DETERMINATION OF PESTICIDE RESIDUES AND CANNABIS POTENCY IN MARIJUANA AND CANNABIS FOODS

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BACKGROUND:

As of July 2015, 23 states and Washington D.C. in USA have legalized the medical use of marijuana, while 4 states and Washington D.C. have legalized the recreational use of marijuana. As a result, many testing labs are looking for fast, reliable, and cost-effective methods to determine cannabis potency and chemical residues, (e.g. pesticides, mycotoxins, and heavy metals) in marijuana and cannabis foods (more informally known as edibles). This poster utilizes the advantages of the QuEChERS (acronym for Quick, Easy, Cheap, Effective, Rugged, and Safe) to extract 35 pesticides and 3 cannabinoids, including tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN), in edibles and seized marijuana, followed by either serial dilutions for cannabis potency analysis, or a dispersive solid phase extraction (dSPE) cleanup for pesticide residue analysis.

The proprietary extraction salts are designed to enhance phase separation and partitioning of pesticides and cannabinoids into the extraction solvent layer, while the proprietary dSPE sorbent mixture removes the majority of matrix co-extractives including lipids, organic acids, sugars, artificial colors, and natural pigments (e.g. chlorophyll and anthocynins) in various sample matrices tested in this study, resulting in clean extracts for instrumental detection.

EXPERIMENTAL:

QuEChERS Products	
Description	**UCT Part Number**
Pouches containing a proprietary blend of QuEChERS salts for THC Potency and Pesticide Testing, 50-mL centrifuge tubes included	ECQUUS10-MP
2-mL centrifuge tubes containing proprietary blend of dSPE sorbents for Pesticide Testing in Edibles and Marijuana	ECQUUS10-2CT

(a) Sample pre-treatment
1. For hard candies, cookies, chocolate bars, and marijuana samples, grind to fine powders using a SPEX 6770 freezer mill.

Figure 1: Hard candy before (left) and after (right) freezer mill grinding

2. For gummy samples, cut into slim pieces. Although freezer mill can grind gummies to powder at low temperature with the use of liquid nitrogen, it returns to gel state when temperature goes up to room temperature, thus gummy samples were cut instead of grinded.

3. For sodas, degas for 30 min by sonication.

Figure 2: Degassing of Reef cola (left) and Orange kush (right)

(b) QuEChERS extraction
1. Weigh 1 g of the pre-treated samples (hard candies, gummies, brownies, cookies, chocolate bars, oil, and marijuana) into 50-mL centrifuge tubes, add internal standard (optional) and 10 mL of reagent water, and hydrate for 1 hr using a horizontal shaker. For sodas, add 10 mL of the degassed sample and internal standard (optional) to 50-mL centrifuges.
2. Add 10 mL of acetonitrile (MeCN) with 1% acetic acid.
3. Add QuEChERS extraction salts from pouches (ECQUUS10-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. For gummy samples, add 2 metal balls and shake for 10 min at 1000 stroke/min.
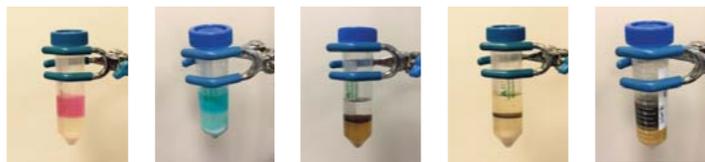5. Centrifuge at 3000 rcf for 5 min.

Figure 3: Samples after QuEChERS extraction (from left to right: hard candies, gummies, soda, chocolate, and marijuana)

(c) dSPE cleanup for pesticide residue analysis
1. Transfer 1 mL of the supernatants to 2-mL dSPE tubes (ECQUUS10-2CT).
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.

Figure 4: Comparison of QuEChERS extracts before and after dSPE cleanup (from left to right: hard candies, gummies, and marijuana)

(d) Make 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 ppb.
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).

RESULTS:

A. Pesticide residue analysis
Matrix-matched calibration curves were generated using post spiked blank extracts of homogenized green tea leaves to semi-quantify the pesticide residues in edibles and seized marijuana. Appropriate amounts of the pesticide spiking solutions (0.1 and 1 ppm) were added into the tea extracts to generate 6-point matrix-matched calibration curves with concentrations at 5, 10, 25, 50, 100, and 250 ng/mL (TPP as IS at 200 ng/mL). The responses were found to be linear (R² > 0.99) over the concentration range. The limit of quantitation (LOQ) of this method was found to be 5 ng/mL in the extract or 50 ng/g in the marijuana and edibles, and 5 ng/mL in the soda samples.

Table 1: Accuracy and precision of pesticides in spiked tea samples

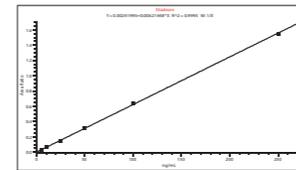| Compound	Spiked at 10 ng/mL Recovery%	Spiked at 10 ng/mL RSD% (n=6)	Spiked at 50 ng/mL Recovery%	Spiked at 50 ng/mL RSD% (n=6)
Methamidophos	80	11	83	12
Acephate	81	14	93	12
Aldicarb_sulfoxide	93	13	95	23
Oxydemeton_methyl	74	16	80	23
Dichrotophos	90	15	75	14
Pymetrozine	57	20	59	10
Dimethoate	105	16	87	12
Triethylphosphorothioate	97	14	82	14
Carbendazim	98	15	74	12
Dichlorvos	97	12	97	11
Fenamiphos_sulfone	121	11	108	15
Fenamiphos_sulfoxide	99	14	96	16
Simazine	121	14	107	14
Carbaryl	93	10	103	14
Tebuthiuron	105	9	105	17
Thiabendazole	70	7	78	8
Famphur	101	14	101	13
Flutriafol	92	14	96	10
Thionazin	103	11	99	12
Atrazine	99	24	95	13
DEET	105	30	97	12
Malathion	102	23	115	14
Triadimefon	97	21	101	18
Bifenazate	154	23	98	21
Pyrimethanil	83	14	84	16
Acetochlor	96	16	101	12
Sulfotep	100	15	99	13
Tebuconazole	85	2	87	5
Zoxamide	83	3	91	5
Diazinon	92	4	92	3
Cyprodinil	77	5	77	3
Pyraziphos	94	4	97	3
Ethion	92	3	92	5
Profenofos	87	8	88	6
Chlorpyrifos	90	9	93	9

Figure 5: Matrix-matched calibration curve of Diazinon (R² = 0.9995)

Table 2: Pesticide residues detected in edibles and seized marijuana

Sample	Detected pesticides (semi-quantitatively)
Elixirs (beverage)	14 ng/g Bifenazate
Orange kush (soda)	10 ng/g Bifenazate
Reef cola (soda)	Not detected
CBD oil	1321 ng/g Bifenazate
Cookie and cream (bar)	Not detected
Fantastic brownies	97 ng/g Bifenazate
Mint milk chocolate	Not detected
Monkey bar	Not detected
Mixed drops (hard candy)	Not detected
Nectarbee (hard candy)	Not detected
Sour gummies	Not detected
Sour fruit ring (gummy)	Not detected
Sweet'n sours (gummy)	Not detected
Marijuana sample 1	414 ng/g Methamidophos
Marijuana sample 2	2498 ng/g DEET
Marijuana sample 3	530 ng/g DEET
Marijuana sample 4	120 ng/g DEET, 1385 ng/g Chlorpyrifos
Marijuana sample 5	72 ng/g Fenamiphos sulfone, 449 ng/g DEET, 4927 ng/g Chlorpyrifos
Marijuana sample 7	176 ng/g Carbaryl, 691 ng/g DEET, 71 ng/g Malathion

B. Cannabis potency determination by standard addition method
Example: Cookie and cream bar, labeled with 30 mg of THC in 45 grams (equals to 667 µg/g) After QuEChERS extraction of 1 g of the ground cookie and cream sample into 10 mL MeCN, the concentration of THC in the supernatant will be 66.7 µg/mL. Serial dilutions (x10x50 = 500) were made to dilute the extract to about 133 ng/mL, then the diluted samples were spiked with 70 (about 50%) and 210 ng/mL (about 150%) cannabinoids. The peak areas were plotted against the diluted sample (0), 50% spiked (70 ng/mL) and 150% spiked (210 ng/mL) samples, a 3-point linear curve (Figure 6) was generated. The concentration in the diluted sample was calculated by dividing the intercept by the slope. With the calculated concentration, the peak areas were re-plotted (Figure 7) and a linear curve with R² of 0.9999 was obtained, indicating that the standard addition method is effective for accurate analyte quantitation.
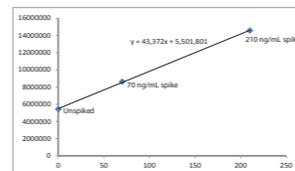

Figure 6: Plot of peak area against the unspiked sample (0), and samples spiked at 50% (70 ng/mL) and 150% (210 ng/mL) of cannabinoids
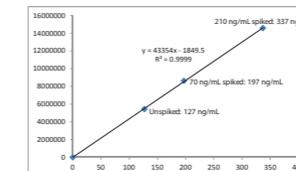

Figure 7: Re-plot of peak area against the actual concentrations: unspiked sample (127 ng/mL), and samples spiked at 50% (197 ng/mL) and 150% (337 ng/mL) of cannabinoids

Calculations:
THC conc. in the diluted sample = 5501801/43372 = 127 ng/mL
THC in the cookie and cream bar = 127 ng/mL x 500 x 10 mL/g x 45 g = 29 mg (very close to the labeled 30 mg THC)

Table 3: Comparison of labeled and detected cannabinoids in edibles (unit: mg)

Edibles	CBD Labeled	CBD Detected	CBN Labeled	CBN Detected	THC Labeled	THC Detected
CBD oil	500	483	<5	ND	5	12
Reef cola					10	7
Orange kush (soda)					10	6
Elixirs (Sarsaparilla)					90	60
Nectarbee hard candy					10	6
Mixed drops (hard candy)	NA	ND	NA	ND	100	49
Fantastic brownie					10	14
Cookie and cream					30	29
Monkey bar					100	69
Mint milk chocolate					100	74
Sour gummies					100	95
Sour fruit ring (gummy)					10	8
Sweet'n sours (gummy)	NA	28	NA	ND	100	31

Table 4: Cannabinoid (%) in seized marijuana samples

Marijuana	THC%	CBN%
Marijuana sample 1	1.1	0.4
Marijuana sample 2	0.9	0.3
Marijuana sample 3	0.9	0.2
Marijuana sample 4	2.3	0.2
Marijuana sample 5	1.5	0.3
Marijuana sample 6	0.9	0.2
Marijuana sample 7	1.1	0.4

Figure 8: Chromatogram of the diluted mint milk chocolate sample (2000 times dilution of the QuEChERS extract) spiked with 70 ng/mL of cannabinoids

CONCLUSIONS:

A fast and effective method was developed for the determination of pesticide residues and cannabis potency in edibles and seized marijuana. Pesticide residues and cannabinoids were extracted using a proprietary blend of QuEChERS salts followed by either a dSPE cleanup for pesticide analysis, or serial dilutions for cannabinoid potency test. Pesticides were found to be present in both edibles (sodas, oil, and brownie) and seized marijuana (6 of the 7 tested samples) at varied concentrations. The detected amounts of cannabinoids were compared to those listed on the labels of the cannabis infused food products, where a small portion of the tested products were accurately labeled, while others were either higher or lower than labeled.

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