Application Note

Approaches for the Analysis of Pesticide Residues in Cannabis

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Introduction

For pesticide analysis of cannabis, many labs have adopted the "quick, easy, cheap, effective, rugged & safe" (QuEChERS) method. There are several issues which may be encountered with this approach, including highly pigmented extracts, interference/ contamination from co-extracted cannabinoids, and poor peak shapes for early eluting peaks in HPLC analysis.

In this work, an approach for analysis of many of the pesticides required for testing by the state of Oregon was developed. Analysis of this list requires the use of both GC and LC. Topics such as sample cleanup and HPLC column and mobile phase selection were studied in order to enable analysis of a single set of QuEChERS extracts by GC/MS/MS and LC/MS/MS.

Experimental

Dried cannabis* was spiked at 50 ng/g with pesticides. 1.9 g samples were then hydrated for 30 min with 10 mL of water, and extracted by QuEChERS using 10 mL of acetonitrile and citrate/sodium bicarbonate salts. 1 mL of extract was subjected to QuEChERS cleanup using both Supel™QuE Verde and PSA/C18/GCB sorbent blends.

After cleanup, the same set of sample extracts was analyzed by LC/MS/MS and GC/MS/MS. Quantitation of spikes was performed by external standard calibration using matrix-matched standards. Separate calibration curves were used for each cleanup.

*Dried cannabis was supplied courtesy of Dr. Hari H. Singh, Program Director at the Chemistry & Physiological Systems Research Branch of the National Institute on Drug Abuse at the National Institute of Health.

Results & Discussion

HPLC method optimization

- HPLC method conditions were optimized (Table 1) to separate the elution ranges of the targeted pesticides and the co-extracted cannabinoids. An RP-Amide phase column with an acetonitrile based gradient provided the best separation, with the major cannabinoids THC and CBD eluting after the last pesticide in the screen (Figure 1). This would then allow flow to be diverted to waste after the last pesticide elutes, preventing a majority of the cannabinoids from entering and contaminating the detector.
- High aqueous starting conditions for the HPLC gradient are necessary for retention of the more polar pesticides. Injecting QuEChERS extracts of 100% acetonitrile into these conditions produces poor peak shapes. Peak shape was improved through use of a column containing packing with a fully porous particle architecture, and by attaching a guard column (Figure 2). Both measures provide additional mixing, and the use of a guard column is recommended to prolong the life of the analytical column.

Table 1. Optimized HPLC method for analysis of pesticides in acetonitrile-based extracts of cannabis.

column:	Ascentis® RP-Amide, 10 cm x 2.1 mm I.D., 3.0 µm with RP-Amide guard, 2 cm x 2.1 mm I.D., 5 µm
mobile phase:	[A] 5 mM ammonium formate, 0.1% formic acid in 95:5 water:acetonitrile; [B] 5 mM ammonium formate, 0.1% formic acid in 5:95 water:acetonitrile
gradient:	10% B held for 1 min; to 100% B in 13 min; held at 100% B for 6 min; to 10% in 0.5 min; held at 10% B for 6 min
flow rate:	0.4 mL/min
column temp.:	30°C
injection:	5 µL

Figure 1. TIC (LC/MS) of cannabis extract spiked with pesticides; over-layed with EIC (m/z 314.5) showing elution of major cannabinoids in the sample.

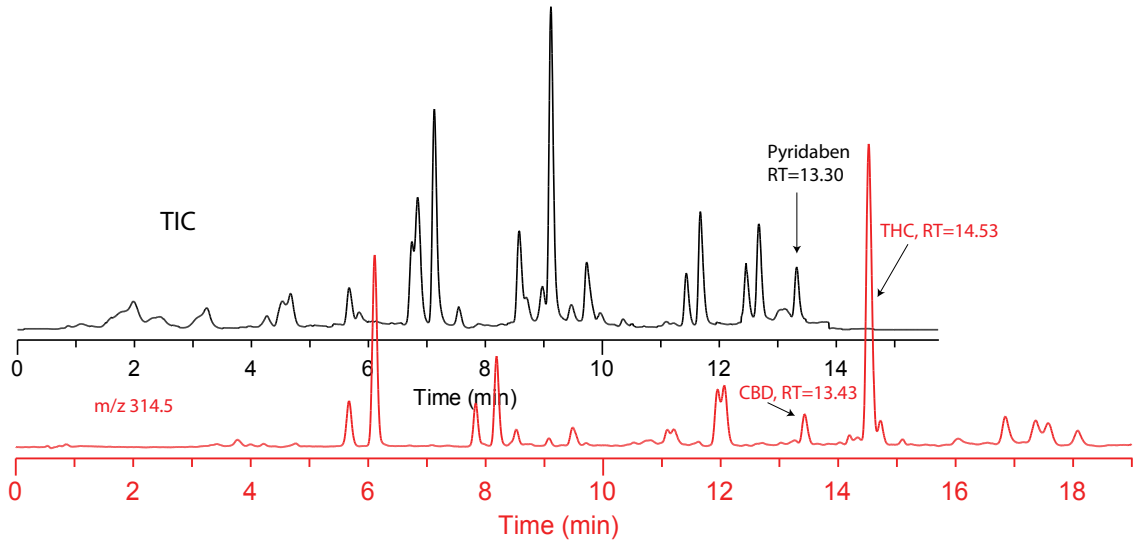
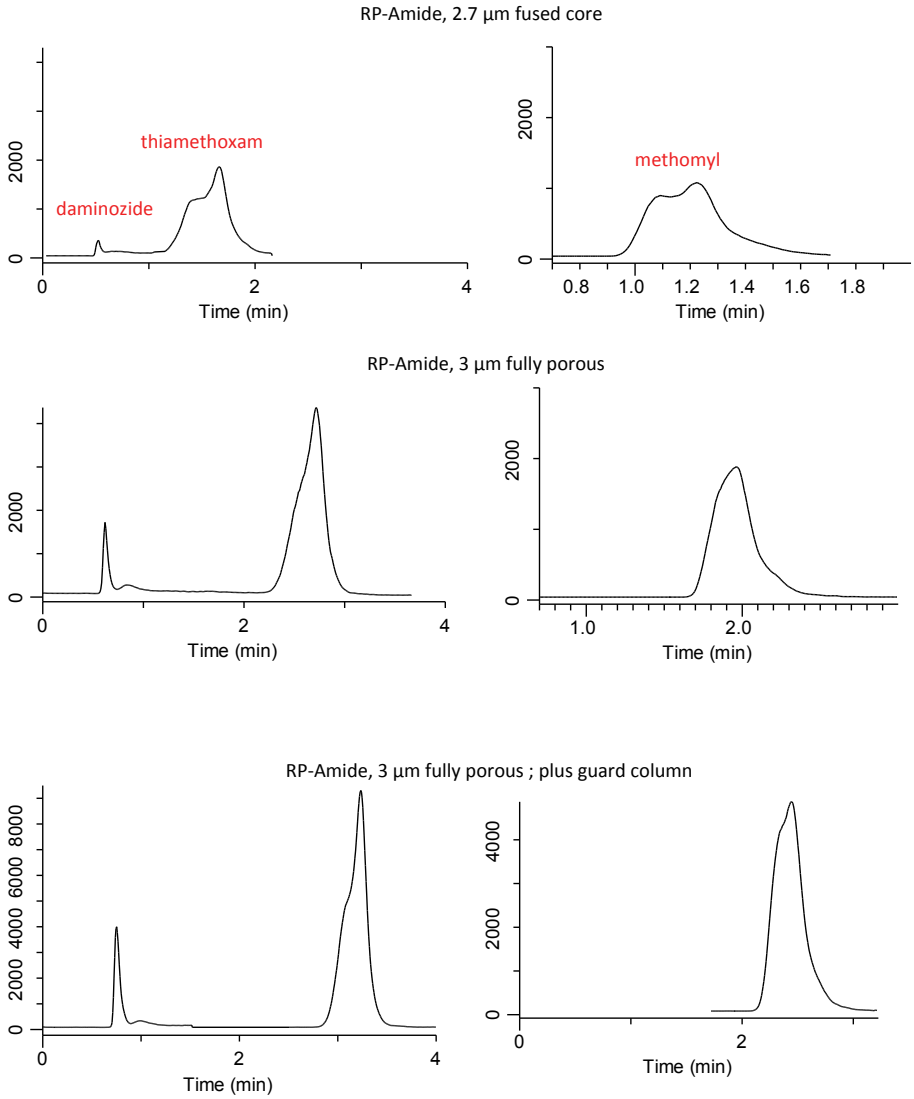


Figure 2. Effect on peak shape of early eluting pesticides when injecting 100% organic into high aqueous starting conditions: 2.7 µm fused core RP-Amide phase vs. 3 µm fully porous RP-Amide, vs. 3 µm fully porous RP-Amide plus guard column.

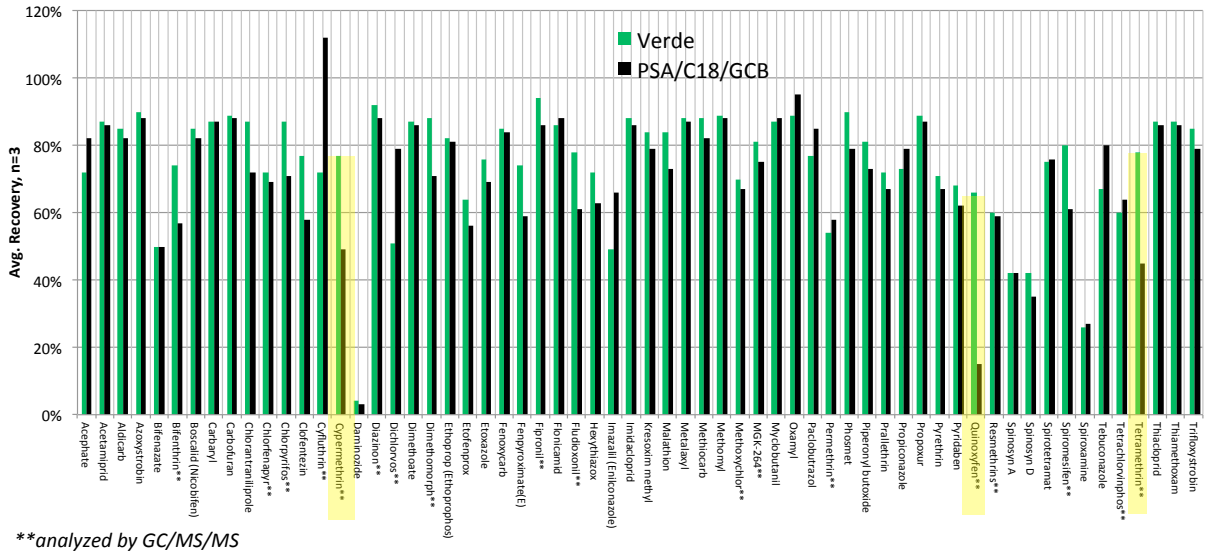


Sample Cleanup & Pesticide Recoveries

Sample cleanup using both Supel QuE Verde and PSA/C18/GCB reduced the color of the extracts from dark green to pale yellow. Co-extracted cannabinoids were reduced with cleanup, but high levels were still present. Levels were slightly lower after cleanup with Supel QuE Verde.

Overall recoveries of the spiked pesticides were higher using Supel QuE Verde. Recoveries of several pesticides, including the planar structured quinoxyfen, were significantly better than PSA/C18/GCB (Figure 3). Supel QuE Verde contains PSA, Z-Sep+ and a low surface area carbon that has much weaker retention of planar structured pesticides than traditional GCB.

Figure 3. Comparison of recoveries of pesticides from cannabis samples spiked at 50 ng/g; QuEChERS extraction and cleanup using Supel QuE Verde and PSA/C18/GCB.



***analyzed by GC/MS/MS*

Conclusions

For sample cleanup of QuEChERS extracts of cannabis, Supel QuE Verde can be substituted directly for PSA/C18/GCB. Both cleanups reduced green color; however Verde produced a slightly lower GC/MS background and lower levels of co-extracted cannabinoids.

Pesticide recoveries were better overall using Verde than PSA/C18/GCB; especially for several compounds.

For LC/MS/MS analysis, the RP-Amide phase combined with an acetonitrile based gradient produced better separation between the pesticides and major cannabinoids than other phase chemistries, including C18. This separation would allow a switch to waste on the LC/MS/MS system after elution of the last pesticide, which would in turn prevent some of the cannabinoids from entering and contaminating the detector.

Using a 3.0 µm Ascentis RP-Amide in combination with a guard column improved peak shapes of the early eluting pesticides, allowing for the direct injection of QuEChERS extracts without the need for dilution or solvent exchange into aqueous prior to LC analysis.

Product #	Product Description
1.00029	Acetonitrile hypergrade for LC-MS LiChrosolv®. CAS 75-05-08, molar mass 41.05 g/mol, and chemical formula CH_3CN., hypergrade for LC-MS LiChrosolv®
565301-U	Ascentis® RP-Amide HPLC Column 3 µm particle size, L × I.D., 10 cm × 2.1 mm
565372-U	Ascentis® RP-Amide Supelguard™ Guard Cartridge 5 µm particle size, L × I.D., 2 cm × 2.1 mm, pkg of 2 ea
29654-U	Certified Vial Kit, Low Adsorption (LA), 2 mL, pk of 100 volume 2 mL, amber glass vial (with marking spot), natural PTFE/silicone septa (with slit), thread 9 mm
55278-U	QuEChERS Shaker and Rack Starter Kit AC/DC input 115 V (USA Compatible Plug)
55227-U	Supel™ QuE Citrate Extraction Tube, pk of 50, suitable for EN 15662:2008 per BS
55248-U	Supel™ QuE Empty Centrifuge Tube with Lid, centrifuge tube volume 50 mL, pk of 50, suitable for EN 15662:2008 per BS
55447-U	Supel™ QuE Verde Tube, centrifuge tube volume 2 mL, pack of 100 ea

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