DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

DLA 17/2014

Patulin in apple juice

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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. 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 validity of the particular testing method.
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.

2. Realisation

2.1 Test material

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

2.1.1 Homogeneity

The calculation of the repeatability standard deviation of the participants for patulin was used as an indicator of homogeneity. The result is comparable to the repeatability standard deviation of the method ASU § 64 LFGB 31.00-20/ cloudy apple juice (14). The repeatability standard deviation of the participants is given in the documentation.
Additionally 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.

2.2 Test

Two portions of test material were sent to every participating laboratory in the 18th week of 2014. The testing method was optional. The tests should be finished at 13. June 2014 the latest.

2.3 Results

The participants submitted their results in standard forms, which have been handed out with the samples. The finally calculated concentrations of patulin as average of duplicate determinations of both numbered samples was used for the statistical evaluation.
Queried and documented were single results, recovery and the testing method used.

3. Evaluation

3.1 Assigned value

Because the analysed material was no certified reference material the robust mean of the submitted results was used as assigned value X (6). The distribution of submitted results showed no hint for bimodal distribution or other reasons for a higher variability.

3.2 Standard deviation

For comparison to the target standard deviation a robust standard deviation (S_x) was calculated (6).

3.3 Outliers

Statistical outliers were determined by Mandel´s-H-Statistic (significance level: 5%) (5). Detected outliers were stated for information only, when z-score simultaneously was < -2 or > 2.

3.4 Target standard deviation

The target standard deviation of the assigned value is determined according to the following methods.

3.4.1 General model (Horwitz)

The relative target standard deviation in % of the assigned value is calculated according to the following equation.

$$\hat{\sigma} \ (\%) = 2^{(1-0,5\log X)}$$

Out of this is calculated the target standard deviation in µg/kg

$$\hat{\sigma} = X * \hat{\sigma} \ (\%) / 100.$$

3.4.2 Precision experiment

Using the reproducibility standard deviation σ_R and the repeatability standard deviation σ_r of a precision experiment the between-laboratories standard deviation (σ_L) can be calculated :

$$\sigma_L = \sqrt{(\sigma_R^2 - \sigma_r^2)} \quad .$$

And then, using the number of replicate measurements n, each participant is to perform, the standard deviation for proficiency assessment is calculated:

$$\hat{\sigma} = \sqrt{(\sigma_L^2 + (\sigma_r^2/n))} \quad .$$

From the precision data of ASU § 64 LFGB L 31.00-20 (14) for the quantitative estimation with HPLC/ cloudy apple juice (x=26µg/l), a target standard deviation of 30,7 % for patulin is received. This target standard deviation was given only for information in the evaluation.

3.5 z-Score

To assess the results of the participants the z-score is used. It indicates about which multiple of the target standard deviation ($\hat{\sigma}$) the result (x) of the participant is deviating from the assigned value (X)(6).

Participants' z-scores were derived as:

$$z = (x - X) / \hat{\sigma} ;$$

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

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

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

Following the Horrat-value the results of a proficiency-test (PT) can be considered convincing, if the quotient of robust standard deviation and target standard deviation 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.

The results of this PT show sufficient comparability.

3.7 Standard uncertainty

The assigned value X has a standard uncertainty u_X which depends on the analytical method, differences between the analytical methods used, the test material, the number of participant laboratories and perhaps on other factors. The standard uncertainty (u_X) for this PT is calculated as follows (6).

$$u_X = 1{,}25 * S^x/\sqrt{(p)}$$

If $u_X \leq 0{,}3 * \hat{\sigma}$ the standard uncertainty of the assigned value needs not be included in the interpretation of the results of the PT (6). The Quotient $u_X/\hat{\sigma}$ is reported in the characteristics of the test.

4. Results

All following tables are anonymized. With the delivering of the evaluation-report the participants are informed about their individual evaluation-number.

For comparison the maximum levels for patulin (EC-VO 1881/2006):

Food	**Maximum level**
1. Fruit juices, concentrated fruit juices as reconstituted and fruit nectars	50 µg/kg
2. Spirit drinks, cider and other fermented drinks derived from apples or containing apple juice	50 µg/kg
3. Solid apple products, including apple compote, apple puree intended for direct consumption	25 µg/kg
3. Apple juice and solid apple products, including apple compote and apple puree, for infants and young children	10 µg/kg

In the upper table – test - the characteristics are listed:

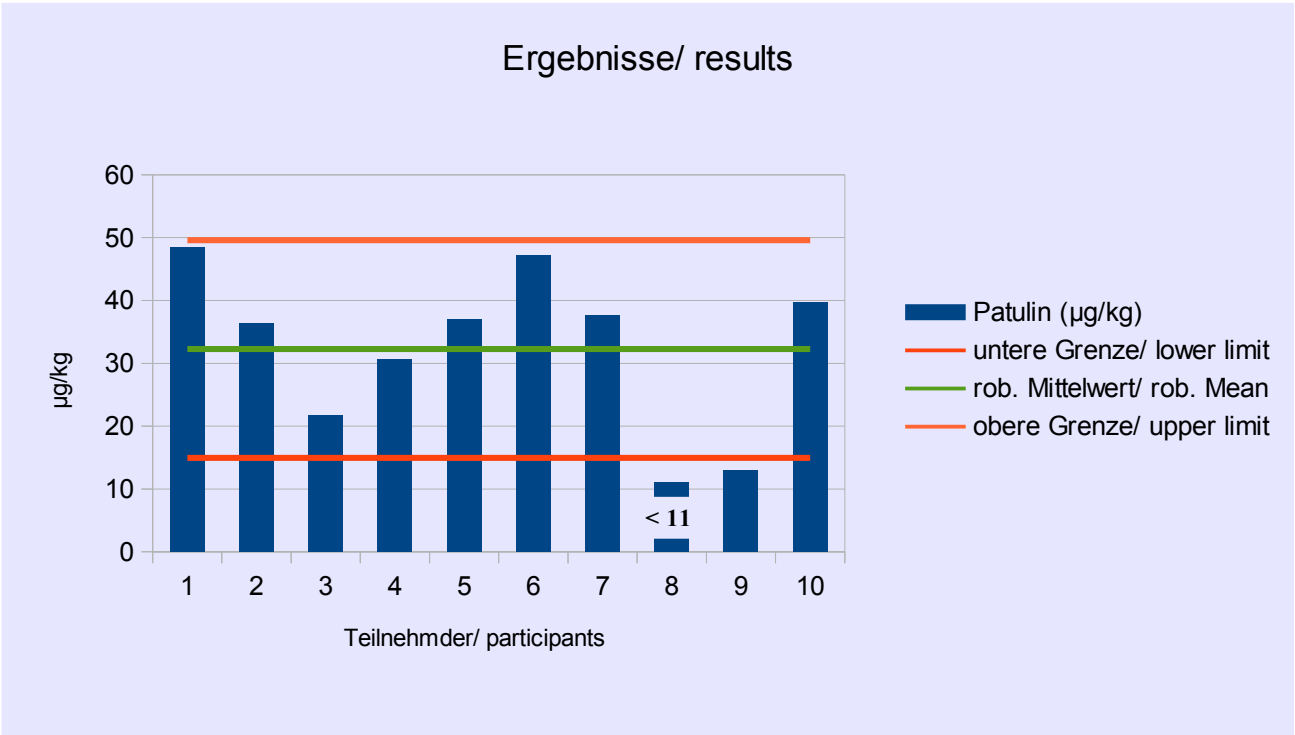
Number of results	
Number of outliers	
Mean	
Median	
Robust mean (X)	
Robust standard deviation (S^x)	
Target standard deviation($\hat{\sigma}$)(according to Horwitz)	
Target standard deviation (ASU § 64 LFGB L 31.00-20) only for information	
Lower limit of target range (X - 2 $\hat{\sigma}$)	
Upper limit of target range (X + 2 $\hat{\sigma}$)	
Quotient $S^x/\hat{\sigma}$	
standard uncertainty u_x	
Quotient $u_X/\hat{\sigma}$	
Number of results in the target range	

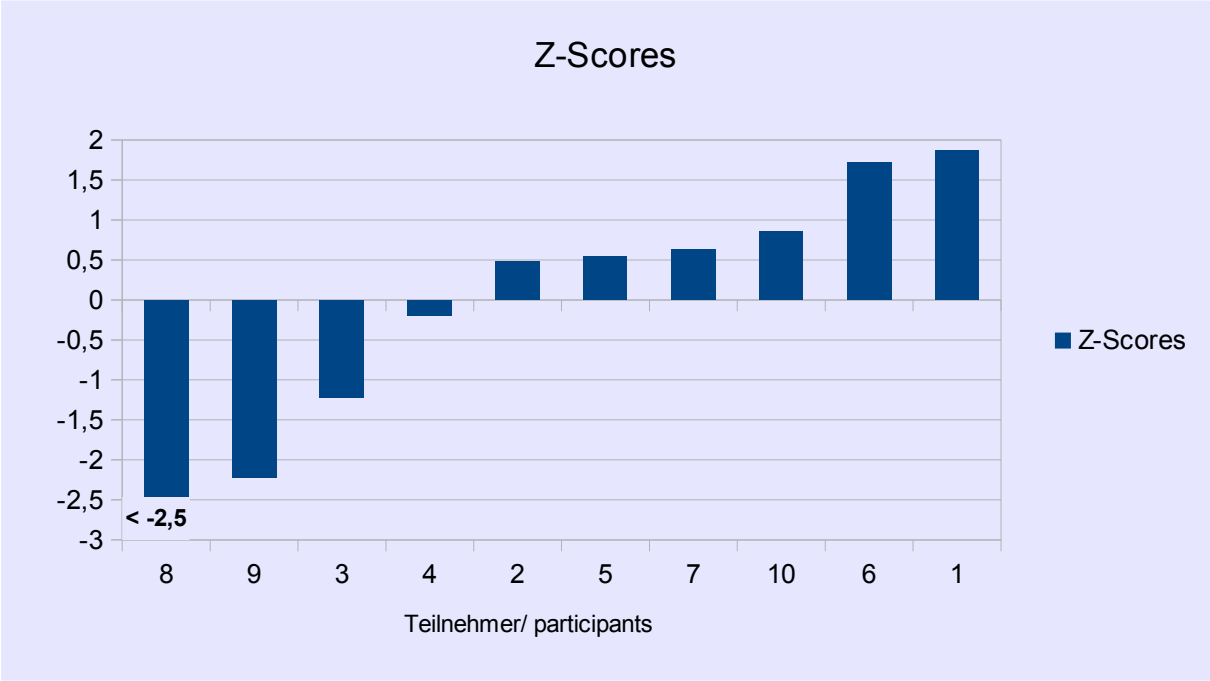
In the lower table - Laboratories - the individual results of the participating laboratories are listed:

Evaluation number	Result	Deviation	z-Score	Remark

4.1 Patulin in µg/kg

Characteristics	
Number of results	10
Number of outliers	0
Mean	32,3
Median	36,7
Robust mean (X)	32,3
Robust standard deviation (S^x)	14,9
Target standard deviation($\hat{\sigma}$)(Horwitz)	8,7
Target standard deviation (ASU § 64 LFGB L 31.00-20) for information	9,9
Lower limit of target range (X - 2 $\hat{\sigma}$)	15,0
Upper limit of target range (X + 2 $\hat{\sigma}$)	49,6
Quotient $S^x/\hat{\sigma}$	1,7
standard uncertainty u_x	5,9
Quotient $u_X/\hat{\sigma}$	0,68
Number of results in the target range	8 (80%)





Laboratories

Teilnehmer/ participants	Patulin (µg/kg)	Abweichung/ deviation	Z-Scores	Bemerkungen/ remarks
1	48,49	16,2	1,9	
2	36,4	4,12	0,5	
3	21,7	-10,6	-1,2	
4	30,6	-1,69	-0,2	
5	37	4,72	0,5	
6	47,2	14,9	1,7	
7	37,7	5,42	0,6	
8	< 11	< -21,3	**< -2,5**	result outside target range
9	13,06	-19,3	**-2,2**	
10	39,7	7,42	0,9	

5. Documentation

5.1 Primary data

5.1.1 Patulin

Teilnehmer/ partcipants	Patulin	Probe/ sample A	Probe/ sample B	Wiederfindung/ recovery	Bemerkungen/ remarks
	µg/kg	µg/kg	µg/kg	in %	
1	48,49	47,03	49,94	100	The sample material was unusually cloudy and after centrifugation we recieved strong sedimentation.
2	36,4	37,5	35,2	85,6	
3	21,7	23,1	20,2	85	
4	30,6	32,8	28,3	100	
5	37	37	36		
6	47,2	37,5	56,9		
7	37,7	37,7	37,7	89	
8	< 11	< 11	< 11	n.d.	lowest calibration point 11 µg/L, strong interfe- rences caused by crystalisation in both samples
9	13,06	12,39	13,72	95	
10	39,7	38,0	41,4	59	

5.2 DLA-portion-numbers and homogeneity

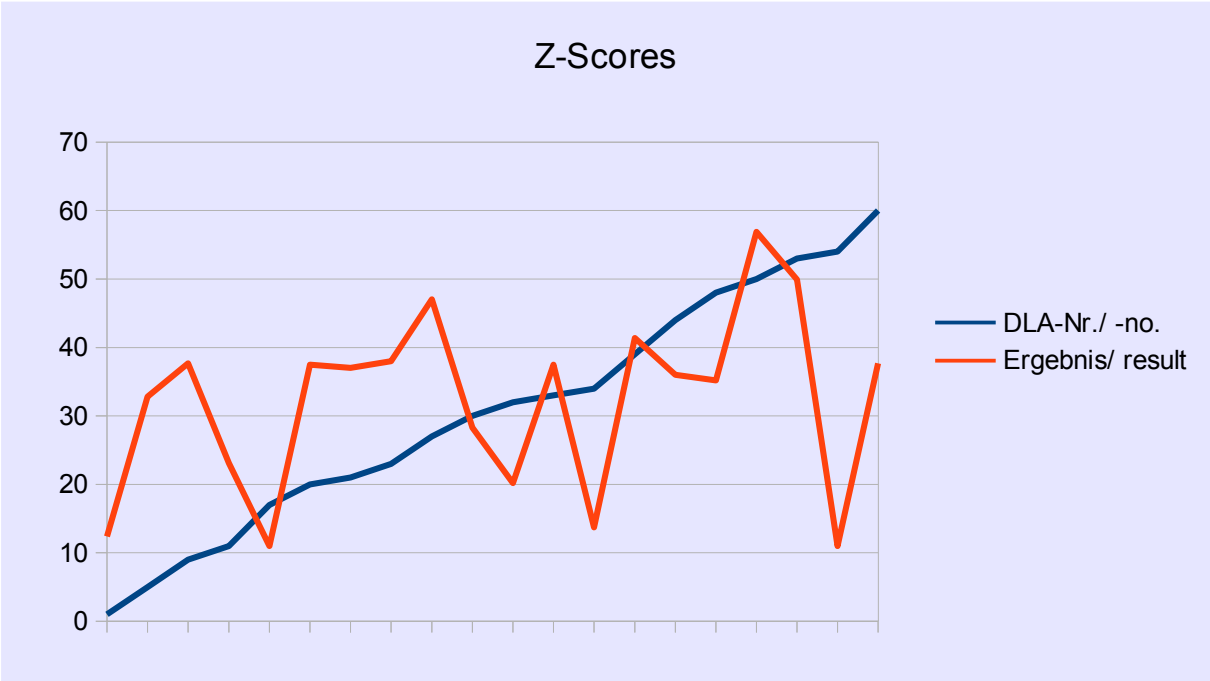
5.2.1 Repeatability standard deviation of participants

The repeatability standard deviation was calculated. The results are documented in 5.1.
It is 6,5 µg/kg = 20,1 % of X (Patulin).

Thus the repeatability standard deviation was comparable to ASU L31.0-20 for cloudy apple juice with a patulin content of 26 µg/kg. There the repeatability standard deviation of 14 % was estimated.

5.2.2 Comparison of sample number/test result

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



5.3 Analytical methods

Teilnehmer/ participants	Methode/ method	Wiederfindung mit gleicher Matrix/ recovery with same matrix	Akkreditiert/ accredited	Hinweise/ remarks
1	5,0g sample with 5,0ml Water; addition of Patulin C13 ISTD 25µg/kg 15min centrifugation; supernatant cleaning with Oasis HLB SPE-cartridge; elution with TBME/Ethylacetat 90/10; Evaporation of the solvent; add in 15% Acetonitril/water; membrane filtration; HPLC-MS/MS Acquity BEH Shield gradient separation ESI negativ MRM (3 tran-sitions)	no	yes	The sample material was unusually cloudy and after cen-trifugation we recieved strong sedi-mentation.
2	Determination of patulin by LC-MS/MS after QUECHERS extraction	jes	no	
3	§ 64 LFGB L31.00-20	no	yes	
4	Extraction with Ethylacetat, determination with LC-MS/MS	-	no	
5	Extraction with Ethylacetat and HPLC		no	
6	HPLC-UV	yes	yes	
7	Patulin (HPLC)	yes	yes	
8	In house method according to "Mycotoxins from Silage Samples Using Strata-X" Fa. Phenomenex"	no	no	lowest cali-bration- point 11 µg/L, strong interfe-rences caused by crystalisation in both samples
9	In house method	yes	yes	
10	In house method (HPLC-MS/MS), PV 805212	yes	yes	

6. Index of participant laboratories

Teilnehmer/ participant	Ort/ location
	Germany
	Belgium
	Germany
	Germany
	Germany
	Germany
	Germany
	Germany
	The Netherlands
	Germany

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

7. Index of literature

1. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
2. Verordnung / Regulation 882/2004/EU; Verordnung über amtliche Kontrollen / Regulation on official controls
3. 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
4. Richtlinie / Directive 1993/99/EU; über zusätzliche Maßnahmen im Bereich der amtlichen Lebensmittelüberwachung / on additional measures concerning the official control of foodstuffs
5. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung
6. DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Chemistry Laboratories ; Pure Appl Chem, 78, 145 – 196 (2006)
9. Evaluation of analytical methods used for regulation of food and drugs; W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
10. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
11. 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)
12. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
13. 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)
14. 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)
15. EG-VO 401-2006 zur Festlegung der Probenahmeverfahren und Analysemethoden für die amtliche Kontrolle des Mykotoxingehalts von Lebensmitteln

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