# ROUTINE ANALYSIS OF CANNABIS FOR PESTICIDES AND MYCOTOXINS USING UPLC-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*

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## INTRODUCTION

The increased use of both medical and recreational cannabis in combination with its expanding legal acceptance in many US states has led to increased demand for 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 a 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 to rapidly monitor pesticides and mycotoxins in cannabis matrix to meet California regulations (Figure 1).

With the variety of residues to be monitored as well as the continued possibility of new ones being added, method generation can be a tedious task. In this study, methods for LC-MS/MS were utilized from a software database and method manager, Quanpedia™, eliminating the need for method development for the California pesticide and mycotoxin lists (Figure 2).


Waters App Note: 720006373EN

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

## SAMPLE PREPARATION

### Initial Extraction

- 0.5 g ground cannabis bud weighed into 50 mL centrifuge tube
- 5 mL acetonitrile added
- Process with Geno Grinder for 2min @ 1500 rpm
- Remove 1 mL aliquot for dSPE

### dSPE

- 2 mL tube with 150 mg MgSO4, 50 mg PSA, 50 mg C18, 7.5 mg graphitized carbon
- 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]

### LC Conditions

| | |
|---|---|
| UPLC: | ACQUITY™ UPLC™ H-Class |
| Separation mode: | Gradient |
| Column: | XBridge C18 2.1 x 150 mm, 2.5 µm |
| Solvent A: | 5 mM Ammonium formate with 0.020 % formic acid in water |
| Solvent B: | Methanol |
| Flow rate: | 0.400 mL/min |
| Column temp.: | 50 °C |
| Injection volume: | 5 µL |

| Time (min) | %A | %B | Curve |
|---|---|---|---|
| 0.00 | 98% | 2% | - |
| 0.20 | 98% | 2% | 6 |
| 4.00 | 30% | 70% | 6 |
| 10.00 | 30% | 70% | 6 |
| 12.00 | 1% | 99% | 6 |
| 15.00 | 1% | 99% | 6 |
| 15.01 | 98% | 2% | 1 |
| 17.00 | 98% | 2% | 1 |

### MS Conditions

| | |
|---|---|
| MS: | Xevo™ TQ-Smicro |
| Ionization mode: | ESI + / ESI - |
| Capillary voltage: | 3.0 kV (+); 2.5 kV (-) |
| Cone Voltage: | Various V |
| Collision Energy: | Various eV |
| Desolvation temp: | 550 °C |
| Source temp.: | 150 °C |
| Desolvation Gas Flow: | 800 (L/Hr) |
| Cone Gas: | 50 (L/Hr) |

## PESTICIDES AND MYCOTOXINS ANALYSIS BY UPLC-MS/MS

Canada and individual US states have defined different requirements for pesticide residue testing in cannabis. The list of pesticides varies from state to state as well as from country to country.

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/kg. Representative MRM chromatograms for selected pesticides are displayed in Figure 3.

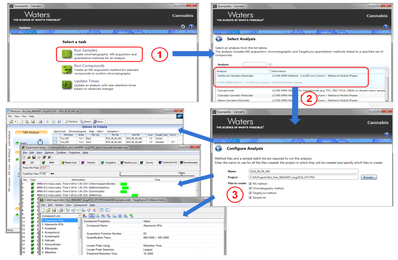


Figure 2. *Rapid implementation of LC, GC, MS and data processing methods using Quanpedia method database.*

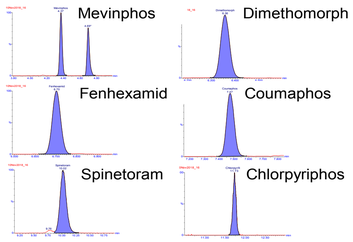


Figure 3. *Representative MRM chromatograms for (1) mevinphos isomers, (2) dimethomorph, (3) fenhexamid, (4) coumaphos, (5) spinetoram, (6) chlorpyriphos spiked at a level of 0.10 µg/kg in cannabis flower .*

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/kg

Figure 4 shows the chromatograms of cannabis matrix spiked at 0.02 µg/kg which is the action level set by the State of California for mycotoxins testing.

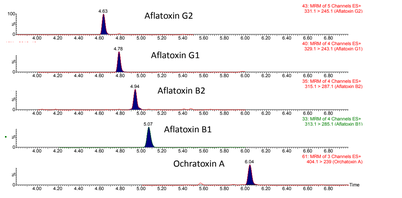


Figure 4. *Representative MRM chromatograms for aflatoxins B1, B2, G1, G2 and ochratoxin A spiked at a level of 0.02 µg/kg in cannabis matrix.*

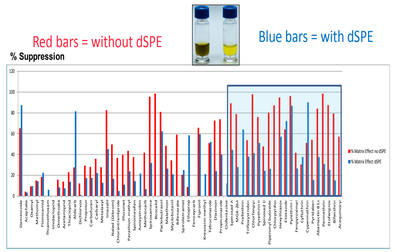


*Figure 6. Matrix suppression at the 200 µg/kg level; the red bars indicate suppression observed without dSPE and the blue bars indicate suppression after dSPE cleanup. The shaded area indicates the compounds that co-elute with cannabis resin constituents*

## RESULTS AND DISCUSSION

Waters Quanpedia method database was used to automatically create the LC, GC, MS and data processing methods (See Figure 2 for the various target pesticides to be monitored using the transitions.)

Users can quickly generate pre-defined LC-MS/MS and GC-MS/MS methods in just three steps, which greatly reduces the level of potential error and the complexity involved in method development for large numbers of target analytes.

As a result, it decreases the amount of work, time, and resources required for laboratories to set up methods. Additionally, 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.

The LC-MS/MS method contained 67 compounds (62 pesticides and 5 mycotoxin) and the GC-MS/MS method contained 54 compounds, fully covering the California requirements for pesticide and mycotoxin residue analysis.

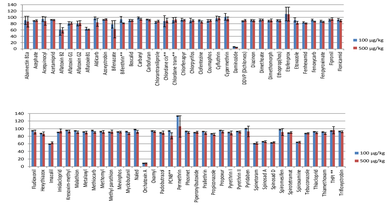


Figure 5. *Recoveries for most of the pesticides were in the range of 80% to 120% (n=6)*

### Linearity

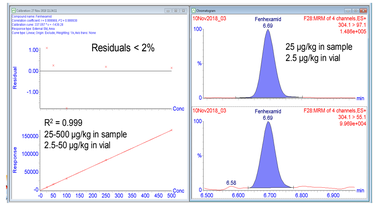


*Figure 7. . Representative example of a quantitation curve for fenhexamid demonstrating a linear range from 25 to 500 µg/kg (2.5 to 50 µg/kg in vial concentration) cannabis resin constituents*

Method recovery was assessed by spiking pesticides at the 0.1µg/kg and 0.5µg/kg levels in a cannabis flower matrix and comparing the response to that observed from spiked matrix blanks (matrix matched standards). As shown in Figure 5, the recoveries observed for most pesticides were in the range of 80-120%.

Matrix suppression was determined at the 200 µg/kg level by comparison of the response observed in matrix matched standards to response observed in solvent standards. Matrix suppression data are presented in Figure 6.

The dSPE cleanup provided significant reduction of suppression for most compounds. Those compounds that co-elute with cannabis resin constituents (retention times from 9 to 12 minutes) showed the greatest suppression after dSPE cleanup.

The recovery of daminozide, ochratoxin A from the PSA sorbent are decreased due to the interaction of this compound with the PSA sorbent. Analysis of these compounds should be performed before dSPE.

For linearity and sensitivity evaluation, matrix matched calibration curves were generated and an example of the quantitation curve and respective MRM chromatograms for fenhexamid is shown in Figure 7.

## 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 pesticides 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

For detail on GC MS/MS analysis for pesticides in cannabis, please see Wednesday Poster 154

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

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*1Waters Corporation, Milford, MA USA; 2Waters Corporation, Beverly, MA, USA; 3ProVerde Laboratories, Milford, MA, USA J. , Milford, MA, USA*

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