DETERMINATION OF PESTICIDE RESIDUES IN MARIJUANA BY QuEChERS AND LC-MS/MS

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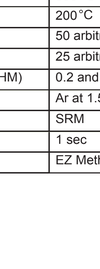
INTRODUCTION

Marijuana is one of the most highly abused drugs in the world. Although the concentrations of the active ingredient (THC) have been determined by many laboratories, few studies have looked at possible organic contaminants such as pesticides in marijuana. The medical use of marijuana has been legalized in many states in the USA. With this limited legalization and use of marijuana, the determination of pesticide residues becomes important. Patients taking medical marijuana in conjunction with other therapies may be more vulnerable to toxic substances, such as pesticides.

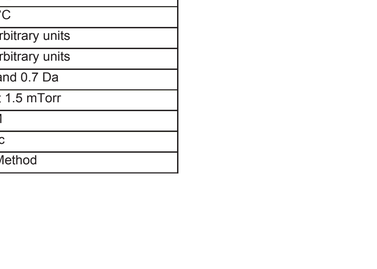
This poster presents a robust and efficient method using the AOAC QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) and LC-MS/MS techniques to determine pesticide concentrations in marijuana samples. A small amount of marijuana sample (approximately 1 gram) was hydrated by 14 mL of DI water. The pesticide residues were extracted into acetonitrile (MeCN) with 1 % acetic acid (HAc). The phase separation was achieved by the addition of magnesium sulfate and sodium acetate. The MeCN extract was then purified with dispersive solid phase extraction (dSPE) using primary secondary amines (PSA) and a specially designed sorbent: ChloroFiltr®. PSA removes organic acids, lipids, and sugars, while ChloroFiltr® removes chlorophyll without loss of planar pesticides, which normally occurs when graphitized carbon black is used for pigment cleanup.

PESTICIDE ANALYSIS

Pesticide Extraction and Cleanup Materials	
50 mL tubes (UCT part#: RFV0050CT)	50 mL polypropylene centrifuge tubes
Extraction salts (UCT part#: ECMSSA50CT-MP)	Mylar pouch with 6 g MgSO4 and 1.5 g NaOAc
15 mL tubes with PSA and ChloroFiltr® (UCT part#: ECMPSGG15CT)	15 mL centrifuge tube with 900 mg MgSO4, 300 mg PSA and 150 mg ChloroFiltr®



dSPE Tube



QuEChERS Extraction Kit

Procedure:

1. QuEChERS extraction

a) Weigh 1 g of the ground marijuana sample into 50-mL centrifuge tubes (UCT part#: RFV0050CT). Prepare 7 unfortified samples for the matrix blank and matrix matched standards; and 5 fortified samples each at two spiking levels.
b) Add 14 mL of DI water to each tube, and hydrate the samples for 1 hr using a horizontal shaker.
c) Add 15 mL of MeCN with 1% HAc, cap and shake for 1 min at 1000 stroke/min using a SPEX 2010 Geno/Grinder.
d) Add the salts from the pre-packed Mylar pouch (6 g MgSO4 and 1.5 g NaOAc) (UCT part#: ECMSSA50CT-MP), vortex for 10 sec to break up salt agglomerates.
e) Shake for 1 min at 1000 stroke/min using the SPEX Geno/Grinder.
f) Centrifuge at 5000 rpm for 5 min.

2. dSPE cleanup

a) Transfer 10 mL of the supernatant to 15-mL dSPE tube containing 900 mg MgSO4, 300 mg PSA and 150 mg ChloroFiltr® (UCT part#: ECMPSGG15CT).
b) Shake for 2 min at 1000 stroke/min using the SPEX Geno/Grinder.
c) Centrifuge at 5,000 rpm for 5 min.
d) Transfer 5 mL of the cleaned extract into a small test tube (75mm x 12 mm), concentrate to dryness under a gentle stream of nitrogen at 35 °C.
e) Reconstitute in 667 µL of 1:1 (v:v) DI water with 0.1% formic acid: MeCN, vortex for 30 sec, and filter with a 0.2 µm syringe filter.
f) The samples are ready for LC-MS/MS analysis.

Marijuana Extract (Before dSPE Cleanup)

Marijuana Extract (After dSPE Cleanup)

LC-MS/MS method

HPLC conditions		
HPLC: Thermo Scientific Dionex UltiMate 3000® LC System		
Column: Thermo Scientific, Accucore aQ®, 100 x 2.1 mm, 2.6 µm		
Guard Column: Thermo Scientific, Accucore aQ®, 10 x 2.1 mm, 2.6 µm		
Column Temperature: 40 °C		
Column Flow Rate: 0.200 mL/min		
Auto-samplerTemperature: 10 °C		
Injection Volume: 10 µL		
Gradient Program: Mobile Phase A:0.3 % formic acid and 0.1% ammonia formate in water Mobile Phase B:0.1 % formic acid in MeOH		
Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	99	1
1.5	99	1
3.5	20	80
10	10	90
12	0	100
15	0	100
15.2	99	1
20	99	1
Divert mobile phase to waste from 0 - 0.5 and 15 - 20 min to prevent ion source contamination.		

MS parameters	
Polarity	ESI +
Spray voltage V	4000 V
Vaporizer Temperature	300 °C
Ion transfer capillary	200°C
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	25 arbitrary units
Q1 and Q3 peak width (FWHM)	0.2 and 0.7 Da
Collision gas and pressure	Ar at 1.5 mTorr
Scan type	SRM
Cycle time	1 sec
Acquisition method	EZ Method

SRM transitions							
Name	Rt (min)	Precursor Ion	Product Ion 1	CE 1	Product Ion 2	CE 2	S-lens (V)
Pymetrozine	1.24	218.03	104.94	18	175.98	16	70
Carbendazim	6.32	192.09	160.08	17	132.08	29	81
Dicrotophos	6.41	238.01	126.58	17	108.60	33	73
Acetachlor	6.42	269.42	111.86	15	71.69	33	72
Thiabendazole	6.56	202.06	175.07	24	131.06	31	103
Tebuthiuron	7.30	228.95	171.63	17	115.59	26	72
Simazine	7.32	201.40	67.68	33	103.60	24	85
Carbaryl	7.39	201.96	144.63	7	126.63	30	40
Atrazine	7.67	215.96	173.60	16	67.65	35	79
DEET	7.70	191.95	118.63	15	90.66	28	92
Pyrimethanil	8.08	200.12	107.06	23	183.14	22	66

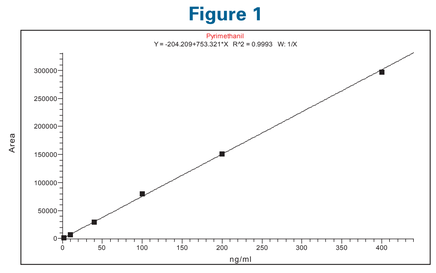
SRM transitions							
Name	Rt (min)	Precursor Ion	Product Ion 1	CE 1	Product Ion 2	CE 2	S-lens (V)
Malathion	8.12	331.01	126.86	12	98.57	23	60
Bifenazate	8.22	300.93	169.82	15	197.62	5	51
Tebuconazole	8.74	308.01	69.66	29	124.56	35	97
Cyprodinil	8.80	226.12	93.05	33	77.03	40	88
Diazinon	8.90	305.14	169.08	14	153.09	15	89
Zoxamide	8.92	335.81	158.51	38	186.50	20	102
Pyrazophos	9.00	374.10	222.13	20	194.06	20	104
Profenofos	9.65	372.30	302.37	19	143.48	35	104
Chlorpyrifos	10.30	349.99	197.94	17	96.89	32	69
Abamectin	11.35	890.49	304.40	18	306.68	15	102
Bifenthrin	12.89	440.04	180.42	11	165.21	39	66

Results:

Matrix-matched calibration curves

Matrix-matched calibration curves were generated using the negative marijuana (male stem) extracts that were prepared by the procedure described above. Appropriate volumes of the 0.1 and 2 ppm pesticide standard solutions were spiked into the sample extracts to generate 6-point matrix-matched calibration curves with pesticide concentrations of 2, 10, 40, 100, 200, and 400 ng/g. The responses were found to be linear over the concentration range (R2 > 0.995). The limit of detection (LOD) of this method was found to be 2 ng/g, and limit of quantitation (LOQ) to be 10 ng/g. Figure 1. provides a selected calibration curve.

Figure 1



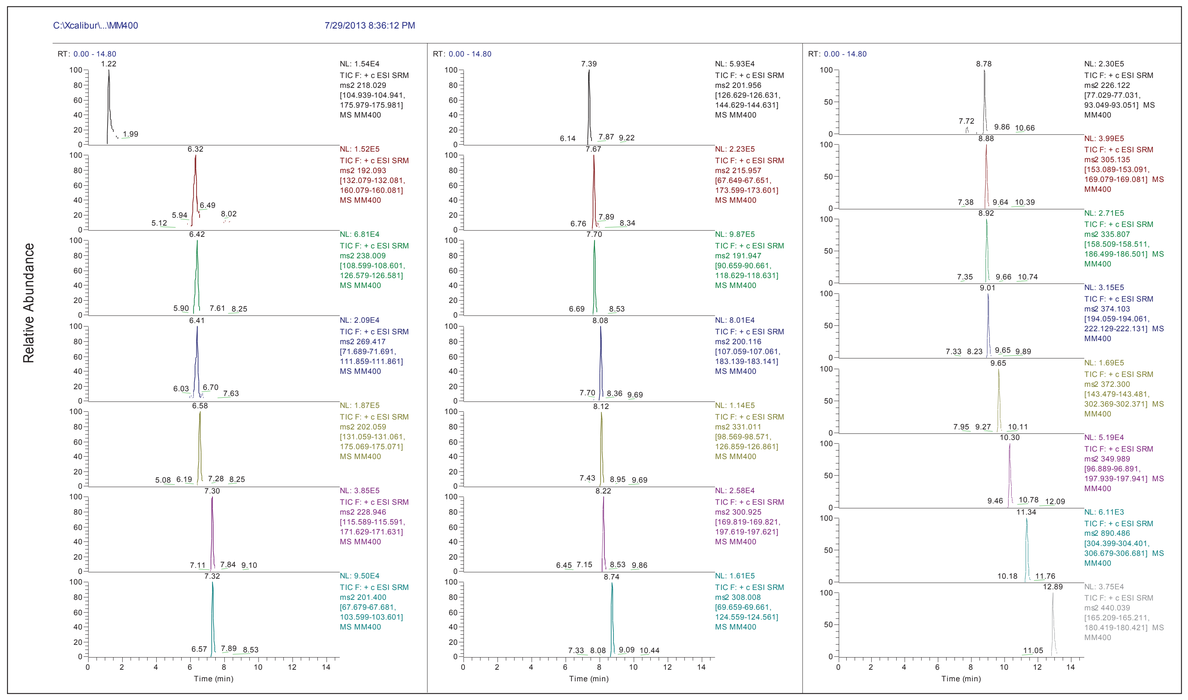
Accuracy and Precision

The negative marijuana sample was used for recovery study at two spiking levels: 10 and 50 ng/g. The recoveries were ranged from 77.8 to 125.6%, all pesticides were within the recovery range of 70-120% set by the EU residue analysis, with the only exception for bifenazate (125.6% at 50 ng/g spike). The relative standard deviations (RSD) of all pesticides were less than 9.5 %, which met the RSD requirement of ≤ 20%.

Analysis of Real Marijuana Samples

This newly developed method was applied to real marijuana samples obtained from a medical examiner's office. Due to the limited sample size available (0.7577 - 1.2102 g), each marijuana sample was extracted only once and the pesticide concentrations were determined against the matrix matched calibration curves generated by the negative marijuana plant samples.

Representative Pesticide Chromatogram



Pesticide Concentration Detected in Seized Marijuana Samples (ng/g)

Analyte	MS1 0.8517g	MS2 0.8657g	MS3 0.7928g	MS5 0.7947g	MS6 0.8647g	MS7 0.8208g	MS9 0.9957g	MS11 0.8500g	MS12 1.2102g	MS13 0.7577g	MS14 0.9630g	MS16 1.0775g	MS20 0.9040g	MS21 0.9042g	MS24 0.9558g
Pymetrozine	< 2.3	< 2.3	< 2.5	< 2.5	< 2.3	< 2.4	< 2.0	< 2.4	< 1.7	< 2.6	< 2.1	< 1.9	< 2.2	< 2.2	< 2.1
Carbendazim	92198	45349	162280	84049	44762	17637	48788	688	45826	58039	42173	13.5	24300	< 2.2	35838
Acetachlor	< 2.3	< 2.3	< 2.5	< 2.5	< 2.3	< 2.4	< 2.0	< 2.4	< 1.7	< 2.6	< 2.1	< 1.9	< 2.2	< 2.2	< 2.1
Dicrotophos	< 2.3	< 2.3	< 2.5	< 2.5	< 2.3	< 2.4	< 2.0	< 2.4	< 1.7	< 2.6	< 2.1	< 1.9	< 2.2	< 2.2	< 2.1
Thiabendazole	< 2.3	14.6	28.8	78.0	24.2	9.7	5.4	< 2.4	49.6	20.3	14.9	< 1.9	< 2.2	< 2.2	20.4
Tebuthiuron	< 2.3	< 2.3	< 2.5	< 2.5	< 2.3	< 2.4	< 2.0	< 2.4	< 1.7	< 2.6	< 2.1	< 1.9	< 2.2	< 2.2	< 2.1
Simazine	< 2.3	< 2.3	< 2.5	< 2.5	< 2.3	< 2.4	< 2.0	< 2.4	< 1.7	< 2.6	< 2.1	< 1.9	< 2.2	< 2.2	< 2.1
Carbaryl	< 2.3	< 2.3	< 2.5	< 2.5	< 2.3	< 2.4	< 2.0	< 2.4	< 1.7	< 2.6	< 2.1	< 1.9	< 2.2	< 2.2	< 2.1
Atrazine	< 2.3	< 2.3	< 2.5	< 2.5	< 2.3	< 2.4	< 2.0	< 2.4	< 1.7	< 2.6	< 2.1	< 1.9	< 2.2	< 2.2	< 2.1
DEET	144	191	156	235	107	223	136	132	109	151	118	11.4	117	651	140
Pyrimethanil	< 2.3	< 2.3	< 2.5	< 2.5	< 2.3	< 2.4	< 2.0	< 2.4	< 1.7	< 2.6	< 2.1	< 1.9	< 2.2	< 2.2	< 2.1
Malathion	< 2.3	< 2.3	< 2.5	< 2.5	< 2.3	< 2.4	< 2.0	< 2.4	< 1.7	< 2.6	< 2.1	< 1.9	< 2.2	562	< 2.1
Bifenazate	< 2.3	< 2.3	< 2.5	< 2.5	< 2.3	< 2.4	< 2.0	< 2.4	< 1.7	< 2.6	< 2.1	< 1.9	< 2.2	< 2.2	< 2.1
Tebuconazole	< 2.3	< 2.3	< 2.5	< 2.5	< 2.3	< 2.4	< 2.0	< 2.4	< 1.7	< 2.6	< 2.1	< 1.9	< 2.2	< 2.2	< 2.1
Cyprodinil	< 2.3	< 2.3	< 2.5	< 2.5	< 2.3	< 2.4	< 2.0	< 2.4	< 1.7	< 2.6	< 2.1	< 1.9	< 2.2	< 2.2	< 2.1
Diazinon	< 2.3	< 2.3	< 2.5	< 2.5	< 2.3	< 2.4	< 2.0	< 2.4	< 1.7	< 2.6	< 2.1	< 1.9	< 2.2	< 2.2	< 2.1
Zoxamide	< 2.3	< 2.3	< 2.5	< 2.5	< 2.3	< 2.4	< 2.0	< 2.4	< 1.7	< 2.6	< 2.1	< 1.9	< 2.2	< 2.2	< 2.1
Pyrazophos	< 2.3	< 2.3	< 2.5	< 2.5	< 2.3	< 2.4	< 2.0	< 2.4	< 1.7	< 2.6	< 2.1	< 1.9	< 2.2	< 2.2	< 2.1
Profenofos	< 2.3	< 2.3	< 2.5	< 2.5	< 2.3	< 2.4	< 2.0	< 2.4	< 1.7	< 2.6	< 2.1	< 1.9	< 2.2	< 2.2	< 2.1
Chlorpyrifos	10.8	154	48.0	184	64.6	45.5	15.2	37.8	151	135	45.6	< 1.9	12.7	< 2.2	135
Abamectin	< 2.3	< 2.3	< 2.5	< 2.5	< 2.3	< 2.4	< 2.0	< 2.4	< 1.7	< 2.6	< 2.1	< 1.9	< 2.2	< 2.2	< 2.1
Bifenthrin	< 2.3	< 2.3	< 2.5	< 2.5	< 2.3	< 2.4	< 2.0	< 2.4	< 1.7	< 2.6	< 2.1	< 1.9	< 2.2	< 2.2	< 2.1

THC ANALYSIS

THC Extraction Materials	
THC (1 mg/mL) THC - D3 (0.1 mg/ mL)	Lipomed (Cambridge MA)
CLEAN SCREEN® THC Part # CSTHC206	UCT, Inc (Bristol, PA)
Select pH Buffer Pouch pH 7 Phosphate Buffer Part # SPHPHO7001-5	UCT, Inc (Bristol, PA)
Methanol Hexanes Ethyl acetate	J.T.Baker (Rahway, NJ)
Formic acid	Sigma-Aldrich (St Louis, MO)

Marijuana Sample Preparation

1. Add 100 mg of Marijuana sample into a clean glass sample tube
2. Add 5 mL of methanol and cap
3. Sonicate for approximately 60 minutes at room temperature
4. Centrifuge for 10 minutes at 3000 rpm
5. Aliquot 500 µL - 1 mL of methanol extract into a clean glass sample tube
6. Add internal standard (THC-d3) and mix.
7. Add 4 mL of 0.1 M phosphate buffer (pH 7) and mix.

Solid Phase Extraction

1. Condition CLEAN SCREEN® CSTHC206 extraction column

a) Add 1 x 3 mL CH_3OH and draw through the column until the liquid is at the top of the sorbent bed
b) Add 1 x 3 mL D.I. H_2O and draw through the column until the liquid is at the top of the sorbent bed
c) Add 1 x 1 mL 0.1 M phosphate buffer (pH 7.0) and draw through the column until the liquid is at the toof the sorbent bed

NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

2. Apply Sample (Part # CSTHC206)

a) Load sample onto the column at a flow rate of 1 to 2 mL/minute.

3. Wash the column

a) Add 1 x 3 mL D.I. H_2O to the column and ; aspirate to waste.
b) Add 1 x 3 mL 0.1 M phosphate buffer (pH 7.0) to the column: aspirate waste.
c) Dry column under full flow using a Positive Pressure Manifold for 5 minutes.

4. Elute THC

a) Add 1 x 3 mL hexane/ethyl acetate/ acetic acid (49:49: 2)
b) Collect eluate at 1 to 2 mL / minute.

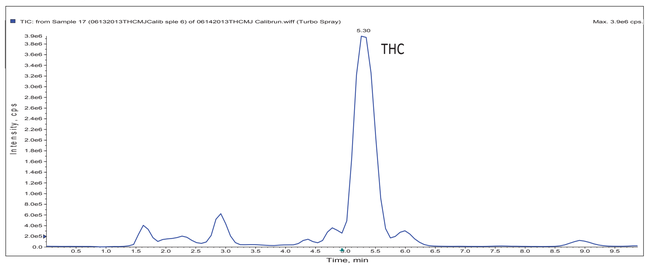
5. Dry Eluate

a) Evaporate to dryness at < 40°C under nitrogen.
b) Dissolve residue in 200 µL of mobile phase.

HPLC conditions
HPLC: Agilent Technologies 1200 HPLC System
Column: UCT, Inc. - SELECTRA® DA HPLC column, 100mm x 2.1mm (5µm) (SLDA100ID21-5UM)
Column Temperature: 40 °C
Column Flow Rate: 0.5 mL/min
Auto-samplerTemperature: 25 °C
Injection Volume: 10 µL
Mobile Phase: D.I. Water w/ 0.1% formic acid: Methanol w/ 0.1% formic acid) (25:75)
AB Sciex 4000 Qtrap in positive MRM mode

SRM transitions							
Compound	Precursor Ion	Product Ion 1	CXP /volts	Product Ion 2	CXP /volts	DP/ volts	EP/ volts	CE/ volts
THC	315.2	193.2	29	123.1	45	4	18.8	4
*THC-D3	318.2	196.2	29	123.2	43	4	18.8	4

Chromatogram of Extracted Marijuana



CONCLUSIONS

An efficient and easy to use method was developed for the determination of pesticide residues in marijuana samples. Pesticide residues in marijuana samples were extracted using an AOAC QuEChERS approach, followed by dSPE cleanup using MgSO4, PSA, and ChloroFiltr®. MgSO4 absorbed any residual water remaining in the MeCN extract, PSA removed organic acids, lipids, and sugars, while ChloroFiltr® retained chlorophyll, resulting in clean extract for LC-MS/MS analysis. Good linearity, low LOD/LOQ's, and satisfactory accuracy and precision data for analyzed pesticides were obtained. Results indicate that this method is suitable for pesticide analysis in marijuana samples. The active ingredient, THC, in the marijuana samples was extracted and analyzed separately. Results ranged from 3% to 9% by mass.

The method has been successfully applied for the analysis of 15 seized marijuana samples. Pesticide residues were detected in all of the marijuana samples tested. Extremely high levels of carbendazim, a fungicide, were reported in this study, demonstrating that pesticides/fungicides were used for higher marijuana production or preservation of harvested marijuana. Thus, it is recommended that marijuana designated for medical use should be inspected for pesticide residues before taken by patients.

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