Determination of *Fusarium* Mycotoxins in Wheat, Maize and Animal Feed Using an Online TurboFlow and Orbitrap LC/MS Method

Ebru Ates and Klaus Mittendorf – Food Safety Response Center, Thermo Fisher Scientific, Dreieich, Germany

Method 63559

Key Words
Mycotoxins, food safety, Transcend system, Exactive, TurboFlow technology, Orbitrap, method validation

1. Schematic of Method

1. Weigh 5 g of homogenized sample into a 50 mL bottle.

Homogenized sample, 5 g

2. Add 20 mL of extraction solvent (water 0.1% FA/ACN (43:57)) and shake for 45 minutes.

Extraction

3. Filter sample through 0.2 µm nylon microfilter.

Filtration

4. Place the vial in autosampler of TLX-HRMS.

TurboFlow - Orbitrap LC/MS

2. Introduction

Mycotoxins are secondary metabolites produced by fungal infection of agricultural crops while in the field or during harvest, drying or subsequent storage. These compounds are very stable and cannot be readily destroyed by heating or food processing, although some processes, such as the milling of grains, can reduce the level in the end product. Good Agricultural Practices (GAP) are important to ensure that fungal infection is minimized and food and feed are produced with the lowest levels of mycotoxins achievable. Approximately 400 mycotoxins are known, but only a few are regulated by legislation.

The general approach to the analysis of mycotoxins involves liquid extraction, solid-phase extraction (SPE) or immunoaffinity-column (IAC) cleanup followed by HPLC with fluorescence detection or LC-MS (LC-MS/MS). The requirement for high sensitivity and the problems of matrix interferences either necessitate a lengthy cleanup process or require the use of high-specificity detection such as LC-MS/MS. However, even though direct analysis is possible without cleanup, dirty extracts can result in ion suppression and the need for frequent cleaning of the instrument source in LC-MS/MS.

Thermo Scientific TurboFlow technology is an online, automated sample cleanup and pre-concentration technique that enables the direct injection of food extracts that saves time by eliminating manual sample preparation. High-resolution mass spectrometry enables the determination of accurate masses with < 5ppm mass accuracy. Additional compound identification is given by fragmentation ions produced in a higher collision dissociation cell (HCD fragments).

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A method using the Thermo Scientific Transcend TLX system with TurboFlow™ technology, which combines chromatography with high-resolution mass spectrometry, was developed and validated in house for the determination of deoxynivalenol, zearalenone, T-2 toxin, HT-2 toxin, fumonisins B_1 and B_2 in maize (corn), wheat and animal feed. Linearity range, limit of detection (LOD), limit of quantification (LOQ), recovery, repeatability, intermediate precision and accuracy were established by analysis using certified reference materials and the performance of successful proficiency testing.

3. Scope

The TurboFlow method based on online sample cleanup and high-resolution mass spectrometric detection can be applied to the determination of *Fusarium* mycotoxins (deoxynivalenol, zearalenone, T-2 toxin, HT-2 toxin and fumonisins B_1 and B_2) below the legislative limits[1-3] in maize, wheat and animal feed. The method replaces cleanup techniques involving numerous manual sample preparation steps, such as the purification of sample extracts using immunoaffinity cleanup cartridges.

4. Principle

This method uses TurboFlow technology for online cleanup of the sample. Finely ground and homogenous sample (5 g) is extracted for 45 minutes with a mixture of water containing 0.1% formic acid (FA)/acetonitrile (ACN) (43:57). After filtration with a 0.2 µm nylon filter into an LC-vial, the sample is injected into the Transcend TLX™ system, an online chromatography–reversed phase chromatography system (TLX-LC) coupled with high-resolution mass spectrometric (HRMS) detection on a Thermo Scientific Exactive Orbitrap mass spectrometer. TurboFlow technology serves as a novel sample preparation technique in food analysis due to its special flow profile, size exclusion, and reversed phase column chemistry. This enables very effective separation of matrix and target compounds, resulting in relatively clean sample extracts. Identification of mycotoxins is based on accurate mass determination at a resolution power of 100,000 and additional HCD fragments with mass deviation below 5 ppm.

5. Reagent List

5.1	Acetonitrile, Fisher Chemical Optima grade, for LC-MS	
5.2	Water, Optima™ grade, for LC-MS	
5.3	Methanol, Optima grade, for LC-MS	
5.4	Formic acid (FA)	
5.5	Thermo Scientific Pierce LTQ ESI positive ion calibration solution	
5.6	Thermo Scientific Pierce LTQ ESI negative ion calibration solution	

6. Calibration Standards

6.1	Deoxynivaneol (DON)	Sigma-Aldrich®
6.2	Zearalenone (ZON)	Sigma-Aldrich
6.3	T-2 toxin (T-2)	Sigma-Aldrich
6.4	HT-2 toxin (HT-2)	Sigma-Aldrich
6.5	Fumonisin B1 (FB1)	Sigma-Aldrich
6.6	Fumonisin B2 (FB2)	Sigma-Aldrich

7. Standard Preparation

7.1 Stock standard solutions of mycotoxins

Stock standard solutions (10 µg/mL) are prepared individually by dissolving the analytes in methanol. Solutions are stored at -20 °C. The standard stock solution is used for spiking, as different spiking levels are required for method validation of each mycotoxin.

8. Apparatus

8.1	Transcend TLX-1 system
8.2	Exactive™ Orbitrap™ mass spectrometer
8.3	Column oven, HotDog 5090 *(Prolab GmbH, Switzerland)*
8.4	Precision balance
8.5	Sartorius® analytical balance *(Sartorius GmbH, Germany)*
8.6	Thermo Scientific Barnstead Easypure II water
8.7	Elmasonic® S 40 (H) ultrasonic bath, *(ELMA® Hans Schmidbauer GmbH & Co. KG, Germany)*
8.8	Vortex shaker
8.9	Vortex standard cap
8.10	IKA® HS 501 digital shaker *(IKA-Werke GmbH & Co. KG, Germany)*

9. Consumables

9.1	Thermo Scientific Hypersil GOLD column, 50 x 4.6 mm, 5 µm particle size
9.2	TurboFlow Cyclone MCX column, 0.5 x 50 mm
9.3	LC vials
9.4	LC vial caps
9.5	Thermo Scientific Finnpipette 10–100 µL pipette
9.6	Finnpipette™ 100–1000 µL pipette
9.7	Finnpipette 500–5000 µL pipette
9.8	Pipette holder
9.9	Fisherbrand Pasteur Pipet, soda lime glass, 150 mm
9.10	Pipette suction device
9.11	Pipette tips 0.5–250 µL, 500/box
9.12	Pipette tips 1–5 mL, 75/box
9.13	Pipette tips 100–1000 µL, 200/box
9.14	Disposable plastic syringe, 1 mL
9.15	Nylon filter 0.2 µm

10. Glassware

10.1	Beaker, 25 mL
10.2	Volumetric flask, 10 mL
10.3	Volumetric flask, 100 mL
10.4	Volumetric flask, 1000 mL
10.5	Amber bottle, 50 mL

11. Procedure

11.1 Chemical preparation

Extraction solvent is prepared by mixing 1000 mL acetonitrile with 750 mL of water containing 0.1% FA.

11.2 Sample preparation and spiking

As no blank certified reference materials are available, a number of samples of maize, wheat and animal feed are analyzed to be used as blank material for spiking purposes. These samples, with trace levels (below LOD) of target mycotoxins, are used as blank materials for the validation study. Spiking is performed at three different levels with mycotoxin standard solutions.

To prepare the spiked sample, 500 g of matrix is homogenized by a laboratory blender and ground to a fine powder using a mortar and pestle. A sample of 5 g (±0.01 g) is weighed, put into a 50 mL amber flask and spiked with the appropriate amount of mycotoxin standard. Spiked samples are stored for 30 minutes in the dark for equilibration of mycotoxins. After the addition of 20 mL of extraction solvent, the bottles are closed and shaken for 45 minutes in the laboratory shaker. Samples are filtered through a nylon filter (0.2 µm) and injected into the TLX-HRMS system.

12. TLX-LC Conditions

TurboFlow methods are performed on a Transcend TLX-1 system. The LC conditions are as follows:

TurboFlow column:	TurboFlow Cyclone MCX, 0.5 x 50 mm
Analytical column:	Hypersil GOLD™, 50 x 4.6 mm, 5 µm particle size
Mobile phases:	A: Water (0.1 % formic acid) C: Methanol (0.1 % formic acid)
Total run time:	18 minutes

The autosampler sample holder temperature is kept at 10 °C. Sample injection volume is 10 µL with a 100 µL injection syringe. The injection syringe is rinsed as described in the injector settings. The gradient program is presented in Table 1.

Table 1. Gradient program table in Thermo Scientific Aria software for TurboFlow Method coupled with an analytical column

Step			Loading Pump[a]					Cut-in Loop		Eluting Pump[b]				
Step	Start (min)	Time (s)	Flow (mL/min)	Grad	A (%)	B (%)	C (%)	Tee	Loop	Flow (mL/min)	Grad	A (%)	B (%)	C (%)
1. Loading	0	90	1.5	Step	100	0	0	===	Out	0.45	Step	99	0	1
2. Transferring	1.30	1	0.3	Step	85	0	15	T	In	0.2	Step	99	0	1
3. Transferring/ HPLC	1.31	59	0.3	Step	85	0	15	T	In	0.2	Ramp	80	0	20
4. Washing/ HPLC	2.30	360	1.5	Step	85	0	15	===	In	0.6	Ramp	0	0	100
5. Washing/ HPLC	8.30	130	1.5	Step	100	0	0	===	In	0.6	Step	0	0	100
6. Washing/ HPLC	10.40	160	1.5	Step	0	0	100	===	In	0.6	Step	0	0	100
7. Loop filling/ equilibrating	13.20	120	1.5	Step	10	0	90	===	In	0.5	Step	99	0	1
8. Equilibrating	15.20	160	1.5	Step	100	0	0	===	Out	0.5	Step	99	0	1

[a]Mobile phases for the TurboFlow method:
A: Water (0.1% FA)
C: Methanol (0.1 % FA)

[b]Mobile phases for the analytical method:
A: Water (0.1% FA)
C: Methanol (0.1 % FA)

The injector settings are as follows:

Injector:	CTC injector *(CTC Analytics AG, Switzerland)* with 100 µL injection syringe volume
Wash solvents for the autosampler:	
Wash 1:	Methanol
Wash 2:	5% Methanol in water
Pre-clean syringe with wash 1 [steps]:	2
Clean injector (TX) with wash 1 [steps]:	2
Get sample (SEQ Tray: SEQ Index):	SEQ Volume

After injecting sample (syringe content) to TX:

Clean syringe with wash 1 [steps]:	7
Clean injector (TX) with wash 1 [steps]:	7
Clean syringe with wash 2 [steps]:	7
Clean injector (TX) with wash 2 [steps]:	7

Injection volume:	10 µL
Tray temperature:	10 °C
Column oven:	40 °C

13. Mass Spectrometric Conditions

MS analysis is carried out using the Exactive Orbitrap high-resolution benchtop mass spectrometer controlled by Aria™ MX software version 1.1. Data acquisition and processing is performed using Thermo Scientific Xcalibur 2.1 software. The Exactive MS was calibrated in positive and negative mode every 48 hours.

The MS Conditions are as follows:

Ionization:	Heated electrospray (HESI)
Polarity:	Positive/negative switching mode
Sheath gas flow rate [arb]:	60
Aux gas flow rate [arb]:	20
Spray voltage [kV]:	3.60
Capillary temperature [°C]:	260
Capillary voltage [V]:	60
Tube lens voltage [V]:	120
Skimmer voltage [V]:	25
Heater temperature [°C]:	250
Scan mode:	Full scan
Scan range [m/z]:	100–900
Microscans:	1
Resolution:	100,000
AGC target:	Balanced

14. Calculation of Results

14.1 Identification

Identification of mycotoxins was indicated by the presence of accurate mass ions obtained at a resolving power of 100,000 FWHM at *m/z* 200 and a mass accuracy window below 5 ppm, corresponding to the retention times of appropriate standards. Additional mass confirmation was given by the simultaneous detection of HCD fragments. Theoretical masses and detected masses in standards in methanol are listed in Table 2. Detected masses of target standards in maize, wheat and animal feed are listed in Table 3. Accurate mass deviation was determined to be below -1.8 ppm.

Table 2. Theoretical and found accurate masses in standards in methanol and fragment ions detected by HCD fragmentation

Mycotoxin	Molecular Formula	Exact Molecular Mass (*m/z*)	Adduct	Found [in MeOH]	Mass Deviation (ppm)	Fragment Ion Formula	Fragment Ion Exact Mass (*m/z*)	eV HCD Fragmentation
DON	$C_{15}H_{20}O_6Na$	319.1152	$+Na^+$	319.1150	+0.6	$C_{14}H_{17}O_4$	249.1124	20
T-2	$C_{24}H_{34}O_9Na$	489.2095	$+Na^+$	489.2095	0	$C_{17}H_{21}O_5$	305.1397	20
HT-2	$C_{22}H_{32}O_8Na$	447.1989	$+Na^+$	447.1989	0	$C_{17}H_{17}O_4$	285.1095	20
FB-1	$C_{34}H_{60}NO_{15}$	722.3957	$+H^+$	722.3955	+0.3	$C_{22}H_{42}NO_2$	352.3227	20
FB-2	$C_{34}H_{60}NO_{14}$	706.4008	$+H^+$	706.4006	+0.3	$C_{22}H_{42}NO$	336.3276	20
ZON	$C_{18}H_{21}O_5$	317.1395	$-H^+$	317.1397	-0.6	C_9H_7O	131.0492	20

Table 3. TLX-HRMS Exact masses and mass deviation in maize, wheat and animal feed

Mycotoxin	Adduct	Found Maize (*m/z*)	Mass Deviation (ppm)	Found Wheat (*m/z*)	Mass Deviation (ppm)	Found Animal Feed (*m/z*)	Mass Deviation (ppm)
DON	$+Na^+$	319.1151	+0.3	319.1151	-0.3	319.1150	+0.6
T-2	$+Na^+$	489.2098	-0.6	489.2098	-0.6	489.2101	-1.2
HT-2	$+Na^+$	447.1993	-0.9	447.1994	-1.1	447.1997	-1.8
FB-1	$+H^+$	722.3962	-0.7	722.3965	-1.1	722.3969	-1.7
FB-2	$+H^+$	706.4010	-0.3	706.4008	0	706.4015	-1.0
ZON	$-H^+$	317.1397	-0.6	317.1398	-0.9	317.1396	-0.3

14.2 Quantification

By comparing peak areas of the samples with those of external matrix-matched calibration standards, quantification of the mycotoxins was carried out. Calibration curves were plotted as relative peak areas (analyte) as a function of concentrations. The mycotoxin concentration (cMyco) in the samples was determined from the equation:

$$C_{Myco} = (A-b)/a$$

C_{Myco} – mycotoxin concentration in µg/kg
A – peak area of the mycotoxin
b – y-intercept
a – slope of calibration curve

15. Method Performance

Single laboratory method performance characteristics were established by spiking experiments in three matrices (maize, wheat and animal feed) with mycotoxin standards. Method recovery and precision was assessed at three different spiking levels (50%, 100% and 200% of the legislative limit for mycotoxins). Method accuracy was confirmed by the analysis of representative, certified reference materials. Other validation parameters included linearity range, LOD, LOQ, intermediate precision and matrix effect. A TLX-HRMS chromatogram of target compounds in maize is presented in Figure 1.

15.1 Specificity

The specificity was confirmed based on the presence of accurate parent masses and HCD fragment ion at the correct retention time corresponding to the mycotoxin standards in methanol (Table 4). The retention times were within ±2.5%. A minimum of 12 data points were required for each peak.

Table 4. Retention time comparison in matrices (specificity), ±2.5% deviation allowed

Mycotoxin	Retention Time (min)			
	Methanol	Maize	Wheat	Animal Feed
DON	2.97	2.98	2.98	2.96
T-2	8.01	8.03	8.02	8.01
HT-2	7.63	7.64	7.63	7.62
FB-1	7.58	7.59	7.56	7.53
FB-2	8.19	8.18	8.15	8.13
ZON	8.39	8.40	8.38	8.38

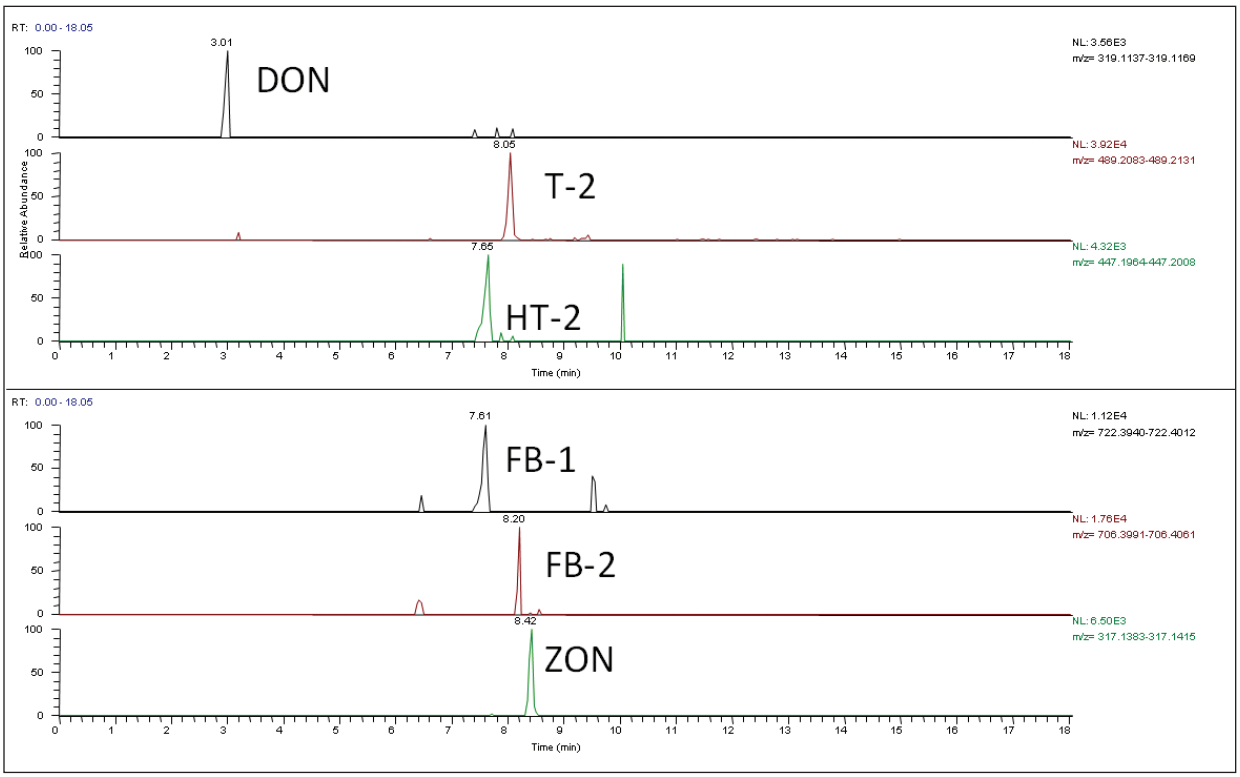

Figure 1. TLX-LC-HRMS chromatogram of DON (200 µg/kg), T-2 (20 µg/kg), HT-2 (20 µg/kg), FB_{-1} (75 µg/kg), FB_{-2} (75 µg/kg) and ZON (10 µg/kg) spiked in maize sample

15.2 Linearity & calibration curve

The linearity of the calibration curves was checked in standard solutions by monitoring the molecular ion of each mycotoxin analyzed (Table 5). In all cases, the correlation coefficients of linear functions were >0.985. The calibration curves were created from eight calibration standards, which were injected in duplicate in each batch starting from zero up to the highest calibration concentration.

Table 5. Linearity ranges of mycotoxin standards in solvent, maize, wheat and animal feed

Mycotoxin	Linearity Range (µg/kg)				
	Methanol		Maize	Wheat	Animal Feed
DON	125– 2190	225–2500	125–2190	125–2190	225–2500
T-2	12–400	12–400	12–400	12–400	12–400
HT-2	12–400	12–400	12–400	12–400	12–400
FB-1	50–500	500–5000	50–500	50–1250	500–5000
FB-2	50–500	500–5000	50–500	50–1250	500–5000
ZON	5–250	25–630	5–250	5–250	25–630

15.3 Recovery, precision and intermediate precision

Method recovery, precision and intermediate precision were evaluated by recovery studies in which maize, wheat and animal feed were spiked at three concentration levels (50%, 100% and 200% of the legislative limit of mycotoxin). Six replicates were prepared for each experiment in accordance with EU guidelines.[4] The samples were spiked as listed in Table 6. Determined concentration (µg/kg), recovery, and relative standard deviation (% RSD) were calculated (Tables 7a-c). Intermediate precision was determined by spiking maize, wheat and animal feed at one level (100% legislative limit) repeated on three days with six replicates (Table 8). Recovery and %RSD values were in the range of 71.6% – 120.2% and 1% – 19%, respectively. The intermediate precisions were found to be below 19%, demonstrating method repeatability. These results conformed to the requirements of Regulation EC 401/2006.[5]

Table 6. Spiking levels for maize, wheat and animal feed at 50%, 100% and 200% of legislative limit

Mycotoxin	Maize and Wheat Spiking Levels (µg/kg)			Animal Feed Spiking Levels (µg/kg)		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
DON	250	500	1000	450	900	1800
T-2	25	50	100	25	50	100
HT-2	25	50	100	25	50	100
FB-1	100	200	400	625	1250	2500
FB-2	100	200	400	625	1250	2500
ZON	25	50	100	50	100	200

Table 7a. Average determined concentration, recovery, and relative standard deviation in maize at three different concentration levels (n=6 each level)

Mycotoxin	Maize								
	Level 1 (µg/kg)	Level 2 (µg/kg)	Level 2 (µg/kg)	Level 1 (%)	Level 2 (%)	Level 3 (%)	Level 3 (%RSD)	Level 2 (%RSD)	Level 3 (%RSD)
DON	215.7	512.6	952.8	86.3	102.5	95.3	18	10	10
T-2	24.8	50.7	103.8	99.3	101.5	103.8	8	3	1
HT-2	26.3	48.6	102.2	105.1	97.3	102.2	4	2	3
FB-1	95.4	193.1	407.6	95.4	96.6	101.9	5	5	2
FB-2	94.7	181.5	376.3	94.7	90.7	94.1	10	10	4
ZON	26.2	50.4	102.8	104.7	100.8	102.8	4	5	2

Table 7b. Average determined concentration, recovery and relative standard deviation in wheat at three different concentration levels (n=6 each level)

Mycotoxin	Wheat								
	Level 1 (µg/kg)	Level 2 (µg/kg)	Level 2 (µg/kg)	Level 1 (%)	Level 2 (%)	Level 3 (%)	Level 3 (%RSD)	Level 2 (%RSD)	Level 3 (%RSD)
DON	247.8	513.4	1087.0	99.1	102.7	108.7	19	19	5
T-2	17.8	46.9	111.6	71.2	93.8	111.6	2	19	12
HT-2	26.9	49.9	98.9	107.5	99.8	98.9	4	5	4
FB-1	82.8	167.3	335.0	82.8	83.6	83.7	7	6	3
FB-2	99.4	183.1	386.6	99.4	91.6	96.6	13	10	5
ZON	29.8	52.4	102.4	119.1	104.9	102.4	4	4	2

Table 7c. Average determined concentration, recovery and relative standard deviation in animal feed at three different concentration levels (n=6 each level)

Mycotoxin	Animal Feed								
	Level 1 (µg/kg)	Level 2 (µg/kg)	Level 2 (µg/kg)	Level 1 (%)	Level 2 (%)	Level 3 (%)	Level 3 (%RSD)	Level 2 (%RSD)	Level 3 (%RSD)
DON	518.7	1024.2	2163.8	115.3	113.8	120.2	15	19	15
T-2	19.0	47.2	95.1	76.0	94.3	95.1	13	3	4
HT-2	26.2	49.3	94.3	104.9	98.5	94.3	8	3	5
FB-1	598.0	1227.7	2456.8	95.7	98.2	98.3	7	6	3
FB-2	571.9	1200.4	2320.3	91.5	96.0	92.8	7	10	6
ZON	58.1	103.6	201.5	116.2	103.6	100.8	2	3	4

Table 8. Average intermediate precision as RSD (%) – mid level (n= 6 on each day) – one level repeated on three days

Mycotoxin	Intermediate %RSD		
	Maize	Wheat	Animal Feed
DON	19	14	19
T-2	4	12	9
HT-2	6	7	6
FB-1	5	7	6
FB-2	7	10	13
ZON	4	5	4

15.4 Accuracy

Quality control materials were analyzed for the determination of method accuracy. All results were in the satisfactory range, thus confirming the accuracy of the method (Table 9). Additionally, the successful participation in a proficiency test confirmed the accuracy of the method (Table 10).

Table 9. Results of quality control materials

QC Material	Matrix	Target Analyte Assigned Value µg/kg (Satisfactory Range)	Found µg/kg (%RSD)
FAPAS 2273	Maize	ZON - 43.7 (24.5-63)	24.9 (1)
FAPAS 2275	Maize	FB 1 - 501 (323-679)	622.7 (7)
		FB 2 - 369 (232-506)	437.2 (6)
FAPAS 2278	Wheat	ZON - 27.7 (15.5-40)	26.1 (4)
FAPAS 2268	Wheat	DON - 618 (405-830)	453.9 (4)
FAPAS 2276	Feed	ZON - 129 (73-184)	100.2 (11)
FAPAS 2258	Feed	DON - 991 (674-1309)	794.1 (11)

Table 10. Results of proficiency testing

PT Material	Matrix	Target Analyte	Assigned Value (µg/kg)	Found (µg/kg)	z-Score
FAPAS 2276	Feed	T-2	T-2 331	373.8	0.7
FAPAS 2258	Feed	HT-2	HT-2 431	458.0	0.3

15.5 Limits of detection (LOD) and quantification (LOQ)

With HRMS detection, no constant noise is detectable and the target peak disappears after a certain concentration level. Therefore, it is not possible to determine the LOD and LOQ values by evaluation of the signal-to-noise ratio as can be done with MS/MS detection. Instead, the LOQ level was defined as 2.5 x LOD as the lowest calibrant, which was possible to integrate with a standard deviation below 20%. The values for mycotoxins detected in matrix had to fulfill requirements to test for compliance with regulatory limits. The individual LOD and LOQ values of mycotoxins are listed in Table 11.

Table 11. LOD, LOQ and maximum limits for all matrices

Mycotoxin	Methanol		Maize		
	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Regulated Limit (µg/kg)
DON	30	75	50	125	500
T-2	2	4	3	8	50
HT-2	2	6	4	10	50
FB-1	12	30	18	45	200
FB-2	14	35	20	50	200
ZON	1	3	2	5	50

Mycotoxin	Wheat			Animal Feed		
	LOD (µg/kg)	LOQ (µg/kg)	Regulated Limit (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Regulated Limit (µg/kg)
DON	48	120	500	150	375	900
T-2	3	8	50	4	10	50
HT-2	4	10	50	5	12	50
FB-1	16	40	200	32	80	1250
FB-2	20	50	200	28	70	1250
ZON	2	5	50	10	25	100

16. Conclusion

A generic method for the determination of deoxynivalenol, zearalenone, T-2 toxin, HT-2 toxin and fumonisins B_1 and B_2 from complex matrices of maize, wheat and animal feed was developed. Rather than using different extraction solvents for efficient extraction of target compounds with different chemical properties, a mixture of water containing 0.1% FA/ACN (43:57) enabled satisfactory recovery (71.6%–120.1%) of all target compounds. By using automated cleanup, more than 200 samples were analyzed without any maintenance of the Transcend TLX- HRMS system. Sample cleanup with the TurboFlow column followed by HPLC analysis took 18 minutes. In comparison to disposable cleanup cartridges, the TurboFlow column was used for a minimum of 500 injections of extracted samples. Relative standard deviations and intermediate precision below 19% demonstrated good repeatability. Certified reference materials, which have been analyzed as representative samples of maize, wheat and animal feed for target compounds, and successful proficiency testing demonstrated method accuracy. The results confirm that this method can be used for routine analysis with respect to legislative limits of regulated *Fusarium* mycotoxins.

17. References

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