DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

DLA 11/2014

Aflatoxins in Peanut

Dienstleistung Lebensmittel Analytik GbR
Pinnberg 5
22927 Großhansdorf - Germany

proficiency-testing@dla-lvu.de **www.dla-lvu.de**

Coordinator: Dr. G. Wichmann

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 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 peanut butter with a natural content of aflatoxins and glucose added for the homogeneity test. Approximately 2 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

The calculation of the repeatability standard deviation of the participants for total aflatoxins was used as an indicator of homogeneity. The result is similar to the repeatability standard deviation of the official method ASU § 64 LFGB 23.05-2 for pistachio paste (13). The repeatability standard deviation of the participants is given in the documentation.

To verify the homogeneity of the test material glucose was added before homogenisation additionally. The homogeneity was examined with glucose/ HPLC. The homogeneity is considered verified with a standard deviation of < 4%, see documentation (5.2.2).

2.2 Test

Two portions of test material were sent to every participating laboratory in the 8th week of 2014. The testing method was optional. The tests should be finished at 4. April 2014 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 aflatoxin B1 and total aflatoxins as average of duplicate determinations of both numbered samples was used for the statistical evaluation.
Queried and documented were single results, recovery and the used testing method for aflatoxin B1, B2, G1 and G2.
One participant submitted no result, all other at least one result.

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 Thompson (11) 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 23.05-2 for a comparable aflatoxin content/peanut butter (13) results in a relative target standard deviation of 18,3 % for aflatoxin B1 and of 25,1 % for total aflatoxin. 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.

In comparison to the year before for aflatoxin B_1 and for the total aflatoxins a sufficient comparability was achieved:

proficiency test	**Quotient** $S^x/\hat{\sigma}$	
	Aflatoxin B_1	Total aflatoxins
DLA 11-2010 (in nutmeg)	2,0	1,6
DLA 11-2011 (in nutmeg)	2,3	2,3
DLA 11-2012 (in nutmeg)	2,0	2,1
DLA 10-2012 (in peanut)	3,5	3,5
DLA 10-2013 (in nutmeg)	1,9	2,3
DLA 10-2014 (in peanut)	3,0	2,6

Using the target standard deviation of ASU § 64 LFGB 23.05-2 for a comparable aflatoxin content the quotient is similar. In this respect the results show a sufficient comparability.

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. In the present proficiency test a quotient > 0,3 was received. However the quotient $S^x/\hat{\sigma}$ and the repeatability standard deviation of the participants gave no reason for an objection.

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 upper table – test - the characteristics are listed:

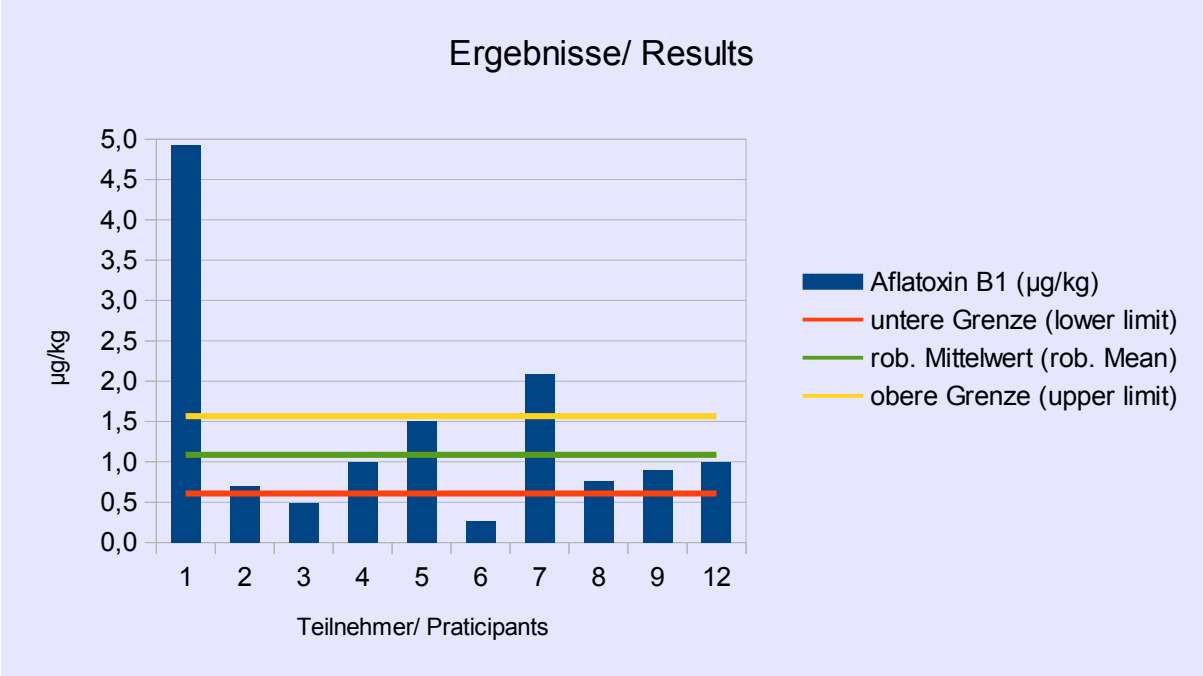
Number of results	
Number of outliers	
Mean	
Median	
Robust mean (X)	
Robust standard deviation (S^x)	
Target standard deviation($\hat{\sigma}$)(Horwitz/Thompson)	
Target standard deviation (ASU §64 LFGB 23.05-2) for information	
Lower limit of target range (X - 2 $\hat{\sigma}$)	
Upper limit of target 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	

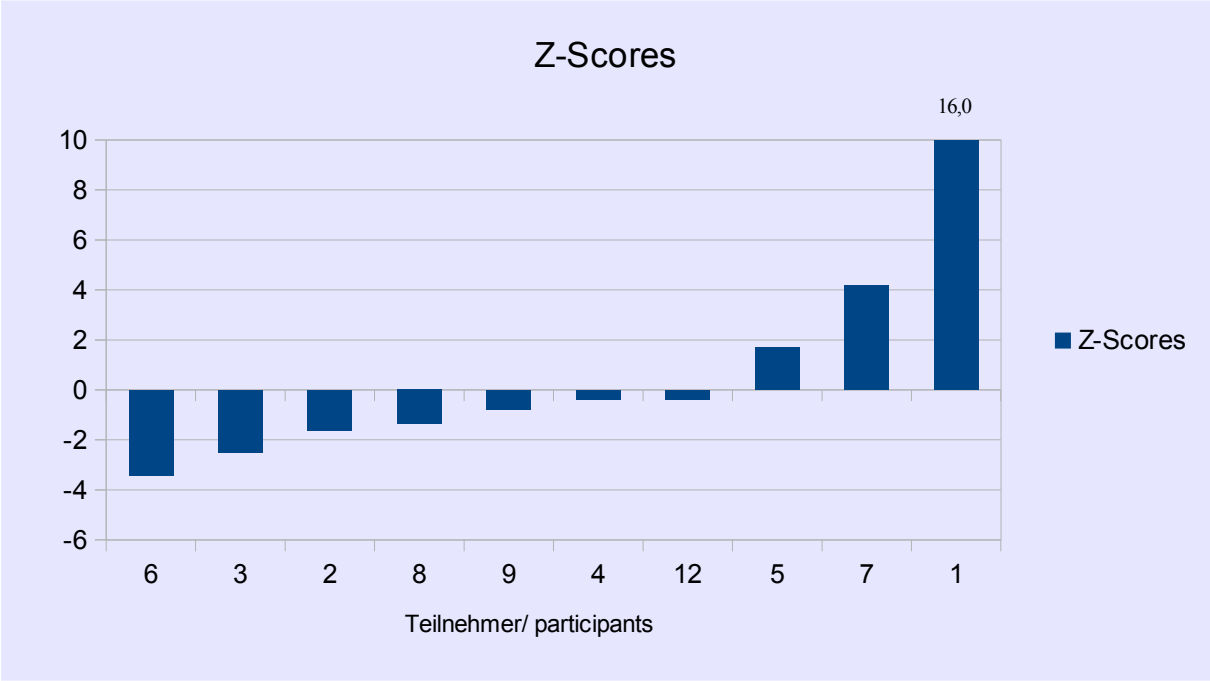
In the lower table - Laboratories - the individual results of the participating laboratories are listed:

Evaluation number	Result	Deviation	z-Score	Remark

4.1 Aflatoxin B_1 in µg/kg

Characteristics	
Number of results	10
Number of outliers	1
Mean	1,4
Median	1,0
Robust mean (X)	1,1
Robust standard deviation (S^x)	0,7
Target standard deviation($\hat{\sigma}$)	0,24
Target standard deviation (L 23.05-2 for information)	0,20
Lower limit of target range (X - 2 $\hat{\sigma}$)	0,6
Upper limit of target range (X + 2 $\hat{\sigma}$)	1,6
Quotient $S^x/\hat{\sigma}$	3,0
Standard uncertainty u_x	0,3
Quotient $u_X/\hat{\sigma}$	1,2
Number of results in the target range	6 (60%)





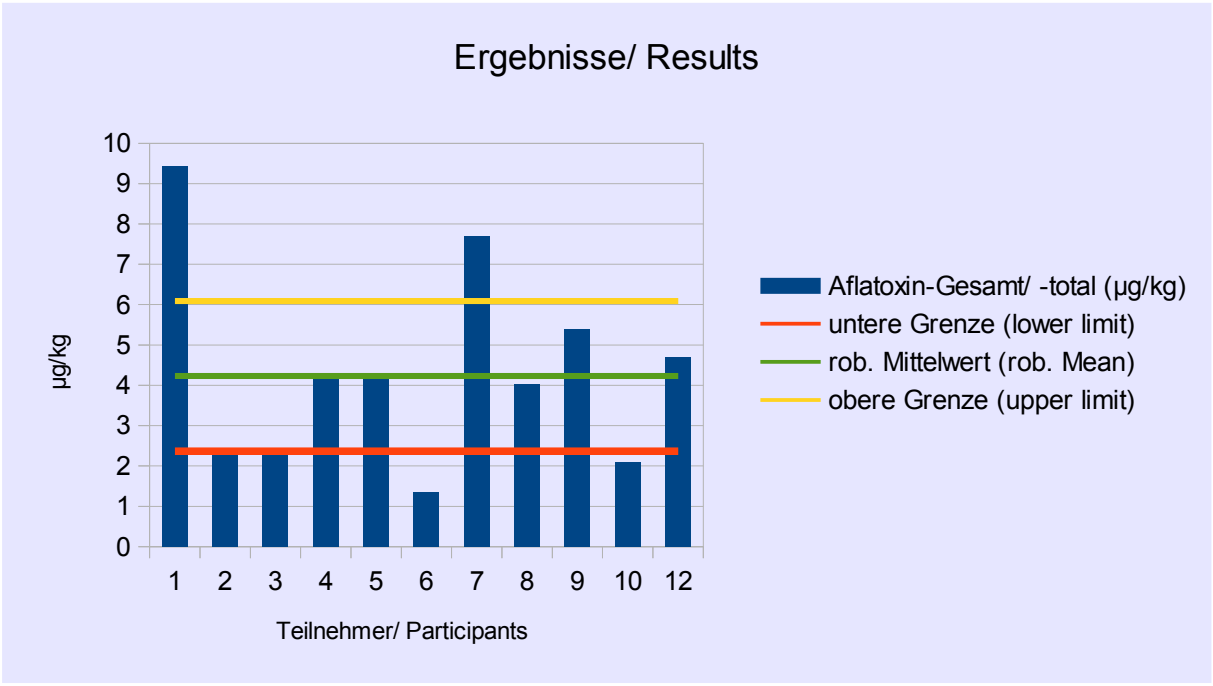
Laboratories

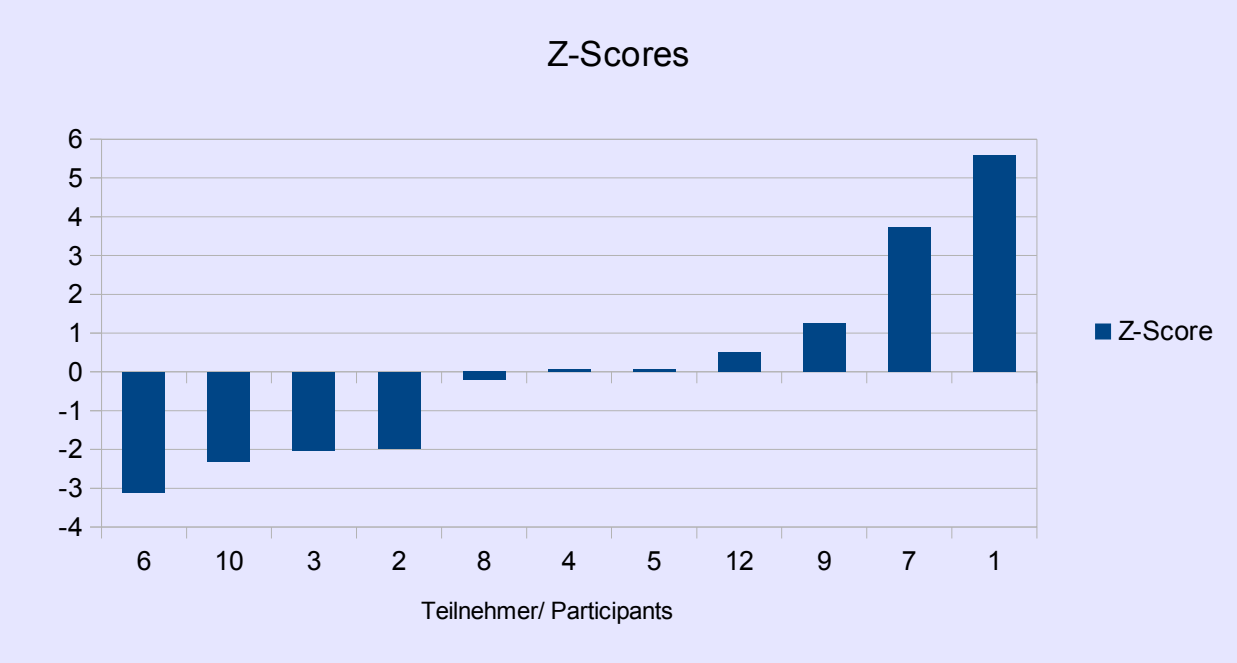
Teilnehmer/ participant	Aflatoxin B1 (µg/kg)	Abweichung/ deviation	Z-Score	Bemerkung/ remark
1	4,93	3,84	**16,0**	**Ausreißer/ outlier**
2	0,7	-0,39	-1,6	
3	0,49	-0,60	**-2,5**	
4	1	-0,09	-0,4	
5	1,5	0,41	1,7	
6	0,27	-0,82	**-3,4**	
7	2,09	1,00	**4,2**	
8	0,76	-0,33	-1,4	
9	0,9	-0,19	-0,8	
12	1,0*	-0,09	-0,4	

** = The mean was calculated by DLA*

4.2 Total aflatoxins in µg/kg

Characteristics	
Number of results (without no. 14)	11
Number of outliers	1
Mean	4,4
Median	4,3
Robust mean (X)	4,2
Robust standard deviation (S^x)	2,5
Target standard deviation($\hat{\sigma}$)	0,9
Target standard deviation (L 23.05-2 for information)	1,0
Lower limit of target range (X - 2 $\hat{\sigma}$)	2,4
Upper limit of target range (X + 2 $\hat{\sigma}$)	6,1
Quotient $S^x/\hat{\sigma}$	2,6
Standard uncertainty u_x	0,9
Quotient $u_X/\hat{\sigma}$	1,0
Number of results in the target range	7 (64%)





Laboratories

Teilnehmer/ participant	Aflatoxin-Gesamt/ -total (µg/kg)	Abweichung/ deviation	Z-Score	Bemerkung/ remark
1	9,42	5,19	**5,6**	**Ausreißer/ outlier**
2	2,4	-1,83	-2,0	
3	2,35	-1,88	-2,0	
4	4,3	0,07	0,1	
5	4,3	0,07	0,1	
6	1,34	-2,89	**-3,1**	
7	7,70	3,47	**3,7**	
8	4,03	-0,20	-0,2	
9	5,4	1,17	1,3	
10	2,08*	-2,15	**-2,3**	
12	4,7*	0,47	0,5	

** = The mean was calculated by DLA*

5. Documentation

5.1 Primary data

5.1.1 Aflatoxin B_1 in µg/kg

Teilnehmer/ participants	Aflatoxin B1	Probe/ sample A	Probe/ sample B	Wiederfindung/ recovery	Bemerkung/ remark
	µg/kg	µg/kg	µg/kg	in %	
1	4,93	4,98	4,88		Ausreißer/ outlier
2	0,7	0,52	0,87	92,1	
3	0,49	0,50	0,47	94,6	
4	1	1	0,96	103	
5	1,5	1,6	1,4	65	
6	0,27	0,30	0,24		
7	2,09	2,07	2,11	89,2	
8	0,76	0,74	0,77	80	
9	0,9	0,9	0,9	168	
10	n.b.	n.d.	n.d.		
11	n.b.	n.d.	n.d.		
12	1,0*	1,2	0,82	100	

** = The mean was calculated by DLA*

5.1.2 Aflatoxin B_2 in µg/kg

Teilnehmer/ participant	Probe/ sample A	Probe/ sample B	Wiederfindung/ recovery
	µg/kg	µg/kg	In %
1	0,92	0,58	
2	0,23	0,31	94,1
3	0,20	0,20	97,0
4	< 0,5	<0,5	116
5	0,54	0,45	62
6			
7	0,70	0,68	88,6
8	0,16	0,24	82
9	0,6	0,5	118
10			
11			
12	<0,5	<0,5	105
mean	0,48	0,42	
median	0,54	0,45	
standard deviation	0,043	0,019	

5.1.3 Aflatoxin G_1 in µg/kg

Teilnehmer/ participant	Probe/ sample A	Probe/ sample B	Wiederfindung/ recovery
	µg/kg	µg/kg	%
1	1,08	1,77	
2	1,6	2,8	102,1
3	1,27	1,34	99,2
4	2,6	2,7	109
5	2	1,8	119
6			
7	3,77	4,06	90,4
8	2,47	2,82	82
9	2,9	3,2	157
10			
11			
12	3,4	2,4	100
mean	2,34	2,54	
median	2,47	2,7	
standard deviation	0,93	0,83	

5.1.4 Aflatoxin G_2 in µg/kg

Teilnehmer/ participant	Probe/ sample A	Probe/ sample B	Wiederfindung/ recovery
	µg/kg	µg/kg	%
1	3,33	1,30	
2	0,39	0,54	103,8
3	0,35	0,38	96,7
4	0,51	0,5	118
5	0,4	0,4	88
6			
7	1,00	1,00	72,7
8	0,37	0,49	82
9	0,9	0,9	119
10			
11			
12	0,89	0,7	105
mean	0,90	0,69	
median	0,51	0,54	
standard deviation	0,95	0,31	

5.1.5 Total aflatoxins in µg/kg

Teilnehmer/ participant	Aflatoxin -Gesamt/ -total	Probe/ sample A	Probe/ sample B	Wiederfindung/ recovery	Bemerkung/ remark
	µg/kg	µg/kg	µg/kg	in %	
1	9,42	10,31	8,53		Ausreißer/ outlier
2	2,4	2,7	2		
3	2,35	2,31	2,38		
4	4,3	4,1	4,2		
5	4,3	4,5	4,1	88	
6	1,34	1,43	1,26		
7	7,70	7,54	7,85	---	
8	4,03	3,74	4,32	82	
9	5,4	5,3	5,6		
10	2,08*	1,65	2,5	60%	
11					
12	4,7*	5,4	3,9		

** = The mean 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 described in chapter 5.1.1.
It is 0,82 µg/kg = 19,5 % of X (Total aflatoxins).

In the ASU L23.05-2 the relative repeatability standard deviation of 20 % (Aflatoxin B_1) for a aflatoxin content x = 2,9 µg/kg was determined for pistachio paste (13).

5.2.2 Homogeneity testing before PT

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

Sample no.	**Glucose**		
37	21,1	g/100g	
25	20,7	g/100g	
16	22,0	g/100g	
10	22,1	g/100g	
4	22,8	g/100g	
mean	**21,7**		
standard deviation	**0,84**	**3,9**	**%**

5.3 Analytical methods

Teilnehmer/ participants	Methode/ method	Wiederfindung mit gleicher Matrix/ recovery with same matrix	Akkreditiert/ accredided	Sonstige Hinweise/ remarks
1	Determination HPLC with IAC.	no	no	
2	AT-010-003 (HPLC)	yes	yes	
3	IAC-HPLC-fluorescence detector	yes	yes	
4	LC/MS/MS	no	no	
5	5 g sample with 25 mL MeOH (80%) extraction and degrease with 12,5 mL n-hexan. 7 mL MeOH extract with mit PBS-buffer diluted to 50 mL and cleaning with IAC. The analyte was eluted with 2 mL MeOH und detected with HPLC-FLD.	yes	no	
6	HPLC	no	no	
7	PV 2.019 /004-06	yes	yes	
8	Chemical analysis of aflatoxins according to PV 3055 (L 23.05-2 (2004-7))	yes	yes	
9	5 g sample, chloroform extraction, cleaning of 0,25 g sample with IAC from 5% MeOH in water extract. IAC.: EASI-extract from r-biopharm, derivatisation with LC Tech UVE. HPLC-separation: Merck Superspher 60m RP Select-B 250x4mm. Fluorometric measurement: Ex=360nm, Em=435nm; Reference material from r-biopharm.	yes	no	To verify the recorery rate 5 g sample (18 and 33) was spiked with 2,0 µg of each of the 4 aflatoxins.
10	ELISA Veratox HS Aflatoxin-Kit	no	yes	
11	Ridarscreen kit		no	Kit screens for aflatoxin M1 only
12	HPLC-FL	yes	yes	

*IAC = Immuno Affinity Column

6. Index of participant laboratories

Teilnehmer/ participants	Ort/ location
	Switzerland
	Germany
	Germany
	New Zealand
	Belgium
	Germany
	Switzerland
	Germany
	Germany
	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 23.05-2 (Jan. 2012): Bestimmung von Aflatoxin B_1 und der Summe von Aflatoxin B_1, B_2, G_1 und G_2 in Erdnüssen, Pistazien, Feigen und Paprikapulver

Printed on 100% Recycling-paper