Featured Application: *Mycotoxins in Peanut Powder on Raptor Biphenyl*

5.5 Minute LC-MS/MS Analysis of Mycotoxins in Peanut Powder

- Fast analysis for higher sample throughput.
- Excellent separation improves accuracy for 12 regulated mycotoxins.
- Quick and easy sample preparation (dilute-filter-shoot).

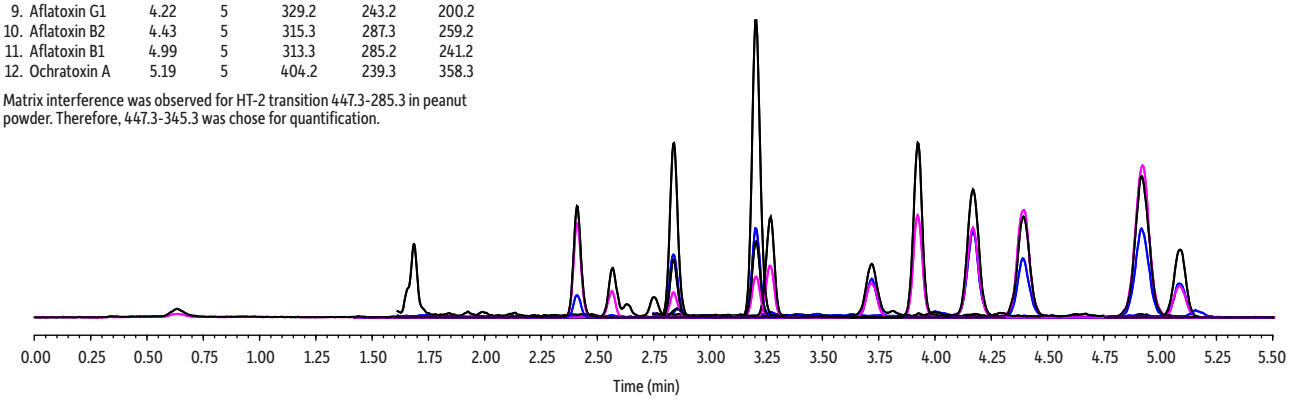
Certain fungi that can grow on agricultural products produce toxic metabolites known as mycotoxins. Modern food processing procedures cannot completely remove these compounds if they are present, so strict monitoring protocols have been established. Although a universal method for the analysis of mycotoxins would allow highly efficient screening, it is very challenging to develop such a method due to differences in physiochemical properties of mycotoxins, extraction efficiencies, and matrix effects. Zhang et al. published a multi-lab study [1] aimed at providing labs with an analytical procedure that could be broadly applied to the analysis of a variety of mycotoxins in many different matrices. Using that work as inspiration, we developed the following LC-MS/MS method that resolves 12 FDA regulated mycotoxins within the pressure limits of traditional HPLC instruments.

In this example, mycotoxins were analyzed in a peanut powder matrix. The use of a relatively short column format, the selectivity of the Biphenyl stationary phase, and the efficiency of 2.7 µm Raptor superficially porous particles provided excellent separations in a fast 5.5 minute analysis (total cycle time of 7 minutes). A coeluting matrix compound that shared the most abundant MRM transition for mycotoxin HT-2 (447.3-285.3) was observed, so a less abundant transition (447.3-345.3) was selected for quantitation. To increase sensitivity, an ammonium buffer was used to promote better ionization of mycotoxins. The Raptor Biphenyl column worked very well for the 12 mycotoxins studied in the cited work, but for longer compound lists containing isobaric mycotoxins with similar structures, the Raptor FluoroPhenyl phase may be necessary to provide adequate chromatographic resolution. The selectivity of the Raptor Fluorophenyl column is demonstrated in an analysis of 20 mycotoxins that can be found by visiting www.restek.com and entering LC_FS0511 in the search.

This method showed excellent precision and accuracy for the 12 FDA regulated mycotoxins that were evaluated during a validation study that covered a variety of matrices (including multiple sources of cornmeal and brown rice flour, in addition to the peanut powder example shown here). Restek would like to thank Dr. Zhang for his technical support during this project.

Peaks	t_R (min)	Conc. (ng/g)	Precursor Ion	Product Ion 1	Product Ion 2
1. Deoxynivalenol	0.62	50	297.3	249.3	231.2
2. Fumonisin B1	2.45	50	722.5	352.4	334.5
3. HT-2	2.60	50	447.3	345.3	285.3
4. Fumonisin B3	2.85	50	706.5	336.4	318.4
5. Fumonisin B2	3.23	50	706.5	336.3	141.2
6. T2	3.31	50	489.3	245.2	387.4
7. Aflatoxin G2	3.74	5	331.2	313.3	189.3
8. Zearalenone	3.96	50	319.3	283.3	187.2
9. Aflatoxin G1	4.22	5	329.2	243.2	200.2
10. Aflatoxin B2	4.43	5	315.3	287.3	259.2
11. Aflatoxin B1	4.99	5	313.3	285.2	241.2
12. Ochratoxin A	5.19	5	404.2	239.3	358.3

Matrix interference was observed for HT-2 transition 447.3-285.3 in peanut powder. Therefore, 447.3-345.3 was chose for quantification.



LC_FS0526

Column: Raptor Biphenyl (cat.# 9309A52); Dimensions: 50 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Guard Column: Raptor Biphenyl EXP guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252); Temp.: 40 °C; Inj. Vol.: 5 µL; **Mobile Phase:** A: Water, 2 mM ammonium formate, 0.1% formic acid; B: Methanol, 2 mM ammonium formate, 0.1% formic acid; **Gradient (%B):** 0.00 min (30%), 0.6 min (30%); 0.7 min (50%); 3.00 min (70%); 4.5 min (75%); 5.0 min (90%); 5.2 min (90%); 5.21 min (75%); 6.00 min (75%); 6.01 min (30%); 7.00 min (30%); **Flow:** 0.5 mL/min; **Detector:** MS/MS; Ion Mode: ESI+; Mode: MRM; **Instrument**: UHPLC; **Notes:** Weighed 1.00 gram of peanut powder in a 50 mL centrifuge tube and added 2.00 mL of water. Vortexed at 3000 rpm for 5 min followed by the addition of 4.0 mL of extraction solvent (50:50 water:acetonitrile, v/v). The tube was then vortexed at 3000 rpm for 5 min followed by centrifugation for 15 min at 4200 rpm. 475 µL of the supernatant was filtered through a Thomson SINGLE StEP Nano filter vial (0.2 µm, cat.# 25882). The sample was then fortified with 25 µL of a standard solution prepared in water at 1000 ng/mL (100 ng/mL for aflatoxins and ochratoxin A) as part of the matrix-matched calibration curve. Vortexed at 3000 rpm for 1 min prior to analysis.; **Notes:** Want even better performance when analyzing metal-sensitive compounds? Check out Inert LC columns at www.restek.com/inert

References

1. K. Zhang, M.R. Schaab, G. Southwood, E.R. Tor, L.S. Aston, W. Song, B. Eitzer, S. Majumdar, T. Lapainis, H. Mai, K. Tran, A. El-Demerdash, V. Vega, Y. Cai, J.W. Wong, A.J. Krynitsky, T.H. Begley, A collaborative study: determination of mycotoxins in corn, peanut butter, and wheat flour using stable isotope dilution assay (SIDA) and liquid chromatography-tandem mass spectrometry (LC-MS/MS), Journal of Agricultural and Food Chemistry, 65 (33) (2017) 7138-7152. https://www.ncbi.nlm.nih.gov/pubmed/27983809.

Raptor Biphenyl LC Columns (USP L11)

Length	2.1 mm cat.#	3.0 mm cat.#	4.6 mm cat.#
1.8 µm Columns			
30 mm	9309232	—	—
50 mm	9309252	930925E	—
100 mm	9309212	930921E	—
150 mm	9309262	—	—
2.7 µm Columns			
30 mm	9309A32	9309A3E	9309A35
50 mm	9309A52	9309A5E	9309A55
100 mm	9309A12	9309A1E	9309A15
150 mm	9309A62	9309A6E	9309A65
5 µm Columns			
30 mm	—	930953E	—
50 mm	9309552	930955E	9309555
100 mm	9309512	930951E	9309515
150 mm	9309562	930956E	9309565
250 mm	—	—	9309575

Want even better performance when analyzing mycotoxins? Check out Inert LC columns at **www.restek.com/inert**

Raptor Inert Biphenyl HPLC Columns

- Inert LC column technology reduces nonspecific binding of chelating analytes, enabling sensitive analysis and smooth integration of peaks.
- Ideal for the analysis of metal-sensitive compounds, such as mycotoxins.
- Increased response and analyte recovery, allowing lower detection limits.
- Improved peak shape without additional passivation or mobile phase additives.
- Part of Restek's Raptor Biphenyl column line featuring 2.7 µm SPP core-shell silica.

ID	Length	Particle Size	Units	Cat.#
2.1 mm	100	2.7 µm	ea.	9309A12-T
3.0 mm	100	2.7 µm	ea.	9309A1E-T
2.1 mm	50	2.7 µm	ea.	9309A52-T
3.0 mm	50	2.7 µm	ea.	9309A5E-T

Raptor EXP Guard Column Cartridges

- Free-Turn architecture lets you change cartridges by hand without breaking inlet/outlet fluid connections—no tools needed.
- Patented titanium hybrid ferrules can be installed repeatedly without compromising high-pressure seal.
- Auto-adjusting design provides ZDV (zero dead volume) connection to any 10-32 female port.

Description	Particle Size	qty.	5 x 2.1 mm cat.#	5 x 3.0 mm cat.#	5 x 4.6 mm cat.#
Raptor Biphenyl EXP Guard Column Cartridge	UHPLC	3-pk.	9309U0252	9309U0253	
Raptor Biphenyl EXP Guard Column Cartridge	2.7 µm	3-pk.	9309A0252	9309A0253	9309A0250
Raptor Biphenyl EXP Guard Column Cartridge	5 µm	3-pk.	930950252	930950253	930950250

Maximum cartridge pressure: 1,034 bar/15,000 psi* (UHPLC), 600 bar/8,700 psi (2.7 µm); 400 bar/5,800 psi (5 µm)

* For maximum lifetime, recommended maximum pressure for UHPLC particles is 830 bar/12,000 psi.

Hybrid Ferrule U.S. Patent No. 8201854, EXP Holders U.S. Patent No. 8696902, EXP2 Wrench U.S. Patent No. D766055. Other U.S. and Foreign Patents Pending. The EXP, Free-Turn, and the Opti- prefix are registered trademarks of Optimize Technologies, Inc.

EXP Direct Connect Holder

Description	qty.	cat.#
EXP Direct Connect Holder for EXP Guard Cartridges (includes hex-head fitting & 2 ferrules)	ea.	25808

Maximum holder pressure: 20,000 psi (1,400 bar)
Hybrid Ferrule U.S. Patent No. 8201854, EXP Holders U.S. Patent No. 8696902, EXP2 Wrench U.S. Patent No. D766055. Other U.S. and Foreign Patents Pending. The EXP, Free-Turn, and the Opti- prefix are registered trademarks of Optimize Technologies, Inc.

RESTEK
Pure Chromatography

Questions? Contact us or your local Restek representative (www.restek.com/contact-us).

www.restek.com

Lit. Cat.# FFSS2971A-UNV