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

SpinFiltr® ChloroFiltr®

UCT Part Numbers

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

SLPFPP100ID21-3UM
Selectra® PFPP
100 × 2.1 mm, 3 µm

ECQUSF154CT
SpinFiltr® dSPE cleanup tube
50 mg $MgSO_4$, 150 mg endcapped C18, 150 mg ChloroFiltr® and 150 mg PSA

SLPFPPGDC20-3UM
Selectra® PFPP guard column
10 x 2.0 mm, 3 µm

SLGRDHLDR
Selectra® Guard cartridge holder

Introduction:

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. Pesticide and mycotoxin 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. With the widespread legalization of cannabis, UCT is presenting this simple method which would be beneficial for any research or production facility wanting to implement regulatory testing.

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Extract Cannabis Flower Procedure:

1. Sample Preparation

a) 10 grams of cannabis flower was ground and homogenized using 2 mL DI water.

b) The sample was mixed well in a Spex 2010 Geno/Grinder® for 10 min.

c) The sample was thoroughy mixed and vortexed to achieve homogeneity.

d) 10 different samples were weighed at 1 gram each.

e) 5 samples were spiked at low (5 ng) and 5 samples at high (25 ng) fortification levels.

2. Extraction Procedure

a) Place each prepared sample in a 50 mL centrifuge tube.

b) Add 10 µL of Internal Standard(s).

c) Add 5 mL of DI water to each sample and vortex mix well to ensure the analyte concentration is distributed as equally as possible throughout sample.

d) Add 10 mL of acetonitrile containing 2% formic acid.

e) Add the contents of the ECMSSC-MP Mylar pouch (4 g $MgSO_4$ and 1 g NaCl) and shake for 10 minutes using the Spex 2010 Geno/Grinder®.

f) The sample is centrifuged at ≥ 3000 × g for 5 minutes.

3. Cleanup Procedure

a) Transfer 1 mL aliquot of supernatant into **ECQUSF154CT** dSPE cleanup tube containing 50 mg $MgSO_4$, 150 mg Endcapped C18, 150 mg Chlorofiltr® and 150 mg PSA.

b) Vortex the sample for 30 seconds.

c) Centrifuge the sample at ≥ 3000 × g for 5 minutes.

d) Transfer the purified and filtered sample extract into an autosampler vial for analysis on ABSciex 6500+ Triple Quad LC-MS/MS.

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LC-MS/MS Parameters:

Instrumentation	
HPLC column	UCT Selectra® PFPP, 100 × 2.1 mm, 3 µm (p/n: **SLPFPP100ID21-3UM**)
Guard column	UCT Selectra® PFPP, 10 × 2.0 mm, 3 µm (p/n: **SLPFPPGDC20-3UM**)
Guard column holder	UCT Selectra® Guard Cartridge Holder (p/n: **SLGRDHLDR**)
Column temperature	40°C
Flow rate	0.400 µL/min
Injection volume	2 µL

Gradient Program		
Time (min)	**% Mobile Phase A** 10 mM Ammonium Formate with 0.1% Formic Acid in DI Water	**% Mobile Phase B** Acetonitrile
0.0	98	2
12.0	0	100
13.0	0	100
13.1	98	2
16.5	98	2

MS Conditions	
MS/MS System	ABSCIEX QTrap 6500+
Ionization Mode	Electrospray Ionization in positive mode (ESI^+)
Ion Spray Voltage (IS)	+4500.00
Temperature (TEM)	300°C
Curtain Gas (CUR)	40
Ion Source Gas 1 (GS1)	50
Ion Source Gas 2 (GS2)	50

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MRM Table				
Analyte	**RT**	**Precursor Ion**	**Fragment 1**	**Fragment 2**
Abamectin	10.80	890.2	567.2	305.0
Acephate	2.80	183.9	143.1	95.0
Acetamiprid	6.00	223.0	126.0	90.0
Aldicarb	6.40	208.0	116.1	89.2
Aflatoxin B1	6.90	313.0	285.0	241.0
Aflatoxin B2	6.70	315.0	287.0	259.0
Aflatoxin G1	6.70	329.0	243.0	200.0
Aflatoxin G2	6.40	331.0	245.1	189.0
Azoxystrobin	8.70	404.0	371.9	329.1
Bifenazate	9.00	301.0	198.1	170.1
Boscalid	8.80	342.9	306.9	140.0
Carbaryl	7.36	202.0	145.0	126.9
Carbofuran	7.05	222.0	165.1	123.0
Chlorantraniliprole	8.10	483.8	452.8	285.8
Chlordane	10.10	410.0	186.0	144.9
Chlorpyrifos	10.70	349.8	197.9	96.8
Clofentezine	10.00	302.9	138.0	102.0
Coumaphos	9.80	362.9	227.0	306.8
Cyfluthrin (Baythroid)	5.20	450.9	190.9	127.0
Daminozide	1.20	161.0	143.1	61.1
Diazinon	9.76	305.0	169.1	97.0
Dichlorvos	6.74	220.9	109.0	78.9
Dimethoate	5.67	229.9	199.0	124.9
Dimethomorph E	8.50	388.0	300.9	165.1
Dimethomorph Z	8.70	388.0	300.9	165.1
Ethoprophos	8.74	243.0	131.0	96.9
Etofenprox	11.66	394.1	177.1	107.0
Etoxazole	10.87	360.0	141.0	113.0
Fenhexamid	8.63	302.0	97.1	55.0
Fenoxycarb	9.13	302.0	116.2	88.0
Fenpyroximate	10.93	422.1	366.0	135.0
Flonicamid	4.73	229.9	203.0	174.1
Fludioxonil	8.48	266.0	229.0	158.0
Hexythiazox	10.70	353.0	228.1	168.1
Imazalil	6.99	296.9	158.9	201.0
Imidacloprid	5.60	256.0	175.2	209.0
Kresoxim-Methyl	9.41	314.0	223.1	116.0
Malathion	8.96	330.9	127.0	125.0
Metalaxyl	7.42	280.0	220.1	160.1
Methiocarb	8.29	226.0	169.0	121.1
Methomyl	4.47	163.0	88.0	58.2
Mevinphos E	5.42	225.0	127.0	193.0
Mevinphos Z	5.93	225.0	127.0	193.0
Myclobutanil (Systhane)	8.55	289.0	70.0	124.9
Naled	7.84	380.8	126.9	109.1
Ochratoxin A -	8.12	404.1	239.0	358.0
Oxamyl	4.35	237.0	71.9	89.9
Paclobutrazol	8.23	294.0	70.0	125.0
Phosmet (Imidan)	8.54	317.9	160.0	133.0
Piperonyl butoxide	10.28	356.1	177.0	119.0
Prallethrin (Isomer mix)	8.90	301.0	198.0	170.1
Propiconazole (Tilt)	9.28	341.9	159.0	69.1
Propoxur (Baygon)	6.95	210.0	110.9	65.0
Pyridaben	11.23	365.0	309.0	147.2
Spinetoram J	9.28	748.3	142.0	98.1
Spinetoram L	9.61	760.3	142.1	97.9
Spinosad-Spinosyn A	8.76	732.3	142.1	98.0
Spinosad-Spinosyn D	9.11	746.3	142.1	98.0
Spiromesifen	11.04	388.1	273.2	255.1
Spirotetramat	8.35	374.1	302.0	216.1
Spiroxamine	7.71	298.2	144.1	100.2
Tebuconazole	8.90	308.0	70.1	125.0
Thiacloprid	6.36	252.9	126.0	90.0
Thiamethoxam	4.94	291.9	211.1	132.1
Trifloxystrobin	10.08	409.0	186.0	145.1

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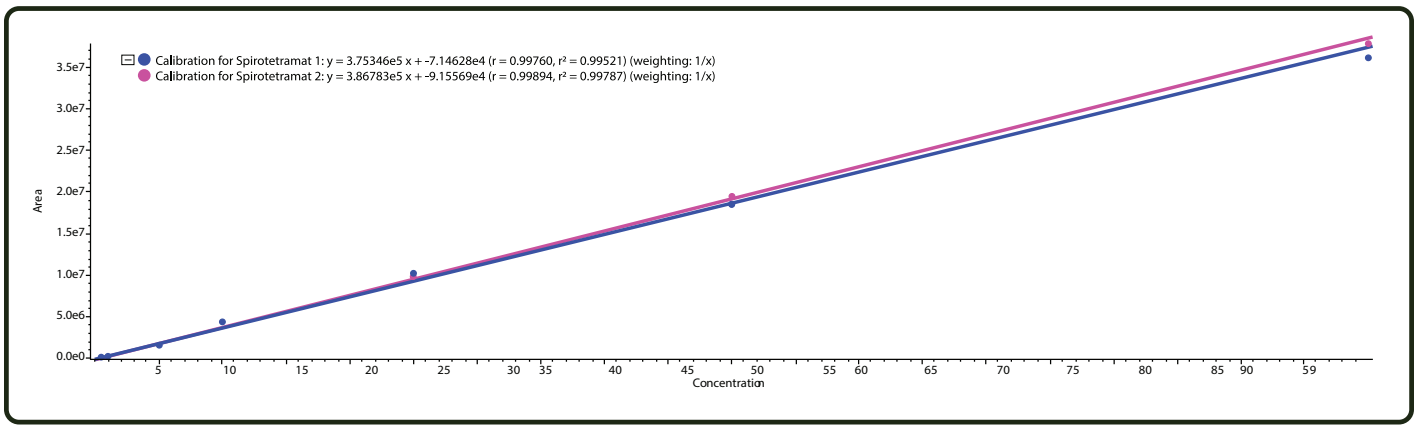

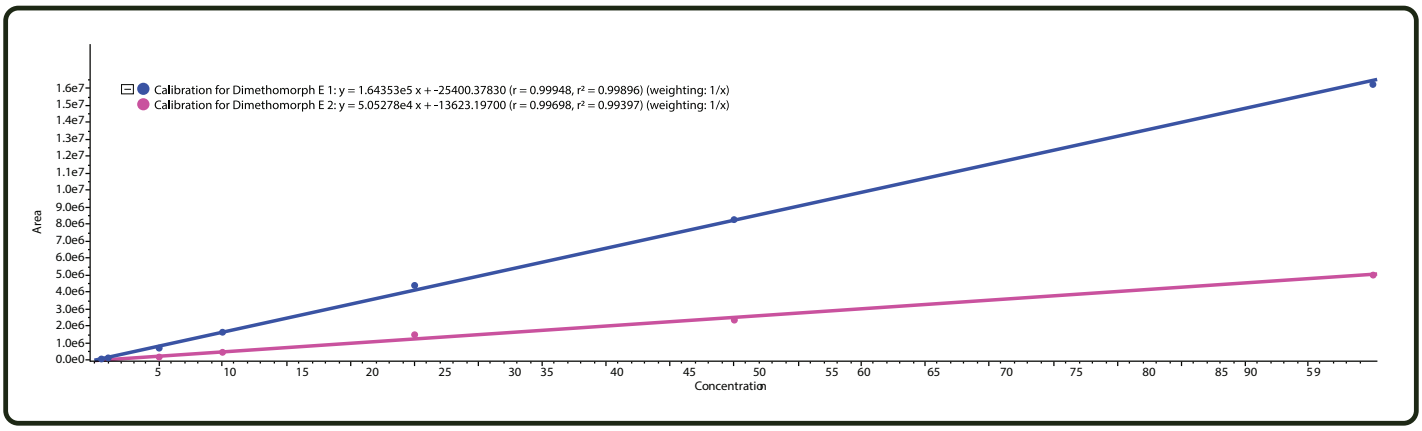

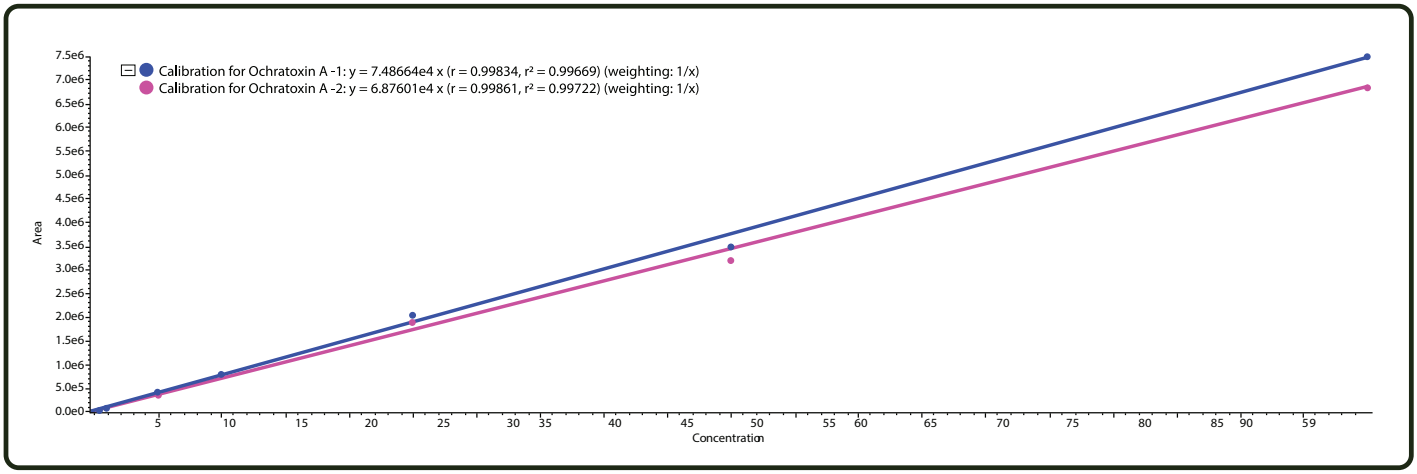

Calibration Curve Examples - Spirotetramat, Dimethomorph E and Ochratoxin A
7 point calibration curve prepared at 0.5, 1, 5, 10, 25, 50 and 100 ng/mL.

Results:

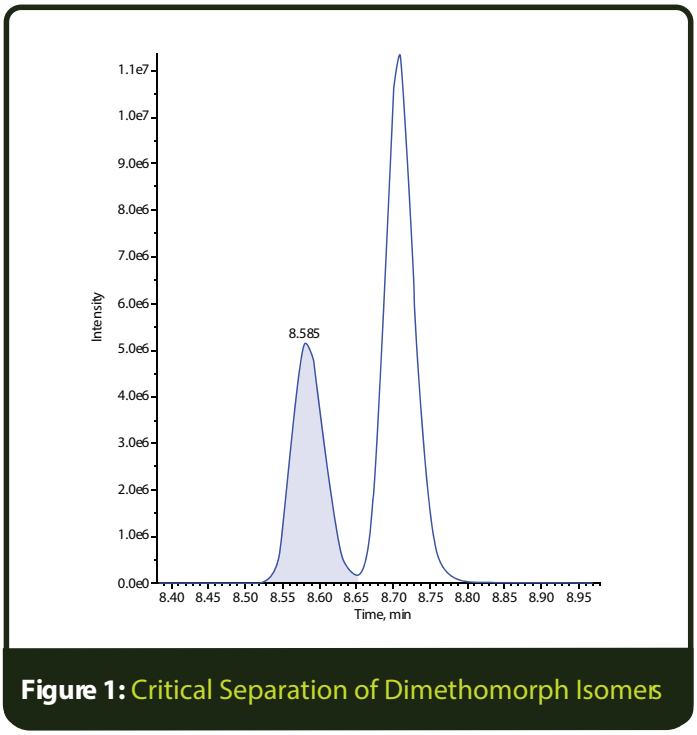

Figure 1: Critical Separation of Dimethomorph Isomers

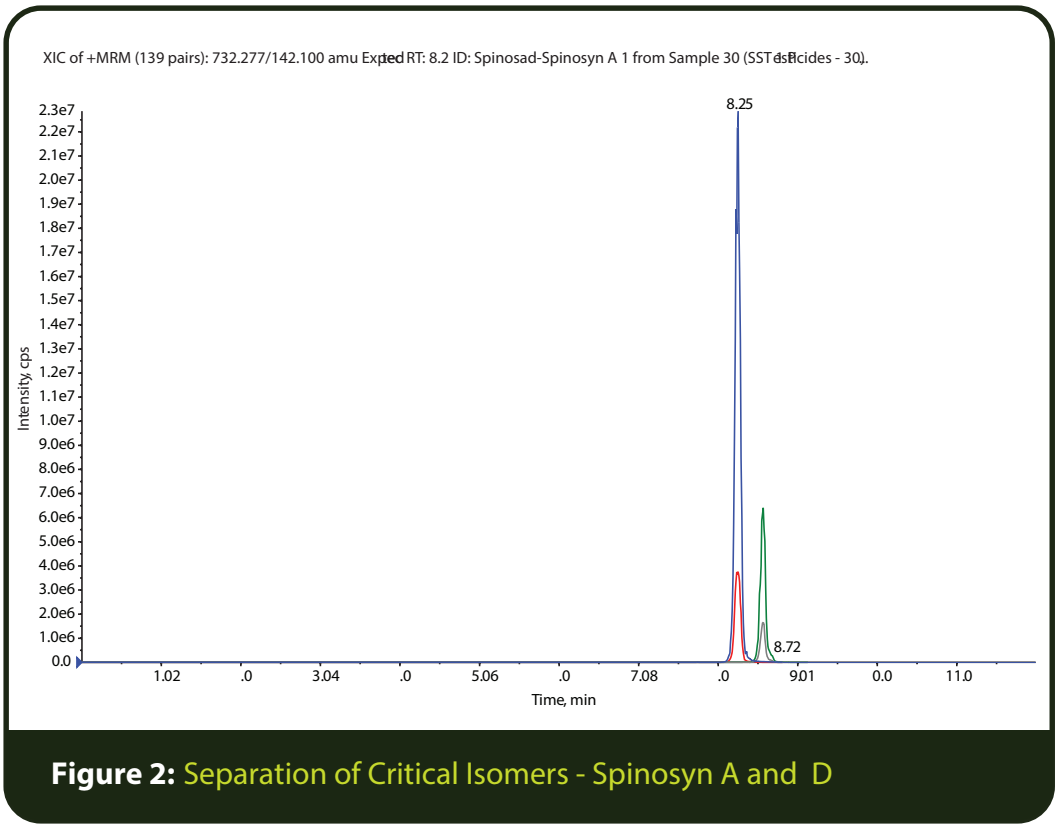

Figure 2: Separation of Critical Isomers - Spinosyn A and D

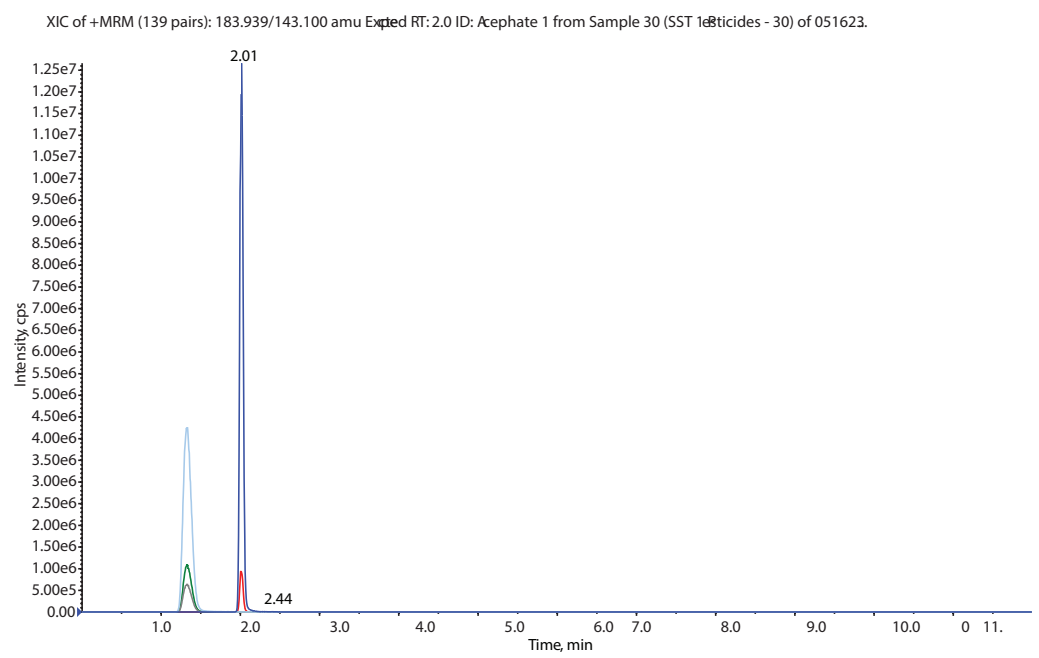

Figure 3: Retention of Early Eluting Analytes - Daminozide and Acephate

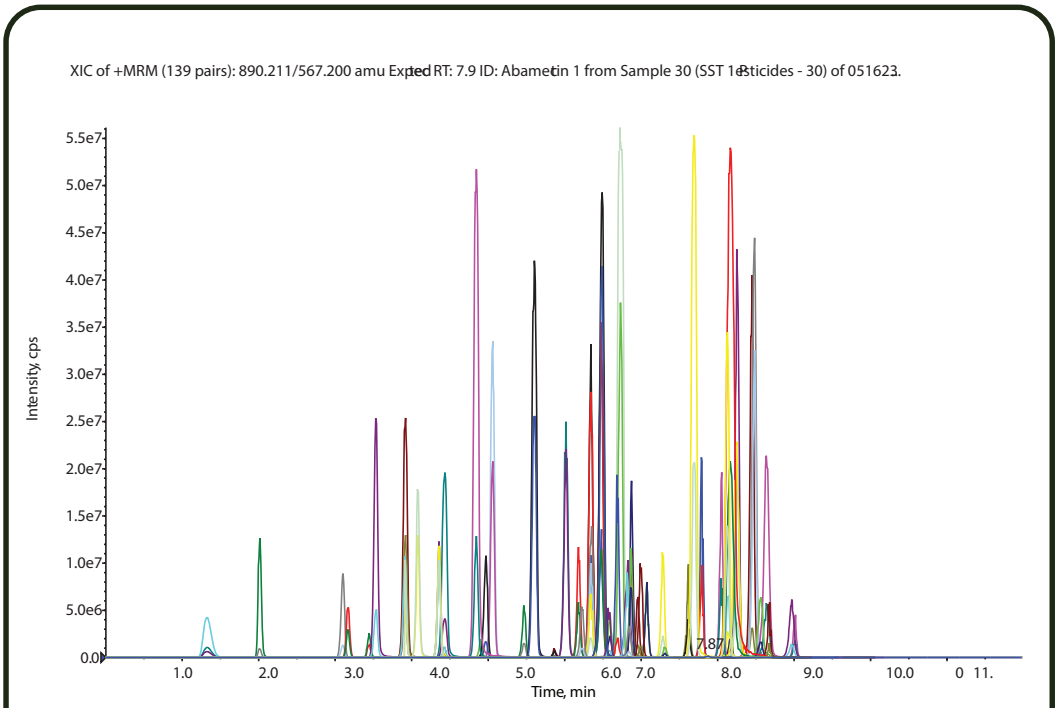

Figure 4: Pesticides Panel TIC

	Low Fortification		High Fortification	
Analyte	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Abamectin	78	5.4	84	6.3
Acephate	73	8.1	81	3.4
Acetamiprid	82	3.8	85	9.7
Aldicarb	62	5.4	75	8.6
Aflatoxin B1	73	2.8	79	5.4
Aflatoxin B2	71	5.1	82	4.7
Aflatoxin G1	69	6.9	78	2.9
Aflatoxin G2	81	2.6	74	8.1
Azoxystrobin	84	7.6	88	4.3
Bifenazate	78	6.2	85	4.1
Boscalid	82	9.4	91	3.6
Carbaryl	73	2.7	83	5.8
Carbofuran	89	4.1	92	6.2
Chlorantraniliprole	85	3.1	94	4.3
Chlordane	72	5.4	81	6.9
Chlorpyrifos	76	7.2	87	5.1
Clofentezine	84	5.8	89	2.6
Coumaphos	86	1.7	94	5.1
Cyfluthrin (Baythroid)	ND	-	55	10.8
Daminozide	45	12.6	59	59
Diazinon	87	3.4	94	94
Dichlorvos	ND	-	68	11.4
Dimethoate	81	4.6	89	6.9
Dimethomorph E	91	1.9	95	5.2
Dimethomorph Z	94	2.7	91	3.8
Ethoprophos	67	7.9	75	6.5
Etofenprox	93	2.1	95	3.6
Etoxazole	91	5.7	89	4.8
Fenhexamid	84	2.4	87	6.1
Fenoxycarb	96	6.3	88	1.7
Fenpyroximate	89	4.6	86	2.9
Flonicamid	75	5.6	84	3.1
Fludioxonil	81	7.1	78	6.2

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	Low Fortification		High Fortification	
Analyte	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Hexythiazox	86	4.3	89	3.8
Imazalil	89	1.4	82	4.8
Imidacloprid	74	8.3	81	6.1
Kresoxim-Methyl	ND	-	78	10.5
Malathion	88	4.7	91	2.4
Metalaxyl	82	5.9	84	3.6
Methiocarb	76	9.4	82	6.7
Methomyl	68	6.9	76	5.1
Mevinphos E	79	5.7	88	9.4
Mevinphos Z	83	6.3	87	4.6
Myclobutanil (Systhane)	89	6.7	84	2.9
Naled	ND	-	74	9.3
Ochratoxin A	64	7.8	72	4.0
Oxamyl	81	5.9	85	2.2
Paclobutrazol	85	5.4	94	2.7
Phosmet (Imidan)	79	7.9	88	4.8
Piperonyl butoxide	83	4.5	79	6.7
Prallethrin (Isomer mix)	77	5.1	84	4.8
Propiconazole (Tilt)	75	5.6	86	3.1
Propoxur (Baygon)	71	8.7	77	4.9
Pyridaben	81	4.6	89	6.9
Spinetoram J	81	5.4	78	9.1
Spinetoram L	80	1.4	85	6.2
Spinosad-Spinosyn A	73	2.7	86	5.8
Spinosad-Spinosyn D	77	3.9	81	7.4
Spiromesifen	62	13	59	18.1
Spirotetramat	81	5.5	85	6.9
Spiroxamine	76	5.7	87	9.4
Tebuconazole	73	4.1	79	2.8
Thiacloprid	88	7.6	91	5.2
Thiamethoxam	84	6.9	78	4.3
Trifloxystrobin	87	2.2	92	5.1

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Conclusion:

This application note outlines a QuEChERS method for the simultaneous analysis of cannabis for 67 pesticides and 5 mycotoxins residues in cannabis flower. 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 analysis.

The SpinFiltr® dSPE tube uses $MgSO_4$, PSA, C18 and ChloroFiltr® for sample cleanup. ChloroFiltr® is a unique 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® PFPP column, coupled to tandem mass spectrometry (LC-MS/MS) is used for analysis of the pesticides. The injection time is 17 minutes with 11 minutes of scan time. The method achieved good separation of all analytes in the panel, especially some critical isomers as Dimethomorph E and Z, Spinosyn A and D, and Mevinphos E and Z.

The method was evaluated by fortifying cannabis samples with each compound at low and high concentrations (n=5 each). The average recovery obtained was predominantly in the range of 70-100% and the RSD ≤20%. For some analytes, lower recoveries were obtained due to polarity, as in the case of Daminozide or sensitivity, as in the case of Cyfluthrin.

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