Ensuring the safety of cannabis and cannabis products

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Introduction

Forty years ago a paper published showed that under favourable conditions, *Aspergillus* moulds could flourish and produce aflatoxins on marijuana. Uncured or inadequately processed marijuana could offer the right conditions for mould growth. There appears to have been little follow-up at the time. Legalisation of consumption of cannabis products in Canada and also in several States of the USA, for medicinal purposes and in some cases recreationally now brings safety to the fore. Cannabis now needs to be scrutinised for residues and contaminants to the same extent as food or pharmaceutical products. This means applying the same safety standards for levels of mycotoxins as apply to foodstuffs and conducting routine monitoring to ensure standards are maintained for products placed on the market.

Fortunately, in terms of analysis, immunoaffinity clean-up is already well established in Official Methods (AOAC International and CEN Standards) for use in the analysis of a diverse range of complex matrices for all regulated mycotoxins including aflatoxins and ochratoxin A. These immunoaffinity column methods have been rigorously validated and have been applied to a variety of botanical products such as herbal medicines, which have matrix similarities to marijuana.

R-Biopharm has previously demonstrated that immunoaffinity columns provide excellent clean-up of marijuana samples. In this study, IMMUNOPREP® ONLINE affinity cartridges were used in conjunction with a RIDA®CREST ICE handling system for automated analysis prior to FLD-HPLC detection. Two affinity cartridges were run simultaneously resulting in increased sample throughput, faster turnaround times, improved quality control and greater accuracy whilst meeting the increasing pressures of a busy testing lab.

Method

Cannabis energy drink:

1. Measure 20 ml of sample and sonicate for 30 mins.
2. AFT: dilute 1 ml of supernatant with 6 ml of 3 % Triton X-100 in water.
   OTA: dilute 1 ml of supernatant with 6 ml of 3 % Tween 20 in water.
3. Adjust to around pH 6.8 - 7.0 using 1 M sodium hydroxide.
4. Inject 0.5 - 1 ml onto the RIDA®CREST system.

Cannabis capsule:

1. Weigh 5 capsules into a centrifuge tube, add 5 ml of water (50 °C) & vortex for 5 mins.
2. Add 15 ml of 100 % acetonitrile and shake for a further 90 minutes on a shaker.
3. Centrifuge at 4,000 rpm for 10 mins.
4. AFT: dilute 1 ml of supernatant with 6 ml of 3 % Triton X-100 in water.
   OTA: dilute 1 ml of supernatant with 6 ml of 3 % Tween 20 in water.
5. Inject 0.5 - 1 ml onto the RIDA®CREST system.

Cannabis products:

1. Dependant on the matrix to be analysed weigh 1 -2 g and add to a centrifuge tube.
2. Add 20 ml of 75 % acetonitrile and mix at 2,000 rpm for 90 minutes on a shaker.
3. Centrifuge at 4,000 rpm for 10 mins.
4. AFT: dilute 1 ml of supernatant with 6 ml of 3 % Triton X-100 in water.
   OTA: dilute 1 ml of supernatant with 9 ml of 3 % Tween 20 in water.
5. Inject 0.5 - 1 ml onto the RIDA®CREST system.

Note: each cartridge was re-used up to 15 times.

Results

Sample	Total aflatoxin (AFT)		Ochratoxin A (OTA)	
	Spike level (ppb)	Average % recovery (% RSD)	Spike level (ppb)	Average % recovery (% RSD)
Cannabis plant	20	105 (3)	20	98 (2)
CBD oil	20	100 (2)	20	97 (1)
CBD capsules	20	108 (3)	20	98 (1)
CBD tea	20	110 (3)	20	81 (5)
Brownies with THC	5	106 (4)	5	107 (3)
Butter cookies with THC	5	117 (3)	5	109 (4)
Energy drink with hemp extract	5	88 (4)	5	76 (6)

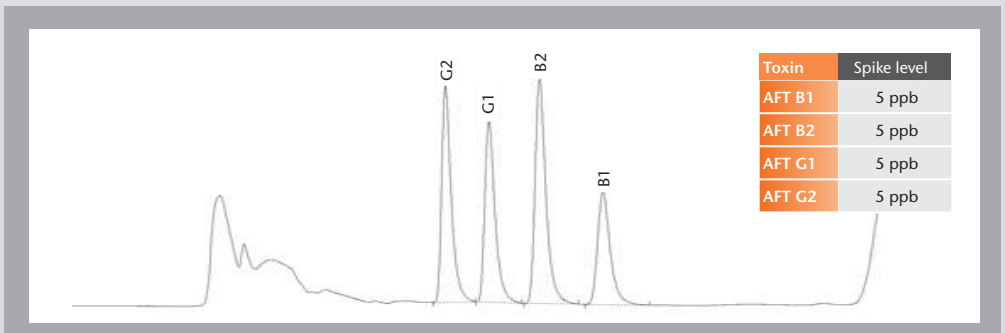

Fig. 2: example aflatoxin chromatogram for cannabis gelatine capsule

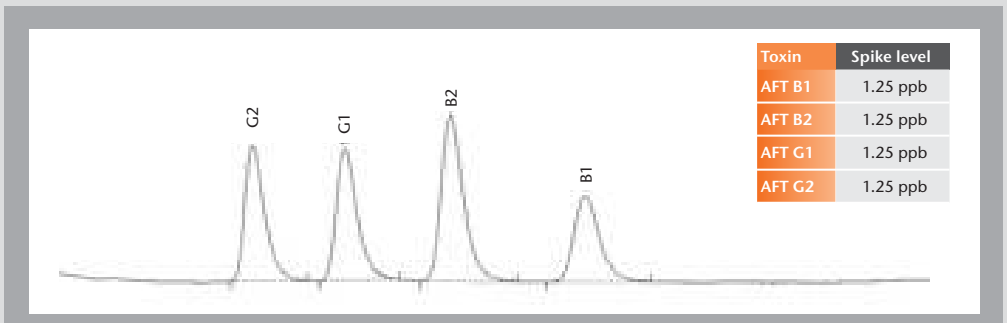

Fig. 1: example aflatoxin chromatogram for cannabis energy drink

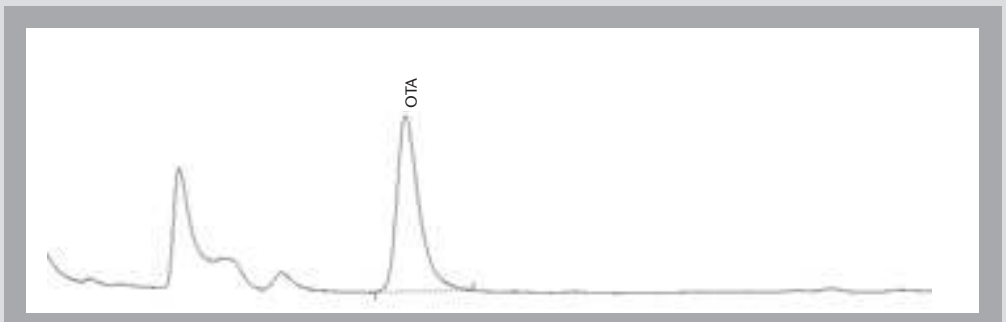

Fig. 3: example ochratoxin chromatogram for cannabis plant (spiked at 20 ppb)

Conclusion

- The results from the validation demonstrate that EU Method Performance Criteria as set out in EC 401/2006 has been exceeded for precision and accuracy demonstrating the methods are fit for purpose.
- For aflatoxin recoveries ranged from 88 - 117 % with % RSD ranging 2 - 4 %. For ochratoxin recoveries ranged 76 - 109 % with % RSD ranging from 1 - 6 %.
- Precision and accuracy are enhanced due to automation of the application of sample extract or standard solutions onto the cartridge. In addition, automated online immunoaffinity clean-up using IMMUNOPREP® ONLINE cartridges reduces sample handling time, enabling analysts to focus on less routine tasks.
- Maximum sample throughput was established using the 2-cartridge model enabling the analysis of 50 samples in a single overnight run.

03/2019