

Mycotoxin Analysis in Peanut Butter Using Captiva EMR—Lipid Cleanup and LC/MS/MS

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Abstract

Several countries regulate the levels of mycotoxins in foods. With peanut butter, contamination by mycotoxins can occur during the cultivation or storage of peanuts. However, the high fat and protein content of peanut butter can be a considerable challenge in the accurate quantitation of low-level mycotoxins in this matrix. This Application Note describes the determination of 13 multiclass mycotoxins in peanut butter using a Quick Easy Cheap Effective Rugged Safe (QuEChERS) workflow followed by Agilent Captiva EMR—Lipid cartridge cleanup. Due to the high selectivity of the Captiva EMR—Lipid sorbent, excellent recoveries (78.7 to 119.1 %) and precision (<17 %) were achieved for all mycotoxins. This simple and robust methodology requires minimal equipment and expertise, which promotes easy implementation in food laboratories.

Introduction

Mycotoxins are produced as secondary metabolites from fungal species that grow on various crops such as grain, corn, and nuts. Contamination of peanut butter can originate from peanuts that were contaminated with mycotoxins prior to harvest or during storage¹. Many countries, including the United States and in Europe^{2,3}, have regulations for mycotoxins in foodstuffs. Detection and measurement of mycotoxins can be accomplished using various immunoassays or LC/MS methods combined with a sample preparation technique such as immunoaffinity, solid phase extraction (SPE), or stable isotope dilution⁴. However, high fat, complex samples such as peanut butter can be especially problematic due to the relatively low concentrations of target analytes and high concentrations of matrix components, such as proteins and lipids, which interfere with analysis. Immunoaffinity cartridges are expensive, and often specific to the analyte, class, or sample type. Other cleanup products struggle to effectively and selectively remove matrix co-extractives, especially lipids, causing poor reproducibility, matrix effects, and accumulation on the instrument.

Agilent Captiva EMR–Lipid, a lipid removal product, combines size exclusion and hydrophobic interaction to selectively capture lipid hydrocarbon chains without the loss of target analytes. Available in 3- and 6-mL volumes, Captiva EMR–Lipid cartridges provide a simple pass-through cleanup, delivering selective lipid removal from fatty sample extracts for multiclass, multiresidue analysis. A QuEChERS extraction was used for the extraction of 13 mycotoxins from peanut butter. QuEChERS is known for high extraction efficiency for a wide range of analyte classes, but can also extract a large amount of matrix. The Captiva EMR–Lipid cartridges provide high lipid removal and allow accurate quantitation of the target mycotoxins. The method was validated for peanut butter at three spike levels for aflatoxins (AF-B1, B2, G1, G2, and M1), ochratoxins (OTA and OTB), fumonisins (FB1, FB2, and FB3), zearalenone (ZON), mycophenolic acid (MPA), and sterigmatocystin (STC). The method delivered excellent recovery, precision, and sensitivity for trace mycotoxins in this high-fat matrix.

Experimental

Sample preparation

- Captiva EMR–Lipid 3-mL tubes (p/n 5190-1003)
- QuEChERS original extraction salts (p/n 5982-5550)
- VacElut SPS 24 vacuum manifold (p/n 12234022)

LC configuration and parameters

Configuration	
Agilent 1290 Infinity II high-speed pump (G7120A)	
Agilent 1290 Infinity II multisampler (G7167B)	
Agilent 1290 Infinity II multicolumn thermostat (G7116B)	
Analytical column	• Agilent InfinityLab Poroshell 120, EC-C18, 2.1 × 100 mm, 2.7 μm, LC column (p/n 695775-902) • Agilent InfinityLab Poroshell 120, EC-C18, 2.1 × 5 mm, 2.7 μm, guard column (p/n 821725-911)
Column temperature	40 °C
Injection volume	5 μL
Mobile phase A	5 mM Ammonium formate in H ₂ O + 0.1 % formic acid
Mobile phase B	1:1 Acetonitrile:methanol + 0.1 % formic acid
Flow rate	0.5 mL/min
Gradient	Start at 5 %B, Hold 1 minute, Then ramp from 50 to 60 %B at 4 minutes, Then to 98 %B at 7 minutes, Hold 1 minute
Post time	2 minutes
Needle wash	1:1:1 H ₂ O:ACN:isopropanol for 10 seconds
Vials	2-mL vial (p/n 5190-4044) PTFE cap (p/n 5182-0725) Insert (p/n 5183-2086)

MS/MS configuration

Configuration	
Agilent 6490 triple quadrupole LC/MS with Agilent Jet Stream	
MS/MS mode	Dynamic MRM
Ion mode	Positive/negative
Drying gas temperature	250 °C
Drying gas flow	8 L/min
Nebulizer pressure	40 psi
Sheath gas temperature	350 °C
Sheath gas flow	11 L/min
Capillary voltage	5,000 V
EMV	500 V(+) 0 V(-)
Nozzle voltage	1,500 V(+) 0 V(-)

MS/MS parameters

Compound	Precursor ion	Quantifier ion (CE)	Qualifier ion (CE)	Fragment (V)	Retention time (min)
Aflatoxin M1	329.1	313.0 (24)	115.1 (88)	135	1.842
Aflatoxin G2	331.1	313.0 (24)	115.1 (88)	165	1.916
Aflatoxin G1	329.1	243.2 (24)	200.0 (44)	175	2.018
Aflatoxin B2	315.1	287.0 (28)	259.0 (32)	175	2.104
Aflatoxin B1	313.1	285.2 (24)	128.1 (84)	170	2.223
Fumonisin B1	722.4	352.3 (36)	334.4 (44)	200	2.810
Ochratoxin B	370.0	205.0 (16)	120.1 (96)	120	3.200
Mycophenolic acid	321.1	302.9 (4)	206.9 (20)	90	3.235
Fumonisin B3	706.4	336.3 (36)	318.5 (40)	200	3.676
Zearalenone	317.1	175 (24)	131 (28)	175	4.217
Fumonisin B2	706.4	336.3 (36)	318.5 (40)	200	4.398
Ochratoxin A	404.1	239.0 (24)	120.1 (96)	120	4.398
Sterigmatocystin	325.0	310.0 (24)	281 (40)	150	4.525

Chemicals and reagents

Food samples bought from a local grocery store were used for method quantitation and matrix-removal studies. Standards and internal standards were purchased as premixed solutions from Sigma-Aldrich (St Louis, MO, USA) or Romer Labs (Getzersdorf, Austria). LC solvents were bought from Honeywell (Muskegon, MI, USA).

Validation study

The validation of mycotoxins in peanut butter was carried out in batches consisting of two double blanks, two blanks, six calibrators, and three QC levels. QCs were prespiked as shown in Table 1 in replicates of six ($n = 6$), and injected in between two sets of calibration curves. Calibration curves were generated using six levels as follows:

- 0.25, 1, 5, 10, 20, and 40 ng/mL for AF-B1, AF-B2, AF-G1, AF-G2, MPA, OTA, STC, and ZON
- 0.125, 0.5, 2.5, 5, 10, and 20 ng/mL for AF-M1 and OTB
- 1.25, 5, 25, 50, 100, and 200 ng/mL for FB1, FB2, and FB3

Isotopically labeled internal standard $^{13}\text{C}_{17}$ -AF-B1 was spiked at 5 ng/mL.

Sample preparation detailed procedure

Calibrators and QCs were prespiked at appropriate levels, and thoroughly soaked into the peanut butter matrix (5 g) for at least one hour before extraction. Next, 10 mL of water were added and allowed to soak into the sample. The sample was extracted with 10 mL of acetonitrile with 2 % formic acid and QuEChERS original salts (4 g MgSO_4 , 1 g NaCl) using vertical shaking on a Geno/Grinder for 10 minutes. This was followed by centrifugation at 5,000 rpm for five minutes. The upper acetonitrile layer (8 mL) was transferred to a

Table 1. Sample QC concentrations.

Analyte	LQ (ng/g)	MQ (ng/g)	HQ (ng/g)
Aflatoxin B1 (AF-B1)	2	10	20
Aflatoxin B2 (AF-B2)	2	10	20
Aflatoxin G1 (AF-G1)	2	10	20
Aflatoxin G2 (AF-G2)	2	10	20
Aflatoxin M1 (AF-M1)	1	5	10
Fumonisin B1 (FB1)	10	50	100
Fumonisin B2 (FB2)	10	50	100
Fumonisin B3 (FB3)	10	50	100
Mycophenolic acid (MPA)	2	10	20
Ochratoxin A (OTA)	2	10	20
Ochratoxin B (OTB)	1	5	10
Sterigmatocystin (STC)	2	10	20
Zearalenone (ZON)	2	10	20

clean 15-mL tube, diluted with 2 mL of water (20 % water by volume), and vortexed. The extract (2.5 mL) was loaded onto a 3-mL Captiva EMR–Lipid tube, and allowed to flow under gravity. Once the extract had completely eluted through the Captiva EMR–Lipid tube (approximately 10 minutes), vacuum was applied and ramped from 1–10 in. Hg to drain the tube. For prespiked samples, 0.500 mL of eluent was transferred to autosampler tubes, and 0.300 mL of 5 mM ammonium formate with 0.1 % formic acid was added. Matrix-matched calibrants were prepared by transferring 0.500 mL of blank eluent to autosampler tubes, and 0.270 mL of 5 mM ammonium formate with 0.1 % formic acid and 0.030 mL of appropriate working standards.

Results and discussion

Linearity

The data were processed with Agilent MassHunter quantification software. Calibration curves gave R² values between 0.993 and 0.998 for 13 mycotoxins using linear regression fit and 1/x² weighting. The accuracy of all calibrators was within ±10 % of expected values.

Accuracy and precision results

The study produced outstanding results, as shown by the summary in Table 2. Recovery for all QCs was 70 to 120 %, and %RSD was <20 at all levels, with most <10. Due to poor extractability using acetonitrile, fumonisins were the only challenging class of mycotoxin in this study. Optimization revealed that the addition of 2 % formic acid greatly enhanced analyte solubility without adversely affecting other classes.

Table 2. Recovery and precision results for 13 mycotoxins in peanut butter (n = 6).

Analyte	Peanut butter					
	LQ		MQ		HQ	
	%Recov.	%RSD	%Recov.	%RSD	%Recov.	%RSD
Aflatoxin M1	100.9	6.4	93.5	5.4	95.6	6.3
Aflatoxin G2	103.4	9.4	101.3	6.3	101.0	6.5
Aflatoxin G1	103.7	5.4	99.7	6.0	103.0	5.6
Aflatoxin B2	105.7	3.1	97.0	4.4	99.2	4.9
Aflatoxin B1	99.5	7.6	95.2	3.1	98.6	4.0
Fumonisin B1	119.1	12.3	108.6	4.9	114.1	10.1
Ochratoxin B	109.8	6.0	102.1	4.9	102.2	5.0
Mycophenolic acid	113.8	7.5	100.0	4.4	101.3	4.3
Fumonisin B3	94.4	16.2	102.1	11.7	113.6	9.1
Zearalenone	103.0	8.3	101.2	3.0	97.2	6.8
Fumonisin B2	78.7	10.2	90.1	8.7	92.7	9.5
Ochratoxin A	103.3	5.9	98.2	5.4	100.2	4.8
Sterigmatocystin	90.3	5.7	84.6	3.9	87.3	4.1

EMR–Lipid mechanism

The EMR–Lipid selectivity is attributed to the combined mechanism of size exclusion and hydrophobic interaction. Lipids possess a linear, unbranched hydrocarbon chain, which is sufficiently small enough to enter the EMR–Lipid sorbent. Once inside the sorbent, the lipids are trapped in place by hydrophobic interaction. Most analytes do not contain a linear, unbranched hydrocarbon chain, and will not enter the sorbent, remaining in solution for analysis. Shorter hydrocarbon chains (<six carbons) are not as strongly bound by EMR–Lipid, and are not removed as efficiently as longer lipids. The unique EMR–Lipid mechanism is well suited to multiclass, multiresidue analysis where matrix interferences are targeted instead of diverse groups of analytes.

Monitoring matrix removal by GC/MS

Peanut butter contains both protein and various classes of lipids. Proteins are effectively removed during the acetonitrile-based QuEChERS extraction. Although the validation is accomplished using LC/MS, the GC/MS full scan comparison of sample cleanups can give valuable information regarding the removal of matrix and lipids. Figure 1 shows the GC/MS full scan chromatogram of peanut butter before and after cleanup with Captiva EMR–Lipid. The black trace is the chromatogram generated from no sample cleanup, and represents lipids as well as other matrix co-extractives. Peanut butter after Captiva EMR–Lipid cleanup (green) shows 60.4 % removal, calculated using Equation 1. While later-eluting matrix is completely removed, early-eluting matrix is significantly reduced but not completely removed.

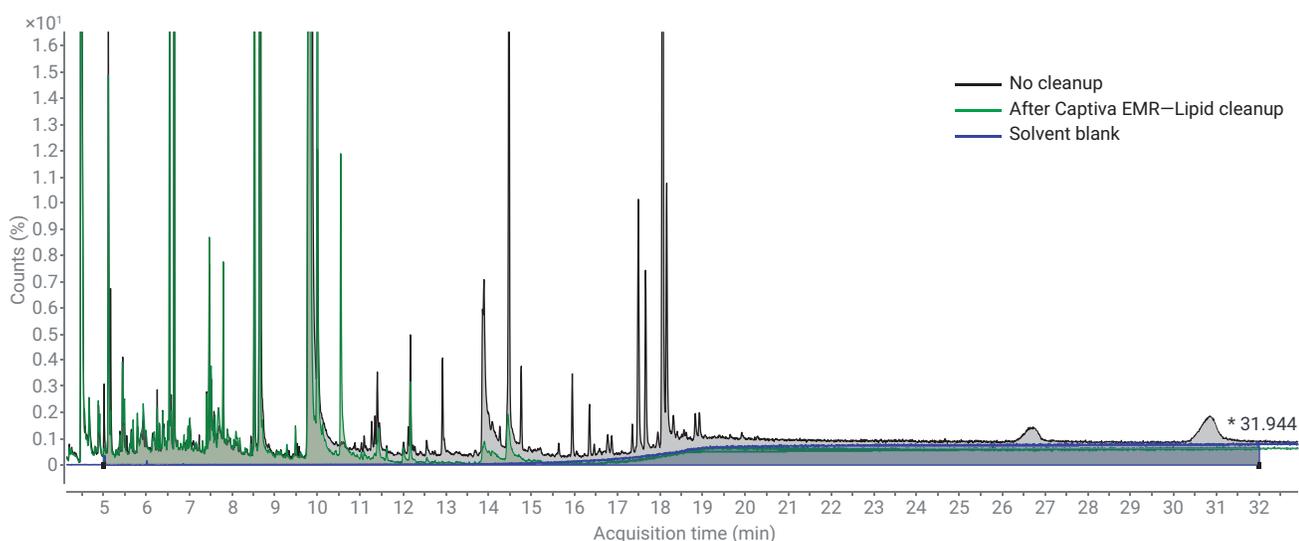


Figure 1. Matrix removal evaluation using a GC/MS full scan chromatogram comparison of peanut butter sample before cleanup and after Captiva EMR–Lipid cleanup.

$$\% \text{ Matrix Removal} = \frac{(\text{Peak Area}_{\text{Blank no cleanup}} - \text{Peak Area}_{\text{Blank Captiva cleanup}})}{(\text{Peak Area}_{\text{Blank no cleanup}} - \text{Peak Area}_{\text{Reagent blank}})} \times 100$$

Equation 1. Calculation for percent matrix removal using total peak area from chromatograms.

Conclusion

This work demonstrates that Captiva EMR–Lipid is an easy and effective cleanup option for multiclass mycotoxin analysis. Validation of peanut butter gave excellent recovery (78.7 to 119.1 %), precision (<17 %), and sensitivity down to 1 ng/g. Efficient cleanup was demonstrated through a GC/MS full scan. Matrix removal for lipids and analyte recovery was high for a wide variety of applications, some of which extends beyond the scope of this work^{5,6}. Captiva EMR–Lipid represents a new generation in selective lipid cleanup for multiclass, multiresidue analysis, and is ideal for laboratories looking to simplify sample preparation while improving method performance.

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