Application News

No. HPLC-019

High Performance Liquid Chromatography

Quantitation of Aflatoxins B1, B2, G1, G2 and Ochratoxin A in Cannabis by LC Using Prominence-i and the RF-20Axs Fluorescence Detector

■ Introduction

Aflatoxins and ochratoxins are varieties of mycotoxins produced as secondary metabolites from mold species within the genera *Aspergillus*. Studies have found that many mycotoxins, including Alfatoxin G1 G2, Alfatoxin B1 B2, and Ochratoxin A, are immunosuppressive, carcinogenic, neurologically toxic, and hepatotoxic [1,3]. The mold itself can also cause diseases such as lung infection and aspergillosis [1,3]. Considering the great risk to consumer health posed by the presence of mycotoxins and the possibility of mold growth in cannabis [1,2], the development of accurate testing methods for mycotoxins in cannabis are of utmost importance.

As more states adopt policy changes allowing for medical or recreational use of marijuana, more regulations regarding testing of cannabis products have been implemented. Existing methods include ELISA, qPCR, and MALDI-ToF-MS; each one has their own advantages and disadvantages in terms of cost, range, preparation time, and accuracy. For instance, ELISA is not accurate and often gives false positive or negative results, and additional expensive instrumental tests need to be conducted to confirm the ELISA results. Moreover, due to the high level of matrix complexity of cannabis products, the mycotoxin testing in cannabis is even more challenging. This study focuses on developing a powerful detection method that allows cannabis testing labs to confidently report concentrations below regulatory limits – Aflatoxins B1, B2, G1 and G2 combined to 20 ppb and 20 ppb for Ochratoxin A alone.

■ Experimental

Sample Preparation

One gram of sample was weighed out in a 50 ml centrifuge tube and ground using a SPEX grinder until homogenized. 10 mL of methanol: water (80:20) was then added to the tube. The mixture was shaken vigorously for 10 minutes using the SPEX Geno Grinder, and then centrifuged at 3600 rpm for 3 minutes. 2.0 mL of the supernatant was transferred to a new tube containing 8.0 mL of 1x PBS/2% Tween Wash Buffer and mixed well. All 10 ml of the diluted extract was passed through the AflaOchra column (see Figure 1) at a rate of 1-2 drops per second until air came through the column—it is important that the flow rate does not exceed 1-2 drops per second when using these columns because doing so could reduce recovery. Likewise, the column was washed with 10 mL of PBS/2% Tween buffer, followed by 10 mL of HPLC grade water. Finally, the column was washed with 2 mL of methanol, 1 mL at a time and combined in the same tube.

Figure 1: Afla-Ochra Column for extraction/clean-up

Standards and Calibration

The standards used to build the calibration curve were ran in triplicate using the high-sensitivity mode at 2, 5, 10, 14, 20, and 50 ppb for the Aflatoxins and 10, 14, 20, and 50 ppb for Ochratoxin A. All standards were spiked in matrix and extracted so the actual concentrations of the injected material were 0.2, 0.5, 1.0, 1.4, 2, and 5ppb for the Aflatoxins. The Ochratoxin A does not need as low of a detection limit because the limit is 20 ppb by itself; the Aflatoxins is 20 ppb for the sum of the four.

Analytical Conditions

Column	NexLeaf CBX for Potency 2.7 um x 150 mm x 4.6 mm
Mobile Phase	A: Water with 0.1% Formic Acid, B: Methanol with 0.1% Formic Acid, C: Acetonitrile with 0.1% Formic Acid
Time Program	Conc. A/Conc. B/Conc. C = 65/30/5 (0.00 – 2.00) → ramp to 23/40/37 (2.00 – 10.00) → 23/40/37 (10.00 – 12.00) → 65/30/5 (12.01 – 14.00)
Flow Rate	1.3 mL/min
Column Temp.	50 °C
Injection Volume	10 uL
Detection	RF-20AXS, Channel 1: Ex= 365nm EMm=450nm, Ch2: Ex=336, Em=464, Ch3: Ex=330 Em=460, Ch4: Ex=350 Em=450, High Sensitivity Mode
Cell Temp.	30 °C

The same column and LC system (i-Series LC-2030) as used here are also used for cannabis potency analysis, so a cannabis lab can expand their analysis capabilities by adding just the RF-20AXS to their current setup.

■ Results and Discussion

Channel 1, excitation of 365 nm and emission at 450 nm, provided the best response for all Aflatoxins and Channel 3, excitation of 330 nm and emission at 460 nm, was chosen for Ochratoxin A, though Channel 2 was also suitable (see Figure 2). Aflatoxin G1 had the lowest response of all the Aflatoxins, but Channel 1 still provided quality data for analysis even at the lowest concentration of 2 ppm. Calibration curve R^2 values for each compound were 0.998 or greater, indicating great linearity.

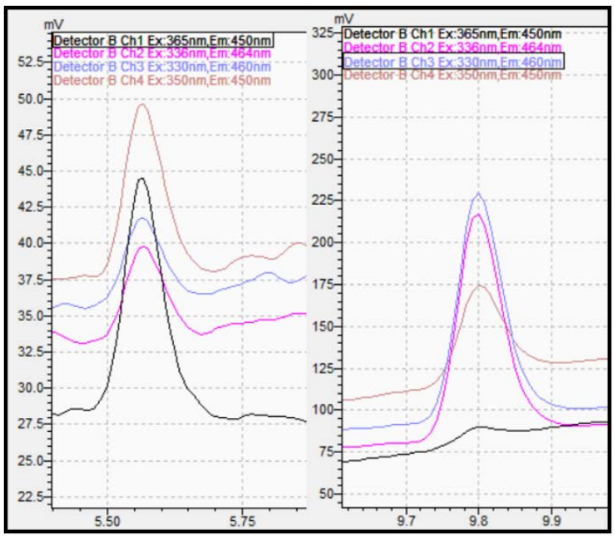

Figure 2: Chromatography of Aflatoxin G1 2 ppm (left) and Ochratoxin A 10 ppm (right)

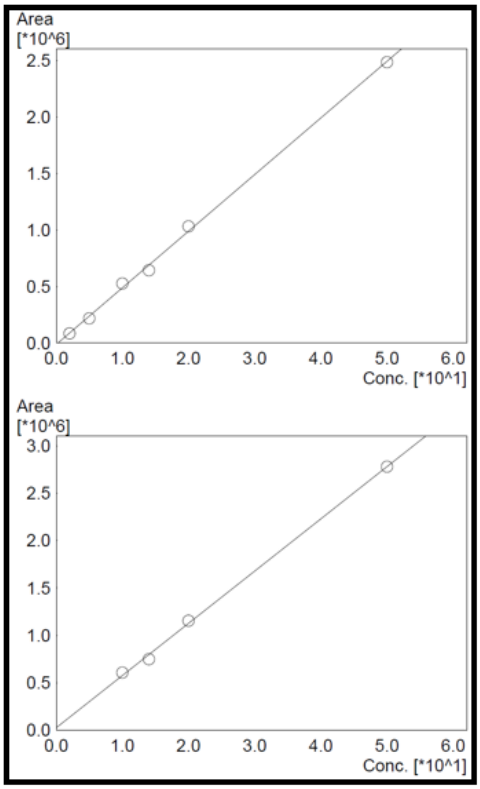

Figure 3: Calibration curves for Aflatoxin G1 (top) and Ochratoxin A (bottom)

Samples were spiked at 10 ppb of mycotoxins and run as unknowns. The chromatograms are shown in Figure 4 and the recovery results, indicating the high accuracy of the method, are shown in Table 1.

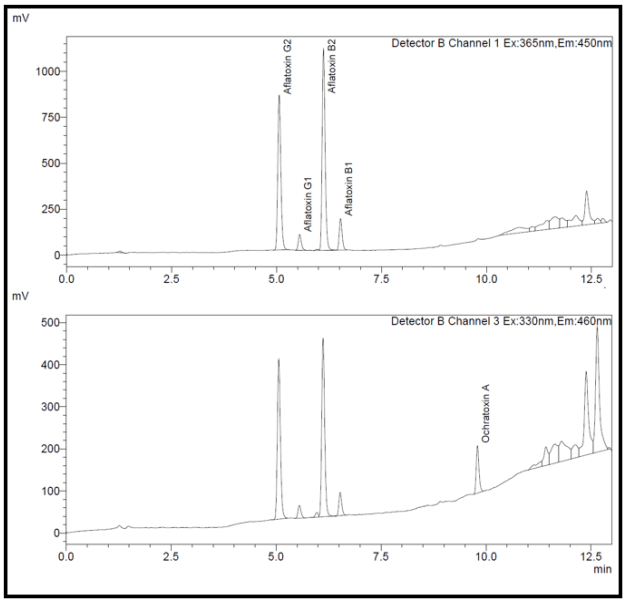

Figure 4: Full chromatogram showing Channels 1 (top) and 3 (bottom) for 10 ppb spiked sample

Table 1: 10 ppb spiked sample results

Mycotoxin	Concentration (ppb)	Recovery (%)	Concentration (ppb)	Recovery (%)
Aflatoxin G2	8.022	80.22	8.380	83.80
Aflatoxin G1	8.534	85.34	8.958	89.58
Aflatoxin B2	7.901	79.01	8.363	83.63
Aflatoxin B1	7.704	77.04	8.044	80.44
Ochratoxin A	8.781	87.81	10.925	109.25

■ Conclusion

A new fluorescence method has been developed to test these Mycotoxins in cannabis. This method is sensitive and robust and requires only the simple addition of our RF-20AXS detector to the i-Series LC-2030.

■ References

1) Hazenkamp A. An evaluation of the quality of medicinal grade cannabis in the Netherlands. Cannabinoids. 2006; 1(1):1-9.
2) Llewellyn G. C., O'Rear C. E. Examination of fungal growth and aflatoxin production on marihuana. Mycopathologia. 1977; 62(2): 109-112.
3) Sedmikova M., Reisnerova H., Dufkova Z., Barta I., Jilek F. Potential hazard of simultaneous occurrence of aflatoxin B1 and ochratoxin A. Veterinary Medicine - Czech. 2001; 46(6): 169-174.