DETERMINATION OF PESTICIDES IN RED WINE BY QuEChERS EXTRACTION, QUICK QuEChERS CLEANUP AND LC/MS/MS DETECTION

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INTRODUCTION

Red wine is a rich source of phenolic compounds, antioxidants that have heart-healthy and anticancer benefits [1]. The application of pesticides, such as fungicides and insecticides to improve grape yields, is common practice in vineyards. Pesticide residues may remain in the grapes after harvest and in the wines that are made from them. Therefore, it is important to analyze for the presence of pesticide residues in red wines. The analysis of pesticide residues in red wine is challenging due to the complexity of the matrix which contains alcohol, organic acids, sugars, phenols and pigments, such as anthocyanins. QuEChERS (acronym for Quick, Easy, Cheap, Effective, Rugged, and Safe) is a promising analytical approach that was first published in 2003 by Anastassiades et al. for the analysis of pesticide residues in vegetables and fruits [2]. The QuEChERS procedure involves the extraction of pesticides into acetonitrile (MeCN) with the aid of salts and buffers, followed by dispersive solid phase extraction (dSPE) to clean up co-extractives.

The aim of this study was to use a QuEChERS extraction, but develop a clean-up approach that is easier and faster than the dSPE used in QuEChERS. This sample clean-up method is based on the filter and clean concept: the red wine extract is passed through a push thru cartridge containing magnesium sulfate ($MgSO_4$) and primary and secondary amine (PSA). The co-extractives are retained onto the sorbents. The cleaned extract is collected in an auto-sampler vial and injected directly into LC/MS/MS for analysis. The clean-up procedure takes less than one minute per sample. Eight pesticides were selected for this study. Polarities of the pesticides were very different, with the logarithm of the octanol water partition coefficient (LogP) ranging from -0.779 to 5.004. Among the eight pesticides, Cyprodinil is the most common pesticide detected in grapes, while Chlorpyrifos, Diazinone, and Methamidophos are also frequently found in grapes [3].

Classes, structures, LogP and pKa values of the eight pesticides selected in this study

Compound	Class	Structure	LogP	pKa
Methamidophos	insecticide		-0.779	-0.58
Carbendazim	fungicide		1.52	5.66, 11.62
Thiabendazole	fungicide and parasiticide		2.47	3.40, 10.53
Pyrimethanil	fungicide		2.558	4.41
Cyprodinil	fungicide		3.012	4.22
Diazinone	insecticide		3.766	1.21
Pyrazophos	fungicide and insecticide		2.810	-1.37
Chlorpyrifos	insecticide		5.004	-5.28

EXPERIMENTAL

Materials

50 mL polypropylene centrifuge tube (UCT cat#: RFV0050CT)

Mylar Pouch containing 4000 mg $MgSO_4$ and 2000 mg NaCl (UCT cat#: ECQUUS2-MP)

Quick QuEChERS mini-cartridge containing 110 mg $MgSO_4$ and 180 mg PSA (UCT cat#: ECPURMPSMC)

Procedures

QuEChERS extraction

a. Add 10 mL red wine to 50 mL polypropylene centrifuge tubes.
b. Spike with appropriate amounts of target analytes for fortified samples, vortex 30 sec and equilibrate for 15 min.
c. Add 10 mL MeCN, vortex 30 sec.
d. Add salts in Mylar pouch ($MgSO_4$ and NaCl), shake vigorously for 1 min.
e. Centrifuge at 5000 rpm for 5 min, the supernatant is ready for cleanup.

Quick QuEChERS cleanup

a. Load 1 mL red wine extract with polypropylene syringe.
b. Pass the extract slowly through Quick QuEChERS mini-cartridge ($MgSO_4$ and PSA).
c. Collect 0.5 mL cleaned extract into 2 mL auto-sampler vial.
d. Add 10 µL 5 ppm triphenyl phosphate as internal standard, the extract is ready for LC/MS/MS analysis.

Photos showing the cleanup procedure:

a. Load 1 mL red wine extract, attach to Quick QuEChERS mini-cartridge

b. Pass red wine extract through the mini-cartridge slowly, collect 0.5 mL cleaned extract

Comparison of mini-cartridges before (left) and after (right) cleanup of 1 mL red wine extract

Red wine extract | Red wine extract after Quick QuEChERS cleanup

INSTRUMENTAL

LC: Thermo Accela 1250 pump with PAL auto-sampler

LC Conditions

Column	Guard column: Restek C18, 2.1 x 20 mm Column: Sepax HP-C18, 2.1 x 100 mm, 3 µm, 120 Å
Column Temperature	Ambient
Injection Volume	10 µL at 15° C
Mobile Phase	A: 0.1% formic acid in Milli-Q-water B: 0.1% formic acid in LC/MS grade methanol
Flow Rate	200 µL/min

LC Gradient Program

Time	%A	%B
0	95	5
1	95	5
3	50	50
8	5	95
14	5	95
14.2	95	5
16	95	5

MS/MS: Thermo TSQ Vantage tandem MS

MS Conditions

Ion source:	Heated ESI
Ion polarity:	Positive
Spray voltage:	3000 V
Sheath gas pressure:	N_2 @ 40 psi
Auxiliar gas pressure:	N_2 @ 10 psi
Ion transfer capillary temperature:	350C
Scan type:	SRM (0-16 min.)
CID conditions:	Ar @ 1.5 mTorr

SRM transitions

Compound	Parent ion 1	Product ion 1	CE	Product ion 2	CE	S-Lens	Dwell time (s)
Methamidophos	142.044	94.090	14	125.050	16	59	0.15
Carbendazim	192.093	132.080	29	160.080	17	81	0.10
Thiabendazole	202.059	131.060	31	175.070	31	103	0.10
Pyrimethanil	200.116	107.060	23	183.140	22	66	0.10
Cyprodinil	226.122	77.030	40	93.050	33	88	0.10
TPP (IS)	327.093	77.020	37	152.070	33	98	0.10
Diazinone	305.135	153.090	15	169.08	14	89	0.10
Pyrazophos	374.103	194.060	20	222.130	20	104	0.10
Chlorpyrifos	349.989	96.890	32	197.940	17	69	0.10

RESULTS AND DISCUSSIONS

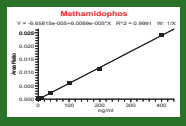
Matrix matched calibration, limit of detection (LOD), and limit of quantification (LOQ)

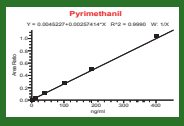
Calibration curves were obtained by analysis of matrix matched standards, which were prepared by spiking appropriate amounts of 2 ppm pesticide mixture to blank red wine extracts after Quick QuEChERS cleanup. Six matrix matched standards at 2, 10, 40, 100, 200, and 400 ng/mL levels were analyzed. The responses were linear over the calibration range. LOD and LOQ are the concentrations that give signal-to-noise ratio (S/N) of 3 and 10 respectively. In this study they were estimated according to the S/N values of the lowest matrix matched calibration level of 2 ng/mL.

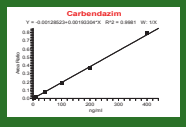
Matrix matched calibration, LOD and LOQ

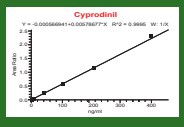
Compound	Linearity range (ng/mL)	R^2	LOD (ng/mL)	LOQ (ng/mL)
Methamidophos	2-400	0.9991	0.15	0.49
Carbendazim	2-400	0.9981	0.40	1.33
Thiabendazole	2-400	0.9940	0.09	0.31
Pyrimethanil	2-400	0.9990	0.01	0.05
Cyprodinil	2-400	0.9995	0.17	0.57
Diazinone	2-400	0.9982	0.06	0.21
Pyrazophos	2-400	0.9976	0.08	0.27
Chlorpyrifos	2-400	0.9981	0.10	0.32

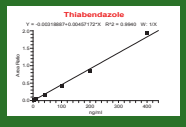
Calibration Curves

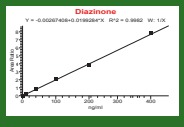


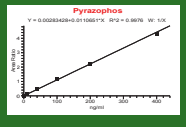


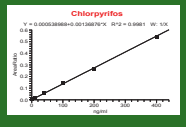












ACCURACY AND PRECISION DATA

Red wine samples fortified with 10, 50, and 100 ng/mL target pesticides were extracted with QuEChERS and cleaned up with Quick QuEChERS mini-cartridges. Recoveries ranged from 81.6% to 112.2% with overall recovery of 97.0%. Relative standard deviations (RSD) based on four replicates for three spiking levels were less than 10.8%. The recovery and RSD data indicated that this method is accurate and precise for the determination of pesticide residues in red wine samples.

Graphitized carbon black (GCB), which is often used to clean up pigments in vegetables and other food samples, is known to retain planar compounds, such as Carbendazim, Thiabendazole, Pyrimethanil and Cyprodinil, resulting in low recoveries of those pesticides. In this study PSA instead of GCB was used to clean up red wine pigments. The recoveries of the four planar pesticides included in this study were not affected (≥85%).

Accuracy and Precision Data

Compound	Fortified at 10 ng/mL		Fortified at 50 ng/mL		Fortified at 100 ng/mL	
	Recovery%	RSD% (n=4)	Recovery%	RSD% (n=4)	Recovery%	RSD% (n=4)
Methamidophos	93.7	3.4	81.6	5.8	84.2	3.5
Carbendazim	105.7	10.8	100.1	10.6	90.5	7.6
Thiabendazole	91.2	4.9	87.9	6.8	85.0	4.0
Pyrimethanil	112.2	2.7	107.0	3.2	102.8	4.9
Cyprodinil	104.3	3.2	99.9	6.1	100.2	4.9
Diazinone	104.9	104.9	96.6	6.6	99.2	6.8
Pyrazophos	99.9	4.0	96.6	5.6	91.3	4.1
Chlorpyrifos	91.7	4.6	99.5	5.2	97.2	3.8

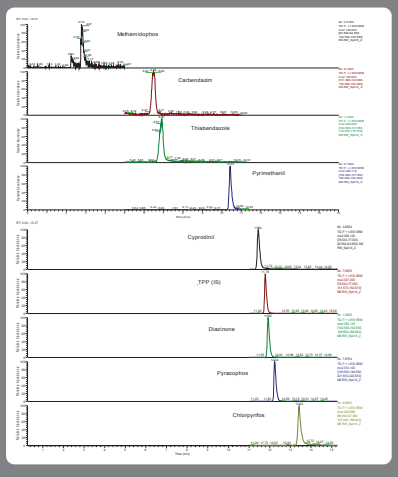
APPLICATION TO REAL SAMPLES

Six red wine samples were tested using this simple, fast, and novel method. Carbendazim was detected at 10.2, 8.7, and 2.3 ng/mL in red wine sample 4, 5, and 6 respectively. The detected concentrations are much lower than the EU or Japan regulated levels (ppm) in grapes.

Pesticides detected in red wine samples (ng/mL)

Compound	Sample 1 (Santa Carolina)	Sample 2 (Liberty Creek)	Sample 3 (Rossi)	Sample 4 (Great Wall)	Sample 5 (Rosemount)	Sample 6 (Two Hands)
Methamidophos	< 2	< 2	< 2	< 2	< 2	< 2
Carbendazim	< 2	< 2	< 2	**10.2**	**8.7**	**2.3**
Thiabendazole	< 2	< 2	< 2	< 2	< 2	< 2
Pyrimethanil	< 2	< 2	< 2	< 2	< 2	< 2
Cyprodinil	< 2	< 2	< 2	< 2	< 2	< 2
Diazinone	< 2	< 2	< 2	< 2	< 2	< 2
Pyrazophos	< 2	< 2	< 2	< 2	< 2	< 2
Chlorpyrifos	< 2	< 2	< 2	< 2	< 2	< 2

Chromatograms of red wine sample 1 fortified with 10 ng/mL pesticides



CONCLUSIONS

An easy, fast, novel, and efficient clean-up method for red wine samples was successfully developed in this study. Pesticide residues in red wine samples were extracted into acetonitrile using QuEChERS. Clean-up was accomplished by passing 1 mL of red wine extract through a push thru cartridge containing $MgSO_4$ and PSA. $MgSO_4$ adsorbed water remaining in the extract, while PSA removed organic acids, sugars, and pigments. This clean-up method, based on the filter and clean concept, takes less than one minute per sample. Combined with the QuEChERS extraction, this method is an excellent choice for high throughput laboratories.

References:
[1] http://www.mayoclinic.com/health/red-wine/HB00089
[2] M. Anastassiades, S.J. Lehotay, D. Stajnbaher and F.J. Schenck, J. AOAC Int. 86(2), 412-431 (2003)
[3] http://www.whatsonmyfood.org/food.jsp?food=GR

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Determination of Organophosphate Pesticides in Urine Using a 'Filter And Shoot' (FASt®) Extraction and LC-MS/MS

UCT Part Numbers:
CSFAS203 - CLEAN SCREEN FASt® 200 mg / 3 mL
SLAQ100ID21-3UM - Selectra Aqueous C18, 100 x 2.1mm, 3µm
SLAQGDC20-3UM - Selectra Aqueous C18, Guard Cartridge**,** 10 x 2.1mm, 3µm
SLGRDHLDR – Guard Cartridge Holder

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Organophosphate pesticides (OP) are a diverse group of compounds. Derived from phosphoric acid they exhibit varied physicochemical properties. They are used extensively as nerve poisons to kill target pests (usually insects). However, their toxicity extends to mammals and they can adversely affect the human nervous system, even at low exposure levels. For example, in 2013, 23 Indian students were killed from cooking oil contaminated with monocrotophos. OP pesticides are unstable and breakdown relatively quickly through hydrolysis and exit the human body via urine; thus monitoring OP pesticides and their metabolites in urine can indicate any recent exposures.

Extracting OP pesticides can be a challenge due to their varied physicochemical properties. Liquid/Liquid (L/L), Solid Phase Extraction (SPE), Supported Liquid Extraction, and QuEChERS work for mid to non-polar compounds, but not for polar compounds due to insufficient analyte partitioning between the aqueous and organic phases or retention on typical reverse phase sorbents.

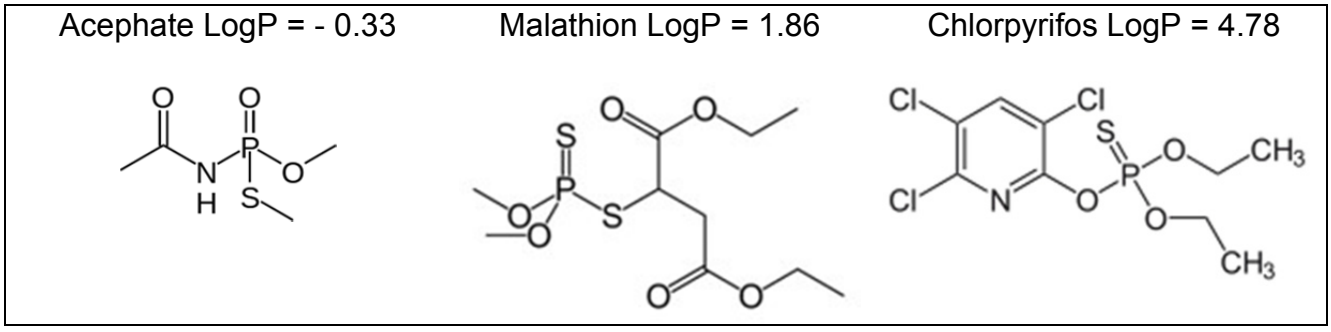

Figure 1. Examples of OP pesticides showing varied structures polarities

In this application a simple, fast sample preparation approach for LC/MS analysis of 13 OP pesticides in urine samples was conducted. This method efficiently retains the unwanted matrix components and particulates to the sorbent and frits

while allowing the analytes of interest to pass through the sorbent bed, and collected for direct LC-MS/MS analysis.

Sample Preparation:

1. Hydrolyze urine sample with beta-glucuronidase if there are any glucuronide- or sulfate- conjugated metabolites.
2. Mix 0.5 mL* urine sample with 0.5 mL acetonitrile containing internal standard(s), vortex for 1 min.
3. Apply the mixed sample to FASt column (or well plate), apply a low vacuum and collect the filtrate.
4. Mix 200 µL filtrate with 800 µL reagent water**, vortex and analyze by LC-MS/MS.

*: Less sample volume can be used for 96-well plate application.
**: Water dilution was needed for better retention of a couple polar OP pesticides, which is not necessary if only mid to non-polar compounds are analyzed.

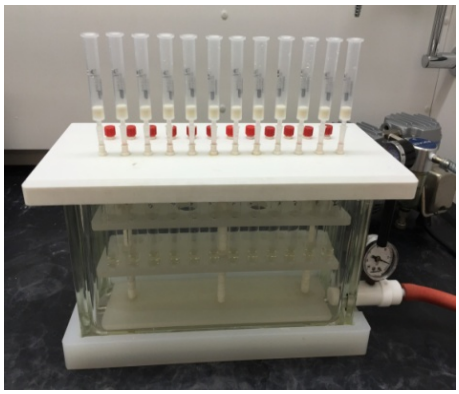

Figure 2.
FASt Setup

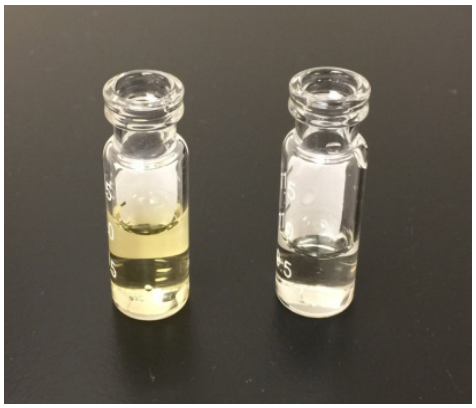

Figure 3.
Urine Sample: Before and after Extraction

LC-MS/MS method:

HPLC: Thermo Scientific Dionex UltiMate 3000® LC System
Mass Spec: Thermo Scientific TSQ Vantage tandem MS
Polarity: ESI +
Column: UCT, Selectra®, aQ C18, 100 x 2.1 mm, 3 µm
Guard column: UCT, Selectra®, aQ C18, 10 x 2.0 mm, 3 µm
Column temperature: 40 °C

Column flow rate: 0.300 mL/min		
Auto-sampler temperature: 10 °C		
Injection volume: 10 µL		
Gradient program: **Mobile phase A:** 20 mM ammonium formate in water **Mobile phase B:** 0.1 % formic acid in MeOH		
Time (min)	**Mobile phase A (%)**	**Mobile phase B (%)**
0	100	0
0.5	100	0
3	50	50
4.5	50	50
6	35	65
9	35	65
13	5	95
15	5	95
15.1	100	0
19	100	0
Divert mobile phase to waste from 0 - 2 and 16 - 19 min to prevent ion source contamination.		

Results:

Retention Times, SRM Transitions & Linearity

Compound	RT (min)	Parent ion	Product ion 1	Product ion 2	Linearity (R^2)
Methamidophos	3.53	142.0	93.7	124.6	0.9997
Acephate	4.36	183.9	142.6	94.6	0.9990
Dicrotophos	5.88	237.9	126.6	71.7	0.9994
o,o,o-triethylphosphorothioate	6.20	199.0	124.6	78.6	0.9991
Dimethoate	6.19	230.0	124.6	170.6	0.9990
Famphur	8.76	325.9	216.6	92.6	0.9980
Malathion	10.23	330.9	126.7	98.6	0.9963
Sulfotep	12.08	322.9	96.6	114.5	0.9977
Diazinon	12.82	305.0	168.7	152.7	0.9995
TPP (IS)	13.14	327.0	151.6	76.7	NA
Pyrazophos	13.42	374.0	221.6	193.6	0.9945
Profenofos	14.06	372.9	127.6	304.4	0.9980
Ethion	14.33	384.9	142.5	96.5	0.9974
Chlorpyrifos	14.54	350.0	96.6	199.3	0.9988

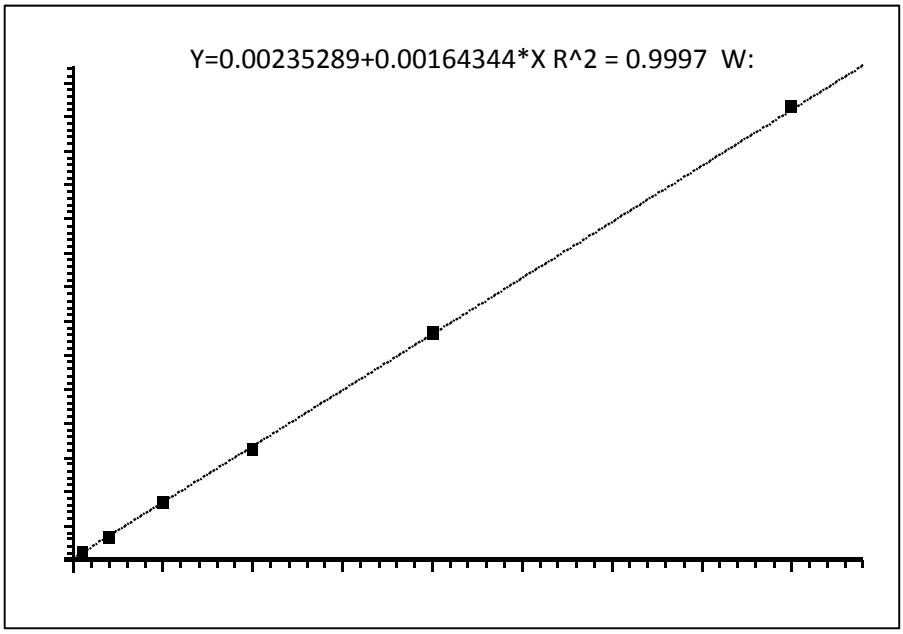

Figure 4. Calibration Curve of Methamidophos

Recovery and RSD Data – Spiked Urine Samples

Compound	Spiked at 10 ng/mL		Spiked at 50 ng/mL		Spiked at 200 ng/mL	
	Recovery%	RSD% (n=6)	Recovery%	RSD% (n=6)	Recovery%	RSD% (n=6)
Methamidophos	**96.3**	2.4	**110.6**	2.8	**101.1**	14.2
Acephate	**92.5**	7.8	**92.1**	7.5	**87.8**	9.6
Dicrotophos	**96.2**	5.9	**103.5**	2.0	**94.3**	5.7
o,o,o-triethylphosphorothioate	**102.0**	11.4	**112.6**	3.4	**101.0**	3.7
Dimethoate	**103.2**	4.7	**109.7**	4.3	**104.4**	2.5
Famphur	**106.5**	9.5	**112.3**	2.6	**106.5**	3.4
Malathion	**104.9**	7.3	**110.4**	1.7	**105.6**	3.1
Sulfotep	**87.3**	8.1	**93.4**	3.7	**92.1**	6.4
Diazinon	**94.8**	5.8	**103.7**	1.1	**104.1**	2.3
Pyrazophos	**104.6**	8.7	**114.8**	0.9	**101.6**	3.4
Profenofos	**90.7**	6.2	**100.8**	4.3	**101.1**	6.8
Ethion	**84.9**	6.6	**101.2**	2.4	**98.7**	8.3
Chlorpyrifos	**91.4**	5.6	**99.5**	7.5	**104.7**	5.5
Overall mean	**96.6**	**6.9**	**105.0**	**3.4**	**100.2**	**5.8**

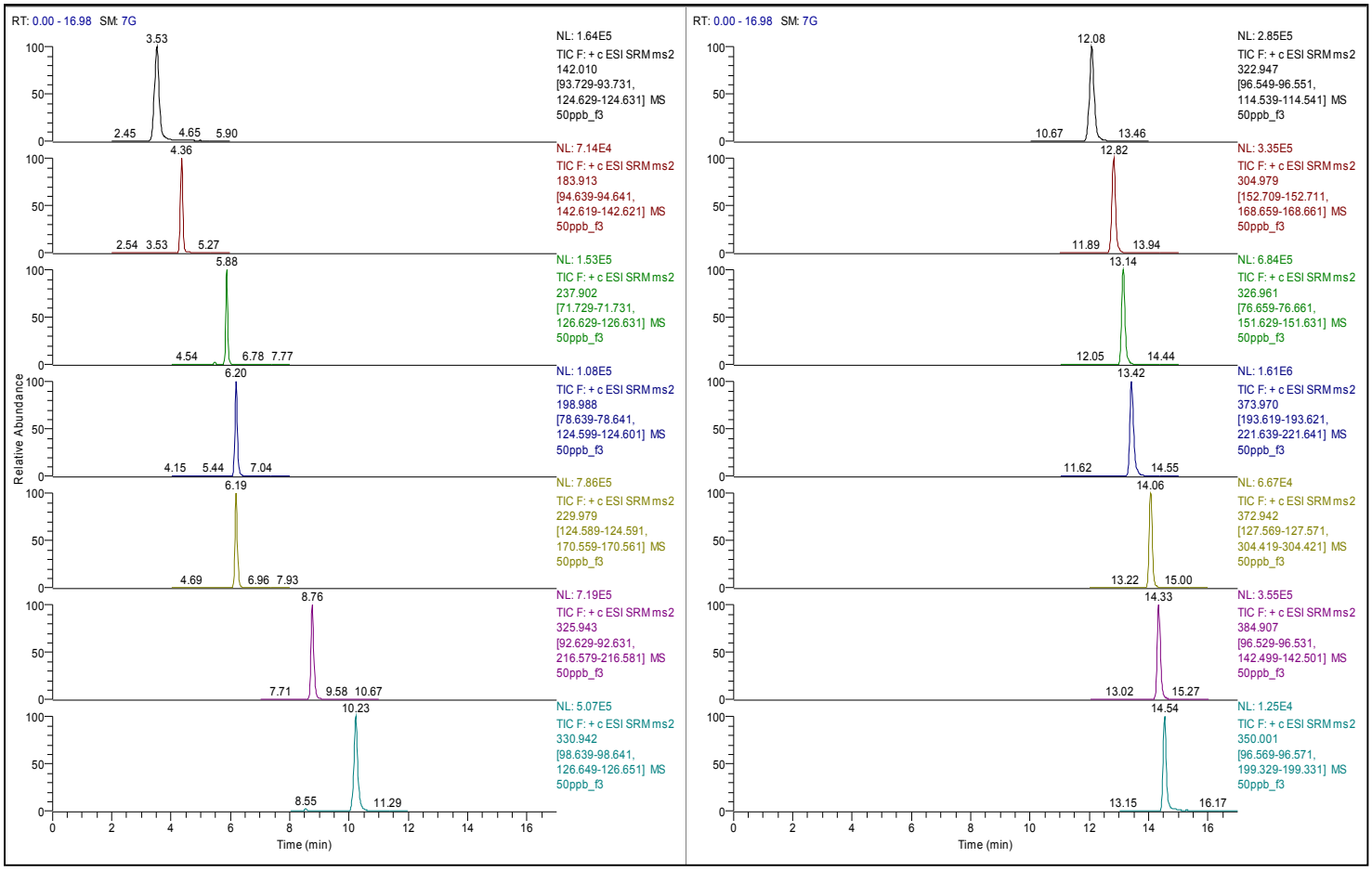

Figure 5. Chromatogram of a 50 ng/mL Solvent Standard

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A Modified QuEChERS Method for the Determination of Organochlorine Pesticides in Serum

UCT Part Numbers:
ECQUUS1115CT - 800 mg $MgSO_4$ and 200 mg NaCl in 15 mL centrifuge tube
ECPURMPSMC - Quick QuEChERS - medium push through cartridge with 110mg $MgSO_4$ and 180mg PSA
GCLGN4MM-5 – Agilent system GC liner, 4mm splitless gooseneck, 4mm ID x 6.5mm OD x 78.5mm)

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Summary:

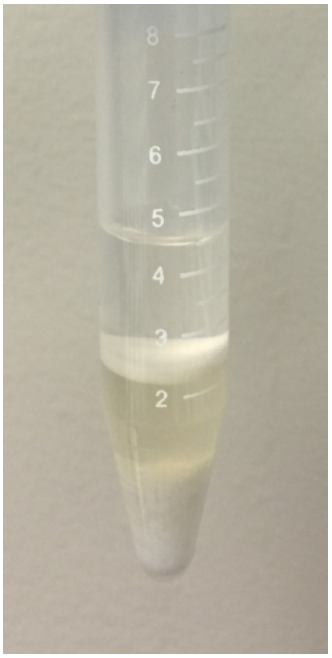
Organochlorine (OC) pesticides are hydrocarbons with multiple chlorine substitutions. They are primarily used as insecticides. OC pesticides do not break down easily as the chlorine-carbon bonds are very strong. As such they remain in the environment long after application and bio-accumulate in organisms long after exposure. They are toxic and some are known or suspected human carcinogens, such as 4,4'-DDT, 4,4'-DDD, and the lindanes. Many OC pesticides are banned or have restricted use. These chemicals are frequently found in human body tissue, blood, fat and breast milk. This application employs a modified QuEChERS extraction and a fast, push-through cartridge clean-up for the determination of OC pesticides in human serum.

1 mL of serum sample is mixed with 1 mL of reagent water and extracted with 2 mL of acetonitrile (MeCN). 800 mg magnesium sulfate ($MgSO_4$) and 200 mg sodium chloride (NaCl) are used to enhance the phase separation and the partition of the target analytes into the MeCN layer. After shaking and centrifugation, 1.5 mL of the supernatant is purified using a push through cartridge containing 110 mg $MgSO_4$ and 180 mg PSA. $MgSO_4$ absorbs any residual water in the extract, while PSA removes the matrix co-extractives, such as the lipids, resulting in a clean extract for instrumental analysis.

Procedure:

1. **QuEChERS Extraction**
   a) Add 2 mL MeCN containing triphenyl phosphate as internal standard (IS) to a 15-mL centrifuge tube pre-packed with 800 mg $MgSO_4$ and 200 mg NaCl (**ECQUUS1115CT**).
   b) Add 1 mL serum sample and 1 mL reagent water into the 15-mL centrifuge tube, and shake for 1 min manually or use a Spex 2010 Geno-Grinder at 1000 strokes/min.
   c) Centrifuge at 3000 g for 5 min.

2. **Push Though Cartridge Cleanup**
   a) Load 1.5 mL supernatant using a disposable plastic syringe and attach a push through cartridge (**ECPURMPSMC**) to the Luer lock tip of the syringe.
   b) Push the syringe plunger slowly, discard the first 3-5 drops (to remove any trapped airborne contaminants in the cartridge), then collect 0.5 mL extract into a 2-mL auto-sampler vial.
   c) The samples are ready for GC/MS (or GC-ECD) analysis.



Serum sample after QuEChERS extraction


Push through cartridge cleanup

GC/MS Method:

GC/MS	Agilent 6890N GC coupled to a 5975C MSD
Injection	1 µL splitless injection at 250 ºC
GC liner	4 mm splitless gooseneck (**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 50 ºC, hold for 1 min; ramp at 10ºC/min to 310 ºC, hold for 3 min. Acquire data from 8 to 24 min.
Temperatures	Transfer line 280 ºC Source 250 ºC Quadrupole 150 ºC

Compound Name	RT (min)	SIM Ions (25 ms)			R^2
TPP (IS)	22.643	326	325	77	NA
alpha lindane	15.911	181	219	109	0.9996
beta lindane	16.449	181	219	109	0.9995
gamma lindane	16.639	181	219	109	0.9998
delta lindane	17.195	181	219	109	0.9998
Heptachlor	18.185	100	272	237	0.9996
Aldrin	18.913	66	263	293	0.9998
Heptachlor epoxide	19.691	353	81	237	0.9995
trans Chlordane	20.155	373	237	272	0.9997
cis Chlordane	20.418	373	237	272	0.9998
Endosulfan I	20.418	237	195	339	0.9997
4,4'-DDE	20.812	246	318	176	1.0000
Dieldrin	20.951	79	263	277	0.9987
Endrin	21.357	263	81	281	0.9995
Endosulfan II	21.551	195	237	339	0.9995
4,4'-DDD	21.619	235	165	199	0.9992
Endosulfan sulfate	22.281	272	274	387	0.9984
4,4'-DDT	22.338	235	165	199	0.9973
Endrin ketone	23.186	317	67	319	0.9983
Methoxychlor	23.352	227	228	152	0.9970

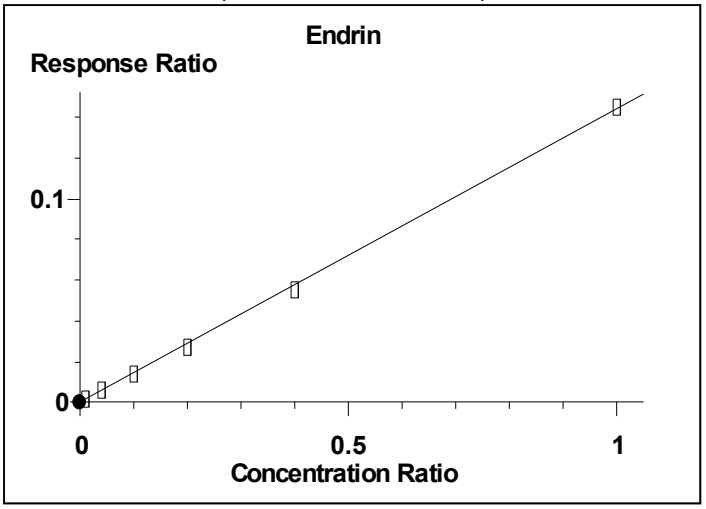
Results:

Recovery and RSD% of Spiked Serum Samples

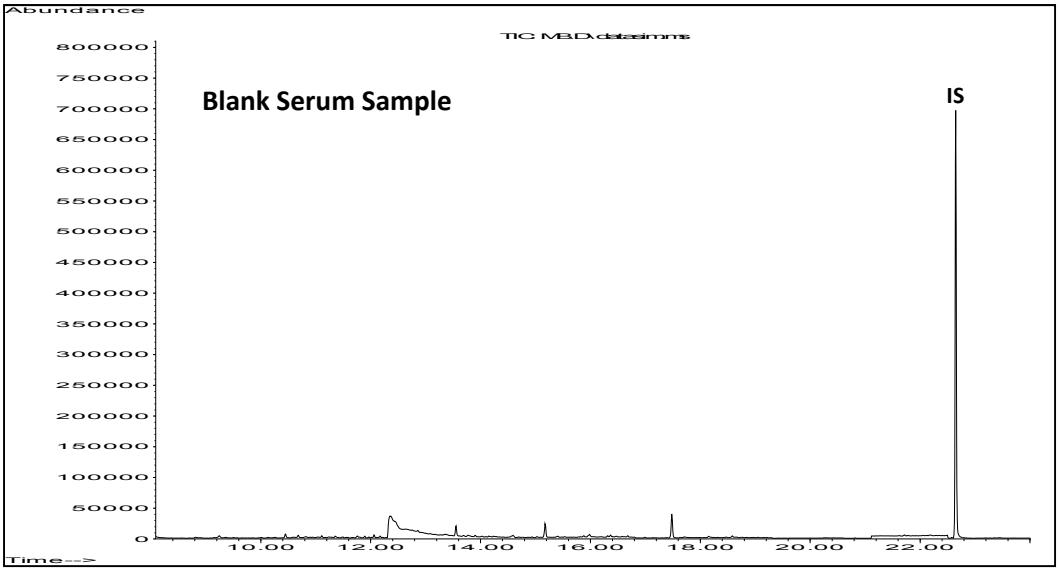
Compound Name	Spiked at 40 ng/mL		Spiked at 200 ng/mL		Spiked at 1000 ng/mL	
	Recovery%	RSD% (n=5)	Recovery%	RSD% (n=5)	Recovery%	RSD% (n=5)
alpha lindane	**106.5**	1.8	**95.7**	3.2	**100.1**	1.1
beta lindane	**105.5**	0.7	**93.7**	5.0	**100.3**	2.5
gamma lindane	**110.2**	1.9	**97.6**	3.3	**101.2**	1.3
delta lindane	**101.4**	1.5	**94.5**	3.5	**101.0**	1.0
Heptachlor	**103.0**	3.8	**89.7**	7.4	**92.6**	2.8
Aldrin	**87.5**	5.6	**81.3**	3.0	**87.4**	2.3
Heptachlor epoxide	**98.7**	2.2	**90.2**	3.2	**99.6**	2.5
trans Chlordane	**90.1**	3.2	**84.3**	3.7	**92.7**	2.6
cis Chlordane	**90.8**	3.4	**83.4**	3.4	**92.5**	1.4
Endosulfan I	**96.9**	3.1	**88.3**	4.0	**94.4**	1.3
4,4'-DDE	**87.9**	5.7	**81.7**	4.1	**87.2**	2.1
Dieldrin	**101.5**	0.7	**89.2**	3.4	**95.1**	4.2
Endrin	**98.7**	1.6	**91.5**	1.4	**98.1**	1.3
Endosulfan II	**95.5**	6.7	**89.8**	5.0	**96.7**	1.2
4,4'-DDD	**77.8**	2.6	**79.2**	3.1	**94.6**	1.1
Endosulfan sulfate	**86.2**	2.0	**85.4**	2.5	**100.0**	0.7
4,4'-DDT	**61.6**	4.7	**65.2**	3.1	**87.0**	5.1
Endrin ketone	**91.7**	2.2	**89.4**	3.8	**99.5**	1.4
Methoxychlor	**69.8**	2.3	**74.1**	3.0	**96.0**	0.7
Overall mean	**92.7**	**2.9**	**86.5**	**3.6**	**95.6**	**1.9**

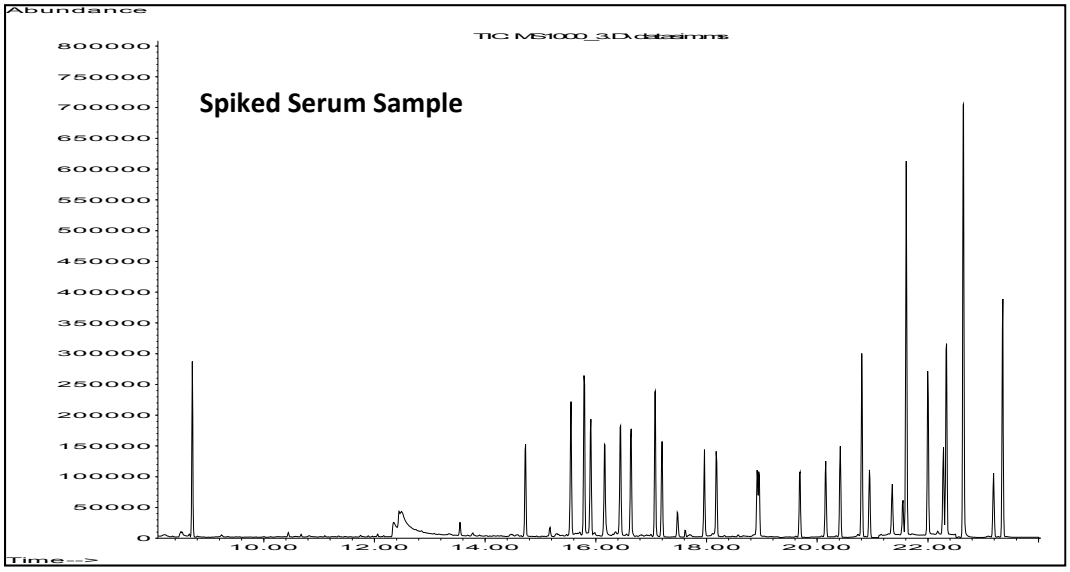
Matrix Matched Calibration Curve

(Endrin, R^2 = 0.9995)



Chromatograms





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Determination of Pesticide Residues in Blueberries by AOAC QuEChERS Approach and Dispersive SPE Cleanup with a Novel Sorbent ChloroFiltr®

UCT Part Numbers:
ECMSSA50CT-MP - Mylar pouch containing 6 g $MgSO_4$ and 1.5 g NaOAc with 50-mL centrifuge tubes included
CUMPSGGC182CT - 2 mL centrifuge tube containing 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18, and 50 mg ChloroFiltr®
SLAQ100ID21-3UM - Selectra® Aqueous C18, 100 x 2.1 mm, 3 µm
SLAQGDC20-3UM - Selectra® Aqueous C18, Guard column, 10 x 2.1 mm, 3 µm
SLGRDHLDR - Guard Cartridge Holder

Summary:

Blueberry has been ranked as one of the healthiest fruits for its high antioxidant content that helps combating free radicals, which could damage DNA and cellular structures [1]. Application of pesticides during plant cultivation is common to increase product yield, therefore it is valuable developing effective analytical methods for the determination of pesticide residues in blueberries, which however is challenging due to matrix complexity as blueberries are rich in anthocyanins, sugars, polyphenols, vitamins, minerals, and other interfering components.

This application outlines a simple, fast, and cost-effective method for the determination of multi-class pesticides, including one of the most problematic pesticides, pymetrozine in blueberries. The acetate buffered AOAC QuEChERS protocol demonstrated higher extraction efficiency for pymetrozine than the other 2 QuEChERS protocols (the EN citrate buffered or the original unbuffered), thus was selected for the extraction of pesticide residues in blueberries. 15 g of homogenized blueberries were extracted with 15 mL of acetonitrile (MeCN) containing 1% acetic acid (HAc). 6 g magnesium sulfate ($MgSO_4$) and 1.5 g sodium acetate (NaOAc) were employed to enhance phase separation and partition of pesticides into the MeCN layer. After shaking and centrifugation, 1 mL of the supernatant was transferred to a 2-mL dSPE tube containing the optimized cleanup sorbents of 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18, and 50 mg

ChloroFiltr®. Residual water was absorbed by $MgSO_4$, anthocynins, polyphenols, sugars and organic acids were removed by PSA, lipids and other non-polar interferences were retained by C18, while chlorophylls were removed by ChloroFiltr®, resulting in clean extract for LC/MS/MS analysis. UCT's aqueous C18 HPLC column was used for analyte retention and separation, which showed superior retention especially for several very polar pesticides, such as methamidophos and acephate.

Procedure:

1. **QuEChERS extraction**
    a) Weigh 15 ± 0.3 g of homogenized blueberry sample into 50-mL centrifuge tubes.
    b) Add triphenyl phosphate (TPP) as internal standard (IS) (optional), and appropriate amounts of spiking solution to fortified samples.
    c) Add 15 mL of MeCN with 1% HAc. Cap and shake for 1 min at 1000 strokes/min using a Spex 2010 Geno-Grinder.
    d) Add salts (6 g $MgSO_4$ and 1.5 g NaOAc) from pouch (**ECMSSA50CT-MP**) to the 50-mL tube, and vortex for 10 sec to break up salt agglomerates.
    e) Shake for 1 min at 1000 strokes/min using the Geno-Grinder.
    f) Centrifuge at 3000 rcf for 5 min.

2. **dSPE cleanup**
    a) Transfer 1 mL of the supernatant to a 2-mL dSPE tube (**CUMPSGGC182CT**).
    b) Shake for 1 min at 1000 strokes/min using the Spex 2010 Geno-Grinder.
    c) Centrifuge at 3000 rcf for 5 min.
    d) Transfer 0.2 mL of the cleaned extract into a 2-mL auto-sampler vial, add 0.2 mL of reagent water, and vortex for 30 sec.
    e) The samples are ready for LC-MS/MS analysis.

LC-MS/MS Method:

HPLC: Thermo Scientific Dionex UltiMate 3000® LC System		
Column: UCT, Selectra®, aQ C18, 100 x 2.1 mm, 3 µm		
Guard column: UCT, Selectra®, aQ C18, 10 x 2.0 mm, 3 µm		
Column temperature: 40 °C		
Column flow rate: 0.300 mL/min		
Auto-sampler temperature: 10 °C		
Injection volume: 2 µL		
Gradient program:		
Time (min)	**A%** (10 mM ammonium acetate in DI water)	**B%** (0.1% formic acid in MeOH)
0	100	0
1	50	50
3.5	50	50
6	5	95
9	5	95
9.1	100	0
14	100	0

Divert mobile phase to waste from 0 – 1.5 and 11.5 – 14 min to prevent ion source contamination.

MS parameters	
Instrumentation	Thermo Scientific TSQ Vantage tandem MS
Polarity	ESI +
Spray voltage	3500 V
Vaporizer temperature	450 °C
Ion transfer capillary	350 °C
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	40 arbitrary units
Q1 and Q3 peak width	0.4 and 0.7 Da
Collision gas and pressure	Ar at 1.5 mTorr
Cycle time	0.5 sec
Acquisition method	EZ Method (scheduled SRM)

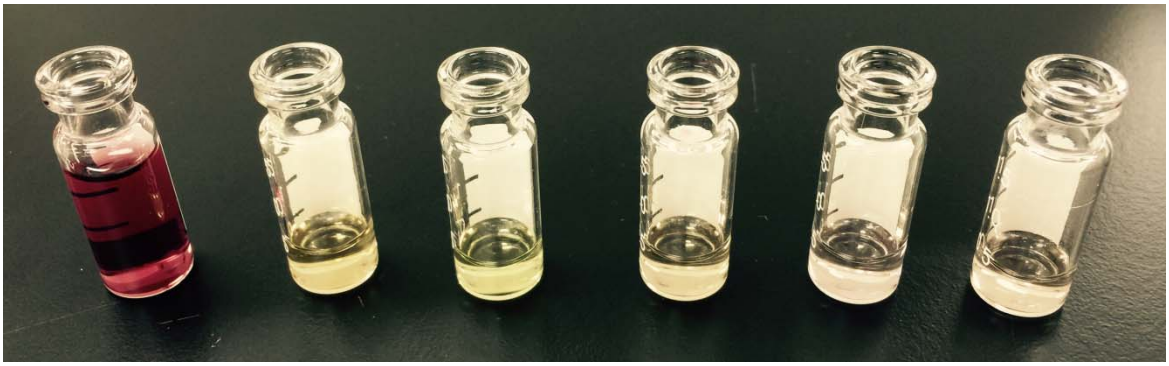
SRM Table						
Compound	**Precursor**	**Product 1**	**CE1**	**Product 2**	**CE2**	**S-lens RF**
Metamidophos	142.0	94.1	14	125.0	13	50
Acephate	184.0	143.0	6	95.0	25	33
Aldicarb sulfoxide	207.1	89.1	13	69.1	16	32
Oxydemeton methyl	247.0	169.0	13	109.0	27	57
Pymetrozine	218.1	105.1	20	176.1	17	63
Dichrotophos	238.1	112.1	12	127.0	18	52
Triethylphosphorothioate	199.0	125.0	16	143.0	14	55
Dimethoate	230.0	125.0	22	171.0	15	50
Carbendazim	192.1	160.1	18	132.1	29	60
Dichlorvos	220.9	109.0	17	127.0	13	62
Thiabendazole	202.0	175.1	25	131.1	31	70
Fenamiphos sulfone	336.1	266.0	19	188.0	26	75
Fenamiphos sulfoxide	320.1	233.0	24	108.1	40	60
Simazine	202.1	132.0	19	124.1	16	66
Tebuthiuron	229.1	172.1	16	116.0	26	55
Carbaryl	202.1	145.1	11	127.1	30	38
Flutriafol	302.1	70.1	17	123.0	28	69
Famphur	326.0	217.0	20	93.0	30	68
Thionazin	249.0	113.0	23	97.0	28	58
DEET	192.1	119.1	17	91.1	29	64
Atrazine	216.1	174.1	16	68.1	34	66
Malathion	331.0	127.0	12	99.0	25	55
Triadimefon	294.1	197.1	14	69.1	20	65
Pyrimethanil	200.1	107.1	24	183.1	23	68
Acetochlor	270.1	224.1	10	148.1	18	58
Sulfotep	323.0	97.0	37	115.0	30	60
Tebuconazole	308.1	70.1	21	125.0	33	66
Zoxamide	336.0	187.0	21	159.0	38	74
Diazinon	305.1	169.1	20	153.1	20	68
TPP (IS)	327.1	152.1	35	77.1	38	95
Cyprodinil	226.1	93.1	33	77.1	43	70
Pyrazophos	374.1	222.1	20	194.1	31	100
Profenofos	372.9	302.9	17	128.0	42	73
Ethion	385.0	142.9	26	199.0	6	56
Chlorpyrifos	349.9	97.0	32	197.9	19	67

Results:

Selection of dSPE cleanup sorbents:

Different sorbent mixtures (A - E) were packed in 2-mL dSPE centrifuge tubes for blueberry extract cleanup:

A. 150 mg $MgSO_4$ and 50 mg PSA
B. 150 mg $MgSO_4$ and 150 mg PSA
C. 150 mg $MgSO_4$, 50 mg PSA, and 50 mg C18
D. 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18, and 7.5 mg GCB
E. 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18, and 50 mg ChloroFiltr®



Photographs, from left to right: crude blueberry extract, and extracts cleaned with sorbent mixture A, B, C, D, and E, respectively.

Illustrated in the above picture, cleanup of blueberry extracts with PSA only (A and B) or PSA and C18 (C) is inefficient for complete pigment removal. With the addition of either GCB (D) or ChloroFiltr® (E), colorless extracts were obtained; therefore sorbent mixtures D and E were selected for the recovery study.

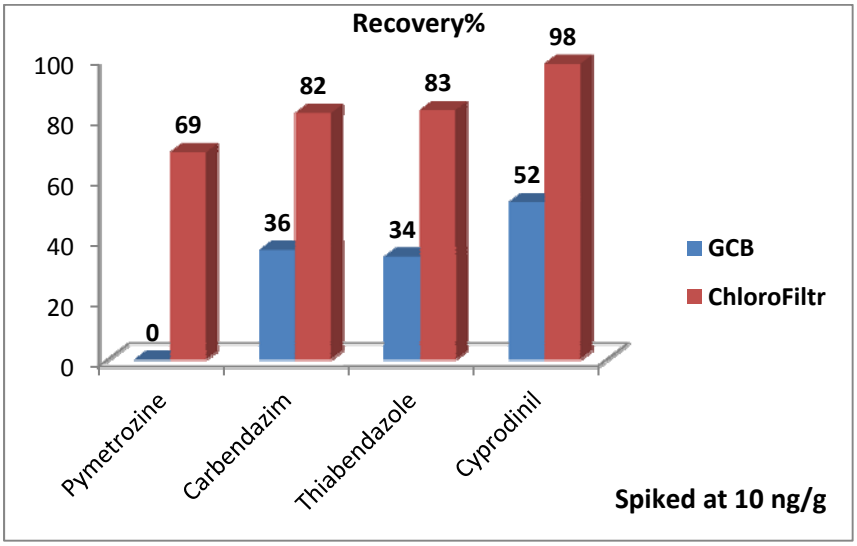
Recovery study:

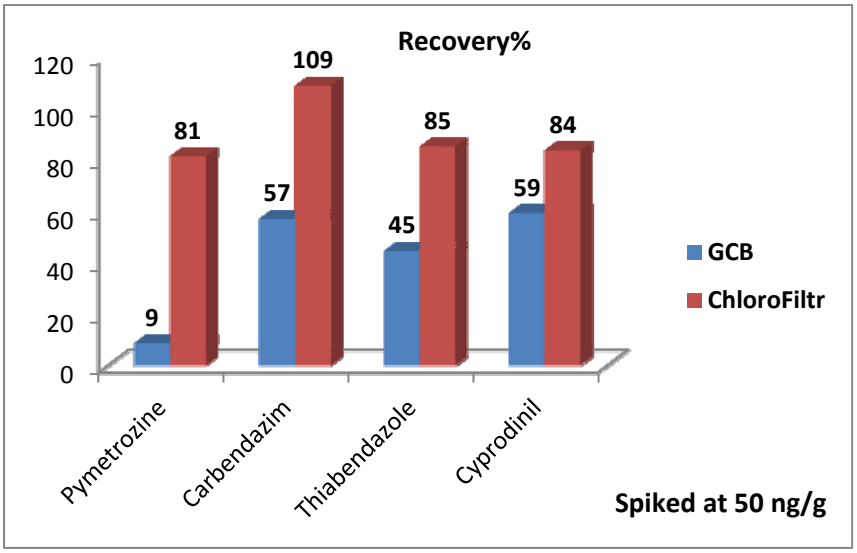
Blueberry samples were fortified with 10 ng/g and 50 ng/g of pesticides, and underwent the AOAC QuEChERS extraction and dSPE cleanup with 150 mg MgSO4, 50 mg PSA, 50 mg C18, and 7.5 mg GCB (D) or 50 mg ChloroFiltr® (E) as described above. The mean recoveries and RSD% of 6 replicated samples are listed in the table below.

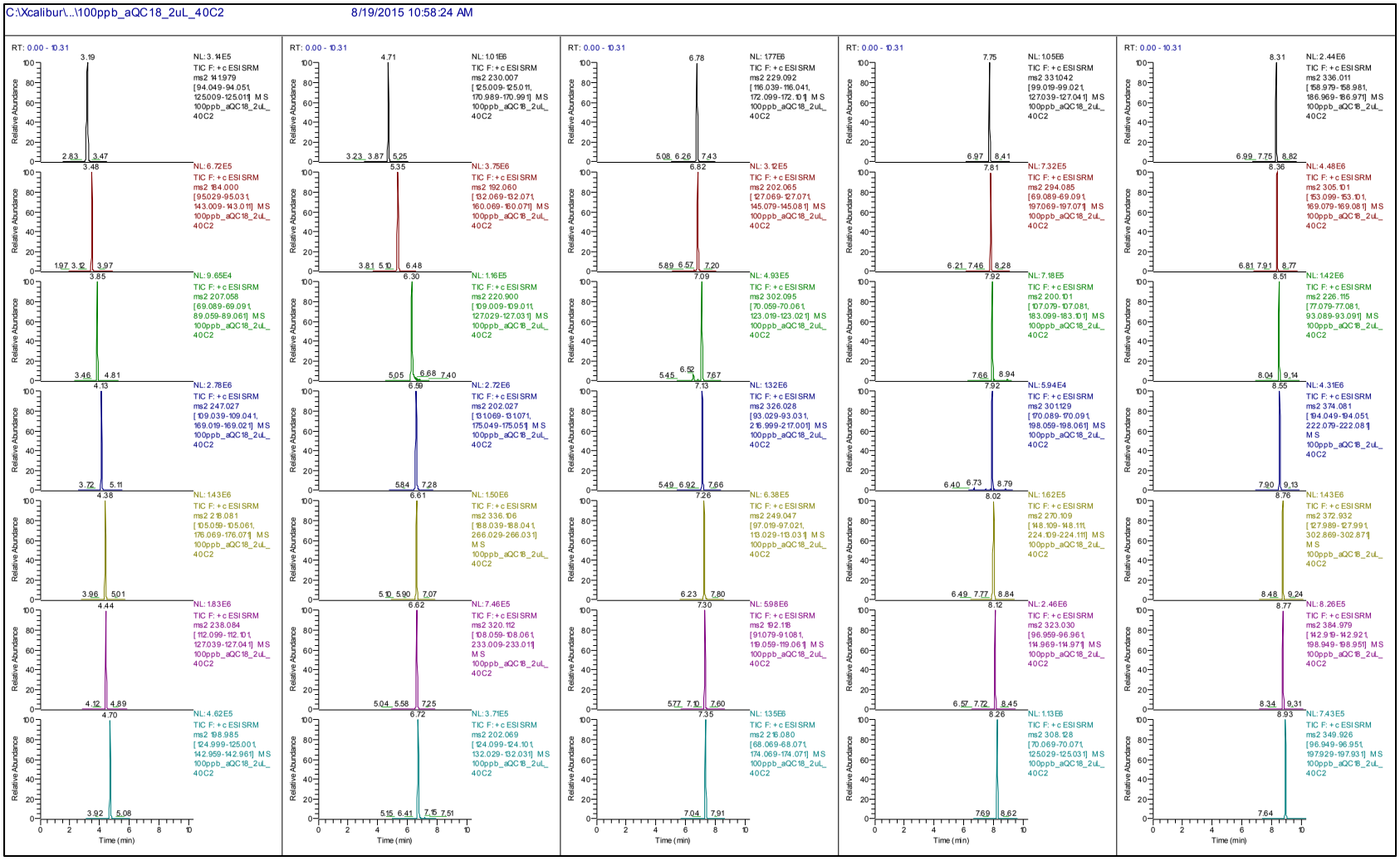
Accuracy and Precision of Pesticides in Spiked Blueberries

Compound	dSPE cleanup with PSA/C18/GCB				dSPE cleanup with PSA/C18/ChloroFiltr®			
	Spiked at 10 ng/g		Spiked at 50 ng/g		Spiked at 10 ng/g		Spiked at 50 ng/g	
	Recovery%	RSD%	Recovery%	RSD%	Recovery%	RSD%	Recovery%	RSD%
Methamidophos	**82.8**	2.5	**92.8**	1.0	**85.0**	3.7	**89.3**	1.2
Acephate	**82.8**	11.0	**83.1**	12.8	**86.1**	11.3	**89.5**	13.3
Aldicarb_sulfoxide	**87.0**	6.5	**95.6**	12.7	**103.4**	11.5	**82.1**	12.9
Oxydemeton_methyl	**96.6**	5.9	**94.0**	7.4	**96.2**	5.9	**84.2**	8.2
Dichrotophos	**65.7**	20.1	**103.6**	10.7	**91.6**	13.3	**102.3**	15.6
Pymetrozine	**0.0**	na	**8.7**	9.4	**68.8**	11.6	**81.3**	13.4
Dimethoate	**62.8**	16.1	**103.0**	6.2	**101.1**	10.9	**94.9**	10.6
Triethylphosphorothioate	**65.0**	11.9	**99.9**	6.7	**96.4**	12.5	**95.8**	10.4
Carbendazim	**36.3**	9.9	**57.0**	11.1	**81.7**	5.4	**108.7**	9.5
Dichlorvos	**91.0**	6.1	**103.3**	1.8	**95.4**	4.9	**91.5**	3.8
Fenamiphos_sulfone	**96.1**	1.9	**104.1**	1.6	**97.2**	5.5	**97.9**	9.4
Fenamiphos_sulfoxide	**94.5**	2.3	**99.2**	1.4	**101.0**	4.2	**94.9**	10.5
Simazine	**94.5**	7.3	**103.7**	3.1	**99.4**	8.0	**94.5**	6.0
Carbaryl	**103.5**	3.3	**104.2**	3.1	**95.4**	3.7	**98.7**	6.0
Tebuthiuron	**95.6**	2.0	**101.1**	1.9	**97.9**	1.9	**97.7**	6.7
Thiabendazole	**34.1**	7.0	**44.5**	7.7	**82.7**	2.9	**85.1**	9.8
Famphur	**103.3**	3.1	**109.4**	1.4	**98.1**	10.0	**102.7**	1.7
Flutriafol	**96.4**	2.3	**105.9**	0.9	**92.1**	3.5	**97.4**	1.4
Thionazin	**104.3**	2.8	**105.6**	1.8	**90.8**	14.9	**97.6**	4.3
Atrazine	**116.9**	2.7	**116.1**	1.8	**84.6**	12.3	**86.6**	11.5
DEET	**127.6**	3.8	**112.9**	2.5	**85.1**	24.9	**84.7**	18.8
Malathion	**94.5**	7.5	**108.2**	2.0	**95.4**	0.9	**104.2**	3.7
Triadimefon	**89.9**	3.3	**104.3**	2.0	**91.4**	5.5	**99.0**	1.6
Pyrimethanil	**62.6**	9.3	**72.8**	3.8	**81.9**	5.9	**89.3**	2.3
Acetochlor	**95.7**	3.7	**105.2**	2.8	**102.1**	5.7	**97.8**	2.4
Sulfotep	**94.2**	3.3	**107.7**	1.7	**96.3**	3.0	**106.3**	1.2
Tebuconazole	**93.0**	3.5	**102.6**	1.7	**87.3**	2.1	**93.5**	1.8
Zoxamide	**99.0**	2.5	**109.1**	1.1	**89.1**	2.1	**96.3**	2.2
Diazinon	**93.8**	2.4	**103.1**	0.9	**93.4**	3.5	**96.4**	1.1
Cyprodinil	**52.2**	5.9	**59.2**	6.6	**98.1**	3.4	**83.6**	1.7
Pyrazophos	**70.2**	5.5	**76.1**	7.5	**94.5**	2.0	**99.3**	1.3
Ethion	**96.8**	4.0	**100.9**	6.1	**95.9**	3.3	**93.6**	2.0
Profenofos	**91.0**	3.6	**98.9**	3.9	**88.1**	2.5	**87.3**	1.6
Chlorpyrifos	**88.7**	2.1	**98.5**	3.4	**94.6**	0.9	**89.1**	2.2

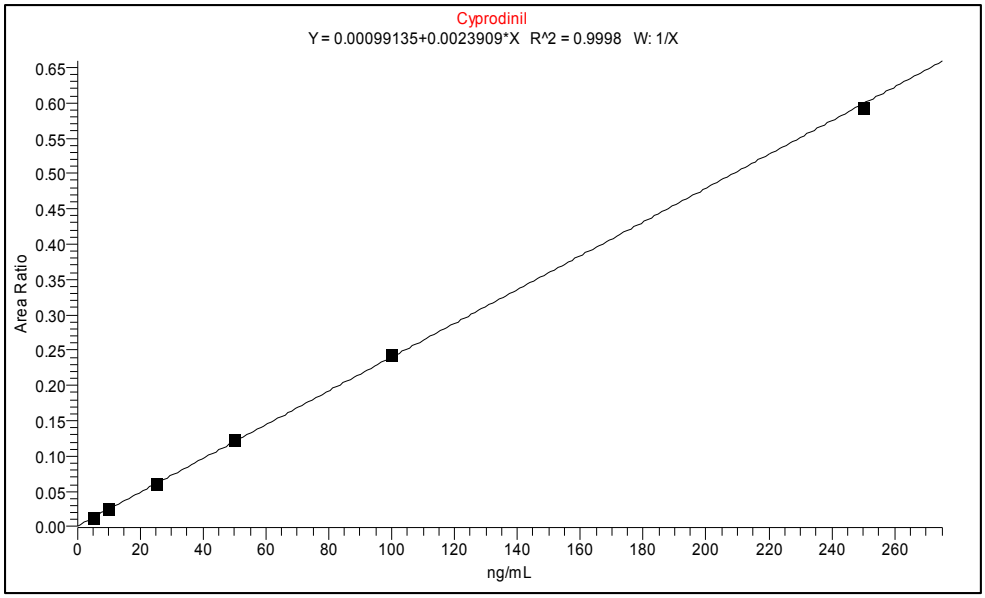
The recoveries of several pesticides such as pymetrozine, carbendazim, thiabendazole, and cyprodinil, were found be adversely affected by GCB, but unaffected when ChloroFiltr® was used; therefore, this sorbent combination was selected for blueberry extract cleanup in the final optimized procedure. The graphs below demonstrate the recovery comparison using GCB versus ChloroFiltr® at 2 contrasting levels (10 and 50 ng/g).







Chromatograms of 34 Pesticides and TPP (IS) in 1:1 MeCN:H_2O (100 ppb) using UCT Aqueous C18 HPLC Column
(Compound order can be found in the SRM table.)



Matrix-matched Calibration Curve of Cyprodinil (R^2 = 0.9998)

References:

[1] http://www.whfoods.com/genpage.php?tname=foodspice&dbid=8

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Method 523: Determination of Triazine Pesticides and their Degradates in Drinking Water by Gas Chromatography/Mass Spectrometry (GC/MS) Version 1.0

UCT Part Numbers:
EC5232506 (250 mg GCB, 6 mL cartridge)

EPA Method 523

August 2012

Method Summary

This is a gas chromatography/mass spectrometry (GC/MS) method for the determination of triazine pesticides and their degradation products in finished drinking waters. Samples are pH adjusted, dechlorinated with ammonium acetate and protected from microbial degradation with 2-chloroacetamide during collection. Analytes are extracted from a **250 mL sample** using 250 mg carbon cartridges.

The following compounds can be determined using this method:

Analyte	CASRN
Atrazine	1912-24-9
Atrazine-desethyl	6190-65-4
Atrazine-desethyl-desisopropyl	3397-62-4
Atrazine-desisopropyl	1007-28-9
Cyanazine	21725-46-2
Propazine	139-40-2
Simazine	122-34-9
Terbuthylazine-desethyl	30125-63-4
Terbuthylazine	5915-41-3
Prometon	1610-18-0
Prometryn	7287-19-6
Ametryn	834-12-8

Procedure

1. Sample Preparation

a) Allow samples to reach room temperature prior to extraction

b) Add an aliquot of the Surrogate Primary Dilution Standards (PDS) to each sample

c) Fortify Laboratory Fortified Blanks, Laboratory Fortified Sample Matrices, or LFSM Duplicates, with an appropriate volume of analyte PDS and the atrazine-desethyl-desisopropyl stock standard

d) Cap and invert each sample several times to mix

e) Proceed with sample extraction using SPE carbon cartridges

2. Cartridge Cleaning & Conditioning

a) Set up extraction cartridges on the SPE vacuum manifold

b) Using low vacuum (approximately 1 to 2 inches Hg), rinse each cartridge with two 6 mL aliquots of DCM drawing completely through

c) Rinse each cartridge with a 6 mL aliquot of MeOH

d) Draw MeOH to the top of the cartridge frit

Note: Do not let the cartridge dry after addition of MeOH

e) Add a 6 mL aliquot of reagent water (RW) to the cartridge

f) Draw RW to the top of the cartridge frit

3. Sample Extraction

a) Add an additional 4 mL of RW to each cartridge

b) Attach sample transfer lines to the cartridges. The additional volume prevents the SPE cartridge bed from going dry while the dead volume in the transfer lines is being filled

c) Extract 250 mL of sample at a cartridge flow rate of 10 mL/minute

d) Dry the cartridges under high vacuum for 10 seconds

e) Release vacuum, then add a 0.25 mL aliquot of MeOH to each cartridge

f) Draw the MeOH to waste, then dry cartridge under full vacuum for 10 minutes

4. Sample Elution

a) Place 15 mL conical tubes into the manifold for collection
b) Add 2 mL of EtOAc to the cartridge and elute dropwise
c) Add 2 x 6 mL aliquots of 9:1 DCM/MeOH to cartridge
d) Allow the cartridge beds to briefly soak in solvent, then draw the solvent through the cartridges
e) Dry the eluate by passing it through approximately 3 grams of anhydrous Na_2SO_4 collecting it in a 40 mL centrifuge tube. Pre-rinse the Na_2SO_4 with a 1 mL aliquot of 3:1 DCM/EtOAc
f) Rinse with 1 mL aliquot of 3:1 DCM/EtOAc collecting it in the centrifuge tube
g) The dried extracts may be stored overnight in the 40 mL tubes at -10 ºC
h) Warm the 40 mL tubes to 35 °C in a water bath under a stream of N_2 and evaporate solvent to less than 1 mL but no less than 0.5 mL
i) Transfer the concentrated eluate to 1 mL volumetric tubes
j) Rinse the conical tube with a small volume of EtOAc, and transfer the rinse to the volumetric
k) Add IS solution and adjust to volume
l) Transfer the extracts to autosampler vials for analysis or store in a freezer ≤ -10 ºC

Complete details at Office of Water (MLK 140) EPA Document No. 815-R-11-002 February 2011 http://www.epa.gov/safewater/

Potency and Pesticide Content in Medical vs. Recreational Marijuana

UCT Part Numbers:
ECQUUS950CT-MP - QuEChERS salts for THC Potency and Pesticide Testing 50 mL Centrifuge Tubes Included
ECQUUS142CT- Dispersive SPE Sorbent Blend for Pesticide Testing in Edibles 2 mL Centrifuge Tubes Included
SLAQ100ID21-3UM- Selectra® Aqueous C18 HPLC Column 100 x 2.1 mm, 3µm
SLAQGDC20-3UM – Selectra® Aqueous C18 Guard Column 10 x 2.1, mm, 3µm
SLGRDHLDR - Guard Column Holder

As of January 2016, 24 states and the District of Columbia have legalized the medical use of marijuana, while 4 states and the District of Columbia have legalized the recreational use of marijuana. As a result, both forensic toxicology and cannabis-specific testing laboratories are looking for fast, reliable, and cost-effective methods to determine cannabis potency and pesticides in medical and/or recreational marijuana. This application utilizes the advantages of QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) to extract for both pesticides and cannabinoids including tetrahydrocannabinol (THC), cannabidiol (CBD), tetrahydrocannabinolic acid (THCA-A) and cannabinol (CBN) in marijuana, followed by either serial dilutions for cannabis potency analysis, or a dispersive solid phase extraction (dSPE) cleanup for pesticide residue analysis. This hybrid method allows for the QuEChERs approach, which is extensively used in the food testing industry, to be utilized in a medico-legal setting.

Figure 1: Seized recreational marijuana samples

Procedure

(a) Recommended Sample Pre-treatment

1. Grind marijuana sample to fine powder using a SPEX 6770 freezer mill.

(b) QuEChERS Extraction

1. Weigh 1 g of the pre-treated marijuana into 50-mL centrifuge tubes, add internal standard, and 10 mL of methanol, and hydrate for 3 hr at 60 °C.
2. Add 10 mL of acetonitrile (MeCN) with 1% acetic acid.
3. Add QuEChERS extraction salts from pouches (**ECQUUS950CT-MP**), and vortex for 10 sec to break up salt agglomerates.
4. Shake for 1 min at 1000 stroke/min using a SPEX Geno/Grinder.
5. Centrifuge at 3000 rcf for 5 min.

(c) dSPE cleanup for pesticide residue analysis

1. Transfer 1 mL of the supernatants to 2-mL dSPE tube (**ECQUUS142CT**).
2. Shake for 1 min at 1000 stroke/min using the SPEX Geno/Grinder.
3. Centrifuge at 3000 rcf for 5 min.
4. Transfer 200 µL extract to the 2-mL auto-sampler vials, add 200 µL of DI water, and vortex for 30 sec.

Figures 2 and 3: Recreational marijuana sample following QuEChERS extraction; Comparison of QuEChERS extracts before (left) and after (right) dSPE cleanup.

(d) Apply serial dilutions for cannabinoid analysis

1. Perform serial dilutions (200 to 20,000 times depending on the cannabinoid concentration in different samples) of the QuEChERS extracts to 100 to 200 ng/mL.
2. Spike the diluted samples with 50 and 150% of the target cannabinoids, which are used to quantify the cannabinoid concentration according to the standard addition method.

(e) Analyze by LC-MS/MS

1. Analyze samples by LC/MS/MS (Thermo Scientific UltiMate 3000 LC system coupled to TSQ Vantage tandem MS) equipped with an UCT Aqueous C18 HPLC column (**SLAQ100ID21-3UM**).

Instrument Parameters (Pesticides)

HPLC: Thermo Scientific Dionex UltiMate 3000® LC System		
Column: UCT, Selectra®, aQ C18, 100 x 2.1 mm, 3 µm		
Guard column: UCT, Selectra®, aQ C18, 10 x 2.0 mm, 3 µm		
Column temperature: 40 °C		
Column flow rate: 0.300 mL/min		
Auto-sampler temperature: 10 °C		
Injection volume: 2 µL		
Gradient program:		
Time (min)	**A%** (10 mM ammonium acetate in DI water)	**B%** (0.1% formic acid in MeOH)
0	100	0
1	50	50
3.5	50	50
6	5	95
9	5	95
9.1	100	0
14	100	0

MS parameters	
Instrumentation	Thermo Scientific TSQ Vantage tandem MS
Polarity	ESI +
Spray voltage	3500 V
Vaporizer temperature	450 °C
Ion transfer capillary temperature	350 °C
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	40 arbitrary units
Q1 and Q3 peak width (FWHM)	0.4 and 0.7 Da
Collision gas and pressure	Ar at 1.5 mTorr
Cycle time	0.5 sec
Acquisition method	EZ Method (scheduled SRM)

SRM Table						
Compound	**Precursor**	**Product 1**	**CE1**	**Product 2**	**CE2**	**S-lens RF**
Metamidophos	142.0	94.1	14	125.0	13	50
Acephate	184.0	143.0	6	95.0	25	33
Aldicarb sulfoxide	207.1	89.1	13	69.1	16	32
Oxydemeton methyl	247.0	169.0	13	109.0	27	57
Pymetrozine	218.1	105.1	20	176.1	17	63
Dichrotophos	238.1	112.1	12	127.0	18	52
Triethylphosphorothioate	199.0	125.0	16	143.0	14	55
Dimethoate	230.0	125.0	22	171.0	15	50
Carbendazim	192.1	160.1	18	132.1	29	60
Dichlorvos	220.9	109.0	17	127.0	13	62
Thiabendazole	202.0	175.1	25	131.1	31	70
Fenamiphos sulfone	336.1	266.0	19	188.0	26	75
Fenamiphos sulfoxide	320.1	233.0	24	108.1	40	60
Simazine	202.1	132.0	19	124.1	16	66
Tebuthiuron	229.1	172.1	16	116.0	26	55
Carbaryl	202.1	145.1	11	127.1	30	38
Flutriafol	302.1	70.1	17	123.0	28	69
Famphur	326.0	217.0	20	93.0	30	68
Thionazin	249.0	113.0	23	97.0	28	58
DEET	192.1	119.1	17	91.1	29	64
Atrazine	216.1	174.1	16	68.1	34	66
Malathion	331.0	127.0	12	99.0	25	55
Triadimefon	294.1	197.1	14	69.1	20	65
Pyrimethanil	200.1	107.1	24	183.1	23	68
Bifenazate	301.1	170.1	18	198.1	6	48
Acetochlor	270.1	224.1	10	148.1	18	58
Sulfotep	323.0	97.0	37	115.0	30	60
Tebuconazole	308.1	70.1	21	125.0	33	66
Zoxamide	336.0	187.0	21	159.0	38	74
Diazinon	305.1	169.1	20	153.1	20	68
TPP (IS)	327.1	152.1	35	77.1	38	95
Cyprodinil	226.1	93.1	33	77.1	43	70
Pyrazophos	374.1	222.1	20	194.1	31	100
Profenofos	372.9	302.9	17	128.0	42	73
Ethion	385.0	142.9	26	199.0	6	56
Chlorpyrifos	349.9	97.0	32	197.9	19	67

Instrument Parameters (Cannabinoids)

HPLC: Thermo Scientific Dionex UltiMate 3000® LC System		
Column: UCT, Selectra®, aQ C18, 100 x 2.1 mm, 3 µm		
Guard column: UCT, Selectra®, aQ C18, 10 x 2.0 mm, 3 µm		
Column temperature: 40 °C		
Column flow rate: 0.300 mL/min		
Auto-sampler temperature: 10 °C		
Injection volume: 5 µL		
Gradient program:		
Time (min)	**A%** (10 mM ammonium acetate in DI water)	**B%** (0.1% formic acid in MeOH)
0	40	60
0.5	40	60
3	5	95
7	5	95
7.1	40	60
10	40	60

Divert mobile phase to waste from 0 – 3 and 8 – 10 min to prevent ion source contamination.

MS parameters	
Instrumentation	Thermo Scientific TSQ Vantage tandem MS
Polarity	ESI +
Spray voltage	3500 V
Vaporizer temperature	450 °C
Ion transfer capillary temperature	350 °C
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	40 arbitrary units
Q1 and Q3 peak width (FWHM)	0.4 and 0.7 Da
Collision gas and pressure	Ar at 1.5 mTorr
Cycle time	0.5 sec
Acquisition method	EZ Method (scheduled SRM)

SRM Table						
Compound	**Precursor**	**Product 1**	**CE1**	**Product 2**	**CE2**	**S-lens RF**
CBD	315.0	193.1	20	123.0	30	77
CBN	311.1	223.1	19	293.2	14	73
THC	315.2	193.1	19	123.1	31	73
THCA-A	357.0	245.1	33	313.2	26	93

Results/Discussion

Pesticides

Due to inconsistent regulations among states that have legalized medical marijuana, as well as states that have decriminalized recreational marijuana, a wide panel of commonly encountered pesticides was selected for this application. DEET, recognized by the EPA as not evoking health concerns to the general public when applied topically, was found on all medical marijuana samples tested[1]. An average of 28 ng/g of DEET was found on medical samples analyzed. Limited research as to possible side effects, if any, of having this pesticide present within volatilized medical grade product is available. Seized street grade marijuana was found to have a variety of pesticides at concentrations higher than what was observed in the medical grade product. Results for selected samples, each coming from separate criminal cases, are shown below:

Sample	Pesticides Detected	Supplemental Info
1	414 ng/g Methamidophos	Discontinued use in commercial settings in 2009[2]
2	2496 ng/g DEET	89% more DEET present compared to medical samples
3	120 ng/g DEET 1385 ng/g Chlorpyrifos	Chlorpyrifos: EPA released proposal in October 2015 to revoke its use[1]
4	6527 ng/g Chlorpyrifos 449 ng/g DEET 72 ng/g Fenamiphos sulfone	Fenamiphos sulfone: restricted use pesticide due to its high acute toxicity[3]
5	178 ng/g Carbaryl 691 ng/g DEET 71 ng/g Malathion	Carbaryl: "likely" to be carcinogenic in humans[1] Malathion: Mosquito control, low risk to human health[1]

Cannabinoids

Tetrahydrocannabinolic acid (THCA-A) is the non-psychoactive precursor to THC. Within fresh plant material, up to 90% of available THC is found in this form[4]. Under intense heating such as when cannabis is smoked, THCA-A is progressively decarboxylated to the psychoactive THC form. Cannabis researchers have begun further research into THCA-A's potential therapeutic properties, such as anti-inflammatory capabilities, antispasmodic treatments and as use as an analgesic[5]. On account of this, the medical marijuana samples specifically were tested for this compound in addition to other commonly noted cannabinoids. On average, 17% of the total weight in each medical marijuana sample came from the presence of THCA-A. In both medical and recreational samples, percentage of THC contribution ranged from 0.9-1.7.

Conclusion

A fast and effective method was developed for the determination of pesticide residues and cannabis potency in recreational and medical marijuana samples. Pesticide residues and cannabinoids were extracted using the QuEChERS approach, followed by either an additional cleanup using a proprietary blend of dSPE sorbents for pesticide analysis, or serial dilutions for cannabinoid potency testing.

References:

1. Environmental Protection Agency
2. Centers for Disease Control and Prevention
3. Paranjape, Kalyani. The Pesticide Encyclopedia. Wallingford, Oxfordshire: CABI, 2015. Print
4. Jung, Julia, Markus R. Meyer, Hans H. Maurer, Christian Neusüß, Wolfgang Weinmann, and Volker Auwärter. "Studies on the Metabolism of the Δ9-tetrahydrocannabinol Precursor Δ9-tetrahydrocannabinolic Acid A (Δ9-THCA-A) in Rat Using LC-MS/MS, LC-QTOF MS and GC-MS Techniques." Journal of Mass Spectrometry J. Mass Spectrom. 44.10 (2009): 1423-433. Web.
5. Galal, Ahmed, Desmond Slade, Waseem Gul, Abir El-Alfy, Daneel Ferreira, and Mahmoud Elsohly. "Naturally Occurring and Related Synthetic Cannabinoids and Their Potential Therapeutic Applications." *RPCN Recent Patents on CNS Drug Discovery* 4.2 (2009): 112-36. Web.

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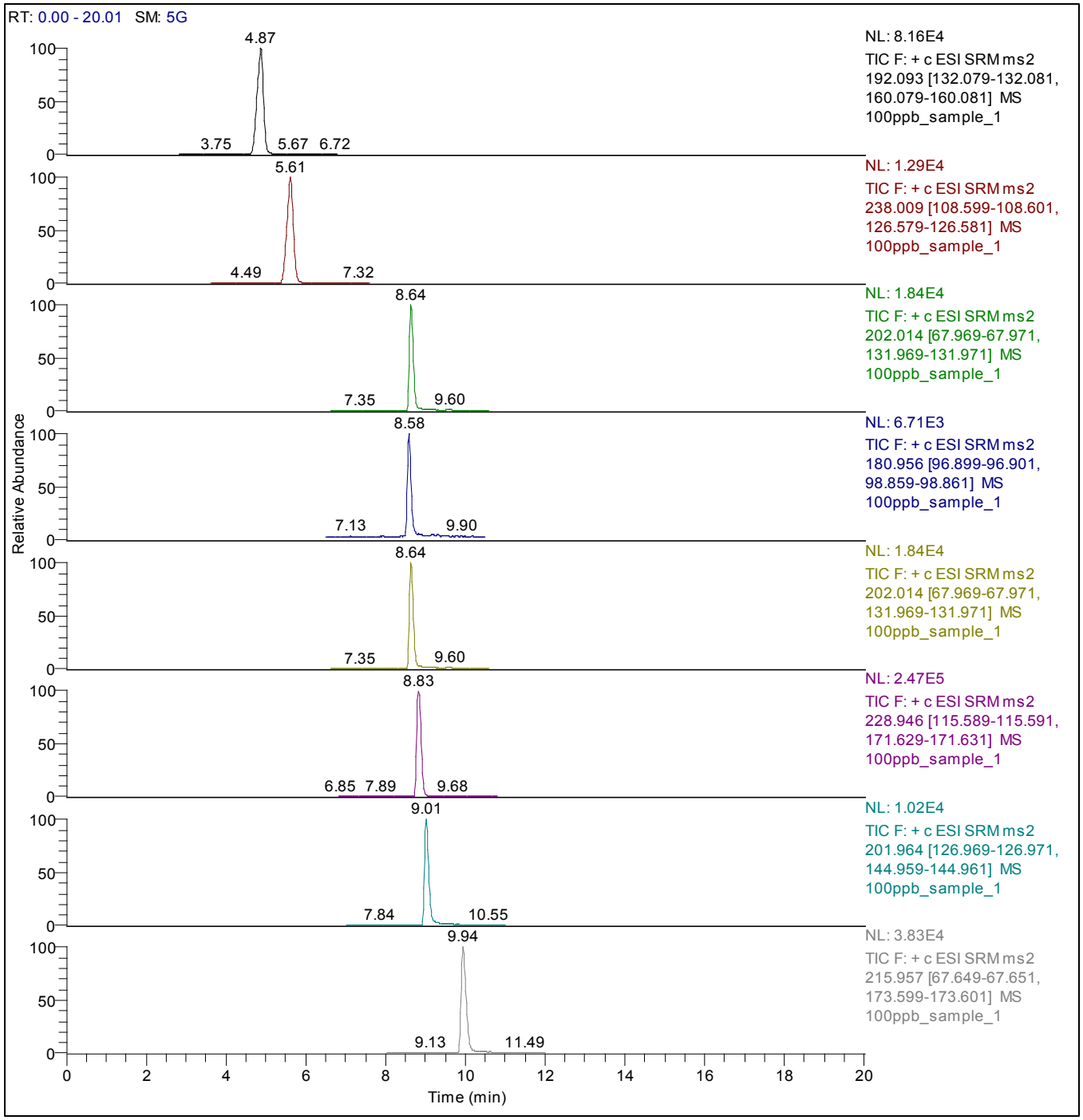
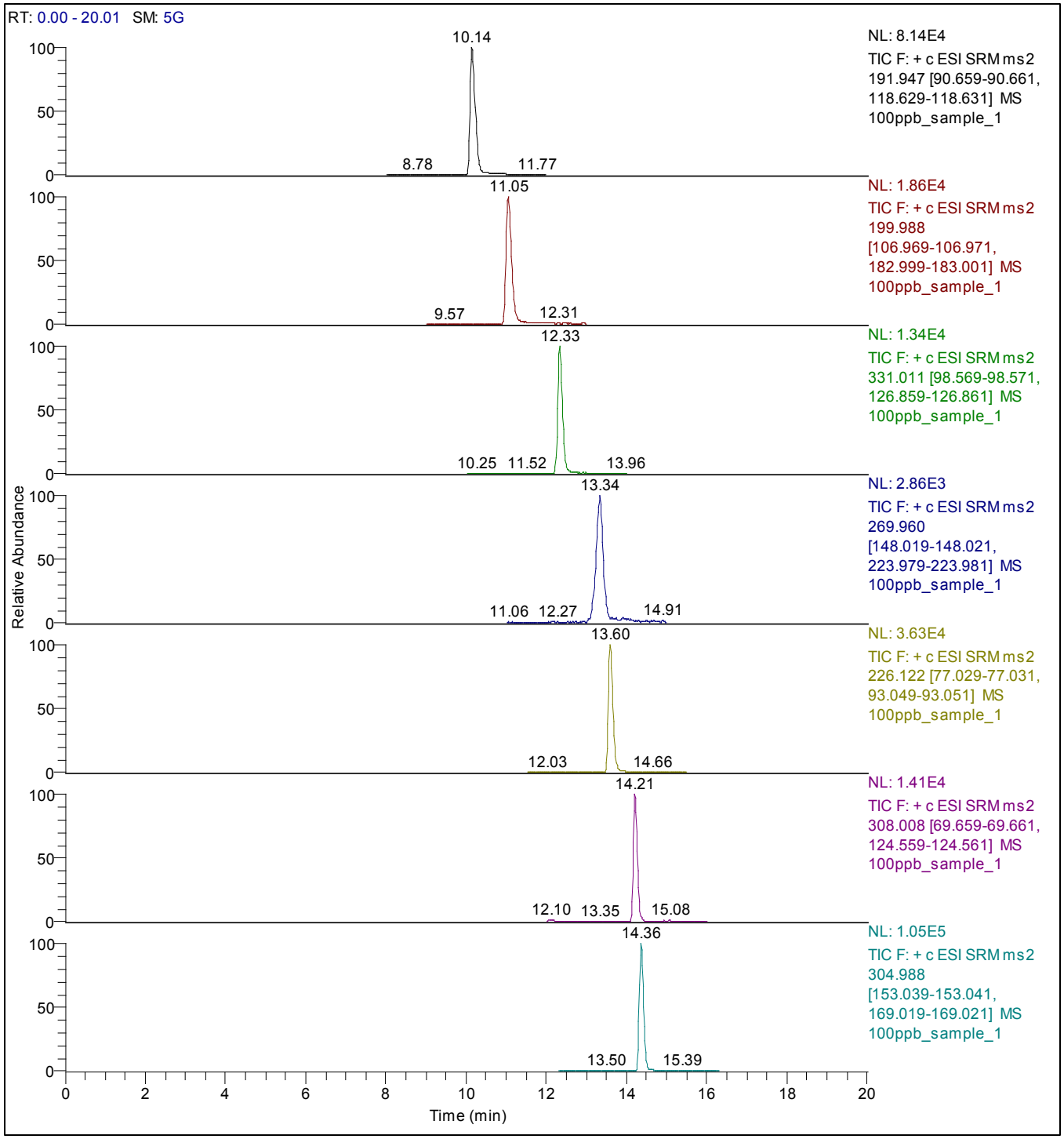
21 PESTICIDES ON SELECTRA C18 BY LC-MS/MS

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8.78
11.77
NL: 8.14E4
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11.05
9.57
12.31
NL: 1.86E4
TIC F: + c ESI SRM ms2 199.988 [106.969-106.971, 182.999-183.001] MS 100ppb_sample_1
12.33
10.25
11.52
13.96
NL: 1.34E4
TIC F: + c ESI SRM ms2 331.011 [98.569-98.571, 126.859-126.861] MS 100ppb_sample_1
13.34
11.06
12.27
14.91
NL: 2.86E3
TIC F: + c ESI SRM ms2 269.960 [148.019-148.021, 223.979-223.981] MS 100ppb_sample_1
13.60
12.03
14.66
NL: 3.63E4
TIC F: + c ESI SRM ms2 226.122 [77.029-77.031, 93.049-93.051] MS 100ppb_sample_1
14.21
12.10
13.35
15.08
NL: 1.41E4
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14.36
13.50
15.39
NL: 1.05E5
TIC F: + c ESI SRM ms2 304.988 [153.039-153.041, 169.019-169.021] MS 100ppb_sample_1
Relative Abundance
Time (min)
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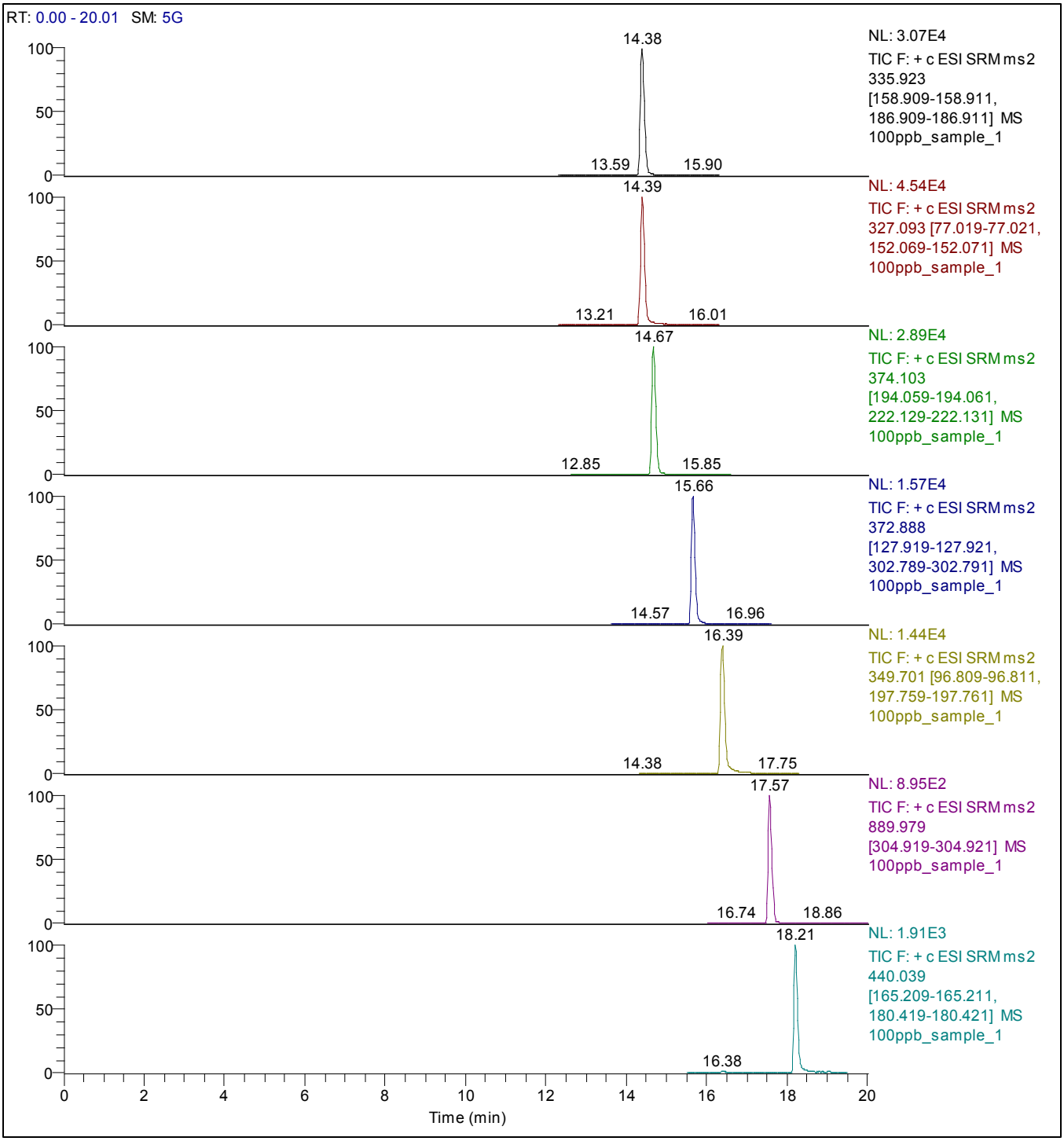
2
4
6
8
10
12
14
16
18
20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13.59
15.90
NL: 3.07E4
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14.39
13.21
16.01
NL: 4.54E4
TIC F: + c ESI SRM ms2 327.093 [77.019-77.021, 152.069-152.071] MS 100ppb_sample_1
14.67
12.85
15.85
NL: 2.89E4
TIC F: + c ESI SRM ms2 374.103 [194.059-194.061, 222.129-222.131] MS 100ppb_sample_1
15.66
14.57
16.96
NL: 1.57E4
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14.38
17.75
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17.57
16.74
18.86
NL: 8.95E2
TIC F: + c ESI SRM ms2 889.979 [304.919-304.921] MS 100ppb_sample_1
18.21
16.38
NL: 1.91E3
TIC F: + c ESI SRM ms2 440.039 [165.209-165.211, 180.419-180.421] MS 100ppb_sample_1
100
50
0
0
2
4
6
8
10
12
14
16
18
20
Time (min)

LC-MS/MS Conditions	
HPLC	Thermo Scientific™ Dionex™ Ultimate™ 3000 LC system
Detector	Thermo Scientific™ TSQ Vantage™ tandem mass spectrometer
Ionization mode	ESI^+
HPLC column	UCT Selectra®C18, 100 × 2.1 mm, 3 µm (p/n: SLC-18100ID21-3UM)
Guard column	UCT Selectra®C18, 10 × 2.0 mm, 3 µm (p/n: SLC-18GDC20-3UM)
Column temp.	40°C
Injection volume	3 µL
Autosampler temp.	10°C
Wash solvent	MeOH:ultrapure water (1:1, v/v)
Mobile phase A	0.1% ammonium formate + 0.3% formic acid
Mobile phase B	methanol + 0.1% formic acid
Flow rate	300 µL/min

MRM Transitions				
Analyte	**t_R (min)**	**Precursor ion**	**Product ion 1**	**Product ion 2**
Carbendazim	4.9	192.09	132.08	160.08
Dicrotophos	5.6	238.01	108.60	126.58
Thiabendazole	8.6	202.06	131.06	175.07
DIMP	8.6	180.96	96.90	98.86
Simazine	8.6	202.01	67.97	131.97
Tebuthiuron	8.8	228.95	115.59	171.63
Carbaryl	9.0	201.96	126.97	144.96
Atrazine	9.9	215.96	67.65	173.60
DEET	10.1	191.95	90.66	118.63
Pyrimethanil	11.0	199.99	106.97	183.00
Malathion	12.3	331.01	98.57	126.86
Acetochlor	13.3	269.96	148.02	223.98
Cyprodinil	13.6	226.12	77.03	93.05
Tebuconazole	14.2	308.01	69.66	124.56
Diazinon	14.3	304.99	153.04	169.02
TPP	14.4	327.09	77.02	152.07
Zoxamide	14.4	335.92	158.91	186.91
Pyrazophos	14.7	374.10	194.06	222.13
Profenofos	15.7	372.89	127.92	302.79
Chlorpyrifos	16.4	349.70	96.81	197.76
Abamectin	17.6	889.98	304.92	751.21
Bifenthrin	18.2	440.04	165.21	180.42

Analysis of 136 pesticides in avocado using a modified QuEChERS method with LC-MS/MS and GC-MS/MS*

UCT Part Numbers:
ECMSSA50CT-MP (6 g of $MgSO_4$ and 1.5 g anhydrous sodium acetate)
CUMPSC18CT (2 mL dispersive cleanup tubes containing 150 mg of anhydrous $MgSO_4$, 50 mg of PSA, and 50 mg endcapped C-18)

July 2013

Summary

A simple, high-throughput modified QuEChERS screening method for the analysis of 136 pesticides in highly fat rich avocado is described. The average recoveries for 79 pesticides by LC-MS/MS at 10, 50, and 200 ng/g fortifying levels were 86% or better (with maximum RSD at 9.2%). GC-MS/MS analysis demonstrated 70% recovery or better (RSD < 18%) from 57 pesticides at the same spike levels.

Table of Pesticides Evaluated for this Method

	Name	Class
Fungicides	Pyrachlostrobin	Strobilurin
	Chlorothalonil	OC
	Pyrimethanil	Anilnopyrimidine
	Imazalil	Imidazole
	o-Phenylphenol	Phenol
	Procymidone	Dicarboximide
	Tebuconazole	Triazole
	Thiabendazole	Benzimidazole
	Tolyfluanid	N-Trihalomethylthio
	Hexachlorobenzene	OC
Insecticides	Bifenthrin	Pyrethroid
	Aminocarb	Carbamate
	Chlorpyrifos	Pyridine OP
	Chlorpyrifos-methyl	Pyridine OP
	Diclorvos	OP
	DDT	OC
	DDE	OC
	Endosulfan	OC
	Ethion	OP
	Methamidophos	OP
	Acephate	OP
	Permethrin	Pyrethroid
	Acetamiprid	Neonicotinoid
Herbicides	Prometryn	Triazine
	Linuron	Phenylurea
	Trifluralin	Dinitroaniline

OC=organochlorine OP=organophosphate

Procedure

1. Sample Preparation

a) Add 3 g of homogenized sample to a 50 mL centrifuge tube
b) Add fortification and/or internal standards
c) Add 5 mL of reagent water and 25 mL of 1% acetic acid in acetonitrile (MeCN) to each sample tube
d) Cap tube and shake for 10 minutes with an SPEX 2000 Geno grinder (or equivalent) @ 1000 stroke/min
e) Add one **ECMSSA50CT** packet to each sample tube and shake for additional 10 min @ 1000 strokes/min
f) Centrifuge @ 3000 rpm for 10 min

2. Sample Clean-up for LC

a) Transfer 1 mL of supernatant to an autosampler vial
b) Sample is ready for LC-MS/MS analysis *(if sample clean-up is desired, see Sample Clean-up for GC below)*

3. Sample Clean-up for GC

a) Pipette 1 mL of supernatant into **CUMPSC18CT** tube
b) Vortex for 1 min
c) Centrifuge @ 2000 rpm for 10 min
d) Sample is ready for GC analysis

Note: Extract a clean matrix and clean-up with the steps above. This extract must be used to prepare matrix-matched calibration standards. Matrix-matching is necessary for this procedure.

LC-MS/MS Parameters
(Equivalent instrumentation may be used)

HPLC Conditions
LC: Shimadzu with two LC 20AD pumps
MS: 4000 Q-TRAP mass spectrometer AB Sciex
Autosampler: Sil-20AC autosampler
Column: Ultra Aqueous C18 column (3 µm, 100 x 2.1 mm) Restek
Guard Column: (10 x 2.1 mm) Restek
Column Oven: CTO-20AC column oven (Shimadzu)
Separation Temp: 50 °C
Software: Analyst software version 1.4
Mobile Phase: **A** 4 mM ammonium formate and 0.1% formic acid in water, **B** 4 mM ammonium formate and 0.1% formic acid in methanol
Mobile Phase Program: Gradient start at 5% B (0.0 - 0.4 min); flow rate of 0.5 mL/min. 60% B at 5 min, then 95% B at 12.5 min, hold until 14.5 min, and concluded by column equilibration at initial condition for 3 min. Total run time18 min.
Injection Volume: 1.0 µL
MS/MS Conditions
Electrospray: positive ion
Ion Transition: 60 sec each analyte
Curtain gas (CUR): 30 psi
Ion Spray V: 4500 volts
Nebulizer Gas (GSI): 60 psi
Heater Gas (GS2) 60 psi
Source Temp (TEM): 350 °C

GC/MS Parameters
(Equivalent instrumentation may be used)

GC Conditions
GC: Agilent 7890A GC,
MS: 7000 triple-quadrupole MS, MassHunter software (version B.05.00412)
Autosampler: 7693 autosampler
Column: two HP-5ms Ultra Inert capillary columns from Agilent (0.25 mm ID x 15m, 0.25 µm film thickness) connected at backflush union
Column Head Pressure: 12.772 psi
Oven Temperature: initial 60° C for 1 min, 40°/min to 170° C , then 10°/min to 310° C. Hold 1.2 min. Total run time 19 min.
Column Flow Rate: 1.335 mL/min He
Injector: 60° C for 0.2 min, ramp to 280° C @ 600° C/min
Autosampler: TriPlus Thermo Fisher Scientific
Back Flush: column 1 for 2 minutes at 310°
Injection Volume: 1.0 µL splitless mode
MS Parameters
Ion Source & Transfer Temp: 300 °C
Electron Multiplier V: 1400 V by auto tune
Collision gas: He & N_2 @ 1.5 and 2.25 mTorr, respectively

Retention Time (RT) and MRM Conditions for LC-MS/MS Analysis

Compound dependent parameters:
DP = declustering potential, CE = collision energy, EP = entrance potential, CXP = collision cell exit potential

Q1	Q3	RT (min)	Analyte	DP	EP	CE	CXP
184.1	143	2.4	**Acephate. 1**	61	10	13	4
184.1	49	2.4	**Acephate. 2**	61	10	33	4
223	126	5.2	**Acetamiprid. 1**	61	10	29	12
223	99	5.2	**Acetamiprid. 2**	61	10	53	18
228.1	186.1	7	**Ametryn. 1**	71	10	21	4
228.1	96	7	**Ametryn 2**	71	10	35	4
209.1	152	3.1	**Aminocarb.1**	71	10	21	8
209.1	137.1	3.1	**Aminocarb.2**	71	10	35	10
318	160.1	7.1	**Azinphos-methyl**	41	10	13	10
318	132	7.1	**Azinphos-methyl**	41	10	21	10
224.1	109	5.8	**Bendiocarb 1**	61	10	27	20
224.1	167.1	5.8	**Bendiocarb 2**	61	10	15	12
440.1	181.2	13.6	**Bifenthrin 1**	51	10	39	14
440.1	166.1	13.6	**Bifenthrin 2**	51	10	65	10
343	307	7.8	**Boscalid.1**	91	10	27	4
343	140	7.8	**Boscalid.2**	91	10	27	4
197	117.2	4.4	**Chlordimeform**	81	10	41	18
197	89	4.4	**Chlordimeform**	81	10	71	14
350	198	12.3	**Chlorpyriphos**	56	10	25	10
350	97	12.3	**Chlorpyriphos**	56	10	47	10
362.8	227	10.2	**Coumaphos**	71	10	37	12
362.8	306.9	10.2	**Coumaphos**	71	10	25	18
241.1	214.2	5.7	**Cyanazine**	66	10	27	18
241.1	104.1	5.7	**Cyanazine**	66	10	47	4
199.1	89.1	7.3	**Cycluron**	50	10	21	4
199.1	89	7.3	**Cycluron**	50	10	21	4
292	70	8	**Cyproconazole A1**	66	10	39	12
292	125	8	**Cyproconazole A2**	66	10	45	8
292.1	70.1	8.4	**Cyproconazole B1**	66	10	39	12
292.1	125.1	8.4	**Cyproconazole B2**	66	10	45	8
318.1	182	6.7	**Desmedipham.1**	41	10	19	12
318.1	136	6.7	**Desmedipham.2**	41	10	33	10
305	169.1	9.9	**Diazinon**	86	10	31	10
305	153.1	9.9	**Diazinon**	86	10	29	8
350	123	8.3	**Dichlorfluanid 1**	21	10	41	10
350	224	8.3	**Dichlorfluanid 2**	21	10	21	10
220.8	127.1	5.9	**Dichlorvos**	71	10	27	22

220.8	109.1	5.9	**Dichlorvos**	71	10	25	18
238.1	112.1	4.6	**Dicrotophos.1**	66	10	19	8
238.1	193	4.6	**Dicrotophos.2**	66	10	15	14
406.1	251.1	11.6	**Difenoconazole 1**	81	10	37	16
408.2	253.1	11.6	**Difenoconazole 2**	76	10	33	4
230	199	4.6	**Dimethoate.1**	50	10	14	15
230	125	4.6	**Dimethoate.2**	50	10	27	8
388.1	301	8.1	**Dimethomorph A1**	66	10	25	4
388.1	165.1	8.1	**Dimethomorph A2**	66	10	45	4
388.2	301.1	8.4	**Dimethomorph B1**	66	10	25	4
388.2	165.2	8.4	**Dimethomorph B2**	66	10	45	4
224.1	167	4.7	**Dioxacarb.1**	51	10	13	10
224.1	123	4.7	**Dioxacarb.2**	51	10	23	24
330	121.1	9.5	**Epoxiconazole. 1**	66	10	29	10
330	101.1	9.5	**Epoxiconazole. 2**	66	10	69	18
162	119	8.4	**Ethiolate. 1**	106	10	23	20
162	120.1	8.4	**Ethiolate. 2**	106	10	19	20
384.8	199.2	12	**Ethion. 1**	51	10	15	18
384.8	142.9	12	**Ethion. 2**	51	10	39	24
287.1	121.1	7.1	**Ethofumesate. 1**	81	10	23	8
287.1	259.1	7.1	**Ethofumesate. 2**	81	10	15	16
394.2	177.3	13.6	**Etofenprox NH_4 +1**	46	10	21	12
394.2	107.2	13.6	**Etofenprox NH_4 +2**	46	10	61	18
337	124.9	9.4	**Fenbuconazole.1**	81	10	41	8
337	70	9.4	**Fenbuconazole.2**	81	10	39	12
302.1	88	9.2	**Fenoxycarb.1**	66	10	31	6
302.1	116.1	9.2	**Fenoxycarb.2**	66	10	17	8
304	147	7.2	**Fenpropimorph.1**	66	10	39	4
304	117	7.2	**Fenpropimorph.2**	66	10	71	4
266	229	7.6	**Fludioxinil.1**	41	10	23	14
266	227.1	7.6	**Fludioxinil.2**	41	10	13	14
376	307	8.5	**Fluquinconazole.1**	71	10	33	4
376	349	8.5	**Fluquinconazole.2**	71	10	25	4
324.1	262.1	7.5	**Flutolanil.1**	76	10	27	16
324.1	242.1	7.5	**Flutolanil.2**	76	10	37	14
314.1	70	10.3	**Hexaconazole.1**	56	10	41	12
314.1	159	10.3	**Hexaconazole.2**	56	10	41	14
297	159	6.5	**Imazalil.1**	66	10	33	14
297	201	6.5	**Imazalil.2**	66	10	27	12
249.1	160	7.7	**Linuron.1**	61	10	23	4
249.1	182.1	7.7	**Linuron.2**	61	10	21	4
331	127.1	7.5	**Malathion. 1**	46	10	17	10

331	99.1	7.5	**Malathion. 2**	46	10	31	10
142	94	1.7	**Methamidophos.1**	55	10	20	4
142	125	1.7	**Methamidophos.2**	55	10	19	8
284.2	252.2	8.7	**Metolachlor. 1**	56	10	21	10
284.2	176.2	8.7	**Metolachlor. 2**	56	10	33	10
166.2	109.1	5.6	**Metolcarb. 1**	36	10	15	10
166.2	94.2	5.6	**Metolcarb. 2**	36	10	37	10
225.1	127.1	4.7	**Mevinphos-E.1**	55	10	20	8
225.1	193.2	4.7	**Mevinphos-E.2**	55	10	10	13
225	127	5.2	**Mevinphos-Z.1**	55	10	20	8
225	193.1	5.2	**Mevinphos-Z.2**	55	10	10	13
224.1	127.1	4.1	**Monocrotophos.1**	51	10	23	12
224.1	98	4.1	**Monocrotophos.2**	51	10	17	4
215.1	126.1	6.4	**Monolinuron.1**	51	10	23	4
215.1	99	6.4	**Monolinuron.2**	51	10	41	4
289	70	8.3	**Myclobutanil.1**	71	10	37	12
289	125	8.3	**Myclobutanil.2**	71	10	47	8
315	252.1	7.4	**Nuarimol.1**	81	10	31	16
315	81	7.4	**Nuarimol.2**	81	10	45	14
214	124.9	3	**Omethoate.1**	46	10	29	4
214	182.8	3	**Omethoate.2**	46	10	17	4
284.1	159	10.4	**Penconazole.1**	71	10	39	10
284.1	70	10.4	**Penconazole.2**	71	10	37	12
318	160	7.1	**Phosmet.1**	51	10	19	10
318	133	7.1	**Phosmet.2**	51	10	49	10
356.2	177.2	12.1	**Piperonyl butoxide 1**	51	10	19	10
356.2	119.1	12.1	**Piperonyl butoxide 2**	51	10	51	8
239.2	72.1	5.9	**Pirimicarb.1**	66	10	35	12
239.2	182.1	5.9	**Pirimicarb.2**	66	10	23	12
376	308	10.9	**Prochloraz.1**	46	10	17	10
376	70	10.9	**Prochloraz.2**	46	10	45	12
242.2	158.1	7.8	**Prometryn.1**	71	10	35	4
242.2	200.1	7.8	**Prometryn.2**	71	10	19	4
212.2	169.9	6.6	**Propachlor. 1**	66	10	23	30
212.2	93.9	6.6	**Propachlor. 2**	66	10	39	16
368.2	231.1	12.6	**Propargite.1**	46	10	15	14
368.2	175.1	12.6	**Propargite.2**	46	10	23	12
342.1	159	10.6	**Propiconazole.1**	61	10	39	10
342.1	69	10.6	**Propiconazole.2**	61	10	37	12
210.1	111	5.8	**Propoxur.1**	39	10	19	6
210.1	168.1	5.8	**Propoxur.2**	39	10	11	11
218.1	125	6	**Pyracarbolid.1**	61	10	27	8

218.1	97	6	**Pyracarbolid.2**	61	10	41	18
388	194	10.5	**Pyraclostrobin.1**	31	10	19	4
388	163	10.5	**Pyraclostrobin.2**	31	10	29	4
365	147	13.3	**Pyridaben.1**	46	10	31	4
365	309	13.3	**Pyridaben.2**	46	10	19	4
200	107	7.7	**Pyrimethanil.1**	71	10	33	4
200	82	7.7	**Pyrimethanil.2**	71	10	35	4
308.1	162.1	12.9	**Quinoxyfen.1**	81	10	65	10
308.1	197.1	12.9	**Quinoxyfen.2**	81	10	45	12
226.2	170.1	6.5	**Secbumeton.1**	50	10	35	4
226.2	100	6.5	**Secbumeton.2**	50	10	35	4
298.2	144.2	7.9	**Spiroxamine.1**	76	10	29	12
298.2	100.1	7.9	**Spiroxamine.2**	76	10	47	18
323	115	8.9	**Sulfotep. 1**	46	10	39	10
323	97.1	8.9	**Sulfotep. 2**	46	10	45	10
308.2	70	9.9	**Tebuconazole.1**	81	10	49	12
308.2	125	9.9	**Tebuconazole.2**	81	10	51	8
334	117	12.1	**Tebufenpyrad.1**	71	10	47	4
334	145	12.1	**Tebufenpyrad.2**	71	10	37	4
230.3	174.2	7.7	**Terbutylazine 1**	41	10	27	10
230.3	68	7.7	**Terbutylazine 2**	41	10	59	10
372.1	159	8.8	**Tetraconazole.1**	76	10	45	10
372.1	70	8.8	**Tetraconazole.2**	76	10	47	12
202.1	175.1	4.9	**Thiabendazole.1**	85	10	35	12
202.1	131.2	4.9	**Thiabendazole.2**	85	10	45	8
364	237.9	9.5	**Tolyfluanid.1**	6	10	19	10
364	137.1	9.5	**Tolufluanid.2**	6	10	37	10
294	197.1	7.8	**Triadimefon.1**	66	10	23	14
294	225	7.8	**Triadimefon.2**	66	10	19	8
296.1	70	8	**Triadimenol.1**	46	10	31	12
296.1	227.1	8	**Triadimenol.2**	46	10	19	14
314	162	8.3	**Triazophos 1**	56	10	25	10
314	119	8.3	**Triazophos 2**	56	10	49	10
190	163	5.8	**Tricyclazole 1**	81	10	33	10
190	136	5.8	**Tricyclazole 2**	81	10	41	12
409	186	11.2	**Trifloxystrobin. 1**	31	10	23	4
409	206	11.2	**Trifloxystrobin. 2**	31	10	21	4
346.1	278.1	11.7	**Triflumizole. 1**	51	10	15	8
346.1	73	11.7	**Triflumizole. 2**	51	10	27	6
346.1	278.1	11.8	**Triflumizole. 1**	51	10	15	8
346.1	73	11.8	**Triflumizole. 2**	51	10	27	6

GC-MS/MS Conditions for GC-amenable Pesticides

Analyte	Precursor 1	Product 1	Collision Energy	Precursor 2	Product 2	Collision Energy	RT (min)
Amitraz	293.1	162	6	293.1	132	25	14.77
Benfluralin	292	160	22	292	206	12	7.29
BHC-alpha	219	183	7	181	145	15	7.64
BHC-beta	219	183	8	217	181	7	8.03
BHC-delta	219	183	8	217	181	7	8.51
BHC-gamma	219	183	8	217	181	7	8.04
Bromopropylate	338.9	182.9	18	342.9	184.9	18	13.89
Cadusafos	159	97	24	158	81	15	7.44
Chlorothalonil	265.9	133	53	265.9	169.9	28	8.59
Chlorpyrifos-methyl	285.9	93	24	285.9	208	15	9.13
Cypermethrin	181	152	30	163	127	4	16.56
Dacthal	298.9	164.9	54	300.9	222.9	30	10.04
DEF	202	147	2	202	113	18	11.57
Dieldrin	262.9	192.9	40	262.9	190.9	38	11.7
Dinitramine	261	195	23	261	241	10	8.4
Endosulfan Sulfate	271.9	236.9	15	271.9	116.9	48	13
Endosulfan-I	240.9	205.9	15	195	159	8	11.25
Endosulfan-II	195	159	8	240.9	205.9	15	12.25
Endrin	262.9	192.9	40	262.9	190.9	38	12.1
EPN	157	110	14	185	110.1	25	13.92
Etridiazole	210.9	182.9	9	210.9	139.9	26	5.87
Fenarimol	219	107	12	251	139	15	15.06
Fenvalerate 1	167	125	12	125	89	23	17.38
Fenvalerate 2	167	125	12	125	89	23	17.58
Fluvalinate 1	250	55	18	250	200	24	17.55
Fluvalinate 2	250	55	18	250	200	24	17.6
Heptachlor	352.8	262.8	15	352.8	281.9	18	10.6
Hexachlorobenzene	283.9	213.9	40	283.8	248.9	22	7.78
L-Cyhalothrin	197	141	13	181	152	29	14.85
Iprodione	314	56	24	314	245	10	13.68
Methyl Parathion	263	109	12	263	79	32	9.13
MGK-264	164	80	32	164	98	12	10.42
Napropamide	271.1	72	15	271.1	128	2	11.39
o,p'-DDT	235	165	30	235	199	18	12.42
o,p'-Methoxychlor	227	121	15	121	78	26	13.19
o-phenylphenol	170	115.1	45	170	141	30	6.27
Oxadixyl	163	132	10	163	117	30	12.42
p,p'-DDE	246	176	35	318	246	25	11.6
p,p'-DDT	235	165	30	235	199	18	13.01

Parathion	291	109	10	291	81	35	9.96
Pentachloroaniline	262.9	191.9	25	264.9	193.9	28	8.91
Pentachlorobenzene	249.9	214.9	21	249.9	141.9	50	6.38
Permethrin-cis	183	153	18	183	115	30	15.62
Permethrin-trans	183	153	18	183	115	30	15.74
Phosalone	182	75	36	182	111	17	14.56
Pirimiphos-methyl	290	125	24	290	233	10	9.58
Procymidone	283	96	10	283	67	37	10.83
Profenofos	336.9	266.9	14	336.9	188	32	11.53
Pronamide	173	74	50	173	109	30	8.18
Propanil	161	99	30	217	161	7	8.93
Pyriproxifen	136	41.1	18	136	78.1	32	14.6
Quinalphos	157	102	28	146	118	10	10.72
Tetradifon	353.9	159	12	353.9	227	9	14.39
Tolclofos-methyl	265	93	26	265	109	52	9.22
Triallate	268	183.9	20	268	226	12	8.56
Trifluralin	306	264	7	306	160	25	7.25
Vinclozolin	212	172	16	187	124	22	9.1

Average Recovery and RSD of 79 Pesticides Spiked in Avocado at Three Concentrations via LC-MS/MS Analysis

	10 ng/g spike level N=5		50 ng/g spike level N=5		200 ng/g spike level N=5	
Analyte	**Recovery %**	**RSD %**	**Recovery %**	**RSD %**	**Recovery %**	**RSD %**
Acephate	104.9	5.0	82.6	11.8	92.6	6.3
Acetamiprid	102.7	6.7	84.6	8.9	96.4	3.9
Ametryn	99.8	3.9	84.3	11.4	91.4	6.1
Aminocarb	104.4	2.4	83.9	10.3	93.4	5.3
Azinphos-methyl	115.0	7.3	87.7	11.1	98.3	5.6
Bifenthrin	104.7	6.1	85.1	10.7	93.9	8.8
Boscalid	121.6	7.1	105.6	14.3	85.7	6.0
Chlordimeform	120.2	6.7	88.5	15.9	95.2	3.9
Chlorpyrifos	102.3	9.3	86.7	12.7	91.9	4.4
Coumaphos	99.0	5.7	81.9	11.2	91.8	4.4
Cyanazine	115.0	2.9	87.1	13.2	87.1	13.2
Cycluron	121.0	4.6	91.9	10.9	103.0	3.8
Cyproconazole A	140.0	9.2	86.7	14.3	93.9	6.0
Cyproconazole B	116.6	8.3	115.2	34.6	102.5	5.1
Desmedipham	110.8	5.9	108.3	29.9	103.4	6.8
Diazinon	112.0	3.7	87.1	11.7	95.3	4.7
Dichlorfluanid	99.8	9.1	84.1	11.0	92.3	4.6
Dichlorvos	83.2	18.8	77.2	9.3	86.8	4.0
Dicrotophos	80.8	14.8	74.7	5.7	93.8	9.5

Difenoconazole	103.6	3.2	84.1	11.9	92.6	5.2
Dimethoate	111.6	5.1	87.3	12.6	100.3	6.9
Dimethomorph A	103.3	4.6	83.9	12.3	92.8	4.2
Dimethomorph B	97.1	5.3	90.3	9.3	98.1	4.9
Dioxacarb	116.0	8.6	86.6	9.5	100.6	5.1
Epoxiconazole	97.2	4.0	83.5	12.1	92.9	5.3
Ethiolate	107.5	4.9	86.5	11.6	98.7	6.8
Ethion	102.0	8.3	88.8	15.8	94.0	8.7
Ethofumesate	98.3	6.5	83.3	11.4	92.4	5.5
Fenbuconazole	107.4	16.9	84.3	14.2	96.3	6.6
Fenoxycarb	104.1	14.4	92.0	12.3	102.3	7.2
Fenpropimorph	105.0	7.1	82.1	11.2	94.1	4.9
Fludioxinil	110.6	8.4	82.0	11.7	92.0	6.2
Fluquinconazole	118.0	13.6	83.9	16.9	102.5	8.9
Flutolanil	146.2	7.5	90.4	18.0	97.6	5.1
Hexaconazole	109.0	4.9	85.8	13.7	93.3	3.5
Imazalil	117.0	4.2	88.4	14.6	100.9	9.5
Linuron	123.4	8.6	94.5	13.9	97.7	6.3
Malathion	103.2	12.4	87.4	14.2	97.1	5.3
Methamidophos	113.0	2.3	83.1	15.9	93.3	7.7
Metolachlor	102.5	2.5	81.7	11.3	94.4	6.3
Metolcarb	100.1	5.9	83.3	13.3	93.5	4.6
Mevinphos-E	108.1	8.2	84.1	11.0	90.4	3.1
Mevinphos-Z	99.6	14.7	83.9	9.3	91.1	4.8
Monocrotophos	97.0	3.3	82.5	8.7	90.4	4.5
Monolinuron	105.0	4.8	85.1	11.8	93.1	5.4
Myclobutanil	110.4	3.1	87.0	11.6	93.0	4.5
Nuarimol	111.2	12.6	91.8	7.8	96.5	4.5
Omethoate	137.0	15.4	83.8	10.9	98.6	7.3
Penconazole	113.4	7.9	88.4	13.6	96.4	5.8
Phosmet Piperonyl	104.8	3.1	85.4	8.3	96.0	6.4
butoxide	106.0	4.1	83.0	10.4	91.5	6.6
Pirimicarb	104.3	2.9	84.5	11.1	93.0	5.6
Prochloraz	124.2	29.9	83.9	10.7	92.6	5.8
Prometryn	101.0	8.5	85.6	10.4	95.7	5.4
Propachlor	101.0	4.5	81.2	12.6	92.2	5.5
Propargite	109.2	6.7	84.2	7.0	91.8	5.5
Propiconazole	106.2	7.1	85.0	13.2	97.3	9.7
Propoxur	97.0	5.1	83.8	10.3	92.4	4.2
Pyracarbolid	101.3	3.2	82.9	13.4	93.0	5.7
Pyraclostrobin	109.6	7.6	83.8	10.9	93.0	5.3
Pyridaben	95.2	7.1	78.6	10.2	85.8	5.5
Pyrimethanil	107.0	15.4	91.2	12.0	93.3	6.5
Quinoxyfen	105.6	6.5	84.6	9.3	92.0	3.1

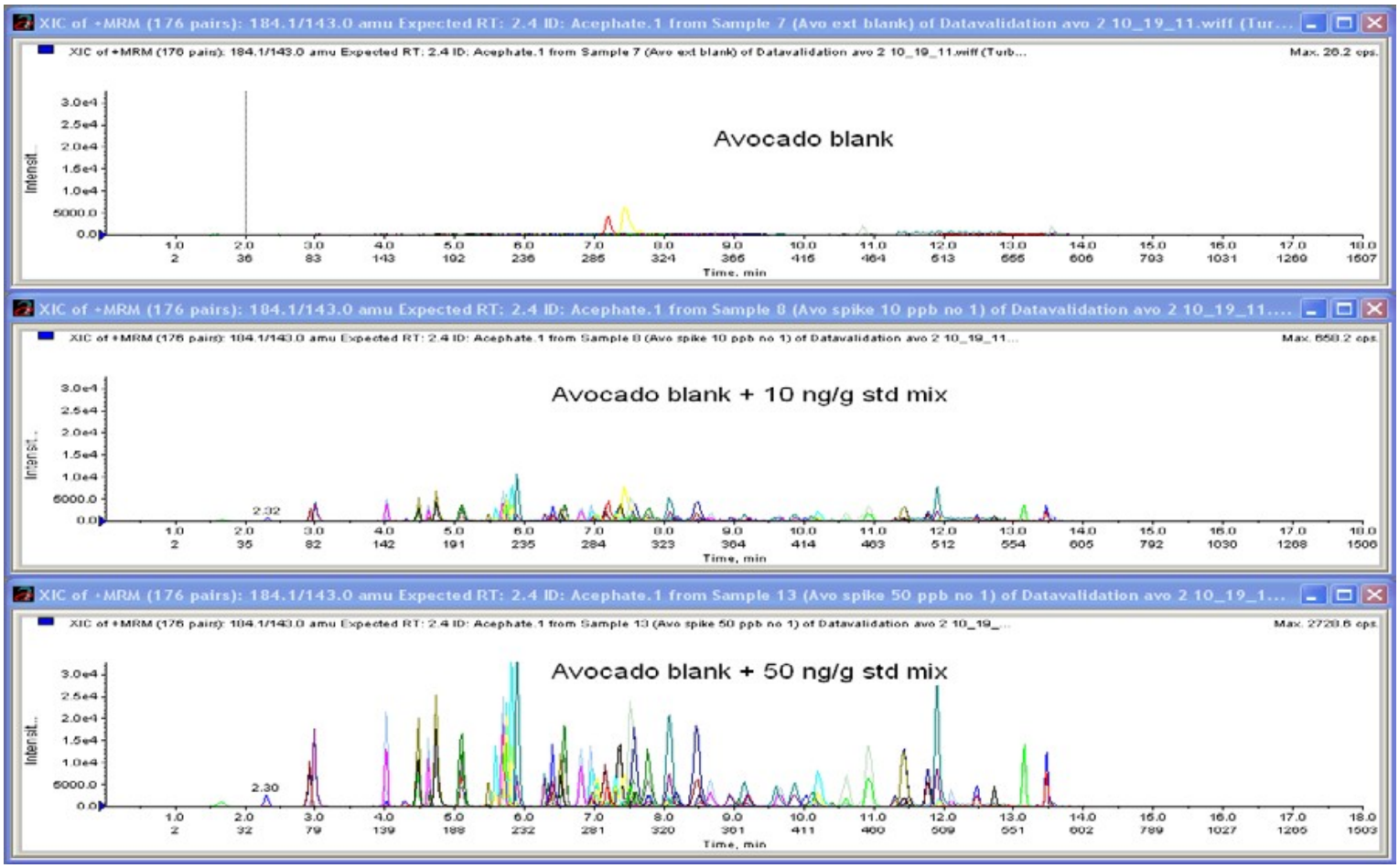
Secbumeton	103.8	5.8	82.2	8.7	92.7	5.1
Spiroxamine	104.6	4.3	83.4	12.6	94.5	6.1
Sulfotep	108.2	7.7	84.7	11.8	91.7	5.6
Tebuconazole	110.6	5.9	88.2	9.8	102.7	9.6
Tebufenpyrad	106.8	10.9	81.9	11.6	95.3	5.9
Terbutylazine	101.4	5.9	84.0	8.8	93.4	4.3
Tetraconazole	112.4	10.7	89.4	5.6	104.0	5.9
Thiabendazole	110.6	4.2	84.9	10.2	94.4	7.3
Tolyfluanid	129.6	4.2	86.7	9.6	89.8	6.0
Triadimefon	95.9	16.4	86.8	7.0	99.9	6.1
Triazophos	102.9	25.3	89.1	7.7	102.9	6.6
Tricyclazole	104.3	4.5	84.0	9.1	93.3	4.2
Trifloxystrobin	96.8	4.2	82.7	10.6	90.9	5.9
Triflumizole	101.5	5.7	84.0	11.1	92.1	4.7
Average	**107.1**		**86.1**		**94.8**	
Std. Dev	**9.9**		**5.8**		**4.0**	
RSD %	**9.2**		**6.7**		**4.2**	

Average Recovery and RSD of 57 Pesticides Spiked in Avocado at Three Concentrations with GC-MS/MS Analysis

Analyte	10 ng/g spike level N=5		50 ng/g spike level N=5		200 ng/g spike level N=5	
	Recovery %	RSD %	Recovery %	RSD %	Recovery %	RSD %
Amitraz	31.8	12.7	38.3	18.0	58.0	7.2
Benfluralin	81.3	9.4	68.5	12.5	91.3	4.8
BHC-alpha	74.9	5.2	76.1	11.9	95.7	3.5
BHC-beta	93.4	12.2	73.2	20.4	103.5	2.7
BHC-delta	70.5	4.8	76.5	12.0	95.4	4.1
BHC-gamma	84.2	12.2	73.1	20.5	101.7	3.5
Bromopropylate	60.2	15.7	69.2	13.7	97.1	5.1
Cadusafos	69.8	3.4	68.8	11.4	92.0	3.1
Chlorothalonil	70.4	28.2	52.2	14.2	81.9	19.4
Chlorpyrifos-methyl	79.0	9.0	73.7	12.4	92.3	7.5
Cypermethrin	130.7	11.0	104.2	10.3	92.3	5.9
Dacthal	70.1	7.5	71.1	14.5	90.2	3.4
DEF	57.1	18.9	61.6	11.0	94.2	6.6
Dieldrin	83.0	26.3	73.8	11.3	94.4	3.6
Dinitramine	92.2	6.5	77.7	12.0	95.2	4.6
Endosulfan Sulfate	106.9	14.2	69.2	22.4	106.2	5.8
Endosulfan-I	91.4	31.7	72.6	16.2	92.2	11.3
Endosulfan-II	78.2	7.3	70.6	9.2	100.0	5.9
Endrin	99.7	12.6	73.4	11.9	100.0	5.7

EPN	66.7	26.7	68.5	13.9	107.5	4.8
Etofenprox	82.8	8.9	78.8	11.6	89.0	4.8
Etridiazole	104.7	7.0	68.7	15.1	110.4	11.2
Fenarimol	63.2	7.7	65.8	15.3	96.9	6.6
Fenvalerate 1	72.2	27.7	76.9	14.3	102.9	7.7
Fenvalerate 2	75.4	20.2	63.9	22.5	92.3	3.9
Fluvalinate 1	58.4	31.4	65.0	17.9	99.6	5.2
Fluvalinate 2	51.5	37.4	57.5	27.5	81.7	11.9
Heptachlor	65.4	17.7	69.7	13.3	95.1	6.1
Hexachlorobenzene	60.6	9.1	61.6	11.9	81.0	6.1
L-Cyhalothrin	66.3	13.9	75.2	9.3	98.0	6.2
Iprodione	37.0	82.8	68.7	14.1	92.7	16.9
Methyl Parathion	75.0	14.1	77.0	13.8	95.6	5.2
MGK-264	74.1	10.1	70.8	11.7	97.7	2.0
Napropamide	74.4	10.2	74.7	15.4	103.7	4.9
o,p'-DDT	94.2	20.3	62.1	29.8	119.2	23.1
o,p'-Methoxychlor	80.5	12.3	84.9	18.5	112.0	15.3
o-phenylphenol	105.0	17.9	76.7	11.3	83.6	5.1
Oxadixyl	64.6	8.6	73.9	13.4	76.6	6.6
p,p'-DDE	61.5	7.4	67.2	14.3	89.0	4.7
p,p'-DDT	NA	NA	NA	NA	NA	NA
Parathion	58.5	14.6	66.4	13.3	94.2	4.6
Pentachloroaniline	71.3	5.0	70.0	11.7	89.9	3.8
Pentachlorobenzene	70.5	4.6	68.2	13.0	85.4	3.8
Permethrin-cis	89.9	12.5	62.1	13.8	93.6	4.8
Permethrin-trans	98.5	14.1	74.7	34.7	111.6	9.1
Phosalone	74.4	15.0	75.6	11.0	108.0	8.5
Pirimiphos-methyl	77.7	11.5	72.2	12.7	92.5	2.1
Procymidone	76.8	5.0	75.6	11.6	98.5	13.5
Profenofos	52.2	37.2	95.1	6.5	89.6	3.7
Pronamide	71.3	8.6	71.7	15.7	93.2	5.2
Propanil	72.4	9.0	72.2	13.8	96.1	6.4
Pyriproxifen	64.8	7.4	67.9	13.4	96.1	6.4
Quinalphos	79.5	15.8	67.5	13.4	91.1	5.0
Tetradifon	66.3	5.9	72.1	11.3	88.4	8.5
Tolclofos-methyl	81.6	3.7	75.4	10.9	94.5	3.7
Triallate	70.3	4.4	67.4	17.1	92.3	4.7
Trifluralin	63.9	9.2	70.8	10.4	95.5	5.7
Vinclozolin	71.5	11.0	70.6	9.8	101.3	6.5
Average	**73.9**		**70.2**		**94.3**	
Std. Dev	**15.0**		**7.9**		**17.0**	
RSD %	**20.3**		**11.3**		**18.0**	

Reconstructed LC-MS/MS Chromatogram of Avocado Blank, Avocado Blank Fortified at 10 ng/g, and Avocado Blank Spiked with 50 ng/g Standard Mix Sample Concentration is 0.12 G Sample/Ml Solvent With 1 µL Injection Volume



* Adapted from: 'Analysis of 136 Pesticides in Avocado Using a Modified QuEChERS Method with LC-MS/MS and GC-MS/MS'
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Determination of Pesticides in Bananas by QuEChERS and LC-MS/MS

UCT Part Numbers:
Enviro-Clean® RFV0050CT (50 mL centrifuge tubes)
Enviro-Clean® ECMSSA50CT-MP (Mylar pouch containing 6 g $MgSO_4$ and 1.5 g NaOAc)
Enviro-Clean® CUMPSC18CT (2 mL dSPE tube with 150 mg MgSO4, 50 mg PSA and 50 mg C18)

July 2013

Summary:

This application describes a simple, fast, and cost-effective method for the determination of multi-class pesticides in bananas including one of the most difficult compounds, pymetrozine. The method employs the AOAC version of the QuEChERS procedure, in which 15 g of the homogenized banana sample is hydrated with 5 mL of reagent water to give a sample with > 80% water content. The hydrated sample is extracted by 15 mL of acetonitrile (MeCN) with 1% (v/v) acetic acid (HAc), followed by the addition of 6 g anhydrous magnesium sulfate ($MgSO_4$) and 1.5 g sodium acetate (NaOAc). After shaking and centrifugation, 1 mL of the supernatant is transferred to a 2-mL dSPE tube containing 150 mg $MgSO_4$, 50 mg PSA, and 50 mg C18. The $MgSO_4$ absorbs residual water; PSA removes organic acids and sugars, while the C18 removes fatty acids and other non-polar interferences in the sample. The result is a clean extract for LC-MS/MS analysis.

Matrix matched calibration curves were constructed for pesticide quantification. The responses for all 24 pesticides were linear with R^2 ranging from 0.9939 to 0.9998 over the concentration range of 2 to 400 ng/g.

Recoveries were excellent with an average recovery of 97% and an average relative standard deviation of about 6.25% using the data from spiked matrix samples with concentrations of 10ng/g and 50ng/g.

The results from this application indicate this pesticide method is suitable in bananas especially when pymetrozine is required to be analyzed.

Procedure:

1. **QuEChERS extraction**
    a) Weigh 15 ± 0.15 g of peeled and homogenized banana sample into a 50-mL centrifuge tube (**RFV0050CT**). Prepare 5 fortified samples, each at two spiking levels.
    b) Add 5 mL of reagent water to each tube (to increase the water content in banana from 74% to > 80%).
    c) Add 30 µL of 50-ppm triphenyl phosphate (TPP) internal standard (IS) solution to all samples, and appropriate amounts of 2-ppm pesticide working solution to fortified samples.
    d) Add 15 mL of MeCN with 1%(v/v) HAc. Cap and shake for 1 min at 1000 strokes/min using a Spex 2010 Geno-Grinder.
    e) Add salts (6 g MgSO4 and 1.5 g NaOAc) from pouch (**ECMSSA50CT-MP**), and vortex for 10 sec to break up any salt agglomerates.
    f) Shake for 1 min at 1000 strokes/min using Spex 2010 Geno-Grinder.
    g) Centrifuge at 5000 rpm (or 3830 rcf) for 5 min.

2. **dSPE cleanup**
    a) Transfer 1 mL of the supernatant to 2 mL dSPE tube (**CUMPSC18CT**).
    b) Shake for 2 min at 1000 strokes/min using Spex 2010 Geno-Grinder.
    c) Centrifuge at 10,000 rpm (or 15,300 rcf) for 5 min.
    d) Transfer 0.3 mL of the cleaned extract into a 2-mL auto-sampler vial, add 0.3 mL of reagent water, and vortex for 30 sec.
    e) The samples are ready for LC-MS/MS analysis.

LC-MS/MS method:

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-sampler Temperature: 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	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

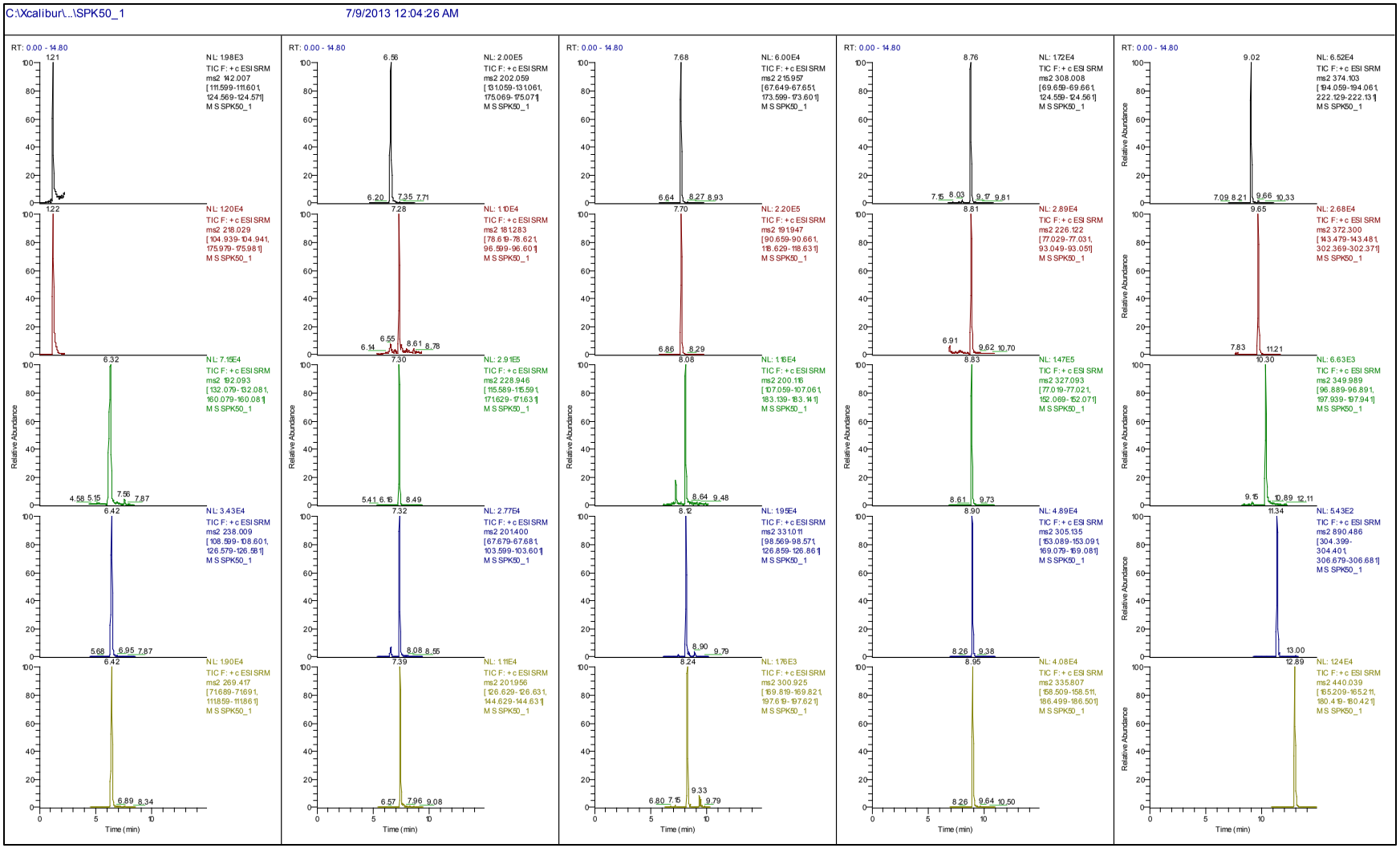
Name	Rt (min)	Precursor ion	Product ion 1	CE 1	Product ion 2	CE 2	S-lens (V)
Methamidophos	1.21	142.007	124.57	14	111.6	5	60
Pymetrozine	1.22	218.029	104.94	18	175.98	16	70
Carbendazim	6.32	192.093	160.08	17	132.08	29	81
Dicrotophos	6.42	238.009	126.58	17	108.60	33	73
Acetachlor	6.42	269.417	111.86	15	71.69	33	72
Thiabendazole	6.56	202.059	175.07	24	131.06	31	103
DIMP	7.28	181.283	96.60	13	78.62	32	44
Tebuthiuron	7.30	228.946	171.63	17	115.59	26	72
Simazine	7.32	201.400	67.68	33	103.60	24	85
Carbaryl	7.39	201.956	144.63	7	126.63	30	40
Atrazine	7.68	215.957	173.60	16	67.65	35	79
DEET	7.70	191.947	118.63	15	90.66	28	92
Pyrimethanil	8.08	200.116	107.06	23	183.14	22	66
Malathion	8.12	331.011	126.86	12	98.57	23	60
Bifenazate	8.24	300.925	169.82	15	197.62	5	51
Tebuconazole	8.76	308.008	69.66	29	124.56	35	97
Cyprodinil	8.81	226.122	93.05	33	77.03	40	88
TPP (IS)	8.83	327.093	152.07	33	77.02	37	98
Diazinon	8.90	305.135	169.08	14	153.09	15	89
Zoxamide	8.95	335.807	158.51	38	186.50	20	102
Pyrazophos	9.02	374.103	222.13	20	194.06	20	104
Profenofos	9.65	372.300	302.37	19	143.48	35	104
Chlorpyrifos	10.30	349.989	197.94	17	96.89	32	69
Abamectin	11.34	890.486	304.40	18	306.68	15	102
Bifenthrin	12.89	440.039	180.42	11	165.21	39	66

Results:

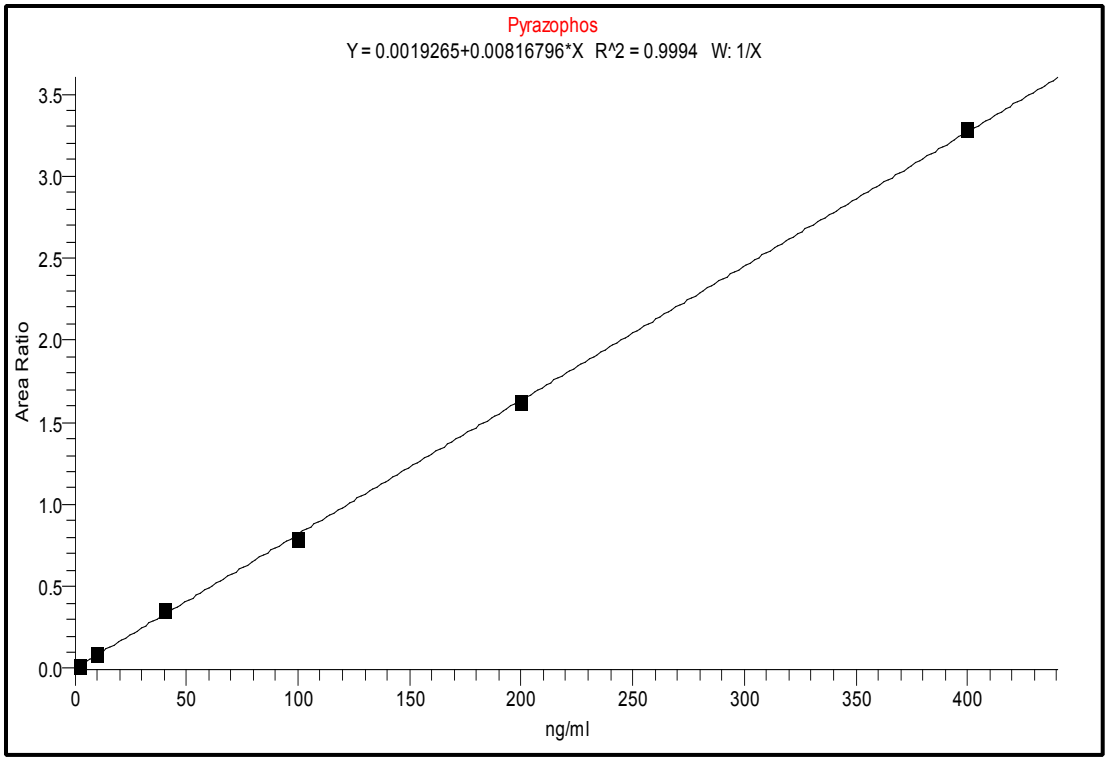
Recovery and RSD% Data Obtained from Fortified Banana Samples

Analytes	Spiked at 10 ng/g		Spiked at 50 ng/g	
	Recovery%	RSD% (n=5)	Recovery%	RSD% (n=5)
Methamidophos	**97.3**	5.9	**100.2**	4.6
Pymetrozine	**96.5**	4.7	**99.3**	3.8
Carbendazim	**103.5**	3.3	**107.3**	5.3
Dicrotophos	**101.8**	4.1	**104.8**	4.8
Acetachlor	**121.0**	2.8	**126.2**	4.5
Thiabendazole	**133.8**	5.8	**111.0**	4.9
DIMP	**89.2**	6.0	**92.1**	7.7
Tebuthiuron	**105.2**	7.9	**112.2**	5.1
Simazine	**96.3**	4.6	**101.2**	4.8
Carbaryl	**93.3**	10.8	**96.4**	7.1
Atrazine	**97.6**	12.8	**101.5**	7.1
DEET	**86.9**	12.8	**93.6**	7.3
Pyrimethanil	**100.6**	8.0	**97.0**	5.7
Malathion	**103.9**	2.6	**100.2**	4.8
Bifenazate	**84.4**	13.7	**85.4**	3.2
Tebuconazole	**90.0**	1.2	**88.2**	1.5
Cyprodinil	**97.3**	3.1	**96.0**	1.8
Diazinon	**104.1**	1.7	**99.8**	2.9
Zoxamide	**104.3**	2.7	**98.9**	4.4
Pyrazophos	**105.4**	3.3	**106.1**	5.2
Profenofos	**95.8**	8.8	**96.4**	8.7
Chlorpyrifos	**86.8**	14.3	**90.7**	12.3
Abamectin	**81.7**	7.8	**80.6**	16.3
Bifenthrin	**90.9**	2.6	**88.4**	7.8
Mean	**98.7**	6.3	**98.9**	5.9

Chromatograms of a Fortified Banana Sample (50 ng/g)



Matrix Matched Calibration Curves of Pyrazophos (R^2=0.9994)



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Multi-residue Pesticide Analysis of Botanical Dietary Supplements using SPE Clean-up and GC-Triple Quadrupole MS/MS*

UCT Part Numbers:
ECPSACB256 (500 mg PSA, 250 mg GCB,6 mL cartridge)
ECMSSC50CT-MP (4000mg $MgSO_4$, 1000mg NaCl)

May 2013

Summary

A screening method for the analysis of 310 pesticides, isomers of organohalogen, organophosphorus, organonitrogen and pyrethroid pesticide metabolites in a variety of dried botanical dietary supplements, spices, medicinal plants, herbals, teas, and phyto-medicines is described. Acetonitrile/water is added to the dried botanical along with anhydrous MgSO4 and NaCl for extraction. This is followed by clean-up using a tandem SPE cartridge consisting of graphitized carbon black (GCB) and primary- secondary amine sorbent (PSA). Pesticides in the study were spiked at 10, 25, 100 and 500 µg/kg. Mean pesticide recoveries were 97%, 91%, 90% and 90%. Percent RSDs were 15%, 10%, 8%, and 6% respectively.

Some Pesticides Screened by this Method

Azoxystrobin	Chlorpyrifos	DDT
Diazinon	Dimethomorph	Hexachlorobenzene
Hexachlorocyclohexanes	methamidophos	Pentachloroaniline
Pentachloroanisole	Pentachlorobenzene	Pentachlorothioanisole
Quinoxyfen	Quintozene	Tecnazene
Tetraconazole	Tetramethrin	

Prepare stock solutions of individual standards by dissolving 25−100 mg of pesticide in 25 mL of toluene.

Procedure

1. Botanical Preparation

a) Add dry botanical powder (1.00 ± 0.02 g) to the 50 mL centrifuge tube
b) Add 10 mL water and 10 mL extraction solvent (60 µg/L of the internal standard, tris-(1,3-dichloroisopropyl)phosphate in acetonitrile)
c) Shake vigorously to insure the botanical is completely wetted
d) Allowed to stand for 15 minutes
e) Add the contents of **ECMSSC50CT-MP** pouch to each centrifuge tube
f) Shake vigorously after addition to disperse the salts
g) Shake samples vigorously for 1 minute
    a. Centrifuge at 4500 rpm (4200g) x 5 min

2. Solid-phase Clean-up

a) Condition **ECPSACB256** cartridge(s) on a manifold using 3 x 6 mL acetone
b) Do not let cartridge go to dryness after last acetone wash
c) Insert 15 mL disposable centrifuge tubes in the vacuum manifold
d) Add a layer of anhydrous sodium sulfate to the top of each cartridge
e) Add a 1.25 mL aliquot of the extract to the cartridge
f) Allow to percolate through the cartridge. Apply low vacuum if needed
g) Rinse cartridge with 1 mL of acetone and continue to collect

3. Cartridge Elution

a) Elute cartridge with 12 mL of 3:1 acetone:toluene
b) Reduce extract to approximately 100 µL with a gentle N_2 stream in a water bath at 50-55 °C
c) Add 0.5 mL toluene, QC standards (50 µL of deuterated polycyclic aromatic hydrocarbons mixture, 500 µg/L), and 25 mg of magnesium sulfate
d) Centrifuge at 3500 rpm x 5 min
e) Divide the toluene extracts between two GC vials with 250 µL vial inserts keeping one vial as a reserve spare

4. GC-MS/MS Analysis

GC-MS/MS Parameters

(Equivalent equipment may be used)

GC: TRACE Ultra Gas Chromatograph
MS: TSQ Quantum triple quadrupole
Autosampler: TriPlus (Thermo Fisher Scientific)
Column: 30 m x 0.25 188 mm id HP-5MS fused silica capillary column (Agilent Technologies, Santa Clara, CA, USA)
Guard Column: deactivated 5 m × 0.25 mm I.D, Restek Corp., Bellefonte, PA
Oven Temperature: Program, initial 105° C for 3 min, 130° C/ @ 10° C/min, 200° C @ 4°C/min, 290° C @ 8° C/min. Hold 6 min.
Column Flow Rate: 1.4 mL/min He
Injector: PTV 100° C for 0.05 min, ramp 12° C/sec to 280° C
Autosampler: TriPlus Thermo Fisher Scientific
Auto-sampler Temperature: 10 °C
Injection Volume: 2.0 µL splitless mode **Injection Liner:** 2 mm id x 120 mm open baffled fused silica deactivated **Ion Source & Transfer T:** 250°C and 280°C, respectively
Electron Multiplier V: auto-tune approx. 1400 V
Ar Collision gas: 1.5 mTorr
Cycle Time: 0.5 sec
Q1 entrance mass width (FWHM): 0.7 amu.
Stock pesticide standards: Full scan 50-550 m/z

There is not complete agreement over which transitions for a given pesticide are optimal for foods or dietary supplements. Reference information on SRM transitions for these analytes is provided in references.[1-4]

Representative Recoveries (RSD) and Percent LOQ's in Each Botanical Matrix

Representative Recoveries (mean, n = 4) ± percent relative standard deviation (RSD) for pesticides by botanical, at 10 and 500 µg/kg and the number not detected (ND) at each fortification concentration

Botanical		10 µg/kg	ND	500 µg/kg	ND
Astralagus	Astragalis membranaceus	94 ±13	68	92 ±3	15
Bitter Orange Peel	Citrus aurantium	112 ±15	63	90 ±5	23
Black Cohosh Root	Cimicifuga racemosa	84 ±11	39	82 ±4	14
Chamomile	Matricaria chamonmilla	87 ±11	68	91 ±4	29
Cinnamon	Cinnamon verum	63 ±26	149	101 ±7	9
Comfrey Root	Symphytum officinale	89 ±18	69	83 ±10	15
Dong Quai	Angelica sinensis	107 ±19	156	97 ±8	16
Echinacea	Echinacea purpurea	97 ±16	61	101 ±8	11
Fenugreek	Trigonella foenum	99 ±14	82	81 ±7	11
Garlic	Allium sativum	98 ±18	78	87 ±6	15
Ginger	Zingiber	103 ±14	211	104 ±6	59
Gingko Biloba	Ginko biloba	99 ±16	89	80 ±7	14
Ginseng	Panax quinquefolius	88 ±11	64	86 ±6	8
Green Tea		91 ±13	43	79 ±6	11
Hoodia	Hoodia gordonii	104 ±19	94	93 ±5	20
Hops	Humulus lupulus	111 ±10	233	102 ±6	53
Jasmine	Jasminum odoratissmum	100 ±14	65	84 ±4	10
Kava Kava	Piper methysticum	111 ±10	164	100 ±4	59
Licorice Root	Glycyrrhiza glabra	93 ±14	43	87 ±6	15
Milk Thistle	Silybum marianum	90 ±13	73	77 ±10	17
Psyllium	Plantago psyllium	99 ±11	39	95 ±4	16
Saw Palmetto	Serenoa serrulata	103 ±13	111	98 ±7	13
St. John's Wort	Hpericum perforatum	93 ±10	100	83 ±6	16
Valerian Root	Valeriana wallichii	101 ±19	68	94 ±10	13

* Adapted from, Douglas G. Hayward, Jon W. Wong, Feng Shi, Kai Zhang, Nathaniel S. Lee, Alex L. DiBenedetto, & Mathew J. Hengel. "Multi-residue Pesticide Analysis of Botanical Dietary Supplements using Salt-out Acetonitrile Extraction, Solid- phase extraction clean-up column and Gas Chromatography-Triple Quadrupole Mass Spectrometry" DOI: 0.1021/ac400481w

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Multiresidue Analysis in Cereal Grains Using Modified QuEChERS Method with UPLC-MS/MS and GC-TOFMS*

UCT Part Number:
ECMSSC50CT-MP (50 mL centrifuge tube, 4 g anhydrous. magnesium sulfate, 1 g NaCl)
CUMPS15C18CT (150 mg anhydrous magnesium sulfate, 150 mg PSA and 50 mg C18)

February 2010

This QuEChERS procedure is specifically developed for cereal grains (corn, oats, rice and wheat) using ultra pressure liquid chromatography-tandem mass spectrometry UPLC MS/MS and automated direct sample introduction GC-TOFMS to achieve good recoveries of over 150 analytes

Pesticide Reference Standards (Chemservice (West Chester, PA)

- Prepare individual pesticide stock solutions (2000 - 5000 µg/mL) in ethyl acetate or acetonitrile (MeCN) and store at -18° C
- Prepare two composite pesticide stock solutions, MIX-1 and MIX-2 at 10 µg/mL in MeCN
- Add 0.1% acetic acid to prevents degradation of base-sensitive analytes in MeCN

Isotopically Labeled Internal Standards (Cambridge Isotope Laboratories, Inc. (Andover, MA))

Prepare 5 µg/mL in acetone

- atrazine (ethylamine-d5)
- carbofuran (ring-^{13}C6)
- dimethoate (o,o-dimethyl-d6)
- 2,4-DDT (ring-^{13}C6)
- α-HCH (^{13}C6)
- parathion (diethyl-d10)

QC Working Solution

- trans-permethrin (phenoxy-^{13}C6) (1 and 5 µg/mL in acetone)

Procedure

1. Sample Preparation

a) Thoroughly homogenize a sample of grain products using a laboratory mill to a flour-like consistency
b) Place appropriate weight** of sample into the 50 ml centrifuge tube
c) Add 10 mL of deionized water (15 mL for rice) and 10 mL of acetonitrile
d) Add 200 µL of ISTD standard solution
e) Vortex tube to disperse sample and standard for 1 hour using a wrist action shaker
f) Add the contents of the **ECMSSC50CT-MP** pouch into the centrifuge tube
g) Immediately seal tube and vortex for 1 minute
h) Centrifuge @ rcf >3,000 for 10 minutes

2. Sample Clean-up

a) Transfer a 1 mL aliquot to a 2 mL **CUMPS15C18CT** tube
b) Vortex for 30 seconds
c) Centrifuge for 5 minutes
d) Transfer 300 µL of the supernatant into the chamber of a Mini-UniPrep syringeless filter vial (Whatman) and add 30 µL 1 µg/mL QC solution*
e) Mix thoroughly
f) Transfer 125 µL of the extract in the Mini-UniPrep vial into a deactivated glass insert placed in a GC autosampler vial and cap the vial with a heat treated septum (overnight at 250° C)
g) Press the 0.2 µm polyvinylidine fluoride (PVDF) filter of the Mini-UniPrep to filter the extract for the UPLC-MS/MS analysis
h) Add 30 µL of QC standard solution
i) Sample is now ready for analysis

3. Analysis UPLC-MS/MS

- Acquity UPLC interfaced to a Quattro Premier triple-quad mass spectrometer (Water's Corp.) MassLynx software v 4.1 or equivalent
- **Column:** Acquity UPLC BEH C18 (50 x 2.1 mm, 1.7 µm particle size, 130 Å pore size) or equivalent

- **Temperature:** 40°C
- **Injection Volume:** 2 µL

Binary Mobile Phase:

- **A** 10 mM ammonium formate in water (pH 3, adjusted with formic acid)
- **B** 10 mM ammonium formate in methanol

Gradient:

Flowrate: 450 µL/minute

Time minutes	% B
0	30
4	30
7.5	60
8.5	60
10.5	100
12.5	100
12.6	30
15.0	30

MS Determination

- Electrospray (ESI) positive mode combined with monitoring of the two most abundant MS/MS (precursor f product) ion transitions.

The MS source conditions:

- capillary voltage of 1.7 kV
- extractor voltage of 4.0 V
- RF lens at 0.9 V
- source temperature of 130° C
- desolvation temperature of 350° C
- collision gas (argon) pressure of 4.31 x 10-3 mbar
- desolvation gas (N2) flow of 600 L/h
- cone gas (N2) flow of 100 L/h

4. **For GC amenable pesticides use automated DSI-GC-TOF Mass Analyzer**

GC Column: Use a combination of a 20 m x 0.25 mm id x 0.25 µm film thickness RTX-5 ms column and a 1m x 0.1 mm id x 0.1 µm film thickness RTX-pesticide 2 column (Restek). This translates into a 1.68 m x 0.1 mm id "virtual" column setting in the ATAS Evolution software or equivalent

Oven Temperature Program (start after a 4.5 minutes solvent vent period):

- 60° C, hold for 4 minutes then ramped to 180° at 20° C/minutes, then ramp 5°C/minutes to 230° C, then 20°C/minutes to 280° C, and finally ramp to 300° C at 40° C/minutes, and hold for 12 minutes. The total run time is 35 minutes.

Automated DSI-GC-TOFMS Analysis.

- Agilent 6890 GC equipped with a secondary oven and nonmoving quad-jet dual stage modulator for two-dimensional comprehensive GC/GC chromatography or equivalent
- Pegasus 4D (Leco Corp., St. Joseph, MI) TOF mass spectrometer or equivalent
- Inject using CombiPAL autosampler (Leap Technologies, Carrboro, NC) or equivalent
- Automated DSI accessory (LINEX) with an Optic 3 programmable temperature vaporizer (PTV) inlet (ATAS-GL International, Veldhoven, The Netherlands) or equivalent
- Leco Chroma TOF (version 3.22) software for GC TOFMS control and data acquisition/processing or equivalent
- CombiPAL Cycle Composer with macro editor (version 1.5.2) and ATAS Evolution software (version 1.2a) to control the automated DSI process and PTV (including column flow) or equivalent

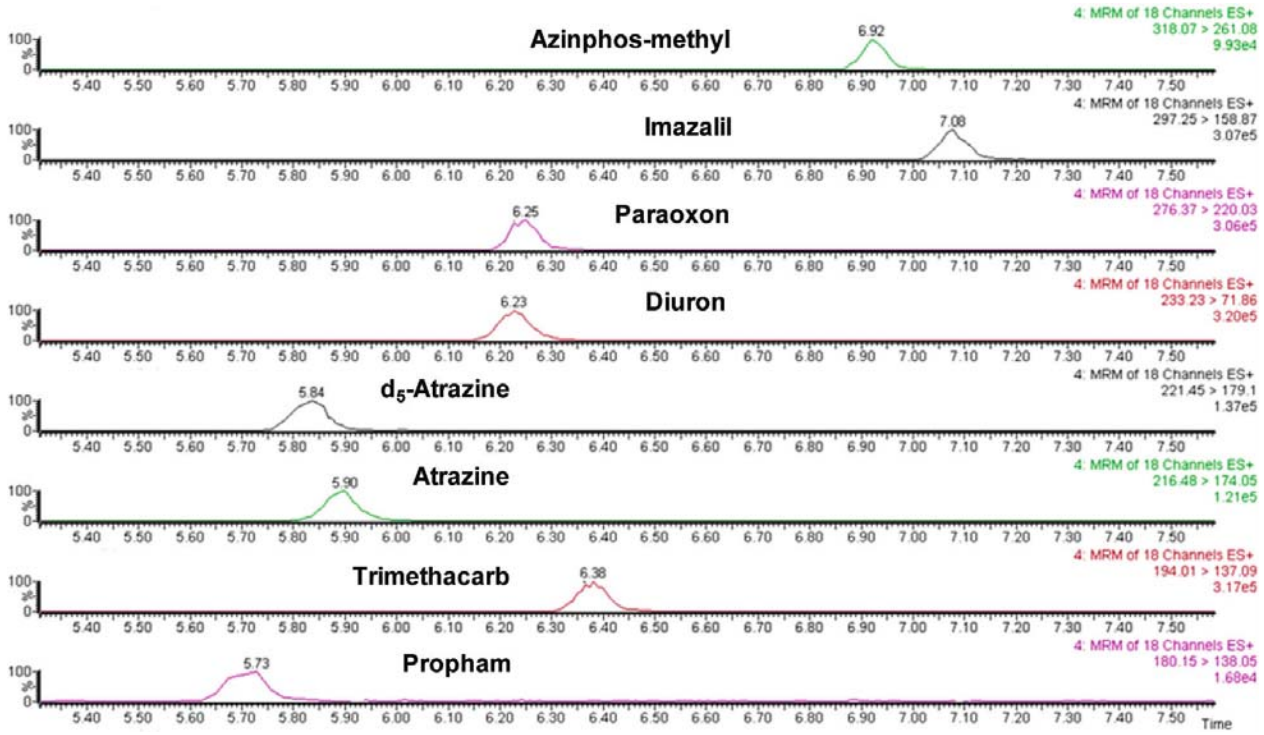
Automated DSI Injection:

- Inject 10 µL into a disposable microvial (1.9 mm i.d., 2.5 mm o.d., 15 mm, (Scientific Instrument Services, Ringoes, NJ), Siltek deactivated (Restek Bellefonte, PA) or equivalent
- Wash with acetone heated at 250° C
- Place in a LINEX DMI tapered liner
- The liner is then transferred into the Optic inlet

Optic 3 PTV Conditions:

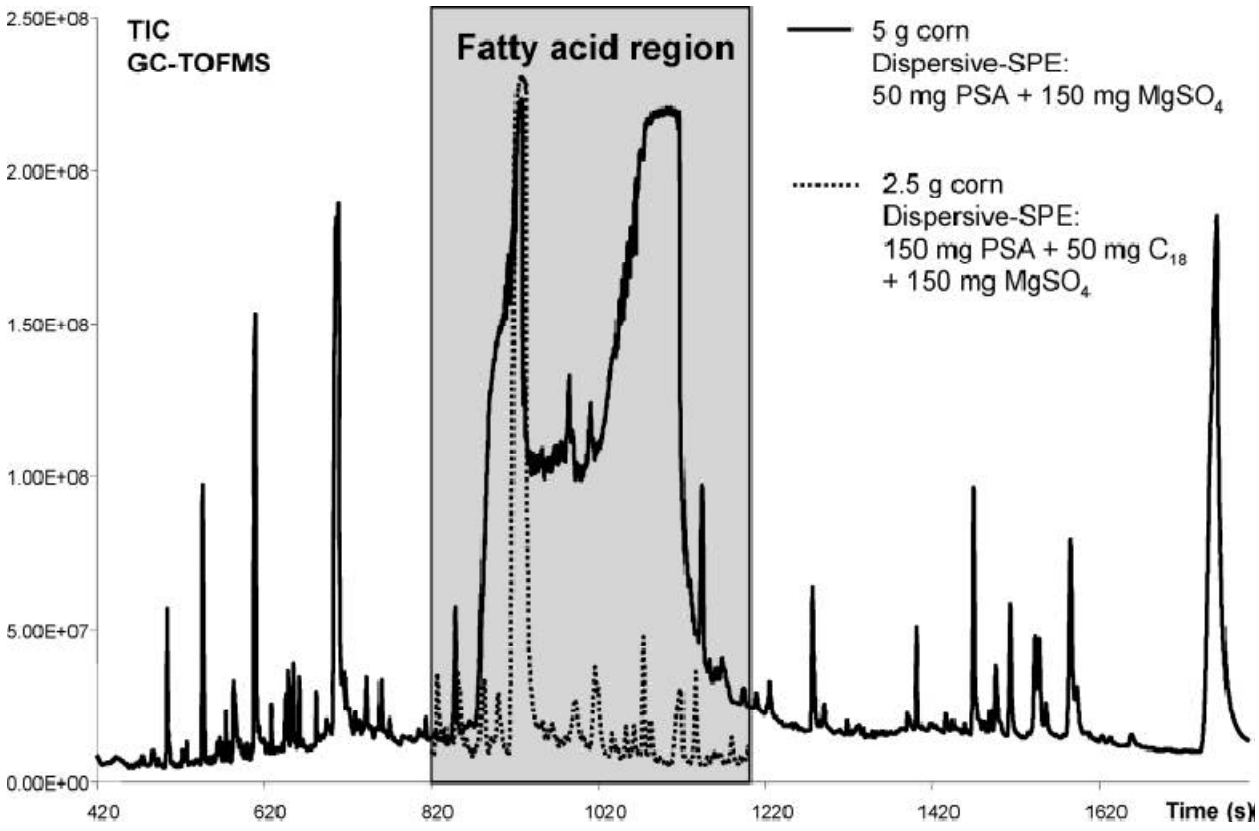
- Solvent vent at an injector temperature of 100° C for 4.5 minutes
- Initial column flow of 0.8 mL/minutes and a split flow of 50 mL/minutes,
- Follow by a splitless transfer of analytes for 4 minutes. The injector temperature was ramped to 280° C (at 16° C/s) Column flow changed to 1.5 mL/minutes (kept constant for the entire GC run). After the splitless period, the split flow adjusted 50 mL/minutes for 6 minutes. After 6 minutes reduce split flow to 25 mL/minutes and decrease injector temperature to 250° C

Shown Below are the UPLC-MS/MS Extracted Ion Chromatograms of Selected Pesticides Spiked at 25 ng/g in Wheat Extract



Total Ion Chromatogram

DSI-LVI-GC-TOFMS analysis of a corn extract prepared using 5 g of sample, original QuEChERS (with 10 mL of water addition for swelling), and 50 mg of PSA in the dispersive SPE step. The highlighted region of the chromatogram is saturated with fatty acids. The dotted trace represents optimized analysis using 2.5 g of corn sample using dispersive SPE with 150mg of PSA and 50 mg
of C_{18}



*Summarized from Mastovska et al, "Pesticide Multiresidue Analysis in Cereal Grains Using Modified QuEChERS Method Combined with Automated Direct Sample Introduction GC-TOFMS and UPLC-MS/MS Techniques" ," J of Agricultural and Food Chemistry, Full article may be found at http://forums.unitedchem.com/

** Corn 2.5 g, oat 3.5 g, rice 5.0 g, wheat 5.0 g

Listing of chemical suppliers and instrument manufacturers does not constitute endorsement by UCT

Determination of Pesticides in Coffee with QuEChERS Extraction and Silica Gel SPE Cleanup

UCT Part Numbers:
ECMSSC50CT-MP - 50-mL centrifuge tube and Mylar pouch containing 4000 mg $MgSO_4$ and 1000 mg NaCl
CUSIL156 – Clean-Up® silica gel, 500mg/6mL column
GCLGN4MM-5 - GC liner, 4mm splitless gooseneck, 4mm ID x 6.5mm OD x 78.5mm

April 2015

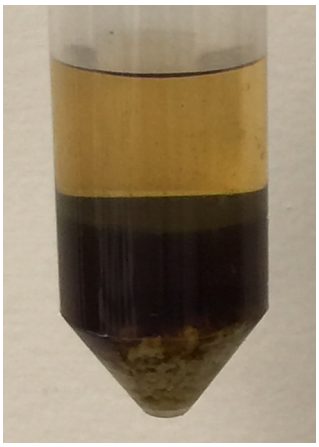
Summary:

Coffee is one of the most widely consumed beverages in the world, partly due to the stimulating effect of its caffeine content. Like most crops, the application of pesticides in coffee cultivation is a common practice in order to increase production yields. To ensure food safety it is important to test pesticide residues in coffee. However, analysis of pesticides in coffee is challenging because it contains a large amount of caffeine as well as acidic and polyphenolic matrix components that are typically co-extracted with the analytes of interest. These matrix components are difficult to remove during sample extraction and cleanup which can cause complications during instrumental analysis. Caffeine, in particular, can significantly compromise GC analysis.

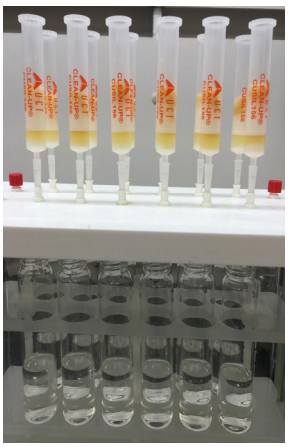
QuEChERS is a well-established method for extraction of pesticide residues in fruit and vegetables, but dispersive-SPE cleanup is not adequate for coffee cleanup as large amounts of caffeine remain in the final extract. To overcome some of the limitations of existing methods there is a need to develop a sample preparation procedure that minimizes matrix effects while reducing the amount of caffeine in the final sample extract. This application details an optimized method for the extraction and cleanup of pesticide residues from coffee using a QuEChERS extraction procedure followed by a silica gel SPE cleanup. Twenty representative pesticides, most of which are commonly used pesticides on coffee farms [1], were evaluated in this study. GC-MS was used for pesticide detection and quantification.

Procedure:

1. Add 10 mL brewed coffee (pH adjusted to about 8 with 1 N NaOH) and 10 mL acetonitrile (MeCN) to a 50-mL centrifuge tube.
2. Add the QuEChERS extraction salts from the Mylar pouch (**ECMSSC50CT-MP**) to the 50-mL tube, and shake vigorously for 1 min manually or using a Spex 2010 Geno-Grinder at 1000 strokes/min.
3. Centrifuge at 3000 g for 5 min, transfer 5 mL supernatant to a clean test tube, add 1.5 mL toluene, and evaporate to about 1 mL.
4. Add about ½ inch of anhydrous sodium sulfate to a silica gel SPE cartridge (**CUSIL156**), and attach the SPE cartridge to a glass block or positive pressure manifold.
5. Wash the SPE cartridge with 6 mL dichloromethane, soak for 1 min, drain to waste, and dry the SPE cartridge for 1 min under full vacuum or pressure.
6. Condition the SPE cartridge with 2 x 6 mL n-hexane by gravity.
7. Insert glass collection container into the manifold, load the 1 mL concentrated sample onto the SPE cartridge, rinse the test tube with 6 mL of 15% acetone in n-hexane and apply the rinsate to the SPE cartridge, and collect.
8. Continue to elute with 3 x 6 mL of 15% acetone in n-hexane by gravity.
9. Add 1.5 mL ethyl acetate to the eluate container and evaporate to 1 mL.
10. Add internal standard, vortex for 30 sec, and inject 1 µL into the GC/MS for analysis.



QuEChERS extraction

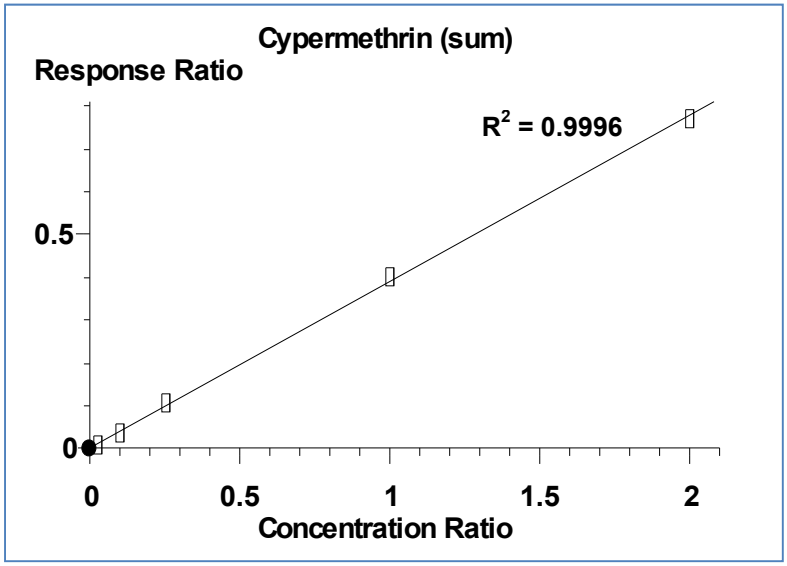


Silica gel SPE cleanup

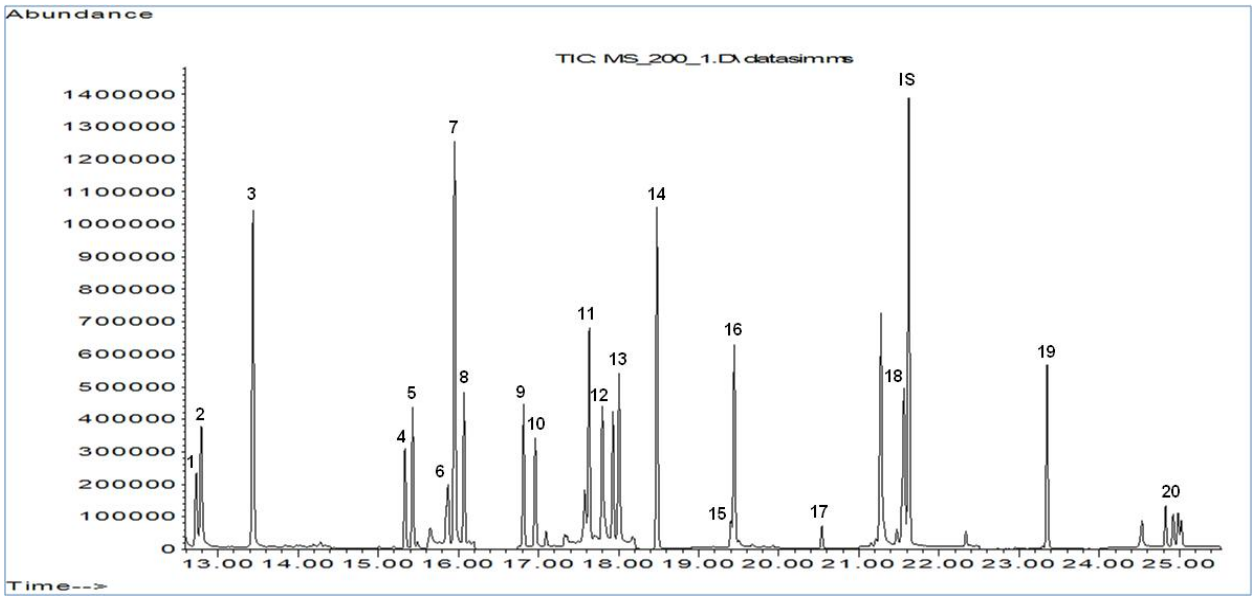
GC-MS Conditions	
Instrumentation	Agilent 6890N GC coupled to a 5975C MSD
Column	Restek Rxi®-5Sil MS (30m × 0.25mm × 0.25µm)
Carrier gas	Helium (1.2 mL/min)
GC inlet temp.	250°C
Injection volume	1 µL (splitless)
Temp gradient	60°C for 1 min, 10°C/min to 310°C, hold for 2 min; 28 min total
Transfer line temp	280°C
Ion source temp	250°C
Ionization mode	EI (70 eV)
Acquisition mode	Selective ion monitoring (SIM)

Compound Name	RT (min)	SIM Ions (25 ms dwell time)			R^2
TPP (IS)	21.625	326	325	77	NA
Carbaryl	12.630	144	115	116	0.9992
Tebuthiuron	12.725	156	171	74	0.9991
DEET	13.389	119	190	91	0.9977
Simazine	15.320	201	186	173	0.9989
Atrazine	15.400	200	215	173	0.9992
Diazinon	15.819	137	179	304	0.9986
Pyrimethanil	15.927	198	199	77	0.9980
Disulfoton	16.050	88	89	97	0.9986
Acetochlor	16.798	146	162	223	0.9975
Methyl parathion	16.935	109	125	263	0.9998
Malathion	17.618	125	173	93	0.9987
Chlorpyrifos	17.787	197	97	314	0.9983
Triadimefon	17.990	57	208	181	0.9982
Cyprodinil	18.456	224	225	210	0.9975
Endosulfan I	19.397	241	195	339	0.9984
Flutriafol	19.426	123	219	164	0.9970
Endosulfan II	20.518	195	241	339	0.9986
Tebuconazole	21.559	125	250	83	0.9999
Pyrazophos	23.362	221	232	373	0.9987
Cypermethrin (sum of 4 isomers)	25.000	163	181	209	0.9996

Results:



Matrix matched calibration curve of cypermethrin (5 - 400 ng/mL)



SIM chromatogram of an extracted coffee sample fortified with 200 ng/mL pesticides. Peaks: 1) carbaryl; 2) tebuthiuron; 3) DEET; 4) simazine; 5) atrazine; 6) diazinon; 7) pyrimethanil; 8) disulfoton; 9) acetochlor; 10) methyl parathion; 11) malathion; 12) chlorpyrifos; 13) triadimefon; 14) cyprodinil; 15) endosulfan I; 16) flutriafol; 17) endosulfan II; 18) tebuconazole; 19) pyrazophos; 20) cypermethrin (sum of 4 isomers).

Recovery and RSD% from Spiked Coffee Samples

Compound Name	Spiked at 20 ng/mL		Spiked at 200 ng/mL	
	Recovery%	RSD% (n=5)	Recovery%	RSD% (n=5)
Carbaryl	**100.2**	5.0	**98.7**	1.6
Tebuthiuron	**95.3**	6.3	**99.9**	2.4
DEET	**102.4**	5.3	**99.1**	2.5
Simazine	**103.5**	5.4	**98.6**	1.2
Atrazine	**103.6**	6.5	**97.9**	2.4
Diazinon	**124.4**	9.9	**99.6**	2.2
Pyrimethanil	**106.4**	6.3	**101.6**	1.2
Disulfoton	**88.1**	7.1	**92.5**	2.2
Acetochlor	**103.3**	5.6	**98.7**	1.6
Methyl parathion	**91.3**	6.3	**97.9**	1.9
Malathion	**103.0**	7.7	**99.9**	3.6
Chlorpyrifos	**103.6**	6.9	**99.4**	1.3
Triadimefon	**109.3**	5.1	**101.5**	1.6
Cyprodinil	**106.4**	6.8	**102.4**	1.0
Endosulfan I	**114.0**	6.2	**98.2**	1.7
Flutriafol	**74.5**	11.6	**87.9**	4.7
Endosulfan II	**103.7**	6.1	**99.5**	1.3
Tebuconazole	**92.7**	8.5	**101.8**	1.5
Pyrazophos	**98.0**	7.5	**101.4**	1.4
Cypermethrin (sum)	**97.0**	5.1	**101.7**	1.0

References:

[1] http://www.coffeehabitat.com/2006/12/pesticides_used_2/

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Pesticides in Fatty Matrices Extraction

UCT Part Numbers:
ECPSAC1856 (500 mg endcapped C18, 500 PSA, 6 mL cartridge)
CUMPSC18CT (150 mg $MgSO_4$, 50 mg PSA and 50 mg C18 in a 2 mL centrifuge tube)
ECMAG00D (500 g organic free $MgSO_4$ anhydrous)
ECNACL05K (5 kg NaCl)

January 2011

Procedure

1. Sample Preparation

a) Weigh 20.0 ± 0.10 grams (g) of homogenized sample into a 250 mL plastic centrifuge bottle, tared on a balance capable of weighing to 0.01 grams
b) Fortify each sample with process control spiking (PCS) solution
c) Add 50 mL of ethyl acetate (EtOAc) to each tube
d) Fortify each sample with internal standard (ISTD) spiking solution
e) Reduce sample material particle size by using a high speed disperser for approximately 1 minute
f) Add 2 g of anhydrous $MgSO_4$ **(ECMAG00D)** and 0.5 g anhydrous NaCl **(ECNACL05K)**

Note: Carefully add the reagents to the tube to avoid contaminating the threads or rims of the tubes otherwise leaks may result

g) Seal the tube and shake vigorously for approximately 1 minute either mechanically or by hand. Make sure the solvent interacts well with the entire sample and that crystalline agglomerates are broken up
h) Cool the sample in a -20 ºC freezer for approximately 30 minutes
i) Centrifuge at 10,000 RCF for 5 minutes
j) Decant at least 50 mL of the EtOAc layer into a 50 mL glass graduated centrifuge tube using a funnel and filter paper. Allow the extract to come to room temperature and adjust the volume with EtOAc to 50 mL using a Pasteur pipette
k) Concentrate the extract under a stream of nitrogen with a 70º C water bath until the volume remains constant (this will be ~ 3 mL and will take about 1 hour)

l) Dilute to 20 mL with acetonitrile (MeCN) and cap with a glass stopper, vortex for 1 minute

m) Freeze at -70 ºC for 30 minutes

n) Centrifuge the extract while frozen for 3 minutes (The MeCN will thaw during centrifugation)

o) Directly after centrifugation in step n), filter > 15 mL of the MeCN layer of the extract with a 0.45 µm syringe filter into a 15 mL glass centrifuge tube

p) Allow the extract to come to room temp, adjust the volume to 15 mL, and concentrate to 2.25 mL under a stream of nitrogen with a 70 ºC water bath

2. LC-MS/MS Analysis

a) Transfer 1 mL of extract to a 2 mL mini-centrifuge tube **CUMPSC18CT**

b) Vortex for 1 minute and centrifuge

c) Transfer to auto sampler vial. Sample is now ready for analysis

3. GC Analysis

a) For GC analyses, use the dual layer cartridge **ECPSAC1856**

b) Add approximately 0.75 – 0.80 grams (~ 0.6 cm = 0.25 inches) of anhydrous $MgSO_4$ added to the top of the cartridge

c) Condition the SPE cartridge by adding one cartridge volume (4.0 mL) of MeCN using a UCT positive pressure SPE manifold

d) Elute to waste

e) Place a labeled 15 mL graduated disposable plastic centrifuge tube below the cartridge in the positive pressure SPE manifold

f) Quantitatively transfer 1 mL of the sample extract from step 15 to the SPE cartridge

g) Elute SPE cartridge in a dropwise manner (Regulated Flow Pressure = 35 psi) into a labeled 15 mL graduated glass centrifuge tube using MeCN

h) Collect the eluate while washing the SPE cartridge **three times** with **4 mL of eluant**.

i) After the last 4 mL portion of eluant has passed through the cartridge move the switch of the positive pressure SPE manifold from “Regulated Flow” to “Full Flow/Dry” to dry the SPE cartridge for approximately 1 minute

j) Using an N-Evap (or equivalent) with the water bath set at 50°C and N_2 flow set at <10 liters per minute (LPM) (typical setting is 2 – 6 LPM), evaporate the sample to approximately 0.5 mL

k) Add 3 mL of toluene to the centrifuge tube containing the sample

l) Evaporate again to < 0.5 mL. (This is to insure all other solvents have been removed from the sample.)

m) Bring the volume to 1.0 mL with toluene and vortex to mix solvent into sample

n) Analyze by GCMS-EI and GCMS-NCI

QuEChERS Pesticide Analysis for Fresh Produce by GC/MS/MS*

UCT Product Number:
ECMSSC50CTFS-MP (6 g $MgSO_4$, 1.5 g NaCl)
ECQUEU1115CT (1.2 g $MgSO_4$, 0.4 g PSA, 0.4 g GCB)
ECMSC1850CT (1500 mg $MgSO_4$, 500 mg endcapped C18)
ECMAG00D (organic free $MgSO_4$ anhydrous)

October 12, 2010

This modified QuEChERS procedure uses GC-MS/MS for analysis of organohalogen, organophosphorus, and pyrethroid pesticides in produce. It is an improvement over the traditional QuEChERS procedure since the sample extracts are in toluene instead of acetonitrile and cleaner due to additional clean-up procedures. In addition, the method uses smaller sample sizes and less solvent than standard multiresidue procedures, and the solid-phase dispersive steps involving GCB/PSA/C18 provide sufficient clean-up for GC-MS/MS analysis.

1. **Sample Extraction**
   a) Combine 15 g of cryo-ground sample with 15 mL acetonitrile
   b) Add contents of **ECMSSC50CTFS-MP**
   c) Shake by hand for 2 minutes
   d) Add IS (500 µL of 3.4 µg/mL solution of tris(1,3-dichloroisopropyl) phosphate
   e) Centrifuge 4500 rpm for 5 minutes
2. **Clean-Up**
   a) Transfer upper layer (12 mL) to a clean centrifuge tube **ECMSC1850CT** containing 0.5 grams C_{18} and 1.2 g $MgSO_4$
   b) Shake for 1 minute and centrifuge @ 4500 rpm for 5 minutes
   c) Transfer 9 mL of supernatant to extraction tube containing **ECQUEU1115CT**
   d) Vortex 15 seconds
   e) Add 3 mL toluene
   f) Shake the centrifuge tube for 2 minutes
   g) Centrifuge @ 4500 rpm for 5 minutes
   h) Transfer extract to clean tube
   i) Reduce 6 mL volume to < 100 µL using N_2 in an evaporator (35°C)
   j) Add 1.0 mL toluene and QC standard (20 µg/mL deuterated polycyclic hydrocarbons) along with 50 mg anhydrous $MgSO_4$
   k) Centrifuge @ 1500 rpm for 5 minutes

l) Transfer 1.0 mL of extract to ALS vials for analysis

Note:

- Use matrix-matched calibration standards in toluene rather than standards prepared in solvent. This will compensate for matrix enhancement effects
- Coextractives in the sample matrix have been shown to cause an enhancement of the pesticide peak response in the matrix compared to that of the same amount of the pesticide in the matrix-free solvent

GC-MS/MS Tandem Mass Spectrometry

Varian CP-3800 series gas chromatograph coupled with a Varian 1200 L triple-quadrupole mass spectrometer with a CTCCOMBI PAL autosampler (Varian Inc., Palo Alto, CA)

- Column: Deactivated guard column (5 m x 0.25 mm i.d., Restek Corp.) Varian 30 m x 0.25 mm x 0.25 µm, VF-5 fused silica capillary analytical column
- Head pressure 13.2 psi with 1.2 mL/min flow rate
- He carrier gas
- Column temperature programmed as follows:
    - initial temperature 105° C for 6 min
    - increased to 130° C at 10° C/min
    - ramp to 230° C at 4° C/min and to 290° C at 1° C/min
    - Hold for 5.5 min.
    - Total run time 45 min.
- Injector temperature: 280° C
- injection volume: 1.0 µL in splitless mode
- Ion source and transfer line temperatures are 240° and 300° C, respectively
- Set Electron multiplier voltage to 1400V by automatic tuning
- Use argon collision gas for all MS/MS
- Pressure in the collision cell 1.8 mTorr

Table of Analytes Covered in this Method

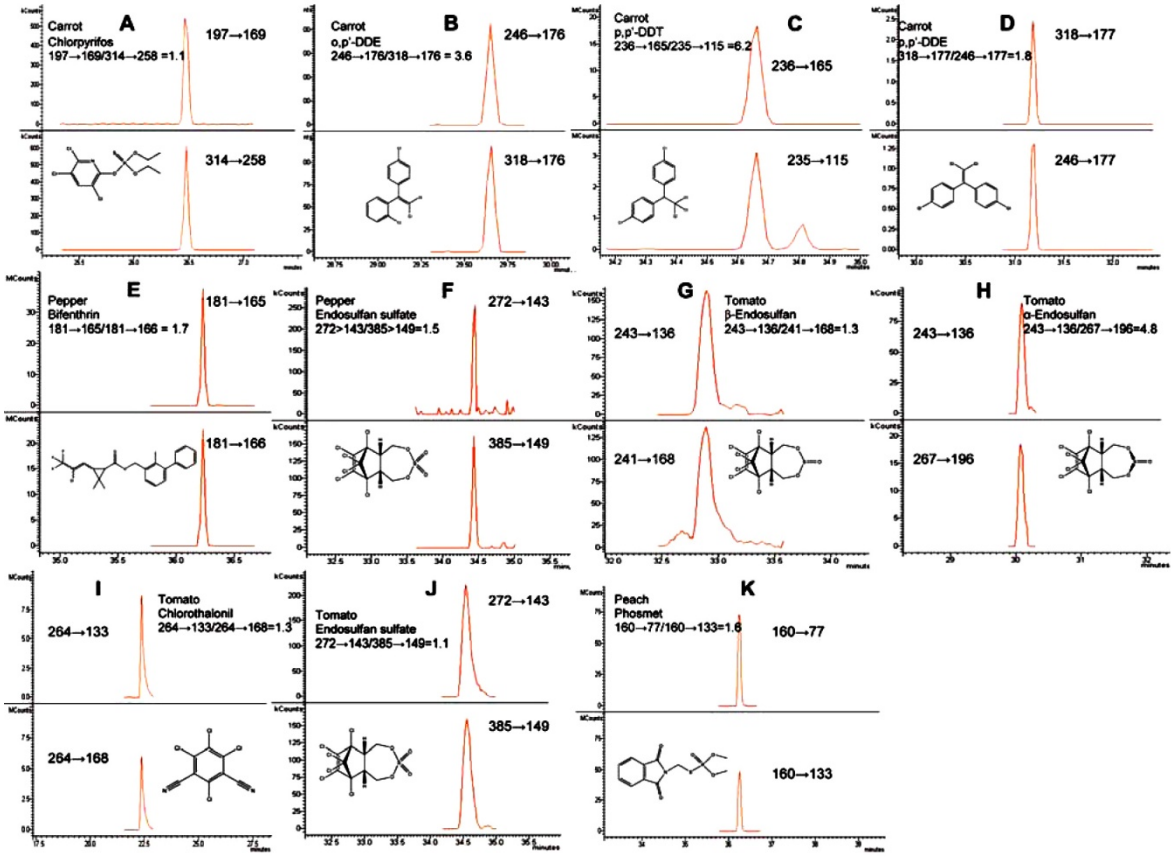
Analytes		
acenaphthene-d_{10}	Diamidafos (nellite)	*p,p'*-methoxychlor
acrinathrin	Diazinon	metolachlor
akton	Dibutyl chlorenate	mevinphos
alachlor	Dicapthon	mirex
aldrin	Dichlobenil	naphthalene-d_8
allethrin	Dichlofenthion	*cis*-nonachlor
atrazine	Dichlofluanid	*trans*-nonachlor
azamethiophos	3,4'-dichloroaniline	parathion
azinphos-ethyl	4,4'-dichlorobenzophenone	parathion-methyl
azinphos-methyl	Dichlorvos	pentachloroaniline
α-BHC	Dicloran	pentachlorobenzene
β-BHC	Dieldrin	pentachlorobenzonitrile
δ-BHC	Dimethachlor	Pentachlorphenyl methyl ester
benfluralin	dioxabenzofos	pentachlorothioanisole
bifenthrin	Dioxathion	*cis*-permethrin
bromophos	Disulfoton	*trans*-permethrin
bromophos-ethyl	Ditalimfos	phenanthrene-d_{10}
bromopropylate	Edifenphos	phenothrin
captafol	α-endosulfan	phorate
captan	β-endosulfan	phosalone
carbophenothion	Endosulfan ether	phosmet
***cis*-chlordane**	Endosulfan sulfate	phenthoate
***trans*-chlordane**	Endrin	pirimiphos-ethyl
α-chlordene	Endrin aldehyde	pirimiphos-methyl
β-chlordene	Endrin ketone	procymidone
γ-chlordene	EPN	profenofos
β-chlorfenvinphos	Ethalfluralin	propachlor
chlorobenzilate	Ethion	propazine
chloroneb	Ethoprop	propetamphos
chlorothalonil	Etridazole	propyzamide
chlorpyrifos	Famphur	prothiophos
chlorpyrifos-methyl	Fenamiphos (ronnel)	pyraclofos
chlorthiophos	Fenarimol	pyrazophos
chrysene-d_{12}	Fenchlorphos	pyridaphenthion
coumaphos	Fenitrothion	quinalphos
cyanazine	Fensulfothion	quintozene
cyanophos	Fenthion	resmethrin
Cyfluthrin 1	Fenvalerate 1	simazine
Cyfluthrin 2	Fenvalerate 2	sulfotep-ethyl
Cyfluthrin 3	Fluchloralin	sulprofos
Cyfluthrin 4	Flucythrinaten 1	tebupirimfos
λ-cyhalothrin	Flucythrinate 2	propachlor
Cypermethrin 1	Fluridone	propazine
Cypermethrin 2	Fluvalinate 1	Tecnazene (TCNB)
Cypermethrin 3	Fluvalinate 2	tefluthrin
Cypermethrin 4	Folpet	temephos
Dacthal (DCPA)	Fonophos	terbufos
***o,p'*-DDD**	Heptachlor	terbuthylazine
***p,p'*-DDD**	Heptachlor epoxide	2,3,5,6-tetrachloroaniline
***o,p'*-DDE**	hexachlorobenzene	tetrachlorvinphos
***p,p'*-DDE**	Iprobenfos (IBP)	tetramethrin
***o,p'*-DDT**	Iprodione	thiometon
***p,p'*-DDT**	Isazophos	tolclofos-methyl
DEF (tribufos)	Isofenfos	tolyfluanid
deltamethrin	Jodfenphos (iodofenphos)	triallate
demeton-S	Leptophos	triazophos
demeton-S-methyl	Lindane (BHC)	trifluralin
dialifor	Malathion	triphenyl
Diallate 1	methidathion	tris(1,3-dichloroisopropyl) phosphate
Diallate 2	*o,p'*-methoxychlor	vinclozolin

Problems with pesticides with low (<70%) recoveries or large variances (SD > 20%) may be attributed to the following issues:

- early eluting analytes
- sensitivity to pH changes
- prone to volatility loss (i.e., 3,4'-dichloroaniline, dichlorvos, diclobenil, and etridazole),
- strongly adsorbed to the PSA or GCB sorbents (i.e., chlorothalonil, endrin aldehyde, hexachlorobenzene, pentachlorobenzene, pentachlorobenzonitrile, and tachlorothioanisole)
- difficult to ionize by mass spectrometric detection (i.e., captafol, captan, dichlofluanid, folpet, and tolylfluanid).
- Highly nonpolar or late-eluting pesticides such as temephos and fluridone may also be problematic

For recovery data, target, qualifier and transition ions please reference original paper*

Reconstructed GC-MS/MS chromatograms of various commodities containing various pesticides including chlorpyrifos (A), o,p′-DDE (B), p,p′-DDT (C), and p,p′-DDE (D) present in carrot; bifenthrin (E) and endosulfan sulfate (F) present in bell pepper; β- (G) and R- (H) endosulfan, endosulfan sulfate (I) and chlorothalonil (J) present in tomato; and phosmet (K) in peach. Included are the transitions from precursor to product ions and the relative ion ratios between the two transitions, primary (top) and secondary (bottom), which are used for pesticide identification



Reagents and Materials

Pesticide standards may be obtained from:

- U.S. Environmental Protection Agency National Pesticide Standard Repository (U.S. EPA, Ft. Meade, MD)
- ChemServices (West Chester, PA), Sigma/Aldrich/Fluka Chemicals (St. Louis, MO),
- Crescent Chemicals (Islandia, NY)
- tris(1,3-dichloroisopropyl) phosphate from TCI America (Portland, OR)
- Quality control standards, naphthalene-d8, acenaphthalened10, phenanthrene-d10, and chrysene-d12 (Sigma/Aldrich/Fluka Chemicals (Milwaukee, WI).

*Adapted and used by permission from Jon W. Wong, Kai Zhang, “Multiresidue Pesticide Analysis In Fresh Produce By Capillary Gas Chromatography-Mass Spectrometry/Selective Ion Monitoring (GC-MS/SIM) and -Tandem Mass Spectrometry”, (GC-MS/MS), J Agric. Food Sci., DOI: 10.1021/Jf903854n

Listing of instrument manufacturers and standards suppliers does not constitute endorsement by UCT. Equivalent systems may be used

QuEChERS-Based LC/MS/MS Method for Multiresidue Pesticide Analysis in Fruits and Vegetables*

UCT Product Number:
EC4MSSA50CT-MP (4 g anhydrous $MgSO_4$, 1.0 g Sodium Acetate)
ECMS12CPSA415CT (1.2 g anhydrous $MgSO_4$, 400 mg PSA)

July 2011

A high-throughput, QuEChERS analytical method (LC-MS/MS) is described for the part per trillion (ppt) determination of 191 pesticides in orange, peach, spinach and ginseng. Pesticide classes include carbamates, polar organophosphates, phenylureas, anilides, benzoyl phenylureas, conazoles, macrocyclic lactone, neonicotinoids, strobilurines, and triazines. This method was validated by the U.S. Food and Drug Administration (FDA).

Analytes Covered in this Method

Table 1

Analyte	CASRN		Analyte	CASRN
Acephate	30560-19-1		Imazalil	35554-44-0
Acetamiprid	135410-20-7		Imidacloprid	138261-41-3
Acibenzolar-S-	135158-54-2		Indoxacarb	173584-44-6
Alanycarb	83130-01-2		Ipconazole	125225-28-7
Aldicarb	116-06-3		Iprovalicarb	140923-17-7
Aldicarb sulfone	1646-88-4		Isoprocarb	2631-40-5
Aldicarb sulfoxide	1646-87-3		Isoproturon	34123-59-6
Ametryn	834-12-8		Isoxaflutole	141112-29-0
Aminocarb	2032-59-9		Ivermectin	70288-86-7
Amitraz	33089-61-1		Kresoxim-methyl	143390-89-0
Avermectin B_{1a}	65195-55-3		Linuron	330-55-2
Avermectin B_{1b}	65195-56-4		Lufenuron	103055-07-8
Azoxystrobin	131860-33-8		Mefenacet	73250-68-7
Benalaxyl	71626-11-4		Mepanipyrim	110235-47-7
Bendiocarb	22781-23-3		Mepronil	55814-41-0
Benfuracarb	82560-54-1		Mesotrione	104206-82-8
Benzoximate	29104-30-1		Metalaxyl	57837-19-1
Bifenazate	149877-41-8		Metconazole.1	125116-23-6
Bitertanol	55179-31-2		Methabenzhiazuron	18691-97-9
Boscalid	188425-85-6		Methamidophos	10265-92-6
Bromuconazole 46	116255-48-2		Methiocarb	2032-65-7
Bromuconazole 47	116255-48-2		Methomyl	16752-77-5
Bupirimate	41483-43-6		Methoprotryne	841-06-5
Buprofezin	953030-84-7		Methoxyfenozide	161050-58-4
Butafenacil	134605-64-4		Metobromuron	3060-89-7

Butocarboxin	34681-10-2		Metribuzin	21087-64-9
Butoxycarboxin	34681-23-7		Mevinphos-E	813-78-5
Carbaryl	63-25-2		Mevinphos-Z	7786-34-7
Carbendazim	10605-21-7		Mexacarbate	315-18-4
Carbetamide	16118-49-3		Monocrotophos	6923-22-4
Carbofuran	1563-66-2		Monolinuron	1746-81-2
Carbofuran, 3OH-	16655-82-6		Moxidectin	113507-06-5
Carboxin	5234-68-4		Myclobutanil	88671-89-0
Carfentrazone-ethyl	128639-02-1		Neburon	555-37-3
Chlorfluazuron	71422-67-8		Nitenpyram	150824-47-8
Chlorotoluron	15545-48-9		Novaluron	116714-46-6
Chloroxuron	1982-47-4		Nuarimol	63284-71-9
Clethodim	99129-21-2		Omethoate	1113-02-6
Clofentezine	74115-24-5		Oxadixyl	77732-09-3
Clothianidin	210880-92-5		Oxamyl	23135-22-0
Cyazofamid	120116-88-3		Paclobutrazol	76738-62-0
Cycluron	2163-69-1		Penconazole	66246-88-6
Cymoxanil	57966-95-7		Phenmedipham	13684-63-4
Cyproconazole A	94361-06-5		Picoxystrobin	117428-22-5
Cyproconazole B	94361-07-6		Piperonyl butoxide	51-03-6
Cyprodinil	121552-61-2		Pirimicarb	23103-98-2
Desmedipham	13684-56-5		Prochloraz	67747-09-5
Diclobutrazol	75736-33-3		Promecarb	2631-37-0
Dicrotophos	141-66-2		Prometon	1610-18-0
Diethofencarb	87130-20-9		Prometryn	7287-19-6
Difenoconazole	119446-68-3		Propamocarb	24579-73-5
Diflubenzuron	35367-38-5		Propargite	2312-35-8
Dimethoate	60-51-5		Propham	122-42-9
Dimethomorph A	110488-70-5		Propiconazole	60207-90-1
Dimethomorph B	2274-67-1		Propoxur	114-26-1
Dimoxystrobin	149961-52-4		Pymetrozine	123312-89-0
Diniconazole	83657-24-3		Pyracarbolid	24691-76-7
Dioxacarb	6988-21-2		Pyraclostrobin	175013-18-0
Diuron	330-54-1		Pyridaben	96489-71-3
Doramectin	117704-25-3		Pyrimethanil	53112-28-0
Emamectin B_{1a}	155569-91-8		Pyriproxyfen	95737-68-1
Epoxiconazole	133855-98-8		Quinoxyfen	124495-18-7
Eprinomectin B_{1a}	123997-26-2		Rotenone	83-79-4
Etaconazole	60207-93-4		Secbumeton	372137-35-4
Ethiofencarb	29973-13-5		Siduron	26259-45-0
Ethiprole	181587-01-9		Simetryne	1014-70-6
Ethofumesate	26225-79-6		Spinosyn A	168316-95-8
Etoxazole	153233-91-1		Spirodiclofen	148477-71-8
Famoxadone	131807-57-3		Spiromefesin	283594-90-1
Fenamidone	161326-34-7		Spiroxamine	118134-30-8

Fenarimol	60168-88-9		Sulfentrazone	122836-35-5
Fenazaquin	120928-09-8		Tebuconazole	107534-96-3
Fenbuconazole	114369-43-6		Tebufenozide	112410-23-8
Fenhexamid	126833-17-8		Tebufenpyrad	119168-77-3
Fenoxycarb	79127-80-3		Tebuthiuron	34014-18-1
Fenpropimorph	67564-91-4		Teflubenzuron	83121-18-0
Fenpyroximate	134098-61-6		Terbumeton	33693-04-8
Fenuron	134098-61-6		Terbutryn	886-50-0
Fludioxinil	131341-86-1		Tetraconazole	112281-77-3
Flufenacet	142459-58-3		Thiabendazole	148-79-8
Flufenoxuron	101463-69-8		Thiacloprid	111988-49-9
Fluometuron	2164-17-2		Thiamethoxam	153719-23-4
Fluoxastrobin	361377-29-9		Thidiazuron	51707-55-2
Fluquinconazole	136426-54-5		Thiobencarb	28249-77-6
Flusilazole	85509-19-9		Thiofanox	39196-18-4
Flutolanil	66332-96-5		Thiophanate-methyl	23564-05-8
Flutriafol	76674-21-0		Triadimefon	43121-43-3
Forchlorfenuron	68157-60-8		Triadimenol	55219-65-3
Formetanate HCl	22259-30-9		Tricyclazole	41814-78-2
Fuberidazole	3878-19-1		Trifloxystrobin	141517-21-7
Furalaxyl	57646-30-7		Triflumizole	99387-89-0
Furathiocarb	65907-30-4		Triflumuron	64628-44-0
Hexaconazole	79983-71-4		Triticonazole	131983-72-7
Hexythiazox	78587-05-0		Vamidothion	2275-23-2
Hydramethylnon	67485-29-4		Zoxamide	156052-68-5

Deuterium Isotope Internal Standards	
D10-Diazinon	D6-diuron
D6-Dichlorvos	D6-Linuron
D6-Dimethoate	D6-Malathion

CDN-Isotopes (Montreal, QC, Canada)

Analytical Stock Solutions

Prepare separate stock solutions of analytical standards, including the isotope labeled internal standards (ILIS) for individual compounds.

- Weigh 10-75 mg each and dissolve in 10 or 25 mL of acetonitrile, methanol, or methanol/water (50:50 v/v) in volumetric flasks
- Prepare intermediate solutions in 100mL volumetric flasks by mixing stock solutions

- Prepare five levels of matrix-matched calibration standards from intermediate solutions by using sample matrix extract and matrix buffer (20 mM ammonium formate) in concentrations of 1, 5, 10, 50, and 100 ppb
- Add the ILIS solution prior to sample preparation and use as an internal standard in the quantitative analysis

Procedure

1. Sample Preparation--orange, peach, spinach

a) Weigh 10 ± 0.1 g of cryoground sample into 50 mL centrifuge tube
b) Add 10 mL of 1% acetic acid in acetonitrile and contents of **EC4MSSA50CT-MP** pouch
c) Shake by hand then add 200 µL of surrogate solution and a steel ball
d) Place on a Geno/Grinder shaker (or equivalent) for 1 min @ 1000 strokes/minute
e) When shaking is complete centrifuge @ 4500 rpm for 5 min
f) Transfer 9 mL of supernatant to a 15 mL centrifuge tube containing **ECMS12CPSA415CT**
g) Shake on Geno/Grinder for 1 min @ 500 strokes/min
h) Centrifuge @ 4500 rpm for 5 min
i) Transfer 2.0 mL of supernatant to injection vials for analysis. Filter cloudy extracts using 0.2 nylon or PTFE membrane filter directly into the LC autosampler vials

2. Calibration Standards-- orange, peach, spinach

a) Prepare matrix-matched calibration standards by mixing 300 µL of 0.0167, 0.033, 0.067, 0.167, and 0.333 ppm standard solutions. Use 200 µL of matrix blank extracts and 500 µL of 20 mM ammonium formate sample buffer
b) Add 500 µL of sample buffer just prior to sample analysis
c) Filter cloudy extracts using 0.2 nylon or PTFE membrane filter directly into the LC autosampler vials
d) Filtered samples should be clear and can be stored in a freezer until analysis

1a. Sample Preparation--ginseng

a) Prepare ginseng samples by using 1.0 ± 0.05 g of ginseng
a) Add 10 mL of HPLC-grade water and a steel ball bearing
b) Shake on a GenoGrinder at 1000 strokes/min for 1 minute
c) Add 10 mL of 1% acetic acid in acetonitrile, 200 µL of surrogate solution and contents of **EC4MSSA50CT-MP** pouch
d) Shake by hand
e) Place on a Geno/Grinder shaker (or equivalent) for 1 min @ 1000 strokes/minute
f) When shaking is complete centrifuge @ 4500 rpm for 5 min
g) Transfer 9 mL of supernatant to a 15 mL centrifuge tube containing **ECMS12CPSA415CT**
h) Shake on Geno/Grinder for 1 min @ 500 strokes/min
i) Centrifuge @ 4500 rpm for 5 min
j) Transfer 2.0 mL of supernatant to injection vials for analysis. Filter cloudy extracts using 0.2 nylon or PTFE membrane filter directly into the LC autosampler vials

2a. Calibration Standards--ginseng

a) Prepare matrix-matched calibration standards by adding 100 µL of 0.033, 0.067, 0.167, 0.333,0.8, 1.6 ppm standard solutions to 400 µL of ginseng blank extracts
b) Add 500 µL of sample matrix buffer just prior to analysis to achieve matrix-matched calibration standards of 1.67, 3.33, 6.67, 16.7, 33.3, 80, and 160 ppb, respectively
c) Filter using 0.2 m Nylon or PTFE membrane filters
d) Filtered samples should be clear and can be stored in a freezer until analysis

3. Sample Analysis

a) HPLC analysis with Shimadzu Prominence/20 series (Columbia, MD) or equivalent interfaced to an ABSciex (Forest City, CA) 4000QTrapmass spectrometer through an ESI interface (IonSpray)
b) Acquire MRM data in positive ion mode

c) Identify target pesticides using two specific MRM transitions for each pesticide to achieve an identification point (IP) of 4

d) Quantify using either external standard calibration (NRCG) or internal standard calibration (FDA and MOE) with $^{2}H_{10}$-diazinon as IS

e) Use N_2 of 99% purity from a nitrogen generator (Parker Balston, Haverhill, MA) in the ESI source and the collision cell

f) Restek LC column (Bellefonte, PA; Ultra Aqueous, C-18, 100 x 2.1 mm, 3 µm) and guard column (Ultra Aqueous, C-18 cartridges, 10 x 2.1 mm in guard cartridge holder) or equivalent

g) Curtain, collision, nebulizer, auxiliary gases, and source temperature of the ESI source were set at 15, 6, 35, and 45 psi and 450° C, respectively

h) Ion spray voltage: 5200

i) Declustering potential (DP), collision energy (CE), and collision cell exit potential (CXP) are optimized by direct infusion. The two most intense ion pairs of each analyte are chosen for the analysis. Values of DP, CE, and CXP and the two specific, most intense MRM pairs are listed in Table 3. Principal component analysis (PCA) is carried out using Infometrix Pirouette 4 (Bothell, WA)

j) Table 2 lists mobile phases, column temperatures, injection volume, flow rate, and LC gradient parameters

Table 2

HPLC Gradient Elution Parameters	
Mobil Phase	**A**: 5 mM ammonium formate, 0.1% formic acid in water
	B: 5 mM ammonium formate, 0.1% formic acid in MeOH
Column Temperature	35° C
Flow rate	0.3 mL/min
Total run time	14.0 min
Gradient program	10% B at 0 min, hold for 1 min 5% B at 0 min 20% B at 0 min to 98%
Injection volume	20 µL

Table 3

DP: declustering potential, V; CE: collision energy, V; CXP: collision cell exit potential						
Pesticide	Formula	Mol Wt	MRM Transitions #1 & #2	DP	CE	CXP
Carbofuran, 3OH-	$C_{12}H_{15}NO_4$	237	238→163 / 181	66	21	16
Acephate	$C_4H_{10}NO_3PS$	183	184→143 / 49	61	13	4
Acetamiprid	$C_{10}H_{11}N_3ClN_4$	223	223→126 / 99	61	29	12
Acibenzolar-S-methyl	$C_8H_6N_2OS_2$	210	211→136 / 140	46	39	9
Alanycarb	$C_{17}H_{25}N_3O_4S_2$	400	400→238 / 91	35	14	4
Aldicarb sulfoxide	$C_7H_{14}N_2O_3S$	206	207→132 / 89	30	10	8
Aldicarb	$C_7H_{14}N_2O_2S$	190	208→116 / 89	36	11	10
Aldicarb sulfone	$C_7H_{14}N_2O_4S$	222	223→86 / 148	52	21	5
Ametryn	$C_9H_{17}N_5S$	227	209→152 / 137	71	21	8
Aminocarb	$C_{11}H_{16}N_2O_2$	208	209→152 / 137	71	21	8
Amitraz	$C_{19}H_{23}N_3$	293	294→163 / 107	46	21	4
Avermectin B1a	$C_{48}H_{72}O_{14}$	873	895→751 / 449	176	61	20
Avermectin B1b	$C_{48}H_{70}O_{14}$	859	890→567 / 305	76	23	18
Azoxystrobin	$C_{22}H_{17}N_3O_5$	403	404→372 / 344	51	19	4
Benalaxyl	$C_{20}H_{23}NO_3$	325	326→148 / 294	71	31	8
Bendiocarb	$C_{11}H_{13}NO_4$	223	224→109 / 167	61	27	20
Benfuracarb	$C_{20}H_{30}N_2O_5S$	411	411→195 / 252	50	30	4
Benzoximate	$C_{18}H_{18}ClNO_5$	364	364→199 / 105	51	13	14
Bifenazate	$C_{17}H_{20}N_2O_3$	300	301→170 / 198	61	29	10
Bitertanol	$C_{20}H_{23}N_3O_2$	337	338→70 / 269	51	31	12
Boscalid	$C_{18}H_{12}Cl_2N_2O$	343	343→307 / 140	91	27	4
Bromuconazole 46	$C_{13}H_{12}BrCl_2N_3O$	377	378→159 / 70	61	37	14
Bromuconazole 47	$C_{13}H_{12}BrCl_2N_3O$	377	378→159 / 70	61	37	14
Bupirimate	$C_{13}H_{24}N_4O_3S$	316	317→166 / 108	86	33	12
Buprofezin	$C_{16}H_{23}N_3OS$	305	306→201 / 116	46	17	4
Butafenacil	$C_{20}H_{18}ClF_3N_2O_6$	475	492→331 / 349	61	35	20
Butocarboxin	$C_7H_{14}N_2O_2S$	190	213→75 / 116	50	20	5
Butoxycarboxin	$C_7H_{14}N_2O_4S$	222	223→106 / 166	45	15	8
Carbaryl	$C_{12}H_{11}NO_2$	201	202→145 / 127	56	15	10
Carbendazim	$C_9H_9N_3O_2$	191	192→160 / 132	80	24	10
Carbetamide	$C_{12}H_{16}N_2O_3$	236	237→192 / 118	56	13	12
Carbofuran	$C_{12}H_{15}NO_3$	221	222→123 / 165	66	31	22
Carboxin	$C_{12}H_{13}NO_2S$	235	484→452 / 285	66	23	14
Carfentrazone-ethyl	$C_{13}H_{10}Cl_2F_3N_3O_3$	412	412→346 / 366	81	31	4
Chlorfluazuron	$C_{20}H_9Cl_3F_5N_3O_3$	541	540→158 / 383	91	27	4
Chlorotoluron	$C_{10}H_{13}ClN_2O$	213	213→72 / 46	61	31	4
Chloroxuron	$C_{15}H_{15}ClNO_2$	291	291→72 / 218	65	30	4
Clethodim	$C_{17}H_{26}ClNO_3S$	360	360→164 / 268	61	29	10
Clofentezine	$C_{14}H_8Cl_2N_4$	303	303→138 / 102	61	23	8
Clothianidin	$C_6H_8ClN_5O_2S$	250	250→169 / 132	51	17	4
Cyazofamid	$C_{13}H_{13}ClN_4O_2S$	325	325→108 / 261	61	21	10
Cycluron	$C_{11}H_{22}N_2O$	198	199→89 / 72	50	21	4
Cymoxanil	$C_7H_{10}N_4O_3$	198	199→128 / 111	60	13	4
Cyproconazole A	$C_{15}H_{18}ClN_3O$	292	292→70 / 125	66	39	12
Cyproconazole B	$C_{15}H_{18}ClN_3O$	292	292→70 / 125	66	39	12
Cyprodinil	$C_{14}H_{15}N_3$	225	226→93 / 77	101	51	16
Desmedipham	$C_{16}H_{16}N_2O_4$	300	318→182 / 136	41	19	12
Diclobutrazol	$C_{15}H_{19}Cl_2N_3O$	328	328→70 / 158	81	49	12
Dicrotophos	$C_8H_{16}NO_5P$	237	238→112 / 193	66	19	8
Diethofencarb	$C_{14}H_{21}NO_4$	267	268→226 / 124	61	15	14

Difenoconazole	$C_{19}H_{17}Cl_2N_3O_3$	406	406→251 / 253	81	37	16
Diflubenzuron	$C_{14}H_9Cl_2FN_2O_2$	311	311→158 / 141	71	23	10
Dimethoate	$C_5H_{12}NO_3PS_2$	229	230→199 / 125	50	14	15
Dimethomorph A	$C_{21}H_{22}ClNO_4$	388	388→301 / 165	66	25	4
Dimethomorph B	$C_{21}H_{22}ClNO_4$	388	388→301 / 165	66	25	4
Dimoxystrobin	$C_{19}H_{22}N_2O_3$	326	327→205 / 116	40	15	4
Diniconazole	$C_{15}H_{17}Cl_2N_3O$	326	326→70 / 158	86	51	12
Dioxacarb	$C_{11}H_{13}NO_4$	223	224→167 / 123	51	13	10
Diuron	$C_9H_{10}Cl_2N_2O$	233	233→72 / 72	56	33	4
Doramectin	$C_{50}H_{74}O_{14}$	899	921→777 / 449	71	65	15
Fenpyroximate	$C_{24}H_{27}N_3O_4$	422	422→366 / 135	56	23	4
Emamectin B_{1a}	$C_{49}H_{75}NO_{13}$	886	886→158 / 82	111	51	10
Epoxiconazole	$C_{17}H_{13}ClFN_3O$	330	330→121 / 101	66	29	10
Eprinomectin B_{1a}	$C_{50}H_{75}NO_{14}$	914	914→186 / 154	76	27	12
Etaconazole	$C_{14}H_{15}Cl_2N_3O_2$	328	328→159 / 205	46	37	10
Ethiofencarb	$C_{11}H_{15}NO_2S$	225	226→106 / 164	41	21	4
Ethiprole	$C_{13}H_9Cl_2F_3N_4OS$	397	397→350 / 255	81	29	24
Ethofumesate	$C_{13}H_{18}O_5S$	286	287→121 / 259	81	23	8
Etoxazole	$C_{21}H_{23}F_2NO_2$	359	360→141 / 57	76	45	4
Famoxadone	$C_{22}H_{18}N_2O_4$	374	392→331 / 238	31	15	4
Fenamidone	$C_{17}H_{17}N_3OS$	311	312→92 / 236	66	39	16
Fenarimol	$C_{17}N_{12}Cl_2N_2O$	331	331→268 / 81	61	31	4
Fenazaquin	$C_{20}H_{22}N_2O$	306	307→161 / 147	71	25	12
Fenbuconazole	$C_{19}H_{17}ClN_4$	337	337→124 / 70	81	41	8
Fenhexamid	$C_{14}H_{17}Cl_2NO_2$	302	302→97 / 55	66	35	18
Fenoxycarb	$C_{17}H_{19}NO_4$	301	302→88 / 116	66	31	6
Fenpropimorph	$C_{20}H_{33}NO$	303	304→147 / 117	66	39	4
Fenuron	$C_9H_{12}N_2O$	164	165→72 / 46	56	25	4
Fludioxinil	$C_{12}H_6F_2N_2O_2$	248	266→229 / 227	41	23	14
Flufenacet	$C_{14}H_{13}F_4N_3O_2S$	363	364→152 / 194	51	29	10
Flufenoxuron	$C_{21}H_{11}ClF_6N_2O_3$	489	489→158 / 141	86	29	10
Fluometuron	$C_{10}H_{11}F_3N_2O$	232	233→72 / 46	71	37	12
Fluoxastrobin	$C_{21}H_{16}ClFN_4O_5$	459	459→427 / 188	55	28	4
Fluquinconazole	$C_{16}H_8Cl_2FN_5O$	376	376→307 / 349	71	33	4
Flusilazole	$C_{16}H_{15}F_2N_3Si$	315	316→247 / 165	81	27	16
Flutolanil	$C_{17}H_{16}F_3NO_2$	323	324→262 / 242	76	27	16
Flutriafol	$C_{16}H_{13}F_2N_3O$	301	302→70 / 123	66	37	12
Forchlorfenuron	$C_{12}H_{10}ClN_3O$	248	248→129 / 93.	52	25	4
Formetanate HCl	$C_{11}H_{15}N_3O_2$	221	222→165 / 120	60	21	12
Fuberidazole	$C_{11}H_8N_2O$	184	185→157 / 65	81	33	14
Furalaxyl	$C_{17}H_{19}NO_4$	301	302→95 / 242	56	41	18
Furathiocarb	$C_{18}H_{26}N_2O_5S$	382	383→195 / 252	76	27	12
Hexaconazole	$C_{14}H_{17}Cl_2N_3O$	314	314→70 / 159	56	41	12
Hexaflumuron	$C_{16}H_8Cl_2F_6N_2O_3$	461	461→158 / 141	56	25	4
Hexythiazox	$C_{17}H_{21}ClN_2O_2S$	353	353→228 / 168	61	23	14
Hydramethylnon	$C_{25}H_{24}F_6N_4$	494	495→323 / 151	146	45	20
Imazalil	$C_{14}H_{14}Cl_2N_2O$	297	297→159 / 201	66	33	14
Imidacloprid	$C_9H_{10}ClN_5O_2$	256	256→209 / 175	61	23	12
Indoxacarb	$C_{22}H_{17}ClF_3N_3O_7$	528	528→203 / 218	86	55	12
Ipconazole	$C_{18}H_{24}ClN_3O$	334	334→70 / 125	76	55	12
Iprovalicarb	$C_{18}H_{28}N_2O_3$	320	321→119 / 203	66	29	8
Isoprocarb	$C_{11}H_{15}NO_2$	193	194→95 / 137	61	23	16
Isoproturon	$C_{12}H_{18}N_2O$	206	207→72 / 46	66	29	4
Isoxaflutole	$C_{15}H_{12}F_2NO_4S$	359	377→251 / 360	36	41	16
Ivermectin	$C_{48}H_{74}O_{14}$	875	897→754 / 610	65	65	8
Kresoxim-methyl	$C_{18}H_{19}NO_4$	313	314→116 / 206	51	21	4
Linuron	$C_9H_{10}Cl_2N_2O_2$	249	249→160 / 182	61	23	4

Lufenuron	$C_9H_{10}Cl_2N_2O_2$	511	511→158 / 141	61	27	4
Mefenacet	$C_{16}H_{14}N_2O_2S$	298	299→148 / 120	56	21	10
Mepanipyrim	$C_{14}H_{13}N_3$	223	224→106 / 77	86	37	8
Mepronil	$C_{17}H_{19}NO_2$	269	270→119 / 228	76	33	8
Mesotrione	$C_{14}H_{13}NO_7S$	339	357→228 / 288	60	31	9
Metalaxyl	$C_{15}H_{21}NO_4$	279	280→220 / 192	61	21	14
Metconazole.1	$C_{17}H_{22}ClN_3O$	319	320→70 / 125	81	51	12
Methabenzhiazuron	$C_{10}H_{11}N_3OS$	221	222→165 / 150	51	21	4
Methamidophos	$C_2H_8NO_2PS$	141	142→94 / 125	55	20	4
Methiocarb	$C_{11}H_{15}NO_2S$	225	226→169 / 121	61	13	12
Methomyl	$C_5H_{10}N_2O_2S$	162	163→88 / 106	35	12	5
Methoprotryne	$C_{11}H_{21}N_5OS$	271	272→240 / 198	50	27	4
Methoxyfenozide	$C_{22}H_{28}N_2O_3$	368	369→149 / 313	56	25	10
Metobromuron	$C_9H_{11}BrN_2O_2$	259	259→170 / 148	56	23	4
Metribuzin	$C_8H_{14}N_4OS$	214	215→84 / 187	71	29	4
Mevinphos-Z	$C_7H_{13}O_6P$	224	225→127 / 193	55	20	8
Mevinphos-E	$C_7H_{13}O_6P$	224	225→127 / 193	55	20	8
Mexacarbate	$C_{12}H_{18}N_2O_2$	222	223→166 / 151	66	23	12
Monocrotophos	$C_7H_{14}NO_5P$	223	224→127 / 98	51	23	12
Monolinuron	$C_9H_{11}ClN_2O_2$	215	215→126 / 99	51	23	4
Moxidectin	$C_{37}H_{53}NO_8$	640	662→549 / 467	90	45	16
Myclobutanil	$C_{15}H_{17}ClN_4$	289	289→70 / 125	71	37	12
Neburon	$C_{12}H_{16}Cl_2N_2O$	275	275→88 / 114	56	23	4
Nitenpyram	$C_{11}H_{15}ClN_4O_2$	271	271→225 / 126	51	17	14
Novaluron	$C_{17}H_9ClF_8N_2O_4$	493	493→158 / 141	71	27	4
Nuarimol	$C_{17}H_{12}ClFN_2O$	315	315→252 / 81	81	31	16
Omethoate	$C_5H_{12}NO_4PS$	213	214→124 / 182	46	29	4
Oxadixyl	$C_{14}H_{18}N_2O_4$	278	279→219 / 132	61	17	14
Oxamyl	$C_7H_{13}N_3O_3S$	219	237→72 / 90	36	25	4
Paclobutrazol	$C_{15}H_{20}ClN_3O$	294	294→70 / 125	66	49	12
Penconazole	$C_{13}H_{15}Cl_2N_3$	284	284→159 / 70	71	39	10
Phenmedipham	$C_{16}H_{16}N_2O_4$	300	301→136 / 168	50	26	4
Picoxystrobin	$C_{18}H_{16}F_3NO_4$	367	368→145 / 205	56	27	4
Piperonyl butoxide	$C_{19}H_{30}O_5$	338	356→177 / 119	51	19	10
Pirimicarb	$C_{11}H_{18}N_4O_2$	238	239→72 / 182	66	35	12
Prochloraz	$C_{15}H_{16}Cl_3N_3O_2$	377	376→308 / 70	46	17	10
Promecarb	$C_{12}H_{17}NO_2$	207	208→109 / 151	36	23	8
Prometon	$C_{10}H_{19}N_5O$	225	226→142 / 86	76	33	10
Prometryn	$C_{10}H_{19}N_5S$	241	242→200 / 158	71	19	4
Propamocarb	$C_9H_{20}N_2O_2$	188	189→102 / 144	61	25	8
Propargite	$C_{19}H_{26}O_4S$	350	368→231 / 175	46	15	14
Propham	$C_{10}H_{13}NO_2$	179	180→138 / 120	36	13	10
Propiconazole	$C_{15}H_{17}Cl_2N_3O_2$	342	342→159 / 69	61	39	10
Propoxur	$C_{11}H_{15}NO_3$	209	210→111 / 168	39	19	6
Pymetrozine	$C_{10}H_{11}H_5O$	217	218→105 / 78	71	27	4
Pyracarbolid	$C_{13}H_{15}NO_2$	217	218→125 / 97	61	27	8
Pyraclostrobin	$C_{19}H_{18}ClN_3O_4$	388	388→194 / 163	31	19	4
Pyridaben	$C_{19}H_{25}ClN_2OS$	365	365→147 / 309	46	31	4
Pyrimethanil	$C_{12}H_{13}N_3$	199	200→107 / 82	71	33	4
Pyriproxyfen	$C_{20}H_{19}NO_3$	321	322→96 / 185	46	21	4
Quinoxyfen	$C_{15}H_8Cl_2FNO$	308	308→162 / 197	81	65	10
Rotenone	$C_{23}H_{22}O_6$	394	395→213 / 192	91	33	14
Secbumeton	$C_{10}H_{15}N_5O$	225	226→170 / 100	50	35	4
Siduron	$C_{14}H_{20}N_2O$	232	233→137 / 94	66	21	4
Simetryne	$C_8H_{15}N_5S$	213	214→124 / 144	51	27	4
Spinosyn A	$C_{41}H_{65}NO_{10}$	732	748→142 / 98	86	45	8
Spirodiclofen	$C_{21}H_{24}Cl_2O_4$	411	411→313 / 71	71	17	8

Spiromefesin	$C_{23}H_{30}O_4$	370	371→273 / 255	71	19	8
Spiroxamine	$C_{18}H_{35}NO_2$	297	298→144 / 100	76	29	12
Sulfentrazone	$C_{11}H_{10}Cl_2F_2N_4O_3S$	387	387→307 / 146	81	27	4
Tebuconazole	$C_{16}H_{22}ClN_3O$	308	308→70 / 125	81	49	12
Tebufenozide	$C_{22}H_{28}N_2O_2$	352	353→133 / 297	51	25	10
Tebufenpyrad	$C_{18}H_{24}ClN_3O$	334	334→117 / 145	71	47	4
Tebuthiuron	$C_9H_{16}N_4OS$	228	229→172 / 116	46	21	4
Teflubenzuron	$C_{14}H_6Cl_2F_4N_2O_2$	381	381→141 / 158	66	53	4
Terbumeton	$C_{10}H_{19}N_5O$	225	226→170 / 100	76	27	12
Terbutryn	$C_{10}H_{19}N_5S$	241	242→186 / 68	71	27	12
Tetraconazole	$C_{13}H_{11}Cl_2F_4N_3O$	372	372→159 / 70	76	45	10
Thiabendazole	$C_{10}H_7N_3S$	201	202→175 / 131	85	35	12
Thiacloprid	$C_{10}H_9ClN_4S$	253	253→126 / 99	71	31	10
Thiamethoxam	$C_8H_{10}ClN_5O_3S$	292	292→211 / 181	61	19	12
Thidiazuron	$C_9H_8N_4OS$	220	221→102 / 127	66	21	4
Thiobencarb	$C_{12}H_{16}ClNOS$	258	258→125 / 89	56	27	8
Thiofanox	$C_9H_{18}N_2O_2S$	218	219→76 / 57	36	20	8
Thiophanate-methyl	$C_{12}H_{14}N_4O_4S_2$	342	343→151 / 311	61	29	14
Triadimefon	$C_{14}H_{16}ClN_3O_2$	294	294→197 / 225	66	23	14
Triadimenol	$C_{14}H_{18}ClN_3O_2$	296	296→70 / 227	46	31	12
Tricyclazole	$C_9H_7N_3S$	189	190→163 / 136	81	33	10
Trifloxystrobin	$C_{20}H_{19}F_3N_2O_4$	408	409→186 / 206	31	23	4
Triflumizole	$C_{15}H_{15}ClF_3N_3O$	346	346→278 / 73	51	15	8
Triflumuron	$C_{15}H_{10}ClF_3N_2O_3$	359	359→156 / 139	51	23	4
Triticonazole	$C_{17}H_{20}ClN_3O$	318	318→70 / 125	66	45	12
Vamidothion	$C_8H_{18}NO_4PS_2$	287	288→146 / 118	61	19	10
Zoxamide	$C_{14}H_{16}Cl_3NO_2$	337	336→187 / 159	45	35	15
D10-Diazinon	$C_{12}D_{10}H_{11}N_2O_3PS$	314	315→170	50	29	4
D6-Dimethoate	$C_5D_6H_6NO_3PS_2$	235	236→131	50	30	4
D6-diuron	$C_9D_6H_4Cl_2N_2O$	239	239→78	90	30	4
D6-Linuron	$C_9D_6H_4Cl_2N_2O_2$	255	255→166	90	30	4
D6-Dichlorvos	$C_4D_6H_1Cl_2O_4P$	227	227→115	70	27	4
D6-Malathion	$C_{10}D_6H_{13}O_6PS_2$	330	337→291	55	12	4

*Summarized with permission from Wong, Jon, Hao, Chunyan, Zhang, Kai, et al., J. Agric. Food Chem. 2010, 58, 5897–5903 5897, DOI:10.1021/jf903849n

Listing of instrument manufacturers does not constitute endorsement by UCT

QuEChERS Extraction and Clean-Up of Pesticides from Olive Oil

UCT Part Number:
CUMPS2CT (150 mg anhydrous $MgSO_4$ & 50 mg PSA)

April 2009

1. Sample Extraction

a) In a suitable vial, add 1.5 mL of olive oil
b) Add 1.5 mL of hexane
c) Add 6 mL of acetonitrile
d) Shake for 30 minutes
e) Allow layers to phase separate for 20 minutes
f) Collect acetonitrile layer (top layer)
g) Repeat steps c) through f) and combine acetonitrile layers

2. Sample Clean-up

a) Add 1 ml of combined acetonitrile to CUMPS2CT
b) Shake for 2 minutes by hand
c) Centrifuge at 3000 rpm for 2 minutes
d) Remove solvent layer
e) Analyze by HPLC using MS detection

QuEChERS Sample Preparation For The Analysis Of Pesticide Residues In Olives*

UCT Product Number:
ECMSSC50CT-MP (4 g $MgSO_4$, 1.0 g NaCl)
ECQUEU122CT (2 mL centrifuge tube, 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18 and 50 mg GCB)
CUMPSC1875CB2CT – For better recovery of planar pesticides
(2 mL centrifuge tube, 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18, 7.5 mg GCB)

April 1, 2011

Summary

This application is a summary of the original paper "Evaluation of the QuEChERS sample preparation approach for the analysis for pesticide residues in olives"*. It describes the use of QuEChERS for the extraction and clean-up of 16 pesticide residues contained in olives. LC-MS/MS with positive ESI was used for pesticides that are difficult to detect by GC-MS. Matrix matched calibration standards were used to compensate for matrix effects. The method achieves acceptable quantitative recoveries of 70–109% with RSDs <20% for DSI-GC-MS and 88–130% with RSDs <10% for LC-MS/MS, and LOQ at or below the regulatory maximum residue limits. Analyte protectants were used with DSI to improve analyte peak shapes and intensities.

Analytes Covered in this Method

Analyte	CASRN
Ometholate	1113-02-6
Dimethoate	60-51-5
Simazine	122-34-9
Diazinon	65863-03-8
p,p'-DDE	82413-20-5
Diuron	56449-18-4
Carbaryl	63-25-2
Malathion	121-75-5
Fenthion	55-38-9
Methidathion	950-37-8
Napropamide	15299-99-7
Oxyfluorfen	42874-03-3
Carfentrazone-ethyl	128639-02-1
Phosmet	732-11-6

Pyriproxyfen	95737-68-1
Deltamethrin	64121-95-5

1. Sample Extraction

a) Weigh 10 g of homogenized sample into a 50 mL centrifuge tube

b) Add 10 mL of acetonitrile (MeCN)

c) Add contents of **ECMSSC50CT-MP**

d) Shake vigorously by hand for 1 minute

e) Centrifuge@ 3450 rcf for 1 minute

2. Dispersive Clean-Up

a) Transfer 1 mL the supernatant to a micro-centrifuge tube **ECQUEU122CT or CUMPSC1875CB2CT**

b) Mix for 20 s

c) Centrifuge @ 3450 rcf for 1 minute

d) Transfer 400µL of supernatant to an autosampler vial

e) Add 25 µL of TPP solution (10 g/mL triphenylphosphate on MeCN with 1.6% formic acid

f) Shake for 5 s

g) Extract is ready for analysis

3. Automated DSI-GC-MS Analysis

GC-MS was performed using an Agilent (Little Falls, DE, USA) 5890 Series II GC and 5972 MS instrument. Injection was performed using a Combi-PAL autosampler (CTC Analytics, Zwingen, Switzerland) using second generation automated DSI accessory (Linex) in combination with an Optic 3 PTV (Atas-GL International BV, Veldhoven, NL)

Note: Equivalent instrumentation and analytical columns can be used

Analyte Protectant Solution

(95% or better purity, prepare at 10:1:1 mg/mL in 7:3 water/MeCN, Sigma or Fluka)

- 3-ethoxy-1,2-propanediol
- D-sorbitol
- L-gulonic acid
- c-lactone

A quality check standard solution of 16 µg/mL triphenylphosphate (TPP) is prepared in MeCN containing 1.6% formic acid (FA)

For analysis by DSI-GC-MS, 20 µL of the analyte protectant solution was added to all the final extracts and matrix-matched calibration standards by transfer of 400 µL of extract into an autosampler vial and adding 25 µL of TPP solution

Conditions:

- Injection volume 10 µL
- 100° C (held 3.5 min with 50:1 split ratio)
- Ramp at 5° C/s to 280°C (use splitless for 3.5 min, then 50:1 split until 9 min, then change split flow to 20:1 and cool injector temperature to 150° C)

GC Separation:

- Varian VF-5 EZ-guard column (30 m x 0.25 mm id x 0.25 lm film thickness) with an integrated retention gap (5 m x 0.25 mm) at the inlet and an additional 1 m of uncoated capillary at the MS entrance
- He carrier gas @ 1 mL/ min

Oven temperature program:

- Start at 3.5 min (after sample introduction)
- 80° C hold for 3.5 min
- Ramp to 230° C at 108° C/min
- Then ramp to 300° C at 45° C/min, hold for 10 min.
- MS transfer line temperature at 290° C
- Electron ionization (EI) at –70 eV in SIM and full-scan (50–600 m/z) modes in different experiments

Agilent Chemstation for data acquisition/processing and GC-MS control, and Cycle Composer and Atas Evolution software are used to control the automated DSI process and PTV. The pesticide analytes in GC-MS and SIM ions are shown in the table below.

GC-MS SIM Conditions for the Monitored Pesticides

Pesticide	Start time (min)	t_R (min)	m/z (% relative abundance)	
			Quantitation ion	Qualifier ions
Dimethoate	4.5	15.89	87 (100)	125 (45), 93 (54), 58 (19)
Simazine		16.00	201 (78)	173 (41), 186 (51), 158 (25)
Diazinon	16.09	16.18	179 (100)	137 (98), 304 (47), 152 (70)
Diuron		16.52	72 (100)	232 (38), 234 (26), 187 (11)
Carbaryl	17.49	17.70	144 (100)	115 (33), 116 (26), 145 (15)
Malathion		18.03	173 (94)	125 (100), 93 (93),127 (75)
Fenthion	18.1	18.27	278 (100)	125 (37), 109 (33), 79 (19)
Methidathion	19.05	19.29	145 (88)	93 (40), 125 (27), 302 (19)
Napropamide	13.39	19.58	271 (26)	72 (100), 128 (63)
p,p'-DDE		19.367	318 (64)	246 (100), 248 (64), 316 (56)
Oxyfluorfen		19.71	361 (38)	252 (100), 300 (35), 280 (14)
Carfentrazone-ethyl	20	20.28	312 (100)	330 (65), 340 (63), 376 (31)
TPP	20.38	20.96	326 (100)	325 (87). 77 (88). 215 (20)
Phosmet		21.17	160 (100)	133 (15), 104 (15), 193 (4)
Pyriproxyfen	21.30	21.50	136 (100)	226 (12), 185 (6)
Deltamethrin	22.8	23.59	253 (85)	181 (100), 251 (44), 152 (20)

4. LC-MS/MS Analysis

Suggested Instrumentation: Agilent 1100 HPLC (consisting of vacuum degasser, autosampler Model WPALS, and a binary pump) equipped with a Prodigy ODS-3 (150 mm x 3 mm) and 5 µ particle size analytical column coupled to a ODS–C18 (4 mm x 2 mm and 5 µ particle size) guard column from Phenomenex (Torrance, CA, USA).

- Column temperature: 30° C
- Injection volume 5 µl.
- Mobile phase A water, B MeCN, both with 0.1% FA
- Gradient program:
  - Flow rate 0.3 mL/min
  - 25% solvent B linear gradient to 100% over the first 5 min
  - Hold for 7 min until 12 min
  - 11-min post run column wash

The LC system is connected to an API 3000 triple-quadrupole mass spectrometer (Applied Biosystems, Toronto, Canada) operated in ESI positive mode. Optimizations of the mass analyzer parameters were done by infusion of 1µg/mL analyte solutions at 10 µL/min with a

syringe pump (Harvard Apparatus, Holliston, MA, USA) using the autotune function.

Note: Equivalent instrumentation and analytical columns can be used

Final MS/MS conditions include:

- N_2 pressure 55 psi
- nebulizer gas setting 14
- curtain gas setting 11
- collision gas setting 12
- 4200 V ionspray voltage
- ESI temperature 525° C
- focusing potential 100 V
- entrance potential 10 V
- 0.15 s dwell time

The pesticide analytes by LC-MS/MS are shown in the table below with respective analytical ions

LC-MS/MS Conditions for the Monitored Pesticides

(Quantitation ion is shown as first mass)

Pesticide	Start time (min)	t_R (min)	Precursor ion (m/z)	Product ions (m/z)
Omethoate	2.5	2.68	214.0	183.2, 125.2
Dimethoate	5	6.83	230.0	199.1, 125.1
Simazine	7.6	7.98	202.0	124.2, 132.2
Carbaryl		8.48	202.2	145.1, 127.1
Diuron		8.67	233.1	72.2, 160.1
Phosmet	9	9.27	318.0	160.2, 133.2
Methidathion		9.28	303.0	145.1, 85.1
Malathion		9.64	331.0	127.2, 285.2
TPP	9.8	10.18	327.0	77.2, 152.0

*Adapted and used by permission from Cunha, Sara C., Lehotay, Steven J., Mastovska, Katerina, Fernandes, Jos O., Beatriz, Maria, Oliveira, P. P., Sep. Sci. 2007, 30, 620 – 632,DOI 10.1002/jssc.200600410

Listing of instrument manufacturers and standards suppliers does not constitute endorsement by UCT. Equivalent systems may be used

A Summary of US FDA LIB 4465: Collaboration of the QuEChERS Procedure for the Multiresidue Determination of Pesticides in Raw Agricultural Commodities by LC/MS/MS

UCT Product Numbers:

ECMSSC50CTFS-MP (6000 mg anhydrous magnesium sulfate, 1500 mg sodium chloride)

CUMPS2CT (150 mg anhydrous magnesium sulfate, 50 mg PSA)

ECMS12CPSA415CT (1200 mg anhydrous magnesium sulfate, 400 mg PSA)

March 2013

Method Summary

The analysis of fruits and vegetables for 173 pesticides using a single level calibration standard has been demonstrated to be an effective screening tool and can be completed in less than 20 minutes with overall accuracy of 105% and precision of 3% RSD. Pesticides are selected from a broad range of classes representing *carbamates, mectins, azoles, neonicitinoids, benzimidazoles, phenylureas, strobulirums, organophosphorous, anilides, tetrazines, anilides, benzoylphenylureas, and others.*

Procedure

Sample Preparation

Samples are composited by grinding in a vertical cutter mixed with dry ice

1. **Sample Extraction**
   a) Weigh 15 g of hydrated sample into the 50 ml centrifuge tube
   a) Add 15 mL acetonitrile (ACN)

   Note: Adjust ACN volume of spike samples to account for spike solution volume to maintain ratio of 1g sample/mL of ACN, e.g. for 5 ml spike volume add 10 mL ACN to 15 g sample

   b) Shake for 1 min
   c) Add internal standard
   d) Add spike standard if needed

e) Add the contents of pouch **ECMSSC50CTFS**

f) Shake 1 min

g) Centrifuge @ ~4500 rpm for 5 min

2. PSA Cleanup

a) Transfer 1.0 mL of extract to **CUMPS2CT** (or alternative, step b)

b) Transfer all extract to **ECMS12CPSA415CT**

c) Vortex and centrifuge

d) Dilute 0.5 mL extract to 5.0 mL with LC-MS aqueous buffer

e) Filter through 0.2 or 0.45 µm Nylon filter

f) Sample is ready for analysis

LC-MS/MS---Instrumentation

- AB Sciex 4000 QTrap: scheduled MRM in the positive ionization mode
- Shimadzu High Pressure HPLC System
- LC-20AD Pump
- Sil-20AC Autosampler
- CTO-20AC Column oven

HPLC Columns

- Ultra Aqueous C18, 3 µm, 100 x 2.1 mm with 10 x 2.1 mm guard column (Restek)

HPLC Instrument Parameters

Equilibration time (min)	1.5
Injection volume (µL)	20
Total Flow (mL/min)	0.5
Rinsing volume (µL)	200
Rinsing speed (µL/sec)	35
Sampling speed (µL/sec)	15
Cooler temperature (°C)	15
Column oven temp (°C)	40

Standards

Pesticide standard mixes may be purchased from AccuStandards and consist of 9 mixes of 20-25 analytes (total of 196 compounds)

The following injection and spiking standards were prepared in acetonitrile from the 3.0 µg/mL mixture of all standards:

Injection Standard: 200 ng/mL

Internal Standard: 200 ng/mL BDMC

Spike standards: 3000, 1200, 300, and 60 ng/mL

HPLC Mobile Phase Composition

Pump A: Water with 4 mM ammonium formate and 0.1 % formic acid
Pump B: Methanol with 4 mM ammonium formate and 0.1 % formic acid

Time	Parameter
Min	% B
0.0	5
1.0	5
9.0	95
11.3	95
12.0	5
13.4	5
13.5	stop

Mass Spectrometer Parameters

Typical MS Settings

MRM Detection Window (sec)	60
Target Scan Time (sec)	.5
Resolution Q1	unit
Resolution Q2	unit
MR Pause (msec)	5
Collision gas	med
Curtin gas (mL/min)	30
Exit Potential (volts)	10
Ion Source gas 1 (mL/min)	50
Ion Source gas 2 (mL/min)	50
Interface heater	on
Ion Spray (Volts)	5000
Turbo Spray T (°C)	400

MS/MS Transition Parameters

Compound	Transition 1					Transition 2				
	Q1	Q2	DP	CE	EXP	Q1	Q2	DP	CE	EXP
3-Hydroxycarbofuran	238.1	163	66	21	15	238.1	181	66	16	11
Acephate	184.1	143	61	13	5	184.1	49	61	33	6
Acetamiprid	223	126	60	29	10	223	99	60	51	14
Acibenzolar-S-methyl	211	136	46	39	8	211	140	46	31	8
Alanycarb	400.1	238.2	35	14	5	400.1	91.1	35	40	5
Aldicarb+NH4	208.1	116	35	11	10	208.1	89	35	23	16
AldicarbSulfoxide	207.1	132.1	30	10	8	207.1	89.1	30	19	6

Aldoxycarb	223.1	86.1	52	21	5	223.1	148	52	13	9
Aminocarb	209.1	152	71	21	8	209.1	137.1	71	35	10
Amitraz	294.2	163.2	46	21	4	294.2	107.1	46	57	4
AvermectinB1a+NH4	890.9	567.7	75	23	18	890.9	305.4	72	35	22
AvermectinB1b+Na	876.5	291	41	35	4	876.5	145	41	43	4
Azoxystrobin	404.1	372.1	51	19	5	404.1	344.1	51	27	5
BDMC	260	122	52	34	5	260	107	52	54	5
Benalaxyl	326.2	148.1	71	31	8	326.2	294.1	71	17	10
Bendiocarb	224.1	109	61	27	20	224.1	167.1	61	15	12
Benfuracarb	411.2	195.1	50	30	5	411.2	252.1	50	19	5
Bentazon	241	199	76	19	8	241	107	76	39	8
Benzoximate	364	199	51	13	13	364	105	51	35	4
Bifenazate	301.1	170.1	59	30	9	301.1	198.1	59	21	10
Bitertanol	338.2	70	51	31	12	338.2	269.2	48	13	14
Boscalid	343	307	90	27	7	343	140	90	27	6
BromuconazoleA	378	159	61	39	12	378	70	61	43	12
BromuconazoleB	378.1	159.1	61	39	12	378.1	70.1	61	43	12
Bupirimate	317	166.1	86	33	12	317	108	86	37	10
Buprofezin	306.2	201.1	46	17	5	306.2	116.2	46	21	5
Butafenacil+NH4	492.1	331	58	33	16	492.1	349	61	21	12
Butocarboxim+Na	213.1	75	50	21	6	213.1	116	50	13	6
Butoxycarboxim	223.1	106	45	15	8	223.1	166	45	11	5
Carbaryl	202.1	145	57	15	9	202.1	127	54	41	8
Carbendazim	192.2	160.2	80	24	10	192.2	132.1	80	41	7
Carbetamide	237.1	192	55	13	10	237.1	118.1	56	19	10
Carbofuran	222.1	123	66	31	19	222.1	165.1	66	19	11
Chlorantraniliprole	484	452.9	66	23	14	484	285.9	66	19	16
Chlorfluazuron	540	158	91	27	4	540	383	91	28	4
Chlorotoluron	213.1	72.2	61	31	5	213.1	46.2	61	27	5
Chloroxuron	291.1	72.4	65	34	5	291.1	218.1	65	30	5
Clethodim	360.1	164	61	28	9	360.1	268.1	61	17	8
Clofentezine	303	138	65	22	8	303	102	65	51	14
Clothianidin	250	169	51	17	4	250	132	51	21	10
Cyazofamid	325	108	60	20	9	325	261.1	60	15	13
Cycluron	199.1	89.1	50	21	5	199.1	72.2	50	21	4
Cyflufenamid	413.1	295.1	56	23	8	413.1	223.1	56	33	14
Cymoxanil	199	128	60	13	5	199	111	60	25	5
CyproconazoleA	292	70	63	37	10	292	125	63	43	8
CyproconazoleB	292.1	70.1	63	37	10	292.1	125.1	63	43	8
Cyprodinil	226	93	95	49	13	226	77	95	64	12
Cyromazine	167.1	85.1	62	27	15	167.1	125.1	62	27	8
Desmedipham+NH4	318.1	182	42	19	10	318.1	136	39	34	9
Diclobutrazol	328.1	70	81	49	12	328.1	158.9	81	49	10
Dicrotophos	238.1	112.1	66	19	8	238.1	193	66	15	13
Diethofencarb	268.1	226.1	60	15	12	268.1	124	61	45	8
Difenoconazole	406.1	251.1	80	37	13	408.2	253.1	76	33	5
Diflubenzuron	311	158.2	71	23	10	311	141.1	71	45	10
Dimethoate	230	199	49	16	12	230	125	50	27	8
DimethomorphA	388.1	301	66	25	5	388.1	165.1	66	45	5
DimethomorphB	388.2	301.1	66	25	5	388.2	165.2	66	45	5

Dimoxystrobin	327.1	205	40	15	5	327.1	116	40	35	5
Dinotefuran	203.1	129.2	51	19	8	203.1	157.2	51	13	14
Dioxacarb	224.1	167	51	13	10	224.1	123	51	23	21
Diuron	233.1	72	56	33	5	235.1	72.1	56	38	10
Doramectin+NH4	916.9	593.6	68	20	16	916.9	331.5	65	33	22
Emamectin	886.5	158.1	111	51	10	886.5	82.1	111	127	13
Eprinomectin	914.5	186.2	77	27	12	914.5	154.2	77	58	10
Ethaboxam	321	183.1	86	33	12	321	200.1	86	39	12
Ethiofencarb	226.1	106.9	41	21	5	226.1	164.1	41	11	5
Ethiprole	397.3	350.9	81	29	24	397.3	255.2	81	49	16
Ethirimol	210.2	140.1	81	31	8	210.2	98.1	81	39	18
Etoxazole	360.1	141	76	45	5	360.1	57.2	76	45	5
Famoxadone+NH4	392	331	32	15	6	392	238	37	23	6
Fenamidone	312.1	92	66	39	16	312.1	236.1	66	21	14
Fenazaquin	307.1	161.1	68	27	10	307.1	147	68	28	9
Fenbuconazole	337	124.9	81	41	8	337	70	81	39	12
Fenhexamid	302	97	75	34	14	302	55	75	67	9
Fenobucarb	208.1	95.1	61	21	18	208.1	152.1	61	13	10
Fenoxycarb	302.1	88	65	30	6	302.1	116.1	65	17	7
Fenpyroximate	422	366.1	56	23	5	422	135.1	56	43	5
Fenuron	165.1	72.1	56	25	5	165.1	46	56	29	5
Flonicamid	230.1	203.1	55	35	4	230.1	174	55	35	4
Flubendiamide	683	408	56	17	12	683	274	56	43	16
Fludioxinil+NH4	266	229	41	23	14	266	227.1	41	13	14
Flufenoxuron	489	158	86	29	10	489	141.1	86	63	8
Fluometuron	233.1	72.1	71	37	12	233.1	46	71	35	4
Fluoxastrobin	459.2	427.2	55	28	5	459.2	188	55	35	5
Flusilazole	316.1	247.1	78	27	14	316.1	165.1	78	38	9
Flutolanil	324.1	262.1	74	26	14	324.1	242.1	74	34	12
Flutolanil+NH4	341.1	242.1	61	35	4	341.1	262.1	61	35	4
Flutriafol	302.1	70.1	66	37	12	302.1	123	66	41	8
Forchlorfenuron	248	129.1	52	25	5	248	93.1	52	48	5
Formetanate	222.1	165	71	22	9	222.1	93	76	53	14
Fuberidazole	185	157	81	33	13	185	65	81	67	11
Furathiocarb	383.1	195.1	74	26	10	383.1	252.1	74	19	14
Halofenozide	331.1	275	41	11	16	331.1	105.1	41	25	8
Hexaflumuron	461.1	158.2	56	25	5	461.1	141.1	56	65	5
Hexythiazox	353.1	228	63	23	12	353.1	168	63	36	9
Hydramethylnon	495.2	323.2	146	45	18	495.2	151.1	146	95	8
Imazalil	297	159	65	34	12	297	201	65	29	10
Imidacloprid	256	209.1	61	23	10	256	175.1	61	28	10
Indoxacarb	528	203	89	54	10	528	218	86	33	14
Ipconazole	334.2	70	74	52	10	334.2	125	74	50	17
Iprovalicarb	321.2	119	66	29	8	321.2	203.1	66	13	13
Isoprocarb	194.1	95	60	23	13	194.1	137	60	13	10
Isoproturon	207.2	72.1	66	29	5	207.2	46.1	66	31	5
Isoxaflutole	360.1	251.1	62	24	9	360.1	220.1	62	50	9
Isoxaflutole+NH4	377	251.1	56	29	14	377	69	56	35	12
Ivermectin+NH4	892.8	569.7	70	21	20	892.8	713.8	71	15	24
Kresoxim-methyl	314	116	51	21	4	314	206	51	13	4

Linuron	249.1	160	60	23	5	249.1	182.1	60	21	5
Lufenuron	511.1	158.1	61	27	5	511.1	141.2	61	67	5
Malathion	331	127	71	19	8	331	285	71	11	16
Mandipropamide	412.1	328.1	81	21	10	412.1	356.1	81	17	10
Mepanipyrim	224	106	86	37	8	224	77	86	59	14
Metaflumizone	507.1	178.1	101	39	12	507.1	287.1	101	37	16
Metalaxyl	280.1	220.2	60	20	12	280.1	192.2	60	26	10
Metconazole	320.1	70	81	51	12	320.1	125	81	59	10
Methamidophos	142	94	54	20	5	142	125	54	19	7
Methiocarb	226.1	169.1	61	13	11	226.1	121.1	61	27	8
Methomyl	163.1	88.1	35	12	6	163.1	106	35	13	6
Methoxyfenozide	369.1	149.1	56	24	9	369.1	313.2	56	13	10
Metobromuron	259	170.2	56	23	4	259	148.2	56	21	4
Mevinphos-E	225.1	127.1	51	20	7	225.1	193.2	51	10	10
Mevinphos-Z	225	127	51	20	7	225	193.1	51	10	10
Mexacarbate	223.2	166.1	64	23	10	223.2	151	64	35	9
Monocrotophos	224.1	127.1	53	23	10	224.1	98	53	17	5
Monolinuron	215.1	126.1	51	23	5	215.1	99	51	41	5
Moxidectin	640.5	528.5	61	12	16	640.5	498.5	61	17	16
Myclobutanil	289	70	71	37	12	289	125	71	47	8
Novaluron	493	158.1	71	27	5	493	141.1	71	69	5
Nuarimol	315	252.1	75	31	13	315	81	75	44	12
Omethoate	214	124.9	46	29	5	214	182.8	46	17	5
Oxadixyl	279.1	219.1	61	17	13	279.1	132.1	61	43	21
Oxamyl+NH_4	237.1	72.1	36	25	5	237.1	90.1	36	12	6
Paclobutrazol	294	70	62	46	10	294	125	58	49	8
Pencycuron	329.1	125	76	37	22	329.1	218.1	76	25	14
Phenmedipham	301.1	136	50	26	5	301.1	168.1	50	14	4
Phorate Sulfone	293.1	97.1	36	41	5	293.1	171.1	36	17	5
Picoxystrobin	368	145	56	27	4	368	205	56	15	4
PiperonylButox+NH_4	356.2	177.2	49	22	9	356.2	119.1	49	46	8
Pirimicarb	239.2	72.1	64	35	10	239.2	182.1	64	23	10
Prochloraz	376	308	45	17	10	376	70	45	44	12
Promecarb	208.1	109	37	23	8	208.1	151	37	13	10
Propamocarb	189.2	102	60	25	8	189.2	144	61	19	13
Propargite+NH4	368.2	231.1	46	15	13	368.2	175.1	46	23	12
Propiconazole	342.1	159	62	40	9	342.1	69	62	36	10
Propoxur	210.1	111	39	19	6	210.1	168.1	39	11	10
Pymetrozine	218	105	71	27	5	218	78	71	47	5
Pyracarbolid	218.1	125	59	27	8	218.1	97	59	40	14
Pyraclostrobin	388	194	31	19	5	388	163	31	29	5
Pyridaben	365	147	46	31	5	365	309	46	19	5
Pyrimethanil	200	107	71	33	5	200	82	71	35	5
Pyriproxyfen	322	96	45	21	5	322	185	45	29	5
Rotenone	395.1	213.1	90	32	12	395.1	192.1	90	34	10
Siduron	233.3	137.2	66	21	5	233.3	94	66	31	5

Spinetoram A	748.5	142.2	86	45	8	748.5	98.1	86	109	18
Spinetoram B	760.5	142.2	96	41	10	760.5	98.1	96	101	18
SpinosynA	732.5	142.2	111	43	10	732.5	98.1	111	103	16
Spirodiclofen	411.3	313.3	72	23	8	411.3	71.3	71	33	10
Spiromesifen	371.2	273.2	73	16	6	371.2	255.2	74	33	4
Spiromesifen+NH_4	388.2	273.2	41	19	12	388.2	255.2	41	39	16
Spirotetramat	374.2	330.2	66	23	8	374.2	302.2	66	25	20
Spiroxamine	298.2	144.2	72	28	10	298.2	100.1	72	46	14
Sulfentrazone	387	307.1	81	27	5	387	146	81	57	5
Tebuconazole	308.2	70	81	49	11	308.2	125	81	51	8
Tebufenozide	353.2	133	54	24	9	353.2	297.2	54	14	9
Tebuthiuron	229.1	172.4	46	21	5	229.1	116.1	46	35	5
Teflubenzuron	381.1	141.2	66	52	5	381.1	158.2	66	23	5
Temephos	467	419.1	101	29	12	467	405	101	23	12
Thiabendazole	202.1	175.1	84	35	10	202.1	131.2	84	45	8
Thiacloprid	253	126	68	30	9	253	99	68	60	14
Thiamethoxam	292	211	64	18	10	292	181	64	32	10
Thidiazuron	221.1	102.1	57	28	6	221.1	128.2	57	22	7
Thiophanate-methyl	343	151.1	61	29	14	343	311	61	17	10
Triadimefon	294	197.1	63	22	12	294	225	63	19	8
Triadimenol	296.1	70	46	31	12	296.1	227.1	46	19	14
Trichlorfon	256.9	109.1	66	25	20	256.9	127	66	25	8
Tricyclazole	190	163	81	33	10	190	136	81	41	11
Trifloxystrobin	409	186	31	23	5	409	206	31	21	5
Triflumizole	346.1	278.1	51	15	8	346.1	73	51	27	6
Triflumuron	359.1	156.2	52	23	6	359.1	139	52	44	6
Triticonazole	318.1	70	63	42	10	318.1	125	63	41	8
Vamidothion	288	146	61	19	10	288	118	61	33	10
Zoxamide	336.1	187	55	33	11	336.1	159	53	39	12

Adapted from: Sack, Chris*, Smoker, Michael, KAN, Lenexa, Chamkasem, Narong, SRL, Thompson, Richard, Satterfield, Greg, ARL, MacMahon, Shaun, Masse, Claude NERL, Mercer, Greg, Neuhaus, Barbara, PRL-NW, Cassias, Irene, Chang, Eugene, Lin, Yi, PRL-SW, Wong, Jon, Zhang, Kai, CFSAN*, Development and Validation of a Multiresidue Determination for Pesticides by LC-MS/MS* DFS/ORA/FDA No. 4464 Pesticidesand *Collaboration of the QuEChERS Procedure for the Multiresidue Determination of Pesticides by LC-MS/MS In Raw Agricultural Commodities*, DFS/ORA/FDA, No. 4465 Pesticides

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Determination of Pesticides in Red Wine by QuEChERS Extraction, Quick QuEChERS Clean-up, and LC/MS/MS Detection

UCT Part Numbers:
RFV0050CT (50 mL polypropylene centrifuge tube)
ECQUUS2-MP (Mylar Pouch contains: 4000 mg $MgSO_4$, 2000 mg NaCl)
ECPURMPSMC (Quick QuEChERS cartridge, 110 mg $MgSO_4$, 180 mg PSA)

The analysis of pesticide residues in red wines is challenging due to the complexity of the matrix, which contains organic acids, sugars, phenols, and pigments, such as anthocyanins. A simple, faster, and easy to use method is developed for the determination of pesticide residues in red wines.

Eight pesticides with a wide range of polarities (LogP from -0.779 to 5.004) were selected as target analytes. Excellent accuracy and precision data were achieved using this method. Recoveries of planar pesticides, such as Carbendazim and Thiabendazole were not affected since PSA was used for clean-up instead of GCB. PSA removed organic acids, sugars and pigments from the red wine extract. Six red wine samples were extracted using this method. Cyprodinil and Carbendazim were detected in the red wine samples tested, with minimum reporting limits of 1.5 ng/mL.

Procedure

1. Extraction

a) Add 10 mL of red wine sample to a 50 mL polypropylene centrifuge tube **(RFV0050CT)**
b) Spike with the appropriate amount of target analytes for fortified samples
c) Vortex 30 sec, then equilibrate for 15 min
d) Add 10 mL of acetonitrile, vortex 30 sec
e) Add salts in Mylar pouch (**ECQUUS2-MP)**
f) Shake vigorously for 1 min
g) Centrifuge at 5000 rpm for 5 min at 20° C
h) Supernatant is ready for clean-up

2. Quick QuEChERS Clean-up

a) Draw 1 mL of supernatant into a disposable polypropylene syringe
b) Pass the supernatant slowly through the Quick QuEChERS cartridge (**ECPURMPSMC**)

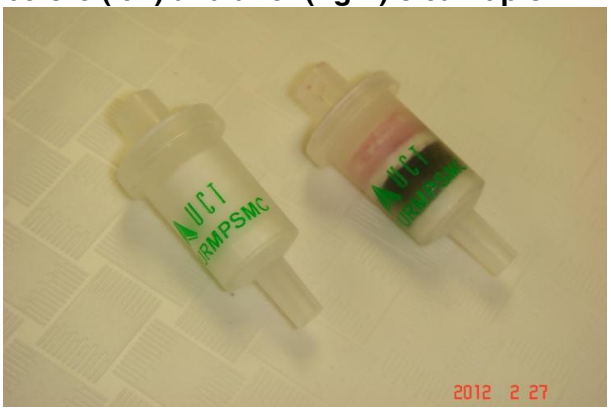
c) Collect 0.5 mL of the cleaned extract into a 2 mL auto-sampler vial

d) Add 10 µL 5 ppm TPP as internal standard (IS)

e) Samples are ready for LC/MS/MS analysis

Clean-up red wine extract with Quick QuEChERS

Quick QuEChERS before (left) and after (right) clean-up of 1 mL red wine extract



3. LC/MS/MS Detection

LC: Thermo Accela 1250 pump with PAL auto-sampler

LC Conditions

Column	Guard column: Restek C18, 2.1 x 20 mm Column: Sepax HP-C18, 2.1 x 100 mm, 3 µm, 120 Å
Column Temperature	Ambient
Injection Volume	10 µL at 15° C
Mobile Phase	A: 0.1% formic acid in Milli-Q-water B: 0.1% formic acid in methanol
Flow Rate	200 µL/min

LC Gradient Program

Time	%A	%B
0	95	5
1	95	5
3	50	50
8	5	95
14.2	95	5
16	95	5

MS/MS: Thermo TSQ Vantage tandem MS

MS Conditions

Ion source:	Heated ESI
Ion polarity:	ESI +
Spray voltage:	3000 V
Sheath gas pressure:	N_2 @ 40 psi
Auxiliary gas pressure:	N_2 @ 10 psi
Ion transfer capillary temperature:	350 °C
Scan type:	SRM (0-16 min)
CID conditions:	Ar @ 1.5 mTorr

SRM transitions

Compound	Parent	Product ion 1	CE	Product ion 2	CE	S-Lens	Dwell time (s)
Methamidophos	142.044	94.090	14	125.050	16	59	0.15
Carbendazim	192.093	132.080	29	160.080	17	81	0.10
Thiabendazole	202.059	131.060	31	175.070	31	103	0.10
Pyrimethanil	200.116	107.060	23	183.140	22	66	0.10
Cyprodinil	226.122	77.030	40	93.050	33	88	0.10

TPP (IS)	327.093	77.020	37	152.070	33	98	0.10
Diazinon	305.135	153.090	15	169.08	14	89	0.10
Pyrazophos	374.103	194.060	20	222.130	20	104	0.10
Chlorpyrifos	349.989	96.890	32	197.940	17	69	0.10

Matrix matched calibration, LOD and LOQ

Compound	Linearity range (ng/mL)	R^2	LOD (ng/mL)	LOQ (ng/mL)
Methamidophos	2-400	0.9991	0.15	0.49
Carbendazim	2-400	0.9981	0.40	1.33
Thiabendazole	2-400	0.9940	0.09	0.31
Pyrimethanil	2-400	0.9990	0.01	0.05
Cyprodinil	2-400	0.9995	0.17	0.57
Diazinon	2-400	0.9982	0.06	0.21
Pyrazophos	2-400	0.9976	0.08	0.27
Chlorpyrifos	2-400	0.9981	0.10	0.32

Accuracy and Precision Data

Compound	Fortified at 10 ng/mL		Fortified at 50 ng/mL		Fortified at 100 ng/mL	
	Recovery%	RSD% (n=4)	Recovery%	RSD% (n=4)	Recovery%	RSD% (n=4)
Methamidophos	93.7	3.4	81.6	5.8	84.2	3.5
Carbendazim	105.7	10.8	100.1	10.6	90.5	7.6
Thiabendazole	91.2	4.9	87.9	6.8	85.0	4.0
Pyrimethanil	112.2	2.7	107.0	3.2	102.8	4.9
Cyprodinil	104.3	3.2	99.9	6.1	100.2	4.9
Diazinon	104.9	5.6	102.0	6.6	99.2	6.8
Pyrazophos	99.9	4.0	96.6	5.6	91.3	4.1
Chlorpyrifos	91.7	4.6	99.5	5.2	97.2	3.8

Pesticides detected in red wine samples (ng/mL)

Pesticide	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Methamidophos	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5
Carbendazim	< 1.5	< 1.5	< 1.5	**10.2**	**8.7**	**2.3**
Thiabendazole	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5
Pyrimethanil	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5
Cyprodinil	**1.7**	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5
Diazinon	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5
Pyrazophos	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5
Chlorpyrifos	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5

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Using a QuEChERS Approach for the Determination of Pesticide Residues in Soil

UCT Part Numbers:
ECQUEU750CT-MP – 4000 mg MgSO4, 1000 mg NaCl, 500 mg Sodium Citrate dibasic sesquihydrate, 1000 mg Sodium Citrate tribasic dehydrate
CUMPSC18CT – 150 mg MgSO4, 50 mg PSA, 50 mg endcapped C18
SLC-18100ID21-3UM – Selectra® C18 HPLC Column 100 x 2.1mm, 3 µm
SLC-18GDC20-3UM - Selectra® C18 Guard Cartridge, 10 x 2.0mm, 3 µm

June 2014

Introduction

The use of pesticides in agriculture and households is widespread. To ensure food safety and prevent the unnecessary exposure of consumers to pesticides it is important to test for these residues in surveillance plans. While the greatest source of pesticide exposure comes from residues that remain in final food products, they can also be found in environmental samples such as water and soil. As a consequence, any pesticides that are present in soil can potentially be incorporated into growing crops. Contaminated soil also represents a serious environmental problem as the pesticides can be transported to other environmental systems such as ground water and air.

Due to the wide range of pesticides used in agriculture, the development of fast multi-residue methods that simultaneously determine a wide range of pesticides is essential. One of the most widely used multi-residue methodologies is the QuEChERS approach. This offers many advantages including speed, cost, ease of use, good performance characteristics and wide applicability range (matrices and analytes).

Soil is a complex matrix consisting of organic and inorganic material. It possesses many active sites (polar, non-polar and ionic) that are capable of retaining pesticides and other residues. Compared to other matrices commonly encountered in pesticide residue analysis (e.g. fruits and vegetable), soil samples are more difficult to extract and require longer extraction times due to the stronger interactions that may occur between the soil and the pesticides.

The aim of this study was to evaluate the effectiveness of the QuEChERS extraction and cleanup approach for the analysis of pesticides in soil. 21 pesticides, comprising various chemical properties, were used for the study. LC-MS/MS was used for detection and quantitation.

NOTE: It is possible for certain compounds to be covalently bound to the soil. These bound residues can only be removed using an acid or base hydrolysis step prior to extraction. However, if a hydrolysis step is employed, this may have a detrimental effect on pH sensitive analytes. Investigating this issue was outside the scope of this study and it was not evaluated.

QuEChERS procedure

Sample Extraction

1. Weigh 10g soil sample (≥70% H_2O content) into a 50mL centrifuge tube. Alternatively, weigh 3g air-dried soil sample into a 50mL tube and add 7mL H_2O, vortex briefly, and allow to hydrate for 30 min.
2. Add 10 mL of acetonitrile to each sample.
3. Shake (manually or mechanically) or vortex samples for 5 min to extract pesticides. (In this study a Spex SamplePrep Geno/Grinder 2010 operated at 1500 rpm was used).
4. Add the contents of an **ECQUEU750CT-MP** Mylar pouch (citrate buffered salts) to each centrifuge tube.
5. Immediately shake samples for at least 2 min.
6. Centrifuge for 5 min at ≥3000 rcf.

Sample Cleanup

1. Transfer a 1 mL aliquot of supernatant to a 2mL **CUMPSC18CT** dSPE tube ($MgSO_4$, PSA & C18).
2. Vortex samples for 0.5 - 1 min.
3. Centrifuge for 2 min at high rcf (e.g. ≥ 5000).
4. Filter purified supernatant through a 0.2 µm syringe filter directly into a sample vial.
5. Analyze samples by LC-MS/MS.

Analytical Procedure

HPLC Conditions	
Instrumentation	Thermo Scientific™ Dionex™ Ultimate™ 3000 LC system
HPLC column	UCT Selectra® C18, 100 × 2.1 mm, 3 µm (p/n: **SLC-18100ID21-3UM**)
Guard column	UCT Selectra® C18, 10 × 2.0 mm, 3 µm, (p/n: **SLC-18GDC20-3UM**)
Column temp.	40°C
Injection volume	3 µL
Autosampler	10°C
Wash solvent	MeOH:ultrapure water (1:1, v/v)
Mobile phase A	0.1% ammonium formate + 0.3% formic acid
Mobile phase B	methanol + 0.1% formic acid
Flow rate	300 µL/min
Run time	25 min (including 5 min re-equilibration)
Divert valve	Mobile phase was sent to waste for the initial 3 min and during re-equilibration to reduce ion source contamination.

MS Conditions	
Instrumentation	Thermo Scientific™ TSQ Vantage™ tandem mass spectrometer
Ionization mode	ESI^+
Spray voltage	4500 V
Vaporizer temperature	450°C
Capillary temperature	225°C
Sheath gas pressure	55 arbitrary units
Auxiliary gas pressure	25 arbitrary units
Ion sweep gas	0 arbitrary units
Declustering potential	0 V
Q1 and Q3 peak width	0.2 and 0.7 Da
Collision gas	argon
Collision gas pressure	1.5 mTorr
Acquisition method	EZ method (SRM)
Cycle time	1 sec

MRM Transitions							
Analyte	**t_R (min)**	**Precursor ion**	**Product ion 1**	**CE 1**	**Product ion 2**	**CE 2**	**S-lens (V)**
Carbendazim	4.9	192.09	132.08	29	160.08	17.0	81
Dicrotophos	5.6	238.01	108.60	33	126.58	17.0	73
Thiabendazole	8.6	202.06	131.06	31	175.07	24.0	103
DIMP	8.6	180.96	96.90	12	98.86	14.0	38
Simazine	8.6	202.01	67.97	32	131.97	17.0	104
Tebuthiuron	8.8	228.95	115.59	26	171.63	17.0	72
Carbaryl	9.0	201.96	126.97	29	144.96	6.00	40
Atrazine	9.9	215.96	67.65	35	173.60	16.0	79
DEET	10.1	191.95	90.66	28	118.63	15.0	92
Pyrimethanil	11.0	199.99	106.97	23	183.00	22.0	97
Malathion	12.3	331.01	98.57	23	126.86	12.0	60
Acetochlor	13.3	269.96	148.02	15	223.98	10.0	64
Cyprodinil	13.6	226.12	77.03	40	93.05	33.0	88
Tebuconazole	14.2	308.01	69.66	29	124.56	35.0	97
Diazinon	14.3	304.99	153.04	16	169.02	16.0	100
TPP	14.4	327.09	77.02	37	152.07	33.0	98
Zoxamide	14.4	335.92	158.91	36	186.91	19.0	89
Pyrazophos	14.7	374.10	194.06	20	222.13	20.0	104
Profenofos	15.7	372.89	127.92	41	302.79	17.0	99
Chlorpyrifos	16.4	349.70	96.81	29	197.76	20.0	81
Abamectin	17.6	889.98	304.92	25	751.21	35.0	112
Bifenthrin	18.2	440.04	165.21	39	180.42	11.0	66

Accuracy & Precision Data				
Analyte	**20 ng/g (n=6)**		**100 ng/g (n=6)**	
	Mean (%)	**RSD (%)**	**Mean (%)**	**RSD (%)**
Abamectin	74.9	11.17	71.8	6.28
Acetochlor	93.9	7.32	97.5	3.19
Atrazine	95.3	5.16	98.1	1.30
Bifenthrin	94.9	12.90	90.9	10.32
Carbaryl	95.2	7.13	93.9	3.53
Carbendazim	69.6	8.55	81.6	5.06
Chlorpyrifos	89.5	6.36	93.1	3.96
Cyprodinil	93.2	9.12	94.1	1.78
DEET	107.3	6.75	101.1	0.67
Diazinon	94.4	7.53	98.2	1.36
Dicrotophos	91.0	6.61	99.1	3.35
DIMP	82.5	6.74	88.1	1.47

Accuracy & Precision Data (cont)				
Analyte	**20 ng/g (n=6)**		**100 ng/g (n=6)**	
	Mean (%)	**RSD (%)**	**Mean (%)**	**RSD (%)**
Malathion	52.3	9.29	78.1	1.78
Profenofos	79.5	8.76	88.6	2.75
Pyrazophos	80.5	8.01	93.9	2.63
Pyrimethanil	90.2	4.88	92.2	2.36
Simazine	92.4	7.74	98.9	2.77
Tebuconazole	88.5	6.69	93.1	3.08
Tebuthiuron	100.7	7.39	101.1	2.14
Thiabendazole	52.8	5.61	63.1	6.80
Zoxamide	92.4	7.92	99.4	2.11
Note: TPP was used as an internal standard. Matrix-matched calibration curves were used for quantification.				

Figure 1. LC-MS/MS chromatogram of 21 pesticides and internal standard (TPP):

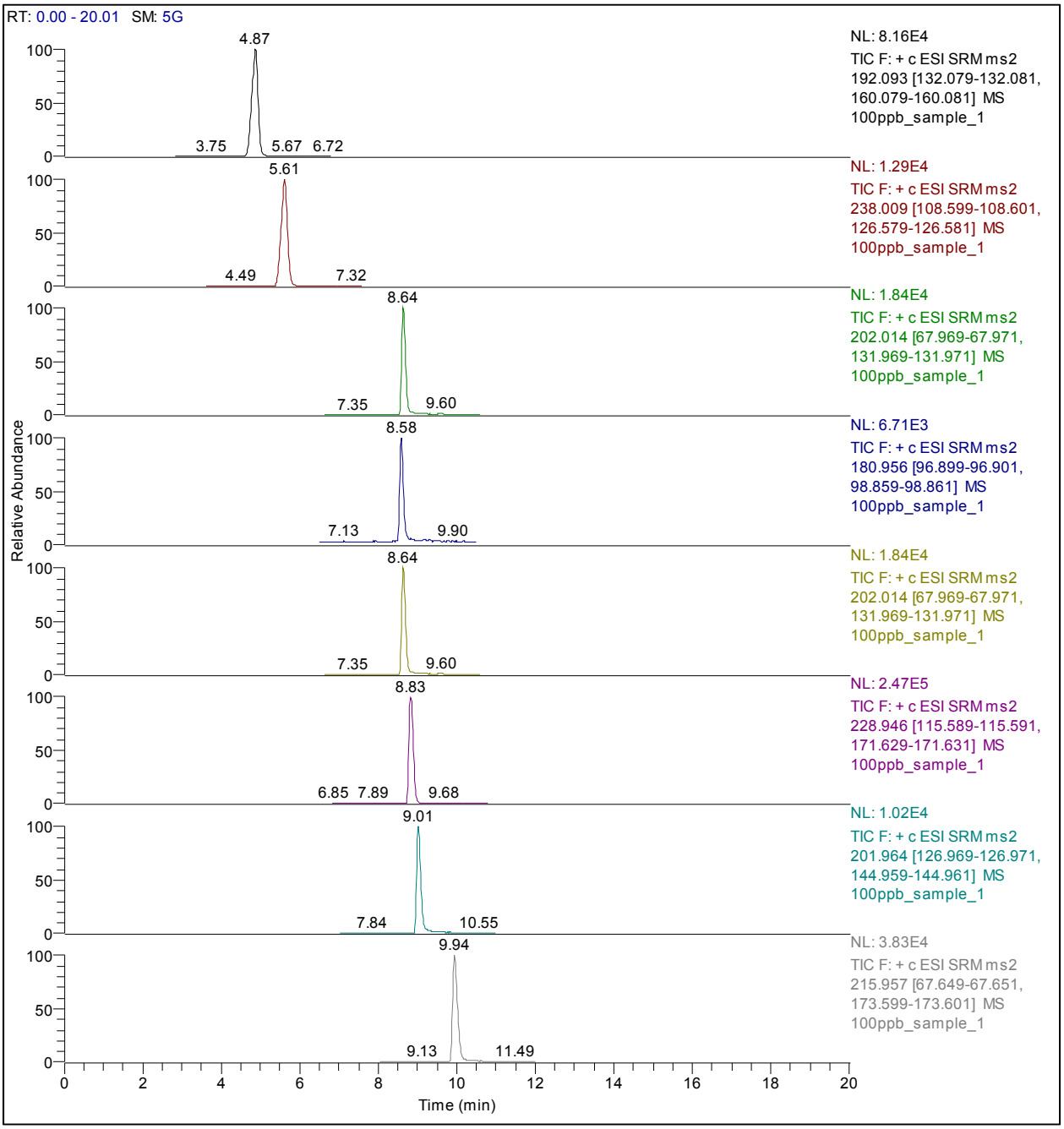


Figure 1 continued.

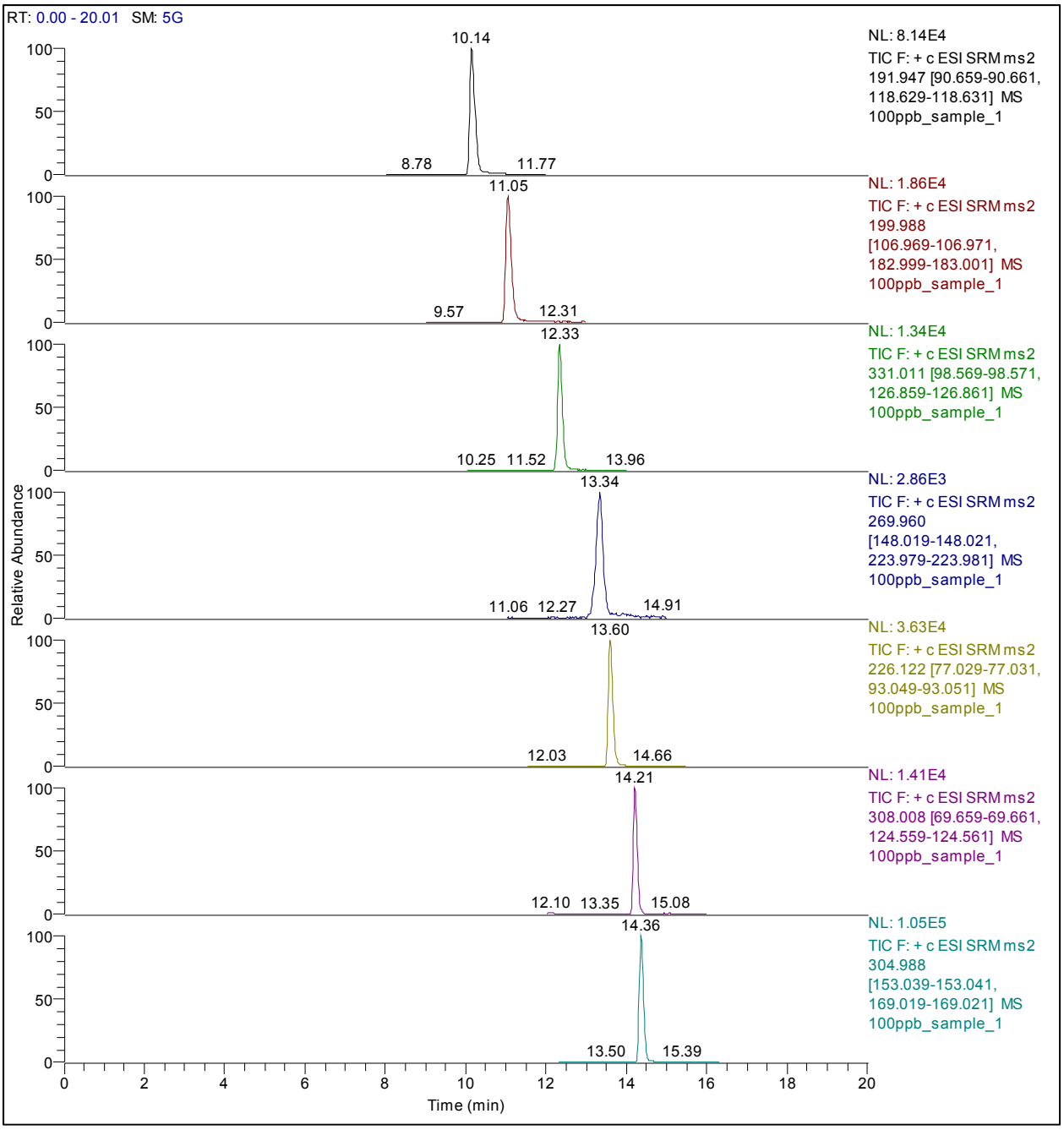
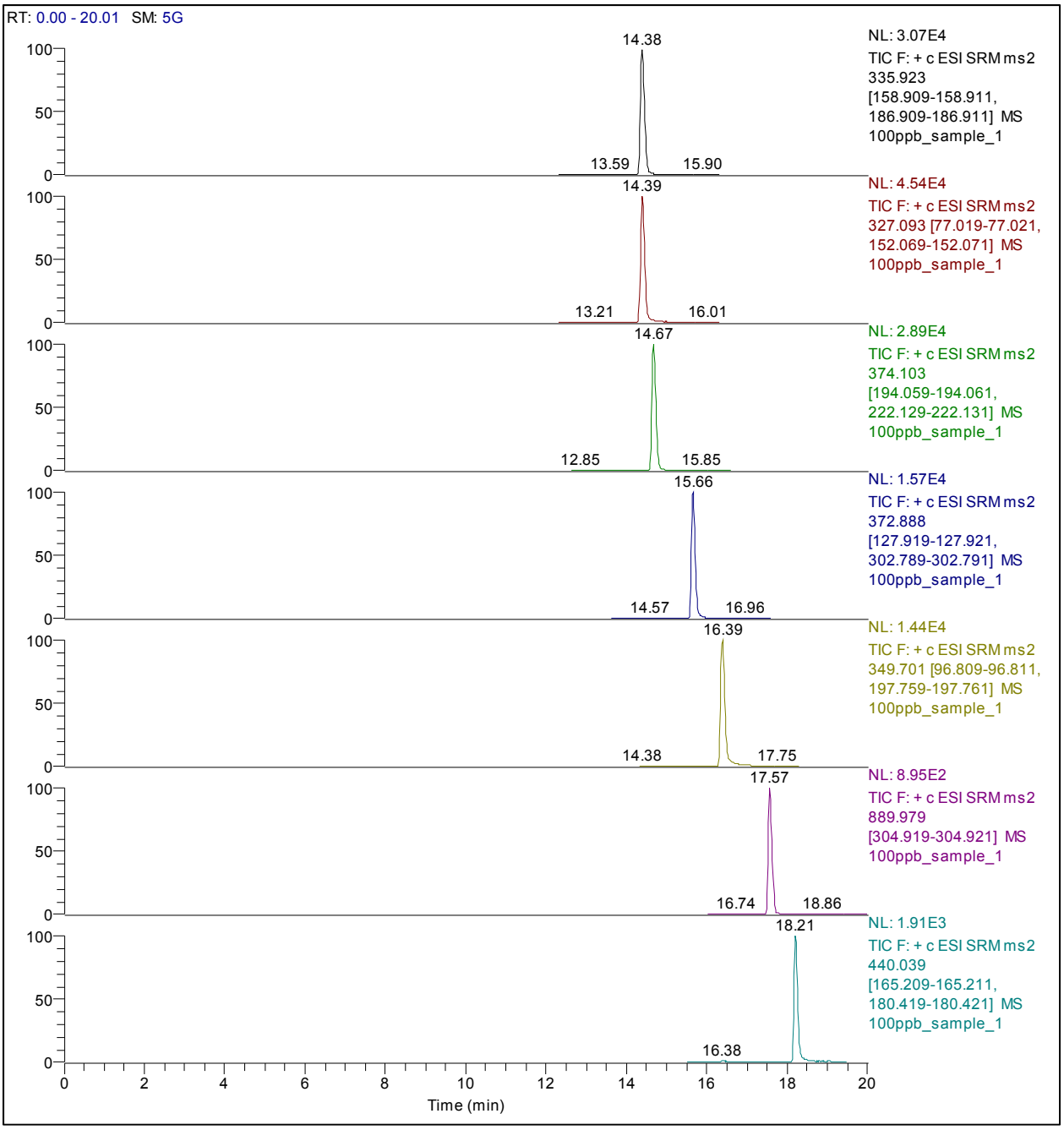


Figure 1 continued.



Results & Discussion

The vast majority of pesticides included in the study could be efficiently extracted from soil using the QuEChERS approach. Neutral pesticides, in particular, could be readily extracted using acetonitrile in combination with the citrate buffered QuEChERS salts. Thiabendazole on the other hand gave low, though reproducible, recovery throughout the study. Thiabendazole is a basic compound that is positively charged at low pH and is capable of being retained on the soil through ionic interactions, particularly by humic/fulvic acids. In addition, it is a planar pesticide and could potentially be retained by strong hydrophobic interactions on the soil (e.g. similar to analyte retention on graphitized carbon black (GCB)).

In the dispersive-SPE cleanup step, using a combination of PSA/C18 yields cleaner extracts than using PSA alone and should be used whenever possible. In this study, no major variation in results was observed between PSA and PSA/C18. In fact the PSA/C18 gave slightly better results, possibly due to reduced matrix effects.

Using UCT's Selectra® C18 HPLC column resulted in good retention and separation of the 21 pesticides and internal standard in less than 20 min. 6-point matrix-matched calibration curves (10, 20, 100, 200, 500 and 1000 ng/mL) were used to obtain the most accurate results possible. Linearity in detector response was observed over the concentration ranges investigated with correlation coefficients (R^2 values) greater than 0.99 for all 21 analytes. As outlined in the Accuracy and Precision Data table, the majority of results were found be within an acceptable recovery range of 70-110 % and have RSD values <10 %, demonstrating that the method meets acceptable performance criteria.

In conclusion, the QuEChERS sample preparation method provides a fast and simple approach for extracting and analyzing 21 pesticides in soil while achieving acceptable recovery and reproducibility. The use of UCT's Selectra® C18 HPLC column provided good chromatographic separation for all analytes included in the study.

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Determination of Pesticides in Strawberries Using QuEChERS Extraction, Quick QuEChERS Clean-up and GC/MS Detection

UCT Part Numbers:

ECQUEU750CT-MP: 4000 mg magnesium sulfate, 1000 mg sodium chloride, 500 mg sodium citrate dibasic sesquihydrate and 1000 mg sodium citrate tribasic dihydrate

ECPURMPSMC: Quick QuEChERS push-through cartridge containing 110 mg $MgSO_4$ and 180 mg PSA

Original: July 12, 2012
Revised: April 23, 2013

Procedure

1) Extraction

a) Add thoroughly homogenized strawberry sample (10 g) to a 50-mL centrifuge tube

b) Add 10 mL acetonitrile

c) Add the contents of pouch **ECQUEU750CT-MP**

d) Immediately shake vigorously for 1 min

e) Centrifuge at ≥ 3000 rcf for 5 min (≤ 20° C)

f) Supernatant is ready for clean-up

2) Quick QuEChERS Clean-up

a) Load 1 mL of supernatant into a disposable syringe

b) Pass the supernatant slowly through a Quick QuEChERS push-through cartridge (**ECPURMPSMC**)

c) Collect 0.5 mL of purified extract directly into a GC autosampler vial

d) Add triphenyl phosphate (200 ng/mL) as internal standard (can use alternative ISTD)

e) Samples are ready for GC/MS analysis

3) GC/MS Detection

Thermo TRACE GC Ultra gas chromatograph coupled to a Thermo ISQ single quadrupole mass spectrometer and TriPlus autosampler.

GC-MS Conditions	
Column	Restek Rtx-5MS, 30 m x 0.25 mm x 0.25 µm
Carrier Gas	Helium
Flow Rate	1.2 mL/min
Ramp	55°C hold for 1 min; 20°C/min to 300°C; hold for 4 min
Injection Volume	1 µL
Injection mode	Splitless
Injector Temperature	220°C
Ion Source Temperature	200°C
Transfer Line Temperature	250°C
MS Operation	SIM and Full Scan

Clean-up of Strawberry Extract with Quick QuEChERS Cartridge

Accuracy and Precision Data					
Compound	**200 ng/mL (n = 5)**		**500 ng/mL (n = 5)**		**Overall Recovery**
	Recovery (%)	**RSD (%)**	**Recovery (%)**	**RSD (%)**	**(n = 10) (%)**
Semi-volatiles					
DDE	83.5	6.76	90.1	5.25	86.8
DDD	79.9	6.38	86.3	5.20	83.1
DDT	84.2	3.62	91.0	5.27	87.6
α-Lindane	91.2	4.60	107.5	12.01	99.3
β-Lindane	87.6	11.29	94.7	3.28	91.2
γ-Lindane	89.4	5.92	107.8	9.07	98.6
δ-Lindane	88.3	5.67	97.6	3.59	92.9
cis-Chlordane	87.0	7.14	96.0	2.24	91.5
trans-Chlordane	78.3	13.22	95.9	6.43	87.1
Heptachlor	80.3	5.89	93.9	1.73	87.1
Heptachlor-epoxide	94.5	5.24	101.1	3.86	97.8
Endosulfan-sulfate	94.1	8.34	102.0	2.56	98.0
Endosulfan I	88.9	8.93	99.8	4.06	94.4
Endosulfan II	97.4	4.39	102.3	8.74	99.8
Aldrin	83.3	4.41	97.3	4.60	90.3
Dieldrin	79.8	8.61	95.6	5.50	87.7
Endrin	84.8	6.30	93.5	3.52	89.2
Endrin-ketone	95.4	6.00	96.7	6.29	96.0
Methoxychlor	89.0	8.78	89.7	9.14	89.4
Organphosphates					
Dichlorvos	80.5	6.88	91.1	3.78	85.8
Chlorpyrifos	78.4	7.20	84.7	7.10	81.6
Mevinphos	66.1	5.23	68.8	21.12	67.5
Ethoprop	81.4	5.99	91.0	5.12	86.2
Paraoxon-methyl	65.2	8.89	72.1	7.30	68.7
Stirofos	67.9	12.06	77.3	5.49	72.6
Disulfoton-sulfone	56.6	9.10	74.4	4.73	65.5

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Determination of Pesticides in Strawberries by QuEChERS Extraction, Quick QuEChERS Clean-up, and GC/MS Detection

UCT Part Numbers:
ECQUEU750CT-MP: (4000 mg magnesium sulfate, 1000 mg sodium chloride, 500 mg sodium citrate dibasic sesquihydrate, 1000 mg sodium citrate tribasic dihydrate)
ECPURMPSMC: (Quick QuEChERS push thru cartridge contains: 110 mg $MgSO_4$, 180 mg PSA)

July 2012

Procedure

1. Extraction

a) Add homogenized and hydrated strawberry sample (10 g) to a 50 mL centrifuge tube

b) Add 10 mL acetonitrile, vortex 30 sec

c) Add the contents of pouch (**ECQUEU750CT-MP)**

d) Shake vigorously for 1 min

e) Centrifuge at >1500 rcf for 1 min at 20° C

f) Supernatant is ready for clean-up

2. Quick QuEChERS Clean-up

a) Load 1 mL of supernatant into a disposable syringe

b) Pass the supernatant slowly through Quick QuEChERS cartridge (**ECPURMPSMC**)

c) Collect 0.5 mL cleaned extract into a GC vial

d) Add triphenyl phosphate as internal standard (200 ng/mL)

e) Samples are ready for GC/MS analysis

Clean-up of Strawberry Extract with Quick QuEChERS

3. GC/MS Detection

Thermo TRACE GC Ultra gas chromatograph coupled with a Thermo ISQ single quadrupole mass spectrometer and TriPlus autosampler

GC/MS Conditions (Using a matrix matched calibration)

Column	Rtx-5MS, 30 m x 0.25 mm x 0.25 µm
Carrier Gas	Helium
Flow Rate	1.2 mL/min
Ramp	55°C for 1 min, 20°C/min to 300°C, hold for 4 min
Injector Temperature	220°C
Injection Volume	1 µL in splitless mode
Ion Source Temperature	200°C
Transfer Line Temperature	250°C
MS Operation	SIM and Full Scan

Accuracy and Precision Data

Compound	Fortified at 10 ng/mL		Fortified at 50 ng/mL		Fortified at 100 ng/mL	
	Recovery%	RSD% (n=4)	Recovery%	RSD% (n=4)	Recovery%	RSD% (n=4)
Methamidophos	93.7	3.4	81.6	5.8	84.2	3.5
Carbendazim	105.7	10.8	100.1	10.6	90.5	7.6
Thiabendazole	91.2	4.9	87.9	6.8	85.0	4.0
Pyrimethanil	112.2	2.7	107.0	3.2	102.8	4.9
Cyprodinil	104.3	3.2	99.9	6.1	100.2	4.9
Diazinon	104.9	5.6	102.0	6.6	99.2	6.8
Pyrazophos	99.9	4.0	96.6	5.6	91.3	4.1
Chlorpyrifos	91.7	4.6	99.5	5.2	97.2	3.8

Determination of Pesticide Residues in Tea: An AOAC Collaborative Study

UCT Part Numbers:
RFV0050CT - 50 mL centrifuge tubes
ECPSACB506 - 6 mL SPE cartridge with 500 mg GCB and 500 mg PSA
ECSS25K - Sodium sulfate, anhydrous, ACS grade, granular 60 mesh
AD0000AS - Cartridge adaptors
RFV0025P - 25 mL empty reservoirs

October 2014

Summary:

Tea is one of the most widely consumed beverages in the world [1]. The application of pesticides in tea cultivation is a common practice in order to increase production yields. Therefore it is important to test the teas for pesticide residues to ensure they are safe for human consumption. However, tea is one of the most complex matrices, which makes the extraction and cleanup of pesticides in tea very challenging. Dr. Guo-Fang Pang and his colleagues at the Chinese Academy of Inspection and Quarantine have developed an efficient and sensitive method to quantitatively determine multiclass pesticide residues in tea [2]. The method employs a solvent extraction using acetonitrile (MeCN), followed by a solvent reduction and a cleanup using solid phase extraction (SPE) cartridge packed with 500 mg each of graphitized carbon black (GCB) and primary secondary amine (PSA), the pesticides are then eluted with MeCN:toluene (3:1, v/v), concentrated down and analyzed by GC/MS, GC/MS/MS or LC/MS/MS.

Matrix matched calibration curves were constructed using organic green and Oolong teas, the responses for 20 representative pesticides were linear with R^2 ranging from 0.9960 to 1.0000. Excellent recoveries (89.5-116% for green tea & 79.3-107% for Oolong tea), and relative standard deviations (RSD% < 10%) were obtained using this simple yet effective method.

Procedure:

a) Weigh 5 ± 0.01 g of homogenized tea sample into a 50-mL centrifuge tube (UCT part#: **RFV0050CT**), add 15 mL of MeCN, and homogenize at 13500 rpm/min for 1 min using an IKA T-25 homogenizer.

b) Centrifuge at 5000 rpm/min for 5 min. Transfer the supernatant to a large test tube (20 x 150 mm).

c) Repeat the extraction with 15 mL of MeCN, and combine the supernatants.

d) Concentrate the extract to about 1 mL using a TurboVap evaporator at 40 ºC under a gentle stream of nitrogen.

e) Add about 2 cm of anhydrous sodium sulfate (Na_2SO_4, UCT part#: **ECSS25K**) to the 6 mL, dual layer SPE cartridge (UCT part#: **ECPSACB506**).

f) Connect a 25 mL empty reservoir (UCT part#: **RFV0025P**) to the top of the dual layer SPE cartridge using cartridge adaptor (UCT part#: **AD0000AS**).

g) Condition the cartridge with 10 mL of MeCN: toluene (3:1, v/v). Do not let the cartridge go dry from this point on.

h) Insert a 50-mL glass vial into the vacuum manifold. Apply the concentrated extract (from Step d) to the cartridge. Wash the test tube with 2 x 3 mL of MeCN: toluene (3:1 v/v) and transfer the rinses to the cartridge, apply a low vacuum to pass the rinse through the SPE cartridge and collect.

i) Continue to elute the extracts from the SPE cartridge with 25 mL of 3:1 MeCN: toluene.

j) Remove the 50-mL vial from the manifold, and concentrate the eluate to about 0.5 mL using TurboVap at 40 ºC under a gentle stream of nitrogen.

k) Add 40 µL of the internal standard solution, and appropriate amounts of pesticide working solution for matrix matched standards and evaporate to dryness under a gentle stream of nitrogen at 35 ºC.

l) Reconstitute with 1.5 mL of n-hexane (or initial mobile phase for LC/MS/MS analysis), vortex for 30 sec. and filter with a 0.2 µm syringe filter. The extract is now ready for instrumental analysis.

GS/MS method:

GC/MS: Agilent 6890N GC coupled to a 5975C MSD
Injector: 1 µL splitless injection at 280 ºC, 40 mL/min purge flow at 1.5 min
Liner: 4 mm splitless gooseneck (UCT part#: **GCLGN4MM**), packed with deactivated glass wool
GC capillary column: Restek Rtx®-1701, 30m x 0.25mm x 0.25µm
Oven temperature: Initial temperature at 40 ºC, hold for 1 min; ramp at 30 ºC/ min to 130 ºC; ramp at 5 ºC/ min to 250 ºC, ramp at 10 ºC/ min to 290 ºC, and hold for 5 min.
Solvent delay: 15.5 min
Carrier gas: Ultra-high pure Helium at a constant flow of 1.2 mL/min
MSD: Transfer line: 280 ºC; MS Source (ESI): 250 ºC; MS Quad: 150 ºC
Tune file: atune

Retention times, quantifying and qualifying ions with ion ratios

Peak No.	Pesticide	Retention time (min)	Quantify ion (ion ratio)	Qualify ion 1 (ion ratio)	Qualify ion 2 (ion ratio)
IS	Heptachlor epoxide	22.44	353(100)	355(81)	351(52)
1	Trifluralin	15.71	306(100)	264(85)	335(7)
2	Tefluthrin	17.67	177(100)	197(28)	161(4)
3	Pyrimethanil	17.73	198(100)	199(51)	200(6)
4	Propyzamide	19.39	173(100)	255(22)	240(10)
5	Pirimicarb	19.44	166(100)	238(20)	138(7)
6	Fenchlorphos	20.22	285(100)	287(69)	270(6)
7	Dimethenamid	20.21	154(100)	230(49)	203(25)
8	Tolclofos-methyl	20.35	265(100)	267(37)	250(11)
9	Pirimiphos-methyl	20.78	290(100)	276(87)	305(64)
10	2,4'-DDE	23.10	246(100)	318(35)	176(25)
11	Bromophos-ethyl	23.52	359(100)	303(83)	357(75)
12	4,4'-DDE	24.34	318(100)	316(78)	246(128)
13	Procymidone	25.22	283(100)	285(65)	255(13)
14	Picoxystrobin	25.37	335(100)	303(44)	367(7)
15	Quinoxyfen	27.63	237(100)	272(41)	307(32)
16	Chlorfenapyr	28.12	247(100)	328(57)	408(46)
17	Benalaxyl	28.23	148(100)	206(28)	325(5)
18	Bifenthrin	29.02	181(100)	182(15)	141(4)
19	Diflufenican	29.26	266(100)	394(21)	267(15)
20	Bromopropylate	29.90	341(100)	183(54)	339(51)

Results:

Linearity parameters of Green and Oolong tea

Pesticide	Linearity range (µg/kg)	Green tea R^2	Oolong tea R^2
Trifluralin	80-1200	0.9998	0.9963
Tefluthrin	40-600	0.9998	0.9995
Pyrimethanil	40-600	0.9999	0.9996
Propyzamide	40-600	0.9992	0.9999
Pirimicarb	40-600	0.9960	0.9999
Fenchlorphos	80-1200	0.9998	0.9991
Dimethenamid	16-240	0.9999	0.9996
Tolclofos-methyl	40-600	0.9998	0.9990
Pirimiphos-methyl	40-600	0.9988	1.0000
2,4'-DDE	160-2400	0.9996	0.9987
Bromophos-ethyl	40-600	0.9999	0.9988
4,4'-DDE	160-2400	0.9998	0.9985
Procymidone	40-600	0.9999	0.9991
Picoxystrobin	80-1200	0.9998	0.9985
Quinoxyfen	40-600	1.0000	0.9990
Chlorfenapyr	320-4800	1.0000	0.9997
Benalaxyl	40-600	0.9999	0.9991
Bifenthrin	40-600	0.9999	0.9971
Diflufenican	40-600	0.9999	0.9990
Bromopropylate	80-1200	0.9999	0.9968

Recovery and RSDs obtained from the spiked Green tea

Pesticide	Spiked (µg/kg)	Rec% 1	Rec% 2	Rec% 3	Rec% 4	Rec% 5	Ave	RSD% (n=5)
Trifluralin	200	91.5	91.5	88.5	91.5	88.5	**90.3**	1.8
Tefluthrin	100	93.1	93.1	93.1	93.1	93.1	**93.1**	0.0
Pyrimethanil	100	90.1	90.1	87.1	90.1	90.1	**89.5**	1.5
Propyzamide	100	99.1	99.1	99.1	99.1	99.1	**99.1**	0.0
Pirimicarb	100	114.1	105.1	120.1	120.1	120.1	**116**	5.7
Fenchlorphos	200	93.0	94.5	91.5	93.0	91.5	**92.7**	1.4
Dimethenamid	40	97.7	97.7	90.2	97.7	90.2	**94.7**	4.3
Tolclofos-methyl	100	93.1	93.1	90.1	93.1	93.1	**92.5**	1.5

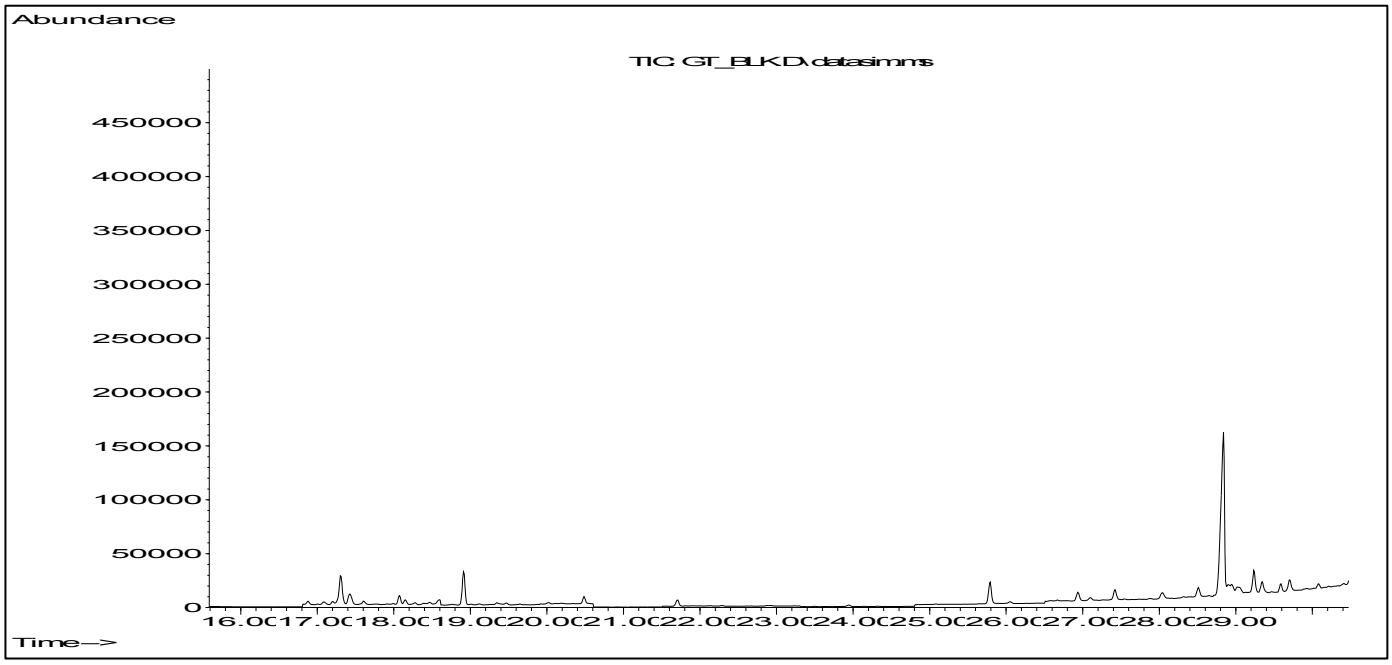
Pirimiphos-methyl	100	93.1	96.1	90.1	93.1	93.1	**93.1**	2.3
2,4'-DDE	400	93.0	94.5	90.8	93.0	91.5	**92.6**	1.6
Bromophos-ethyl	100	93.1	96.1	93.1	93.1	93.1	**93.7**	1.4
4,4'-DDE	400	93.0	94.5	92.3	93.0	92.3	**93.0**	1.0
Procymidone	100	96.1	96.1	93.1	96.1	93.1	**94.9**	1.7
Picoxystrobin	200	94.5	96.0	93.0	94.5	94.5	**94.5**	1.1
Quinoxyfen	100	90.1	90.1	90.1	93.1	90.1	**90.7**	1.5
Chlorfenapyr	800	94.5	96.7	92.6	94.1	94.1	**94.4**	1.6
Benalaxyl	100	96.1	96.1	96.1	96.1	96.1	**96.1**	0.0
Bifenthrin	100	93.1	96.1	93.1	93.1	93.1	**93.7**	1.4
Diflufenican	100	93.1	96.1	93.1	93.1	90.1	**93.1**	2.3
Bromopropylate	200	94.5	96.0	93.0	94.5	94.5	**94.5**	1.1

Recovery and RSDs obtained from the spiked Oolong tea

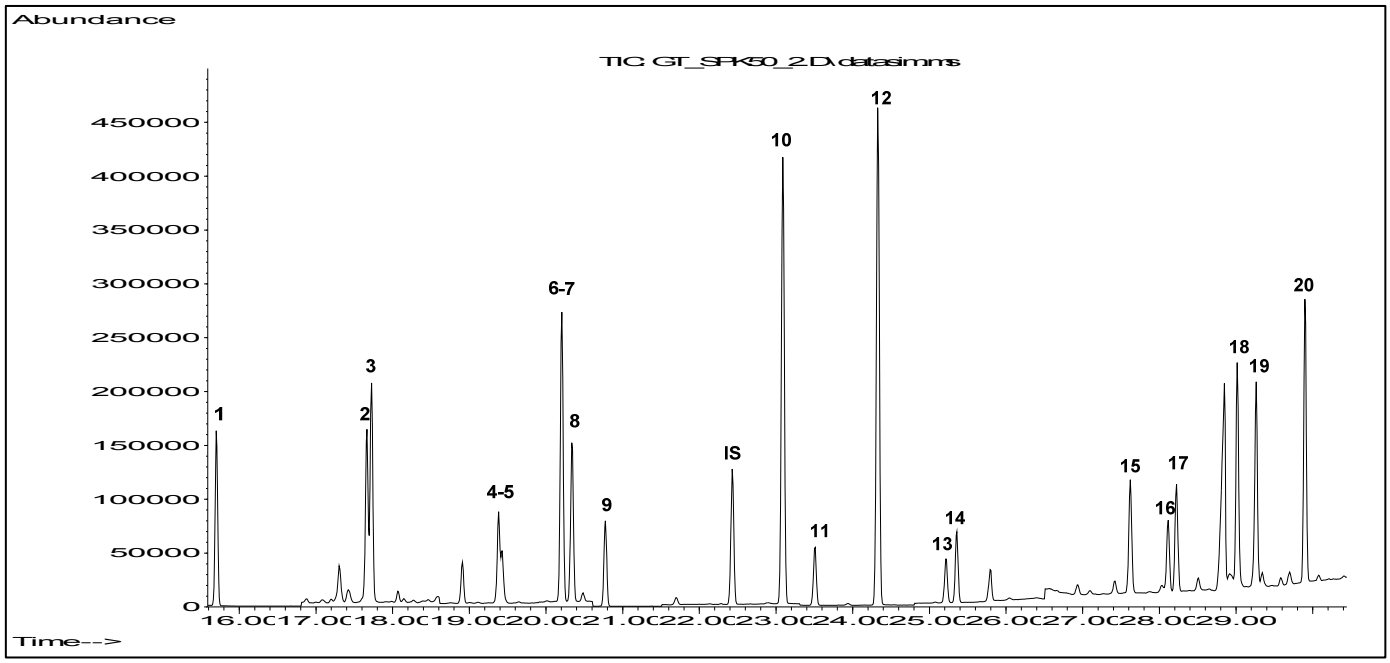
Pesticide	**Spiked** (µg/kg)	**Rec% 1**	**Rec% 2**	**Rec% 3**	**Rec% 4**	**Rec% 5**	**Ave**	**RSD%** (n=5)
Trifluralin	200	84.0	91.5	87.0	85.5	87.0	**87.0**	3.2
Tefluthrin	100	81.1	87.1	87.1	84.1	81.1	**84.1**	3.6
Pyrimethanil	100	78.1	81.1	81.1	78.1	78.1	**79.3**	2.1
Propyzamide	100	81.1	87.1	84.1	84.1	81.1	**83.5**	3.0
Pirimicarb	100	99.1	114.1	102.1	111.1	108.1	**107**	5.8
Fenchlorphos	200	81.0	87.0	84.0	85.5	82.5	**84.0**	2.8
Dimethenamid	40	82.7	90.2	82.7	82.7	82.7	**84.2**	4.0
Tolclofos-methyl	100	81.1	87.1	84.1	87.1	84.1	**84.7**	3.0
Pirimiphos-methyl	100	84.1	90.1	87.1	90.1	87.1	**87.7**	2.9
2,4'-DDE	400	85.5	87.0	89.3	87.8	84.0	**86.7**	2.3
Bromophos-ethyl	100	90.1	90.1	90.1	90.1	90.1	**90.1**	0.0
4,4'-DDE	400	85.5	87.8	84.8	86.3	84.0	**85.7**	1.7
Procymidone	100	87.1	87.1	87.1	87.1	78.1	**85.3**	4.7
Picoxystrobin	200	87.0	87.0	88.5	90.0	79.5	**86.4**	4.7
Quinoxyfen	100	93.1	102.1	96.1	99.1	87.1	**95.5**	6.0
Chlorfenapyr	800	87.0	91.5	91.9	94.9	84.7	**90.0**	4.5
Benalaxyl	100	93.1	96.1	96.1	96.1	90.1	**94.3**	2.8
Bifenthrin	100	90.1	93.1	96.1	93.1	87.1	**91.9**	3.7
Diflufenican	100	87.1	87.1	90.1	87.1	81.1	**86.5**	3.8
Bromopropylate	200	84.0	91.5	91.5	90.0	82.5	**87.9**	4.9

Chromatograms:

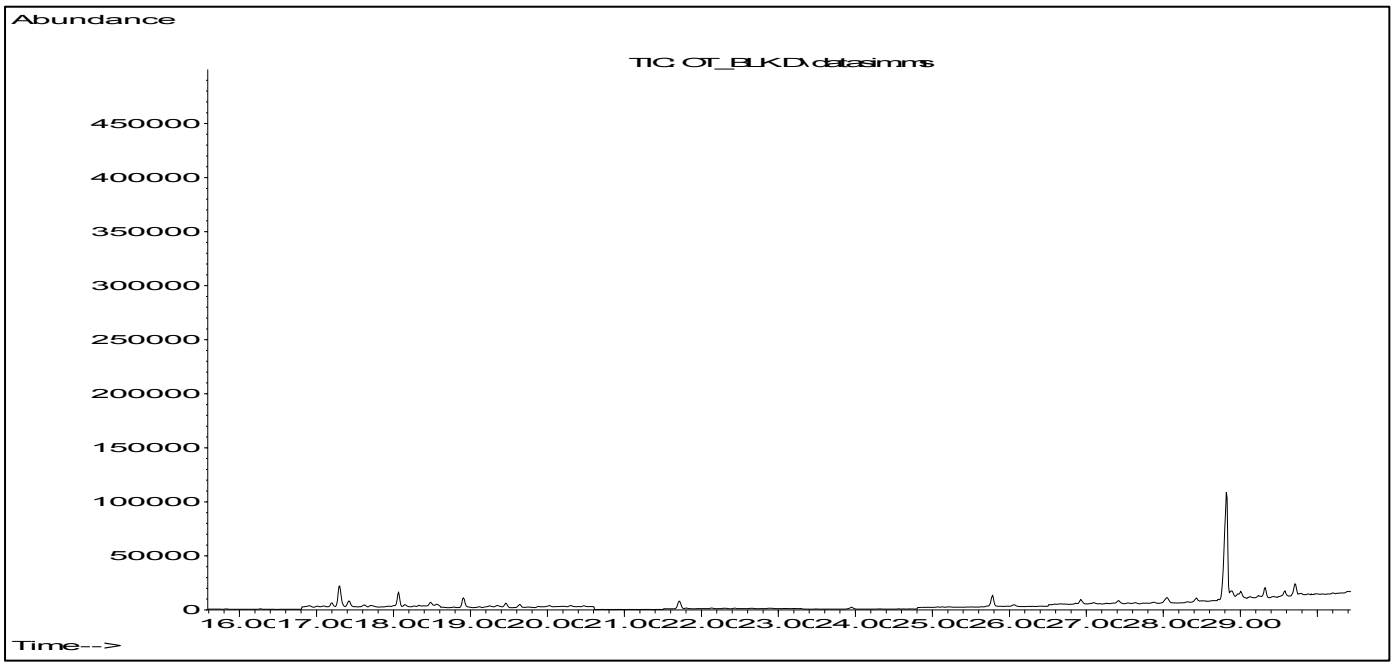
(a) Chromatogram of blank Green tea



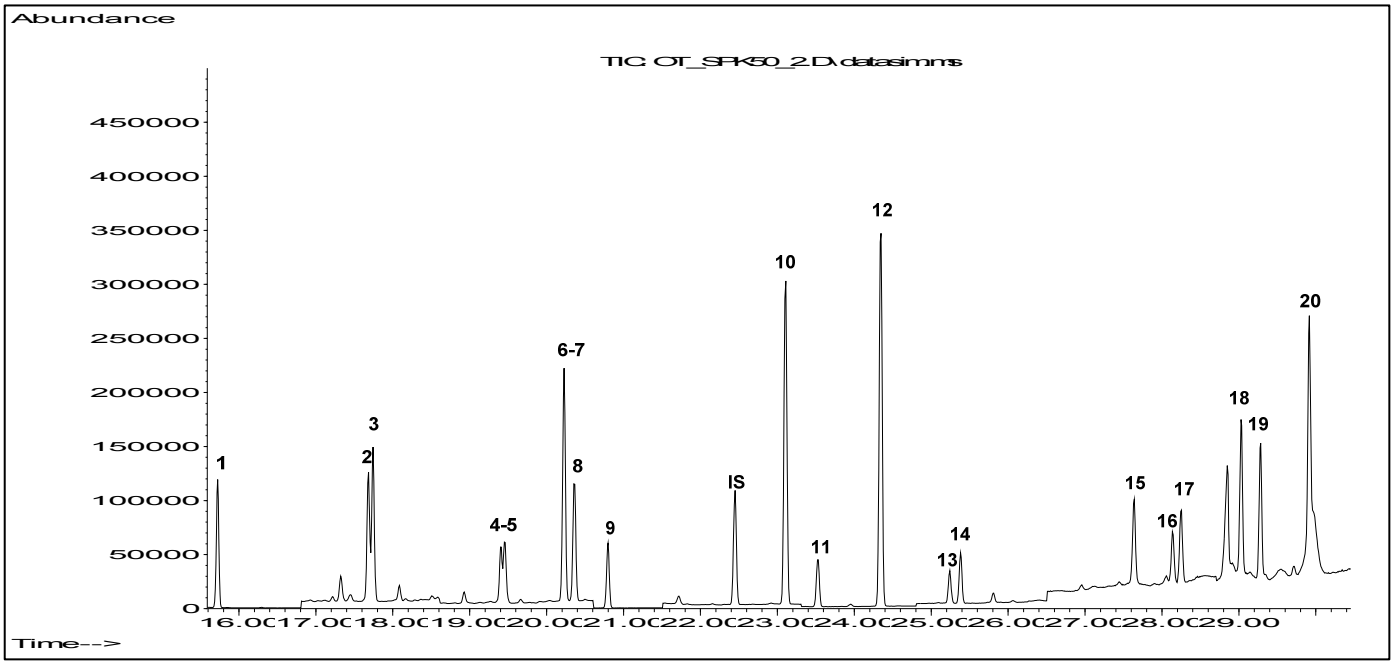
(b) Chromatogram of spiked Green tea



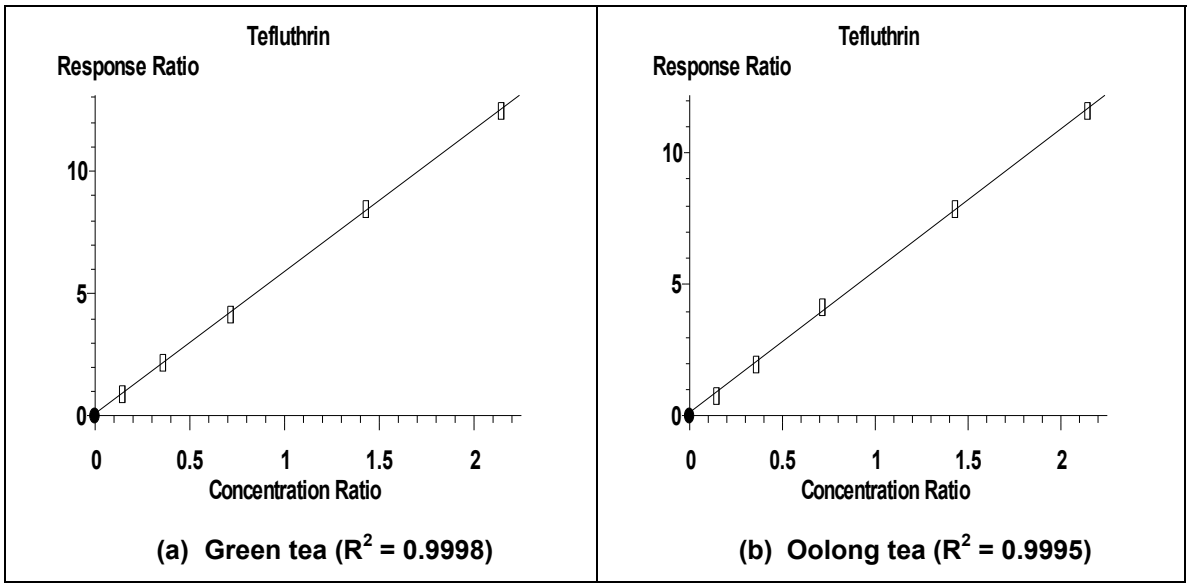
(c) Chromatogram of blank Oolong tea



(d) Chromatogram of spiked Oolong tea



Peak list in chromatograms (b) and (d): 1. Trifluralin; 2. Tefluthrin; 3. Pyrimethanil; 4. Propyzamide; 5. Pirimicarb; 6. Fenchlorphos; 7. Dimethenamid; 8. Tolclofos-methyl; 9. Pirimiphos-methyl; 10. 2,4'-DDE; 11. Bromophos-ethyl; 12. 4,4'-DDE; 13. Procymidone; 14. Picoxystrobin; 15. Quinoxyfen; 16. Chlorfenapyr; 17. Benalaxyl; 18. Bifenthrin; 19. Diflufenican; and 20. Bromopropylate.



Example calibration curves of Tefluthrin in Green and Oolong teas

References:

[1] http://en.wikipedia.org/wiki/Tea

[2] Pang GF, Fan CL, Zhang F, Li Y, Chang QY, Cao YZ, Liu YM, Li ZY, Wang QJ, Hu XY, and Liang P. High-throughput GC/MS and HPLC/MS/MS techniques for the multiclass, multiresidue determination of 653 pesticides and chemical pollutants in tea. J AOAC Int. 2011, 94(4), 1253-1296.

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Extraction of Pesticides from Tomato Using the QuEChERS Approach

(This method is applicable to all pigmented fruit and vegetables)

UCT Product Number:
ECQUEU750CT-MP (4000 mg magnesium sulfate anhydrous, 1000 mg sodium chloride, 500 mg sodium citrate dibasic sesquihydrate, 1000 mg sodium citrate tribasic dihydrate)
ECQUEU32CT (2 mL micro-centrifuge tube with 150 mg magnesium sulfate anhydrous, 25 mg primary secondary amine bonded phase (PSA) and 2.5 mg graphitized carbon black)
ECQUEU515CT (15 mL centrifuge tube with 900 mg magnesium sulfate anhydrous, 150 mg primary secondary amine (PSA) bonded phase and 15 mg graphitized carbon black)

March 2010

Procedure

1. Sample Preparation

a) Add 15g of homogenized and hydrated tomato product (> 80% moisture) to a centrifuge tube
b) Add 15 mL acetonitrile including internal standard
c) Shake or vortex for 30 seconds
d) Add contents of a package of **ECQUEU750CT-MP** to centrifuge tube
e) Immediately, shake vigorously for 2 minutes
f) Centrifuge for 2 minutes at 3450 rcf
g) Draw 1 or 6 mL of supernatant for clean-up

2. Clean-Up

a) For 1 mL of supernatant, use product **ECQUEU32CT**
b) For 6 mL of supernatant, use product **ECQUEU515CT**
c) Add supernatant to centrifuge tube and shake vigorously for 1 minute
d) Centrifuge for 2 minutes at 3450 rcf

3. Analysis by GC (suggested)

a) Transfer an aliquot of supernatant from step 2 to a centrifuge tube

b) Add TPP solution and 1 mL of toluene

c) Evaporate using nitrogen at 50°C to approximately 0.3 to 0.6 mL.

d) Bring to 1 mL final volume with toluene

e) Inject 8 µL on LVI/GC/MS

4. Analysis by LC (suggested)

a) Transfer 0.25 mL of supernatant from step 2 to a LC vial.

b) Add TPP solution and 0.86 mL of 6.7 mM formic acid

c) Analyze by LC/MS/MS

References:
QuEChERS Method EN 15662

*Anastassiades, et al (*2003) "Fast and Easy Multiresidue method employing acetonitrile extraction partitioning and dispersive solid-phase extraction for the determination of pesticide residues in product" Journal of AOAC International Vol 86 no. 2

Solid-Phase Extraction of Pesticides in Water using Graphitized Carbon Black (GCB)

UCT Part Numbers:
EUCARB1M6 (1000 mg GCB (non-porous, 120/400 mesh), 6 mL)
AD0000AS (cartridge adaptor)
RFV0075P (reservoirs, 75 mL)

May 2013

Graphitized carbon black (GCB) is a reverse phase and anion exchange sorbent. GCB retains non-polar compounds, such as organochlorine pesticides, and some very polar compounds, such as surfactants, which are difficult to retain by other reverse phase sorbents. This simple SPE method uses UCT's proprietary, treated GCB for the determination of pesticides in water providing excellent recovery.

Procedure

1. Cartridge Preparation

a) Transfer 100 mL of aqueous sample to a glass container
b) Adjust pH to less than 2 using 6N HCl
c) Spike as necessary
d) Connect **RFV0075P** reservoirs to the top of the **EUCARB1M6** cartridges using **AD0000AS** adaptor
e) Wash cartridges with 10 mL dichloromethane (DCM)
f) Draw full vacuum to remove all DCM
g) Add 10 mL methanol and draw down to top of frit
h) Add 10 mL reagent water and draw down to top of frit
i) Do not let cartridges go dry after step g)

2. Extraction

a) Add samples to the reservoirs adjusting vacuum to give a drop-wise flow, about 10 mL/min
b) Rinse sample containers using 10 mL reagent water and add rinsate to cartridges
c) Dry cartridges using full vacuum for 10 min

3. Elution

a) Insert test tubes in the manifold then elute cartridges using 5 mL ethyl acetate dropwise followed by 5 mL of DCM dropwise

b) Dry extracts by passing through anhydrous Na_2SO_4

c) Rinse test tubes with DCM and add to Na_2SO_4

d) Concentrate extracts to 1 mL using a gentle stream of N_2 at 35 °C

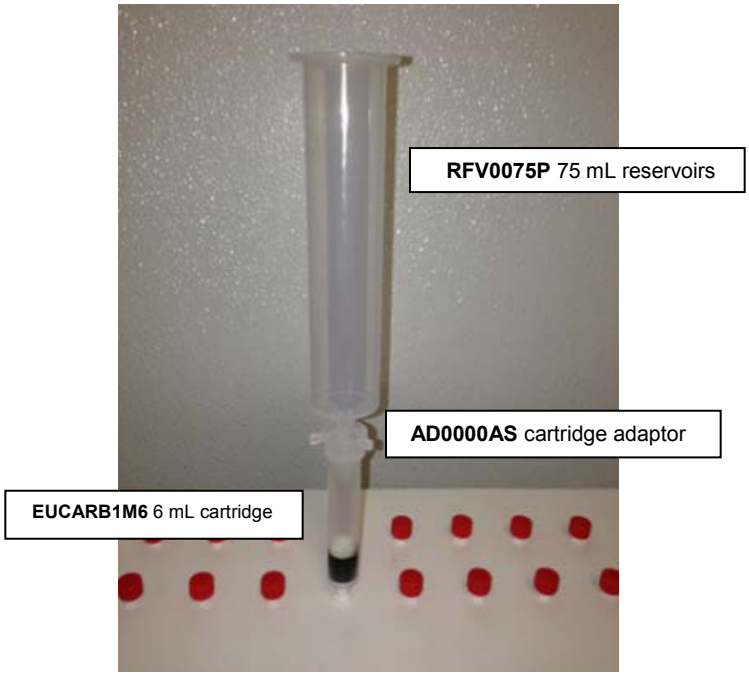
e) Add IS prior to GC/MS analysis

4. Analysis

Parameters

GCMS: Agilent 6890N GC coupled with 5975C MSD
MSD Injector: 1 µL splitless injection at 250 ºC
Injection Vol: 1 µL
Liner: 4 mm splitless gooseneck liner with deactivated glass wool (UCT: GCLGN4MM)
Column: Restek Rxi®-5sil MS 30m x 0.25mm x 0.25µm
Guard Column: 10 m
Column Flow Rate: 1.2 mL/min
Carrier Gas: He
Full Scan: 45-500 amu
Temperature Program: Initial T 55 ºC hold for 1 min; ramp at 10 ºC/min to 200 ºC; ramp at 7 ºC/min to 300 ºC; hold for 0.21 min.

Detail of Reservoir, Adaptor, and Cartridge Setup



Accuracy and Precision Data

Compound	Intra-day (n=4)		Inter-day (n=17)	
	Rec%	RSD	Rec%	RSD
alpha Lindane	93	2.1	89	9.3
beta Lindane	96	1.9	91	8.8
gamma Lindane	93	1.7	92	8.3
delta Lindane	95	3.3	89	11.7
Heptachlor	97	3.2	91	11.1
Aldrin	95	1.5	84	12.9
Heptachlor epoxide	102	2.4	97	12.0
trans-Chlordane	93	3.8	90	8.8
Endosulfan I	94	5.0	91	8.4
cis-Chlordane	96	3.3	91	9.7
p,p'-DDE	91	3.5	89	8.8
Dieldrin	98	1.9	93	9.4
Endrin	100	2.1	95	11.8
Endosulfan II	105	1.4	97	10.3
p,p'-DDD	98	2.2	92	9.8
Endrin aldehyde	95	5.4	92	9.3
Endosulfan sulfate	102	3.8	97	10.2
p,p'-DDT	99	3.0	94	9.6

Endrin ketone	106	2.1	99	10.9
Methoxychlor	105	2.7	99	10.5
Dichlofluanid	107	2.8	98	10.8
Dicofol	95	0.7	86	11.6
Tolyfluanide	106	3.1	98	11.6
Captan	119	4.2	105	13.4
Folpet	107	3.9	95	10.0
Overall average	**99**	**2.8**	**93**	**10.4**

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Pesticide Residue Analysis in Whole Milk by QuEChERS and LC-MS/MS

UCT Part Numbers:
Enviro-Clean® RFV0050CT (50 mL centrifuge tubes)
Enviro-Clean® ECMSSA50CT-MP (Mylar pouch containing 6 g $MgSO_4$ and 1.5 g NaOAc)
Enviro-Clean® CUMPSC18CT (2 mL dSPE tube with 150 mg MgSO4, 50 mg PSA and 50 mg C18)

July 2013

Summary:

This application describes a cost-effective and easy to use method for the determination of pesticide residues in whole milk. The method employs the AOAC version of QuEChERS. This procedure provides better analytical results than either the original or EN versions of the QuEChERS procedure in extracting a few sensitive pesticides; such as pymetrozine and hexazinone (Velpar).

15 mL of whole milk is extracted using 15 mL of acetonitrile (MeCN) with 1%(v/v) acetic acid (HAc); 6 g magnesium sulfate ($MgSO_4$) and 1.5 g sodium acetate (NaOAc) are added into the mixture to enhance the phase separation and the extraction of pesticides. After shaking and centrifugation, 1 mL of the supernatant is purified by 2-mL dSPE tube containing 150 mg $MgSO_4$, 50 mg PSA, and 50 mg C18. $MgSO_4$ absorbs residual water in the extract, PSA removes organic acids and carbohydrates, while C18 retains fatty acids and cholesterol. The pesticides in the cleaned extract are detected and quantified by LC-MS/MS.

Matrix matched calibration curves were constructed for pesticide quantification. The responses for all 24 pesticides were linear with R^2 ranged from 0.9954 to 0.9997 over the concentration range of 2 to 400 ng/mL. Excellent recoveries and relative standard deviations were obtained, indicating that this method is suitable for pesticide analysis in whole milk samples, especially when pymetrozine and hexazinone are being analyzed.

Procedure:

1. **QuEChERS extraction**
    a) Transfer 15 mL of whole milk into 50-mL centrifuge tube (**RFV0050CT**).
    b) Add 30 µL of 50-ppm triphenyl phosphate (TPP) internal standard (IS) solution to all samples, and appropriate amounts of 2 ppm pesticide working solution to fortified samples.
    c) Add 15 mL of MeCN with 1% HAc. Cap and shake 1 min at 1000 strokes/min using a Spex 2010 Geno-Grinder.
    d) Add salts, 6 g MgSO4 and 1.5 g NaOAc from pouch (**ECMSSA50CT-MP**), and vortex for 10 sec to break up salt agglomerates.
    e) Shake 1 min at 1000 strokes/min using Spex 2010 Geno-Grinder.
    f) Centrifuge at 3830 rcf for 5 min.

2. **dSPE cleanup**
    a) Transfer 1 mL of the supernatant to 2-mL dSPE tube (**CUMPSC18CT**).
    b) Shake 2 min at 1000 strokes/min using Spex 2010 Geno-Grinder.
    c) Centrifuge at 15,300 rcf for 5 min.
    d) Transfer 0.3 mL of the cleaned extract into 2-mL auto-sampler vial, add 0.3 mL of reagent water, and vortex for 30 sec.
    e) The samples are ready for LC-MS/MS analysis.

Whole Milk Samples Extracted by the AOAC QuEChERS Procedure

LC-MS/MS method:

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-sampler Temperature: 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	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

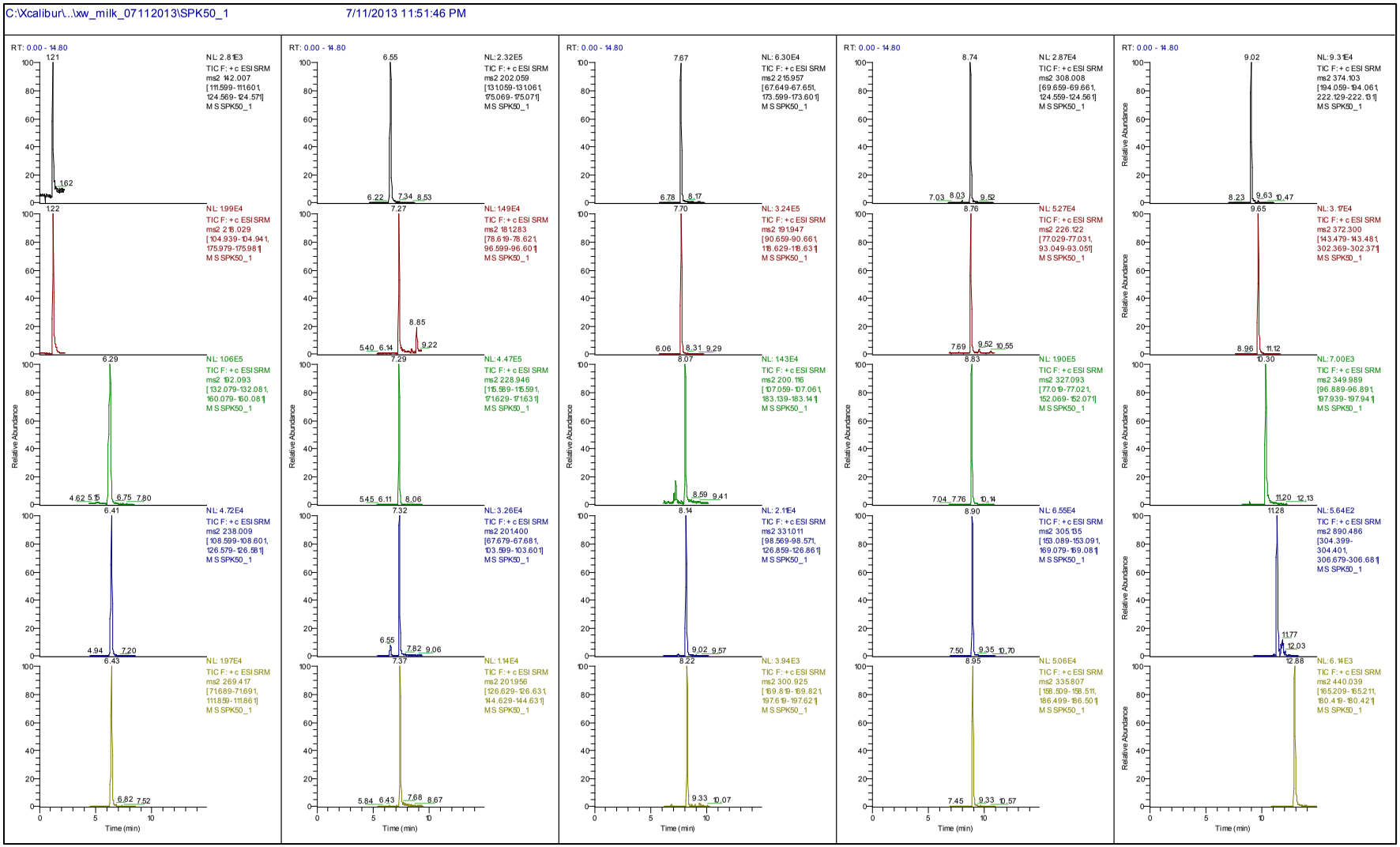
Name	Rt (min)	Precursor ion	Product ion 1	CE 1	Product ion 2	CE 2	S-lens (V)
Methamidophos	1.21	142.007	124.57	14	111.6	5	60
Pymetrozine	1.22	218.029	104.94	18	175.98	16	70
Carbendazim	6.29	192.093	160.08	17	132.08	29	81
Dicrotophos	6.41	238.009	126.58	17	108.60	33	73
Acetachlor	6.43	269.417	111.86	15	71.69	33	72
Thiabendazole	6.55	202.059	175.07	24	131.06	31	103
DIMP	7.27	181.283	96.60	13	78.62	32	44
Tebuthiuron	7.29	228.946	171.63	17	115.59	26	72
Simazine	7.32	201.400	67.68	33	103.60	24	85
Carbaryl	7.37	201.956	144.63	7	126.63	30	40
Atrazine	7.67	215.957	173.60	16	67.65	35	79
DEET	7.70	191.947	118.63	15	90.66	28	92
Pyrimethanil	8.07	200.116	107.06	23	183.14	22	66
Malathion	8.14	331.011	126.86	12	98.57	23	60
Bifenazate	8.22	300.925	169.82	15	197.62	5	51
Tebuconazole	8.74	308.008	69.66	29	124.56	35	97
Cyprodinil	8.76	226.122	93.05	33	77.03	40	88
TPP (IS)	8.83	327.093	152.07	33	77.02	37	98
Diazinon	8.90	305.135	169.08	14	153.09	15	89
Zoxamide	8.95	335.807	158.51	38	186.50	20	102
Pyrazophos	9.02	374.103	222.13	20	194.06	20	104
Profenofos	9.65	372.300	302.37	19	143.48	35	104
Chlorpyrifos	10.30	349.989	197.94	17	96.89	32	69
Abamectin	11.28	890.486	304.40	18	306.68	15	102
Bifenthrin	12.88	440.039	180.42	11	165.21	39	66

Results:

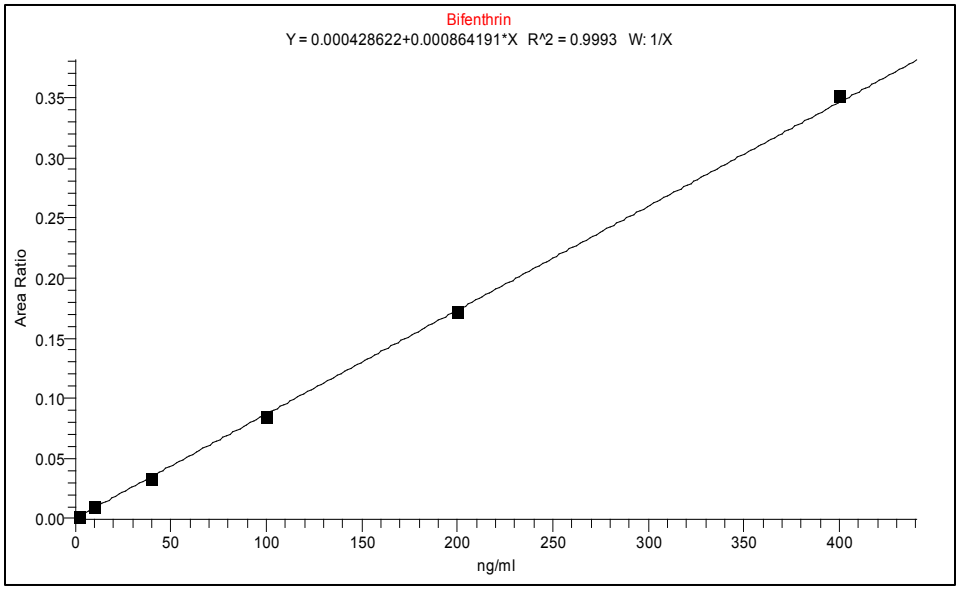
Recovery and RSD% Obtained from 5 Replicated Fortified Milk Samples

Analytes	Spiked at 10 ng/mL		Spiked at 50 ng/mL	
	Recovery%	RSD% (n=5)	Recovery%	RSD% (n=5)
Methamidophos	**85.2**	5.8	**100.3**	5.1
Pymetrozine	**93.9**	5.2	**97.3**	5.4
Carbendazim	**100.4**	3.8	**102.8**	3.1
Dicrotophos	**102.3**	2.1	**106.5**	2.9
Acetachlor	**119.9**	3.6	**128.8**	2.9
Thiabendazole	**99.8**	2.1	**103.8**	2.3
DIMP	**90.3**	3.2	**93.1**	4.7
Tebuthiuron	**108.6**	3.0	**113.3**	2.7
Simazine	**102.6**	1.6	**105.1**	2.7
Carbaryl	**95.6**	5.3	**97.1**	4.0
Atrazine	**99.1**	2.0	**102.8**	3.0
DEET	**103.6**	2.4	**106.4**	3.4
Pyrimethanil	**91.0**	4.7	**92.3**	4.0
Malathion	**100.7**	3.8	**99.1**	3.0
Bifenazate	**85.6**	9.1	**81.0**	8.7
Tebuconazole	**91.0**	2.7	**91.9**	3.5
Cyprodinil	**94.2**	2.1	**95.6**	3.1
Diazinon	**96.8**	2.6	**97.7**	3.5
Zoxamide	**100.4**	3.0	**101.9**	3.0
Pyrazophos	**100.3**	1.6	**104.0**	2.0
Profenofos	**90.9**	2.8	**93.0**	3.9
Chlorpyrifos	**94.2**	4.9	**87.8**	4.5
Abamectin	**81.3**	7.7	**86.6**	4.2
Bifenthrin	**77.8**	3.1	**75.8**	2.1
Overall mean	**96.1**	3.7	**98.5**	3.7

Chromatograms of a Fortified Whole Milk Sample at 50 ng/mL



Matrix Matched Calibration Curves of Bifenthrin (R^2=0.9993)



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