Detection of Mycotoxins in Corn Meal Extract Using Automated Online Sample Preparation with Liquid Chromatography-Tandem Mass Spectrometry

Yang Shi, Catherine Lafontaine, Timothy B. Haney, and François A. Espourteille
Thermo Fisher Scientific, Franklin, MA, USA

Thermo SCIENTIFIC

Overview

Purpose: To develop a rapid and sensitive automated online sample preparation LC-MS/MS method to detect and quantify multiple mycotoxins in corn meal extract and also to shorten assay time and increase throughput.

Methods: Automated online sample preparation using Thermo Scientific TurboFlow technology coupled with the Thermo Scientific TSQ Vantage triple stage quadrupole mass spectrometer.

Results: Two quick, automated online sample preparation LC-MS/MS methods have been developed that are sensitive enough to detect mycotoxins in corn meal extract.

Introduction

Since the discovery of aflatoxin in 1960, mycotoxins research has received considerable attention. Mycotoxins are a group of naturally occurring toxic substances produced by certain molds, which can contaminate food and feed. The inhalation or absorption of mycotoxins into the body may cause sickness, such as kidney, liver damage, and cancers, or even death in man or animals.[1] From a food safety perspective, the aflatoxins, ochratoxin A, patulin, fumonisins, trichothecenes and zearalenone are the mycotoxins of major concern. Many countries now monitor mycotoxin levels in food products. Liquid chromatography-mass spectrometry (LC/MS) is currently a common analytical approach for the quantification of mycotoxin contamination.[2] Sample preparation for LC-MS/MS analysis can be time- and labor-intensive, often involving pH modification, solid phase extraction, multi-step extract clean-up, and pre-concentration.[3] The strict regulation published by the European Union (EU) in 1999 asking for lower detection limits and higher method reliability presented a new analytical challenge.[4]

In this study we describe an easy, comprehensive, LC-MS/MS method using a Thermo Scientific Transcend TLX-1 system powered by TurboFlow™ technology to analyze multiple mycotoxin residues in corn meal extract. Figure 1 illustrates a typical Transcend™ TLX-1 system with the TSQ Vantage™ triple stage quadrupole mass spectrometer.

FIGURE 1. Typical Layout of a Transcend TLX-1 system with a TSQ Vantage triple stage quadrupole mass spectrometer

Methods

The matrix standard curve

Five grams of corn meal purchased from a local grocery store was extracted using 25 mL of 70 % methanol in water followed by 60 minutes of ultra-sonication. The extract sat overnight at room temperature. The resulting solution was then centrifuged at 6000 RPM for 20 minutes. The supernatant was used to prepare the matrix calibrators and QC samples. Each milliliter of supernatant corresponds to 0.2 g solid corn meal powder as the unit of conversion.

The analyte stock mix solutions were prepared in methanol. Table 1 lists selected reaction monitoring (SRM) transitions and stock concentrations for individual analytes. The remaining three compounds, deoxynivalenol (DON), nivalenol (NIV) and 3-acetyl-DON (3-AcDON) were analyzed under negative electrospray ionization (ESI) mode.

LC/MS Methods using positive ESI mode (Method A):

TurboFlow Method Parameters

Column: TurboFlow Cyclone-P column 0.5 x 50 mm
Injection Volume: 10 µL
Solvent A: 10 mM ammonium acetate in water
Solvent B: 0.1% formic acid in acetonitrile (ACN)
Solvent C: 1:1:1 ACN: isopropanol: acetone (v:v:v) with 0.3% formic acid

HPLC Method Parameters

Analytical Column: Thermo Scientific Hypersil GOLD column 2.1 x 100 mm, 1.9µm
Solvent A: 0.1% formic acid in water
Solvent B: 0.1% formic acid in ACN

Mass Spectrometer Parameters

MS: TSQ Vantage triple stage quadrupole mass spectrometer
Ionization Source: heated electrospray ionization (H-ESI)
Spray Voltage: 5KV
Sheath Gas (N2): 50 arbitrary units
Auxiliary Gas (N2): 20 arbitrary units
Vaporizer Temp: 209 °C
Capillary Temp: 270 °C
Collision Gas: 1.5mTorr

LC/MS Methods using negative ESI mode (Method B):

TurboFlow Method Parameter

Column: research column A 0.5 x 50 mm
Injection Volume: 10 µL
Solvent A: water
Solvent B: methanol
Solvent C: 0.1 % ammonium hydroxide
Solvent C: 45:45:10 ACN: isopropanol: acetone (v:v:v)

HPLC Method Parameters

Analytical Column: Hypersil GOLD™ column 2.1 x 50 mm, 1.9µm
Solvent A: 0.1% formic acid in water
Solvent B: 0.1% formic acid in ACN

Mass Spectrometer Parameters

MS: TSQ Vantage triple stage quadrupole mass spectrometer
Ionization Source: H-ESI
Spray Voltage: 4.5KV
Sheath Gas (N2): 50 arbitrary units
Auxiliary Gas (N2): 20 arbitrary units
Vaporizer Temp: 250 °C
Capillary Temp: 270 °C
Collision Gas: 1.5mTorr

TABLE 1. The list of analytes and their MS transitions

Compounds	Parent ion (m/z)	Primary product ion (m/z)	Secondary product ion (m/z)
Aflatoxins B1	313	241	285
Aflatoxins B2	315	259	287
Aflatoxins G1	329	243	283
Aflatoxins G2	331	245	275
Zearalenone (ZEA)	319	187	185
Ochratoxin A (OTA)	404	239	221
Fumonisins B1 (FB1)	722	334	352
Fumonisins B2 (FB2)	706	336	318
Deoxynivalenol (DON)	295	138	265
Nivalenol (NIV)	311	281	205
3-Acetyl-DON (3-AcDON)	337	307	173

Red: Negative ESI mode

The LC method schematic views from Thermo Scientific Aria OS software are shown in Figures 2 and 3.

FIGURE 2. Method A view in Aria OS software

Step	Start	Sec	Flow	Grad	%A	%B	%C	%D	Tee	Loop	Flow	Grad	%A	%B
1	0.00	45	2.00	Step	100.0	-	-	-	====	out	0.30	Step	98.0	2.0
2	0.75	5	0.10	Step	100.0	-	-	-	====	out	0.30	Step	98.0	2.0
3	0.83	120	0.10	Step	100.0	-	-	-	T	in	0.30	Step	98.0	2.0
4	2.83	5	2.00	Step	100.0	-	-	-	====	out	0.30	Step	98.0	2.0
5	2.92	90	2.00	Step	-	-	100.0	-	====	out	0.30	Ramp	60.0	40.0
6	4.42	220	2.00	Step	-	100.0	-	-	====	out	0.30	Ramp	30.0	70.0
7	8.08	220	2.00	Step	-	-	100.0	-	====	out	0.30	Ramp	2.0	98.0
8	11.75	45	2.00	Step	-	100.0	-	-	====	in	0.30	Step	2.0	98.0
9	12.50	180	2.00	Step	100.0	-	-	-	====	out	0.30	Step	98.0	2.0

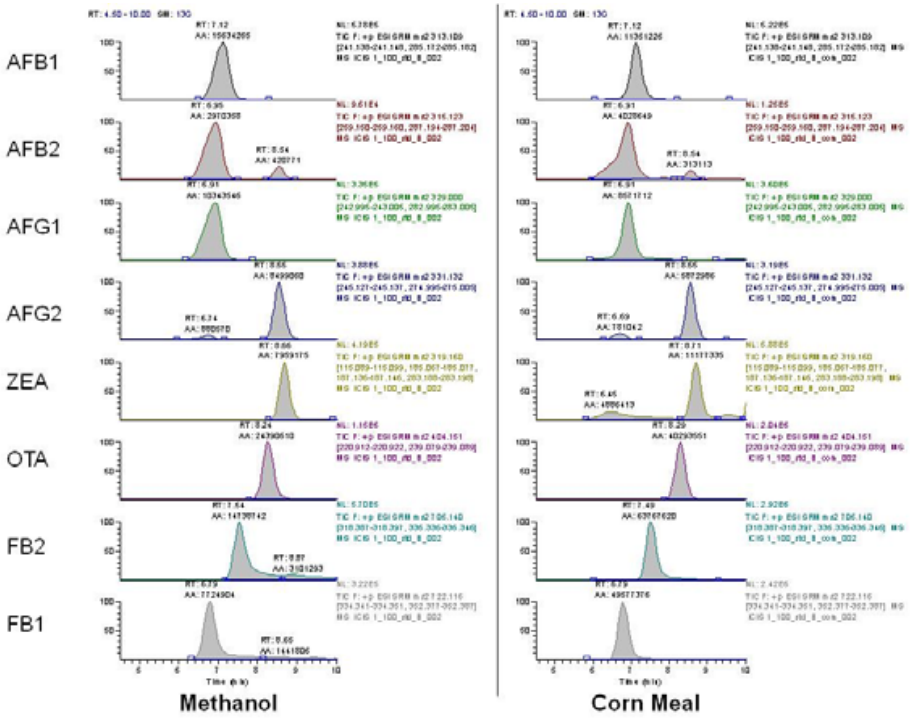
FIGURE 3. Method B view in Aria OS software

Step	Start	Sec	Flow	Grad	%A	%B	%C	%D	Tee	Loop	Flow	Grad	%A	%B
1	00:00	45	1.50	Step	100.0	-	-	-	====	out	0.30	Step	98.0	2.0
2	00:45	60	0.07	Step	100.0	-	-	-	T	in	0.50	Step	98.0	2.0
3	01:45	45	1.50	Step	-	100.0	-	-	====	out	0.30	Ramp	20.0	80.0
4	02:30	45	1.50	Step	-	100.0	-	-	====	out	0.30	Ramp	10.0	90.0
5	03:15	45	1.50	Step	-	50.0	50.0	-	====	out	0.30	Ramp	2.0	98.0
6	04:00	30	1.50	Step	-	-	-	100.0	====	out	0.30	Step	2.0	98.0
7	04:30	15	1.50	Step	-	100.0	-	-	====	out	0.30	Step	2.0	98.0
8	04:45	30	1.50	Step	-	5.0	95.0	-	====	in	0.30	Step	98.0	2.0
9	05:15	150	1.50	Step	100.0	-	-	-	====	out	0.30	Step	98.0	2.0

Results and Discussion

Figure 4 shows the comparison of chromatograms of 8 positive analytes at 1:100 dilutions in methanol and corn meal extract, indicating excellent chromatographic separation in both solvent standard and matrix. Matrix-matched calibration standards showed linear response of two orders of magnitude (r2 > 0.99) for 6 of them (Table 2). Significant signal enhancement was observed for FB1 and FB2 due to matrix-induced ionization variability, which was previously reported by other researchers.[5] In future work, the isotope-labeled internal standard might be used to compensate for the matrix interference.

FIGURE 4. The comparison of chromatograms of 8 positive analytes in methanol and corn flour extract (1:100 dilution of stock mixture)



Because DON, NIV and 3-AcDON have a better signal response under negative ionization mode, a separate LC-MS/MS method was developed. Figure 5 shows the chromatograms of DON, NIV and 3-AcDON identified at 100 ng/mL fortified in the corn meal extract. Figure 6 presents the linear fit calibration curves for DON and NIV, indicating excellent linear fits over the dynamic range. Table 3 summarizes detection, quantitation limits and standard curve linearity for 3 analytes analyzed in negative ion mode. For all analytes, the quantitation limits obtained using the present methodology exceed the EU's maximum level standards.[6] To the best of our knowledge, this is the first application of its type to detect these 3 compounds using an online sample clean-up technique coupled to tandem mass spectrometry.

FIGURE 5. Selected chromatograms of DON, NIV and 3-AcDON detected at 100 ng/mL fortified in the corn meal extract

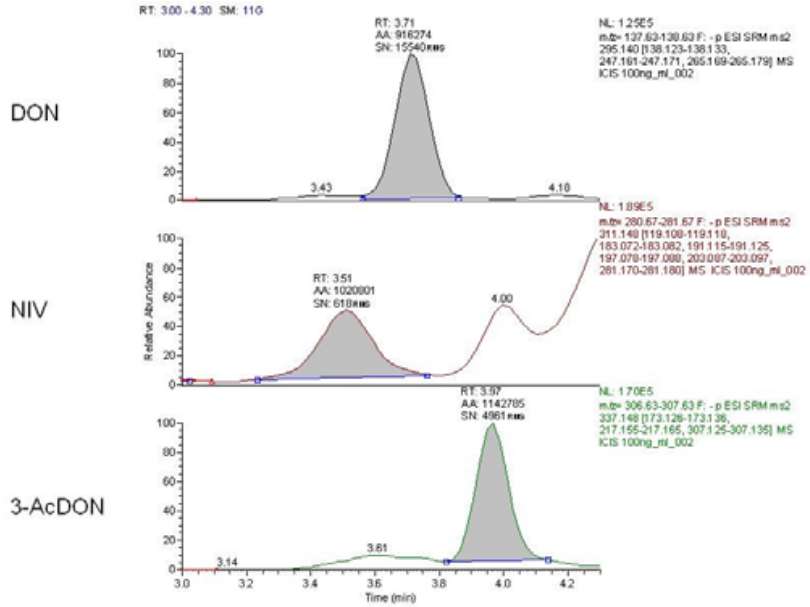


FIGURE 6. Calibration curves for DON and NIV

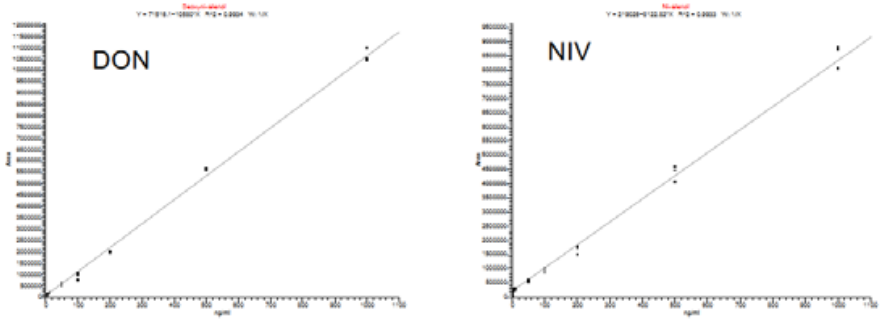


TABLE 2. Limit of detection (LOD), limit of quantitation (LOQ) and standard curve linearity for analytes detected in positive ion mode

Compounds	LOD (ng/g)	LOQ (ng/g)	r^2
B1	0.25	0.50	0.9956
G1	0.25	0.50	0.9910
OTA	5.00	5.00	0.9937
ZEA	50.00	50.00	0.9955
FB1	12.50	12.50	0.9984
FB2	12.50	12.50	0.9965

TABLE 3. LOD, LOQ and standard curve linearity for analytes detected in negative ion mode

Compounds	LOD (ng/g)	LLOQ (ng/g)	r^2
Deoxynivalenol (DON)	5.00	25.00	0.9934
Nivalenol (NIV)	25.00	25.00	0.9933
3-Acetyl-DON (3-AcDON)	25.00	25.00	0.9925

Conclusion

Developing a rapid and sensitive quantitative method is always a major goal for mycotoxins analysis.[7] Two quick, automated online sample preparation LC-MS/MS methods have been developed that are sensitive enough to detect mycotoxins in corn meal extract. By eliminating manual sample preparation, the reliability of this methodology was improved significantly. The sample throughput could be improved by multiplexing the two methods on different LC channels using a Transcend TLX-2 (or TLX-4) system. Future work will focus on the application of this methodology on various food matrices and references.

References

1. Pitt, J.I. What are mycotoxins? Australian Mycotoxin Newsletter. 1996, 7(4), 1.
2. Spanjer, M.C., Rensen, P.M., Scholten, J.M. LC-MS/MS multi-method for mycotoxins after single extraction, with validation data for peanut, pistachio, wheat, maize, cornflakes, raisins and figs. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 2008, 25, 472-89.
3. Shephard, G.S., Determination of mycotixins in human foods, Chem. Soc. Rev., 2008, 37, 2468-77.
4. DS/CEN/CR 13505, Food analysis-Biotoxins –Criteria of analytical methods of mycotoxins, European Committee for Standardization, May 20, 1999.
5. Li, W., Herrman, T.J., Dai, S. Y., Rapid Determination of Fumonisins in Corn-Based Products byLiquid Chromatography/Tandem Mass Spectrometry, J. AOAC Int., 2010, 93, 1472-81.
6. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R1881 :20100701:EN:PDF. Accessed on Mar. 15, 2011
7. Rahmani, A., Jinap, S., Soleimany, F., Quantitative and qualitative analysis of mycotoxins, Compr. Rev. Food Sci. Food Safety, 2009, 8, 202-251

www.thermoscientific.com

Thermo Fisher Scientific, San Jose, CA USA is ISO Certified.

Africa-Other +27 11 570 1840
Australia +61 3 9757 4300
Austria +43 1 333 50 34 0
Belgium +32 53 73 42 41
Canada +1 800 530 8447
China +86 10 8419 3588
Denmark +45 70 23 62 60
Europe-Other +43 1 333 50 34 0
Finland/Norway/Sweden +46 8 556 468 00
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9434
Italy +39 02 950 591
Japan +81 45 453 9100
Latin America +1 561 688 8700
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Russia/CIS +43 1 333 50 34 0
South Africa +27 11 570 1840
Spain +34 914 845 965
Switzerland +41 61 716 77 00
UK +44 1442 233555
USA +1 800 532 4752

PN63610_E 06/12S

Thermo SCIENTIFIC

Part of Thermo Fisher Scientific