

## Analysis of Mycotoxins in Hemp and Hemp-Containing Edible Products

The Agriculture Improvement Act (2018 Farm Bill) makes hemp production and distribution legal under federal law. Hemp has wide range of possible applications, including production of fibers, paper, certain foods, supplements, cosmetics and even the recently FDA-approved drug Epidiolex. Legally available hemp and hemp products need to be tested for presence of pesticides, heavy metals, residual solvents and other harmful substances. Similarly classified crops are routinely tested for Mycotoxins, including Aflatoxins and Ochratoxin A.

Pickering Laboratories developed an easy and sensitive method to analyze Aflatoxins B1, B2, G1, G2 and Ochratoxin A in hemp and hemp-containing edible products. Mycotoxins are isolated using immunoaffinity clean-up columns and analyzed with fluorescence detection. To increase sensitivity of Aflatoxins B1 and G1, an in-line photochemical reactor (UVE™) is installed before the detector. This method utilizes standard HPLC equipment and allows laboratories to easily determine these Mycotoxins at low ppb levels.

### Method

#### Isolation of Aflatoxins B1, B2, G1, G2 and Ochratoxin A

Blend 1 g of finely ground sample with extraction solution (10 mL of methanol/water 80:20) using a handheld homogenizer. Centrifuge for 10 min. Mix 2 mL of the extract with 10 mL of PBS buffer (containing 2% Tween 20).

Load 10 mL of diluted extract on AflaOchra HPLC immunoaffinity column at a flow rate of 1-2 drops/sec. Wash the column with 10 mL of PBS buffer (containing 2% Tween 20) followed by 10 mL of DI water at a flow rate of 1-2 drops/sec. Elute the toxins with two 1 mL portions of methanol at a flow rate of 1 drop/sec.

Evaporate the solution to about 0.3 mL at 55 °C and bring the volume to 1.5 mL with water/methanol solution (50:50). Filter into an injection vial.

#### Analytical Conditions

**Analytical Column:** MYCOTOX™ Reversed-phase Column, 4.6 x 250 mm, P/N 1612124

**Guard Column:** Reversed-phase guard cartridge, P/N 18ECG001

**HPLC Eluant:** Sodium Phosphate Buffer (Cat #1700-1108), Methanol, Acetonitrile

**Flow Rate:** 1 mL/min

**Injection Volume:** 100 µL

**Post-Column Photochemical Reactor:** UVE™ (Cat # 10519 (240V), Cat # 10742 (120 V))

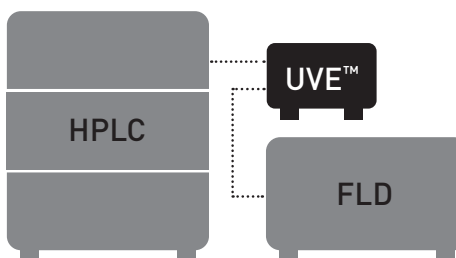
**FLD:** Excitation 365 nm, Emission 430 nm for Aflatoxins  
Excitation 333 nm, Emission 477 nm for Ochratoxin A

HPLC Gradient			
Time	1700-1108 %	Methanol %	Acetonitrile %
0	57	28	15
13	57	28	15
16	30	45	25
25	30	45	25

Equilibration time: 10 min

#### Calibration

The 5-point calibration curves were built in the ranges of 0.325-3.25 ppb for B1, 0.088- 0.882 ppb for B2, 0.310-3.099 ppb for G1, 0.099-0.996 ppb for G2 and 1-10 ppb for Ochratoxin A. Correlation coefficient was R<sup>2</sup> >0.999 for all toxins. All calibration standards were prepared in methanol/water (50:50).



Flow Diagram for UVE™ Photochemical Reactor

Recoveries

Toxins	Spike Levels, ng/g	CBD-Containing Tincture		CBD-Containing Chocolate		CBD Oil		Hemp Pre-Rolls		Full-Spectrum CBD Chews	
		Recoveries %	RSD %	Recoveries %	RSD %	Recoveries %	RSD %	Recoveries %	RSD %	Recoveries %	RSD %
Aflatoxin G2	1.932	91.85	2.54	78.89	1.42	88.67	7.32	84.66	10.82	93.90	6.85
Aflatoxin G1	6.198	92.50	7.86	85.89	1.21	96.35	1.24	93.56	7.05	97.93	2.42
Aflatoxin B2	1.764	92.99	5.30	88.73	2.88	99.31	1.84	94.28	6.77	101.63	1.72
Aflatoxin B1	6.498	84.73	3.51	87.88	0.56	91.62	3.08	85.78	7.50	91.97	1.99
Ochratoxin A	20.16	92.31	4.50	91.86	4.17	89.75	2.26	85.68	5.08	86.97	5.27

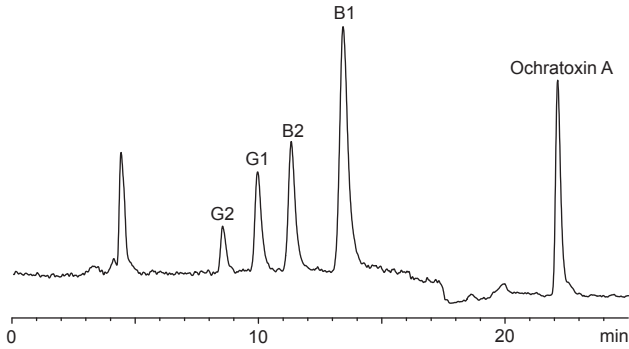


Fig 1. Chromatogram of hemp pre-roll spiked with 6.5 ng/g of Aflatoxin B1; 1.8 ng/g of Aflatoxin B2; 6.1 ng/g of Aflatoxin G1; 1.9 ng/g of Aflatoxins G2 and 20.1 ng/g of Ochratoxin A

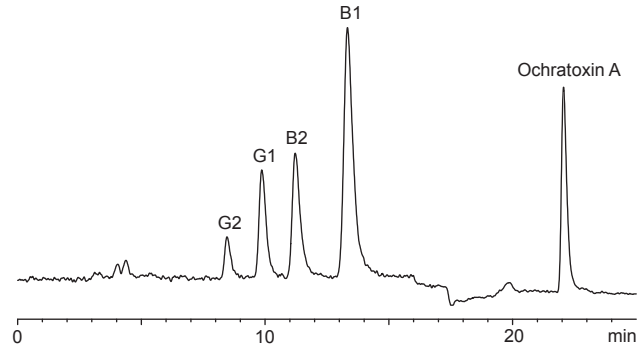


Fig 2. Chromatogram of hemp-containing chews spiked with 6.5 ng/g of Aflatoxin B1; 1.8 ng/g of Aflatoxin B2; 6.1 ng/g of Aflatoxin G1; 1.9 ng/g of Aflatoxins G2 and 20.1 ng/g of Ochratoxin A

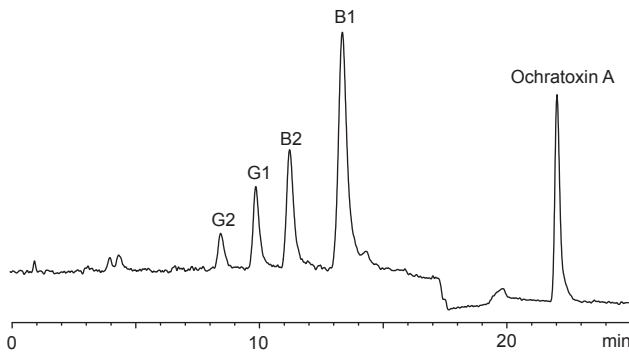


Fig 3. Chromatogram of full spectrum hemp tincture spiked with 6.5 ng/g of Aflatoxin B1; 1.8 ng/g of Aflatoxin B2; 6.1 ng/g of Aflatoxin G1; 1.9 ng/g of Aflatoxins G2 and 20.1 ng/g of Ochratoxin A

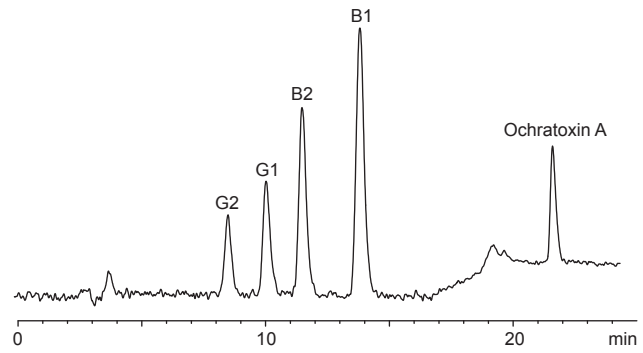


Fig 4. Low-level Mycotoxins standard containing 0.81 ppb of Aflatoxin B1; 0.22 ppb of Aflatoxin B2; 0.31 ppb of Aflatoxin G1; 0.24 ppb of Aflatoxins G2 and 2.52 ppb of Ochratoxin A

