Determination of 35 Pesticides and 3 Cannabinoids in Marijuana Edibles

UCT Part Numbers

ECQUUS950CT-MP
QuEChERS salts for THC Potency & 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 (100 x 2.1 mm, 3µm)

SLAQGDC20-3UM
Selectra® Aqueous C18 Guard (10 x 2.1mm, 3µm)

SLGRDHLDR
Guard Cartridge Holder

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Summary:

As of January 2016, in the United States of America, 24 states and the District of Columbia have legalized the medical use of marijuana, while four states and the District of Columbia have legalized the recreational use of marijuana. Although the federal government still classifies any use or possession of the drug as illegal, all fifty states are starting to see an increase in the number of edible marijuana samples within their borders. As a result, many forensic toxicology labs are looking for fast, reliable and cost-effective methods to determine cannabis potency and pesticides in edibles. The pros and cons of legalization are still heavily debated throughout the country, but all scientists agree that uniform testing policies and procedures need to be established as soon as possible and that overall sample clean-up is the main issue within these analyses.

This application utilizes the advantages of QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) to extract 35 pesticides and 3 cannabinoids including tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN) in edibles, followed by either serial dilution for cannabis potency analysis, or a dispersive solid phase extraction (dSPE) cleanup for pesticide residue analysis. This hybrid method allows the QuEChERs technique, which is extensively used in the food testing industry, to be utilized in a forensic setting.

Sample Pretreatment:

For hard candies and chocolate, grind to a fine powder using a SPEX 6770 freezer mill (Figure 1).

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

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 should be cut instead of ground. For sodas, degas for 30 min by sonication (Figure 2).

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

QuEChERS Procedure:

Sample Extraction:

1. Weigh 1 g of the pre-treated samples (hard candies, gummies, brownies, chocolate, and oil) 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 (**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. 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).

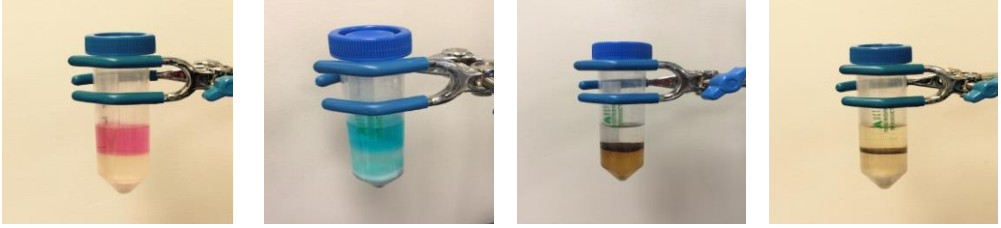

Figure 3: Samples after QuEChERS extraction (from left to right: hard candies, gummies, soda, and chocolate)

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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 (Figure 4).

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

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 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.

LC-MS/MS Parameters:

HPLC Parameters (Cannabinoids)		
HPLC System: Thermo Scientific™ Dionex™ Ultimate™ 3000		
HPLC Column: UCT Selectra® AQ C18, 100 x 2.1 mm, 3 µm		
Guard cartridge: UCT Selectra® AQ C18, 10 x 2.1 mm, 3 µm		
Column temperature: 40 °C		
Flow rate: 0.3 mL/min		
Autosampler temperature: 10 °C		
Injection volume: 5 µL		
Gradient program:		
Time (min)	**A%** (10 mM Ammonium Acetate)	**B%** (0.1% Formic Acid in MEOH)
0.0	40	60
0.5	40	60
3.0	5	95
7.0	5	95
7.1	40	60
10.0	40	60

HPLC Parameters (Pesticides)		
HPLC System: Thermo Scientific™ Dionex™ Ultimate™ 3000		
HPLC Column: UCT Selectra® AQ C18, 100 x 2.1 mm, 3 µm		
Guard cartridge: UCT Selectra® AQ C18, 10 x 2.1 mm, 3 µm		
Column temperature: 40 °C		
Flow rate: 0.3 mL/min		
Autosampler temperature: 10 °C		
Injection volume: 2 µL		
Gradient program:		
Time (min)	**A%** (10 mM Ammonium Acetate)	**B%** (0.1% Formic Acid in MEOH)
0.0	100	0
1.0	50	50
3.5	50	50
6.0	5	95
9.0	5	95
9.1	100	0
14.0	100	0

MS parameters (Both)	
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	Argon at 1.5 mTorr
Cycle time	0.5 sec
Acquisition method	EZ Method (scheduled SRM)

SRM Table (Cannabinoids)						
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

SRM Table (Pesticides)						
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

Results:

Pesticide residue analysis

Table 1: Accuracy and Precision of Pesticides in Spiked Samples

Compound	Spiked at 10 ng/mL		Spiked at 50 ng/mL	
	Recovery%	RSD% (n=6)	Recovery%	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**	13	**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	**86**	3	**91**	5
Diazinon	**92**	4	**92**	3
Cyprodinil	**77**	5	**77**	3
Pyrazophos	**94**	4	**97**	3
Ethion	**92**	3	**92**	5
Profenofos	**87**	8	**88**	6
Chlorpyrifos	**90**	9	**93**	9

6-point matrix-matched calibration curves with concentrations at 5, 10, 25, 50, 100, and 250 ng/mL were generated. The responses were found to be linear ($R^2 > 0.99$) over the concentration range. The limit of quantitation (LOQ) of this method was found to be 50 ng/g in the edibles, and 5 ng/mL in the soda samples.

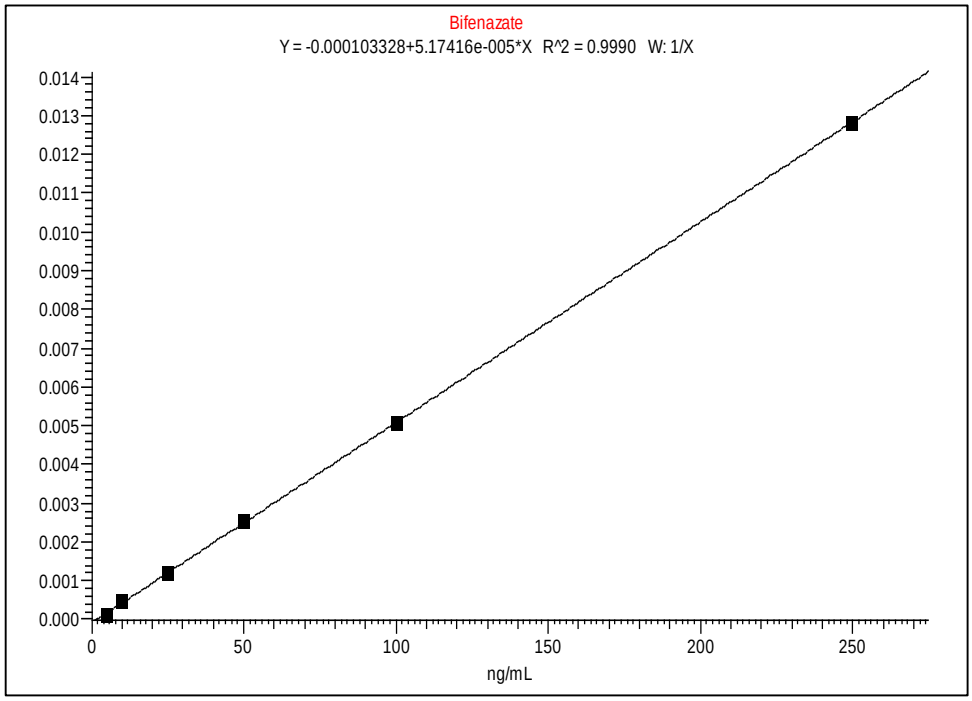

Figure 5: Matrix-matched calibration curve of Bifenazate ($R^2 = 0.9990$)

Table 2: Pesticide residues detected in edibles

Brand	Product	Detected pesticides
Keef Cola	Keef Cola	Not detected
Keef Cola	Orange Kush	10 ng/mL Bifenazate
Dixie Brands	Elixir	14 ng/mL Bifenazate
Nectar Bee	Cherry Lime Hard Candy	Not detected
Nectar Bee	Sour Fruit Ring Strawberry	Not detected
Wana	Sour Gummies	Not detected
EdiPure	Sweet 'n Sours	Not detected
EdiPure	Mixed Drops	Not detected
Growing Kitchen	Fantastic brownie	97 ng/g Bifenazate
Incredibles	Mile High Mint	Not detected
Incredibles	Cookie and Cream	Not detected
Incredibles	Monkey Bar	Not detected
Elite Botanicals	CBD Oil	1221 ng/g Bifenazate

Cannabis potency Analysis

Example: Cookie and cream bar, labeled with 30 mg of THC in 45 grams (equals 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 R^2 of 0.9999 was obtained, indicating that the standard addition method is effective for accurate analyte quantitation.

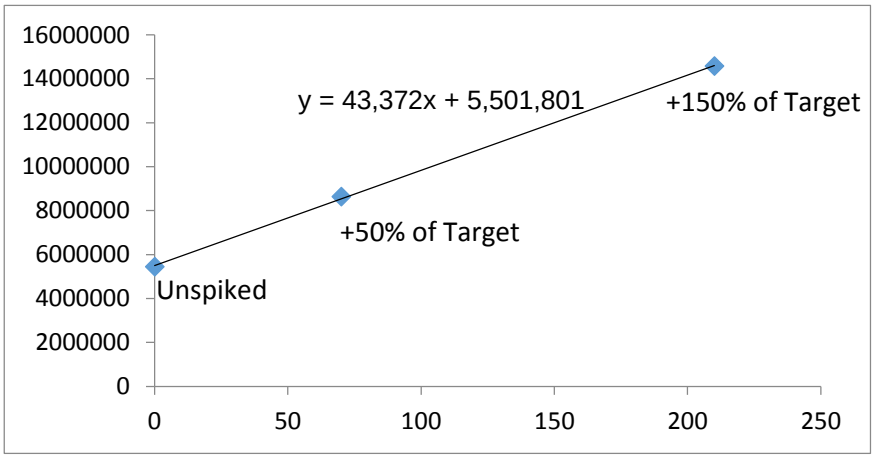

Figure 6: Plot of peak area against the unspiked sample (0), and samples spiked at 50% (70 ng/mL) and 150% (210 ng/mL) of cannabinoids.

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 x 10^{-6} mg/ng = 29 mg (very close to the labeled 30 mg THC).

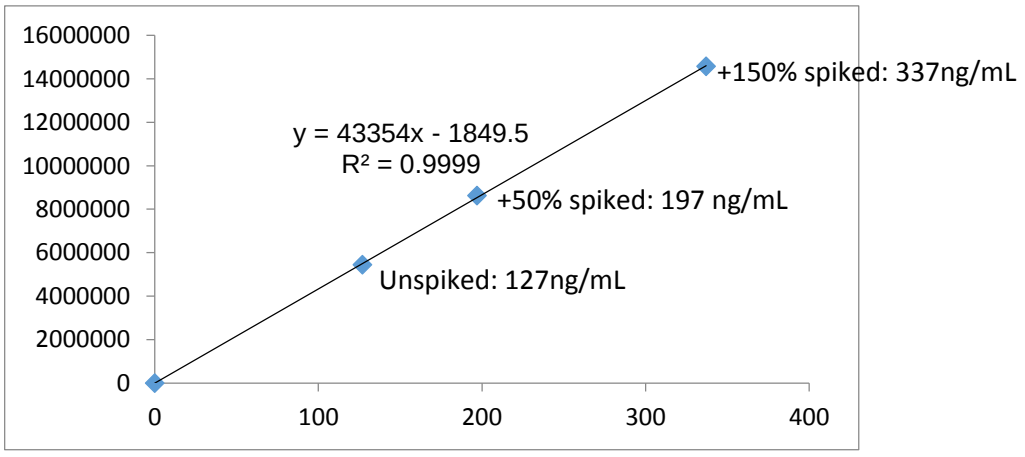

Figure 7: Re-plot of peak area against the actual concentrations: unspiked sample (127 ng/mL), and samples spiked at 50% (197 ng/mL) and 150% (337 ng/mL) of cannabinoids

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

Brand	Edibles	CBD		CBN		THC	
		Labeled	Detected	Labeled	Detected	Labeled	Detected
Keef Cola	Keef Cola	NA	ND	NA	ND	10	7
Keef Cola	Orange Kush	NA	ND	NA	ND	10	6
Dixie Brands	Elixir	NA	ND	NA	ND	90	60
Nectar Bee	Cherry Lime Hard Candy	NA	ND	NA	ND	10	6
Nectar Bee	Sour Fruit Ring Strawberry	NA	ND	NA	ND	10	8
Wana	Sour Gummies	NA	ND	NA	ND	100	95
EdiPure	Sweet 'n Sours	NA	28	NA	ND	100	31
EdiPure	Mixed Drops	NA	ND	NA	ND	100	49
Growing Kitchen	Fantastic brownie	NA	ND	NA	ND	10	14
Incredibles	Mile High Mint	NA	ND	NA	ND	100	74
Incredibles	Cookie and Cream	NA	ND	NA	ND	100	29
Incredibles	Monkey Bar	NA	ND	NA	ND	100	69
Elite Botanicals	CBD Oil	500	493	<5	ND	5	12

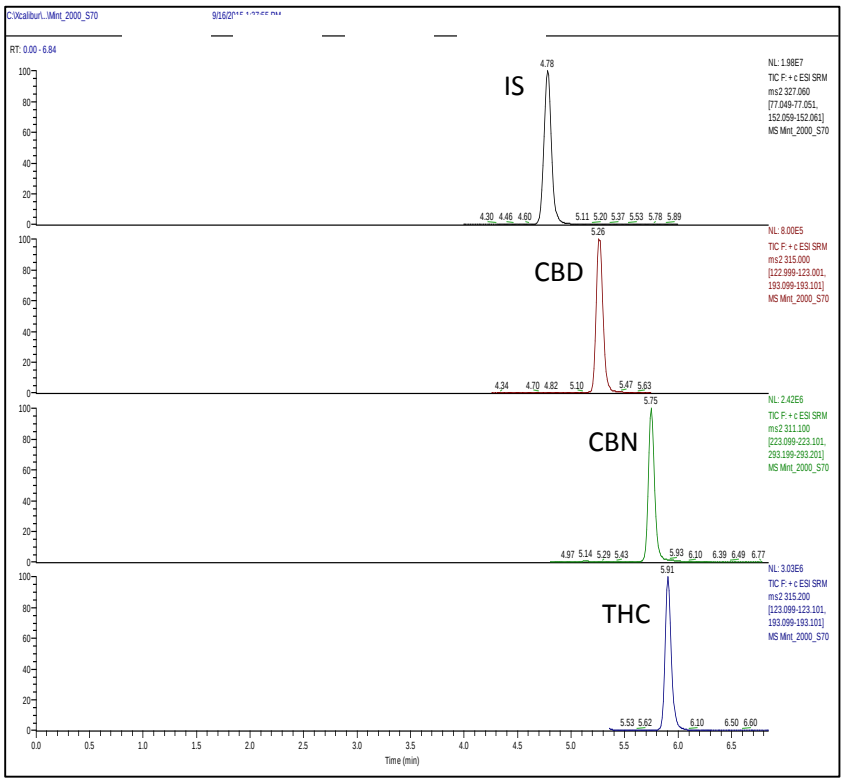

Figure 8: Chromatogram of the diluted mint milk chocolate sample (2000 times dilution of the QuEChERS extract) spiked with 70 ng/mL of cannabinoids

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Conclusion:

A fast and effective method was developed for the determination of pesticide residues and cannabis potency in edibles. Pesticide residues and cannabinoids were extracted using the QuEChERS approach, followed by either a proprietary blend of dSPE sorbents for pesticide analysis, or serial dilutions for cannabinoid potency test. Bifenazate, commonly used to control mites on agricultural products, was found to be present in two soda products as well as oil and brownie edibles. The detected amounts of cannabinoids were compared to those listed on the labels of the cannabis infused food products. Of the tested products, 23% were accurately labeled within (+/-) 10% of expected concentrations, while others were either higher or lower than claimed amounts.

ACKNOWLEDGEMENT

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