Extraction of Cannabinoids in Marijuana and Edibles by QuEChERS

UCT Part Numbers:
ECQUEU750CT-MP - QuEChERS, Mylar packs containing 4 g magnesium sulfate, 1 g sodium chloride, 0.5 g sodium citrate dibasic sesquihydrate, and 1 g sodium citrate tribasic; includes 50-mL centrifuge tubes
CSTHC206 - Clean Screen® THC extraction column, 200mg/6mL
SPHPHO7001-5 – Select pH Buffer Pouch, pH 7.0 100 mM phosphate buffer
GCLGN4MM-5 - GC liner, 4mm splitless gooseneck, 4mm ID x 6.5mm OD x 78.5mm

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Summary:

Medical marijuana has been legalized in multiple states across the USA [1]. As a result, many testing labs are seeking fast and reliable analytical methods to determine the cannabinoid potency in marijuana and cannabis infused foods (more informally known as edibles) [2]. This application utilizes the advantages of the QuEChERS technique to extract cannabinoids in marijuana and cannabis containing foods. This is followed by either a dilution for marijuana samples or a solid phase extraction (SPE) cleanup for various complex food samples. This method results in clean extracts for instrumental detection.

1 gram homogenized food or 100 mg marijuana sample is hydrated with 10 mL reagent water for 30 min. The cannabinoids are then extracted into 10 mL of acetonitrile (MeCN) with the aid of EN15662 QuEChERS extraction salts for analyte partitioning and phase separation. After centrifugation the supernatant is either diluted or undergoes a secondary SPE cleanup before analysis.

Three representative cannabinoids, tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN) were selected for analysis in this study. The accuracy and precision obtained from spiked negative bread samples were excellent. The method was applied to 6 seized marijuana samples, and the cannabinoid contents and phenotypic indexes (THC/CBD and (THC+CBN)/CBD ratios) were reported.

Procedure:

1. Extraction

a) Weigh 1 g of homogenized food sample (or 100 mg marijuana) into a 50-mL centrifuge tube.
b) Add 10 mL of reagent water, let soak and shake occasionally for 30 min.
c) Add 10 mL of MeCN, shake or vortex for 1 min (10 min for marijuana).
d) Add the EN15662 extraction salts in pouch (**ECQUEU750CT-MP**), and shake for 1 min manually or use a Spex 2010 Geno-Grinder at 1000 strokes/min.
e) Centrifuge at 3000 g for 5 min. Target analytes were extracted into the upper MeCN layer (phase separation shown below).

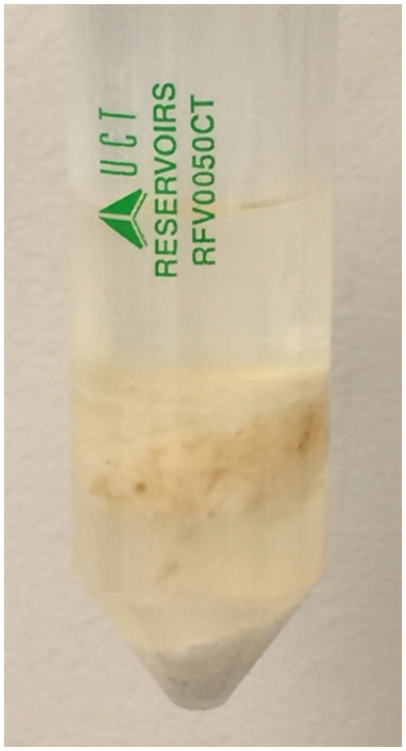


Figure 1. Bread sample after extraction

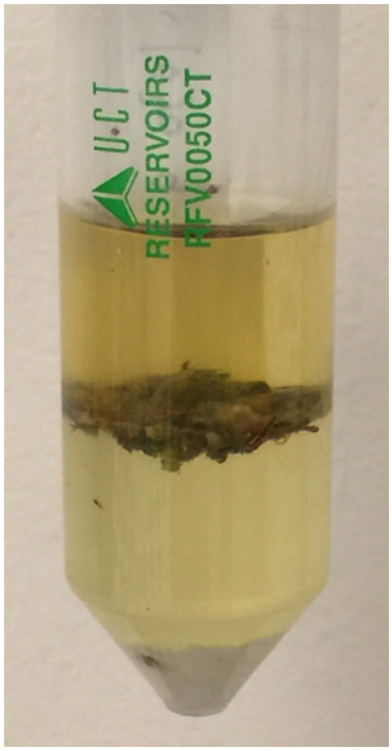


Figure 2. Marijuana sample after extraction

2. Dilution or Cleanup

OPTION 1: For cannabinoids in marijuana:
Mix 5 µL of the supernatant with 1 mL n-hexane for GC, GC/MS or GC/MS/MS analysis; or 1 mL mobile phase (50% organic) for HPLC, LC/MS or LC/MS/MS analysis.

Note: *For detection using GC or HPLC detectors which are less sensitive or selective than MS detectors either dilute the extract less (i.e. take more supernatant) or use an additional SPE cleanup to remove any remaining interfering peaks affecting analyte integration.*

OPTION 2: For cannabinoids in food samples:

a) Transfer 0.1 to 1 mL supernatant (volume depends on the cannabinoid contents in the sample and the instrument sensitivity) to a clean test tube.
b) Dilute 10 times with pH 7 phosphate buffer*.
c) Place the SPE columns (UCT p/n **CSTHC206**) into a glass block or positive pressure manifold, condition the SPE columns with 3 mL 1:1 n-hexane:ethyl acetate, soak 1 min, then drain to waste and allow to dry for 1 min. This is followed by the addition of 3 mL methanol and 3 mL reagent water. Allow these to draw through the column by gravity.
d) Load the sample by gravity.
e) Rinse the test tube with 3 mL reagent water, apply the rinsate to the SPE columns, and drain to waste.
f) Repeat step e) with 3 mL n-hexane**, then dry the SPE columns under full vacuum or pressure for 5 min.
g) Insert a collection rack with test tubes into the manifold, and elute the SPE columns with 3 x 1.5 mL of 1:1 n-hexane:ethyl acetate by gravity.
h) Evaporate the eluates to dryness under a gentle stream of nitrogen at 40°C.
i) Reconstitute in 1 mL n-hexane for GC, GC/MS or GC/MS/MS analysis, or 1 mL mobile phase (50% organic) for HPLC, LC/MS or LC/MS/MS analysis.

** pH 7 phosphate buffer is prepared by dissolving the salts in pouch (UCT p/n **SPHPHO7001-5**) with 1 L of reagent water.*

*** Insignificant analyte loss (< 2%) was observed with 3 mL n-hexane wash in this study, analysts may need to optimize the n-hexane volume for different matrices and cannabinoid contents.*

GC/MS method:

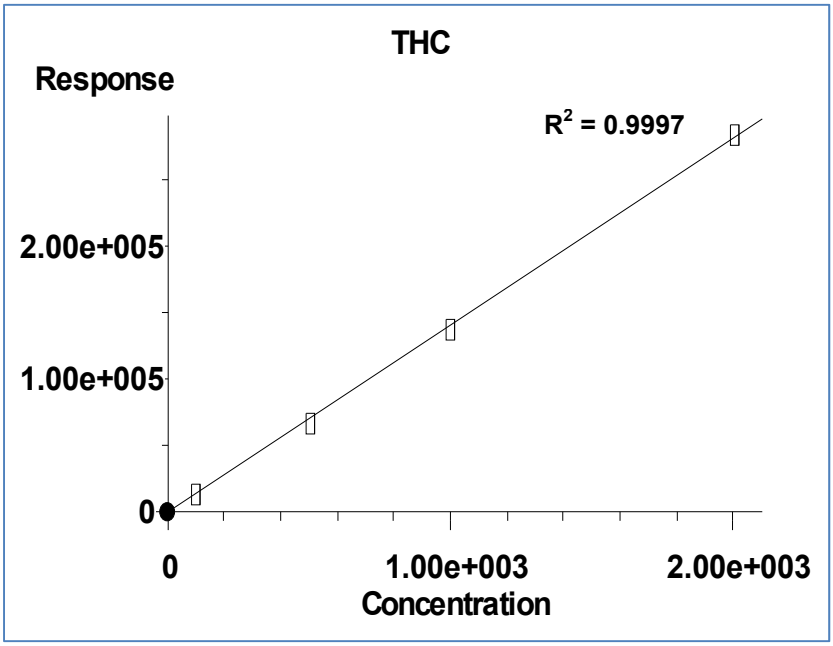
GC/MS	Agilent 6890N GC coupled to a 5975C MSD
Injection	2 µL splitless injection at 250 ºC
GC liner	4 mm splitless gooseneck (UCT p/n **GCLGN4MM-5**), packed with deactivated glass wool
GC column	Restek Rxi®-5sil MS 30m x 0.25mm, 0.25µm with 10m integrated guard column
Carrier gas	Ultra high purity Helium at a constant flow of 1.2 mL/min
Oven temp. program	Initial temperature at 60 ºC, hold for 1 min; ramp at 30 ºC/min to 310 ºC, hold for 2.67 min. Acquire data from 8 to 10 min.
Temperatures	Transfer line 280 ºC Source 250 ºC Quadrupole 150 ºC

Analyte	**Retention (min)**	**SIM ions (50 ms)**			**Linearity (R^2)**
CBD	9.078	231.1	246.1	314.2	0.9998
THC	9.357	299.1	231.1	314.2	0.9997
CBN	9.539	295.1	238.1	310.2	0.9998

Results:

Matrix effect was determined by comparing the calibration curve slopes of the matrix matched standards (bread samples) against those of the calibration standards prepared in n-hexane, which were found to be insignificant (≤ 20% for all of the 3 analytes), thus solvent standards (0, 100, 500, 1000 and 2000 ppb) were used to generate calibration curves using the external standard calibration technique. The responses were found to be linear over the analytical range (R^2 ≥ 0.9997 for 0 - 2000 ppb).

Calibration Curve of THC



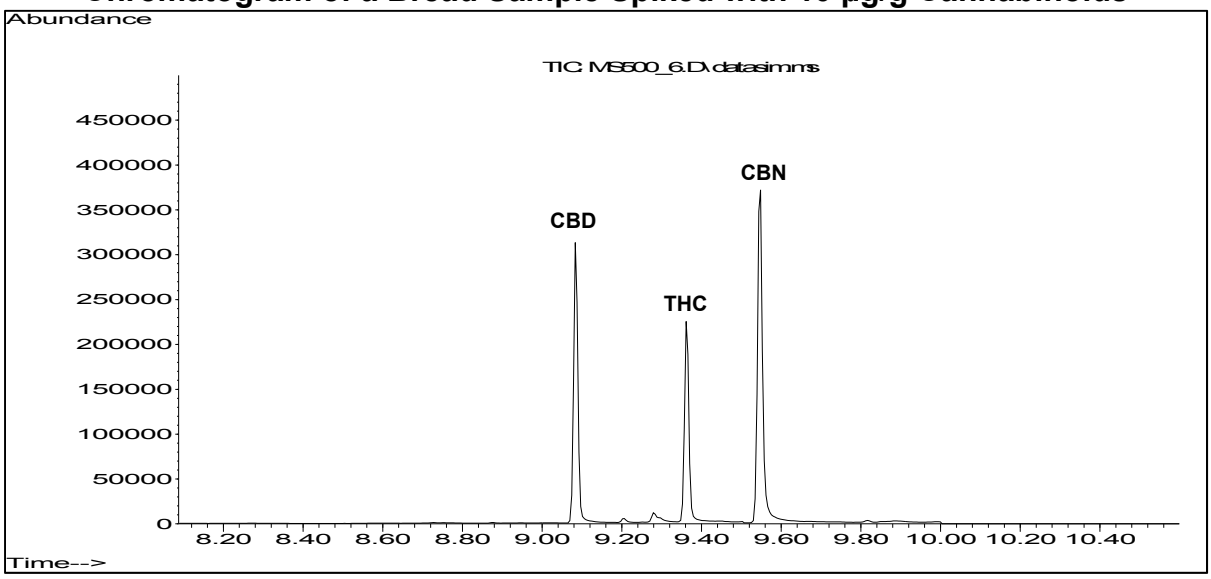
Recovery and RSD% from Spiked Bread Sample

Analyte	Recovery%	RSD% (n=6)
CBD	92.6	3.0
THC	93.1	4.6
CBN	96.3	2.0

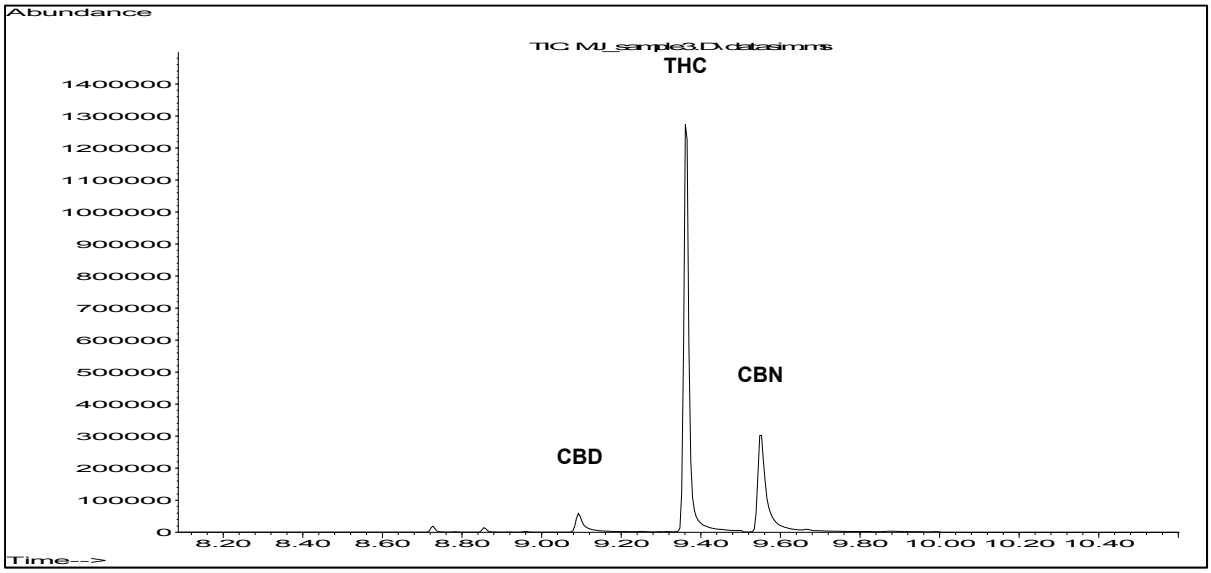
Cannabinoid Content and Phenotypic Index of 6 Seized Marijuana Samples

Marijuana	Cannabinoids (% dry weight)			Phenotypic index	
	CBD%	THC%	CBN%	THC/CBD	(THC+CBN)/CBD
Sample 1	0.16	2.63	0.80	16.9	22.0
Sample 2	0.15	4.47	1.11	29.1	36.3
Sample 3	0.37	5.59	1.22	15.0	18.3
Sample 4	0.54	5.63	1.51	10.5	13.3
Sample 5	0.13	1.02	1.16	7.7	16.5
Sample 6	0.14	2.09	0.60	15.0	19.3

Chromatogram of a Bread Sample Spiked with 10 µg/g Cannabinoids



Chromatogram of a Seized Marijuana Sample



References:

[1] http://medicalmarijuana.procon.org/view.resource.php?resourceID=000881

[2] http://en.wikipedia.org/wiki/Cannabis_foods

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