Analysis of Mycotoxins in Cannabis Plant Material and Derivative Products by Immunoaffinity Enrichment LC-MS/MS

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INTRODUCTION

Mycotoxins are regulated in many commodities worldwide. Cannabis and its derivative products have remained challenging matrices when analyzing for the regulated mycotoxins, Aflatoxin B1, B2, G1, G2 and Ochratoxin A. Therefore, the main objective of this study was to develop a robust, comprehensive and effective method for the analysis of the regulated mycotoxins in cannabis plant material and derivative products using immunoaffinity columns coupled with LC-MS/MS. The validated method uses an AflaOchra™ immunoaffinity column clean-up procedure that is streamlined for the analysis of cannabis plant material, topicals, tinctures, edibles and concentrates. Immunoaffinity columns were provided by VICAM®, A Waters Business. The validated method includes data for linearity, limit of detection, limit of quantitation, accuracy, precision, matrix effect and analytical system robustness.

METHODS

Purpose

The purpose of this study was to develop a robust and efficient method for detection of multiple matrices in cannabis using LC-MC/MS coupled with immunoaffinity columns.

Materials

Separate Aflatoxin B1, B2, G1, and G2 (3 ug/mL) standards were purchased from (Supelco, USA). Ochratoxin standard (50 ug/mL) was purchased from (Supelco, USA). LC-MS grade water was used as a purified water source (Fisher, USA). LC-MS grade 100% methanol was used in extraction solution (Fisher, USA).

AflaOchra® immunoaffinity columns (2 different lots) were supplied by (VICAM, A Waters Business, USA). Dried hemp and cannabis flower/bud, tinctures, topicals and edible material was collected and analyzed for this study within an ISO 17025 accredited, licensed MMJ analytical laboratory (ProVerde Labs, USA).

Methods

All sample types were weighed out at 0.5 gram sample and placed in an extraction vessel. The appropriate spike volumes of a combined Mycotoxin Stock Solution (MTSS) was then added to the dried sample within each extraction vessel. Aflatoxin standard at various levels were allowed to dry on to the surface of the sample for at least 30 minutes to1 hour.

Sample Extraction

Add a small scoop of Yttria Zirconia Micro Milling beads to a 15 ml Polypropylene conical tube. The beads should fill tube to the first line (1 mL mark) on the tube.

Weigh 500 mg of homogenized Cannabis flower sample (100 mg for concentrate samples) into the 15 mL Polypropylene conical tube.

Add 2 mL LCMS grade Isopropanol to the tube and vortex for 1.5 minutes. For concentrates, make sure sample is fully dissolved.

Shake on a SPEX geno-mill stroke type shaker 1min at 1500 RPM.

Add 5 mL of 60/40 Methanol/LCMS grade water to the conical tube.

Shake on a SPEX geno-mill stroke type shaker 1min at 1500 RPM.

Centrifuge at 5000 RPM (4696 x g) for 5 minutes. Pipette off top layer to clean vial.

Pipet 4.2 mL extract into a clean vessel. Dilute extract up to 50 mL 0.1%Tween 20/ PBS. Mix well.

Filter precipitate.

Load onto the AflaOchra column and follow IAC procedure.

LC-MS/MS Method Parameters

Column: 2.1 X 100 mm C18 column X-Bridge® (Waters)

Mobile phase A: 5mM Ammonium Formate with 0.02% Formic Acid in Water

Mobile phase B: 5mM Ammonium Formate with 0.02% Formic Acid in MeOH

Flow rate: 0.5 mL/minute

Column Temperature: 300C

Injection volume: 10 µL

Gradient:

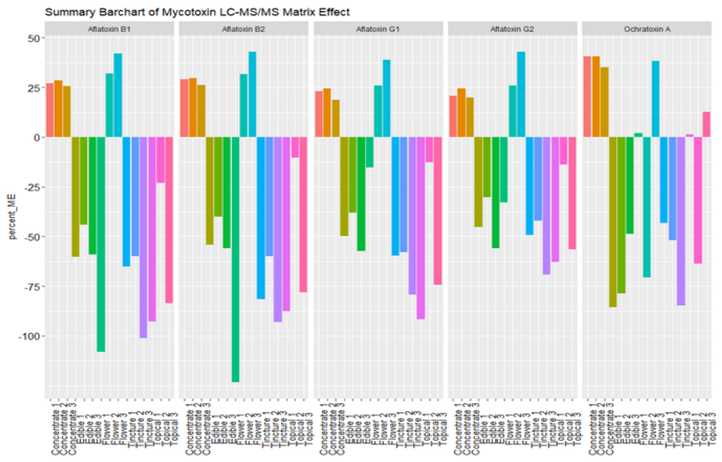
Time (min)	Flow	%A	%B
0	0.5	98	2
0.2	0.5	98	2
3.0	0.5	1	99
4.0	0.5	1	99
4.1	0.5	98	2
5	0.5	98	2

RESULTS

Matrix effect

Matrix effect was investigated in three example samples of five representative matrix classes, plant material, oleoresins, edible oils, topical products, and edible products. Blank sample extracts are spiked with analytes (post-extraction) at the same concentration levels as the solvent based calibrators. Overall matrix effect was calculated as the ratio of the slope of the matrix matched calibration curve to that of the solvent reference. Negative matrix effect indicates suppression and positive matrix effect indicates enhancement.

RESULTS



Summary Bar Chart of Mycotoxin LC-MS/MS Matrix Effect, 3 ng/mL toxins in solvent vs. extract

Accuracy

Accuracy was assessed for three example samples from each of five representative matrix classes including, Cannabis plant material, Cannabis oleoresins/concentrates, edible oils, topical products, and assorted edible products. Blank samples are spiked with analytes (pre-extraction) in duplicate at three different concentration levels (3 - 60 ng/g), allowed to stand at least 30 - 60 min, then extracted and analyzed per SOP. A single point standard addition calibration was used to compensate for matrix effects. The sample extract was split into two 285 µL aliquots, one is spiked with 15 µL of additional diluent, the other spiked with 15 µL of standard solution. The calibrator prepared in this way was used for quantification by the method of standard additions.

Recovery is calculated as
%Recovery=(Conc Found)/(Conc Spiked)*100

and averaged across 6 replicates, two at each of three concentration levels. Cannabis oeleoresins/concentrates are tested based on a 100 mg sample weight and are spiked at correspondingly higher concentrations.

In the accuracy tables below, three different cannabis samples for each matrix were spiked in duplicate, pre-extraction, with analytes at 3 different concentrations. *The % recovery average is an average of the triplicate sample matrices at each level.

Cannabis Plant Material Accuracy Recoveries

Aflatoxin G_2 Target (ppb)	3	6	12
% Recovery Average*	96.06	98.92	99.87
Standard Deviation	5.49	6.40	18.67
% CV	5.72	6.47	18.70

Aflatoxin G_1 Target (ppb)	3	6	12
% Recovery Average*	95.97	97.23	96.24
Standard Deviation	6.38	5.30	18.51
% CV	6.65	5.45	19.23

Aflatoxin B_2 Target (ppb)	3	6	12
% Recovery Average*	94.11	91.80	99.61
Standard Deviation	3.51	6.48	12.68
% CV	3.73	7.05	12.73

Aflatoxin B_1 Target (ppb)	3	6	12
% Recovery Average*	81.01	87.57	90.97
Standard Deviation	4.23	11.09	8.27
% CV	5.22	12.66	9.09

Ochratoxin A Target (ppb)	9	18	36
% Recovery Average*	96.06	87.58	93.40
Standard Deviation	5.49	2.20	11.18
% CV	5.72	2.51	11.97

Concentrate Accuracy Recoveries

Aflatoxin G_2 Target (ppb)	5	10	20
% Recovery Average*	89.33	82.89	81.35
Standard Deviation	15.05	8.65	1.18
% CV	16.85	10.44	1.45

Aflatoxin G_1 Target (ppb)	5	10	20
% Recovery Average*	86.20	77.02	79.37
Standard Deviation	5.05	4.42	1.90
% CV	5.86	5.75	2.40

Aflatoxin B_2 Target (ppb)	5	10	20
% Recovery Average*	79.21	80.37	79.11
Standard Deviation	4.55	4.62	3.38
% CV	5.75	5.74	4.27

Aflatoxin B_1 Target (ppb)	5	10	20
% Recovery Average*	74.17	68.83	70.24
Standard Deviation	8.76	4.65	5.71
% CV	11.81	6.76	8.13

Ochratoxin A Target (ppb)	15	30	60
% Recovery Average*	78.17	70.50	93.40
Standard Deviation	0.48	4.87	11.18
% CV	0.62	6.90	11.97

Tincture Accuracy Recoveries

Aflatoxin G_2 Target (ppb)	3	6	12
% Recovery Average*	108.17	102.02	92.95
Standard Deviation	7.11	4.60	13.76
% CV	6.58	4.51	14.80

Aflatoxin G_1 Target (ppb)	3	6	12
% Recovery Average*	107.91	105.91	97.99
Standard Deviation	3.98	4.02	13.41
% CV	3.69	3.79	13.69

Aflatoxin B_2 Target (ppb)	3	6	12
% Recovery Average*	106.92	104.75	95.17
Standard Deviation	9.16	6.79	11.96
% CV	8.57	6.49	12.56

Aflatoxin B_1 Target (ppb)	3	6	12
% Recovery Average*	94.54	96.32	93.78
Standard Deviation	2.85	3.79	12.22
% CV	3.01	3.93	13.03

Ochratoxin A Target (ppb)	9	18	36
% Recovery Average*	93.11	93.48	83.13
Standard Deviation	9.31	17.74	17.85
% CV	10.00	18.98	21.47

RESULTS

Topical Accuracy Recoveries

Aflatoxin G_2 Target (ppb)	3	6	12
% Recovery Average*	95.00	99.53	99.55
Standard Deviation	10.98	9.57	6.42
% CV	6.56	9.61	6.45

Aflatoxin G_1 Target (ppb)	3	6	12
% Recovery Average*	100.46	106.48	107.24
Standard Deviation	6.60	4.47	1.95
% CV	6.57	4.20	1.82

Aflatoxin B_2 Target (ppb)	3	6	12
% Recovery Average*	101.98	109.22	103.58
Standard Deviation	9.41	0.92	0.50
% CV	9.23	0.84	0.48

Aflatoxin B_1 Target (ppb)	3	6	12
% Recovery Average*	93.19	96.94	104.56
Standard Deviation	4.98	2.54	2.93
% CV	5.34	2.62	2.80

Ochratoxin A Target (ppb)	9	18	36
% Recovery Average*	90.23	89.43	82.51
Standard Deviation	7.59	10.49	11.39
% CV	8.41	11.73	13.76

Edible Accuracy Recoveries

Aflatoxin G_2 Target (ppb)	3	6	12
% Recovery Average*	95.45	102.80	104.26
Standard Deviation	24.08	6.54	4.66
% CV	25.23	6.36	4.47

Aflatoxin G_1 Target (ppb)	3	6	12
% Recovery Average*	92.26	104.64	108.54
Standard Deviation	24.29	2.39	0.19
% CV	26.33	2.28	0.18

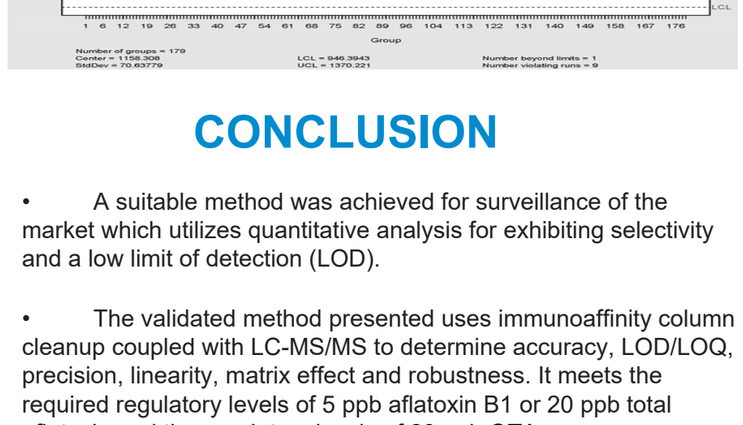
Aflatoxin B_2 Target (ppb)	3	6	12
% Recovery Average*	97.29	110.73	109.63
Standard Deviation	17.01	3.49	0.95
% CV	17.49	3.15	0.86

Aflatoxin B_1 Target (ppb)	3	6	12
% Recovery Average*	90.91	100.29	100.10
Standard Deviation	11.69	3.66	1.70
% CV	12.86	3.65	1.70

Ochratoxin A Target (ppb)	9	18	36
% Recovery Average*	91.09	100.72	90.60
Standard Deviation	12.94	7.35	1.43
% CV	14.21	7.30	1.58

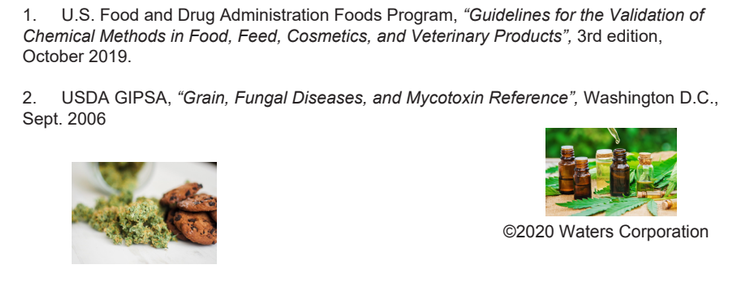
Precision

Repeatability precision was assessed for one sample from each matrix class, Cannabis plant material, Cannabis oleoresins/ concentrates, edible oils, topical products, and assorted edible products. Six (6) replicate blank samples are spiked with analytes (pre-extraction) at the L2 concentration level (6 - 30 ng/g), allowed to stand at least 60 min, then extracted and analyzed per SOP. The 30 repeatability replicates are processed by the same analyst on multiple days and analyzed on the same LC-MS system. A calibration curve in solvent is injected on each day of sample analysis and used for quantification.



Robustness

Upper control limit (UCL), Control limit (CL) and Lower control limit (LCL) are graphed for 179 consecutive injections. Include in this graph below for analytical system robustness is the number of injections that fall beyond the limits and the number of violating runs based on the Western Electric Rules for flagging trends for Aflatoxin G2.



CONCLUSION

- A suitable method was achieved for surveillance of the market which utilizes quantitative analysis for exhibiting selectivity and a low limit of detection (LOD).
- The validated method presented uses immunoaffinity column cleanup coupled with LC-MS/MS to determine accuracy, LOD/LOQ, precision, linearity, matrix effect and robustness. It meets the required regulatory levels of 5 ppb aflatoxin B1 or 20 ppb total aflatoxin and the regulatory levels of 20 ppb OTA.
- LC-MS/MS with immunoaffinity provides a solution for the analysis of difficult cannabis matrices by demonstrating effective matrix cleanup, method robustness, accuracy and precision while meeting required regulatory levels.

References

1. U.S. Food and Drug Administration Foods Program, *"Guidelines for the Validation of Chemical Methods in Food, Feed, Cosmetics, and Veterinary Products"*, 3rd edition, October 2019.

2. USDA GIPSA, *"Grain, Fungal Diseases, and Mycotoxin Reference"*, Washington D.C., Sept. 2006

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