Potency and Pesticide Content in Medical vs. Recreational Marijuana

UCT Part Numbers:
ECQUUS950CT-MP - QuEChERS salts for THC Potency and Pesticide Testing 50 mL Centrifuge Tubes Included
ECQUUS142CT- Dispersive SPE Sorbent Blend for Pesticide Testing in Edibles 2 mL Centrifuge Tubes Included
SLAQ100ID21-3UM- Selectra® Aqueous C18 HPLC Column 100 x 2.1 mm, 3µm
SLAQGDC20-3UM – Selectra® Aqueous C18 Guard Column 10 x 2.1, mm, 3µm
SLGRDHLDR - Guard Column Holder

As of January 2016, 24 states and the District of Columbia have legalized the medical use of marijuana, while 4 states and the District of Columbia have legalized the recreational use of marijuana. As a result, both forensic toxicology and cannabis-specific testing laboratories are looking for fast, reliable, and cost-effective methods to determine cannabis potency and pesticides in medical and/or recreational marijuana. This application utilizes the advantages of QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) to extract for both pesticides and cannabinoids including tetrahydrocannabinol (THC), cannabidiol (CBD), tetrahydrocannabinolic acid (THCA-A) and cannabinol (CBN) in marijuana, followed by either serial dilutions for cannabis potency analysis, or a dispersive solid phase extraction (dSPE) cleanup for pesticide residue analysis. This hybrid method allows for the QuEChERs approach, which is extensively used in the food testing industry, to be utilized in a medico-legal setting.

Figure 1: Seized recreational marijuana samples

Procedure

(a) Recommended Sample Pre-treatment

1. Grind marijuana sample to fine powder using a SPEX 6770 freezer mill.

(b) QuEChERS Extraction

1. Weigh 1 g of the pre-treated marijuana into 50-mL centrifuge tubes, add internal standard, and 10 mL of methanol, and hydrate for 3 hr at 60 °C.
2. Add 10 mL of acetonitrile (MeCN) with 1% acetic acid.
3. Add QuEChERS extraction salts from pouches (**ECQUUS950CT-MP**), and vortex for 10 sec to break up salt agglomerates.
4. Shake for 1 min at 1000 stroke/min using a SPEX Geno/Grinder.
5. Centrifuge at 3000 rcf for 5 min.

(c) dSPE cleanup for pesticide residue analysis

1. Transfer 1 mL of the supernatants to 2-mL dSPE tube (**ECQUUS142CT**).
2. Shake for 1 min at 1000 stroke/min using the SPEX Geno/Grinder.
3. Centrifuge at 3000 rcf for 5 min.
4. Transfer 200 µL extract to the 2-mL auto-sampler vials, add 200 µL of DI water, and vortex for 30 sec.

Figures 2 and 3: Recreational marijuana sample following QuEChERS extraction; Comparison of QuEChERS extracts before (left) and after (right) dSPE cleanup.

(d) Apply serial dilutions for cannabinoid analysis

1. Perform serial dilutions (200 to 20,000 times depending on the cannabinoid concentration in different samples) of the QuEChERS extracts to 100 to 200 ng/mL.
2. Spike the diluted samples with 50 and 150% of the target cannabinoids, which are used to quantify the cannabinoid concentration according to the standard addition method.

(e) Analyze by LC-MS/MS

1. Analyze samples by LC/MS/MS (Thermo Scientific UltiMate 3000 LC system coupled to TSQ Vantage tandem MS) equipped with an UCT Aqueous C18 HPLC column (**SLAQ100ID21-3UM**).

Instrument Parameters (Pesticides)

HPLC: Thermo Scientific Dionex UltiMate 3000® LC System		
Column: UCT, Selectra®, aQ C18, 100 x 2.1 mm, 3 µm		
Guard column: UCT, Selectra®, aQ C18, 10 x 2.0 mm, 3 µm		
Column temperature: 40 °C		
Column flow rate: 0.300 mL/min		
Auto-sampler temperature: 10 °C		
Injection volume: 2 µL		
Gradient program:		
Time (min)	**A%** (10 mM ammonium acetate in DI water)	**B%** (0.1% formic acid in MeOH)
0	100	0
1	50	50
3.5	50	50
6	5	95
9	5	95
9.1	100	0
14	100	0

MS parameters	
Instrumentation	Thermo Scientific TSQ Vantage tandem MS
Polarity	ESI +
Spray voltage	3500 V
Vaporizer temperature	450 °C
Ion transfer capillary temperature	350 °C
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	40 arbitrary units
Q1 and Q3 peak width (FWHM)	0.4 and 0.7 Da
Collision gas and pressure	Ar at 1.5 mTorr
Cycle time	0.5 sec
Acquisition method	EZ Method (scheduled SRM)

SRM Table						
Compound	**Precursor**	**Product 1**	**CE1**	**Product 2**	**CE2**	**S-lens RF**
Metamidophos	142.0	94.1	14	125.0	13	50
Acephate	184.0	143.0	6	95.0	25	33
Aldicarb sulfoxide	207.1	89.1	13	69.1	16	32
Oxydemeton methyl	247.0	169.0	13	109.0	27	57
Pymetrozine	218.1	105.1	20	176.1	17	63
Dichrotophos	238.1	112.1	12	127.0	18	52
Triethylphosphorothioate	199.0	125.0	16	143.0	14	55
Dimethoate	230.0	125.0	22	171.0	15	50
Carbendazim	192.1	160.1	18	132.1	29	60
Dichlorvos	220.9	109.0	17	127.0	13	62
Thiabendazole	202.0	175.1	25	131.1	31	70
Fenamiphos sulfone	336.1	266.0	19	188.0	26	75
Fenamiphos sulfoxide	320.1	233.0	24	108.1	40	60
Simazine	202.1	132.0	19	124.1	16	66
Tebuthiuron	229.1	172.1	16	116.0	26	55
Carbaryl	202.1	145.1	11	127.1	30	38
Flutriafol	302.1	70.1	17	123.0	28	69
Famphur	326.0	217.0	20	93.0	30	68
Thionazin	249.0	113.0	23	97.0	28	58
DEET	192.1	119.1	17	91.1	29	64
Atrazine	216.1	174.1	16	68.1	34	66
Malathion	331.0	127.0	12	99.0	25	55
Triadimefon	294.1	197.1	14	69.1	20	65
Pyrimethanil	200.1	107.1	24	183.1	23	68
Bifenazate	301.1	170.1	18	198.1	6	48
Acetochlor	270.1	224.1	10	148.1	18	58
Sulfotep	323.0	97.0	37	115.0	30	60
Tebuconazole	308.1	70.1	21	125.0	33	66
Zoxamide	336.0	187.0	21	159.0	38	74
Diazinon	305.1	169.1	20	153.1	20	68
TPP (IS)	327.1	152.1	35	77.1	38	95
Cyprodinil	226.1	93.1	33	77.1	43	70
Pyrazophos	374.1	222.1	20	194.1	31	100
Profenofos	372.9	302.9	17	128.0	42	73
Ethion	385.0	142.9	26	199.0	6	56
Chlorpyrifos	349.9	97.0	32	197.9	19	67

Instrument Parameters (Cannabinoids)

HPLC: Thermo Scientific Dionex UltiMate 3000® LC System		
Column: UCT, Selectra®, aQ C18, 100 x 2.1 mm, 3 µm		
Guard column: UCT, Selectra®, aQ C18, 10 x 2.0 mm, 3 µm		
Column temperature: 40 °C		
Column flow rate: 0.300 mL/min		
Auto-sampler temperature: 10 °C		
Injection volume: 5 µL		
Gradient program:		
Time (min)	**A%** (10 mM ammonium acetate in DI water)	**B%** (0.1% formic acid in MeOH)
0	40	60
0.5	40	60
3	5	95
7	5	95
7.1	40	60
10	40	60

Divert mobile phase to waste from 0 – 3 and 8 – 10 min to prevent ion source contamination.

MS parameters	
Instrumentation	Thermo Scientific TSQ Vantage tandem MS
Polarity	ESI +
Spray voltage	3500 V
Vaporizer temperature	450 °C
Ion transfer capillary temperature	350 °C
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	40 arbitrary units
Q1 and Q3 peak width (FWHM)	0.4 and 0.7 Da
Collision gas and pressure	Ar at 1.5 mTorr
Cycle time	0.5 sec
Acquisition method	EZ Method (scheduled SRM)

SRM Table						
Compound	**Precursor**	**Product 1**	**CE1**	**Product 2**	**CE2**	**S-lens RF**
CBD	315.0	193.1	20	123.0	30	77
CBN	311.1	223.1	19	293.2	14	73
THC	315.2	193.1	19	123.1	31	73
THCA-A	357.0	245.1	33	313.2	26	93

Results/Discussion

Pesticides

Due to inconsistent regulations among states that have legalized medical marijuana, as well as states that have decriminalized recreational marijuana, a wide panel of commonly encountered pesticides was selected for this application. DEET, recognized by the EPA as not evoking health concerns to the general public when applied topically, was found on all medical marijuana samples tested[1]. An average of 28 ng/g of DEET was found on medical samples analyzed. Limited research as to possible side effects, if any, of having this pesticide present within volatilized medical grade product is available. Seized street grade marijuana was found to have a variety of pesticides at concentrations higher than what was observed in the medical grade product. Results for selected samples, each coming from separate criminal cases, are shown below:

Sample	Pesticides Detected	Supplemental Info
1	414 ng/g Methamidophos	Discontinued use in commercial settings in 2009[2]
2	2496 ng/g DEET	89% more DEET present compared to medical samples
3	120 ng/g DEET 1385 ng/g Chlorpyrifos	Chlorpyrifos: EPA released proposal in October 2015 to revoke its use[1]
4	6527 ng/g Chlorpyrifos 449 ng/g DEET 72 ng/g Fenamiphos sulfone	Fenamiphos sulfone: restricted use pesticide due to its high acute toxicity[3]
5	178 ng/g Carbaryl 691 ng/g DEET 71 ng/g Malathion	Carbaryl: "likely" to be carcinogenic in humans[1] Malathion: Mosquito control, low risk to human health[1]

Cannabinoids

Tetrahydrocannabinolic acid (THCA-A) is the non-psychoactive precursor to THC. Within fresh plant material, up to 90% of available THC is found in this form[4]. Under intense heating such as when cannabis is smoked, THCA-A is progressively decarboxylated to the psychoactive THC form. Cannabis researchers have begun further research into THCA-A's potential therapeutic properties, such as anti-inflammatory capabilities, antispasmodic treatments and as use as an analgesic[5]. On account of this, the medical marijuana samples specifically were tested for this compound in addition to other commonly noted cannabinoids. On average, 17% of the total weight in each medical marijuana sample came from the presence of THCA-A. In both medical and recreational samples, percentage of THC contribution ranged from 0.9-1.7.

Conclusion

A fast and effective method was developed for the determination of pesticide residues and cannabis potency in recreational and medical marijuana samples. Pesticide residues and cannabinoids were extracted using the QuEChERS approach, followed by either an additional cleanup using a proprietary blend of dSPE sorbents for pesticide analysis, or serial dilutions for cannabinoid potency testing.

References:

1. Environmental Protection Agency
2. Centers for Disease Control and Prevention
3. Paranjape, Kalyani. The Pesticide Encyclopedia. Wallingford, Oxfordshire: CABI, 2015. Print
4. Jung, Julia, Markus R. Meyer, Hans H. Maurer, Christian Neusüß, Wolfgang Weinmann, and Volker Auwärter. "Studies on the Metabolism of the Δ9-tetrahydrocannabinol Precursor Δ9-tetrahydrocannabinolic Acid A (Δ9-THCA-A) in Rat Using LC-MS/MS, LC-QTOF MS and GC-MS Techniques." Journal of Mass Spectrometry J. Mass Spectrom. 44.10 (2009): 1423-433. Web.
5. Galal, Ahmed, Desmond Slade, Waseem Gul, Abir El-Alfy, Daneel Ferreira, and Mahmoud Elsohly. "Naturally Occurring and Related Synthetic Cannabinoids and Their Potential Therapeutic Applications." *RPCN Recent Patents on CNS Drug Discovery* 4.2 (2009): 112-36. Web.

Acknowledgements: Lisa Mundy, Philadelphia OCME, for her assistance with this comparison study and application.

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 • 800.385.3153 • 215.781.9255 • www.unitedchem.com • Email: methods@unitedchem.com