Agricultural

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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

Analysis of Tobacco Alkaloids

UCT Part Number:
EUBCX1HL2Z (200 mg benzenesulfonic acid high load, 10 ml)

October 2012

Tobacco alkaloids are extracted with a strong cation exchange sorbent using an acidic buffer, filtered and further acidified. Extraneous compounds are removed by washing the sorbent, yielding clean chromatography without loss of target analytes.

Procedure

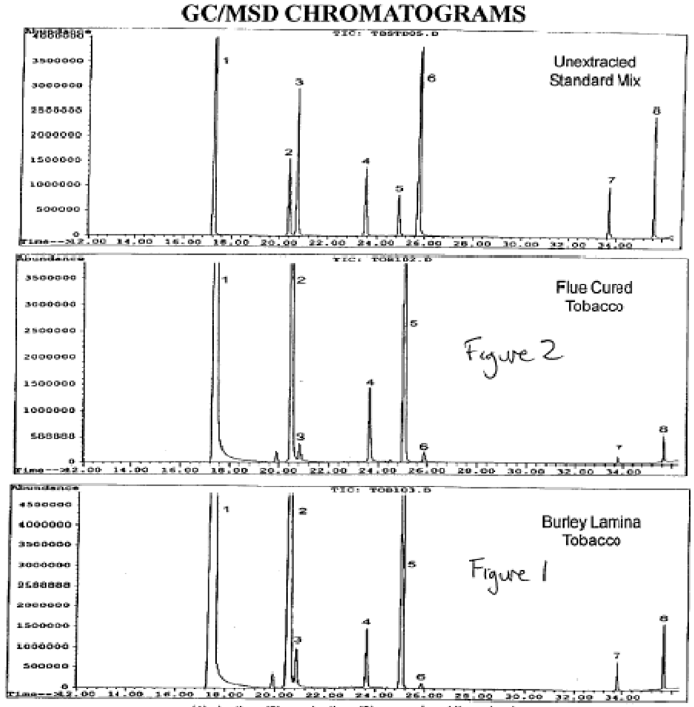
1. **Sample Preparation**
   a) Weigh 100 mg of finely ground tobacco in a screw cap vial
   b) Add 6 mL 0.1M sodium acetate buffer (pH 4.5) and 100 µL IS (d4- nornicotine, 1 µg/µL)
   c) Mix on rotating shaker for 10 minutes
   d) Filter extract through 20 micron frit filter column
   e) Add 300 µL glacial acetic acid and mix
2. **Sample Extraction**
   a) Condition column **EUBCX1H2Z** with 3 mL of MeOH:1.0M acetic acid (80:20)
   b) Pour sample onto column, draw through at 1-2 mL/min
   c) Wash column with 3 mL of MeOH:1.0M acetic acid (80:20)
   d) Dry column for 5-10 min using full vacuum
3. **Elution**
   a) Elute alkaloids with 3 mL CH_2Cl_2/isopropanol/NH_4OH (70:26:4) by gravity
   b) Evaporate eluant to dryness with N_2 and low heat (< 40° C)
   c) Reconstitute with 200 µL ethyl acetate
   d) Analyze on GC/FID/NPD or GC/MSD

Results

Alkaloid	pKa	Flue Cured Tobacco	Burley Lamina Tobacco
	n=15/mean	mg/gram/CV	mg/gram/CV
Mysomine	NA	48/6.2	189/7.9
Nicotine	7.94	39406/6.2	39119/8.8
Nornicotine	9.46	1381/3.5	5429/5.2
Anatasine	9.20	229/5.2	183/8.7
Anatabine	8.23	1932/2.3	1774/2.3
2,3'-dipyridyl	4.25	54/8.9	30/11.2
Cotinine	4.88	20/11.2	52/12.4
Formylnornicotine	NA	31/11.9	145/12.4

The average of 15 separate runs show excellent reproducibility

GC/MSD CHROMATOGRAMS



(1) nicotine, (2) nornicotine, (3) myosmine, (4) anabasine, (5) anatabine, (6) 2,3'-dipyridyl, (7) cotinine, (8) formylnornicotine

Instrument:	Agilent 5890GC/5971MSD
GC column:	Rtx-5 Amine, 30 m x 0.25 mm i.d. x 1.0 µm film
Injector:	1 µL sample at 10:1 split, 250° C
Temp program:	Initial 120° C, hold 1 min, ramp 2.5° C/min to 200° C, ramp 20° C/min to 280° C, hold 1 min.
MSD conditions:	SIM monitoring, EI mode, 295° C

DCN-216111-60

Analysis of Glyphosate and Glufosinate by Solid-Phase Anion Exchange Extraction with GC/MS or LC/MS Analysis

UCT Part Number:
EUQAX2M6 (1000 mg, QAX2, 6 mL cartridge)

September 2009

Analyte	CASRN	Common Name
Glyphosate[1]	1071-83-6	Roundup®,
Glyfosinate[2]	51276-47-2	Basta®, Challenge®)

1. **Sample Preparation**
   a) Adjust water sample pH to 6 or higher with buffer
2. **Cartridge Conditioning**
   a) Place **EUQAX2M6** cartridges(s) on manifold
   b) Add 5 mL of methanol to the cartridge
   c) Slowly draw methanol through leaving enough to cover cartridge frit
   d) Rinse using 10 mL of pH 6 or higher buffer leaving a layer of buffer on frit

Note: Do not let the cartridge dry out after addition of methanol otherwise repeat

3. **Extraction Protocol**
   a) Draw a known volume of sample water through the cartridge, usually 100-500 mL

Note: Sample volume is determined by the analytical quantitation limit

   b) Adjust vacuum so that flow is approximately 1 - 3 mL per minute
   c) Wash sorbent using 10 ml of pH 6 buffer
   d) Dry the cartridge by drawing full vacuum for 10 minutes
4. **Analyte Elution**
   a) Elute using 5 mL of 1 mol/L HCl/methanol solution (4/1)
   b) Add eluant to the cartridge then draw through at 1 mL/minute
   c) Evaporate to dryness with N_2 flow in a water bath heated to 50°C

5. GC Analysis

a) Add 50 µL of **MTBSTFA** (N-methyl-N-(tert-butyldimethylsilyl) triflouroacetamide) and 50 µL of dimethylformamide for derivatization

b) Sonicate at room temperature for 2 minutes (critical)

c) Quantitatively transfer to GC vial and cap

d) Heat to 80°C for a minimum of 30 minutes

e) Cool to room temperature

f) Sample is ready for analysis

6. LC Analysis (**Alternative Analysis Procedure**—no derivatization needed)

a) After step 4) c) dissolve the dry residue using 100 µL of methanol

b) Quantitatively transfer to an LC vial then cap

c) Sample is ready for LC analysis

[1] N-phosphonomethyl glycine
[2] *RS*-2-amino-4-(hydroxyl-methyl-phosphoryl)butanoic acid

DCN-218030-118

Extraction of Phenoxyacetic Acid Herbicides From Soil By LC-MS/MS

UCT Part Number:
EEC181M6 (endcapped C18 - 1000 mg/6 mL)
ECUNIC18 (Universal C18 1100mg/83 mL)

April 2009

1. Sample Pretreatment

a) Prepare an acid washed beaker*
b) Add 10-100 grams of soil sample
c) Add enough DI H_2O to form a loose slurry
d) Insert a magnetic stir bar and extract for 15 minutes
e) Adjust pH to 2 using 50% aqueous sulfuric acid (H_2SO_4)
f) Continue extraction for 15 minutes adjusting pH as needed
g) Filter sample through previously acidified filter media

***Note:** Acid washed glassware must be used in this procedure. Soda lime glassware must be avoided as it may interfere with the analysis

2. Condition C18 SPE Cartridge

a) Add 5 mL CH_3OH and wait 1 minute
b) Add 5 mL DI H_2O

Note: Aspirate at low vacuum setting. Do not let cartridge dry out otherwise repeat steps a) and b)

3. Add Sample

a) Adjust vacuum and load cartridge at 10 mL/minute flow rate

4. Dry Cartridge

a) Dry cartridge for 10 minutes at full vacuum

5. Elute Phenoxyacetic acid Herbicides

a) Place a clean collection vial in manifold

b) Add 5 mL of CH_3OH and wait 1 minute

c) Add a second 5 mL volume of CH_3OH

d) Adjust vacuum and collect at 1-2 mL/ minute

6. Dry Eluate

a) Evaporate to dryness at < 40˚C using N_2

b) Reconstitute in 100 µL of mobile phase for **LC-MS/MS**

- Inject 10-100 µL

HPLC Analysis and Instrumentation Requirements

Guard Column: C18 10mm x 2.6mm with 0.5 µm frit

Analytical Column:
- C18 100 mm x 2 mm 5 µm particle ODS-Hypersil
- C18 100 mm x 2 mm 3 µm particle MOS2-Hypersil or equivalent

HPLC/MS Interface:
- Micromixer 10-µL interface HPLC column system with HPLC post-column addition solvent

Interface:
- Thermospray ionization interface and source capable of generating both positive and negative ions and have a discharge electrode or filament

Mass Spectrometer System:
- A single quadrupole mass spectrometer capable of scanning from 1 to 1000 amu
- Scanning from 150 to 450 amu in 1.5 sec. or less using 70 volts (nominal) in positive or negative electron modes
- Capable of producing a calibrated mass spectrum for polyethylene glycol (PEG 400, 600, or 800, average mol. wts.) or other compounds used as a calibrant
- Use PEG 400 for analysis of chlorinated phenoxyacid compounds. PEG is introduced via the Thermospray interface circumventing the HPLC

Thermospray Temperatures:
Vaporizer Control: 110°C to 130°C
Vaporizer Tip: 200°C to 215°C
Jet: 210°C to 220°C
Source Block: 230°C to 265°C

Recommended HPLC Chromatographic Conditions

Chlorinated Phenoxyacid Compounds A=0.1 M ammonium acetate/methanol

Initial Mobile Phase %	**Initial Time** minutes	**Final** minutes	**Final Mobile Phase** %	**Time** minutes
75A/25	2	15	40/60	
40A/60	3	5	75/25	10

Limits of Detection in the Positive and Negative Ion Modes for HPLC Analysis of Chlorinated Phenoxyacid Herbicides and Esters

Compound	**Positive Ion Mode Quantitation LOD**		**Negative Ion Mode Quantitation LOD**	
	Ion	**ng**	**Ion**	**ng**
Dalapon	Not detected		141 $(M^{-}H)^{-}$	11
Dicamba	238 $(M^{+}NH_4)^{+}$	13	184 $(M^{-}HCl)^{-}$	3.0
2,4-D	238 $(M^{+}NH_4)^{+}$	2.9	184 $(M^{-}HCl)^{-}$	50
MCPA	218 $(M^{+}NH_4)^{+}$	120	199 $(M^{-}1)^{-}$	28
Dichloroprop	252 $(M^{+}NH_4)^{+}$	2.7	235 $(M^{-}1)^{-}$	25
MCPP	232 $(M^{+}NH_4)^{+}$	5.0	213 $(M^{-}1)^{-}$	12
2,4,5- T	272 $(M^{+}NH_4)^{+}$	170	218 $(M^{-}HCl)^{-}$	6.5
2,4,5-TP Silvex	286 $(M^{+}NH_4)^{+}$	160	269 $(M^{-}1)^{-}$	43
Dinoseb	228 $(M^{+}NH_4^{-}NO)^{+}$	24	240 (M-)	19
2,4-DB	266 $(M^{+}NH_4)^{+}$	3.4	247 (M-1)-	110
2,4,5-D, butoxy ethanol ester	321 $(M^{+}H)^{+}$	1.4	185 $(M^{-}C_6H_{13}O_1)^{-}$	
2,4,5-T,butoxy ethanol ester	372 $(M^{+}NH_4)^{+}$	0.6	195 $(M^{-}C_8H_{15}O_3)^{-}$	
2,4,5-T, butyl ester	328 $(M^{+}NH_4)^{+}$	8.6	195 $(M^{-}C_6H_{11}O_2)^{-}$	
2,4-D, ethyl hexyl ester	350 $(M^{+}NH_4)^{+}$	1.2	161 $(M^{-}C_{10}H_{19}O_3)^{-}$	

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

DCN-900840-157

QuEChERS Multiresidue Pesticide Method for the Determination of Multiple Pesticides in Wines*

UCT Part Number:
ECQUVIN50CT (50 mL centrifuge tube, 8.0 grams anhydrous $MgSO_4$ & 2 grams NaCl)
ECMPSCB15CT (900 mg anhydrous $MgSO_4$, 300 mg PSA &150 mg GCB)

February 2010

This method summary describes a multi-residue pesticide method for the determination of 72 pesticides in wines. Pesticides are extracted using acetonitrile saturated with magnesium sulfate and sodium chloride followed by a dispersive solid-phase clean-up with primary-secondary amine (PSA) and graphitized carbon black (GCB) sorbents.

Analysis is performed using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) in ESI (positive) mode.

1. Sample Preparation

a) Add 20 mL acetonitrile (ACN) and *internal standard* 250 µL Fluconazole (10 µg/L) to **ECQUVIN50CT**
b) Quantitatively add 20.0 mL of wine
c) Shake for approximately 2 minutes
d) Centrifuge at 4500 rpm for 5 minutes (use refrigerated centrifuge if available)
e) Transfer 9.0 mL of top layer and add to **ECMPSCB15CT** (900 mg anhydrous $MgSO_4$, 300 mg PSA &150 mg GCB)
f) Vortex tube for approximately 10 seconds
g) Open tube and add 3.0 mL of toluene and shake for 1 minute
h) Centrifuge the tube for 5 minutes @ 4500 rpm
i) Quantitatively transfer 2.0 mL of supernatant to a glass centrifuge tube
j) Evaporate to dryness at < 40 ˚C using N_2
k) Add 500 µL of ACN and 25 µL of *surrogate standard* (benzanilide - 20.0 µg/L) for QC and 500 µL of 20 mM ammonium acetate in 1% ACN to the dried extract
l) Vortex for approximately 5 seconds and filter into autosampler vial using 17mm, 0.2 µm nylon membrane cartridges attached to a disposable syringe

2. UPLC/MS/MS Analysis

UPLC Conditions:

Column: Water's Acquity UPLC BEH C_{18} column 100 x 2.1 mm, 1.7 µm particle or equivalent

Flowrate: 0.2 mL/minute

Injection volume: 3 µL

Analytical Standards: Matrix Matched

Gradient Program:

Time	% Acetonitrile	% 10mM Ammonium Acetate
0	10	90
10	90	10
14.5	90	10
14.6	10	90
20.1	10	90

Triple Quadrupole MS Conditions--electrospray ionization mode (ESI+)

Capillary Voltage: 1.5 kV

Source Temperature: 120 °C

N_2 Flow: cone 50 L/h, desolvation 800 L/h

Collision Gas: Argon

Dwell Time: 10 µS for multiple reaction monitoring (MRM) experiments

Collision Cell Pressure: 5.9 x 10^{-3} mbar

Summary of MS/MS Conditions

Pesticide	Molecular Weight	CV (V)	Quantification Transition
Acephate	183.17	20	184.0→143.0
Acetamiprid	222.67	30	223.4→126.1
Acibenzolar S-methyl	210.27	35	211.1→136.0
Aldicarb	190.27	12	208.1→116.0
Aldicarb sulfone	222.27	15	240.0→222.9
Aldicarb sulfoxide	206.26	15	224.2→206.9
Atrazine	215.69	35	215.9→173.85
Avermectin B_{1b}	873.09	20	876.6→553.4
Avermectin B_{1a}	873.09	20	890.7→567.5
Azoxystrobin	403.30	25	404.0→372.1
Benalaxyl	325.41	26	326.1→148.1
Benfuracarb	410.53	20	411.2→190.0
Benzanilide	197.24	30	198.1→105.1
Bifenazate	300.35	20	301.3→170.2
Bitertanol	337.42	20	338.2→ 99.1
Buprofezin	305.44	25	306.3→201.2
Carbaryl	201.22	22	202.1→145.1
Carbendazim	191.19	30	192.0→160.0
Carbofuran	221.26	26	222.1→123.1
Chloroxuron	290.75	35	291.0→ 72.2
Cyprodinil	225.29	45	226.1→ 93.0
Cyromazine	166.19	25	167.2→ 85.1
Diclobutrazol	328.24	30	328.1→70.2
Dimethoate	229.26	20	230.1→199.0
Dimethomorph	387.86	35	388.0→301.1
Dimoxystrobin	326.39	20	327.1→206
Dinotefuran	202.20	20	203.5→14.0
Diuron	233.10	30	233.0→72.1
Ethofumesate	286.35	30	286.9→258.9
Famoxadone	374.39	-32	373.2→ 282
Fenamidone	311.40	25	312.2→236.2
Fenbuconazole	336.82	35	337.1→125.0
Fenhexamid	302.20	65	301.9→261.9
Fenpropimorph	304.49	40	304.4→147.1
Fluconazole	306.27	30	307.2→220
Fludioxinil	248.19	-45	247.0→180.0
Furathiocarb	382.48	30	383.2→195.1
Hexaconazole	314.21	35	314.0→ 70.2
Imazalil	297.18	35	297.1→159.0
Imidacloprid	255.65	25	256.1→175.0
Ipconazole	333.86	35	334.1→70.2

Iprovalicarb	320.43	24	321.2→119.0
Kresoxim-methyl	313.35	20	314.1→116.0
Mepanipyrim	223.28	30	224.4→ 77.3
Metalaxyl	279.34	25	280.1→220.1
Methamidophos	141.13	22	142.0→ 94.0
Methomyl	162.21	20	163.0→ 88.0
Methoxyfenozide	368.47	15	369.5→149.0
Mevinphos	224.15	22	225.1→192.8
Myclobutanil	288.78	35	289.1→ 70.2
Omethoate	213.14	20	214.1→183.0
Oxadixyl	278.31	20	279.1→219.1
Piperonyl butoxide	338.45	17	356.2→177.0
Prochloraz	376.67	20	376.1→308.0
Propamocarb	188.27	30	189.1→102.1
Propargite	350.48	20	368.1→231.0
Propiconazole	342.22	35	342.0→159.0
Propoxur	209.24	20	210.0→111.0
Pyraclostrobin	387.83	23	388.0→194.0
Pyridaben	364.94	22	365.3→309.1
Pyrimethanil	199.25	40	200.1→107.0
Quinoxyfen	308.14	50	307.8→196.8
Rotenone	394.42	40	395.3→213.2
Simazine	201.66	30	202.2→131.4
Spinosyn A	731.97	40	732.6→142.2
Spinosyn D	746.00	30	746.6→142.2
Spiroxamine	297.48	30	298.2→144.0
Tebuconazole	307.82	30	308.2→ 70.2
Thiabendazole	201.25	35	202.0→175.0
Triadmimefon	293.75	30	294.0→197.1
Trifloxystrobin	408.38	25	409.0→186.0
Triflumizole	345.75	20	346.0→278.1
Vamidothion	287.34	20	288.1→146.0
Zoxamide	336.54	35	336.0→187.0

Schematic Diagram of Sample Preparation Steps

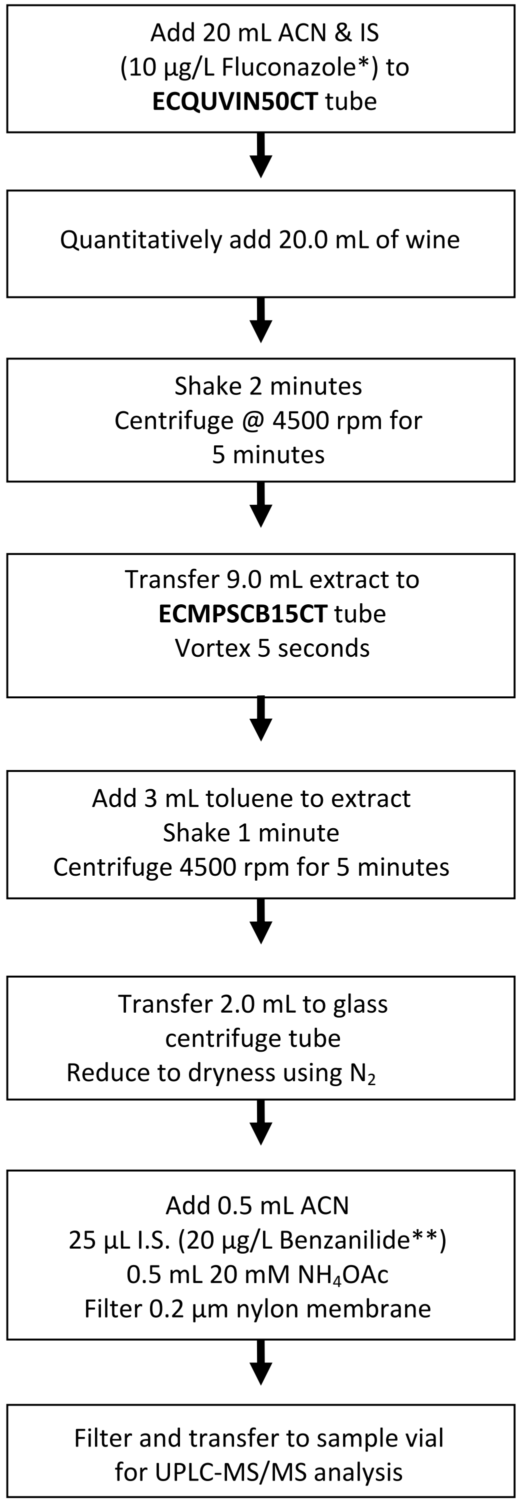


Table of Average Pesticide Recoveries at 100 g/L Spike

(average values with SD, n=4)

	Pesticide Recovery	
	Red Wine @ 100 µg/L	**White Wine @ 100 µg/L**
Acephate	84±4	79±3
Acetamiprid	83±8	97±7
Acibenzolar S-methyl	80±15	45±5
Aldicarb	92±5	82±5
Aldicarb sulfone	91±7	83±4
Aldicarb sulfoxide	83±8	80±1
Atrazine	92±5	83±5
Avermectin B_{1b}	94±12	107±13
Avermectin B_{1a}	82±8	80±6
Azoxystrobin	93±5	86±4
Benalaxyl	92±5	84±4
Benfuracarb	ND	ND
Benzanilide	69±7	70±8
Bifenazate	86±4	86±11
Bitertanol	92±5	86±4
Buprofezin	91±4	88±6
Carbaryl	77±4	76±4
Carbendazim	126±7	106±7
Carbofuran	90±4	86±4
Chloroxuron	75±5	72±2
Cyprodinil	38±2	56±5
Cyromazine	89±6	83±5
Diclobutrazol	89±6	82±4
Dimethoate	88±7	84±4
Dimethomorph	95±5	85±4
Dimoxystrobin	85±5	74±6
Dinotefuran	88±4	78±5
Diuron	74±12	90±1
Ethofumesate	92±10	95±14
Famoxadone	87±3	86±5
Fenamidone	88±5	80±5
Fenbuconazole	133±21	90±11
Fenhexamid	91±5	83±4
Fenpropimorph	86±6	84±4
Fluconazole	112±4	101±2
Fludioxinil	91±2	87±8
Furathiocarb	81±4	77±4
Hexaconazole	90±2	77±7
Imazalil	89±5	83±5

Imidacloprid	94±6	87±4
Ipconazole	89±5	83±5
Iprovalicarb	94±6	87±4
Kresoxim-methyl	85±5	86±5
Mepanipyrim	76±6	94±12
Metalaxyl	94±5	85±5
Methamidophos	82±6	74±5
Methomyl	90±4	81±4
Methoxyfenozide	102±5	89±5
Mevinphos	84±5	71±4
Myclobutanil	96±8	90±4
Omethoate	82±4	75±4
Oxadixyl	94±3	88±4
Piperonyl butoxide	94±5	87±4
Prochloraz	84±3	84±5
Propamocarb	80±3	80±5
Propargite	93±6	86±2
Propiconazole	94±4	86±5
Propoxur	89±5	82±4
Pyraclostrobin	77±6	76±4
Pyridaben	85±4	83±4
Pyrimethanil	79±6	75±4
Quinoxyfen	70±5	68±3
Rotenone	81±3	85±9
Simazine	85±9	88±7
Spinosyn A	88±7	83±4
Spinosyn D	87±4	80±3
Spiroxamine	92±5	84±4
Tebuconazole	90±4	83±5
Thiabendazole	71±3	75±5
Triadmimefon	89±8	84±7
Trifloxystrobin	90±8	84±4
Triflumizole	88±6	86±3
Vamidothion	86±4	83±6
Zoxamide	86±4	80±4

*Adapted from Kai Zhang, Jon W. Wong et al, Multiresidue Pesticide Analysis of Wines by Dispersive Solid-phase Extraction and Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry *Journal of Agricultural and Food Chemistry*

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)

March 12012

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**)

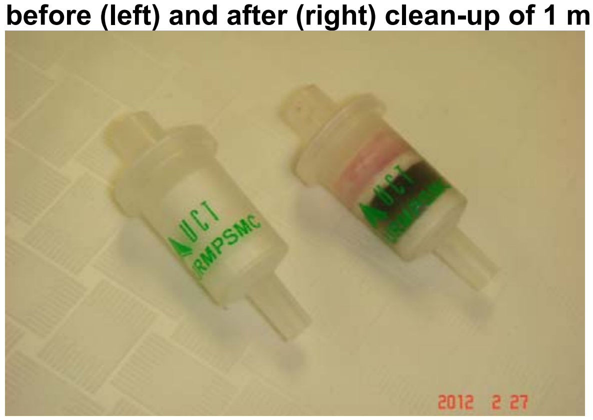
c) Collect 0.5 mL of the cleaned extract into a 2 mL auto-sampler vial

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

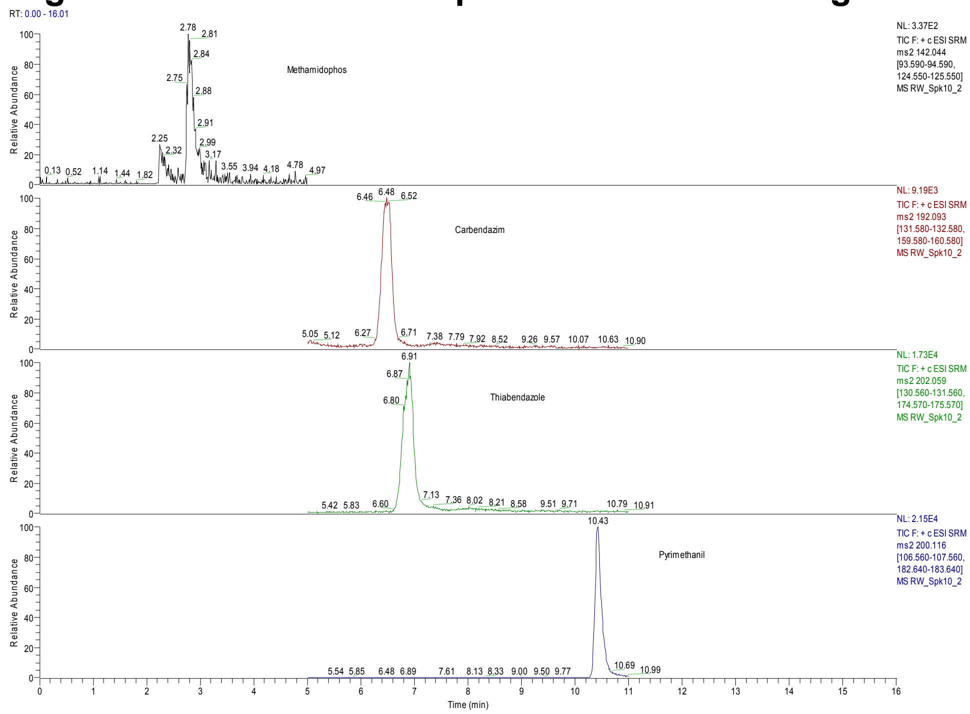
Compound	Linearity range (ng/mL)	R^2	LOD (ng/mL)	LOQ (ng/mL)
Methamidophos	2-400	0.9991	0.15	0.49
Carbendazim	2-400	0.9981	0.40	1.33
Thiabendazole	2-400	0.9940	0.09	0.31
Pyrimethanil	2-400	0.9990	0.01	0.05
Cyprodinil	2-400	0.9995	0.17	0.57
Diazinon	2-400	0.9982	0.06	0.21
Pyrazophos	2-400	0.9976	0.08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

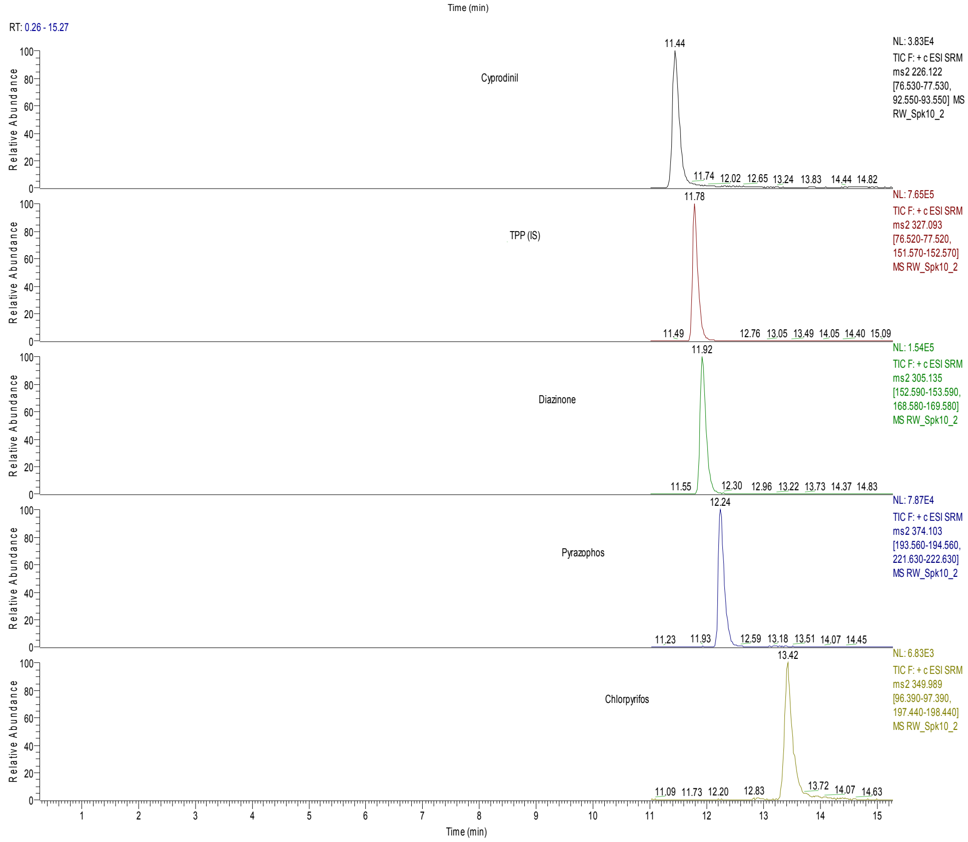
Accuracy and Precision Data

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

Chromatograms of Red Wine Sample Fortified With 10 ng/mL Pesticides





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

Analysis of Flukicides/ Anthelmintics in Animal Tissue Using QuEChERS and LC/MS/MS

UCT Part Number:

ECMSSC50CT-MP (4000 mg $MgSO_4$, 1000 mg NaCl)
ECMSC1850CT (1500 mg $MgSO_4$, 500 mg endcapped C18)

Revised March 27 2013

Procedure

1. Extraction

a) To 10 g of homogenized/hydrated sample in a 50 mL centrifuge tube add 10 mL acetonitrile

b) Add internal standard (100 ng/g Cyprodinil + 2,4-D)

Note: Isotopically labeled internal standards are now commercially available.

c) Shake for 1 minute

d) Add contents of ECMSSC50CT-MP pouch (4 g of anhydrous magnesium sulfate and 1g sodium chloride) to the centrifuge tube

e) Immediately shake for 1 minute

f) Centrifuge for 5 minutes at 3450 rcf

2. Sample Clean-Up

a) Add a 3 mL aliquot of supernatant from (step 1 f) to **ECMSC1850CT**

b) Shake for 1 minute

c) Centrifuge for 1 minute at 3450 rcf

3. Analysis

a) Transfer 0.5 mL of cleaned extract into a autosampler vial

b) Add QC spike (100 ng/mL TPP).

Note: Isotopically labeled internal standards are now commercially available.

c) Inject onto LC-MS/MS

d) Use ESI^+ and/or ESI^- mode depending upon specific analytes of interest

Note:
Abamectin, doramectin and ivermectin form sodium adducts ($[M+23]^+$) when acids are used as mobile phase additive in MS analysis. It is advisable to use ammonium formate or ammonium acetate as mobile phase buffer and monitor the ammonium adduct ($[M+18]^+$) for these three compounds. It is essential to use ammonium buffer in the organic mobile phase as the avermectins elute at 100% organic content. In addition, ammonium formate is more soluble in organic solvent than ammonium acetate.

MS amenable acids can be used for the aqueous mobile phase, which should be at a low pH (≤4) to get the best results. The aqueous mobile phase may also include ammonium buffer, although it is not an essential requirement.

Albendazole-sulfone and hydroxy-mebendazole are prone to isobaric interference as they have similar precursor and product ions that can't be distinguished using triple quadrupole instruments. It is therefore necessary to chromatographically separate these two compounds.

39 Flukicides/Anthelmintics

ESI+		ESI-
ISTD Triphenylphosphate	**QC Spike** Cyprodinil	**ISTD** 2,4D
Abamectin	Albendazole	Bithionol
Doramectin	Albendazole-sulfoxide	Clorsulon
Emamectin	Albendazole-sulfone	Closantel
Eprinomectin	Albendazole-amino-sulfone	Niclosamide
Moxidectin	Cambendazole	Nitroxynil
Ivermectin	Flubendazole	Oxyclozanide
Selamectin	Flubendazole, amino	Rafoxanide
Dichlorvos	Flubendazole, hydroxy	Triaclabendazole-sulfoxide
Coumaphos	Mebendazole	
Coumaphos-oxon	Mebendazole, amino	Albendazole-amino-sulfone
Haloxon	Mebendazole, hydroxy	
Morantel	Oxibendazole	
Levamisole	Thiabendazole	
Fenbendazole	Thiabendazole, 5-hydroxy	
Fenbendazole-sulfone	Triclabendazole	
Fenbendazole-sulfoxide (oxfendazole)		

Adapted from Kinsella, Lehotay et al, "New method for the Analysis of Anthelmintics in Animal Tissue"

Streamlined Method for the Determination of More Than 100 Veterinary Drugs in Animal Tissue Using Dispersive-SPE Clean-up and LC- MS/MS Detection

UCT Part Numbers:
ECMSC1850CT (500 mg C18 and 1500 mg $MgSO_4$)

August 2012

More than 100 veterinary drugs can be extracted and analyzed using this fast and easy multi-class, multi-residue method.

Procedure

1. Extraction

a) Weigh 2 g of homogenized tissue sample into a 50 mL centrifuge tube
b) Add 10 mL of MeCN/water (4/1 v/v)
c) Shake or vortex for 5 min
d) Centrifuge for 5 min at >3700 rcf
e) Supernatant is ready for clean-up

2. dSPE Clean-up

a) Transfer the supernatant into product **ECMSC1850CT**
b) Add 10 mL hexane that has been pre-saturated with MeCN
c) Shake or vortex for 30 sec
d) Centrifuge for 5 min at >3700 rcf
e) Aspirate hexane to waste
f) Evaporate 5 mL of the extract under nitrogen at 45ºC to <0.7 mL
g) Add 0.1% formic acid to reach a final volume of 1 mL (1 g/mL sample equivalent)
h) Transfer sample to a HPLC vial (filter with PVDF if desired)
i) Sample is ready for LC-MS/MS analysis

3. **LC-MS/MS analysis**

MS: Waters TQD
HPLC: Waters Acquity UHPLC

LC Parameters

Guard	Agilent Eclipse Plus C18, 5 µm, 4.6 x 12.5 mm
Column	Waters Acquity HSS T3 (C18), 1.8 µm, 2.1 x 100 mm
Flow	0.5 ml/min
Injection Vol	20 µl
Oven	40 ˚C
Equilibration Time	3.3 min
Autosampler Temperature	4 ˚C

Mobile Phase

Aqueous A: 0.1 % formic acid in water/MeCN (95/5 v/v)
Organic B: 0.1 % formic acid in MeCN

Time	%B
0	0.2
0.1	0.2
8.0	99.8
9.5	99.8
9.6	0.2
13	0.2

For analysis of late eluting compounds, 50 µL/min of 27 mM ammonium formate in MeOH:MeCN (75:25) is infused from 5.05 to 9.45 min using the instrument's infusion syringe to enhance the signal of the late-eluting anthelmintics.

MS Instrument Settings

Capillary voltage	3000 V
Extractor voltage	3 V
Desolvation temperature	450°C
Source temperature	150°C
Dwell time	5 msec

Analyte	Drug class	RT (min)	Precursor ion	Cone V	Product 1	Collision energy (V)	Product 2	Collision energy (V)
Desacetyl Cephapirin	β-Lactam	0.69	382.1	32	152	28	124.2	48
Florfenicol Amine	Phenicol	0.68	248.1	25	230.2	10	130.1	35
Sulfanilamide	Sulfonamide	1.19	173	40	92.9	20	75.9	36
Amoxicillin	β-Lactam	1.47	366.1	20	114	22	349.3	10
Salbutamol	β -Agonist	1.46	240.2	20	148.2	20	222.3	10

Zilpaterol	β -Agonist	1.46	262.3	27	244.3	12	185.2	30
Cimaterol	β -Agonist	1.51	220	16	143	24	115.9	34
DCCD	β -Lactam	1.72	549.1	40	183	30	241.1	20
Lincomycin	Lincosamide	1.87	407.3	20	126.1	30	359.2	20
Sulfadiazine	Sulfonamide	2	251.1	30	156.1	15	108	20
Ampicillin	β -Lactam	2.01	350.1	26	106.1	24	114	30
Desethylene Ciprofloxacin	Fluoroquinolone	2.06	306.2	35	288.2	20	245.2	20
Sulfathiazole	Sulfonamide	2.1	256.1	25	156.1	15	108	25
Sulfapyridine	Sulfonamide	2.18	250.1	32	156.1	18	108.1	28
Norfloxacin	Fluoroquinolone	2.16	320.2	36	276.2	18	233.1	26
Tulathromycin	Macrolide	2.17	806.8	38	72	56	577.5	24
Oxytetracline	Tetracycline	2.21	461.2	25	426.4	20	443.4	15
Ciprofloxacin	Fluoroquinolone	2.22	332.2	35	245.2	25	288.4	20
Ractopamine	β -Agonist	2.27	302.2	26	164	16	107	32
Sulfamerazine	Sulfonamide	2.3	265.1	28	91.9	28	155.9	16
Danofloxacin	Fluoroquinolone	2.31	358.1	28	96	26	314.2	18
Tetracyline	Tetracycline	2.35	445.2	30	154.1	30	410.2	20
Enrofloxacin	Fluoroquinolone	2.38	360.2	35	316.4	20	245.3	25
2-Quinoxalinecarboxylic Acid	Other	2.43	175	22	129	16	131	16
Sulfamethizole	Sulfonamide	2.55	271.1	28	156.1	16	92	30
Sulfamethazine	Sulfonamide	2.54	279.1	35	186.1	20	156.1	20
Sulfamethazine-13C6 (IS)		2.54	285.2	32	186.1	18	124.1	26
Cefazolin	Cephalosporin	2.56	455.1	20	156	16	323.2	12
Sulfamethoxypyridazine	Sulfonamide	2.58	281.1	30	156.1	20	126.2	20
Difloxacin	β -Lactam	2.62	400.3	35	356.4	20	299.2	30
Sarafloxacin	Fluoroquinolone	2.58	386.1	20	342.2	20	299.2	30
Clenbuterol	β -Agonist	2.56	277.2	25	259.2	10	132.1	30
Pirlimycin	Lincosamide	2.74	411.3	30	112.2	40	363.3	20
Chlortetracycline	Tetracycline	2.84	479.2	30	154.1	30	444.3	20
Clindamycin	Lincosamide	2.89	425.3	45	126.2	40	377.4	20
Gamithromycin	Macrolide	2.91	777.8	62	83	54	116	50
Sulfachloropyridazine	Sulfonamide	2.95	285	28	156.1	16	108	26
Tilmicosin	Macrolide	3.06	869.8	45	174.2	35	696.6	35
Sulfadoxine	Sulfonamide	3.1	311.2	35	156.1	20	108.1	30
Sulfamethoxazole	Sulfonamide	3.11	254	26	92.1	30	156	18
Sulfaethoxypyridazine	Sulfonamide	3.14	295.1	30	156.1	20	140.2	20
Florfenicol	Phenicol	3.15	358.1	24	241	18	206	28
Chloramphenicol	Phenicol	3.36	323.1	16	275	16	165	26
Erythromycin	Macrolide	3.49	734.8	30	158.2	36	115.9	54
Sulfadimethoxine	Sulfonamide	3.57	311.1	35	156.1	20	108	30
Sulfaquinoxaline	Sulfonamide	3.59	301.1	34	156.1	18	108	28
Prednisone	Corticosteroid	3.67	359.2	22	341.1	10	146.9	26
Tylosin	Macrolide	3.66	916.8	45	174.2	35	101.1	35
Penicillin G-d7 (IS)		3.86	342.1	46	183.1	26	160.1	24
Penicillin G	β -Lactam	3.86	335.1	18	176	16	160.1	18
Beta/Dexa-methasone	Corticosteroid	4.11	393.2	20	373.2	10	147.1	28

Sulfanitran	Sulfonamide	4.16	336.2	26	156	12	134.1	28
Sulfabromomethazine	Sulfonamide	4.21	357.1	35	92	30	156.1	25
Zeranol (Î±-Zearalanol)	Other	4.37	323.2	16	305.2	10	189.1	24
Oxacillin	β -Lactam	4.39	402.1	22	160	20	243.1	18
Atrazine (QC)		4.49	216.1	34	174	18	103.9	30
Cloxacillin	β -Lactam	4.66	436.2	22	160.1	12	277.1	16
Nafcillin	β -Lactam	4.79	415.2	20	199.1	14	171.1	38
Oxyphenylbutazone	NSAID	4.83	325.2	26	120.1	24	148.2	30
Flunixin	NSAID	4.86	297.1	42	279.1	22	109	50
Flunixin-d3 (IS)		4.82	300.1	40	282.1	24	112	54
Dicloxacillin	β -Lactam	5.03	470.2	22	160.1	14	311.1	16
Phenylbutazone	NSAID	5.93	309.1	28	120	20	91.8	30
Melengesterol Acetate	Other	6.3	397.4	30	279.3	20	337.5	15
2-thiouracil	Thyreostat	0.85	128.9	32	111.9	12	69.9	18
2-mercapto-1-	Thyreostat	1.14	114.9	40	87.9	16	73.9	16
6-methyl-2-thiouracil	Thyreostat	1.22	142.9	32	83.9	18	125.9	14
Metronidazole-OH	Nitroimidazole	1.42	188	22	123	14	126	18
Dipyrone	Tranquilizer	1.6	218.1	24	96.9	12	187	10
Dimetridazole-OH	Nitroimidazole	1.63	158	22	140	12	93.9	22
Metronidazole	Nitroimidazole	1.63	172	26	127.9	14	81.9	24
5-hydroxythiabendazole	Anthelmintic	1.7	218	50	190.9	26	147	32
Albendazole 2-amino-sulfone	Anthelmintic	1.85	240	36	133	28	198	20
Ronidazole	Nitroimidazole	1.85	201	18	139.9	10	54.8	20
Levamisole	Anthelmintic	1.86	205	40	178	22	90.9	34
Dimetridazole	Nitroimidazole	1.86	142	32	95.9	16	80.9	24
Thiabendazole	Anthelmintic	1.94	202	44	174.9	26	130.9	32
6-propyl-2-thiouracil	Thyreostat	2.15	171	38	154	18	112	20
2-mercaptobenzimidazole	Thyreostat	2.3	150.9	42	92.8	20	118	22
Azaperone	Tranquilizer	2.34	328.3	34	165	20	122.9	36
Orbifloxacin	Fluoroquinolone	2.39	396.2	36	352.2	18	295.1	24
Albendazole sulfoxide	Anthelmintic	2.44	282.1	28	240	14	207.3	24
Xylazine	Tranquilizer	2.48	221.1	42	164	26	147	24
Ipronidazole-OH	Nitroimidazole	2.54	186.1	22	168	14	121.8	20
Morantel	Anthelmintic	2.6	221.1	50	122.9	36	163.9	28
2-amino-Mebendazole	Anthelmintic	2.63	238.1	50	104.9	26	132.9	36
6-phenyl-2-thiouracil	Thyreostat	2.73	205	38	103	26	187.9	18
2-amino-Flubendazole	Anthelmintic	2.77	256	50	122.9	28	94.9	38
Cambendazole	Anthelmintic	2.83	303.1	34	261.1	18	217	28
Bacitracin	Other	2.87	475.3	26	85.9	24	199.1	30
Carazolol	Tranquilizer	2.9	299.3	34	116	20	97.9	22
Doxycycline	Tetracycline	2.91	445.3	28	428.2	20	97.9	46
Oxibendazole	Anthelmintic	2.95	250.1	34	218.1	18	175.9	28
Oxfendazole	Anthelmintic	3.01	316.1	40	158.9	32	191	22
Albendazole sulfone	Anthelmintic	3.02	298.1	38	266	20	159	36
Ipronidazole	Nitroimidazole	3.2	170.1	34	124	18	109	24
Clorsulon	Flukicide	3.39	377.7	24	341.8	12	241.9	20

Haloperidol	Tranquilizer	3.53	376.2	40	165	24	122.9	42
Acetopromazine	Tranquilizer	3.55	327.2	32	86	20	254	22
Promethazine	Tranquilizer	3.58	285.2	24	85.9	16	198	20
Fenbendazole sulfone	Anthelmintic	3.65	332.1	40	300	22	158.9	38
Albendazole	Anthelmintic	3.65	266.1	34	234	20	191.1	32
Mebendazole	Anthelmintic	3.7	296.1	36	264.1	20	104.9	36
Flubendazole	Anthelmintic	3.9	314.1	38	282	22	94.9	50
Propionylpromazine	Tranquilizer	3.91	341.2	32	85.9	22	268.1	24
Chlorpromazine	Tranquilizer	4.04	319.2	32	86	20	246	22
Triflupromazine	Tranquilizer	4.26	353.2	34	85.9	22	280	28
Fenbendazole	Anthelmintic	4.33	300.1	38	268	20	158.9	36
Oleandomycin triacetate	Macrolid	4.37	814.7	38	200.1	30	98	48
Nitroxynil	Flukicide	4.41	288.8	40	126.8	20	115.9	34
Virginiamycin M1	Other	4.49	526.4	26	508.3	12	108.9	44
Ketoprofen	Tranquilizer	4.71	255.1	28	104.9	24	209	14
Haloxon	Anthelmintic	5.28	415.1	44	272.9	34	210.9	36
Triclabendazole sulfoxide	Flukicide	5.37	372.8	36	357.8	18	212.9	30
Emamectin benzoate	Anthelmintic	5.49	886.8	52	158	40	126	46
Diclofenac	Tranquilizer	5.55	296	20	214.9	20	250	12
Triclabendazole	Flukicide	5.99	359	52	343.9	26	274	38
Novobiocin	Other	6.05	613.5	30	189	28	132.9	64
Oxyclozanide	Flukicide	6.08	399.6	38	363.8	14	175.9	24
Niclosamide	Flukicide	6.2	325	36	170.9	30	289	16
Tolfenamic acid	Tranquilizer	6.23	262.1	22	244	14	180	40
Bithionol	Flukicide	6.76	352.9	36	160.8	24	191.2	28
Eprinomectin	Anthelmintic	7.44	914.8	18	186.1	20	154	40
Abamectin	Anthelmintic	7.94	890.8	16	305.3	28	145	42
Closantel	Flukicide	8.07	660.9	70	126.8	54	344.8	32
Doramectin	Anthelmintic	8.3	916.9	22	331.3	26	113	56
Moxidectin	Anthelmintic	8.32	640.5	16	528.3	8	498.2	10
Rafoxanide	Flukicide	8.5	623.9	62	126.1	48	344.8	30
Selamectin	Anthelmintic	8.62	770.7	36	145	30	112.9	40
Ivermectin $[M+Na]^+$	Anthelmintic	8.77	897.8	82	183	58	329.2	56

Accuracy and Precision

A multi-day, multi-analyst validation demonstrated that the final method is suitable for screening of 113 analytes, identifying 98 and quantifying 87 out of the 127 tested drugs at or below US regulatory tolerance levels in bovine muscle. Overall, the method demonstrated reasonably good quantitative performance with recoveries ranging between 70–120% for 87 out of 127 analytes, and recovery of < 50% for only 20 analytes. 85 analytes gave RSDs ≤ 20% and 100 analytes gave RSDs ≤ 25%.

Adapted from L. Geis-Asteggiante, S.J. Lehotay, A.R. Lightfield, T. Dutko, C. Ng, L. Bluhm, Ruggedness testing and validation of a practical analytical method for >100 veterinary drug residues in bovine muscle by ultrahigh performance liquid chromatography tandem mass spectrometry, Journal of Chromatography A 1258 (2012) 43-54.

Acrylamide by QuEChERS Extraction with LC/MS/MS Detection

UCT Product Number:
ECMSSC50CT-MP (4000 mg $MgSO_4$, 1000 mg NaCl, in Mylar pouch)
CUMPS15C18CT (150 mg, $MgSO_4$, 150 mg PSA and 50 mg endcapped C18, 2 mL centrifuge tube)

August 2012

Acrylamide is a neurotoxin and classified as a probable human carcinogen and genotoxicant

Procedure

1. Extraction

a) Add 5 g of homogenized sample to a 50 mL centrifuge tube
b) Fortify with ISTD
c) Add 10 mL of reagent water, vortex
d) Allow >15 minutes for hydration
e) Add 10 mL of acetonitrile, vortex
f) Add salts from Mylar pouch **ECMSSC50CT-MP**
g) Shake vigorously for 1 min
h) Centrifuge at 5000 rpm for 10 min
i) Supernatant is ready for clean-up

2. Clean-up

a) Add 1 mL of supernatant to the 2 mL centrifuge tube **CUMPS15C18CT**
b) Vortex for 30 sec
c) Centrifuge at 5000 rpm for 10 min
d) Transfer 500 µL of extract into LC vial for analysis

LC/MS/MS Instrumentation

- **LC:** Thermo Accela 1250 pump
- **Column:** Sepax C18, 150 mm x 2.1 mm, 3µm
- **Guard:** Restek C18 2.1 x 20mm
- **Column Temperature:** Ambient
- **Injection:** 20 µL at 15° C
- **Mobile Phase:** A: water; B: methanol
- **Flow Rate:** 200 µL/min

Mobile Phase Program

Time	Mobile Phase
0 min	100% A
3 min	100% A
5 min	100% B
6 min	100% B
7 min	100% A
12 min	100% A*

* divert to waste for 0-1 min & 3-12 min

MS/MS: Thermo TSQ Vantage

- **Ion source:** APCI
- **Ion polarity:** positive mode
- **Discharge Current:** 22V
- **Declustering voltage:** 11 V
- **Sheath gas:** N_2 at 10 arbitrary units
- **Auxiliary gas:** N_2 at 15 arbitrary units
- **Vaporizer Temp:** 380 °C
- **Ion Transfer Cap. Temp:** 250 °C
- **Scan Type:** SRM
- **Dwell Time:** 150 ms
- **CID Pressure:** 0.5 mTorr

SRM Transitions				
Analyte	**Parent ion**	**Product ion 1**	**Collision Energy**	**S-lens**
Acrylamide	72.0	55.0	9	43
	72.0	27.0	20	43
$^{13}C_3$-Acrylamide	75.1	58.0	10	50

Acrylamide Recovery

Matrix n = 5	Analyte Conc. ng/mL	Recovery %	Mean Conc. ng/mL	SD (%)	RSD (%)
French Fries	**50**	106	53	6.1	11.56
	250	106	265	9.8	3.71
Potato Chips	**50**	111	56	9.4	16.84
	250	103	257	32.2	12.54
Multigrain Cereal	**50**	98	49	5.9	11.93
	250	93	232	9.2	3.97

DCN-211380-242

Trichothecene Type A & B Analysis in Wheat and Corn Using the QuEChERS Approach*

UCT Part Number:
ECMSSC50CT-MP (50 mL centrifuge tube, 4 g anhydrous magnesium sulfate, 1 g NaCl)
CUMPS2CT (150 mg anhydrous magnesium sulfate and 50 mg PSA

January 2010

An extraction and purification method for the simultaneous LC-MS determination of five mycotoxins is described including three type A, diacetoxyscirpenol (DAS), T-2 toxin and HT-2 toxin, and two type B trichothecenes, deoxynivalenol (DON) and nivalenol (NIV). These mycotoxins are responsible for a wide range of disorders in animals. They have been found to inhibit proteins synthesis and to have immunosuppressive and cytotoxic effects. Health risks associated with human exposure to *Fusarium* toxins are recognized worldwide and depend on concentration in a particular diet. The major dietary sources of trichothecenes are cereal products wheat and corn. The analysis has been optimized using a modified QuEChERS approach.

Procedure

1. **Sample Preparation**
   a) Thoroughly homogenize a sample of grain products using a laboratory mill
   b) Weigh 5 g of sample into the 50 ml centrifuge tube
   c) Add 10 mL of methanol:acetonitrile (85:15) into 50 mL centrifuge tube
   d) Shake to disperse solvent
   e) Add the contents of the **ECMSSC50CT-MP** pouch containing 4 g anhydrous magnesium sulfate, 1 g sodium chloride to the centrifuge tube
   f) Vortex for 1 minute then centrifuge @ 4,000 rpm for 10 minutes

2. **Sample Clean-up**
   a) Transfer a 1 mL aliquot to a 2 mL **CUMPS2CT** tube (150 mg anhydrous magnesium sulfate and 50 mg PSA)
   b) Shake for 1 minute
   c) Centrifuge for 10 minutes @ 4,000 rpm
   d) Filter extract through a 0.45 µm filter into an LC injection vial if supernatant is not clear
   e) Sample is now ready for analysis

3. Analysis

a) MSD detection with atmospheric pressure ionization (API) configured for electrospray positive ion mode

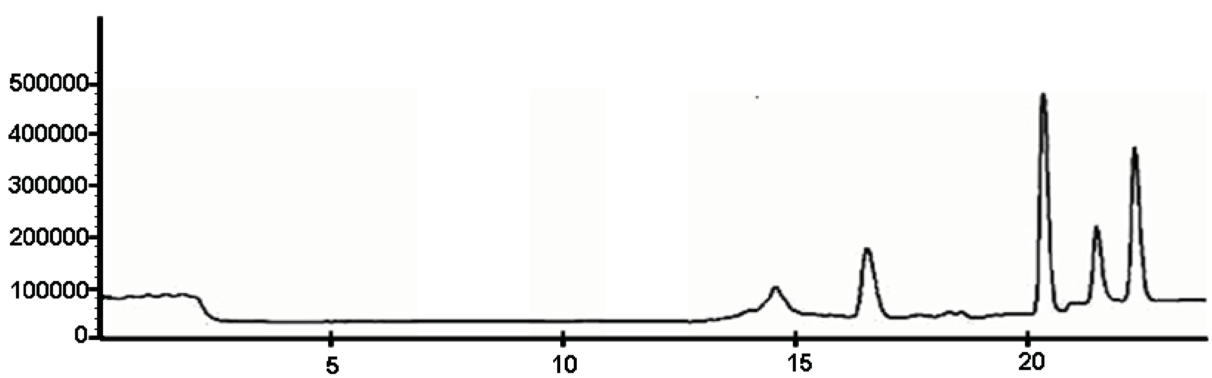
b) Analytical column: Luna C18 (250mm x 4.6 mm x 5 µm) or equivalent may be used but may change elution times

c) Mobile phase A: 1% formic acid in water, B: 1% formic acid in methanol

d) Gradient, Flow 0.5 mL/minute, Initial 40%B, 10 minutes 90% B until 25 minutes

Mass Ions for Mycotoxins [Na+M]	
Ion	**M/Z**
NIV	355
DON	319
DAS	389
HT2	447
T2	489

Chromatogram Showing Elution of Mycotoxins
Peaks in order of elution: NIV, DON, DAS, HT-2, T-2



*Modified from Sospedra et al, "Use of the Modified Quick, Easy, Cheap, Effective, Rugged and Safe Sample Preparation Approach for the Simultaneous Analysis of Type A and B Trichothecenes in Wheat Flour," J of Chromatography A

DCN-102201-182

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

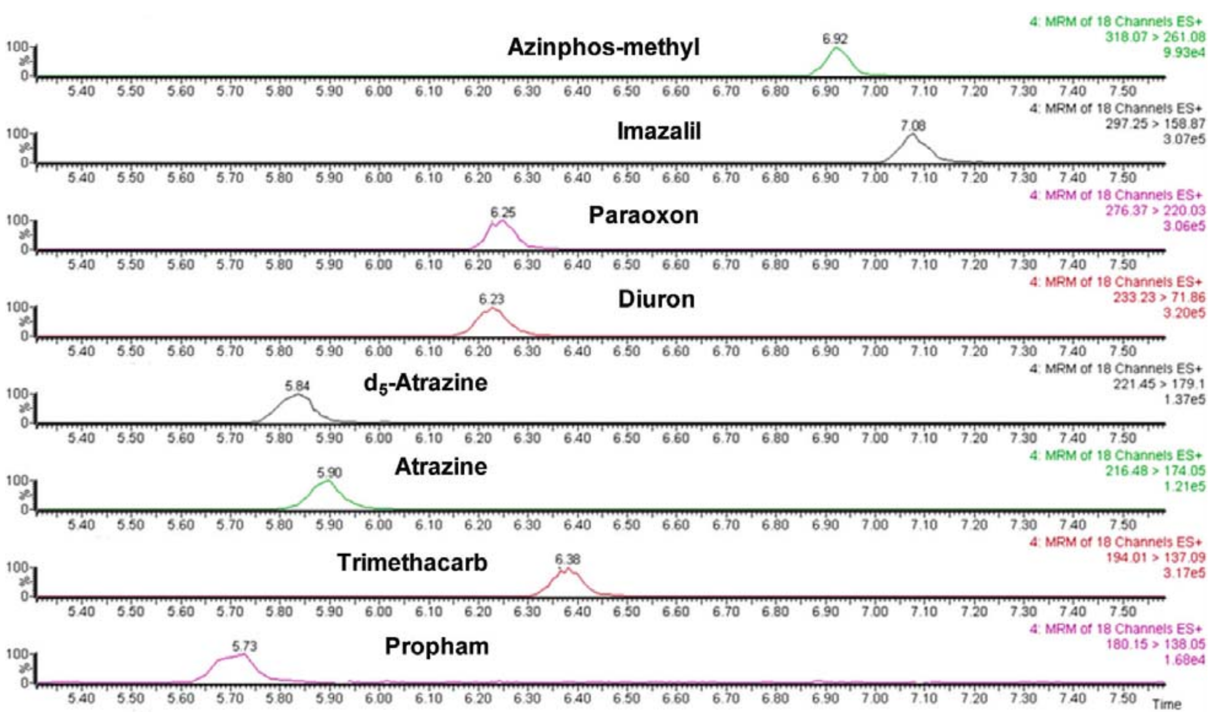
Automated DSI Injection:

- 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:

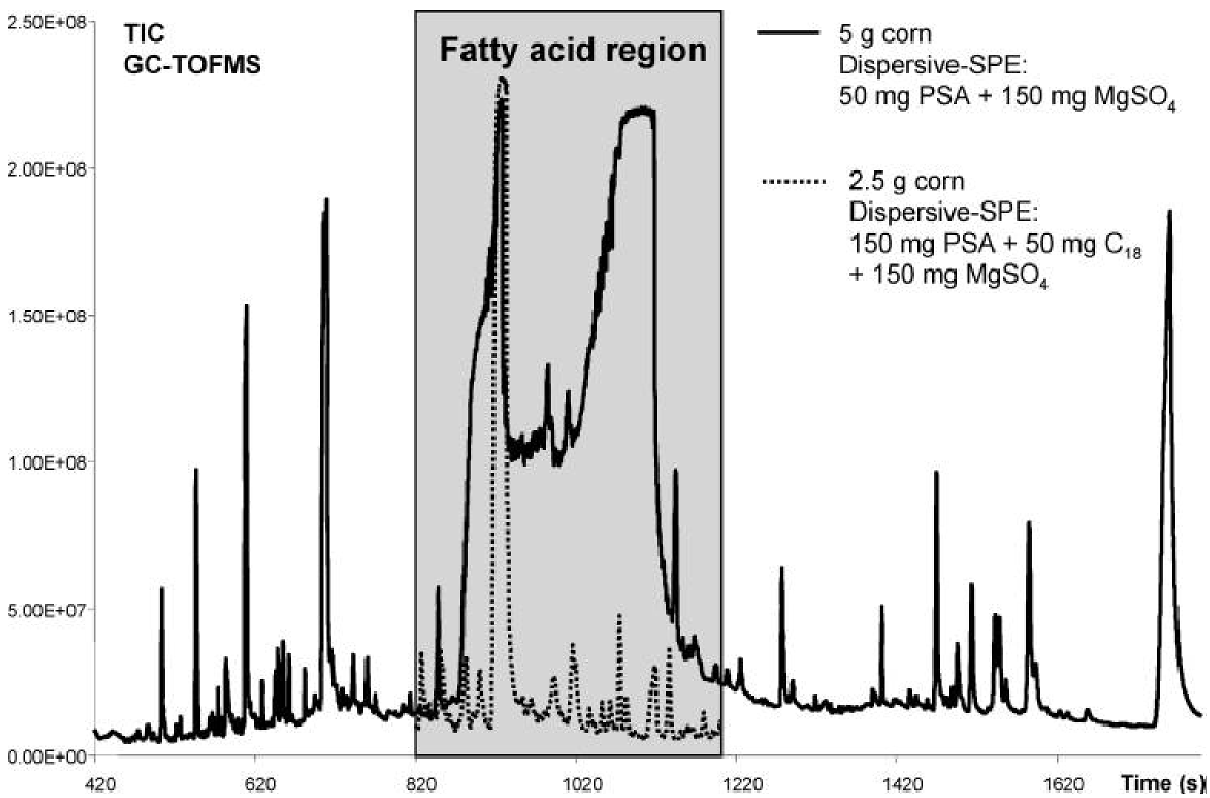
- Solvent vent at an injector temperature of 100° C for 4.5 minutes
- Initial column flow of 0.8 mL/minutes and a split flow of 50 mL/minutes,
- Follow by a splitless transfer of analytes for 4 minutes. The injector temperature was ramped to 280° C (at 16° C/s) Column flow changed to 1.5 mL/minutes (kept 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

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

Extraction of Polycyclic Aromatic Hydrocarbons from Fish Using the QuEChERS Approach

UCT Product Number:
ECMSSC-MP (4000 mg Magnesium Sulfate, 1000 mg sodium chloride)
ECMPSC1815CT (900 mg MgSO4, 300 mg PSA and 150 mg endcapped C18)
ECPAHFR50CT (ENVIRO-CLEAN® PAH 50 mL centrifuge tubes)

June 2010

The QuEChERS approach is used for the extraction of 16 PAH compounds from fish with analysis by LC with fluorescence detection

1. Extraction: Protect samples from exposure to light to avoid degradation

a) Transfer 5 grams of homogenized fish to a 50 mL centrifuge tube
b) Add 10 mL of acetonitrile
c) Mix by shaking
d) Add the contents of pouch containing 4 grams $MgSO_4$ and 1 gram of NaCl to the centrifuge tube
e) Immediately vortex the mixture for 3 minutes
f) Centrifuge samples for 3 minutes at 3400 rpm
g) Recover the clear supernatant for clean-up

2. Clean-up, Dispersive Solid-phase (dSPE)

a) Use 3 mL of the supernatant for clean-up
b) Add supernatant to centrifuge tube **ECMPSC1815CT**
c) Shake for 1 minute
d) Centrifuge for 1 minute at 3400 rpm
e) Filter supernatant through 0.20 µm PTFE membrane filter
f) Samples are ready for analysis

3. Analysis Conditions

Separation of the compounds is performed in a C18 column (CC 150/4Nucleosil 100-5 C18 PAH, 150 x 4.0 mm; 5 µm particle size; Macherey-Nagel, Duren, Germany) or equivalent maintained at room temperature

a) Inject 15 µL
b) The initial composition of the mobile phase is 50% of ACN and 50% water
c) Program A linear gradient to 100% in 15 minutes
d) Hold 13 minutes
e) Initial conditions are achieved within 1 minute and maintained for 6 min before next run
f) Total run time 40 minutes
g) Flow rate 0.8 mL/minute

Fluorescence wavelength program:

Each compound is detected at its optimum excitation/emission wavelength pair:

315/260 nm naphthalene, acenaphthene and fluorene

366/260 nm Phenanthrene

430/260 nm anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene and dibenzo(a,l)pyrene

505/290 nm (indeno(1,2,3-cd)pyrene)

***Adapted from**, Ramalhosa, Maria Joao et al, "Analysis of polycyclic aromatic hydrocarbons in fish: evaluation of a quick, easy, cheap, effective, rugged, and safe extraction method", J. Sep. Sci. 2009, 32, 3529 – 3538

Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Seafood Using GC/MS

UCT Part Numbers:
ECQUUS2-MP (4 g of muffled anh. $MgSO_4$ and 2 g of NaCl)
ECPAHFR50CT (50 mL centrifuge tubes, PAHs removed)
EUSILMSSM26 (6 mL, 1g silica gel cartridge with 200 mg of muffled anhydrous sodium sulfate on top)

January 2012

This method is used for the determination of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in fish and seafood--oyster, shrimp, and mussel. Benzo[*a*]pyrene is the main analyte of interest. GC/MS instrumentation is used for analysis.

PAH Analytes Covered in this Method

PAH	Abbreviation	PAH	Abbreviation
Anthracene	Ant	**Indeno[1,2,3-*cd*]pyrene**	IcdP
Benz[*a*]anthracene	BaA	**Naphthalene**	Naph
Benzo[*a*] pyrene	BaP	**Phenanthrene**	Phe
Benzo[*b*]fluoranthene	BpF	**Pyrene**	Pyr
Benzo[*k*]fluoranthene	BkF	**3-Methylchrysene**	3-MC
Benzo[*g,h,i*]perylene	BghiP	**1-Methylnaphthalene**	1-MN
Chrysene	Chr	**1-Methylphenanthrene**	1-MP
Dibenz[*a,h*]anthracene	DBahA	**2,6 Dimethylnaphthalene**	2,6-DMN
Fluoranthene	Flt	**1,7-Dimethylphenanthrene**	1,7-DMP
Fluorene	Fln		

Procedure

1. Extraction

a) To the 50 mL polypropylene centrifuge tube add 10 ± 0.1 g of homogenized seafood sample

b) Add 50 µL of 1 µg/mL ^{13}C-PAHs solution

c) Vortex sample for 15 sec and then equilibrate for 15 min

d) Add 5 mL of reagent water and 10 mL of ethyl acetate (EtOAc)

e) Shake for 1 min

2. Partition

a) Add the contents of pouch **ECQUUS2-MP**. Tightly seal the tube to ensure that salts do not get into the screw threads

b) Shake for 1 min

c) Centrifuge at > 1,500 rcf for 10 min

d) Remove 5 mL aliquot of the upper ethyl acetate layer, add 50 µL of isooctane as a keeper

e) Evaporate all ethyl acetate until only isooctane and co-extracted sample fat remain

f) Reconstitute with 1 mL of hexane

3. Clean-Up

a) Condition a silica SPE column **EUSILMSSM26** (**Note 1**) (1 g of silica gel with approx. 0.2 g of muffled anh. sodium sulfate on the top) with 6 mL of hexane:dichloromethane (3:1 v/v) and 4 mL of hexane

b) Apply the reconstituted extract to the silica SPE cartridge (**Note 2**)

c) Elute with 10 mL of hexane:dichloromethane (3:1 v/v) and collect the eluent

d) Add 0.5 mL isooctane to the eluent as a keeper and evaporate to 0.5 mL to remove hexane and dichloromethane from the final extract

e) Transfer the final extract into an autosampler vial for GC/MS analysis

Notes:

1. The fat capacity of the 1-g silica gel SPE column is approx. 0.1 g. If the ethyl acetate extract aliquot contains more than 0.1 g of fat, use a smaller aliquot to avoid sample breakthrough
2. Ethyl acetate should not be present in the extract applied to the silica cartridge as it affects extract polarity and potential retention of fat and analytes on the silica gel.

GC Conditions for the Analysis of PAHs

Column	BPX-50 (30 m x 0.25 mm i.d. x 0.25 µm film thickness)
Oven Temperature Program	80°C (hold for 4.3 min), 30°C/min to 220°C, 2°C/min to 240°C, and 10°C/min to 360°C (hold for 17 min)
He Flow Rate	1.3 mL/min (hold for 19 min), then 50 mL/min to 2 mL/min (hold for 16 min)
Injection Technique	PTV solvent vent
Injection Volume	1 x 8 µL
Vent Time	2.3 min
Vent Flow	50 mL/min
Vent Pressure	50 psi
Inlet Temperature Program	50°C (hold for 2.3 min), then 400°C/min. to 300°C

MS Conditions

Any GC-MS instrument (single quadrupole, triple quadrupole, time-of-flight or ion trap) with electron ionization (EI) may be used

MS Ions (*m*/*z*) for Quantification and Identification of Target PAHs for Single-stage MS Instruments

Analyte PAH's	Abbreviation	Confirmation Ions *(m/z)*	Quantification Ions *(m/z)*
Anthracene	Ant	177	178
Benz[*a*]anthracene	BaA	226	228
Benzo[*a*] pyrene	BaP	253	252
Benzo[*b*]fluoranthene	BpF	253	252
Benzo[*k*]fluoranthene	BkF	253	252
Benzo[*g,h,i*]perylene	BghiP	277	276
Chrysene	Chr	226	228
Dibenz[*a,h*]anthracene	DBahA	276	278
Fluoranthene	Flt	200	202

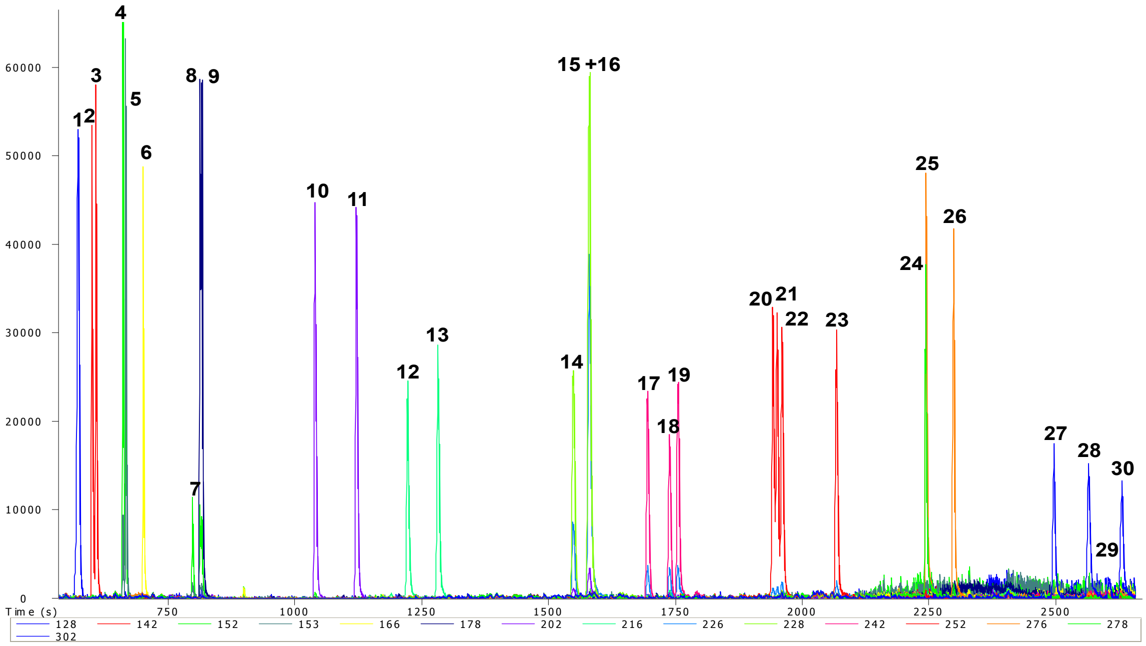
Fluorene	Fln	165	166
Indeno[1,2,3-cd]pyrene	IcdP	277	276
Naphthalene	Naph	127	128
Phenanthrene	Phe	177	178
Pyrene	Pyr	200	202
3-Methylchrysene	3-MC	241	242
1-Methylnaphthalene	1-MN	115	142
1-Methylphenanthrene	1-MP	189	192
2,6 Dimethylnaphthalene	2,6-DMN	141	156
1,7-Dimethylphenanthrene	1,7-DMP	191	206

MS Ions (*m*/*z*) for Quantification and Identification of Target ^{13}C-PAHs for Single-stage MS Instruments

Analyte PAH's	Abbreviation	Confirmation Ions *(m/z)*	Quantification Ions *(m/z)*
Naphthalene ($^{13}C_6$)	Naph- $^{13}C_6$	133	134
Fluorene ($^{13}C_6$)	Fln- $^{13}C_6$	171	172
Phenanthrene ($^{13}C_6$)	Phe- $^{13}C_6$	183	184
Anthracene ($^{13}C_6$)	Ant- $^{13}C_6$	183	184
Fluoranthene ($^{13}C_6$)	Flt-$^{13}C_6$	205	208
Pyrene ($^{13}C_6$)	Pyr-$^{13}C_6$	208	205
Benz[*a*]anthracene ($^{13}C_6$)	BaA- $^{13}C_6$	232	234
Chrysene ($^{13}C_6$)	Chr- $^{13}C_6$	232	234
Benzo[*b*]fluoranthene ($^{13}C_6$)	BbF- $^{13}C_6$	259	258
Benzo[*k*]fluoranthene ($^{13}C_6$)	BkF- $^{13}C_6$	259	258
Benzo[*a*]pyrene ($^{13}C_4$)	BaP- $^{13}C_4$	257	256
Indeno[1,2,3-*cd*]pyrene ($^{13}C_6$)	IcdP- $^{13}C_6$	283	282

Dibenz[*a,h*]anthracene ($^{13}C_6$)	DBahA- $^{13}C_6$	282	284
Benzo[*g,h,i*]perylene ($^{13}C_{12}$)	BghiP- $^{13}C_{12}$	289	288

An Example Chromatogram of A GC Separation of PAH's and Their Alkyl Homologues In A Standard Solution Mixture At 25 ng/mL In Isooctane



1 – naphthalene, 2 – 2-methylnaphthalene, 3 – 1-methylnaphthalene, 4 – acenaphthylene, 5 – acenaphthene, 6 – fluorene, 7 – dibenzothiophene, 8 – phenanthrene, 9 – anthracene, 10 – fluoranthene, 11 – pyrene, 12 – 1-methylpyrene, 13 – benzo[*c*]fluorene, 14 – benz[*a*]anthracene, 15 – cyclopenta[*c,d*]pyrene, 16 – chrysene, 17 – 1-methylchrysene, 18 – 5-methylchrysene, 19 – 3-methylchrysene, 20 – benzo[*b*]fluoranthene, 21 – benzo[*k*]fluoranthene, 22 – benzo[*j*]fluoranthene, 23 – benzo[*a*]pyrene, 24 – dibenz[*a,h*]anthracene, 25 – indeno[1,2,3-*cd*]pyrene, 26 – benzo[*g,h,i*]perylene, 27 – dibenzo[*a,l*]pyrene, 28 – dibenzo[*a,e*]pyrene, 29 – dibenzo[*a,h*]pyrene, 30 – dibenzo[*a,i*]pyrene

*The analyst should refer to Katerina Mastovska, Wendy R. Sorenson, Covance Laboratories Inc
Jana Hajslova, Institute of Chemical Technology, Prague "Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Seafood using Gas Chromatography-Mass Spectrometry: A Collaborative Study"

References

Lucie Drabova, Kamila Kalachova, Jana Pulkrabova, Tomas Cajka, Vladimir Kocourek and Jana Hajslova. "Rapid Method for Simultaneous Determination of Polycyclic Aromatic Hydrocarbons (PAHs), Polychlorinated Biphenyls (PCBs) and Polybrominated Diphenyl Ethers (PBDEs) in Fish and Sea Food Using GC-TOFM," ICT document, Prague, Czech Republic, 2010.

QuEChERS Analysis of Miticides and Other Agrochemicals in Honey Bees, Wax or Pollen*

UCT Part Number:
ECMSSA50CT-MP (6000 mg $MgSO_4$ and 1500 mg sodium acetate)
CUMPSC18CT (150 mg $MgSO_4$, 50 mg PSA, 50 mg C18)
ECPSACB256 (dual layer cartridge 250 mg GCB, 500 mg PSA)
ECMAG00D (organic free magnesium sulfate anhydrous)

March 2012

An analytical method using QuEChERS type procedures for 121 different pesticide residues is described. Extracts of wax, beebread, and adult bees or brood can also be analyzed for metabolites of primary miticides and insecticides using this method. This includes the oxon and phenolic metabolites of coumaphos, chlorferone, the sulfoxide and sulfone metabolites of aldicarb, and the toxic olefin and 5-hydroxy metabolites of imidacloprid.

Sample Collection and Preservation

- Wrap in aluminum foil and store on dry ice until placement in a -80° C freezer as soon as possible
- Beebread and brood can be removed from the combs at room temperature and then stored along with the remaining beeswax at -20° C until processed

1. Sample Preparation

a) Weigh 3 grams beebread (or comb wax) into a 50 mL centrifuge tube
b) Add 100 µL of a process control spiking solution
c) Add 27 mL of extraction solution*
d) *44% DI water, 55% acetonitrile & 1% glacial acetic acid

Brood and adults are extracted without using DI water

e) Add 100 µL of an internal standard
f) For beebread, reduce particle size by using a high speed disperser for 1 minute
g) For comb wax melt the sample at 80° C in a water bath followed by cooling to RT
h) Add the contents of **ECMSSA50CT-MP** pouch to the mixture

i) Seal tube and shake vigorously for 1 minute

j) Centrifuge for 1 minute

2. Clean-Up for LC/MS-MS

a) Transfer 1 mL of supernatant to **CUMPSC18CT** micro centrifuge tube

b) Vortex for 1 minute and centrifuge

c) Transfer supernatant to an autosampler vial for LC analysis

3. Clean-Up for GC/MS

a) Prepare a dual layer solid-phase extraction cartridge **ECPSACB256** by adding about 80 mg of anhydrous magnesium sulfate (ECMAG00D) to the top of the cartridge

b) Condition cartridge by adding 4.0 mL of acetone/toluene (7:3 v:v)

c) Using a positive pressure or vacuum manifold, elute solvent to waste

d) Add 2 mL of supernatant to the cartridge

e) Elute cartridge using 3 x 4 mL of acetone/toluene & 7:3, (v:v) into a 15 mL graduated glass centrifuge tube

f) Using an analytical evaporator @ 50°C, dry eluate to a final volume of 0.4 mL

g) Sample is ready for analysis

4. Analysis—by LC or GC

LC analysis is necessary for neonicotinoids, other polar pesticides and their metabolites

For LC analysis

a) Analysis by LC/MS-MS use a 3.5 µm 2.1 X 150 mm Agilent Zorbax SB-C18 (or equivalent)

b) Agilent 1100 LC with a binary pump interfaced to a Thermo-Fisher TSQ Quantum Discovery triple quadrupole MS 9 (or equivalent)

For GC analysis

a) For analysis use Agilent 6890 (or equivalent) GC equipped with a 0.25 mm, 30 m J&W DB-5MS (2 µm film) capillary column

b) Interface to an Agilent 5975 triple quadrupole MS (or equivalent)

c) Use GC/MS in the electron impact and negative chemical ionization modes

Pesticides Representatives Found in Wax Samples	
Aldicarb sulfoxide	Flutolanil
Aldicarb sulfone	Fluvalinate
Allethrin	Heptachlor
Atrazine	Heptachlor epoxide
Azinphos methyl	Hexachlorobenzene
Azoxystrobin	Imidacloprid
Bendiocarb	Iprodione
Bifenthrin	Malathion
Boscalid	Metalaxyl
Captan	Methidathion
Carbaryl	Methoxyfenozide
Carbendazim	Metribuzin
Carbofuran	Norflurazon
Carbofuran, 3-hydroxy	Oxyfluorfen
Carfentrazone ethyl	Parathion methyl
Chlorfenapyr	p-Dichlorobenzene
Chlorferone (coumaphos)	Pendimethalin
Chlorothalonil	Permethrin
Chlorpyrifos	Phosmet
contrast	Piperonyl butoxide
Coumaphos	Prallethrin
Coumaphos	Pronamide
Cyfluthrin	Propiconazole
Cyhalothrin	Pyraclostrobin
Cypermethrin	Pyrethrins
Cyprodinil	Pyridaben
DDE p,p'	Pyrimethanil
Deltamethrin	Pyriproxyfen
Diazinon	Quintozene
Dicofol	Spirodiclofen
Dieldrin	Tebufenozide
Dimethomorph	Tebuthiuron
DMA (amitraz)	Tefluthrin
DMPF (amitraz)	Tetradifon
Endosulfan I	Thiabendazole
Endosulfan II	Thiacloprid
Endosulfan sulfate	Triadimefon
Esfenvalerate	Tribufos
Ethion	Trifloxystrobin
Ethofumesate	Trifluralin
Fenamidone	Vinclozolin
Fenbuconazole	
Fenhexamid	
Fenpropathrin	
Fipronil	

*Summarized and adapted from: Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, et al. (2010) ***High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health***. PLOS ONE 5(3): e9754. doi:10.1371/journal.pone.0009754

DCN-011308-189

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

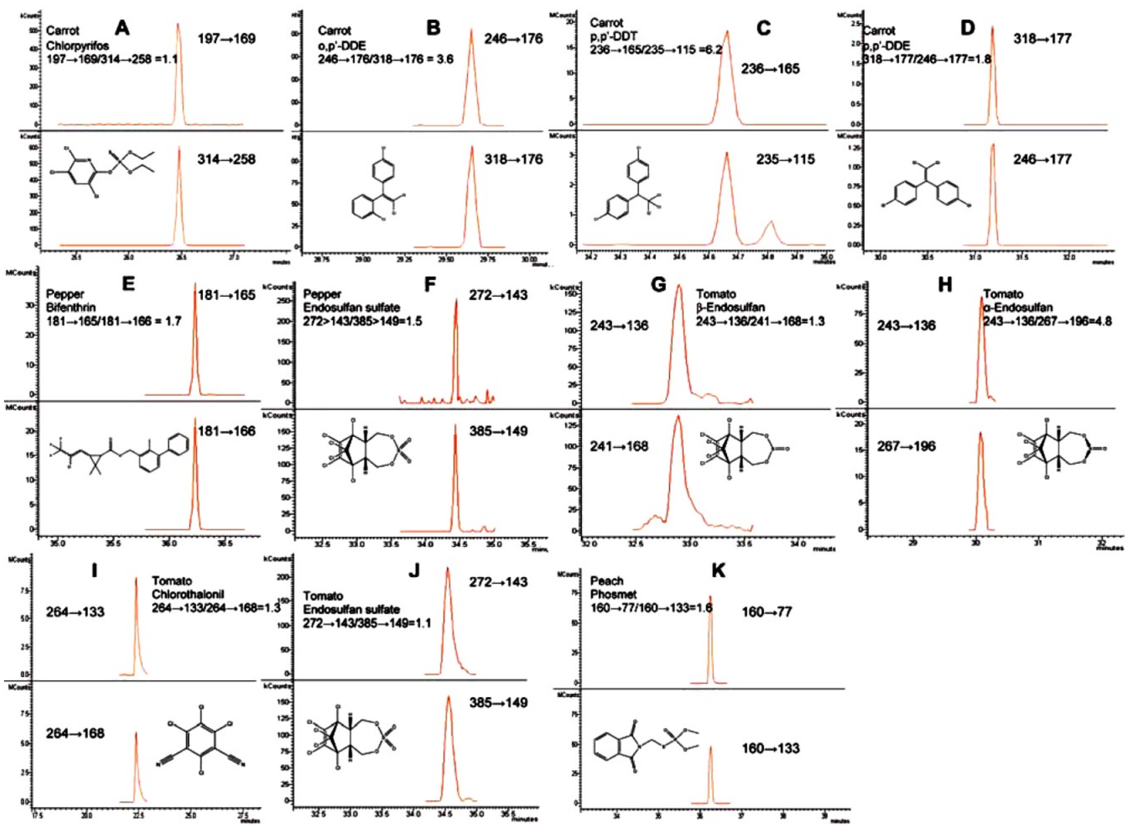
Analytes		
acenaphthene-d$_{10}$	Diamidafos (nellite)	*p,p'*-methoxychlor
acrinathrin	Diazinon	metolachlor
akton	Dibutyl chlorenate	mevinphos
alachlor	Dicapthon	mirex
aldrin	Dichlobenil	naphthalene-d$_8$
allethrin	Dichlofenthion	*cis*-nonachlor
atrazine	Dichlofluanid	*trans*-nonachlor
azamethiophos	3,4'-dichloroaniline	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

Analysis of Cyromazine in Poultry Feed Using a QuEChERS Approach

UCT Product Number:
ECMSSA50CT-MP (6 g anhydrous $MgSO_4$ and 1.5 g Na Acetate)
EEC18156 (500 mg endcapped C18, 6 mL cartridge)

October 2010

Introduction
This summary outlines a QuEChERS procedure for the analysis of the insecticide cyromazine (Trigard or Larvadex) in poultry feed by LC-MS/MS. Processing time is significantly faster than EPA method AG-555 and uses less solvent. Modifications include adding glacial acetic acid to the acetonitrile to increase extraction efficiency.

Procedure

1. Sample Preparation

a) Homogenize 2 grams of poultry feed and add to a 50 mL centrifuge tube
b) Add 10 mL of acetonitrile/acetic acid (75:25)
c) Sonicate at 50/60 Hz for 15 minutes
d) Add the contents of **ECMSSA50CT-MP** pouch and shake for 1 minute
e) Centrifuge at 3400 rpm for 10 minutes
f) Transfer 1 mL of supernatant to a calibrated test tube and add 9 mL of water: acetonitrile (95:5) with 0.1% acetic acid

2. Sample Clean-up

a) Add the 10 mL from 1) f) above to a **EEC18156** cartridge and elute dropwise
b) Filter eluant using a 0.45 µm Teflon filter (Millipore, Billerica, MA) or equivalent
c) Transfer 2 mL of eluant to an HPLC vial for analysis by LC-MS/MS

3. Analysis LC-MS/MS

Waters Alliance 2695 HPLC (Waters) coupled with a micromass Quattro Micro triple-quadrupole mass spectrometer (Micromass, Manchester, U.K.) or equivalent

HPLC conditions:

Guard column (Alltima, C18, 5 µm, 2.1 x 7.5 mm, Deerfield, IL) or equivalent
Analytical column (Alltima, C18, 5 µm, 2.1 x 250 mm, Waters) or equivalent
Mobile phase: (A) acetonitrile with 0.1% formic acid and (B) water with 0.1% formic acid
Gradient:

- 0-2 min, 5%A
- 2-5 min from 5 to 10% A
- 5-5.5 min from 10 to 90% A
- 5.5-8 min 90 to 5% A
- 8-10 min, from 90 to 5% A
- 10-12 min, 5% A

Flow rate 0.2 mL/minute
Injection volume: 25 µL

- **Mass Spectrometer**
  - Positive ion mode electrospray ionization
  - Monitor the ion transition of the parent ion (m/z 167) to the product ion (m/z 85) in multiple reaction monitoring (MRM)

Mass Spectrometry Conditions for Cyromazine Quantitation

Capillary Voltage	3.1 kV
Cone Voltage	65 V
Collision Energy	21-24 V
Source Temperature	120° C
Desolvation Temperature	350° C
Cone Gas Flow	135 L/h
Desolvation Gas Flow Rate	750 L/h
Collision Gas	Argon
Parent Ion	(m/z) 167
Product Ion	(m/z) 85

*Summarized with permission from Xia, Kang, Atkins, Jack et al, "Analysis of Cyromazine in Poultry Feed Using the QuEChERS Method Coupled with LC-MS/MS" J. Agric. Food Chem, DOI:10.1021/jf9034282

Listing of instrument manufacturers does not constitute endorsement by UCT

DCN-012101¬196

Determination of Anthelmintic Drug Residues in Milk Using Ultra High Performance Liquid Chromatography- Tandem Mass Spectrometry*

UCT Products:

ECMSSC50CT-MP	(4000 mg anhydrous $MgSO_4$, 1000 mg NaCl)
ECMSC1850CT	(1500 mg anhydrous $MgSO_4$ and 500mg C18)

Revised March 27 2013

Introduction

A modified QuEChERS-based method is used with an additional concentration step to detect 38 anthelmintic residues (nematicides, flukicides, endectocides) in milk at ≤ 1µg/kg using UHPLC-MS/MS detection. The drugs covered by this method include benzimidazoles, avermectins and flukicides.

Procedure

1. **Sample Preparation**

   a) Weigh 10.0 g milk into a 50 mL centrifuge tube

   b) Add IS and allow to sit for 15 minutes

   c) Add 10 mL acetonitrile (MeCN) and the contents of **ECMSSC50CT-MP** pouch

   d) Shake vigorously, then centrifuge for 12 minutes @ ≥ 3,500 rcf

2. **Dispersive Sample Clean-up**

   a) Add the supernatant to **ECMSC1850CT**

   b) Vortex sample for 30 seconds

   c) Centrifuge for 10 minutes @ ≥ 3000 rcf

   d) Transfer 5 mL of supernatant to an evaporation tube

   e) Add 0.25 mL DMSO (keeper solvent) and vortex briefly

   f) Evaporate the MeCN @ 50° C using nitrogen evaporation to 0.25 mL

   g) Filter extract using 0.2 µm PTFE syringe filter

   h) Sample is ready for UHPLC-MS/MS analysis

3. **Analysis UHPLC-MS/MS**

- Waters Acquity UPLC system (Milford MA; USA) or equivalent
- Analytical column HSS T3 C18 (100 × 2.1 mm, particle size 1.8 µm) (or equivalent) with appropriate guard column
- Column temperature: 60° C
- Pump flow rate of 0.6 mL/min
- Binary gradient:

  mobile phase A 0.01% formic acid in water:MeCN (90:10, v/v)

  mobile phase B 5mM ammonium formate in MeOH:MeCN (75:25 v/v)

  Gradient profile:

  0 – 0.5 min, 100% A

  5 min, 50% A

  7 min, 10% A

  8.5 min, 10% A

  8.51 min, 0% A

  9.5 min, 0% A

  9.51 min, 100% A

  13 min, 100% A
- Injection volume 5 µL
- Waters Quattro Premier XE triple quadrupole mass spectrometer
- Electrospray ionization (ESI) interface using fast polarity switching
- System controlled by MassLynx™ software and data was processed using TargetLynx™ Software (Waters)

Note:
Ammonium formate is used in the organic mobile phase because abamectin, doramectin and ivermectin form sodium adducts ([M+23]+) when acids are used. In this case, the ammonium adducts ([M+18]+) should be monitored for these three compounds and not the protonated precursor ions.

MS amenable acids can be used for the aqueous mobile phase, which should be at a low pH (≤4) to get the best results. It is essential to use ammonium buffer in the organic mobile phase as the avermectins elute at 100% organic content. The aqueous mobile phase may also include ammonium buffer, although it is not an essential requirement. Additionally, ammonium formate is more soluble in organic solvent than ammonium acetate.

Albendazole-sulfone and hydroxy-mebendazole are prone to isobaric interference as they have similar precursor and product ions that can't be distinguished using triple quadrupole instruments. It is therefore necessary to chromatographically separate these two compounds.

Standards, Internal Standards, Stock Solutions & Suppliers

Sigma-Aldrich

Analyte*	Abbreviation	Analyte	Abbreviation
Abamectin	ABA	**Ivermectin**	IVER
Albendazole	ABZ	**Levamisole**	LEVA
Bithionol	BITH	**Morantel**	MOR
Clorsulon	CLOR	**Niclosamide**	NICL
Closantel	CLOS	**Nitroxynil**	NITR
Coumaphos	COUM	**Oxfendazole**	OFZ
Doramectin	DORA	**Oxyclozanide**	OXY
Emamectin	EMA	**Rafoxanide**	RAF
Fenbendazole	FBZ	**Thiabendazole**	TBZ
Haloxon	HAL		

Witega Laboratories Berlin-Aldershof GmbH (Berlin, Germany)

Analyte**	Abbreviation
Albendazole-2-amino-sulfone	ABZ-NH_2-SO_2
Albendazole sulfone	ABZ-SO_2
Albendazole-sulfoxide	ABZ-SO
Amino-oxibendazole	OXI-NH_2
5-hydroxy-thiabendazole	5-OH-TBZ
Fenbendazole-sulfone	FBZ-SO_2
Triclabendazole	TCB
Triclabendazole-sulfone	TCB-SO_2
Triclabendazole sulfoxide	TCB-SO

Deuterated forms of these standards are available from Witega & QUCHEM (Belfast, UK)

Janssen Animal Health (Beerse, Belgium)

Analyte**	Abbreviation
Amino-flubendazole	FLU-NH2
Amino-mebendazole	MBZ-NH2
Hydroxy-flubendazole	FLU-OH
Hydroxy-mebendazole	MBZ-OH
Flubendazole	FLU
Mebendazole	MBZ

Greyhound Chromatography and Allied Chemicals, (Merseyside, UK)

Analyte**	Abbreviation
Coumaphos-oxon	COUM-O

QMX Laboratories (Essex, UK)

Analyte**	Abbreviation
Cambendazole	CAM
Oxibendazole	OXI

Merial Animal Health (Lyon, France)

Analyte**	Abbreviation
Eprinomectin	EPR

Fort Dodge Animal Health (Princeton, NJ, USA)

Analyte**	Abbreviation
Moxidectin	MOXI

Non-Isotopically Labeled Internal Standards Used

Internal Standard	Abbreviation & Source
Selamectin	SELA (Pfizer Animal Health)
Salicylanide	SALI (Sigma-Aldrich)
4-nitro-3-(trifluoromethyl)phenol	TFM (Sigma-Aldrich)
Ioxynil	IOX (Sigma-Aldrich)

Primary Stock Standard Solutions:

- 4,000 µg/mL from the certified standard materials-- ABZ, ABZ-SO, ABZ-SO2, ABZ-NH2- SO2, FBZ, OFZ, FBZ-SO2, EPR, CLOS, OXY, NITR, CLOR, BITH and MOR
- The remaining standards are prepared at concentrations of 2,000 µg/mL
- All internal standards are prepared at concentration of 1,000 µg/mL
- Avermectins were prepared in MeCN
- Flukicides, CAM, LEVA and TCB metabolites are prepared in MeOH
- Benzimidazoles are prepared in DMSO

Intermediate working standard mix solutions:

- 100 µg/mL for OXY, CLOR, BITH and MOR
- 50 µg/mL in MeOH for the remaining analytes

Prepare working IS as follows:

- 20 µg/mL for SELA and TCB-NH2, 4 µg/mL for LEVA-D5, TBZ164 D3 and IOX
- 2 µg/mL for the remaining analytes in MeOH- D

Primary, intermediate and working standard solutions are stable for at least six months when stored at -20°C

*Adapted and used with permission from Whelen, M., Kinsella, B., "Determination Of Anthelmintic Drug Residues In Milk Using Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry With Rapid Polarity Switching", doi:10.1016/j.chroma.2010.05.007, CHROMA 351049, J. of Chromatography A

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

EURL-FV Multiresidue Method Using QuEChERS
by GC-QqQ/MS/MS & LC-QqQ/MS/MS for Fruits & Vegetables

UCT Product Number:
ECQUEU750CT 50 mL centrifuge tube contains: **(**4 g $MgSO_4$, 1 g NaCl, 0.5 grams Na Citrate Dibasic Sesquihydrate, 1 g Na Citrate Tribasic Dihydrate)
ECMPS15CT 15 mL centrifuge tube contains: (900 mg $MgSO_4$ & 150 mg PSA) *(other configurations are available)*

January 2011

This summary of the European Union Reference Laboratory Residue method describes a QuEChERS approach for the analysis of 138 pesticides included in the Coordinated Multiannual Community Control Programme for 2010, 2011 and 2012 (Commission Regulation (EC) No 901/2009). Analysis is developed for **avocado, carrot, orange and pepper**.

Samples are prepared according to the Quality Control procedure established in the "Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed" (Document No. SANCO/10684/2009)

Procedure

1. Sample Preparation (for pesticides analyzed by **HPLC-MS/MS**)

a) Homogenize the sample using a food processor according the typical QuEChERS procedures
b) Weigh 10 g ± 0.1 g of sample into a 50 mL centrifuge tube
c) Add 10 mL of acetonitrile
d) Shake vigorously or vortex for 1 minute to disperse contents
e) Centrifuge for 5 minutes @ 4000 rpm

2. Clean-Up

a) Transfer 6 mL of the supernatant to product **ECMPS15CT**
b) Shake vigorously or vortex for 1 minute
c) Centrifuge @ 6000 rpm for 2 minutes
d) Transfer 1 mL of extract to a test tube and add 220 µL of acetonitrile
e) Using a 0.45µ Teflon syringe filter, transfer extract to an LC injection vial

f) Sample is ready for HPLC analysis

1A. Sample Preparation (For pesticides analyzed by **GC-MS/MS**)

a) Procedure is the same as for HPLC analysis through steps 2) c)

b) Transfer 1 mL of extract to a test tube

c) Evaporate to dryness

d) Add 1 mL of cyclohexane:acetone (9:1) to the dried extract

e) Shake or vortex until completely dissolved

f) Filter extract using a 0.45μ Teflon syringe filter into a GC vial

g) Sample is ready for analysis by GC-MS/MS

1) Instrumentation and Analytical Conditions for the LC/QqQ (MS/MS) System

- LC-MS/MS System 3200 Q TRAP, Applied Biosystems
- Column: Atlantis T3 2.1x100 mm, 3 μm
- Column temperature: 40 ºC
  - Mobile phase A: $H2O$, 2 mM ammonia formate, 0.1 1% formic acid
  - Mobile phase B: methanol
  - Injection volume: 10μL
  - Autosampler temperature: 10º C
  - Analysis time: 18 min.

HPLC Flow Rate and Elution Gradient Table

Time (min)	A (%)	B (5)	Flow (μL/min)
0.0	95	5	300
1.0	95	5	300
1.1	70	30	300
10.0	0	100	300
13.0	0	100	300
13.1	95	5	300

3. Instrumentation and Analytical Conditions for the GC/QqQ (MS/MS)

- GC: Agilent 7890 Series or equivalent
- Autosampler: Agilent 7683 Injector and sample tray
  - Inlet: Splitless
  - Carrier gas: He
  - Inlet pressure: 22.73 psi
  - Inlet temperature: 250ºC

- Injection volume: 1 µL

- Analytical column: Agilent J&W HP-5ms 30 m x 250 µm x 0.25 µm or equivalent
- Retention time locking: Chlorpyrifos methyl locked to 16.596 min
- Spectrometer: Agilent 7000B Series
- Source temperature: 280ºC
- Quadrupole temperature: Q1 and Q2 = 150ºC
- Collision gas flows: N_2 at 1.5 mL/min, He at 25 mL/min

GC Oven Temperature Program

	Rate (°C/min)	Value (°C)	Hold Time (min)	Run Time (min)
Initial		70	2	2
Ramp 1	25	150	0	5.2
Ramp 2	3	200	0	21.9
Ramp 3	8	280	10	41.9

Spike Level with Method Validation Results

Pesticide	Mean **0.01 mg/Kg**	RSD **0.01 mg/Kg**	Mean **0.1 mg/Kg**	RSD **0.1 mg/Kg**	Technique **w MS/MS**
Acephate	88	7	89	3	HPLC
Acetamiprid	84	10	102	4	HPLC
Acrinathrin	103	12	91	13	GC
Aldicarb(RD)	105	13	98	5	HPLC
Amitraz(RD)	89	8	80	7	HPLC
DMPF	84	7	92	5	HPLC
DMF	75	5	103	7	HPLC
Azinphos-methyl	86	17	100	5	HPLC
Azoxystrobin	87	6	104	4	HPLC
Bifenthrin	95	9	92	10	GC
Bitertanol	118	11	103	8	HPLC
Boscalid	98	8	100	4	HPLC
Bromopropylate	95	11	94	12	GC
Bupirimate	96	6	94	10	GC
Buprofezin	87	8	89	8	GC
Cadusafos	88	16	96	6	HPLC
Captan	76	11	85	9	GC
Carbaryl	110	9	97	5	HPLC
Carbendazim(rd)	96	6	95	5	HPLC
Carbofuran(rd)	91	14	103	4	HPLC
Chlorfenvinphos	99	8	86	27	GC

Chlorothalonil	56	4	67	3	GC
Chlorpropham(RD)	99	7	95	7	GC
Chlorpyriphos	98	6	92	7	GC
Chlorpiriphos-methyl	96	5	93	7	GC
Clofentezin(RD)	93	27	88	9	HPLC
Clothianidin	109	8	98	7	HPLC
Cyfluthrin(RD)	104	13	97	16	GC
Cypermethrin(RD)	109	11	109	16	GC
Cyproconazole	97	13	95	5	HPLC
Cyprodinil	97	7	91	9	GC
Deltamethrin	106	14	95	16	GC
Diazinon	99	7	91	7	GC
Dichlofluanid	68	5	71	2	GC
Dichlorvos	85	9	98	6	HPLC
Dicloran	111	17	97	8	GC
Difenoconazole	94	9	99	5	HPLC
Dimethoate(RD)	86	11	99	5	HPLC
Dimethomorph	107	10	97	5	HPLC
Diphenylamine	101	8	86	7	GC
Endosulfan(rd)	92	10	89	10	GC
Epoxiconazole	86	8	101	3	HPLC
Ethion	114	9	102	10	GC
Etofenprox	95	13	94	14	GC
Ethoprophos	98	4	92	6	GC
Fenarimol	98	12	90	13	GC
Fenazaquin	89	9	88	11	GC
Fenbutatin oxide		-	78	4	HPLC
Fenbuconazole	106	12	100	5	HPLC
Fenhexamid	79	9	94	5	HPLC
Fenitrothion	116	10	103	7	GC
Fenoxycarb	105	24	98	8	HPLC
Fenpropathrin	99	10	96	11	GC
Fenpropimorph	81	7	99	3	HPLC
Fenthion(RD)	92	7	90	8	GC
Fenthion sulfoxide	86	15	92	10	HPLC
Fenvalerate	96	15	93	15	GC
Fipronil(RD)	98	5	91	11	GC
Fludioxinil	132	3	96	2	HPLC
Flufenoxuron	83	18	110	10	HPLC
Fluquinconazole	94	21	99	7	HPLC
Flusilazole	98	8	93	10	GC
Flutriafol	99	8	102	5	HPLC
Folpet	59	21	65	13	GC
Formetanate(RD)	87	10	95	4	HPLC
Fosthiazate	79	20	114	8	HPLC

Hexaconazole	95	12	96	4	HPLC
Hexythiazox	104	15	97	12	HPLC
Imazalil	88	6	97	6	HPLC
Imidacloprid	96	17	100	6	HPLC
Indoxacarb(RD)	90	40	113	9	HPLC
Iprodione	106	13	94	13	GC
Iprovalicarb	105	8	99	4	HPLC
Kresoxim-methyl	103	14	107	5	HPLC
Lambda-cyhalothrin(RD)	108	12	96	13	GC
Linuron	97	29	99	6	HPLC
Lufenuron	132	16	110	12	HPLC
Malathion(RD)	107	6	100	9	GC
Mepanipyrim(RD)	103	12	95	12	GC
Metalaxyl(rd)	111	11	94	8	GC
Metconazole	101	9	95	4	HPLC
Methamidophos	91	13	89	6	HPLC
Methidathion	115	6	101	9	GC
Methiocarb(RD)	109	33	104	15	HPLC
Methomyl(RD)	109	7	106	5	HPLC
Methoxyfenozide	125	12	99	13	HPLC
Monocrotophos	79	10	98	6	HPLC
Myclobutanil	89	11	91	11	GC
Oxadixyl	94	12	88	9	GC
Oxamyl	96	8	96	5	HPLC
Oxydemeton-methyl(RD)	94	7	97	4	HPLC
Paclobutrazole	91	13	100	5	HPLC
Parathion	119	6	101	9	GC
Parathion-methyl(RD)	109	6	100	7	GC
Pencycuron	100	18	101	6	HPLC
Penconazole	97	11	97	5	HPLC
Pendimethalin	116	8	96	8	GC
Permethrin(rd)	98	12	93	13	GC
Phenthoate	109	7	99	8	GC
Phosalone	111	14	99	14	GC
Phosmet(RD)	100	10	99	7	HPLC
Pyraclostrobin	95	11	110	4	HPLC
Pirimicarb(RD)	92	6	94	7	GC
Pirimiphos-methyl	111	8	94	9	GC
Prochloraz(RD)	88	8	95	5	HPLC
Procymidone	94	8	93	8	GC
Profenofos	100	10	97	10	GC
Propamocarb(RD)	68	9	69	6	HPLC
Propargite	112	10	104	7	HPLC
Propiconazole	94	9	91	10	GC
Propyzamide	99	5	96	7	GC

Prothioconazole	78	24	33	12	HPLC
Pyridaben	95	14	92	13	GC
Pyrimethanil	119	13	95	6	GC
Pyriproxyfen	103	16	95	13	GC
Quinoxyfen	114	8	87	10	HPLC
Spinosad(RD)	97	11	98	4	HPLC
Spiroxamine	124	15	80	15	HPLC
Taufluvalinate	102	13	96	17	GC
Tebuconazole	113	11	95	11	GC
Tebufenozide	124	45	97	13	HPLC
Tebufenpyrad	92	11	94	12	GC
Teflubenzuron	96	31	103	14	HPLC
Tefluthrin	89	5	89	7	GC
Tetraconazole	107	10	91	8	GC
Tetradifon	87	12	90	13	GC
Thiabendazole	92	9	93	8	HPLC
Thiamethoxam(RD)	84	16	101	5	HPLC
Thiacloprid	86	8	105	4	HPLC
Thiophanate-methyl	69	13	104	6	HPLC
Tolclofos-methyl	91	4	97	5	GC
Tolyfluanid(RD)	69	9	72	10	GC
Triadimenol(RD)	-	-	105	29	HPLC
Triazophos	117	7	102	11	GC
Trichlorfon	75	19	106	7	HPLC
Trifloxystrobin	93	13	103	7	HPLC
Triflumuron	-	-	121	6	HPLC
Trifluralin	92	3	88	6	GC
Triticonazole	104	14	97	5	HPLC
Vinclozolin(RD)	97	7	95	7	GC
Zoxamide	79	17	112	5	HPLC

Summarized and adapted from “EURL-FV Multiresidue Method using QuEChERS followed by GC-QqQ/MS/MS and LC-QqQ/MS/MS for Fruits and Vegetables,” European Reference Laboratory in Pesticide Residue, 2009

Extraction of Pyrethrin and Pyrethroid Pesticides from Fish Using the QuEChERS Approach

UCT Product Number:
EC4MSSA50CT-MP (4000 mg $MgSO_4$ and 1000 mg sodium acetate)
CUMPSC18CT (150 mg $MgSO_4$, 50 mg PSA and 50 mg endcapped C18)

January 2011

The QuEChERS approach is used for the determination of trace levels of natural pyrethrins and synthetic pyrethroids (cypermethrin & deltamethrin) in fish.

1. Extraction

a) Weigh 10 grams of homogenized fish into a 50 mL centrifuge tube

b) Add 500 ng *cis*-permethrin (phenoxy-$^{13}C_6$) surrogate standard

c) Add 10 mL 1% acetic acid in acetonitrile

d) Add the contents of pouch **EC4MSSA50CT-MP**

e) Shake vigorously for 1 minute then centrifuge

2. Clean-up, Dispersive Solid-phase (dSPE)

a) Transfer 1 mL of supernatant to a 2 mL micro-centrifuge tube **CUMPSC18CT**

b) Shake for 1 minute then centrifuge

c) Transfer 0.5 mL of extract to a graduated tube then evaporate to near dryness

d) Add 50 ng *trans*-permethrin (phenoxy-$^{13}C_6$) and bring to exactly 0.5 mL with trimethyl phosphate (TMP)

e) Add $MgSO_4$ to the 0.2 mL mark then vortex

f) Transfer supernatant to injection vial for analysis

3. Analysis

a) Use GC/MS in CI mode

b) Column: HP-5, 30m X 0.32 mm with 0.25 µm film (or equivalent)

c) Splitless mode @ 240°

GC Oven program:

- Initial 80°C, hold 1 minute
- 50°C/min to 200°C
- 5°C/min to 285°C
- 50°C/min to 325°C, hold 5 minutes
- Transfer line 250°C

MS Conditions:

- Source 150°C
- Methane reagent gas
- Selected Ion Monitoring Mode

Calibration using matrix matching may be required

***Adapted from** Roscoe, Veronica, Judge, Judy, Rawn, Dorothea F.K., "Application of the QuEChERS Extraction Method for the Analysis of Pyrethrin and Pyrethroid Pesticides in Fin and non-Fin Fish", Health Products and Food Program, Winnipeg, Manitoba and Bureau of Chemical Safety, Food Research Division, Ottawa, Ontario, Canada, Florida Pesticide Residue Workshop, July 2009

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

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, neonicotinoids, benzimidazoles, phenylureas, strobulirums, organophosphorus, 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
b) 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

c) Shake for 1 min
d) Add internal standard
e) Add spike standard if needed
f) Add the contents of pouch **ECMSSC50CTFS**

g) Shake 1 min

h) Centrifuge @ ~4500 rpm for 5 min

2. PSA Clean-up

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
Aldicarb Sulfoxide	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
Butoxycarboxin	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 Pesticides and *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

DCN-316140-263

Modified QuEChERS Procedure for Analysis of Bisphenol A in Canned Food Products

UCT Product Number:

ECQUEU750CT-MP (pouch contains 4000 mg $MgSO_4$, 1000 mg NaCl, 500 mg Na citrate dibasic sesquihydrate, 1000 mg Na citrate tribasic)
ECPSAC1856 (500 mg of PSA and 500 mg endcapped C18, 6 mL cartridge)
ECPSACB6 (400 mg PSA and 200 mg GCB, 6 mL cartridge)
SMTBSTFA-1-1 (MTBSTFA w/1% TBDMCS)
ECSS10K (Sodium sulfate, anhydrous, ACS Grade, Granular 60 Mesh)

July 2011

Procedure

(Developed for tuna, baby food, pineapple and tea)

1. Sample Preparation

a) Weigh 10 g homogenized sample into a 50 mL QuEChERS tube

b) Spike with 25 ng/g internal standard: Bisphenol A d16 (BPA d16)

c) Add 10 mL MeCN

d) Add the contents of **ECQUEU750CT-MP**

e) Shake vigorously for 2 min

f) Centrifuge at 3500 rpm for 3 min

2. Clean-up (products without pigments)

a) Attach **ECPSAC1856** to the vacuum manifold

b) Add 3 g muffled Na_2SO_4 to the cartridge

c) Rinse with 2 x 2 mL MeCN

d) Insert test tubes into the manifold

e) Load 5 mL of the supernatant to the cartridge

f) Turn on vacuum to collect extract dropwise

g) Transfer 1 mL of cleaned extract into a 5 mL test tube

h) Concentrate to dryness by N_2 at 35 ºC

3. Clean-up (products with pigments)

a) Attach ECPSACB6 to the vacuum manifold

b) Add 3 g muffled Na_2SO_4 to the cartridge

c) Rinse with 2 x 2 mL MeCN

d) Insert test tubes into the manifold

e) Load 5 mL of the supernatant to the cartridge

f) Turn on vacuum to collect extract dropwise

g) Transfer 1 mL of cleaned extract into a 5 mL test tube

h) Concentrate to dryness by N_2 at 35 ºC

4. Derivatization

a) Add 50 µL pyridine to the dried test tube and vortex

b) Add 50 µL MTBSTFA/1%TBDMCS and cap the test tube

c) Heat at 75º C for 30 min

d) Cool then concentrate to dryness by N_2 at 35 ºC

e) Reconstitute with 75 µL toluene and 25 µL surrogate: 2 ppm triphenyl phosphate (TPP)

f) Vortex

g) Transfer to auto-sampler vial with 100 µL insert

h) Inject 1 µL to GC/MS

5. GC/MS Analysis

GC/MS

- Agilent 6890N GC coupled with 5975C MSD, equipped with 7683 auto sampler. Chemstation software for data acquisition and analysis. Equivalent instrumentation may be used

Injector

- 1 µL splitless injection at 250 ºC, split vent of 30 mL/min at 1 min

Liner

- 4 mm splitless gooseneck, 4mmID x 6.5mmOD x 78.5mm (UCT #: **GCLGN4MM**)

Glass wool for liner

- Restek® Deactivated Wool

GC capillary column

- Restek® Rxi-5sil MS 30m x 0.25mm x 0.25µm

Oven temperature program

- Initial oven temperature of 100 ºC, hold for 1 min
- Ramp at 20 ºC/min. to 300 ºC, hold for 1 min
- Ramp at 40 ºC/min. to 320 ºC, hold for 2.5 min

Total run time 15 min. Data acquisition begins at 9 min

Carrier Gas

- He constant flow 1.2 mL/min

MSD Conditions

- Aux temperature: 280 ºC, MS Source: 230 ºC, MS Quad: 150 ºC

Simultaneous Scan/SIM:
Scan range: 50-500

SIM:
Group 1: 9.0 min.: 326.1, 325.1 (Triphenyl phosphate)
Group 2: 10.5 min.: 441.3, 456.3, 442.3 (derivatized: Bisphenol A-2TBDMS)
452.4, 470.4, 453.4 (derivatized: Bisphenol A d16-2TBDMS)

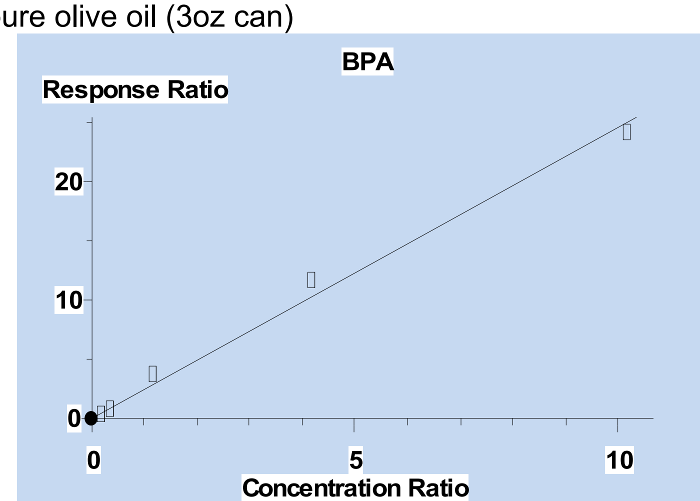
Dwell time: 100 ms for all ions
Matrix matched curves are generated with the adjustment of the concentrations in the blank and baby food sample

Experimental Data

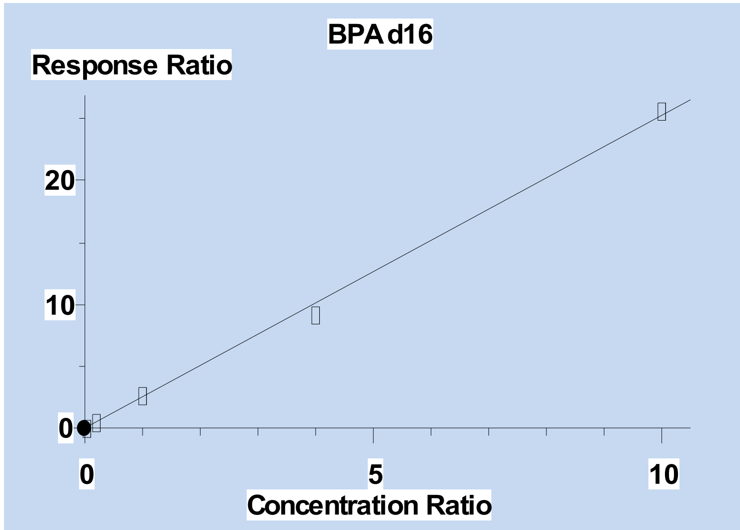
Calculations

C_{blank} and C_{sample} were calculated by equations 1 and 2:
Equation 1: $A_{sample}/A_{blank}=(C_{sample}+C_{blank})*R_1\%/C_{blank}*R_1\%$
Equation 2: $A_{spiked\ sample}/A_{sample} =(C_{sample}+C_{blank}+C_{spike})*R_2\%/(C_{sample}+C_{blank})*R_2\%$

Tuna: Tuna in pure olive oil (3oz can)



BPA: Linear dynamic range: 1-500 ng/mL (conc. per 1 mL extract) R^2=0.9933

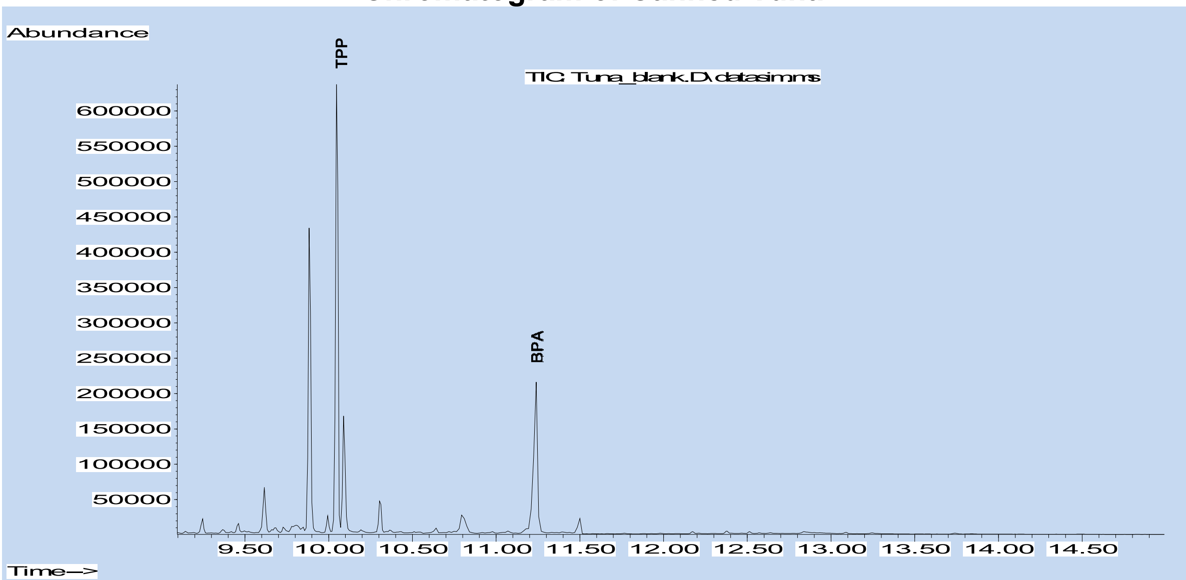


BPA d16: Linear dynamic range: 1-500 ng/mL (conc. per 1 mL extract) R^2=0.9983

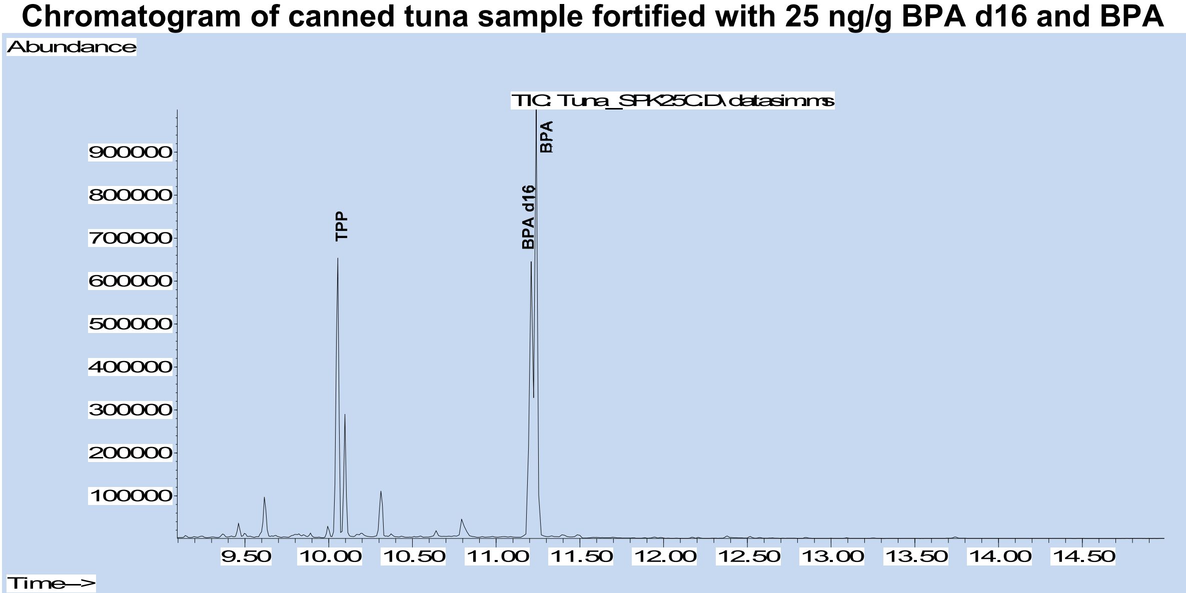
Bisphenol A in Canned Tuna

	BPA in blank	**BPA in tuna**	**Tuna fortified with 25 ng/g BPA**	**Tuna fortified with 50 ng/g BPA**
Analyte	Conc. (ng/g) (ng/mL)	Conc. (ng/g)	Recovery% ± RSD% (n=3)	Recovery% ± RSD% (n=3)
BPA d16	0	0	74.0 ± 6.4	76.5 ± 5.9
BPA	0.56	6.64	86.6 ± 7.5	100 ± 8.0

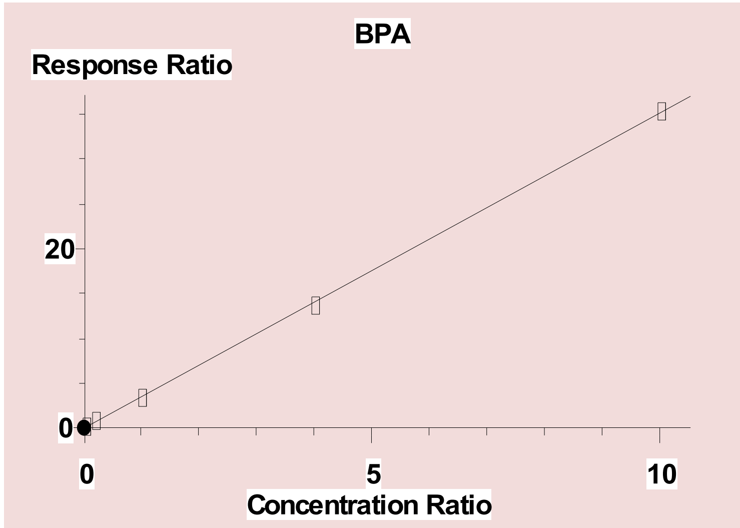
Chromatogram of Canned Tuna



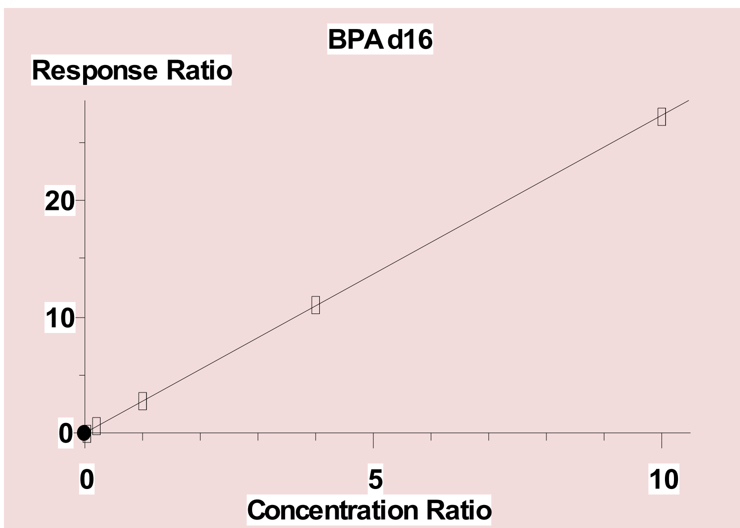
Chromatogram of canned tuna sample fortified with 25 ng/g BPA d16 and BPA



Baby Food: Peaches, Stage 2



BPA: Linear dynamic range: 1-500 ng/mL (conc. per 1 mL extract) R^2=0.9998

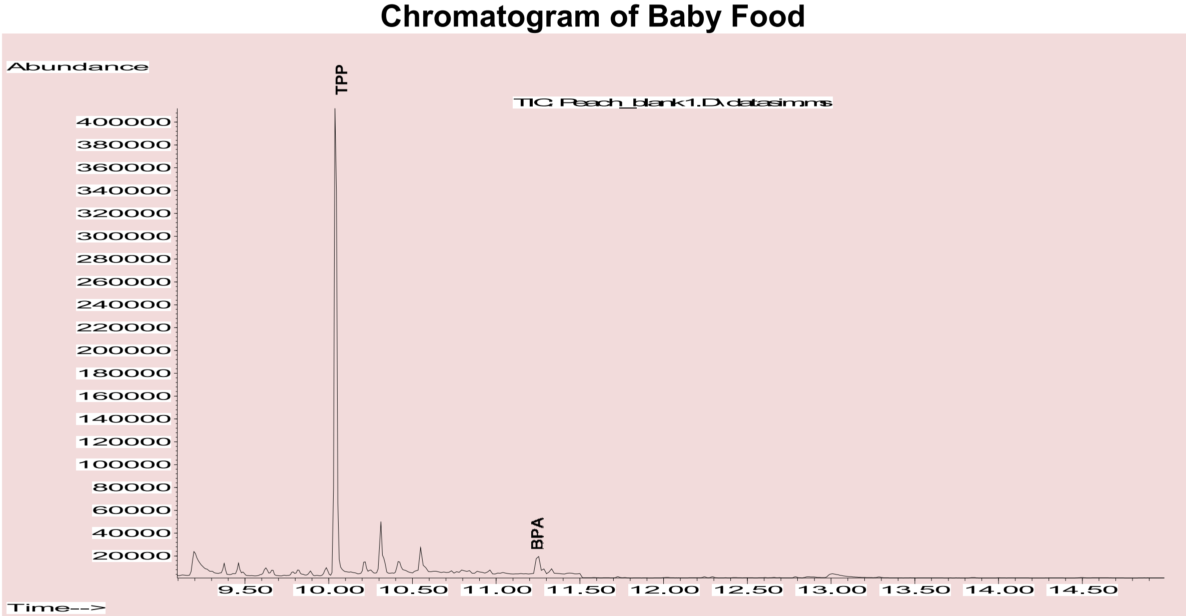


BPA d16: Linear dynamic range: 1-500 ng/mL (conc. per 1 mL extract) R^2=0.9999

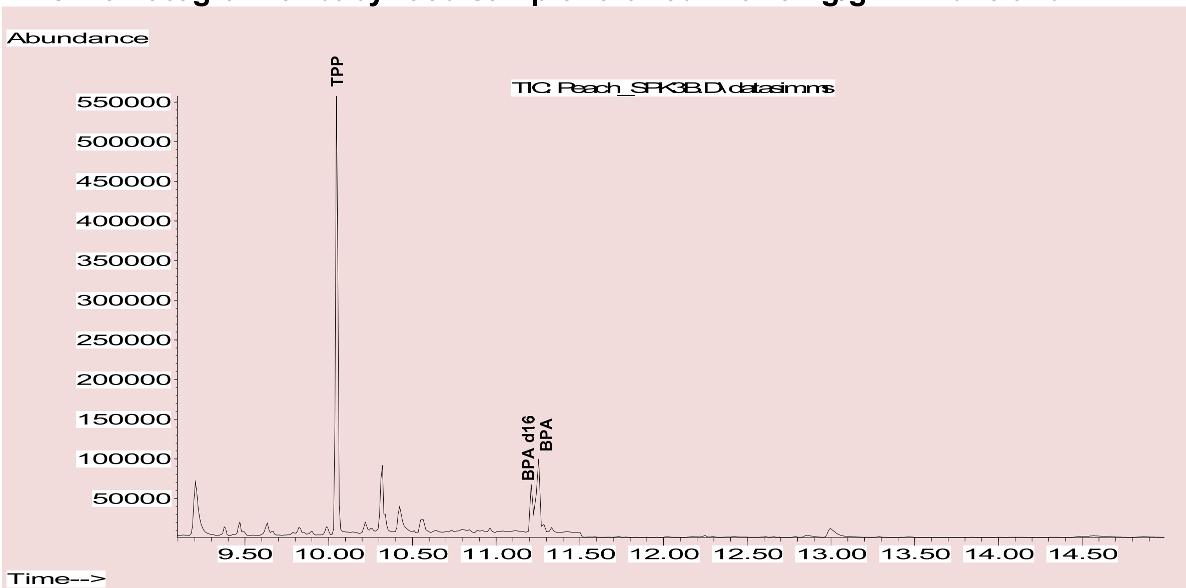
Bisphenol A in Baby Food

	BPA in blank (ng/g)	**BPA** in baby food (ng/g)	**Baby food** fortified with 3 ng/g BPA	**Baby food** fortified with 10 ng/g BPA
Analyte	Conc. (ng/mL)	Conc. (ng/g)	Recovery% ± RSD% (n=3)	Recovery% ± RSD% (n=3)
BPA d16	0	0	98.0 ± 4.7	98.8 ± 7.7
BPA	0.33	< 1 ng/g (0.33 ng/g)	99.2 ± 2.0	95.3 ± 11

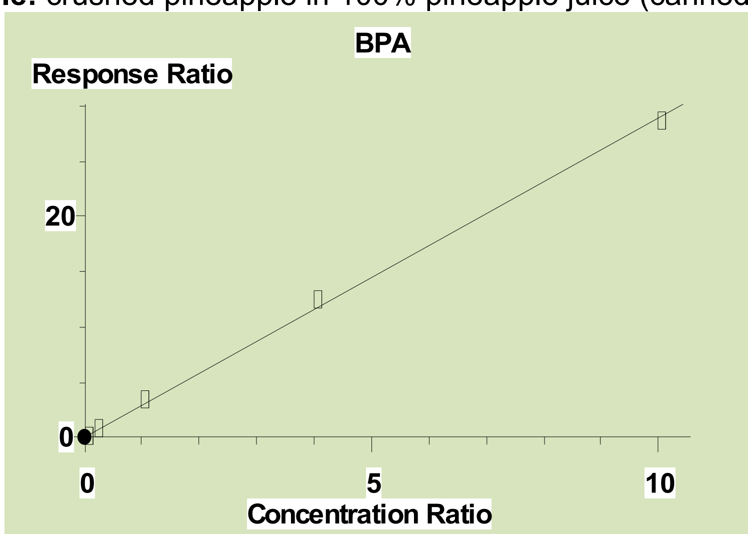
Chromatogram of Baby Food



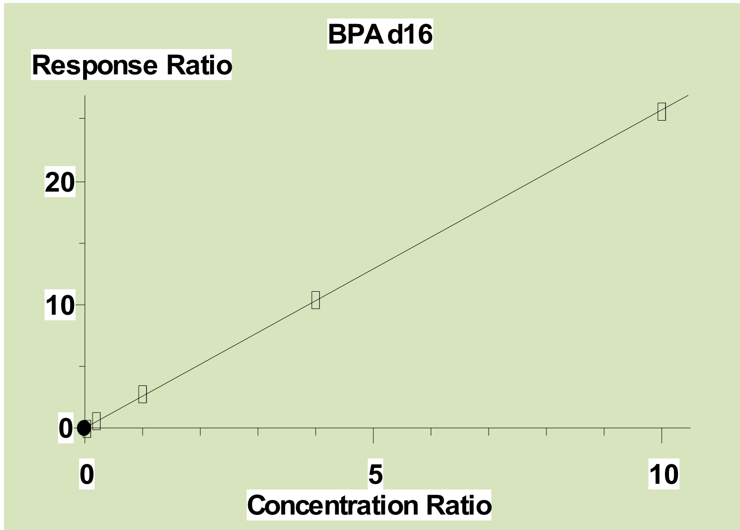
Chromatogram of baby food sample fortified with 3 ng/g BPA d16 and BPA



Pineapple: crushed pineapple in 100% pineapple juice (canned), pH=3 .



BPA: Linear dynamic range: 1-500 ng/mL (conc. per 1 mL extract) R^2=0.9989

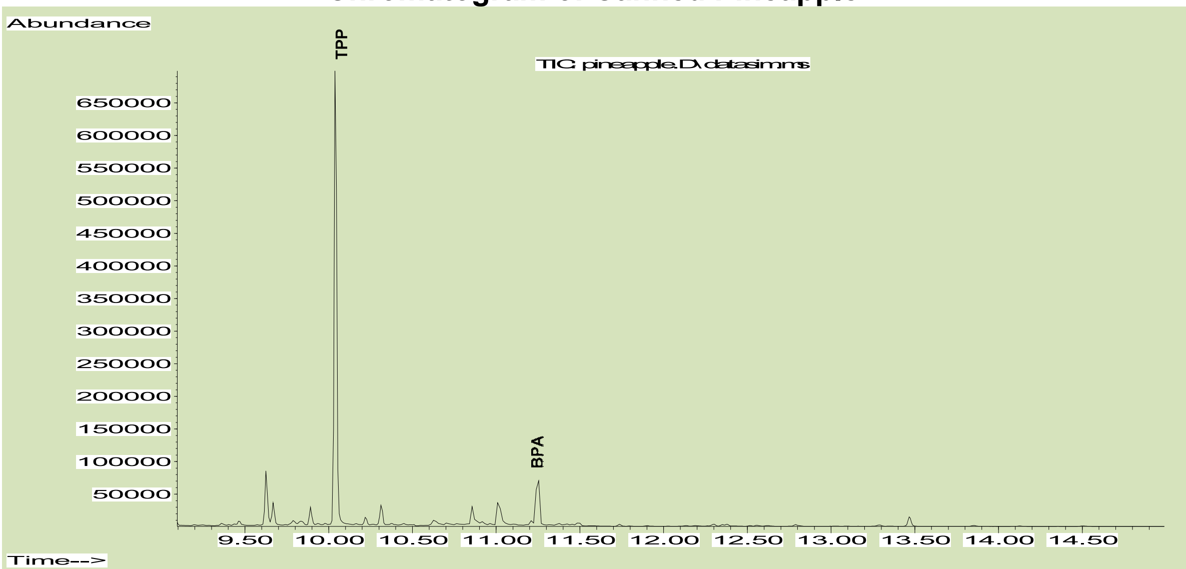


BPA d16: Linear dynamic range: 1-500 ng/mL (conc. per 1 mL extract) R^2=0.9999

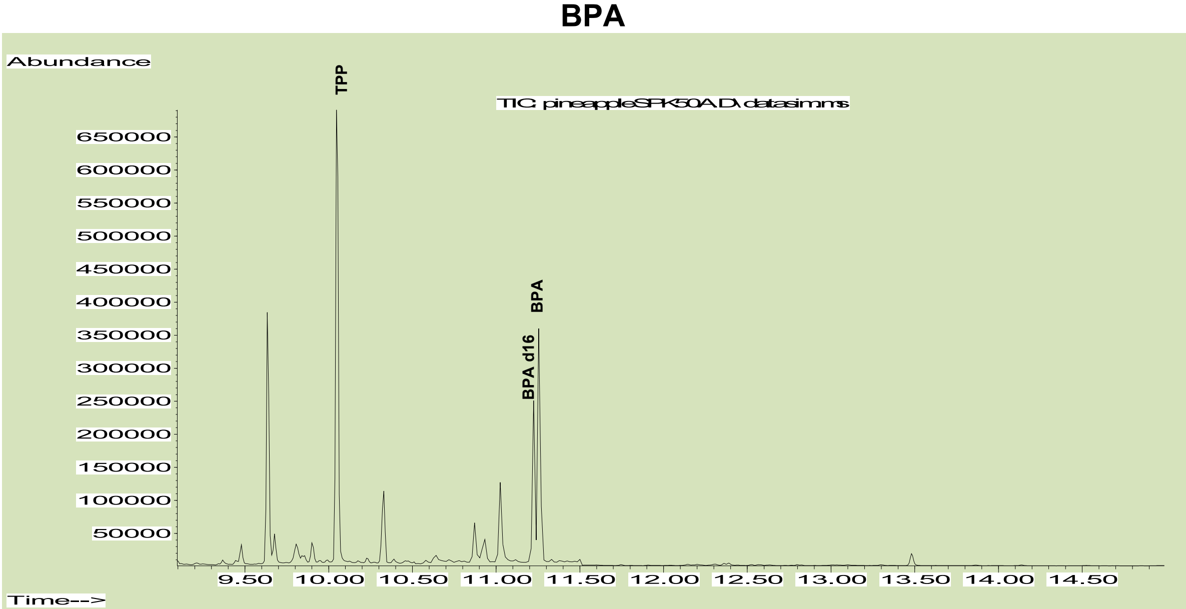
Bisphenol A in Canned Pineapple

	BPA in blank	**BPA in pineapple**	**Pineapple fortified with 5 ng/g BPA**	**Pineapple fortified with 25 ng/g BPA**
Analyte	Conc. (ng/g) (ng/mL)	Conc. (ng/g)	Recovery% ± RSD% (n=3)	Recovery% ± RSD% (n=3)
BPA d16	0	0	112 ± 2.3	93.4 ± 6.1
BPA	0.33	1.65	112 ± 5.7	96.1 ± 5.7

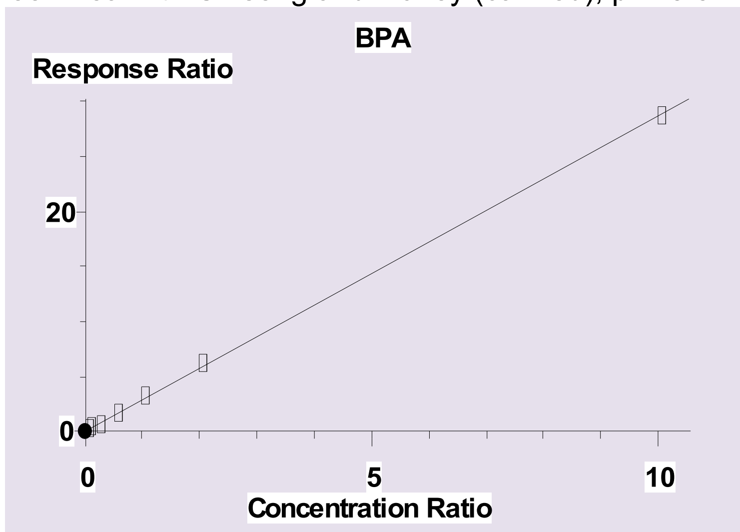
Chromatogram of Canned Pineapple



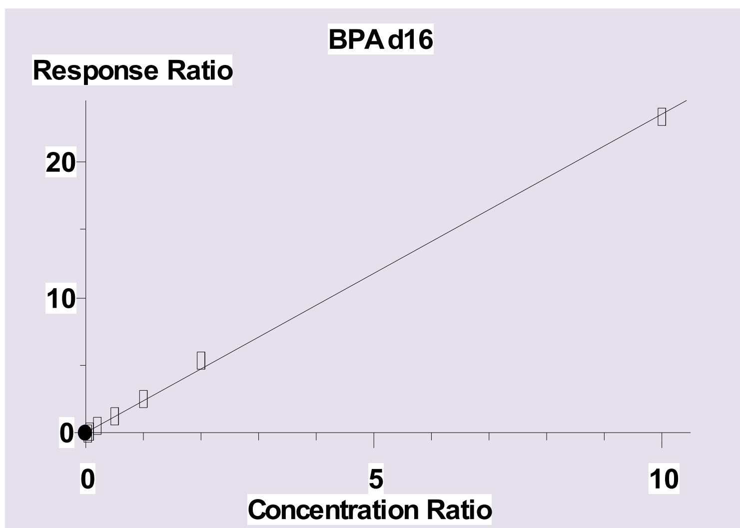
Chromatogram of canned pineapple sample fortified with 5 ng/g BPA d16 and BPA



Tea Sample: Green Tea with Ginseng and Honey (canned), pH=3.5



BPA: Linear dynamic range: 1-500 ng/mL (conc. per1 mL extract) R^2=0.9998

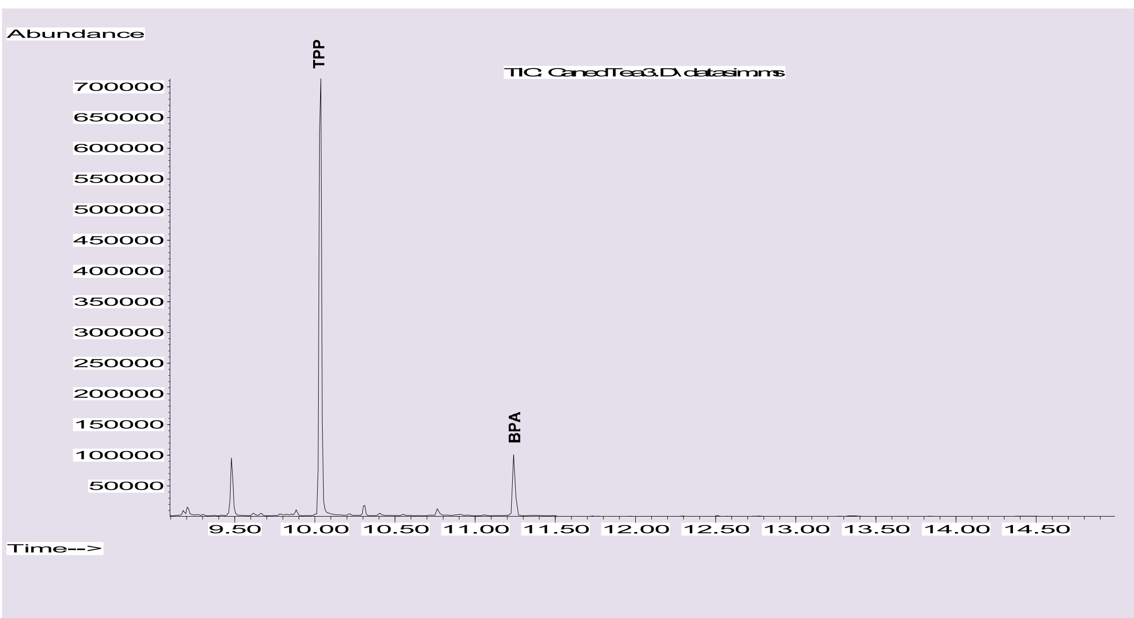


BPA d16: Linear dynamic range: 1-500 ng/mL (conc. per 1 mL extract) R^2=0.9993

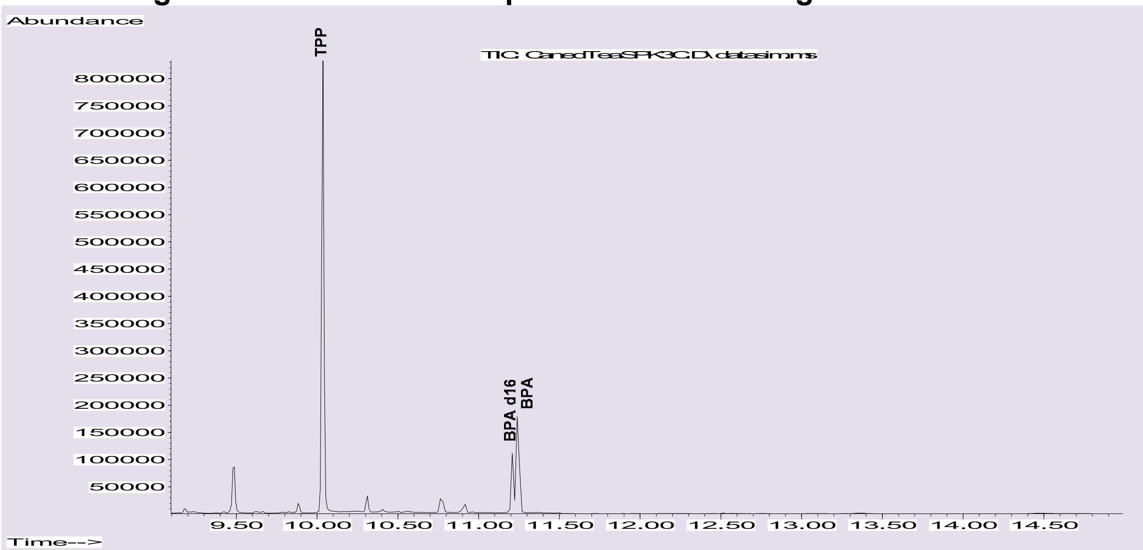
Bisphenol A in Canned Tea

	BPA in blank (ng/g)	BPA in tea (ng/g) (ng/g)sample	Tea fortified with 3 ng/mL BPA	Tea fortified with 10 ng/mL BPA
Analyte	Conc. (ng/mL)	Conc. (ng/mL)	Recovery% ± RSD% (n=3)	Recovery% ± RSD% (n=3)
BPA d16	0	0	120 ± 5.9	107 ± 2.7
BPA	0.46	2.28	104 ± 8.2	90.0 ± 5.8

Chromatogram of Canned Tea Sample



Chromatogram of canned tea sample fortified with 3 ng/mL BPA d16 and BPA



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

Determination of Carbendazim in Orange Juice Using QuEChERS with LC/MS/MS Detection

UCT Part Numbers:

ECQUEU750CT-MP: Pouch contains: 4000 mg $MgSO_4$, 1000 mg NaCl, 500 mg Na citrate dibasic sesquihydrate and 1000 mg Na citrate tribasic dihydrate

CUMPSC18CT: 2 mL centrifuge tube contains: 150 mg $MgSO_4$, 50 mg PSA, 50 mg endcapped C18

February 1, 2012

Introduction

The planar fungicide carbendazim (CASRN 10605-21-7) can be used to control mold on citrus crops but is not approved for use in the US or on imported products. Concentrations in citrus products can be rapidly and accurately determined using a QuEChERS extraction with dSPE clean-up. LOD and LOQ for this method are 0.4 and 1.4 ng/mL, respectively.

Procedure

1. **Extraction**
   a) Add 10 mL of orange juice to a 50 mL centrifuge tube
   b) Add 10 mL acetonitrile then vortex
   c) Add the contents of **ECQUEU750CT-MP**
   d) Shake vigorously for 1 min
   e) Centrifuge at 5000 rpm for 5 min at 20° C
   f) Supernatant is ready for clean-up

2. **Dispersive Clean-up**
   a) Add 1 mL of supernatant to **CUMPSC18CT** tube
   b) Shake sample(s) for 1 min
   c) Centrifuge at 10,000 rpm for 5 min
   d) Transfer 0.5 mL to 2 mL autosampler vial
   e) Add 25 μL 1 ppm TPP, vortex
   f) Samples are ready for analysis

3. LC/MS//MS Analysis

Instrumentation: Thermo Accela HPLC with autosampler

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	10µL at 15° C
Mobile Phase	A: 0.1% formic acid in water B: 0.1% formic acid in methanol
Flow Rate	200 µL/min

LC Gradient Program

Time	%A	%B
0	50	50
3	0	100
8	0	100
9	50	50
14	50	50

MS Conditions

Thermo TSQ Vantage MS

Ion source:	Heated ESI
Ion polarity:	ESI +
Spray voltage:	3000 V

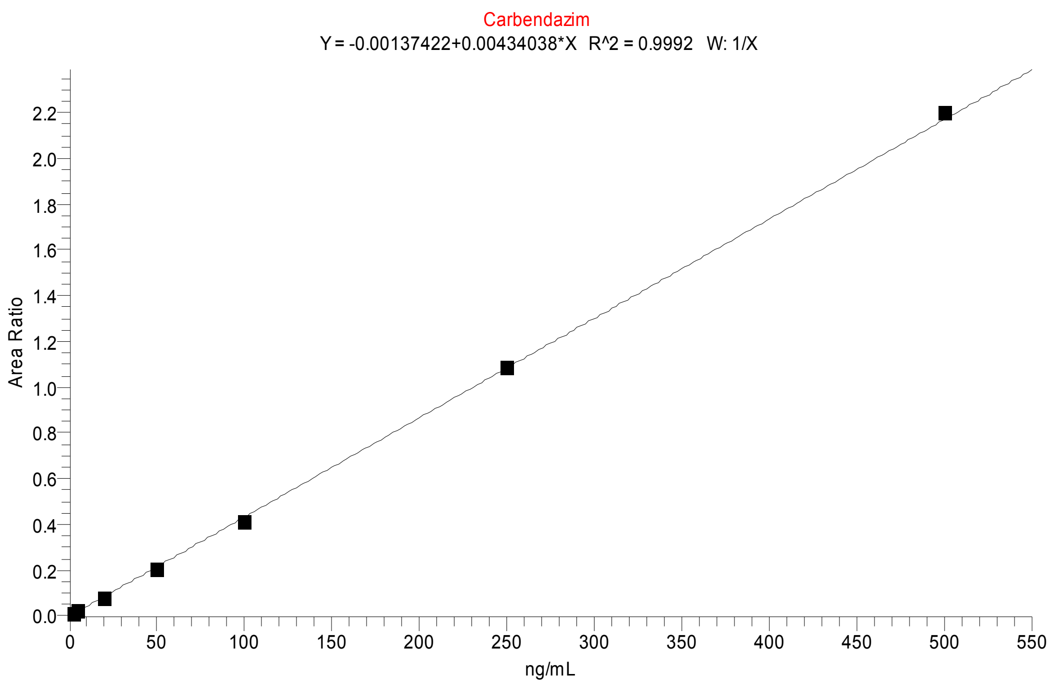
Sheath gas pressure:	N_2 @ 40 psi
Auxiliary gas pressure:	N_2 @ 10 psi
Ion transfer capillary temperature:	350 °C
Scan type:	SRM (0-10 min)
CID conditions:	Ar @ 1.5 mTorr

SRM transitions

Compound	Parent	Product ion 1	CE	Product ion 2	CE	S-Lens	Dwell time (s)
Carbendazim	192.093	132.080	29	160.080	17	81	0.20
TPP (IS)	327.093	77.020	37	152.070	33	98	0.10

Matrix Matched Calibration Curve

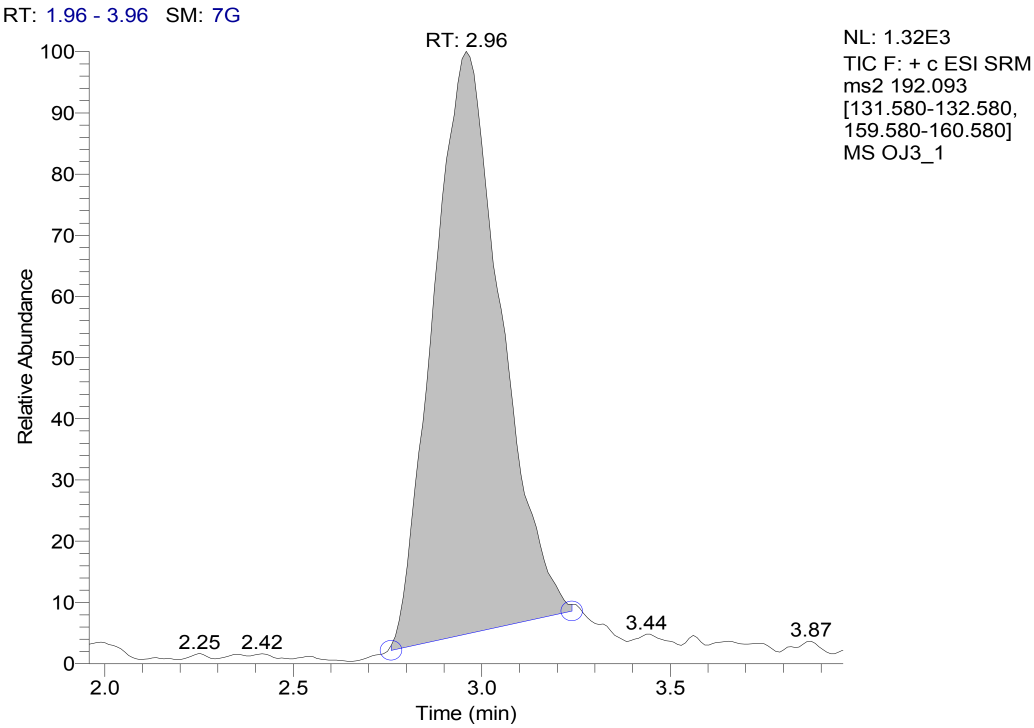
Dynamic linearity range is from 2 to 500 ng/mL with R^2=0.9992



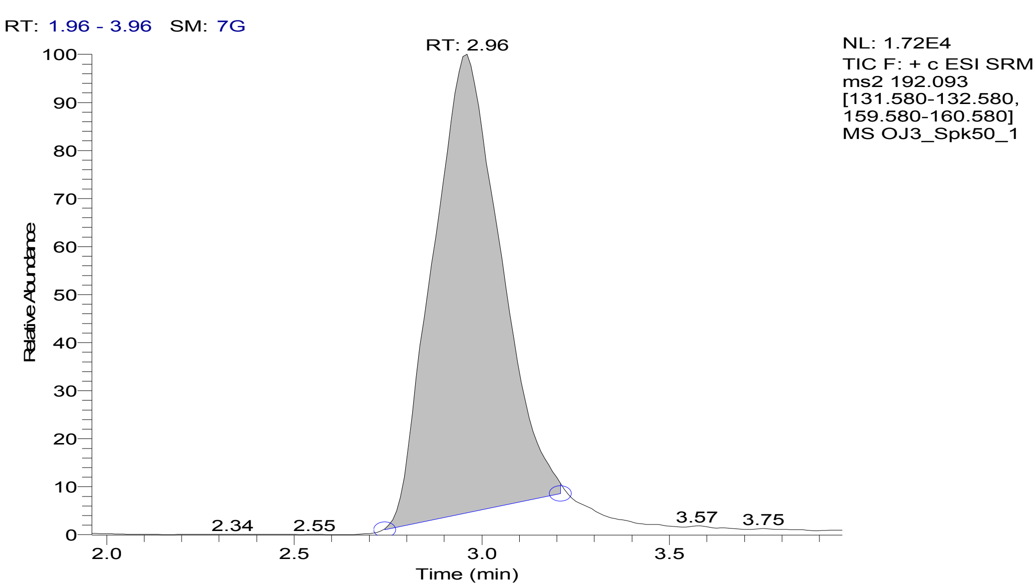
Accuracy and Precision of Carbendazim Data from Spiked Orange Juice Sample

Fortified (ng/mL)	Recovery%	RSD % n=4
10	96.6	4.5
50	100.2	3.4
250	103.7	2.1

Chromatogram of Orange Juice Sample and Fortified at 50 ng/mL



50 ng/mL Fortified



DCN-212020-230

Determination of Pesticide Residues in Marijuana and Tea by QuEChERS and LC/MS/MS

UCT Part Numbers:
ECPAHFR50CT (50 mL centrifuge tubes)
CUMPSGG2CT (2 mL dSPE tube with 150 mg MgSO4, 50 mg PSA and 50 mg ChloroFiltr®)
ECQUUS2-MP (pouch containing 4 g $MgSO_4$ and 2 g NaCl)

April 2013

Summary

Various pesticides can be rapidly determined in dried tea leaves or marijuana using this simple method. Samples are hydrated in water and then extracted by QuEChERS and dSPE clean-up using ChloroFiltr®. Results are determined by LC/MS/MS.

Internal standard

Prepare a 10 ppm TPP solution by mixing 20 µL of the 5000 ppm TPP solution with 10 mL of MeCN

Transfer all standards to amber glass vials and store at -20°C until needed

Procedure

1. QuEChERS Extraction

a) Weigh 2 g of the homogenized tea or marijuana into a 50 mL centrifuge tube
b) Add 10 mL of reagent water to each tube and hydrate the samples for 1 hour using a horizontal shaker
c) Add 100 µL of the 10 ppm TPP solution to all samples
d) Add 10 mL of acetonitrile (MeCN). Vortex for 1 min
e) Add contents of **ECQUUS2-MP** pouch then shake vigorously for 1 min
f) Centrifuge at 5000 rpm for 5 min

2. dSPE clean-up

a) Transfer 1 mL of the supernatant to 2 mL dSPE tube **CUMPSGG2CT**

b) Shake for 30 seconds

c) Centrifuge at 10,000 rpm for 5 min

d) Transfer 0.3 mL of the cleaned extract into a 2 mL auto-sampler vial

e) Add 0.3 mL of reagent water

f) Vortex, then filter using a 0.45 µm syringe filter

g) The samples are ready for LC/MS/MS analysis

Instrumentation
Thermo Scientific Dionex Ultimate 3000 LC system coupled to a TSQ Vantage® triple quadrupole mass spectrometer

HPLC Parameters

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	1.24	142.007	124.57	14	111.6	5	60
Carbendazim	6.37	192.093	132.08	29	160.08	17	81
Dicrotophos	6.46	238.009	126.58	17	108.60	33	73
Acetachlor	6.48	269.417	111.86	15	71.69	33	72
Thiabendazole	6.61	202.059	131.06	31	175.07	24	103
DIMP	7.30	181.283	96.60	13	78.62	32	44
Tebuthiuron	7.32	228.946	115.59	26	171.63	17	72
Simazine	7.34	201.400	67.68	33	103.60	24	85
Carbaryl	7.41	201.956	126.63	30	144.63	7	40
Atrazine	7.68	215.957	67.65	35	173.60	16	79
DEET	7.72	191.947	118.63	15	90.66	28	92
Pyrimethanil	8.10	200.116	107.06	23	183.14	22	66
Malathion	8.12	331.011	98.57	23	126.86	12	60
Bifenazate	8.22	300.925	169.82	15	197.62	5	51
Tebuconazole	8.73	308.008	69.66	29	124.56	35	97
Cyprodinil	8.81	226.122	77.03	40	93.05	33	88
TPP (IS)	8.81	327.093	77.02	37	152.07	33	98
Diazinon	8.87	305.135	153.09	15	169.08	14	89

Zoxamide	8.90	335.807	186.50	20	158.51	38	102
Pyrazophos	8.99	374.103	194.06	20	222.13	20	104
Profenofos	9.59	372.300	302.37	19	143.48	35	104
Chlorpyrifos	10.23	349.989	96.89	32	197.94	17	69
Abamectin	11.20	890.486	304.40	18	306.68	15	102
Bifenthrin	12.77	440.039	165.21	39	180.42	11	66

Accuracy and Precision Data Obtained from the Fortified Tea Samples

Analyte	Spiked at 2 ng/mL		Spiked at 10 ng/mL		Spiked at 50 ng/mL	
	Rec%	RSD (n=6)	Rec%	RSD (n=6)	Rec%	RSD (n=6)
Methamidophos	112.5	7.3	100.7	1.5	85.9	10.1
Carbendazim	nd	nd	87.8	14.6	79.5	13.2
Dicrotophos	114.2	3.3	102.3	3.4	93.9	11.0
Acetachlor	108.3	10.4	111.3	4.6	105.5	6.5
Thiabendazole	86.7	6.0	84.0	2.9	73.5	10.8
DIMP	111.7	6.7	109.3	2.7	102.4	7.8
Tebuthiuron	113.3	3.6	108.3	2.4	100.4	8.1
Simazine	110.8	6.0	118.8	2.1	105.6	10.2
Carbaryl	115.8	5.0	122.0	2.1	111.2	10.4
Atrazine	124.2	7.8	117.5	2.6	105.2	9.8
DEET	149.2	12.8	125.5	3.1	106.3	10.1
Pyrimethanil	99.2	5.9	98.0	5.0	90.3	2.6
Malathion	143.3	9.5	125.0	6.7	110.2	4.4
Bifenazate	114.2	12.2	106.8	6.7	98.2	3.8
Tebuconazole	72.5	7.2	80.2	5.4	79.1	4.7
Cyprodinil	90.8	5.4	77.5	2.7	74.1	3.2
Diazinon	108.3	7.5	99.5	1.9	97.7	4.3
Zoxamide	95.0	7.4	92.2	1.3	90.1	3.4
Pyrazophos	90.0	5.0	91.7	2.4	88.3	5.1
Profenofos	96.7	7.8	80.8	4.5	75.0	5.3
Chlorpyrifos	80.0	7.9	82.5	2.7	81.6	5.4
Abamectin	99.2	8.1	89.8	4.0	82.2	9.4
Bifenthrin	89.2	7.5	119.8	7.6	126.5	19.0

nd: < LOQ, not determined

Crude Tea and Marijuana Extracts Before, and After dSPE Clean-up Using 150 mg $MgSO_4$, 50 mg PSA, and 50 mg ChloroFiltr®

Figure 1a Tea extracts before (left) and after dSPE clean-up (right)

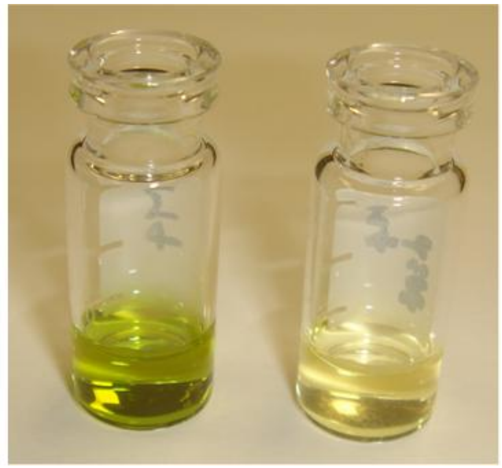


Figure 1b Marijuana (Sample #4) extracts before (left) and after dSPE clean-up (right)

DCN-316140-262

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

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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ChloroFiltr®: A Novel Sorbent for Chlorophyll Removal using QuEChERS

UCT Part Numbers:
ECPAHFR50CT (50 mL polypropylene centrifuge tube)
ECQUUS2-MP (Mylar pouch with 4000 mg $MgSO_4$ and 2000 mg NaCl)
CUMPSGGC182CT (2 mL centrifuge tube with 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18 and 50 mg ChloroFiltr®)

January 2013

Spinach and other highly pigmented vegetables contain chlorophylls, carotenoids, xanthophylls, and anthocyanins. Chlorophylls have the greatest adverse effect on GC systems due to their non-volatile characteristics. This QuEChERS procedure uses ChloroFiltr® to significantly reduce chlorophylls without sacrificing the recoveries of planar pesticides.

Procedure

1. QuEChERS Extraction

a) Homogenize 500 g of spinach in a food processor for 1-2 minutes
b) Weigh 10 grams of homogenized spinach sample into 50 mL centrifuge tube
c) Spike with 100 µL of 50 ppm triphenyl phosphate* as internal standard (IS)
d) Add 10 mL of acetonitrile then shake for 1 min
e) Add contents of Mylar pouch **ECQUUS2-MP** then shake vigorously for 1 min
f) Centrifuge at 5,000 rpm for 5 min
g) Supernatant is ready for clean-up

*50 ppm TPP solution: mix 50 µL of 5000 ppm TPP solution with 4.95 mL of MeCN

2. dSPE Clean-up

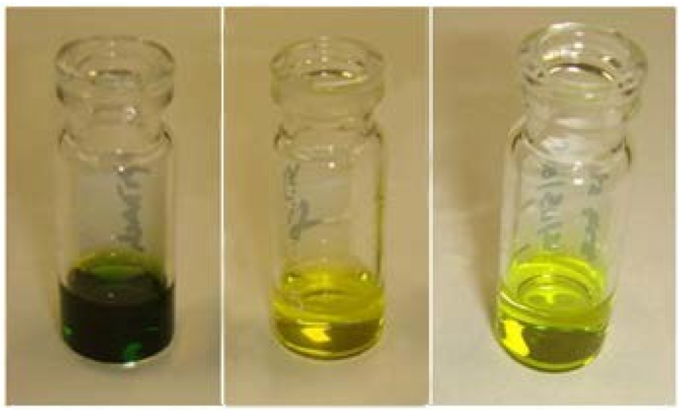
a) Transfer 1 mL of the extract to the 2 mL **CUMPSGGC182CT** (ChloroFiltr) dSPE micro centrifuge tube
b) Shake for 30 sec
c) Centrifuge at 3,000 rpm for 5 min
d) Transfer 0.4 mL of the supernatant to a 2 mL autosampler vial
e) Sample is ready for LC/MS/MS analysis

MS Parameters	
MS instrument	Thermo TSQ Vantage triple
Polarity	ESI +
Spray Voltage	3000 V
Vaporizer Temperature	350 °C
Ion Transfer Capillary	300 °C
Sheath Gas Pressure	40 arbitrary units
Auxiliary Gas Pressure	10 arbitrary units
Q1 and Q3 Peak Width	0.7 Da
Collision Gas and Pressure	Argon at 1.5 mTorr

HPLC Conditions	
HPLC system	Thermo Accela 1250 LC equipped with PAL auto-sampler
LC Column	Sepax HP-C18, 2.1*100 mm, 3 μm
Guard column	Restek C18, 2.1*20 mm, 3 μm
Column temperature	ambient
Auto-sampler Temp.	15 °C
Injection volume	10 μL
Mobile phase A	0.1% formic acid in Milli-Q water
Mobile phase B	0.1% formic acid in methanol
Flow rate	200 μL/min

Gradient Program		
Time (min)	**Mobile phase A**	**Mobile phase B**
0	95	5
1	95	5
3	50	50
8	5	95
14	5	95
14.2	95	5
16	95	5

SRM Transitions							
Compound	**Precursor ion**	**Product ion 1**	**CE 1**	**Product ion 2**	**CE 2**	**S-Lens**	**Dwell time**
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



Crude spinach extract — dSPE cleanup with ChloroFiltr — dSPE cleanup with GCB

Extract cleaned with ChloroFiltr® (Middle) is less green than that cleaned with GCB (Right), indicating that ChloroFiltr® is slightly more efficient in Chlorophyll removal.

Comparison of Pesticide Recoveries and RSDs Obtained by dSPE Clean-up of Spinach Sample using ChloroFiltr® and GCB (n=4)				
Pesticide	**ChloroFiltr®**		**GCB** (7.5 mg)	
	Recovery%	**RSD%**	**Recovery%**	**RSD%**
Carbendazim	87.1	1.0	71.2	4.0
Thiabendazole	93.2	1.9	55.9	2.6
Pyrimethanil	97.3	1.2	85.0	1.2
Cyprodinil	91.2	0.5	79.3	3.1
Diazinon	104.5	2.3	100.0	0.6
Pyrazophos	92.0	0.9	92.7	1.6
Chlorpyrifos	95.6	2.5	96.3	2.1

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
	Dichlorvos	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**	

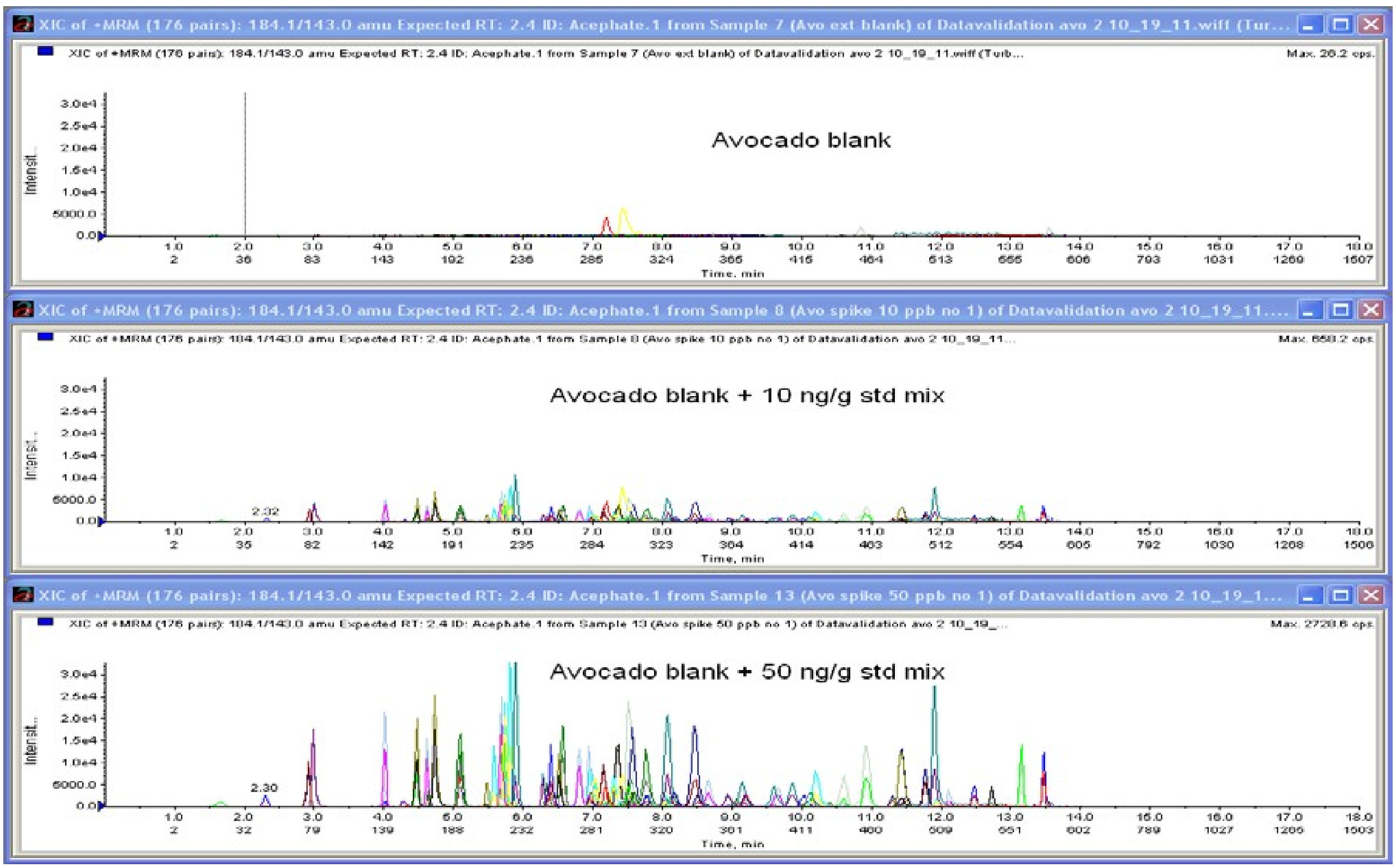
Average Recovery and RSD of 57 Pesticides Spiked in Avocado at Three Concentrations with GC-MS/MS Analysis

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'
Narong Chamkasem [a], Lisa W. Ollis [a], Tiffany Harmon [a], Sookwang Lee [a] and Greg Mercer [b]
a Southeast Regional Laboratory, U.S. Food and Drug Administration, 60 Eighth Street, N.E., Atlanta, GA, 30309
b Pacific Regional Laboratory – Northwest, U.S. Food and Drug Administration, 22201 23rd Drive, S.E., Bothell, WA 98021

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QuEChERS Determination of 2, 4-Dichlorophenoxyacetic Acid (2, 4-D) and Other Acidic Herbicides by LC-MS/MS

UCT Part Numbers:

ECQUEU750CT-MP (4000 mg $MgSO_4$, 1000 mg NaCl, 500 mg Na Citrate dibasic sesquihydrate, 1000 mg Na Citrate tribasic dihydrate)
ECMSC1850CT contains (500 mg C18 and 1500 mg $MgSO_4$)

August 2013

The pesticide 2,4-dichlorophenoxyacetic acid (2,4-D; Agent Orange; CASRN 94-75-7; pKa 2.73) along with other acidic pesticides can be extracted and analyzed using this QuEChERS approach.

Procedure

1. Sample Preparation

a) Weigh 10 g of homogenized, hydrated sample into a 50 mL centrifuge tube
b) Add ISTD
c) Add NH_4OH dropwise with shaking until pH 12
d) Cap and let sit for 30 min at room temperature
e) Neutralize sample by evaporation of NH_4. Dry N_2 may be used
f) Add 10 mL of acetonitrile
g) Shake
h) Add contents of **ECQUEU750CT-MP** pouch to centrifuge tube
i) Immediately shake or vortex for 2 min
j) Centrifuge for 5 min at >1500 rcf
k) Remove 5 - 10 mL aliquot and add to **ECMSC1850CT** tube
l) Shake vigorously or vortex for 2 min
m) Centrifuge for 5 min at > 1500 rcf
n) Pass extract through a 0.45 µm syringe filter into sample vial
o) Analyze by LC-MS/MS

Instruments

MS: AB Sciex 5500 QTrap
HPLC: Shimadzu LC-20AD pump, Sil-20AC autosampler & CTO-20AC column oven

LC Parameters

Guard Column	Agilent Eclipse Plus C18, 5 µm, 4.6 x 12.5 mm
Column	Agilent Eclipse Plus C18, 1.8 µm, 4.6 x 75 mm
Flow	0.5 ml/min
Injection Vol	10 µl
Oven	40 ˚C
Equilibration Time	0 min
Rinsing Vol	200 µl
Rinsing Speed	35 µl/sec
Sampling Speed	15 µl/sec
Cooler Temperature	15 ˚C

Mobile Phase

Aqueous A: 0.1 % formic acid in water
Organic B: 0.1 % formic acid in methanol

Time	%B
0	10
3.0	70
8.0	95
12.0	95
12.5	10
14.5	10

MS Instrument Settings

MRM Window	40 sec
Target Scan	0.25 sec
Cycle	0.5 sec
Resolution	Unit
PosNeg Switch	50 mS
Pause	5 mS
Curtin Gas	50 ml/min
Collision	High
Ion Spray Voltage	4500 volts
Turbospray Temperature	450 ˚C
Ion Source Gas 1	50 ml/min
Ion Source Gas 2	80 ml/min
Interface Heater	On

Analyte	ESI	RT	Q1	Q2	DP	EP	CE	CXP
2,3,6-TBA	-	5.9	224.6	180.8	-30	-10	-12	-18
2,3,6-TBA	-	5.9	222.6	178.9	-30	-10	-12	-18
2,4,5-T	-	7.7	252.6	194.9	-35	-10	-20	-18
2,4,5-T	-	7.7	254.8	196.9	-60	-10	-18	-18
2,4,5-TB	-	8.7	282.9	196.8	-35	-10	-20	-18
2,4,5-TB	-	8.7	280.8	194.8	-35	-10	-16	-18
2,4-D	-	6.8	218.7	160.9	-20	-10	-20	-18
2,4-D	-	6.8	220.6	162.9	-35	-10	-20	-18
2,4-DB	-	7.7	246.6	160.9	-20	-10	-18	-18
2,4-DB	-	7.7	248.6	162.9	-35	-10	-18	-18
4-CPA	-	6	184.7	126.9	-15	-10	-20	-18
4-CPA	-	6	186.7	128.9	-40	-10	-20	-18
Acifluorfen	-	8.2	359.8	316	-25	-10	-14	-18
Acifluorfen	-	8.2	359.8	194.9	-25	-10	-38	-18
Bromoxynil	-	6.4	273.4	78.8	-25	-10	-62	-18
Bromoxynil	-	6.4	275.4	80.8	-20	-10	-60	-18
Chloramben	-	5.2	203.6	159.9	-35	-10	-10	-18
Chloramben	-	5.2	205.6	161.9	-35	-10	-12	-18
Dalapon	-	4.4	140.6	96.9	-50	-10	-12	-18
Dalapon	-	4.4	142.6	98.9	-40	-10	-12	-18
Dicamba	-	6	218.7	175	-45	-10	-10	-18
Dicamba	-	6	220.4	176.9	-30	-10	-10	-18
Dicamba	-	6	218.7	35	-45	-10	-40	-18
Dichloroprop	-	7.4	232.6	160.9	-20	-10	-18	-18

Dichloroprop	-	7.4	234.5	162.9	-20	-10	-18	-18
Diclofop	-	9	324.9	253	-25	-10	-22	-18
Diclofop	-	9	326.9	255	-25	-10	-22	-18
Fenac	-	7.2	192.7	35	-55	-10	-35	-18
Fenac	-	7.2	194.7	35	-55	-10	-35	-18
Haloxyfop	-	8.4	359.8	288.1	-40	-10	-20	-18
Haloxyfop	-	8.4	361.9	290	-35	-10	-20	-18
MCPA	-	6.8	198.6	140.9	-20	-10	-20	-18
MCPA	-	6.8	200.7	142.9	-30	-10	-20	-18
MCPB	-	7.8	226.8	141	-30	-10	-18	-18
MCPB	-	7.8	228.5	143	-20	-10	-18	-18
Mecoprop	-	7.4	212.7	141	-20	-10	-22	-18
Mecoprop	-	7.4	214.6	143	-30	-10	-24	-18
PCP	-	10	264.4	35	-50	-10	-66	-18
PCP	-	10	262.4	35	-60	-10	-68	-18
Quizalofop	-	8.4	343	271	-20	-10	-22	-18
Silvex	-	8.2	266.6	194.8	-30	-10	-18	-18
Silvex	-	8.2	266.6	158.9	-30	-10	-40	-18
Triclopyr	-	7.2	255.4	197.9	-35	-10	-18	-18
Triclopyr	-	7.2	253.3	195.8	-35	-10	-18	-18
Diflufenzopyr	-	6.5	333	128	-50	-10	-34	-18