Screening for Pesticide Residues in Cannabis using a High Resolution Accurate Mass GC/Q-TOF and a Highly Sensitive GC/QQQ

Philip L. Wylie and Jessica Westland
Agilent Technologies
Wilmington, DE

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Introduction

Recent Headlines about Pesticides in Cannabis

"First tests are in, and 1 in 5 marijuana samples in California isn't making grade" *The Orange County Register,* July 25, 2018

"Vape Company Fails Pesticide Test, Issues First Product Recall in Legal Market" *Merry Jane, Tuesday, August 21, 2018*

"Regulators investigating Maryland medical marijuana grower for alleged pesticide use" *The Baltimore Sun, August 21, 2018*

"Toxic pesticide use rising at illegal California pot farms" *The associated Press, May 29, 2018*

Objectives

- Study different extraction procedures for pesticide residues
- Observe the effects of diluting extracts 500:1
- Spike cannabis samples with CA + OR pesticides; extract, dilute and analyze by Agilent 7010 GC/TQ and Agilent 7250 GC/Q-TOF

Experimental

Extraction Procedure

Spike 66 CA + OR Pesticides

ACN Extraction
Weigh ~1.0 g of sample into a 50-mL centrifuge tube
Add ceramic homogenizer, cap the tube, and mechanically shake it for 2 minutes (1,500 strokes/min)
Add 15 mL of acetonitrile (ACN) to each tube
Spike Surrogate Standards
Cap the tube, and mechanically shake for 2 minutes (1,500 strokes/min)
Transfer solvent to unconditioned SPE Cartridge
SampliQ C18 Endcapped (p/n 5982-1365)
Bond Elut Plexa (p/n 12259506)
6 mL cartridges elute into 16 x 100 mm collection tubes
Collection tube aliquot transferred to designated 50 mL centrifuge tube
Rinse the centrifuge tube with 5 mL of ACN
Cap the tube, and mechanically shake for 2 minutes (1,500 strokes/min)
Transfer solvent to specified unconditioned SPE Cartridge
Rinse the centrifuge tube with 5 mL of ACN
Cap the tube, and mechanically shake for 2 minutes (1,500 strokes/min)
Cetrifuge the tube for 5 min at 5000 rpm
Transfer solvent to specified unconditioned SPE Cartridge
Collection tube aliquot transferred to designated 50 mL centrifuge tube
Bring the collected eluent to a 25 mL final volume with ACN in the tube
An aliquot of each 25 mL extraction was added to a GC analysis vial
Dilution - no dSPE cleanup
Add 50 µL extract to 950 µL ACN
Put in vial for analysis
GC/MS/MS
Add 100 µL of extract to
900 µL of hexane/acetone (1:1) to a 2-mL dSPE cartridge (p/n 5982-0028)
Vortex the tube for 60 seconds, then centrifuge for 2 minutes at 2,000 rpm
Add available supernatant to a vial for analysis
dSPE
Add 300 µL of supernatant to 300 µL of hexane/acetone (1:1 v/v)
Put in vial for analysis

Try 2 different SPE cleanups

25:1 dilution - Pests at 100ppb

20:1 dilution - Pests at 5 ppb

2 dilutions – 1:10 & 1:2 - Pests at 5 ppb

Experimental

GC/TQ and GC/Q-TOF Analysis

An Agilent 7890/7010 GC/TQ was used to analyze matrix matched calibration standards and extracts. Of the 66 spiked compounds, 46 could be analyzed by the GC/TQ. The others require LC/TQ. This method has been published [1].

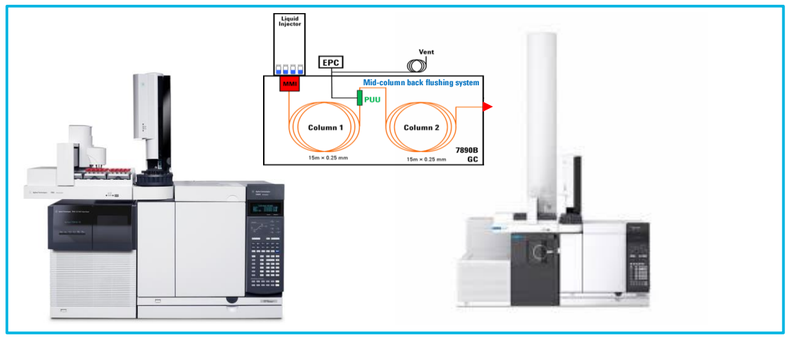

Figure 1. Agilent 7890/7010 GC/TQ (L) and 7890/7250 GC/Q-TOF (R). Configuration for mid-column backflushing (top).

Instrument Conditions

Select GC/TQ Conditions	
Column 1	**Agilent HP-35MS, 15 m x 0.25 mm x 0.25 µm**
Column 2	**Agilent HP-5MS, 15 m x 0.25 mm x 0.25 µm**
Column flow rates	**col 1 = 1.2 mL/min, col 2 = 1.25 mL/min**
Oven Temp program	**70 °C (1 min), 35°C/min to 180°C (0 min), 10°C/min to 200°C (0 min), 8°C/min to 300°C (4.5 min)**
Injection volume	**2 µL splitless**
Sandwich Injection	**L1 = Standard in solvent; L2 = Clean cannabis extract**
TQ mode	**MRM**
Select GC/Q-TOF Conditions	
Column 1 and 2	**Agilent HP-5MS UI, 15 m x 0.25 mm x 0.25 µm**
Column flow rates	**col 1 = 1.094 mL/min, col 2 = 1.294 mL/min**
Oven Temp program	**60°C (1 min), 40°C/min to 170°C (0 min), 10°C/min to 310°C (3 min)**
Injection volume	**2 µL cold splitless**
Q-TOF mode	**TOF Only**

Results and Discussion

Cleanup comparison

Figure 2 shows GC/TOF TICs for three different extracts. All used the C-18 SPE. Figure 2A shows the cannabis extract after the 25:1 dilution. Figures 2B and 2C show the extracts with & without dSPE (respectively) & after a further 20:1 dilution.

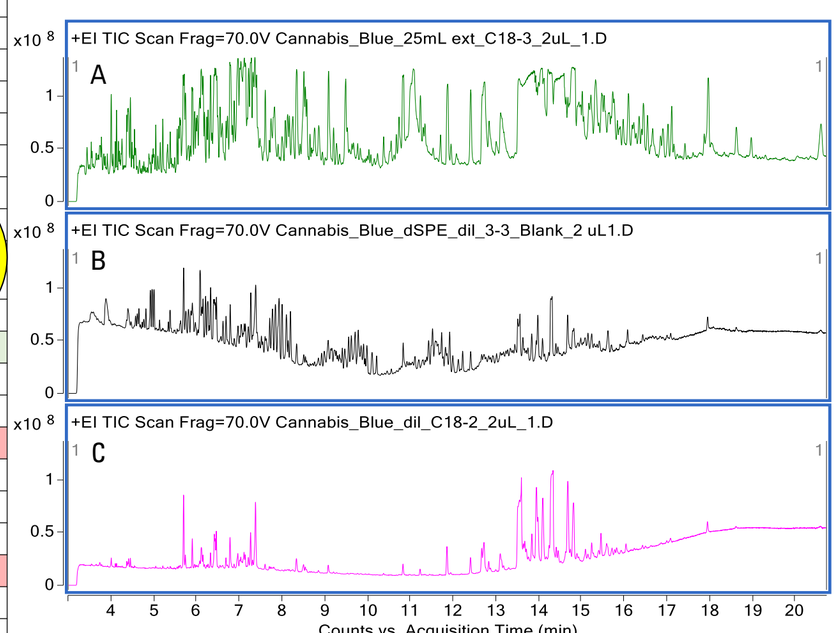

Figure 2. GC/Q-TOF TICs of cannabis extracts A) after C18 SPE with 25:1 dilution, B) C18 SPE + dSPE & 500 X total dilution, and C) C18 SPE & 500 X total dilution (equivalent to 1 g cannabis in 500 mL/ACN).

Results and Discussion

Identifying Pesticides using GC/Q-TOF with the Find by Fragments Algorithm in MassHunter Qualitative SW

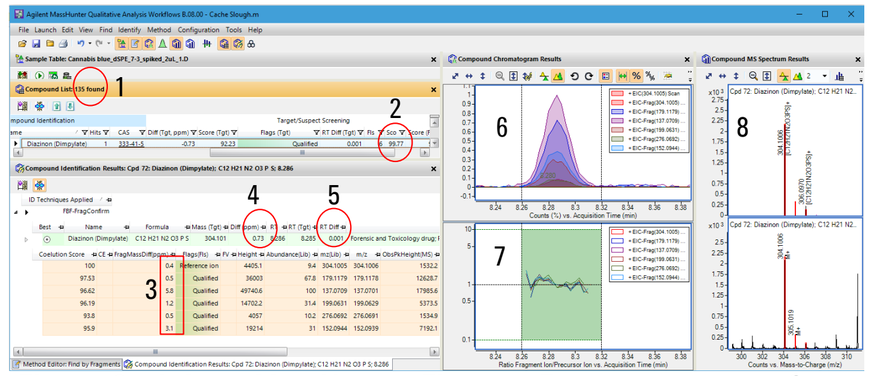

Figure 3. Agilent MassHunter Find by Fragments results for Diazinon at 5 ppb after C18 SPE and dSPE. 1) Number of qualified peaks, 2) How well ion ratios match database spectrum, 3) Fragment mass accuracy, 4) Mass accuracy for $[M^*]^+$, 5) Difference between database and measured RT, 6) Extracted ions, 7) Coelution plot, 8) Molecular ion isotope pattern.

Targeted pesticide analysis by GC/TQ. 46 Pesticides found with reasonable ($R^2 \geq 0.99$) calibration curves (Some with quadratic fit)

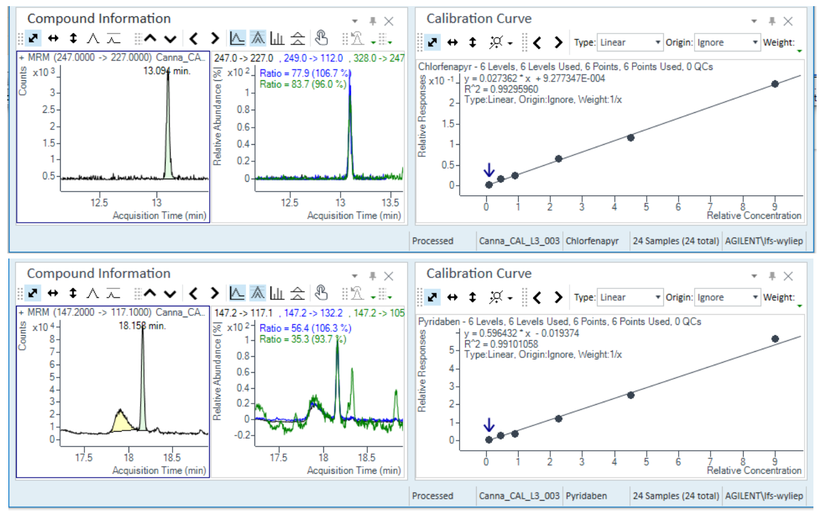

Figure 4. MRMs (quant + 2 qualifiers) & calibration curves (4.5 – 450 ppb) for Chlorfenapyr and Pyridaben in cannabis matrix.

Conclusions

- Our assumption is that illegal pesticides will be used, sometimes on legal cannabis and often on black market cannabis.
- Our best sample prep method (so far) for GC/TQ: C18 SPE + dSPE (50 mg PSA, 50 mg C18EC, 7.5 mg GCB, 150 mg MgSO4) + 500:1 dilution.
- GC/Q-TOF may require less dilution – 20:1 or 25:1. Experiments planned.
- Of the 66 pesticides spiked into cannabis, GC/TQ found 46 at 4.5 ppb in dilute cannabis matrix. The other 22 require LC/TQ.
- Of the 66 pesticides spiked into cannabis, GC/Q-TOF found evidence for 40 at 5 ppb in dilute cannabis matrix. A few responses were below the detection limit. Others require LC/MS.
- The GC/Q-TOF can screen for ~1000 pesticides and environmental contaminants. When standards are available, one can quantify the hits. Or, new hits can be added to the GC/TQ method.

References

[1] A novel comprehensive strategy for residual pesticide analysis in cannabis flower, **Agilent Technologies Application Note No 5991-9030EN, March 9, 2018.**