Determination of Mycotoxins in Peanuts With Enhanced Matrix Removal—Lipid by LC/MS/MS

Application Note

Food Testing

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Abstract

This application note describes a method for the quantitative determination of 12 mycotoxins in peanut. The sample was initially extracted and cleaned up using an Agilent Bond Elut EMR—Lipid dispersive SPE (dSPE) product. The resultant solution was then analyzed with an Agilent 1290 Infinity LC system coupled to an Agilent 6460 Triple Quadrupole Mass Spectrometer. The method provides limits of quantification (LOQs) for these mycotoxins in peanut, which are lower than the available maximum residue levels (MRLs) currently regulated by China and other regulatory organizations [1,2]. The dynamic calibration ranges for these compounds are obtained from 0.15 to 500 ng/mL. The overall recoveries range from 80 to 110 %, with RSD values below 10 %.

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Introduction

Mycotoxins are toxic secondary metabolites produced by various mold species growing on many agricultural commodities and processed food, either in the field or during storage [3]. Many organizations regulate the levels of mycotoxins in crops such as cereal, corn, milk, and edible oils [4]. The MRLs for aflatoxin B1 in peanut are 8.0 µg/kg, which is indicated in the commission regulation (EC) No 1881/2006 [2].

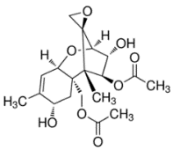
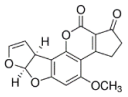
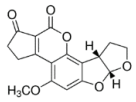
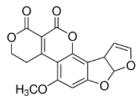
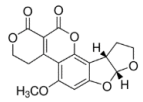
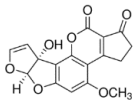
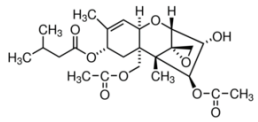
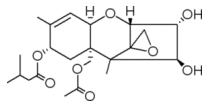
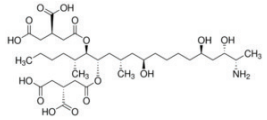
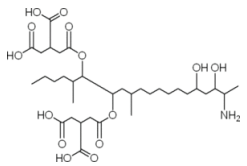
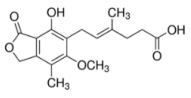
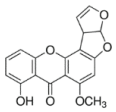
Agilent Bond Elut Enhanced Matrix Removal—Lipid (EMR—Lipid) is a novel sorbent material that selectively removes major lipid classes from the sample without unwanted analyte retention. Removal of lipid interferences from complex matrixes is especially important for techniques such as QuEChERS and protein precipitation. Since these simple sample preparation methods typically do not remove a large percentage of lipids, these co-extractives remain in the final sample extract with the target analytes. These co-extractives cause chromatographic anomalies, poor data precision and accuracy, and increased maintenance issues [5]. This application note uses Agilent Bond Elut QuEChERS EN extraction kits to extract mycotoxins from peanuts, and Agilent Bond Elut EMR—Lipid dSPE to provide further cleanup. Table 1 shows the chemical information for the target mycotoxin compounds.

Experimental

Reagents and chemicals

All reagents were MS grade. Methanol, acetonitrile, and water were from Honeywell. The formic acid and mycotoxin standard compounds neosolaniol (NEO), aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), aflatoxin M1 (AFM1), T2 toxin, HT-2 toxin, fumonisin B1 (FB1), fumonisin B2 (FB2), mycophenolic acid (MPA), and sterigmatocystin (ST) were purchased from Beijing J&K Scientific.

Table 1. Chemical Information for Target Mycotoxins

Name	CAS number	Molecular formula	Structure
Neosolaniol NEO	36519-25-2	$C_{19}H_{26}O_8$	
Aflatoxin B1 AFB1	1162-65-8	$C_{17}H_{12}O_6$	
Aflatoxin B2 AFB2	7220-81-7	$C_{17}H_{14}O_6$	
Aflatoxin G1 AFG1	1165-39-5	$C_{17}H_{12}O_7$	
Aflatoxin G2 AFG2	7241-98-7	$C_{17}H_{14}O_7$	
Aflatoxin M1 AFM1	6795-23-9	$C_{17}H_{12}O_7$	
T2 toxin	21259-20-1	$C_{24}H_{34}O_9$	
HT-2 toxin	26934-87-2	$C_{22}H_{32}O_8$	
Fumonisin B1 FB1	116355-83-0	$C_{34}H_{59}NO_{15}$	
Fumonisin B2 FB2	116355-84-1	$C_{34}H_{59}NO_{14}$	
Mycophenolic acid MPA	24280-93-1	$C_{17}H_{20}O_6$	
Sterigmatocystin ST	10048-13-2	$C_{18}H_{12}O_6$	

Equipment and materials

- Agilent 1290 Infinity LC system
- Agilent 6460 Triple Quadrupole LC/MS/MS system
- Agilent Bond Elut QuEChERS EN extraction kits (p/n 5982-5650)
- Agilent Bond Elut EMR—Lipid dSPE (p/n 5982-1010)
- Agilent Bond Elut EMR—Lipid $MgSO_4$ polish pouch (p/n 5982-0102)
- Agilent ZORBAX Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm (p/n 959757-902)
- AgieInt Bond Elut QuEChERS homogenizers (p/n 5982-9313)
- Spex sample preparation 2010 Geno/Grinder (Metuchen, NJ, USA)

Sample extraction and cleanup

The homogenized peanut sample (5.0 g) was weighed into a 50 mL centrifuge tube. To this tube two ceramic homogenizers, 10 mL of water with 0.2 % formic acid, and 10 mL of acetonitrile were added. Next, the tube was vortexed for 1 minute. Then, an EN QuEChERS extraction salt packet was added to the tube. The sample was then shaken on a Geno Grinder for 1 minute, followed by centrifugation at 4,000 rpm for 5 minutes.

To activate the sorbent, 5 mL of water was added to an Agilent EMR—Lipid tube, before vortexing for 10 seconds. Then 5 mL of the upper layer of the QuEChERS extracted sample were transferred to this tube. The resulting cloudy solution was shaken on a Geno Grinder for 1 minute and centrifuged at 4,000 rpm for 5 minutes. The supernatant solution was then transferred to an empty 50 mL centrifuge tube. Next anhydrous magnesium sulfate from an EMR—Lipid $MgSO_4$ final polish pouch and two ceramic homogenizers were then added to this extract. Then, the tube was shaken on a Geno Grinder for 1 minute and centrifuged at 4,000 rpm for 5 minutes. Finally, 300 µL of the supernatant was transferred to a sample vial with 700 µL of water, before vortexing for 10 seconds.

Figure 1 shows the entire sample preparation workflow.

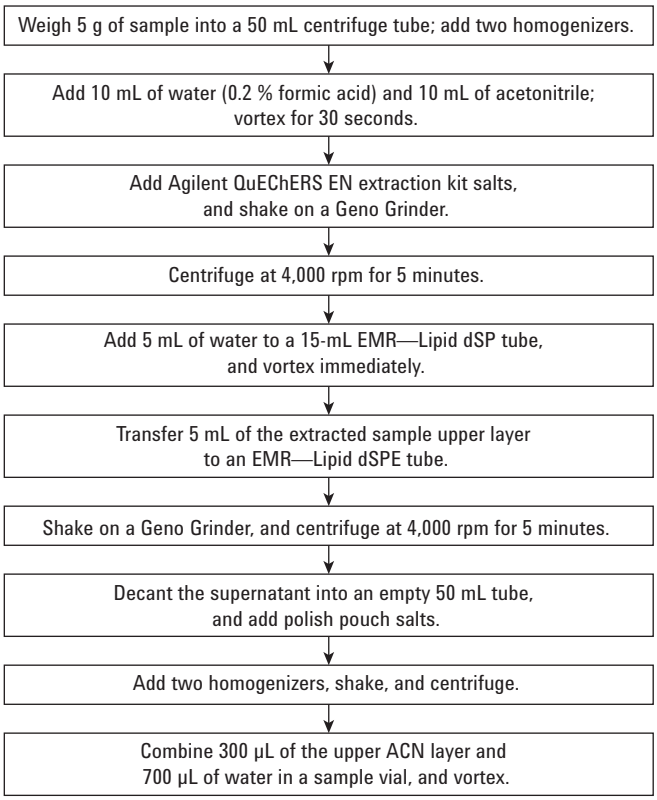


Figure 1. Sample preparation procedure using Agilent Bond Elut EMR—Lipid for the analysis of mycotoxins in peanuts.

Instrument conditions

HPLC conditions

Parameter	Value	
Column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm	
Flow Rate	0.4 mL/min	
Column temperature	40 °C	
Injection volume	10 µL	
Mobile phase	A) Water (0.1 % formic acid) B) Methanol (0.1 % formic acid)	
Gradient:	Time (min)	%B
	0	30
	5	35
	5.5	55
	10	70
	12	90
Gradient program for lipid evaluation	Time (min)	%B
	0	5
	3	30
	10	100
	13	100

MS conditions

These standard compounds were monitored in positive mode. Table 2 shows the source conditions, and Table 3 shows the MRM channels.

Table 2. MS Source Parameters for Selected Compounds

Parameter	Value
Gas temperature	300 °C
Gas flow	6 L/min
Nebulizer	45 psi
Sheath gas temperature	350 °C
Sheath gas flow	12 L/min
Nozzle voltage	Positive: 0 V
Capillary	Positive: 3,500 V

Table 3. Masses Monitored in MRM for Selected Compounds

Compound	RT (min)	Precursor ion	Frag. voltage (V)	Quant ion (CE/V)	Qual ion (CE/V)	Ionization mode
NEO	0.99	400.2	90	215.0 (10)	185.0 (14)	Pos.
AFG2	2.66	331.2	160	313.1 (23)	245.1 (30)	Pos.
AFM1	2.88	329.1	140	273.1 (23)	259.1 (24)	Pos.
AFG1	3.40	329.2	150	243.1 (27)	311.1 (20)	Pos.
AFB2	4.28	315.2	160	287.0 (24)	254.1 (32)	Pos.
AFB1	5.32	313.2	160	241.1 (38)	285.1 (20)	Pos.
HT-2	6.68	447.2	135	345.2 (12)	285.0 (16)	Pos.
FB1	6.87	722.4	180	334.3 (42)	352.3 (36)	Pos.
MPA	7.09	321.1	90	206.9 (20)	302.9 (4)	Pos.
T2	7.43	484.3	125	215.2 (14)	305.3 (6)	Pos.
ST	8.22	325.0	150	281.0 (40)	310.0 (24)	Pos.
FB2	8.40	706.5	180	336.3 (36)	318.3 (38)	Pos.

MS conditions for lipid evaluation

Source parameters

Same as noted previously

Precursor ion scan

Parameter	Value
Product ion	184
MS1 from	100
MS1 to	1,000
Scan time	40
Frag mode	Fixed
Frag	135 V
CE	40 V
Cell acc	7 V

Results and Discussion

Matrix effects

Peanut has various matrix components such as fat, protein, carbohydrates, and vitamins. This complex matrix makes sample preparation more challenging especially since these target compounds are present in low levels. Figure 2 shows the overlay chromatogram for a peanut sample with QuEChERS extraction without cleanup, and with C18/PSA or EMR—Lipid dispersive SPE cleanup.

Using the EMR—Lipid cleanup procedure, lipids and fat were mostly removed. There is no significant ion suppression or enhancement for most of mycotoxins in this study, only NEO and AFM1 show nearly 20 % ion suppression.

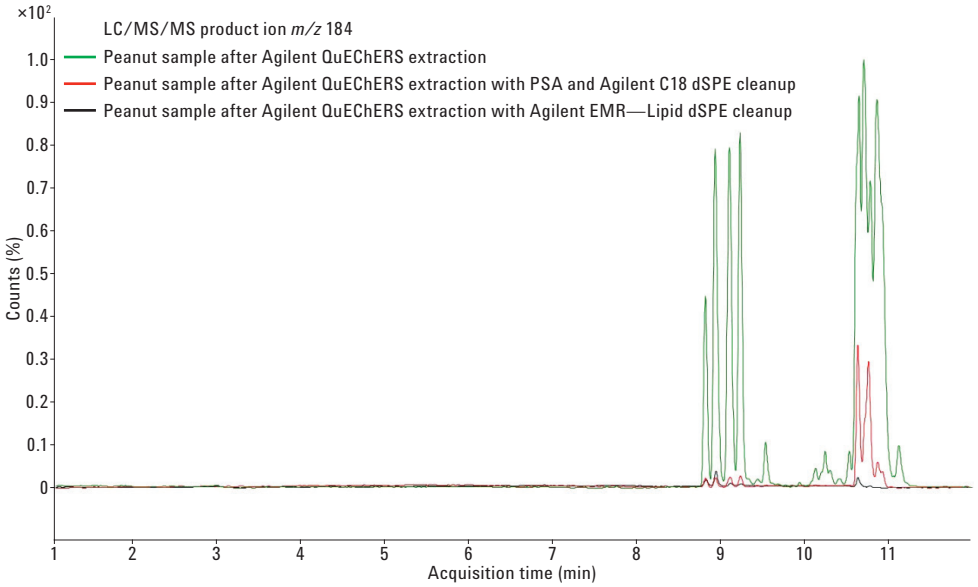


Figure 2. Overlay of chromatograms of peanut samples after extraction with or without cleanup.

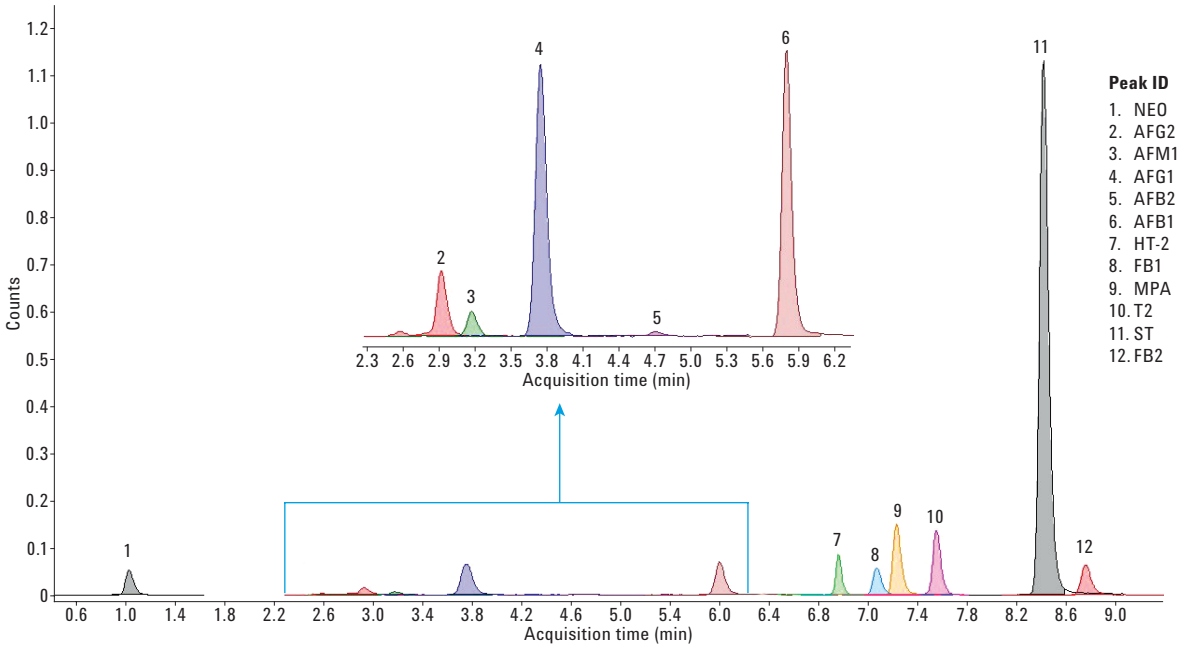


Figure 3. Chromatogram of mycotoxins in a peanut sample after Agilent QuEChERS extraction and Agilent EMR—Lipid dispersive SPE cleanup.

Linearity and limit of detection

The linearity calibration range for all the mycotoxins was 0.15 to 500 ng/mL. Matrix blanks were created by taking peanut through the entire sample preparation procedure. The calibration curves were generated by plotting the responses of analytes to the concentration of analytes. Table 4 shows the linear regression equation, correlation coefficient (R^2), and LOQ for each mycotoxin in this study. LOQs were determined experimentally based on method performance, and are shown in Table 4. European and Chinese maximum levels are 8 or 20 µg/kg.

Recovery and reproducibility

The recovery and reproducibility of this method were determined at three levels, with peanut spiked to a concentration from 4 to 100 ng/g. The analysis was performed with six replicates at each level. The samples were extracted using the procedure in this study. Recoveries of the prespike samples were calculated based on their responses against the matrix-matched calibration curve. Figure 4 and Figure 5 show the recovery and relative standard deviation (RSD) data. The recovery for all 12 mycotoxins at three levels were between 80 to 110 %, with RSDs below 10 %.

Table 4. The LOQ and Linearity of Compounds

Mycotoxin	LOQ (ng/mL)	Linear equation	R^2	Linear range (ng/mL)
NEO	0.75	y = 846.653549x – 116.653431	0.99955	0.75–500
AFG2	0.15	y = 1541.675396x + 79.667158	0.99922	0.15–100
AFM1	0.15	y = 630.536405x + 0.160324	0.99907	0.15–100
AFG1	0.15	y = 7505.657839x – 408.181580	0.99927	0.15–100
AFB2	0.30	y = 141.721922x + 10.841360	0.99944	0.30–100
AFB1	0.15	y = 8247.572097x – 197.129776	0.99954	0.15–100
HT-2	0.75	y = 845.218679x + 126.174819	0.99987	0.75–500
FB1	0.75	y = 1251.068201x – 905.352983	0.99914	0.75–500
MPA	0.75	y = 2460.137601x – 1029.055233	0.99872	0.75–500
T2	0.75	y = 2203.724000x – 520.251804	0.99949	0.75–500
ST	0.75	y = 19781.753359x – 1961.684850	0.99996	0.75–500
FB2	0.75	y = 1855.863338x – 1718.525612	0.99854	0.75–500

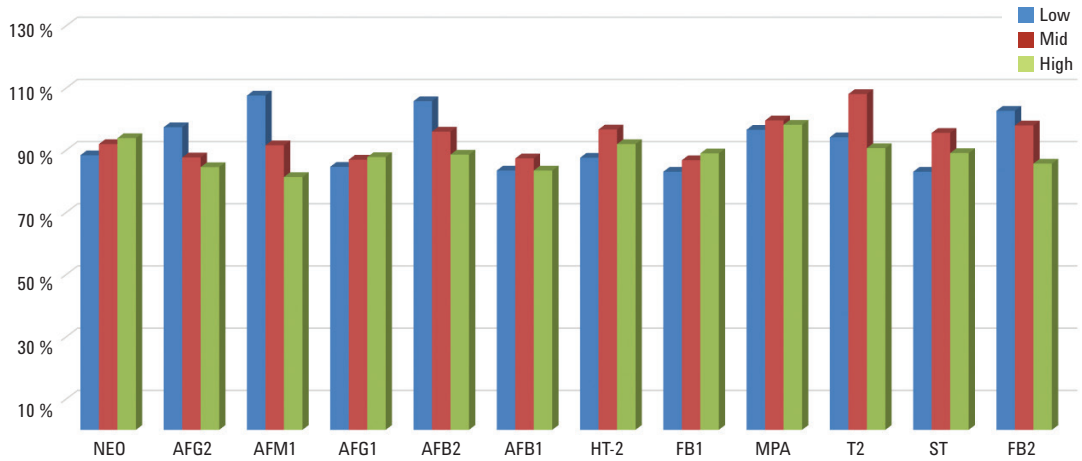


Figure 4. Recovery for 12 mycotoxins in peanut using Agilent EMR—Lipid cleanup. Low: 4 ng/g for aflatoxins (B1,B2,G1,G2,M1), 20 ng/g for other mycotoxins. Mid: 8 ng/g for aflatoxins (B1,B2,G1,G2,M1), 40 ng/g for other mycotoxins. High: 20 ng/g for aflatoxins (B1,B2,G1,G2,M1),100 ng/g for other mycotoxins.

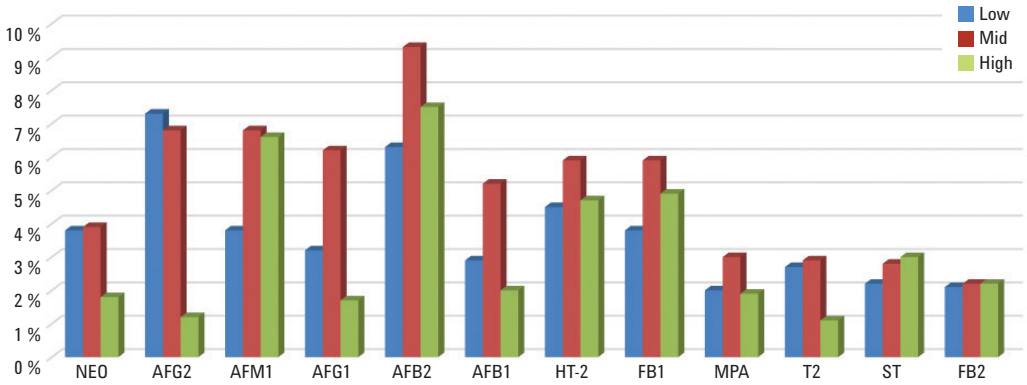


Figure 5. RSD for 12 mycotoxins in peanut using Agilent EMR—Lipid cleanup. Low: 4 ng/g for aflatoxins (B1,B2,G1,G2,M1), 20 ng/g for other mycotoxins. Mid: 8 ng/g for aflatoxins (B1,B2,G1,G2,M1), 40 ng/g for other mycotoxins. High: 20 ng/g for aflatoxins (B1,B2,G1,G2,M1), 100 ng/g for other mycotoxins.

Conclusion

A method for the quantitation of 12 common mycotoxins in peanut was developed using the Agilent EMR—Lipid cleanup with Agilent QuEChERS extraction and LC/MS/MS analysis. This study shows that QuEChERS and EMR—Lipid can be used in combination as an effective method for extraction and purification of mycotoxins in high fat samples such as peanut. The recovery and reproducibility results based on matrix spiked standards exceed the requirements for mycotoxin residue determination in peanut under current regulations. The interference and matrix effect are minimal, and do not interfere with the quantification of any target compound.

References

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Printed in the USA
October 20, 2016
5991-7381EN

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