DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

DLA 14/2014

Ochratoxin A in coffee

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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. 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 principles of the DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2009.

2. Realisation

2.1 Test material

The test material was a mixture of different sorts of roasted coffee with a natural content of ochratoxin A and fructose (1%) added for the homogeneity test. Approximately 2,5 kg of the material were homogenized and then packaged lightproof in portions to approximately 50 g. The portions were numbered chronologically. The material was checked for homogeneity.

2.1.1 Homogeneity

To verify the homogeneity of the test material fructose was added before homogenisation. The homogeneity was examined with HPLC. The homogeneity is considered verified with a standard deviation of 6,1 %, see documentation (5.2.2).

The calculation of the repeatability standard deviation of the participants was used as an indicator of homogeneity. The result is similar to the repeatability standard deviation of the official method ASU § 64 LFGB L15.03-1. 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 ochratoxin A. 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 46th week of 2014. The testing method was optional. The tests should be finished at 9. January 2015 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 ochratoxin A as mean of duplicate determinations of both numbered samples were used for the statistical evaluation.

Queried and documented were single results, recovery and the used testing method . All participants submitted their results in time.

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

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

For analytes with a content below 120 µg/kg after the evaluation of a lot of mycotoxin-proficiency testing schemes after 1997 Thompson suggested for the target standard deviation a steady value of 22 % (11), analogical

$$\hat{\sigma} = 0,22 \ C / mr$$

with $\hat{\sigma}$ = Target standard deviation for contents < 120 µg/kg
C = measured value, expressed as a dimensionless mass ratio
mr = dimensionless mass ratio.

The target standard deviation according to Horwitz/Thompson was used.

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:

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

The precision data of the method ASU § 64 LFGB 15.03-1 for a comparable ochratoxin A content/ roasted coffee(14) result in a relative target standard deviation of 13,9 % for ochratoxin A. This target standard deviation is given 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:

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

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.

3.7 Standard uncertainty

The assigned value X has a standard uncertainty u_X that 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 to 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

For information: the legal value is at a maximum of 5,0 µg/kg (Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs).

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

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($\hat{\sigma}$)(Horwitz/Thompson)	
Target standard deviation (ASU § 64 LFGB 15.03-1 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 line with previous DLA-practice the results are evaluated according to Horwitz/Thompson. Additional the results are evaluated according to Horwitz in accordance to the EG-GL 401-2006 (15). In the evaluation the results are shown consecutive equivalent.

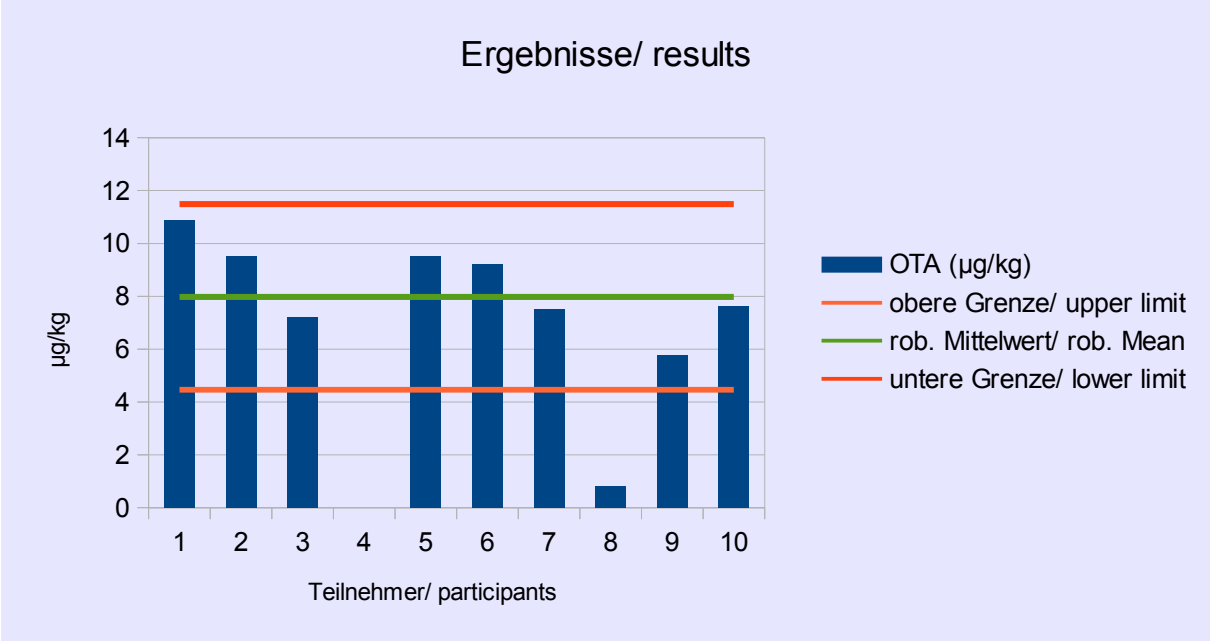
In the lower table - Laboratories - the individual results of the participating laboratories are listed:

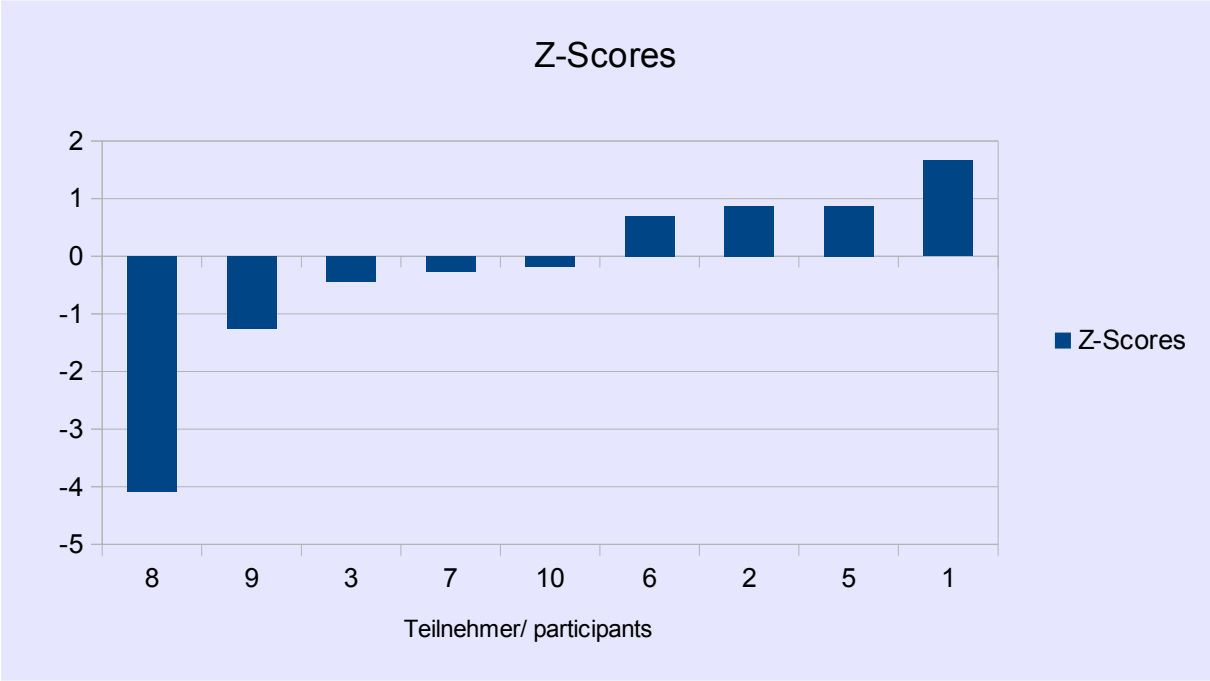
Evaluation number	Result	Deviation	z-Score	Remark

4.1 Ochratoxin A in µg/kg

Participant no. 4 was not considered in the statistical evaluation.

Characteristics	
Number of results	9
Number of outliers	1
Mean	7,6
Median	7,6
Robust mean (X)	8,0
Robust standard deviation (S^x)	2,3
Target standard deviation($\hat{\sigma}$) (Horwitz/Thompson)	1,8
Target standard deviation (ASU § 64 LFGB 15.03-1 for information)	1,1
Lower limit of target range (X - 2 $\hat{\sigma}$)	4,5
Upper limit of target range (X + 2 $\hat{\sigma}$)	11,5
Quotient $S^x/\hat{\sigma}$	1,3
Standard uncertainty u_x	0,94
Quotient $u_X/\hat{\sigma}$	0,5
Number of results in the target range	8 (89%)





Laboratories

Teilnehmer/ participants	OTA	Abweichung/ deviation	Z-Score	Bemerkung/ remark
	µg/kg	µg/kg		
1	10,9	2,92	1,7	
2	9,5	1,52	0,9	
3	7,2	-0,78	-0,4	
4	> 54		(> 25)*	
5	9,5	1,52	0,9	
6	9,2	1,22	0,7	
7	7,5	-0,48	-0,3	
8	0,8	-7,16	**-4,1**	**Ausreißer/ outlier**
9	5,8	-2,19	-1,2	
10	7,6	-0,34	-0,2	

** only for information*

5. Documentation

5.1 Primary data

Teilnehmer/ paticipants	Ergebnis/ result	DLA-Nr Probe A/ DLA no. Sample A	DLA-Nr Probe B/ DLA no. Sample B	Ergebnis A/ result A	Ergebnis B/ result B	Wiederfindungsrate/ recovery
	µg/kg			µg/kg	µg/kg	in %
1	10,9	10	39			
2	9,5	22	43	9,2	9,9	73,2
3	7,2	2	13	7,2	7,2	95%
4	>54	20	48	>54	>54	
5	9,5*	6	23	9,23	9,75	79,8
6	9,2	25	40			
7	7,5	37	4	7,5	7,4	97
8	0,82	15	30	0,83	0,8	
9	5,79	14	27	5,94	5,64	105
10	7,64	21	38	7,67	7,6	90

** The mean of the results was calculated by DLA*

5.2 Homogeneity

5.2.1 Repeatability standard deviation of participants

The repeatability standard deviation of the single results was calculated as documented in chapter 5.1.
It is 0,35 µg/kg = 4,4 % of X (Ochratoxin A).

In the ASU L15.03-1 a relative repeatability standard deviation of 11 % for a Ochratoxin A (medium content) and 2% for Ochratoxin A (high level) was determined for roasted coffee.

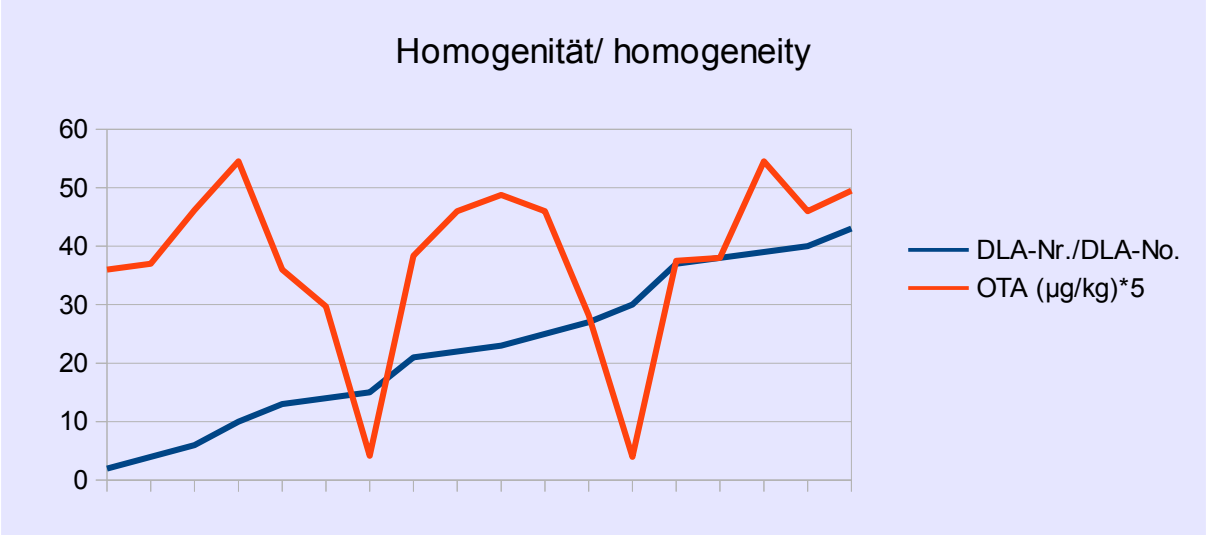
5.2.2 Homogeneity testing before PT

Probe/ sample	Fructose		
9	1,13	g/100g	
16	1,16	g/100g	
24	1,00	g/100g	
35	1,13	g/100g	
45	1,17	g/100g	
Mittelwert/ mean	1,1		
Standardabw./ standard deviation	0,07	6,1	%

To verify the homogeneity of the test material fructose was added before homogenisation additionally. The homogeneity was examined with HPLC.

5.2.3 Comparison of sample number/ test result

The comparison of the increasing sample-numbers and measured Ochratoxin A results (*5) shows homogeneity:



5.3 Analytical methods

Teilnehmer/ participant	**Methode/ method**	**Wiederfindung** mit gleicher Matrix/ recovery with the same matrix	**Akkreditiert/ accredited**	**Bemerkung/ remark**
1	HPLC		no	
2	Ochratoxin A, (HPLC-FLD), PV 805091:	yes	yes	
3	HPLC-FL	yes	yes	
4	ELISA: RIDASCREEN® Ochratoxin A 30/15 (R1311) in combination with RIDA Ochratoxin A columns (R1303), R-Biopharm.		no	Dilution factor 30. Resulting measuring range of RIDASCREEN Ochratoxin A 30/15: 1,5-54 ppb
5	HPLC-FLD	no	yes	
6	35.1 Determination of Ochratoxin A in roasted coffee		no	Mean of three-fold determination
7	ASU-L 15.03-1	yes	yes	
8	P313 020 03 HPLC		yes	
9	Ochratoxin A: wet chemical extraction and Clean Up with IAC. Determination with HPLC/FLD; Basic standard: for feedstuff EN 16007, for food EN 14132, EN 15829 and EN 15835	yes	yes	
10		yes	yes	LCMSMS after IAC-cleanup

*IAC = Immuno Affinity Column

6. Index of participant laboratories

Teilnehmer/ participant	Ort/ location	Land/ country
		Austria
		Spain
		Germany
		Belgium
		Spain
		Germany
		Netherlands
		Germany
		Germany
		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. ASU §64 LFGB 30.00-5: Bestimmung von Ochratoxin A in Korinthen, Rosinen, Sultaninen, gemischtem Trockenobst und getrockneten Feigen (Jan. 2011)
14. ASU §64 LFGB 15.03-1: Bestimmung von Ochratoxin A in Gerste (Jan. 2010)
15. EG-VO 401-2006 zur Festlegung der Probenahmeverfahren und Analysemethoden für die amtliche Kontrolle des Mykotoxingehalts von Lebensmitteln

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