SCREENING FOR PESTICIDE RESIDUES IN CANNABIS USING A HIGH RESOLUTION ACCURATE MASS GC/Q-TOF

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Agilent

Trusted Answers

Introduction

Cannabis is:

- Considered to be a schedule 1 drug by the US government
  - same as heroin, LSD, ecstasy…
- Legal for medical and recreational use in 9 states & DC
- Legal for medical use in 29 states

Regulations are state-by-state

No uniformity in pesticide regulations

California legalized recreational use – Jan. 1, 2018

Canada will legalize recreational use this summer

California is the state with the most comprehensive testing requirements

Black market competes with legalized cannabis pesticides widely used on black market cannabis

Figure 1. Mess and contamination left behind by illegal cannabis growers.

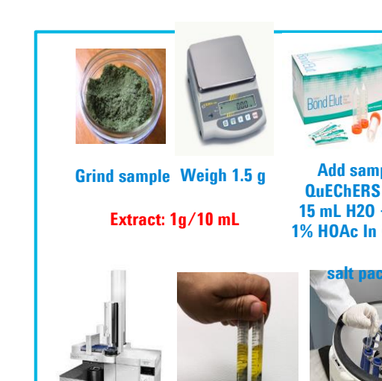
There are estimated to be 50,000 illegal cannabis growing sites deep in the California forests. Growers contaminate the cannabis & pollute the environment with pesticides.

Experimental

Extraction Procedure: Modified QuEChERS

Figure 2.Extraction procedure

A second set of extractions used 1 g of cannabis & 3 mL each of acetonitrile & water & an additional back extraction with a second aliquot of water. This gave a much dirtier extract (Figure 4).



GC Parameters	
Injection volume	1 µL
MMI Temperature	280°C (Splitless)
Columns	Two 15 m X 0.25 mm x 0.25 µm HP-5MS UI
He Carrier gas	Col 1 = 0.979 mL/min; Col 2 = 1.179 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)
RT Locking	Chlorpyrifos locked to 9.954 min
Backflushing	3 min post run at 310°C and −7.87mL/min
Q-TOF Parameters	
Mode	TOF
Acquisition range	40 – 550 u
Acquisition rate	5 Hz
Collision gas	N_2 at 1.5 mL/min

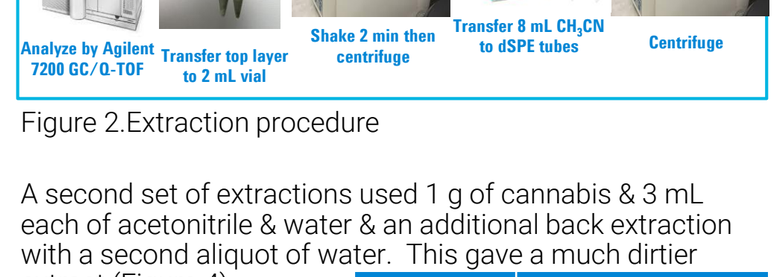


Figure 3. GC configuration for backflushing column 1

Results and Discussion

Cannabis Extracts are Dirty! High Concentrations of Terpenes and Cannabinoids

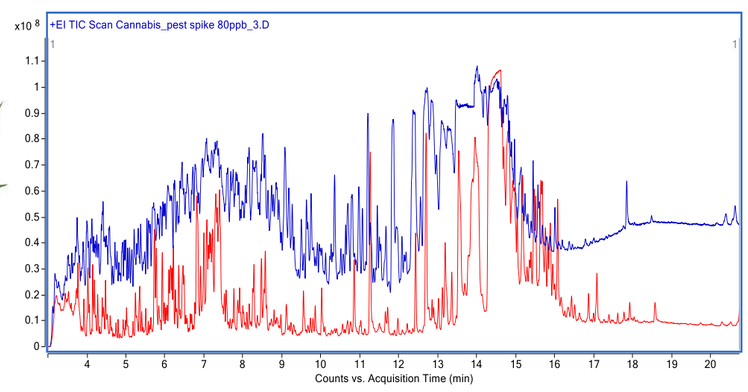


Figure 4. Chromatograms of two different cannabis extracts. Red: used procedure shown in Figure 2. Blue: used the modifications described below Figure 2.

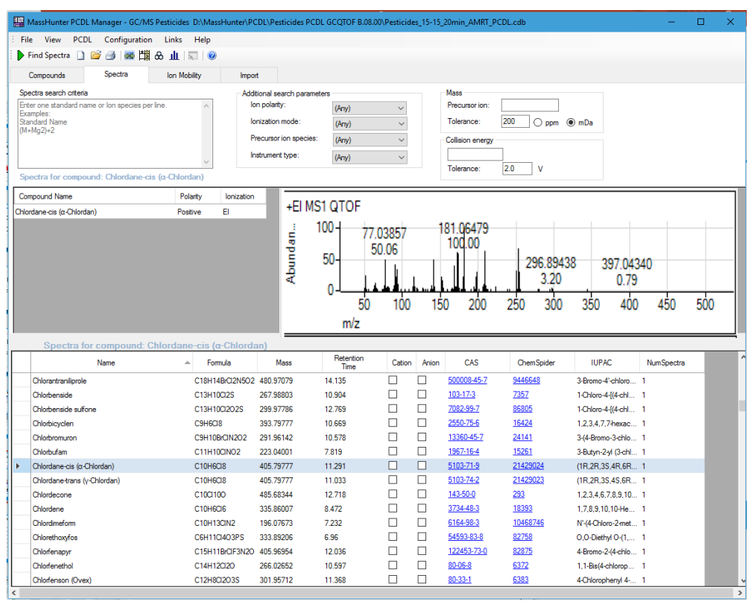


Figure 5. Exact mass Pesticide Personal Compound Database and Library containing HRAM spectra for >850 pesticides and related contaminants.

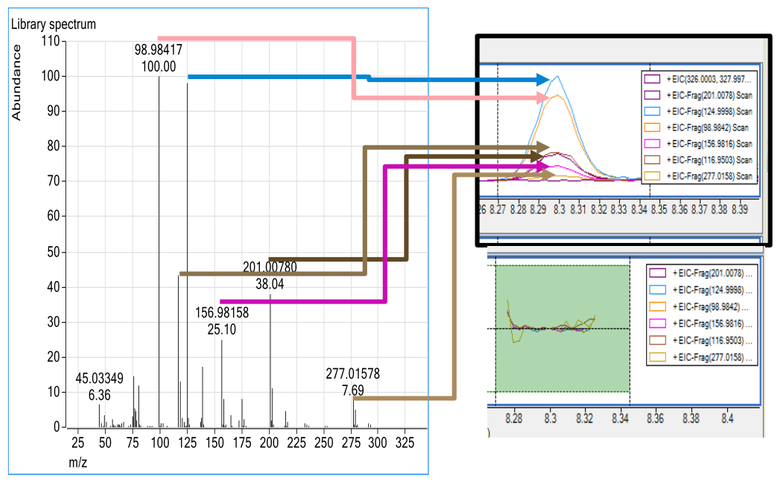


Figure 6. Software extracts six exact mass ions at the locked RT for each compound in the PCDL. EICs with the same RT and peak shape are "qualified." Other information is available: RT difference from PCDL, # of qualified ions, fragment ratio score, mass accuracy, and more.

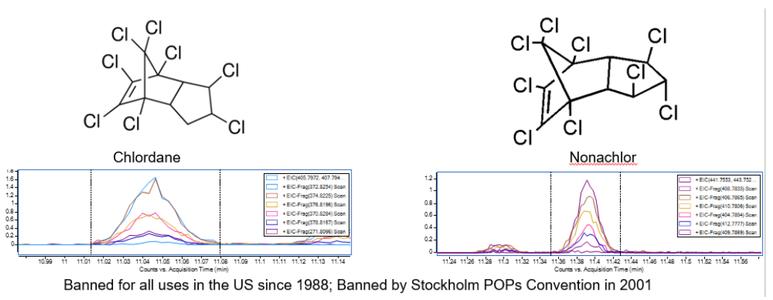


Figure 7. Banned pesticides found in two confiscated samples

Pesticides & contaminants found in 16 confiscated cannabis samples

Pesticides & Metabolites	Pesticides & Metabolites
DEET	Benzyl Benzoate
Cypermethrins	gamma-Cyhalothrin
Tributyl phosphate *	2-Phenylphenol
Acrinathrin	Cyphenothrin
Jasmone	Pentachloroanisole (Pentachlorophenol metabolite)
Empenthrin	
Dichlorvos	**Fire Retardants**
trans-Chlordane	Tris(3-chloropropyl)phosphate*
cis-Chlordane	Tris(β-chloropropyl)phosphate*
trans-Nonachlor	
Diphenylamine	
2,6-Diisopropylnaphthalene*	**PAHs**
Terbucarb	Phenanthrene*
Fenpropathrin	Fluoranthene*
Myclobutanil	Pyrene*
Carbaryl	Yellow highlight = on California's Forbidden List
1-Naphthol (Carbaryl metabolite)	Blue: California has a Max Residue Limit

Quantitative Analysis

Spiking samples

Cannabis grown without pesticides at U. Mississippi

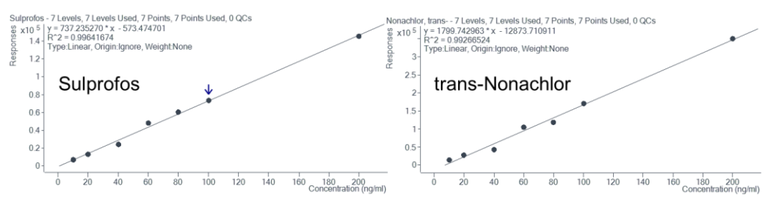
Spiked with ~200 pesticides @ 10, 20, 40, 60, 100 & 200 ng/g before extraction

Chromatogram of cannabis extract shown in Figure 4 (blue)

Matrix interferences interfered with many pesticides

Reasonable calibration curves for 83 pesticides

R^2 for most >0.99



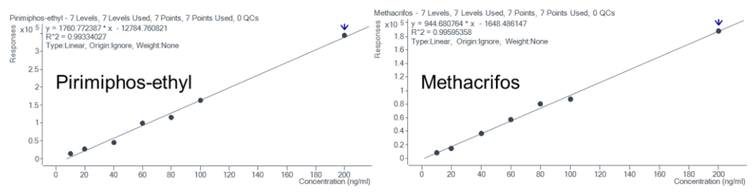


Figure 8. Calibration curves for select pesticides analyzed by GC/Q-TOF. Interferences from coextracted endogenous compounds prevented many pesticides from being quantified.

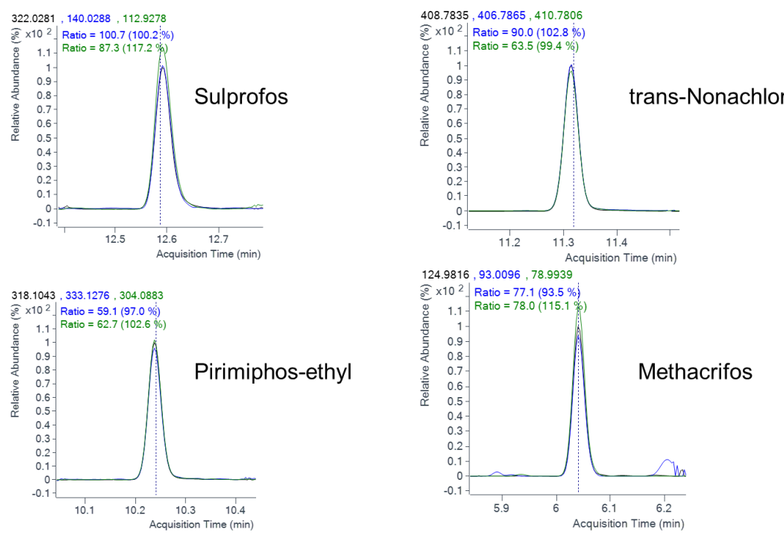


Figure 9. Quant and qualifier ions for selected pesticides analyzed by GC/Q-TOF

The Role of GC/QQQ

Cannabis testing labs use GC/QQQ for pesticide residue testing because of its selectivity and lower cost.[1] One can quantify more than 200 pesticides but one only "sees" those compounds on the target list

Next Steps for Cannabis Analysis by GC/Q-TOF

Improve extraction process

Dilute extracts by at least 100X

Analyze samples using an Agilent 7250 GC/Q-TOF with

- Improved sensitivity
- Much higher resolution
- Larger linear dynamic range
- Low energy ionization

Try MS/MS with the Q-TOF

Conclusions

Cannabis Extraction

- Two methods tried
  1. QuEChERS extraction with 10:1 dilution
  2. Modified QuEChERS with 3:1 dilution
- Both methods resulted in an extract with a lot of matrix
- Will try other extraction strategies with 100:1 dilution

Qualitative Analysis

- 22 different pesticides identified in 16 confiscated marijuana samples
- OP fire retardants and PAHs are ubiquitous in the environment and may be environmental or processing contamination
- Some pesticides were probably missed because of matrix

Quantitative Analysis

- Method developed for 82 pesticides
- Heavy matrix interfered with many pesticides
  Better cleanup and sample dilution will probably help

References

[1] L. Asanuma, D. Miller, R. Jordan, M. Churley, and A. Macherone, A novel comprehensive strategy for residual pesticide analysis in cannabis flower, Agilent Application Note 5991-9030EN.