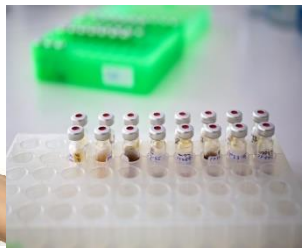
Bundesinstitut für Risikobewertung

Report on the 2020 Proficiency Test of the German National Reference Laboratory for Mycotoxins and Plant Toxins

Determination of Alternaria toxins in tomato products



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Determination of alternaria-toxins in tomato products

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Abstract

The German Federal Institute for Risk Assessment (BfR) in its function as a national reference laboratory for mycotoxins and plant toxins in food and feed (NRL), conducted a proficiency tests (PT) for the analysis of the *Alternaria* toxins altenuene (ALT), alternariol (AOH), alternariol methyl ether (AME), tentoxin (TEN) and tenuazonic acid (TEA) in 2019. A total of twelve laboratories participated in the interlaboratory comparison, all of which are either active as national reference laboratories or official laboratories. Eight participants came from Germany, the other participants from other European countries.

Three tomato products (juice, ketchup, tomato purée) and a control standard, which contained all five of the above toxins, were investigated. All laboratories used liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) for detection.

Ten of the twelve participants submitted result data sets, which were assessed as being sufficiently precise in over 80 % of the sample/analyte combinations submitted (z score $|z| \leq 2$).

1 Study design

In April 2019, the laboratories received one sample of tomato purée (BfR_PU), one sample of tomato ketchup (BfR_KE) and one sample of tomato juice (BfR_JU) each, along with a control standard as a thin film (CONT_AT). The contents and concentrations of ALT, AOH, AME, TEN and TEA were to be determined in all samples. The analytical method could be chosen freely.

The samples were shipped on 15/4/2019, the submission deadline for results was initially 7/6/2019. The deadline for the submission of results was extended to 17/6/2019, and all participants were informed of this by email on 7/5/2019. An additional extension to the submission deadline period was granted to Laboratory LC0011 on 13/6/2019 by request. At the time of the request, all the other participants had already disclosed a result. The result from LC0011 was eventually submitted on 25/6/2019 and considered for the present evaluation.

The laboratories who participated are listed in Table 1. The accompanying sample documents can be found in appendix 6.5.

Table 1: List of participants

National participants
German Federal Institute for Risk Assessment (BfR), NRL für Mykotoxine und Pflanzentoxine, Berlin, Germany
Chemisches und Veterinäruntersuchungsamt (CVUA) Rheinland, Hürth, Germany
Chemisches und Veterinäruntersuchungsamt (CVUA) Sigmaringen, Sigmaringen, Germany
Chemisches und Veterinäruntersuchungsamt (CVUA) Westfalen, Arnsberg, Germany
Hamburger Landesinstitut für Lebensmittelsicherheit, Gesundheitsschutz und Umweltuntersuchungen, Hamburg, Germany
Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei (LLLF) Mecklenburg-Vorpommern, Rostock, Germany
Landesuntersuchungsamt (LUA) Rheinland-Pfalz, Institut für Lebensmittelchemie, Trier, Germany
Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES), Lebensmittel- und Veterinärinstitut, Braunschweig, Germany
International participants
General Chemical State Laboratory (GCSL), Piraeus, Greece
Kantonales Laboratorium Thurgau, Switzerland
Public Analyst's Laboratory (PAL), Dublin, Ireland
Service Commun des Laboratoires, Laboratoire de Rennes – SCL35, Rennes, France

2 Samples

2.1 Preparation of the samples

The raw materials for the production of samples were tomato juice, tomato ketchup and tomato purée acquired from retail. *Alternaria* toxins could already be detected in all samples on purchasing (Table 2). The samples were spiked to obtain the intended target concentrations. Partial quantities of samples were mixed with ethanol solutions of *Alternaria* toxins. These partial samples were mixed with the total sample quantities of 2.5 to 3 kg each. The percentage of ethanol added to the total sample quantity was between 0.02 and 0.08% (v/m) and considered as insignificant to the analysis.

The samples were mixed thoroughly with a mixer. The whole amount of sample was filled to approx. 50 g in plastic sample containers (Figure 1). Three samples were stored at -80°C for the stability experiment, all the other samples were stored at -20°C until shipping.

Figure 1: Preparation of sample BfR_JU (tomato juice)

Table 2: Spiking of samples: the concentration before spiking was determined by the BfR in three repeated measurements, the resulting overall concentration was then calculated as the sum of the initial and the nominally added concentration.

Analyt	Concentration before spiking [µg/kg]			Nominal concentration added [µg/kg]			Resulting nominal overall concentration [µg/kg]		
	BfR_JU	BfR_PU	BfR_KE	BfR_JU	BfR_PU	BfR_KE	BfR_JU	BfR_PU	BfR_KE
AOH	0.6	7.6	1.6	2.4	-	12.2	3.0	7.6	13.8
AME	0.2	1.0	0.4	4.7	17	1.5	4.9	18	1.9
ALT	<LOD	<LOD	<LOD	9.1	10	2.1	9.1	10	2.1
TEN	<LOD	0.4	<LOD	42	12.1	112	42	12.5	112
TEA	39	97	26	164	603	53	203	700	79

The control standards were produced by diluting each standard solution, placed in pre-treated amber glass screwtop vials (2 ml) and reduced to dryness with a rotation vacuum concentrator. Participants were instructed to reconstitute the thin film by adding 1 ml of the same solvent which was used to produce the calibration solutions in each laboratory.

2.2 Homogeneity

The homogeneity of the samples was inspected in accordance with the IUPAC Harmonised Protocol [Thompson, 2006]. Ten randomly selected sample bottles were investigated for each tomato product, and each bottle was analysed twice. Ten vials with two injections each were investigated for the homogeneity inspection of the thin film CONT_AT. The content and concentration of all *Alternaria* toxins were analysed under repeatability conditions, using HPLC-MS/MS. The BfR method was used for analysis.

Sufficient homogeneity under the criteria in accordance with DIN 13528 (B2.2) was confirmed for all sample/analyte combinations. The sample/analyte combination BfR_PU/AOH demonstrated significant fluctuations in the F-test between the median values of samples, which could not be traced back to incorrect measurements. However, sufficient homogeneity could be confirmed for this sample as well. In accordance with DIN 13528 (Chapter B2.2), the experimental material is sufficiently homogeneous when the standard deviation between the test material samples is less than 0.3 times the target standard deviation from the interlaboratory study (Table 5).

Table 3: Determination of homogeneity

	TEA	AOH	ALT	TEN	AME
	BfR_JU				
n	20	20	20	20	20
Mean [µg/kg]	198.8	3.2	10.2	43.5	5.3
Standard deviation [µg/kg]	6.7	0.2	0.6	1.2	0.1
Relative standard deviation	3.4 %	7.3 %	6.1 %	2.9 %	2.8 %
Test value F	0.55	0.91	0.18	0.45	0.90
$F < F_{krit}$	Yes	Yes	Yes	Yes	Yes
	BfR_PU				
n	20	20	20	20	20
Mean [µg/kg]	644.1	7.2	10.3	12.3	17.7
Standard deviation [µg/kg]	17.1	0.6	0.8	0.3	0.5
Relative standard deviation	2.7 %	8.0 %	7.9 %	2.7 %	2.8 %
Test value F	0.77	3.35	0.63	0.99	1.09
$F < F_{krit}$	Yes	No	Yes	Yes	Yes
Ss		0.431			
$0{,}3 * s_{target}$		0.474			
	BfR_KE				
n	20	20	20	20	20
Mean [µg/kg]	77.5	13.2	2.3	118.4	1.7
Standard deviation [µg/kg]	2.2	0.6	0.3	2.5	0.0
Relative standard deviation	2.9 %	4.9 %	13.9 %	2.1 %	2.8 %
Test value F	1.47	0.85	0.33	0.91	0.64
$F < F_{krit}$	Yes	Yes	Yes	Yes	Yes
	CONT_AT				
n	20	20	20	20	20
Mean [µg/kg]	40.3	17.0	12.8	20.6	35.3
Standard deviation [µg/kg]	1.1	0.5	0.6	0.4	0.8
Relative standard deviation	2.7 %	3.0 %	5.0 %	2.1 %	2.3 %
Test value F	2.41	0.80	1.73	0.63	2.50
$F < F_{krit}$	Yes	Yes	Yes	Yes	Yes

n: number of measurements

F_{krit} 95 %: critical value for an error probability of 5 %, in all cases the value (for n=20) is F_{krit}= 3,020

S_{target}: $_{Target}$ standard deviation for this sample/analyte combination

Ss: Standard deviation between samples

2.3 Stability

The sample stability was assessed with an ‘isochronous study’ (Lamberty et al., 1998). Three samples stored at -80°C and six samples stored at -20°C were used for this study. The isochronous study was performed under the assumption that no loss of analytes takes place at -80°C. The samples are considered to be stable for the experiment period at the storage temperature of -20°C when the difference between the concentrations measured at -80°C and -20°C is less than 0.3 times the target standard deviation.

To simulate an unexpected temporary thawing of the samples, three of the samples stored at -20°C were thawed for 24 hours at room temperature and in the dark after 68 or 69 days. The samples were then frozen again at -20°C until the analysis.

After the end of the interlaboratory comparison (118 or 120 days after production), all samples were analysed in one measuring sequence under repeatability conditions. The BfR method was used for this analysis.
The average concentration and standard deviation for each type of storage condition were calculated for all materials (appendix 6.1). A sufficient stability for all analytes for the duration of the proficiency test could be derived from the stability study when the samples were stored at -20°C. The samples that were thawed for a day were also assessed as being sufficiently stable.

2.4 Shipping

The samples were frozen at -20°C and shipped in styrofoam containers with two cooling elements. Delivery was carried out by a courier service. Eleven of the twelve participants confirmed receipt within 24 hours. Only laboratory LC0013 received the package delayed on 17/4/2019, after two days, and disclosed that cooling had ceased for the samples at this point. This break in the cold chain was not identified as a problem, as the samples demonstrated sufficient stability even after being warmed up for one day (2.3). This assessment was retrospectively confirmed by the z-score of this laboratory, which was consistently very low (chapter 4.1).

3 Statistical evaluation

Statistical evaluation was carried out based on results submitted by 12 participants. The complete analysis results from the laboratories are listed in appendix 6.12.

Some participants submitted incomplete results data sets. Laboratory LC0011 did not disclose any results for the ALT analytes, and no results for AOH were disclosed for the BfR_PU, BfR_KE and BfR_JU samples. Laboratory LC0011 did not submit any results for TEN for the CONT_AT sample. Laboratory LC0013 only disclosed results for the three tomato product samples, but not for the CONT_AT control standard.

3.1 Procedure for statistical evaluation

Three parallel tests were required for each sample/analyte combination. Laboratories that only specified one measured value were excluded from the calculation of statistical parameters in line with the stipulations of ISO 13528. Before the statistical calculation, Mandel's statistic was used to check whether the results of laboratories showed significant deviation from those of the other laboratories. If significant deviations were found, the results of these laboratories were not used for calculation of the statistical parameters.
Statistical evaluation of the interlaboratory comparison was performed using robust evaluation methods in line with ISO 13528. The measured results do not need to be normally distributed for the use of a robust evaluation method. Results with right-skewed distributions and a breakpoint of between 30 and 50 % can also be evaluated.

The repeatability standard deviation and the reproducibility standard deviation were determined using the Q-method, while the robust mean value was calculated with the help of the Hampel estimator using the ProLab software. The estimation technique according to Hampel et al. [Hampel, 1986] excludes laboratory results from the calculation of the robust mean value if these results show more than 4.5-fold deviation of the reproducibility standard deviation from the mean value.

The standard error (u_x) of the robust mean value according to Hampel was calculated using formula 1:

$$u_x = \sqrt{\left(\frac{s_R^2 - s_r^2}{p} + \frac{s_r^2}{p * \bar{n}}\right)} \qquad (1)$$

s_R: Reproducibility standard deviation measured using the Q-method
s_r: Repeatability standard deviation measured using the Q-method
p: Number of mean values
n: Average number of laboratory measurements per analyte/sample combination

There was no certified level for any of the samples. The robust mean values calculated from the laboratory mean values were used as target values for the corresponding samples. This method was also chosen for the target value of the thin film standard, as random errors in the production of the solution can result in deviations from the original weight.

The Horwitz standard deviation (sH_a) based on the formula modified by Thompson [Thompson, 2000] served as the target standard deviation. In dependence on the analyte level or concentration, the sH_a is calculated using the following formulas:

With levels < 120 µg/kg

$sH_a = 0.22 * c$ (2)

With levels 120 µg/kg ≤ c < 138 g/kg

$sH_a = 0.02 * c^{0.8495}$ (3)

c: Level of the analyte [kg(analyte)/kg(sample)] or [kg/dm³ (control solution)]
sH_a: Absolute standard deviation according to Horwitz, modified by Thompson [kg/kg] or [kg/dm³]

The Horwitz Ratio [Horwitz, 2006] is used to assess the interlaboratory comparison.

$$HorRat = \frac{RSD_R}{sH_r} \quad (4)$$

HorRat: Horwitz Ratio
RSD_R: Relative reproducibility standard deviation calculated from the laboratory results [%]
sH_r: Relative target standard deviation according to Horwitz, modified according to Thompson [%]

Assessment of the performance capability of the laboratories is based on the z-scores [Thompson, 1993] according to formula 5:

$$z = \frac{x - SW}{s_{target}} \quad (5)$$

x: Mean value for the analyte levels from the individual tests performed by the laboratory [µg/kg or ng/mL]
SW: Target value for analyte level [µg/kg] or [ng/mL] of the test material
s_{target}: Target standard deviation [µg/kg] or [ng/mL], corresponds to sH_a

The upper or lower tolerance limits (TLs) form the tolerance range and were calculated for assessment of the laboratories using the following formula:

$TG = SW \pm (2 * s_{target})$ [µg/kg] or [ng/mL] (6)

The proxy z-score (Pereboom et al., 2019) was calculated to evaluate the results which fell below the LOQ given by the participants. The proxy z-score is calculated analogously to formula 5 by replacing the mean value of the analyte contents with the limit of quantification.

A proxy z-score below -2 indicates an incorrect negative finding. A proxy z-score above +2 indicates that the LOQ of the laboratory is high with regards to the target value and the limits of quantification of the other participants

The proxy z-score is shown for information purposes and is not used for laboratory assessment.

3.2 Graphic compatibility test results as per Mandel

Mandel's k statistic was used to assess differences between the variances of the individual laboratories. If the k values of a laboratory for multiple analytes in one sample or for one analyte in multiple samples are higher than the corresponding critical k values of the sample/analyte combination, this is an indication that this laboratory shows a systematically higher variance for the sample or the analyte compared to the other laboratories. Deviations of laboratory mean values compared to the mean values of the other laboratories are assessed with the help of Mandel's h statistic. If the h values of a laboratory for one analyte in multiple samples or for

multiple analytes in one sample are higher than the corresponding critical h values for the sample/analyte combination, it can be concluded that there are systematic deviations in the mean values of this laboratory in the sample or for this specific analyte.

Graphic representations of the Mandel's h-statistic and Mandel's k-statistic can be found in the diagrams in the appendices 6.7 and 6.8. The critical values for the significance level of 5 % are represented as a yellow line in the diagram, and the significant level of 1 % as a red line. Statistically deviating laboratory values are accordingly marked as yellow bars (significance level 5 %) and red bars (significance level 1 %).

Laboratories which stood out due to significant deviations ($\alpha = 0.01$) in the Mandel's h-statistic or Mandel's k-statistic were not included in the calculation of the statistical parameters. The excluded laboratory/analyte combinations and laboratory/sample combinations are represented in Table 4. LC0011 demonstrated significant deviations from the measured values for all analytes for the CONT_AT sample, as did LC0006 for the BFR_KE sample. These laboratories were excluded from the calculation of statistical parameters for CONT_AT and BFR_KE. Compared to the values from the other laboratories, LC0014 demonstrated significant deviations from Mandel's k-values for TEA, and LC0011 demonstrated significant deviations from Mandel's h-values for AME, in all samples investigated. LC0014 and LC0011 were therefore excluded for the calculation of statistical parameters for the relevant analytes in all samples. Laboratory LC0014 could only measure concentrations for AME over the LOQ in the BFR_PU sample. These results demonstrated significantly higher values in the Mandel's k-statistic, compared to the k-values of the other laboratories. Both laboratories were therefore excluded from the calculation of statistical parameters for each sample/analyte combination.

Table 4: Exclusion of laboratory/sample combinations and laboratory/analyte combinations due to significant deviations in the Mandel's h-statistic and/or Mandel's k-statistic

Labor	Probe/Analyt-	Mandel's-Statistik	Modus
LC0011	CONT_AT	h (all analytes)	Sample[1]
LC0006	BFR_KE	k (all analytes)	Sample[1]
LC0014	TEA	k (all samples)	Analyte[2]
	AME	k (BFR_PU)	Analyte[2]
LC0011	AME	k (all samples)	Analyte[2]

[1]Sample: considering all analytes in one sample
[2]Analyte: considering specific analytes in all samples

3.3 Results of the statistical calculations

The statistical parameters were calculated using the method described in chapter 3.1. The results can be found in Table 5 and Table 6.

Table 5: Statistical parameters for all matrix samples and the control standard

	Tomato juice (BfR_JU)					Tomato puree (BfR_PU)					Tomato ketchup (BfR_KE)					CONT_AT ***)				
	TEA	ALT	AOH	AME	TEN	TEA	ALT	AOH	AME	TEN	TEA	ALT**)	AOH	AME	TEN	TEA	ALT	AOH	AME	TEN
Robust mean *) [µg/kg]	**233.3**	**10.8**	**3.2**	**5.2**	**49.4**	**755.6**	**11.2**	**7.2**	**17.4**	**13.9**	**87.4**	**(2.3)**	**12.7**	**1.6**	**135.6**	**42.4**	**15.1**	**15.8**	**35.1**	**23.9**
Target standard deviation sH [µg/kg]	46.5	2.4	0.7	1.2	10.9	126.1	2.5	1.6	3.8	3.1	19.2	(0.5)	2.8	0.4	29.3	9.3	3.3	3.5	7.7	5.3
Reproducibility standard deviation sR [µg/kg]	28.9	2.1	0.5	0.6	5.6	74.4	2.4	0.6	1.2	1.4	9.5	(0.5)	1.1	0.3	13.7	6.7	2.0	1.7	2.7	2.9
Repeatability standard deviation. sr [µg/kg]	6.4	0.6	0.2	0.2	1.7	14.0	0.5	0.4	0.5	0.5	1.4	(0.3)	0.4	0.1	2.4	1.0	0.4	0.2	0.5	0.5
Relative target standard deviation [%]	19.91	22.0	22.0	22.0	22.00	16.7	22.0	22.0	22.0	22.0	22.0	(22.0)	22.0	22.0	21.6	22.0	22.0	22.0	22.0	22.0
Relative reproducibility standard deviation (RSDR) [%]	**12.40**	**19.39**	**14.67**	**12.17**	**11.26**	**9.85**	**21.21**	**8.03**	**7.01**	**10.14**	**10.86**	**(20.9)**	**9.00**	**15.75**	**10.09**	**15.78**	**12.96**	**10.60**	**7.79**	**12.22**
Relative repeatability standard deviation (RSDr) [%]	**2.72**	**5.15**	**4.73**	**2.85**	**3.33**	**1.86**	**4.21**	**5.13**	**2.68**	**3.88**	**1.62**	**(10.80)**	**3.06**	**4.12**	**1.74**	**2.37**	**2.73**	**1.44**	**1.49**	**1.96**
Reproducibility limit, R (2,80 X sR) [µg/kg]	81.0	5.9	1.3	1.8	15.6	208.3	6.7	1.6	3.4	4.0	26.6	(1.4)	3.2	0.7	38.3	18.7	5.5	4.7	7.7	8.2
Repeatability limit, r (2,80 X sr) [µg/kg]	17.8	1.6	0.4	0.4	4.6	39.3	1.3	1.0	1.3	1.5	4.0	(0.7)	1.1	0.2	6.6	2.8	1.2	0.6	1.5	1.3
Rel. reproducibility limit [%]	34.72	54.29	41.07	34.08	31.53	27.57	59.37	22.49	19.61	28.38	30.40	(58.58)	25.20	44.10	28.24	44.18	36.29	29.67	21.83	34.23
Rel. repeatability limit [%]	7.63	14.43	13.25	7.98	9.34	5.19	11.79	14.37	7.51	10.87	4.52	(30.23)	8.57	11.54	4.88	6.63	7.65	4.02	4.17	5.47
Lower limit of tolerance [µg/kg]	140.4	6.1	1.8	2.9	27.7	503.5	6.3	4.0	9.7	7.8	49.0	(1.3)	7.1	0.9	77.0	23.8	8.5	8.9	19.7	13.4
Upper limit of tolerance [µg/kg]	326.2	15.6	4.6	7.5	71.2	1007.8	16.1	10.4	25.1	20.1	125.9	(3.3)	18.3	2.4	194.2	61.1	21.8	22.8	50.6	34.5
Standard error of robust mean [µg/kg]	8.58	0.68	0.16	0.20	1.56	22.16	0.78	0.17	0.37	0.39	2.98	(0.22)	0.35	0.08	4.08	2.10	0.61	0.53	0.85	0.92
Lower confidence interval of robust mean [µg/kg]	216.1	9.5	2.9	4.8	46.3	711.3	9.6	6.9	16.7	13.2	81.5	(1.9)	12.0	1.5	127.4	38.2	13.9	14.8	33.4	22.1

Continuation of Table 5: Statistical parameters for all matrix samples and the control standard

	Tomato juice (BfR_JU)					Tomato puree (BfR_PU)					Tomato ketchup (BfR_KE)					CONT_AT ***)				
	TEA	ALT	AOH	AME	TEN	TEA	ALT	AOH	AME	TEN	TEA	ALT**)	AOH	AME	TEN	TEA	ALT	AOH	AME	TEN
Number of participants that reported results	12	11	11	12	12	12	11	11	12	12	12	11	11	12	12	11	10	11	11	10
Number of participants that reported results, without outliers	11	9	8	10	12	11	9	8	10	12	10	4	10	9	11	10	10	10	10	10
Number of values reported	36	33	33	36	36	36	33	33	36	36	36	31	33	36	36	30	29	30	30	29
Number of values, without outliers	33	27	24	30	36	33	27	24	30	36	30	12	30	27	33	29	29	29	29	29
HORRAT (sR/sH)	**0.62**	**0.88**	**0.66**	**0.56**	**0.51**	**0.59**	**0.97**	**0.37**	**0.32**	**0.46**	**0.49**		**0.41**	**0.72**	**0.47**	**0.72**	**0.59**	**0.48**	**0.35**	**0.56**

*) Target value
**) The figures for altenuene in tomato ketchup are only given for the sake of completeness; this sample/analyte combination has not been included in the assessment
***) Unit [ng/mL] and [%]

Table 6: Overview of mean sample concentrations for all laboratories

	Tomato juice (BfR_JU)					Tomato puree (BfR_PU)					Tomato ketchup (BfR_KE)					CONT_AT				
	TEA	ALT	AOH	AME	TEN	TEA	ALT	AOH	AME	TEN	TEA	ALT	AOH	AME	TEN	TEA	ALT	AOH	AME	TEN
Unit	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml
LC0001	227	17.63	3.53	6.43	45.9	735	20.17	7.03	18.0	14.1	89.6	< 5.00	12.5	2.14	129	39.1	17.1	17.3	35.5	26.6
LC0002	213	9.89	3.29	5.23	44.5	709	9.67	7.53	17.6	12.6	85.7	2.30	13.6	1.46	122	38.4	11.7	16.5	33.7	21.0
LC0003	201	11.03	2.99	4.59	46.3	676	12.03	7.07	16.1	13.8	78.8	2.56	12.4	1.38	135	39.3	14.4	15.5	30.3	22.2
LC0004	254	10.02	3.18	4.60	54.5	844	9.71	7.24	15.9	15.4	99.8	2.41	12.3	1.43	157	50.5	16.1	15.8	35.3	25.9
LC0005	206	12.50	3.63	5.33	53.2	668	14.07	8.33	18.4	14.7	80.6	< 5.00	13.4	2.37	146	27.2	15.1	13.8	37.3	24.4
LC0006	222	< 15.00	< 4.00	5.90	45.1	718	< 15.00	< 8.00	18.2	12.2	85.7	< 7.50	13.2	< 2.00	121	48.9	16.4	18.1	39.5	24.8
LC0007	242	11.20	2.91	5.07	49.1	833	11.47	7.00	17.2	14.3	92.0	1.93	12.3	1.51	131	42.1	13.8	15.2	33.5	23.1
LC0008	242	8.39	< 0.90	5.56	46.7	755	9.47	< 1.40	18.9	13.6	89.5	< 2.21	3.3	1.69	127	49.6	17.6	16.7	37.4	27.5
LC0011	273			8.48	72.8	851			33.4	22.5	128.6			3.49	128	266		29.2	46.2	
LC0013	235	11.40	3.35	4.93	52.5	725	11.57	7.23	16.8	13.8	83.6	< 10.00	13.5	1.67	140	45.0	14.2	14.9	33.8	22.5
LC0014	377	< 5.00	< 5.00	< 10.00	55.3	1630	< 10.00	< 5.00	24.3	17.0	105.7	< 5.00	12.3	< 5.00	137					
LC0015	251	9.63	2.40	4.84	48.7	798	9.90	6.44	16.9	12.7	85.7	< 2.90	11.9	1.54	141	38.9	14.4	14.7	34.4	21.4

4 Discussion

4.1 Assessment of the proficiency of the laboratories

The proficiency of the laboratories is assessed with the aid of the z-score. The z-score was calculated using the formulas mentioned in chapter 3.1. No z-scores were calculated for altenuene in sample BfR_KE, as quantified results were available from only four laboratories. The statistical figures for this sample/analyte combination are listed for information purposes. The z-scores calculated are summarised in Table 7 and graphically represented in Figure 2.

The assessment of the laboratories was carried out in accordance with ISO 13528, under which the results were given a z-score

$|z| \leq 2$ as acceptable
$2 < |z| < 3$ as questionable (highlighted in yellow)
$|z| \geq 3$ as unacceptable (highlighted in red).

Seven of the 12 participants achieved a sufficiently accurate z-score of $|z| \leq 2$ for all 19 sample/analyte combinations assessed. Moreover, laboratory LC0006 also achieved consistently satisfactory results with z-scores of $|z| \leq 2$ for 14 out of 14 results submitted. For the five results below the limit of quantification, LC0006 achieved a satisfactory, positive proxy z-score below 2, i.e. the assigned value of these sample/analyte combinations was in fact lower than the concentration the laboratory reported as LOQ and that LOQ fell within the z-score range up to 2.

Only two of the participating laboratories (LC0011 and LC0014) achieved sufficiently accurate results for less than 80 % of the sample/analyte combinations (Table 8).

Table 7: z-Scores

	CONT_AT					Tomato puree					Tomato ketchup					Tomato juice				
Labor-code	TEA	ALT	AOH	AME	TEN	TEA	ALT	AOH	AME	TEN	TEA	ALT	AOH	AME	TEN	TEA	ALT	AOH	AME	TEN
LC0001	-0.4	0.6	0.4	0.0	0.5	-0.2	3.6	-0.1	0.2	0.1	0.1		-0.1	1.4	-0.2	-0.1	2.9	0.5	1.1	-0.3
LC0002	-0.4	-1.0	0.2	-0.2	-0.6	-0.4	-0.6	0.2	0.1	-0.4	-0.1		0.3	-0.5	-0.5	-0.4	-0.4	0.2	0.0	-0.5
LC0003	-0.3	-0.2	-0.1	-0.6	-0.3	-0.6	0.3	-0.1	-0.4	-0.1	-0.5		-0.1	-0.7	0.0	-0.7	0.1	-0.3	-0.6	-0.3
LC0004	0.9	0.3	0.0	0.0	0.4	0.7	-0.6	0.0	-0.4	0.5	0.6		-0.1	-0.6	0.7	0.5	-0.3	0.0	-0.5	0.5
LC0005	-1.6	0.0	-0.6	0.3	0.1	-0.7	1.2	0.7	0.3	0.3	-0.4		0.3	2.0	0.4	-0.6	0.7	0.7	0.1	0.3
LC0006	0.7	0.4	0.6	0.6	0.2	-0.3	(1.5)	(0.5)	0.2	-0.6	-0.1		0.2	(1.0)	-0.5	-0.3	(1.7)	(1.2)	0.6	-0.4
LC0007	0.0	-0.4	-0.2	-0.2	-0.2	0.6	(0.1)	(-0.1)	0.0	0.1	0.2		-0.1	(-0.4)	-0.2	0.2	0.2	-0.4	-0.1	0.0
LC0008	0.8	0.7	0.3	0.3	0.7	0.0	-0.7	(-3.7)	0.4	-0.1	0.1		-3.4	0.1	-0.3	0.2	-1.0	(-3.2)	0.3	-0.3
LC0011	23.9		3.8	1.4		0.8			4.2	2.8	2.1			5.2	-0.3	0.9			2.8	2.2
LC0013	0.3	-0.3	-0.3	-0.2	-0.3	-0.2	0.2	0.0	-0.2	-0.1	-0.2		0.3	0.1	0.1	0.0	0.2	0.3	-0.3	0.3
LC0014						6.9	(-0.5)	(-1.4)	1.8	1.0	1.0		-0.1	(9.3)	0.0	3.1	(-2.4)	(2.6)	(4.2)	0.5
LC0015	-0.4	-0.2	-0.3	-0.1	-0.5	0.3	-0.5	-0.5	-0.1	-0.4	-0.1		-0.3	-0.3	0.2	0.4	-0.5	-1.1	-0.3	-0.1

|z| ≤ 2 Result acceptable(not highlighted)
2 < |z| < 3 Result questionable (highlighted in yellow)
|z| ≥ 3 Result unacceptable (highlighted in red)

Values marked with green frame: proxy z-Score is given in brackets for samples below limit of quantitation

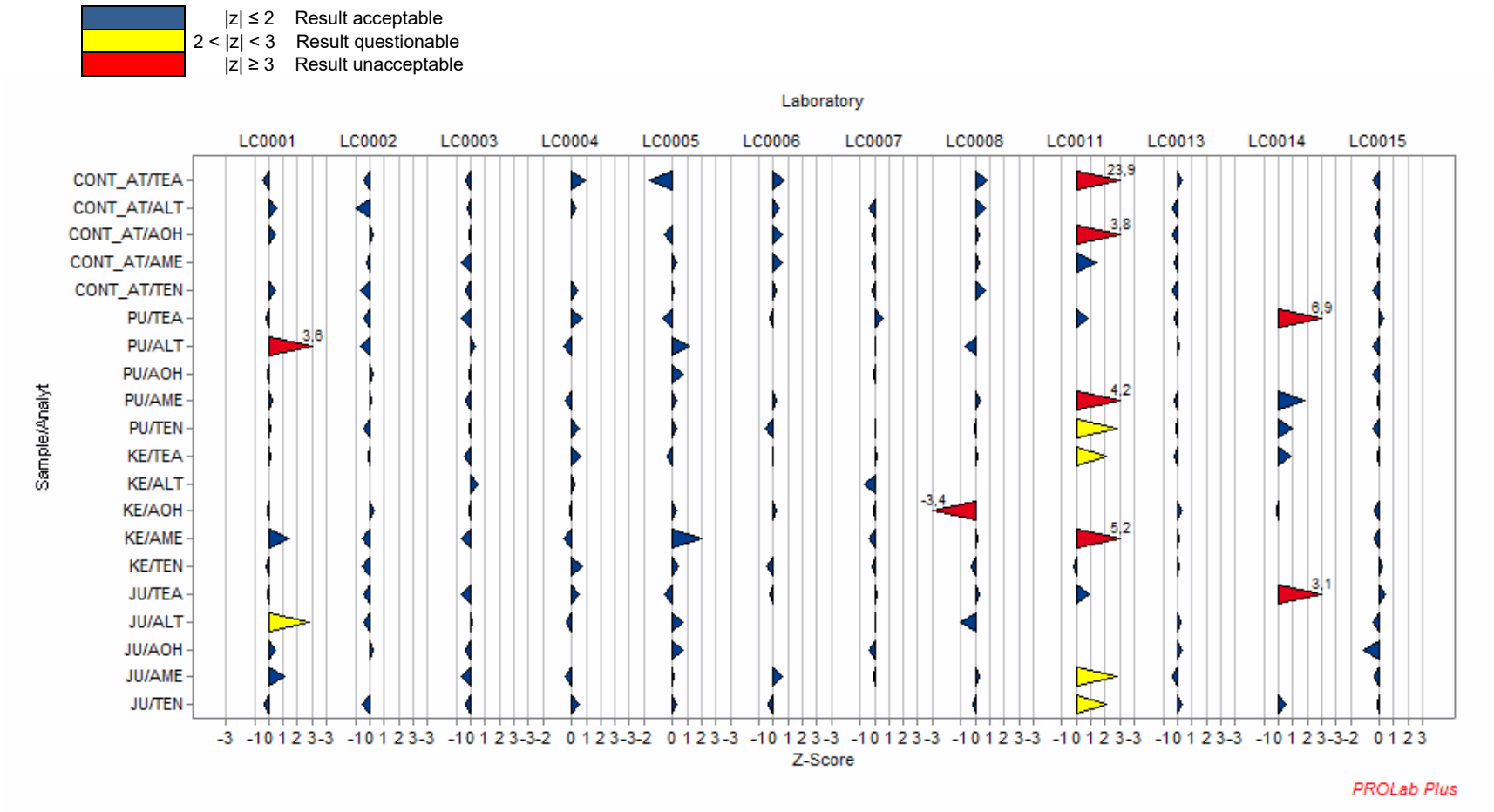

Figure 2: Representation of the z-scores achieved for all sample/analyte combinations

Table 8: Number of z-scores achieved in each category

Lab code	Number of results submitted	Number of results ≥ LOQ	Number |z|≤2	Percentage |z|≤2 [%]	Number 2<|z|<3	Number |z|≥3	Number of results < LOQ	Number $\|z_{proxy}\| \leq 2$	Number $\|z_{proxy}\|$ 2–3	Number $\|z_{proxy}\| > 3$
LC0001	19	19	17	89	1	1	0			
LC0002	19	19	19	100	0	0	0			
LC0003	19	19	19	100	0	0	0			
LC0004	19	19	19	100	0	0	0			
LC0005	19	19	19	100	0	0	0			
LC0006	19	14	14	100	0	0	5	5		
LC0007	19	19	19	100	0	0	0			
LC0008	19	17	16	94	0	1	2			2
LC0011	12	12	4	33	4	4	0			
LC0013	19	19	19	100	0	0	0			
LC0014	14	8	6	75	0	2	6	2	2	2
LC0015	19	19	19	100	0	0	0			

Proxy z-scores are for information purposes and were not assessed.

Laboratory LC0011, which only submitted 12 of the 19 (60 %) requested results, showed noticeable differences to the other participants. Only 33 % (4 out of 12) of the reported results were sufficiently precise. This laboratory commented that the chromatographic method used required further development (see Appendix 6.10).

Laboratory LC0014 did not disclose any results for the CONT_AT control solution. Of the 14 results reported (73 %), six results (43 %) were labelled with '< LOQ' or < 'LOD'. Of the remaining eight results, six (75%) were sufficiently precise.

4.2 Methods used

The participants were requested to provide detailed information on the analytical methods they used (Appendix 6.9). All laboratories analysed the *Alternaria* toxins using liquid chromatography and tandem mass spectrometry (HPLC-MS/MS). Seven laboratories used the analytical method that was validated in a method validation study within CEN/TC275/WG5 and is available as a draft method. Three laboratories used the NRL analytical method provided by the BfR (Appendix II). Three laboratories stated that they deviated from the validated methods in one or more work steps (Table 9). Two laboratories used their own methods.

Table 9: Methods used

Lab code	Method used	Addition of isotope-labelled standards	Deviations from the method	Type of deviation (see Appendix 6.9)
LC0001	CEN	To sample	No	
LC0002	NRL	To extract	Yes	Extraction and clean-up adjusted
LC0003	other	To sample		
LC0004	CEN	To sample	No	
LC0005	NRL	To final test solution	No	
LC0006	CEN	To sample	Yes	Matrix matched calibration instead of external calibration
LC0007	NRL	To final test solution	No	
LC0008	CEN	To sample	Yes	Reconstitution of dry sample adjusted
LC0011	CEN	To sample	No	
LC0013	CEN	To sample	No	
LC0014	other	-		
LC0015	CEN	To sample	No	

CEN: Draft CEN/TC275/WG5-WI275285 (prEN 17521)
NRL: Analytical method of the German NRL for mycotoxins and plant toxins at BfR (Appendix II)

All laboratories except LC0014 used isotope-labelled standards. Laboratory LC0014 used the standard addition method instead, which can help to compensate for loss of analytes, similar to an isotope-labelled standard.

The majority of laboratories using the CEN draft method or the NRL method were in a position to achieve a satisfactory z-score for all sample/analyte combinations.

The two laboratories using in-house methods achieved sufficiently accurate results, with a z-score of $|z| \leq 2$ for 100% (LC0003) and 75% (LC0014) of the results submitted.

4.3 Analytical standards used

Ten out of 12 participants used commercially acquired certified standard solutions and/or thin films as a reference for determining concentrations (see Appendix 6.11). A significant majority used products from the same manufacturer. Laboratory LC0002 manufactured the required standard solutions from crystalline solid material and checked the concentration with the aid of molar extinction coefficients which were taken from scientific literature. Laboratory LC0001 used these extinction coefficients to check the concentrations of some of the commercial certified standard solutions used.

In the experiment with the CONT_AT control standard, all laboratories except laboratory LC0011 achieved acceptable results ($|z| \leq 2$). This means that the comparability of the available commercial analytical standards is high. The use of crystalline standards, after photometric concentration determination, also led to acceptable results.

Laboratory LC0011 used standards from the same manufacturer as the majority of the other laboratories. The laboratory noted various problems when carrying out the analysis, especially unsatisfactory calibration (see Appendix 6.10), meaning that the outliers reported can be traced back to that. In this regard, it is worth noting that this laboratory could not correctly determine the concentration of TEA in the CONT_AT control standard (z-score 23.9), yet was able to achieve a sufficiently accurate result for TEA in tomato products in two out of three cases. This discrepancy cannot be explained by a faulty standard.

4.4 Recovery correction

The participants were instructed to correct the results for recovery. The recoveries reported by the laboratories can be found in Table 14 (Appendix 6.3). The methods used to determine recovery were not asked about in the survey. Participant LC0003 noted that the recovery was calculated from the ratio of the peak area of the internal standard in the sample and the peak area of the internal standard in the matrix-free calibration solution (Appendix 6.10).

The four participants LC0001, LC0004, LC0006 and LC0013 stated in the survey that the results were not corrected with the recovery. These four participants used the draft method prepared within CEN/TC275/WG5-WI275285(E). This method prescribes the addition of an isotope-labelled standard at the beginning of the analysis, which enables an intrinsic recovery rate correction.

Laboratories LC0008 and LC0014 indicated a recovery rate correction without reporting the numerical recovery values. Laboratory LC0008 also used the draft method as per CEN/TC275/WG5-WI275285 (E). Laboratory LC0014 determined concentrations using a standard addition method, which can also provide a recovery rate correction.

The analysis results were processed as reported by the laboratories in the statistical calculations, i.e. no subsequent recovery rate corrections were made by the organisers.

Laboratory LC0006 noted that six commercial samples were purchased for the purpose of determining recovery. All six samples were naturally contaminated with *Alternaria* toxins and were therefore not considered suitable for determining recovery (Appendix 6.10).

Participant LC0001 noted that a very low recovery of the isotope-labelled standards was observed for several analytes. The peak areas of the isotope-labelled standards measured in the sample amounted to approximately 1%, 2%, 0.5%, 4% and 99% of the peak areas in the matrix-free calibration solution for ALT, AOH, AME, TEN and TEA respectively. The isotope-labelled standard was added to the sample before extraction in the method used. Therefore it cannot be deduced with certainty which working step of the method caused this signal loss. As high losses are usually not expected during sample extraction and clean-up using the specified CEN method draft, ion suppression in the mass spectrometry detector appears to be the most likely cause. This conclusion conforms with the laboratory's comment that high signal losses were also observed when using the method of the BfR.

Despite the considerable signal loss for the first four analytes mentioned, laboratory LC0001 achieved a satisfactory z-score of $|z| \leq 2$ in 17 out of 19 cases. After the conclusion of the proficiency test, this laboratory disclosed upon request that it traced the unsatisfactory results of the altenuene analysis to the aforementioned high signal loss.

Laboratory LC0002 reported a higher recovery of the internal standard than laboratory LC0001, with values of around 50-110%. The internal standard recoveries for laboratory LC0003 were within the range of 80-110%. It can be concluded from these examples that the extremely high signal loss reported by laboratory LC0001 during the analysis of *Alternaria* toxins in tomato products is not a common phenomenon.

4.5 Conclusion

In summary, it can be confirmed that good results were achieved by the laboratories participating in this proficiency test. The analysis of *Alternaria* toxins has been mastered by the majority of laboratories. The reproducibility standard deviation remains below the target standard deviation (capped Horwitz function) for all sample/analyte combinations, meaning that HorRat values below 1 have been reached (Table 5).

5 Literature

Hampel, F., Ronchetti, E. M., Rousseeuw, P. J., Stahel, W. 1986. Robust Statistics. The Approach Based on Influence Functions. Vol. Wiley, New York.

Horwitz, W., Albert, R. 2006. The Horwitz Ratio (HorRat): A useful index of method performance with respect to precision. J.of AOAC International 89(4):1095-1109.

ISO 13528:2015 Statistical methods for use in proficiency testing interlaboratory comparisons

Lamberty, A., Schimmel, H. & Pauwels, J. 1998 The study of the stability of reference materials by isochronous measurements. Fresenius J Anal Chem 360, 359–361 https://doi.org/10.1007/s002160050711

Pereboom, D. P. K. H., Dam, R. C. J., van Rijk, T. C., de Nijs, M., & Mol, J. G. J. 2019. Proficiency test for deoxynivalenol (DON), acetyl-DONs and DON-3G in cereals: EURL-PT_MP01 (2018). (RIKILT report; No. 2019.007). Wageningen: RIKILT Wageningen University & Research. https://doi.org/10.18174/476812

Thompson, M. 2000. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing. Analyst 125:385-386.

Thompson, M., Ellison, S.L.R., and Wood, R. 2006: The International Harmonized Protocol for the proficiency testing of analytical chemistry laboratories (IUPAC Technical Report). Pure Appl. Chem. 78(1), 145-196

Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs

6 Appendix I

6.1 Stability

Table 10: Concentrations and statistical parameters for the analysis of stability for sample BfR_JU

Tomato juice BfR_JU	TEA			AOH			ALT			TEN			AME		
Temperature of storage	-80°C	-20°C	1 day RT	-80°C	-20°C	1 day RT	-80°C	-20°C	1 day RT	-80°C	-20°C	1 day RT	-80°C	-20°C	1 day RT
Concentration [µg/kg]	212	209	203	3.55	3.15	3.64	10.0	9.98	8.80	45.9	46.0	44.6	5.38	5.39	5.18
	208	206	205	3.36	3.37	3.04	9.81	8.96	10.2	46.4	46.1	45.7	5.35	5.15	5.16
	213	209	205	3.33	3.44	3.63	9.66	9.70	8.78	46.6	45.6	45.4	5.35	5.24	5.02
n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Mean [µg/kg]	211	208	204	3.41	3.32	3.43	9.82	9.55	9.26	46.3	45.9	45.2	5.36	5.26	5.12
Standard deviation [µg/kg]	2.9	1.7	1.0	0.12	0.15	0.34	0.18	0.52	0.82	0.3	0.2	0.6	0.02	0.12	0.09
Difference to -80°C [µg/kg]		2.8	6.7		0.09	0.02		0.28	0.56		0.38	1.07		0.09	0.24
0.3*tH		12.8	12.8		0.22	0.22		0.65	0.65		3.05	3.05		0.35	0.35
Difference < 0.3*tH		Yes	Yes		Yes	Yes		Yes	Yes		Yes	Yes		Yes	Yes

Total length of storage: 118 days
„1 day RT“: Samples were stored at -20°C for 69 days, thawed for one day at room temperature and stored at -20°C again

Table 11: Concentrations and statistical parameters for the analysis of stability for sample BfR_KE

Tomato ketchup BfR_KE	TEA			AOH			ALT			TEN			AME		
Temperature of storage	-80°C	-20°C	1 day RT	-80°C	-20°C	1 day RT	-80°C	-20°C	1 day RT	-80°C	-20°C	1 day RT	-80°C	-20°C	1 day RT
Concentration [µg/kg]	80.8	80.0	76.9	14.8	14.7	13.9	2.27	2.29	2.30	125	122	123	1.52	1.61	1.52
	77.5	83.3	73.6	14.9	14.5	13.3	2.20	2.33	2.27	125	130	119	1.42	1.41	1.44
	78.3	78.5	76.7	14.4	15.0	14.0	2.46	2.03	2.11	123	122	121	1.46	1.42	1.45
n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Mean [µg/kg]	79	81	76	14.68	14.73	13.73	2.31	2.22	2.23	124.0	124.5	120.7	1.46	1.48	1.47
Standard deviation [µg/kg]	1.7	2.4	1.9	0.26	0.25	0.38	0.14	0.16	0.10	0.9	4.8	2.3	0.05	0.11	0.04
Difference to -80°C [µg/kg]		1.7	3.2		0.05	0.95		0.09	0.08		0.50	3.33		0.01	0.00
0.3*tH		5.2	5.2		0.97	0.97		0.15	0.15		8.15	8.15		0.10	0.10
Difference < 0.3*tH		Yes	Yes		Yes	Yes		Yes	Yes		Yes	Yes		Yes	Yes

Total length of storage: 118 days
„1 day RT“: Samples were stored at -20°C for 68 days, thawed for one day at room temperature and stored at -20°C again

Table 12: Concentrations and statistical parameters for the analysis of stability for sample BfR_PU

Tomato puree BfR_PU	TEA			AOH			ALT			TEN			AME		
Temperature of storage	-80°C	-20°C	1 day RT	-80°C	-20°C	1 day RT	-80°C	-20°C	1 day RT	-80°C	-20°C	1 day RT	-80°C	-20°C	1 day RT
Concentration [µg/kg]	687	672	681	7.25	7.05	7.71	10.1	9.68	9.14	13.0	12.8	12.9	19.0	16.8	18.3
	691	675	674	7.65	7.65	7.87	9.79	10.2	9.55	13.1	12.4	12.3	18.0	18.4	17.7
	673	702	663	7.63	7.94	7.37	10.2	10.4	9.77	13.1	13.5	12.6	18.2	18.9	17.4
n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Mean [µg/kg]	683	683	673	7.51	7.54	7.65	10.02	10.09	9.49	13.0	12.9	12.6	18.4	18.0	17.8
Standard deviation [µg/kg]	9.5	16.7	9.1	0.23	0.45	0.26	0.20	0.38	0.32	0.1	0.6	0.3	0.6	1.1	0.4
Difference to -80°C [µg/kg]		0.5	10.7		0.03	0.14		0.07	0.53		0.15	0.45		0.35	0.58
0.3*tH		34.7	34.7		0.50	0.50		0.66	0.66		0.86	0.86		1.21	1.21
Difference < 0.3*tH		Yes	Yes		Yes	Yes		Yes	Yes		Yes	Yes		Yes	Yes

Total length of storage: 118 days

„1 day RT“: Samples were stored at -20°C for 68 days, thawed for one day at room temperature and stored at -20°C again

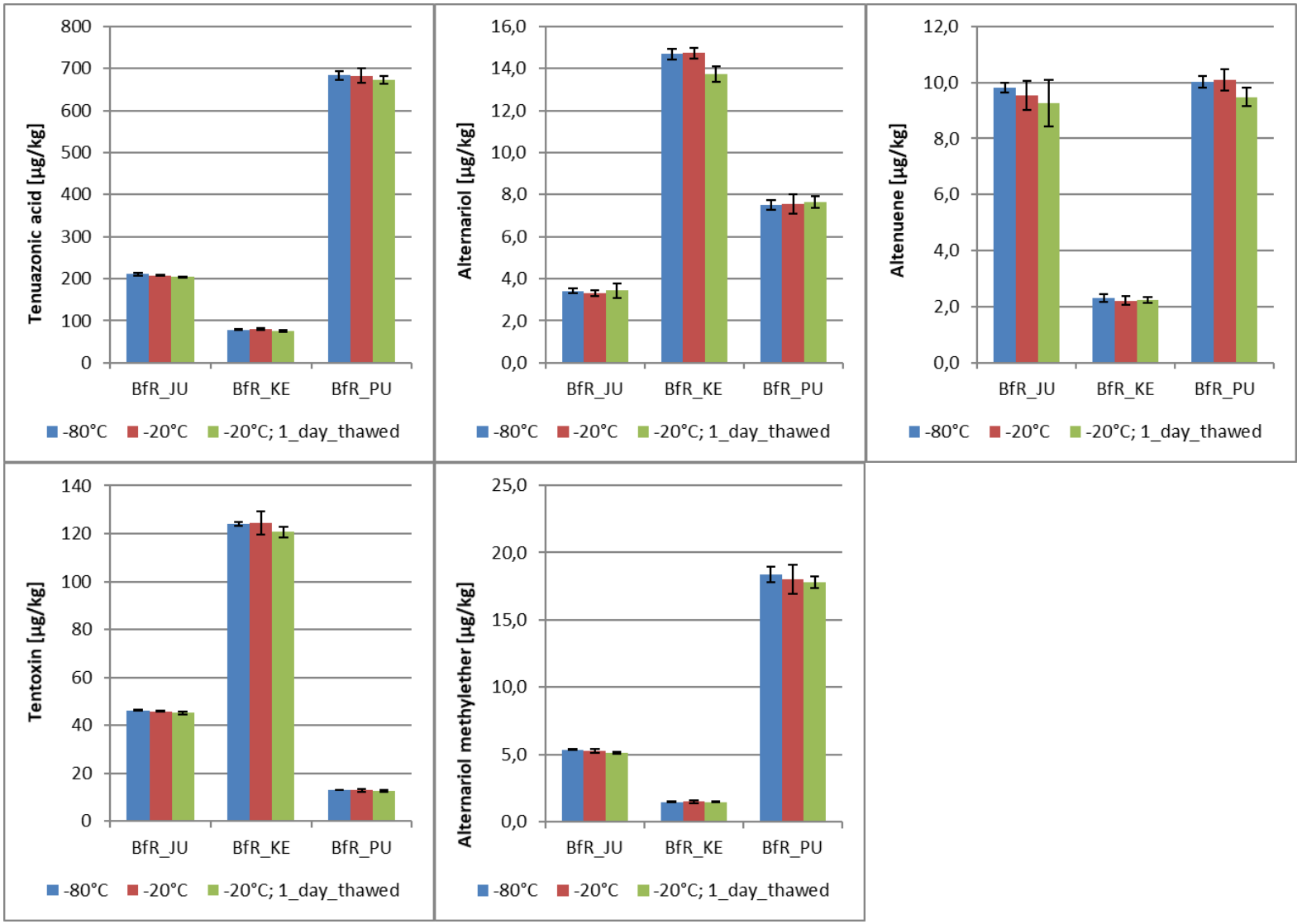

Figure 3: Assessment of stability (mean concentrations for 3 replicates; bars: standard deviation); “1 day thawed” = Samples were stored at -20°C, thawed for one day and stored at -20°C again.

6.2 Limits of detection and quantitation (as reported by participants)

Table 13: Limits of detection (LOD) and limits of quantitation (LOQ)

	Analyt	LC0001		LC0002		LC0003		LC0004		LC0005		LC0006		LC0007		LC0008		LC0011		LC0013		LC0014		LC0015	
		LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD
CONT_AT	ALT	5.0	2.5			0.5	0.25	3.6	0.9															1.2	0.4
	AME	2.0	1			0.1	0.05	2.3	0.6															0.5	0.16
	AOH	3.0	1			0.3	0.13	2.5	0.7															0.3	0.1
	TEA	25.0	10			0.5	0.25	31.4	8.1															0.5	0.15
	TEN	10.0	2			0.3	0.13	11.2	2.9															0.2	0.05
BFR_JU	ALT	5.0	2.5	0.9	0.3	2.0	1	3.6	0.9	5.0	2.5	15.0	7.5	1.0	0.3	4.0	1.98						5	2.9	0.9
	AME	2.0	1	0.5	0.1	0.4	0.2	2.3	0.6	2.0	1	5.0	2	0.3	0.1	0.1	0.031	1.0	1				5	0.8	0.2
	AOH	3.0	1	0.7	0.2	1.0	0.5	2.5	0.7	2.0	1	8.0	4	0.3	0.1	1.8	0.9						5	0.8	0.2
	TEA	25.0	10	6.0	2	2.0	1	31.4	8.1	2.0	1	15.0	7.5	0.5	0.2	1.9	0.931	10.0	10			20.0	10	1.1	0.3
	TEN	10.0	2	0.4	0.1	1.0	0.5	11.2	2.9	2.0	1	5.0	2	0.3	0.1	0.2	0.106	5.0	5			5.0	2	1.6	0.5
BFR_KE	ALT	5.0	2.5	1.0	0.4	2.0	1	3.6	0.9	5.0	2.5	15.0	7.5	1.8	0.5	4.4	2.21			10.0		10.0	5	2.9	0.9
	AME	2.0	1	0.7	0.2	0.4	0.2	2.3	0.6	2.0	1	5.0	2	0.6	0.2	0.1	0.029	1.0	1			10.0	5	0.8	0.2
	AOH	3.0	1	3.0	0.9	1.0	0.5	2.5	0.7	2.0	1	8.0	4	0.6	0.2	3.0	1.52					10.0	5	0.8	0.2
	TEA	25.0	10	6.0	2	2.0	1	31.4	8.1	2.0	1	15.0	7.5	1.0	0.4	2.1	1.05	10.0	10			30.0	10	1.1	0.3
	TEN	10.0	2	0.7	0.2	1.0	0.5	11.2	2.9	2.0	1	5.0	2	0.6	0.2	0.2	0.086	5.0	5			5.0	2	1.6	0.5
BFR_PU	ALT	5.0	2.5	0.9	0.3	2.0	1	3.6	0.9	5.0	2.5	15.0	7.5	1.8	0.5	1.9	0.95					10.0	5	2.9	0.9
	AME	2.0	1	0.9	0.3	0.4	0.2	2.3	0.6	2.0	1	5.0	2	0.6	0.2	0.1	0.026	1.0	1			10.0	5	0.8	0.2
	AOH	3.0	1	2.0	0.6	1.0	0.5	2.5	0.7	2.0	1	8.0	4	0.6	0.2	1.4	0.7					10.0	5	0.8	0.2
	TEA	25.0	10	30.0	8	2.0	1	31.4	8.1	2.0	1	15.0	7.5	1.0	0.4	2.1	1.05	10.0	10			20.0	10	1.1	0.3
	TEN	10.0	2	0.8	0.3	1.0	0.5	11.2	2.9	2.0	1	5.0	2	0.6	0.2	0.2	0.103	5.0	5			5.0	2	1.6	0.5

6.3 Recoveries (as reported by participants)

Table 14: Recoveries (REC) and recovery correction

Sample	Analyte	LC0001	LC0002	LC0003	LC0004	LC0005	LC0006	LC0007	LC0008	LC0011	LC0013	LC0014	LC0015
		REC [%]	REC [%]	REC [%]	REC [%]	REC [%]	REC [%]	REC [%]	REC [%]	REC [%]	REC [%]	REC [%]	REC [%]
CONT_AT	ALT	100			100						100		100
	AME	100			100						100		100
	AOH	100			100						100		100
	TEA	100			100						100		100
	TEN	100			100						100		100
BFR_JU	ALT	100	105	93	100	98.2		91.2			100		88.6
	AME	100	101	98	100	97.3		95.7		132.5	100		89.7
	AOH	100	101	96	100	103		91.5			100		81.1
	TEA	100	95	87	100	98.6		91.9		126.9	100		70.8
	TEN	100	101	85	100	99.7		96.8		105.7	100		91.6
BFR_KE	ALT	100	101	92	100	98.2		90.1			100		88.6
	AME	100	102	107	100	97.3		98.8		128.4	100		89.7
	AOH	100	102	101	100	103		93.7			100		81.1
	TEA	100	87	90	100	98.6		90.5		116	100		70.8
	TEN	100	99	82	100	99.7		96.8		132.9	100		91.6
BFR_PU	ALT	100	101	88	100	98.2		90.1			100		88.6
	AME	100	98	86	100	97.3		98.8		111.8	100		89.7
	AOH	100	100	93	100	103		93.7			100		81.1
	TEA	100	90	93	100	98.6		90.5		102.9	100		70.8
	TEN	100	97	80	100	99.7		96.8		117.6	100		91.6
Recovery correction applied ?		No	Yes	Yes	No	Yes	No	Yes	Yes	Yes	No	Yes	Yes
Method		CEN	NRL	other	CEN	NRL	CEN	NRL	CEN	CEN	CEN	other	CEN

LC0003: REC=Ratio of peak area of internal standard in sample and peak area of internal standard in matrix free calibration

6.4 Measurement uncertainty (as reported by participants)

Table 15: Measurement uncertainty (MU)

Sample	Analyte	LC0001	LC0002	LC0003	LC0004	LC0005	LC0006	LC0007	LC0008	LC0011	LC0013	LC0014	LC0015
		MU [%]	MU [%]	MU [%]	MU [%]	MU [%]	MU [%]	MU [%]	MU [%]	MU [%]	MU [%]	MU [%]	MU [%]
CONT_AT	ALT			3.48	10						35		3.9
	AME			9.37	10						35		4.2
	AOH			5.58	10						35		4.2
	TEA			9.55	10						35		4.4
	TEN			14.4	10						35		6.8
BFR_JU	ALT	84		1.15	10		11.1				35	13	28.3
	AME	42	40	5.55	10		4			16.6	35	48	19
	AOH	30	41	16.5	10						35	28	40.5
	TEA	3	36	5.81	10		12.6			16.6	35	20	14.5
	TEN	3	40	12.4	10		15.3			16.6	35	25	18.6
BFR_KE	ALT		43	20.9	10						35	16	112.6
	AME	17	42	4.89	10					16.6	35	48	49.6
	AOH	18	41	5.96	10		13.3				35	22	19.1
	TEA	6	36	5.36	10		11.5			16.6	35	20	14.5
	TEN	2	36	6.75	10		16.4			16.6	35	12	19.9
BFR_PU	ALT	33	40	10.9	10						35	13	31.6
	AME	14	40	12.3	10		7.5			16.6	35	48	19
	AOH	35	41	7.66	10						35	28	19.1
	TEA	4	30	5.84	10		7.2			16.6	35	12	14.5
	TEN	4	40	7.92	10		15.3			16.6	35	25	19.9

6.5 Accompanying documents

Bundesinstitut für Risikobewertung

German Federal Institute for Risk Assessment • PO Box 12 69 42 • 10609 Berlin

German Federal Institute for Risk Assessment
PO Box 12 69 42
10609 Berlin, GERMANY
Tel. +49 30 18412-0
Fax +49 30 18412-99099
bfr@bfr.bund.de
www.bfr.bund.de/en

Reference number and date of original message	Reference number (please include in reply)	Tel. extension/ fax number	Date	Date Org. unit/contact
	85-60-0201-10/001-10477719	-28502	15/04/2019	85/Dr. Bahlmann

Proficiency Test 2019 "Alternaria toxins in tomato products"

Dear

Enclosed with the present document you receive the samples for the proficiency test for alternaria toxins in tomato products. For further instructions and your laboratory code, please see the enclosed document "Annex 1".

Please use the form "Annex 2" to confirm the receipt of samples upon arrival.

For the submission of results you will receive an e-mail containing the two data files **LC0011.LAB** and **LC0011.LA2**. In addition, please fill out the file "**Method_parameters_BfR2019_Alternaria_PT_LCxxxx.xls**" to report the parameters of the analytical method used. Instructions how to use these data files can be found in attachment "Annex 3". Please send back the completed data files **LC0011.LAB** and **LC0011.LA2** and the excel sheet **Method_parameters_BfR2019_Alternaria_PT_LCxxxx.xls**

until the **7th of June 2019**

by e-mail to:
nrl_mykotoxine@bfr.bund.de

or to the following address:
German Federal Institute of Risk assessment
FG 85 – Plant toxins and mycotoxins, NRL for Mycotoxins
Max-Dohrn-Str. 8 - 10
10589 Berlin
Germany

or send a **Fax: +49 30 18412-628502**

Please note the regulations of the Federal Institute for Risk Assessment (BfR) for the exchange of material. A document containing the "General Terms and Conditions for the Exchange of Materials – Provision by the BfR (Material Transfer-Terms and Conditions – Part A)" can be retrieved directly under the link http://www.bfr.bund.de/cm/349/mt-terms-and-conditions-part-a.pdf.

With best regards,

Dr. Arnold Bahlmann

Annex

Annex 1: Protocol
Annex 2: Confirmation of receipt
Annex 3: User guide for data reporting

Annex 1: Protocol

1. **Your Lab code: LC0011**

2. You receive three samples of tomato products and one thin film standard.

Sample	
Tomato juice	BFR_JU
Tomato ketchup	BFR_KE
Tomato purée	BFR_PU
Thin film standard	CONT_AT

3. Please acknowledge receipt and report the conditions of the test material by returning the reply form (Annex 2).
   If necessary, you will receive a replacement sample.

4. In order to ensure comparability of measurements you are required to store the samples immediately after receipt in a freezer at a temperature of -18 °C or below until analysis.

5. Samples and dissolved thin film standards have to be tempered to room temperature and need to be mixed completely before usage.

6. The concentration of the thin film standard is in the usual range of calibration. To dissolve the thin film standard, please proceed as follows: Add accurately **1,00 mL** of solvent to the vial. Use the same solvent mixture which is used for your calibration. Dissolve the standard by thorough mixing (e.g. 10 minutes vortex mixing). After these steps, the solution is ready for analysis. If the concentration is outside your calibration function, suitable dilution steps need be conducted.

7. You may freely choose the method of analysis.

8. **3 replicates** of each sample and thin film standard are mandatory.

9. Please record the mass in [g] with an accuracy of three significant digits. The analytical results should be reported in [µg/kg] for tomato products and [ng/mL] for the control standard with an accuracy of three significant digits (Example: [34.5] or [3.45]).

10. Please provide details about your equipment and measurement conditions by filling the excel data sheet (Method_parameters_BfR2019_Alternaria_PT_LCxxxx.xls). In addition, please report any irregularities that occurred during analysis and might affect the result.

11. Please send one chromatogram selected as being representative for the analysis of samples and control standard. Please mark all relevant peaks used for quantification.

12. Please report all data electronically. Further instructions regarding how to report your data can be found in Annex 3.

Please address any queries to Dr. Arnold Bahlmann

Phone: +49 30 18412 28502
or nrl_mykotoxine@bfr.bund.de.

Annex 2:

Proficiency test: Alternaria toxins in tomato products

Confirmation of receipt

Name			
Institute			
Lab code	**LC0011**	**Date of receipt**	

Test material		**Condition on arrival**
Tomato juice (BFR_JU)	o.k. ☐	damaged ☐
Tomato ketchup (BFR_KE)	o.k. ☐	damaged ☐
Tomato puree (BFR_PU)	o.k. ☐	damaged ☐
Control standard as thin film (CONT_AT)	o.k. ☐	damaged ☐

Date **Signature**

Please send this form to:

Fax: +49 30 18412 628502

or as a pdf-file to:

e-mail: nrl_mykotoxine@bfr.bund.de

Annex 3: User Guide for Data Reporting – Electronic Recording

You will receive the following two template files. Please use these templates to report your results:

LC0011.LAB (Results table, **LC0011** is your lab code)
LC0011.LA2 (configuration file, includes sample information and legend)

To enter your data you need the configurable data input module called "RingDat".
Please download the file "RingDat.exe" from the following URL address: http://quodata.de/fileadmin/RingDat/ringdat4_en.zip. The download is free of charge.

To report your data, please proceed as follows:

1. Copy the provided data files on a selected directory on your hard disk.

2. If desired, you may voluntarily report results obtained from more than one analytical method. If so, please rename the file from **LC0011.LAB** to **LC0011_1.LAB, LC0011_2.LAB** and so on. Skip this point, if you want to submit a single data set.

3. Open the file RINGDAT4.exe with a double-click.

4. Click on "Open" in the menu bar. A menu window will appear where you can find the file **LC0011.LAB** in the corresponding directory. Open that file now, the table "Proficiency Test: Alternaria toxins in tomato products 2019" will appear.

5. Enter your analytical results (stated in µg/kg eg. ng/ml) into column "MWx" (x = 1;2;3) with an accuracy of three significant digits (e.g. 45.3 or 4.,53). Please enter " <(LOD) "(LOD = value of limit of detection, e.g. "<< 0.10") into column "MWx" if an analyte is not detectable. Enter your limit of detection (LOD) into column "LOD" (e.g. "0.10"). If you get levels below the limit of quantification (LOQ), please enter "< (LOQ)" (LOQ = value of LOQ, e.g. "< 100") into column "MWx" and in column "LOQ" the limit of quantification (e.g. "100"). If a substance has not been analysed, please do not enter anything, not even a value, in column LOD or LOQ.
   Enter recovery in column REC and measurement uncertainty in column MU.

6. If you have not analysed the selected sample or analyte, do not fill in anything in the row.

7. Save your file by using the command "Save data". If you have finished data entry, finalize the file using "Finish input".

8. Reduce every column to a minimum size, still allowing for legibility though, by positioning the cursor on top of the column and drawing the width to a smaller scale. It is necessary to change the display format to make a print-out of the result table (protocol) by clicking on “Protocol”. Otherwise an error message may be displayed.

9. Please send the signed result table (protocol) to the address given below.

Please return the templates after inserting your data

LC0011.LAB,
LC0011.LA2,
Method_parameters_BfR2019_Alternaria_PT_LCxxxx.xls

by email to
nrl_mykotoxine@bfr.bund.de

Please send the result table (protocol) with your signature to the address given below.

Fax: +49 30 18412-628502
e-Mail: nrl_mykotoxine@bfr.bund.de

Please submit your results until **7th June 2019**.

6.6 Graphic representations of the results of all participants for all samples

The following graphs display the calculated mean results for each laboratory.

The graphs include the following parameters:

Sample:	sample
Statistical method:	statistical method used for determination of precision (DIN 38402 A45 is equivalent to ISO/TS 20612 or ISO 13528 Annex C)
Rel. sT:	relative target standard deviation, according to Horwitz and modified by Thompson [Thompson, 2000] (sH_r)
Rel. sR:	relative reproducibility standard deviation (RSD_R)
Rel. sr:	relative repeatability standard deviation (RSD_r)
s_R:	reproducibility standard deviation (yellow bar)
s_r:	repeatability standard deviation (yellow bar)
Red lines:	upper and lower limits of tolerance (robust mean +/- 2*sT, corresponds with |z| = 2.00)
Blue line (‘Mean’):	robust mean
Green area:	confidence area of the target value
Blue columns/squares:	reported value of the participant, Mean within limits of tolerance
Red arrow:	values far outside the scale (see assigned value), mean value exceeds tolerance limit
Green column, red square:	reported value of the participant, mean exceeds limit of tolerance
E:	mean result exceeding the limits of tolerance (green bar)
Blue triangles (e.g. Fig. 10):	measured result of the participant below LOQ („<BG“) or below LOD („<<NG“), blunt end of the triangle indicates the value for LOQ or LOD, respectively.

6.6.1 Graphic representations of the results for sample control solution (CONT_AT)

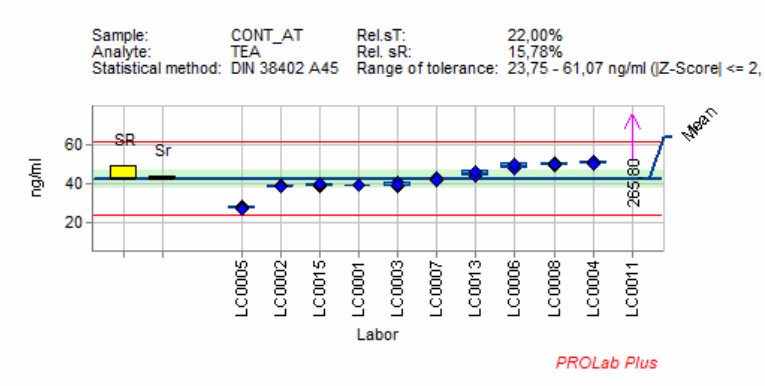

Figure 4: Tenuazonic acid (TEA) / CONT_AT

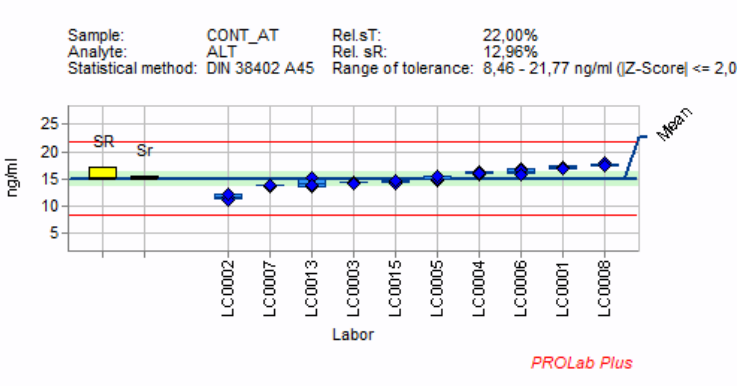

Figure 5: Altenuene (ALT) / CONT_AT

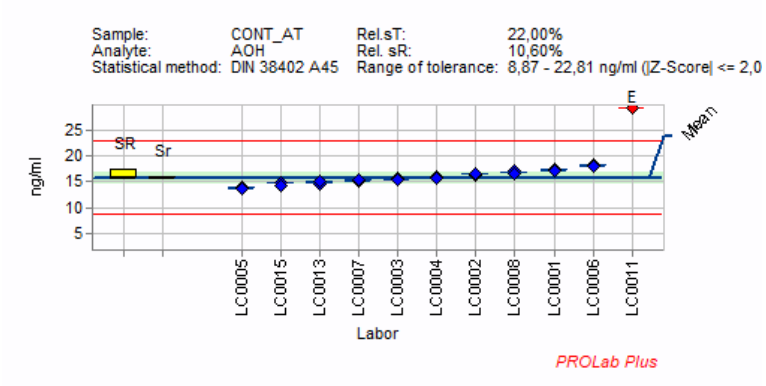

Figure 6: Alternariol (AOH) / CONT_AT

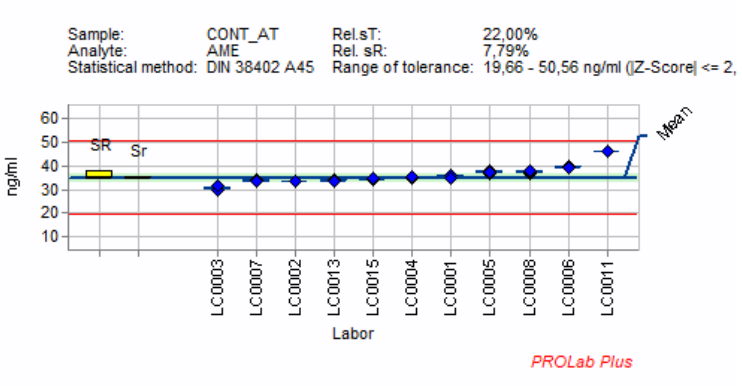

Figure 7: Alternariol methyl ether (AME) / CONT_AT

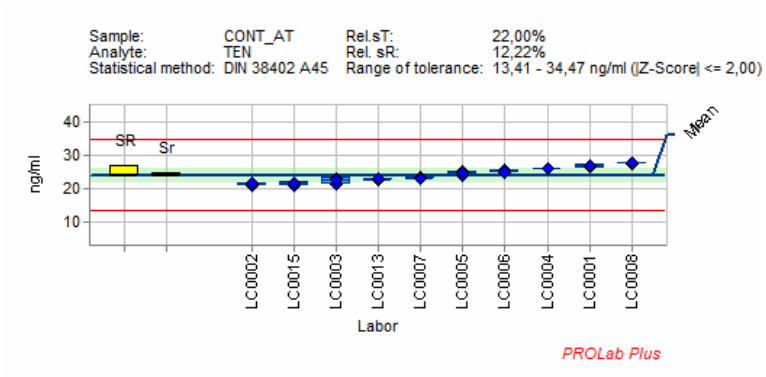

Figure 8: Tentoxin (TEN) / CONT_AT

6.6.2 Graphic representations of the results for sample tomato puree (BfR_PU)

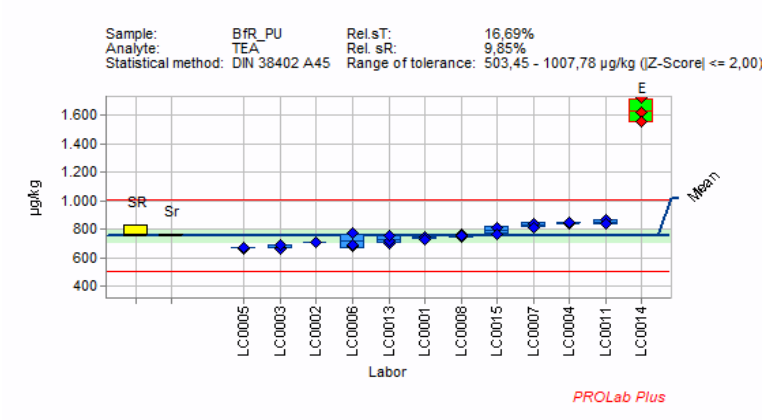

Figure 9: Tenuazonic acid (TEA) / BfR_PU

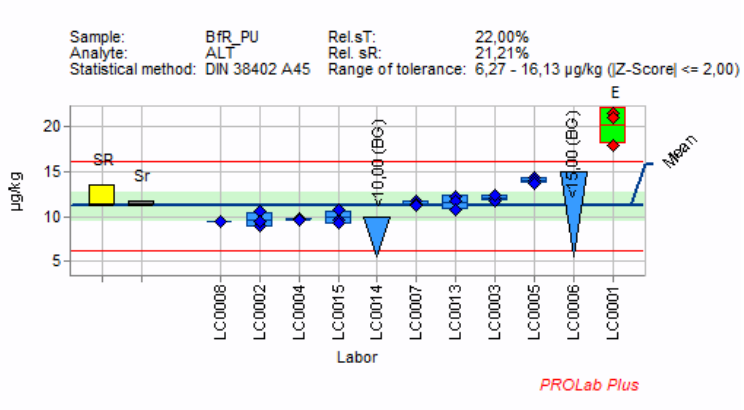

Figure 10: Altenuene (ALT) / BfR_PU

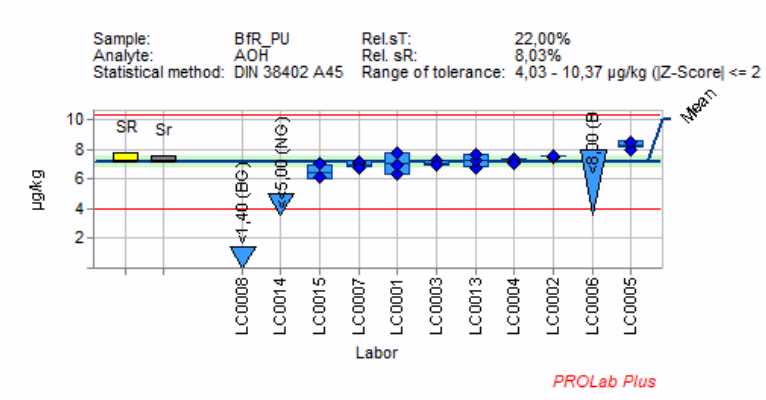

Figure 11: Alternariol (AOH) / BfR_PU

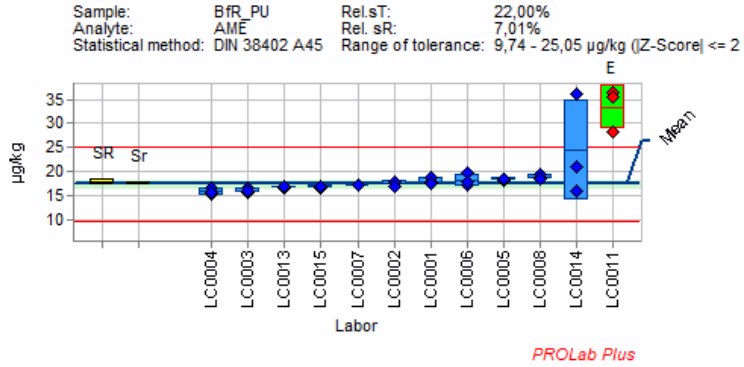

Figure 12: Alternariol methyl ether (AME) / BfR_PU

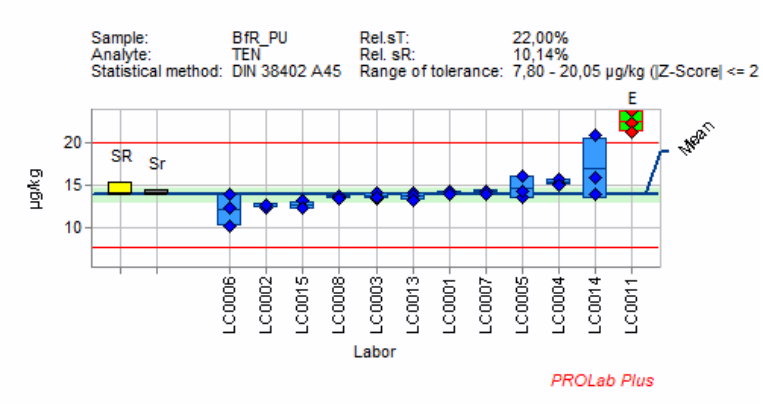

Figure 13: Tentoxin (TEN)/ BfR_PU

6.6.3 Graphic representations of the results for sample tomato ketchup (BfR_KE)

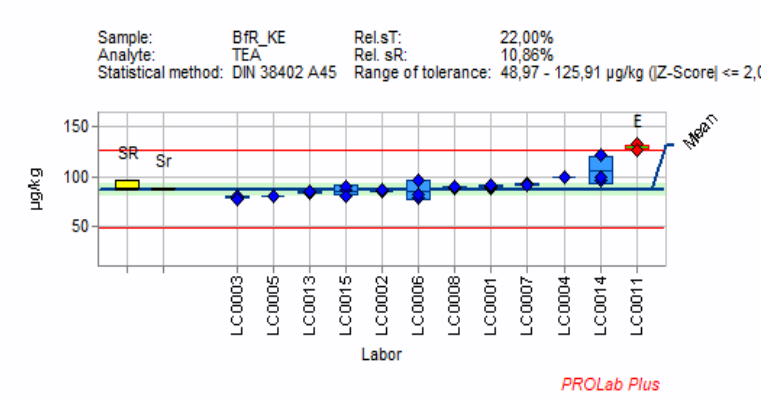

Figure 14: Tenuazonic acid (TEA) / BfR_KE

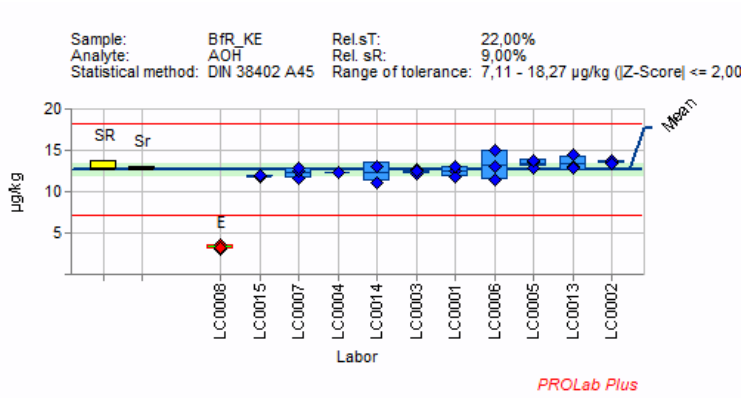

Figure 15: Alternariol (AOH) / BfR_KE

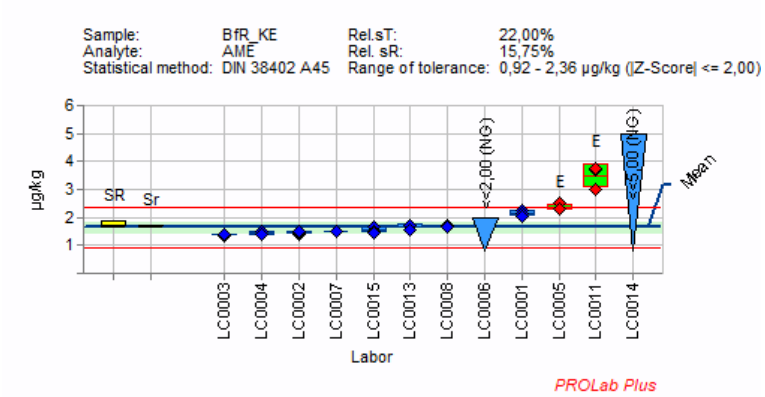

Figure 16: Alternariol methyl ether (AME) / BfR_KE

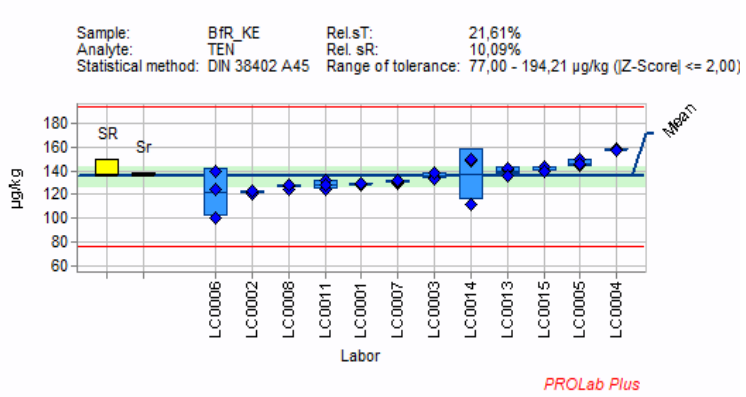

Figure 17: Tentoxin (TEN)/ BfR_KE

6.6.4 Graphic representations of the results for sample tomato juice (BfR_JU)

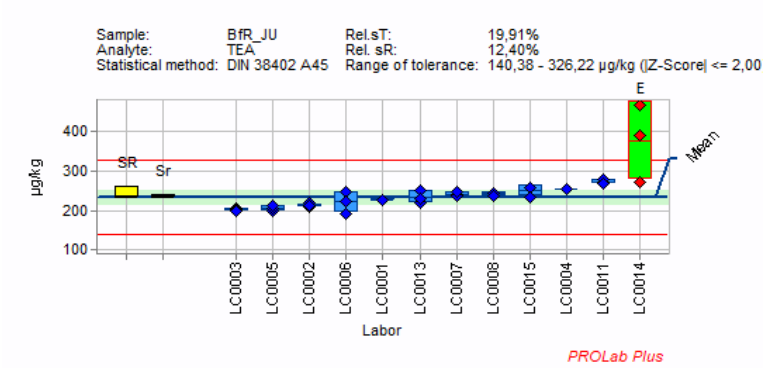

Figure 18: Tenuazonic acid (TEA) / BfR_JU

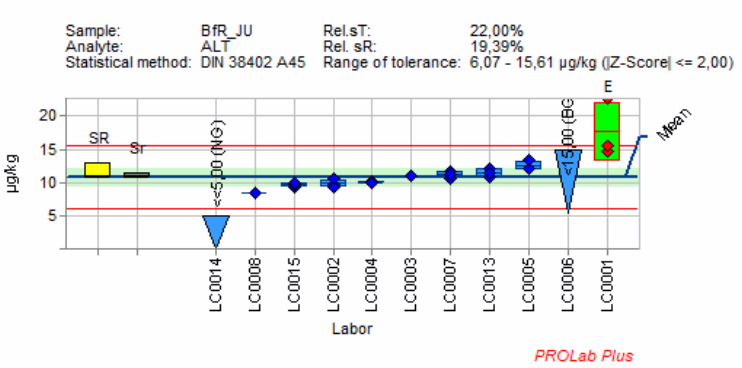

Figure 19: Altenuene (ALT) / BfR_JU

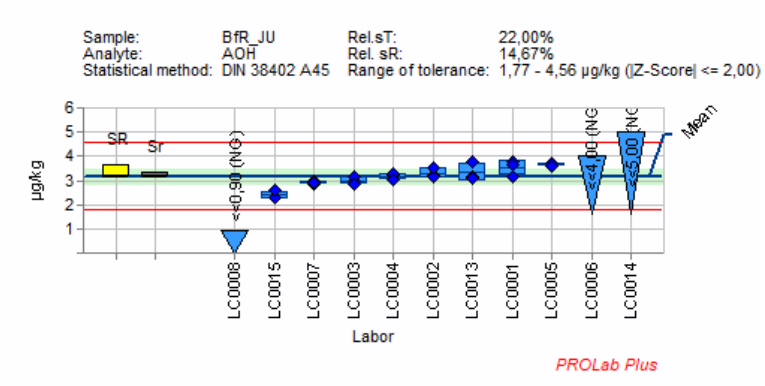

Figure 20: Alternariol (AOH) / BfR_JU

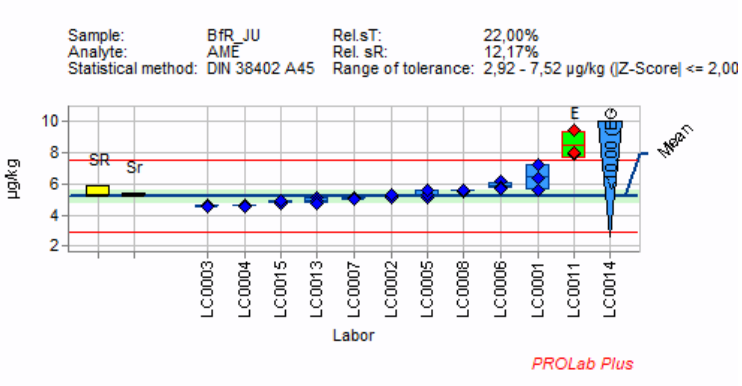

Figure 21: Alternariol methyl ether (AME) / BfR_JU

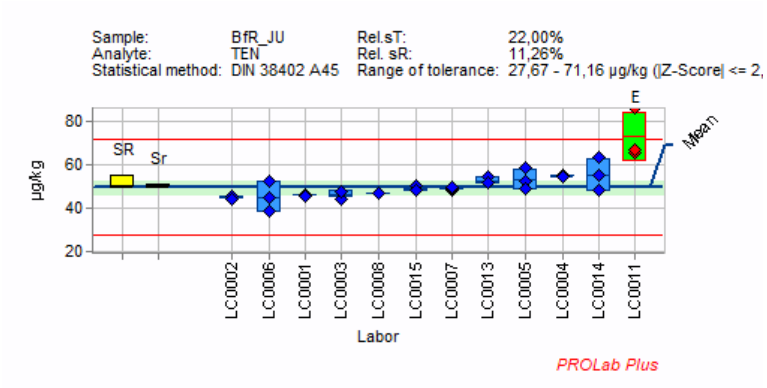

Figure 22: Tentoxin (TEN)/ BfR_JU

6.7 Mandel's statistic – grouped per analyte

In this chapter graphs for Mandel's h and Mandel's k statistics are shown including the reported results from all participants. The red line indicates the critical value for Mandel's k and Mandel's h statistics using α = 0.01. The yellow line shows k and h using α = 0.05; Red bars depict laboratories that exceed the critical value of k or h using α = 0.01; yellow bars depict laboratories that exceed the critical value of k or h using α = 0.05.

In the figures below, the bars correspond to: bar 1: CONT_AT, bar 2: BfR_PU, bar 3: BfR_KE, bar 4: Bf'R_JU .

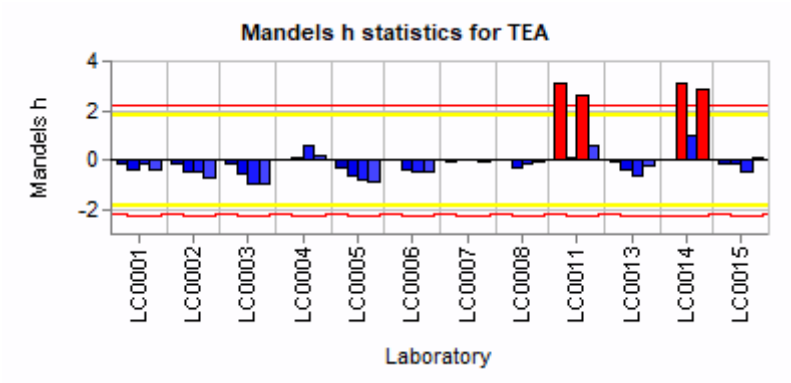

Figure 23: Mandel h statistics / tenuazonic acid (TEA)

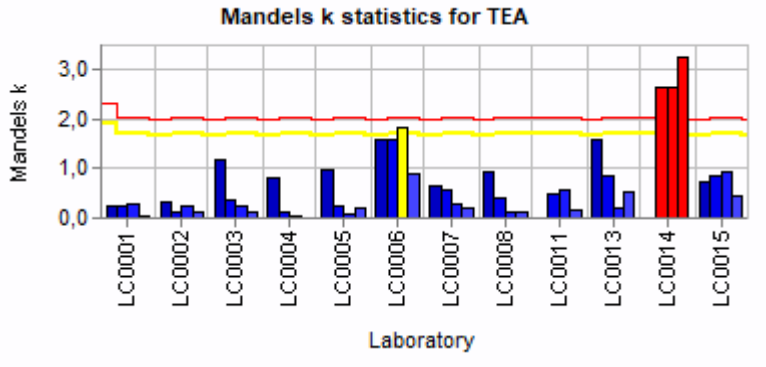

Figure 24: Mandel k statistics / tenuazonic acid (TEA)

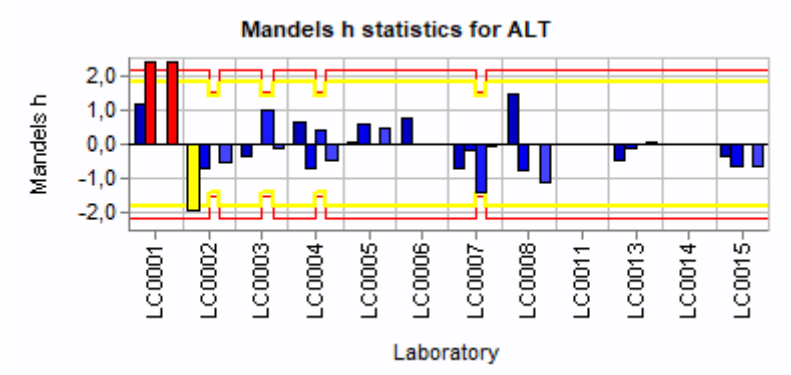

Figure 25: Mandel h statistics / altenuene (ALT)

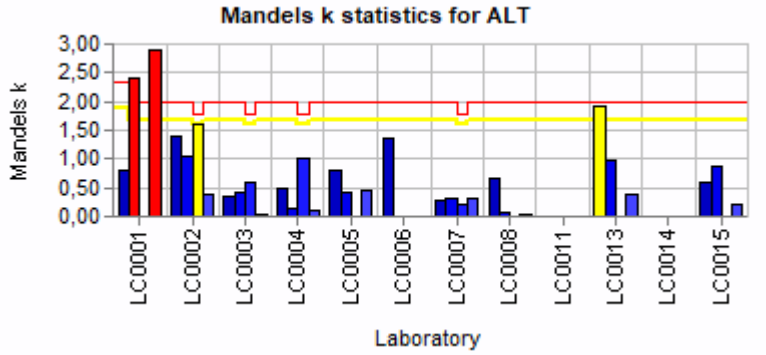

Figure 26: Mandel k statistics / altenuene (ALT)

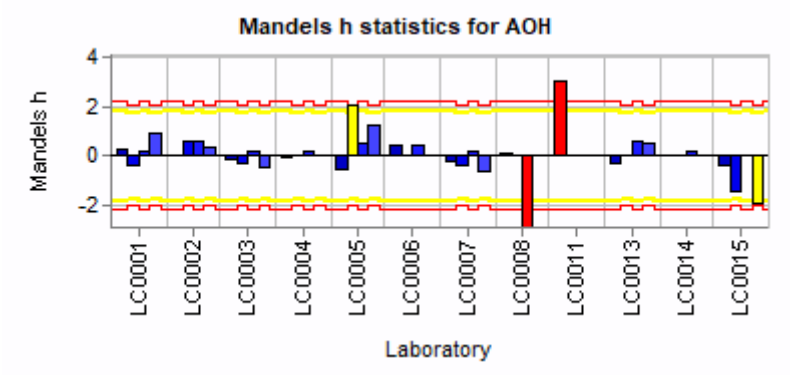

Figure 27: Mandel h statistics / alternariol (AOH)

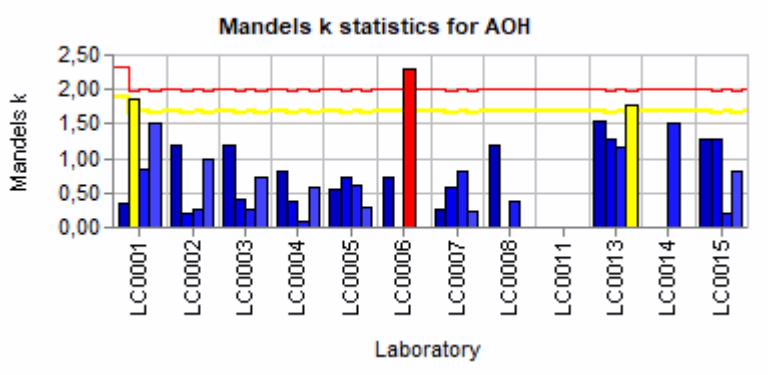

Figure 28: Mandel k statistics / alternariol (AOH)

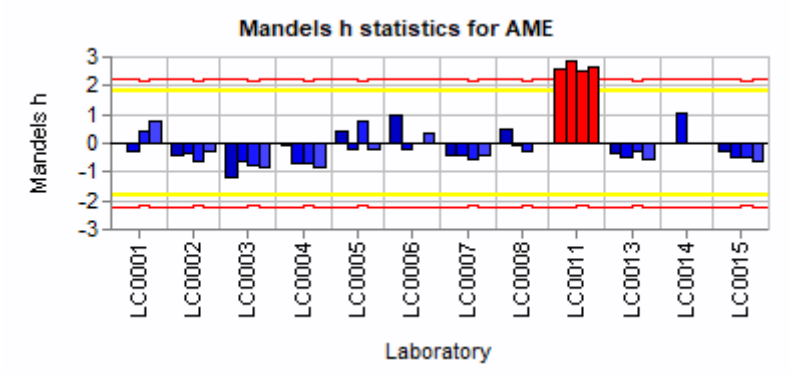

Figure 29: Mandel h statistics / alternariol methyl ether (AME)

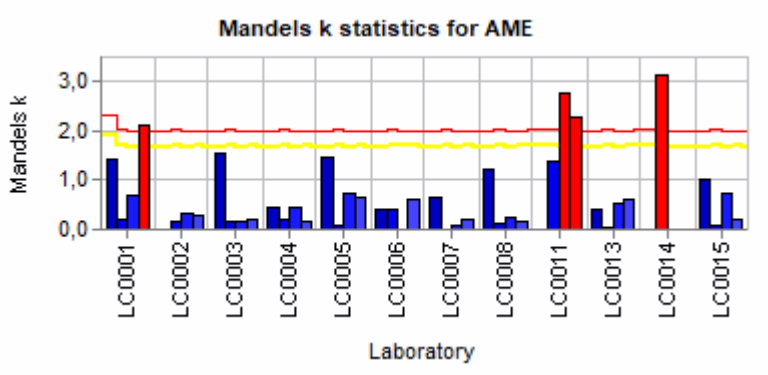

Figure 30: Mandel k statistics / alternariol methyl ether (AME)

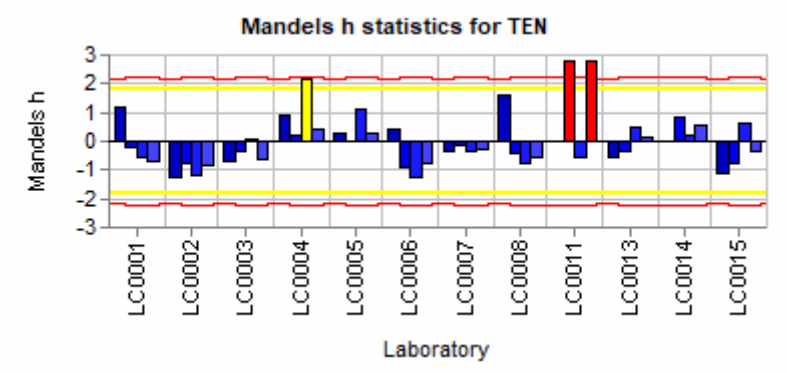

Figure 31: Mandel h statistics / tentoxin

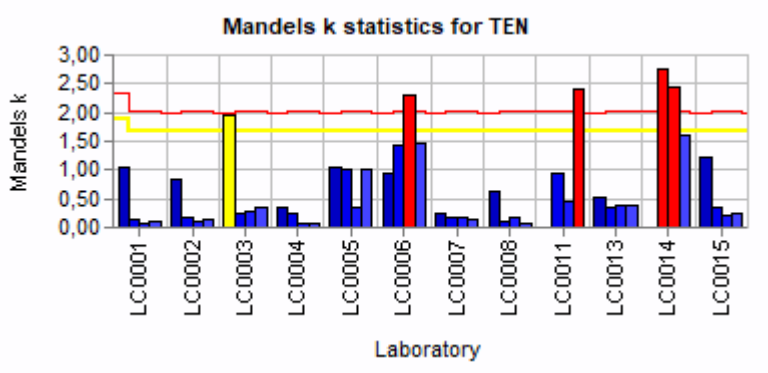

Figure 32: Mandel k statistics / tentoxin

6.8 Mandel's statistic – grouped per sample

In the figures below, the bars correspond to: bar 1: TEA, bar 2: ALT, bar 3: AOH, bar 4: AME, bar 5: TEN .

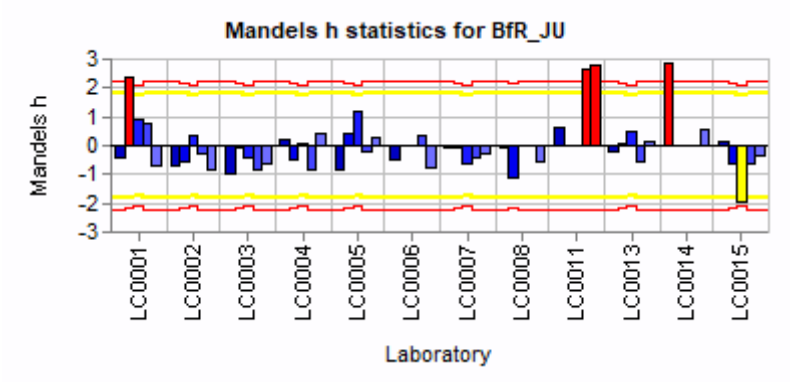

Figure 33: Mandel h statistics / BfR_JU

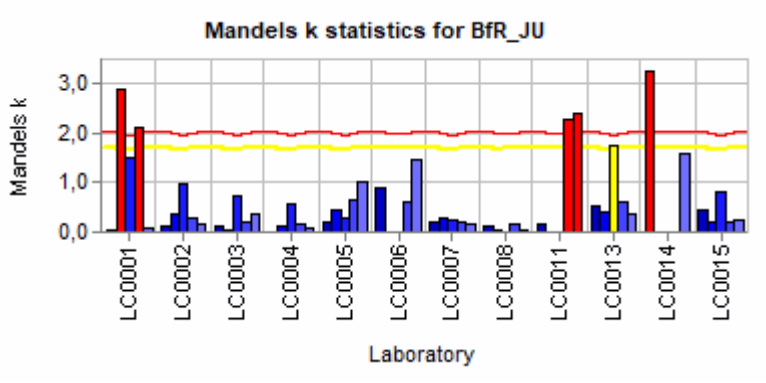

Figure 34: Mandel k statistics / tentoxin BfR_JU

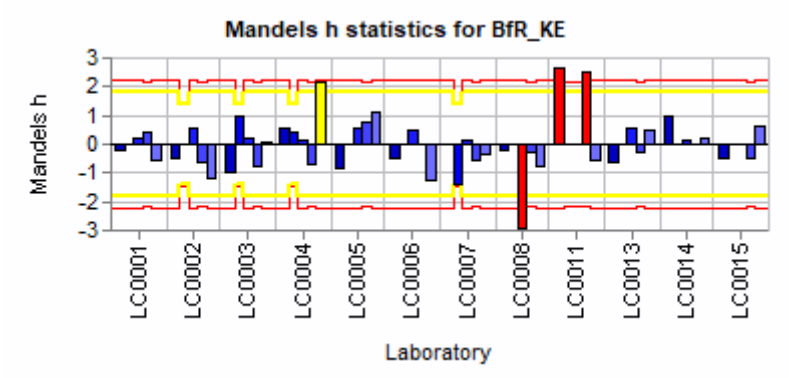

Figure 35: Mandel h statistics / BfR_KE

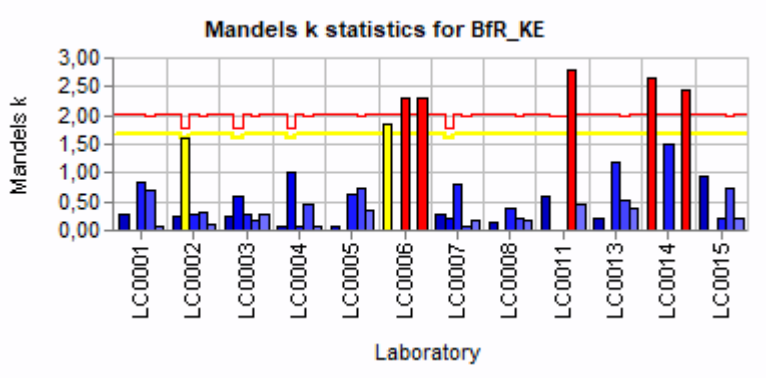

Figure 36: Mandel k statistics / BfR_KE

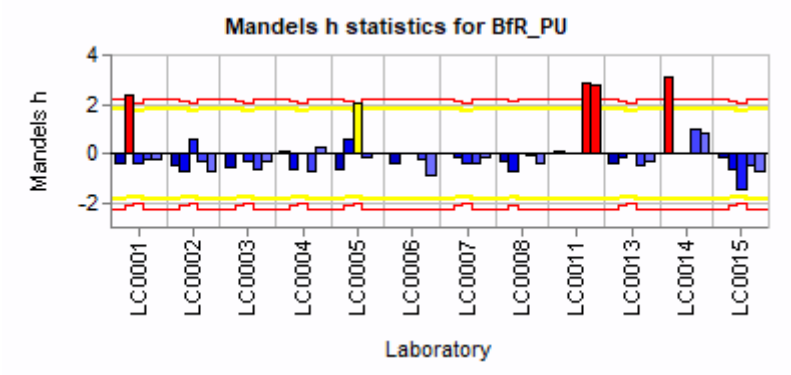

Figure 37: Mandel h statistics / BfR_PU

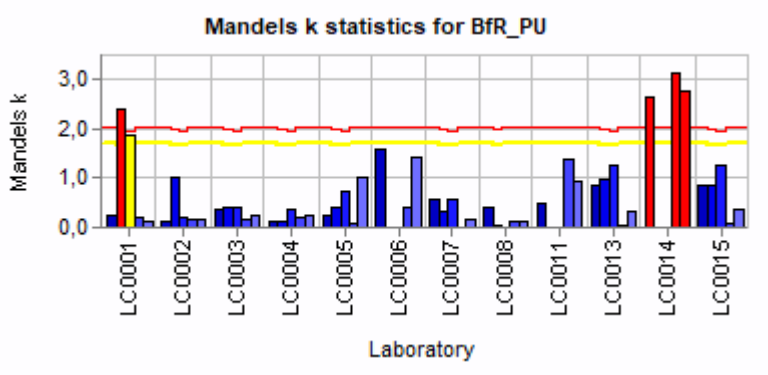

Figure 38: Mandel k statistics / BfR_PU

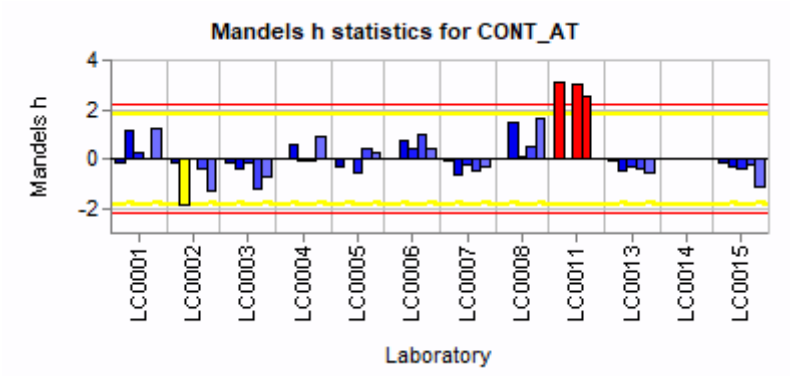

Figure 39: Mandel h statistics / CONT_A

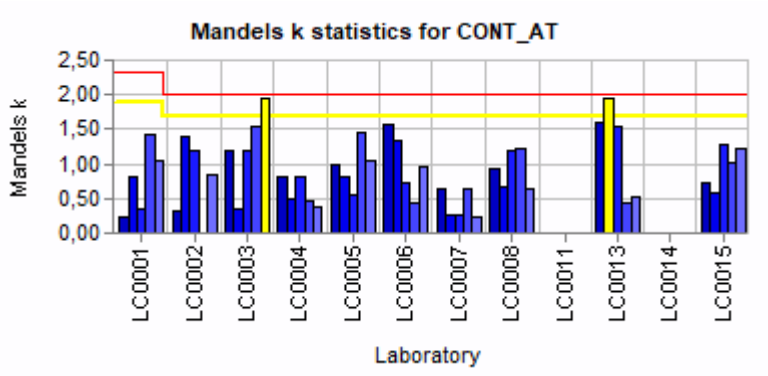

Figure 40: Mandel k statistics / CONT_A

6.9 Analytical methods used by the participants

Table 16: Analytical methods used (as reported by participants in the questionnaire)

	LC0001	LC0002	LC003	LC0004	LC0005	LC0006
Analytical method	CEN/TC275/WG5	German NRL	other	CEN/TC275/WG5	German NRL	CEN/TC275/WG5
Extraction Clean-Up						
Sample amount BFR_PU [g]	2	5	4.0	2.00	5	2
BFR_KE [g]	2	5	4.0	2.00	5	2
BFR_JU [g]	2	10	2.0	2.00	10	2
Extraction		Ketchup and puree samples were diluted with 6 and 5 ml water, respectively. 10 ml acetonitrile containing 2% formic acid were added to all three types of samples. Samples were vigorously shaken for 30 min and centrifuged at 3500 g for 10 minutes at room temperature.	- weigh sample in 50 ml centrifuge tube and add ISTD - adjust pH of sample to ca. 3.4 (using formiate buffer pH 3.4 and formic acid solution) - extract with 20 ml (at 2 g sample amount) or 40 ml (at 4 g sample amount) ethyl acetate, shake - after centrifugation take off 5 ml ethyl acetate supernatant and transfer to a graduated centrifuge tube containing 1.5 ml aquous 0.025 % acetic acid solution - evaporate ethyl acetate using vacuum concentrator with an aquous residue of 1.5 ml remaining - add 0.5 ml of acetonitrile and fill up to 2 ml with 0.025 % acetic acid, if necessary - after shaking and centrifugation, transfer 1 ml of clear supernatant into PP microvial for measurement	No deviations from the method		

Continuation of Table 16: Analytical methods used (as reported by participants in the questionnaire)

	LC0001	LC0002	LC003	LC0004	LC0005	LC0006
Clean Up		2 ml of supernatant was transfered to a 15 ml centrifuge tube. 20 µl of solution containing isotopically labeled analytes and salt (0.4 g MgSO4, 0.1 g NaCl) were added to each tube and the mixture was vortexed thoroughly immediately. Samples were centrifuged at 3500 g for 5 min. 550 µl of the upper (acetonitrile) phase were transferred to a silanized glass tube. The solution was dried under nitrogen and reconstituted in 500 µl acetontrile+aquous ammonium acetate solution (pH9) 25+75 (v+v). The test solution was transfered to a microcentrifuge tube and centrifuged at 17000g. The supernatant was filtered (0,22 µm PTFE) and injected.				
Derivatization						
HPLC						
HPLC_MS	Agilent 6470	SCIEX API4000	API 5500 QTrap	ABSciex Qtrap 6500	Sciex 6500 QTrap +	4000 QTRAP, Fa. Sciex
Instrument	UPLC	HPLC	HPLC	HPLC	HPLC	HPLC
Separation column						
Type	ACQUITY UPLC HSS T3 1.8µm	Phenomenex Gemini NX-C18	Waters , XBridge	Phenomenex Gemini 5µm NX-C18 110A	Phenomenex Gemini NX-C18	Phenomenex Kinetex EVO C18
Dimension	2.1 x 150 mm	100 x 2.1 mm, 5 µm	100mm x 3mm ; 3.5µm	100 x 2mm	100 x 2.1 mm; 5 µm	100 x 3.0 mm, 2.6µm

Continuation of Table 16: Analytical methods used (as reported by participants in the questionnaire)

	LC0001	LC0002	LC003	LC0004	LC0005	LC0006
Separation condition						
Solvent A	H2O + 5 mmol Ammonium acetate pH8	aquous ammonium acetate solution, 1 mM, pH 9	Ammonium hydrogen carbonate 1mM in MeOH/ H2O 5/95	5 mM ammonium acetate buffer pH 8 H2O	Ammonium acetate 1mM, pH9	1 mM ammonium acetate (pH 9)
Solvent B	MeOH	MeOH+2-Propanol 90+10 (v+v)	MeOH	MeOH	MeOH / 2-Propanol (9:1), v:v	MeOH / Isopropanol (90:10, v:v)
HPLC-program	0 min 90% A 10%B 1 min 90% A 10%B 10 min 0% A 10% B 17.0 min 0% A 100% B 17.2 min 90% A 10% B	0 min 100% A; 0.1 min 60% A; 3 min 25% A; 3.5 min 5% A; 6.5 min 5% A; 6.6 min 100% A	0 min 100% A 0,5 min 55% A 5 min 45% A 10 min 5% A 16 min 5% A 17 min 100% A bis 23 min 100% A	t(min) A % B % 0 10 90 1 10 90 10 100 0 12 100 0 12.20 10 90 16 10 90	t(min) A % B % 0 100 8.6 100 2 100 14 100 2.1 60 5 25 5.5 5 8.5 5	
Flow rate [mL/min]	0.3	0.4	0.5	0.3	0.4	0.4
MS system	other	Triple-Quad (LC-MS/MS)	Triple-Quad (LC-MS/MS)	other	other	Triple-Quad (LC-MS/MS)
Measurement conditions						
Ionisation-method LC-MS	ESI	ESI	ESI	ESI	ESI	ESI
Scan type	MRM	MRM	MRM	MRM	MRM	MRM
Polarity						
Quantification						
Type of calibration				Matrix-Matched		
Internal standard	Yes	Yes	Yes	Yes	Yes	Yes
Calibration range	AME, ALT, AOH: 1-75µg/kg TEN: 2-150µg/kg TEA: 10-750µg/kg	TEA: 2.5 - 125 ng/ml; other: 1.0 - 50 ng/ml	ALT, TEN: 0.1 - 50 ng/ml AOH: 0.16 - 82 ng/ml AME: 0.05 - 25 ng/ml TEA: 0.4 - 200 ng/ml	No deviations from the method	2.5 ng/mL - 50 ng/mL	ng/ml (= µg/kg); TEA:0.00 – 800ng/ml TEN:0.00 – 400ng/ml ALT, AOH, AME: 0.00 - 80 ng/ml

Continuation of Table 16: Analytical methods used (as reported by participants in the questionnaire)

	LC0001	LC0002	LC003	LC0004	LC0005	LC0006
Recovery correction	no	Yes	Yes	no	Yes	no
Quality management						
LOD/LOQ-method	single-to-noise ratio	EURL guidance	single-to-noise ratio	calibration curve	single-to-noise ratio	single-to-noise ratio
LOD/LOQ-method	with matrix	with matrix	without matrix	without matrix	without matrix	with matrix
Instructions for PT sufficient?		yes		yes	yes	

Continuation of Table 16: Analytical methods used (as reported by participants in the questionnaire)

	LC0007	LC0008	LC0011	LC0013	LC0014	LC0015
Analytical method	German NRL	CEN/TC275/WG5	CEN/TC275/WG5	CEN/TC275/WG5	other	CEN/TC275/WG5
Extraction Clean-Up						
Sample amount BFR_PU [g]	5.00	2	2	1.97	4	2
BFR_KE [g]	5.00	2	2	1.95	4	2
BFR_JU [g]	10.0	2	2	1.99	4	2
Extraction				The 3 replicates were done on 3 different weights. The other weights are: PU: MW2 = 2.05g / MW3 = 2.06g KE: MW2 = 2.04g / MW3 = 2.06g JU: MW2 = 2.02g / MW3 = 2.00g	4g Sample + 16g H2O: homogenize 1g of them + Standard-Addition-Solution + 5ml Methanol: homogenize shaking with 350 rpm for 40 minutes	
Clean Up		6.6 of CEN method (reconstitution): instead of 400 µL MeOH ACN was used instead of 600 µL HPLC buffer A water acidified with formic acid (0.1 %) was used		For extraction and sample preparation/clean up, we followed strictly the protocol provided for the collaborative study of october 2018 (internal standard solution is added on 2g of sample).	Centrifugation for 10 minutes with 4000 rpm. decantation add 0.1 ml derivatization-reagent (2.4- Dinitrophenylhydrazin 0.58% in 2 M HCl) shaking (60 min by 300 rpm) add 0.5 ml stop-reagent (Undecanal/Methanol: 5:95) shaking 30 min with 300 rpm add with 50 mmol ammonium formiate pH 3	

Continuation of Table 16: Analytical methods used (as reported by participants in the questionnaire)

	LC0007	LC0008	LC0011	LC0013	LC0014	LC0015
Derivatization					Cleanup with extraction-column (Strata-XL), elute with methanol, drying with nitrogen. Resolve in 0.5 ml methanol and add 0.5 ml water centrifugation and filtration (0.22 µm PTFE)	
HPLC						
HPLC_MS	Sciex QTrap 5500	Waters TQ-XS	Waters Xevo TQ-Xs	Waters TQ-MS	TSQ Quantum Ultra (Thermo Scientific)	API SCIEX 3200
Instrument	HPLC	UPLC	UPLC	UPLC	HPLC	HPLC
Separation column						
Type	Phenomenex Gemini NX C18	Waters Acquity UPLC BEH C18 1.7 µm	BEH C18	Waters XSelect HSS T3	Column: Hypersil GOLD aQ (Thermo Scientific)	
Dimension	150 x 2.0 mm ; 5µm	2.1 x 50 mm	1.7um 2.1 x 100mm	100 x 2.1 mm (2.5 µm)		
Separation condition						
Solvent A	Ammonium acetate solution 1 mmol/L; pH 9	H2O + 0.1 % FA	5 mM ammonium acetate buffer at pH ~8.0	5 mM ammonium acetate buffer at pH 8.0	MeOH/H2O 1:9 (10 mM ammonium formiate + 0.05% formic acid)	
Solvent B	MeOH	ACN + 0.1 % FA	MeOH	MeOH	MeOH 100%	
HPLC-program	t(min) A% B% 0 75 25 0.1 75 25 6.0 5 75 11 5 95 12 75 25	t(min) A % B % Initial 90 10 3 90 10 10 30 70 10.10 10 90 12 10 90 12.10 90 10 15 90 10	t(min) A% B% Intial 90 10 1.0 90 10 7.5 0 100 10 0 100 10.2 90 10 12.0 90 10	t(min) A % B % 0.0 95 5 10.0 0 100 18.0 0 100 18.5 95 5 23.0 95 5	t(min) A % B % 0 100 0 0.05 100 0 12 5.6 94.4 24 5.6 94.4 26 100 0 36 100 0	
Flow rate [mL/min]	0.35	0.5	0.3	0.3	0.25	

Continuation of Table 16: Analytical methods used (as reported by participants in the questionnaire)

	LC0007	LC0008	LC0011	LC0013	LC0014	LC0015
MS system	other	Triple-Quad (LC-MS/MS)	other	Triple-Quad (LC-MS/MS)	other	other
Measurement conditions						
Ionisation-method LC-MS	ESI	ESI	ESI	ESI	ESI	ESI
Scan type	MRM	MRM	MRM	MRM	MRM	MRM
Polarity						
Quantification						
Type of calibration				Standard addition	Standard addition	
Internal standard	Yes	Yes	Yes	Yes		Yes
Calibration range	0.05 - 100 ng/ml	ALT, AOH, AME : 1, 5, 10, 25, 50, 90 µg/kg TEN: 5, 25, 50, 125, 250,450 µg/kg TEA : 10, 50, 100, 250, 500, 900 µg/kg	AME, AOH, ALT: 1-100ppb TEN: 5-500ppb, TEA: 10-1000ppb	AOH/AME/ALT: 1-100 ng/ml TEN: 5-500 ng/ml TEA: 10-1000 ng/ml	1-Point (optimated of the concentration in the sample): automatically recovery included	TEA: 10-1000 ng/ml, TEN: 5-500 ng/ml, ALT: 1-100 ng/ml, AOH: 1-100 ng/ml, AME: 1-100 ng/ml
Recovery correction	Yes	Yes	Yes	no	Yes	no
Quality management						
LOD/LOQ-method	calibration curve	single-to-noise ratio	single-to-noise ratio	single-to-noise ratio	single-to-noise ratio	single-to-noise ratio
LOD/LOQ-method	without matrix	with matrix	without matrix	with matrix	with matrix	without matrix
Instructions for PT sufficient?	Yes	Yes		Yes	yes	YES

6.10 Comments of the participants

LC0001: „Absolute recovery ISTD ALT = 1% (very low response); AOH = 2%, AME = 0.5%, TEA = 99%, TEN = 4 %“
(translated from German, original comment: „Absolute Wiederfindung ISTD: ALT = 1% (sehr schlechter response); AOH = 2%, AME = 0,5%, TEA = 99%, TEN = 4%“

LC0003: „1.) The calibration range was adjusted to the level of toxin, i.e. for very low levels the very high calibration standards were not used.“ (translated from German, original comment: „1.) Der Kalibrierbereich wurde an den Toxingehalt angepasst, d.h. bei sehr niedrigen Gehalten wurden die sehr hohen Kalibrierstandards nicht verwendet.“)

„2.) All results are only corrected via ISTD (spiked at the beginning!) and were not corrected additionally with the recovery of additional experiments.”(translated from German, original comment: „2.) Alle Ergebnisse sind nur über den ISTD (am Anfang dotiert!) korrigiert und wurden nicht zusätzlich über die WDF-Raten von Zusatzversuchen hochgerechnet.“)

„3.) The recoveries are the recoveries of the ISTD in the samples related to the external calibration standards.” (translated from German, original comment: „3.) Die WDF-Raten sind die jeweiligen WDF des ISTD in den Proben bezogen auf die ext. Kalibrierstandards.“)

„4.) The reported LOD and LOQ are the reporting limits specified in our test method, the LOD calculated by S/N =3 are much lower in some cases.” (translated from German, original comment: „4.) Die mitgeteilten NG und BG sind unsere für dieses Prüfverfahren festgelegten Berichtsgr, die über s/n =3 berechneten NG liegen teilweise deutlich niedriger.“)

„5.) The reported MU is the expanded MU calculated from the confidence level of the three measured values.” (translated from German, original comment: „5.) Die mitgeteilte MU ist die erweiterte MU berechnet aus dem VB der 3 Messwerte.“)

LC0006: „ - According to the CEN method quantification is performed with a calibration in solvents. - We quantified with calibration in solvents and external matrix matched calibration.” (translated from German, original comment:” - Nach der CEN-Methode wird mit einer Lösungsmittelkalibrierung quantifiziert. - Wir haben mit LM-Kalibrierung und externer Matrixkalibrierung quantifiziert.“

„- Recovery could not be reported because all tomato products purchased in retail stores, such as tomato juice, tomato paste, two different samples of tomato ketchup and two different samples of tomato purees, were contaminated with *Alternaria* toxins.
- Problem: these contaminated matrices were used to prepare the calibration.
- no recovery correction possible with/without ISTD
- blank material for matrix calibration and for control samples should be made available (experiences of other particpants?)

(translated from German, original comment:” - Angaben zur Wiederfindung nicht möglich, da sämtliche selbst im Handel erworbenen Tomatenprodukte wie Tomatensaft, Tomatenmark, 2 verschiedene Ketchups und 2 verschiedene passierte Tomaten mit Alternaria-Toxinen belastet waren. - Problem: mit diesen belasteten Matrices wurden auch die Kalibrierungen angesetzt. - keine WDF-Korrektur möglicswertung mit/ohne ISTDs - Es sollte Blankmaterial für die Matrixkalibrierung

und für die Kontrollproben zur Verfügung gestellt werden (Erfahrung anderer Teilnehmer ?)

LC0011: “Method needs further development chromatography for AOH & ALT poor. Unable to achieve a satisfactory curve.

Control std was only run once and ISTD was not added. Correlation co-efficient not sufficient to integrate not using internal std.

The ISTD we was "gifted" to us by the JRC for method development. We are in the process of ordering new standards”

LC0014: “We are sorry, that we didn't analysed Cont_AT. It was a communication error laboratory intern”

6.11 Analytical standards used (as reported by the participants)

Table 17: Analytical standards used for the quantitation of altenuene

Lab code	Certified standard used? (Y/N)	Manufacturer of standard	Was the applied standard checked photometrically? (Y/N)	Value and reference for molar extinction coefficient used (if applicable)	Name of internal standard used (if applicable)
LC0001	YES	LGC*	YES	30000 (EtOH)	
LC0002	NO	ASCA	YES	30000 (EtOH); Montemurro, Visconti in Alternaria: Biology, Plant Diseases and Metabolites, 1992, p. 449	Altenuene-d6
LC0003	YES	HPC Standard	NO		Altenuen-d6
LC0004	YES	Romer Labs (Biopure)	NO		
LC0005	YES	Romer Labs (Biopure)	NO		Altenuen-d6
LC0006	YES	ASCA	NO		Altenuen-d6
LC0007	YES	Romer Labs (Biopure)	NO		ALT-d6
LC0008	YES	Romer Labs (Biopure)	NO		ALT-d6
LC0011	YES	Romer Labs (Biopure)	NO		Supplied By JRC
LC0013	YES	Romer Labs (Biopure)	NO	-	ALT-D6
LC0014	YES	LGC Standards GmbH*	NO		
LC0015	The standards used, were supplied from Joint Research Centre for the MVS "Determination of Alternaria Toxins in Wheat, Tomato puree and sunflower seeds by solid phase extracion clean-up and liquid chromatography with tandem mass spectrometric detection"				Altenuene (methoxy-d3,methyl-d3) (ALT d6)

*Product from LGC Standards identical with product from Romer Labs (Biopure)

Table 18: Analytical standards used for the quantitation of alternariol

Lab code	Certified standard used? (Y/N)	Manufac-turer of standard	Was the applied standard checked photo-metrically? (Y/N)	Value and reference for molar extinction coefficient used (if applicable)	Name of internal standard used (if applicable)
LC0001	YES	LGC*	NO		
LC0002	NO	TRC, CA	YES	40600 (ACN); Asam et al., J. Agric. Food Chem. 2009, 57 (12), 5152-5160	Alternariol-d3
LC0003	YES	Romer Labs (Biopure)	NO		Alternariol-d3
LC0004	YES	Romer Labs (Biopure)	NO		
LC0005	YES	Romer Labs (Biopure)	NO		Alternariol-d3
LC0006	YES	ASCA	NO		Alternariol-d3
LC0007	YES	Romer Labs (Biopure)	NO		AOH-d3
LC0008	YES	Romer Labs (Biopure)	NO		AOH-d3
LC0011	YES	Romer Labs (Biopure)	NO		Supplied By JRC
LC0013	YES	Romer Labs (Biopure)	NO	-	AOH-D3
LC0014	YES	Romer Labs (Biopure)	NO		
LC0015	The standards used, were supplied from Joint Research Centre for the MVS "Determination of Alternaria Toxins in Wheat, Tomato puree and sunflower seeds by solid phase extracion clean-up and liquid chromatography with tan-dem mass spectrometric detection"				Alternariol-(me-thyl-d3) (AOH d3)

* Product from LGC Standards identical with product from Romer Labs (Biopure)

Table 19: Analytical standards used for the quantitation of alternariol methyl ether

Lab code	Certified standard used? (Y/N)	Manufacturer of standard	Was the applied standard checked photometrically? (Y/N)	Value and reference for molar extinction coefficient used (if applicable)	Name of internal standard used (if applicable)
LC0001	YES	LGC*	YES	47600 (ACN)	
LC0002	NO	Sigma-Aldrich	YES	47600 (ACN); Asam et al., J. Agric. Food Chem. 2009, 57 (12), 5152-5160	Alternariol-methylether-d3
LC0003	YES	Romer Labs (Biopure)	NO		Alternariolmonomethylether-d3
LC0004	YES	Romer Labs (Biopure)	NO		
LC0005	YES	Romer Labs (Biopure)	NO		AME-d3
LC0006	YES	ASCA	NO		Alternariolmonomethylether-d3
LC0007	YES	Romer Labs (Biopure)	NO		AME-d3
LC0008	YES	Romer Labs (biopure)	NO		AME-de
LC0011	YES	Romer Labs (Biopure)	NO		Supplied By JRC
LC0013	YES	Romer Labs (Biopure)	NO	-	AME-D3
LC0014	YES	Romer Labs (Biopure)	NO		
LC0015	The standards used, were supplied from Joint Research Centre for the MVS "Determination of Alternaria Toxins in Wheat, Tomato puree and sunflower seeds by solid phase extracion clean-up and liquid chromatography with tandem mass spectrometric detection"				Alternariol-9-monomethyl ether-(1-methyl-d3) (AME d3)

* Product from LGC Standards identical with product from Romer Labs (Biopure)

Table 20: Analytical standards used for the quantitation of tentoxin

Lab code	Certified standard used? (Y/N)	Manufacturer of standard	Was the applied standard checked photometrically? (Y/N)	Value and reference for molar extinction coefficient used (if applicable)	Name of internal standard used (if applicable)
LC0001	YES	LGC*	YES	20700 (EtOH)	
LC0002	NO	Sigma-Aldrich	YES	20700 (EtOH); Meyer et al., J. Am. Chem. Soc. 1975, 97 (13), 3802-3809	Tentoxin-d3
LC0003	YES	Romer Labs (Biopure)	NO		Tentoxin-d3
LC0004	YES	Romer Labs (Biopure)	NO		
LC0005	YES	Romer Labs (Biopure)	NO		Tentoxin-d3
LC0006	YES	ASCA	NO		Tentoxin-d3
LC0007	YES	Romer Labs (Biopure)	NO		TEN-d3
LC0008	YES	Romer Labs (Biopure)	NO		TEN-d3
LC0011	YES	Romer Labs (Biopure)	NO		Supplied By JRC
LC0013	YES	Romer Labs (Biopure)	NO	-	TEN-D3
LC0014	YES	Romer Labs (Biopure)	NO		
LC0015	The standards used, were supplied from Joint Research Centre for the MVS "Determination of Alternaria Toxins in Wheat, Tomato puree and sunflower seeds by solid phase extracion clean-up and liquid chromatography with tandem mass spectrometric detection"				Tentoxin d3 (TEN d3)

* Product from LGC Standards identical with product from Romer Labs (Biopure)

Table 21: Analytical standards used for the quantitation of tenuazonic acid

Lab code	Certified standard used? (Y/N)	Manufacturer of standard	Was the applied standard checked photometrically? (Y/N)	Value and reference for molar extinction coefficient used (if applicable)	Name of internal standard used (if applicable)
LC0001	YES	HPC	YES	12980 (MeOH)	
LC0002	NO	Sigma-Aldrich	YES	12980 (MeOH); Shephard et al., J. Chromatogr. 1991, 566 (1), 195-205	Tenuazonic acid 13C2
LC0003	YES	Romer Labs (Biopure)	NO		Tenuazonic acid-13C2
LC0004	YES	Romer Labs (Biopure)	NO		
LC0005	YES	Romer Labs (Biopure)	NO		13C2 Tenuazonsäure
LC0006	YES	ASCA	NO		Tenuazonsäure-13C2
LC0007	YES	Romer Labs (Biopure)	NO		13C2-TEA
LC0008	YES	Romer Labs (biopure)	NO		13C-TEA
LC0011	YES	Romer Labs (Biopure)	NO		Supplied By JRC
LC0013	YES	Romer Labs (Biopure)	NO	-	13C2-TEA
LC0014	YES	Romer Labs (Biopure)	NO		
LC0015	The standards used, were supplied from Joint Research Centre for the MVS "Determination of Alternaria Toxins in Wheat, Tomato puree and sunflower seeds by solid phase extracion clean-up and liquid chromatography with tandem mass spectrometric detection"				Tenuazonic acid-(acetyl-13C2) (TEA 13C2)

6.12 List of the results reported by the participants

Table 22: List of the results reported by the participants

Sample	Analyte	Lab code	MEAN	S	ME 1	ME 2	ME 3	Z-Score	LOQ	LOD	Unit	Sample code
CONT_AT	ALT	LC0001	17.050	0.354	17.30	16.80		0.58	5.0	2.5	ng/ml	CONT_AT
CONT_AT	AME	LC0001	35.450	0.778	36.00	34.90		0.04	2.0	1	ng/ml	CONT_AT
CONT_AT	AOH	LC0001	17.250	0.071	17.30	17.20		0.40	3.0	1	ng/ml	CONT_AT
CONT_AT	TEA	LC0001	39.050	0.212	39.20	38.90		-0.36	25.0	10	ng/ml	CONT_AT
CONT_AT	TEN	LC0001	26.550	0.495	26.20	26.90		0.50	10.0	2	ng/ml	CONT_AT
JU	ALT	LC0001	17.633	4.406	22.70	14.70	15.50	2.85	5.0	2.5	µg/kg	BfR_JU27
JU	AME	LC0001	6.430	0.805	7.24	6.42	5.63	1.05	2.0	1	µg/kg	BfR_JU27
JU	AOH	LC0001	3.530	0.310	3.77	3.64	3.18	0.52	3.0	1	µg/kg	BfR_JU27
JU	TEA	LC0001	227.000	1.732	225.00	228.00	228.00	-0.14	25.0	10	µg/kg	BfR_JU27
JU	TEN	LC0001	45.933	0.416	45.60	46.40	45.80	-0.32	10.0	2	µg/kg	BfR_JU27
KE	ALT	LC0001			NB	NB	NB		5.0	2.5	µg/kg	BfR_KE09
KE	AME	LC0001	2.143	0.111	2.26	2.13	2.04	1.41	2.0	1	µg/kg	BfR_KE09
KE	AOH	LC0001	12.467	0.651	12.50	11.80	13.10	-0.08	3.0	1	µg/kg	BfR_KE09
KE	TEA	LC0001	89.567	1.457	88.40	89.10	91.20	0.11	25.0	10	µg/kg	BfR_KE09
KE	TEN	LC0001	128.667	0.577	129.00	128.00	129.00	-0.24	10.0	2	µg/kg	BfR_KE09
PU	ALT	LC0001	20.167	1.973	21.50	21.10	17.90	3.64	5.0	2.5	µg/kg	BfR_PU29
PU	AME	LC0001	18.000	0.721	17.80	18.80	17.40	0.16	2.0	1	µg/kg	BfR_PU29
PU	AOH	LC0001	7.027	0.730	6.31	7.00	7.77	-0.11	3.0	1	µg/kg	BfR_PU29
PU	TEA	LC0001	735.000	7.810	730.00	744.00	731.00	-0.16	25.0	10	µg/kg	BfR_PU29
PU	TEN	LC0001	14.100	0.173	14.00	14.30	14.00	0.06	10.0	2	µg/kg	BfR_PU29
CONT_AT	ALT	LC0002	11.700	0.600	11.70	11.10	12.30	-1.03			ng/ml	CONT_AT
CONT_AT	AME	LC0002	33.700	0.000	33.70	33.70	33.70	-0.18			ng/ml	CONT_AT
CONT_AT	AOH	LC0002	16.450	0.250	16.45	16.20	16.70	0.18			ng/ml	CONT_AT
CONT_AT	TEA	LC0002	38.400	0.300	38.10	38.70	38.40	-0.43			ng/ml	CONT_AT
CONT_AT	TEN	LC0002	21.000	0.400	21.00	20.60	21.40	-0.56			ng/ml	CONT_AT
JU	ALT	LC0002	9.893	0.559	9.78	9.40	10.50	-0.40	0.9	0.3	µg/kg	BfR_JU15
JU	AME	LC0002	5.227	0.104	5.26	5.11	5.31	0.00	0.5	0.1	µg/kg	BfR_JU15
JU	AOH	LC0002	3.293	0.203	3.23	3.13	3.52	0.18	0.7	0.2	µg/kg	BfR_JU15
JU	TEA	LC0002	213.333	4.163	218.00	210.00	212.00	-0.43	6.0	2	µg/kg	BfR_JU15
JU	TEN	LC0002	44.500	0.693	45.30	44.10	44.10	-0.45	0.4	0.1	µg/kg	BfR_JU15
KE	ALT	LC0002	2.300	0.437	1.95	2.79	2.16	0.00	1.0	0.4	µg/kg	BfR_KE15
KE	AME	LC0002	1.460	0.050	1.41	1.46	1.51	-0.49	0.7	0.2	µg/kg	BfR_KE15
KE	AOH	LC0002	13.567	0.208	13.50	13.80	13.40	0.31	3.0	0.9	µg/kg	BfR_KE15
KE	TEA	LC0002	85.700	1.308	84.80	85.10	87.20	-0.09	6.0	2	µg/kg	BfR_KE15
KE	TEN	LC0002	122.000	1.000	122.00	121.00	123.00	-0.46	0.7	0.2	µg/kg	BfR_KE15
PU	ALT	LC0002	9.673	0.850	8.93	10.60	9.49	-0.62	0.9	0.3	µg/kg	BfR_PU41
PU	AME	LC0002	17.600	0.529	17.80	18.00	17.00	0.05	0.9	0.3	µg/kg	BfR_PU41
PU	AOH	LC0002	7.533	0.076	7.48	7.50	7.62	0.21	2.0	0.6	µg/kg	BfR_PU41
PU	TEA	LC0002	709.000	3.464	707.00	707.00	713.00	-0.37	30.0	8	µg/kg	BfR_PU41
PU	TEN	LC0002	12.633	0.208	12.70	12.40	12.80	-0.42	0.8	0.3	µg/kg	BfR_PU41
CONT_AT	ALT	LC0003	14.367	0.153	14.40	14.50	14.20	-0.23	0.5	0.25	ng/ml	CONT_AT
CONT_AT	AME	LC0003	30.333	0.839	29.80	29.90	31.30	-0.62	0.1	0.05	ng/ml	CONT_AT
CONT_AT	AOH	LC0003	15.533	0.252	15.50	15.80	15.30	-0.09	0.3	0.13	ng/ml	CONT_AT
CONT_AT	TEA	LC0003	39.267	1.102	40.40	38.20	39.20	-0.34	0.5	0.25	ng/ml	CONT_AT
CONT_AT	TEN	LC0003	22.233	0.929	21.20	23.00	22.50	-0.32	0.3	0.13	ng/ml	CONT_AT
JU	ALT	LC0003	11.033	0.058	11.10	11.00	11.00	0.08	2.0	1	µg/kg	BfR_JU33
JU	AME	LC0003	4.587	0.075	4.51	4.66	4.59	-0.55	0.4	0.2	µg/kg	BfR_JU33
JU	AOH	LC0003	2.987	0.146	2.94	3.15	2.87	-0.26	1.0	0.5	µg/kg	BfR_JU33
JU	TEA	LC0003	201.333	3.512	205.00	201.00	198.00	-0.69	2.0	1	µg/kg	BfR_JU33
JU	TEN	LC0003	46.333	1.677	44.40	47.20	47.40	-0.28	1.0	0.5	µg/kg	BfR_JU33
KE	ALT	LC0003	2.557	0.158	2.42	2.52	2.73	0.51	2.0	1	µg/kg	BfR_KE20
KE	AME	LC0003	1.383	0.025	1.36	1.41	1.38	-0.70	0.4	0.2	µg/kg	BfR_KE20
KE	AOH	LC0003	12.433	0.208	12.20	12.60	12.50	-0.09	1.0	0.5	µg/kg	BfR_KE20
KE	TEA	LC0003	78.833	1.250	78.80	80.10	77.60	-0.45	2.0	1	µg/kg	BfR_KE20

Continuation of Table 22: List of the results reported by the participants

Probe	Analyt	Labor-code	MEAN	s	ME 1	ME 2	ME 3	Z-Score	LOQ	LOD	Unit	Sample code
KE	TEN	LC0003	135.333	2.517	135.00	133.00	138.00	-0.01	1.0	0.5	µg/kg	BfR_KE20
PU	ALT	LC0003	12.033	0.351	12.00	11.70	12.40	0.34	2.0	1	µg/kg	BfR_PU09
PU	AME	LC0003	16.067	0.569	16.70	15.60	15.90	-0.35	0.4	0.2	µg/kg	BfR_PU09
PU	AOH	LC0003	7.067	0.159	6.96	7.25	6.99	-0.09	1.0	0.5	µg/kg	BfR_PU09
PU	TEA	LC0003	675.667	11.590	670.00	668.00	689.00	-0.63	2.0	1	µg/kg	BfR_PU09
PU	TEN	LC0003	13.767	0.306	14.10	13.50	13.70	-0.05	1.0	0.5	µg/kg	BfR_PU09
CONT_AT	ALT	LC0004	16.067	0.208	16.30	15.90	16.00	0.29	3.6	0.9	ng/ml	CONT_AT
CONT_AT	AME	LC0004	35.267	0.252	35.00	35.50	35.30	0.02	2.3	0.6	ng/ml	CONT_AT
CONT_AT	AOH	LC0004	15.800	0.173	15.90	15.90	15.60	-0.01	2.5	0.7	ng/ml	CONT_AT
CONT_AT	TEA	LC0004	50.467	0.764	50.30	51.30	49.80	0.86	31.4	8.1	ng/ml	CONT_AT
CONT_AT	TEN	LC0004	25.900	0.173	25.70	26.00	26.00	0.37	11.2	2.9	ng/ml	CONT_AT
JU	ALT	LC0004	10.023	0.166	10.20	9.87	10.00	-0.34	3.6	0.9	µg/kg	BfR_JU05
JU	AME	LC0004	4.600	0.056	4.66	4.55	4.59	-0.54	2.3	0.6	µg/kg	BfR_JU05
JU	AOH	LC0004	3.177	0.117	3.20	3.28	3.05	0.01	2.5	0.7	µg/kg	BfR_JU05
JU	TEA	LC0004	254.333	0.577	254.00	254.00	255.00	0.45	31.4	8.1	µg/kg	BfR_JU05
JU	TEN	LC0004	54.500	0.346	54.30	54.90	54.30	0.47	11.2	2.9	µg/kg	BfR_JU05
KE	ALT	LC0004	2.410	0.281	2.39	2.70	2.14	0.22	3.6	0.9	µg/kg	BfR_KE05
KE	AME	LC0004	1.427	0.072	1.51	1.39	1.38	-0.58	2.3	0.6	µg/kg	BfR_KE05
KE	AOH	LC0004	12.333	0.058	12.30	12.30	12.40	-0.13	2.5	0.7	µg/kg	BfR_KE05
KE	TEA	LC0004	99.833	0.289	99.50	100.00	100.00	0.64	31.4	8.1	µg/kg	BfR_KE05
KE	TEN	LC0004	157.333	0.577	158.00	157.00	157.00	0.74	11.2	2.9	µg/kg	BfR_KE05
PU	ALT	LC0004	9.707	0.114	9.80	9.74	9.58	-0.61	3.6	0.9	µg/kg	BfR_PU16
PU	AME	LC0004	15.867	0.737	16.70	15.30	15.60	-0.40	2.3	0.6	µg/kg	BfR_PU16
PU	AOH	LC0004	7.237	0.142	7.21	7.39	7.11	0.02	2.5	0.7	µg/kg	BfR_PU16
PU	TEA	LC0004	844.333	3.512	841.00	844.00	848.00	0.70	31.4	8.1	µg/kg	BfR_PU16
PU	TEN	LC0004	15.367	0.306	15.30	15.70	15.10	0.47	11.2	2.9	µg/kg	BfR_PU16
CONT_AT	ALT	LC0005	15.067	0.351	14.70	15.10	15.40	-0.01			ng/ml	CONT_AT
CONT_AT	AME	LC0005	37.267	0.802	36.50	38.10	37.20	0.28			ng/ml	CONT_AT
CONT_AT	AOH	LC0005	13.767	0.115	13.70	13.90	13.70	-0.59			ng/ml	CONT_AT
CONT_AT	TEA	LC0005	27.233	0.907	28.20	27.10	26.40	-1.63			ng/ml	CONT_AT
CONT_AT	TEN	LC0005	24.400	0.500	23.90	24.90	24.40	0.09			ng/ml	CONT_AT
JU	ALT	LC0005	12.500	0.693	12.10	12.10	13.30	0.69	5.0	2.5	µg/kg	BfR_JU07
JU	AME	LC0005	5.333	0.252	5.10	5.30	5.60	0.10	2.0	1	µg/kg	BfR_JU07
JU	AOH	LC0005	3.633	0.058	3.60	3.70	3.60	0.67	2.0	1	µg/kg	BfR_JU07
JU	TEA	LC0005	206.000	6.557	200.00	205.00	213.00	-0.59	2.0	1	µg/kg	BfR_JU07
JU	TEN	LC0005	53.167	4.790	52.10	49.00	58.40	0.34	2.0	1	µg/kg	BfR_JU07
KE	ALT	LC0005			NB	NB	NB		5.0	2.5	µg/kg	BfR_KE30
KE	AME	LC0005	2.367	0.115	2.50	2.30	2.30	2.03	2.0	1	µg/kg	BfR_KE30
KE	AOH	LC0005	13.433	0.473	13.60	12.90	13.80	0.27	2.0	1	µg/kg	BfR_KE30
KE	TEA	LC0005	80.633	0.379	80.90	80.20	80.80	-0.35	2.0	1	µg/kg	BfR_KE30
KE	TEN	LC0005	146.467	2.970	149.80	144.10	145.50	0.37	2.0	1	µg/kg	BfR_KE30
PU	ALT	LC0005	14.067	0.351	14.40	14.10	13.70	1.16	5.0	2.5	µg/kg	BfR_PU06
PU	AME	LC0005	18.433	0.208	18.20	18.60	18.50	0.27	2.0	1	µg/kg	BfR_PU06
PU	AOH	LC0005	8.333	0.289	8.00	8.50	8.50	0.71	2.0	1	µg/kg	BfR_PU06
PU	TEA	LC0005	668.333	7.371	660.00	674.00	671.00	-0.69	2.0	1	µg/kg	BfR_PU06
PU	TEN	LC0005	14.733	1.332	13.60	14.40	16.20	0.27	2.0	1	µg/kg	BfR_PU06
CONT_AT	ALT	LC0006	16.367	0.586	16.80	16.60	15.70	0.38			ng/ml	CONT_AT
CONT_AT	AME	LC0006	39.533	0.231	39.40	39.80	39.40	0.57			ng/ml	CONT_AT
CONT_AT	AOH	LC0006	18.067	0.153	17.90	18.20	18.10	0.64			ng/ml	CONT_AT
CONT_AT	TEA	LC0006	48.933	1.457	47.30	50.10	49.40	0.70			ng/ml	CONT_AT
CONT_AT	TEN	LC0006	24.833	0.451	25.30	24.40	24.80	0.17			ng/ml	CONT_AT
JU	ALT	LC0006			NB	NB	NB		15.0	7.5	µg/kg	BfR_JU16
JU	AME	LC0006	5.897	0.237	6.16	5.83	5.70	0.59	5.0	2	µg/kg	BfR_JU16
JU	AOH	LC0006			NN	NN	NN		8.0	4	µg/kg	BfR_JU16
JU	TEA	LC0006	221.667	27.574	248.00	193.00	224.00	-0.25	15.0	7.5	µg/kg	BfR_JU16

Continuation of Table 22: List of the results reported by the participants

Probe	Analyt	Labor-code	MEAN	s	ME 1	ME 2	ME 3	Z-Score	LOQ	LOD	Unit	Sample code
JU	TEN	LC0006	45.100	6.909	38.40	52.20	44.70	-0.40	5.0	2	µg/kg	BfR_JU16
KE	ALT	LC0006			NN	NN	NN		15.0	7.5	µg/kg	BfR_KE06
KE	AME	LC0006			NN	NN	NN		5.0	2	µg/kg	BfR_KE06
KE	AOH	LC0006	13.200	1.752	11.50	15.00	13.10	0.18	8.0	4	µg/kg	BfR_KE06
KE	TEA	LC0006	85.733	9.886	96.90	78.10	82.20	-0.09	15.0	7.5	µg/kg	BfR_KE06
KE	TEN	LC0006	121.333	20.133	100.00	140.00	124.00	-0.49	5.0	2	µg/kg	BfR_KE06
PU	ALT	LC0006			NB	NB	NB		15.0	7.5	µg/kg	BfR_PU30
PU	AME	LC0006	18.167	1.361	17.10	19.70	17.70	0.20	5.0	2	µg/kg	BfR_PU30
PU	AOH	LC0006			NB	NB	NB		8.0	4	µg/kg	BfR_PU30
PU	TEA	LC0006	717.667	51.733	777.00	682.00	694.00	-0.30	15.0	7.5	µg/kg	BfR_PU30
PU	TEN	LC0006	12.167	1.861	10.20	13.90	12.40	-0.57	5.0	2	µg/kg	BfR_PU30
CONT_AT	ALT	LC0007	13.833	0.115	13.90	13.70	13.90	-0.39			ng/ml	CONT_AT
CONT_AT	AME	LC0007	33.533	0.351	33.20	33.90	33.50	-0.20			ng/ml	CONT_AT
CONT_AT	AOH	LC0007	15.233	0.058	15.20	15.20	15.30	-0.17			ng/ml	CONT_AT
CONT_AT	TEA	LC0007	42.067	0.603	42.00	42.70	41.50	-0.04			ng/ml	CONT_AT
CONT_AT	TEN	LC0007	23.067	0.115	23.00	23.20	23.00	-0.17			ng/ml	CONT_AT
JU	ALT	LC0007	11.200	0.458	11.60	10.70	11.30	0.15	1.0	0.3	µg/kg	BfR_JU11
JU	AME	LC0007	5.070	0.079	5.16	5.01	5.04	-0.13	0.3	0.1	µg/kg	BfR_JU11
JU	AOH	LC0007	2.910	0.046	2.95	2.86	2.92	-0.37	0.3	0.1	µg/kg	BfR_JU11
JU	TEA	LC0007	242.000	6.000	242.00	236.00	248.00	0.19	0.5	0.2	µg/kg	BfR_JU11
JU	TEN	LC0007	49.100	0.721	48.50	48.90	49.90	-0.03	0.3	0.1	µg/kg	BfR_JU11
KE	ALT	LC0007	1.927	0.057	1.99	1.91	1.88	-0.74	1.8	0.5	µg/kg	BfR_KE19
KE	AME	LC0007	1.507	0.012	1.52	1.50	1.50	-0.36	0.6	0.2	µg/kg	BfR_KE19
KE	AOH	LC0007	12.300	0.624	11.60	12.50	12.80	-0.14	0.6	0.2	µg/kg	BfR_KE19
KE	TEA	LC0007	92.033	1.474	91.50	93.70	90.90	0.24	1.0	0.4	µg/kg	BfR_KE19
KE	TEN	LC0007	130.667	1.528	129.00	131.00	132.00	-0.17	0.6	0.2	µg/kg	BfR_KE19
PU	ALT	LC0007	11.467	0.252	11.70	11.50	11.20	0.11	1.8	0.5	µg/kg	BfR_PU20
PU	AME	LC0007	17.233	0.058	17.20	17.20	17.30	-0.04	0.6	0.2	µg/kg	BfR_PU20
PU	AOH	LC0007	7.003	0.229	7.11	7.16	6.74	-0.13	0.6	0.2	µg/kg	BfR_PU20
PU	TEA	LC0007	833.000	19.053	844.00	844.00	811.00	0.61	1.0	0.4	µg/kg	BfR_PU20
PU	TEN	LC0007	14.267	0.231	14.40	14.40	14.00	0.11	0.6	0.2	µg/kg	BfR_PU20
CONT_AT	ALT	LC0008	17.567	0.289	17.90	17.40	17.40	0.74			ng/ml	CONT_AT
CONT_AT	AME	LC0008	37.433	0.666	37.00	37.10	38.20	0.30			ng/ml	CONT_AT
CONT_AT	AOH	LC0008	16.733	0.252	17.00	16.70	16.50	0.26			ng/ml	CONT_AT
CONT_AT	TEA	LC0008	49.600	0.854	50.50	48.80	49.50	0.77			ng/ml	CONT_AT
CONT_AT	TEN	LC0008	27.500	0.300	27.20	27.50	27.80	0.68			ng/ml	CONT_AT
JU	ALT	LC0008	8.393	0.055	8.45	8.39	8.34	-1.03	4.0	1.98	µg/kg	BfR_JU13
JU	AME	LC0008	5.560	0.060	5.62	5.50	5.56	0.29	0.1	0.03	µg/kg	BfR_JU13
JU	AOH	LC0008			NN	NN	NN		1.8	0.9	µg/kg	BfR_JU13
JU	TEA	LC0008	242.333	3.786	244.00	245.00	238.00	0.19	1.9	0.93	µg/kg	BfR_JU13
JU	TEN	LC0008	46.700	0.265	46.50	46.60	47.00	-0.25	0.2	0.11	µg/kg	BfR_JU13
KE	ALT	LC0008			NN	NN	NN		4.4	2.21	µg/kg	BfR_KE18
KE	AME	LC0008	1.687	0.035	1.72	1.69	1.65	0.14	0.1	0.03	µg/kg	BfR_KE18
KE	AOH	LC0008	3.297	0.293	3.08	3.63	3.18	-3.36	3.0	1.52	µg/kg	BfR_KE18
KE	TEA	LC0008	89.533	0.681	90.30	89.00	89.30	0.11	2.1	1.05	µg/kg	BfR_KE18
KE	TEN	LC0008	126.667	1.528	127.00	125.00	128.00	-0.31	0.2	0.09	µg/kg	BfR_KE18
PU	ALT	LC0008	9.470	0.046	9.51	9.48	9.42	-0.70	1.9	0.95	µg/kg	BfR_PU05
PU	AME	LC0008	18.900	0.436	18.70	19.40	18.60	0.39	0.1	0.03	µg/kg	BfR_PU05
PU	AOH	LC0008			NB	NB	NB		1.4	0.7	µg/kg	BfR_PU05
PU	TEA	LC0008	754.667	12.583	768.00	743.00	753.00	-0.01	2.1	1.05	µg/kg	BfR_PU05
PU	TEN	LC0008	13.633	0.153	13.60	13.80	13.50	-0.09	0.2	0.1	µg/kg	BfR_PU05
CONT_AT	ALT	LC0011									ng/ml	CONT_AT
CONT_AT	AME	LC0011	46.200		46.20			1.44			ng/ml	CONT_AT
CONT_AT	AOH	LC0011	29.200		29.20			3.83			ng/ml	CONT_AT
CONT_AT	TEA	LC0011	265.800		265.80			23.94			ng/ml	CONT_AT

Continuation of Table 22: List of the results reported by the participants

Probe	Analyt	Labor-code	MEAN	s	ME 1	ME 2	ME 3	Z-Score	LOQ	LOD	Unit	Sample code
CONT_AT	TEN	LC0011									ng/ml	CONT_AT
JU	ALT	LC0011									µg/kg	BfR_JU17
JU	AME	LC0011	8.477	0.872	7.90	9.48	8.05	2.83	1.0	1	µg/kg	BfR_JU17
JU	AOH	LC0011									µg/kg	BfR_JU17
JU	TEA	LC0011	272.983	5.465	272.96	278.46	267.53	0.85	10.0	10	µg/kg	BfR_JU17
JU	TEN	LC0011	72.767	11.340	85.83	65.46	67.01	2.15	5.0	5	µg/kg	BfR_JU17
KE	ALT	LC0011									µg/kg	BfR_KE14
KE	AME	LC0011	3.490	0.434	3.71	3.77	2.99	5.15	1.0	1	µg/kg	BfR_KE14
KE	AOH	LC0011									µg/kg	BfR_KE14
KE	TEA	LC0011	128.580	3.098	126.59	132.15	127.00	2.14	10.0	10	µg/kg	BfR_KE14
KE	TEN	LC0011	128.407	3.952	132.29	124.39	128.54	-0.25	5.0	5	µg/kg	BfR_KE14
PU	ALT	LC0011									µg/kg	BfR_PU19
PU	AME	LC0011	33.367	4.588	36.50	35.50	28.10	4.17	1.0	1	µg/kg	BfR_PU19
PU	AOH	LC0011									µg/kg	BfR_PU19
PU	TEA	LC0011	851.063	16.321	840.38	869.85	842.96	0.76	10.0	10	µg/kg	BfR_PU19
PU	TEN	LC0011	22.473	1.234	22.36	21.30	23.76	2.79	5.0	5	µg/kg	BfR_PU19
CONT_AT	ALT	LC0013	14.233	0.839	13.70	15.20	13.80	-0.27			ng/ml	CONT_AT
CONT_AT	AME	LC0013	33.833	0.231	34.10	33.70	33.70	-0.17			ng/ml	CONT_AT
CONT_AT	AOH	LC0013	14.867	0.321	14.50	15.10	15.00	-0.28			ng/ml	CONT_AT
CONT_AT	TEA	LC0013	45.000	1.480	46.00	43.30	45.70	0.28			ng/ml	CONT_AT
CONT_AT	TEN	LC0013	22.533	0.252	22.30	22.50	22.80	-0.27			ng/ml	CONT_AT
JU	ALT	LC0013	11.400	0.600	10.80	12.00	11.40	0.23			µg/kg	BfR_JU18
JU	AME	LC0013	4.930	0.231	5.17	4.91	4.71	-0.25			µg/kg	BfR_JU18
JU	AOH	LC0013	3.347	0.360	3.76	3.18	3.10	0.26			µg/kg	BfR_JU18
JU	TEA	LC0013	234.667	15.822	252.00	221.00	231.00	0.03			µg/kg	BfR_JU18
JU	TEN	LC0013	52.533	1.721	54.50	51.80	51.30	0.29			µg/kg	BfR_JU18
KE	ALT	LC0013			NB				10.0		µg/kg	BfR_KE11
KE	AME	LC0013	1.670	0.079	1.73	1.70	1.58	0.10			µg/kg	BfR_KE11
KE	AOH	LC0013	13.467	0.896	14.50	13.00	12.90	0.28			µg/kg	BfR_KE11
KE	TEA	LC0013	83.567	1.097	83.20	82.70	84.80	-0.20			µg/kg	BfR_KE11
KE	TEN	LC0013	139.667	3.215	141.00	142.00	136.00	0.14			µg/kg	BfR_KE11
PU	ALT	LC0013	11.567	0.808	12.30	11.70	10.70	0.15			µg/kg	BfR_PU44
PU	AME	LC0013	16.800	0.100	16.70	16.90	16.80	-0.16			µg/kg	BfR_PU44
PU	AOH	LC0013	7.233	0.495	6.74	7.23	7.73	0.02			µg/kg	BfR_PU44
PU	TEA	LC0013	725.000	27.622	699.00	722.00	754.00	-0.24			µg/kg	BfR_PU44
PU	TEN	LC0013	13.767	0.451	14.20	13.80	13.30	-0.05			µg/kg	BfR_PU44
CONT_AT	ALT	LC0014									ng/ml	CONT_AT
CONT_AT	AME	LC0014									ng/ml	CONT_AT
CONT_AT	AOH	LC0014									ng/ml	CONT_AT
CONT_AT	TEA	LC0014									ng/ml	CONT_AT
CONT_AT	TEN	LC0014									ng/ml	CONT_AT
JU	ALT	LC0014			NN	NB	NN	10.0		5	µg/kg	BfR_JU37
JU	AME	LC0014			NB	NB	NB	10.0		5	µg/kg	BfR_JU37
JU	AOH	LC0014			NN	NN	NN	10.0		5	µg/kg	BfR_JU37
JU	TEA	LC0014	376.667	99.279	271.00	391.00	468.00	3.09	20.0	10	µg/kg	BfR_JU37
JU	TEN	LC0014	55.333	7.506	55.00	63.00	48.00	0.54	5.0	2	µg/kg	BfR_JU37
KE	ALT	LC0014			NN	NB	NN		10.0	5	µg/kg	BfR_KE04
KE	AME	LC0014			NN	NN	NN		10.0	5	µg/kg	BfR_KE04
KE	AOH	LC0014	12.333	1.155	11.00	13.00	13.00	-0.13	10.0	5	µg/kg	BfR_KE04
KE	TEA	LC0014	105.667	14.224	96.00	99.00	122.00	0.95	30.0	10	µg/kg	BfR_KE04
KE	TEN	LC0014	136.667	21.385	112.00	148.00	150.00	0.04	5.0	2	µg/kg	BfR_KE04
PU	ALT	LC0014			NB	NB	NB		10.0	5	µg/kg	BfR_PU04
PU	AME	LC0014	24.333	10.408	16.00	36.00	21.00	1.81	10.0	5	µg/kg	BfR_PU04
PU	AOH	LC0014			NN	NB	NB		10.0	5	µg/kg	BfR_PU04
PU	TEA	LC0014	1630.333	85.172	1618.00	1552.00	1721.00	6.94	20.0	10	µg/kg	BfR_PU04

Continuation of Table 22: List of the results reported by the participants

Probe	Analyt	Labor-code	MEAN	s	ME 1	ME 2	ME 3	Z-Score	LOQ	LOD	Unit	Sample code
PU	TEN	LC0014	17.000	3.606	16.00	14.00	21.00	1.01	5.0	2	µg/kg	BfR_PU04
CONT_AT	ALT	LC0015	14.433	0.252	14.40	14.20	14.70	-0.21	1.2	0.4	ng/ml	CONT_AT
CONT_AT	AME	LC0015	34.400	0.557	33.90	34.30	35.00	-0.09	0.5	0.16	ng/ml	CONT_AT
CONT_AT	AOH	LC0015	14.700	0.265	14.80	14.90	14.40	-0.33	0.3	0.1	ng/ml	CONT_AT
CONT_AT	TEA	LC0015	38.933	0.666	38.50	38.60	39.70	-0.37	0.5	0.15	ng/ml	CONT_AT
CONT_AT	TEN	LC0015	21.367	0.577	21.70	20.70	21.70	-0.49	0.2	0.05	ng/ml	CONT_AT
JU	ALT	LC0015	9.633	0.310	9.28	9.76	9.86	-0.51	2.9	0.9	µg/kg	BfR_JU02
JU	AME	LC0015	4.843	0.075	4.84	4.77	4.92	-0.33	0.8	0.2	µg/kg	BfR_JU02
JU	AOH	LC0015	2.400	0.165	2.31	2.30	2.59	-1.10	0.8	0.2	µg/kg	BfR_JU02
JU	TEA	LC0015	250.667	13.577	258.00	259.00	235.00	0.37	1.1	0.3	µg/kg	BfR_JU02
JU	TEN	LC0015	48.700	1.136	47.90	50.00	48.20	-0.07	1.6	0.5	µg/kg	BfR_JU02
KE	ALT	LC0015			NB	NB	NB		2.9	0.9	µg/kg	BfR_KE16
KE	AME	LC0015	1.543	0.114	1.67	1.51	1.45	-0.26	0.8	0.2	µg/kg	BfR_KE16
KE	AOH	LC0015	11.867	0.153	12.00	11.70	11.90	-0.29	0.8	0.2	µg/kg	BfR_KE16
KE	TEA	LC0015	85.667	5.006	87.10	89.80	80.10	-0.09	1.1	0.3	µg/kg	BfR_KE16
KE	TEN	LC0015	141.000	1.732	140.00	143.00	140.00	0.18	1.6	0.5	µg/kg	BfR_KE16
PU	ALT	LC0015	9.903	0.703	9.64	10.70	9.37	-0.53	2.9	0.9	µg/kg	BfR_PU26
PU	AME	LC0015	16.867	0.231	16.60	17.00	17.00	-0.14	0.8	0.2	µg/kg	BfR_PU26
PU	AOH	LC0015	6.443	0.500	6.14	7.02	6.17	-0.48	0.8	0.2	µg/kg	BfR_PU26
PU	TEA	LC0015	798.000	27.731	815.00	813.00	766.00	0.34	1.1	0.3	µg/kg	BfR_PU26
PU	TEN	LC0015	12.667	0.462	13.20	12.40	12.40	-0.41	1.6	0.5	µg/kg	BfR_PU26

CONT_AT: Control solution

JU: Tomato juice BFR_JU

KE: Tomato ketchup BFR_KE

PU: Tomato puree BFR_PU

TEN: Tentoxin

ALT: Altenuene

AOH: Alternariol

AME: Alternariol methyl ether

TEA: Tenuazonic acid

Mean: Mean

S: Standard deviation

ME1 bis ME3: Reported values 1 to 3

LOQ: Limit of quantitation

LOD: Limit of detection

NB: Not quantified (<LOQ)

NN: Not detected (<LOD)

7 Appendix II

7.1 Analytical method of the German NRL for mycotoxins and plant toxins (German version)

The following method for the determination of *Alternaria* toxins in tomato juice and tomato products was developed and validated at the national reference laboratory for mycotoxins and plant toxins at the German Federal Institut for Risk Assessment. This method was distributed to the national participants of the proficiency test via the website Fachinformationssystem für Verbraucherschutz und Lebensmittelsicherheit (FIS-VL) hosted by the German Federal Office of Consumer Protection and Food Safety (BVL).

Prüfvorschrift zur

Bestimmung von Alternariatoxinen in Tomatensaft und Tomatenprodukten

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1. Zweck und Anwendungsbereich

Dieses Analyseverfahren legt ein hochleistungsflüssigchromatographisches Verfahren (HPLC) mit massenspektrometrischer Detektion (MS/ MS) zur Bestimmung der Alternaria-Toxine (ATs) (Altenuen (ALT), Alternariol (AOH), Alternariolmethylether (AME), Tentoxin (TEN), Tenuazonsäure (TEA)) in Tomatenprodukten (Tomatensaft, -soße, -ketchup, -mark, getrocknete Tomaten, passierte Tomaten) fest.

2. Prinzip

Die ATs werden mittels modifizierten QuEChERS-Ansatzes aus der wässrigen Matrix extrahiert. Nach Phasentrennung mit Hilfe von Salzzugabe und anschließender Zentrifugation wird ein Aliquot des organischen Extraktes zur Trockene eingedampft und in wässrig-methanolischer Injektionslösung rückgelöst. Die ATs werden mittels HPLC-MS/MS quantitativ bestimmt.

Sicherheits-Hinweis

Alternariol und Alternariolmonomethylether sind von der EFSA als genotoxisch eingestuft. Acetonitril ist gesundheitsschädlich (Xn), Methanol giftig (T), Methyl-tert-butylether wirkt reizend (Xi) und ist leicht entzündlich - Vorsicht vor elektrostatischer Aufladung! Während der gesamten Untersuchung sollten Sicherheitsbrille und Handschuhe getragen werden und alle Arbeitsschritte während der Proben- oder Standardbehandlung sollten unter einem Laborabzug durchgeführt werden.

3. Reagenzien

3.1 Allgemeines

Falls nicht anders angegeben, werden bei der Untersuchung nur analysenreine Chemikalien und destilliertes Wasser oder Wasser der Qualität 1 nach EN ISO 3696 verwendet. Kommerziell erhältliche Chemikalien mit gleichen Eigenschaften, wie die hier aufgelisteten, dürfen verwendet werden. Die Lösemittel müssen in HPLC-Qualität vorliegen.

3.2 Acetonitril (LiChrosolv, hypergrade for LC-MS, oder mit einem vergleichbaren Reinheitsgrad)

z. B.: von Merck (Artikelnr.: 1.00029.2500)

3.3 Methanol (LiChrosolv, hypergrade for LC-MS, oder mit einem vergleichbaren Reinheitsgrad)

z. B.: von Merck (Artikelnr.: 1.06035.2500)

3.4 2-Propanol (LiChrosolv, hypergrade oder vergleichbar)

3.5 Ammoniaklösung (25 %, zur Analyse)

Wegen des hohen Dampfdrucks im Kühlschrank zu lagern.
z. B.: von Merck (Artikelnr.: 1.05432.1000)

3.6 Ammoniumacetat (zur Analyse)

z.B.: von Merck (Artikelnr.: 1.01116.0500)

3.6.1 Ammoniumacetatlösung (pH 9), 1 mM

77 mg Ammoniumacetat werden in 1 L Wasser (3.11) gelöst. Der pH-Wert wird mit Ammoniaklösung (3.5) auf pH 9 eingestellt. Hierzu ist ein auf den pH-Bereich 7 – 10 kalibriertes pH-Meter zu verwenden

3.6.2 Ammoniumacetatlösung, 2 mM, für Nachsäuleninfusion

154 mg Ammoniumacetat werden in 1 L Methanol (3.3) gelöst und entgast. Die Einstellung eines pH-Wertes ist nicht erforderlich.

3.7 Magnesiumsulfat (anhydrous, ≥97%, reagent grade)

z.B.: von Sigma-Aldrich (Artikelnr.: 208094-500G)

3.8 Methyl-tert-butylether (LiChrosolv, for liquid chromatography, oder mit einem vergleichbaren Reinheitsgrad)

z.B.: von Merck (Artikelnr.: 1.01845.2500)

3.9 Natriumchlorid (for analysis)

z.B.: von Merck (Artikelnr.: 1.06404.0500)

3.10 ortho-Phosphorsäure, konzentriert (pro analysis, w= 85%)

z.B.: von Fisher (Artikelnr. 0/0515/PB08)

3.11 Wasser, bidestilliert

3.12 Mobile Phase für die HPLC-MS/MS

Eluent A: 100 Volumenteile Ammoniumacetat-Lösung (3.6.1)
Eluent B: 90 Volumenteile Methanol, 10 Volumenteile 2-Propanol
Post Column: 100 Volumenteile (3.6.2), wird über zweite HPLC Pumpe direkt in die ESI-Quelle über T-Stück mit 200 µL/min gefördert

Hinweis: Als Eluent A kann auch eine wässrige Triethylamin-Lösung (1 mM, pH 8) verwendet werden. Dieser Eluent ist bei herkömmlichen C18- und gealterten NX-C18 Säulen besser geeignet für die Chromatographie der Tenauzonsäure. Es ist zu beachten, dass TEA zu einer Ionensuppression im positiven Ionisationsmodus führen kann.

3.13 Salzmischung zur Phasentrennung

4 g Magnesiumsulfat (3.7) und 1 g Natriumchlorid (3.9) auf 0,05 g genau in ein Röhrchen einwiegen. Bis zum Gebrauch verschließen.

3.14 Injektionslösung

Acetonitril (3.2) : Ammoniumacetatlösung (3.6.1) / 25:75 (v/v)

3.15 Stammlösungen Alternariatoxine

Zertifizierte Standardlösungen können kommerziell erworben oder durch Einwaage der kristallinen Festsubstanz hergestellt werden.
Zur Herstellung der Stammlösung aus der Festsubstanz werden zwischen 0,5 mg und 1 mg der kristallinen Festsubstanz in einen 1 mL Messkolben eingewogen und diese in Ethanol mit Hilfe von Ultraschall gelöst. Nach Temperieren auf Raumtemperatur wird der Messkolben bis zur Eichmarke mit ebenfalls temperiertem Ethanol aufgefüllt. Die Konzentrationen liegen zwischen 0,5 mg/mL für AME und 1 mg/mL für alle anderen Analyten. Die Überprüfung des Standardmaterials und der nominellen Standardkonzentrationen erfolgt mittels UV/VIS-Spektralphotometrie. Die Stammlösungen werden hierfür auf nominelle 10 µg/mL verdünnt (AOH und AME auf 5 µg/mL), damit die zu erwartende Extinktion unter 1,0 liegt und sich damit näherungsweise im linearen Bereich befindet.
Als Lösungsmittel wird jeweils das in der Literatur für den genannten Extinktionskoeffizienten gültige verwendet. Im Falle von anderen als Ethanol wird zuerst die pipettierte Menge an Ethanol vollständig verdampft und danach mit dem jeweiligen Lösungsmittel der verwendete Messkolben bis zur Kalibrierung aufgefüllt. Nach Messung der Extinktion werden die Konzentrationen gemäß dem Lambert-Beer'schen Gesetz berechnet (s.u.). Bei Abweichungen der gemessenen Konzentration von der nominellen wird die nominelle Konzentration der Stammlösung korrigiert. Die Stammlösungen werden stets bei –30°C gelagert. Die Stammlösungen sind unter diesen Lagerbindungen erfahrungsgemäß sehr lange stabil. Vor Verwendung sollten die Stammlösungen UV-spektroskopisch überprüft werden.

Tabelle 23: Standardsubstanzen

Toxin / Name	CAS Nr.
TEA / Tenuazonsäure	610-88-8
AOH / Alternariol	641-38-3
AME / Alternariol-monomethylether	23452-05-3
TEN / Tentoxin	28540-82-1
ALT / Altenuen	29752-43-0

Mit Hilfe der UV/VIS-Spektren können die Substanzen identifiziert und durch die Extinktionskoeffizienten der einzelnen Toxine die Konzentrationen berechnet werden. Dieser Schritt kann bei Verwendung eines zertifizierten Standards entfallen.

Die Konzentrationen wurden über das Lambert-Beer'sche Gesetz berechnet:

$E = \varepsilon * c * d$ (Gleichung 7)
$\rightarrow c = E / (\varepsilon * d)$ (Gleichung 8)

mit:
E: Extinktion (einheitenlos)
ε: Extinktionskoeffizient [L/(mol*cm)]
c: Konzentration [mol/L]
d: Schichtdicke der Quarzküvette [cm hier 1]
Umrechnung Konzentration: c [mol/L] * M [g/mol] = c [g/L] (*1000 = µg/mL)

Nach den in der Literatur angegebenen Wellenlängen wurde für jedes Toxin die Adsorption gemessen und mit Hilfe der Extinktionskoeffizienten die wahren Konzentrationen berechnet.

Tabelle 24: Parameter für Konzentrationsbestimmung

	ALT	AOH	AME	TEN	TEA
Molmasse in g/mol	292,3	258,2	272,3	414,5	197,2
Wellenlänge in nm	240 (EtOH)	258 (EtOH)	256 (ACN)	282 $(EtOH)^{+}$	277 (MeOH)
Extinktionskoeffizient in l/(mol*cm)	30000*	38000**	47600***	20700	12980^{++}

* Montemurro, Visconti in Alternaria: Biology, Plant Diseases and Metabolites, p. 449
** Montemurro, Visconti in Alternaria: Biology, Plant Diseases and Metabolites, p. 449
*** Asam et al., J. Agric. Food Chem. 2009, 57, 5152
\+ Meyer et al., J. Am. Chem. Soc. 1975, 97 (13), 3802
++ Shephhard et al., J. Chromatogr. 1991, 566, 195

3.16 Standardgemisch

Aus den überprüften Stammlösungen (3.15) (Konzentration ca 1 mg/mL) werden Arbeitslösungen (Konzentration ca 10 µg/mL) in Ethanol hergestellt.
Aus diesen Arbeitslösungen wird in einem 20 mL Messkolben ein Standardlösungsgemisch aus allen Analyten, in Acetonitril, hergestellt (Konzentration ca. 125 ng/mL). Diese Lösung kann für die Herstellung einer Matrixkalibrierung (5.6.2) verwendet werden.
Wird statt einer Matrixkalibrierung eine externe Kalibrierung verwendet, wird das Standardlösungsgemisch (Konzentration ca. 125 ng/mL) stattdessen in Injektionslösung (3.14) hergestellt.

Hinweis: Das Standardlösungsgemisch kann als Dünnfilm vorbereitet werden. Hierzu wird aus den Arbeitslösungen (Konzentration ca 10 µg/mL) in einem 20 mL Messkolben ein Standardlösungsgemisch aus allen Analyten, in Ethanol, hergestellt (Konzentration ca. 250 ng/mL). Dieses Standardlösungsgemisch wird in 4-ml-Braunglasvials in Aliquote á 2 mL aufgeteilt und diese unter Rotation, Wärme und Unterdruck an einem Rotations-Vakuum-Konzentrator (4.12) zur Trockene zu Dünnfilmen eingedampft.
Die Dünnfilme werden bei -18°C gelagert und bei Bedarf in 4 mL Injektionslösung (3.14) für eine externe Kalibrierung (5.6.1) oder in 4 mL Acetonitril (3.2) für die Herstellung einer Matrixkalibrierung (5.6.2) durch Ultraschall und Schütteln rückgelöst. Die rückgelösten Dünnfilme ergeben Lösungen mit Konzentrationen von ca 125 ng/mL je Analyt.

3.17 Stammlösungen isotopenmarkierte interne Standards

Zu allen fünf Alternariatoxinen sind isotopenmarkierte Standards kommerziell verfügbar (s. Tabelle 25). Die isotopenmarkierten Standards können analog zu den nativen Toxinen in Ethanol oder Methanol gelöst werden.

Tabelle 25: Liste kommerziell erhältlicher isotopenmarkierter Substanzen

Toxin / Name
$^{13}C_2$ Tenuazonsäure
Alternariol-d_3
Alternariol-monomethylether-d_3
Tentoxin-d_3
Altenuen-d_6

3.18 Mischlösung isotopenmarkierter Standards (1 µg/mL)

Aus den Stammlösungen (3.17) wird eine Mischlösung hergestellt, die alle isotopenmarkierten Toxinen in der Endkonzentration 1 µg/mL enthält. Als Lösungsmittel kann z. B. Acetonitril verwendet werden.

4. Geräte und Hilfsmittel

Für die Durchführung sind laborübliche Geräte erforderlich, insbesondere die folgenden:

4.1 Direktverdrängerpipetten,

mit 1 mL, 200 µL, 100 µL und 50 µL und 10 µL mit geeigneten Plastikspitzen, alternativ Glaspipetten

4.2 Handy Step (oder vergleichbares)

Einwegaufsätze mit verschiedenen Volumina (50 ml für Extraktionsmittel) 2,5 mL und 1,25 mL

4.3 Analysenwaage

4.4 UV-Spektralphotometer

Doppelstrahlgerät, für Messungen zwischen 250 nm bis 350 nm, zur Überprüfung der Konzentration der Stammlösung

4.5 Quarzküvetten,

mit optischer Länge von 1 cm

4.6 Laborzentrifugen

Passend für 50 ml Zentrifugengfäße (3500 g), für Mikrozentrifugengefäße (21000 g)

4.7 Extraktionsgefäß/Zentrifugengefäß 50 ml

z.B.: 50 mL PP-Falcons

4.8 verschließbare Röhrchen zum Lagern der Salzmischung

z.B.: 15 mL PP-Falcons

4.9 Mikrozentrifugengefäße

PP-Mikrozentrifugengefäße 1,5 ml (z. B. von Eppendorf)

4.10 Geeignete mechanische Schüttelgeräte

zur Extraktion z. B. Überkopfschüttler, Turbula oder Taumelmischer; Vortexmischer

4.11 Verdampfungsgerät, das eine Temperatur von 50 °C mit einem gleichmäßigen Luft- oder Stickstoffstrom aufrechterhält.

4.12 Vakuumkonzentrator

z. B. Rotations-Vakuum-Konzentrator RVC 2-33 IR mit Kühlfalle Alpha 2-4 LD plus

4.13 Messermühle

z. B. Grindomix, Thermomix

4.14 Einweg Spritzenvorsatzfilter, Porengröße 0,2 µm (optional)

PTFE Material;
Geeignet sind auch HPLC-Vials mit integriertem Filter, z. B. Mini Uni Prep von Whatmann PTFE Filter 0,2 µm (Artikelnr. UN 203NPEORG)

Wichtig: andere Materialien wie PVDF, Teflon oder Nylon halten einzelne Analyten zurück!

4.15 Reagenzgläser aus Glas, 10 ml, silanisiert

Alle verwendeten Reagenzgläser werden vor Gebrauch silanisiert. Die Silanisierung kann mit 5%iger Surfasillösung (100 mL Surfasil™ + 1900 ml Cyclohexan (p.a.)) erfolgen.

Hinweis: Die zwingende Notwendigkeit der Silanisierung ist im BfR nicht geprüft worden.

4.16 HPLC-Vials

Aus Braunglas, etwa 2 ml Fassungsvermögen, verschließbar mit Crimpverschlüssen oder gleichwertig.

4.17 HPLC-Gerät

bestehend aus:

4.17.1 HPLC-Pumpe,

Geeignete HPLC-Pumpe, die pulsationsfrei mindestens binäre Gradienten erzeugen kann, bei für die Analysensäule geeigneten Durchflussraten.

4.17.2 Analytische Umkehrphasen-Trennsäule:

Phenomenex Gemini NX-C18 100 x 2.1 mm, 5µm Material

Hinweis: Die Ethyl-Brücken im NX-C18 Säulenmaterial verbessern gegenüber herkömmlichem C18-Säulenmaterial die Retention und Peakform von Tenuazonsäure.

4.17.3 Vorsäule (optional)

geeignet für die angewendete Analysensäule, 10 mm Länge, sowie Javelin-Filter (2.1 mm I.D.).

4.17.4 Autosampler

geeignet zur Injektion entsprechender Volumen mit ausreichender Wiederholpräzision.

4.17.5 MS/MS-System, inkl. Datenerfassungssystem

Tandem Massenspektrometer, z. B. SCIEX API 4000

4.17.6 Nachsäuleninfusion (optional)

Zusätzliche Pumpe für eine post column Zugabe von Lösungsmittel, Zugabe in den HPLC-Flow vor der ESI-Quelle über T-Stück; permanente Flussförderung von 200 µL/min muss ermöglicht werden; z. B. über zweite isokratische HPLC Pumpe oder quarternäre Pumpe.
Die Nachsäuleninfusion erhöht die Sensitivität für den Nachweis von TeA etwa um den Faktor 2 und kann wahlweise weggelassen werden.

5. Durchführung

Alle Schritte sind zügig nacheinander durchzuführen. Erst nach Abnahme des Zentrifugates ist die Probenaufarbeitung beendet.

5.1 Probenvorbereitung

5.1.1 Tomatensaft

10 g Tomatensaft werden in ein 50 mL Zentrifugengefäß (4.7) eingewogen

5.1.2 Ketchup, Tomatenmark, Tomatensoße, passierte Tomaten

5 g Probe werden in ein 50 mL Zentrifugengefäß (4.7) eingewogen und mit 5 mL Wasser versetzt sowie 10 min turbulent geschüttelt (4.10)

.

5.1.3 getrocknete Tomaten

Die getrockneten Tomaten werden zunächst grob zerkleinert und danach homogenisiert, z. B. mit einer Messermühle (4.13). 2 g werden in ein 50 mL Zentrifugengefäß (4.7) eingewogen, mit 8 mL Wasser versetzt und über Nacht bei 4°C gekühlt eingeweicht. Die Probe wird 30 min im Ultraschallbad behandelt und anschließend 10 min geschüttelt (4.10).

5.2 Einstellung des pH-Wertes

Der Probe bzw. dem Probengemisch werden 120 µL Phosphorsäure (3.10) zugegeben. Das Gefäß wird verschlossen und 5 min turbulent geschüttelt.

5.3 Extraktion

Es werden nacheinander 5 mL Acetonitril (3.2) und 5 mL MTBE (3.8) zur Probe gegeben, das Extraktionsgefäß wird fest verschlossen und mindestens 2 min turbulent geschüttelt. Anschließend wird die Probe 10 min im Ultraschallbad behandelt.

5.4 Phasentrennungen

Die Salzmischung (3.13) wird erst vor Zugabe geöffnet und zügig in das Extraktionsgefäß zur Probe gegeben. Das Extraktionsgefäß wird sofort verschlossen und entweder kurz mit einem Vortexgerät behandelt oder per Hand turbulent kurz geschüttelt. Dieser Schritt ist zügig auszuführen, da sonst die Gefahr des Verklumpens besteht. Wenn mehrere Proben zusammen aufgearbeitet werden, muss der unter 5.4 beschriebene Schritt mit den Proben nacheinander durchgeführt werden. Anschließend wird 5 min turbulent geschüttelt, danach 5 min bei 3500 g zentrifugiert.

5.5 Herstellen der Probenmesslösung für die HPLC-Analyse

1 ml (V_1) des Zentrifugates wird mittels Vollpipette oder Direktverdrängungspipette (4.1) in ein 10 mL Reagenzglas (4.15) pipettiert. Es wird bei 50°C unter Stickstoffstrom vorsichtig zur Trockene eingedampft (4.11). Der Rückstand wird zuerst mit 250 µL Acetonitril (3.2) rückgelöst, indem das Reagenzglas 10 min im Ultraschallbad behandelt wird. Anschließend werden 740 µL 1 mM Ammoniumacetat-Lösung (pH 9) (3.6.1) zupipettiert. Es werden 10 µL des internen Standards (3.18) zugegeben. Diese Probenmesslösung wird 10 min im Ultraschallbad behandelt sowie 10 min geschüttelt.

Im Fall einer Trübung ist die Probenmesslösung in ein Mikrozentrifugengefäß (4.9) zu überführen und bei 21.000 g und 0 °C zu zentrifugieren. Der klare Überstand ist in ein Vial zu überführen. Alternativ kann die trübe Probenmesslösung mit einem 0,2 µm PTFE-Spritzenfilter (4.13) direkt in ein Vial filtriert werden. Hierbei sind ausschließlich PTFE-Filter zu verwenden!

Hinweis: Bei ausreichender Sensitivität des Massenspektrometers kann das Volumen V_1 reduziert werden, z. B. auf 250 µL. Hierdurch wird die Rekonstitution verbessert und Matrixeffekte werden verringert.

5.6 HPLC-MS/MS-Bestimmung

Vorbemerkung: Bei der ESI-LC-MS/MS Technik kann es durch Matrixbestandteile, welche mit den zu identifizierenden Analyten gleichzeitig in die Ionenquelle eintreten, zu einer Unterdrückung oder Verstärkung des Signals führen (Suppression/Enhancement). Bei der dargestellten QuEChERS-Aufarbeitung ist insbesondere bei AOH und AME in allen Matrizes mit einer hohen Ionensuppression von bis zu 80% zu rechnen. Eine Kompensation der Matrixeffekte ist daher nötig.

Im Zuge der Validierung wurden eine Wiederfindungskalibrierung und eine Matrixkalibrierung zum Ausgleich des Matrixeffektes bei der Ionisierung vergleichen:

- Bei der Wiederfindungskalibrierung wurde eine Blankprobe mit sieben verschiedenen Gehalten dotiert. Nach Abdampfen der Dotierungslösung wurden diese sieben Proben gemäß der Prüfvorschrift aufgearbeitet. Die Sollkonzentration dieser sieben Proben wurde für die Kalibrierung benutzt.
- Bei der Matrixkalibrierung (s. 5.6.2) wurde eine Blankprobe gemäß der Prüfvorschrift aufgearbeitet. Die resultierende Probenmesslösung wurde aliquotiert und mit sieben Konzentrationen dotiert.

Im Ergebnis wurde festgestellt, dass beide Verfahren vergleichbare Ergebnisse lieferten. Daher sieht diese Prüfvorschrift die Matrixkalibrierung gegenüber der wesentlich aufwändigeren Wiederfindungskalibrierung vor.
Gegebenenfalls kann das Standardadditionsverfahren (5.6.3) benutzt werden.

5.6.1 Externe Kalibrierung

Die externe Kalibrierung kann zur Gehaltsbestimmung verwendet werden, wenn isotopenmarkierte Standards zur Verfügung stehen. Stehen keine isotopenmarkierte Standards zur Verfügung, ist eine Matrixkalibrierung zu verwenden (5.6.2).

Tabelle 26: Pipettierschema für die externe Kalibrierung

Name	Konzentration [ng/mL]	Zu pipettierendes Volumen des Standardgemischs (3.16)* in HPLC-Vial	Zu pipettierendes Volumen der Injektionslösung (3.14)	Zu pipettierendes Volumen des internen Standards (3.18)
KAL 1	2,50	20 µL	970 µL	10 µL
KAL 2	5,00	40 µL	950 µL	10 µL
KAL 3	10,00	80 µL	910 µL	10 µL
KAL 4	25,00	200 µL	790 µL	10 µL
KAL 5	50,00	400 µL	590 µL	10 µL

*für die externe Kalibrierung muss das Gemisch in Injektionslösung (3.14) gelöst vorliegen

5.6.2 Matrixkalibrierung

Um die Matrixeffekte auszugleichen und somit eine korrekte Gehaltsermittlung der Toxine zu gewährleisten, sollte eine geeignete Matrixkalibrierung verwendet werden.
Hierfür wird eine zur Probe passende, geeignete toxinfreie Matrix ausgewählt und diese wie unter 5.1 – 5.4 aufgearbeitet. Für fünf Matrixkalibrierstandards werden in fünf Reagenzgläser (4.15) je 1 mL desselben Probenextrakts pipettiert. In diese Reagenzgläser werden die in Tabelle 27 beschriebenen Volumina an Standardgemisch für den Abdampfungsschritt zupipettiert. Danach wird wie unter 5.5 beschrieben zur Trockene eingedampft und rückgelöst.

Tabelle 27: Pipettierschema für die Matrixkalibrierung

Name	Konzentration [ng/mL]	Zu pipettierendes Volumen des Standardgemischs (3.16)* in Reagenzglas (4.15)	
KAL 1	2,50	20 µL	Zugabe von je 1 mL Blank-Proben-extrakt (5.6.2), danach Abdampfen und Rekonstitution in je 1 mL Injektionslösung (3.14), Transfer der Lösung in HPLC-Vial
KAL 2	5,00	40 µL	
KAL 3	10,00	80 µL	
KAL 4	25,00	200 µL	
KAL 5	50,00	400 µL	

*für die Matrixkalibrierung muss das Gemisch in Methanol (3.3) gelöst vorliegen

5.6.2.1 Selbst hergestellter Tomatensaft

Da Tenuazonsäure so gut wie immer in kommerziell erhältlichen Tomatenprodukten enthalten ist, kann ersatzweise geeigneter toxinfreier Tomatensaft aus frischen, unversehrten Tomaten hergestellt werden. Hierzu werden die Tomaten gewaschen und die Schale, Kerne sowie der Strunk mittels Passiersieb vom Saft getrennt. Der Wasseranteil des so erhaltenen Saftes wird durch Kochen reduziert bis die Konsistenz des Saftes kommerziell erhältlichem Saft ähnelt.

5.6.3 Standardadditionsverfahren

Die Matrixeffekte können innerhalb derselben Matrix variieren. Im Einzelfall kann eine Gehaltsbestimmung mittels Standardadditionsverfahren erforderlich sein.

Hierzu werden die Proben wie unter 5.1 - 5.4 beschrieben aufgearbeitet. Von dem in 5.4 hergestelltem Zentrifugat wird 1 ml wie in 5.5 beschrieben weiterbearbeitet. Etwa 5 mL des Zentrifugates werden in ein verschließbares, dichtes Aufbewahrungsgefäß pipettiert und bei -30°C bis zur weiteren Verwendung gelagert.

Der Gehalt an Alternariatoxinen wird zunächst näherungsweise über eine Matrixkalibrierung (5.6.2) bestimmt. Basierend auf den so erhaltenen Gehalten wird eine Standardaddition mit den drei Konzentrationsstufen 50%, 100% und 200 % durchgeführt. Die Aufstockung der drei Konzentrationsstufen erfolgt durch Zugabe entsprechender Volumina aus Arbeitslösungen. Diese Arbeitslösungen werden durch geeignete Verdünnungen (1 µg/ml – 10 µg/ml) der Stammlösungen (3.15) hergestellt.

Die Konzentration der Analyten in der Probe lässt sich grafisch als x-Achsenabschnitt ermitteln, indem die Konzentration der vier Probenmesslösungen (0%, 50%, 100%, 200%) gegen die gemessenen Peakfläche aufgetragen werden.

5.6.4 Bestimmung Alternariatoxine in der Probenmesslösung

Aliquote Anteile der Probenmesslösungen sind unter denselben Bedingungen wie die Kalibrierlösungen in den Chromatographen zu injizieren.

5.6.5 Identifizierung der Peaks

Der Peak des jeweiligen ATs in der Probenmesslösung ist durch Vergleich seiner Retentionszeit mit der durchschnittlichen Retentionszeit des jeweiligen AT in den Kalibrierlösungen zu identifizieren. Die Abweichungen der Retentionszeiten sollten nicht größer als +/-0,2 min sein. Weiter wird der Analyt über das Ion Ratio (Verhältnis zwischen dem ersten Masse zu Ladungsübergang (m/z; Quant)) und dem zweiten Masse zu Ladung Übergang (m/z Qual)) identifiziert. Die Abweichung zwischen der Ion Ratio der Probe und der Standards sollte nicht größer als 30% sein.

Die Konzentration der jeweiligen ATs in der Untersuchungslösung muss innerhalb des Kalibrierbereichs liegen. Wenn die Konzentration in der Probenmesslösung die Konzentration der höchsten Kalibrierlösung überschreitet, muss die Probenmesslösung mit der Injektionslösung verdünnt werden, um den Kalibrierbereich wieder zu erreichen. Wird eine externe Kalibrierung verwendet, muss bei der Verdünnung der Probenmesslösung zusätzlich eine proportionale Menge interner Standard (3.18) zugegeben werden, so dass die Konzentration der isotopenmarkierten Alternariatoxine in der Probenmesslösung konstant bleibt.

5.6.6 HPLC-Betriebsbedingungen (informativ)

<u>Injektionssystem:</u>
Autosampler Model: Agilent 1100 Thermo Autosampler
Syringe Size (µL): 100
Injection Volume (µL): 5.00
Draw Speed (µL/min): 200.0
Eject Speed (µL/min):200.0
Temperature: 10 °C
Wash program:

Nach Aufziehen der Injektionslösung wird die Autosamplernadel durch Eintauchen in Acetonitril (Vial-Position 91) und anschließend in Injektionslösung (Vial-Position 92) äußerlich gereinigt, um Verschleppungen nach der Injektion im Nadelsitz zu vermeiden. Dies ist insbesondere für AME wichtig, da AME deutliche Verschleppungen im Injektionssystem hervorrufen kann („carry over“).

<u>Eluenten Gradient:</u>
Pump Model: Agilent 1100 Capillary Pump

Tabelle 28: Gradientenprogramm

Step	Total Time(min)	Flow Rate(µL/min)	Eluent A (%)	Eluent B (%)	
1	-5	400	100	0	Equilibrie-rung
2	0	400	100	0	
3	0,1	400	60	0	
4	3	400	25	75	
5	3,5	400	5	95	
6	6,5	400	5	95	
7	6,6	400	100	0	
8	7	400	100	0	

Programm Säulenofen:
Agilent Column Oven Model: Agilent 1100 Oven mit Säulenschaltventil
Temperature (°C): 40.00
Programm:

Step	Total Time (min)	8-Port-Ventil-Stellung
1 (Equilibrierung)	5.00	left (waste)
2	1.80	right (MS)
3	6.00	left (waste)

LC-MS/MS-Parameter:

Tabelle 29: Allgemeine Einstellungen des Massenspektrometers (Periode 1 Experiment 1)

Collision Gas	6
Curtain Gas	40
Ion source Gas 1 (GS1)	50
Ion source Gas 2 (GS2)	50
Ion Spray Voltage (IS)	-3000
Temperature (TEM)	550
Interface Heater (ihe)	On

Tabelle 30: Masse zu Ladungs Übergänge und Messparameter der einzelnen Toxine

Q1 Mass (Da)	Q3 Mass (Da)	Retention Time (min)	Analyt	DP (volt)	EP (volt)	CE (volt)	CXP (volt)
196,1	139,0	2,25	Tenuazonic acid 1	-50	-10	-26	-9
	112,0		Tenuazonic acid 2	-50	-10	-32	-7
198,1	141,0	2,25	^{13}C-TEA 1	-50	-10	-26	-7
	114,0		^{13}C-TEA 2	-50	-10	-32	-9
257,0	213,0	3,22	Alternariol 1	-80	-10	-33	-11
	147,0		Alternariol 2	-80	-10	-46	-9
260,0	216,0	3,22	AOH-d_3 1	-80	-10	-33	-11
	150,0		AOH-d_3 2	-80	-10	-46	-9
291,1	214,0	3,52	Altenuen 1	-50	-10	-28	-11
	229,1		Altenuen 2	-50	-10	-20	-13
297,1	217,0	3,52	ALT-d_6 1	-50	-10	-28	-11
	235,1		ALT-d_6 2	-50	-10	-20	-13
413,1	141,0	4,25	Tentoxin 1	-85	-10	-28	-7
	271,0		Tentoxin 2	-85	-10	-22	-11
416,1	141,0	4,25	TEN-d_3 1	-85	-10	-28	-7
	274,0		TEN-d_3 2	-85	-10	-22	-11
271,0	256,1	4,92	Alternariolmethylether 1	-75	-10	-30	-11
	228,0		Alternariolmethylether 2	-75	-10	-40	-17
274,0	259,1	4,92	AME-d_3 1	-75	-10	-30	-11
	231,0		AME-d_3 2	-75	-10	-40	-17

Tabelle 31: Legende Abkürzungen

DP	declustering potential
EP	entrance potential
CE	collision energy
CXP	collision cell exit potential

6. Berechnung

Die Toxinmenge in ng/mL, die in der auf die Säule injizierten Probenmesslösung enthalten ist, wird direkt aus der Matrixkalibrierkurve berechnet.

$c_{AT} = x = (y - t) / m$ (Gleichung 9)

Dabei ist

c_{AT} die errechnete Konzentration an Analyt im gemessenen Probenextrakt [ng/mL]

Die Massenkonzentration an AT in der Probe ω_{pat}, in ng je g (entspricht µg je kg) wird nach Gleichung 10 berechnet:

$$\omega_{AT} = (m_{AT} * 10) / m_{Einwaage} \quad \text{(Gleichung 10)}$$

Dabei ist:

m_{AT} die über die Kalibrierung berechnete Masse an AT, die in der auf die Säule injizierten Probenmesslösung enthalten ist, in Nanogramm;

10 Verdünnungsfaktor des Probenextraktes (1 mL von 10 mL Extraktionslösung wurde zur Trockene eingedampft und in 1 mL Injektionslösung rückgelöst)
Bemerkung: Es wird davon ausgegangen, daß die Menge an AT aus der Probe durch die Phasentrennung komplett in 10 mL Extraktionsmittel übergeht.

$m_{Einwaage}$ die Probeneinwaage in g

7. Angabe der Ergebnisse

Im Untersuchungsbericht sind mindestens anzugeben:

a) alle notwendigen Informationen zur Identifizierung der Probe (Art, Herkunft, Bezeichnung der Probe);
b) ein Verweis auf die verwendete Methode;
c) Datum und Art der Probenahme (wenn bekannt);
d) Datum des Probeneinganges;
e) Datum der Untersuchung;
f) die Ergebnisse und Einheiten, in denen sie angegeben werden;
g) ob die Wiederholgrenze eingehalten wurde; falls vorhanden
h) alle Besonderheiten, die während der Untersuchung festgestellt wurden;
i) alle Arbeitsschritte, die nicht in diesem Verfahren festgelegt sind, wahlweise vorgenommen wurden und das Ergebnis möglicherweise beeinflusst haben.

Die Angabe der Ergebnisse erfolgt mit einer Nachkommastelle in µg/kg Lebensmittel.
Es ist anzugeben, ob die erhaltenen Messergebnisse um die Wiederfindung korrigiert wurden.

8. Anhang

8.1 Beispielchromatogramm

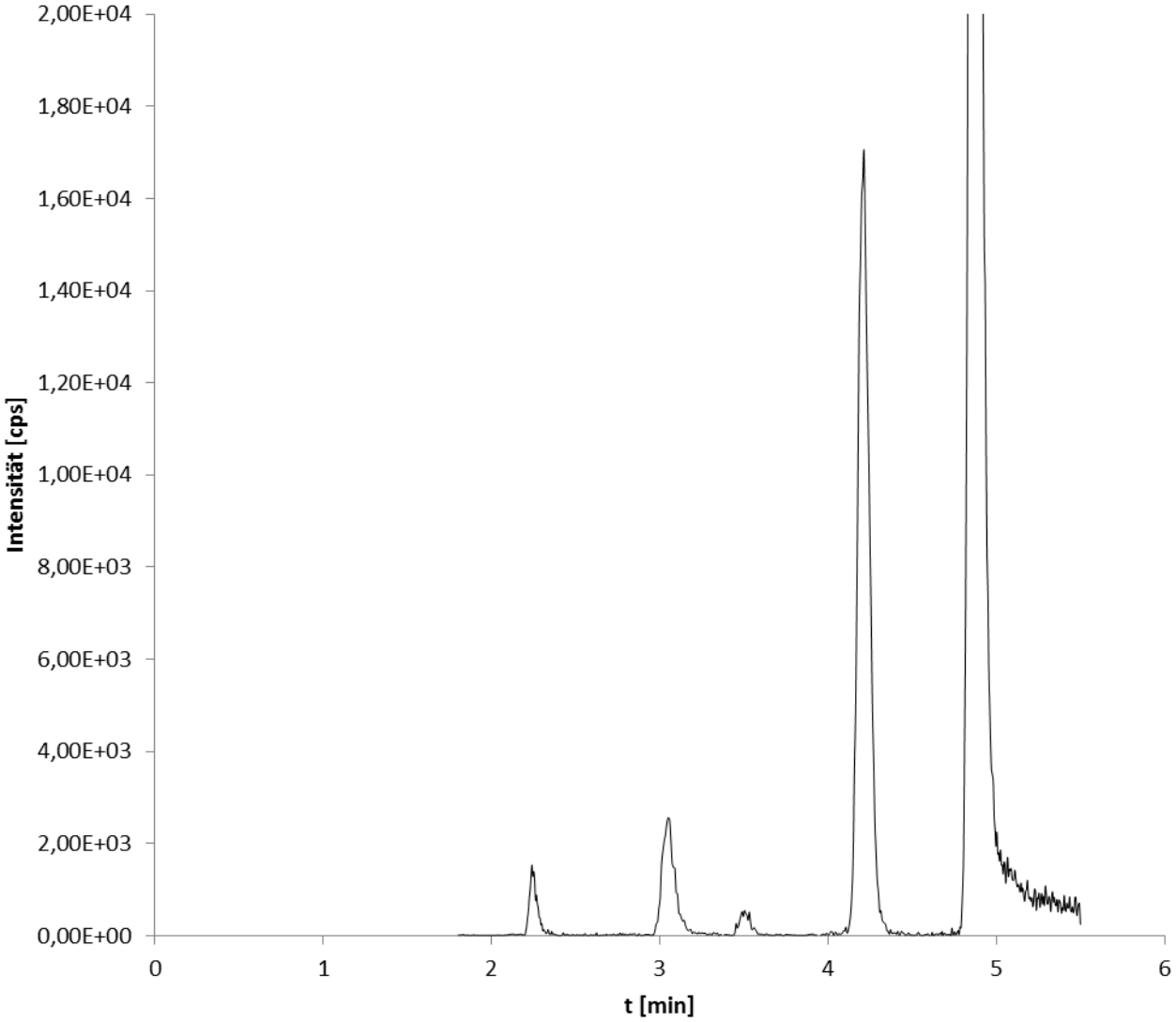

Abbildung 1: Chromatogramm einer matrixfreien Kalibrierlösung (2,5 ng/ml), Peakzuordnung: TeA (RT 2,25 min), AOH (RT 3,22 min), ALT (RT 3,52 min), TEN (4,25 min), AME (4,92 min)

8.2 Ablaufschema

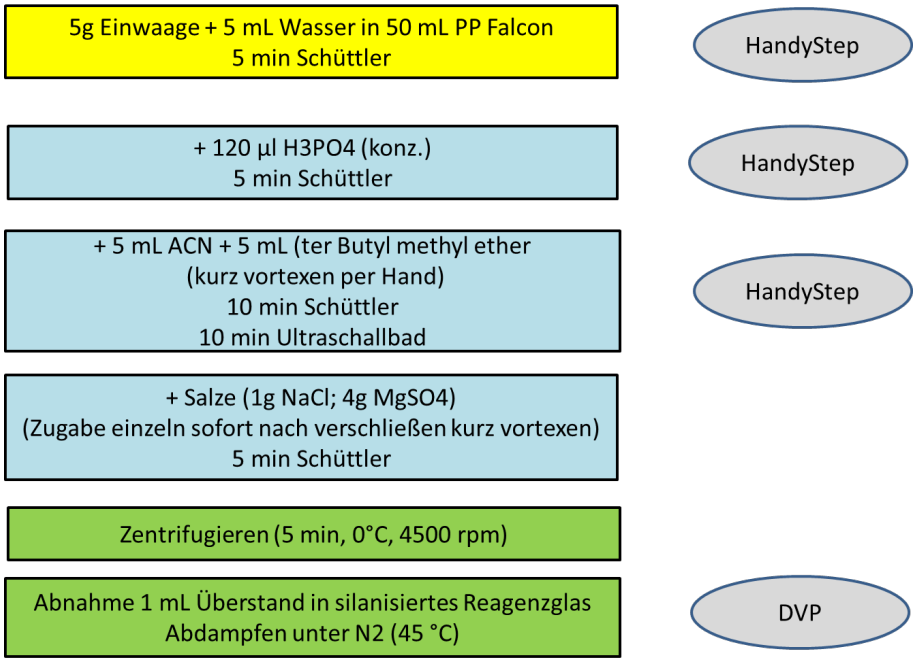

Abbildung 2: Schema der Probenvorbereitung für Tomatenprodukte

9. Literatur

- Payá P, Anastassiades M, Mack D, et al. (November 2007). "Analysis of pesticide residues using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass spectrometric detection".Anal Bioanal Chem 389 (6): 1697–714
- Scientific Opinion on the risks for animal and public health related to the presence of Alternaria toxins in feed and food, European Food Safety Agency (EFSA), 2011

8 List of figures

9 List of tables