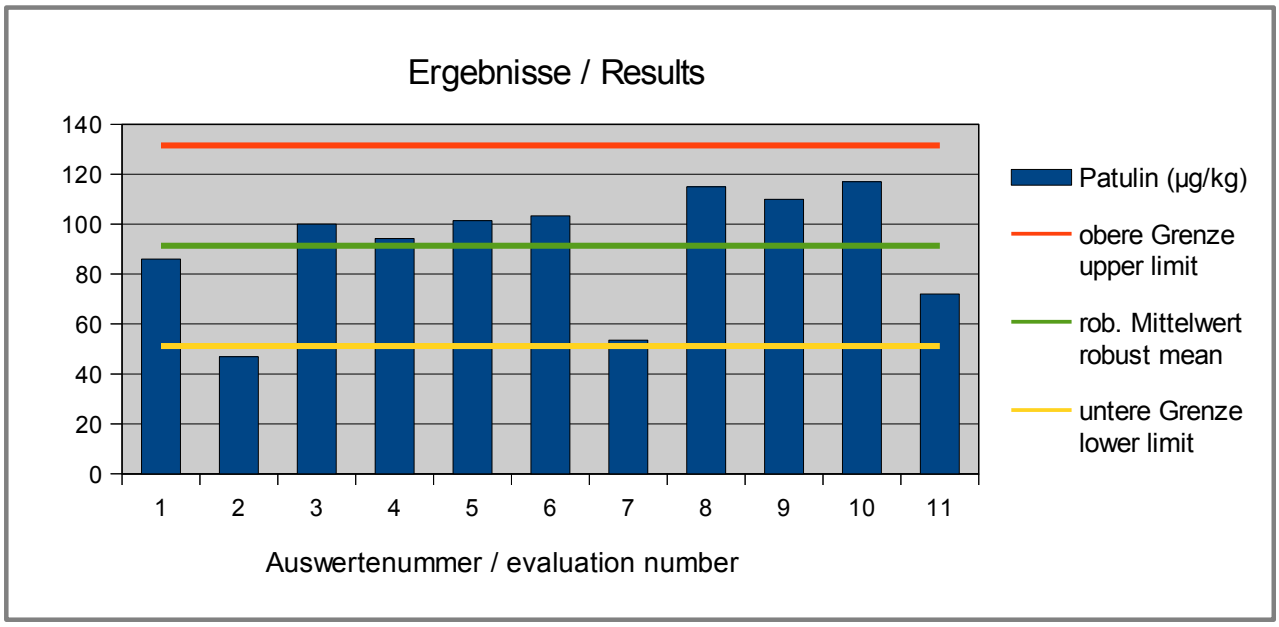


Abb. 1: Ergebnisse Patulin
Fig. 1: Results Patulin

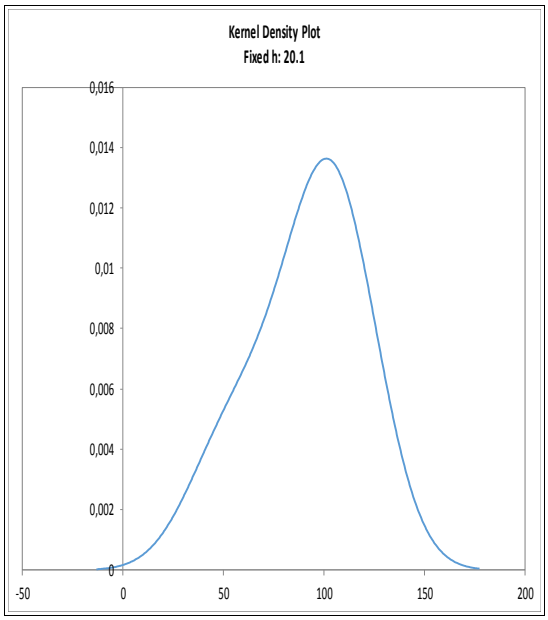


Abb. 2: Kern Dichte Plot der Ergebnisse Patulin mit h = Zielstandardabweichung (20,1 µg/kg)
Fig. 2: Kernel density plot of the patulin results with h = target standard deviation (20,1 µg/kg)

Comments:
The kernel density plot shows nearly a normal distribution of results (with a slight shoulder at 50 ug/kg).

Ergebnisse der teilnehmenden Institute:
Results of Participants:

Auswerte-nummer Evaluation number	Patulin (µg/kg)	Abweichung [µg/kg] Deviation [µg/kg]	Z-Score σ_{pt}	z-Score (Info)	Hinweis Remark
1	86,0	-5,35	-0,3	-0,3	
2	46,9	-44,5	-2,2	-2,1	
3	100	8,65	0,4	0,4	
4	94,3	2,92	0,1	0,1	
5	101	10,0	0,5	0,5	
6	103	11,9	0,6	0,6	
7	53,5	-37,9	-1,9	-1,8	Results converted by DLA*
8	115	23,6	1,2	1,1	
9	110	18,6	0,9	0,9	
10	117	25,6	1,3	1,2	
11	72,0	-19,4	-1,0	-0,9	

** The result was converted to [17] of µg/l in µg/kg.*

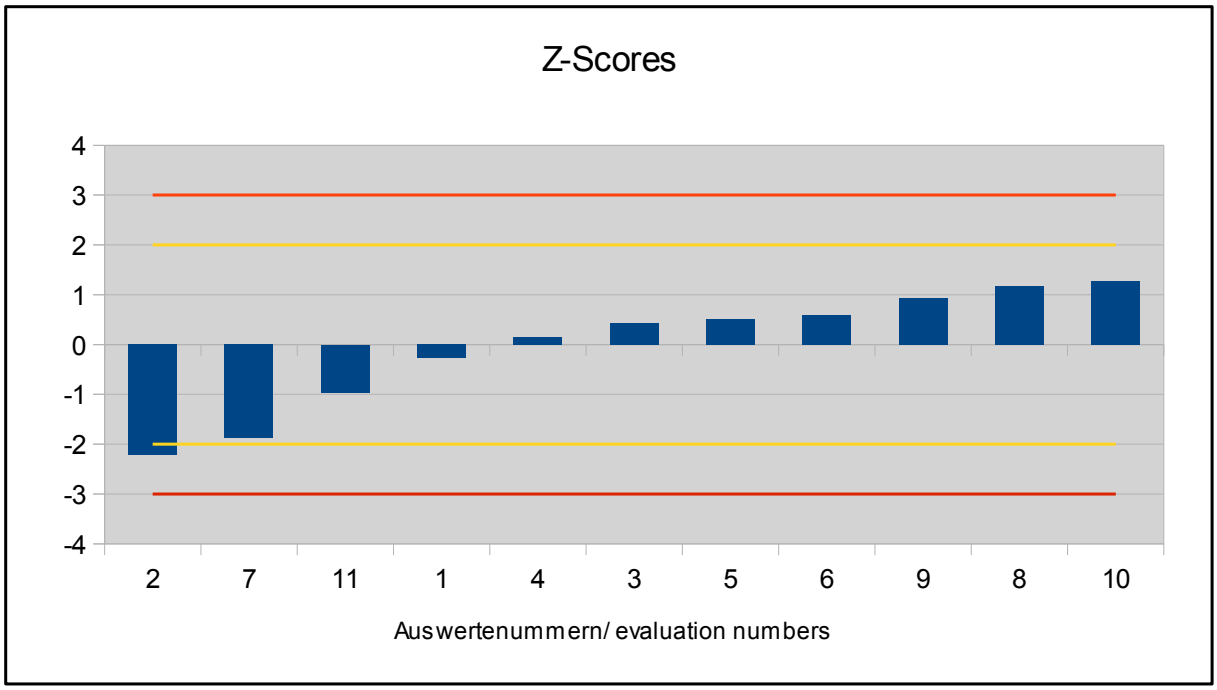


Abb. 3: Z-Scores Patulin
Fig. 3: Z-Scores Patulin

5. Documentation

5.1 Primary data

5.1.1 Patulin

Teilnehmer/ participant	Ergebnis/ result	DLA-Probe A/ sample A	DLA-Nr Probe B/ sample B	Ergebnis Probe A/ result sample A	Ergebnis Probe B/ result sample B	Wieder-findungsrate / recovery
	µg/kg			µg/kg	µg/kg	in %
1	86	3	42	86	86	93
2	46,9	7	36	45,1	48,7	113
3	100	16	56	104	102	100
4	94,27	17	30			100
5	101,39	34	58		.	108,04
6	103,3	5	28	104,2	102,4	
7	51,4*	23	46	51,2*	51,5*	104
8	115	12	45	112	118	73
9	110	24	50	115	106	81
10	117	9	21	111,3	121,8	86,5
11	72	69	75	69	75	100

*** Result was given in µg/l.**

5.2 Homogeneity

5.2.1 Homogeneity testing before PT

To verify the homogeneity of the test material the content of sorbic acid was determined in a 5-fold determination by HPLC/UV (ASU §64 LFGB L 15.00-9).

Probe/ sample	Sorbinsäure/ sorbic acid		
1	153	mg/l	
2	154	mg/l	
3	154	mg/l	
4	153	mg/l	
5	154	mg/l	
Mittelwert/ mean	**153,6**	**mg/l**	
Standardabw./ standard deviation	**0,55**	**0,4**	**%**

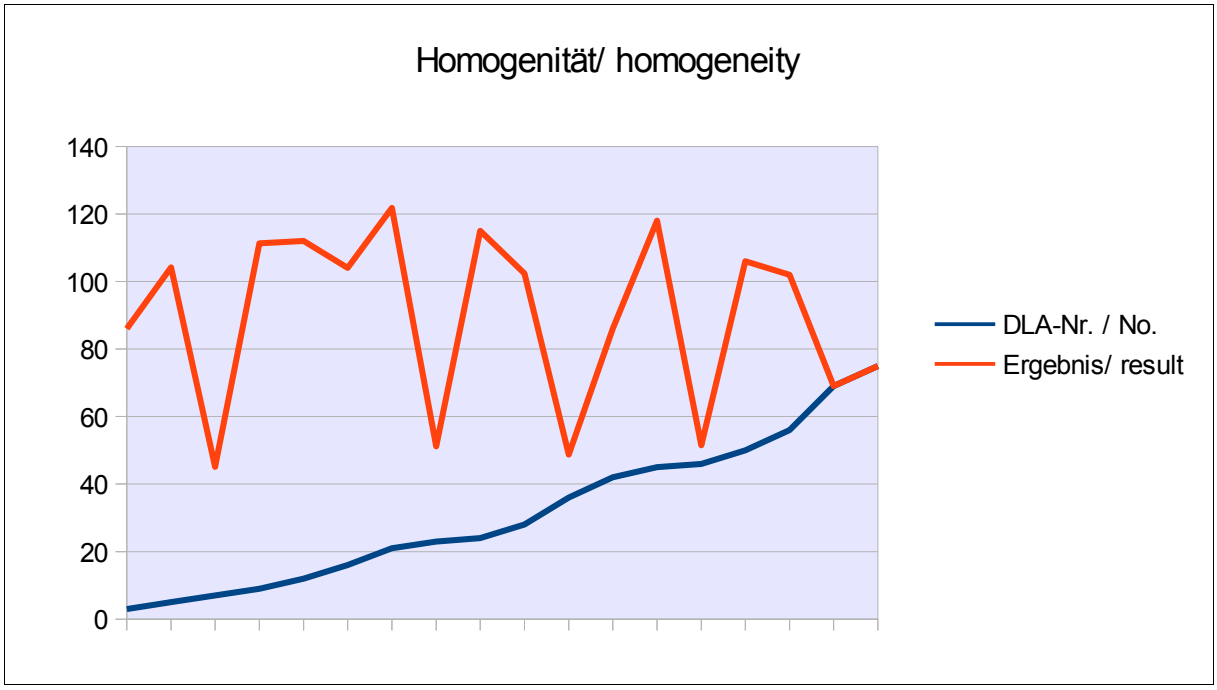
5.2.2 Repeatability standard deviation of participants

The repeatability standard deviations were calculated with the data documented in chapter 5.1, see also statistic data 4.1 .
It is 5,61 µg/kg = 5,1 % of X_{pt} (Patulin).

In the ASU L48.03-2 and ASU L31.00-20 the relative repeatability standard deviations were determined in a comparable range for apple juice.

5.2.3 Comparison of sample number/test result

The comparison of the increasing sample-numbers and measured patulin-results shows a sufficient homogeneity.



5.3 Analytical methods

To the participants:

5.3.1 Patulin

Teilnehmer/ Participant	**Methode/ method**	**Wiederfindung** mit gleicher Matrix/ recovery with the same matrix	**Akkreditiert/ accrededed**	**Sonstige Hinweise/ remarks**
		yes/no	yes/no	
1	DIN EN 1417 mod.	yes	yes	
2		yes	yes	
3	Patulin LM (LC/MS-MS)	yes	yes	Recovery with C13-Patulin, inlet temperature 21°C
4	§ 35 LMBG 31.00-20	yes (internal standard)	no	
5	Determination of Patulin with HPLC-DAD after cleaning with solid phase extraction	yes	yes	Recovery included
6	Determination of Patulin with LC-MS/MS	yes	yes	measurement uncertainly $U_{(k=2)}$ = 34%
7	SOP Patulin; modif. according to ISO 8128-1	yes	no	Results in µg/L; analysed samples after enzymatic pretreatment with reduced chemical quantity
8	Determination of Patulin in apple juice and other fruit- and vegetabel juices, also for infants and young children, with HPLC (PV3065 (2014-05))	yes (with similar matrix apple juice)	yes	
9	after DIN 15890, HPLC-DAD	yes	yes	
10	ASU §64 LFGB 31.00 - 20	yes	no	
11	LC/MS/MS after liquid/ liquid extraction	yes	no	

6. Index of participant laboratories

Teilnehmer/ Participant	Ort/ Town	Land/ country
		Austria
		Deutschland
		Deutschland
		Belgium
		Deutschland
		Deutschland
		Deutschland
		Deutschland
		Deutschland
		Deutschland
		Deutschland

[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)
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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. EG-VO 401-2006 zur Festlegung der Probenahmeverfahren und Analysemethoden für die amtliche Kontrolle des Mykotoxingehalts von Lebensmitteln
15. ASU §64 LFGB L31.00-20, Bestimmung von Patulin in klarem und trüben Apfelsaft und Apfelpüree, HPLC-Verfahren mit Reinigung durch Flüssig/Flüssig-Verteilung (Dezember 2004)
16. ASU §64 LFGB L48.03-03, Bestimmung von Patulin in Fruchtsaft und Obstbrei für Säuglinge und Kleinkinder, HPLC-Verfahren mit Reinigung durch Flüssig/Flüssig-Verteilung (Februar 2014)
17. Leitsätze des Deutschen Lebensmittelbuchs für Fruchtsäfte (2002)
18. ISO 8128-1:1993 Apple juice, apple juice concentrates and drinks containing apple juice – Dertermination of patulin content – Part 1: Method using high-performance liquid chromatography