DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

DLA 16/2014

Aflatoxins + Ochratoxin A in spice mixture

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 spice mixture (main constituents are paprika (45%) and curcuma (45%)) with a natural content of ochratoxin A and aflatoxins and glucose (3,5%) added for the homogeneity test. Approximately 9 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 paprika (14). 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. The homogeneity was examined with glucose/ HPLC. The homogeneity is considered verified with a standard deviation of 4,3 %, see documentation (5.2.2).

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 30th week of 2014. The testing method was optional. The tests should be finished at 5. September 2014 the latest. One participant submitted the results after consultation later.

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, aflatoxin B1 and total aflatoxins 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 for aflatoxin B1, B2, G1, G2, total aflatoxins and ochratoxin A.
Two participants submitted no results, 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 Horwitz/Thompson (11) and additional according to Horwitz (9/13) 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/paprika(14) and ochratoxin A/sultans result in a relative target standard deviation of 14,6 % for aflatoxin B1, 26 % for total aflatoxin and of 13,5% for ochratoxin A. This target standard deviation is given for information in the evaluation.

Because the method was optional, the target standard deviation according to Horwitz/Thompson (11) and additional according to Horwitz (9/13) was used.

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. 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 or Horwitz)	
Target standard deviation 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	

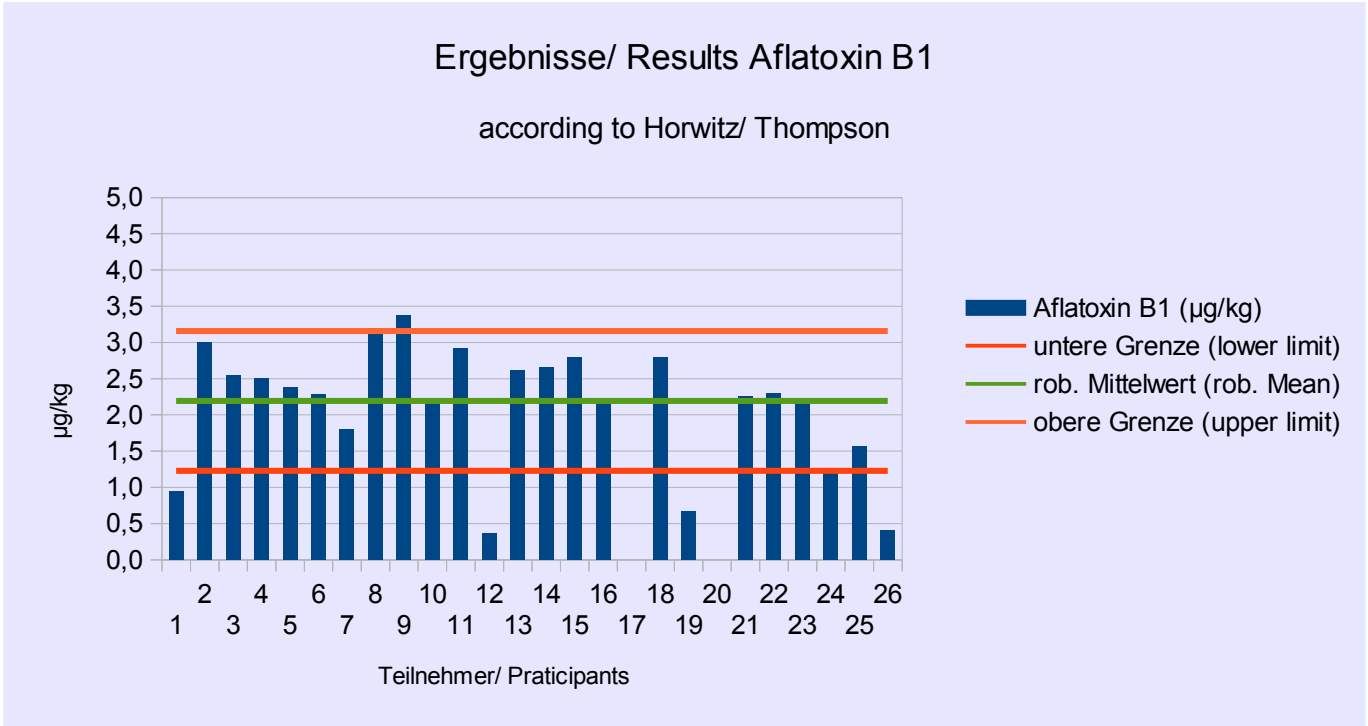
In line with previous DLA-practice the results are evaluated according to Horwitz/Thompson. Additional the results are evaluated according to Horwitz in accordance to the EG-GL 401-2006 (13). In the evaluation the results are shown consecutive equivalent.

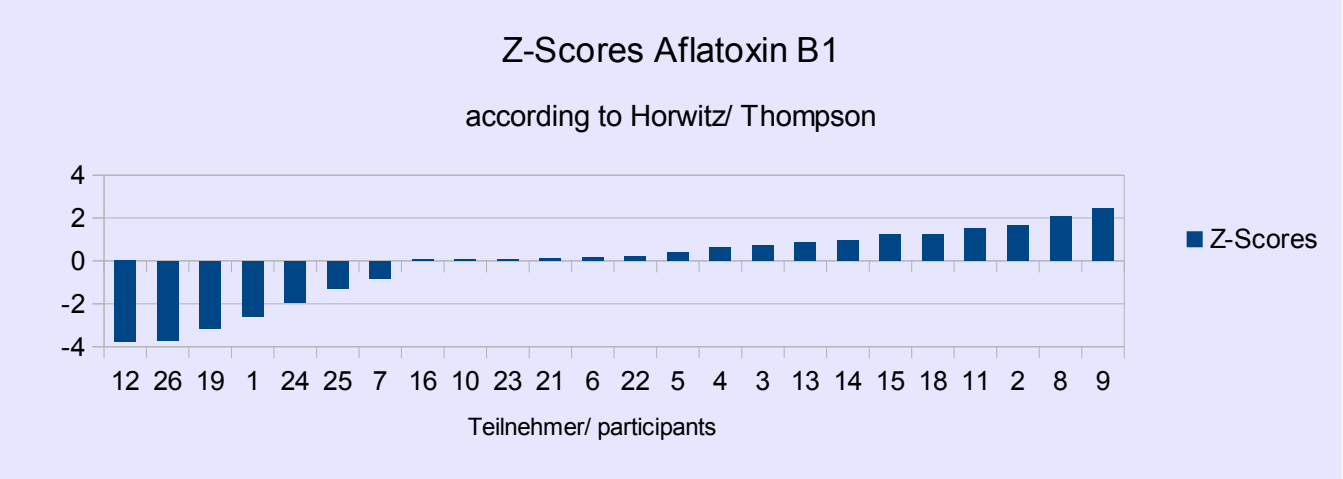
In the lower table - Laboratories - the individual results of the participating laboratories are listed:

Evaluation number	Result	Deviation	z-Score	Remark

4.1 Aflatoxin B_1 according to Horwitz/Thompson in µg/kg

Characteristics	
Number of results	24
Number of outliers	2
Mean	2,14
Median	2,29
Robust mean (X)	2,19
Robust standard deviation (S^x)	0,85
Target standard deviation($\hat{\sigma}$) (Horwitz/Thompson)	0,48
Target standard deviation (L 23.05-2 for information)	0,32
Lower limit of target range (X - 2 $\hat{\sigma}$)	1,23
Upper limit of target range (X + 2 $\hat{\sigma}$)	3,16
Quotient $S^x/\hat{\sigma}$	1,8
Standard uncertainty u_x	0,22
Quotient $u_X/\hat{\sigma}$	0,4
Number of results in the target range	18 (75%)





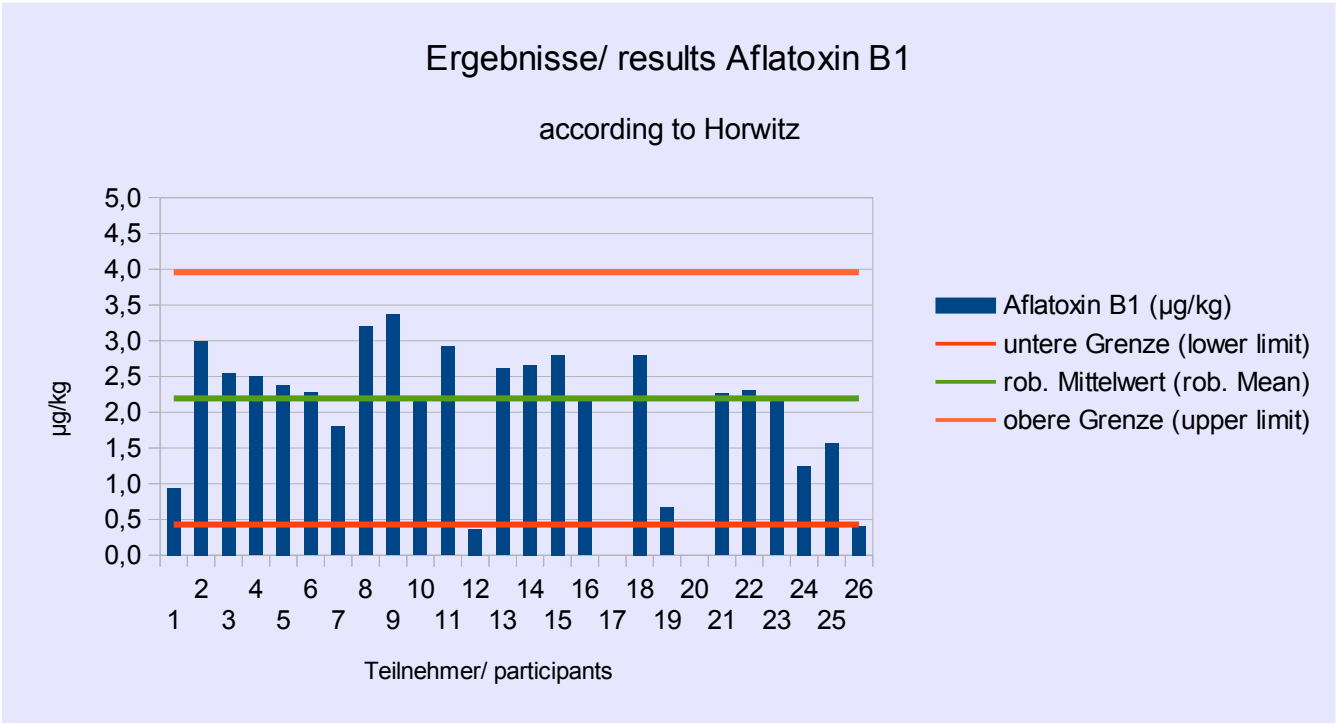
Laboratories

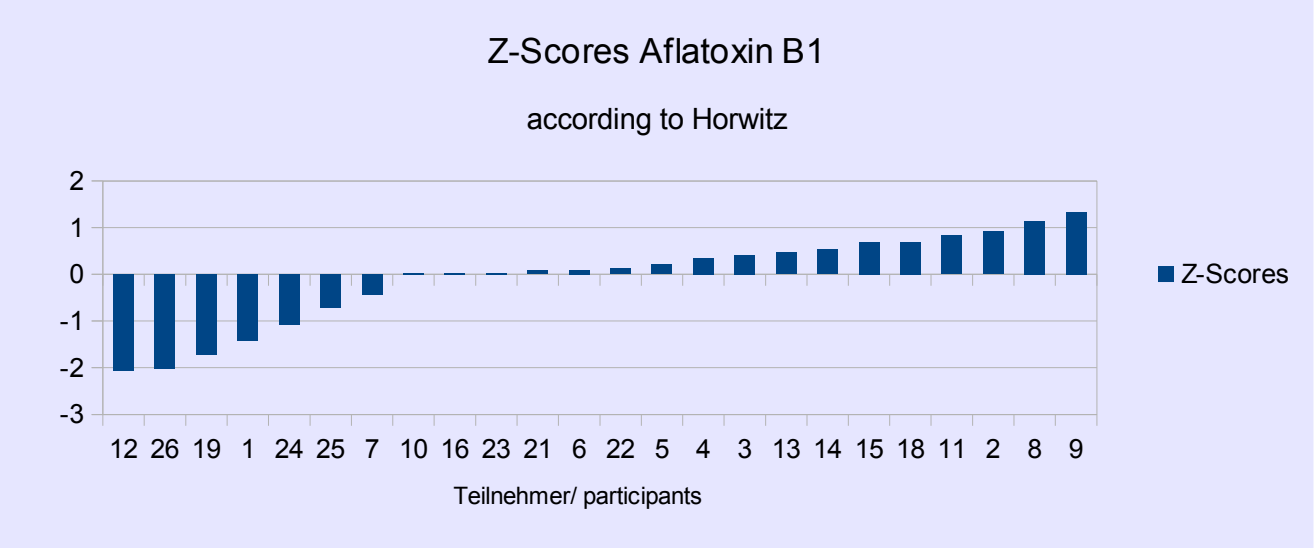
Teilnehmer/ participant	Aflatoxin B1	Abweichung/ deviation	Z-Score	Bemerkungen/ remarks
	µg/kg	µg/kg		
1	0,94	-1,25	**-2,6**	
2	3	0,81	1,7	
3	2,55	0,36	0,7	
4	2,5	0,31	0,6	
5	2,38	0,19	0,4	
6	2,28	0,09	0,2	
7	1,8	-0,39	-0,8	
8	3,20	1,01	**2,1**	
9	3,37	1,18	**2,4**	
10	2,22	0,03	0,1	
11	2,92*	0,73	1,5	A=4,93;B=0,91
12	0,37	-1,82	**-3,8**	Ausreißer/ outlier
13	2,61	0,42	0,9	
14	2,66	0,47	1,0	
15	2,8	0,61	1,3	
16	2,22	0,03	0,1	
17				
18	2,80	0,61	1,3	
19	0,67	-1,52	**-3,2**	
20				
21	2,26	0,07	0,1	
22	2,3	0,11	0,2	
23	2,22	0,03	0,1	
24	1,25	-0,94	-2,0	
25	1,57	-0,62	-1,3	
26	0,41	-1,78	**-3,7**	Ausreißer/ outlier

** = The mean was calculated by DLA*

4.2 Aflatoxin B_1 according to Horwitz in µg/kg

Characteristics	
Number of results	24
Number of outliers	1
Mean	2,14
Median	2,29
Robust mean (X)	2,19
Robust standard deviation (S^x)	0,85
Target standard deviation($\hat{\sigma}$) (Horwitz)	0,88
Target standard deviation (L 23.05-2 for information)	0,32
Lower limit of target range (X - 2 $\hat{\sigma}$)	0,43
Upper limit of target range (X + 2 $\hat{\sigma}$)	3,96
Quotient $S^x/\hat{\sigma}$	1,0
Standard uncertainty u_x	0,22
Quotient $u_X/\hat{\sigma}$	0,2
Number of results in the target range	23 (96%)





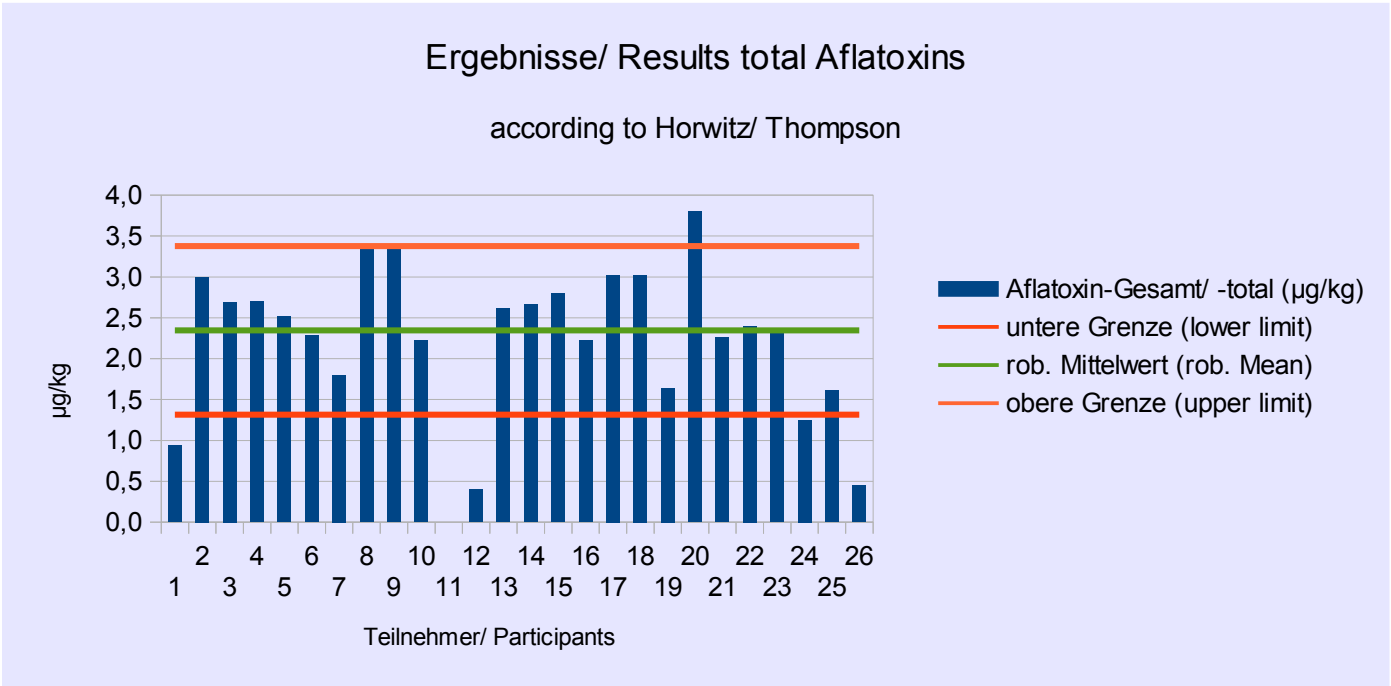
Laboratories

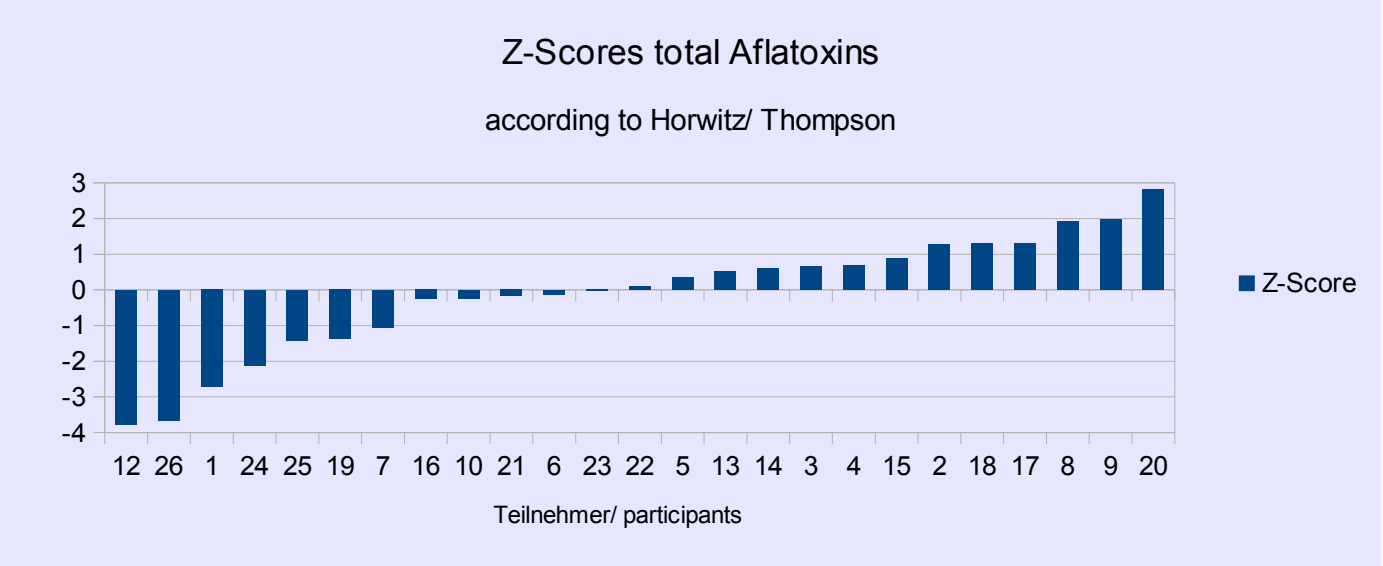
Teilnehmer/ participant	Aflatoxin B1	Abweichung/ deviation	Z-Score	Bemerkungen/ remarks
	µg/kg	µg/kg		
1	0,94	-1,25	-1,4	
2	3	0,81	0,9	
3	2,55	0,36	0,4	
4	2,5	0,31	0,3	
5	2,38	0,19	0,2	
6	2,28	0,09	0,1	
7	1,8	-0,39	-0,4	
8	3,20	1,01	1,1	
9	3,37	1,18	1,3	
10	2,22	0,03	0,0	
11	2,92*	0,73	0,8	A=4,93;B=0,91
12	0,37	-1,82	**-2,1**	Ausreißer/ outlier
13	2,61	0,42	0,5	
14	2,66	0,47	0,5	
15	2,8	0,61	0,7	
16	2,22	0,03	0,0	
17				
18	2,80	0,61	0,7	
19	0,67	-1,52	-1,7	
20				
21	2,26	0,07	0,1	
22	2,3	0,11	0,1	
23	2,22	0,03	0,0	
24	1,25	-0,94	-1,1	
25	1,57	-0,62	-0,7	
26	0,41	-1,78	-2,0	

** = The mean was calculated by DLA*

4.3 Total aflatoxins according to Horwitz/Thompson in µg/kg

Characteristics	
Number of results (without no. 14)	25
Number of outliers	2
Mean	2,29
Median	2,40
Robust mean (X)	2,35
Robust standard deviation (S^x)	0,83
Target standard deviation($\hat{\sigma}$)(Horwitz/Thompson)	0,52
Target standard deviation (L 23.05-2 for information)	0,61
Lower limit of target range (X - 2 $\hat{\sigma}$)	1,31
Upper limit of target range (X + 2 $\hat{\sigma}$)	3,38
Quotient $S^x/\hat{\sigma}$	1,6
Standard uncertainty u_x	0,21
Quotient $u_X/\hat{\sigma}$	0,4
Number of results in the target range	20 (80%)





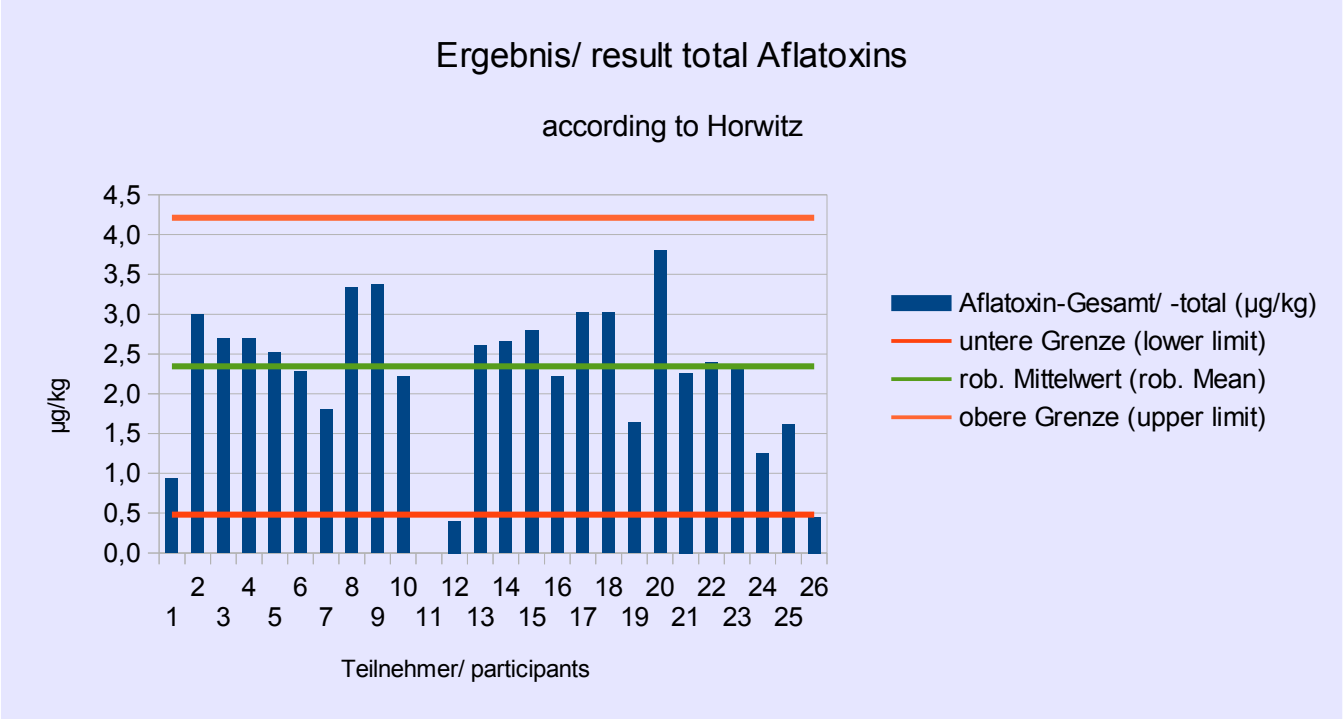
Laboratories

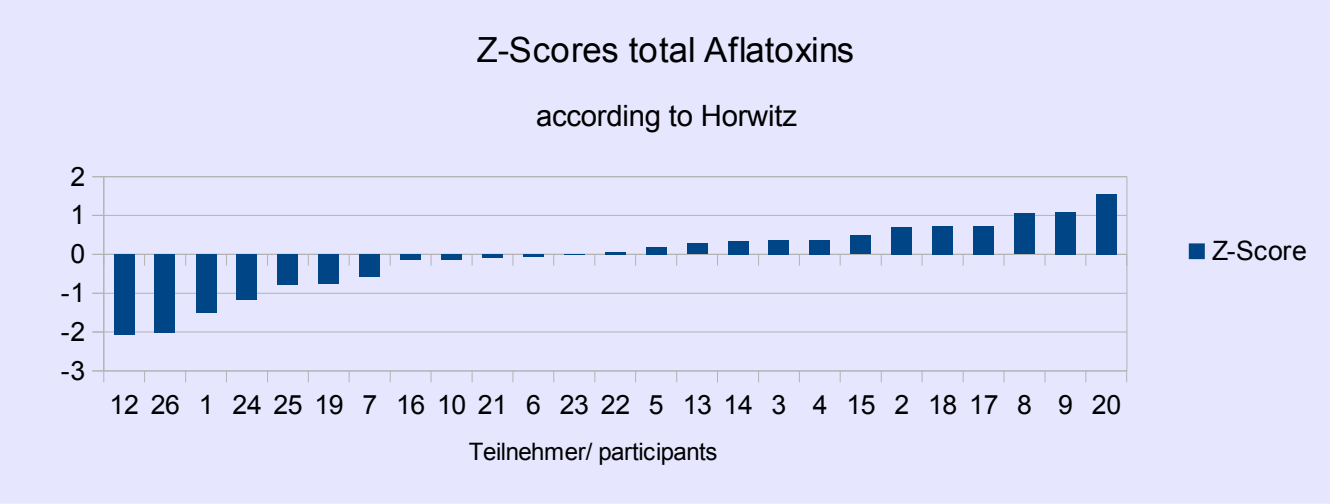
Teilnehmer/ participant	Aflatoxin -Gesamt/ -total	Abweichung/ deviation	Z-Score	Bemerkungen/ remarks
	µg/kg	µg/kg		
1	0,94	-1,41	**-2,7**	
2	3	0,65	1,3	
3	2,69	0,34	0,7	
4	2,7	0,35	0,7	
5	2,52	0,17	0,3	
6	2,28	-0,07	-0,1	
7	1,8	-0,55	-1,1	
8	3,33	0,98	1,9	
9	3,37	1,02	2,0	
10	2,22	-0,13	-0,2	
11				
12	0,4	-1,95	**-3,8**	Ausreißer/ outlier
13	2,61	0,26	0,5	
14	2,66*	0,31	0,6	
15	2,8	0,45	0,9	
16	2,22	-0,13	-0,2	
17	3,03	0,68	1,3	
18	3,02	0,67	1,3	
19	1,64	-0,71	-1,4	
20	3,8	1,45	**2,8**	
21	2,26	-0,09	-0,2	
22	2,4	0,05	0,1	
23	2,33	-0,01	0,0	
24	1,25	-1,10	**-2,1**	
25	1,61	-0,74	-1,4	
26	0,45	-1,90	**-3,7**	Ausreißer/ outlier

** = The mean was calculated by DLA*

4.4 Total aflatoxins according to Horwitz in µg/kg

Characteristics	
Number of results (without no. 14)	25
Number of outliers	1
Mean	2,29
Median	2,40
Robust mean (X)	2,35
Robust standard deviation (S^x)	0,83
Target standard deviation($\hat{\sigma}$) (Horwitz)	0,93
Target standard deviation (L 23.05-2 for information)	0,61
Lower limit of target range (X - 2 $\hat{\sigma}$)	0,48
Upper limit of target range (X + 2 $\hat{\sigma}$)	4,21
Quotient $S^x/\hat{\sigma}$	0,9
Standard uncertainty u_x	0,21
Quotient $u_X/\hat{\sigma}$	0,2
Number of results in the target range	24 (96%)





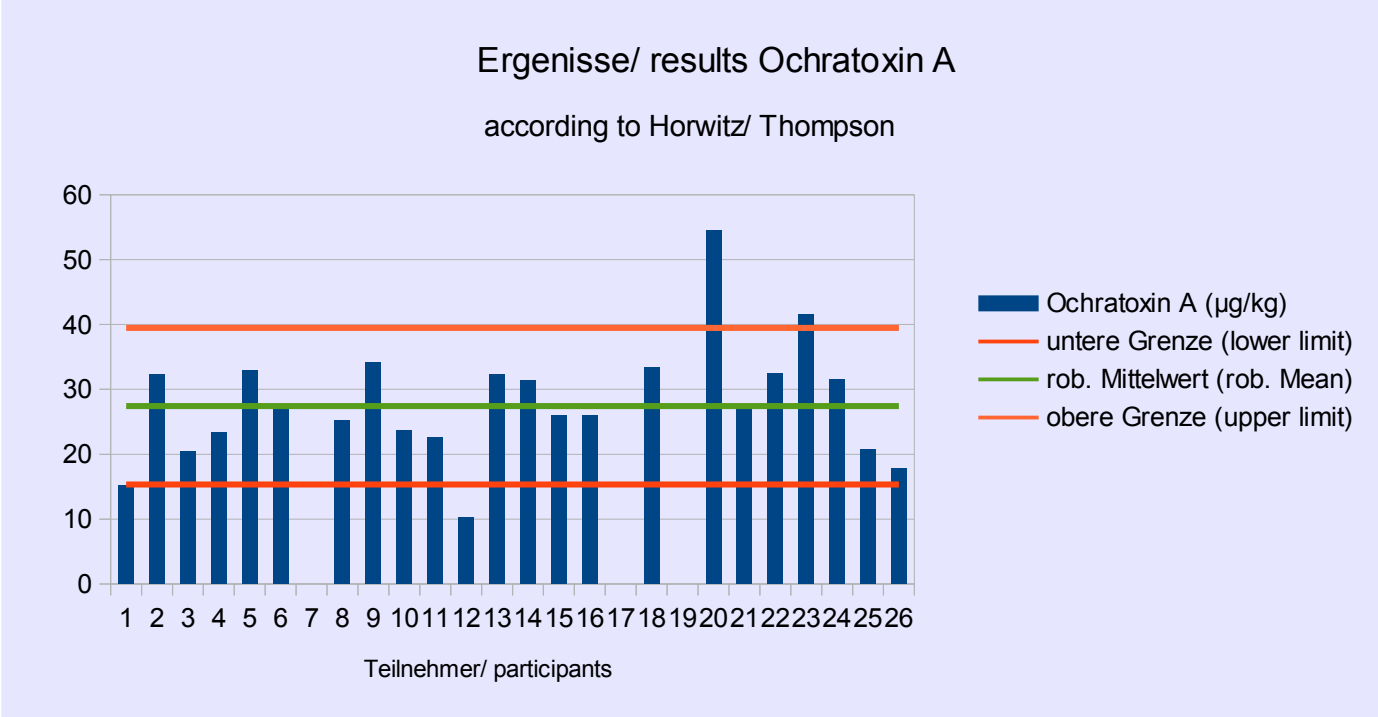
Laboratories

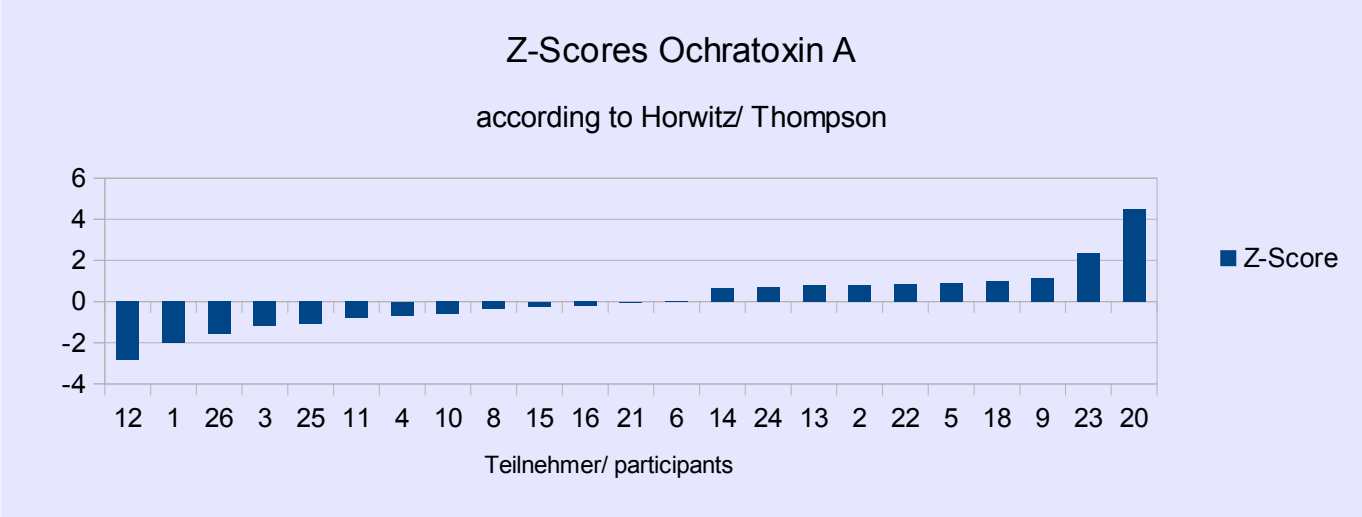
Teilnehmer/ participant	Aflatoxin-Gesamt/ -total	Abweichung/ deviation	Z-Score	Bemerkungen/ remarks
	µg/kg	µg/kg		
1	0,94	-1,41	-1,5	
2	3	0,65	0,7	
3	2,69	0,34	0,4	
4	2,7	0,35	0,4	
5	2,52	0,17	0,2	
6	2,28	-0,07	-0,1	
7	1,8	-0,55	-0,6	
8	3,33	0,98	1,1	
9	3,37	1,02	1,1	
10	2,22	-0,13	-0,1	
11				
12	0,4	-1,95	**-2,1**	Ausreißer/ outlier
13	2,61	0,26	0,3	
14	2,66*	0,31	0,3	
15	2,8	0,45	0,5	
16	2,22	-0,13	-0,1	
17	3,03	0,68	0,7	
18	3,02	0,67	0,7	
19	1,64	-0,71	-0,8	
20	3,8	1,45	1,6	
21	2,26	-0,09	-0,1	
22	2,4	0,05	0,1	
23	2,33	-0,01	0,0	
24	1,25	-1,10	-1,2	
25	1,61	-0,74	-0,8	
26	0,45	-1,90	-2,0	

** = The mean was calculated by DLA*

4.5 Ochratoxin A according to Horwitz/Thompson in µg/kg

Characteristics	
Number of results	23
Number of outliers	2
Mean	27,9
Median	27,2
Robust mean (X)	27,4
Robust standard deviation (S^x)	7,6
Target standard deviation($\hat{\sigma}$) (Horwitz/Thompson)	6,0
Target standard deviation (L 30.00-5 for information)	3,7
Lower limit of target range (X - 2 $\hat{\sigma}$)	15,4
Upper limit of target range (X + 2 $\hat{\sigma}$)	39,5
Quotient $S^x/\hat{\sigma}$	1,3
Standard uncertainty u_x	2,0
Quotient $u_X/\hat{\sigma}$	0,3
Number of results in the target range	20 (87%)





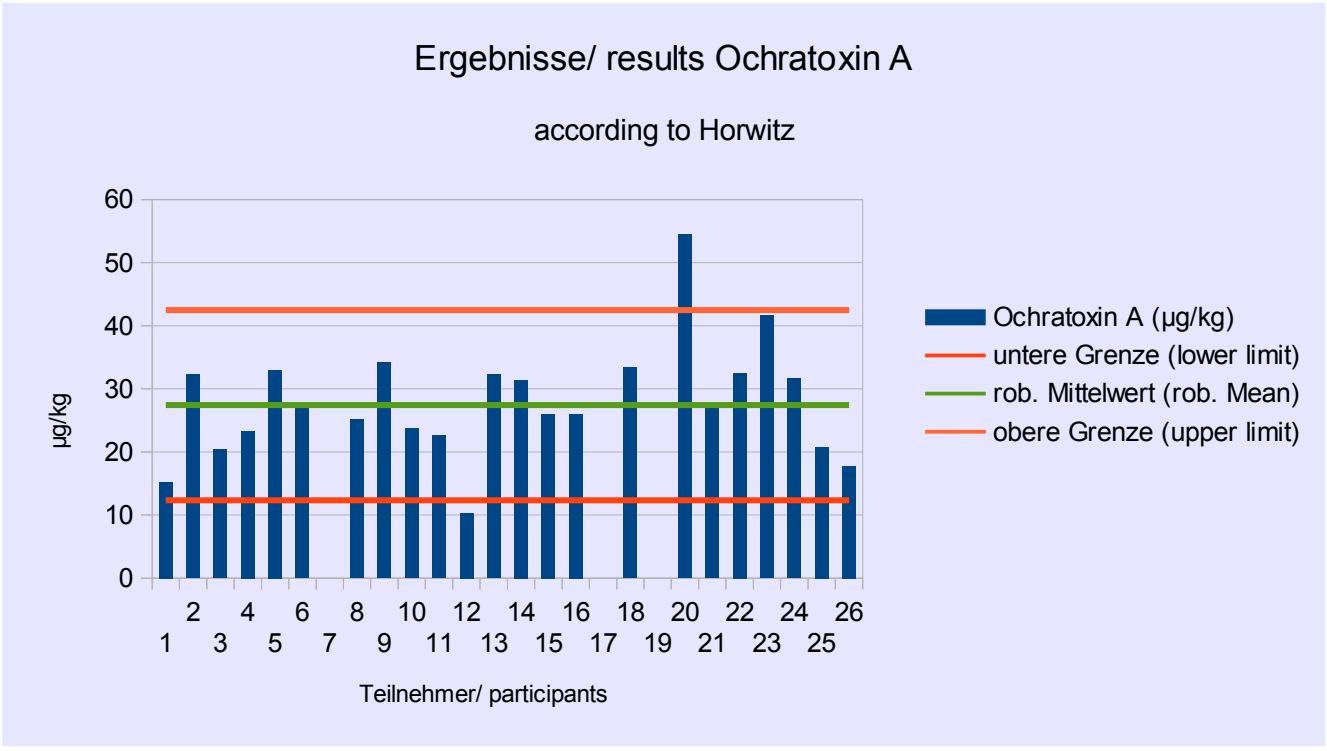
Laboratories

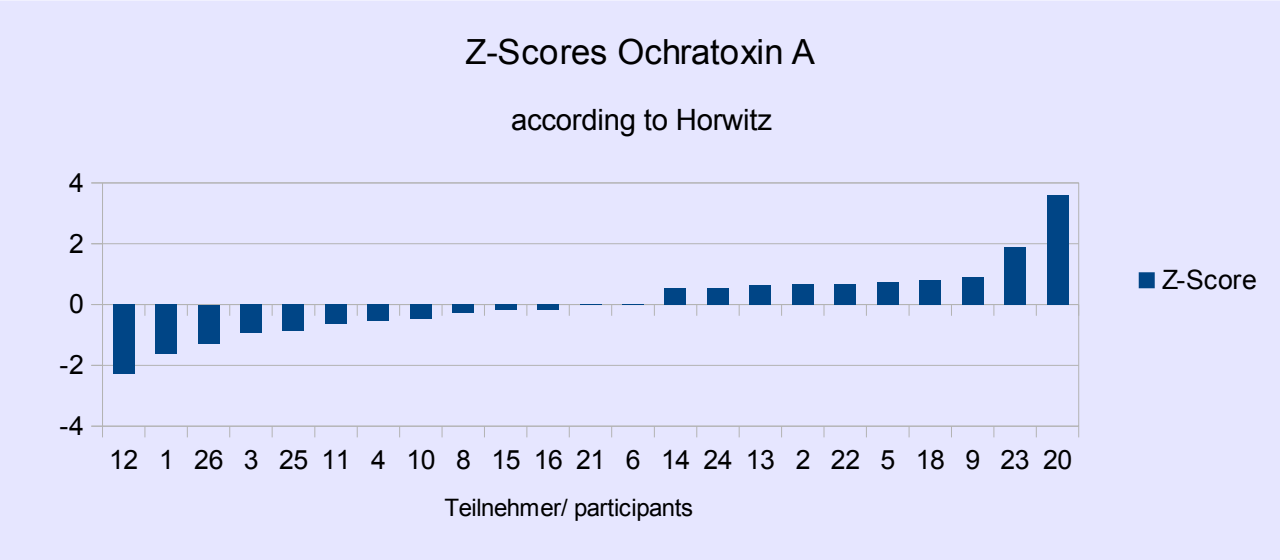
Teilnehmer/ participant	Ochratoxin A	Abweichung/ deviation	Z-Score	Bemerkungen/ remarks
	µg/kg	µg/kg		
1	15,2	-12,22	-2,0	
2	32,3	4,88	0,8	
3	20,38	-7,04	-1,2	
4	23,33	-4,09	-0,7	
5	33	5,58	0,9	
6	27,35	-0,07	0,0	
7				
8	25,2	-2,22	-0,4	
9	34,2	6,78	1,1	
10	23,7	-3,72	-0,6	
11	22,6*	-4,82	-0,8	
12	10,31	-17,11	**-2,8**	Ausreißer/ outlier
13	32,26	4,84	0,8	
14	31,4	3,98	0,7	
15	26	-1,42	-0,2	
16	26,03	-1,39	-0,2	
17				
18	33,38	5,96	1,0	
19				
20	54,5	27,08	**4,5**	Ausreißer/ outlier
21	27,2	-0,22	0,0	
22	32,5	5,08	0,8	
23	41,6	14,18	**2,4**	
24	31,6	4,18	0,7	
25	20,8	-6,62	-1,1	
26	17,8	-9,62	-1,6	

** = The mean was calculated by DLA*

4.6 Ochratoxin A according to Horwitz in µg/kg

Characteristics	
Number of results	23
Number of outliers	2
Mean	27,9
Median	27,2
Robust mean (X)	27,4
Robust standard deviation (S^x)	7,6
Target standard deviation($\hat{\sigma}$) (Horwitz)	7,5
Target standard deviation (L 30.00-5 for information)	3,7
Lower limit of target range (X - 2 $\hat{\sigma}$)	12,3
Upper limit of target range (X + 2 $\hat{\sigma}$)	42,5
Quotient $S^x/\hat{\sigma}$	1,0
Standard uncertainty u_x	2,0
Quotient $u_X/\hat{\sigma}$	0,3
Number of results in the target range	21 (91%)





Laboratories

Teilnehmer/ participant	Ochratoxin A	Abweichung/ deviation	Z-Score	Bemerkungen/ remarks
	µg/kg	µg/kg		
1	15,2	-12,22	-1,6	
2	32,3	4,88	0,6	
3	20,38	-7,04	-0,9	
4	23,33	-4,09	-0,5	
5	33	5,58	0,7	
6	27,35	-0,07	0,0	
7				
8	25,2	-2,22	-0,3	
9	34,2	6,78	0,9	
10	23,7	-3,72	-0,5	
11	22,6*	-4,82	-0,6	
12	10,31	-17,11	**-2,3**	Ausreißer/ outlier
13	32,26	4,84	0,6	
14	31,4	3,98	0,5	
15	26	-1,42	-0,2	
16	26,03	-1,39	-0,2	
17				
18	33,38	5,96	0,8	
19				
20	54,5	27,08	**3,6**	Ausreißer/ outlier
21	27,2	-0,22	0,0	
22	32,5	5,08	0,7	
23	41,6	14,18	1,9	
24	31,6	4,18	0,6	
25	20,8	-6,62	-0,9	
26	17,8	-9,62	-1,3	

** = 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/ samle A	Probe/ sample B	Wiederfindung/ recovery
	µg/kg	µg/kg	µg/kg	in %
1	0,94	0,92	0,95	100
2	3	2,86	3,13	63
3	2,55	2,4	2,69	98
4	2,5	2,62	2,37	94
5	2,38	2,44	2,32	68
6	2,28	2,21	2,35	98
7	1,8	1,89	1,84	
8	3,20	3,23	3,17	91
9	3,37	3,30	3,43	90/90
10	2,22	2,19	2,25	61
11	2,92*	4,93	0,91	98
12	0,37	0,39	0,34	96
13	2,61	2,56	2,66	97,7
14	2,66	2,6	2,72	100
15	2,8	3,1	2,4	38
16	2,22	2,25	2,19	68,9
17				
18	2,80	2,78	2,83	72
19	0,67			
20				
21	2,26	2,27	2,24	90
22	2,3	2,3	2,2	70,5
23	2,22	2,35	2,1	111,4
24	1,25	1,28	1,21	81,4
25	1,57	1,75	1,38	80
26	0,41	0,41		87

** = The mean was calculated by DLA*

5.1.2 Aflatoxin B_2 in µg/kg

Teilnehmer/ participants	Probe/ sample A	Probe/ sample B	Wiederfindung/ recovery
	µg/kg	µg/kg	in %
1	<0,5	<0,5	105
2	< 0,2	< 0,2	93
3	0,14	0,14	
4	0,21	0,2	89,3
5	0,14	0,14	69
6	<0,4	<0,4	
7			
8	0,14	0,12	113
9	< 0,18	< 0,18	90/90
10			
11			
12	0,03	0,03	104
13			
14	LOQ: <0,5	LOQ: <0,5	
15	<0,5	<0,5	66
16	<LOQ	<LOQ	
17			
18	0,22	0,22	82
19			
20			
21			
22	0,1	0,1	
23	0,12	0,11	108,8
24	< 0,2	< 0,2	84,4
25	0,11	0,09	
26			
Mean	0,16	0,13	
Median	0,14	0,12	
Standard deviation	0,012	0,005	

5.1.3 Aflatoxin G_1 in µg/kg

Teilnehmer/ participants	Probe/ sample A	Probe/ sample B	Wiederfindung/ recovery
	µg/kg	µg/kg	in %
1	<0,5	<0,5	105
2	< 0,2	< 0,2	74
3	<0,1	<0,1	
4	0	0	93,2
5	< LOQ	< LOQ	72
6	<0,4	<0,4	
7			
8	< 0,13	< 0,13	-
9	< 0,15	< 0,15	80/80
10			
11			
12	< 0,04	< 0,04	
13			
14	LOQ: <0,5	LOQ: <0,5	
15	<0,5	<0,5	52
16	<LOD	<LOD	
17			
18	<LOQ	<LOQ	77
19			
20			
21			
22	<0,1	<0,1	
23	0	0	
24	< 0,2	< 0,2	79,3
25			
26			

5.1.4 Aflatoxin G_2 in µg/kg

Teilnehmer/ paticipants	Probe/ sample A	Probe/ sample B	Wiederfindung/ recovery
	µg/kg	µg/kg	in %
1	<0,5	<0,5	105
2	< 0,2	< 0,2	86
3	<0,1	0,1	
4	0	0	87,3
5	< LOQ	< LOQ	63
6	<0,4	<0,4	
7			
8	< 0,13	< 0,13	-
9	< 0,18	< 0,18	87/87
10			
11			
12	< 0,02	< 0,02	
13			
14	LOQ: <0,5	LOQ: <0,5	
15	<0,5	<0,5	64
16	<LOD	<LOD	
17			
18	<LOQ	<LOQ	74
19			
20			
21			
22	<0,1	<0,1	
23	0	0	
24	< 0,2	< 0,2	77,5
25			
26			

5.1.5 Total aflatoxins in µg/kg

Teilnehmer/ participants	Gesamt-/ Total-Aflatoxin	Probe/ sample A	Probe/ sample B	Wiederfindung/ recovery
	µg/kg	µg/kg	µg/kg	in %
1	0,94	0,92	0,95	
2	3	2,86	3,13	
3	2,69	2,54	2,83	
4	2,7	2,83	2,57	
5	2,52	2,58	2,46	-
6	2,28	2,21	2,35	
7	1,8			
8	3,33	3,37	3,29	-
9	3,37	3,30	3,43	n.a.
10	2,22	2,19	2,25	
11				
12	0,4	0,42	0,37	107
13	2,61	2,56	2,66	97,7
14	2,66*	2,6*	2,72*	
15	2,8	3,1	2,4	
16	2,22	2,25	2,19	
17	3,03	3,2	2,85	
18	3,02	3,00	3,05	
19	1,64			
20	3,8	3,5	4,2	95
21	2,26			
22	2,4	2,4	2,3	
23	2,33	2,47	2,2	111,3
24	1,25	1,28	1,21	
25	1,61	1,86	1,47	
26	0,45	0,45		

** = The mean was calculated by DLA/ n.a. = nicht anwendbar/ not applicable*

5.1.5 Ochratoxin A in µg/kg

Teilnehmer/ participants	Ochratoxin A	Probe/ sample A	Probe/ sample B	Wiederfindung/ recovery
	µg/kg	µg/kg	µg/kg	in %
1	15,2	15,4	15	100
2	32,3	33,3	31,4	96
3	20,38	20,66	20,09	128
4	23,33	23,03	23,62	83,5
5	33	34	31	72
6	27,35	27,91	26,78	90
7				
8	25,2	26,1	24,3	94
9	34,2	33,1	35,3	77/62
10	23,7	21,9	25,5	92
11	22,6*	22,3	22,8	99
12	10,31	11	9,61	75
13	32,26	31,56	32,95	88
14	31,4	31,1	31,7	100
15	26	25	26	100
16	26,03	26,04	26,02	105
17				
18	33,38	33,24	33,53	76
19				
20	54,5	62,5	47,5	95
21	27,2	25,96	28,52	81
22	32,5	34,8	30,2	129
23	41,6	42	41,2	93,3
24	31,6	31,85	31,34	93
25	20,8	19,52	21,99	
26	17,8		17,8	40

** = 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 documented in chapter 5.1.1.
It is 0,26 µg/kg = 10,9 % of X (Total aflatoxins) and
3,70 µg/kg = 13,2 % of X (Ochratoxin A).

In the ASU L23.05-2 the relative repeatability standard deviation of 12 % (total Aflatoxin) for a total aflatoxin content x = 2,0 µg/kg was determined for paprika (13).

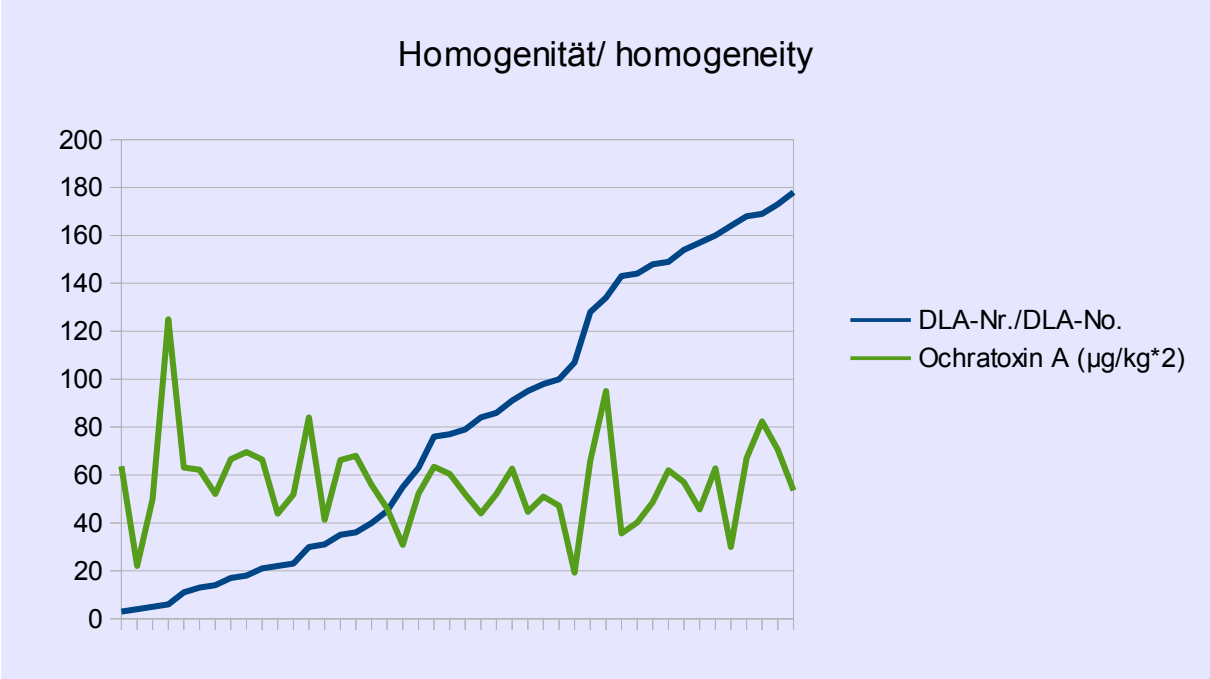
5.2.2 Homogeneity testing before PT

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

Sample no.	**Glucose**		
129	3,46	g/100g	
74	3,55	g/100g	
43	3,60	g/100g	
10	3,85	g/100g	
166	3,49	g/100g	
Mean	**3,6**		
Standard deviation.	**0,16**	**4,3**	**%**

5.2.3 Comparison of sample number/ test result

The comparison of the increasing sample-numbers and measured Ochratoxin A results (*2) shows homogeneity:



5.3 Analytical methods

Teilnehmer/ participant	Methode/ method	Wiederfindung mit gleicher Matrix/ recovery with the same matrix	Akkreditiert/ accredited	Bemerkung/ remark
		yes/no		
1	HPLC	no (paprika)	no	
2	IAC-LC-MS/MS	yes (matrix spiked)	yes	Final result is corrected with the recovery. Recovery is mean of sample A and B.
3/ Aflatoxins	SOP Q171-03, complies with DIN EN ISO 16050	yes	yes	Sample preparation was performed different to the instruction of the IAC alfa prep R with detergents: Extraction with 80% methanol and PBS buffer with 0,5% tween 20 for extract dilution.
3/ Ochrat. A	SOP Q02-04, complies with DIN EN 16007	yes	yes	Sample preparation was performed different to the instruction of the ochraprep spices of R-biopharm: Extraction with 80% methanol and PBS buffer with 0,1% tween 20 for extract dilution.
4	IAC-HPLC-fluorescence detection	no	yes	Recovery with 60/40 paprika-/curry powder
5/ Aflatoxins	Aflatoxins (HPLC-FLD), PV 805050	yes	yes	
5/ Ochrat. A	Ochratoxin A (HPLC-FLD), PV 805090	yes	yes	
6/ Aflatoxins	PRM 0 54.3 0029 01; cleaning with IAC, HPLC-FLD with post column deri-vatisation using Kobra cell	yes (similar)	yes	
6/ Ochrat. A	PRM 0 54.3 0011 03; Cleaning with dichloro-methane and with IAC; HPLC-FLD	yes (similar)	yes	

Teilnehmer/ participant	Methode/ method	Wiederfindung mit gleicher Matrix/ recovery with the same matrix	Akkreditiert/ accredited	Bemerkung/ remark
7	HPLC-FLD with post column derivatisa-tion using Kobra cell and RP-C18 column.		yes	
8/ Aflatoxins	Extraction with acetone/ water (85/15), cleaning with IAC; measuring with LC-MS/MS	yes	yes	
8/ Ochrat. A	Extraction with sodium hydrogen car-bonate solu-tion (1%), cleaning with IAC, measuring with LC-MS/MS	yes	yes	
9	SOP 592, determination of the mycotoxins Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2, Ochratoxin A and Zearalenon in snus and tobacco with HPLC tandem Mass Spektro-metry	yes	yes, for the matrix snus and tobacco	Final result is the mean of result A and B. The recovery was only determined for the single substances. Results were calculated with matrix matched calibration, therefore the recovery is included. Recovery for sample A/ sample B
10/ Aflatoxins	Analogous to ASU § 64 LFGB L 23.05-2, modified.	yes	yes	
10/ Ochrat. A	Extraction with 1 % sodium hydro-gen carbonate solution and cleaning with IAC, HPLC with FLD.	yes	yes	Detection after electrochemical deriva-tisation (KOBRA cell) to reduce interfering matrix. There was no derivatisation of ochratoxin A.
11	HPLC-FL	no	yes	
12	HPLC-FLD	no	yes	

Teilnehmer/ participant	Methode/ method	Wiederfindung mit gleicher Matrix/ recovery with the same matrix	Akkreditiert/ accredited	Bemerkung/ remark
13	Determination with HPLC-FLD after isolation with IAC.	yes	yes	
14	In house method: LCMS	yes	yes	
15	IAC, HPLC/FLD	yes	yes	
16	Wet homogeni-sation, IAC, HPLC	no	yes	
17	Neogen ELISA			
18	IAC, HPLC-FL	yes	no	
19	HPLC, AV/36.017	no	no	
20	ELISA of Fa r-biopharm	yes	yes	
21	In house method: HPLC FLD	yes	yes	
22	HPLC	yes	yes	
23/ Aflatoxins	§64 LFGB 23.05-2 analogous	yes	no	
23/ Ochrat. A	§64 LFGB 30.00-5 analogous	yes	no	
24/ Aflatoxins	Aflatoxins: Extraction with MeOH, cleaning with IAC, deriva-tisation with tri-fluoroacetic acid, separa-tion and quantifica-tion with HPLC/ fluo-rescence detection.	yes	yes	
24/ Ochrat. A	Ochratoxin: Extraction with sodium hydrogen carbonate, cleaning with IAC, separa-tion and quantifica-tion with HPLC/ fluo-rescence detection.	yes	yes	

Teilnehmer/ participant	Methode/ method	Wiederfindung mit gleicher Matrix/ recovery with the same matrix	Akkreditiert/ accredited	Bemerkung/ remark
25/ Aflatoxins	IAC, HPLC, FLD; Kobra cell	no	yes	
25/ Ochrat. A	HPLC, FLD, IAC cleaning	no	yes	
26		no	yes	Recovery NOT used in calculation.

*IAC = Immuno Affinity Column

6. Index of participant laboratories

Teilnehmer/ participant	Ort/ location
	Australia
	Germany
	Germany
	Germany
	Germany
	Germany
	Germany
	Germany
	Germany
	Germany
	Germany
	Germany
	Germany
	Germany
	Italien
	Belgien
	Germany
	Germany
	Germany
	Germany
	Germany
	Germany
	Austria
	Germany
	Germany
	Germany
	South Africa
	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. EG-VO 401-2006 zur Festlegung der Probenahmeverfahren und Analysemethoden für die amtliche Kontrolle des Mykotoxingehalts von Lebensmitteln
14. 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
15. ASU §64 LFGB 30.00-5: Bestimmung von Ochratoxin A in Korinthen, Rosinen, Sultaninen, gemischtem Trockenobst und getrockneten Feigen (Jan. 2011)
16. ASU §64 LFGB 15.03-1: Bestimmung von Ochratoxin A in Gerste (Jan. 2010)
17. Report on the 2007 Proficiency Test for the Determination of Ochratoxin A in Capsicum ssp (Paprika Powder), J.Stroka et al., JRC Scientific and Technical Reports, European Commission EUR 23382 EN, European Communities, 2008

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