FOOD & BEVERAGE

Approaches to the Analysis of the Oregon List of Pesticides in Cannabis Using QuEChERS Extraction and Cleanup

Edited article, refer to SigmaAldrich.com/Analytix (Issue 5) for full version.

Katherine K. Stenerson, Principal R&D Scientist, katherine.stenerson@milliporesigma.com

Introduction

Consumption of cannabis and / or cannabis-based products is currently legal in some form in 33 US states plus the District of Columbia. Testing of the plant materials and products is required by many of these states; however, the specific test methods and target compound lists are not mandated in all cases. In October of 2016, the state of Oregon took a major step forward by requiring that all labs testing cannabis be accredited by the Oregon Environmental Laboratory Accreditation Program (ORLEP) and licensed by the Oregon Liquor Control Commission (OLCC).[1] Consequently, Oregon Administrative Rules (OAR) list specific contaminants to be tested in marijuana samples, along with action levels.[2] The pesticides on this list include carbamate, organophosphorus, macrocylic lactone, neonicotinoid, pyrethroid, and triazole fungicides as well as others. Action levels per OAR vary from 0.2 to 1 µg/g, depending on the specific pesticide. In addition, the state of California, which legalized recreational cannabis in 2016, requires testing for a list of pesticides similar to that on the OAR list, plus 8 additional.[3]

Due to its ease of use and applicability to a wide range of pesticides, the "quick, easy, cheap, effective, rugged & safe" (QuEChERS) approach has been adopted by many testing laboratories for use on cannabis. After extraction, incorporation of a cleanup step is important for removing pigments, as well as other contaminants. QuEChERS cleanup using a mixture of primary secondary amine (PSA), C18 and graphitized carbon black (GCB) is often chosen for this purpose. PSA will remove acidic interferences, C18 hydrophobic interferences and GCB retains some pigments – specifically the green color imparted by chlorophyll. This mixture of sorbents thus retains a wide range of contaminants; however it also has potential to reduce recoveries of target pesticides which are susceptible to hydrophobic retention on C18, or planar enough in structure to be strongly retained by GCB. In previous work done by the author in 2015 with cannabis, an alternative sorbent mix, Supel™ QuE Verde, was evaluated for cleanup in the analysis of various pesticides, and found to offer an advantage with regards to background reduction and recovery.[4] This sorbent mix contains PSA, Z-Sep+, and ENVI-Carb Y. Z-Sep+ is a zirconia coated silica functionalized with C18. The zirconia retains by Lewis acid/base interactions, and has been found to retain certain fatty compounds as well as some pigments. ENVI-Carb Y is a specially manufactured graphitized carbon that is engineered to have weaker retention of small, planar molecules such as certain pesticides. This mixture offers a better balance than traditional PSA/C18/GCB with regards to removal of pigmentation and pesticide recovery. In this application, the pesticide list tested in 2015 has been expanded to include many of those on the OAR list described above. Supel™ QuE Verde was compared directly to PSA/C18/GCB for cleanup and analysis of spiked replicates of cannabis plant material analyzed by LC-MS/MS and GC-MS/MS. Column and mobile phase selection for LC-MS/MS was done based on several factors, which will be described. For the additional pesticides included on the OAR list, Supel™ QuE Verde was found to yield better overall recovery than PSA/C18/GCB.

Experimental

Extraction

Dried cannabis* was pulverized using a IKA T10 Ultra Turrax mixer. 1.9 g was weighed into a 50 mL centrifuge tube and spiked with pesticides at 50 ng/g. After a 10 min equilibration time, the sample was mixed with 10 mL of deionized water and allowed to sit for 30 minutes. 10 mL of acetonitrile was added, and the sample was shaken at 2500 rpm for 30 minutes. The contents of the Supel™ QuE Citrate tube (55227-U) were added, and the sample shaken for 1 minute. The sample was then centrifuged at 5000 rpm for 5 min, and the supernatant removed for 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.*

Cleanup

1 mL of extract was added to a 2 mL tube containing the mixture of cleanup sorbents. Two different sorbent mixtures were used:

1. PSA/C18/GCB/MgSO4 (400 mg/400 mg/ 400 mg/1200 mg)
2. Supel™ QuE Verde (55447-U)

Samples were shaken for 1 minute, centrifuged at 5000 rpm for 3 minutes, and the supernatant removed for analysis.

Analysis

Samples were analyzed by LC-MS/MS and GC-MS/MS using the conditions listed in **Tables 1** and **2**. The same extracts were run on both systems. Pesticides that did not yield response by LC-MS/MS were attempted by GC-MS/MS. Quantitation was done against a 5-point matrix-matched calibration curve prepared in unspiked cannabis extract. Separate curves were prepared for each cleanup. No internal standards were used, thus all recovery values reported are absolute.

Table 1. LC-MS/MS conditions

column:	Ascentis® RP-Amide, 10 cm x 2.1 mm I.D., 3.0 µm (565301-U) with RP-Amide Supelguard cartridge, 2 cm x 2.1 mm I.D., 5 µm (565372-U)
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
detector:	MRM*
injection:	5 µL
sample:	QuEChERS extract in acetonitrile

Table 2. GC-MS/MS analysis conditions

column:	SLB®-5ms, 20 m x 0.18 mm I.D., 0.18 µm (28564-U)
oven:	50 °C (2 min), 8 °C/min to 325 °C (10 min)
inj. temp.:	250 °C
carrier gas:	helium, 1.2 mL/min constant flow
detector:	MRM*
MSD interface:	325 °C
injection:	1 µL, pulsed splitless (50 psi until 0.75 min, splitter open at 0.75 min)
liner:	4 mm I.D. FocusLiner with taper

*see online version of article on SigmaAldrich.com/Analytix (Issue 5) for MRM listing

Results and Discussion

HPLC column and mobile phase selection. Typical cannabis samples analyzed by testing labs contain high levels of cannabinoids, often in the range of 20-25% by weight. These compounds will coextract with the pesticides during the QuEChERS process. The acidic forms can be partially retained by some cleanup sorbents (specifically PSA and Z-Sep+), however the neutral forms are not retained well by cleanup sorbents used for pesticide testing. In the case of LC-MS/MS analysis, these co-extracted cannabinoids can build up on the detector, requiring more frequent system maintenance. In this application, column and mobile phase selection were based on conditions that would force elution of the cannabinoids as late as possible in the run, ideally after the pesticides. Under these conditions, the diverter valve on the LC-MS/MS system could be set to flow to waste after elution of the last pesticide. This will then prevent a majority of the cannabinoids from entering the detector.

To facilitate the appropriate HPLC conditions, a screening experiment was designed to study elution of the major cannabinoids compared to the targeted pesticides on several different column chemistries, and using both acetonitrile and methanol based gradients. The columns screened were as follows:

1. Ascentis® Express C18
2. Ascentis® Express RP-Amide
3. Ascentis® Express Phenyl-Hexyl
4. Ascentis® Express Biphenyl
5. Ascentis® Express F5

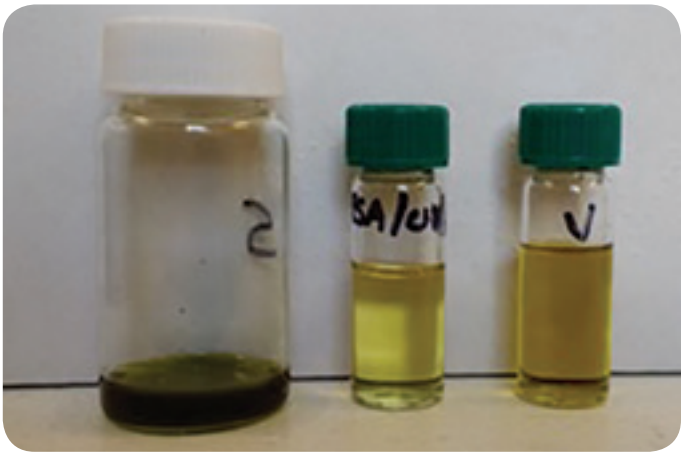
All columns were 10 cm x 2.1 mm I.D., 2.7 µm. The Ascentis® Express Fused-Core version of these chemistries was initially chosen for both efficiency and speed. The HPLC conditions were similar to those listed in **Table 1**, with UV used for detection, and ammonium formate omitted from the mobile phase. Samples were injected in 100% acetonitrile, to emulate samples resulting from the QuEChERS extraction, and as expected, this resulted in poor peak shapes of the earliest eluting pesticides on all five columns. Using an acetonitrile gradient, the Ascentis® Express RP-Amide yielded the least amount of overlap between the pesticide and cannabinoid elution ranges. In addition, comparing acetonitrile to methanol, using the former in the gradient eluted the pesticides faster, resulting in less overlap with the cannabinoids.

To simplify the method as much as possible, the same QuEChERS extract was analyzed by both HPLC and GC. However, as indicated previously in the column screening experiment, injection of 100% acetonitrile into the high aqueous starting conditions of the gradient produced poor peak shapes for the early eluting pesticides. To improve the peak shapes of these compounds, a 3 µm Ascentis® RP-Amide was substituted for the 2.7 µm Ascentis® Express RP-Amide. Installation of a guard column further improved peak shape most likely due to increased retention and improved mixing of the sample with the

mobile phase. (For chromatograms see online version of article on **SigmaAldrich.com/analytix** - Issue 5.) In addition, when working with high background samples, use of a guard column is highly recommended to extend the life of the analytical column.

Background reduction. A comparison of the cannabis extracts before and after cleanup with PSA/C18/GCB and Verde is shown in **Figure 1**. As expected, the co-extracted chlorophyll generated an extract with a deep green color. After cleanup, a majority of the green color was removed, with the extracts appearing yellowish in color. The Verde cleaned extract was slightly darker than the PSA/C18/GCB cleaned extract. Analysis of the extracts by GC/MS in full scan mode is shown in **Figure 2**. The data showed a similar peak pattern between uncleaned and cleaned extracts (both cleanups), but a difference in the amplitude of background peaks (indicated in shaded regions). The predominant peaks eluting in these regions are terpenes (earlier) and cannabinoids (later). Overall reduction in background was compared by summation of total peak area for each chromatogram. Compared to no cleanup, Verde was slightly better than PSA/C18/GCB (35% vs. 31% reduction in background). Specifically in the highlighted regions, Verde showed lower peak amplitudes.

Figure 1. QuEChERS extracts of cannabis before and after cleanup



Elution of cannabinioids. Using the optimized HPLC conditions described previously in the final LC-MS/MS analysis of the cannabis extracts, minimal overlap was observed between two of the major cannabinoids present in the samples and the later eluting pesticides. **Figure 3** shows an EIC of m/z 314.5, taken from a full scan LC/MS analysis of a cannabis extract compared to a TIC of the pesticides of interest in the analysis. The EIC represents the molecular ion of the two major cannabinoids detected in the sample extract; tetrahydrocannabinol and cannabidiol. As indicated, the last pesticide analyzed, pyridaben, eluted just before cannabidiol. The most abundant cannabinoid present, THC, eluted well after. Column flow could be switched to waste after elution of pyridaben, preventing some of the CBD and all of the THC from entering the MS. Other cannabinoids; specifically CBG, CBN, CBDA, CBC, CBGA, and THCAA, are known to elute after CBD on the RP-Amide phase. Thus, if present in the cannabis sample, all of these could also be diverted to waste as well.

Pesticide recoveries. The pesticides included in this study represented a majority of those on the OAR list. Two pesticides from this list, avermectin B1a and naled

Figure 3. EIC, m/z 314.5 from full scan analysis of cannabis extract (green), compared to a TIC of MRM analysis for pesticides (purple).

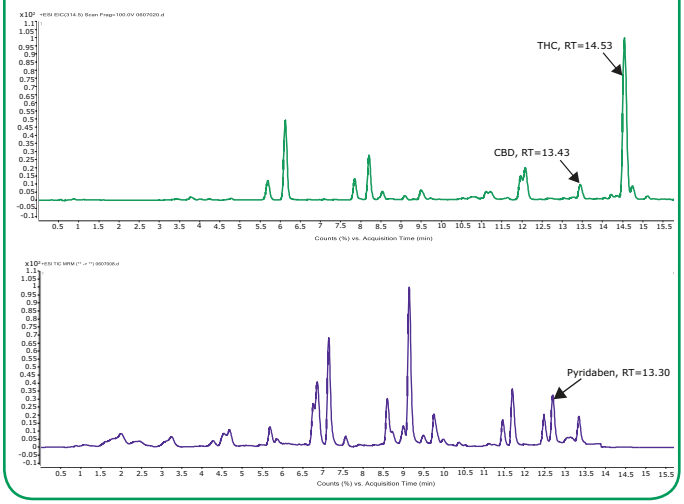
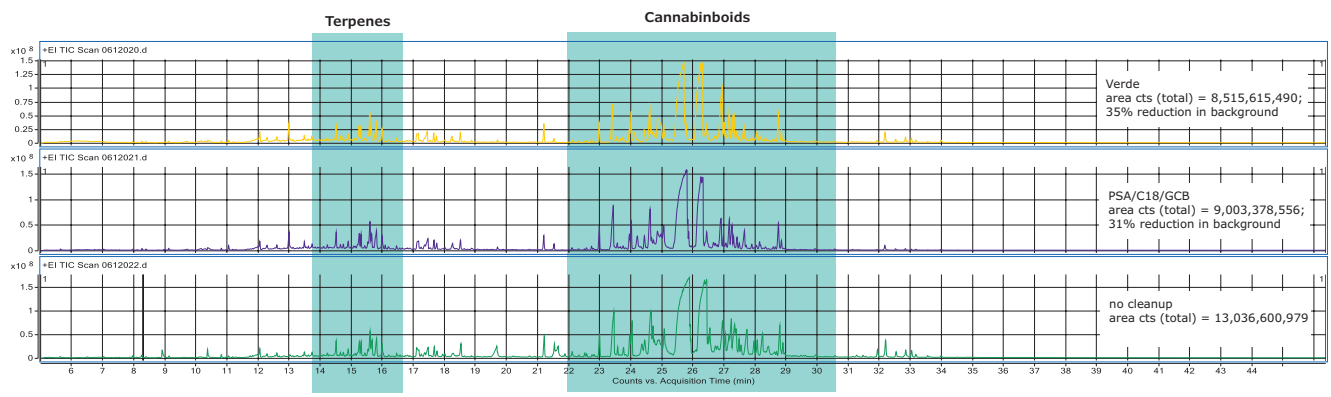


Figure 2. GC/MS scan comparisons of the cannabis extracts



were not analyzed due to lack of response. Avermectin is prone to sodium and potassium adduct formation. The presence of ammonium formate in the mobile phase should reduce this occurrence (as it is monitored as an ammonium adduct). However, even with these measures, others have also reported issues with low level detection of this compound.[4,5] Naled is susceptible to adsorption by PSA, and thus did not make it through the cleanup process with either sorbent mix.

Comparing spike data from the two cleanup methods (**Figure 4**) Supel™ QuE Verde exhibited better overall performance than PSA/C18/GCB. Several pesticides (**Table 3**), specifically bifenthrin, chlorantraniliprole, clofentezin, fenproximate, fludioxinil and hexythiazox showed notably better recoveries using Verde. Although none of these are completely planar in structure, it is possible that recovery was reduced using PSA/C18/GCB

Figure 4. Overall method performance for pesticides spiked at 50 ng/g in cannabis; Supel™ QuE Verde compared to PSA/C18/GCB.

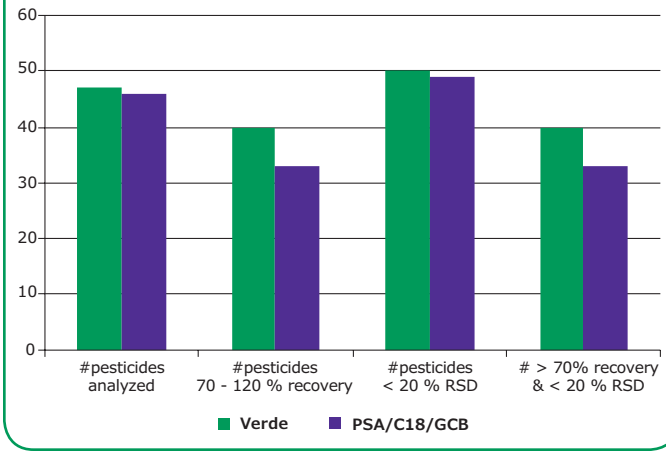


Table 3. Recovery and reproducibility summary; 50 ng/g spiked replicates

Cleanup:	Verde		PSA/C18/GCB		
	% Rec	% RSD	% Rec	% RSD	Analysis
Acephate	72%	12%	82%	6%	LC
Acetamiprid	87%	5%	86%	0.9%	LC
Aldicarb	85%	15%	82%	7%	LC
Azoxystrobin	90%	4%	88%	2%	LC
Bifenazate (D 2341)	50%	6%	50%	2%	LC
Bifenthrin*	74%	4%	57%	9%	GC
Boscalid (Nicobifen)	85%	3%	82%	5%	LC
Carbaryl	87%	7%	87%	2%	LC
Carbofuran	89%	5%	88%	2%	LC
Chlorantraniliprole	87%	6%	72%	1%	LC
Chlorfenapyr	72%	3%	69%	16%	GC
Chlorpyrifos*	87%	5%	71%	5%	GC
Clofentezin	77%	6%	58%	4%	LC
Cyfluthrin*	72%	6%	112%	11%	GC
Cypermethrin*	77%	16%	49%	25%	GC
Daminozide	4%	45%	3%	64%	LC
Diazinon*	92%	3%	88%	10%	GC
Dichlorvos	31%	23%	matrix		LC
Dimethoate	87%	4%	86%	0.4%	LC
Ethoprop (Ethoprophos)	82%	3%	81%	3%	LC
Etofenprox	59%	15	55	8	GC
Etoxazole	76%	2%	69%	2%	LC
Fenoxycarb	85%	6%	84%	2%	LC
Fenpyroximate(E)	74%	3%	59%	2%	LC
Fipronil*	94%	5%	86%	2%	GC
Flonicamid	86%	11%	88%	3%	LC
Fludioxonil	78%	5%	61%	12%	GC
Hexythiazox	72%	3%	63%	4%	LC

Cleanup:	Verde		PSA/C18/GCB		
	% Rec	% RSD	% Rec	% RSD	Analysis
Imazalil (Enilconazole)	49%	5%	66%	2%	LC
Imidacloprid	88%	4%	86%	2%	LC
Kresoxim methyl	84%	13%	79%	7%	LC
Malathion	84%	5%	73%	6%	LC
Metalaxyl	88%	4%	87%	2%	LC
Methiocarb (Mercaptodimethur)	88%	6%	82%	2%	LC
Methomyl	89%	5%	88%	2%	LC
MGK-264	81%	2%	75%	5%	GC
Myclobutanil	87%	3%	88%	2%	LC
Oxamyl	89%	4%	95%	2%	LC
Paclobutrazol	77%	3%	85%	0.5%	LC
Permethrin	54%	2%	58%	6%	GC
Phosmet (Imidan)	90%	5%	79%	7%	LC
Piperonyl butoxide	81%	5%	73%	2%	LC
Prallethrin	72%	10%	67%	7%	LC
Propiconazole	73%	7%	79%	7%	LC
Propoxur	89%	5%	87%	1%	LC
Pyrethrin	71%	6%	67%	29%	LC
Pyridaben	68%	5%	62%	1%	LC
Spinosyn A	42%	10%	42%	2%	LC
Spinosyn D	42%	9%	35%	19%	LC
Spirotetramat	75%	1%	76%	1%	LC
Spiromesifen*	80%	5%	61%	12%	GC
Spiroxamine	26%	1%	27%	3%	LC
Tebuconazole	67%	4%	80%	1%	LC
Thiacloprid	87%	5%	86%	1%	LC
Thiamethoxam	87%	5%	86%	3%	LC
Trifloxystrobin	85%	5%	79%	2%	LC

**See reference 4.*

due to hydrophobic retention on the GCB; which has a higher surface area than the carbon used in the Supel™ QuE Verde mix.

Several pesticides exhibited poor recoveries after both cleanup techniques:

- **bifenazate**: Recovery was around 50% after both cleanups. Bifenazate is susceptible to oxidation to bifenazate-diazine[8], which may have occurred to some degree during the extraction and cleanup process.
- **daminozide:** Very low recovery after both cleanups. This compound is a carboxylic acid, and is thus retained by PSA (present in both cleanups).
- **dichlorvos:** Matrix interference prevented analysis of this pesticide in the PSA/C18/GCB extracts. In the Supel™ QuE Verde cleaned extracts, the peak could be detected, but recoveries were low and variable. The low recovery using Verde is most likely due to retention on the Z-Sep+ portion of the sorbent. This same behavior has been observed in the past with this compound when using zirconia sorbents by both the author and others.[9]
- **etofenprox:** This is a very hydrophobic pesticide (log p= 7.1) and may exhibit poor extraction efficiency and/or retention by the C18 and Z-Sep+ portions of the cleanup sorbents (although less so on the later).
- **imazalil:** This is a relatively polar pesticide, which can be retained by PSA (present in both cleanups). Recovery issues have been observed by others with this compound when using Supel™ QuE Verde for cleanup as well as other zirconia containing sorbent mixtures.[9,10]
- **spinosyn A & D:** Lower recoveries of these large, macrocyclic lactones have been observed when using C18, carbon and zirconia containing sorbents.[11] In the case of zirconia, the use of citrate buffering in the QuEChERS extraction has been observed to increase recovery, possibly by displacement of the analytes from the zirconia.[9]
- **spiroxamine:** Recovery was very low, and about the same level after both cleanups. This could indicate an issue with extraction efficiency.

Conclusions

In the analysis of a majority of the pesticide list required by the state Oregon for cannabis, several recommendations can be made:

- QuEChERS extraction and cleanup can be used; and both LC-MS/MS and GC-MS/MS will be required for analysis.
- Cleanup using Supel™ QuE Verde can be substituted for PSA/C18/GCB. Both cleanups will reduce the green color of the extracts; however Verde was found to produce a slightly lower GC/MS background. Cannabinioids were co-extracted with the pesticides. Significant levels were still present after both cleanups, although slightly less after Verde. Pesticide recovery using Verde was found to be better overall, especially for several pesticides.
- Compared to C18, the Ascentis® RP-Amide column provided less overlap between the elution ranges of the targeted pesticides and the co-extracted cannabinoids. 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 the MS.
- In the LC-MS/MS analysis, a high percentage of aqueous was necessary in the starting mobile phase conditions to increase retention of the more polar pesticides. As a result, injecting extracts in 100% organic resulted in distorted peak shapes for early eluting peaks. Switching from a 2.7 µm Ascentis® Express RP-Amide to a 3 µm Ascentis® RP-Amide (fully porous particle) improved these peak shapes.
- A guard column prior to the Ascentis® RP-Amide will further improve peak shapes when injecting 100% organic, and is recommended to prolong the life of the analytical column.

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