

Targeted Screening of Fungal and Plant Metabolites in Wheat, Corn, and Animal Feed Using Automated Online Sample Preparation Coupled to Orbitrap LC-MS

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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 min.

Extraction

3. Filter sample through 0.2 µm nylon microfilter.

Filtration

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

TLX-HRMS

Data Analysis



It is a big challenge to analyze all these toxins with a single method, as most of the compounds are not commercially available as analytical standards. The only approach that can be employed is to perform targeted screening using databases of accurate masses, aimed at searching in full scan spectra. High-resolution mass spectrometry has the capability of acquiring mass spectrometric data with very high resolving power, in case of Thermo Scientific™ Orbitrap™ mass analyzers typically >140,000 (FWHM) and with a mass accuracy of <3 ppm. This enables the separation of compounds with similar accurate masses and helps to distinguish the target compound from matrix interferences. This method is an extension of a previously validated method for the quantification of *fusarium* mycotoxins (DON, T2, HT2, FB₁, FB₂, and ZON) in corn, wheat, and animal feed.⁴ It can be applied, for targeted screening of 21 fungal and plant metabolites with automated online sample cleanup utilizing a Thermo Scientific™ Transcend™ system coupled to a Thermo Scientific™ Exactive™ high-resolution mass spectrometer. This method has been validated according to current legislation.^{5,6} Full scan data processing was performed using Thermo Scientific™ ExactFinder™

2. Introduction

Mycotoxins are secondary metabolites produced by fungal infection of agricultural crops in the field, during harvest, drying, or subsequent storage. Mycotoxins are very stable compounds that cannot be readily destroyed by heating or during food processing, although there can be reductions in levels during milling of grains, for example. Approximately 400 mycotoxins are known today, but only a few of them are regulated by legislation.¹⁻³ Besides the detection of the mycotoxins, it is also important to analyze their biosynthetic precursors, degradation products, and related masked forms, which are indicative of fungal contamination of food and feed. On the other hand, plants themselves can produce toxins as secondary metabolites, such as pyrrolizidine or ergot alkaloids.

software enabling targeted screening of toxins. The criteria for compound identification using ExactFinder software is based on detection of accurate mass at a resolving power of 100,000 (FWHM) at m/z 200 with a minimum of one fragment ion at the correct retention time with a mass deviation <5 ppm and retention time tolerance of $\pm 2.5\%$ for compound confirmation.³ As this method is intended for screening, no further optimization of peak shapes was performed for the additional 16 compounds.

3. Scope

Extracted samples of corn, wheat, and animal feed can be injected directly into an automated online clean-up system coupled to a high-resolution mass spectrometer. This method also enables rapid targeted screening for possible fungal metabolites employing data analysis with ExactFinder software.

4. Principle

This method uses Thermo Scientific™ TurboFlow™ technology for online cleanup of the sample. Finely ground and homogenous sample (5 g) is extracted for 45 min with a mixture of water 0.1% formic acid (FA)/acetonitrile (ACN) (43:57). After filtration with a 0.2 μ m nylon filter into an LC-vial, the sample is injected in the Transcend TLX-1 system, an online chromatography–reversed phase chromatography clean-up system coupled with high-resolution mass spectrometric (HRMS) detection. Data analysis is performed with ExactFinder software using a fungal metabolite database in positive and negative ionization mode. Criteria for compound confirmation and identification are defined.

5. Reagent List

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

6. Standards

- | | |
|--|----------------|
| 6.1 Aflatoxin B ₁ (AFB ₁) | Sigma-Aldrich® |
| 6.2 Aflatoxin B ₂ (AFB ₂) | Sigma-Aldrich |
| 6.3 Aflatoxin G ₁ (AFG ₁) | Sigma-Aldrich |
| 6.4 Aflatoxin G ₂ (AFG ₂) | Sigma-Aldrich |
| 6.5 Apicidin | Sigma-Aldrich |
| 6.6 Deoxynivalenol (DON) | Sigma-Aldrich |
| 6.7 Ergocornine | Römer Labs® |
| 6.8 Fumagillin | Sigma-Aldrich |
| 6.9 Fumonisin B ₁ (FB ₁) | Sigma-Aldrich |
| 6.10 Fumonisin B ₂ (FB ₂) | Sigma-Aldrich |
| 6.11 Fusarenone X | Sigma-Aldrich |
| 6.12 HT-2 toxin (HT2) | Sigma-Aldrich |
| 6.13 Malformin A | Sigma-Aldrich |
| 6.14 Monocrotaline | Römer Labs |

- | | |
|-------------------------|---------------|
| 6.15 Ochratoxin A (OTA) | Sigma-Aldrich |
| 6.16 p-Anisaldehyde | Sigma-Aldrich |
| 6.17 Retrorsine | Römer Labs |
| 6.18 Sterigmatocystin | Sigma-Aldrich |
| 6.19 T-2 toxin (T2) | Sigma-Aldrich |
| 6.20 Tenuazonic acid | Sigma-Aldrich |
| 6.21 Zearalenone (ZON) | Sigma-Aldrich |

7. Standard Preparation

Stock standard solutions of mycotoxins (100 μ g/mL) are prepared individually by dissolving in methanol. Solutions are stored at -20° C.

8. Apparatus

- 8.1 Transcend TLX 1 system
- 8.2 Exactive mass spectrometer
- 8.3 Column oven, HotDog 5090 (Prolab GmbH, Switzerland)
- 8.4 Fisher Scientific™ precision balance
- 8.5 Sartorius® analytical balance (Sartorius GmbH, Switzerland)
- 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™, 50 \times 4.6 mm, 5 μ m
- 9.2 Thermo Scientific™ TurboFlow™ Cyclone™ MCX column, 50 \times 0.5 mm
- 9.3 LC vials
- 9.4 LC vial caps
- 9.5 Thermo Scientific™ Finnpiquette™ 10–100 μ L
- 9.6 Finnpiquette 100–1000 μ L
- 9.7 Finnpiquette 500–5000 μ L
- 9.8 Pipette holder
- 9.9 Pipette Pasteur soda lime glass 150 mm
- 9.10 Pipette suction device
- 9.11 Pipette tips 0.5–250 μ L, 500/box
- 9.12 Pipette tips 1–5 mL, 75/box
- 9.13 Pipette tips 100–1000 μ L, 200/box
- 9.14 Disposable plastic syringe, 1 mL
- 9.15 Nylon filter 0.2 μ m

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

Step			Loading Pump				Cut-in loop		Eluting Pump			
Step	Start [min]	Time [s]	Flow [mL/min]	Grad	A [%]	B [%]	Tee	Loop	Flow [mL/min]	Grad	A [%]	B [%]
1. Loading	0	90	1.5	Step	100	0	====	Out	0.5	Step	99	1
2. Transferring	1:30	1	0.3	Step	85	15	T	In	0.2	Step	99	1
3. Transferring/HPLC	1:31	59	0.3	Step	85	15	T	In	0.2	Ramp	80	20
4. Washing/HPLC	2:30	360	1.5	Step	85	15	====	In	0.6	Ramp	0	100
5. Washing/HPLC	8:30	130	1.5	Step	100	0	====	In	0.6	Step	0	100
6. Washing/HPLC	10:40	160	1.5	Step	0	100	====	In	0.6	Step	0	100
7. Loop filling/equilibrating	13:20	120	1.5	Step	10	90	====	In	0.5	Step	99	1
8. Equilibrating	15:20	160	1.5	Step	100	0	====	Out	0.5	Step	99	1

11. Procedure

11.1 Chemical Preparation

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

11.2 Sample Preparation and Spiking

As no blank materials were available, a number of samples of corn, wheat, and animal feed were analyzed to test whether they could be used as blank material for spiking purposes. These samples, with trace levels (below LOD) of target mycotoxins, were used as blank materials for the method validation. Spiking was performed at two different levels (250 and 500 µg/kg) with solutions of standards.

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 and put into a 50 mL amber flask and spiked with the appropriate amount of standard. Spiked samples are stored for 30 min in the dark for equilibration of the spike. After the addition of 20 mL of extraction solvent, bottles are closed and shaken for 45 min 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

LC Conditions

TurboFlow column: Cyclone MCX, 50 × 0.5 mm

Analytical column: Hypersil GOLD, 50 × 4.6 mm, 5 µm

Total run time: 18 min

Mobile phase: A: Water (0.1% formic acid)

B: Methanol (0.1% formic acid)

The autosampler sample tray 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. Mobile phase composition in loading- and eluting- pump is A) water (0.1% FA) and B) methanol (0.1% FA). Total run time for TLX cleanup and separation on the analytical column is 18 min.

Injector Settings

Injector: CTC Analytics (CTC Analytics AG, Switzerland) with 100 µL injection syringe volume

Wash solvents for the autosampler

Wash 1: Methanol

Wash 2: 5% Methanol

Pre-clean syringe with wash 1: ×2

Clean injector (TX) with wash 1: ×2

Get sample (SEQ Tray: SEQ. Index): SEQ. Volume

Inject sample (Syringe content) to TX

Clean syringe with wash 1: ×7

Clean injector (TX) with wash 1: ×7

Clean syringe with wash 2: ×7

Clean injector (TX) with wash 2: ×7

Injection volume: 10 µL

Tray temperature: 10 °C

Column oven: 40 °C

13. Mass Spectrometric Conditions

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

Mass Spectrometer Conditions	
Ionization:	Heated electrospray (HESI II)
Polarity:	Positive/negative switching mode
Sheath gas flow rate:	60 arb
Aux gas flow rate:	20 arb
Spray Voltage:	3.60 kV
Capillary temperature:	260 °C
Capillary voltage:	60 V
Tube lens voltage:	120 V
Skimmer voltage:	25 V
Heater temperature:	250 °C
Scan mode:	Full scan
Scan range:	100–900 <i>m/z</i>
Microscans:	1
Resolution:	100,000 (FWHM) at <i>m/z</i> 200
AGC target:	1e6
Scan events:	Full scan positive mode <i>m/z</i> 100–900 Full scan negative mode <i>m/z</i> 100–900 HCD fragmentation in positive mode <i>m/z</i> 50–500 HCF fragmentation in negative mode <i>m/z</i> 50–500
Collision energy:	35 eV

14. Database

A database containing more than 600 plant and fungal metabolites and other fungal metabolites comprising their empirical formula, exact mass, polarity, fragment ions (max. 5), and retention time is maintained as an Excel® spreadsheet and converted to a comma separated values (.csv) file (Figure 1). The .csv file is uploaded to the ExactFinder as a compound database which is saved as a

.cdb file. The .cdb file is modified by addition of adduct ions of $[M+H]^+$ and $[M+Na]^+$ (adduct ions can be defined already in the .csv file as well) in positive mode and $[M-H]^+$ in negative mode. Additional adducts that can be chosen from the software are $[M+K]^+$ and $[M+NH_4]^+$. The isotopic pattern match can be defined as an additional identification or confirmation criteria. Two .cdb files are saved, one for data processing in ESI positive mode and one for data processing in ESI negative mode. The sequence is processed once with the database in negative mode and once in positive mode. The database was created based on the work of Senyuva et al.⁷, Nielsen and Smedsgaard⁸, Mol et al.⁹, Cole and Cole¹⁰, and an internal Thermo Scientific database.

14.1 Confirmation and Identification of Toxins

Compound identification criteria by processing the data with the .cdb file database are set to be the accurate mass with a mass tolerance of <5 ppm and a peak threshold of 20,000 units (defined in method development settings screening method in ExactFinder software). Identified compounds are shown as yellow flag in the software. Compound confirmation is deemed as having been achieved with the additional detection of a minimum of one fragment ion at the corresponding retention time with a time tolerance of $\pm 2.5\%$. Confirmed hits are marked with a green flag in the software. An example of data evaluation is demonstrated with T-2 toxin in Figures 2 and 3. In Figure 2, a screen shot of processed data is shown. On the upper window the targeted screening results can be found with information about compound, accurate mass (theoretical and found), mass deviation in ppm, retention time (defined and found), intensity, and fragment ions (green is found, red is not found). On the left hand side there is a list of sequence samples with additional information about compound identification. In the window below chromatogram (left) and spectrum (right) of selected compound can be seen.

Index	Compound Name	Elemental Co	Polarity	Analyte Type	Expected RT	Intensity Thresh	Adduct1	Adduct2	Adduct3	Fragment1	Fragment2	Fragment3	Fragment4	Fragment5
198	195 Dihydroxysterigmatocystin	C18H14O6	+	Parent		1.00E+03								
199	196 Methoxysterigmatocystin	C19H14O6	+	Parent		1.00E+03								
200	197 Sterigmatocystin	C18H12O6	+	Parent	10.1	1.00E+03				310.0463	281.0437			
201	198 Norsolorinic acid	C20H18O7	+	Parent		1.00E+03								
202	199 Parasiticol	C16H14O6	+	Parent		1.00E+03								
203	200 Nivalenol	C15H20O7	+	Parent		1.00E+03								
204	201 Fusarenone X	C17H22O8	+	Parent	4.16	1.00E+03				288.9214	232.9276	176.938		
205	202 Deoxynivalenol	C15H20O6	+	Parent	4.1	1.00E+03				118.9425	132.9584	249.1565	265.1215	281.183
206	203 3-Acetyldeoxynivalenol	C17H22O7	+	Parent		1.00E+03								
207	204 15-O-Acetyl-4-deoxystigmatocystin	C17H22O7	+	Parent		1.00E+03								
208	205 15-Acetoxydeoxystigmatocystin	C17H24O6	+	Parent		1.00E+03								
209	206 3a-Acetyldiacetoxystigmatocystin	C21H28O8	+	Parent		1.00E+03								
210	207 Neosolaniol	C19H26O8	+	Parent		1.00E+03								
211	208 T-2 Triol	C20H30O7	+	Parent		1.00E+03								
212	209 HT-2 Toxin	C22H32O8	+	Parent	9.2	1.00E+03				141.1694	90.9768	203.106	345.13	203.106
213	210 T-2 Toxin	C24H34O9	+	Parent	9.55	1.00E+03				199.1112	387.1399	327.1192	245.1166	
214	211 Iso-T-2 toxin	C24H34O9	+	Parent		1.00E+03								
215	212 Acetyl-T-2 toxin	C26H36O10	+	Parent		1.00E+03								
216	213 Trichodermin	C17H24O4	+	Parent		1.00E+03								
217	214 Trichodermol	C15H22O3	+	Parent		1.00E+03								
218	215 7-a-Hydroxytrichodermin	C15H22O4	+	Parent		1.00E+03								
219	216 Verrucarol	C15H22O4	+	Parent		1.00E+03								
220	217 4,15-Diacetylverrucarol	C19H26O6	+	Parent		1.00E+03								
221	218 Trichothecin	C19H24O5	+	Parent		1.00E+03								
222	219 Trichothecolone	C15H20O4	+	Parent		1.00E+03								
223	220 Isosatratoxin F	C29H34O10	+	Parent		1.00E+03								
224	221 Roridin A	C29H40O9	+	Parent		1.00E+03								

Figure 1. Database template in Excel converted to an .csv file

Overview of analyzed sequence with green, yellow, or red flags

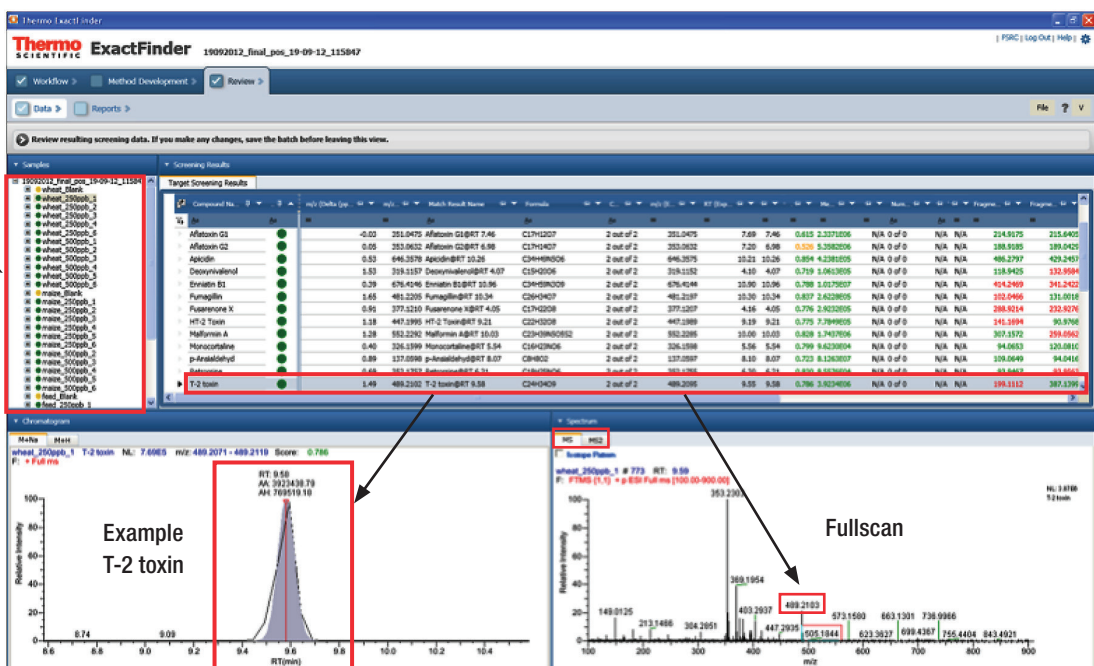
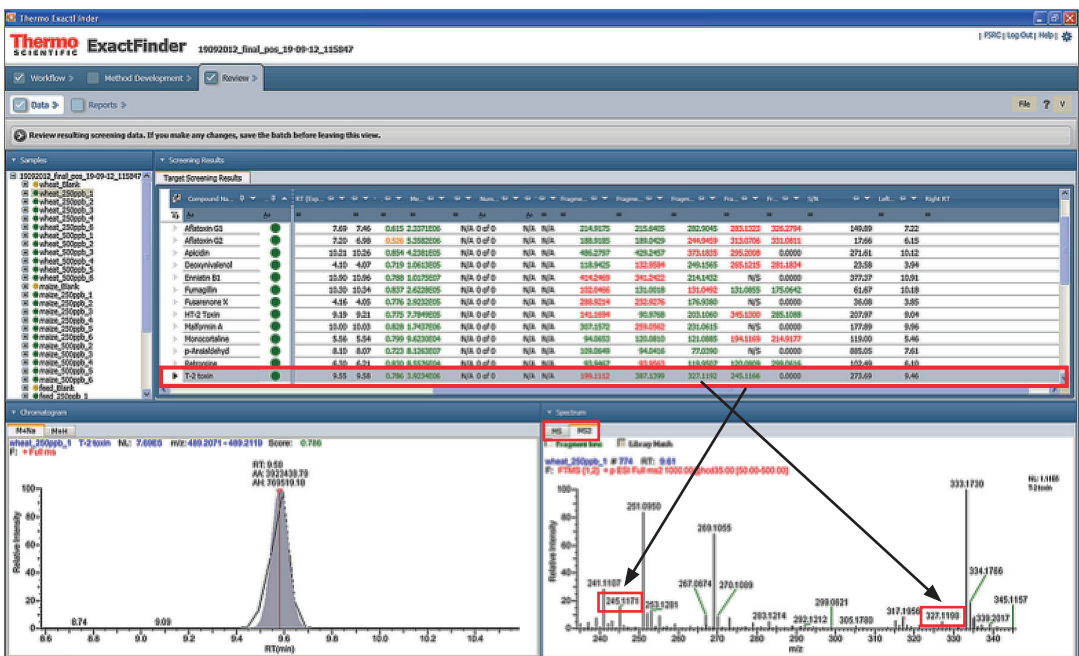


Figure 2. Accurate mass confirmation of T-2 toxin in wheat 250 µg/kg sample in ESI_{pos} mode



HCD-MS2 experiment with fragment ion confirmation

Figure 3. HCD fragment ion confirmation of T-2 toxin in wheat 250 µg/kg sample in ESI_{pos} mode

Figure 3 documents how additional information about fragment ions of T-2 toxin from the HCD experiment can be provided (bottom right).

14.2 Not Detected Compounds

All peaks that cannot be confirmed or identified by attempting to match against reference compounds in the database are marked with red flags and defined as not found.

15. Method Validation

15.1 Specificity

Method specificity is based on the detection of ions with a mass accuracy <5 ppm.² Detected ions, mass deviation from theoretical value, and fragment ions of 21 targeted fungal and plant metabolites are listed in Table 2.

15.2 Quality Control Materials

Six samples of certified reference materials have been prepared according to the section "Sample Preparation and Spiking" to determine the accuracy of compound identification and confirmation by ExactFinder software.

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

Mycotoxins	Molecular Formula	Adduct	Found Molecular Mass in Wheat [m/z] (Δ ppm)	Found Molecular Mass in Corn [m/z] (Δ ppm)	Found Molecular Mass in Feed [m/z] (Δ ppm)	RT in Wheat [min]	RT in Corn [min]	RT in Feed [min]	Fragment Ion 1 [m/z]	Fragment Ion 2 [m/z]	Fragment Ion 3 [m/z]	eV HCD
Apicidin (ESIpso)	C ₃₄ H ₄₉ N ₅ O ₆	Na ⁺	646.3576 (+0.22)	646.3585 (+1.6)	646.3581 (+0.87)	10.29	10.24	10.27	429.2457	373.1835		35
Apicidin (ESIneg)	C ₃₄ H ₄₉ N ₅ O ₆	-H ⁺	622.3618 (+1.21)	622.3615 (+0.82)	622.3619 (+1.4)	10.28	10.25	10.25	462.2748	252.1350		35
AFB ₁	C ₁₇ H ₁₂ O ₆	Na ⁺	335.0530 (+1.23)	335.0530 (+1.2)	335.0531 (+1.38)	8.31	8.18	8.25	197.0118	175.0638		35
AFB ₂	C ₁₇ H ₁₄ O ₆	Na ⁺	337.0681 (-0.33)	337.0684 (+0.35)	337.0688 (+1.53)	7.91	7.8	7.88	259.0603	314.6734		35
AFG ₁	C ₁₇ H ₁₂ O ₇	Na ⁺	351.0474 (-0.23)	351.0477 (+0.42)	351.0481 (+1.54)	7.8	7.76	7.88	215.6405			35
AFG ₂	C ₁₇ H ₁₄ O ₇	Na ⁺	353.0631 (-0.25)	353.0636 (+1.13)	353.0638 (+1.66)	7.6	7.4	7.62	188.9185	331.0811	313.0706	35
DON	C ₁₅ H ₂₀ O ₆	Na ⁺	319.1154 (+0.6)	319.1160 (+2.39)	319.1157 (+1.68)	4.32	4.06	4.15	249.1565	265.1215	281.1834	35
Ergocornine	C ₃₁ H ₃₉ N ₅ O ₅	H ⁺	562.3033 (+1.68)	562.3035 (+1.93)	562.3041 (+2.96)	7.84	7.8	7.6	266.9992	351.0471		35
Fumagillin	C ₂₆ H ₃₄ O ₇	Na ⁺	481.2204 (+1.47)	481.2205 (+1.71)	481.2204 (+1.59)	10.37	10.33	10.36	102.0466	131.0018		35
FB1	C ₃₄ H ₅₉ NO ₁₅	H ⁺	722.3973 (+2.18)	722.3973 (+2.17)	722.3980 (+3.17)	8.64	8.62	8.69	352.3198	334.0913		35
FB2	C ₃₄ H ₅₉ NO ₁₄	H ⁺	706.4020 (+1.62)	706.4025 (+2.39)	706.4030 (+3.01)	9.27	9.22	9.27	336.3253	318.3147		35
Fusarenone X	C ₁₇ H ₂₂ O ₈	Na ⁺	377.1208 (+0.33)	377.1213 (+1.65)	377.1214 (+1.95)	4.0	4.1	4.13	176.9380	232.9276	288.9214	35
HT-2	C ₂₂ H ₃₂ O ₈	Na ⁺	447.1996 (+1.46)	447.1999 (+2.13)	447.2000 (+2.44)	9.24	9.2	9.23	203.1060	285.1088		35
Malformin A (ESIpso)	C ₂₃ H ₃₉ N ₅ O ₅ S ₂	Na ⁺	552.2293 (+1.46)	552.2295 (+1.91)	552.2295 (+1.92)	10.08	10.07	10.06	307.1572	231.0615		35
Malformin A (ESIneg)	C ₂₃ H ₃₉ N ₅ O ₅ S ₂	-H ⁺	528.2324 (+0.86)	528.2324 (+0.74)	528.2326 (+1.13)	9.98	10.09	10.25	141.0658	221.1543		35
Monocrotaline	C ₁₆ H ₂₃ NO ₆	H ⁺	326.1599 (+0.19)	326.1601 (+0.85)	326.1601 (+1.0)	5.58	5.55	5.57	94.0653	120.0810	194.1169	35
OTA	C ₂₀ H ₁₈ NO ₆ Cl	Na ⁺	426.0721 (+1.34)	426.0722 (+1.66)	426.0724 (+2.23)	9.95	9.9	9.94	260.9917	239.0100		35
p-Anisaldehyde	C ₈ H ₈ O ₂	H ⁺	137.0598 (+0.74)	137.0599 (+1.12)	137.0600 (+1.9)	8.15	8.08	8.11	109.0649	94.0416	77.0390	35
Retrorsine	C ₁₆ H ₂₃ NO ₆	H ⁺	352.1756 (+0.33)	352.1758 (+0.94)	352.1760 (+1.54)	6.28	6.23	6.26	93.9467	119.9507	299.0616	35
Sterigmatocystin	C ₁₈ H ₁₂ O ₆	Na ⁺	347.0532 (+1.66)	347.0534 (+2.32)	347.0533 (+2.0)	10.13	10.11	10.1	281.0437	310.0463		35
T-2	C ₂₄ H ₃₄ O ₉	Na ⁺	489.2102 (+1.39)	489.2103 (+1.62)	489.2105 (2.06)	9.61	9.59	9.6	199.1112	387.1399	327.1192	35
Tenuazonic acid	C ₁₀ H ₁₅ NO ₃	H ⁺	198.1129 (+2.09)	198.1130 (+2.77)	198.1131 (+3.09)	8.95	8.86	8.93	124.9913	149.0448		35
ZON (ESI neg)	C ₁₈ H ₂₂ O ₅	-H ⁺	317.1395 (+0.31)	317.1395 (+0.27)	317.1397 (+0.66)	9.98	9.97	9.96	131.0490	175.0391		35

16. Results and Discussion

16.1 Compound Confirmation, Identification, and Not Detected Compounds by ExactFinder Software

Samples of corn, wheat, and animal feed were spiked with fungal metabolite standards at two concentration levels (250 and 500 µg/kg). Each level in each matrix was prepared in six replicates.

Identification of 21 targeted metabolites was sought by processing with the ExactFinder software. Compound confirmation or identification was based on previously defined criteria (see the sections "Confirmation and

Identification of Toxins" and "Not Detected Compounds"). Evaluation of % hits of confirmed, identified, and not found mycotoxins is illustrated graphically in Figure 4 and summarized in Table 3.

Evaluation of targeted screening of 21 fungal and plant metabolites shows an average confirmed/identified rate of 98% in corn, 97% in wheat, and 100% in animal feed. The overall results (Table 3) show 99% identified or confirmed with 1% of not found hits. In wheat, few not found hits (3%) have been found for OTA, fumagillin, ergocornine, DON, and FB1. This can be explained by chromatographic problems such as poor peak shape or matrix interferences.

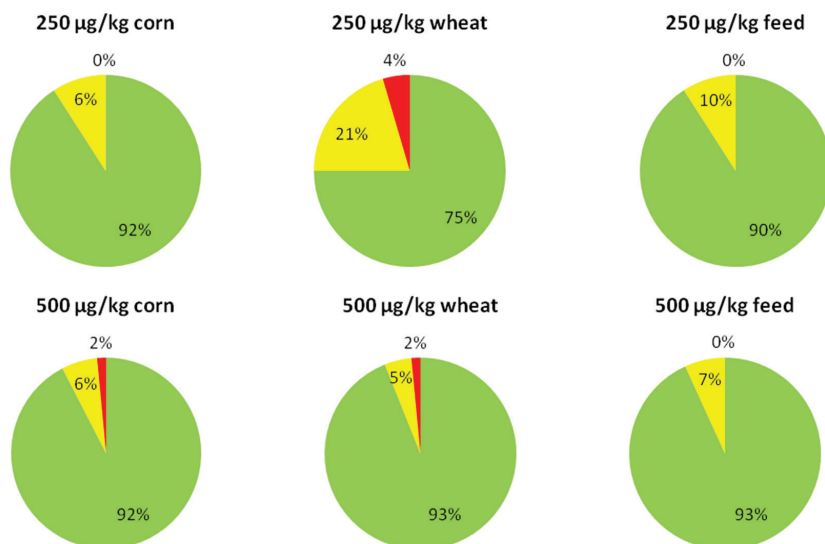


Figure 4. Graphical illustration of % hits of compound confirmation (green), identification (yellow), and not found (red) in corn, wheat, and animal feed at two concentration levels (250 and 500 µg/kg)

Table 3. Evaluation of total confirmed, identified, and not found hits by ExactFinder software

Total Number of Analyzed Samples	Confirmed	Identified	Not Found
756	673	73	10
100%	89%	10%	1%

Table 4. Results of quality control materials

QC Material	Matrix	Target Analyte (Assigned Value µg/kg)	Found
FAPAS T2280	Oat flour	T-2 (220)	Confirmed
		HT-2 (89)	Identified
FAPAS T2268	Breakfast cereal	DON (618)	Confirmed
Römer labs 3020		Ergot alkaloids (331–1349)	Ergosine, Ergocornine Ergometrine, Ergometrinine, Ergosinine, Ergotamine, Ergotaminine, α-Ergocryptine, α-Ergocryptinine
FAPAS T2273	Corn	ZON (44)	Confirmed
FAPAS T2275	Corn	FB1 (501)	Confirmed
		FB2 (369)	Confirmed
FAPAS T2276	Feed	ZON (129)	Confirmed

16.2 Analysis of Quality Control Materials

Quality control materials were analyzed for the determination of compound confirmation (green), identification (yellow), or not found (red) hits. The results are listed in Table 4. Most of the compounds have been confirmed by the software. HT-2 in sample T2280 has only been identified because of the low signal of the present fragment ion. Yellow hits in the ergot alkaloid sample can be explained by the missing information in the database about retention time and fragment ions.

17. Conclusion

This method documents a fast screening method for the detection of fungal metabolites in corn, wheat, and animal feed. Two sets of samples were prepared for each matrix at 250 and 500 µg/kg spiking level. The extracted samples were injected to the Transcend TLX-1 system for automated sample preparation clean up and analyzed with HRAM. Compound identification was based on the detection of a peak with minimum threshold of 20,000 and accurate mass with <5 ppm mass deviation. Compounds were confirmed by additional detection of minimum one fragment ion at the specific retention time. Data processing with ExactFinder software has proved to be an effective tool with 99% of compounds identified and confirmed and 1% not found. The false positive rate was 0%. This method is in compliance with the guidelines of the validation of the screening method in which a reliable method is defined to have a false-compliant rate of <5%.⁶ Additional confirmation of accurate compound confirmation and identification was given by the analysis of certified quality control materials.

18. References

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