Analysis of Pesticide Residues and Mycotoxins in Cannabis using QuEChERS Extraction, ChloroFiltr® dSPE Cleanup and LC-MS/MS Detection

UCT Part Numbers

ECMSSC-MP
Mylar pouch containing 4g $MgSO_4$ and 1g NaCl

ECQUSF54CT
SpinFiltr™ dSPE cleanup tube, 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18 and 50 mg ChloroFiltr®

SLAQ100ID21-3UM
Selectra® Aqueous C18 HPLC column, 100 × 2.1 mm, 3 µm

SLAQGDC20-3UM
Selectra® Aqueous C18 guard cartridge, 10 × 2.1 mm, 3 µm

SLGRDHLDR
Guard cartridge holder

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Summary:

Twenty-eight states and Washington D.C. have passed laws allowing cannabis to be used for medicinal purposes, and in some cases recreationally. With the recent trends in legalization, interest in cannabis and cannabis-based products (e.g. concentrated oils, soda, candy and other edible forms) have dramatically increased. Like any other crop, pesticides are commonly used in cannabis 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 that are ideal for growing cannabis are also conducive to the growth of molds and fungi which can produce carcinogenic mycotoxins, including ochratoxin A and aflatoxins. As a result, testing for the presence of pesticides and mycotoxins in cannabis 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 application note outlines a QuEChERS method for the simultaneous analysis of 47 pesticides and 5 mycotoxins in cannabis, including most of the LC-MS/MS amenable compounds on the Massachusetts and Nevada monitoring lists [1-3]. 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 membrane to simultaneously remove unwanted matrix components and filter the sample prior to LC (or GC) analysis. The SpinFiltr™ dSPE tube uses PSA, C18 and ChloroFiltr® sorbents for sample cleanup. ChloroFiltr® is a unique polymeric sorbent designed for the removal of chlorophyll, and unlike graphitized carbon black (GCB) does not result in the loss of planar analytes. Liquid chromatography, using a Selectra® Aqueous C18 column, coupled to tandem mass spectrometry (LC-MS/MS) is used for the analysis of the pesticides and mycotoxins.

Sample Pretreatment:

Prior to extraction cannabis samples should be ground to a fine powder using cryogenic milling (e.g. SPEX 6775 Freezer/Mill®). For this work a large quantity (100 g) of cannabis was thoroughly blended in a Robot-Coupe® using dry ice to generate a homogenous sample for use during method development and recovery experiments.

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

QuEChERS Procedure:

Sample Extraction:

1. Weigh 1 g of cannabis sample into a 50 mL polypropylene centrifuge tube.
2. Add internal standard(s).
3. Add 10 mL ultrapure water, vortex briefly, and allow sample to hydrate for 15 min (improves extraction efficiency).
4. Add 10 mL acetonitrile containing 2% formic acid.
5. Add the contents of the **ECMSSC-MP** Mylar pouch and shake for a minimum of 5 minutes (by hand or mechanically). For this work a Spex 2010 Geno/Grinder® was used (1500 RPM).
6. Centrifuge the sample at ≥ 3000 rcf for 5 minutes.

SpinFiltr™ dSPE Clean-up:

1. Transfer 1 ml of supernatant to a SpinFiltr™ dSPE cleanup tube (**ECQUSF54CT**).
2. Vortex the sample for 30 seconds.
3. Centrifuge the sample at ≥ 3000 rcf for 5 minutes.
4. Transfer the purified and filtered sample extract into an autosampler vial for analysis.

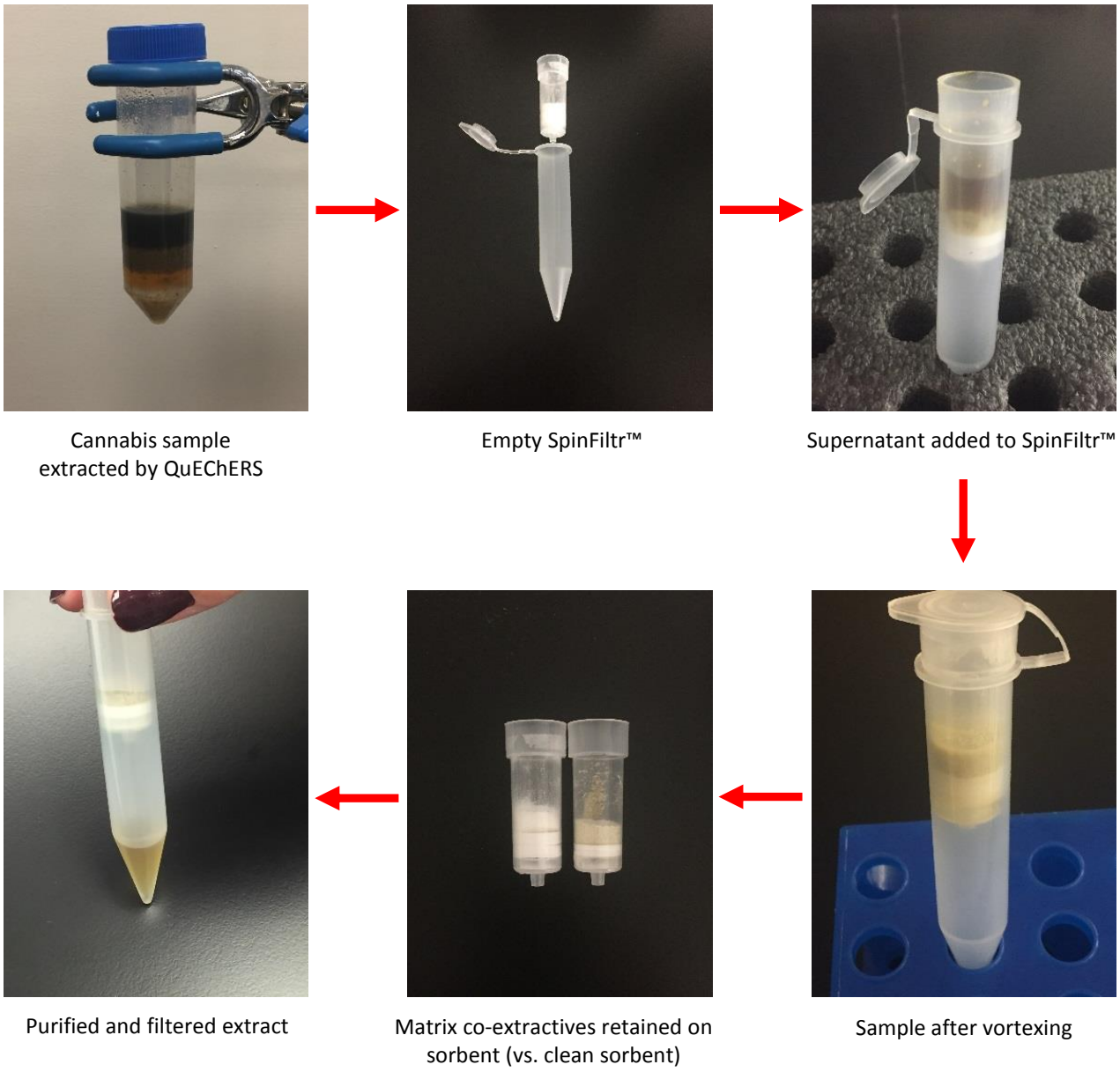

Figure 2. Schematic of the SpinFiltr™ dSPE cleanup procedure.

LC-MS/MS Parameters:

Table 1. Instrumentation	
HPLC system	Thermo Scientific™ Dionex™ Ultimate™ 3000
MS system	Thermo Scientific™ TSQ Vantage™ (MS/MS)
HPLC column	UCT Selectra® Aqueous C18, 100 × 2.1 mm, 3 µm (p/n: SLAQ100ID21-3UM)
Guard column	UCT Selectra® Aqueous C18, 10 × 2.1 mm, 3 µm (p/n: SLAQGDC20-3UM)
Guard column holder	p/n: SLGRDHLDR
Column temperature	40°C
Flow rate	300 µL/min
Injection volume	5 µL

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Table 2. Mobile Phase Gradient		
Time (min)	**% Mobile Phase A (Water + 5mM NH_4HCO_2 + 0.1% formic acid)**	**% Mobile Phase B (Methanol + 5mM NH_4HCO_2 + 0.1% formic acid)**
0.0	100	0
2.0	50	50
5.0	50	50
5.5	40	60
9.0	40	60
12.0	0	100
15.0	0	100
15.1	100	0
20.0	100	0

Table 3. MS Parameters and Retention Times						
Analyte	**RT**	**Parent ion**	**Product 1**	**CE 1**	**Product 2**	**CE 2**
Abamectin ($M+NH_4$)	14.00	890.5	305.0	24	567.2	9
Acephate	3.75	184.0	95.1	23	143.0	6
Acequinocyl	13.75	384.4	119.1	31	177.1	13
Acetochlor	11.00	270.1	148.1	18	224.1	10
Aflatoxin B1	7.65	313.0	241.0	36	285.1	21
Aflatoxin B2	7.25	315.1	259.1	28	287.1	24
Aflatoxin G1	6.65	329.0	199.0	48	243.0	25
Aflatoxin G2	6.35	331.1	189.0	39	245.1	28
Aldicarb sulfoxide	4.35	207.1	69.2	17	89.1	13
Atrazine	8.60	216.1	68.1	34	174.1	16
Bifenazate	10.90	301.1	170.1	18	198.1	6
Carbaryl	7.50	202.1	127.1	29	145.1	11
Chlorpyrifos	13.85	349.9	97.0	32	197.9	19
Cyprodinil	12.45	226.1	77.1	43	93.1	33
DEET	8.60	192.1	91.1	29	119.1	17
Dichlorvos	6.80	220.9	109.1	18	127.1	13
Dichrotophos	5.05	238.1	112.1	12	127.0	18
Dimethomorph	10.80	388.2	165.1	30	301.1	19
Etoxazole	14.00	360.3	113.1	54	141.1	30
Fenamiphos sulfone	7.30	336.1	188.0	26	266.0	19
Fenamiphos sulfoxide	7.45	320.1	108.1	40	233.0	24
Fenhexamid	11.20	302.2	216.2	27	270.2	19
Fenoxycarb	12.30	302.1	88.1	17	116.1	7
Flonicamid (ESI-)	4.50	228.1	81.1	13	146.0	22
Fludioxinil (ESI-)	10.30	247.1	126.1	32	180.1	29
Flutriafol	8.10	302.1	70.1	17	123.0	28
Imazilil	8.10	297.1	159.0	24	201.0	17

Imidacloprid	5.30	256.1	175.1	17	209.1	17
Malathion	9.80	331.0	99.0	25	127.0	12
Metamidophos	3.10	142.0	94.1	14	125.0	13
Myclobutanil	10.80	289.2	70.1	18	125.1	31
Ochratoxin A	10.80	404.0	102.1	63	239.0	22
Oxydemeton methyl	4.70	247.0	109.0	27	169.0	13
Paclobuterol	9.80	294.1	70.1	19	125.0	33
Piperonyl butoxide	13.75	356.3	119.1	31	177.1	12
Pymetrozine	4.50	218.1	105.1	20	176.1	17
Pyrazophos	13.20	374.1	194.1	31	222.1	20
Pyrethrin I ($M+NH_4$)	10.95	346.2	170.1	22	198.1	12
Pyrethrin II ($M+NH_4$)	10.80	390.2	165.1	29	303.1	19
Simazine	7.65	202.1	124.1	16	132.0	19
Spinetroram	13.85	748.6	98.0	37	142.1	29
Spinosyn A	13.55	732.6	97.9	40	142.1	29
Spinosyn D	13.85	746.6	97.9	36	142.1	28
Spiromesifen*	13.95	273.1	187.1	16	255.2	13
Spirotetramat	11.20	374.2	216.1	31	302.2	16
Tebuconazole	12.60	308.1	70.1	21	125.0	33
Tebuthiuron	7.60	229.1	116.0	26	172.1	16
Thiabendazole	5.70	202.0	131.1	31	175.1	25
Thiabendazole-$^{13}C_6$ (IS)	5.70	208.0	137.1	32	181.1	25
Thiamethoxam	4.80	292.1	181.1	21	211.1	11
Triadimefon	10.20	294.1	69.1	20	197.1	14
Triethylphosphorothioate	13.70	199.0	125.0	16	143.0	14
Trifloxystrobin	13.30	409.2	145.0	41	186.1	17
Zoxamide	12.45	336.0	159.0	38	187.0	21

*See Discussion section for an explanation on the choice of ion used for spiromesifen.

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Results:

Table 4. Recovery and Precision Data for Pesticides and Mycotoxins in Cannabis								
(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)

Table 5. Comparison of ChloroFilter® vs GCB

Conc = 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: The sorbent combination was 150mg $MgSO_4$, 50mg PSA, 50mg C18, and either 50mg ChloroFiltr® or 7.5mg GCB. Significant variation in results are highlighted in red.

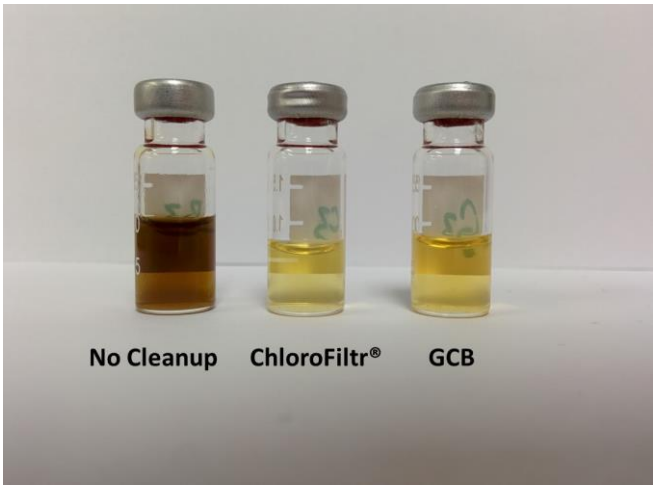

Figure 3. Comparison of dSPE cleanup between ChloroFiltr® and GCB.

Discussion:

Sample preparation was carried out using a QuEChERS-based approach. Acetonitrile containing 2% formic acid and unbuffered QuEChERS salts were used in the extraction step to prevent the acidic ochratoxin A from being retained on the PSA sorbent during dSPE cleanup, although this also contributed to reduced recovery of pymetrozine (a basic analyte). The use of higher pH extraction conditions, including the use of citrate and acetate buffered QuEChERS salts, resulted in lower recovery of ochratoxin A. 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 (Table 5) while sample cleanliness was similar for both sorbents (Figure 3). Furthermore, by utilizing UCT's new SpinFiltr™ product, valuable time was saved during the dSPE cleanup step as the sample extract is purified and filtered simultaneously using the built-in 0.2 µm PTFE filter membrane. Cumulatively, this saves valuable time and cost while providing improved robustness and less instrument downtime. In addition, a larger sample volume can be recovered compared to classical dSPE and the tedious pipetting step and the associated risk of sorbent carryover is eliminated.

Chlormequat and daminozide are included in the Massachusetts monitoring list [1]; however, they are too polar to be included in the QuEChERS extraction procedure (remain in the aqueous phase and do not partition into the organic solvent) and an alternative approach is required for these compounds (e.g. take an aliquot of the sample extract prior to the addition of the QuEChERS salts for direct analysis or for further cleanup). Furthermore, due to their high polarity these compounds can be difficult to retain on a typical reversed-phase HPLC sorbent, which may necessitate alternative HPLC conditions.

Abamectin is prone to sodium (and potassium) adduct formation. The use of an ammonium buffer (acetate or formate) in the organic mobile phase can help to reduce sodium adducting by forming the $[M+NH_4]^+$ adduct. However, the MS signal for abamectin was still quite low and recovery data could only be generated for the highest spiked samples (500 ng/g). Spiromesifen is also an insensitive compound that exhibited a very weak signal for the protonated molecular ion (m/z 371.4). In spite of this, an intense peak at m/z 273.1 was observed (possibly due to in-source fragmentation) and this was successfully used for quantitative purposes. Acequinocyl is an insecticide that is included in both the Massachusetts and Nevada monitoring lists [1,3]. It was originally included in the method but gave unacceptable results and was later omitted. While this compound could be readily detected by LC-MS/MS (as $[M+H]^+$ parent ion) when analyzing neat solvent standards, when conducting spiking experiments very poor results were obtained, including the fortified matrix-matched calibration curve. One explanation for this could be the poor stability of acequinocyl. According to Ying *et al.* [4], acequinocyl undergoes rapid phototransformation in an aquatic environment and rapid hydrolysis under neutral and alkaline conditions, with the major transformation product being hydroxyacequinocyl. In their work, they observed the highest intensity peak at m/z 343.3 owing to the loss of ethenone (CH2=C=O). They also determined that $APCI^+$ provided vastly superior signal response over ESI^+. For the problematic compounds included in this method, different LC-MS/MS parameters, including interface parameters, should be further evaluated in order to obtain better signal intensity and lower detection limits.

Quantitation was performed against a 6-point matrix-matched calibration curve prepared in blank cannabis 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 less than 10%. Notable exceptions include ochratoxin A and pymetrozine. Ochratoxin A is an acidic compound that can get retained on the PSA sorbent during the dSPE cleanup step. Acidic extraction conditions were used to reduce this retention on PSA but unfortunately some of the ochratoxin A was still retained. Further acidification of the extraction solvent (e.g. adding 5% formic acid) might

improve the recovery of ochratoxin A, but it may also negatively affect some of the other compounds included in the method. Low recovery and high variability were obtained for ochratoxin A at the lowest fortification level due to the lower signal intensity obtained at this concentration. Better results were obtained when evaluating the more highly concentrated samples. Pymetrozine is a basic compound and the low recovery observed for this compound is likely caused by the acidic pH conditions used for the QuEChERS extraction procedure, which resulted in insufficient partitioning of pymetrozine into the organic solvent. Acephate, aldicarb sulfoxide, chlorpyrifos, pyrethrin I, spiromesifen and triethylphosphorothioate also exhibited some low recovery and/or elevated RSD values, but this occurred mostly at the lower concentration levels and was primarily caused by reduced sensitivity. Additional LC-MS/MS optimization and the inclusion of suitable isotopically labelled internal standards would further improve the overall performance of the method.

Conclusion:

The method outlined in this application note allows for the simultaneous analysis of 47 pesticides and 5 mycotoxins in cannabis 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 analytes. 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 method was evaluated by fortifying cannabis samples with each compound at four varying concentrations (n=4 each). The average recovery obtained was generally in the range of 70-100% and the reproducibility ≤10%. With the widespread legalization of cannabis, this simple method will be beneficial for any research facility wanting to implement regulatory testing.

References:

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