Multi-Residue Analysis of Mycotoxins with Selective Lipid Removal using Captiva EMR-Lipid

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Introduction

Hard cheeses can be contaminated by mycotoxins through secondary metabolites of unwanted fungal species or through indirect milk contamination. Sample preparation of foods for multi-class mycotoxin analysis include a general extraction such as QuEChERS followed by analysis using LC or GC-MS/MS. The high fat content of cheese can present challenges for the accurate quantitation of mycotoxins at low levels. Many cleanup materials struggle to effectively and selectively remove co-extractives, especially lipids, causing poor reproducibility, matrix effects, and instrument maintenance. Captiva EMR-Lipid overcomes the limitations of traditional sample cleanups by providing an easy to use and selective lipid removal platform for fatty samples with minimal analyte retention. Available in 3 and 6 mL, Captiva EMR-Lipid allows pass-through cleanup of fatty sample extracts. Cleaned extracts can be directly injected onto the LC-MS or post-treated as necessary to meet method requirements. This work describes the validation of 13 mycotoxins in blue and parmesan cheese using a QuEChERS workflow followed by cleanup with Captiva EMR-Lipid.

Experimental

Sample Preparation

EXTRACTION
- Add 2 g of cheese to a 50 mL centrifuge tube
  spike as required
- Add 10 mL water and soak for 5 min
- Add 10 mL ACN with 2% formic acid and shake on Geno/Grinder for 20 min
- Add Original QuEChERS salts, vertical shake 2 min, and centrifuge for 5 min

CLEANUP
- Transfer 8 mL extract to clean tube and add 2 mL water (20% water)
- Load 2.5 mL onto Captiva EMR-Lipid 3 cc tube
- Gravity flow elution for ~10 min then drain tube with 10 in. Hg vacuum for 20 s

CONC.
- Transfer 1.25 mL to test tube and dry under N_2 at 40°C
- Reconstitute with 0.2 mL 85:15 buffer:ACN for LC-MS/MS analysis.

Experimental

Captiva EMR-Lipid Pass-through Workflow

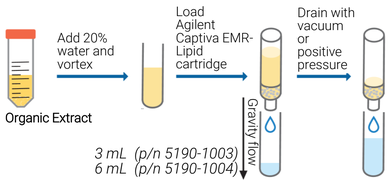


Figure 1. Dilute extract with 20% water, load it onto the EMR-Lipid tube, and elute with gravity.

LC-MS/MS Instrument Conditions

LC-MS/MS: Agilent 1290 Infinity II LC, 6460 Triple Quadrupole LC/MS/MS, Jet Stream ESI
Ionization: Positive/Negative

Table 1. Source Parameters.

Parameter	Value (+)
Gas Temp (°C)	250
Gas Flow (l/min)	8
Nebulizer (psi)	40
Sheath Gas Heater	350
Sheath Gas Flow	11
Capillary (v)	5000

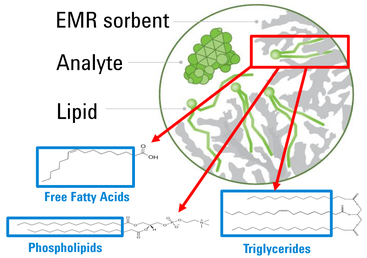
Column: Poroshell C18-EC, 2.1 x 100 mm, 2.7 µm
Injection volume: 5 µL
Mobile Phase:
A: Water, 5 mM Ammonium Formate, 0.1% FA
B: 1:1 ACN:Methanol, 5 mM Formate, 0.1% FA
Flow Rate: 0.5 mL/min

Table 2. LC gradient timetable.

	Time, min	A	B
1	0.00	95.0%	5.0%
2	1.00	60.0%	50.0%
3	4.00	40.0%	60.0%
4	7.00	2.00%	98.0%

Results and Discussion

EMR-Lipid Removal Mechanism



- **Size exclusion** – Unbranched hydrocarbon chains (lipids) enter the sorbent; bulky analytes do not.
- **Sorbent chemistry** – Lipid chains that enter the sorbent are trapped by hydrophobic interactions.

Matrix Removal Evaluation

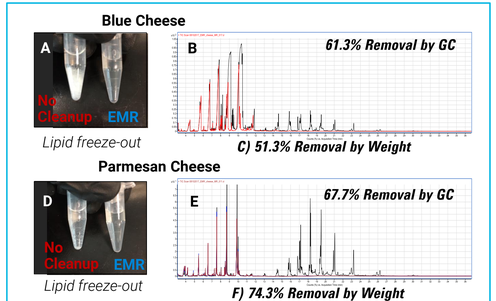


Figure 2. Sorbent mechanism and matrix removal comparison of cheese extracts before and after Captiva EMR-Lipid cleanup.

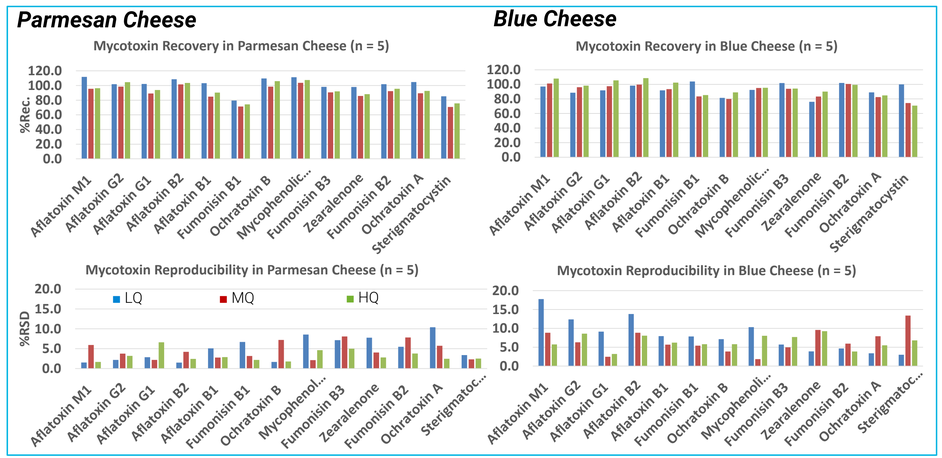


Figure 3. Mycotoxin recovery and precision data in blue and parmesan cheeses.

Results and Discussion

Captiva EMR-Lipid Matrix Removal

The lipid removal mechanism of EMR-Lipid is described as a combination of size exclusion and hydrophobic interaction. Matrix removal was assessed using a variety of techniques, shown in Figure 2. Pictures A and D show precipitated lipids in untreated samples after 1 hour of freezing at 0°C. No precipitates are observed in EMR treated samples. GC-MS fullscan chromatograms B and E show the reduction in matrix profile after EMR treatment. A residue weight comparison (E and F) also indicated significant matrix removal using EMR cleanup.

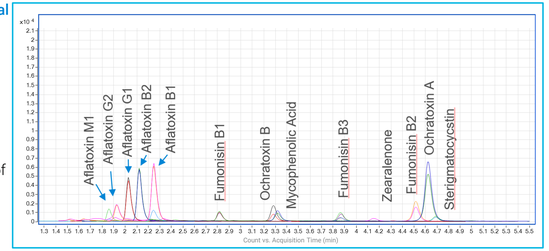


Figure 4. LC/MSMS Chromatogram for 13 mycotoxins in the Study, 5 ppb.

Validation of Mycotoxins in Cheese

Cheese samples were pre-spiked with standards (table 3) and I.S. (^{13}C AF-B1) prior to extraction and prepared in replicates of 5. Blank samples were taken through the sample preparation and post-spiked for matrix matched calibrators. Two calibration curves were generated for each matrix and run before and after the QC's to verify calibration reproducibility. Recoveries were measured between 70.7% -111.8% with %RSD <20 for all compounds with the vast majority <10% (Figure 3). Fumonisins are poorly extracted with 100% ACN, however the ACN with 2% formic acid additive gave good recovery. Blue cheese proved to be fattier than parmesan and required a 2 g sample size. In order to reach the low detection limits, especially for AF-M1, evaporation and reconstitution was used as a final concentration step.

Table 3. Mycotoxin concentrations and calibration ranges.

Analyte	LQ (ng/g)	MQ (ng/g)	HQ (ng/g)	Calibration Range (ng/mL)
Aflatoxin B1	1	5	10	0.25 – 40
Aflatoxin B2	1	5	10	0.25 – 40
Aflatoxin G1	1	5	10	0.25 – 40
Aflatoxin G2	1	5	10	0.25 – 40
Aflatoxin M1	0.5	2.5	5	0.125 – 20
Fumonisin B1	5	25	50	1.25-200
Fumonsin B2	5	25	50	1.25-200
Fumonisin B3	5	25	50	1.25-200
Mycophenolic acid	1	5	10	0.25 – 40
Ochratoxin A	1	5	10	0.25 – 40
Ochratoxin B	0.5	2.5	5	0.125 – 20
Sterigmatocystin	1	5	10	0.25 – 40
Zearalenone	1	5	10	0.25 – 40

Conclusions

- The NEW EMR-Lipid chemistry gave highly effective and efficient lipid removal from high fat cheeses with an easy pass-through cartridge format without the need for conditioning or washing of the sorbent.
- Validation of 13 mycotoxins in cheese gave acceptable mycotoxin recoveries (70-120%) and RSD < 20%.
- Captiva EMR-Lipid allows selective removal of lipids from fatty sample extracts and is amenable to a variety of multi-residue analysis applications including mycotoxins, veterinary drugs, and pesticides.