Analysis of Pesticide Residues in Cannabis Using QuEChERS Extraction and Cleanup Followed by GC/MS/MS Analysis

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Cannabis (marijuana) has been legalized for recreational and/or medical use by 23 states, the District of Columbia, and the territory of Guam. Thus, regulations regarding its production have become a topic of interest. The use of pesticides on cannabis is currently not regulated by the US EPA due to its listing as a Schedule 1 narcotic by the federal government. States that allow cannabis use for recreational and/or medical use have differing regulations regarding pesticides. As indicated in **Table 1**, the scope of regulation varies widely; from regulations on use, testing, and labeling, to no regulation at all.[1]

Table 1. US State Regulations Regarding Pesticide Use and Testing for Cannabis

Regulation	States
None	AK, CA, HI, MI, MT, NM, NY, RI
None – recreational use only	DC, OR
Testing, labeling	AZ
Testing, labeling – medical use only	OR
Labeling – recreational use only	CO
Use, testing, labeling	CT, DE, DC, IL, MA, NV, NH
Use, testing, labeling – medical use only	DC, CO
Use, testing, labeling – recreational use only	WA
Use, testing	ME, MN, NJ
Use	VT
Use – medical use only	WA

There are no official test methods for the determination of pesticide residues in cannabis. However, a potential approach is the use of the "quick, easy, cheap, effective, rugged and safe" (QuEChERS) method outlined in AOAC official method 2007.01. In this application, QuEChERS was used in the extraction of pesticides from dried cannabis. The pesticides studied were selected from lists currently tested by several commercial cannabis testing laboratories. The list included triazole fungicides as well as organophosphorus, organochlorine, and pyrethroid insecticides. Also included was piperonyl butoxide, a pesticide synergist included on the state of Colorado's list of "pesticides for use in marijuana production."[2]

For cleanup of the extracts, two different sorbent mixes were evaluated: (1) PSA/C18/ENVI-Carb™ and (2) Supel™ QuE Verde. The first represents a conventional mix often described as "PSA/C18/GCB" in QuEChERS protocols. PSA removes acidic interferences, while C18 retains hydrophobic interferences. Graphitized carbon blank (GCB or ENVI-Carb in this application) will retain chlorophyll and other colored compounds. However, while effective in removing pigment, GCB also retains target compounds with planar structures. Thus, pesticides with planar structures can be retained, reducing their recoveries. The second sorbent, Supel QuE Verde, contains a mixture of PSA, Z-Sep+ and an improved GCB. Z-Sep+ is a silica that is functionalized with both zirconia and C18. Zirconia will retain some fats and carotenoids, while C18 retains hydrophobic interferences. The improved GCB used in the Supel QuE Verde mix has been optimized to balance chlorophyll removal and improved recoveries of planar pesticides. When used to clean samples containing chlorophyll, this sorbent blend will provide better recovery of planar pesticides than sorbents containing traditional GCB.

Figure 1. Sample Preparation for Extraction of Pesticides from Cannabis

(continued on next page)

Experimental

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. The sample was ground to a fine powder and extracted following the procedure outlined in **Figure 1**. The resulting acetonitrile extract was cleaned using two different sorbents: (1) PSA/C18/ENVI-Carb and (2) Supel QuE Verde. Unspiked and spiked (50 ng/g) samples were prepared. Analysis of the final extracts was done by GC/MS/MS using the GC conditions listed in **Figure 3** and MRMs in **Table 2**. Quantitation was performed against multi-point, matrix-matched calibration curves prepared in unspiked cannabis extract. Separate curves were prepared for each cleanup sorbent.

Results and Discussion

Background

Figure 2 shows cannabis extracts before and after each cleanup. As expected, the PSA/C18/ENVI-Carb sorbent mixture removed the most color. The Supel QuE Verde mix removed the green from the cannabis extract, leaving only yellow pigments, and a much lighter colored extract than no cleanup. Extracts were analyzed by GC/MS in full scan mode to evaluate background (**Figures 3 a-c**). The TIC patterns of the extracts, cleaned and un-cleaned, appear similar. However, the amplitude and area sum of the peaks was the lowest after cleanup with Supel QuE Verde.

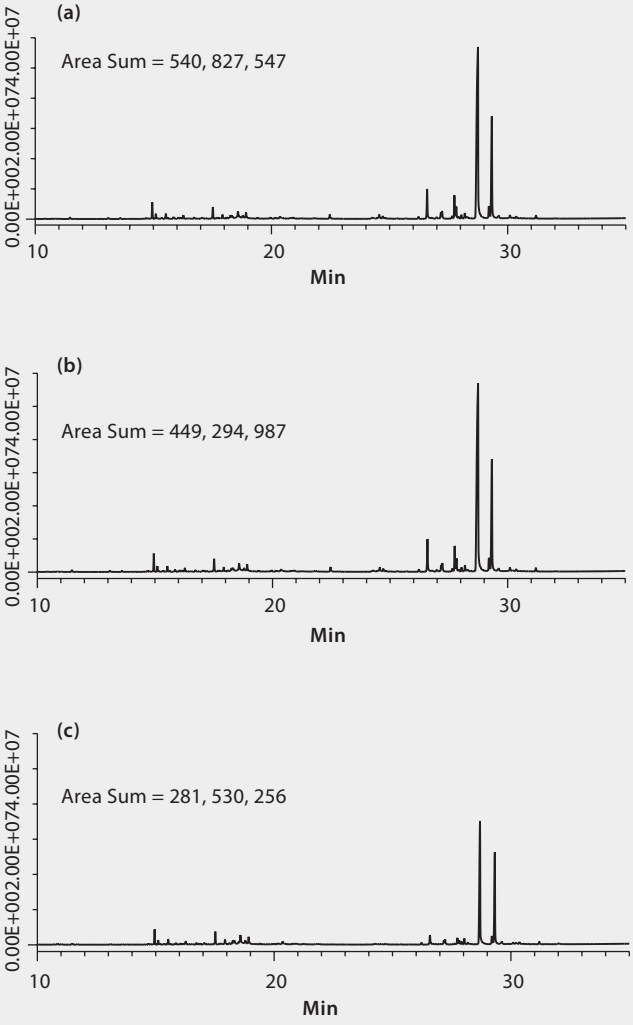
Table 2. MRM Transitions Used for GC/MS/MS Analysis of Pesticides in Cannabis Extracts

	MRM 1	CE	MRM 2	CE
Dichlorvos	185/93	10	145/109	10
Propoxur	110/63	30	110/64	15
Diazinon	137/84	10	199/135	15
Metalaxyl	234/146	20	206/132	20
Malathion	173/99	15	158/125	5
Chloropyrifos	314/258	15	314/286	5
Fipronil	351/255	15	367/213	25
Tetrachlorvinphos	331/109	25	331/79	35
Paclobutrazol	236/125	10	236/167	10
Myclobutanil	179/125	15	179/90	35
Trifloxystrobin	116/89	15	172/145	15
Quinoxyfen	272/237	10	307/237	20
Spiromesifen	272/254	5	272/209	10
Resmethrin	123/81	5	171/128	15
Tebuconazole	250/125	20	127/89	20
Piperonyl butoxide	176/131	15	176/103	25
Tetramethrin	164/107	10	164/77	25
Methoxychlor	227/169	30	127/109	10
Bifenthrin	181/165	25	181/166	10
Phosmet	160/77	25	160/51	40
Etoxazole	141/113	15	141/63	30
Permethrin	163/127	5	183/168	15
Boscalid	140/112	10	140/76	25
Cyfluthrin	206/150	15	206/177	5
Cypermethrin	163/91	10	165/127	5
Dimethomorph	301/165	10	303/165	10

Figure 2. QuEChERS Extracts of Cannabis With and Without Cleanup

Figure 3. GC/MS Scan Analysis of Cannabis Extract Background (a) with no cleanup, (b) after cleanup with Supel QuE PSA/C18/ENVI-Carb, and (c) after cleanup with Supel QuE Verde

column: SLB®-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)
oven: 50 °C (2 min), 8 °C/min to 320 °C (5 min)
inj. temp.: 250 °C
detector: MSD, full scan, m/z 45-500
MSD interface: 320 °C
carrier gas: helium, 1.4 mL/min constant
injection: 1 µL, splitless (splitter open at 0.75 min)
liner: 4 mm I.D., split/splitless type, single taper wool packed FocusLiner™ design



Recovery and Reproducibility

The average recoveries obtained from 50 ng/g spiked cannabis samples are summarized in **Table 3**. Recoveries were generally better using the Supel QuE Verde cleanup for many of the pesticides, especially the pyrethroid pesticides and triazole fungicides. The organophosphorus pesticides showed mixed results, with some such as chloropyrifos showing better recovery from Supel QuE Verde, and others such as phosmet showing better recovery using PSA/C18/ENVI-Carb. Quinoxyfen (highlighted in yellow in **Table 3**) showed very poor recovery after cleanup with PSA/C18/ENVI-Carb. This compound has some planar character to its structure, which resulted in strong retention on the ENVI-Carb, and thus reduced recovery. The pesticide synergist piperonyl butoxide, of interest in relation to cannabis production, showed >70% recovery using both cleanups, but better recovery after Supel QuE Verde compared to PSA/C18/ENVI-Carb cleanup. Reproducibility, determined as % RSD for n=3 spiked replicates, was generally very good for both cleanups. The exceptions were tetramethrin and cypermethrin, which had RSD values >20% after PSA/C18/ENVI-Carb cleanup.

Table 3. Average Pesticide Recoveries and Relative Standard Deviation (RSD) Values for Spiked Replicates of Cannabis

	PSA/C18/ENVI-Carb	Supel QuE Verde
Dichlorvos	79% (8)	51% (4)
Propoxur	90% (5)	90% (3)
Diazinon	88% (10)	92% (3)
Metalaxyl	84% (10)	92% (6)
Malathion	81% (5)	83% (4)
Chloropyrifos	71% (5)	87% (5)
Fipronil	86% (2)	94% (5)
Tetrachlorvinphos	64% (4)	60% (7)
Paclobutrazol	68% (4)	79% (4)
Myclobutanil	67% (2)	78% (3)
Trifloxystrobin	79% (4)	84% (4)
Quinoxyfen	15% (1)	66% (2)
Spiromesifen	61% (12)	80% (5)
Resmethrin	59% (17)	60% (6)
Tebuconazole	50% (3)	58% (2)
Piperonyl butoxide	75% (3)	83% (10)
Tetramethrin	45% (26)	78% (4)
Methoxychlor	67% (3)	70% (3)
Bifenthrin	57% (9)	74% (4)
Phosmet	101% (6)	63% (9)
Etoxazole	82% (4)	76% (6)
Permethrin	58% (6)	54% (2)
Boscalid	81% (6)	65% (2)
Cyfluthrin	112% (11)	72% (6)
Cypermethrin	49% (25)	77% (16)
Dimethomorph	71% (8)	88% (5)
Avg. % Recovery (all pesticides)	71%	75%
Avg. RSD (all pests)	8%	5%

Conclusions

As demonstrated here, QuEChERS is a viable approach for the analysis of pesticide residues in cannabis. Many compounds of interest, including piperonyl butoxide, show good recovery and reproducibility by this extraction technique. For cleanup of QuEChERS extracts of cannabis, the use of graphitized carbon is recommended for removal of chlorophyll. However, as evidence by the recovery of quinoxyfen in **Table 3**, traditional GCBs such as ENVI-Carb will reduce the recovery of pesticides with planar structures. Supel QuE Verde, which contains PSA, Z-Sep+ and an improved GCB, can be used to reduce pigmentation while improving recoveries of these compounds.

Compared to conventional PSA/C18/GCB cleanup, cannabis extracts cleaned with the Supel QuE Verde mixture showed lower GC/MS background, and better recoveries for many pesticides.

References

1. Feldman, J. Pesticide Use in Marijuana Production: Safety Issues and Sustainable Options. *Pesticides and You*, Winter 2014-2015, pp 14-23.
2. Colorado Dept. of Agriculture website. Pesticide Use in Marijuana Production. https://www.colorado.gov/pacific/agplants/pesticide-use-marijuana-production (accessed Nov 2015)

Featured Products

Description	Cat. No.
Supel QuE QuEChERS Products	
Verde Cleanup Tube, 2 mL, pack of 100	55447-U
Verde Cleanup Tube, 15 mL, pack of 50	55442-U
PSA/C18/ENVI-Carb (AOAC) Tube, 2 mL, pack of 100	55289-U
Citrate Extraction Tube, 12 mL, pack of 50	55227-U
Empty Centrifuge Tube, 50 mL, pack of 50	55248-U
Column	
SLB-5ms GC Capillary, 30 m × 0.25 mm I.D., 0.25 µm	28471-U
Solvents and Reagents	
Acetonitrile, UHPLC Plus, for gradient elution, ≥99.9%	34998
Acetic Acid, ACS, reagent grade, .99.7%	650501
Accessories	
QuEChERS Shaker and Rack Starter Kit, USA compatible plug, AC input 115 V	55278-U
QuEChERS Shaker and Rack Starter Kit, EU compatible Schuko plug, AC input 230 V	55278-U
Certified Vial Kit, Low Adsorption (LA), 2 mL, pk of 100	29653-U
Inlet Liner, Split/Splitless Type, Single Taper FocusLiner™ Design (wool packed)	2879901-U

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