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The Movement Towards More Comprehensive Testing of Mycotoxins in Cannabis

Guest Author – David C. Kennedy, PhD, Phenomenex, Inc.

In the USA, cannabis testing requirements and analytical methodology vary widely from state to state owing to the lack of federal criteria. This variation is most pronounced in the area of mycotoxin testing where this highly toxic contaminant does not always receive the priority it deserves. A case is made for the use of more rigorous testing methodologies and an example is provided based upon a QuEChERs-LC-MS/MS approach that provides a high degree of speciation and sensitivity.

Introduction

Mycotoxins are a family of highly toxic secondary metabolites of certain fungi and molds that easily colonize agricultural crops, notably grains and forage. Mycotoxins have long been recognized as a dangerous contaminant in food crops and the human and animal food products derived therefrom [1]. Depending upon water activity and storage conditions, these highly toxic substances have been known to cause many types of illness in both humans and animals, and not infrequently death. Consequently, in the United States, the US Food and Drug Administration (FDA) has established a regulatory limit for mycotoxins of 0.02 micrograms per gram (20 ppb) in both human food and animal feed.

While careful regulation of mycotoxins in the food supply chain has successfully prevented food consumption-related fatalities in humans, significant quantities of raw agricultural

products continue to need to be destroyed owing to mycotoxin contamination.

Mycotoxins and Cannabis

Cannabis (and likewise hemp) is also an agricultural crop that is destined for human consumption – both medicinally and recreationally – in a wide variety of formats. Cannabis is no less subject to mycotoxin contamination than other crops. Some have argued that mycotoxin contamination of cannabis is even more problematic, owing to the agricultural environment, particularly that of grow rooms [2]. Given the level of threat, it would seem reasonable that mycotoxin contamination in cannabis would likewise be heavily regulated. This would certainly be appropriate for those cannabis products destined for human consumption, but even more so for medicinal cannabis, with the heightened risk of impacting patients with weakened immune systems. And, so it is in many parts of the civilized world.

US Complications

However, the situation in the United States is rather different, owing to the unusual legal and regulatory regime that governs the production, use, and testing of cannabis, cannabis products, and cannabis consumption. On the federal level, cannabis is still legally classified as a 'Schedule 1 Drug', inhabiting the same category as heroin and LSD. At the same time, the federal government has allowed the individual states to decide whether or not to legalize cannabis consumption for medicinal and/or recreational purposes. This has led to an incoherent patchwork of local cannabis regulation and enforcement within the 50 states [3]:

- In 11 states both recreational and medical cannabis use is legal
- In 20 states medical use is legal
- In 13 states medical use is not 'legal', but has been 'decriminalized'
- In 6 states all use of cannabis is illegal

This legal bifurcation has a large number of societal impacts, but the specific impacts on cannabis testing have been quite profound. To name just two: 1) there are no federal standards, criteria, or analytical methods that govern cannabis testing, and 2) it is generally illegal to ship cannabis containing materials – including analytical standards, performance evaluation samples, and cannabis samples for testing – across state lines.

These restrictions have stifled the creation of uniform nationwide testing of cannabis quality and safety, a situation quite unlike that which exists for the testing of food safety and quality.

Variation in Mycotoxin Testing

Not surprisingly, since state cannabis testing programs have developed independently, large variations have arisen in the quality, consistency, and efficacy amongst the state programs which govern local cannabis testing. Perhaps nowhere is this variation more problematic than in the testing for mycotoxins. This particularly dangerous contaminant does not everywhere appear to be receiving the attention it deserves.

Although most states appear to recognize the inherent danger of mycotoxins, this is not uniformly reflected in the testing requirements and methodology. Many states require basic testing for mold but do not drill down upon testing for the specific mycotoxins of greatest concern. Several states, notably California, Colorado, and Washington, do have well-defined requirements, but many do not. And, in a few states, those where cannabis use is illegal, there are no testing requirements at all, leaving consumers to deal with the safety threat of illegally obtained cannabis.

Potential Solutions

That the current unsatisfactory mix of cannabis mycotoxin testing requirements and

effectiveness should continue to exist is, I believe, clearly a political challenge, not a technical one. Current measurement technology is fully capable of solving the problem. A brief internet search of the recent analytical chemistry literature will produce many examples of analytical methods that have the ability to identify, speciate and quantify the most important mycotoxins in cannabis matrices at levels of detection adequate to protect health and safety. Any number of potential solutions exist which could better serve as a unified mycotoxin testing model than the chaotic situation which currently exists.

One Example of a Solution

The following is a synopsis of some collaborative work between Phenomenex, Inc and Columbia Laboratories, a cannabis and food testing laboratory located in Portland, Oregon, USA [4]. The method was developed to analyze 13 mycotoxins in cannabis at the low ppb level, including the five primary mycotoxins required in several states (Ochratoxin A, Aflatoxin B1, B2, G1, and G2).

Experimental Conditions and Results

A 0.5 g sample of ground cannabis flowers was soaked in 5 ml of 2% ascorbic acid and extracted with 10 ml of acetonitrile followed by a modified roQ QuEChERs extraction. The extracted sample was centrifuged and the supernatant was diluted five-fold with ammonium formate buffer and filtered through a 0.45 um syringe filter prior to HPLC injection. The samples were analyzed on a 3 um Polar C18 HPLC column (Phenomenex Luna Omega) using the conditions described in Table 1. The mass analyzer used was a SCIEX Triple Quad 5500. Table 2 displays the 13 analytes along with their HPLC retention times and MRM transitions, including the MRM used to quantify each analyte. Figure 1 displays a typical chromatogram for the 13 mycotoxin analytes, all of which are very well separated in a 10-minute run.

Table 1. 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	

Table 2. Mass Spec Parameters.

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)	357.037	281.1	2.51	-55	-10	-18	-15	X
Nivalenol (M-H+HCOOH)	357.037	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	
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	

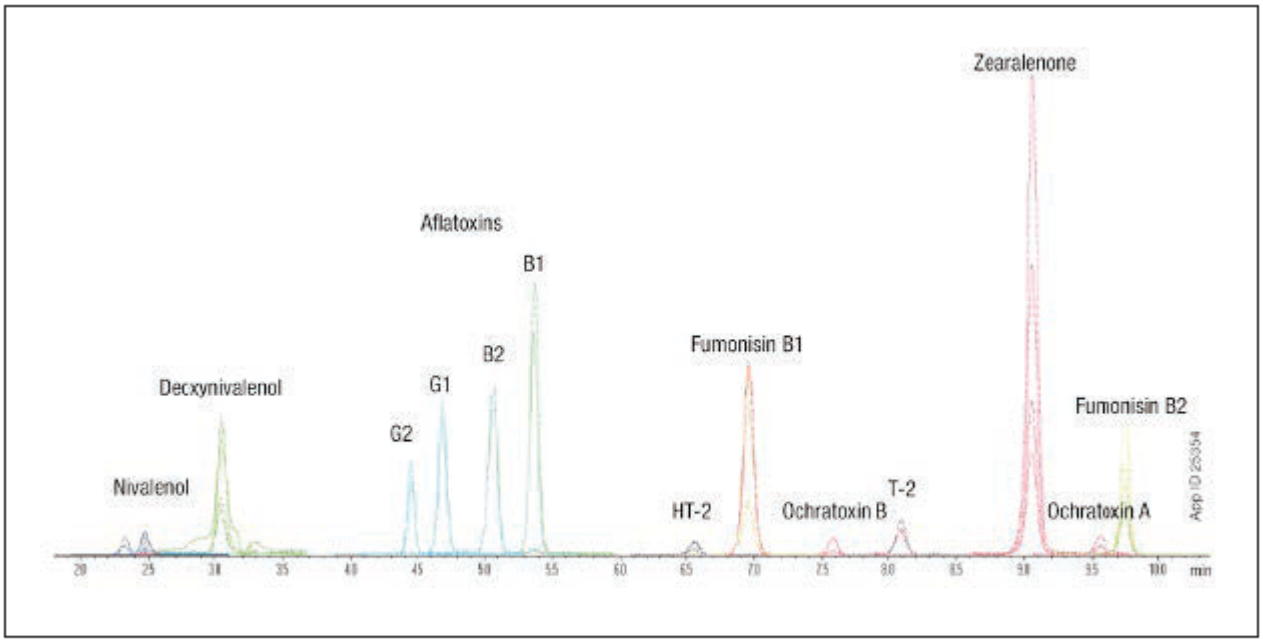

Figure 1. Chromatography of Expanded Mycotoxin Analyte List.

Commentary and Conclusion

The above-described method is just one example of similar LC-MS/MS methods that can readily be found in the literature, any one of which could potentially serve as the basis for a unified national approach to mycotoxin testing that would be far superior to the hodgepodge that currently exists in the US. So, given the need for rigorous and efficacious mycotoxin testing of cannabis and the lack of significant scientific or technology barriers, why has the status quo been so slow to change? Well, institutional and bureaucratic inertia are always handy excuses and, of course, the balkanized legal status of cannabis regulation in the US has clearly not helped the situation. But, given all that, it is still vexing that individual states, particularly those with a more progressive reputation, have not been more assertive in the area of mycotoxin testing.

A very useful review article [5] shows the many different ways that can be used to test for the presence of mycotoxins in cannabis. The author started with simple qualitative test strips

and concluded with advanced tests, like the LC-MS/MS approach described here and acknowledged the advantages of these advanced analytical approaches, but listed the disadvantages as well, such as expensive equipment and the need for highly trained staff. He concluded with the observation that: “This can be a large hurdle to overcome for smaller testing laboratories or new start-ups which may not have the capital to be able to purchase the equipment and employ expert staff.”

The honesty of that observation is much appreciated but it does raise an unsettling question. Are simple economics the reason for the slow adoption of better cannabis mycotoxin testing? Is the desire to let undercapitalized labs ‘have a go’ at cannabis testing a legitimate reason to compromise consumer safety? Surely this simplistic hypothesis is not the root cause for the slowness of adoption of advanced mycotoxin testing and it is indeed important to thoughtfully balance cost and benefit in testing scenarios. However, mycotoxin testing should not be an area where compromises should be made.

Acknowledgments

We wish to acknowledge the contribution of Wes Maguire and his team at Columbia Laboratories, Portland, Oregon, the USA for performing the analytical work.

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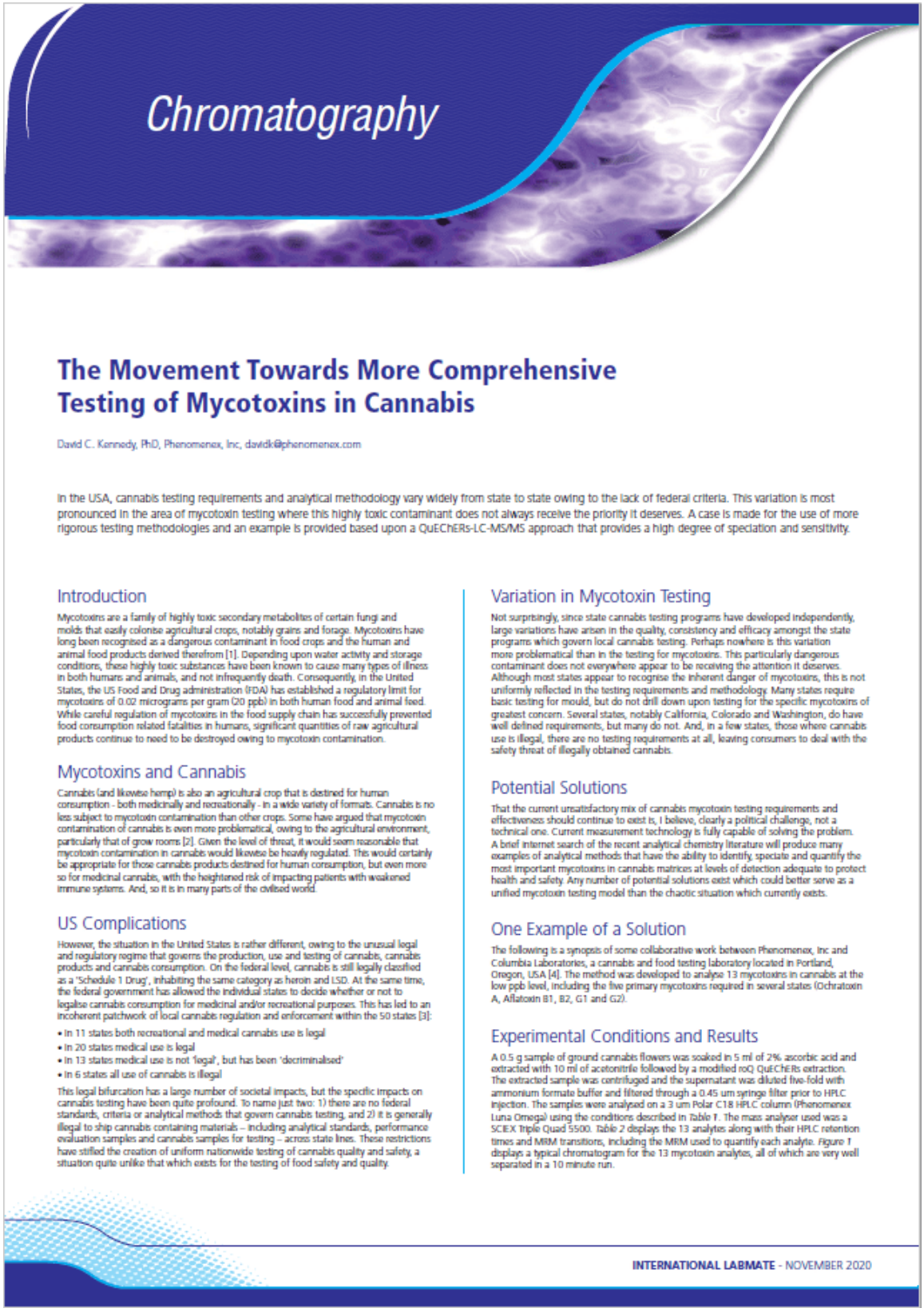

Chromatography

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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

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Compound Name	Q1 Mass	Q3 Mass	Retention Time (min)	Declustering Potential	Entrance Potential	Collision Energy	Collision Cell Exit Potential	Quantifier
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Fotoksin	152.9	53.1	2.37	-90	-10	-82	-9	
Nivalenol	311.02	203	2.52	-105	-10	-14	-37	
Nivalenol (M-H+HCOOH)	357.007	293.1	2.52	-55	-10	-16	-15	X
Nivalenol (M-H+HCOOH)	357.007	159	2.52	-55	-10	-40	-19	
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Fumonisin B1	722.5	352.5	7.02	146	10	49	30	
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Fumonisin B2	707.2	356.3	9.0	176	10	45	32	
Fumonisin B2	707.2	65.1	9.0	176	10	129	26	
Fumonisin B2	707.2	67	9.0	176	10	127	8	
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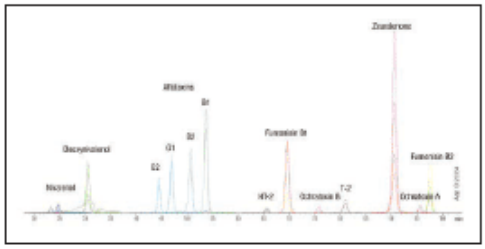

Figure 1. Chromatography of Expanded Mycotoxin Analyte List.

Commentary and Conclusion

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