Supelco®
Analytical Products

Application Note

Sensitive, Quick LC/MS/MS Analysis of Aflatoxins in Cannabis

Using Supel™ Tox AflaZea SPE and an Ascentis® Express Phenyl-Hexyl HPLC Column

Quantitative analysis of mycotoxins commonly involves sampling, sample preparation, extraction, and cleanup followed by chromatographic methods such as GC and HPLC. The matrix complexity of cannabis often makes sample cleanup methods used for common commodities ineffective. In this study, a sample preparation method using Supel™ Tox AflaZea SPE cartridges for cleanup of cannabis extracts prior to LC/MS/MS analysis of aflatoxins was developed. Supel™ Tox AflaZea cartridges utilize the "interference removal" strategy, requiring few processing steps and saving time by eliminating wash steps prior to analyte elution. The Ascentis® Express Phenyl-Hexyl HPLC column provided the selectivity to separate four aflatoxin compounds in cannabis.

Experimental

Dried cannabis sample was obtained courtesy of Dr. Hari H. Singh, Program Director at the Chemistry and Physiological Systems Research Branch of the National Institute on Drug Abuse at the National Institute of Health. The sample was ground to a fine powder and extracted following the procedure outlined in the condition section of **Figure 2**. Pictures of the cannabis samples before, during, and after cleanup are shown in **Figure 1**. Matrix-matched calibration curves were constructed and run along with solvent-based calibration curves to compare ionization effects and sample cleanliness.

Figure 1. Photos of the Cannabis Samples (A) Before Cleanup, (B) On Supel™ Tox AflaZea SPE Cartridge, and (C) After Cleanup

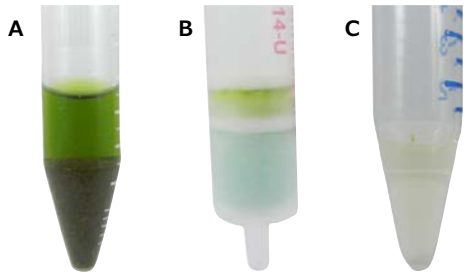
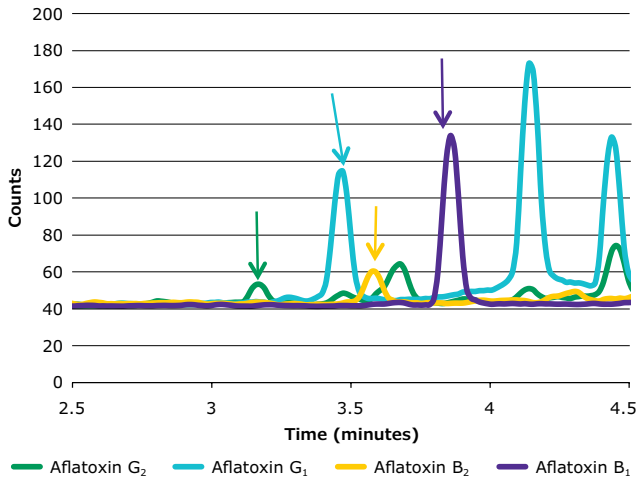


Figure 2. LC/MS/MS Chromatogram of Aflatoxins Spiked Into Cannabis Sample at 24.4 ppb (Aflatoxin B_1 and G_1), and 6.1 ppb (Aflatoxin B_2 and G_2)

sample/matrix:	cannabis extract spiked with Aflatoxin B_2 and G_2 at 6 ppb and Aflatoxin B_1 and G_1 at 24 ppb each
sample pretreatment:	add 10 mL of extraction solvent, acetonitrile:water (86:14), to a 15 mL centrifuge containing 0.5 g of ground cannabis. Mix on a shaker for 30 minutes, and then centrifuge at 3000 rpm for 5 minutes.
SPE tube:	Supel™ Tox AflaZea Cartridge, 6 mL (55314-U)
sample addition:	2 mL of sample extract spiked with the mixture of aflatoxins
elution:	apply strong vacuum and collect the sample into plastic tube
eluate post-treatment:	dilute 0.2 mL of the extract with 0.8 mL distilled water and mix well; use silanized vials for analysis
column:	Ascentis® Express Phenyl-Hexyl, 5 cm x 2.1 mm I.D., 2.7 µm (53334-U)
mobile phase:	(A) 5 mM ammonium formate with 1% formic acid in water; (B) 5 mM ammonium formate with 1% formic acid in methanol
gradient:	Min %A %B 0.0 70 30 3.0 40 60 5.0 0 100 7.0 0 100 7.1 70 30 8.5 70 30
flow rate:	0.40 mL/min
pressure:	380 bar
temp.:	40 °C
det.:	MS, ESI(+), MRM 331.3/189.0, 329.1/243.0, 315.9/259.0, 313.1/241.0
injection:	10 µL
instrument:	Agilent® 1290/6460 LC/MS/MS



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Results and Discussion

Matrix Removal

The chromatographic separation of the aflatoxins was performed on an Ascentis® Express Phenyl-Hexyl HPLC column (**Figure 2**). Cannabis matrix effects were determined by comparison of the calibration curves constructed in solvent versus those in extract. Significant ion suppression was observed in the cannabis samples, and the matrix-matched calibration curves were required for accurate quantitation. The matrix effects can be attributed to the complex cannabis composition and the limited capacity of the Supel™ Tox AflaZea SPE for removal of all of the components.

Analyte recovery values from spiked cannabis extracts fall in the range of 102–127% with RSD below 12% for three replicates (**Table 1**). Excellent recovery values were even observed for Aflatoxins B_2 and G_2 that were spiked at a low level of 6.1 ppb.

Table 1. Percent Recovery for Aflatoxins from Cannabis (*n*=3)

	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2
Recovery (%)	102	109	108	127
RSD% (*n*=3)	8	12	3	9

* versus matrix-matched calibration curve

Conclusions

A sample preparation method utilizing Supel™ Tox AflaZea SPE cartridges was developed for the cleanup of cannabis extracts prior to HPLC analysis. SPE, used according to the standard methodology, contributed to a simple, economical, quick analysis. This SPE methodology, in combination with LC/MS/MS detection and the selectivity of the Ascentis® Express Phenyl-Hexyl HPLC column, allowed for sensitive detection of four aflatoxin compounds in cannabis with recoveries of 102–127% at 6–25 ppb levels. Therefore, this current analytical method utilizing SPE, UHPLC, and MS/MS detection can be used successfully for testing aflatoxins at 5–50 ppb in cannabis.

Featured and Related Products

Description	Cat. No.
SPE Tube	
Supel™ Tox AflaZea SPE Cartridge 6 mL, pack of 30	**55314-U**
HPLC Column	
Ascentis® Express Phenyl-Hexyl 5 cm × 2.1 mm I.D., 2.7 µm particle size	**53334-U**
Standard	
Aflatoxin Mix 4 Solution 0.5 µg/mL B_2 and G_2 in acetonitrile 2 µg/mL B_1 and G_1 in acetonitrile	**34036**
Solvent	
Acetonitrile, for HPLC, ≥99.9%	**34851**

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