Multi-mycotoxin Analysis in Corn-Based Feed by Quadrupole-Orbitrap LC-MS/MS

Ed George[1], Charles Yang[1], Viet Dang[1], Dwyane Schrunk[2], Laura Burns[2], Thermo Fisher Scientific[1], San Jose, California, Iowa State University Diagnostic Laboratory[2], Ames, Iowa

ABSTRACT

Purpose: To demonstrate the productivity, robustness, and accuracy of the Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer coupled with ultra-high-pressure liquid chromatography (UHPLC) for the identification and quantitation of mycotoxins in corn feed matrix.

Methods: A modified QuEChERS sample preparation procedure was used to extract 5g of homogenized sample. 2 µL was injected into a UHPLC coupled to a Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer capable of rapid polarity switching while producing full-scan and MS/MS data.

Results: Data independent (DIA), data dependent (DDA), and targeted MS2 (tMS2) acquisition workflows all showed a high degree of confidence for effective quantitation and confirmation in corn feed for the target mycotoxins. Spectral and fragment matching using highly curated libraries and compound databases were used to easily confirm results.

INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by fungi that can grow on food products and crops[1]. These toxins account for the worldwide annual loss of millions of dollars in human health, animal health, and condemned agricultural products[2]. Many regulatory agencies have set up guidelines for the maximum tolerance levels for mycotoxins in products intended for use as animal feed. Animal producers often test their feed to determine the level of mycotoxin contamination. This information is then used to determine how much to dilute the feed to safely feed their production animals. Liquid chromatography-mass spectrometry (LC-MS) has become a powerful tool for simultaneous analysis of mycotoxins due to their co-occurrence and complexity in feed.

MATERIALS AND METHODS

Sample Preparation

1. Weigh 5 g corn feed powder in 50 mL Teflon tube
2. Add 20 mL 70:30 v/v MeOH:H2O with 0.1% Formic acid
3. Shake on muti-tube vortexer for 30 minutes
4. Centrifuge 5 minutes @ 4700 rpm
5. Filter supernatant through 0.45 µm cellulose filters
6. Transfer 1 mL to A/S vial, inject 2.0µL

UHPLC Conditions

LC System: Thermo Scientific™ Vanquish™ Flex UHPLC System
Column: Thermo Scientific™ Hypersil GOLD aQ, 100 × 2.1 mm, 1.9 µm
Mobile Phase A: Water with 5 mM ammonium formate, 0.1% formic acid, 0.1% acetic acid
Mobile Phase B: Methanol with 5 mM ammonium formate, 0.1% formic acid, 0.1% acetic acid
Run Time: 13 minutes
Injection Volume: 2 µL

Time	Flow (ml/min)	% B
0.0	0.300	5.0
0.5	0.300	5.0
2.0	0.300	20
10	0.300	95
11.5	0.300	95
11.6	0.300	5
13.0	0.300	5

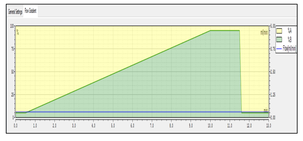

Figure 1: LC Gradient table and profile.

MATERIALS AND METHODS- cont.

HRMS Acquisition Workflows

The analyses were carried out using a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system and a Thermo Scientific™ Orbitrap Exploris™ 120 high resolution accurate mass spectrometer. Three acquisition workflows were compared in this study, as shown below in Figure 2.

Data Independent Acquisition (DIA)
- No target list
- Precursor isolation windows w/ stepped NCE
- MS2 triggered across entire peak

Data Dependent Acquisition (DDA)
- Target inclusion list with retention times
- Specific precursor isolation and NCE
- MS2 trigger on single apex scan

Targeted MS2 (tMS2 or PRM)
- Target inclusion list with retention times
- Specific precursor isolation and NCE
- All fragments are collected in a full scan high resolution mass analysis

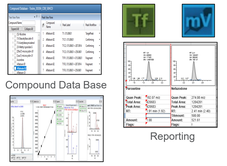

Figure 2: Three acquisition workflows set up on the Orbitrap Exploris 120. All three make use of a compound database for fragment matching and a mass spectral library for confirmation of results. TraceFinder™ software with the spectral library stored in mzVault™ was used.

Mass Spectrometer API and Scan Settings

Spray voltage	3.5 kV POS/2.5 kV NEG
Sheath gas	35 arb
Aux gas	5 arb
Sweep gas	1 arb
Capillary temp.	325 ° C
Vaporizer temp.	320 ° C
Ion polarity	POS/NEG Switching

Full scan range	100-1000 m/z
Full scan resolution	30,000
MS2 resolution	15,000
HCD collision energy:	Stepped 10,50
RF Lens	50
DIA m/z windows	5 @ 200 m/z

Data acquisition and processing

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software (version 5.1), to ensure full automation from instrument setup to raw data collection, data processing, and reporting. Data acquired from the three scan modes were analyzed with an extraction mass tolerance of 5 ppm for both precursor and product ions. Analytes were quantified based on full-scan information. In addition, confirmation of target mycotoxins was performed using MS^2 fragment matching and searches against a highly curated Thermo Scientific™ mzCloud™ mass spectral library. The target compounds are listed below in Table 1.

	Compound	Formula	Adduct	m/z	z	RT Time (min)
1	Aflatoxin B1	C17H12O6	+H	313.0707	1	7.07
2	Aflatoxin B2	C17H14O6	+H	315.0863	1	6.83
3	Aflatoxin G1	C17H12O7	+H	329.0656	1	6.53
4	Aflatoxin G2	C17H14O7	+H	331.0812	1	6.27
5	3-Acetyl DON	C17H22O7	+H	339.1438	1	5.5
6	Fumonisin B1	C34H59NO15	+H	722.3957	1	8.29
7	Fumonisin B2	C34H59NO14	+H	706.4008	1	9.17
8	Fumonisin B3	C34H59NO14	+H	706.4008	1	8.78
9	Ochratoxin A_M+H	C20H18ClNO6	+H	404.0895	1	8.86
10	T-2_M+NH4	C24H34O9	+NH4	484.2541	1	8.39
11	HT-2_M+NH4	C22H32O8	+NH4	442.2435	1	7.84
12	Zearalenone_M+H	C18H22O5	+H	319.154	1	8.95
13	Nivalenol_M+H	C15H20O7	+H	313.1282	1	2.63
14	Nivalenol_M+Na	C15H20O7	+Na	335.1101	1	2.63
15	DON_M+H	C15H20O6	+H	297.1333	1	3.59
16	Alpha-zearalenol_M-H	C18H24O5	-H	319.1551	1	8.86
17	HT-2_M+H	C22H32O8	+H	425.217	1	7.84
18	Zearalenone_M-H	C18H22O5	-H	317.1394	1	8.95

Table 1: Target compounds analyzed in corn feed matrix on the Exploris 120 MS.

RESULTS

Chromatographic separation of the 15 mycotoxins was optimized to achieve good peak symmetry, especially for early-eluting compounds (e.g., 3-acetyldeoxynivalenol and deoxynivalenol at retention times 2.65 min and 3.58 min, respectively). Fumonisins, which are structurally similar to sphinganine containing the backbone precursor of sphingolipids, are less polar and hence elute later (retention time between 8-9 min) on the reversed-phased column. Good separation was obtained for all 15 mycotoxins on the analytical column (Figure 3).

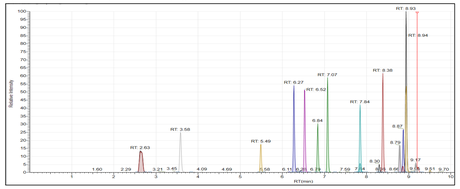

Figure 3: Chromatogram of 15 mycotoxins in spiked corn feed at 10 ppb, for Aflatoxin and Ochratoxin, 40 ppb for Fumonisin and T2, 200 ppb for Zearalenone, HT2, and 3-acetyldeoxynavinelenol, and 400 ppb for nivalenol and α-Zearalenol.

Using the three acquisition modes described previously, the linearity and spike recovery for the targeted compounds were compared (Table 2). Among the 15 mycotoxins studied, HT2 and T2 showed the highest signal intensities for the ammonium adduct. The majority of mycotoxins were easily ionized in positive ionization mode; however, α-Zearalenol and Zearalenone required use of negative ionization mode. The results showed comparable calibration linearity ($r^2 > 0.995$) and good spike recovery (80 – 120%) for all 15 mycotoxins in all three acquisition modes.

		DIA			DDA			tMS2		
Compound	Adduct	r2	Recovery	% RSD	r2	Recovery	% RSD	r2	Recovery	% RSD
3-acetyl-deoxynivalenol	M+H	0.997	104.6 ± 4.0	11.7	0.997	106.5 ± 2.8	7.4	0.998	103.9 ± 1.3	10.2
Aflatoxin B1	M+H	0.998	90.9 ± 1.5	3.7	0.999	92.2 ± 0.9	3.3	0.998	92.1 ± 0.5	3.1
Aflatoxin B2	M+H	0.998	96.1 ± 2.4	2.9	0.999	96.0 ± 0.6	4.8	0.999	95.4 ± 1.3	3.9
Aflatoxin G1	M+H	0.998	95.2 ± 1.4	4.7	0.999	96.3 ± 0.9	2.1	0.998	95.1 ± 0.5	2.9
Aflatoxin G2	M+H	0.998	99.0 ± 1.8	4.8	0.999	100.7 ± 1.1	3.4	0.998	99.2 ± 0.9	4
α-Zearalenol	M-H	0.999	98.9 ± 1.3	11.3	0.999	101.8 ± 1.0	9.4	0.998	96.8 ± 1.5	9.1
Deoxynivalenol	M+H	0.996	98.2 ± 2.2	7.6	0.997	104.0 ± 1.0	7.9	0.993	99.5 ± 1.0	10.7
Fumonisin B1	M+H	0.998	112.1 ± 2.6	9.9	0.998	113.1 ± 1.2	4.8	0.998	108.5 ± 3.7	2.6
Fumonisin B2	M+H	0.997	116.3 ± 4.8	9.2	0.998	119.2 ± 3.9	7.1	0.998	112.6 ± 1.2	3.2
Fumonisin B3	M+H	0.999	107.8 ± 2.5	7.1	0.999	110.0 ± 1.4	9.5	0.997	107.2 ± 2.0	8
HT2-Toxin	M+NH4	0.997	114.8 ± 3.2	6	0.998	111.5 ± 1.0	9.7	0.997	109.6 ± 0.9	6.5
Nivalenol	M+H	0.999	98.8 ± 1.1	9.6	0.999	98.6 ± 0.9	9.2	0.996	97.5 ± 2.5	9
Ochratoxin A	M+H	0.997	88.6 ± 1.7	8.1	0.997	92.6 ± 2.5	7.7	0.999	90.8 ± 1.9	3.3
T2-Toxin	M+NH4	0.998	111.9 ± 2.3	4.6	0.999	115.3 ± 1.1	2.5	0.997	111.4 ± 0.8	3.1
Zearalenone	M-H	0.998	96.2 ± 0.4	2.2	0.999	99.6 ± 0.4	9.4	0.997	95.3 ± 1.5	8.6

Table 2. Polarity, coefficient of determination for linear regression curves, and recovery (mean ± SD%) for mycotoxins spiked into control corn feed using three scan modes.

Mass accuracy and quantitative results during polarity switching

The high field Thermo Scientific™ Orbitrap™ mass analyzer coupled with the fast polarity switching of the Thermo Scientific™ OptaMax™ NG ion source produced excellent mass stability and quantitative results during polarity switching. Figure 4 displays the exact mass measurements across every scan in the peak for Zearalenone in both polarities. This compound had good response in both ionization modes and excellent mass accuracy and stability maintained across the scans within the peaks. In addition, the MS^2 fragments had < 5 ppm mass accuracy in both polarities. In all three scan modes, quantitation of the mycotoxins in the corn feed matrix was excellent.

RESULTS- cont.

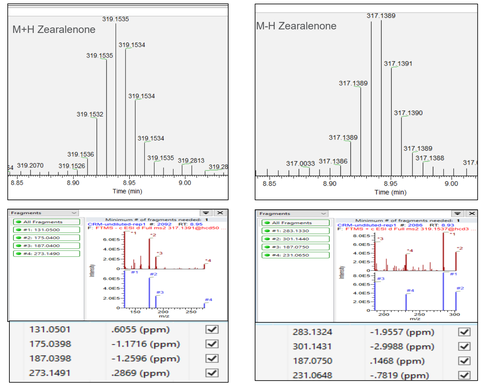

Figure 4. (Top) Exact mass measurements across the peak for Zearalenone during positive and negative switching. The Orbitrap Exploris 120 maintains excellent mass stability and accuracy. MS2 fragment match (Bottom) for each polarity with mass accuracy < 5 ppm.

Excellent sensitivity and linearity (2 – 100 ng/g) were also achieved in each of three different scan modes for Aflatoxin B1 (Figure 5).

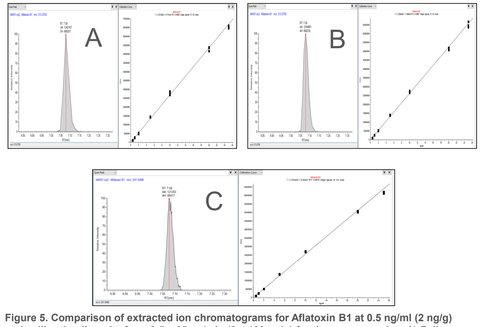

Figure 5. Comparison of extracted ion chromatograms for Aflatoxin B1 at 0.5 ng/ml (2 ng/g) and calibration linearity from 0.5 – 25 ng/mL (2 – 100 ng/g) for three scan modes: A) Full scan used for quantitation in the DIA experiment, B) Full scan used for quantitation in DDA experiment, and C) Primary MS^2 transitions used for quantitation in MS^2 experiment

Quantitation using the three scan modes was also evaluated using a certified reference material (CRM). The measured concentrations for mycotoxins present in the CRM were not significantly different between the three scan modes and were comparable (percent difference < 20%) to the values specified in the CRM (Table 3). The mean measured concentration of Ochratoxin A was > 20% more than its nominal concentration, but there was no statistical difference between the measured and nominal concentrations.

RESULTS- cont.

	Nominal Conc. (ng/g)	Measured concentration (ng/g) DIA	DDA	tMS2
Aflatoxin B1	12.1 ± 2.6	12.3 ± 2.9	14.1 ± 2.8	13.3 ± 3.8
DON	2200 ± 200	2258.2 ± 241.7	2333.7 ± 213.7	2156.3 ± 144.8
Total fumonisins	10200 ± 1000	-	-	-
HT-2	199.9 ± 21.9	230.7 ± 18.0	219.8 ± 24.1	236.5 ± 22.3
Ochratoxin A	9.9 ± 1.9	12.9 ± 1.6	13.0 ± 1.3	12.9 ± 0.6
T-2	254.0 ± 18.6	273.0 ± 32.2	291.3 ± 20.5	277.3 ± 20.1
Zearalenone	347.4 ± 23.6	351.4 ± 15.5	367.4 ± 16.9	336 ± 38.3

Table 3. Nominal and measured concentrations (mean ± SD, ng/g) of mycotoxins in certified reference material using three different scan modes. Results for total fumonisins were excluded due to very high concentrations in the samples, which were outside the calibration ranges.

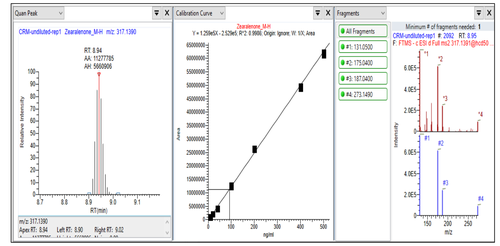

Figure 3. Quantitative result for Zearalenone in negative ESI taken from the data dependent acquisition (DDA) experiment. Plenty of scans across the peak (with polarity switching) and excellent calibration linearity with fragments matching and spectral library search result.

CONCLUSIONS

- The Oribtrap Exploris™ 120 mass spectrometer provided high quality data in all three scan operation modes for both screening and quantitation of mycotoxins in corn feed.
- Method is fit-for-purpose as demonstrated by excellent accuracy and recovery, with polarity switching and confirmation against a highly curated mass spectral library.
- Quantitation accuracy and reproducibility were shown to be excellent from the analysis of incurred residues in a CRM sample.

REFERENCES

1. Bennett, J.W.; Klich, M. Mycotoxins. Clin. Microbiol. Rev. 2003, 16, 497-516.
2. Zain, M.E. Impact of mycotoxins on human and animals. J. Saudi. Chem. Soc. 2011, 15, 129-144.

TRADEMARKS