Simultaneous Determination of *Alternaria* Toxins, Ergot Alkaloid Epimers, and Other Major Mycotoxins in Various Food Matrices by LC-MS/MS

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Abstract & Introduction

Various food commodities are vulnerable to different types of fungal pathogens and could be contaminated with differential classes of mycotoxins as a result. It is ideally to implement a generic method for simultaneous determination of multi-mycotoxins in different food matrices or agricultural products. In this study, a simplified sample preparation procedure and a reliable LC-MS/MS analytical method was developed for comprehensive measurement of 37 regulated and emerging mycotoxins including 5 *Alternaria* toxins, 6 major ergot alkaloids and their corresponding epimers. Four different food matrices (baby wheat cereal, peanut, tomato puree, and blended flour) were chosen for method validation to demonstrate the applicability of this analytical method to a wide range of food types. Sample extraction was performed using a formic acid-acidified 80:20 acetonitrile:water solution followed by extract dry-down and reconstitution in a 50:50 water:methanol solution for injection analysis on a Biphenyl LC column. Chromatographic analysis was performed using MS-friendly acidic mobile phases and completed with a short 11-minute cycling time for proper separation of ergot alkaloid epimers. Method accuracy and precision was evaluated by fortification of food samples at 3 different levels. Accurate quantification was achieved using matrix-matched calibration standards at the range of 0.4 to 400 µg/kg. The recoveries of all mycotoxins (except citrinin) in fortified samples were from 70% to 120%, and the relative standard deviation was less than 20%. The established workflow was simple and fast for multi-mycotoxin determination with a unique benefit of simultaneous analysis of *Alternaria* toxins and ergot alkaloids. Furthermore, a novel inert Biphenyl LC column demonstrated the high degree of Non-Specific Binding (NSB) that occurs between the column's stainless-steel hardware and certain mycotoxins. The implementation of the inert column offers a robust and improved chromatographic performance as it mitigates the NSB for highly adsorptive analytes (e.g. Fumonisins, Aflatoxins, and Tenuazonic acid) leading to better sensitivity and peak shapes without the need of mobile phase additives or sample passivation.

Methods

Table 1: Analytical Conditions (Waters Xevo TQ-S with Acquity UPLC)

Analytical Column	Raptor Biphenyl 2.7µm 100 mm x 2.1 mm id or Raptor Inert Biphenyl 2.7µm 100 mm x 2.1 mm id	
Guard Column	Raptor Biphenyl EXP Guard Column Cartridge 2.7µm, 5 mm x 2.1 mm id	
Mobile Phase A	0.05% formic acid in water	
Mobile Phase B	0.05% formic acid in methanol	
Gradient	**Time (min)**	**%B**
	0.00	25
	5.00	50
	9.00	100
	9.01	25
	11.00	25
Flow Rate	0.4 mL/min	
Injection Volume	5 µL	
Column Temp.	60°C	
Ion Mode	Scheduled MRM in positive ESI	

Food Products

Baby wheat cereal, raw peanut, tomato puree, and flours were purchased from local grocery stores. Baby wheat cereal and tomato puree were used as their original forms. Raw peanut was grinded and stored in the refrigerator. A blended flour was prepared by mixing white rice flour (75%), brown rice flour (5%), millet flour (5%), oat flour (5%), all-purpose wheat flour (5%), and all-purpose gluten free flour (5%) with a handheld blender.

Sample and Matrix-Matched Standards Preparation

Two grams of the sample were weighed into a 50-mL polypropylene centrifuge tube and fortified at 5, 50, and 200 µg/kg with stock standard solution. After sitting at room temperature for 10 minutes, 16 mL of extraction solution (80:20 acetonitrile:water) containing 0.5% formic acid (no formic acid for tomato puree) were added and the tube was stirred to gain homogenous suspension. The extraction was carried out by shaking horizontally on a digital pulse mixer (Glas-Col LLC, Terre Haute, IN) at 800 rpm for 20 minutes. After centrifuging for 5 minutes at 4000 rpm, 1 mL of extract was evaporated to dryness at 45°C under a gentle stream of nitrogen. The dried extract was reconstituted with 1 mL of 50:50 water:methanol solution and a 0.4 mL aliquot was transferred to and filtered using a Thomson SINGLE StEP filter vial with a 0.2 µm PTFE filter. To prepare matrix-matched calibration standards, the non-fortified matrices were extracted and dried down as described for the sample preparation procedure followed by reconstitution in 50:50 water/methanol solution containing 0.05 – 50 ng/mL of analytes which equals to 0.4 – 400 µg/kg of sample concentration.

Results & Discussion

(1) **Chromatographic Performance:** A fast chromatographic method using the Raptor Biphenyl column was established (see **Table 1**) for simultaneous analysis of 38 mycotoxins with a 11-minute total cycling time (**Figure 1**). Analytes were detected with ESI+ and the MRMs were shown in **Table 2**. All epimer pairs of ergot alkaloids were chromatographically separated for definitive and accurate quantification. It was noted that whenever a new Biphenyl column was used, it would need to be rinsed and maintained under the mobile phase overnight to gain an acceptable and quantifiable peak shape for tenuazonic acid.

(2) **Linearity:** It was shown that a consistent and most suitable linearity of all analytes could be obtained with a quadratic regression (1/x weighted). The lowest concentrated standards were varied due to the differential MS ionization of analytes and specific matrix effect of different food matrices. Nevertheless, most analytes were quantifiable at the full range of 0.4 – 400 µg/kg and all compounds showed proper linearity with r^2 >0.997 and deviations <30% (**Table 3**).

(3) **Accuracy & Precision:** For each food sample, 3 batches of analyses were performed on different days with a total of 9 repetition of each fortified level. The average recovery and relative standard deviation (RSD) were shown in **Table 4**. Except citrinin in solid samples, all analytes had the recovery of 72 – 112% of for 3 fortification levels among 4 different types of food matrices. The satisfactory method precision was demonstrated with the %RSD of within 0.5 – 12%. For solid samples, the use of formic acid-containing extraction solution was necessary to obtain adequate recovery for fumonisin Bs but resulted in low recovery (24 – 36%) of citrinin. For food with high water content such as tomato puree, acceptable recovery of both fumonisin Bs (90 – 94%) and citrinin (72 – 77%) were achievable without the addition of formic acid. Due to specific matrix interference, nivalenol could not be measured in baby wheat cereal. The negative impact of matrix interference could also be observed for deoxynivalenol, fusarenon X, and patulin for tomato puree analysis in which the 5 µg/kg fortification sample was not quantifiable.

Figure 1. Chromatogram of Fortified Blended Flour at 50 µg/kg

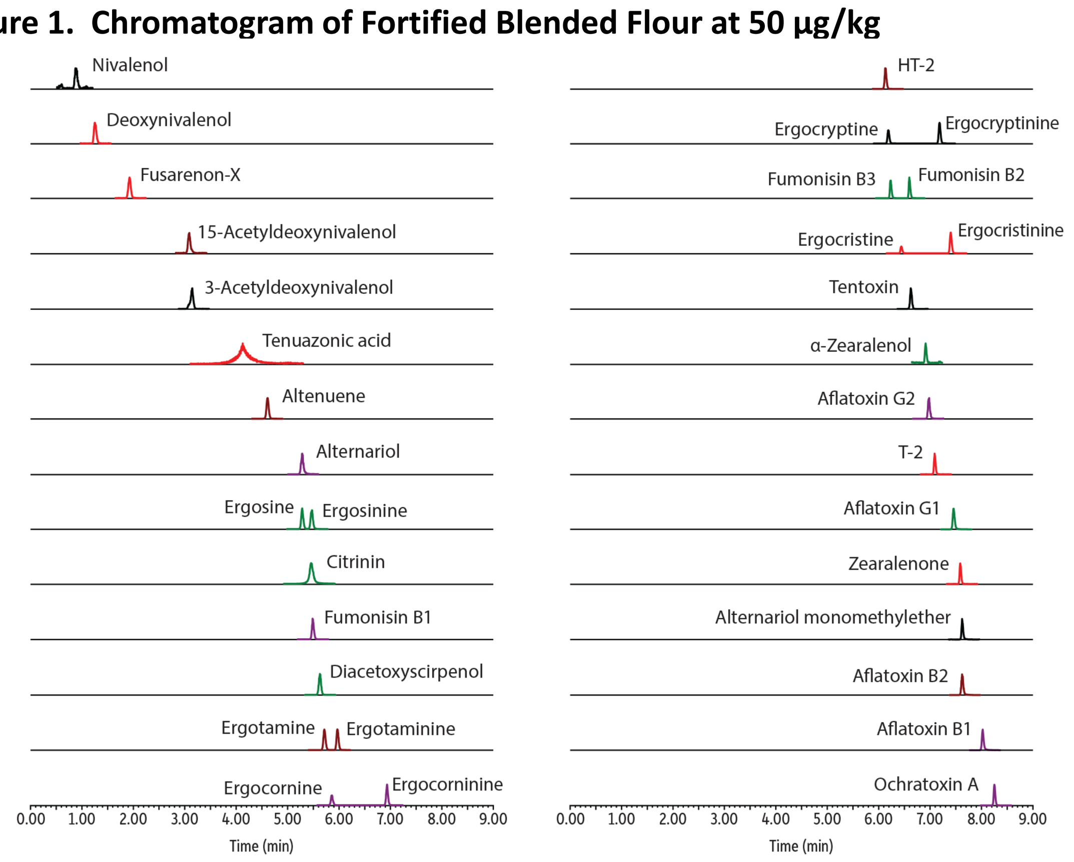


Table 2. MS Transition and Retention Time

Compounds	Retention time (min)	Precursor Ion	Product ion 1	Product ion 2
Aflatoxin B1	8.20	313.2 [M+H]+	241.1	284.9
Aflatoxin B2	7.81	315.1 [M+H]+	287.0	259.0
Aflatoxin G1	7.62	329.1 [M+H]+	199.7	243.0
Aflatoxin G2	7.15	331.2 [M+H]+	189.0	313.0
Ochratoxin A	8.31	404.1 [M+H]+	239.0	358.0
3-Acetyldeoxynivalenol	3.21	339.2 [M+H]+	213.1	231.1
15-Acetyldeoxynivalenol	3.14	339.2 [M+H]+	137.1	321.2
Deoxynivalenol	1.30	297.2 [M+H]+	231.0	249.0
Diacetoxyscirpenol	5.73	384.2 [M+H]+	247.1	307.2
Fumonisin B1	5.63	722.5 [M+H]+	352.3	334.2
Fumonisin B2	6.68	706.4 [M+H]+	336.2	318.3
Fumonisin B3	6.32	706.4 [M+H]+	336.2	318.3
Fusarenon-X	1.98	355.1 [M+H]+	137.1	247.1
HT-2	6.20	447.2 [M+Na]+	345.1	285.1
Nivalenol	0.92	295.1 $[M-H_2O]+$	137.1	91.0
T-2	7.14	489.2 [M+Na]+	387.1	245.1
α-Zearalenol	6.96	303.1 $[M-H_2O]+$	285.1	175.0
Zearalenone	7.65	319.2 [M+H]+	283.1	187.0
Citrinin	5.43	251.2 [M+H]+	233.1	205.1
Patulin	1.03	155.0 [M+H]+	99.0	81.0
Alternariol	5.30	259.0 [M+H]+	185.1	130.0
Alternariol monomethylether	7.69	273.0 [M+H]+	199.1	128.0
Altenuene	4.70	293.2 [M+H]+	257.1	275.2
Tentoxin	6.70	415.2 [M+H]+	312.2	302.2
Tenuazonic acid	4.22	198.1 [M+H]+	125.0	153.1
Ergocornine	6.03	562.4 [M+H]+	268.2	223.2
Ergocorninine	7.07	562.4 [M+H]+	268.2	223.2
Ergocristine	6.56	610.4 [M+H]+	223.2	592.4
Ergocristinine	7.53	576.4 [M+H]+	223.2	592.4
Ergocryptine	6.32	576.4 [M+H]+	268.2	223.2
Ergocryptinine	7.31	576.4 [M+H]+	268.2	223.2
Ergometrine	1.27	326.2 [M+H]+	223.2	208.1
Ergometrinine	1.83	326.2 [M+H]+	223.2	208.1
Ergosine	5.47	548.4 [M+H]+	208.1	223.2
Ergosinine	5.67	548.4 [M+H]+	208.1	223.2
Ergotamine	5.90	582.4 [M+H]+	223.2	268.2
Ergotaminine	6.13	582.4 [M+H]+	223.2	268.2

Table 3. Calibration Ranges

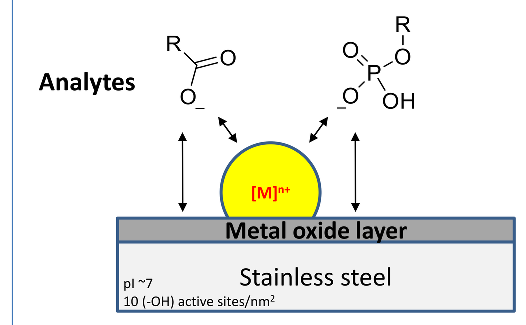
Compound	Baby Wheat Cereal Linear Range (µg/kg)	r^2	Peanut Linear Range (µg/kg)	r^2	Tomato Puree Linear Range (µg/kg)	r^2	Blended Flour Linear Range (µg/kg)	r^2
Aflatoxin B1	0.4 - 400	0.9996	0.4 - 400	0.9998	0.4 - 400	0.9995	0.4 - 400	0.9998
Aflatoxin B2	0.4 - 400	0.9997	0.4 - 400	0.9998	0.4 - 400	0.9996	0.4 - 400	1.000
Aflatoxin G1	0.4 - 400	0.9999	0.4 - 400	0.9997	0.4 - 400	0.9979	0.4 - 400	1.000
Aflatoxin G2	0.4 - 400	0.9997	0.4 - 400	0.9998	0.4 - 400	0.9993	0.4 - 400	0.9998
Ochratoxin	0.4 - 400	0.9998	0.4 - 400	0.9993	0.4 - 400	0.9996	0.4 - 400	1.000
3-Acetyldeoxynivalenol	4.0 - 400	0.9994	2.0 - 400	0.9997	4.0 - 400	0.9982	2.0 - 400	0.9998
Deoxynivalenol	2.0 - 400	0.9998	4.0 - 400	0.9994	8.0 - 400	0.9991	2.0 - 400	0.9995
Diacetoxyscirpenol	0.8 - 400	0.9998	0.4 - 400	0.9995	0.8 - 400	0.9993	0.4 - 400	0.9998
Fumonisin B1	0.4 - 400	0.9999	0.4 - 400	0.9994	0.4 - 400	0.9999	0.4 - 400	0.9999
Fumonisin B2	0.4 - 400	0.9997	0.4 - 400	0.9997	0.4 - 400	0.9998	0.4 - 400	0.9999
Fumonisin B3	0.4 - 400	0.9999	0.4 - 400	0.9997	0.4 - 400	1.000	0.4 - 400	0.9999
Fusarenon-X	4.0 - 400	0.9971	2.0 - 400	0.9971	8.0 - 400	0.9974	2.0 - 400	0.9995
HT-2	0.4 - 400	0.9999	0.4 - 400	0.9997	0.4 - 400	0.9997	0.4 - 400	0.9999
Nivalenol	-	-	8.0 - 400	0.9990	20 - 400	0.9996	8.0 - 400	0.9997
T-2	0.4 - 400	0.9998	0.4 - 400	0.9998	0.4 - 400	0.9992	0.4 - 400	1.000
α-Zearalenol	4.0 - 400	0.9985	2.0 - 400	0.9992	2.0 - 400	0.9979	2.0 - 400	0.9994
Zearalenone	0.8 - 400	0.9998	0.8 - 400	0.9996	0.8 - 400	0.9995	2.0 - 400	0.9998
Citrinin	0.4 - 400	0.9996	0.4 - 400	0.9986	0.4 - 400	0.9984	0.4 - 400	0.9999
Patulin	4.0 - 400	0.9991	4.0 - 400	0.9995	8.0 - 400	0.9997	4.0 - 400	0.9993
Alternariol	0.4 - 400	0.9998	0.4 - 400	0.9990	4.0 - 400	0.9996	0.4 - 400	0.9997
Alternariol monomethylether	0.4 - 400	0.9996	0.4 - 400	0.9995	0.4 - 400	0.9992	0.4 - 400	0.9999
Altenuene	0.4 - 400	0.9999	0.4 - 400	0.9997	2.0 - 400	0.9999	0.4 - 400	1.000
Tentoxin	2.0 - 400	0.9998	0.4 - 400	0.9998	0.8 - 400	0.9998	0.4 - 400	0.9999
Tenuazonic acid	8.0 - 400	0.9994	2.0 - 400	0.9997	8.0 - 400	0.9987	8.0 - 400	0.9992
Ergocornine	0.4 - 400	0.9999	0.4 - 400	0.9998	0.4 - 400	0.9998	0.4 - 400	0.9999
Ergocorninine	0.4 - 400	0.9998	0.4 - 400	0.9997	0.4 - 400	0.9996	0.4 - 400	0.9999
Ergocristine	0.4 - 400	0.9998	0.4 - 400	0.9998	0.4 - 400	0.9997	0.4 - 400	1.000
Ergocristinine	0.8 - 400	0.9999	0.4 - 400	0.9997	0.4 - 400	0.9990	0.4 - 400	0.9999
Ergocryptine	0.4 - 400	0.9999	0.4 - 400	0.9998	0.4 - 400	0.9998	0.4 - 400	0.9999
Ergocryptinine	0.4 - 400	0.9999	0.4 - 400	0.9997	0.4 - 400	0.9988	0.4 - 400	1.000
Ergometrine	0.4 - 400	0.9998	0.4 - 400	0.9999	0.4 - 400	0.9973	0.4 - 400	0.9999
Ergometrinine	0.4 - 400	0.9997	0.4 - 400	0.9999	0.4 - 400	0.9993	0.4 - 400	0.9999
Ergosine	0.4 - 400	0.9995	0.4 - 400	0.9996	0.4 - 400	0.9996	0.4 - 400	0.9999
Ergosinine	0.4 - 400	0.9999	0.4 - 400	0.9997	0.4 - 400	0.9998	0.4 - 400	0.9997
Ergotamine	0.4 - 400	0.9998	0.4 - 400	0.9998	0.4 - 400	0.9995	0.4 - 400	0.9999
Ergotaminine	0.4 - 400	0.9999	0.4 - 400	0.9997	0.4 - 400	0.9998	0.4 - 400	0.9998

Table 4. Recovery & Precision

	Average Recovery (RSD, %)											
	Baby Wheat Cereal			Peanut			Tomato Puree			Blended Flour		
Concentration, µg/kg	5	50	200	5	50	200	5	50	200	5	50	200
Aflatoxin B1	105 (4.8)	100 (3.0)	79.8 (2.6)	98.2 (6.4)	97.0 (5.2)	89.0 (5.7)	92.7 (3.8)	97.6 (5.2)	103 (3.0)	101 (2.8)	95.5 (1.3)	89.0 (1.5)
Aflatoxin B2	110 (1.4)	109 (2.8)	106 (2.3)	102 (5.8)	99.3 (4.7)	91.3 (2.9)	91.7 (4.2)	93.3 (0.9)	94.7 (0.4)	100 (2.3)	101 (0.9)	88.7 (1.3)
Aflatoxin G1	105 (6.1)	107 (1.7)	102 (2.1)	98.2 (4.2)	97.3 (3.2)	91.2 (4.1)	91.3 (1.9)	92.2 (3.6)	93.3 (2.5)	99.3 (1.7)	100 (1.6)	93.6 (2.2)
Aflatoxin G2	108 (3.0)	109 (1.3)	104 (2.2)	104 (5.3)	102 (3.8)	93.5 (1.9)	86.8 (8.3)	96.4 (2.5)	98.5 (2.5)	98.7 (3.1)	102 (2.6)	94.5 (2.0)
Ochratoxin A	109 (1.8)	108 (2.1)	94.5 (1.5)	102 (1.9)	101 (1.1)	97.7 (0.9)	90.9 (3.5)	93.8 (3.3)	101 (5.9)	98.1 (1.6)	98.2 (1.3)	82.8 (1.7)
3- + 15-Acetyldeoxynivalenol	104 (6.3)	108 (1.8)	104 (3.3)	101 (6.5)	95.9 (5.8)	91.0 (4.4)	91.9 (4.3)	98.1 (2.7)	95.0 (1.8)	98.4 (5.2)	101 (2.9)	100 (0.9)
Deoxynivalenol	112 (4.0)	102 (2.6)	95.7 (1.3)	98.1 (3.5)	93.7 (4.8)	88.2 (3.4)	-	90.3 (6.4)	94.5 (2.6)	102 (3.5)	97.5 (2.6)	96.9 (0.8)
Diacetoxyscirpenol	105 (4.0)	107 (1.5)	103 (1.2)	93.2 (4.3)	95.4 (3.9)	93.8 (5.0)	90.9 (3.8)	94.5 (4.7)	94.0 (1.9)	98.1 (6.3)	101 (3.1)	98.7 (1.8)
Fumonisin B1	94.3 (4.6)	94.0 (2.8)	92.3 (2.6)	87.2 (3.1)	88.2 (4.5)	87.8 (6.6)	91.8 (3.6)	91.5 (1.9)	91.9 (0.7)	100 (3.2)	99.6 (1.7)	96.1 (1.2)
Fumonisin B2	93.3 (4.1)	95.1 (4.8)	90.3 (2.9)	95.4 (4.7)	92.5 (2.3)	88.8 (3.9)	89.9 (4.1)	92.9 (2.3)	92.4 (0.8)	104 (2.7)	99.6 (1.4)	94.4 (1.6)
Fumonisin B3	91.8 (4.9)	94.6 (4.9)	91.6 (3.1)	90.6 (2.7)	90.1 (3.8)	87.7 (4.7)	91.1 (3.6)	93.1 (1.8)	91.9 (0.9)	104 (2.2)	99.9 (1.4)	95.9 (1.2)
Fusarenon-X	99.0 (3.9)	100 (2.9)	103 (2.8)	86.9 (7.0)	90.3 (11.0)	88.3 (10.1)	-	92.0 (6.8)	94.3 (1.9)	101 (3.8)	100 (3.7)	98.3 (1.6)
HT-2	110 (2.4)	111 (1.4)	108 (1.1)	100 (2.7)	100 (2.0)	94.3 (3.0)	96.8 (3.1)	96.1 (2.1)	99.0 (1.4)	101 (1.6)	103 (2.2)	98.3 (1.3)
Nivalenol	-	-	-	-	98.3 (6.2)	89.0 (3.6)	-	92.5 (4.5)	93.7 (5.0)	-	95.5 (4.7)	92.9 (2.3)
T-2	111 (2.1)	110 (1.8)	108 (2.8)	99.1 (2.7)	101 (1.7)	95.9 (2.1)	92.0 (6.3)	94.7 (1.3)	98.6 (1.5)	102 (1.3)	103 (1.3)	96.9 (1.3)
α-Zearalenol	100 (4.9)	102 (5.2)	90.1 (5.8)	89.2 (8.1)	93.6 (5.5)	94.7 (3.4)	97.7 (3.2)	88.9 (4.2)	90.0 (3.4)	96.9 (3.7)	99.0 (3.6)	95.0 (3.3)
Zearalenone	110 (6.7)	110 (3.0)	105 (3.7)	98.3 (7.3)	97.4 (2.8)	91.3 (1.5)	95.0 (4.5)	93.6 (2.2)	95.7 (2.0)	101 (3.8)	102 (2.1)	92.3 (1.4)
Citrinin	26.1 (9.2)	26.6 (3.1)	30.1 (3.8)	24.1 (8.7)	25.1 (1.9)	25.8 (3.5)	71.9 (4.7)	76.4 (1.6)	77.1 (1.7)	32.3 (3.5)	32.2 (6.3)	35.8 (4.5)
Patulin	106 (4.6)	95.6 (5.6)	89.2 (5.1)	88.8 (12.0)	83.6 (9.0)	86.0 (7.2)	-	98.9 (3.6)	103 (4.5)	93.6 (4.4)	86.1 (3.1)	92.2 (2.9)
Alternariol	108 (4.1)	108 (1.6)	104 (1.0)	94.2 (3.4)	95.4 (2.4)	96.2 (2.7)	89.3 (4.6)	91.8 (2.5)	91.4 (1.3)	98.4 (2.3)	101 (2.5)	96.3 (3.2)
Alternariol monomethylether	108 (4.1)	109 (2.2)	99.3 (2.7)	93.5 (3.3)	93.5 (3.7)	89.8 (2.4)	91.3 (6.6)	88.7 (5.1)	93.9 (3.9)	104 (2.9)	101 (1.7)	93.7 (1.9)
Altenuene	110 (2.1)	109 (2.1)	105 (2.1)	99.6 (2.0)	99.5 (1.2)	95.4 (1.2)	98.4 (3.4)	92.4 (2.1)	92.8 (1.8)	101 (2.9)	101 (3.1)	98.2 (0.5)
Tentoxin	111 (3.6)	109 (2.5)	103 (1.4)	104 (2.9)	101 (1.1)	95.3 (1.4)	92.5 (6.2)	94.2 (2.2)	95.8 (1.4)	104 (4.2)	105 (2.1)	98.2 (1.9)
Tenuazonic acid	-	85.8 (1.7)	87.4 (6.3)	92.5 (4.7)	91.0 (2.1)	88.5 (2.4)	-	89.3 (4.1)	88.5 (2.0)	-	92.5 (8.8)	90.0 (9.5)
Ergocornine	109 (1.5)	109 (1.4)	102 (1.3)	93.8 (3.5)	93.2 (4.4)	91.2 (3.3)	91.5 (3.0)	93.1 (1.9)	92.9 (0.6)	102 (2.5)	101 (1.9)	97.6 (1.7)
Ergocorninine	109 (3.0)	109 (2.0)	101 (1.9)	105 (3.0)	104 (2.4)	99.5 (3.1)	89.9 (3.8)	92.3 (2.2)	92.5 (3.1)	101 (2.5)	102 (2.6)	95.7 (2.4)
Ergocristine	108 (3.1)	108 (2.9)	101 (4.4)	92.1 (3.8)	91.7 (5.1)	92.0 (2.2)	91.3 (2.9)	94.2 (2.0)	94.3 (0.8)	101 (1.7)	99.8 (2.0)	96.7 (1.8)
Ergocristinine	106 (3.5)	105 (1.4)	101 (0.8)	102 (4.8)	104 (4.3)	102 (4.6)	91.6 (5.9)	94.4 (1.8)	95.6 (2.7)	102 (2.9)	102 (3.0)	99.3 (4.5)
Ergocryptine	107 (2.0)	109 (1.9)	104 (3.4)	95.0 (3.0)	94.7 (4.1)	92.1 (1.7)	90.1 (3.0)	93.5 (2.2)	93.2 (0.7)	99.5 (2.7)	99.9 (1.2)	97.4 (1.4)
Ergocryptinine	106 (1.7)	108 (2.0)	101 (1.1)	103 (5.3)	105 (4.0)	101 (4.2)	91.1 (4.3)	95.1 (1.5)	98.1 (1.6)	101 (2.0)	101 (1.8)	95.4 (1.9)
Ergometrine	92.8 (7.3)	90.0 (4.2)	88.3 (3.6)	101 (2.3)	96.2 (2.6)	86.7 (1.9)	90.7 (3.6)	88.9 (6.1)	87.6 (3.5)	101 (1.8)	99.7 (3.2)	95.3 (1.3)
Ergometrinine	101 (4.2)	99.1 (1.9)	94.3 (0.7)	93.2 (4.3)	95.5 (1.7)	89.1 (2.2)	90.6 (3.9)	90.1 (4.4)	89.7 (1.9)	100 (3.5)	98.5 (1.9)	91.1 (1.9)
Ergosine	108 (2.6)	106 (5.6)	101 (3.2)	90.8 (2.0)	91.8 (2.2)	89.2 (2.6)	91.7 (2.2)	90.4 (3.1)	90.3 (1.5)	99.9 (2.7)	99.1 (3.0)	98.2 (1.1)
Ergosinine	111 (1.8)	109 (0.9)	103 (1.1)	100 (1.1)	102 (2.0)	97.7 (2.2)	92.7 (1.4)	93.6 (2.5)	93.8 (0.9)	99.2 (2.8)	98.4 (2.8)	97.5 (1.0)
Ergotamine	109 (1.9)	108 (1.7)	102 (2.8)	91.0 (2.8)	92.6 (2.8)	89.8 (3.6)	91.1 (2.2)	90.6 (3.7)	90.7 (1.3)	101 (2.9)	100 (3.1)	96.4 (2.2)
Ergotaminine	109 (1.0)	109 (0.7)	101 (0.6)	98.2 (2.0)	101 (1.5)	96.6 (1.3)	93.6 (3.5)	94.7 (1.7)	94.5 (0.6)	101 (2.3)	99.7 (1.3)	97.1 (1.5)

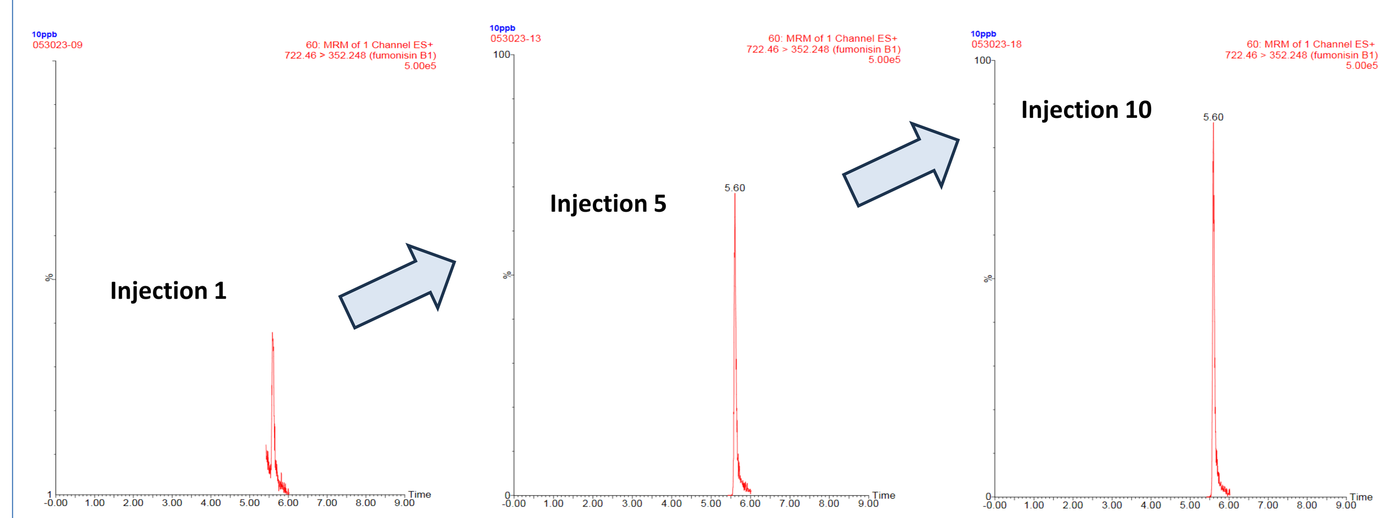
Further Findings

Non-Specific Binding (NSB) was leading to unstable signals, peak tailing, and low sensitivity as the injections progressed for most of the mycotoxins; including Fumonisins (**Figure 2**), Tenuazonic acid, Citrinin, Ochratoxin A, and Diacetoxycispernol. NSB was attributed to the presence of active metal surfaces within the column's hardware and instrument.



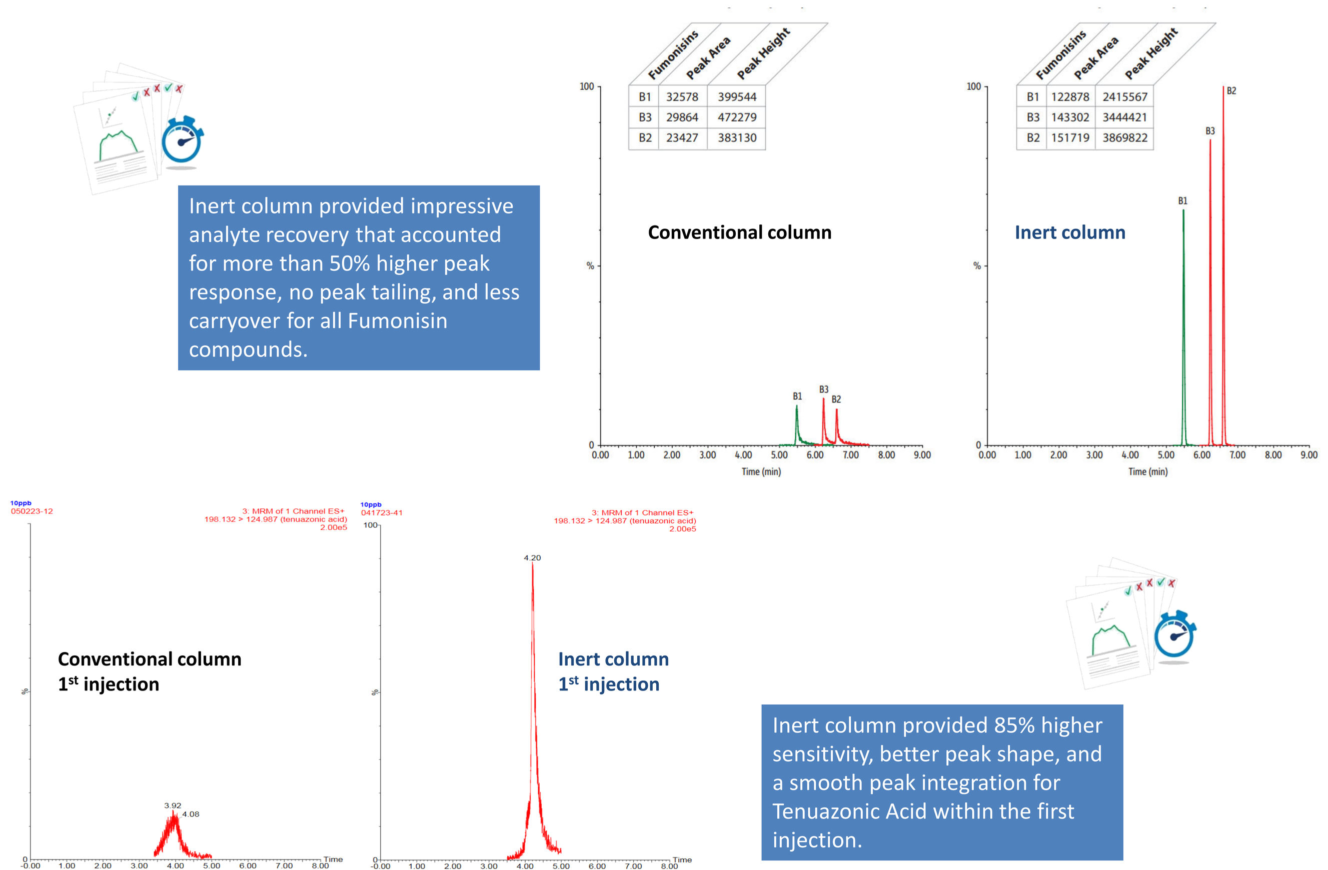
NSB is aggravated under low pH and low ionic strength mobile phases, leading to irreproducible chromatography. Analytes with acidic functional groups or chelating moieties are particularly prone to this surface metal interaction.

Figure 2. Chromatograms of Fumonisin B1 at 10 ng/mL



In order to mitigate NSB, an inert column hardware was implemented; which allows for the efficient and reproducible analysis of metal sensitive compounds. The inert hardware consists of a premium inert coating applied to the stainless-steel surface of the column that guarantees a more consistent chromatography. **Figure 3** shows the overall benefit of switching from a conventional to an inert column without any change in the method itself.

Figure 3. Effect of Inert Column on Selected Mycotoxins at 10 ng/mL



Conclusions

A workflow was established in this study to provide a unique solution for simultaneous determination of *Alternaria* toxins, ergot alkaloid epimers, and other major mycotoxins produced by fungal genus of Aspergillus, Fusarium, and Penicillium. The reported method was rugged, accurate, and precise using a combination of convenient sample preparation procedure and a fast 11-minute chromatographic analysis. Most importantly, this solution could be applied to multi-mycotoxin quantification in a wide variety of food products. Furthermore, the application of inert column hardware aided in the consistent analysis of several mycotoxins that tend to interact with metal surfaces and therefore increasing the sensitivity of such.