Application Note: 523

Detection of Mycotoxins in Corn Meal Extract Using Automated Online Sample Preparation with LC-MS/MS

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Key Words

- Transcend TLX-1
- TurboFlow Technology
- TSQ Vantage
- Food Safety

Introduction

Since the discovery of aflatoxin in 1960, mycotoxin 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 harm, including kidney or liver damage, cancer, 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 and feed products. Liquid chromatography-tandem mass spectrometry (LC-MS/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 or immunoaffinity column clean-up extraction, multi-step extract clean-up, and pre-concentration.[3] The strict regulation published by the European Union 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 Thermo Scientific TurboFlow technology to analyze multiple mycotoxin residues in corn meal extract. Figure 1 illustrates a typical Transcend™ TLX-1 system with the Thermo Scientific TSQ Vantage triple stage quadrupole mass spectrometer.

Goal

Develop a rapid and sensitive automated, online sample preparation LC-MS/MS method to detect and quantify multiple mycotoxins in corn meal extract resulting in a shorter assay time and increased throughput.

Experimental

The matrix standard curve

Five grams of corn meal purchased from a local grocery store were 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.

Figure 1. Thermo Scientific Transcend TLX system with TSQ Vantage triple quadrupole mass spectrometer

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

Table 1. Analytes list

Compounds	Parent *(m/z)*	Primary *(m/z)*	Secondary *(m/z)*	Stock concentration (µg/mL)
Aflatoxins B1	313	241	285	0.050
Aflatoxins B2	315	259	287	0.015
Aflatoxins G1	329	243	283	0.050
Aflatoxins G2	331	245	275	0.015
Zearalenone (ZEA)	319	187	185	10.000
Ochratoxin A (OTA)	404	239	221	1.000
Fumonisins B1 (FB1)	722	334	352	2.500
Fumonisins B2 (FB2)	706	336	318	2.500
Deoxynivalenol (DON)	295	138	265	20.000
Nivalenol (NIV)	311	281	205	20.000
3-Acetyl-DON (3-AcDON)	337	307	173	20.000

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

TurboFlow™ Method Parameters

Column:	TurboFlow Cyclone-P 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 2.1 x 100 mm, 1.9 µm
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
MS Ionization Source:	Heated Electrospray Ionization (H-ESI)
Spray Voltage:	5 KV
Sheath Gas Pressure (N_2):	50 arbitrary units
Auxiliary Gas Pressure (N_2):	20 arbitrary units
Vaporizer Temperature:	209 °C
Capillary Temperature:	270 °C
Collision Gas Pressure:	1.5 mTorr

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)

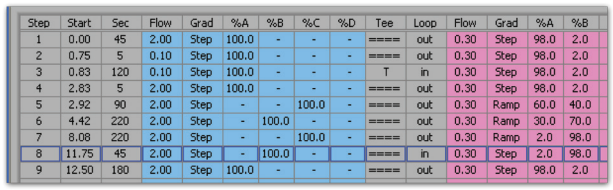
HPLC Method Parameters

Analytical Column:	Hypersil GOLD™ 2.1 x 50 mm, 1.9 µm
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
MS Ionization Source:	H-ESI
Spray Voltage:	4.5 kV
Sheath Gas Pressure (N_2):	50 arbitrary units
Auxiliary Gas Pressure (N_2):	20 arbitrary units
Vaporizer Temperature:	250 °C
Capillary Temperature:	270 °C
Collision Gas Pressure:	1.5 mTorr

The LC method views from Thermo Scientific Aria Operating Software are shown in Figures 2 and 3.



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 2. Method A 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

Figure 3. Method B view in Aria OS software

Results and Discussion

Figure 4 shows the comparison of chromatograms of eight 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 ($r^2 > 0.99$) for six 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.

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 three analytes analyzed in negative ion mode. For all analytes, the quantitation limits obtained using the present methodology comply with the maximum levels in foods defined by European Union.[6] To the best of our knowledge, this is the first application of its type to detect these three compounds using an automated online sample preparation technique coupled to tandem mass spectrometry.

In addition, a lower limit of quantitation (LOQ) could be achieved by increasing sample injection volume since TurboFlow columns can handle larger injections (up to a few hundred microliters) while regular HPLC or UHPLC columns can not.

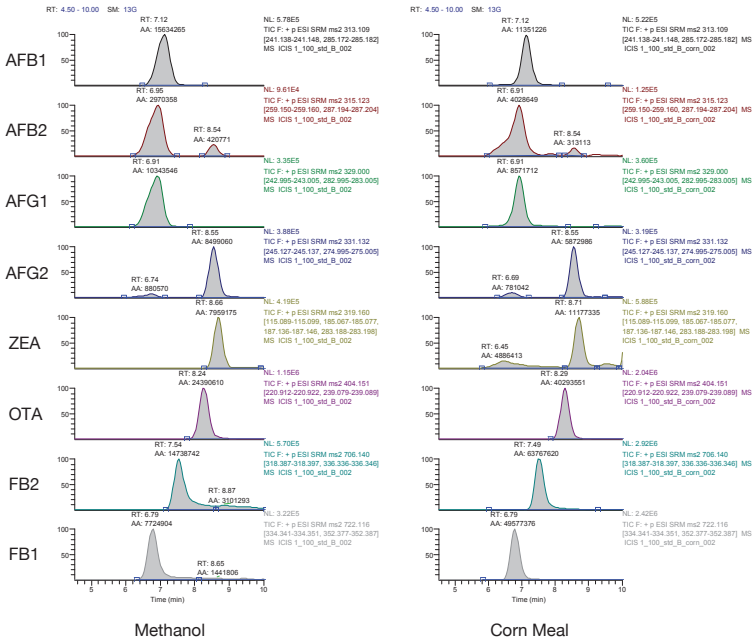


Figure 4. Comparison of chromatograms of 8 SRM analytes in methanol and corn flour extract (1:100 dilution of stock mixture)

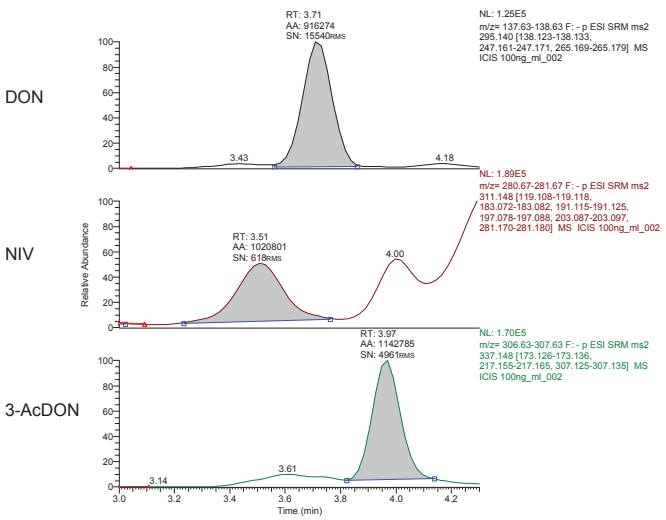


Figure 5 Selected chromatograms of DON, NIV, and 3-AcDON detected at 100 ng/mL fortified in the corn meal extract

Table 2. Limit quantitation (LOQ) and standard curve linearity (r^2) for analytes detected in positive ion mode

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

Table 3. LOQ and standard curve linearity for analytes detected in negative ion mode

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

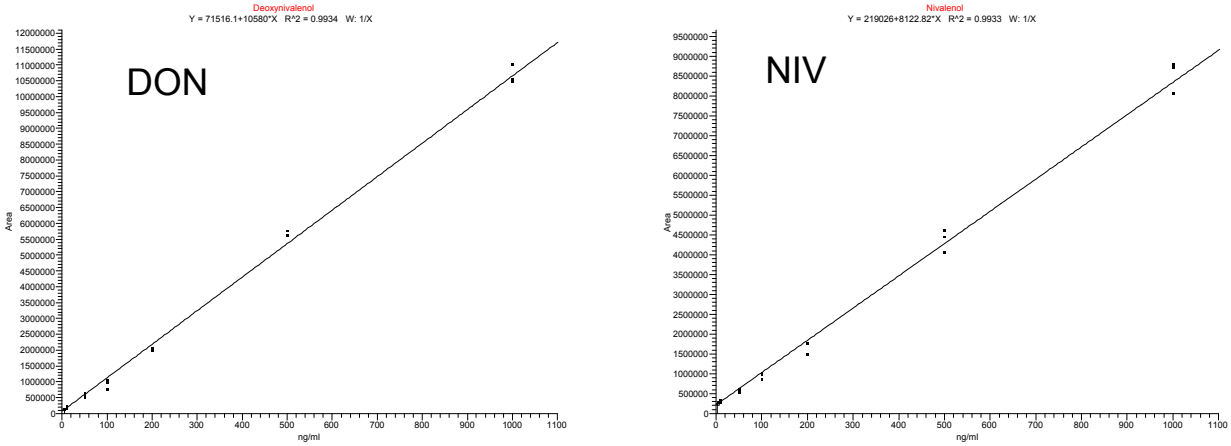


Figure 6. Calibration curves for DON and NIV

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

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