Proficiency Tests

DLA

food
cosmetics
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Evaluation Report

proficiency test

DLA 23/2016

Contaminated Food:
Ochratoxin A in Licorice Powder

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Allgemeine Informationen zur Eignungsprüfung (EP)
General Information on the proficiency test (PT)

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EP-Nummer *PT-Number*	DLA 23/2016
EP-Koordinator *PT-Coordinator*	Dr. Gerhard Wichmann
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report : 26/01/17
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler* Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager) *- gezeichnet / signed G. Wichmann* Datum / Date: 26 January 2017
Unteraufträge *Subcontractors*	Die Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern wird von DLA im Unterauftrag vergeben. The analysis of the content, homogeneity and stability of PT-parameters are subcontracted by DLA.

Inhalt / Content

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 with a natural content of ochratoxin A is a mixture of different liquorice powders and a sweetwood extract (12% share). To the mixture were further added microtracer iron particles (FSS red lake) for homogeneity verification. The raw materials were sieved, combined, homogenized and then sieved again.

Approximately 4 kg of the material was packaged in about 50 grams in metallized PET film bags. The portions were numbered chronologically.

The detectability of ochratoxin A was ensured by preliminary investigations of the material.

The material was checked for homogeneity.

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 10-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].

Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15]. The microtracer analysis of the present PT sample showed probability of 87%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. This gave a HorRat value of 0,7. The results of microtracer analysis are given in the documentation.

The calculation of the **variation coefficient** of the repeatability standard deviation (CV_r) of the participants for ochratoxin A was used as an indicator of homogeneity, see table 1. The result is similar to the repeatability standard deviation of the respective standardized methods (see 3.6.2 (table 2) [18-19]. The repeatability standard deviation of the participants is given in the documentation and in the statistic data (see 4.1). Compared to the previous PTs, the variation coefficients and the quotient S^* / σ_{pt} of the present PT are of the same order of magnitude, see table 3 (3.6.3).

	CV_r	**Quotient S^*/σ_{pt}**
Ochratoxin A	7,51 %	1,7

Table 1: Compilation of the variation coefficients CV_r and the quotient S^*/σ_{pt} of the present PT.

Furthermore, the homogeneity for ochratoxin A was characterized by the **trend line function of participants' results for chronological bottled single samples**. The maximum deviations from the mean value of the trend line was in the range of 40% of the target standard deviation σ_{pt} (s. 5.2 homogeneity) and is to be judged as increased. The reason for this is the relatively large variability between the participants (reproducibility standard deviation) with normal repeatability standard deviation.

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].

2.1.2 Stability

The experience with various DLA reference materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of ochratoxin A in the case of a comparable dry mass (a_W value <0.5) and matrix. The sample material is therefore stable against microbial spoilage at room temperature and dry light-protected storage.

2.2 Sample shipment and information to the test

Two portions of test material were sent to every participating laboratory in the 47th week of 2016. The testing method was optional. The tests should be finished at January 6th 2017 the latest.

With the cover letter along with the sample shipment the following information was given to participants:

In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.

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 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, information on the limit of quantification, the date of the analysis and general points to the method.

From the 10 participants all 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.

In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method. For the method description, see documentation 5.1.

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.

In case single results from participants are available the calculation of the repeatability standard deviation S_r, also known as standard deviation within laboratories S_w, is performed by: [3, 4].

The relative repeatability 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 in case single results from participants are available.

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.

In case single results from participants are available 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 in case single results from participants are available. Its meaning is explained in more detail 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]. Even if a result is e.g. with a factor >10 deviates significantly from the mean value and has an influence on the robust statistics, a result can be excluded from the statistical evaluation [3].

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.

The target standard deviation according to Horwitz/ Thompson was used (see 3.6.1).

For the purpose of information, the target standard deviation of a test for precision is also given (ASU §64 Method: [18]), see 3.6.2 / Table 2.

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)

3.6.2 Precision experiment

Using the reproducibility standard deviation σ_R and the repeatability standard deviation σ_r of a precision experiment (collaborative 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 2 relative repeatability standard deviation (RSD_r) and relative reproducibility standard deviation (RSD_R) were determined in collaborative trials using the specified methods. The target standard deviation is given for information in the evaluation.

Table 2: *Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) from selected precision experiments and resulting target standard deviations σ_{pt}* [18-19]

Parameter	**Matrix**	**Mean (µg/kg)**	**RSD_r**	**RSD_R**	**σ_{pt}**	**Method / Literature**
Ochratoxin A	Sultanas	11,39	5,6 %	14,3 %	13,7 %[1]	IAC/HPLC [18]
Ochratoxin A	Currants	4,51	5,7 %	28,4 %	28,1 %	IAC/HPLC [18]
Ochratoxin A	Raisins	7,55	4,9 %	14,0 %	13,6 %	IAC/HPLC [18]
Ochratoxin A	Barley	4,5	14 %	15 %	11,3 %	IAC/HPLC [19]
Ochratoxin A	Roasted coffee	5,5	2 %	14 %	13,9 %	IAC/HPLC [19]

[1] *Values used in the evaluation (see 4.1)*

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 the target standard deviation according to 3.6.1 were regarded suitable.

Table 3 shows selected characteristics of participants results of the present PT in comparison to the previous year.

Table 3: Characteristics of the present PT (on blue-grey) in comparison to previous PTs since 2013 (SD = standard deviation, CV = coefficient of variation)

Parameter	Matrix (powder)	rob. Mean (µg/kg)	rob. SD (S*) (µg/kg)	rel. SD (CV_{S*}) [%]	Quotient S^*/σ_{pt}	DLA-report
Ochratoxin A	licorice	39,5	13,4	33,9 %	1,5	23-2016
Ochratoxin A	spice	42,1	27,9	66,2 %	1,9	24-2016
Ochratoxin A	Grape juice	11,6	4,13	35,6 %	1,6	25-2016
Ochratoxin A	spice	52,7	31,9	60,5 %	2,0	19-2015
Ochratoxin A	coffee	7,6	2,3	30,3 %	1,3	14-2014
Ochratoxin A	spice	2,35	0,83	35,3 %	0,9	16-2014
Ochratoxin A	licorice	41,5	8,5	20,5 %	0,9	13-2013
Ochratoxin A	spice	26,8	9,1	40,0 %	1,5	14-2013

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

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

The z-score valid for the PT evaluation is designated z-score (σ_{pt}), while the value of z-score (Info) is for information only. The two z-scores are calculated using the different target standard deviations according to 3.6.

3.7.1 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].

3.8 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

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

For warning- and action-signals see 3.7.1.

3.9 Reproducibility coefficient of variation (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^*)
Number with 2 replicates
repeatability standard deviation (S_r)
Repeatability (Cv_r) in %
reproducibility standard deviation (S_R)
Reproducibility (CV_R) in %
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 ($X_{pt} + 2\sigma_{pt}$´) *
Quotient S/σ_{pt} or S*/σ_{pt}'*
Standard uncertainty $U(X_{pt})$
Quotient $U(X_{pt})/\sigma_{pt}$ or $U(X_{pt})/\sigma_{pt}$'
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	**Parameter [Einheit/ Unit]**	**Abweichung**	**Z´-Score** σ_{pt}´	**z-Score** (Info)	**Hinweis**
Evaluation number		**Deviation**			**Remark**

4.1 Ochratoxin A in µg/kg

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	9*
Number of outliers	0
Mean	38,7
Median	40,8
Robust Mean (X)	**39,5**
Robust standard deviation (S*)	**13,4**
Number with 2 replicates	9
Repeatability SD (S_r)	2,91
Repeatability (CV_r)	7,51%
Reproducibility SD (S_R)	13,5
Reproducibility (CV_R)	34,8%
Target range:	
Target standard deviation σ_{pt}	**8,68**
Target standard deviation (for Information)	5,42
lower limit of target range	**22,1**
upper limit of target range	**56,8**
Quotient S/σ_{pt}*	*1,5*
Standard uncertainty $U_{(Xpt)}$	*5,58*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,64*
Results in the target range	*8*
Percent in the target range	*89%*

* Participant no. 4 was excluded from the statistical evaluation since the result was increased by a factor >500 from rob. mean value and because it has a clear influence on the rob. Statistics and is outside the normal distribution, see Fig. 2 / Kernel density plot.

Comments:

The standard target deviation was evaluated using the model of Horwitz/Thompson. The standard deviation "for information" was calculated according to ASU §64 LFGB L30.00-5 (sultanas), see 3.6.2.

The distribution of the results showed a normal variability. The quotient S*/σpt was well below 2.0. The robust standard deviation is comparable to those of prior PTs (see 3.6.3). The comparability of results is given.

Repeatability and reproducibility standard deviation are in the range of established values for the methods used (see 3.6.2).
The quotient $U(X_{pt})/\sigma_p$ (0,64) is increased.

89% of the results were in the target area.

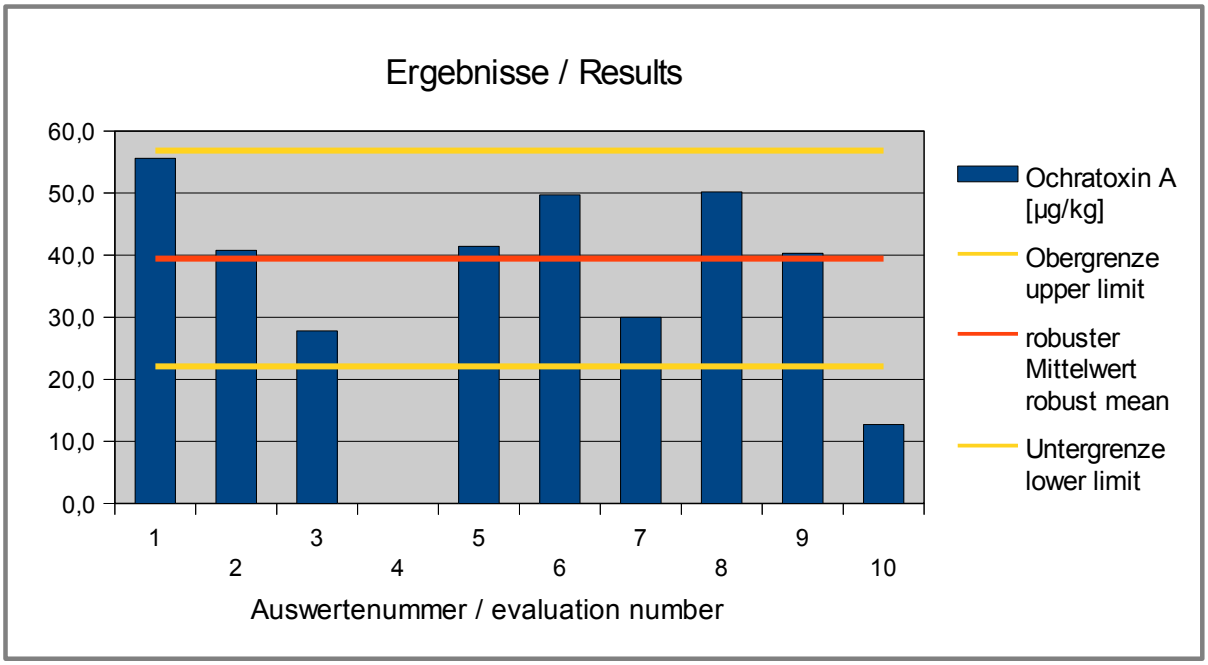

Abb. / Fig. 1: Ergebnisse Ochratoxin A / Results Ochratoxin A

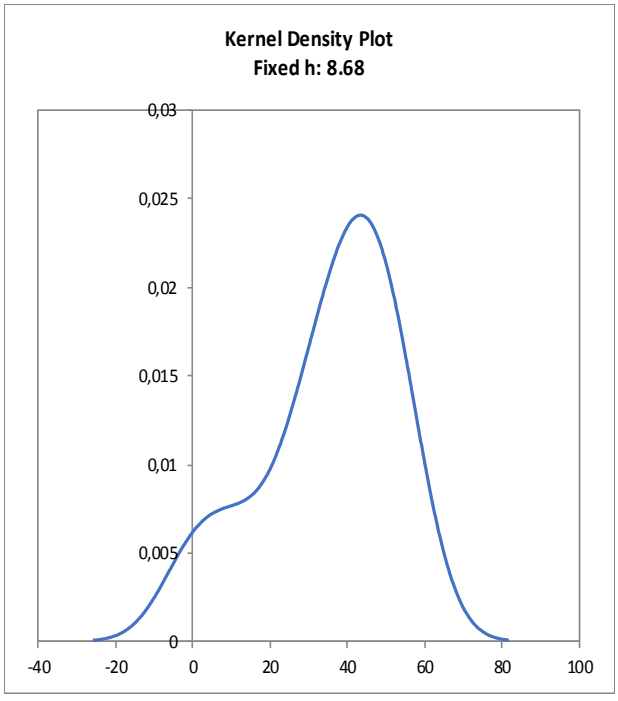

Abb. / Fig. 2:
Kerndichte-Schätzung der Ergebnisse (mit h = σ_{pt} von X_{pt} = 8,68 µg/kg)

Kernel density plot of results (with h = σ_{pt} *of* X_{pt} = 8,68 µg/kg)

Comment:
The kernel density shows a normal distribution of results with a clear shoulder at 0,1 µg/kg, due to the result of no. 4.

Ergebnisse der teilnehmenden Institute:
Results of Participants:

Auswerte-nummer Evaluation number	Ochratoxin A [µg/kg]	Abweichung [µg/kg] Deviation [µg/kg]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	55,6	16,1	1,9	3,0	
2	40,8	1,29	0,1	0,2	
3	27,8	-11,66	-1,3	-2,2	
4	0,0702*				Ergebnis ausgeschlossen/ Result excluded
5	41,4	1,96	0,2	0,4	
6	49,7	10,2	1,2	1,9	
7	30,0	-9,46	-1,1	-1,7	
8	50,2	10,7	1,2	2,0	
9	40,3	0,84	0,1	0,2	
10	12,7	-26,8	-3,1	-4,9	

** Mean calculated by DLA*

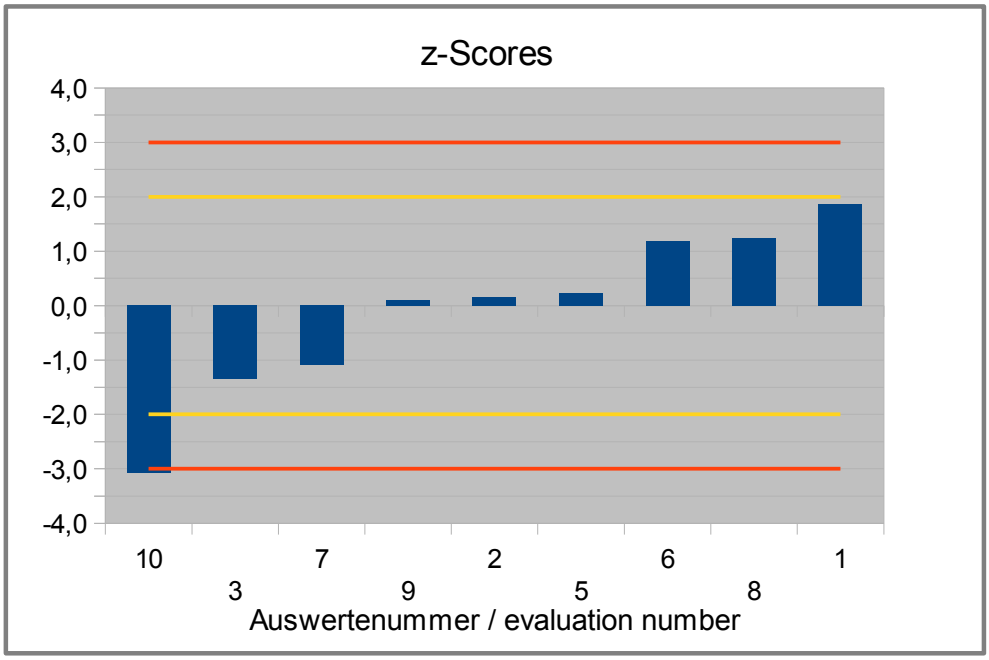

Abb. / Fig. 3: Z-Scores Ochratoxin A

5. Documentation

5.1 Details by participants

5.1.1 Primary data

5.1.1.1 Ochratoxin A

Teilnehmer/ participant	Ergebnis/ result	DLA-Nr Probe A/ sample A	DLA-Nr Probe B/ sample B	Datum der Analyse/ date of the analysis	Ergebnis A/ result A	Ergebnis B/ result 2B	Bestimmungs-grenze/ Limit of determination
	µg/kg			day/month	µg/kg	µg/kg	µg/kg
1	55,6	17	60	05.12.17	54,6	56,5	3
2	40,75	37	68	21.12.17	40,49	41,02	0,3
3	27,8	44	70	21.12.16	25,7	29,9	5 µg/kg
4	0,0702*	0,0706/ no. 66	0,0697/ no. 31	04.01.17	0,0019	0,0021	0,001
5	41,42	10	35	02.01.17	46,83	36,01	1
6	49,7	26	42	29.12.	48,6	50,7	0,5 µg/kg
7	30,0	63	49	04.12.16	29,6	30,4	5
8	50,2	1	76	12.12.	49,2	51,1	2
9	40,3	14	52	5./6.12	39,5	41	1
10	12,7	23	56	04.01.17	13,5	11,9	0.10 ng/mL

** Mean calculated by DLA*

5.1.2 Analytical methods

5.1.2.1 Ochratoxin A

Teilnehmer/ participant	**Wiederfindung** mit gleicher Matrix/ recovery with the same matrix	**Methode/ method**	**Methode ist akkreditiert/ method is accredited**	**Sonstige Hinweise/ further remark**
	ja / nein		ja / nein	
1	no	Extraction with 1% sodium bicarbonate, centrifuged and filtered, clean up with IAC, analysis by HPLC with Fluorescence detection	yes	
2	no	§64 LFGB L15.00-1, mod.	yes	
3	yes	After immunoaffinity treatment with HPLC/ fluorescence mod. after ASU § 64 LFGB.	yes	Unfortunately, OTA appeared as a rider peak
4	yes	Internal method		
5	yes	HPLC-Fluorescence	yes	
6	yes	ASU L 00.00-50a (EG), SOP M 1386, LC-MS/MS	yes	Recovery calculated using internal standards.
7	yes	ASU §64 LFGB L15.03-1 mod.	yes	
8	yes	Ochratoxin A, (HPLC-FLD), PV 805091:	yes	n.a.
9	yes	Determination of Ochratoxin A in licorice with HPLC (PV3077 (2007-01))	yes	
10	no	LC/MS/MS	yes	Cleaning with IAC

IAC = Immunoaffinity column

5.2 Homogeneity

5.2.1 Homogeneity testing before PT

The **mixture homogeneity before bottling** was examined 10-fold by **microtracer analysis.**

Microtracer Homogeneity test
DLA 33-2016

Weight whole sample	4,007	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	19,8	mg/kg

Results of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	9,54	82	17,2
2	10,15	96	18,9
3	9,15	89	19,5
4	9,48	80	16,9
5	9,89	96	19,4
6	9,71	78	16,1
7	10,63	84	15,8
8	8,22	77	18,7
9	9,74	81	16,6
10	9,15	82	17,9

Poisson distribution		
Number of samples	10	
Degree of freedom	9	
mean	84,7	Particles
Standard deviation	6,56	Particles
χ^2 (CHI-Quadrat)	4,58	
Probability	**87**	%
Recovery rate	90	%

Normalverteilung		
Number of samples	10	
Mean	17,7	mg/kg
Standard deviation	1,37	mg/kg
rel. Standard deviation	7,8	%
Horwitz Standard deviation	10,4	%
HorRat value	**0,7**	
Recovery rate	90	%

5.2.2 Comparison of sample number/test results and trend line

By comparison of the **increasing sample numbers** and the measurement results, the homogeneity of the chronological bottled PT item can be characterized with the help of the trend line function:

Name				
Target standard deviation $\sigma_{pt'}$	10,6			µg/kg
Sample numbers	1 - 76			
Total numbers of samples	16*			
Slope	-0,551			
Trend line range	46,6	-	37,8	µg/kg
Deviation trend line	42,2	±	4,41	µg/kg
Percent of $\sigma_{pt'}$	41,8	%		

* Without sample no. 4 + 10

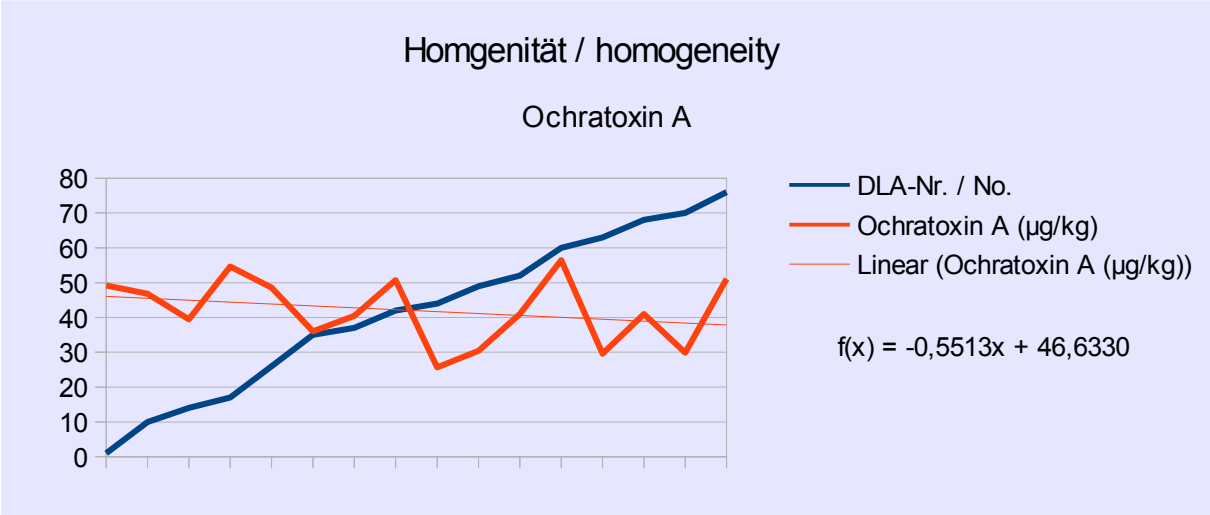

Abb./Fig. 4:
Trendfunktion Probennummern vs. Ergebnisse
trend line function sample number vs. results

6. Index of participant laboratories

Teilnehmer/ participant	Ort/ town	Land/ country
		Germany
		Germany
		USA
		Germany
		Germany
		Germany
		Italy
		Germany
		Ireland
		Germany

[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)
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14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Checking procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
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16. EG-VO 401-2006 zur Festlegung der Probenahmeverfahren und Analysemethoden für die amtliche Kontrolle des Mykotoxingehalts von Lebensmitteln

17. EU-VO 519/2014 zur Änderung der Verordnung (EG) Nr. 401/2006 hinsichtlich der Probenahmeverfahren für große Partien, Gewürze und Nahrungsergänzungsmittel, der Leistungskriterien für die Bestimmung von T-2-Toxin, HT-2-Toxin und Citrinin sowie der Screening-Methoden für die Analyse (v. 16. Mai 2014)
18. ASU §64 LFGB 30.00-5: Bestimmung von Ochratoxin A in Korinthen, Rosinen, Sultaninen, gemischtem Trockenobst und getrockneten Feigen (Jan. 2011)
19. ASU §64 LFGB 15.03-1: Bestimmung von Ochratoxin A in Gerste (Jan. 2010)
20. 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