Expanded Mycotoxins Analysis in Cannabis Matrices by LC-MS/MS

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Background

Mycotoxins are secondary fungal metabolites that can be harmful for human consumption, and the primary five (Ochratoxin A, Aflatoxin B1, B2, G1, and G2) are required testing in several states. Currently, Oregon does not mandate mycotoxin testing, however, given water activity requirements in Oregon along with the heat-stable nature of these compounds, they might persist in cured material. Processing of contaminated cannabis flower or trim with even trace levels of residues may concentrate these down to unsafe levels. Columbia Food Labs has taken the initiative to test for 15 common fungal toxins, above and beyond any current regulatory requirements, to ensure safety and scientific validity within the industry.

Introduction

An expanded list of 15 mycotoxins LC-MS/MS analysis is shown here with a simple solvent and modified QuEChERS salt extraction. The entire workflow is simple and accessible to most cannabis and food residue testing laboratories.

Mass Spec Parameters

Mass Spec Detector: Sciex® Triple Quad™ 5500

Samples were ionized using electrospray with positive/negative ion-mode polarity switching

MRM Transitions

Compound Name	Q1 Mass	Q3 Mass	Retention Time (min)	Declustering Potential	Entrance Potential	Collision Energy	Collision Cell Exit Potential	Quantifier
Patulin	152.9	109	2.37	-90	-10	-14	-13	
Patulin	152.9	81	2.37	-90	-10	-18	-11	X
Patulin	152.9	53.1	2.37	-90	-10	-22	-9	
Nivalenol	311.02	281	2.51	-105	-10	-14	-17	
Nivalenol (M-H+HCOOH)	357.007	281.1	2.51	-55	-10	-18	-15	X
Nivalenol (M-H+HCOOH)	357.007	191	2.51	-55	-10	-40	-16	
Deoxynivalenol (DON)	296.9	248.9	3.08	71	10	17	18	X
Deoxynivalenol (DON)	296.9	203.1	3.08	71	10	21	14	
Deoxynivalenol (DON)	296.9	175	3.08	71	10	27	12	
Deoxynivalenol (DON)	296.9	91	3.08	71	10	65	4	X
Aflatoxin G2	331.014	216.85	4.47	106	10	49	14	
Aflatoxin G2	331.014	188.833	4.47	106	10	57	12	X
Aflatoxin G1	329.059	243.35	4.71	81	10	39	16	
Aflatoxin G1	329.059	311.2	4.71	81	10	31	10	X
Aflatoxin B2	315.1	287.1	5.09	91	10	37	20	
Aflatoxin B2	315.1	259.1	5.09	91	10	41	16	X
Aflatoxin B1	313.039	285	5.41	86	10	31	20	

Experimental Conditions

Sample Preparation:

Cannabis Flower Samples were ground in a Retch GM 200 knife mill, and 0.5 g sample soaked in 5 mL of 2 % ascorbic acid in water in a 50 mL falcon tube. 10 mL acetonitrile were added, followed by a modified EN 15662 QuEChERS salt extraction (4 g $MgSO_4$, 1 g NaCl, 1.5 g sodium citrate), shaken for 5 minutes, then centrifuged at 2500 RPM. The supernatant was diluted 5 x with aqueous ammonium formate buffer and filtered through a 0.45 µm syringe filter prior to injection to HPLC.

LC Method Parameters

Column: Luna® Omega 3 µm Polar C18
Dimensions: 100 x 2.1 mm
Part No.: 00D-4760-AN
Flow Rate: 0.4 mL/min
Mobile Phase: A: 1 mM Ammonium formate + 0.1% Formic acid in Water
B: Methanol

Gradient:

Time (min)	% B
0	5
3	35
10	90
12	90

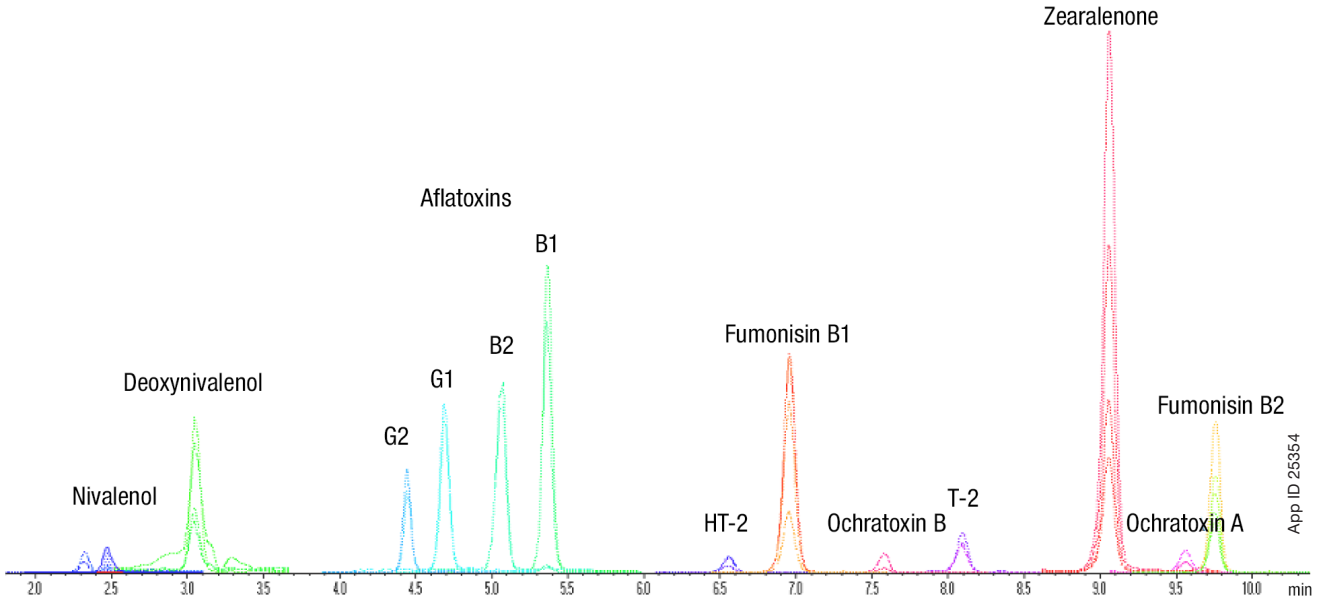
Temperature: 40 °C
Injection Volume: 3 µL
Detection: MS/MS – Sciex Triple Quad 5500

(Cont'd on next page)

MRM Transitions (Cont'd.)

Compound Name	Q1 Mass	Q3 Mass	Retention Time (min)	Declustering Potential	Entrance Potential	Collision Energy	Collision Cell Exit Potential	Quantifier
Aflatoxin B1	313.039	241	5.41	86	10	49	18	X
HT-2 Toxin	442.4	263	6.66	46	10	19	18	X
HT-2-Toxin	442.4	214.9	6.66	46	10	17	14	
HT-2-Toxin	442.4	169	6.66	46	10	35	10	
Fumonisin B1	722.2	334.2	7.02	146	10	53	24	X
Fumonisin B1	722.2	352.2	7.02	146	10	49	30	
Fumonisin B1	722.2	704.2	7.02	146	10	39	20	
Fumonisin B1	722.2	67.1	7.02	146	10	129	10	
Fumonisin B1	722.2	91	7.02	146	10	129	10	
Ochratoxin B	370.1	205	7.68	45	10	27	15	X
Ochratoxin B	370.1	187	7.68	45	10	35	12	
T2-Toxin	484.1	305.1	8.23	51	10	19	20	X
T2-Toxin	484.1	214.9	8.23	51	10	29	14	
T2-Toxin	484.1	185	8.23	51	10	31	12	
Zearalenone (ZON)	319	283.2	9.2	66	10	17	20	X
Zearalenone (ZON)	319	186.9	9.2	66	10	27	12	
Zearalenone (ZON)	319.4	185	9.2	66	10	35	8	
Zearalenone (ZON)	319	69.3	9.2	66	10	37	8	
Ochratoxin A	404.2	238.9	9.65	41	10	33	16	X
Ochratoxin A	404.2	358	9.65	41	10	21	10	
Ochratoxin A	404.2	220.8	9.65	41	10	49	14	
Fumonisin B2	707.2	319.2	9.8	176	10	53	24	X
Fumonisin B2	707.2	355.3	9.8	176	10	45	32	
Fumonisin B2	707.2	55.1	9.8	176	10	129	26	
Fumonisin B2	707.2	67	9.8	176	10	127	8	
Fumonisin B2	707.2	69.1	9.8	176	10	107	10	

Chromatography of expanded Mycotoxins overlayed MRMs.



Conclusion

Analysis of aflatoxins and mycotoxins can be performed using simple, well-characterized techniques and equipment readily available to most testing labs. As new regulations are developed across North America, mycotoxin testing analysis will likely become standard. Expanding the targeted scope to include a wider range of potential fungal contaminants within cannabis and hemp extracts will improve public safety as well as scientific credibility in the cannabis testing community.

Ordering Information

roQ™ QuEChERS Kits

roQ Extraction Kits

Extraction kits contain fifty easy-pour salt packets and fifty 50 mL stand-alone centrifuge tubes

Description	Unit	Part No.
AOAC 2007.01 Method Extraction Kits		
6.0 g $MgSO_4$, 1.5 g NaOAc	50/pk	KS0-8911
EN 15662 Method Extraction Kits		
4.0 g $MgSO_4$, 1.0 g NaCl, 1.0 g SCTD, 0.5 g SCDS	50/pk	KS0-8909
Original Non-buffered Method Extraction Kits		
4.0 g $MgSO_4$, 1.0 g NaCl	50/pk	KS0-8910
6.0 g $MgSO_4$, 1.5 g NaCl	50/pk	KS0-8912

roQ Extraction Salt Packets

Salt packets only. Centrifuge tubes not included.

Description	Unit	Part No.
AOAC 2007.01 Method Extraction Packets		
6.0 g $MgSO_4$, 1.5 g NaOAc	50/pk	KS0-9043
EN 15662 Method Extraction Packets		
4.0 g $MgSO_4$, 1.0 g NaCl, 1.0 g SCTD, 0.5 g SCDS	50/pk	KS0-9041
Original Non-Buffered Method Extraction Packets		
4.0 g $MgSO_4$, 1.0 g NaCl	50/pk	KS0-9042
6.0 g $MgSO_4$, 1.5 g NaCl	50/pk	KS0-9044

APPLICATIONS

Luna® Omega Polar C18 LC Column Ordering Information

1.6 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Polar C18	00A-4748-AN	00B-4748-AN	00D-4748-AN	00F-4748-AN	AJ0-9505
					for 2.1 mm ID

3 µm Minibore and MidBore™ Columns (mm)								SecurityGuard Cartridges (mm)
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0*
Polar C18	00A-4760-AN	00B-4760-AN	00D-4760-AN	00F-4760-AN	00B-4760-Y0	00D-4760-Y0	00F-4760-Y0	AJ0-7600
							for ID:	2.0 - 3.0 mm

3 µm Analytical Columns (mm)					SecurityGuard Cartridges (mm)
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*
Polar C18	00B-4760-E0	00D-4760-E0	00F-4760-E0	00G-4760-E0	AJ0-7601
				for ID:	3.2-8.0 mm

Kinetex® Core-Shell Column Ordering Information

1.7 µm Minibore Columns (mm)				SecurityGuard ULTRA Cartridges‡
Phases	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Biphenyl	00B-4628-AN	00D-4628-AN	00F-4628-AN	AJ0-9209
				for 2.1 mm ID

‡SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

2.6 µm Minibore Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Biphenyl	00A-4622-AN	00B-4622-AN	00D-4622-AN	00F-4622-AN	AJ0-9209
					for 2.1 mm ID

2.6 µm MidBore Columns (mm)				SecurityGuard ULTRA Cartridges‡
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
Biphenyl	00B-4622-Y0	00D-4622-Y0	00F-4622-Y0	AJ0-9208
				for 3.0 mm ID

2.6 µm Analytical Columns (mm)				SecurityGuard ULTRA Cartridges‡
Phases	50 x 4.6	100 x 4.6	150 x 4.6	3/pk
Biphenyl	00B-4622-E0	00D-4622-E0	00F-4622-E0	AJ0-9207
				for 4.6 mm ID

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000
* SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

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