



# 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



## 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).

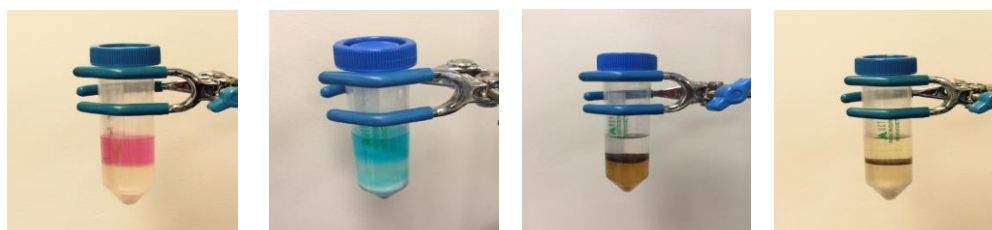


**Figure 2:** Degassing of Reef cola (left) and Orange kush (right)

## 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)



#### 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  $\mu$ L extract to the 2-mL auto-sampler vials, add 200  $\mu$ L 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)		
<b>HPLC System:</b> Thermo Scientific™ Dionex™ Ultimate™ 3000		
<b>HPLC Column:</b> UCT Selectra® AQ C18, 100 x 2.1 mm, 3 $\mu$ m		
<b>Guard cartridge:</b> UCT Selectra® AQ C18, 10 x 2.1 mm, 3 $\mu$ m		
<b>Column temperature:</b> 40 °C		
<b>Flow rate:</b> 0.3 mL/min		
<b>Autosampler temperature:</b> 10 °C		
<b>Injection volume:</b> 5 $\mu$ L		
<b>Gradient program:</b>		
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)		
<b>HPLC System:</b> Thermo Scientific™ Dionex™ Ultimate™ 3000		
<b>HPLC Column:</b> UCT Selectra® AQ C18, 100 x 2.1 mm, 3 µm		
<b>Guard cartridge:</b> UCT Selectra® AQ C18, 10 x 2.1 mm, 3 µm		
<b>Column temperature:</b> 40 °C		
<b>Flow rate:</b> 0.3 mL/min		
<b>Autosampler temperature:</b> 10 °C		
<b>Injection volume:</b> 2 µL		
<b>Gradient program:</b>		
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)	
<b>Instrumentation</b>	Thermo Scientific™ TSQ Vantage™ tandem MS
<b>Polarity</b>	ESI +
<b>Spray voltage</b>	3500 V
<b>Vaporizer temperature</b>	450 °C
<b>Ion transfer capillary temperature</b>	350 °C
<b>Sheath gas pressure</b>	50 arbitrary units
<b>Auxiliary gas pressure</b>	40 arbitrary units
<b>Q1 and Q3 peak width (FWHM)</b>	0.4 and 0.7 Da
<b>Collision gas and pressure</b>	Argon at 1.5 mTorr
<b>Cycle time</b>	0.5 sec
<b>Acquisition method</b>	EZ Method (scheduled SRM)

SRM Table (Cannabinoids)						
Compound	Precursor	Product 1	CE1	Product 2	CE2	S-lens RF
<b>CBD</b>	315.0	193.1	20	123.0	30	77
<b>CBN</b>	311.1	223.1	19	293.2	14	73
<b>THC</b>	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:

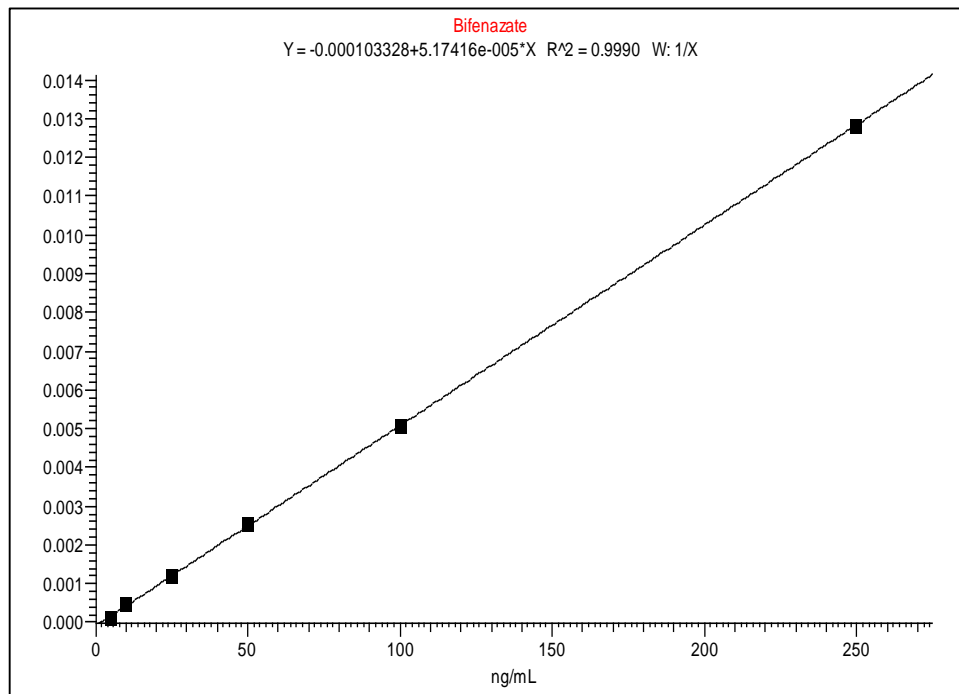
### Pesticide residue analysis

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**

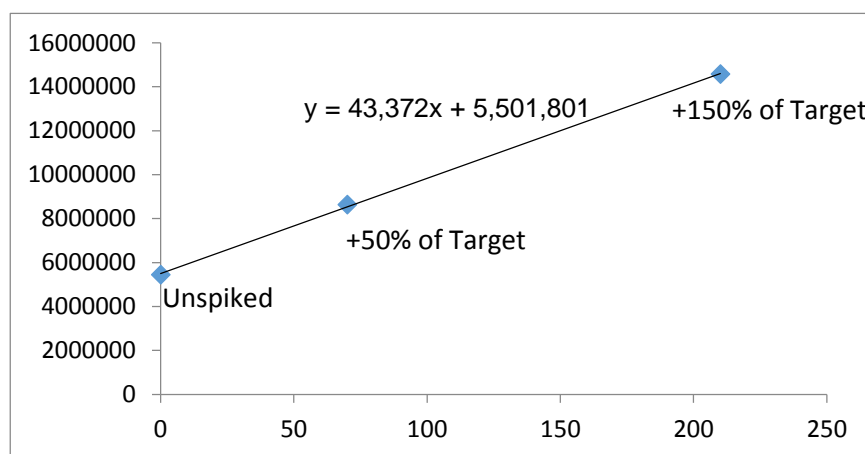
Brand	Product	Detected pesticides
Keef Cola	Keef Cola	Not detected
Keef Cola	Orange Kush	10 ng/mL Bifenazate
Dixie Brands	Elixir	14 ng/mL Bifenazate
Nectar Bee	Cherry Lime Hard Candy	Not detected
Nectar Bee	Sour Fruit Ring Strawberry	Not detected
Wana	Sour Gummies	Not detected
EdiPure	Sweet 'n Sours	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 ( $10 \times 50 = 500$ ) 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.

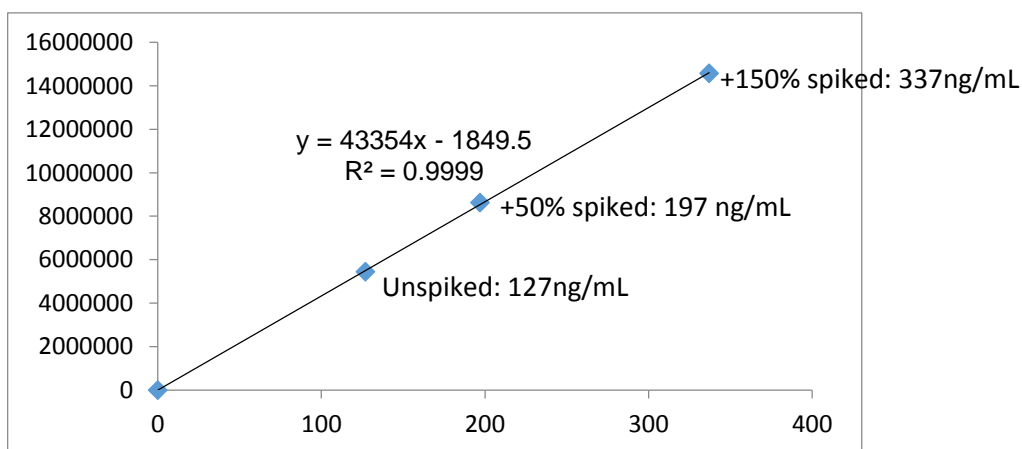


**Figure 6:** Plot of peak area against the unspiked sample (0), and samples spiked at 50% (70 ng/mL) and 150% (210 ng/mL) of cannabinoids.

### Calculations:

THC conc. in the diluted sample =  $5501801/43372 = 127$  ng/mL.

THC in the cookie and cream bar =  $127 \text{ ng/mL} \times 500 \times 10 \text{ mL/g} \times 45 \text{ g} \times 10^{-6} \text{ mg/ng} = 29 \text{ mg}$  (very close to the labeled 30 mg THC).



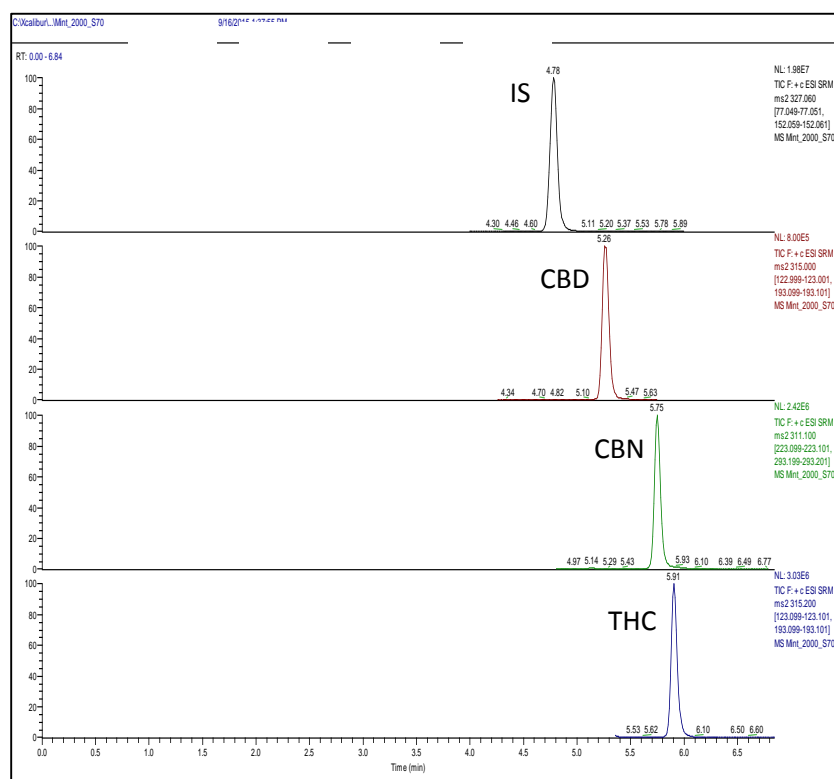
**Figure 7:** Re-plot of peak area against the actual concentrations: unspiked sample (127 ng/mL), and samples spiked at 50% (197 ng/mL) and 150% (337 ng/mL) of cannabinoids





**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**

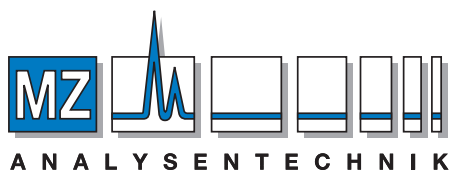


## Conclusion:

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

Keith Tucker (Vice President of Marketing at *SPEX SamplePrep*, LLC) is acknowledged for kindly providing the 6770 Freezer mill and 2010 Geno/grinder. Erik Swiatkowski (UCT) is thanked for his help in grinding samples using the SPEX 6770 freezer mill.



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