Application Note 00394

Multi Component Mycotoxin Analysis using LC/MS/MS

Mahalakshmi Rudrabhatla, Ph.D., Varian, Inc.

Introduction

Mycotoxins are secondary metabolites of molds, contaminating a wide range of crop plants and fruits before or after harvest, the most important mycotoxins being: aflatoxins, type A trichothecenes, type B trichothecenes, ochratoxin A, zearalenone, fumonisins and patulin.

The structures of the mycotoxins are given in figure 1.

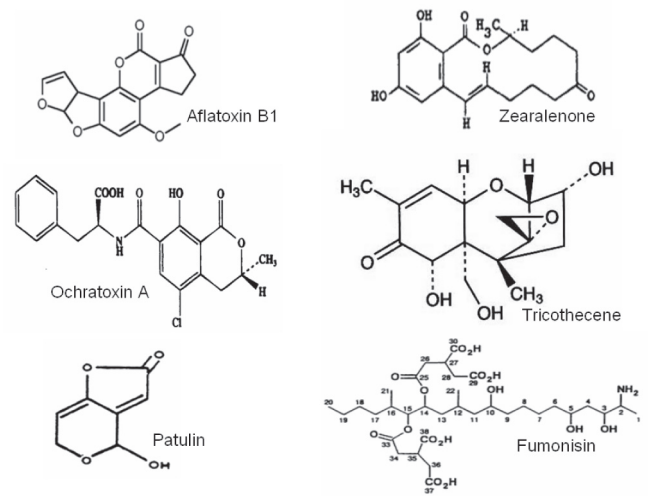

Fig 1 Structures of most relevant types of mycotoxins

Ingestion of food contaminated with mycotoxins was found to induce a variety of toxic responses including kidney damage, liver damage, birth defects and cancer. Mycotoxin contamination is recognized as an unavoidable risk because the formation of fungal toxins is weather dependent and effective prevention is difficult.

According to the Food and Agricultural Organization (FAO) more than 25% of the world's agricultural production is contaminated with mycotoxins. This equates to economic losses estimated at $923 million annually in the US grain industry alone[1]. Most countries have adopted regulations to limit exposure to mycotoxins, having strong impact on food and animal crop trade[2]. The presence of mycotoxins is unavoidable and therefore testing of raw materials and products is required to keep our food and feed safe.

The toxicity and potential health hazards induced by the ingestion of foods contaminated by mycotoxins demands the need for sensitive, robust and rugged analytical methodologies. Current methods include gas chromatography electron capture detection[3], gas chromatography-mass spectrometry (GC/MS)[4], Liquid chromatography (LC)-post column derivitization and fluorescence detection[5], Liquid chromatography mass spectrometry with fast atom bombardment (LC/MS-FAB)[6] and Liquid chromatography tandem mass spectrometry (LC/MS/MS)[7].

LC/MS/MS is highly specific, broadly applicable and is rich in providing both qualitative and quantitative information. Current LC/MS/MS methods are unable to detect all the relevant groups of mycotoxins in a single run. We developed a sensitive LC/MS/MS method for the simultaneous determination of 6 groups of mycotoxins, namely aflatoxins, fumonisins, type A and B trichothecenes, zearalenone and its metabolites, ochratoxin A and patulin and validated the method in a corn matrix.

Instrumentation

- Varian 320-MS LC/MS/MS equipped with an ESI source
- Varian Prostar™ 210 solvent delivery system (2)
- Varian Prostar 430 autosampler

Materials and Reagents

All the reagents and mycotoxins other than Nivalenol were purchased from Sigma-Aldrich Co, St. Louis, MO, Nivalenol was purchased from Romer Labs, 1301 Stylemaster Drive, Union, MO. Fumonisin B3 was provided by Darsa Siantar, ATL Laboratories, 490 N Wiget Lane, Walnut Creek, CA.

Sample Preparation

25 g of corn flakes were ground to a fine powder. The powder was dissolved in 50 ml of 80:20 methanol water. 2.5 g of NaCl was added to it. The mixture was stirred for 2 hrs at high speed on a magnetic stirrer. The extract was then filtered through Whatman No. 1 filter paper. The filtered extract was diluted 5 times with mobile phase buffer A (0.1% acetic acid in 10% methanol in water). Finally, 0.1-450 ppb of multi-mycotoxin mix was spiked into the extract for calibration. All the mycotoxins other than zearalenone were analysed by external standard analysis. Zearalanone was used as an internal standard at 75 ppb. It was used to quantify the target analyte zearalenone.

Conditions

LC Conditions

Column Polaris C-18A 5µm, 150 mm x3.0 mm ID (Varian part No A2000150x030)

Buffer A	0.1% CH_3COOH in 10% methanol
Buffer B	0.1% CH_3COOH in 100% methanol
Injection solvent	0.1% CH_3COOH in 10% methanol
Injection volume	10 µl

LC program

Time	% A	% B	Flow ml/min
00.00	100	0	0.285
25.00	15	85	0.285
25.01	100	0	0.4
35.00	100	0	0.4

Table 1 LC program

API Conditions

Ionization Mode	ESI (positive and negative)
Collision Gas	1.8 mTorr Argon
API Drying Gas	30 psi at 250° C
API Nebulizing Gas	50 psi
SIM Width	0.7 amu
Needle	4500 V
Capillary	Scanning (table 2.0)
Shield	600 V
Detector	1900 V

MS/MS parameters

Name	Capillary (V)	Q1 (m/z)	Ion polarity	Q3 (1) (m/z)	CE1(V)	Q3 (2) (m/z)	CE2 (V)	Dwell (sec)
Nivalenol	59	311.1	[M-H]-	281.0	8.00	191.0	19.0	0.3
Patulin	40	153.1	[M-H]-	108.9	7.00	81.00	10.0	0.3
Deoxynivalenol	40	295.0	[M-H]-	265.0	11.5	138.0	11.5	0.3
Fusarenon X	40	353.0	[M-H]-	187.0	29.0	205.0	9.50	0.3
Aflatoxin G2	90	331.0	[M+H]+	245.0	16.5	275.0	11.5	0.3
Aflatoxin G1	90	329.0	[M+H]+	283.0	25.5	243.0	25.5	0.3
Aflatoxin B2	63	315.0	[M+H]+	287.0	21.5	259.0	10.0	0.3
Aflatoxin B1	63	313.0	[M+H]+	245.0	16.5	241.0	22.0	0.3
Fumonisin B1	54	720.9	[M-H]-	157.0	33.0	563.0	24.0	0.3
Fumonisin B2	95	704.8	[M-H]-	157.0	33.0	548.0	22.0	0.3
Fumonisin B3	95	704.6	[M-H]-	546.0	33.0	159.0	33.0	0.3
T2 toxin	85	484.0	$[M+NH_4]+$	305.0	18.5	117.0	13.5	0.3
OchratoxinA	70	402.7	[M-H]-	358.0	18.5	167.0	34.0	0.3
Zearalanone	85	319.0	[M-H]-	136.0	26.5	187.0	28.5	0.3
α-Zearalenol	90	319.0	[M-H]-	174.0	25.5	159.0	27.5	0.3
β-Zearalenol	100	319.0	[M-H]-	275.0	16.0	187.0	23.0	0.3
Zearalenone (IS)	70	317.0	[M-H]-	185.0	24.5	187.0	18.5	0.3

Table 2 MS/MS scan parameters for the detection of 16 mycotoxins in the positive and negative ESI mode

Discussion

The MS parameters were optimized by infusing 100 ppb of the individual mycotoxin at 10 µl/min flow rate through a syringe pump. The electrospray ionization parameters such as drying gas temperature, drying gas pressure and nebulizing gas pressure were optimized by flow injection. An LC-MS/MS method was developed that achieved good separation and sensitivity for the detection of all the 16 mycotoxins studied. Figure 2 gives the MS chromatogram of the 16 mycotoxins that were separated and detected in a single run.

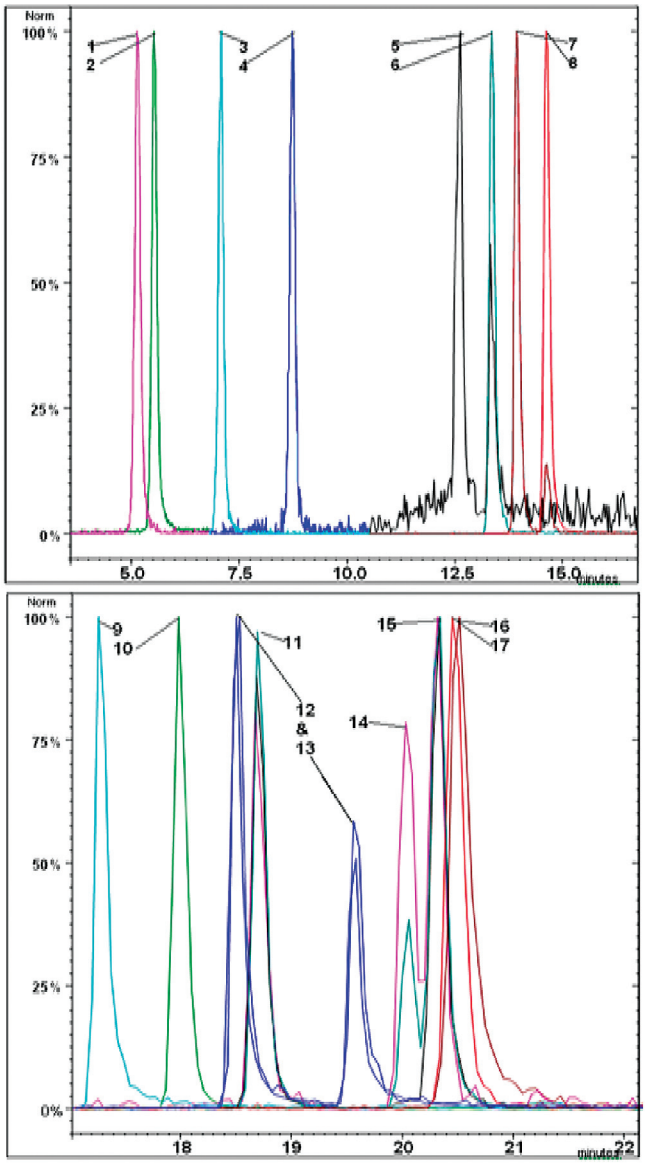

Fig 2 Normalized chromatograms of the 16 mycotoxins in the multi-component method. The numbered label on each chromatogram corresponds to the respective mycotoxins given in Table 3.

In this method, aflatoxins were detected in the positive mode; ZON, α-ZOL, β-ZOL, type A and B trichothecenes (except for T2 toxin) were detected in the negative mode. Polarity switching was incorporated in the last segment to simultaneously determine T2 toxin that elutes in the positive mode and other mycotoxins in the negative mode.

Calibrations of mycotoxins were done in solvent and also in matrix (corn extract). Details are summarized in Table 3.

Best sensitivity was found for aflatoxins B1, B2, G1 and G2 in the positive ESI mode and β-Zol and fumonisins in the negative mode.

S.No	Name	Range (PPB)	Linearity	LOQ (PPB)
1	Nivalenol	1-400	0.999	6.25
2	Patulin	1-400	0.999	1.00
3	Deoxynivalenol	1-400	0.998	3.50
4	Fusarenon X	1-400	0.998	6.25
5	Aflatoxin G2	0.1-192	0.998	0.12
6	Aflatoxin G1	0.1-192	0.998	0.20
7	Aflatoxin B2	0.1-192	0.996	0.12
8	Aflatoxin B1	0.1-192	0.983	0.20
9	Fumonisin B1	0.1-384	0.999	1.75
10	T2 toxin	0.1-384	0.995	0.50
11	α-Zearalenol	0.1-384	0.998	0.50
12	Fumonisin B2	0.1-384	0.998	1.00
13	Fumonisin B3	0.1-384	0.995	1.00
14	β-Zearalenol	0.1-384	0.997	1.00
15	Zearalanone(ISTD)	0.1-384	0.987	1.00
16	Zearalenone	0.1-384	0.994	1.00
17	Ochratoxin A	0.1-384	0.965	0.80

Table 3 Range, linearity and LOQ's of the mycotoxins spiked in solvent

The LOQ's in corn matrix were compared with the detection limits set by FAO 2003[2] (Table 4.0). LOQs are based on a peak-to-peak signal to noise of 10.

Examples of some of the calibration curves generated with the standard mycotoxins in solvent and corn extract are given in figures 4, 5 and 6, respectively.

Patulin

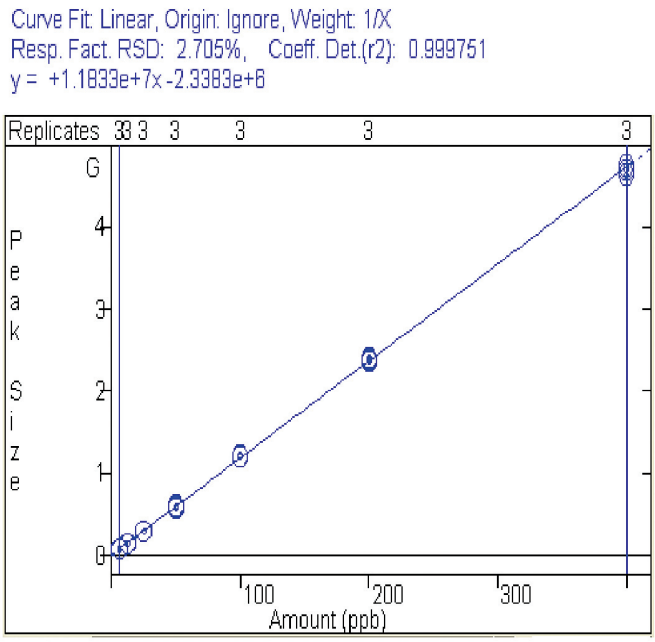

Figure 4 Calibration curve of patulin spiked in solvent

Nivalenol

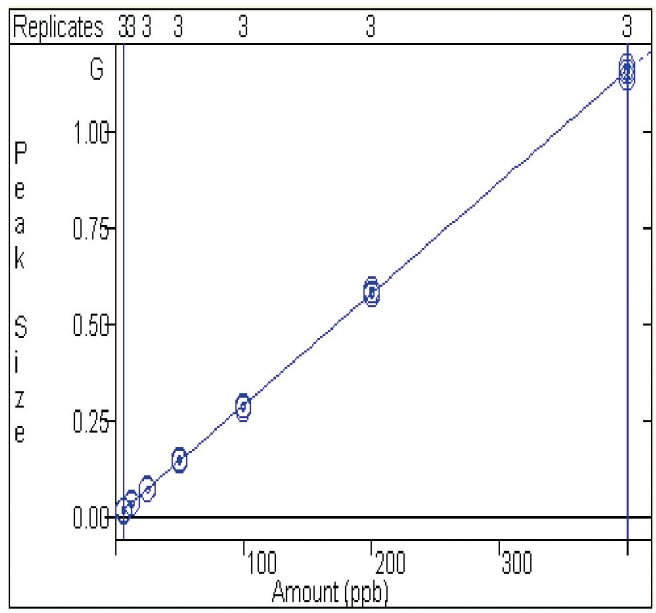

Figure 5 Calibration curve of nivalenol spiked in solvent.

Beta Zearalenol

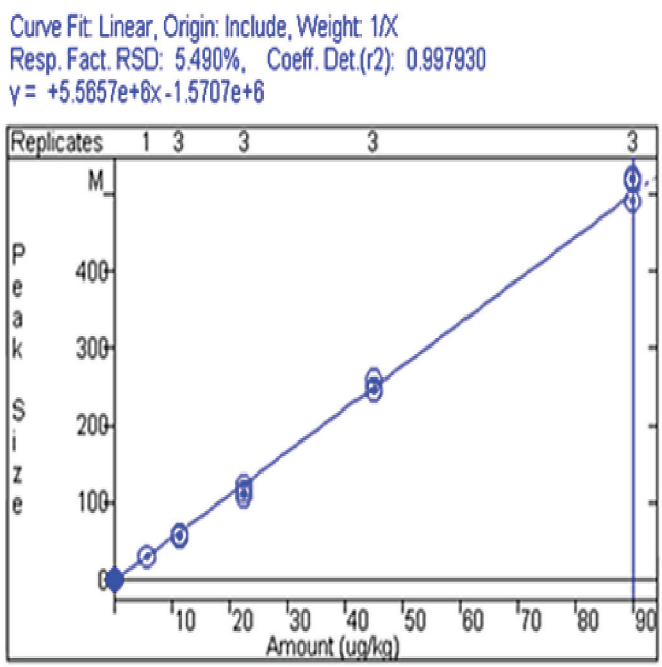

Figure 6 Calibration curve of β-zearalenol spiked in corn extract.

The LOQ's in corn matrix were compared with the detection limits set by FAO 2003 (2) (Table 4.0). LOQs are based on a peak-to-peak signal to noise of 10.

Name	LOQ's (µg/kg) of Mycotoxins in corn extract with the multi component method	World wide limits (ug/kg) for myco-toxins in food. (FAO,2003)
Deoxynivalenol	11.4	300-2000
Aflatoxin B1	1.14	1-35
Aflatoxin B2	0.34	1-35
Afaltoxin G1	1.14	1-35
Aflatoxin G2	0.34	1-35
Fumonisin B1	1.75	1000-3000
Fumonisin B2	8.00	1000-3000
Fumonisin B3	8.00	1000-3000
Zearalenone	10.0	50-100
Ochratoxin A	8.00	3-50
Patulin	2.00	5-100

Table 4 LOQ's of mycotoxins (injection vol=10 µl) in comparison with the world wide regulations for mycotoxins in food and feed in 2003.

All the mycotoxins detected were within the range for the detection limits set by FAO.

Area reproducibility of this method was tested in corn matrix by spiking mycotoxins mix at low concentrations i.e., around the limits of detection and quatitation. The relative standard deviation (RSDs) for different mycotoxins is given in table 5.

	Low Range		Medium range	
Name	**Conc. (µg/kg)**	**RSD%**	**Conc. (µg/kg)**	**RSD%**
Afla B1	7.20	10.4	35.10	5.91
Afla G1	2.16	15.2	10.70	6.52
Afla G2	2.16	11.1	10.70	6.16
DON	7.20	8.42	35.10	6.22
T2	7.20	7.73	35.10	5.08
ZON	7.20	ND	35.10	9.32
β-ZOL	7.20	10.4	3.510	1.79
FB1	14.3	17.5	71.41	6.22
FB2	14.3	17.3	71.41	4.10
FB3	14.3	4.78	71.41	7.60
NIV	14.3	10.1	71.41	7.05

Table 5 Area reproducibility of the spiked mycotoxin mix in corn extract at low levels. (n = 10)

The 320-MS provides excellent reproducibility in this matrix with no clean up, even at very low levels. Since no sample preparation is required, throughput and cost of analysis are greatly reduced.

Conclusions

Performance of the multi component method has been demonstrated in this application with excellent results for all the 16 mycotoxins in a single run.

Due to the high selectivity and sensitivity of the 320-MS, corn samples can be analysed without any prior clean up.

The limit of quantitation for all the 16 mycotoxins analyzed by this multi component method are in agreement with the limits set by FAO.

References

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