Analysis of Pesticide Residues and Mycotoxins in Marijuana using QuEChERS Extraction and ChloroFiltr® dSPE Cleanup

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INTRODUCTION

Twenty-four states and Washington, D.C. have passed laws allowing marijuana to be used for medicinal purposes, and in some cases recreationally. With the recent trends in legalization, interest in marijuana and marijuana-based products (e.g. concentrated oils, soda, candy and other edible forms) have dramatically increased. Like any other crop, pesticides are commonly used in marijuana cultivation to protect plants from pests and improve growth yields. However, pesticide residues can pose significant health risks, especially with chronic exposure. The warm, wet conditions ideal for growing cannabis are also conducive to the growth of molds and fungi which are capable of producing carcinogenic mycotoxins, including aflatoxins and ochratoxin A. As a result, testing for the presence of pesticides and mycotoxins in marijuana is essential to ensure consumer safety. Only a few states have introduced legislation for the analysis of pesticides and mycotoxins, while other states are in the process of implementing legislation. This poster outlines a QuEChERS method for the simultaneous analysis of 48 pesticides and 4 mycotoxins in marijuana, including those listed in the Massachusetts and Nevada regulations. Sample purification is carried out using UCT's new cleanup product SpinFiltr™, which combines the convenience of classical dispersive-SPE (dSPE) with an ultrafiltration tube containing a 0.2 µm filter to simultaneously remove unwanted matrix components and filter the sample prior to LC or GC analysis. The SpinFiltr™ dSPE tube contains PSA, C18 and ChloroFiltr®, a unique polymeric sorbent for the removal of chlorophyll that unlike graphitized carbon black (GCB) does not result in the loss of planar analytes. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is used for the analysis of the pesticides and mycotoxins.

EXPERIMENTAL

Products Used

Product	Description	Part Number
QuEChERS extraction salts	4 g Magnesium Sulfate + 1 g Sodium Chloride in a Mylar pouch	ECMSSC-MP
SpinFiltr™ cleanup tubes	150 mg $MgSO_4$, 50 mg PSA, 50 mg C18 and 50 mg ChloroFiltr® in an ultrafiltration tube containing a 0.2 µm PTFE filter	ECQUSF54CT
HPLC column	Selectra® Aqueous C18, 100 × 2.1 mm, 3 µm	SLAQ100ID21-3UM
Guard cartridge	Selectra® Aqueous C18, 10 × 2.1 mm, 3 µm	SLAQGDC20-3UM
Guard holder	Guard cartridge holder	SLGRDHLDR

Sample Pretreatment:
100 g of marijuana was thoroughly blended in a Robot-Coupe® using dry ice to generate a homogenous sample for the study.

Figure 1. Marijuana sample before (left) and after (right) homogenization with dry ice.

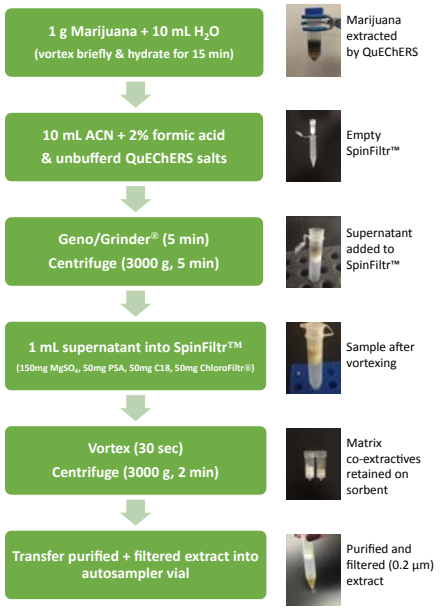

Figure 2. Sample preparation procedure.

Instrumental Conditions

Mass spectrometer	Thermo Scientific™ TSQ Vantage™ (QqQ)
Ionization mode	ESI^+ & ESI^-
HPLC system	Thermo Scientific™ Dionex™ Ultimate™ 3000
HPLC column	Selectra® Aqueous C18, 100 × 2.1 mm, 3 µm
Guard cartridge	Selectra® Aqueous C18, 10 × 2.1 mm, 3 µm
Column temp.	40°C
Mobile phase A	Water + 5mM NH_4HCO_2 + 0.1% formic acid
Mobile phase B	Methanol + 5mM NH_4HCO_2 + 0.1% formic acid
Flow rate	300 µL/min
Gradient	0 min (0% B), 2-5 min (50 %B), 5.5-9 min (60% B), 12-15 min (100% B), 15.1-20 min (0% B)
Injection volume	5 µL

RESULTS AND DISCUSSION

Recovery and Precision Data for Pesticides and Mycotoxins in Marijuana

(n=4)	Recovery	RSD	Recovery	RSD	Recovery	RSD	Recovery	RSD
Mycotoxins								
Conc. in sample	20 ng/g		50 ng/g		100 ng/g		200 ng/g	
Conc. in extract	2 ng/mL		5 ng/mL		10 ng/mL		20 ng/mL	
Aflatoxin B1	67.6	1.92	73.8	1.39	72.4	1.11	79.3	1.23
Aflatoxin B2	67.4	2.26	77.0	2.26	75.3	2.70	81.0	1.55
Aflatoxin G1	69.5	5.37	76.6	1.78	75.1	2.06	80.0	1.71
Aflatoxin G2	75.3	3.72	77.5	1.31	73.3	1.91	79.4	2.42
Ochrotoxin A	22.6	29.38	47.0	5.82	48.6	2.08	52.7	3.19
Pesticides								
Conc. in sample	50 ng/g		100 ng/g		200 ng/g		500 ng/g	
Conc. in extract	5 ng/mL		10 ng/mL		20 ng/mL		50 ng/mL	
Abamectin	ND	ND	ND	ND	ND	ND	88.2	6.50
Acephate	44.9	4.09	65.4	3.72	67.3	3.99	75.7	2.60
Acetochlor	89.7	5.08	86.4	1.71	86.0	1.33	82.7	2.02
Aldicarb sulfoxide	< LOD	< LOD	52.9	5.85	67.2	4.89	72.6	3.19
Atrazine	91.4	1.33	91.1	3.09	88.8	3.13	86.3	2.13
Bifenazate	84.0	3.76	80.4	1.41	78.9	2.57	77.8	2.78
Carbaryl	78.7	2.56	76.0	6.54	89.2	2.04	80.6	0.55
Chlorpyrifos	< LOD	< LOD	79.7*	9.39*	79.7	3.71	85.0	2.60
DEET	92.6	2.38	88.2	3.92	92.0	4.02	84.2	2.13
Dichlorvos	83.4	8.99	81.2	4.44	83.3	3.94	81.7	2.45
Dichrotophos	81.4	2.83	81.0	3.18	85.3	3.35	81.1	2.05
Dimethomorph	85.4	2.98	81.6	3.87	85.0	2.73	81.7	2.03
Etoxazole	74.3	3.05	72.6	1.40	72.7	3.25	72.1	1.42
Fenamiphos sulfone	86.2	5.54	84.2	5.35	89.1	2.74	84.1	1.28
Fenamiphos sulfoxide	81.5	2.65	79.4	3.57	83.0	2.68	78.3	0.96
Fenhexamid	84.3	1.22	82.4	5.55	83.6	2.13	79.4	1.61
Fenoxycarb	85.6	1.72	81.9	3.89	79.5	4.55	80.7	2.08
Flonicamid	82.6	2.74	87.5	3.00	83.8	4.95	80.2	1.79
Fludioxinil	77.8	6.43	76.1	2.87	78.4	3.32	74.6	1.61
Flutriafol	84.7	1.56	77.7	3.08	82.0	2.76	78.1	1.55
Imazilil	92.6	1.19	86.2	4.20	85.2	1.98	78.7	1.26
Imidacloprid	72.7	5.24	76.8	3.22	81.6	1.87	77.9	6.85
Malathion	90.2	4.82	85.0	4.94	98.8	10.72	90.2	6.05
Cyprodinil	75.7	6.88	70.8	3.63	67.8	7.86	69.6	2.77
Metamidophos	71.2	7.19	64.6	1.42	63.4	2.91	62.8	2.94
Myclobutanil	90.5	2.06	83.9	2.78	85.4	3.32	81.6	0.42
Oxydemeton methyl	78.7	5.72	78.5	2.37	82.0	1.90	77.4	2.42
Paclobuterol	80.2	3.71	81.0	4.10	96.5	2.98	100.6	1.75
Piperonyl butoxide	64.2	6.46	69.7	1.92	73.6	5.05	76.0	1.76
Pymetrozine	34.2	4.83	28.7	12.97	24.7	4.55	24.2	9.18
Pyrazophos	79.1	2.60	76.6	7.81	78.6	1.12	83.2	1.27
Pyrethrin I	< LOD	< LOD	< LOD	< LOD	64.7	5.69	81.5	4.27
Pyrethrin II	73.6	6.82	73.2	3.12	79.9	0.37	76.5	1.32
Simazine	61.2	8.96	81.1	1.39	92.3	3.19	83.6	1.30
Spinetoram	84.3	3.19	78.9	5.19	83.8	3.07	79.1	3.68
Spinosyn A	82.0	2.73	78.0	6.75	79.9	3.32	75.8	0.60
Spinosyn D	79.5	2.59	77.2	6.74	81.5	3.23	75.3	0.60
Spiromesifen	37.5	11.95	59.2	3.31	67.3	1.07	67.9	3.08
Spirotetramat	77.2	4.69	73.8	6.37	78.3	2.82	79.1	1.56
Tebuconazole	80.2	3.68	79.3	3.43	78.1	5.70	78.1	1.02
Tebuthiuron	81.7	3.54	76.9	2.86	80.0	3.45	77.1	1.76
Thiabendazole	97.2	3.40	95.8	4.79	100.4	2.44	99.6	1.82
Thiamethoxam	86.1	3.97	80.5	3.78	81.9	4.21	79.8	3.25
Triadimefon	88.4	3.51	86.3	0.58	87.6	2.96	90.5	1.15
Triethylphosphorothioate	< LOD	< LOD	100.1	9.02	89.2	4.40	82.9	2.26
Trifloxystrobin	93.1	1.52	87.4	2.82	83.2	7.31	85.8	0.83
Zoxamide	82.6	4.19	77.6	4.56	77.9	1.51	80.6	1.63
Overall average	**77.1**	**4.62**	**77.3**	**4.05**	**79.3**	**3.35**	**78.7**	**2.31**

*(n=3)

- Unbuffered extraction salts and ACN + 2% formic acid were used to prevent the acidic Ochratoxin A from getting retained on the PSA sorbent. However, the low sample pH also contributed to the reduced recovery of pymetrozine (a basic analyte). Citrate and acetate salts resulted in the loss of Ochratoxin A.

- By utilizing UCT's new SpinFiltr™ product, valuable time was saved during the dSPE cleanup step as the sample is purified and filtered simultaneously. A larger sample volume can be recovered and the tedious pipetting step and the associated risk of sorbent carryover is eliminated. Incorporating a 0.2 µm filter improves robustness and less instrument downtime.

- The use of ChloroFiltr®, a novel polymeric based sorbent designed for the selective removal of chlorophyll, was effective in removing pigments without sacrificing recovery of planar analytes. Overall, better recoveries were obtained with ChloroFiltr® than GCB, although sample cleanliness was similar for both products (Figure 3).

- Quantitation was performed against a 6-point matrix-matched calibration curve prepared in unspiked marijuana extract. With the exception of thiabendazole, no internal standards were used for quantitation. However, for most compounds the absolute recovery was still in the range of 70-100% and the reproducibility was <10%. The inclusion of suitable isotopically labelled internal standards would further improve the performance of the method.

Comparison of ChloroFiltr® vs GCB

Concentration 200 or 500 ng/g	ChloroFilter®		GCB	
(n=4)	Recovery	RSD	Recovery	RSD
Aflatoxin B1	77.6	1.58	70.3	0.91
Aflatoxin B2	78.6	1.04	63.0	0.66
Aflatoxin G1	76.9	1.72	70.0	3.13
Aflatoxin G2	77.6	1.65	70.5	2.05
Ochrotoxin A	53.9	3.30	62.2	3.46
Abamectin	93.0	6.87	ND	ND
Acephate	75.4	3.93	74.8	3.53
Acetochlor	80.7	0.63	74.7	1.14
Aldicarb sulfoxide	70.0	6.09	70.7	2.49
Atrazine	76.6	0.67	62.0	2.55
Bifenazate	74.7	1.66	77.2	0.67
Carbaryl	79.8	0.96	86.3	2.99
Chlorpyrifos	77.1	7.63	**41.0**	**16.75**
DEET	77.3	1.49	69.1	1.05
Dichlorvos	78.3	1.68	73.7	1.38
Dichrotophos	79.4	0.72	75.0	0.96
Dimethomorph	78.5	3.06	70.0	1.31
Etoxazole	70.9	2.10	64.5	1.60
Fenamiphos sulfone	82.0	1.20	76.8	0.51
Fenamiphos sulfoxide	76.7	1.44	72.6	1.23
Fenhexamid	76.2	2.04	73.3	0.75
Fenoxycarb	80.0	1.19	77.9	2.08
Flonicamid	77.4	4.44	69.4	4.78
Fludioxinil	72.3	1.84	71.0	1.30
Flutriafol	76.1	0.83	72.5	1.66
Imazilil	76.1	0.30	70.2	0.70
Imidacloprid	78.0	7.86	70.3	7.13
Malathion	85.8	6.95	78.9	8.48
Cyprodinil	66.6	6.58	**17.0**	3.42
Metamidophos	64.1	9.16	61.2	5.18
Myclobutanil	80.1	2.61	74.7	1.58
Oxydemeton methyl	75.6	1.06	71.2	1.21
Paclobuterol	93.4	3.90	88.0	7.71
Piperonyl butoxide	76.6	1.40	68.2	5.44
Pymetrozine	21.5	28.47	**12.9**	**10.36**
Pyrazophos	79.7	2.89	69.2	2.49
Pyrethrin I	77.5	4.84	70.1	9.29
Pyrethrin II	74.0	2.27	69.6	1.10
Simazine	81.0	0.93	**61.7**	3.20
Spinetoram	77.4	2.70	61.6	1.73
Spinosyn A	73.9	0.56	63.6	2.30
Spinosyn D	73.4	0.56	63.8	2.99
Spiromesifen	66.0	2.08	65.8	2.20
Spirotetramat	75.5	0.59	71.1	0.81
Tebuconazole	76.7	2.32	72.8	1.87
Tebuthiuron	76.0	0.98	77.7	1.38
Thiabendazole (no IS)	60.0	2.67	**19.8**	2.92
Thiamethoxam	78.2	1.20	76.8	4.07
Triadimefon	83.2	3.43	76.8	1.84
Triethylphosphorothioate	82.4	2.77	79.2	6.79
Trifloxystrobin	82.5	2.77	69.6	2.60
Zoxamide	78.4	3.81	77.2	2.40
Overall average	**75.6**	**3.18**	**67.6**	**3.14**

Note: dSPE cleanup includes 150mg MgSO , 50mg PSA, 50mg C18, and either 50mg ChloroFiltr® or 7.5mg GCB.

Figure 3. Comparison of dSPE cleanup between ChloroFiltr® and GCB.

CONCLUSIONS

The method outlined above allows for the simultaneous analysis of 48 pesticides and 4 mycotoxins in one simple QuEChERS extraction procedure, thereby saving time, sample and cost. Sample cleanup is carried out by dSPE using UCT's new Spinfilter™ product which purifies and filters the sample in one easy step. Chlorofiltr® dSPE sorbent was used to selectively remove chlorophyll without losing any planar compounds. Analysis of the samples was performed by LC-MS/MS utilizing a Selectra® Aqueous C18 HPLC column which allowed for improved retention of the more polar pesticides included in the method. The developed method was evaluated by fortifying marijuana samples with each compound at four concentrations. With the widespread legalization of marijuana, this simple method will be beneficial for implementing regulatory testing.