Routine Analysis of Cannabis for Pesticides and Mycotoxins using UPLC-MS/MS and GC-MS/MS

Kim Tran,[1] Kari Organtini,[1] Marian Twohig,[1] Peter Alden,[1] Michael S. Young,[1] Narendra Meruva,[1] Kenneth Rosnack,[1] Gordon Fujimoto,[2] Rebecca Stevens,[3] James Roush,[3] and Christopher J. Hudalla[3]
[1]Waters Corporation, Milford, MA USA; [2]Waters Corporation, Beverly, MA, USA; [3]ProVerde Laboratories, Milford, MA, USA

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

INTRODUCTION

- The increased use of both medical and recreational cannabis in combination with its expanding legal acceptance in several US states has led to increased cannabis safety and quality control testing.
- Analytical testing typically includes cannabinoids profiling/potency, mycotoxins, terpenes, residual solvents, metals, and pesticide residue analysis. Pesticides are of particular interest as they are widely used in the cultivation of cannabis plants to safeguard against harmful insects and to promote crop yields.
- In addition to pesticides, cannabis must also be tested for mycotoxins. A robust and rapid test is critical and single simultaneous test for pesticides and mycotoxins is ideal.
- Multi-residue compound detection is routinely performed using tandem quadrupole mass spectrometry (MS/MS) in combination with liquid chromatography (LC) and gas chromatography (GC).
- Tandem quadrupole MS is the detector of choice as it provides high sensitivity and selectivity for simultaneous analysis of hundreds of pesticides at low ng/g (ppb) levels in a single analysis.
- In this study, we present the use of a simple sample extraction and dSPE cleanup where the resulting extract is analyzed by UPLC-MS/MS and/or GC-MS/MS for rapidly monitoring pesticides and mycotoxins in cannabis matrix to meet California regulations (Figure 1).

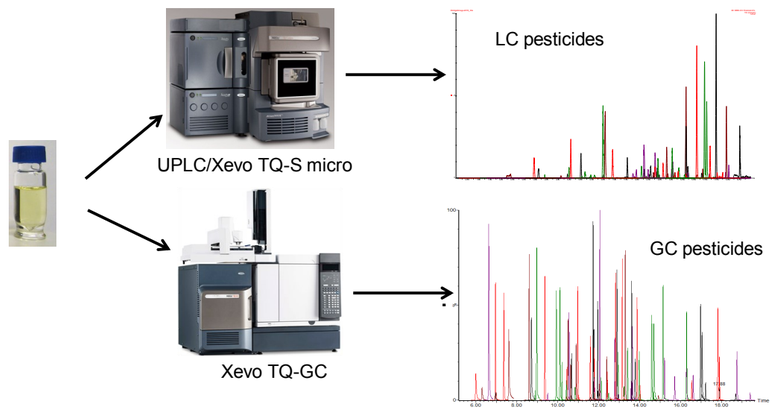

Figure 1. A workflow for multi-residue pesticide analysis by LC-MS/MS and GC-MS/MS

SAMPLE PREPARATION

Initial Extraction

- 0.5 g ground cannabis bud weighed into 15 mL centrifuge tube
- 5 mL acetonitrile added
- Process with Geno Grinder for 5min @ 1500 rpm
- Remove 1 mL aliquot for dSPE

dSPE

- Shake dSPE tube for 1 min
- Centrifuge
- Transfer supernatant to autosampler vial for analysis by LC-MS/MS and GC-MS/MS

Recoveries for most compounds were in the range of 80-120%. Matrix effects were significantly reduced when dSPE was performed following the initial acetonitrile extraction. [1]

QUANPEDIA METHOD DEVELOPMENT ASSISTANCE

- The Quanpedia method database was used to automatically create the LC, GC, MS, and data processing methods (Figure 2).
- Pre-defined LC-MS/MS, and GC-MS/MS methods can be generated in just three steps, which eliminates the level of potential error and the complexity involved in method development for large numbers of target analytes.
- Quanpedia also contains functionality to quickly adjust retention times associated with a method, eliminating the lengthy process of manually adjusting MRM time windows due to retention time shifts.

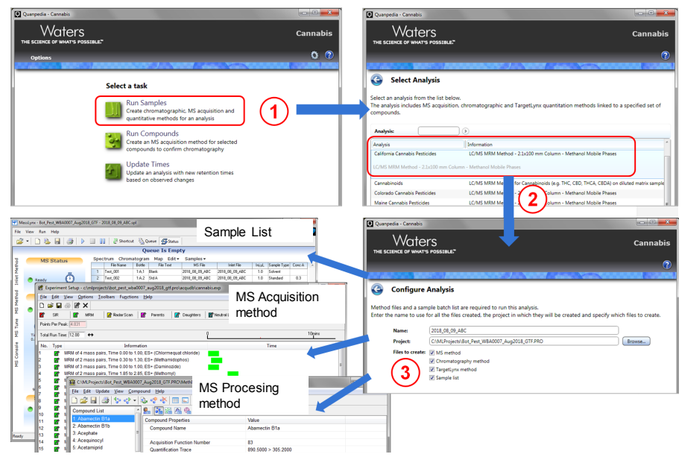

Figure 2. *Rapid implementation of LC, GC, MS and data processing methods using Quanpedia method database.*

UPLC Method

Acquity H Class coupled to TQ-S micro
Column: XBridge C18, 2.1 x 150 mm, 2.5 µm @ 50°C
Mobile Phase A: Water + 5 mM ammonium formate with 0.02% formic acid
Mobile Phase B: MeOH
Flow rate: =0.40 mL/min
Injection volume: 5 µl

Time	% A	% B
0	98	2
0.20	98	2
4.00	30	70
10.00	30	70
12.00	1	99
15.00	1	99

GC Method

Xevo TQ-GC
Column: Rxi-5MS 20 m x 0.18 mm x 0.18 µm
Carrier Gas: Helium
Injector Temp.: 280 °C
Flow rate: 2mL/min
Injection volume: 1 µl

Oven Program:

Rate (°C/min)	Temperature (°C)	Hold (min)
-	60	0.45
18.70	330	2.25

For a complete list of LC, GC and MS parameters please review the referenced application note

PESTICIDES AND MYCOTOXINS ANALYSIS BY UPLC-MS/MS

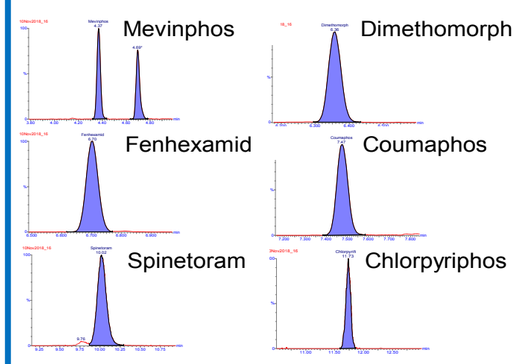

Figure 3. *Representative MRM chromatograms for (1) mevinphos isomers, (2) dimethomorph, (3) fenhexamid, (4) coumaphos, (5) spinetoram, (6) chlorpyriphos spiked at a level of 0.10 µg/g in cannabis flower .*

- US states and Canada have defined different testing requirements for pesticide residue testing in cannabis. The list of pesticides varies with each state.
- The composition and complexity of the matrix varies widely across different cannabis strains. The combination of long lists of pesticides with variable and complex matrices presents a significant challenge in method development.
- Linear calibration curves (R^2>0.990) for all pesticides were obtained over the range tested 0.025 to 0.50 µg/g.
- Representative MRM chromatograms for selected pesticides are displayed in Figure 3.

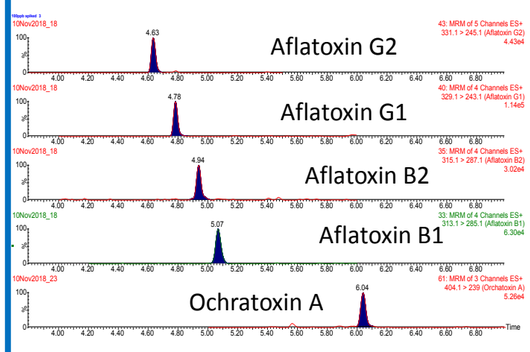

Figure 4. *Representative MRM chromatograms for aflatoxins B1, B2, G1, G2 and ochratoxin A spiked at a level of 0.02 µg/g in cannabis matrix.*

- The LC-MS/MS analysis of mycotoxins can be combined with the analysis of pesticide residues in a single analytical injection, allowing trace level detection of aflatoxins B1, B2, G1, G2, and ochratoxin A.
- The calibration curves for all mycotoxins were linear (R^2>0.990) over the range tested 0.005 to 0.10 µg/g
- Figure 4 shows the chromatograms of cannabis matrix spiked at 0.02 µg/g which is the action level set by the State of California for mycotoxins testing.

PESTICIDES ANALYSIS BY GC-MS/MS

- Analysis for pesticide residues in the cannabis flower extracts also required GC-MS/MS to fully cover the California pesticide regulations.
- Compounds like chlordane, captan (analyzed as its degradant THPI), and pentachloronitrobenzene (PCNB) require GC-MS/MS due to poor ionization using electrospray in LC-MS/MS.
- There was a large subset of compounds that worked well using both techniques. Analysis on both systems allows for increased confidence in results and the GC-MS/MS data can be used as an added confirmatory technique.
- Linearity over the range of 0.025 to 1 µg/g was excellent with R^2 values >0.995 and residuals were within 20%.
- Figure 5 shows the chromatograms of cannabis matrix spiked with the pesticide mix at 0.10 µg/g

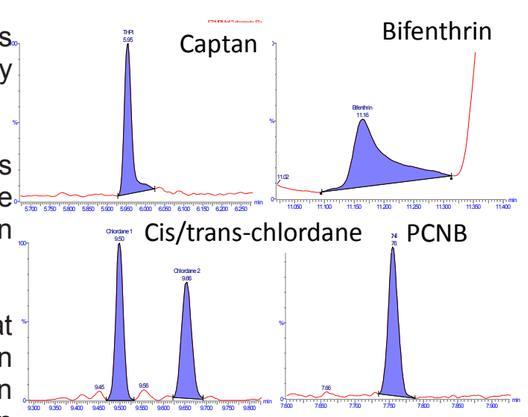

Figure 5. *Representative MRM chromatograms for (1) THPI, captan degradation product; (2) bifenthrin; (3) trans and cis chlordane; (4) PCNB spiked at a level of 0.1 µg/g in cannabis flower 0.7 µg/g for THPI) and extracted using the sample preparation protocol reported.*

CONCLUSION

- This simple sample extraction and dSPE cleanup method followed by UPLC-MS/MS and GC-MS/MS analysis provides a rapid, sensitive, and robust workflow for determination of the California pesticide list and mycotoxins in challenging cannabis matrix. Matrix suppression was significantly reduced using dSPE cleanup for many pesticides; thereby improving the data quality.
- This method is capable of meeting the action levels for the California pesticide list and mycotoxins in cannabis matrix.

REFERENCES
1. Kim Tran, Kari Organtini et al. Analysis of Residual Pesticides and Mycotoxins in Cannabis Using UPLC-MS/MS and GC-MS/MS to Meet California Regulatory Requirements Waters application note 720006465EN http://www.waters.com/webassets/cms/library/docs/720006465en.pdf

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS