The Analysis of Mycotoxins in CBD Oils by LC-MS/MS

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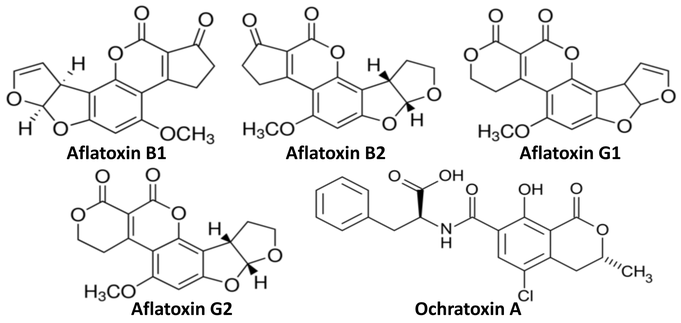
Introduction

Fungi that readily colonize crops such as cannabis are capable of producing secondary metabolites known as mycotoxins that can cause disease and death in humans. The two primary types of mycotoxins associated with cannabis are aflatoxins and ochratoxins (Figure 1). These mycotoxins are produced by the fungal species *Aspergillus flavus* and *Aspergillus parasiticus* for aflatoxins and *Penicillium verrucosum* as well as *Aspergillus ochraceus* for ochratoxins. Crops are susceptible to contamination from seed through storage with most effort focused on mitigation during harvest and storage. The thermal stability of mycotoxins presents a particular challenge as the likelihood is high that existing crop contamination will persist and concentrate during processing into oil and extracts causing levels to rise above state limits.

Figure 1: Chemical Structures of Aflatoxins and Ochratoxin A

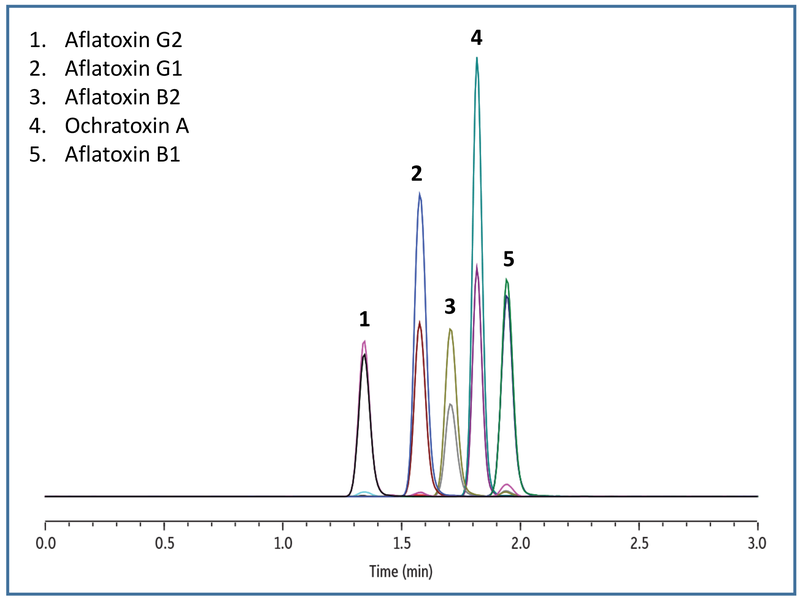


In the analysis of mycotoxins, particularly in the food industry, the use of immunoaffinity columns (IACs) for sample preparation is common. IACs contain monoclonal antibodies that are cross reactive towards specific mycotoxins resulting in a highly selective sample cleanup. When not used, significant matrix interferences have been shown to elute near target mycotoxins resulting in an adverse effect on measured ion ratios. However, IACs also contribute to the overall complexity, time, and cost associated with sample analysis. In this study, a simple pass-through SPE sample cleanup was developed as an alternative to IACs and applied to lipid-rich CBD oils. The simplified pass-through SPE sample cleanup was then paired with a rapid LC-MS/MS analysis with a total cycle time of only three minutes.

Method Development - Chromatography

Before the addition of matrix, chromatograms were developed in solvent only. It was found during compound tuning that the analytes formed both protonated and sodium adducts in a 1:1 ratio in the presence of 0.1% formic acid (v/v) modified mobile phases. In order to increase the signal response, the analytes were driven to their protonated form by the addition of 2 mM ammonium formate to the 0.1% formic acid (v/v) modified mobile phases. Concentrations above 2 mM ammonium formate began to suppress ionization.

Figure 2: Analysis of Mycotoxins in Solvent



During column scouting, it was found that Raptor Biphenyl provided the best separation characteristics. The unique selectivity of the phase combined with the speed of superficially porous particles enabled the analysis to be performed in two minutes with a complete cycle time of only three minutes (Figure 2).

Analytical Method

Table 1: Analytical Conditions

Column:	Raptor Biphenyl 2.7 µm, 50 mm x 2.1 mm (cat.# 9309A52)		
Guard Column:	Raptor Biphenyl EXP Guard Column Cartridge 2.7 µm, 5 x 2.1 mm (cat.# 9309A0252)		
Mobile Phase A:	Water, 2 mM Ammonium Formate, 0.1% Formic Acid		
Mobile Phase B:	Methanol, 2 mM Ammonium Formate, 0.1% Formic Acid		
Time Program:	Time (min.)	Flow (mL/min.)	%B
	0.00	0.7	65
	2.00	0.7	90
	2.01	0.7	65
	3.00	0.7	65
Oven Temp.:	35 °C		
Sample Temp.:	10 °C		
Inj. Volume:	5 µL		
MS/MS:	Shimadzu LCMS-8045		
Ion Mode:	ESI+		

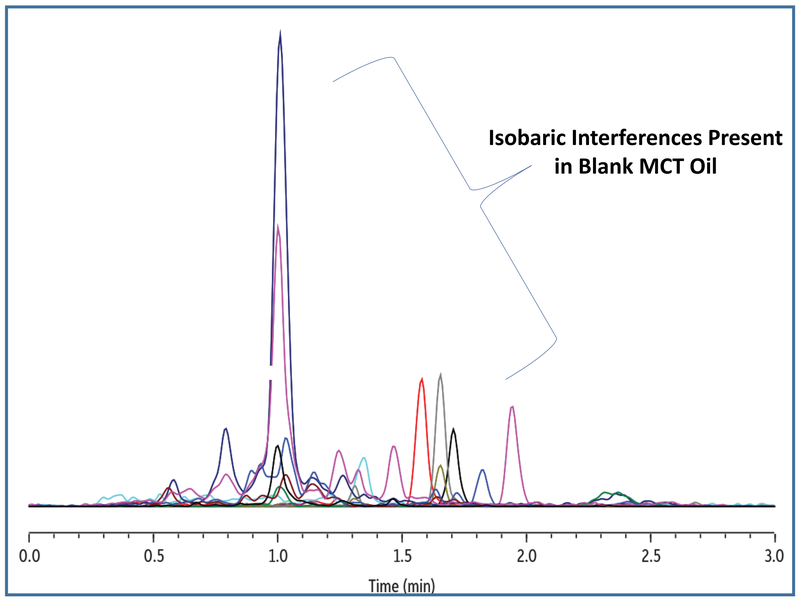
Table 2: Analyte Transitions

Analyte	Precursor Ion	Product Ion (Quantifier)	Product Ion (Qualifier)
Aflatoxin G2	331.2	189.3	115.2
Aflatoxin G1	329.2	243.2	215.3
Aflatoxin B2	315.3	287.2	243.3
Ochratoxin A	404.3	239.1	358.3
Aflatoxin B1	313.2	241.2	128.2

Method Development – Sample Preparation

Initial attempts to simplify sample preparation were based on dilute-and-shoot preparations. Both acetonitrile and methanol were utilized in combination with varying portions of water, buffer salt, and acid in order to produce a clean extract. Regardless of the extraction solvent, this approach always produced chromatograms containing large amounts of isobaric interferences resulting in both false positives for unspiked MCT oil and non-detects for spiked MCT oil (Figure 3).

Figure 3: Isobaric Interferences Present in Blank MCT Oil (Dilute-and-Shoot)



Since oil-based cannabis products rely on carrier oils such as coconut-derived MCT oil and sunflower oil to deliver cannabinoids, these products will always contain high levels of lipids. In order to address these isobaric matrix interferences from lipid-rich MCT oil, a simple pass-through SPE sample cleanup was attempted. A matrix break-through study was peformed using 1mL, 100 mg Resprep Bonded Reversed Phase SPE Cartridges (cat.# 26030) in order to determine the organic composition that enabled the elution of mycotoxins while retaining lipids. It was determined that an extraction solvent of 45:55 Water:Methanol resulted in extracts with isobaric interferences removed while still recovering spiked mycotoxins.

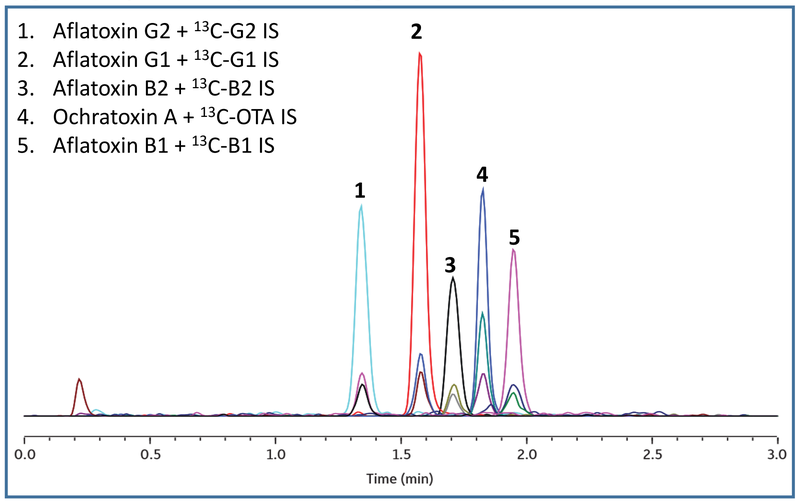
Sample Preparation

Working calibration solutions containing Aflatoxin B1, B2, G1, and G2 and Ochratoxin A were prepared from 50.0 – 5,000 ng/mL in methanol. 0.25 g of MCT oil was spiked to 2.00 – 200 ng/g using 10 µL of the working calibration solution. A working internal standard was prepared using ^{13}C labeled analogs at a concentration of 250 ng/mL in methanol. 10 µL of the working internal standard was aliquoted into the sample followed by vortexing for 10 seconds at 3,000 rpm. 1 mL of 45:55 H2O:MeOH was added to the sample. The sample was vortexed for 30 seconds at 3,000 rpm. The sample was then centrifuged at 3,000 xg for 5 min. at 10 °C. 750 µL of the supernatant was transferred to a conditioned (1 mL 45:55 water:methanol) 1 mL, 100 mg Resprep bonded reversed phase SPE cartridge. The sample was pulled through under vacuum into an autosampler vial for LC-MS/MS analysis.

Results and Discussion

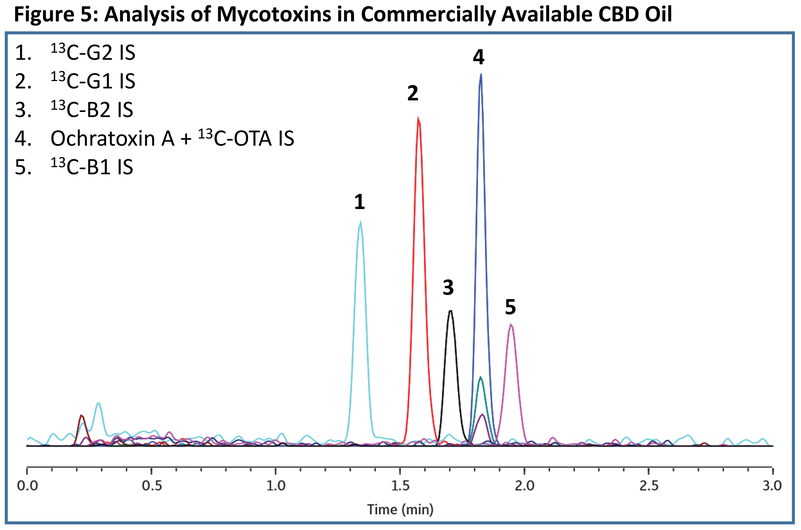
The Resprep bonded reversed phase SPE cartridge effectively removed isobaric matrix interferences resulting in passing ion ratios and S:N > 10:1 for all analytes at a spiked concentration of 2 ng/g (Figure 4). The accuracy of QC samples (including QC LLOQ) ranged from 82.7% – 113% while the %RSD of QC samples (including QC LLOQ) ranged from 0.878% – 15.8% with r^2 values of 0.995 and above.

Figure 4: Mycotoxins Spiked at 2 ng/g in MCT Oil (Pass-through SPE)



The method was applied to a commercially available CBD oil which resulted in the detection of Ochratoxin A with passing ion ratios, with the reported result below the LOQ. (Figure 5).

Figure 5: Analysis of Mycotoxins in Commercially Available CBD Oil



Conclusions

A simple pass-through SPE sample cleanup was developed for the analysis of mycotoxins in lipid-rich CBD oils. Isobaric matrix interferences were removed and measured ion ratios remained consistent between injections and carrier oil types enabling confident identification and quantitation of mycotoxins. The sample preparation was paired with a rapid two minute analysis time using a Raptor Biphenyl analytical column with a total cycle time of only three minutes. Detection limits of 2 ng/g were easily achieved with the use of ammonium formate and formic acid modified mobile phases to drive protonated adduct formation, matrix removal during SPE, and excellent peak shapes.