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ENVIRO-CLEAN®
SOLID PHASE EXTRACTION APPLICATIONS MANUAL

ENVIRO

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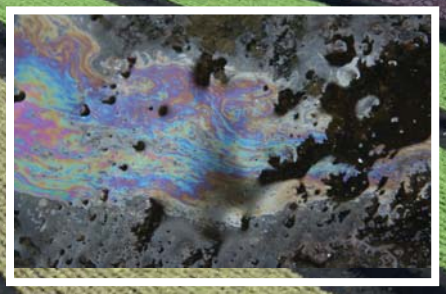

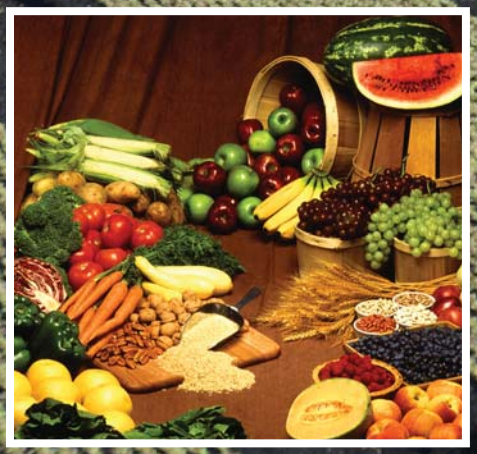

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Extraction Hints

- Verify sample application pH. Analytes that are not in their proper form (i.e., neutral or charged), will not effectively bind to the sorbent and may result in low or erratic recoveries. The pH of deionized water cannot be correctly determined using pH paper. Use of a calibrated pH probe is necessary.

- Always pre-rinse the cartridge with the strongest solution the cartridge will see to ensure the cleanest extraction of your eluate.

- Do not allow the sorbent to completely dry out between conditioning steps or before sample application. To ensure properly solvated cartridges, apply each solvent immediately after the previous solvent. Improperly conditioned cartridges may lead to erratic recoveries.

- Prior to elution, fully dried cartridges will ensure optimal analyte recovery. To confirm cartridge dryness, touch the sides of the cartridge at the sorbent level at full vacuum. Cartridges should feel about room temperature but not cool. If the cartridge feels cool, water is probably present. Continue drying the cartridge unless otherwise specified in the application note.

- Elution rates and soak times specified in the applications are critical for acceptable and consistent recoveries. Hint: When in doubt, slower is always better.

- Always use fresh ammonium hydroxide (NH_4OH). NH_4OH rapidly loses its strength when exposed to air. Weak NH_4OH may lead to erratic recoveries.

- Some analytes are slightly volatile. Closely monitor eluate concentration to prevent loss of analyte. Hint: Higher water bath temperatures and lower nitrogen flow rates usually provide optimal results.

EPA Methods
Drinking Water Analyses

Determination of Phthalate and Adipate Esters in Drinking Water by Liquid-Solid Extraction and Gas Chromatography with Photoionization Detection

UCT Part Numbers:
ECUNIC18 (1100 mg C18, 83 mL cartridge) or **EEC181M6G** (1000 mg C18, 6 mL glass cartridge)
ECSS156 (Drying Cartridge 5 g, Na_2SO_4, 6 mL cartridge)
FLORISIL PR® Clean-up Cartridge EUFLS12M15 (2000 mg,15 mL cartridge)
Alumina Clean-up Cartridge EUALN1M6 (1000 mg, 6 mL cartridge)
ENVIRO-CLEAN® Zero-Blank™ filter cartridge (optional)

EPA Method 506 Revison 1.1*

Procedure

1. Cartridge Activation

a) Place **ECUNIC18** cartridge(s) on the manifold
b) Add a 10 mL aliquot of methylene chloride
c) Slowly draw solvent completely through the cartridge
d) Repeat with a second 10 mL aliquot of methylene chloride
e) Hold for 1 minute then draw through
f) Dry cartridge by drawing air at full vacuum for 2-3 minutes

Optional: To reduce background contamination from laboratory air, use of the **ENVIRO-CLEAN® Zero-Blank™** filter cartridge is highly recommended during drying steps 1) f) and 2) c)

g) Add 10 mL of methanol to the cartridge
h) Draw through leaving a thin layer on the frit

Note: Do not allow the cartridge to go dry otherwise repeat step i

i) Add a second 10 mL portion of methanol
j) Wait 1-2 minutes to activate sorbent then draw through to level of frit
k) Add 10 mL reagent water to the cartridge and draw through until meniscus reaches the top of the frit
l) Cartridge is now ready for sample extraction

2. Sample Extraction

a) Add the 1 liter water sample to the cartridge and draw through over a period of about 20–30 minutes (fast drip)

b) Rinse sample bottle and cartridge with a small volume of reagent water then add to cartridge

c) Dry cartridge under full vacuum for 10 minutes (**Optional**: Use **Zero-Blank™** filter cartridge to prevent airborne contamination)

3. Cartridge Elution

a) Place a collection vial in the vacuum manifold

b) Add 5 mL of methylene chloride to the sample bottle and swirl

c) Using a disposable glass pipette transfer the methylene chloride to the cartridge

d) Collect dropwise

e) Repeat this procedure with another 5 mL of methylene chloride

4. Eluate Drying and Concentration

a) Pour eluant through a 3 gram bed of anhydrous sodium sulfate (or use Drying Cartridge **ECSS156**) and collect

b) Rinse vial and sodium sulfate with a 3 mL aliquot of methylene chloride

c) Repeat rinse using an additional 3 mL aliquot of methylene chloride

d) Evaporate with a gentle stream of N_2 to 1 mL

e) If sample is clean proceed to GC analysis

f) If extract requires clean-up for phthalates esters proceed to Florisil or Alumina clean-up stages

Extract Clean-up---Florisil or Alumina

Clean-up procedures are not required for clean drinking water. Under certain circumstances for dirty water, a Florisil or Alumina clean-up may be needed.

Florisil Column Clean-up for Phthalate Esters

1. Add a 1 cm layer of anhydrous sodium sulfate to the top frit of Florisil cartridge **EUFLS12M15**
2. Flush cartridge with 20 mL of hexane leaving enough to cover the frit
3. Add sample extract to the cartridge then rinse vial with 2 mL of hexane

4. Add 20 mL of hexane to the cartridge and elute. Discard the hexane solution
5. Elute using 20 mL of 20% diethyl ether in hexane into a 500 mL K-D flask** equipped with a 10 mL concentrator tube. Elute at a rate of about 2 mL/minute
6. No solvent exchange is required
7. Concentrate eluate in hot water bath at 85° C to 1 mL
8. Sample is ready for GC analysis

Alumina Column Clean-up (neutral) for Phthalate Esters

1. Add a 1 cm layer of anhydrous sodium sulfate to the top frit of a Alumina cartridge **EUALN1M6**
2. Flush cartridge with 10 mL of hexane leaving enough to cover sodium sulfate
3. Add sample extract to the cartridge. Rinse vial with 2 mL of hexane and add to the cartridge
4. Add 15 mL of hexane to the cartridge and elute. Discard hexane solution
5. Elute at a rate of about 2 mL/minute using 15 mL of 20% diethyl ether in hexane
6. Collect eluate in a 500 mL K-D flask equipped with a 10 mL concentrator tube
7. No solvent exchange is required
8. Concentrate eluate in hot water bath at 85° C to 1 mL
9. Sample is ready for GC analysis

*The analyst should refer to EPA Method 506 “Determination of Phthalate and Adipate Esters in Drinking Water by Liquid-Solid Extraction and Gas Chromatography with Photoionization Detection”, Revision 1.1 Issued 1995, F.K. Kawahara and J.W. Hodgeson, Ed. By D. J. Munch US EPA, National Exposure Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Cincinnati, Ohio 45268
**K-D Flask = Kuderna-Danish Flask

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Determination of Chlorinated Pesticides, Herbicides, and Organohalides by Liquid-Solid Extraction and Electron Capture Gas Chromatography*

UCT Part Numbers:
ECUNIC18 (1100mg C18, 83 mL cartridge)
Or
CEC181M6 (1000mg C18, 6 mL cartridge)

EPA Method 508.1 Rev 2.0

Procedure

1. Condition Cartridge

a) Wash the cartridge with 5 mL of ethyl acetate (EtOAc) and 5 ml of methylene chloride (MeCl2). Let the cartridge soak for 2 min, then drain dry under full vacuum for 1 min after each wash

b) Add 10 mL of methanol (MeOH) to the cartridge then draw through to top of frit

c) Rinse the cartridge with 10 mL of reagent water and draw through to top of frit

Note: Do not let the cartridge go dry after addition of methanol otherwise repeat at step b)

2. Sample Extraction

a) Samples must be dechlorinated and pH adjusted to <2

b) Add 5 mL of methanol to the 1 liter sample and mix well

c) Add 50 µL of surrogate compound to the water sample and mix well

d) Draw the water sample through the cartridge at a fast drip

e) Dry the cartridge by drawing air or N_2 through for about 10 minutes

3. Extract Elution

a) Insert an eluate collection tube in the vacuum manifold

b) Rinse the inside walls of the sample bottle using 10 mL EtOAc then transfer solvent to the cartridge and let soak for 2 min

c) Collect dropwise, turn full vacuum on for 1 min

d) Repeat step b) and c) with 10 mL of $MeCl_2$

4. Sample Drying

a) Dry the eluents with 10-15 grams of anhydrous sodium sulfate held in either a glass wool stopped glass funnel or fritted empty cartridge, and collect in a new tube/vial

b) Rinse the eluate collection tube with 2 x 5 mL portions of $MeCl_2$, and apply the rinse to the sodium sulfate bed and collect

c) Concentrate the extract to about 0.8 mL under a gentle stream of N_2 while in a water bath

d) Rinse the inside walls of the concentrator tube three times with EtOAc during the evaporation

e) Add internal standard (IS), and adjust the final volume to 1.0 mL

f) Sample is ready for GC analysis

For complete details on Method 508.1 , rev 2.0 “Determination of Chlorinated Pesticides, Herbicides, and Organohalides by Liquid-Solid Extraction and Electron Capture Gas Chromatograph”, the analyst is referred to: J. W. Eichelberger rev 1.0, 1994 and J. Munch, rev 2.0, 1995, Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268

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Determination of Ethylene Thiourea (ETU) in Water Using Gas Chromatography with a Nitrogen-Phoshorus Detector

UCT Part Numbers:
CLEAN-ELUTE™ (25,000 mg diatomatious earth, 200 mL cartridge)

EPA Method 509

Method 509 is a gas chromatographic method for the determination of ethylene thiourea (ETU) (CAS 96-45-7) a metabolic byproduct of the ethylene bisdithiocarbonate (EBDC) fungicides in water. Toxicological studies indicate that ETU may produce goitrogenic, tumorigenic, and teratogenic effects in laboratory animals, raising the concern that residues may be found in agricultural commodities. The method uses a packed column of diatomaceous earth to capture the analyte before elution with methylene chloride. Confirmation is made using a nitrogen-phosphorous detector or a mass spectrometer.

Method Summary

A 50 mL water sample is adjusted to ionic strength and pH by the addition of ammonium chloride (NH_4Cl) and potassium fluoride (KF). The sample is poured into a UCT CLEAN-ELUTE™ column and the ETU is eluted from the column using 400 mL of methylene chloride. An excess of a free radical scavenger is added to the eluate. The methylene chloride eluant is concentrated to 5 mL after exchange into ethyl acetate. GC analysis with a nitrogen-phosphorous detector or mass spectrometer is used for quantitation.

Safety

- ETU is a suspected carcinogen. Prepare all standards in a fume hood

Sample Collection and Preservation

- Grab samples must be collected in 60 mL glass containers fitted with Teflon-lined crew caps
- Do not pre-rinse with sample before collection
- After collection shake the sample bottle for 1 minute
- ETU may degrade in water even during refrigeration. Mercuric chloride has been used as a preservative but due to its toxicity and harm to the environment is not recommended
- Store sample on ice or in refrigerator at 4°C and protected from light. Extract as soon as possible after collection

Interferences

Method interferences arise from contaminated glassware, solvents, reagents and other laboratory apparatus in which the sample may come in contact. All reagents and glassware must be shown to be free from interferences under analysis conditions.

- Glassware must be scrupulously clean
- Clean glassware by rinsing with the last solvent used followed by hot water and detergent. Rinse with reagent water, dry and heat in an oven at 400°C for one hour. Do not heat volumetric flasks
- Always use high purity reagents and solvents
- Interfering contamination may occur when a low concentration sample is analyzed after a high concentration sample. Complete rinsing of the syringe using ethyl acetate may be required

Procedure

1. Sample Preparation

a) Pipette 50 mL of the water sample into a clean bottle
b) Add 1.5 grams ammonium chloride (NH_4Cl)
c) Add 25 grams potassium fluoride (KF)
d) Seal bottle and shake until salts are completely dissolved

2. Sample Extraction

a) Add 5 mL of 1000 g/mL of dithiothreitol (DTT, Cleland's Reagent) in ethyl acetate as a free radical scavenger to a 500 mL Kuderna-Danish K-D concentrator tube
b) Support a CLEAN-ELUTE™ 200 mL cartridge using a clamp over a (K-D) tube
c) Add the entire contents of the bottle from step 1) d) above
d) Do not use vacuum but allow the cartridge to stand for 15 minutes

3. Sample Collection

a) Add 400 mL of methylene chloride in 50 mL aliquots to the CLEAN-ELUTE™ column
b) Collect the eluant in the K-D apparatus

4. Extract Concentration (The following steps must be conducted in the fume hood)

a) Add two boiling chips to the K-D apparatus and attach a macro Snyder column
b) Attach a condenser to the Snyder column to collect solvent

c) Place the K-D apparatus in a 65-70°C water bath so that the K-D tube is partially submerged in the water

d) Once liquid volume had been reduced to 5 mL remove from the water bath

e) Continue to reduce the liquid volume to < 1 mL in an analytical evaporator at 35-40°C under a stream of nitrogen

f) Dilute sample to 5 mL with ethyl acetate rinsing the walls of the K-D apparatus

g) Add 50 µL of internal standard and agitate

h) Transfer to a GC vial

i) Sample is ready for analysis

GC Analysis Conditions

Primary Conditions:
Column: 10 m long x 0.25 mm I.D. DB-Wax bonded fused
Carrier Gas: He @ 30 cm/sec linear velocity
Makeup Gas: He @ 30 mL/min flow
Detector Gases: Air @ 100 mL/min flow; H2 @ 3 mL/min flow
Injector Temperature: 220°C
Detector Temperature: 230°C
Oven Temperature: 220°C isothermal
Sample: 2 µL splitless; nine second split delay
Detector: Nitrogen-phosphorus

Confirmation Conditions:
Column: 5 m long x 0.25 mm I.D. DB-1701 bonded fused
Carrier Gas: He @ 30 cm/sec linear velocity
Makeup Gas: He @ 30 mL/min flow
Detector Gases: Air @ 100 m:/min flow; H_2 @ 3 mL/min flow
Injector Temperature: 150°C
Detector Temperature: 270°C
Oven Temperature: 150°C isothermal
Sample: 2 µL splitless; nine second split delay
Detector: Nitrogen-phosphorus

Analyte	Primary Column RT (min)	Confirmation Column RT
ETU	3.5	4.5
THP internal standard	5.1	5.0
PTU surrogate standard	2.7	2.2

*The analyst should refer to EPA Method 509 “Determination of Ehtylene Thiourea (ETU) in Water Using Gas Chromatography with a Nitrogen-Phoshorus Detector”, Revision 1.0 Issued 1992, By DJ Munch and RL Graves, US EPA, National Exposure Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Cincinnati, Ohio 45268
and TM Engel and ST Champagne, Battelle, Columbus Division

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Determination of Nitrosamines in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography with Large Volume Injection and Chemical Ionization Tandem Mass Spectrometry (MS/MS)*

UCT Part Numbers:
EU52112M6 (2000 mg activated coconut carbon, 6 mL)
ECSS156 (6 mL Drying Cartridge with 5 grams anhydrous sodium sulfate)

EPA Method 521

Activated carbon is used for the determination of various nitrosamines in finished drinking water and untreated source waters using GC/MS/MS.

Nitroaromatics, Nitramines and Nitrate Ester Analytes

Analyte	Abbreviation	% Recovery n=3
N-Nitrosodimethylamine	NDMA	95
N-Nitrosomethyldiethylamine	NMEA	98
N-Nitrosodiethylamine	NDEA	95
N-Nitrosodi-n-propylamine	NDPA	90
N-Nitrosodi-n-butylamine	NDBA	94
N-Nitrosopyrollidine	NPYR	76
N-Nitrosopiperidine	NPIP	81

*For complete details on Method 521, September 2004, the analyst is referred to: J.W.Munch & M.V.Bassett, "Determination of Nitrosamines in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography with Large Volume Injection and Chemical Ionization Tandem Mass Spectrometry (MS/MS), National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268

Procedure

1. Cartridge Conditioning

a) Add 3 mL of methylene chloride to the cartridge, then slowly draw all solvent through the cartridge

b) Add 3 mL of methanol to the cartridge, turn on vacuum and draw through

c) Add 3 mL of methanol again and draw through so that the methanol just covers the top of the cartridge frit

Do not let the cartridge go dry after this step otherwise repeat starting at step 1 b)

d) Add 3 mL of reagent water and draw through

e) Repeat water rinse, step d) **5 additional times**

Proper conditioning of the cartridge is essential for good precision and accuracy

2. Sample Extraction

a) Adjust the vacuum setting so that the flow rate is 10 mL/minute

b) After sample extraction, draw air through the cartridge for **10 minutes** at full vacuum

c) After drying, proceed immediately to cartridge elution

3. Cartridge Elution

a) Insert a clean collection tube in the manifold

b) Fill the cartridge with methylene chloride

c) Partially draw the methylene chloride through at low vacuum and then turn vacuum off

d) Allow cartridge to soak for 1 minute

e) Draw the remaining methylene chloride through in dropwise fashion

f) Continue to add methylene chloride to the cartridge as it is being drawn through until a total of 12-13 mL have been added

g) Concentrate the methylene chloride to about 0.9 mL in a water bath near room temperature. Do not concentrate less than 0.5 mL as loss of analyte may occur

Note: Small amounts of residual water from the sample container and SPE cartridge may form an immiscible layer with the extract. To eliminate the water a drying column packed with 5 grams of anhydrous sodium sulfate or use **ECSS15M6** for drying. Wet the cartridge with a small volume of methylene chloride before adding extract. Rinse the drying column with 3 mL of methylene chloride.

4. Sample Analysis

a) Calibrate the MS in EI mode using FC-43

b) Inject into a GC/MS/MS

c) Identify the product ion spectrum to a reference spectrum in a user created data base

Mass Spectral Data

Analyte	Retention Time (min)	Precursor Ion (m/z)	Product/Quantitation Ion (m/z)
NDMA	8.43	75	43(56)
NMEA	11.76	89	61(61)
NDEA	14.80	103	75(75)
NPYR	22.34	101	55(55)
NDPA	22.40	131	89(89)
NPIP	24.25	115	69(69)
NDBA	30.09	159	57(103)
NDMA-d6 surrogate	8.34	81	46(59)
NMEA-d10 IS	14.63	113	81(81)
NDPA-d6 IS	22.07	145	97(97)

Injector Program

Temp (°C)	Rate (°C/min)	Time (min)
37	0	0.72
250	100	2.13
250	0	40

Injector Split Vent Program

Time (min)	Split Status
0	Open
0.70	Closed
2.00	Open

GC Oven Temperature Program

Temperature (°C)	Rate (°C/min)	Hold Time (min)
40	0	3.0
170	4.0	0
250	20.0	3.0

Limits and Lowest Concentration Minimum Reporting Levels

Analyte	DL (ng/L)	LCMRL (ng/L)
NDMA	0.28	1.6
NMEA	0.28	1.5
NDEA	0.26	2.1
NPYR	0.35	1.4
NDPA	0.32	1.2
NPIP	0.66	1.4
NDBA	0.36	1.4

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Determination of 1,4-Dioxane In Drinking Water By Solid Phase Extraction (SPE) AND Gas Chromatography/Mass Spectrometry (GC/MS) With Selected Ion Monitoring (SIM)*

UCT Part Numbers:
EU52112M6 (2 grams coconut carbon in a 6 mL cartridge)

EPA Method 522

1,4-dioxane (CASRN 123-91-1) referred to as dioxane, is a highly water soluble non-biodegradable ether. This is a gas chromatography/mass spectrometry (GC/MS) method for the determination of this analyte in drinking water. Scan mode may be used if appropriate sensitivity is attained. Option 1 is designated for sample volumes of 500 mL. This product can be used with most automated systems.

Sample Collection & Preservation

- Prepare bottles before sample collection with sodium sulfite (CASRN 7757-83-7)
- Open the tap and allow the system to flush until the water temperature has stabilized (approximately three to five min)
- Fill sample bottles. Do not flush out the dechlorination reagent
- Cover the bottle and agitate by hand until the **sodium sulfite** has dissolved
- Add **sodium bisulfate** (CASRN 7681-38-1). Mix until dissolved

Sample Shipment and Storage

- Samples must be chilled during shipment and must not exceed 10° C during the first 48 hours after collection
- Sample temperature must be confirmed to be at or below 10° C when they are received at the laboratory
- Verification of sample dechlorination – Upon the receipt of samples at the laboratory, verify that field samples were dechlorinated at the time of collection. The absence of total chlorine can be verified with a N, N-DIETHYL-P-HENYLENEDIAMINE (DPD) CHLORINE TEST KIT – (Hach model CN-66; cat. # 2231-01 or equivalent)
- Holding time may be up to 28 days

Interfereces:

- All glassware must be meticulously cleaned with detergent and tap water, rinse with tap water, followed by reagent water
- Non-volumetric glassware should be heated in a muffle furnace at 400°C for 2 hours
- Volumetric glassware should be solvent rinsed with DCM or purge and trap grade methanol after washing, and dried in a low temperature oven (<120°C) or air dried
- Interferences may be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware. All items must be routinely demonstrated to be free from interferences (less than 1/3 the MRL for the method analyte) under the conditions of the analysis. **Subtracting blank values from sample results is not permitted**
- **Purge and trap grade methanol must be used** for all steps where methanol is required. Other grades of methanol may contain low molecular weight compounds which may prohibit accurate identification and quantitation of the analyte, SUR and IS
- Matrix interferences may be caused by contaminants that are co-extracted from the sample. The matrix interferences will vary considerably from source to source
- Analyte carry-over may occur when a relatively “clean” sample is analyzed immediately after a sample containing relatively high concentrations of compounds
- Syringes and splitless injection port liners must be cleaned carefully and replaced as needed. After analysis of a sample containing high concentrations of compounds, a LRB should be analyzed to ensure that accurate values are obtained for the next sample
- During automated GC/MS analyses, extracts with positive results that were analyzed immediately following a sample with high concentrations of the analyte, should be reanalyzed after analyzing an acceptable LRB. If the analyte is not detected in extracts analyzed immediately after a high concentration extract, no reanalysis is necessary
- Many silicone compounds may be leached from punctured autosampler vial septa, particularly when particles of the septa sit in the vial. This can occur after repeated injections from the same autosampler vial. If this method is performed in full scan mode, silicone compounds appear as regular chromatographic peaks with similar fragmentation patterns. They can unnecessarily complicate the total ion chromatogram
- High laboratory background levels of 1,4-dioxane have been reported to be associated with air contamination. Contact UCT technical support for possible solutions to air contamination issues.

Safety:

- The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined
- 1,4-Dioxane is classified as a class B2 or probable human carcinogen
- Sodium bisulfate is used as a sample preservative to inhibit microbial growth and decay of 1,4-dioxane

Option 1—100-500 mL samples

1. **Prepare Cartridge** (vacuum manifold use)
   a) Place a **EU52112M6** cartridge on a single or multi-station vacuum manifold
   b) Add 3 mL of dichloromethane (methylene chloride) to the cartridge and draw through
   c) Add 3 mL of methanol and draw through completely
   d) Add 3 mL of DI water and draw through. **Do not let cartridge go dry**
   e) Repeat step d) 5 times without letting the cartridge go dry
2. **Extract Sample**

   a) Add surrogate 1,4-dioxane-d_8 to the 100-500 mL sample water
   b) Add sample water to the cartridge. Adjust vacuum for a 10 mL/minute flow rate
   c) After the entire sample has drawn through, dry cartridge by drawing air at full vacuum. Cartridge will feel room temperature when dry
3. **Cartridge Elution**

   a) Place a clean collection vial in the vacuum manifold
   b) Fill each cartridge with dichloromethane (DCM). Soak sorbent for 1 minute then slowly draw through dropwise
   c) Continue to add DCM to the cartridge until the total volume is 9 mL
   d) Remove collection tube and adjust volume to 10 mL with DCM
   e) Add IS. Adding 5 µl of 1000 µg/mL of IS solution to 10 mL DCM extract will yield 500 ng/mL
   f) Mix by vortexing
   g) Dry extract by adding 2 grams anhydrous sodium sulfate (Na_2SO_4)
   h) Mix well
   i) Transfer aliquot to autosampler vials for analysis

NOTE: This extract cannot be reliably concentrated by nitrogen evaporation because of the volatility of dioxane

Instrument & Conditions

Column: Varian CP-Select 624 CB (6% cyanopropyl phenyl, 94& PDMS, 30 m 0.25mm x 1.4 µm column (or equivalent)

Injector: 200°C (Splitless mode)

Injector Volume: 1 µL

Helium: Carrier gas 99.999% or better

Flow: 1 mL/minute

Oven: 30° C for 1 minute 90°C at 8° C/minute
200° C at 20° C/minute for 4 minutes

MS: Any type of mass spectrometer may be used (i.e., quadrupole, ion trap, time of flight, etc.), although the SIM option may not provide enhanced sensitivity, or be an available option on all instruments

SIM MODE

Segment 1: m/z 46**, 78, 80
Segment 2: m/z 58, 62, 64, 88**, 96**

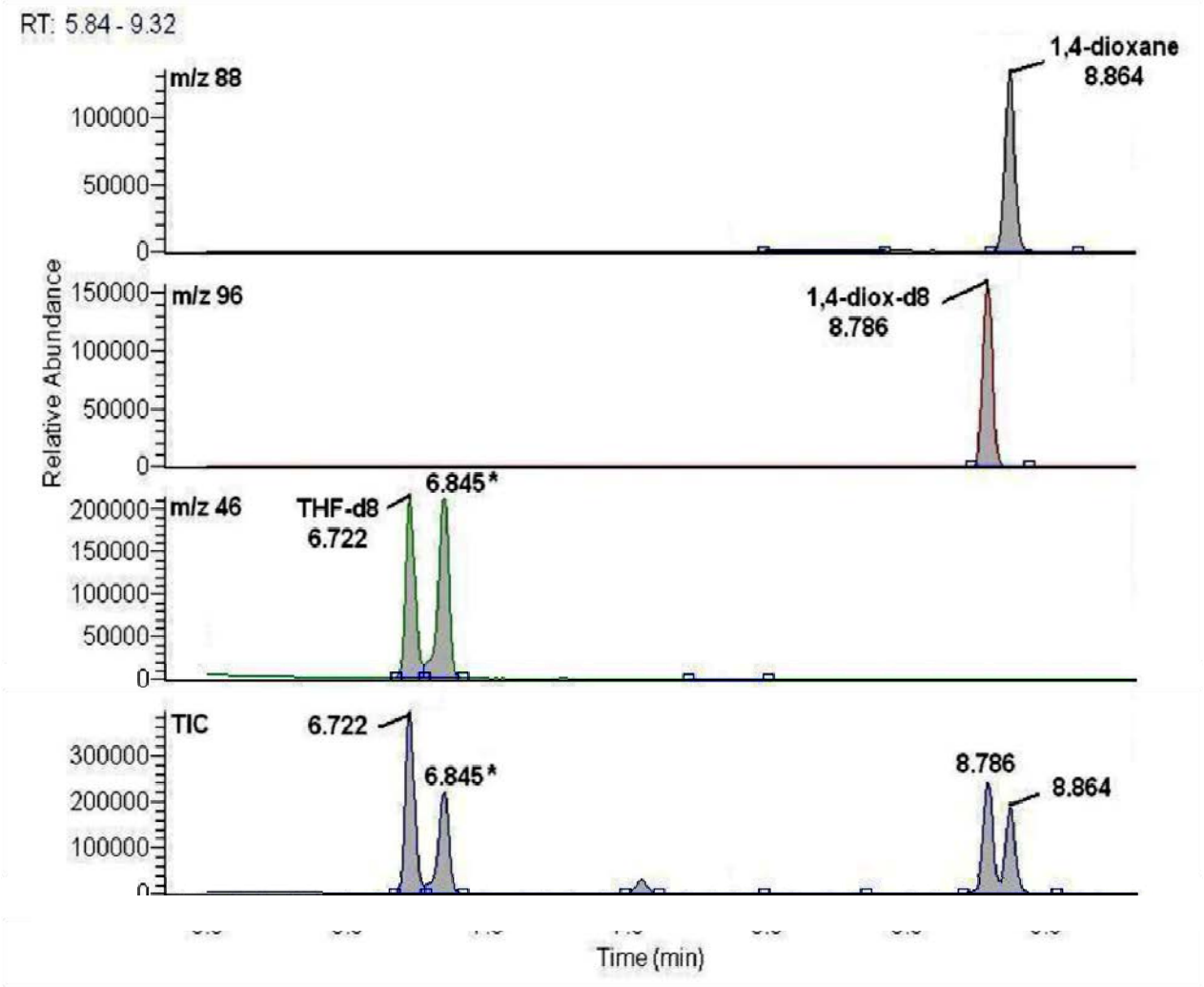
Dwell time 100 µs
Emission current 100 µA

**** quantitation ions**

Retention Times and Quantitation Ions (QIs)

Compound	Retention Time (min)	SIM Ions (*m/z*)
1,4-dioxane	8.85	58, **88**
1,4-dioxane–d_8	8.77	62, 64, 96
THF-d_8 (IS)	6.68	**46**, 78, 80

Reconstructed total ion current chromatogram and mass chromatograms for THF-*d*8 (IS), 1,4-dioxane-*d*8 (SUR), and 1,4-dioxane at 0.5 mg/mL each (the standard is equivalent to an extract of a 10 mg/L aqueous sample). * Peak at 6.845 min is chloroform, a chemical present in DCM



Summarized from Jean W. Munch and Paul E. Grimmett, "Method 522 Determination Of 1,4-Dioxane In Drinking Water By Solid Phase Extraction (SPE) And Gas Chromatography/Mass Spectrometry (GC/MS) With Selected Ion Monitoring (SIM)", Office of Research and Development, National Exposure Laboratory, Cincinnati, OH Version 1.0, September 2008

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

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

EPA Method 523

Method Summary

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

The following compounds can be determined using this method:

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

Procedure

1. Sample Preparation

a) Allow samples to reach room temperature prior to extraction

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

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

d) Cap and invert each sample several times to mix

e) Proceed with sample extraction using SPE carbon cartridges

2. Cartridge Cleaning & Conditioning

a) Set up extraction cartridges on the SPE vacuum manifold

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

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

d) Draw MeOH to the top of the cartridge frit

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

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

f) Draw RW to the top of the cartridge frit

3. Sample Extraction

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

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

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

d) Dry the cartridges under high vacuum for 10 seconds

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

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

4. Sample Elution

a) Place 15 mL conical tubes into the manifold for collection

b) Add 2 mL of EtOAc to the cartridge and elute dropwise

c) Add 2 x 6 mL aliquots of 9:1 DCM/MeOH to cartridge

d) Allow the cartridge beds to briefly soak in solvent, then draw the solvent through the cartridges

e) Dry the eluate by passing it through approximately 3 grams of anhydrous Na_2SO_4 collecting it in a 40 mL centrifuge tube. Pre-rinse the Na_2SO_4 with a 1 mL aliquot of 3:1 DCM/EtOAc

f) Rinse with 1 mL aliquot of 3:1 DCM/EtOAc collecting it in the centrifuge tube

g) The dried extracts may be stored overnight in the 40 mL tubes at -10 ºC

h) Warm the 40 mL tubes to 35 °C in a water bath under a stream of N_2 and evaporate solvent to less than 1 mL but no less than 0.5 mL

i) Transfer the concentrated eluate to 1 mL volumetric tubes

j) Rinse the conical tube with a small volume of EtOAc, and transfer the rinse to the volumetric

k) Add IS solution and adjust to volume

l) Transfer the extracts to autosampler vials for analysis or store in a freezer ≤ -10 ºC

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

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EPA Method 525.2 Revision 2.0
Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Gas Chromatography/Mass Spectrometry

UCT Part Numbers:
ECUNI525 (1500 mg 525 C18, 83 mL cartridge)
or
EC525006-P (1500 mg 525 C18, 6 mL cartridge, PE Frit)

METHOD 525.2

Method 525.2 Analytes

The analyte list for this method is comprised of over 120 compounds representative of several classes of pesticides, polynuclear aromatic hydrocarbons, PCBs, phthalates and adipates and other drinking water pollutants. Recovery ranges from 70-130%. Refer to the published method for compound specific MDL's.

The validation data presented herein were determined on independent lots of UCT ENVIRO-CLEAN® Universal Cartridges. MDLs were not determined on all analytes as part of this validation. In addition to the listed method analytes, recovery data for an extended list of analytes is also included.

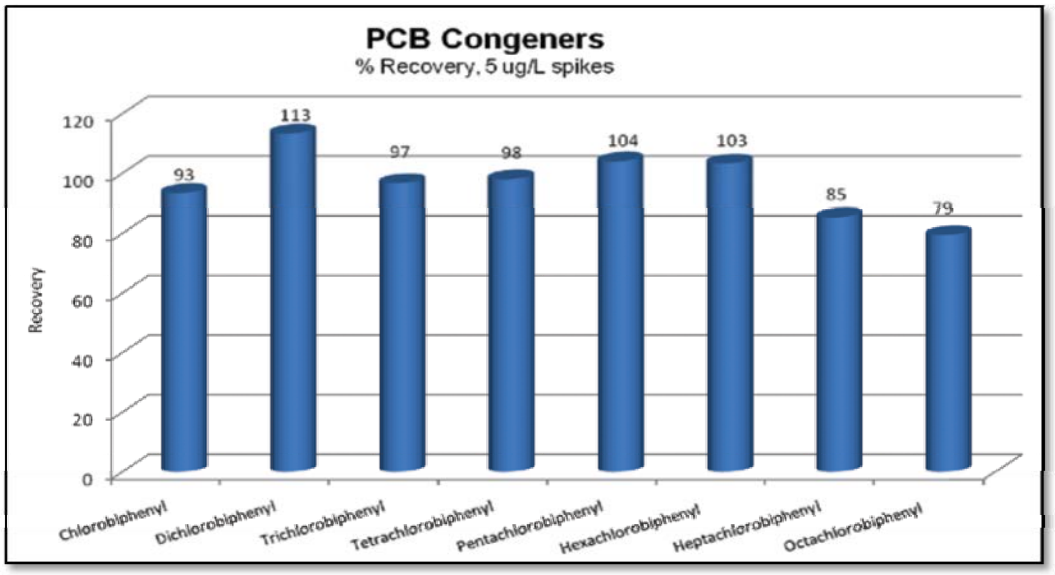
Table of Compounds
Tested using the UCT ENVIRO-CLEAN® Universal Cartridge 525

Analyte	Average 3 Replicates % Recovery	Std Dev
Acenaphthene	100	0.0
2,4-dinitrotoluene	83	NA
2,6-dinitrotoluene	78	NA
4,4"-DDE	91	4.2
4,4"-DDT	94	3.5
4,4'-DDD	94	4.5
Acenaphthylene	96.0	0.012
Acenaphthene	99.1	0.013
Acetochlor	115	0.01
Alachlor	99	0.007
Aldrin	77	4.4
Ametryn	95	4.6
Anthracene	80	0.0
Atraton	84	17.3
Atrazine	111	0.011
Benzo(a) anthracene	75.4	0.049
Benzo(a)pyrene	105	9.9
Benzo(b)fluoranthene	184.9	0.022
Benzo(k)fluoranthene	95.7	0.029
Benzo[g,h,i]perylene	83.1	0.05
BHC, alpha	108	6.9
BHC, beta	97	3.1
BHC,delta	109	7.9
BHC,gamma	102	11.9
bis- (2-ethylhexyl) adipate	95.1	0.033
bis 2 ethylhexyl phthalate	104	0.029
Bromacil	126	0.012

Butachlor	113	0.005
Butylate	103	4.6
Butylbenzylphthalate	97.1	0.02
Caffeine	90.0	0
Captan	86.9	0.273
Carboxin	103	12.9
Chlordane, alpha	97	4.6
Chlordane, gamma	94	2.5
Chlordane, trans nonachlor	115	11.0
Chlorneb	113	11.0
Chlorobenzilate	118	10.0
Chlorpropham	130	0
Chlorpyrifos (Dursban)	107	5.0
Chlorothalonil	117	12.1
Chrysene	100	0.012
Cyanazine (Bladex)	126	0.008
Cycloate	111	15.0
Dacthal (DCPA) methyl ester	118	13.1
Diazinon	135	0.031
Dibenzo[a,h]anthracene	77.4	0.051
Dichlorvos (DDVP)	127	9.5
Dieldrin	96	6.8
Diethylphthalate	99.1	0.071
Dimethoate	106	0.008
Dimethylphthlate	78.6	0.022
Di-n-butylphthalate	113	0.12
Diphenamid	119	0.008
Disulfoton	92.1	0.01
Disulfoton Sulfone	108	12.5
Endosulfan I	116	11.1
Endosulfan sulfate	114	6.8
Endrin	88	0.0
Endrin Aldehyde	97	3.6
Endrin Ketone	90	3.8
EPTC	102	0.005
Ethion	112	0.005
Ethoprophos	109	5.8
Etridiazole (terrazole)	97	1.2
Fenarimol	70	0.0
Fluoranthene	100	0.018
Fluorene	99.7	0.012
Heptachlor	79	8.2
Heptachlor Epoxide Iso A	116	16.3
Hexachlorobenzene	94	17.4
Hexachlorocyclopentadiene	82	8.4
Hexazinone (Velpar)	105	8.1
Indeno[1,2,3-cd]pyrene	77.4	0.16
Isophorone	91	NA
Lindane	127	4.8
methoxychlor	123	7.6
Methyl Paraxon (Parathion)	115	5.0
Metolachlor	111	0.004
Metribuzin	109	0.005
Mevinphos (phosdrin)	117	
	12.1	
MGK 264	121	5.8
Molinate	114	0.013
Naphthalene	90.3	0.013
Napropamide (Devrinol)	115	2.3
Nonachlor, trans	116	11.1
Norflurazon	133	6.1
PCNB (carbaryl)	91.4	0.021
Pebulate	101	1.7
Pentachlorophenol	80	0.017
Permethrin, cis	124	2.1
Permethrin, trans	123	3.1
Perylene-d12	119	0.0
Phenanthrene	96.9	0.014
Phenanthrene-d10	99	6.6

Prometon	78.6	0.008
Prometryn	110	0.012
Pronamide (propyzamide)	101	1.2
Propachlor	113	15.0
Propazine	105	4.6
Pyrene	94.6	0.022
Simazine	91.4	0.005
Simetryn	93	4.6
Stirofos (tetrachlorvinphos)	126	6.9
Thiobencarb	112	0.008
Tebuthiuron	85	33.5
Terbacil	120	3.5
Terbutryn	103	2.3
Triademefon	98	6.9
Tricyclazole	107	5.0
Trifluralin	82	9.7
Trifluran	83	9.2
Trithion (carbofenothion)	101	0.004
Terbufos	95	7.0
Vernolate	107	1.2

PCB Congeners	**Average**	**Std Dev**
2-chlorobiphenyl	93	2.3
2.3-Dichlorobiphenyl	113	15.0
2,4,5-trichlorobiphenyl	97	3.1
2,2,4,4-tetrachlorobiphenyl	98	5.3
2,2,3,4,6-pentachlorobiphenyl	104	2.0
2,2,4,4,5,6-hexachlorobiphenyl	103	3.1
2,2,3,3,4,4,6-heptachlorobiphenyl	85	1.2
Octachlorobiphenyl (BZ#200)	79	1.2



Procedure

1. Wash the extraction apparatus and cartridge

a) Add 10 mL of a 1: 1 mixture of ethyl acetate: methylene chloride (EtOAc: $MeCl_2$) to the reservoir.

b) Draw a small amount through the cartridge with vacuum

c) Turn off the vacuum and allow the cartridge to soak for about 1 minute

d) Draw the remaining solvent through the cartridge to waste

e) Allow the cartridge to dry for 2 minutes under full vacuum

2. Condition Cartridge

a) Add 10 mL of methanol

b) Draw a small amount through the cartridge

c) Let soak for about one minute

d) Draw most of the remaining methanol through the cartridge, leaving 3 to 5 mm of methanol on the surface of the cartridge frit

e) Immediately add 20 mL of reagent water to the cartridge and draw most of the water through leaving 3 to 5 mm on the top of the cartridge frit

Note: Do not let the cartridge dry out after the addition of water

f) Add 5 ml of methanol to the water sample (dechlorinated and pH adjusted to ≤2) and mix well

g) Add the water sample to the cartridge and under vacuum, filter at a rate of approximately 50 mL per minute

h) Drain as much water from sample bottle as possible

i) Dry the cartridge under vacuum for 10 minutes

Note: Exceeding a 10-minute dry time could result in low recoveries. For faster drying, remove the cartridge and tapping the excess moisture from the bottom of the cartridge before continuing vacuum drying

3. Elution

a) Insert a suitable sample tube for eluate collection

b) Add 10 mL of EtOAc to the sample bottle

c) Rinse the sample bottle thoroughly

d) Transfer the solvent to the cartridge with a disposable pipette, rinsing sides of filtration reservoir

e) Draw half of solvent through cartridge then release the vacuum. Allow the remaining solvent to soak the cartridge for about one minute

f) Draw remainder through under vacuum

g) Repeat the solvent rinse of the sample bottle and apparatus using 10 mL of 1:1 EtOAc:$MeCl_2$

h) Using a disposable pipette, rinse down the sides of the cartridge and bottle holder with another 10 mL aliquot of 1: 1 EtOAc:$MeCl_2$

i) Add the rinse to the cartridge, then draw through

4. Dry the combined eluant

a) Use granular anhydrous sodium sulfate

b) Rinse the collection tube and sodium sulfate with 2 x 5 mL portions of $MeCl_2$ and place combined solvent in a concentrator tube

c) Draw through using vacuum

d) Concentrate the extract to 1 mL under gentle stream of nitrogen (may be warmed gently) being careful not to spatter the contents

Note: Do not concentrate to <0.5 mL or loss of analytes could occur. Rapid extract concentration could result in loss of low molecular weight analytes

5. Analyze by GC/MS

Revision 2.0, 1995. Method authors: Eichelberger, J. W., Behymer,T .D. Budde, W L., Munch, J., National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268

This summary highlights major steps in the 525.2 method. Complete details about the preparation and composition of reagent solutions can be found in method and should be referenced by anyone needing complete details. It is available as a part of Supplement 11 from National Technical Information Service (NTIS), Springfield, VA 22161; publication PB 92 207703. (800) 553-6847 or at www.epa.gov/safewater/methods/methods.html

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Semivolatile Organic Compounds In Drinking Water By Solid-Phase Extraction And Capillary Column (GC/MS) **EPA Method 525.3** Version 1.0

UCT Part Number:
ECUNI525 (1500 mg 525 C18, 83 mL cartridge)
or
EC525006-P (1500 mg 525 C18, 6 mL cartridge, PE Frit)

Method Summary

A 1-liter water sample is fortified with surrogate analytes then extracted using a solid phase extraction (SPE) cartridge (ECUNI525). Analytes are eluted from the solid phase with a small amount of organic solvents. The extract is dried using anhydrous sodium sulfate and concentrated to approximately 0.7 mL using N_2. IS are added and the volume adjusted to 1 mL with ethyl acetate. A splitless injection is made into a GC equipped with a capillary column, interfaced to an MS with either scan, SIM or SIS detection used for analysis. **The GC/MS may be calibrated using standards prepared in solvent or using matrix-matched standards.**

Internal standards are added after the extract concentration step. If the analyte pentachlorophenol is being measured, use IS ^{13}C-pentachlorophenol at 1000 µg/mL.

Sample Preservation

Preservation reagents, listed in the table below, are added to each sample bottle as dry solids prior to shipment to the field (or prior to sample collection).

Compound	Amount	Purpose
L-ascorbic acid	0.10 g/L	Dechlorination
Ethylenediaminetetraacetic acid, trisodium salt (EDTA)	0.35 g/L	Inhibit metal-catalyzed hydrolysis of targets
Potassium dihydrogen citrate	9.4 g/L	pH 3.8 buffer, microbial inhibitor

Procedure

1. Cartridge Clean-up

a) Assemble an extraction system

b) Rinse bottle holders and cartridges with 5 mL 1:1 EtOAc:DCM (ethyl acetate:dichloromethane)

c) Draw half the volume through the cartridge and then soak for 1 min

d) Draw remaining solvent through the cartridge

e) Maintain full vacuum for 2 min to dry cartridge

2. Cartridge Conditioning

a) Add 10 ml of methanol to each cartridge

b) Soak for 1 minute

c) Draw through leaving a thin layer of methanol on the cartridge frit

Note: Do not let the cartridge go dry from this point until elution, otherwise recondition

d) Add 10 mL of reagent water to each cartridge

e) Draw through leaving a thin layer of water on the cartridge frit

3. Sample Extraction

a) All field and QC samples, including LRBs and LFBs, must contain preservatives

b) Ensure that sample pH is ≤ 4 (use a pH meter for reagent water)

c) Place sample bottle(s) in holder

d) Adjust vacuum to fast drip flow rate. A flow of 10 mL/min is optimum

e) After entire sample has extracted, rinse bottle with 10 mL reagent water

f) Add rinse to cartridge

g) Rinse cartridge using 10 mL reagent water to remove sample preservatives

h) Dry cartridge for 10 min under full vacuum or nitrogen positive pressure

4. Cartridge Elution

a) Insert 40 mL glass vial in manifold

b) Rinse bottle, holder, and cartridge with 5 mL EtOAc

c) Pour rinsate into cartridge

d) Draw ½ volume through cartridge, soak 1 min then draw through completely

e) Repeat using 5 mL DCM rinse

f) Repeat using 5 mL EtOAc

g) Repeat using 5 mL DCM

5. Extract Drying

a) Pre-rinse a drying tube containing 10-20 g of anhydrous sodium sulfate with DCM

b) Quantitatively transfer the eluant through the sodium sulfate tube and collect

c) Rinse the collection tube 2 x 5 mL of DCM

d) Pass the DCM through the sodium sulfate and collect

6. Extract Concentration

a) Concentrate extract to about 0.7 mL (not < 0.5 mL) under a gentle stream of N_2 in a water bath at 40 °C

b) Transfer to a 1 mL volumetric flask, add IS and bring to volume using EtOAc

7. Analyze by GC/MS

Internal Standards	CASRN	Solvent	PDS conc.
acenaphthene-*d*10 (IS 1)	15067-26-2	acetone	500 µg/mL
phenanthrene-*d*10 (IS 2)	1517-22-2	acetone	500 µg/mL
chrysene-*d*12 (IS 3)	1719-03-5	acetone	500 µg/mL
^{13}C-pentachlorophenol (IS 4)	85380-74-1	methanol	1000 µg/mL

Surrogates	CASRN	Solvent	PDS conc.
1,3-dimethyl-2-nitrobenzene (SUR 1)	81-20-9	acetone	500 µg/mL
triphenyl phosphate (SUR 2)	115-86-6	acetone	500 µg/mL
benzo[*a*]pyrene-*d*12 (SUR 3)	63466-71-7	acetone	500 µg/mL

Instrument Conditions for GC Analysis	
Agilent 5975C MSD with 6890N GC	**Restek RXI-5sil-MS 30m x 0.25 mm x 0.25 µm column**
4-mm i.d. splitless gooseneck injection port liner	**UCT#GCLGN4MM**
Injection Port	**250 °C**
Injection Vol	**1 µL with 1 min split delay**
GC Oven Temp	**Initial: 55 °C, hold 1 min Ramp 10 °C/min to 200 °C Ramp 7 °C/min to final T 320 °C Hold 0.36 min**

Precision and Accuracy Data Obtained for Method 525.3 Analytes Fortified in Reagent Water at Three Concentrations and Extracted Using UCT 525 Universal Cartridges; N=4; Full Scan GC/MS Analyses[a]

Analytes	Fortified Conc. 0.25 µg/L[b]		Fortified Conc. 2.0 µg/L[c]		Fortified Conc. 5.0 µg/L[d]	
	Mean % Recovery	RSD	Mean % Recovery	RSD	Mean % Recovery	RSD
acenaphthylene	101	2.0	93.6	0.51	99.8	0.88
acetochlor	99.0	3.9	93.6	2.1	104	2.1
alachlor	100	7.3	89.8	0.72	92.8	1.0
aldrin	77.0	5.0	78.4	2.9	85.0	3.4
ametryn	105	4.8	93.1	1.3	95.8	1.1
anthracene	106	3.8	92.3	1.0	104	0.71
atraton	112	2.9	90.3	4.1	96.8	2.2
atrazine	111	3.5	96.1	3.2	97.3	1.5
benzo[*a*]anthracene	112	5.1	99.1	3.8	112	3.6
benzo[*a*]pyrene	109	5.5	103	1.7	111	1.2
benzo[*b*]fluoranthene	119	5.0	102	1.2	114	2.4
benzo[*g,h,i*]perylene	112	2.9	102	4.3	113	2.8
benzo[*k*]fluoranthene	105	1.9	103	2.4	113	3.1
BHT	ND[e]		ND		ND	
bromacil	102	9.3	98.9	0.86	103	2.1
butachlor	107	3.6	86.3	1.1	99.4	1.4
butylate	85.0	7.1	83.0	2.2	84.0	3.0
butylbenzylphthalate	122	1.9	95.9	3.7	114	3.6
chlordane, cis	98.0	5.3	102	2.5	101	1.3
chlordane, trans	103	1.9	103	2.3	96.6	0.71
chlorfenvinphos	113	1.8	110	2.5	111	3.9
chlorobenzilate	82.0	9.3	99.8	5.1	94.1	1.3
chloroneb	93.0	2.2	92.0	2.9	100	1.4
chlorothalonil	116	2.8	106	3.8	105	1.5
chlorpropham	109	3.5	93.1	2.5	98.6	1.1
chlorpyrifos	102	5.1	93.4	3.3	97.2	2.5
chrysene	117	1.7	97.1	1.7	114	2.1
cyanazine	99.0	3.9	88.1	4.9	106	2.4
cycloate	102	3.9	87.4	1.0	88.8	1.2
dacthal (DCPA)	105	3.6	102	3.5	101	1.7
DDD, 4,4′-	107	3.6	85.8	0.75	105	1.4
DDE, 4,4′-	99.0	3.9	82.3	1.3	101	1.0
DDT, 4,4′-	116	2.8	87.6	2.8	112	0.83
DEET	103	1.9	98.3	3.2	104	2.0
di(2-ethylhexyl)adipate	112	4.1	96.6	3.1	111	1.8
di(2-ethylhexyl)phthalate	137	3.7	97.6	1.3	110	2.4
dibenzo[*a,h*]anthracene	110	3.6	95.4	2.5	109	1.5
dibutyl phthalate	115	3.3	101	1.5	114	2.6
dichlorvos	104	3.1	91.6	1.7	88.8	2.7
dieldrin	103	1.9	87.4	0.55	98.1	0.39
diethylphthalate	111	1.8	111	2.3	114	1.4
dimethipin	24.0	14	29.5	6.5	24.9	2.5

dimethylphthalate	110	3.6	113	0.25	113	0.78
DIMP	112	5.8	90.0	1.9	93.7	3.0
dinitrotoluene, 2,4-	126	1.8	105	2.6	113	2.5
dinitrotoluene, 2,6-	121	1.7	106	0.71	111	0.67
diphenamid	106	2.2	95.1	0.90	97.8	0.69
disulfoton	79.0	2.5	91.5	8.9	85.3	1.5
endosulfan I	95.0	5.3	88.4	1.3	101	1.0
endosulfan II	103	1.9	89.6	3.5	103	1.0
endosulfan sulfate	112	7.1	96.5	2.4	106	0.75
endrin	89.0	5.7	82.9	3.4	91.3	4.0
EPTC	89.0	2.2	88.0	0.80	85.8	0.60
ethion	106	2.2	100	2.7	108	3.1
ethoprop	110	2.1	91.3	1.6	96.2	1.4
ethyl parathion	117	4.3	97.6	2.3	105	3.5
etridiazole	118	3.4	90.6	2.2	101	1.5
fenarimol	110	4.7	87.1	2.0	91.7	3.4
fluorene	106	3.8	97.5	2.1	101	1.1
fluridone	92.0	5.0	103	4.9	98.6	2.9
HCCPD	92.0	3.5	65.6	1.7	68.0	5.8
HCH, α	101	3.8	92.5	1.3	95.2	0.47
HCH, β	101	3.8	94.0	4.0	102	1.7
HCH, δ	97.0	6.2	96.4	1.3	101	0.49
HCH, γ (lindane)	90.0	4.4	95.6	2.1	97.9	1.8
heptachlor	96.0	3.4	83.1	2.0	86.2	1.2
heptachlor epoxide	104	3.1	86.9	2.0	95.9	1.6
hexachlorobenzene	94.0	5.5	78.4	3.8	93.0	1.9
hexazinone	107	1.9	84.6	1.7	94.6	2.6
indeno[1,2,3-*c,d*]pyrene	113	4.5	95.0	2.1	112	2.4
isophorone	108	3.0	108	3.2	102	1.1
methoxychlor	122	1.9	89.9	1.5	109	0.72
methyl parathion	129	3.0	103	2.1	112	2.5
metolachlor	109	1.8	93.1	1.1	97.8	0.42
metribuzin	116	2.8	97.3	0.30	106	2.5
mevinphos	115	3.3	96.1	3.1	97.0	1.5
MGK 264(a)	94.0	2.5	75.5	1.9	88.3	4.1
MGK 264(b)	94.0	2.5	82.8	0.35	92.3	0.80
molinate	88.0	3.7	89.4	1.2	88.9	2.1
napropamide	105	3.6	89.9	2.4	99.2	1.5
nitrofen	129	3.0	106	2.7	113	3.9
nonachlor, trans	119	3.2	103	2.3	96.2	1.0
norflurazon	106	2.2	91.9	1.6	102	1.6
oxyfluorfen	129	1.6	93.9	2.9	111	3.5
pebulate	85.0	8.0	84.5	1.7	84.7	2.4
pentachlorophenol	104	4.1	100	1.3	96.0	3.6
permethrin, cis	110	3.6	107	1.3	107	2.2
permethrin, trans	115	3.3	96.1	3.1	97.0	1.5
phenanthrene	94.0	2.5	75.5	1.9	88.3	4.1
phorate	94.0	2.5	82.8	0.35	92.3	0.80
phosphamidon	88.0	3.7	89.4	1.2	88.9	2.1
profenofos	105	3.6	89.9	2.4	99.2	1.5
prometon	129	3.0	106	2.7	113	3.9

prometryn	119	3.2	103	2.3	96.2	1.0
pronamide	106	2.2	91.9	1.6	102	1.6
propachlor	129	1.6	93.9	2.9	111	3.5
propazine	85.0	8.0	84.5	1.7	84.7	2.4
pyrene	104	4.1	100	1.3	96.0	3.6
simazine	110	3.6	107	1.3	107	2.2
simetryn	115	3.3	96.1	3.1	97.0	1.5
tebuconazole	94.0	2.5	75.5	1.9	88.3	4.1
tebuthiuron	94.0	2.5	82.8	0.35	92.3	0.80
terbacil	88.0	3.7	89.4	1.2	88.9	2.1
terbutryn	105	3.6	89.9	2.4	99.2	1.5
tetrachlorvinphos	129	3.0	106	2.7	113	3.9
triadimefon	119	3.2	103	2.3	96.2	1.0
tribufos+merphos	106	2.2	91.9	1.6	102	1.6
trifluralin	129	1.6	93.9	2.9	111	3.5
vernolate	85.0	8.0	84.5	1.7	84.7	2.4
vinclozolin	104	4.1	100	1.3	96.0	3.6
PCB Congeners by UPAC#						
2-chlorobiphenyl (1)	75.0	2.7	81.0	2.6	85.0	1.1
4-chlorobiphenyl (3)	85.0	2.4	84.4	2.8	88.9	1.5
2,4′-dichlorobiphenyl (8)	85.0	2.4	82.5	2.2	87.1	0.51
2,2′,5-trichlorobiphenyl (18)	104	3.1	89.3	2.9	95.3	4.0
2,4,4′-trichlorobiphenyl (28)	81.0	2.5	88.6	3.3	92.4	0.48
2,2′,3,5′-tetrachlorobiphenyl (44)	85.0	9.7	91.1	3.1	93.4	1.6
2,2′,5,5′-tetrachlorobiphenyl (52)	84.0	3.9	90.3	4.4	96.4	1.1
2,3′,4′,5-tetrachloroobiphenyl (70)	84.0	3.9	92.4	3.4	97.0	0.94
2,3,3′,4′,6-pentachlorobiphenyl (110)	81.0	2.5	94.3	3.2	97.1	0.90
2,3′,4,4′,5-pentachlorobiphenyl (118)	90.0	2.6	94.5	3.7	98.8	1.1
2,2′,3,4,4′,5′-hexachlorobiphenyl (138)	86.0	4.7	99.0	3.2	105	1.3
2,2′,3,4′,5′,6-hexachlorobiphenyl (149)	87.0	5.8	96.5	4.3	101	1.2
2,2′,4,4′,5,5′-hexachlorobiphenyl (153)	79.0	2.5	96.9	2.8	101	1.1
2,2′,3,4,4′,5,5′-heptachlorobiphenyl (180)	101	2.0	93.1	2.9	91.6	2.2
Surrogate Analytes						
1,3-dimethyl-2-nitrobenzene	92.9	4.0	98.9	3.1	88.8	4.0
benzo[*a*]pyrene-*d*12	112	2.1	101	2.6	101	4.9
triphenyl phosphate	107	2.5	97.9	4.1	104	3.2

a. Data obtained on the instrumentation described in Sect. 13.1.1.4
b. Exceptions to the stated concentration are as follows: Surrogate concentrations are 5.0 µg/L, pentachlorophenol is 1.0 µg/L, c-permethrin is 0.13 µg/L, t-permethrin is 0.38 µg/L, MGK 264 (a) is 0.085 µg/L and MGK 264 (b) is 0.17 µg/L.

c. Exceptions to the stated concentration are as follows: Surrogate concentrations are 5.0 µg/L, pentachlorophenol is 8.0 µg/L, c-permethrin is 1.0 µg/L, t-permethrin is 3.0 µg/L, MGK 264 (a) is 0.67 µg/L and MGK 264 (b) is 1.3 µg/L.
d. Exceptions to the stated concentration are as follows: Surrogate concentrations are 5.0 µg/L, pentachlorophenol is 20.0 µg/L, c-permethrin is 2.5 µg/L, and t-permethrin is 7.5 µg/L, MGK 264 (a) is 1.7 µg/L and MGK 264 (b) is 3.3 µg/L.
e. ND = Not determined.

Precision and Accuracy Data Obtained for Method Analytes Fortified into Finished Drinking Waters from Ground and Surface Water Sources, and Extracted Using UCT 525 Universal Cartridges; N=4; Full Scan GC/MS Analyses[a]

Analytes	Fortified Conc. (µg/L)	Ground Water[b]		Surface Water[c]	
		Mean % Recovery[d]	RSD	Mean % Recovery[d]	RSD
acenaphthylene	2.0	99.3	5.7	95.1	1.8
acetochlor	2.0	97.4	5.5	106	4.9
alachlor	2.0	93.5	3.5	95.0	3.3
aldrin	2.0	94.9	4.0	81.1	0.59
ametryn	2.0	98.9	5.7	93.4	6.2
anthracene	2.0	102	4.7	101	1.6
atraton	2.0	92.6	5.9	87.3	5.3
atrazine	2.0	100	4.6	95.6	1.5
benzo[*a*]anthracene	2.0	104	1.5	102	2.4
benzo[*a*]pyrene	2.0	106	3.2	100	2.7
benzo[*b*]fluoranthene	2.0	104	3.3	100	3.9
benzo[*g,h,i*]perylene	2.0	101	3.9	101	5.3
benzo[*k*]fluoranthene	2.0	103	3.0	101	2.6
BHT	2.0	95.5	1.2	114	1.9
bromacil	2.0	98.9	5.5	104	4.6
butachlor	2.0	96.4	2.9	95.6	3.3
butylate	2.0	87.6	3.2	83.6	1.8
butylbenzylphthalate	2.0	107	4.1	107	2.9
chlordane, cis-	2.0	96.1	6.2	98.0	5.9
chlordane, trans	2.0	94.5	5.9	98.3	7.3
chlorfenvinphos	2.0	93.1	3.9	111	3.4
chlorobenzilate	2.0	101	5.6	97.1	4.3
chloroneb	2.0	104	2.0	108	4.0
chlorothalonil	2.0	108	1.5	110	2.2
chlorpropham	2.0	95.9	5.6	98.1	2.4
chlorpyrifos	2.0	97.8	5.3	103	4.1
chrysene	2.0	106	2.2	100	3.2
cyanazine	2.0	97.4	5.2	91.0	11
cycloate	2.0	92.3	5.6	95.1	1.6
dacthal (DCPA)	2.0	92.1	7.3	107	4.3
DDD, 4,4′-	2.0	93.1	3.7	91.0	2.0
DDE, 4,4′-	2.0	90.0	3.6	85.6	2.7
DDT, 4,4′-	2.0	91.4	3.7	90.4	3.5
DEET	2.0	101	1.8	109	1.1
di(2-ethylhexyl)adipate	2.0	102	5.8	106	3.4
di(2-ethylhexyl)phthalate	2.0	107	2.7	104	2.2

dibenzo[*a,h*]anthracene	2.0	106	6.2	102	2.2
dibutyl phthalate	2.0	110	3.7	107	1.5
dichlorvos	2.0	90.4	7.3	88.8	2.3
dieldrin	2.0	96.0	5.4	96.3	2.6
diethylphthalate	2.0	110	2.5	107	1.1
dimethipin	2.0	29.4	8.7	38.1	4.2
dimethylphthalate	2.0	111	2.4	111	1.0
DIMP	2.0	89.6	5.9	99.6	5.9
dinitrotoluene, 2,4-	2.0	95.6	7.0	105	4.1
dinitrotoluene, 2,6-	2.0	95.6	6.2	101	5.0
diphenamid	2.0	98.5	5.6	101	2.3
disulfoton	2.0	79.6	5.9	103	6.3
endosulfan I	2.0	96.9	7.0	93.0	4.5
endosulfan II	2.0	98.5	5.7	94.3	4.1
endosulfan sulfate	2.0	102	2.8	100	2.1
endrin	2.0	97.9	2.6	88.1	3.4
EPTC	2.0	89.0	7.2	85.8	3.0
ethion	2.0	95.8	6.9	98.9	4.3
ethoprop	2.0	94.9	5.7	103	3.9
ethyl parathion	2.0	97.6	5.9	103	3.0
etridiazole	2.0	104	2.4	104	3.1
fenarimol	2.0	93.5	4.4	86.6	3.3
fluorene	2.0	101	5.1	104	1.8
fluridone	2.0	111	3.9	97.4	6.0
HCCPD	2.0	64.6	6.7	69.1	2.8
HCH, α	2.0	93.5	5.7	91.8	2.4
HCH, β	2.0	97.9	4.2	97.8	1.8
HCH, δ	2.0	102	6.4	94.3	3.7
HCH, γ (lindane)	2.0	100	6.8	90.0	2.1
heptachlor	2.0	91.0	3.0	86.1	1.5
heptachlor epoxide	2.0	98.3	6.2	91.6	1.6
hexachlorobenzene	2.0	90.4	4.0	89.0	2.7
hexazinone	2.0	93.4	3.1	97.0	8.1
indeno[1,2,3-*c,d*]pyrene	2.0	107	6.6	103	4.6
isophorone	2.0	104	3.9	101	2.6
methoxychlor	2.0	94.0	5.6	94.0	2.6
methyl parathion	2.0	100	3.7	110	2.5
metolachlor	2.0	97.5	5.6	97.8	2.1
metribuzin	2.0	102	2.6	120	0.77
mevinphos	2.0	89.9	5.3	96.3	4.5
MGK 264(a)	1.3	92.9	2.3	86.4	4.1
MGK 264(b)	0.67	95.9	4.1	92.6	3.9
molinate	2.0	94.9	5.0	89.1	2.3
napropamide	2.0	95.9	4.6	104	3.1
nitrofen	2.0	111	6.2	96.4	5.3
nonachlor, trans	2.0	105	4.0	98.4	5.3
norflurazon	2.0	98.1	3.8	101	6.4
oxyfluorfen	2.0	91.8	7.7	94.9	5.0
pebulate	2.0	89.3	4.4	84.3	1.0
pentachlorophenol	8.0	98.3	2.6	97.3	2.9
permethrin, cis	1.0	92.6	6.0	108	4.3

permethrin, trans	3.0	91.4	7.1	100	2.3
phenanthrene	2.0	106	3.7	104	2.5
phorate	2.0	95.5	2.0	98.9	4.8
phosphamidon	2.0	100	3.3	114	2.8
profenofos	2.0	98.4	4.4	108	1.7
prometon	2.0	96.9	2.1	88.4	5.7
prometryn	2.0	98.8	4.9	94.6	4.9
pronamide	2.0	95.1	5.5	96.6	3.4
propachlor	2.0	106	1.4	110	3.4
propazine	2.0	101	2.8	96.4	2.5
pyrene	2.0	106	1.2	107	2.2
simazine	2.0	101	3.7	96.1	3.0
simetryn	2.0	98.6	4.2	87.9	3.8
tebuconazole	2.0	94.4	5.4	96.0	5.2
tebuthiuron	2.0	88.5	7.1	101	1.8
terbacil	2.0	101	5.6	95.8	10
terbutryn	2.0	97.3	5.1	87.4	5.1
tetrachlorvinphos	2.0	97.5	6.6	104	5.4
triadimefon	2.0	99.3	2.1	101	3.0
tribufos+merphos	4.0	96.6	7.3	107	2.0
trifluralin	2.0	90.8	2.2	90.0	4.5
vernolate	2.0	88.0	6.0	88.3	1.8
vinclozolin	2.0	98.6	7.0	111	1.7
PCB Congeners by IUPAC#					
2-chlorobiphenyl (1)	2.0	90.8	1.7	96.3	5.6
4-chlorobiphenyl (3)	2.0	96.1	0.65	99.9	5.4
2,4′-dichlorobiphenyl (8)	2.0	97.9	0.87	86.6	6.3
2,2′,5-trichlorobiphenyl (18)	2.0	101	3.8	90.9	7.5
2,4,4′-trichlorobiphenyl (28)	2.0	101	2.2	86.1	4.9
2,2′,3,5′-tetrachlorobiphenyl (44)	2.0	93.0	3.0	88.1	6.9
2,2′,5,5′-tetrachlorobiphenyl (52)	2.0	97.1	3.1	87.8	6.6
2,3′,4′,5-tetrachloroobiphenyl (70)	2.0	107	1.5	88.1	5.2
2,3,3′,4′,6-pentachlorobiphenyl (110)	2.0	107	1.3	92.0	6.3
2,3′,4,4′,5-pentachlorobiphenyl (118)	2.0	108	1.2	91.3	6.4
2,2′,3,4,4′,5′-hexachlorobiphenyl (138)	2.0	110	2.0	93.8	5.3
2,2′,3,4′,5′,6-hexachlorobiphenyl (149)	2.0	106	1.4	91.5	6.0
2,2′,4,4′,5,5′-hexachlorobiphenyl (153)	2.0	108	0.89	91.3	6.3
2,2′,3,4,4′,5,5′-heptachlorobiphenyl (180)	2.0	99.6	1.0	87.6	7.2
Surrogate Analytes					

1,3-dimethyl-2-nitrobenzene	5.0	91.7	7.5	89.0	6.5
benzo[*a*]pyrene-*d*12	5.0	103	2.7	104	3.0
triphenyl phosphate	5.0	104	0.58	112	3.5

a. Data obtained on the instrumentation described in Sect. 13.1.1.4.
b. Tap water from a ground water source with high mineral content. Tap water hardness was 300mg/L as calcium carbonate
c. Tap water from a surface water source. TOC of 2.4 mg/L
d. Recoveries have been corrected to reflect the native amount in the unfortified matrix water.

Toxaphene from Fortified Reagent Water Precision and Accuracy: Extracts Analyzed by SIM

Fortified Concentration 10 µg/L N=4	Mean % Recovery	RSD
Toxaphene	111	1.8

Method 525.3, "Determination Of Semivolatile Organic Chemicals In Drinking Water By Solid Phase Extraction And Capillary Column Gas Chromatography/ Mass Spectrometry (GC/MS)," Ver 1.0, February 2012, Jean W. Munch and Paul E. Grimmett (U.S. EPA, Office of Research and Development, National Exposure Research Laboratory), David J. Munch and Steven C. Wendelken (U.S. EPA, Office of Water, Office of Ground Water and Drinking Water, Technical Support Center) Mark M. Domino (Industrial and Environmental Services, LLC) Alan D. Zaffiro and Michael L. Zimmerman (Shaw Environmental and Infrastructure, Inc.), National Exposure Research Laboratory Office Of Research And Development, U. S. Environmental Protection Agency, Cincinnati, Ohio 45268

Complete details at www.epa.gov/safewater/methods/methods.html

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Determination of the 11 Fourth Unregulated Contaminant Monitoring Rule (UCMR4) Compounds by EPA Method 525.3

UCT Part Numbers:
ECHLD156-P - Enviro-Clean® HL DVB 500 mg, 6 mL cartridge, PE Frit
VMFSTFR12 - Large volume sample transfer tubes
VMF016GL - 16 position glass block manifold
VMF02125 - 12 position large volume collection rack
RFV1F15P - 15 mL reservoirs with 1 frit, 10 micron porosity
ECSS25K - Sodium sulfate, anhydrous, ACS grade, granular, 60 mesh
GCLGN4MM-5 - GC liner, 4mm splitless gooseneck, 4mm ID x 6.5mm OD x 78.5mm

Summary:

The US Environmental Protection Agency (EPA) uses the Unregulated Contaminant Monitoring Program to collect data for contaminants suspected to be present in drinking water but do not have health-based standards set under the Safe Drinking Water Act [1]. Every five years the US EPA reviews and issues a list of contaminants (30 or less) to be monitored; largely based on the Contaminant Candidate List. The US EPA and its contracting laboratories are currently developing and publishing testing methods for the UCMR4 contaminants. These compounds will be monitored by public drinking water systems no later than 2018. Among the EPA issued UCMR4 compound list there are 11 analytes (disulfoton, ethoprop, alpha-Hexachlorocyclohexane, trans- permethrin, cis-permethrin, tribufos, profenofos, oxyfluorfen, vinclozolin, dimethipin, and tebuconazole) that can be analyzed by EPA Method 525.3.

A 1-liter finished drinking water sample is extracted using a 6 mL solid phase extraction (SPE) cartridge containing 500 mg of DVB based polymeric sorbent. The analytes are retained on the sorbent and then eluted with a small amount of organic solvents. The extract is dried using anhydrous sodium sulfate and concentrated to 1 mL. The final extract is analyzed by GC/MS equipped with a capillary column using either full scan or SIM mode. Calibration standards can be prepared in solvent (ethyl acetate) or matrix matched standards obtained by spiking known amounts of target analytes to the 1-mL final extracts from the extracted blank samples.

Sample Preservation:

Preservation reagents, including 0.1 g of L-ascorbic acid (dechlorination), 0.35 g of EDTA (to inhibit metal-catalyzed hydrolysis), and 9.4 g of potassium dihydrogen citrate (pH 3.8 buffer and microbial inhibitor), are added to each 1-L sample bottle prior to shipment to the sampling field.

SPE Procedure:

1. Add surrogates to the 1-L preserved water sample, and target analytes for spiked samples, and mix well.

   Note: Spiking solutions should be prepared in water miscible solvents, such as methanol and acetone.
2. Attach the SPE cartridges (**ECHLD156P**) to the 16 position glass block manifold (**VMF016GL**).
3. Wash the SPE cartridges with 6 mL dichloromethane (DCM), let the sorbent soak for 1 min, draw the DCM through and leave full vacuum on for 1 min. Repeat the wash with 6 mL DCM.
4. Condition the SPE cartridges with 10 mL methanol, draw the methanol through slowly and leave a thin solvent layer above the frit. Equilibrate the SPE cartridges with 10 mL DI water, draw the water through and leave about 1” layer above the frit.
5. Attach the large volume sample transfer tubes (**VMFSTFR12**) to the top of the SPE cartridges. Adjust the vacuum for a fast dropwise flow (about 15 to 20 mL/min).
6. Rinse sample bottles with 10 mL DI water, and pass the rinsates to the SPE cartridges using the transfer tubes to remove the sample preservatives.
7. Dry the SPE cartridges under full vacuum for 10 min.
8. Insert the collection rack (**VMF02125**) with glass vials (40 - 60 mL volumes) into the manifold to collect the SPE eluates.
9. Rinse sample bottles with 10 mL ethyl acetate (EA), draw 1/3 though the SPE cartridges and let soak 2 min before passing the remaining EA through the SPE cartridges in a slow dropwise fashion. Leave full vacuum on for 1 min.
10. Repeat Step 9) with 10 mL DCM.

11. Dry the eluates by passing through the 15 mL reservoirs (**RFV1F15P**) holding about 15 g of anhydrous sodium sulfate (**ECSS25K**) and collect in a new glass vial (40 - 60 mL). Rinse the eluate vials with 10 mL DCM and apply the rinses to the sodium sulfate and collect.
12. Concentrate the dried eluates to about 0.8 mL using a TurboVap under a gentle stream of nitrogen in a water bath of 40 °C.
13. Transfer the concentrated extracts to 2-mL auto-sampler vials, rinse the glass vials with small quantities of EA and transfer the rinses to the 2-mL vials until 1 mL final volume is reached.
14. Add internal standards and inject 1 µL to GC/MS for analysis.

GC/MS method:

GC/MS	Agilent 6890N GC coupled to a 5975C MSD
Injection	1 µL splitless injection at 250 ºC
GC liner	UCT p/n **GCLGN4MM-5 -** 4 mm splitless gooseneck with deactivated glass wool
GC column	Restek Rxi®-5sil MS 30m x 0.25mm, 0.25µm with 10m integrated guard column
Carrier gas	Ultra high purity Helium at a constant flow of 1.2 mL/min
Oven temp. program	Initial temperature at 55 ºC, hold for 1 min; ramp at 10 ºC/min to 200 ºC; ramp at 7 ºC/min to 320 ºC; and hold for 0.36 min
Temperatures	Transfer line 280 ºC; Source 250 ºC; Quadrupole 150 ºC
Full scan range	45 - 500 amu

Results:

Recovery and RSD% of 1-L Drinking Water Spiked with 5 µg/L of Target Analytes

Compound Name	Recovery%	RSD% (n=5)
alpha-Hexachlorocyclohexane	86.2	1.8
Dimethipin	89.4	2.2
Disulfoton	90.7	3.4
Ethoprop	98.9	2.4
Oxyfluorfen	99.5	3.8
Permethrin, cis-	102.4	3.9
Permethrin, trans-	94.6	3.4
Profenofos	105.6	3.7
Tebuconazole	92.9	4.0
Tribufos+Merphos	88.2	3.8
Vinclozolin	89.7	2.5

References:

[1] http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/

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EPA Method 528 Determination of Phenols in Drinking Water by Solid Phase Extraction and GC/MS Detection

UCT Part Numbers:
ECHLD156-P - Enviro-Clean® HL DVB 500 mg, 6 mL cartridge, PE Frit
ECSS15M6 - 5g anhydrous sodium sulfate in 6 mL cartridge
AD0000AS - Cartridge adaptor

Summary:

EPA method 528 determines 12 phenols in finished drinking water using solid phase extraction (SPE) and GC/MS detection. Among the 12 phenols, 10 are listed as priority pollutants by U.S. EPA, the two exceptions are 2-methylphenol and 4-chloro-3-methylphenol. UCT has developed a new polystyrene divinylbenzene material for EPA method 528. One liter of drinking water sample is passed through the SPE cartridge using a large sample delivery tube. Phenols in the water samples are retained to the sorbent by hydrophobic interaction, and are eluted thereafter with dichloromethane (DCM). A drying cartridge packed with anhydrous sodium sulfate is attached to the end of the SPE cartridge in the elution step, eliminating the eluate drying step. This cross linked DVB sorbent demonstrates superior extraction performance with recoveries ranging from 85.1 to 108.4% and minimum lot-to-lot variations < 10% for 7 tested sorbent lots.

Experimental:

Sample pretreatment:

a) De-chlorinate 1 L of drinking water sample with 40-50 mg sodium sulfite if free chlorine is present, and acidify the sample to pH ≤ 2 with 6 N HCl.
b) Spike with appropriate amounts of surrogates, and target analytes for fortified samples.

SPE procedure:

a) Connect the large sample delivery tubes to the top of the SPE cartridges (**ECHLD156**), and attached the cartridges to a SPE manifold.
b) Wash the cartridges with 3 aliquots of 5 mL DCM. Condition the cartridges with 3 aliquots of 5 mL methanol. Do not let the sorbent go dry after applying the 3rd aliquot of methanol. Equilibrate the cartridges with 10 mL of 0.05 N HCl.
c) Insert the stainless steel ends of the sample delivery tubes into the sample containers, and draw the entire sample through the SPE cartridge at a fast drop-wise fashion (about 20 mL/min).
d) Remove the sample delivery tubes from the SPE cartridges and dry the cartridges under full vacuum for 15 min.
e) Attach the drying cartridges (**ECSS15M6**) to the bottom of the SPE cartridges with cartridge adaptors (**AD0000AS**) such that the elution solvent will pass through the SPE cartridge first and then the drying cartridge.
f) Insert test tubes or glass vials into the manifold, elute the SPE cartridges with 5 mL DCM, and repeat with sample bottle rinse using 10 mL DCM.
g) Concentrate the eluate to between 0.7 and 0.9 mL under a gentle stream of nitrogen at 35 ºC.
h) Add internal standards and adjust final volume to 1 mL with DCM. The samples are ready for GC/MS analysis.

GC/MS method:

GC/MS: Agilent 6890N GC with 5975C MSD

Injector: 1 µL splitless injection at 200 ºC

Liner: 4 mm splitless gooseneck liner with deactivated glass wool (**GCLGN4MM**)

GC column: Restek Rxi®-5sil MS 30m*0.25mm*0.25µm with 10 m guard column

Carrier gas: Helium at a constant flow of 1.0 mL/min

Oven: Initial temperature of 40 ºC, hold for 6 min; ramp at 8 ºC/min to 250 ºC.

Solvent delay: 10 min

Tune: dftpp.u

Full Scan: 45-350 amu

Results:

Accuracy and Precision Data

Target Analytes	Spiked (µg/L)	Single lot		Multiple lots (7)	
		Ave Recovery%	RSD% (n=5)	Ave Recovery%	RSD% (n=35)
Phenol	10	**88.2**	2.2	**86.4**	4.0
2-chlorophenol	10	**87.4**	1.3	**85.3**	3.5
2-methylphenol	10	**88.6**	1.5	**86.8**	3.6
2-nitrophenol	10	**85.6**	0.8	**85.5**	3.8
2,4-dimethylphenol	10	**88.4**	1.1	**85.1**	6.5
2,4-dichlorophenol	10	**87.4**	1.3	**86.5**	3.8
4-chloro-3-methylphenol	10	**90.4**	1.0	**89.5**	2.9
2,4,6-trichlorophenol	10	**88.3**	0.6	**87.8**	3.2
2,4-dinitrophenol	10	**103.2**	7.6	**108.4**	5.6
4-nitrophenol	10	**96.5**	1.2	**97.4**	4.2
2-methyl-4,6-dinitrophenol	10	**92.9**	2.5	**97.9**	6.7
Pentachlorophenol	10	**94.3**	1.1	**95.8**	4.7
Surrogates					
2-chlorophenol d4	2.5	**88.7**	1.6	**87.3**	4.7
2,4-dimethylphenol d3	2.5	**88.5**	1.4	**86.9**	6.6
2,4,6-tribromophenol	5.0	**88.7**	0.9	**89.5**	4.3

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EPA Method 529 Determination of Explosives in Drinking Water by Solid Phase Extraction and GC/MS Detection

UCT Part Numbers:
ECHLD156-P - Enviro-Clean® HL DVB 500 mg, 6 mL cartridge, PE Frit
ECSS15M6 - 5 g anhydrous sodium sulfate in 6 mL cartridge
AD0000AS - Cartridge adaptor

Summary:

EPA method 529 determines a variety of explosives and related compounds in finished drinking water. The analytes have sufficient volatility and thermal stability for GC/MS analysis and can be partitioned from aqueous samples onto a DVB solid phase extraction (SPE) sorbent. UCT has developed a novel DVB sorbent for the extraction of explosives in water. One liter of sample is passed through a 6-mL SPE cartridge packed with 500 mg of the DVB sorbent using a sample transfer tube. The explosives are retained on the DVB sorbent and then eluted with ethyl acetate (EtOAc). A drying cartridge packed with 5 grams of anhydrous sodium sulfate is attached to the bottom of the SPE cartridge using a cartridge adaptor in the elution step, eliminating the need of an additional extract drying step. Excellent recoveries and minimum lot-to-lot variations were obtained using this new DVB sorbent.

Procedure

1. Cartridge Conditioning

a) Preserve 1 L of the water sample with 50 mg sodium sulfite (dechlorinating agent), 0.5 g copper sulfate pentahydrate (microbial inhibitor) and 5 g pH 7 Trizma Pre-set Crystals (pH buffer).
b) Spike with appropriate amounts of surrogates, and target analytes for fortified samples.
c) Attach the SPE cartridges onto a multi-position manifold.
d) Wash the SPE cartridges with 5 mL EtOAc - pass 1/3 through the cartridge to wet the sorbent, allow the EtOAc to soak for 1 min before drawing the remaining solvent through. Repeat this process 2 additional times. Dry under full vacuum for 1 min.

e) Condition the cartridges with 3 aliquots of 5 mL methanol each. During this step and in subsequent steps do not allow the sorbent to go dry until instructed to do so in the drying step (step 8).

f) Equilibrate the cartridges with 2 aliquots of 10 mL DI water. After the second addition leave about 4 mL of water in the cartridge. Attach sample transfer lines (available from Restek p/n 26250) to the top of the SPE cartridges (**ECHLD156**).

2. Sample Extraction

a) Insert the weighted ends of the transfer lines into the 1 L sample bottles and draw the entire sample through the SPE cartridge in a fast, drop-wise fashion (10-15 mL/min).

b) Remove the transfer lines from the SPE cartridges and dry the SPE cartridges under full vacuum for 10 min.

c) Attach the drying cartridges (**ECSS15M6**) to the bottom of the SPE cartridges with the cartridge adaptors (**AD0000AS**).

3. Cartridge Elution

a) Insert a collection tube or vial into the manifold underneath each SPE cartridge.

b) Rinse each sample bottle with 5 mL EtOAc, and pull the rinsate through the SPE cartridges slowly using the transfer line. Turn full vacuum on for 1 min to pull all of the elution solvent into the collection container.

c) Remove the transfer line from the SPE cartridge. Add 5 mL EtOAc to the SPE cartridge, pass 1/3 though the cartridge, allow the cartridge to soak for 1 min and then draw the remainder through in a slow drop-wise fashion.

d) Concentrate the eluate to about 0.9 mL under a gentle stream of nitrogen at 40 ºC.

e) Add internal standard and adjust the final volume to 1 mL with EtOAc. The samples are ready for GC/MS analysis.

4. Sample Analysis

GC/MS method:
GC/MS: Agilent 6890N GC coupled with 5975C MSD
Injector: 1-2 µL PTV or cold on-column injection
GC column: Restek Rxi®-5sil MS 30mx0.25mm, 0.25µm with 10m guard column
Carrier gas: Helium at a constant flow of 1.2 mL/min
Oven: Initial temperature at 50 ºC, hold for 1 min; ramp at 8 ºC/min to 210 ºC; ramp at 20 ºC/min to 250 ºC, hold for 2 min.
Tune: bfb.u
Full Scan: 45-250 amu

Results:

Accuracy and Precision Data

Target analytes	Spiked (µg/L)	Single lot		Multiple lots (5)	
		Ave Recovery%	RSD% (n=5)	Ave Recovery%	RSD% (n=25)
Nitrobenzene d5 (Surrogate)	5	**92.4**	3.5	**88.9**	4.3
Nitrobenzene	5	**86.8**	2.7	**88.8**	4.6
2-Nitrotoluene	5	**87.6**	3.6	**89.1**	4.7
3-Nitrotoluene	5	**86.6**	3.6	**87.7**	4.6
4-Nitrotoluene	5	**84.4**	3.3	**87.2**	4.9
1,3-Dinitrobenzene	5	**102.4**	5.3	**99.7**	4.2
2,6-Dinitrotoluene	5	**98.2**	5.7	**97.3**	4.8
2,4-Dinitrotoluene	5	**91.2**	5.3	**92.9**	4.2
1,3,5-Trinitrobenzene	5	**100.0**	9.1	**100.4**	5.5
2,4,6-Trinitrotoluene	5	**103.0**	6.3	**100.9**	5.3
RDX	5	**107.0**	1.7	**111.1**	5.8
4-Amino-2,6-Dinitrotoluene	5	**100.1**	7.5	**99.6**	5.8
3,5-Dinitroaniline	5	**104.3**	5.6	**103.6**	6.3
2-Amino-4,6-Dinitrotoluene	5	**103.3**	5.2	**105.7**	5.0
Tetryl	5	**102.2**	3.7	**105.4**	4.7
Overall mean		**96.6**	**4.8**	**97.2**	**5.0**

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Determination of Phenylurea Compounds In Drinking Water By Solid-Phase Extraction And High Performance Liquid Chromatography With UV Detection

UCT Part Numbers:
EUC18156 (6 mL cartridge with 500 mg C18)
SLC-18150ID46-3UM - Selectra® C18, 150 x 4.6mm, 3µm
SLC-18GDC20-3UM - Selectra® C18, Guard column, 10 x 2.0mm, 3µm
SLGRDHLDR - Guard Cartridge Holder

EPA Method 532 Revision 1.0

This is an HPLC method for the determination of phenylurea pesticides in drinking water. It is applicable to phenylurea compounds that are efficiently extracted from the water using a C18 Solid-Phase cartridge. Accuracy, precision, and method detection limit (MDL) data have been generated for the following compounds* in reagent water and finished ground and surface waters.

Phenylurea Compounds

Analyte	CAS #
Diflubenzuron	35367-38-5
Diuron	330-54-1
Fluometuron	2164-17-2
Linuron	330-55-2
Propanil	709-98-8
Siduron	1982-49-6
Tebuthiuron	34014-18-1
Thidiazuron	51707-55-2

Method Summary

A 500 mL water sample is extracted using an SPE C18 cartridge. The phenylurea pesticides and surrogate compounds are captured by the solid-phase then analytes and surrogates are eluted with methanol. The extract is concentrated to a final volume of 1.0 mL. Analytes are chromatographically separated using an HPLC and detected using UV/Vis. Identification of target and surrogate analytes and quantitation is accomplished by retention times and analyte responses using external standard procedures. Sample extracts with positive results are solvent exchanged and confirmed using a second, dissimilar HPLC column that is also calibrated using external standard procedures.

Safety

- Each chemical compound should be treated as a potential health hazard, and exposure to these chemicals should be minimized using proper handling technique

Sample Collection, Preservation, and Storage

- Grab samples must be collected using 500 mL amber or clear glass bottles fitted with PTFE lined screw caps

- Prior to field shipment, sample bottles must contain, 0.25 g of cupric sulfate and 2.5 g of Trizma crystals to each bottle for each 500 mL of sample collected

- Alternately, the Tris buffer may be prepared by adding 2.35 g of Tris HCl and 0.15 g Tris to the sample bottle in addition to the 0.25 g of cupric sulfate

- When sampling from a cold water tap, remove the aerator so that no air bubbles will be trapped in the sample. Allow the system to flush until the water temperature has stabilized (usually about 3-5 minutes). Collect samples from the flowing system.

- When sampling from an open body of water, fill a 1 quart wide-mouth bottle or 1L beaker with sample. Carefully fill sample bottles from the container

- Sampling equipment, including automatic samplers, must be free of plastic tubing, gaskets, and other parts that may leach interfering analytes into the water sample

- Fill sample bottles, taking care not to over fill bottles so as not to flush out the sample preservation reagents. Samples do not need to be collected headspace free

- After collecting the sample, replace cover then agitate by hand for 1 minute. Keep samples sealed from collection time until extraction

Preservation Reagents

- Cupric Sulfate, $CuSO_4$ $5H_2O$ (ACS Grade or equivalent) – Added as a biocide to guard against potential degradation of method analytes by microorganisms

- Trizma Preset Crystals, pH 7.0 (Sigma # T 3503 or equivalent) – Reagent grade. A premixed blend of Tris [Tris(hydroxymethyl)aminomethane] and Tris HCL [Tris(hydroxymethyl)aminomethane hydrochloride]

- Alternatively, a mix of the two components with a weight ratio of 15.5/1; Tris HCL/Tris may be used. These blends produce a pH of 7.0 at 25°C in reagent water. Tris functions as a buffer, binding free chlorine in chlorinated finished waters and prevents the formation of a copper precipitate

Sample Shipment and Storage

- All samples should be iced during shipment and must not exceed 10° C during the first 48 hours after collection. Samples should be confirmed to be at or below 10°C when they are received at the laboratory

- Samples stored in the lab must be held at or below 6° C until extraction, but should not be frozen

Sample and Extract Holding Times

- Method analytes are stable for 14 days in water samples that are collected, dechlorinated, preserved, shipped and stored as described in above

- Samples must be extracted within 14 days

- Sample extracts may be stored in methanol at 0°C or less for up to 21 days after extraction. Samples that are exchanged into reagent water/acetonitrile (60/40) for confirmational analysis may be stored 7 days at 0° C or less. The combined extract holding time should not exceed
  21 days

Interferences

- All glassware must be meticulously cleaned. Wash glassware with detergent and tap water, rinse with tap water. Follow by a reagent water rinse

- Non-volumetric glassware can be heated in a muffle furnace at 400° C for 2 hours

- Method interferences may also be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware. This may lead to discrete artifacts or elevated baselines in the chromatograms

- All solvents and equipment must be routinely demonstrated to be free from interferences under the conditions of the analysis. **Subtracting blank values from sample results is not permitted in this method**

- Matrix interferences may be caused by contaminants that are co-extracted from the sample

Surrogate Analytes (SUR),

Monuron (CAS #150-68-5) & Carbazole (CAS# 86-74-8)

SUR STOCK SOLUTION (5 to 7 mg/mL)

- Accurately weigh 25 to 35 mg of the neat SUR to the nearest 0.1 mg into a tared, 5 mL volumetric flask. Dilute to the mark with the appropriate solvent, methanol for monuron and acetonitrile for cabazole
- Prepare each compound individually. They will be combined in the SUR primary dilution standard

SUR Primary Dilution Standard (500 µg/mL)

- Prepare the SUR Primary Dilution Standard (PDS) by dilution of the SUR stock standards. Add enough of each of the SUR stock standards to a volumetric flask partially filled with methanol to make a 500 µg/mL solution when filled to the mark with methanol

Analyte Stock Standard Solution

- Prepare analyte stock standard solutions for all compounds in methanol except thidiazuron and Diflubenzuron which are prepared in acetone
- Acetone elutes early in the chromatogram and will not interfere with compound quantitation
- Accurately weigh 10 to 12 mg of neat material to the nearest 0.1 mg in volumetric flasks. Thidiazuron is difficult to dissolve but 10 mg of pure material should dissolve in a 10 mL of acetone. Sonication may be used to help dissolve these compounds.

Analyte Primary Dilution Standard (PDS, 200 µg/mL and 10 µg/mL)

- Prepare the Analyte PDS by dilution of the stock standards. Add enough of each stock standard to a volumetric flask partially filled with methanol to make a 200 µg/mL solution when filled to the mark with methanol
- Once prepared, a dilution of the 200 µg/mL solution may be used to prepare a 10 µg/mL solution used for low concentration spiking. The PDS's can be used for preparation of calibration and fortification solutions
- Use at least 5 calibration concentrations to prepare the initial calibration curve
- The lowest concentration of calibration standard must be at or below the MRL
- In this method, 500 mL of an aqueous sample is concentrated to a 1 mL final extract volume

Preparation of Calibration Curve Standards					
CAL Level	PDS Conc µg/mL	Volume PDS Standard µL	Final Vol CAL Standard mL	Final Conc CAL Standard µg/mL	Equivalent Conc in 500 mL sample µg/L
1	10	25	1	0.25	0.50
2	10	50	1	0.50	1.00
3	200	5.0	1	1.00	2.00
4	200	25	1	5.00	10.0
5	200	50	1	10.0	20.0
6	200	75	1	15.0	30.0

Procedure

1. Cartridge Conditioning

a) Rinse the cartridge(s) using 5 mL of methanol

b) Slowly draw through leaving a layer covering the frit

c) Repeat using a second 5 mL aliquot and slowly draw through. Leave enough methanol to cover the frit

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

d) Add 5 mL of reagent water to the cartridge and draw through to the level of the frit

e) Repeat with a second 5 mL aliquot of reagent water

2. Sample Extraction

a) Add the water sample to the cartridge. Adjust vacuum setting for a flow rate of about 20 mL/minute

b) Rinse the sample container with reagent water and add to the cartridge

c) Once the sample has been extracted, turn vacuum setting to high and draw air through for 15 minutes to dry cartridge

3. Sample Elution

a) Insert a clean collection tube in the vacuum manifold

b) Add about 3-5 mL of methanol to each cartridge

c) Draw through to the top of the frit then turn off vacuum

d) Allow to soak for 30 seconds

e) Add a second 2-3 mL of methanol and draw through cartridge as a drip

f) Repeat methanol addition with a third 1-2 mL portion

4. Extract Concentration

a) Concentrate the extract to about 0.5 mL in a warm water bath 4° C under a gentle stream of N_2

b) Transfer to a 1 mL volumetric flask rinsing the collection tube with methanol

c) Bring to volume with methanol

d) Sample is ready for analysis

5. Solvent Exchange for Confirmation Analysis

a) Samples to be confirmed must be exchanged into reagent water/acetonitrile (60/40)

b) Transfer the remaining 980 µL of the extract to a 1 mL volumetric (or other appropriate collection tube)

c) Mark the sample volume then take the extract to dryness in a warm water bath (at ~ 40° C) under a gentle steam of N_2

d) Reconstitute the residue with a mixture of reagent water/acetonitrile (60/40) to the mark made before the extract was taken to dryness. Redissolve the film as thoroughly as possible. Use of a vortex mixer is recommended. Transfer to an appropriate autosampler vial

e) The best recoveries are obtained when extracts are immediately reconstituted

6. HPLC Analysis—Primary Analysis

Primary HPLC Column: **SLC-18150ID46-3UM -** Selectra® C18, 150 x 4.6mm, 3 µm

HPLC Conditions	
Solvent A	25 mM phosphate buffer
Solvent B	Acetonitrile
40% B	0-9.5 minutes
Linear gradient 40-50% B	9.5-10.0 minutes
Linear gradient 50-60% B	10.0-14.0 minutes
Linear gradient 60-40% B	14.0-15.0 minutes
Flow Rate	1.5 mL/minute
Wavelength	245 nm
Equilibration time prior to next injection = 15 minutes	

Chromatographic Retention Time Data for the Primary Column

Peak Number	Analyte	Retention Time (min)
1	Tebuthiuron	2.03
2	Thidiazuron	2.48
3	Monuron (SUR)	2.80
4	Fluometuron	4.45
5	Diuron	5.17
6	Propanil	8.53
7	Siduron A	8.91
8	Siduron B	9.76
9	Linuron	11.0
10	Carbazole (SUR)	12.8
11	Diflubenzuron	13.9

7. HPLC Analysis--Confirmation Analysis

Confirmation Column: Supelcosil 4.6 × 150 mm packed with 5 um cyanopropyl stationary phase or equivalent

Chromatographic Retention Time Data for the Confirmation Column

Peak Number	Analyte	Retention Time (min)
1	Tebuthiuron	2.56
2	Thidiazuron	3.98
3	Monuron (SUR)	4.93
4	Fluometuron	5.94
5	Diuron	7.67
6	Propanil	9.53
7	Siduron A	10.1
8	Siduron B	10.8
9	Linuron	12.2
10	Carbazole (SUR)	14.3
11	Diflubenzuron	15.2

*The analyst should refer to EPA Method 532 “Determination of Phenylurea Compounds in Drinking Water by Solid-Phase Extraction and High Performance Liquid Chromatography with UV Detection”, Revision 1.0 Issued June 2000, by M. V. Bassett, S.C. Wendelken, T.A. Dattilio, and B.V. Pepich (IT Corporation) D.J. Munch (US EPA, Office of Ground Water and Drinking Water) EPA, National Exposure Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Cincinnati, Ohio 45268

Measurement of Chloroacetanilide and Other Acetamide Herbicide Degradates in Drinking Water by Solid-Phase Extraction and Liquid Chromatography/ Tandem Mass Spectrometry (LC/MS/MS)

UCT Part Number:
EC535156 (500 mg graphitized carbon black (GCB) 90 m^2/g, 6 mL)

EPA METHOD 535

Method Summary

A 250 mL water sample is drawn through and captured on a **EC535156** cartridge containing 0.5 grams of nonporous graphitized carbon. Acetanilide and acetamide compounds are eluted from the cartridge using a small quantity of methanol containing 10 mM ammonium acetate. The methanol extract is concentrated to dryness by blow down with N_2 in a water bath at 65^{O}C then reconstituted with 1 mL of water containing 5 mM ammonium acetate. A 100 µL portion of the aqueous reconstitution is injected into an HPLC fitted with a C18 reverse phase analytical column. Detection occurs by tandem mass spectrometry and is compared to internal standards. A surrogate analyte of known concentration is measured with the same internal standard calibration procedure.

Interferences

Humic and/ or fulvic acid material, if present in the water source, is co-extracted with this method. High concentrations of these compounds can cause enhancement or suppression of the in the electrospray ionization source or low recoveries on the carbon SPE. Total organic carbon (TOC) is a good indicator of these interferences if present in the water sample.

1. Condition Cartridge

a) Rinse the cartridge with 20 mL of 10 mM ammonium acetate/methanol solution

b) Rinse cartridge with 30 mL of reagent water. Do not let water drop below level of cartridge packing

c) Add about 3 mL of reagent water to the top of the cartridge

Note: Do not let the cartridge go dry during any step otherwise start over

2. Sample Addition

a) Add sample water to the cartridge and adjust vacuum so the flow is about 10-15 mL/minute

b) Rinse cartridge with 5 mL of reagent water

c) Draw air or N_2 through the cartridge at high vacuum (10-15 in/Hg) for 3 minutes

3. Extract Elution

All glassware must be meticulously washed to avoid contamination

a) Insert a clean collection tube into the extraction manifold

b) Use 15 mL of 10 mM ammonium acetate/methanol and adjust vacuum to draw through at 5 mL/minute. Solvent will exit the cartridge in a dropwise fashion at this vacuum setting

4. Eluate Drying

a) Concentrate the extract to dryness under a gentle stream of N_2 in a heated water bath at 60°-70° C to remove all of the ammonium acetate/methanol

b) Reconstitute the dried eluate by adding 1 mL of 5 mM ammonium acetate/methanol solution

5. Extract Analysis

a) Establish operating conditions for the liquid chromatograph and mass spectrometer according to Tables 1-4 in Section 17. See Table A below for RT and precursor ions

b) If the analyte peak area exceed the range of the initial calibration curve, the extract may be diluted with 5 mM ammonium acetate/reagent water and adjusting internal standards to compensate for this dilution

Table A
Triple Quadrupole MS/MS Method Conditions

Analyte	Retention Time	Precursor Ion	Product Energy	Collision Energy
Propachlor OA	7.33	206	134	8
Flufenacet OA	8.67	224	152	10
Propachlor ESA	10.01	256	80	25
Flufenacet ESA	10.81	274	80	25
Dimethenamid OA	13.25	270	198	10
Dimethenamid ESA	14.87&15.11	320	80	25
Alachlor OA	15.86	264	160	10
Acetochlor OA	16.34	264	146	10
Alachlor ESA	18.46	314	80	25
Metolachlor OA	18.60	278	206	8
Acetochlor ESA	19.12	314	80	30
Metolachlor ESA	20.95	328	80	25
Dimethachlor ESA (sur)	12.18	300	80	25
Butachlor ESA (IS)	36.95	356	80	25

*For complete details on Method 535 Version 1.1 the analyst is referred to: J. A. Shoemaker and M. V. Bassett, April 2005, National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268

Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid-Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

UCT Part Number:
ECDVB156P- Enviro-Clean® DVB 500 mg, 6 mL cartridge, PE Frit
or
ECHLD156-P - Enviro-Clean® HL DVB 500 mg, 6 mL cartridge, PE Frit

EPA Method 537 Version 1.1

Method 537 Analytes

Analyte	Acronym	CASRN
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	--
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	--
Perfluorobutanesulfonic acid	PFBS	375-73-5
Perfluorodecanoic acid	PFDA	335-76-2
Perfluorododecanoic acid	PFDoA	307-55-1
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorohexanesulfonic acid	PFHxS	355-46-4
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluorononanoic acid	PFNA	375-95-1
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorotetradecanoic acid	PFTA	376-06-7
Perfluorotridecanoic acid	PFTrDA	72629-94-8
Perfluoroundecanoic acid	PFUnA	2058-94-8

Interferences

- **Do not cover glassware with aluminum foil because PFAAs can be potentially transferred from the aluminum foil**

- PFAA standards, extracts and samples should not come in contact with any glass containers or pipettes as these analytes can potentially adsorb to glass

- PFAA analyte, IS and SUR standards commercially purchased in glass ampoules are acceptable however all subsequent dilutions must be prepared and stored in polypropylene containers

- The method analytes in this method can also be found in many common laboratory supplies and equipment, such as PTFE (polytetrafluoroethylene) products, LC solvent lines, methanol, aluminum foil and SPE sample transfer lines. These items must be routinely demonstrated to be free from interferences (less than 1/3 the MRL for each method analyte) under the conditions of the analysis by analyzing laboratory reagent blanks

- Humic and/or fulvic material can be co-extracted during SPE and high levels can cause enhancement and/or suppression in the electrospray ionization source or low recoveries.
  Total organic carbon (TOC) is a good indicator of humic content of the sample

Preservation Reagent

Compound	Amount	Purpose
Trizma®*	5.0 g/L	Buffer & free Cl removal

* Synonym: TRIS HCl (Tris(hydroxymethyl)aminomethane hydrochloride) CASRN 1185-53-1

Internal Standards and Surrogates

Internal Standards Table

Internal Standards	Acronym
Perfluoro-[1,2-$^{13}C_2$]octanoic acid	^{13}C-PFOA
Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate	^{13}C-PFOS
N-deuteriomethylperfluoro-1-octanesulfonamidoacetic acid	d_3-NMeFOSAA

Internal Standard (IS) Primary Dilution Table

IS	Conc. of IS Stock (µg/mL)	Vol. Of IS Stock (µL)	Final Vol. of IS PDS (µL)	Final Conc. of IS PDS (ng/µL)
^{13}C-PFOA	1000	5.0	5000	1.0
^{13}C-PFOS	50	300.0	5000	3.0
d_3-NMeFOSAA	50	400.0	5000	4.0

Surrogates (SUR) Table

Surrogates	Acronym
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	^{13}C-PFHxA
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	^{13}C-PFDA
N-deuterioethylperfluoro-1-octanesulfonamidoacetic acid	d_5-NEtFOSAA

Surrogate Primary Dilution Table

SUR	Conc. Of SUR Stock (µg/mL)	Vol. of SUR Stock (µL)	Final Vol. of SUR PDS (µL)	Final Conc. Of SUR PDS (ng/µL)
^{13}C-PFHxA	50	100.0	5000	1.0
^{13}C-PFDA	50	100.0	5000	1.0
d_5-NEtFOSAA	50	400.0	5000	4.0

Analyte Solvent Dilution Table

Analyte	Analyte Stock Solvent
PFHxA	96:4% (vol/vol) methanol:water
PFHpA	96:4% (vol/vol) methanol:water
PFOA	96:4% (vol/vol) methanol:water
PFNA	96:4% (vol/vol) methanol:water
PFDA	96:4% (vol/vol) methanol:water
PFUnA	96:4% (vol/vol) methanol:water
PFDoA	96:4% (vol/vol) methanol:water
PFTrDA	100% ethyl acetate
PFTA	100% ethyl acetate
PFBS	100% methanol
PFHxS	100% methanol
PFOS	100% methanol
NEtFOSAA	100% methanol
NMeFOSAA	100% methanol

Internal Standard Primary Dilution (IS PDS) Standard

Prepare (or purchase) IS PDS at a suggested concentration of 1-4 ng/µL in 96:4% (vol/vol) methanol:water. Use 10 µL of this 1-4 ng/µL solution to fortify the final 1 mL extracts. This will yield a concentration of 10-40 pg/µL of each IS in the 1 mL extracts.

Procedure

1. Cartridge Preparation

a) Insert a **ECDVB156P** cartridge in a vacuum manifold or automated extraction system

b) Add 15 mL of methanol to the cartridge and slowly draw through under vacuum

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

c) Add 18 mL of reagent water

d) Draw through under vacuum but do not let water level drop below cartridge frit

2. Sample Extraction

a) Adjust vacuum setting to achieve a flow rate of about 10-15 mL/min

b) Draw water through sample cartridge

c) After sample extraction, rinse sample bottles and reservoir with 2 x 7.5 mL aliquots of reagent water and add to cartridge

d) Dry cartridge by drawing air through it for 5 minutes at high vacuum

3. Sample Elution

a) Insert a polypropylene (PP) collection tube in the vacuum manifold

b) Rinse sample bottle and reservoir with 4 mL of methanol and add to cartridge

c) Draw through cartridge in dropwise manner

d) Rinse sample bottle and reservoir with another 4 mL of methanol and add to the cartridge

e) Draw through cartridge in dropwise manner

4. Extract Concentration

a) Concentrate the extract to dryness using a gentle stream of N_2 in a heated water bath 60-65° C

b) Add the appropriate amount of 96:4% (vol/vol) methanol:water solution and the IS PDS to the collection vial

c) Bring the volume to 1 mL and vortex

d) Transfer a small aliquot with a plastic pipette to a polypropylene autosampler vial

NOTE: Do not transfer the entire 1 mL aliquot to the autosampler vial because the polypropylene autosampler caps do not reseal after injection. Do not store the extracts in the autosampler vials as evaporation losses can occur. Extracts can be stored in 15 mL centrifuge tube

5. HPLC Analysis Conditions

LC Method Conditions

Time (min)	% 20 mM	% Methanol
Initial	60.0	40.0
1.0	60.0	40.0
25.0	10.0	90.0
32.0	10.0	90.0
32.1	60.0	40.0
37.0	60.0	40.0
Flow rate of 0.3 mL/min		10 µL injection
Waters Atlantis® dC_{18} 2.1 x 150 mm packed with 5.0 µm C_{18} stationary phase or equivalent		

ESI-MS Conditions	
Polarity	Negative Ions
Capillary Needle Voltage	-3 kV
Cone Gas Flow	98 L/hr
Nitrogen Desolvation Gas	1100 L/hr
Desolvation Gas Temperature	350° C

MS/MS Method Conditions					
Segment	**Analyte**	**Precursor Ion (m/z)**	**Product Ion (m/z)**	**Cone Voltage**	**Collision Energy (v)**
1	PFBS	299	80	40	25
2	PFHxA	313	269	15	10
3	PFHpA	363	319	12	10
3	PFHxS	399	80	40	40
4	PFOA	413	369	15	10
4	PFNA	463	419	12	10
4	PFOS	499	80	40	40
5	PFDA	513	469	15	10
5	NMeFOSAA	570	419	25	20
5	NEtFOSAA	584	419	25	20
5	PFUnA	563	519	15	10
5	PFDoA	613	569	15	10
6	PFTA	663	619	15	10
6	PFTA	713	669	15	10
2	^{13}C-PFHxA	315	270	15	10
5	^{13}C-PFDA	515	470	12	12
5	d_5-NEtFOSAA	589	419	25	20
4	^{13}C-PFOA	415	370	15	10
4	^{13}C-PFOS	503	80	40	40
5	d_3-NMeFOSAA	573	419	25	20

Summarized 15 from: Shoemaker, J.A., Grimmett P.E., Boutin, B.K., Method 537, Determination Of Selected Perfluorinated Alkyl Acids In Drinking Water By Solid-Phase Extraction And Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), Version 1.1, September 2009, National Exposure Research Laboratory, Office Of Research And Development, U. S. Environmental Protection Agency, Cincinnati, Ohio 45268

Listing of instrument manufacturers does not constitute endorsement by UCT

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EPA Method 538: Determination of Selected Organic Contaminants in Drinking Water by Aqueous Direct Injection and LC/MS/MS

UCT Part Numbers:
SLAQ100ID21-3UM - Selectra® Aqueous C18, 100 x 2.1mm, 3µm
SLAQGDC20-3UM - Selectra® Aqueous C18, Guard column, 10 x 2.0mm, 3µm
SLGRDHLDR - Guard Cartridge Holder

Summary:

This application outlines a direct aqueous injection-liquid chromatography/tandem mass spectrometry (DAI-LC/MS/MS) method for the determination of 11 selected organic contaminants in drinking water, including methamidophos, acephate, aldicarb sulfoxide, oxydemeton methyl, dicrotophos, aldicarb, diisopropyl methylphosphonate (DIMP), fenamiphos sulfone, fenamiphos sulfoxide, thiofanox, and quinoline [1]. Dicrotophos, oxydemeton methyl, methamidophos, and acephate are UCMR4 compounds.

An Aqueous C18 HPLC column was utilized for analyte retention and separation. Calibration curves were constructed using calibration standards prepared in reagent water with preservative reagents for analyte quantitation. The responses were linear over the entire analytical ranges ($R^2 \geq 0.9970$). Excellent accuracy (90 - 111%) and precision (RSD% < 20%, n=7) were achieved for fortified reagent water and tap water samples.

Procedure:

1. Preserve drinking water sample with 64 mg/L of sodium omadine (antimicrobial) and 1.5 g/L of ammonium acetate (binding free chlorine).
2. Mix 0.99 mL of the preserved water sample with 10 µL of 0.4-12.5 ng/µL internal standard mixture, and vortex for 30 sec.
3. Inject 50 µL onto LC/MS/MS equipped with an aqueous C18 HPLC column for analysis.

LC-MS/MS method:

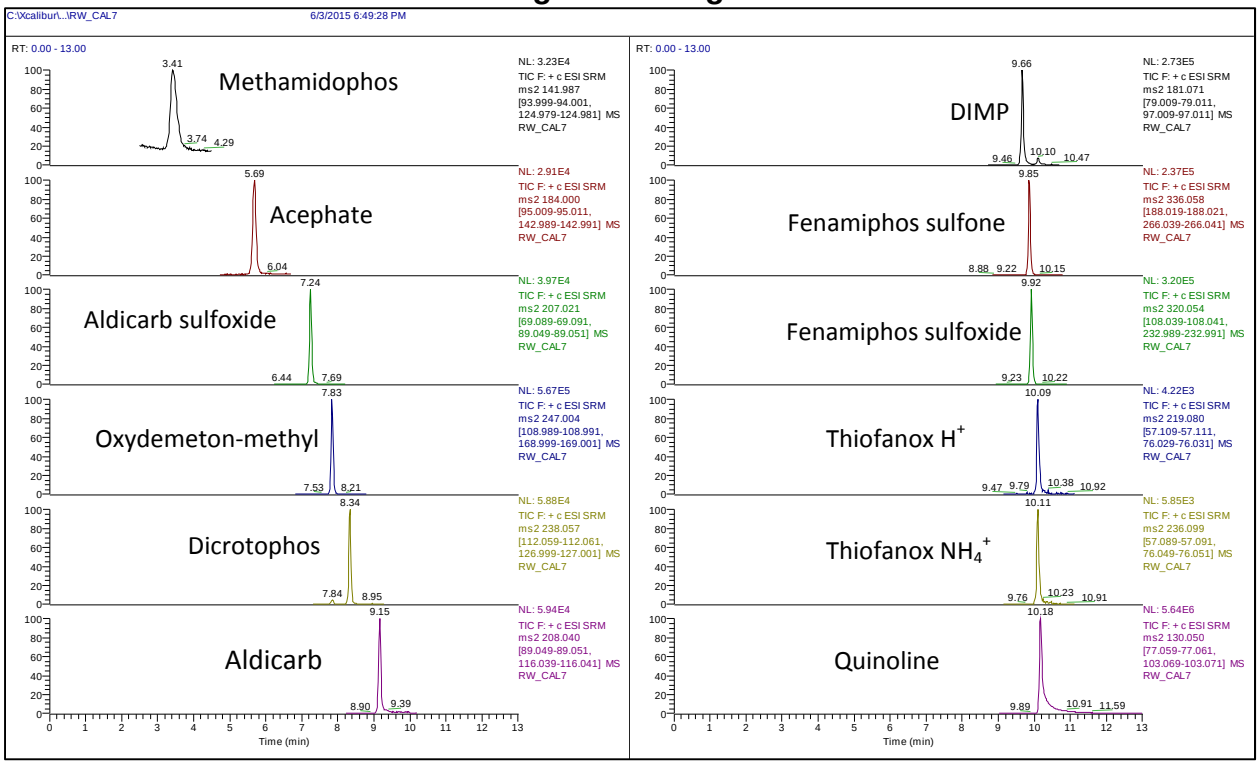
HPLC: Thermo Scientific Dionex UltiMate 3000® LC System		
Column: UCT, Selectra® Aqueous C18, 100 x 2.1 mm, 3 µm		
Guard column: UCT, Selectra® Aqueous C18, 10 x 2.0 mm, 3 µm		
Column temperature: 40 °C		
Column flow rate: 0.300 mL/min		
Auto-sampler temperature: 10 °C		
Injection volume: 50 µL		
Gradient program:		
Time (min)	**A%** (20 mM ammonium formate)	**B%** (MeOH)
0	100	0
2	100	0
9	15	85
12	15	85
12.1	100	0
16	100	0

Divert mobile phase to waste from 0 - 2 and 14 - 16 min to prevent ion source contamination.

MS parameters	
Instrumentation	Thermo Scientific TSQ Vantage tandem MS
Polarity	ESI +
Spray voltage	5000 V
Vaporizer temperature	203 °C
Ion transfer capillary temperature	208 °C
Sheath gas pressure	40 arbitrary units
Auxiliary gas pressure	5 arbitrary units
Q1 and Q3 peak width (FWHM)	0.2 and 1.0 Da
Collision gas and pressure	Ar at 1.5 mTorr
Cycle time	1 sec
Acquisition method	EZ Method (scheduled SRM)

SRM Transitions							
Compound Name	**Rt (min)**	**Precursor**	**Product 1**	**CE 1**	**Product 2**	**CE 2**	**S-lens**
Methamidophos	3.41	142.0	94.0	14	125.0	12	69
Acephate-d6	5.63	190.0	149.0	5	98.0	24	64
Acephate	5.69	184.0	143.0	5	95.0	22	53
Aldicarb sulfoxide	7.24	207.0	89.1	13	69.1	15	60
Oxydemeton-methyl-d6	7.81	253.0	175.0	13	115.0	27	89
Oxydemeton-methyl	7.83	247.0	169.0	13	109.0	27	84
Dicrotophos	8.34	238.1	112.1	11	127.0	18	75
Aldicarb	9.15	208.0	116.0	5	89.1	14	45
DIMP-d14	9.62	195.1	99.0	12	80.0	35	61
DIMP	9.66	181.1	97.0	12	79.0	32	49
Fenamiphos sulfone	9.85	336.1	266.0	16	188.0	26	135
Fenamiphos sulfoxide	9.92	320.1	233.0	24	108.0	39	102
Thiofanox H^+	10.10	219.1	57.1	12	76.0	5	40
Thiofanox $NH4^+$	10.10	236.1	57.1	11	76.1	5	42
Quinoline-d7	10.14	137.1	81.1	33	109.1	26	78
Quinoline	10.18	130.1	77.1	32	103.1	25	101

Chromatogram – Reagent Water



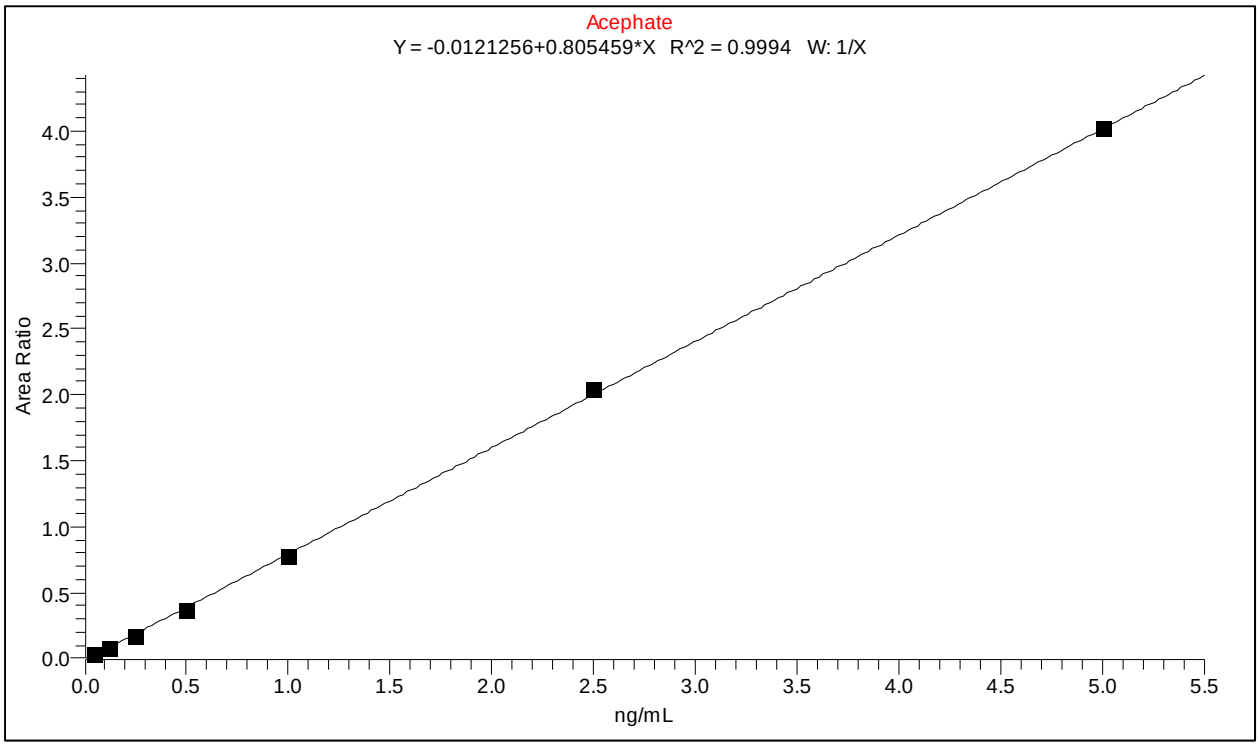
Results:

Analytical Range and Linearity Data

Compound Name	Analytical range (ng/mL)	Linearity (R^2)
Methamidophos	0.05 - 5	0.9982
Acephate	0.05 - 5	0.9994
Aldicarb sulfoxide	0.05 - 5	0.9996
Oxydemeton-methyl	0.05 - 5	0.9996
Dicrotophos	0.05 - 5	0.9973
Aldicarb	0.1 - 10	0.9970
DIMP	0.05 - 5	0.9997
Fenamiphos sulfone	0.05 - 5	0.9996
Fenamiphos sulfoxide	0.05 - 5	0.9989
Thiofanox*	0.2 - 20	0.9993
Quinoline	2 - 200	0.9972

*: For thiofanox, NH_4^+ adduct is more abundant than H^+ adduct, thus was selected for quantitation.

Calibration Curve of Acephate



Accuracy and Precision in Reagent Water Fortified at 0.125 - 5 ng/mL (n=7)

Compound Name	Spiked Conc. (ng/mL)	Avg Recovery%	RSD% (n=7)	Det. Limit (ng/mL)
Methamidophos	0.125	**103.0**	4.9	0.020
Acephate	0.125	**100.0**	6.6	0.026
Aldicarb sulfoxide	0.125	**101.6**	6.5	0.026
Oxydemeton-methyl	0.125	**101.1**	4.4	0.017
Dicrotophos	0.125	**104.8**	5.1	0.021
Aldicarb	0.25	**90.1**	10.7	0.076
DIMP	0.125	**97.2**	6.8	0.026
Fenamiphos sulfone	0.125	**96.9**	6.9	0.026
Fenamiphos sulfoxide	0.125	**97.1**	6.8	0.026
Thiofanox	0.5	**90.9**	18.3	0.261
Quinoline	5	**97.6**	4.6	0.700

Accuracy and Precision in Reagent Water Fortified at 1 - 40 ng/mL (n=7)

Compound Name	Spiked Conc. (ng/mL)	Avg Recovery%	RSD% (n=7)
Methamidophos	1	**100.3**	3.4
Acephate	1	**96.9**	3.9
Aldicarb sulfoxide	1	**103.9**	1.4
Oxydemeton-methyl	1	**95.7**	1.6
Dicrotophos	1	**104.0**	3.9
Aldicarb	2	**99.7**	4.7
DIMP	1	**97.6**	1.5
Fenamiphos sulfone	1	**100.2**	4.7
Fenamiphos sulfoxide	1	**100.9**	3.1
Thiofanox	4	**90.9**	6.5
Quinoline	40	**93.1**	3.7

Accuracy and Precision in Tap Water Fortified at 0.125 - 5 ng/mL (n=7)

Compound Name	Spiked Conc. (ng/mL)	Avg Recovery%	RSD% (n=7)
Methamidophos	0.125	**102.4**	8.7
Acephate	0.125	**98.6**	5.7
Aldicarb sulfoxide	0.125	**100.9**	6.1
Oxydemeton-methyl	0.125	**101.6**	2.8
Dicrotophos	0.125	**102.6**	9.6
Aldicarb	0.25	**94.5**	10.9
DIMP	0.125	**97.6**	3.0
Fenamiphos sulfone	0.125	**95.0**	5.1
Fenamiphos sulfoxide	0.125	**90.6**	8.5
Thiofanox	0.5	**92.7**	10.7
Quinoline	5	**96.7**	2.8

Accuracy and Precision in Tap Water Fortified at 1 - 40 ng/mL (n=7)

Compound Name	Spiked Conc. (ng/mL)	Avg Recovery%	RSD% (n=7)
Methamidophos	1	**102.8**	3.1
Acephate	1	**99.1**	2.0
Aldicarb sulfoxide	1	**110.6**	6.8
Oxydemeton-methyl	1	**98.9**	0.7
Dicrotophos	1	**105.7**	6.6
Aldicarb	2	**100.1**	3.7
DIMP	1	**99.5**	0.8
Fenamiphos sulfone	1	**97.3**	6.3
Fenamiphos sulfoxide	1	**98.0**	4.2
Thiofanox	4	**92.2**	6.9
Quinoline	40	**92.7**	3.8

References:

[1] http://www.epa.gov/nerlcwww/documents/Method538_Final.pdf

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Determination of Small Polar Organic Compounds in Drinking Water by Solid Phase Extraction with Activated Carbon Sorbent

UCT Part Numbers:
EU521163 - 600 mg activated carbon in 3 mL cartridges
AD0000AS - Cartridge adaptors
RFV0075P - 75 mL empty reservoirs
VMF024GL – 24-position glass block vacuum manifold
VMF02024 - Stopcocks
ECSS25K - 25 kg sodium sulfate, anhydrous, ACS grade, granular 60 mesh
GCLGN4MM – Agilent style - 4 mm splitless gooseneck GC liner

Summary:

This application describes a solid phase extraction (SPE) method for the determination of EPA method 541 analytes, including 1-butanol, 1,4-dioxane, 2-methoxyethanol and 2-propen-1-ol in finished drinking water. Among these 4 target analytes, 3 are part of the new UCMR4 (Unregulated Contaminant Monitoring Rule 4) compounds. The fourth compound is 1,4-dioxane, a UCMR3 (Unregulated Contaminant Monitoring Rule 3) compound currently being monitored by EPA method 522. The target analytes and surrogates are extracted from 50 mL preserved water samples onto an activated carbon sorbent packed in 3 mL SPE cartridges. The cartridges are dried and eluted with 5% methanol (MeOH, purge and trap grade) in dichloromethane (DCM, pesticide grade). The water residue in the eluate is removed by anhydrous sodium sulfate (Na_2SO_4) and the extract is injected into a GC/MS for analysis without further concentration due to the high volatilities of the analytes. The GC/MS is equipped with a 30 meter WAX column and operated in selected ion monitoring (SIM) mode for analyte separation and detection. Excellent recoveries and relative standard deviations (RSD) were obtained in reagent and tap water samples spiked with known amount of analytes.

Sample Preservation:

2.5 mg of sodium sulfite and 50 mg of sodium bisulfate are added to each empty sample bottle prior to shipment to the fie

SPE Procedure:

1. **SPE Setup**

a) Connect the 75-mL reservoirs (**RFV0075P**) to the top of the SPE cartridges (**EU521163**) using the cartridge adaptors (**AD0000AS**).

b) Attach the connected cartridges onto a 24-position glass block vacuum manifold (**VMF024GL**) with control stopcocks (**VMF02024**).

2. **Cartridge Conditioning**

a) Rinse the SPE cartridges with 5 mL of 5% MeOH in DCM, pass 1/3 through to wet the sorbent. Soak the sorbent for 1 min then draw the remaining solvent to waste. Apply full vacuum and leave on for 1 min.

b) Repeat Step 2a with 2 mL of MeOH.

c) Condition the SPE cartridges with 2 mL of MeOH. Allow the MeOH to soak the sorbent for 1 min; then draw to waste. Do not allow the sorbent go completely dry.

d) Equilibrate the cartridges with 5 mL of DI water. Draw about half of the water through the cartridge.

3. **Sample Extraction**

a) Add 50 mL water samples into the 75-mL reservoirs, adjust the stopcocks for a slow dropwise flow (about 5 mL/min), and draw the entire sample through.

b) Rinse the sample bottles with 5 mL of DI water and apply the rinses to the reservoirs. After the rinses pass completely through the cartridges, aspirate for 30 sec, and turn off the stopcocks.

c) Remove the reservoirs and cartridge adaptors from the SPE cartridges.

4. **Cartridge Drying**

a) Add 200 µL of MeOH to the SPE cartridges, rinsing the water droplets from the walls of the SPE cartridges, and aspirate at full vacuum for 30 sec.

b) Transfer the SPE cartridges to a drying manifold. Dry the cartridges with inert nitrogen at 5 L/min for 10 min.

5. Cartridge Elution

a) Rinse the stopcocks and tips of the manifold with 2 mL of MeOH to remove any trapped residual water; apply full vacuum for 1 min.

b) Insert a collection rack with small test tubes into the manifold underneath each SPE cartridge.

c) Place the dried SPE cartridges back onto the manifold with stopcocks. Elute the SPE cartridges with 2 x 1.3 mL of 5% MeOH in DCM. Let the elution solvent soak the SPE sorbent for 1 min, then elute by gravity, finally apply a low vacuum to extract all of the elution solvent from the SPE cartridges into the collection tubes. This will yield approximately 2 mL of eluate.

6. Eluate Drying

a) Add 10 µL of the 50 ppm internal standard solution to each extract and 1-2 grams of anhydrous sodium sulfate (**ECSS25K**), vortex for 1 min using a multi-tube vortexer.

b) Let the salt and extract remain in contact for 15 min before transferring the dried extract to a 2 mL auto-sampler vial. Adjust the final volume to 2 mL using 5% MeOH in DCM.

c) The samples are ready for GC/MS analysis.

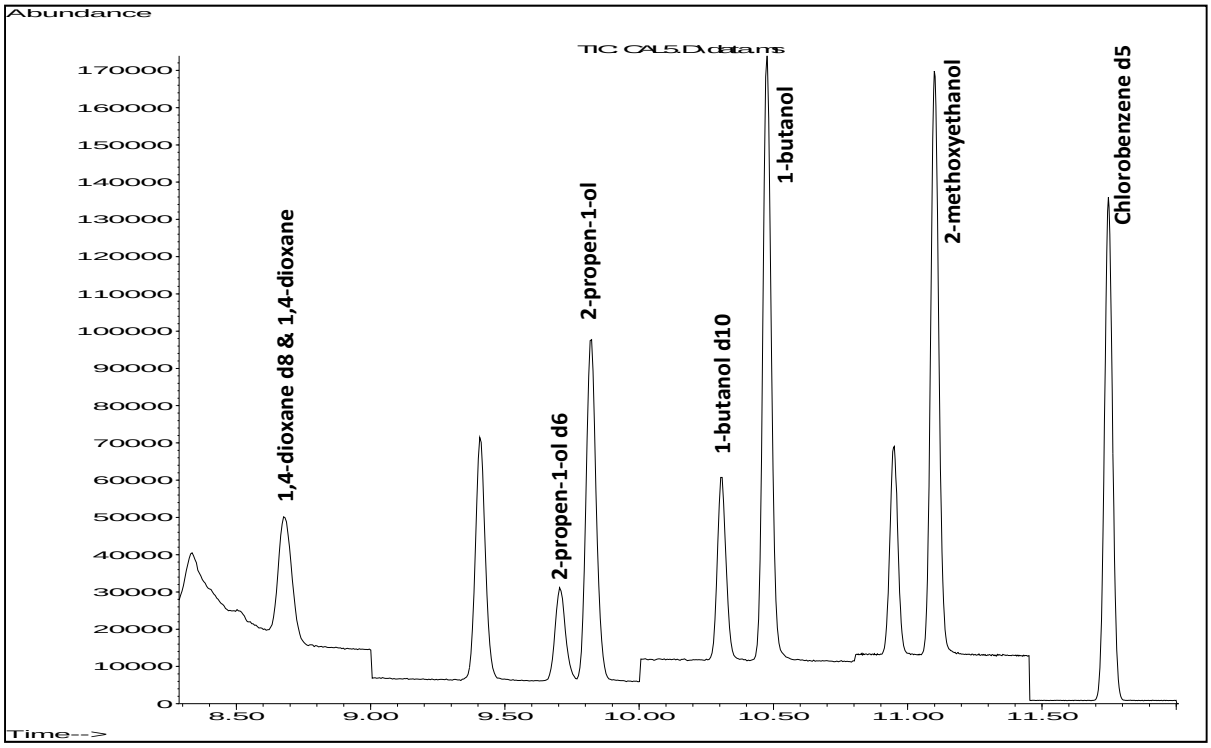
GC/MS Conditions

Parameter	Conditions
Column	Phenomenex ZB-WAX *plus*: 30m x 0.25mm x 0.5µm
Inlet liner (**GCLGN4MM**)	4mm, single gooseneck, with deactivated glass wool
Injection	1 µL at 200 °C, splitless injection, purge flow of 50 mL/min at 0.5 min
Carrier gas and flow rate	Ultra high purity Helium at 0.9 mL/min (constant flow)
GC temperature program	35 °C for 5 min, ramp 10 °C/min to 105 °C, ramp 30 °C/min to 240 °C, hold 3.5 min
Solvent delay	8.2 min (MS filament on from 8.2 to 12 min)
MS source temperature	250 °C
MS quadrupole temperature	150 °C
GC/MS interface	240 °C

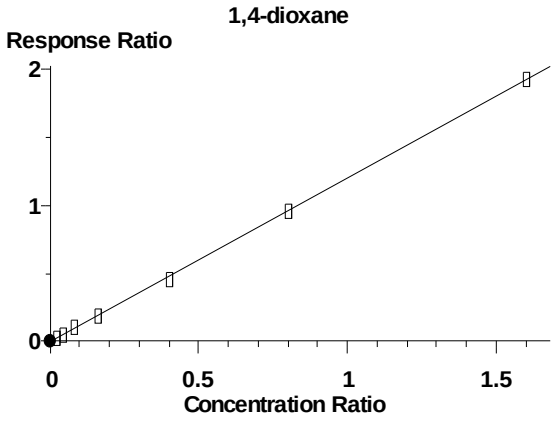
Accuracy and Precision Data

Compound	Fortified reagent water		Fortified tap water	
	Recovery%	RSD% (n=5)	Recovery%	RSD% (n=5)
1,4-dioxane	**89.5**	5.2	**87.7**	1.9
2-propen-1-ol d6	**92.9**	4.6	**82.5**	4.8
2-propen-1-ol	**85.5**	3.4	**84.1**	2.8
1-butanol d10	**101.0**	3.6	**88.0**	5.3
1-butanol	**96.4**	3.3	**88.4**	2.1
2-methoxyethanol	**97.6**	4.0	**92.9**	2.6

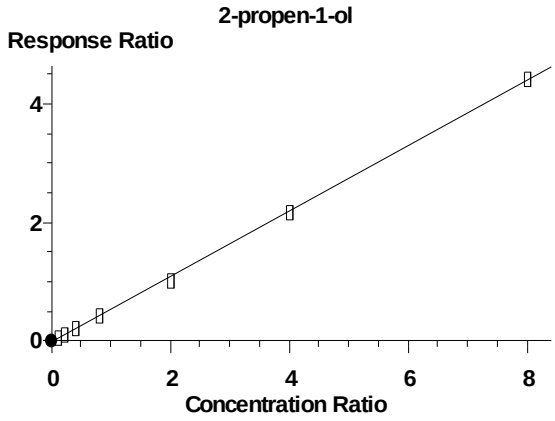
Chromatogram



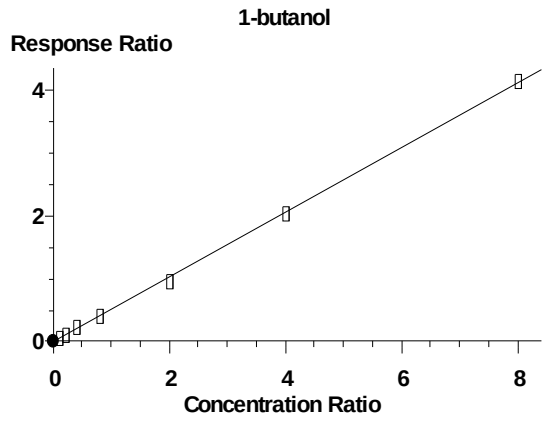
Calibration Curves



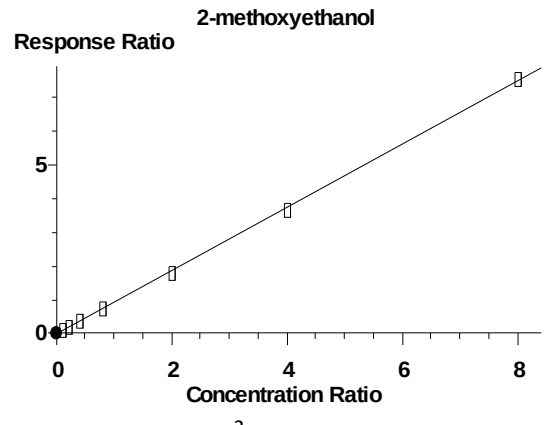
$R^2 = 0.9998$



$R^2 = 0.9997$



$R^2 = 0.9996$



$R^2 = 0.9997$

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EPA Method 545: Determination of Cylindrospermopsin and Anatoxin-a in Drinking Water by Aqueous Direct Injection and LC/MS/MS

UCT Part Numbers:
SLAQ100ID21-3UM - Selectra® Aqueous C18, 100 x 2.1mm, 3µm
SLAQGDC20-3UM - Selectra® Aqueous C18, Guard column, 10 x 2.0mm, 3µm
SLGRDHLDR - Guard Cartridge Holder

Summary:

Cyanotoxins are toxins that are produced by some species of photosynthetic cyanobacteria (also known as blue-green algae) blooming under certain conditions [1], such as stagnant or slow moving warm water with high-level nutrients like phosphates and nitrogen. Cyanotoxins are dangerous to humans and wildlife, affecting their livers (hepatotoxic), nervous systems (neurotoxic), and skin (acutely dermatotoxic). Human exposure to cyanotoxins can occur through ingestion of either the contaminated drinking water or exposed fish and shellfish, in addition to inhalation or dermal contact with the contaminated recreational water [2].

This application note describes a direct aqueous injection and liquid chromatography tandem mass spectrometry (DAI-LC/MS/MS) method for the determination of cylindrospermopsin (hepatotoxin) and anatoxin-a (neurotoxin) in drinking water under EPA Method 545 [3]. These two target analytes are also included in the UCMR4 screening compound list that will be monitored by public drinking water systems soon.

UCT's Aqueous C18 HPLC column was utilized for analyte retention and separation, which had demonstrated excellent consistency in peak area and retention times. 7-point calibration curves were constructed for analyte quantification. The responses were linear ($R^2 \geq 0.9982$) over the analytical range from 0.1 to 10 µg/L. Excellent accuracy (93.6 – 110.3%) and precision (RSD% < 10%, n=7) were achieved in fortified reagent water and tap water samples.

Procedure:

1. Preserve water samples with 1 g/L of sodium bisulfate (antimicrobial) and 0.1 g/L of ascorbic acid (dechlorination).
2. Add 10 µL of 0.5-2 ng/µL internal standard mixture to 2-mL vials, and appropriate amounts of spiking solutions for fortified samples, and bring the final volume to 1 mL with the preserved water samples.
3. Vortex the samples for 30 sec and analyze by LC-MS/MS equipped with an Aqueous C18 HPLC column.

LC-MS/MS method:

HPLC: Thermo Scientific Dionex UltiMate 3000® LC System		
Column: UCT, Selectra®, aQ C18, 100 x 2.1 mm, 3 µm		
Guard column: UCT, Selectra®, aQ C18, 10 x 2.0 mm, 3 µm		
Column temperature: 30 °C		
Column flow rate: 0.300 mL/min		
Auto-sampler temperature: 10 °C		
Injection volume: 50 µL		
Gradient program:		
Time (min)	**A%** (50 mM acetic acid in DI water)	**B%** (MeOH)
0	100	0
1.5	100	0
4.5	70	30
6	70	30
6.1	10	90
7.5	10	90
7.6	100	0
13.5	100	0

Divert mobile phase to waste from 0 – 1.8 and 7 – 13.5 min to prevent ion source contamination.

MS parameters	
Instrumentation	Thermo Scientific TSQ Vantage tandem MS
Polarity	ESI +
Spray voltage	4000 V
Vaporizer temperature	400 °C
Ion transfer capillary temperature	350 °C
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	25 arbitrary units
Q1 and Q3 peak width (FWHM)	0.2 and 0.7 Da
Collision gas and pressure	Ar at 1.5 mTorr
Cycle time	0.6 sec
Acquisition method	EZ Method (scheduled SRM)

Retention Times and SRM Transitions							
Compound	**Rt (min)**	**Precursor**	**Product 1**	**CE 1**	**Product 2**	**CE 2**	**S-Lens RF**
Uracil-d4	2.05	115.1	98.1	16	72.1	14	45
L-phenylalanine-d5	4.58	171.1	125.2	14	106.1	28	47
Cylindrospermopsin	5.40	416.1	194.1	31	176.1	31	106
Anatoxin-a	5.74	166.1	149.1	12	131.0	15	62

Results:

Relative Standard Deviation (RSD) of Peak Area and Retention Times by aQ C18 HPLC Column

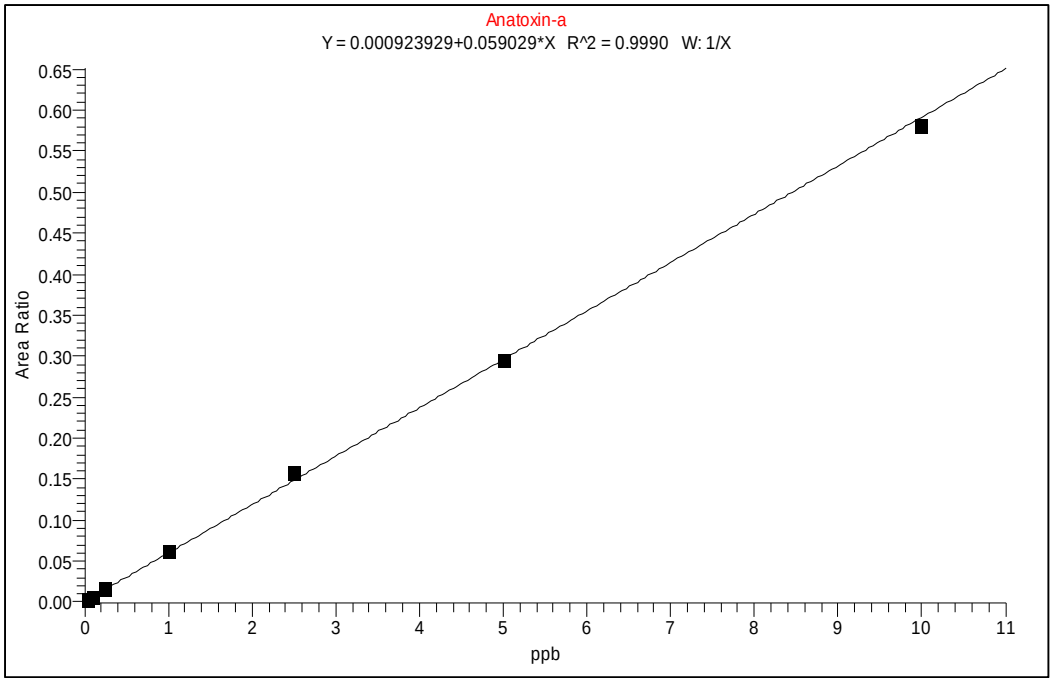
Compound	RSD% (n=44)	
	Peak Area	**Retention Time**
Uracil-d4	3.7	0.1
L-phenylalanine-d5	2.5	0.4
Cylindrospermopsin	ND	0.1
Anatoxin-a	ND	0.3

ND: not determined.

Analytical Range and Linearity Data

Compound	Analytical range	Linearity (R^2)	
	(µg/L)	Reagent water	Tap water
Cylindrospermopsin	0.1 - 10	0.9989	0.9992
Anatoxin-a	0.1 - 10	0.9990	0.9982

Calibration Curve of Anatoxin-a in Reagent Water



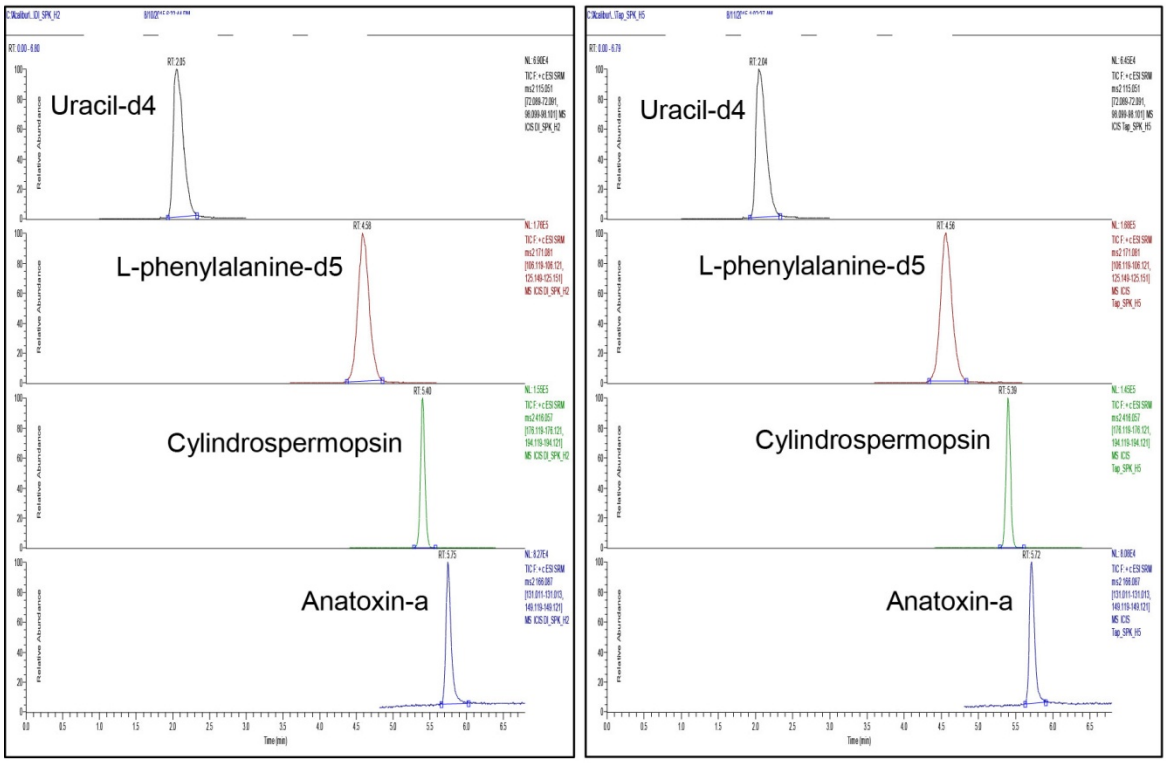
Accuracy and Precision in Fortified Reagent Water (n=7)

Compound	Spiked at 0.1 µg/L		Spiked at 2.5 µg/L	
	Recovery%	RSD%	Recovery%	RSD%
Cylindrospermopsin	**99.8**	4.5	**105.8**	2.3
Anatoxin-a	**101.5**	8.6	**103.1**	2.0

Accuracy and Precision in Fortified Tap Water (n=7)

Compound	Spiked at 0.1 µg/L		Spiked at 2.5 µg/L	
	Recovery%	RSD%	Recovery%	RSD%
Cylindrospermopsin	**100.4**	6.4	**110.3**	0.7
Anatoxin-a	**93.6**	5.2	**106.8**	0.6

Chromatograms of Fortified Water Samples



Reagent Water at 2.5 µg/L **Tap Water at 2.5 µg/L**

References:

[1] http://public.health.oregon.gov/

[2] http://www.waterrf.org/PublicReportLibrary/4548a.pdf

[3] http://water.epa.gov/scitech/drinkingwater/labcert/upload/epa815r15009.pdf

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Determination of Endothall in Drinking Water by Ion-Exchange Extraction, Acid Methanol Methylation and Gas Chromatography/Mass Spectrometry

UCT Part Numbers:
EC548006 (Bio-Rex® 5, 6 mL)
AD0000AS (Cartridge adapters)
RFV0075P (75 mL empty reservoir)
CLTTP050 (Clean-Thru tips)

EPA Method 548.1 Revision 1.0

Procedure

1. Condition Cartridge

a) Remove the seal caps on each end of the **EC548006** cartridge, attach a Clean-Thru tip (to prevent corrosion of manifold lid caused by strong acid) to the bottom of the SPE cartridge, and a 75-mL reservoir to the top of the cartridge adaptor, and place on vacuum manifold

b) Draw each of the following reagents through the cartridge at a rate of 10 mL/minute, slow drip

c) Add 10 mL of methanol and draw through to top of frit

d) Add 10 mL of reagent water and draw through to top of frit

e) Add 10 mL of 10% H_2SO_4 in methanol and draw through to top of frit

f) Add 10 mL of reagent water and draw through to top of frit

g) Add 10 mL 0f 0.1 N NaOH and draw through to top of frit

h) Add 20 mL of reagent water and draw through to top of frit

Note: Do not allow the cartridge to become dry between steps otherwise repeat steps starting with c)

It is critical that the extraction steps be followed exactly in order for the cartridge to effectively function in sample clean up and extraction

2. Sample Addition

a) Fill the reservoir with 70 mL of sample (pH 5.5-7.5) and adjust vacuum for a sample flow rate of 3 mL/minute

b) Add the remaining sample to the reservoir

c) After the sample has been drawn through the cartridge, rinse the sample bottle with 10 mL of methanol, apply the rinse to the reservoir and draw through

d) Dry cartridge for 5 minutes under 10-20 in Hg vacuum

e) Place a culture tube inside the manifold to collect the eluant

3. Extract Elution

a) Elute the cartridge with 8 mL of 10% H_2SO_4 in methanol

b) Follow with 6 mL of methylene chloride (CH_2Cl_2).

c) Elute over a 1 minute period

4. Sample Derivatization and Partition

a) Place a cap on the culture tube and heat at 50° C for 1 hour

b) Pour the contents of the culture tube into a 125 mL separatory funnel rinsing the tube with 2 x 0.5 mL aliquots of methylene chloride

c) Add the rinse to the separatory funnel

d) Add 20 mL of 10% Na_2SO_4 in reagent water to the separatory funnel. Vigorously shake the separatory funnel several times venting the funnel each time

e) Allow the phases to separate then drain the organic layer into a 15 mL graduated centrifuge tube

f) Repeat the above extraction with 2 additional 2 mL aliquots of methylene chloride. Add this to the methylene chloride in the centrifuge tube, then concentrate to 1 mL

g) Sample is ready for GC/MS analysis

5. Analysis

a) Analyze the extract by injecting1- 2 µL of the concentrated extract into a GC/MS

b) Identify endothall by comparison of its mass spectrum to a reference sample

Retention Times and Method Detection Limits

	Retention Time (min)			Method Detection Limits		
Compound	**Column A**	**Column B**	**Column C**	**GC/MS**	**2 µg/L spike**	**FID**
Endothall	16.02	19.85	18.32	1.79		0.7
Acenapththene -d10	14.69					

Column A: DB-5 fused silica capillary for GC/MS, 30 m x 0.25 mm, 0.25 micron film
MS inlet temperature = 200°C
Injector temperature = 200°C
Temperature Program: Hold five minutes at 80°C, increase to 260°C at 10°/min, hold 10 minutes

Column B: FID primary column, RTX Volatiles, 30 m x 0.53 mm I.D., 2 micron film
Detector temperature = 280°C
Injector Temperature = 200°C
Carrier gas velocity = 50 cm/sec
Temperature program: Same as Column A.

Column C: FID confirmation column, DB-5, 30 m x 0.32 mm ID, 0.25 micron film.
Carrier Gas velocity = 27 cm/sec
Same injector, detector, and temperature program as Column A

Interferences

- Major potential interferences in this ion-exchange procedure are other naturally occurring ions such as calcium, magnesium and sulfate. Calcium and magnesium (>100 mg/L) can complex with the endothall anion and make it unavailable for capture as an anion
- Sulfate anions (>250 mg/L) can act as a counter ion displacing anionic endothall on the ion exchange column. Elevated levels of these ions may contribute to reduced recovery of the primary analyte

One or both of the following remedies may be used reduce these interferences:

- Sample dilution to reduce the concentration of these ions (10:1)
- Ethylenediamine tetraacetic acid (EDTA) addition to complex the cations (186 mg/100 mL sample)

For samples containing moderately high levels of these ions, add 186 mg of EDTA per 100 mL sample (0.005 M). For samples containing higher levels of sulfate, sample dilution may be required in addition to the EDTA. Using western surface water as an example (2000 mg/L sulfate) it was successfully analyzed after dilution by a factor of 10 and the addition of 75 mg EDTA per 100 mL of the diluted sample (0.002 M). Samples containing intermediate levels of sulfate can be analyzed with smaller dilution factors. Guidelines on dilution factors and EDTA addition are shown below.

Sulfate mg/L	Dilution Factor	Added EDTA mg/100 mL
<250	1:1	186
250-500	1:2	125
500-1250	1:5	75
>1250	1:10	75

Note: Dilution should not be employed if adequate recovery is attained by the addition of EDTA alone

MDL (spiked at 2 µg/L) and LCS (spiked at 10 µg/L) Results Using UCT's EC548006 Cartridges

MDL 1	1.80
MDL 2	1.56
MDL 3	2.12
MDL 4	1.87
MDL 5	1.60
MDL 6	2.13
MDL 7	1.84
Average	1.85
Standard Deviation	0.22
Ave Recovery%	**92.5**
RSD% (n=7)	**11.9**
Calculated MDL (µg/L)	**0.69**

LCS 1	10.42
LCS 2	10.00
LCS 3	10.25
LCS 4	9.84
Average	10.13
Standard Deviation	0.26
Ave Recovery%	**101.3**
RSD% (n=4)	**2.5**

*For complete details on Method 548.1 "Determination of Endothall in Drinking Water by Ion-Exchange Extraction, Acid Methanol Methylation and Gas Chromatography/Mass Spectrometry", the analyst is referred to: J. W. Hodgeson, August 1992, Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268

Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography with Ultraviolet Detection*

UCT Part Number:
EEC08156 (500 mg C8, 6 mL cartridge) or
ECUNI549 (500 mg C8, 83 mL Universal cartridge)

EPA Method 549.2 Revision 1.0

Analyte	CASRN
Diquat 1,1'-ethylene-2,2'-bipyridium dibromide salt	85-00-7
Paraquat 1,1'-dimethyl-4,4'-bipyridium dichloride salt	1910-42-5

Initial Preparation

- Since diquat and paraquat are ionic analytes there is the potential for adsorption on glass surfaces
- **Use only plastic labware.** Labware must be thoroughly washed and dried before use
- Adjust a 250 mL of sample to pH 7 - 9 with 10 % aqueous sodium hydroxide or 10% aqueous hydrochloric acid solution depending upon initial pH
- Assemble a C8 extraction cartridge in an appropriate manifold apparatus
- If the sample contains particulates, filter through 0.45 µm Nylon membrane filter
- **Ammonium hydroxide is volatile. Make fresh solutions daily from relatively new ammonium hydroxide stock**

Sample Clean-up

- Clean-up procedures may not be necessary for a relatively clean sample matrix
- If the sample contains particulates the entire sample should be passed through a 0.45 mm Nylon of PTFE membrane filter into a plastic container before starting extraction

Stock Standard Solutions

Diquat dibromide and Paraquat dichloride Stock Solutions (1000 mg/L)

1. Dry diquat (diquat dibromide monohydrate) and paraquat (paraquat dichloride tetrahydrate) salts in an oven at 110°C for three hours. Cool in a desiccator
2. Repeat process to a constant weight.
3. Weigh 0.1968 g of dried diquat salt and 0.1770 g of dried paraquat salt
4. Transfer to a silanized glass or polypropylene 100 mL volumetric flask. Add approximately 50 mL of deionized water then dilute to the mark with deionized water

Calibration

In order to closely match calibration standards to samples, process standards by the following method:

- Condition a cartridge according to section 1 below.
- Pass 250 mL of reagent water through the cartridge and discard the water.
- Dry the cartridge by passing 5 mL of methanol through it. Discard the methanol.
- Pass 4.0 mL of the eluting solution through the cartridge and catch in a 5 mL silanized volumetric flask.
- Fortify the eluted solution with 100 µL of the ion-pair concentrate and with 500 µL of the stock standard and dilute to the mark with eluting solution. This provides a 10:1 dilution of the stock.
- Use serial dilution of the calibration standard by the same method to achieve lower concentration standards.

Procedure

The cartridge must be conditioned properly before extraction

1. Condition Cartridge

a) Place C8 cartridge(s) on a vacuum manifold system

b) Draw the following solutions through the cartridge in the stated order. The flow rate through the cartridge should be approximately 10 mL/min

Note: Do not to let the cartridge go dry once starting the addition of solutions

c) Add 5 mL of reagent water to the cartridge and draw through to waste

d) Add 5 ml of methanol to the cartridge and soak for about one minute

e) Apply vacuum to draw most of the methanol through the cartridge. Leave a thin layer on top of the frit

f) Add 5 ml reagent water to the cartridge

g) Apply vacuum and draw most of the water through the cartridge. Leave a thin layer of water on the frit

h) Apply 5 mL of conditioning **Solution A** to the cartridge

Solution A: Dissolve 0.500 grams cetyl trimethyl ammonium bromide and 5 mL of ammonium hydroxide in 500 mL of reagent water. Dilute to 1000 mL

i) Draw a small amount through the cartridge leaving a thin layer on the frit

j) Soak for one minute

k) Use 5 mL of reagent grade water to rinse the **Solution A** from the cartridge. Allow a thin layer of water to remain on the cartridge frit

l) Rinse the cartridge with 10 mL of methanol

m) Rinse the cartridge with 5 mL of reagent grade water

n) Condition the cartridge with 20 mL of **Solution B**

o) **Solution B**: Dissolve 10 g 1-hexanesulfonic acid sodium salt and 10 mL of ammonium hydroxide in 250 mL of DI water then dilute to 500 mL

p) Retain **Solution B** in the cartridge to keep it activated. **Do Not Rinse**

2. Sample Extraction

a) Determine the pH of the sample. Adjust to 7.0 – 9.0 with 10% NaOH or 10% v/v HCl before extracting

b) Using a volumetric flask add 250 mL of the water sample to the reservoir and start the vacuum at a rate of 3 to 6 mL per minute

c) Draw the sample through the cartridge draining as much water from the sample bottle as possible

d) Rinse the cartridge with 5 ml of HPLC grade methanol

e) Draw vacuum through the cartridge for 1 minute to dry

f) Remove the filtration assembly and insert a silanized 5 mL volumetric (plastic vessel is preferred) flask for collection of the eluate

3. Cartridge Elution

a) Add 4.5 ml of **Cartridge Eluting Solution** to the cartridge

b) Allow to soak for one minute

Cartridge Elution Solution: Dissolve 13.5 mL of orthophosphoric acid and 10.3 mL of diethylamine in 500 mL of DI water, then dilute to 1 liter

c) Elute at 1-2 mL (drop by drop) per minute drawing all solution through the cartridge

d) Using cartridge **Ion-pair solution**, add 100 µL to the flask

Ion-pair Concentrate: Dissolve 3.75 grams of 1-hexanesulfonic acid in 15 mL of the **Cartridge Elution Solution** and dilute to 25 mL in a volumetric flask with additional **Cartridge Elution Solution**

e) Bring the eluate to a known volume of 5 mL using **Cartridge Elution Solution**

f) The extract is now ready for HPLC analysis as shown below

4. HPLC Analysis

Mobile Phase – Prepare mobile phase by adding reagents 1-4 to 500 mL DI water:

a) 13.5 mL of orthophosphoric acid

b) 10.3 mL of diethylamine

c) 3.0 g of 1-hexanesulfonic acid, sodium salt

d) Mix and bring to a final volume of 1 L with DI water

HPLC Conditions:
Column: Phenomenex Spherisorb, 3F, 4.6 mm x 100 mm or equivalent
Column Temperature: 35° C
Flow Rate: 2.0 mL/min., Ion-Pair Mobile Phase
Injection Volume: 200 µL
Photodiode Array Detector Settings:
Wavelength Range: 210 - 370 nm
Sample Rate: 1 scan/sec.
Wavelength Step: 1 nm
Integration Time: 1 sec.
Run Time: 5.0 min.
Quantitation Wavelengths: Diquat 308 nm, Paraquat 257 nm

*EPA Method 549.2 Revision 1.0, Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography with Ultraviolet Detection, J.W. Munch (USEPA) and W.J. Bashe (DynCorp/TAI) - Method 549.2, Revision 1.0 (1997),National Exposure Research Laboratory, Office Of Research And Development, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268

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Determination of Polycyclic Aromatic Hydrocarbons in Drinking Water by Liquid-Solid Extraction and HPLC with Coupled Ultraviolet and Fluorescence Detection*

UCT Part Numbers:
ECUNIPAH (2000 mg unendcapped C18, 83 mL cartridge)
Or
EUC1812M15 (2000 mg unendcapped C18, 15 mL cartridge)
CUC181M6 (1000 mg unendcapped C18, 6 mL cartridge)

ECSS25K (Anhydrous Sodium Sulfate)

EPA Method 550.1

Procedure

1. Cartridge Preparation

a) Wash with 4 x 10 mL aliquots of methylene chloride ($MeCl_2$)
b) Wash with 4 x 10 mL aliquots of methanol (MeOH)
c) Wash with 2 x 10 mL aliquots of reagent water

Do not let the cartridge dry out after step 1) c otherwise repeat starting at 1) b

2. Sample Extraction

a) Adjust the vacuum setting for a flow rate of 10-15 mL per minute
b) Add the 1 liter sample to the cartridge
c) Rinse sample bottle with reagent water, add to cartridge and draw through
d) Dry cartridge by drawing full vacuum for 10 minutes

3. Sample Elution and Drying

a) Elute the cartridge dropwise by using 2 x 5 mL aliquots of $MeCl_2$ and collect
b) Rinse sample container with 5 mL of $MeCl_2$, add to cartridge and draw through
c) Prepare a drying column/funnel containing 10-20g sodium sulfate by rinsing with 10 mL of $MeCl_2$ and discard
d) Add the eluate to the drying column, draw through and collect

e) Rinse the eluate vial and the drying column with a 2 x 5 mL aliquot of $MeCl_2$ and collect

4. Sample Evaporation

a) Evaporate the extract using a gentle stream of N_2 with a water bath or heating block temperature of 40°C. Evaporate to about 1.0 mL

b) Add 3.0 mL of acetonitrile (ACN)

c) Concentrate to a final volume of 0.5 mL

5. Sample Analysis

a) Inject 5 - 100 µL into an HPLC

*See "Determination of Polycyclic Aromatic Hydrocarbons in Drinking Water by Liquid-Solid Extraction and HPLC with Coupled Ultraviolet and Fluorescence Detection", W. J. Bashe & T.V. Baker (Technology Applications, Inc, Environmental Monitoring Systems Laboratory, US Environmental Protection Agency, Cincinnati, OH

Determination of Haloacetic Acids and Dalapon in Drinking Water by SPE and GC/ECD*

UCT Part Numbers:
EUQAX156 (Quaternary Amine with Cl^- counter ion, 6 mL cartridge)**
CLTTP050 (CLEAN-THRU® Tips)
AD0000AS (cartridge adapter)
RFV0075P (75 mL reservoir)
ECSS25K (Sodium Sulfate Anhydrous, ACS, 60 mesh)

Method 552.1 Revision 1.0

Summary

Method 552.1 is a gas chromatographic method for determination of haloacetic acid compounds, including Dalapon in drinking water, ground water, raw water and other waters at any intermediate stage of treatment. This method is used over the concentration ranges typically found in drinking water ranging from less than 1 to over 50 µg/L. The observed MDL's may vary according to the particular matrix analyzed and the specific instrumentation employed.
In this procedure, a 100 mL sample of water is adjusted to pH 5 and then extracted using an anion exchange cartridge. Method 552.1 suggests the use of polymeric-based anion exchange resins. This application uses silica-based anion exchange, therefore the conditioning steps have been modified to accommodate this silica-based sorbent. Exceptional recoveries are reported as shown in the data below.

Haloacetic Acids

The following RCRA compounds are determined by method 552.1 in water:

Analyte	Abbreviation	CASRN
Monochloroacetic acid	MCAA	79-11-8
Dichloroacetic acid	DCAA	79-03-9
Trichloroacetic acid	TCAA	66-03-9
Monobromoacetic acid	MBAA	79-08-3
Bromochloroacetic acid	BCAA	5589-96-8
Dibromoacetic acid	DBAA	631-64-1
Dalapon		75-99-0

Safety

- The toxicity or carcinogenicity of each reagent used in this method has not been defined

Sample Collection, Preservation, and Storage

- Collect samples in all glass amber bottles with PTFE lined screw caps
- Prior to sample collection add ammonium chloride (NH_4Cl) to the bottles to produce a concentration of 100 mg/L
- Alternatively, add 1.0 mL of a 10 mg/mL aqueous solution of NH_4Cl during collection then shake well
- Refrigerate at 4°C or keep on ice and away from light until extraction
- Analyze within 48 hours after collection

Interferences

Care and attention to potential interferences will result in better analysis

- Method interferences may be caused by contamination of solvents, reagents, glassware or other lab apparatus
- Glassware must be scrupulously clean
- Heat all glassware except volumetric flasks in a muffle oven at 400°C. Thermally stable compounds such as PCB's may not be eliminated by this treatment. Thorough rinsing with reagent grade acetone may be substituted for the heating step
- Glassware must be rinsed with 1:9 HCl prior to use
- Reduced analyte recoveries may be observed in high ionic strength matrices particularly waters containing sulfate concentrations above 200 mg/L
- Improved recoveries may be obtained by sample dilution but at the expense of higher MDL's
- Tribromoacetic acid has not been included because of problems associated with stability and chromatography
- Organic acids and phenols, especially chlorinated compounds, are the most direct potential interferences with this method
- For each set of samples analyzed, the reagent blank concentration values exceeding
  0.1 µg/L can be subtracted from the sample concentration
- Interfering contamination may occur when a sample with a low concentration of analytes is analyzed following a sample of relatively high concentration. Routine between-sample rinsing of the sample syringe and associated equipment with methyl- *t*-butyl ether (MTBE) can minimize sample cross-contamination

Standard Stock Solution

Prepare a stock solution of each analyte of interest at a concentration of 1-5 mg/mL in MTBE. Method analytes may be obtained as neat materials or ampulized solutions (>99% purity) from commercial suppliers

Prepare Primary Dilution Standard (PPDS)

Prepare PPDS solutions by combining and diluting stock standard solutions with methanol. Prepare stock standard solutions in the 1-2 mg/mL range for all analytes including the surrogate. Aliquots of each stock standard solution (approximately 50-250 µL) are added to 100 mL methanol to yield a primary dilution standard containing the following approximate concentrations of analytes

Analyte	Concentration, µg/mL
Monochloroacetic Acid (MCAA)	3
Monobromoacetic acid (MBAA)	2
Dalapon	2
Dichloroacetic acid (DCAA)	3
2-bromopropionic acid [b]	1
Trichloroacetic acid (TCAA)	1
Bromochloroacetic acid	2
Dibromoacetic acid	1

The primary dilution standards are used to prepare calibration standards of at least three concentration levels (optimally five) of each analyte with the lowest standard being at or near the MDL of each analyte. The concentrations of the other standards should define a range containing the expected sample concentrations or the working range of the detector

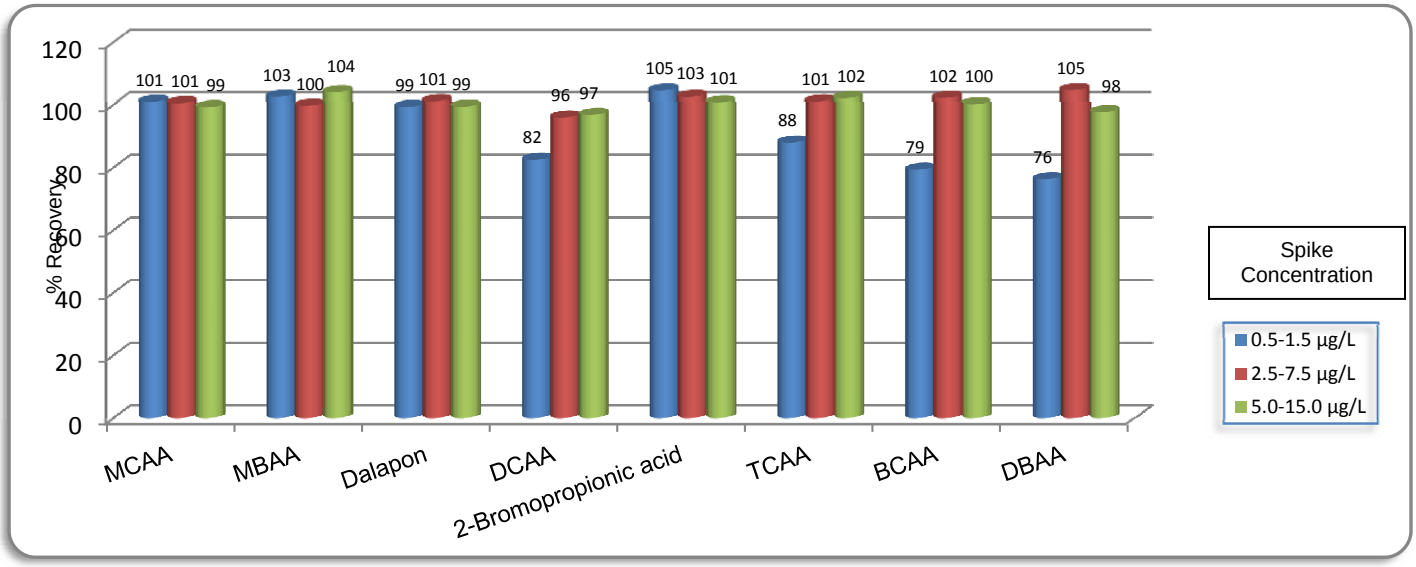
Calibration Standards

- Perform by extracting calibration standards in fortified reagent water using the SPE cartridge
- Prepare a five-point calibration curve 100- mL reagent water samples at pH 5 with 20,
  50, 100, 250, and 500 µL of the primary dilution standard
- Analyze each calibration standard in triplicate including a reagent water blank
- Include the surrogate 2-bromopropionic acid with the calibration standards in the table

Internal Standard Fortifying Solution

- Prepare a solution of 1,2,3-trichloropropane at 1 mg/mL by adding 36 µL of the neat material to 50 mL of MTBE. From this stock standard solution, prepare a primary dilution standard at 10 mg/L by the addition of 1-100 mL MTBE

Analyte Recovery at Various Spike Concentrations (N=7)



Procedure

1. Cartridge Conditioning

a) Assemble a glass block manifold system

b) Place **EUQAX156** cartridge(s) in the bulkhead fittings of the vacuum manifold. If nylon fittings are used, Clean-Thru® tips are recommended to prevent damage to the fittings by sulfuric acid

c) Attach adapters and reservoirs to the cartridges

d) Add a 10 mL aliquot of methanol (MeOH) to the cartridge

e) Draw slowly through at 2 mL/min flow

f) Add 10 mL of reagent water then slowly draw through

Note: After the conditioning steps do not let the cartridge go dry before addition of sample

2. Sample Extraction

a) Check the sample pH (preferably with a pH meter) to assure pH 5 ± 0.5

b) Add 250 µL of the surrogate primary dilution standard to water sample

c) Add the 100 mL sample to the cartridge while drawing through under vacuum

at 2 mL/minute

d) After the sample has passed through the cartridge, draw 10 mL of MeOH through the cartridge to dry the sorbent

3. Cartridge Elution

a) Dissemble the vacuum manifold and insert a screw cap culture tube

b) Add 2 mL of 10% H_2SO_4/MeOH to the cartridge

c) Elute at a rate of 1.5 mL/minute (slow drip)

d) Repeat with a second aliquot of 2 mL of 10% H_2SO_4/MeOH

4. Derivatization and Solvent Partition

a) To each of the elutant samples from 3) d) add 2.5 mL of MTBE

b) Agitate in a vortex mixer for 5 seconds

c) Place the culture tubes containing the MTBE in a heating block at 50° C for 1 hour

d) Remove from heating block and add to each tube 10 mL of a 10% solution of sodium sulfate ($NaSO_4$)

e) Agitate for 10 seconds on a vortex mixer

f) Allow the phases to separate for 5 minutes

g) Transfer the upper MTBE layer to a 15 mL graduated conical centrifuge tube

h) Add another 1 mL portion of MTBE

i) Agitate using a vortex mixer, allow to settle then transfer with a Pasteur pipette
to the other MTBE collected in step g)

j) Add 200 µL IS to each extract then additional MTBE to bring the final volume to 5 mL

k) Transfer a portion of the extract to capped GC vials

l) Sample is ready for analysis by GC-ECD

5. Sample Analysis—Gas Chromatography

a) Inject 2 µL using the recommended conditions listed below

GC/ECD Conditions

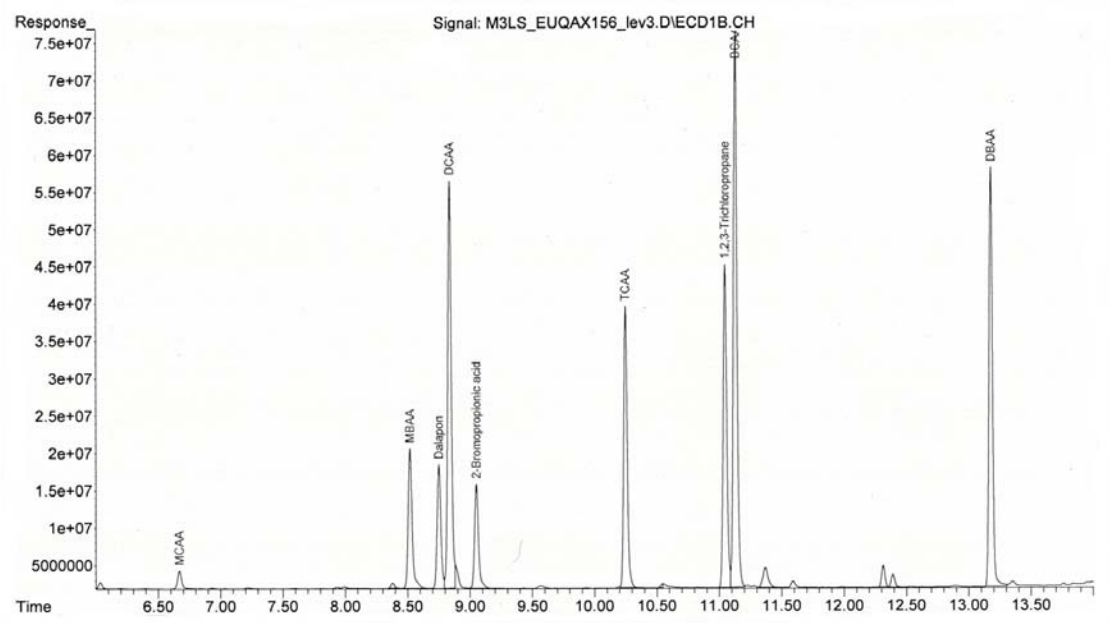
GC: Agilent 6890N coupled with ECD, equipped with 7683 auto sampler and Chemstation software for data acquisition and analysis. Other instruments may be used

GC capillary column: Restek Rtx®-1701, 30m x 0.25mm x 0.25um
Injector: 2 µL splitless injection at 200 ºC, with a split delay of 0.5 min.
Liner: 4 mm splitless gooseneck, 4mm ID x 6.5mm OD x 78.5mm (UCT **GCLGN4MM**)
Oven temperature program:

- Initial oven temperature of 55 ºC
- hold for 5 minutes
- ramp at 7 ºC/min to 115 ºC
- ramp at 40 ºC/min to a final temperature of 280 ºC
- hold for 2.3 minutes
- Total run time 20 minutes

Carrier gas: He flow of 1.5 mL/min
ECD temperature: 280 ºC
Make up: N_2 at 30 mL/min
Date rate: 20 Hz, save data from 6 to 14 minutes

Chromatogram Showing Elution of Haloacetic Acids



Analyte Elution Order	RSD% @ 0.5-1.5 µg/L	MDL µg/L @ 0.5-1.5 µg/L	RSD% @ 2.5-7.5 µg/L	RSD% @ 5.0-15.0 µg/L
MCAA	4.6	0.22	1.9	2.2
MBAA	3.2	0.10	1.6	2.0
Dalapon	1.3	0.04	1.0	1.0
DCAA	1.5	0.06	1.1	1.5
2-bromopropionic acid (surrogate)	2.6	0.04	0.6	1.7
TCAA	1.9	0.03	2.2	1.0
1,2,3-trichloropropane (IS)				
BCAA	10	0.25	2.4	3.4
DBAA	10	0.12	2.1	7.6

*For complete details on Method 552.1, August 1992, the analyst is referred to: Hodgeson, Jimmie W, Becker, David, (Technology Applications, Inc., **"**Determination of Haloacetic acids And Dalapon In Drinking Water by Ion-Exchange Liquid-Solid Extraction and Gas Chromatography with an Electron Capture Detector", Environmental Monitoring Systems Laboratory,, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268

**Other cartridge and sorbent masses are available from UCT

Determination of Benzidines and Nitrogen-Containing Pesticides in Water Liquid-Solid Extraction And Reverse Phase High Performance Liquid Chromatography/Particle Beam/Mass Spectrometry*

UCT Product Number:
ECDVB156P- Enviro-Clean® DVB 500 mg, 6 mL cartridge, PE Frit
ECHLD156-P - Enviro-Clean® HL DVB 500 mg, 6 mL cartridge, PE Frit
ECUNIDVB500 (500 mg, DVB, 83 mL cartridge)

EPA Method 553 Revision 1.1

Procedure

1. Cartridge Preparation

a) Rinse the cartridge(s) with a 10 mL aliquot of methanol
b) Slowly draw methanol to the top of the frit
c) Add a second 10 mL aliquot of methanol and draw through to top of frit
d) Add 10 mL of reagent water to the cartridge and draw through to top of frit

Note: Do not let the cartridge go dry after addition of methanol otherwise repeat at 1) c

2. Sample Extraction

a) Adjust sample pH to 7 using either 1N NaOH or HCl
b) Add water sample to the cartridge and draw through at 20 mL/minute
c) Rinse the sample container with 10 mL of reagent water and add to the cartridge
d) Dry sorbent by drawing full vacuum for 10 minutes
e) Place a clean collection vial in the vacuum manifold

3. Cartridge Elution

a) Add 15 mL of methanol to the sample container, swirl then add to the cartridge
b) Elute the cartridge with 2 x 7.5 mL aliquots of methanol dropwise

4. Extract Concentration

a) Concentrate the extract under a gentle stream of N_2
b) Bring to a known volume after concentration step
c) Sample is now ready for analysis

*The analyst should refer to EPA Method 553 "Determination Of Benzidines and Nitrogen-Containing Pesticides in Water By Liquid-Liquid Extraction or Liquid-Solid Extraction and Reverse Phase High Performance Liquid Chromatography/Particle Beam/Mass Spectrometry", Revision 1.1 Issued August 1992, by Thomas D. Behymer, Thomas A. Bellar, James S. Ho, William L. Budde, US EPA, Office of Ground Water and Drinking Water, EPA, National Exposure Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Cincinnati, Ohio 45268

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EPA Method 608 ATP*: Alternative Test Procedure for the Measurement of Organochlorine Pesticides and Polychlorinated Biphenyls in Wastewater

UCT Part Numbers:
ECUNIC18 - 1100 mg endcapped C18, 83 mL
ECUNIMSS - Enviro Clean Universal Cartridge 20g Muffled Sodium Sulfate
ECCU01K - 1 kg activated copper granules
EUFLS1M6 - 1000 mg PR Grade Florisil®, 6 mL
EUFLSA1M6 - 1000 mg Grade A Florisil®, 6 mL

This is a gas chromatographic (GC) method for determination of compounds listed below in municipal and industrial discharges. The EPA has approved the use of C18 SPE for this method.

Analytes Recovered Using Method 608ATP

Analyte	CAS
Aldrin	309-00-2
α-BHC	319-84-6
β-BHC	319-85-7
γ-BHC (Lindane)	58-89-9
δ-BHC	319-86-8
α-chlordane	5103-71-9
γ-chlordane	5103-74-2
4,4'-DDD	72-54-8
4,4'-DDE	72-55-9
4,4'-DDT	50-29-3
Dieldrin	60-57-1
Endosulfan I	959-98-8
Endosulfan II	33213-65-9
Endosulfan sulfate	1031-07-8
Endrin	72-20-8
Endrin Aldehyde	7421-93-4
Endrin Ketone	53494-70-5
Heptachlor	76-44-8
Heptachlor epoxide	1024-57-3
Methoxychlor	72-43-5
Toxaphene	8001-35-2
PCB-1016	12674-11-2
PCB-1221	1104-28-2
PCB-1232	11141-16-5
PCB-1242	53469-21-9
PCB-1248	12672-29-6
PCB-1254	11097-69-1
PCB-1260	11096-82-5

Procedure

1. Condition Cartridge

a) Insert cartridge(s) into the manifold or automated extraction system

b) Wash with 10 mL of methylene chloride ($MeCl_2$)

c) Soak for 1 minute then draw through to waste

d) Draw air under full vacuum to dry cartridge

e) Add 10 mL of methanol (MeOH) then slowly draw through to top of frit

f) Soak for 1 minute

NOTE: Do not let the cartridge go dry after addition of methanol otherwise repeat at step1) e)

g) Rinse the cartridge with 10 mL of reagent water

h) Draw through leaving a thin layer on top of frit

2. Sample Extraction

a) Adjust 1 liter of sample pH to < 2 using sulfuric acid

b) If sample water is high in suspended solids, allow particulates to settle then slowly decant the water in the bottle. Once most of the water passes through the cartridge add the solids portion

c) Draw the sample water through the cartridge over a 20-30 minute time period (fast drip) by adjusting the vacuum

d) Dry the cartridge by drawing air under full vacuum through for 10 minutes

3. Extract Elution

a) Insert a collection tube into the vacuum manifold

b) Add 5 mL of acetone to the sample bottle then swirl

c) Add this to the cartridge

d) Soak for 1 minute and slowly collect eluate

e) Add 20mL of methylene chloride to the sample bottle cover and shake. Add this to the cartridge

f) Soak for 2 minutes and slowly collect eluate

g) Rinse the inside walls of the sample bottle using 10 mL of methylene chloride then transfer solvent to the cartridge using a disposable pipette rinsing the inside of the cartridge

h) Soak for 2 minutes then collect eluate

4. Sample Drying

a) Pour the combined elutes together through a drying cartridge (ECUNIMSS) pre-rinsed with methylene chloride. Alternatively, use 15-20 grams of sodium sulfate over a bed of glass wool in a glass funnel

b) Rinse the eluate collection tube with 2 x 5 mL of methylene chloride, apply the rinse to the sodium sulfate bed and collect

c) Concentrate sample using a Kuderna-Danish (KD) concentrator while performing solvent exchange into hexane

d) Concentrate sample under a gentle stream of N_2 while gently heating in a water bath. **Other drying techniques may be used**

e) Rinse the inside walls of the concentrator tube two or three times with hexane during the evaporation

f) Adjust the final volume of the extract to 10 mLs

Florisil PR® or Copper Granule Clean-up Procedure (if needed)

Clean-up procedures may not be needed for relatively clean samples. If required, the following procedure can be used to remove polar interferences from organochlorine pesticide and PCB extracts in hexane eluants prior to analysis.

5. Florisil PR® Clean-Up

a) Place a cartridge in a vacuum manifold

b) Pre-rinse the Florisil® column with 10 mL of 90:10 hexane/acetone using gravity flow (a low vacuum may be necessary to start flow)

c) Discard solvent

d) Add a collection tube under the column

e) Add a 2 mL aliquot of the sample extract (in hexane) to the cartridge

f) Collect extract by gravity

g) Add 10 mL of 90:10 hexane/acetone to the cartridge

h) Continue to collect by gravity or low vacuum

i) Gently evaporate the extract to a volume of 1 mL

j) Adjust eluate to a final volume of 2 mL with hexane

k) Sample is now ready for analysis

6. Sulfur Clean-up

a) Place 4 grams of **ECCU01K** copper granules in a glass vial

b) Add 2 mL of liquid sample extract to the vial

c) Seal the glass vial and mix sample with copper for 2 minutes

d) Allow to stand for approximately 10 minutes

e) If sample contains high levels of sulfur, repeat process with 4 grams of fresh copper granules

Note: For the analysis of PCB type analytes, copper may reside in the extract; Copper cleanup may result in low recoveries for several pesticides

7. Analysis--GC/ECD

a) Transfer clean extract to autosampler vial

b) Sample is now ready for analysis

*The EPA has accepted the use of C18 bonded phases in packed cartridge format expanding the method from a disk only approach. For complete details on Method 607ATP, the analyst is referred to: "An alternative test procedure for the measurement of organochlorine pesticides and polychlorinated biphenyls in waste water", Federal register/Vol.60, No.148, August 2, 1995, Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268

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EPA Method 625: Determination of Bases, Neutrals and Acids by Solid Phase Extraction and GC/MS Detection

UCT Part Numbers:
EC82701M15 - 1000 mg 8270 sorbent/15 mL cartridge
or
EC82702M15 - 2000 mg 8270 sorbent/15 mL cartridge
EU52112M6 - 2000 mg activated carbon/6 mL cartridge
or
EU52113M6 - 3000 mg activated carbon/6 mL cartridge
AD0000AS - Cartridge adaptor
VMFSTFR12 - Large volume sample transfer tubes
VMF016GL - 16 position glass block manifold
VMF02125 - 12 position large volume collection rack
RFV1F15P - 15 mL reservoirs with 1 frit, 10 micron porosity
ECSS25K - Sodium sulfate, anhydrous, ACS grade, granular, 60 mesh
GCLGN4MM-5 - GC liner, 4 mm splitless gooseneck, 4mm ID x 6.5mm OD x 78.5mm

EPA method 625 was published many years ago for the analysis of bases, neutrals and acids in municipal and industrial wastewater. The analysis uses liquid-liquid extraction (LLE) and GC/MS detection. This technique requires multiple extractions at 2 different sample pHs and consumes large amounts of organic solvents. EPA method 625.1, an updated version of method 625 has been recently published (by Lemuel Walker, Office of Science and Technology, US EPA) allowing for the use of solid phase extraction (SPE) as an alternative sample preparation technique if the quality control (QC) acceptance criteria are met.

Recently, the Independent Laboratory Institute (ILI) led a working group including regulatory agencies, commercial SPE vendors, analytical testing laboratories and academia. The goal was to validate the performance of SPE and see if it offered comparable extraction results to traditional LLE. Various sample matrices, e.g. reagent water, synthetic wastewater, and TCLP (Toxicity Characteristic Leaching Procedure) buffer samples were investigated. UCT was one of several SPE vendors that participated in this validation study. Clean extracts, low method detection limits and excellent analyte recoveries were obtained using UCT's specially designed SPE sorbents. Results were excellent even for compounds that usually result in low recoveries when using LLE (e.g. 2,4-dinitrophenol and pentachlorophenol).

SPE Procedure:

1. Sample Pretreatment

a) To 200 mL of water sample add 20 mg of sodium thiosulfate if free chlorine is present.

b) Adjust sample pH to < 2 using 6N HCl.

c) Spike with surrogates, and target analytes for fortified samples.

Tip 1: The spiking solutions should be prepared in water miscible solvents, such as methanol and acetone. If water sample turns milky after spiking, make a more diluted spiking solution or mix with 2-3 mL methanol before spiking the sample. This will help prevent low analyte recovery caused by poor water solubility of some analytes.

2. SPE System Setup

a) Connect the carbon cartridge (**EU52112M6** or **EU52113M6**) to the end of the 8270 cartridge (**EC82701M15** or **EC82702M15**) using a cartridge adaptor (**AD0000AS**).

b) Insert a loose plug of deactivated glass wool into the 8270 cartridge to prevent sorbent clogging caused by samples with high particulate content.

c) Attach the connected SPE cartridges to the SPE manifold (**VMF016GL**).

Tip 2: The carbon cartridge is not needed if several very polar analytes, such as 1,4-dioxane, n-nitrosodimethylamine, n-nitrosomethylethylamine, methyl methanesulfonate, ethyl methanesulfonate, and 1-Nitrosopyrrolidine are not being analyzed.

Tip 3: Use SPE cartridges with higher sorbent amounts (**EU52113M6** and **EC82702M15**) for samples with > 500 mL volume or highly spiked (> 20 µg for each target analyte).

3. Cartridge Conditioning

a) Wash the SPE cartridges with 15 mL of dichloromethane (DCM), soak 1 min, and apply full vacuum for 1 min.

b) Condition the SPE cartridges with 10 mL of methanol. Draw most of the way through the column leaving a thin layer (about 0.5 cm) of solvent above the frit. Do not allow cartridges to go dry from this step until instructed to do so in the cartridge drying step.

c) Equilibrate the cartridges with 10 mL of reagent water and 10 mL of 0.05N HCl.

4. **Sample Loading**
    a) Attach the large volume sample delivery tube (**VMFSTFR12**) to the top of the 8270 cartridge, and insert the stainless steel end of the tube into the sample bottle.
    b) Adjust vacuum for a fast dropwise sample flow (about 10-15 mL/min), and draw the entire sample through.
5. **Washing and Drying**
    a) Rinse the sample bottle with 10 mL of reagent water, and apply the rinsate to the SPE cartridges.
    b) Disassemble the transfer tube and the connected SPE cartridges. Dry the 8270 cartridge under full vacuum for 10 min, and the carbon cartridge for 15 min.

Tip 4: Remove as much water as possible, wet sorbents result in low analyte recovery.

6. **Analyte Elution**
    a) Insert the collection rack (**VMF02125**) with 40-60 mL glass vials into the manifold.
    b) Elute the SPE 8270 and carbon cartridges separately. Apply elution solvent to the SPE cartridges, draw 1/3 through, soak 1-2 min, and then draw the remaining solvent through the cartridge in a slow dropwise fashion. Leave full vacuum on for 1 min after each elution.

8270 Cartridge	10 mL 1:1 acetone:n-hexane* (bottle rinse added to 8270 cartridge using transfer tube)
	10 mL DCM
	4 mL ammonium hydroxide (28-30%), drain to waste
	3 x 7 mL DCM
Carbon Cartridge	5 x 3 mL DCM

*: Use 10 mL DCM if Horizon's DryVap is used to dry the SPE eluates, as acetone (water miscible) may cause low recovery for some polar analytes, such as 2,4-dinitrophenol.

Tip 5: Bottle rinse is critical for good recovery of PAHs, which tend to adsorb on the glass wall.

7. Eluate Drying

a) Dry the eluates using a 15-mL reservoir (or a glass funnel stopped with glass wool) holding about 15-20 g of anhydrous Na_2SO_4, pre-rinse the Na_2SO_4 with 10 mL of DCM.

b) Insert the collection rack with 40-60 mL glass vials into the manifold to collect the dried eluates.

c) Pass the eluates through the Na_2SO_4 bed.

d) Rinse the eluate vials with 2 x 5 mL of DCM, transfer the rinses to the Na_2SO_4 bed.

Tip 6: If Na_2SO_4 appears greenish, rinse with more solvent until it turns white.

8. Concentration

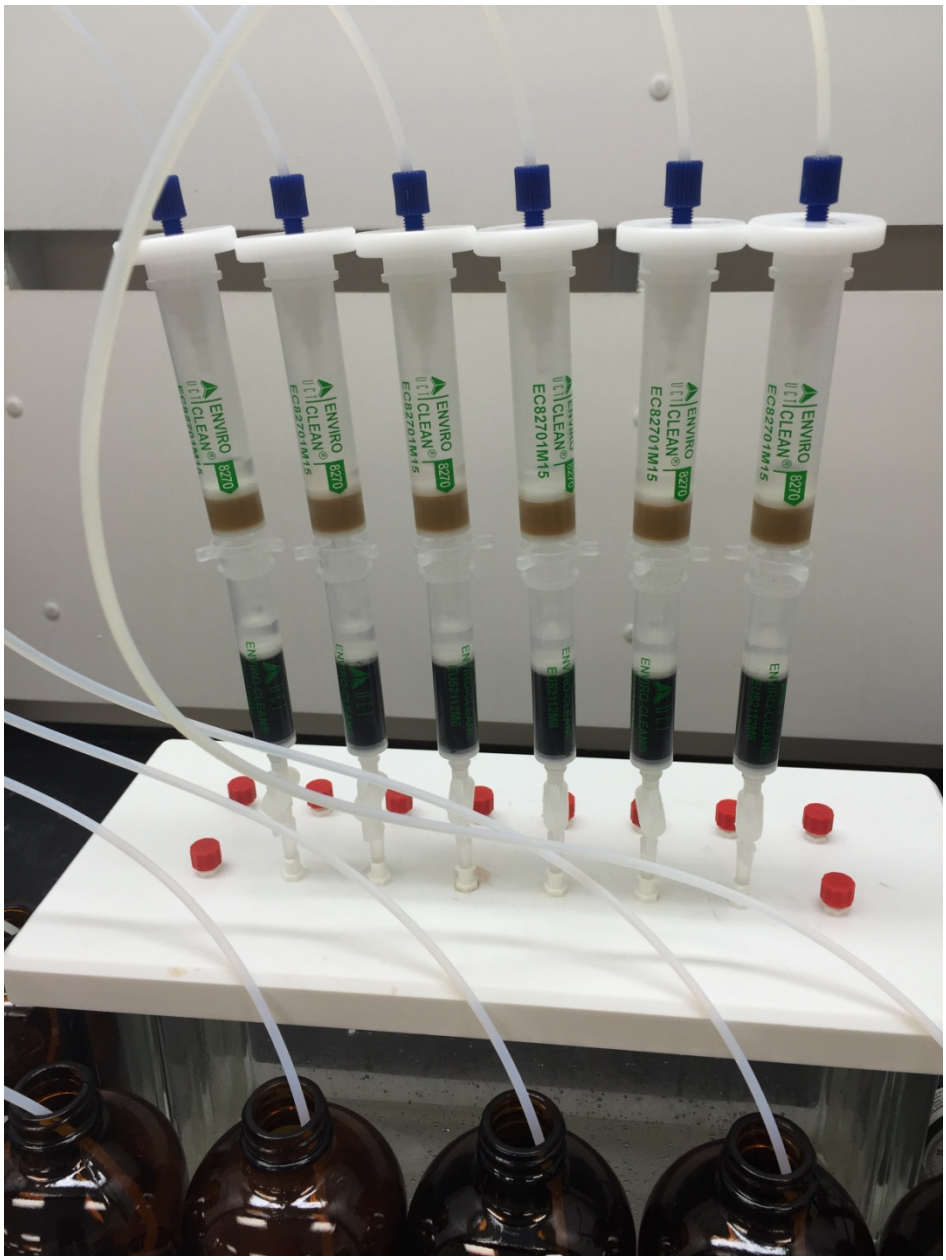
a) Concentrate the eluates to 0.7-0.9 mL under a gentle stream of N_2 at 40 ºC.

b) Add internal standards, transfer the extract to a 2-mL auto-sampler vial, and adjust the final volume to 1 mL.

c) The samples are ready for GC/MS analysis.

GC/MS Method

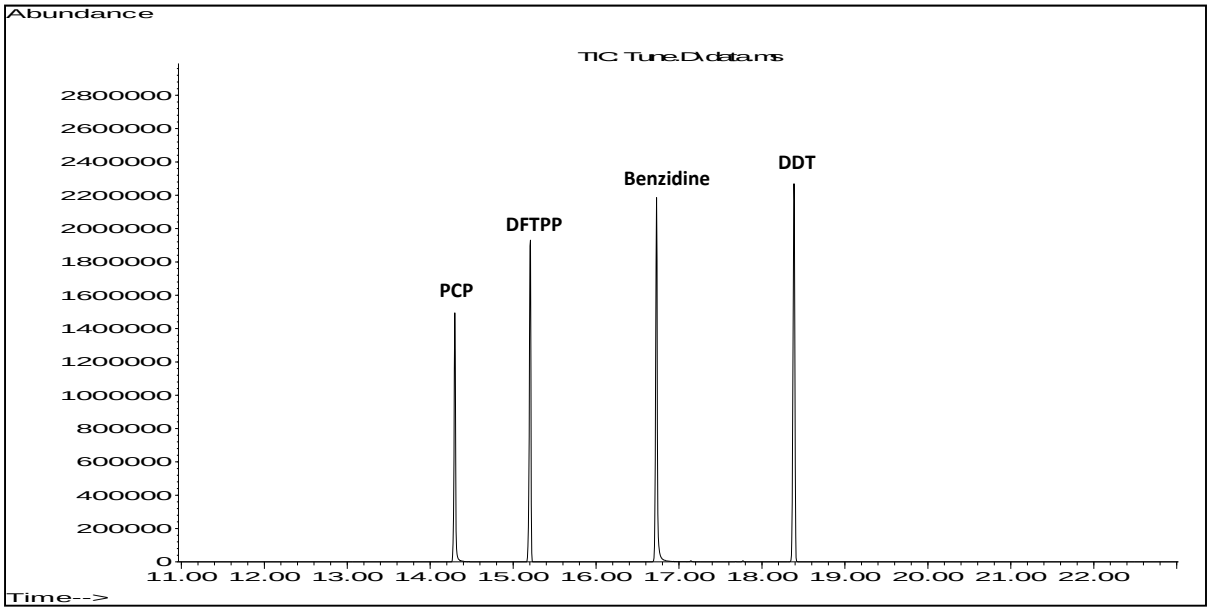
GC/MS	Agilent 6890N GC coupled to a 5975C MSD
Injection	1 µL splitless injection at 250 ºC, split vent of 30 mL/min at 1 min
GC Liner	4 mm splitless gooseneck (GCLGN4MM-5), packed with deactivated glass wool
GC Column	Restek Rxi®-5sil MS 30m x 0.25mm, 0.25µm with 10m integrated guard column
Carrier Gas	Ultra high purity helium at a constant flow of 1.5 mL/min
Oven Temp. Program	Initial temperature at 40 ºC, hold for 3 min; ramp at 15 ºC/min to 240 ºC; ramp at 6 ºC/min to 310 ºC; and hold for 2 min
MSD Temp.	Transfer line 280 ºC; Source 250 ºC; Quadrupole 150 ºC
Full Scan Range	35 - 500 amu



UCT SPE Extraction System

The 8270 cartridge on top captures the majority compounds including bases, neutrals and acids, while the downstream carbon cartridge retains a few very polar compounds, such as 1,4-dioxane, n-nitrosodimethylamine, n-nitrosomethylethylamine, methyl methanesulfonate, ethyl methanesulfonate, and 1-nitrosopyrrolidine.

GC/MS Performance Check using UCT GC Liners (GCLGN4MM-5)



DFTPP tune:

DFTPP tune met the method required criteria.

Tailing factor:

Pentachlorophenol (PCP) = 1.03

Benzidine = 0.88

Tailing factors met the required criteria (< 2).

DDT breakdown:

(DDE + DDD) / (DDE + DDD + DDT) * 100% = 0.4%

DDT breakdown met the required criteria (< 20%).

Target Analyte Recovery from the Spiked DI Water, Synthetic Wastewater (SWW), and TCLP Buffer Samples, including Method Detection Limits (MDL)

Target Analyte	Average Recovery%			MDL
	DI water	SWW	TCLP	(µg/L)
1,4-Dioxane	58	42	50	0.7
Pyridine	59	27	51	0.6
N-nitrosodimethylamine	80	82	83	0.7
2-Picoline	79	82	75	0.7
N-nitrosomethylethylamine	88	92	88	0.9
Methyl methanesulfonate	80	69	68	0.5
N-nitrosodiethylamine	90	99	96	0.7
Ethyl methanesulfonate	93	96	92	1.0
Pentachloroethane	83	73	78	0.7
Aniline	95	86	82	2.1
Phenol	80	102	103	1.1
Bis[2-chloroethyl]ether	89	99	97	0.7
2-Chlorophenol	99	107	101	0.6
1,3-Dichlorobenzene	79	71	76	0.5
1,4-Dichlorobenzene	82	73	78	0.8
Benzyl alcohol	104	93	100	1.8
1,2-Dichlorobenzene	85	78	82	0.9
2-Methylphenol	97	117	103	1.2
Bis[2-chloroisopropyl]ether	89	95	98	0.5
Acetophenone	105	108	107	1.2
1-Nitrosopyrrolidine	95	107	101	1.1
3&4-Methylphenol	106	121	106	3.0
o-Toluidine	94	97	83	1.3
N-nitro-di-n-propylamine	99	111	109	0.9
Hexachloroethane	83	71	76	1.1
Nitrobenzene	96	98	99	1.2
1-Nitrosopiperidine	92	104	103	0.7
Isophorone	95	102	100	1.0
2-Nitrophenol	102	104	100	1.1
2,4-Dimethylphenol	103	107	109	0.8
Bis[2-chloroethoxy]methane	99	108	104	0.8
Benzoic acid	122	116	113	1.1
2,4-Dichlorophenol	103	118	106	0.5
1,2,4-Trichlorobenzene	88	82	88	0.7

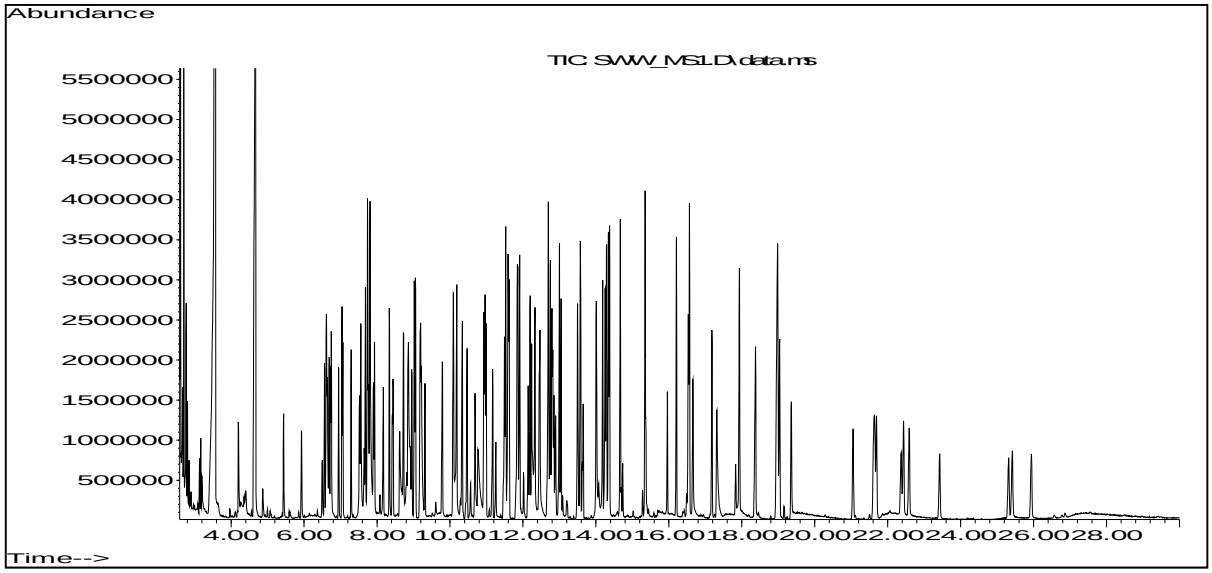
Naphthalene	94	91	90	0.7
2,6-Dichlorophenol	109	114	105	1.6
4-Chloroaniline	103	108	89	1.0
Hexachloropropene	67	42	76	0.8
Hexachlorobutadiene	71	76	79	1.0
N-nitroso di-n-butylamine	97	107	102	2.3
4-Chloro-3-methylphenol	108	120	106	2.2
Isosafrole (cis & trans)	98	96	94	2.1
2-Methylnaphthalene	96	102	91	2.2
1-Methylnaphthalene	97	95	92	2.2
1,2,4,5-Tetrachlorobenzene	88	86	86	1.7
Hexachorocyclopentadiene	70	31	78	1.1
2,4,6-Trichlorophenol	104	96	100	2.0
2,4,5-Trichlorophenol	108	125	106	2.4
Safrole	91	92	95	1.3
1-Chloronaphthalene	101	93	96	2.1
2-Chloronaphthalene	94	80	85	2.3
2-Nitroaniline	106	116	103	2.4
1,4-Naphthalenedione	93	69	71	1.7
Dimethyl phthalate	106	117	106	2.1
Acenaphthylene	100	101	98	1.9
2,6-Dinitrotoluene	104	118	102	1.9
3-Nitroaniline	100	111	97	0.6
3-Nitrophenol	106	70	98	0.9
Acenaphthene	98	96	94	0.7
2,4-Dinitrophenol	123	91	91	2.4
Dibenzofuran	99	97	94	0.8
4-Nitrophenol	102	106	97	3.3
Pentachlorobenzene	90	81	88	0.4
2,4-Dinitrotulene	108	102	103	1.1
1-Naphthalenamine	111	107	88	2.8
2,3,4,6-Tetrachlorophenol	101	102	98	0.8
2-Naphthalenamine	128	119	107	1.2
Diethyl phthalate	107	116	105	1.4
Fluorene	99	98	96	1.4
4-Chlorophenylphenylether	95	93	92	1.4
5-Nitro-o-toluidine	92	99	95	1.3
4-Nitroaniline	110	107	102	1.6
4,6-Dinitro-2-methylphenol	110	72	95	1.6
Diphenylamine	103	113	101	1.5

Azobenzene	100	97	99	1.5
Diallate (cis & trans)	100	104	102	1.7
1,3,5-Trinitrobenzne	119	80	99	1.4
Bromophenoxybenzene	92	92	90	1.1
Phenacetin	110	116	107	1.4
Hexachlorobenzene	94	65	94	1.1
4-Aminobiphenyl	95	89	82	1.6
Pentachlorophenol	105	99	95	2.6
Pronamide	104	116	104	2.3
Pentachloronitrobenzene	98	79	97	1.4
Phenanthrene	106	104	103	1.0
Dinoseb	119	69	100	1.2
Anthracene	107	104	104	1.4
Carbazole	109	117	107	1.2
Dibutyl phthalate	115	121	111	1.3
Isodrin	108	64	103	1.0
Fluoranthene	107	103	106	1.2
Benzidine	80	27	61	0.9
Pyrene	107	103	106	1.1
p-Dimethylaminoazobenzene	89	94	87	0.6
Chlorobenzilate	116	124	109	0.8
Benzyl butyl phthalate	114	115	109	1.1
2-Acetylaminofluorene	114	123	112	1.3
Benz[a]anthracene	105	79	103	1.4
3,3'-Dichlorobenzidine	72	78	68	1.2
Chrysene	103	78	105	0.6
Bis(2-ethylhexyl) phthalate	115	50	112	1.1
Di-n-octyl phthalate	115	45	112	0.5
Benzo[b]fluoranthene	97	54	103	0.7
Benzo[k]fluoranthene	108	64	109	0.6
7,12-Dimethyl benz[a]anthracene	98	55	101	0.7
Benzo[a]pyrene	101	60	106	0.8
3-Methylcholanthrene	105	48	106	0.7
Indeno[123-cd]pyrene	101	48	104	0.9
Dibenz[ah]anthracene	105	48	104	0.8
Benzo[ghi]perylene	102	47	106	0.9
Overall mean	**98**	**90**	**96**	**1.3**

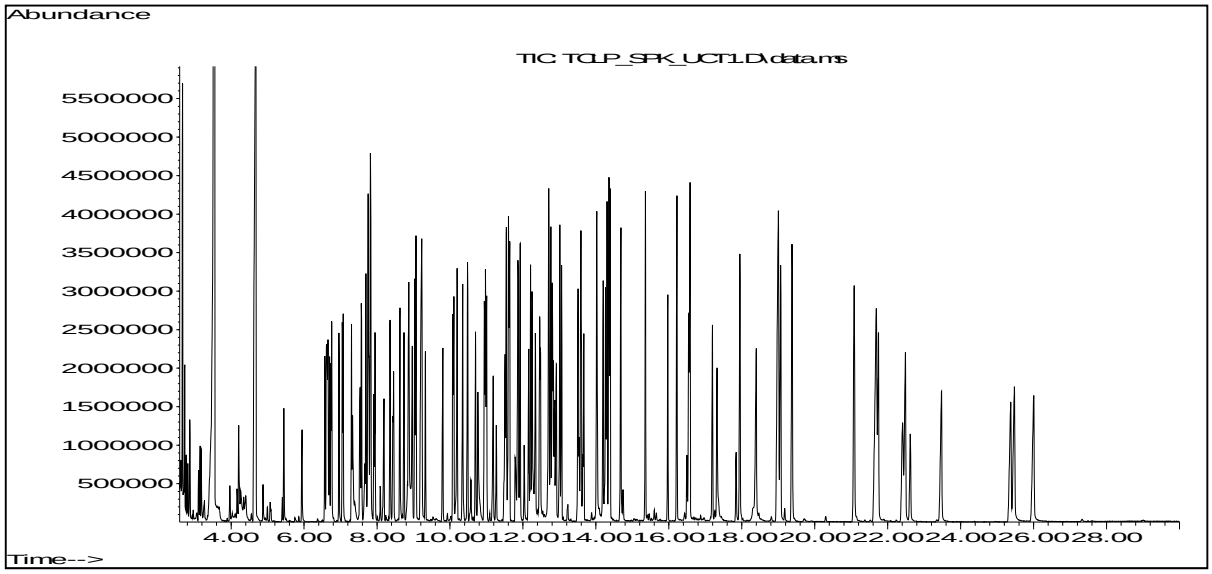
Surrogate Recovery in the Spiked DI Water, Synthetic Wastewater, and TCLP Buffer Samples

Surrogate	Average Recovery%		
	DI water	SWW	TCLP
N-nitrosodimethylamine d6	77	69	81
Phenol d5	80	78	92
Bis(2-chloroethyl) ether d8	89	82	94
2-Chlorophenol d4	95	86	92
4-Methylphenol d8	99	98	98
Nitrobenzene d5	91	81	91
2-Nitrophenol d4	100	87	93
2,4-Dichlorophenol d3	98	95	96
4-Chloroaniline d4	94	85	79
Dimethylphthalate d6	99	95	94
Acenaphthylene d8	93	81	86
4-Nitrophenol d4	111	88	86
Fluorene d10	92	85	89
4,6-Dintro-2-methylphenol d2	119	69	96
Anthracene d10	92	84	90
Pyrene d10	95	86	93
Benzo(a)pyrene d12	95	50	98
Overall mean	**95**	**82**	**91**

GC/MS Chromatograms



200 mL Synthetic Wastewater Spiked with 100 µg/L of Target Analytes



200 mL TCLP Sample Spiked with 100 µg/L of Target Analytes

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EPA Method 1664B:n- Hexane Extractable Material (HEM; Oil and Grease)

UCT Part Numbers**:**
ECUCTVAC6 - Enviro-Clean® 6-Station Vacuum Manifold
ECROCKER400 - Rocker 400 Vacuum Pump 110 volt
ECUCTTRAP20 - 20L Manifold Trap
ECUCTADP - Enviro-Clean® Glass Cartridge Adaptor
ECUNIBHD - Enviro-Clean® Universal White Bottle Holder
ECUNIOGXF- Enviro-Clean® Universal Oil & Grease XF 2000mg/83mL
ECSS25K - Enviro-Clean® Bulk Anhydrous Sodium Sulfate 25kg
ECUNIMSS - Enviro Clean® Universal Cartridge 20g Muffled Sodium Sulfate

Procedure

1. Assemble

a) Connect a 6-station vacuum manifold (ECUCTVAC6) to a 20-L manifold trap (ECUCTTRAP20), and attach the trap to a vacuum pump (ECROCKER400)
b) Attach the glass adapters (ECUCTADP) onto the manifold
c) Connect the bottle holders (ECUNIBHD) to the top of the SPE cartridges (ECUNIOGXF), and attach the cartridges to the glass adaptors on the manifold

2. Prepare Water Sample

a) Adjust the pH of the sample to < 2 by adding 6N HCl or H_2SO_4.
b) If acid was added to the sample in the field, do not add more unless the pH > than 2

Note: Gloves are recommended as skin oils may affect final sample weight

3. Condition the Cartridge

a) Rinse the sides of the cartridge and bottle holder with 10 mL of n-hexane
b) Allow cartridge to soak for 1 minute
c) Draw the hexane through the cartridge using vacuum, leave full vacuum on for 1 min, then release the vacuum.
d) Add 10 mL of methanol to the cartridge, soak for 1 min
e) Slowly draw the methanol through leaving a thin layer on the cartridge frit
f) Add about 80 mL of D.I. Water to the cartridge
g) Draw all of the water through the cartridge to waste, do not let the sorbent go dry

4. Sample Addition

a) Load sample bottle onto the bottle holder

b) Draw the sample through the cartridge under low vacuum. This may take 20 or more minutes depending on the solids in the sample (Note 1). Increase vacuum pressure if necessary. Do not exceed 50 mL/min for optimum recoveries. This is a fast drip, but not a stream

c) After all samples are passed through, turn full vacuum on. Remove the cartridges and tap/swing to remove any excess water from the bottom of the cartridges

d) Replace the cartridges to the manifold, and dry under full vacuum for 10 min

5. Elution

a) Insert extract collection vials with a thin layer (about 0.5-0.8 cm) of anhydrous sodium sulfate (ECSS25K) in the manifold

b) Add a thin layer (about 0.5-0.8 cm) of anhydrous sodium sulfate into the SPE cartridge

c) Rinse the sample bottle with 10 mL of n-hexane

d) Add the hexane to the cartridge

e) Soak cartridges for 2 min. A slow drip of hexane is permissible

f) Turn on vacuum and draw the hexane through the cartridges and into the collection vials, leave vacuum on for 0.5 min

g) Turn off vacuum then repeat steps 5 c) – f) 2 additional times with 10 mL of n-hexane

h) Add 10 mL of hexane to the cartridges, rinsing the bottle holders

i) Soak cartridges for 2 min

j) Draw the hexane through the cartridges and collect, do not allow the solvent to splash into the collection vial

3. Dry the Extract

a) Remove the collection vials from the manifold

b) Dry the extracts by pouring them through the drying cartridges (ECUNIMSS**)** pre-rinsed with 10 mL n-hexane into the pre-weighed vials. Alternatively, use a glass wool stopped glass funnel containing approximately 10-20 g anhydrous sodium sulfate (pre-rinsed with n-hexane). Do not use filter paper

c) Rinse the extract collection vials with hexane and add it to the sodium sulfate and collect. This will rinse the vial and the sodium sulfate. Poor rinsing of the sodium sulfate will result in low recoveries

4. Gravimetric analysis

a) Carefully evaporate the samples using a nitrogen evaporator at 40° C until the extract just reaches dryness

Note: Over drying will result in low recoveries

b) Allow the samples to cool to room temperature in a desiccator before weighing

c) Record the weight difference and report as mg/L of HEM

Notes:

1) If very high solids are present, add a small plug of glass wool to the cartridge prior to extraction to prevent clogging and improve flow. The glass wool must be thoroughly rinsed with hexane as part of the cartridge during the elution step.

2) Stearic acid must be in solution in the spiking solution or low recoveries will result. If small crystals are present in the spiking solution, sonicate or shake until dissolved.

3) If white crystals are present in the sample bottle after elution, the sample pH was not low enough prior to extraction. Repeat with lower pH.

4) HCl will lose strength over time. Sulfuric acid is a good substitute.

5) Any residue that does not rinse from the bottle or elute from the cartridge is not HEM.

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Determination of Diesel Range Organics (DROs) Using SPE and GC/FID by Method 8015D*

UCT Part Number:
ECUNIPAH - ENVIRO-CLEAN®- Universal PAH/DRO 2000 mg/83 mL cartridge

Method Summary

Method 8015D may be used to determine the concentrations of several nonhalogenated volatile organic compounds and semivolatile organic compounds using GC with flame ionization detection. This application outlines a solid phase extraction method using C18 to determine DROs in water sample matrices.

- Diesel Range Organics (**DRO**) corresponds to the range of alkanes from C_{10} to C_{28} and covering a boiling point range of approximately 170° C - 430° C

Sample Collection, Preservation, and Storage

- See sample collection options EPA Method 5035A

Interferences

- Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis
- All these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks
- Specific selection of reagents and purification of solvents by distillation in all-glass systems may be necessary
- Contamination by carryover occurs whenever high-concentration and low-concentration samples are analyzed in sequence. To reduce the potential for carryover, the sample syringe or purging device must be rinsed out between samples with an appropriate solvent
- All glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by rinsing with the last solvent used followed by detergent washing with hot water, and rinses with tap water and organic-free reagent water
- Drain the glassware and dry it in an oven (except volumetric glassware) at 130° C for several hours or rinse it with methanol

- FID is a non-selective detector. There is a potential for many non-target compounds present in samples to interfere with this analysis and potential for analytes to be resolved poorly, especially in samples that contain many analytes.

Reagents and Standards

- Reagent grade chemicals must be used in all tests
- Organic-free reagent water
- Methanol, CH_3OH - Pesticide quality or equivalent
- Methylene Chloride – Pesticide residue quality or equivalent
- Fuels, e.g., diesel - Purchase from a commercial source
- Alkane standard - Standard contains a homologous series of *n*-alkanes for establishing retention times (e.g., C_{10}-C_{28} for diesel)

Stock Standards

- Prepared from pure standard materials or purchased as certified solution
- Standards must be replaced after 6 months

Secondary Dilution Standards

- Using stock standard solutions, prepare secondary dilution standards in methanol, as needed either singly or mixed together
- Secondary dilution standards should be stored with minimal headspace for volatiles
- Check frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards

Recommended GC Columns

The choice of GC column will depend on the analytes of interest, expected concentrations, and intended use of the results. The capillary columns are necessary for petroleum hydrocarbon analyses and are recommended for all other analyses. Other columns may be employed if the analyst can demonstrate acceptable performance.

- Establish the GC operating conditions appropriate for the GC column being utilized and the target analytes specified in the project plan

- Optimize the instrumental conditions for resolution of the target analytes and sensitivity
- Suggested operating conditions and GC programs are given below for the recommend columns:

  (DRO) 30-m x 0.53-mm ID fused-silica capillary column chemically bonded with 5% methyl silicone (DB-5, SPB-5, RTX-5, or equivalent), 1.5-µm film thickness
    - Carrier gas (He) flow rate: 5-7 mL/min
    - Makeup gas (He) flow rate: 30 mL/min
    - Injector temperature: 200° C
    - Detector temperature: 340° C
    - Temperature program: Initial temperature: 45° C, hold for 3 minutes
    - Program: 45° C to 275° C, at 12° C/min
    - Final temperature: 275° C, hold for 12 minutes

SPE Procedure**

1. **Condition Cartridge**
    a) Assemble an SPE manifold system **UCT ECUCTVAC1, ECUCTVAC3 or ECUCTVAC6** (1,3 or 6 station)
    b) Place a **ECUNIPAH** cartridge(s) on the vacuum manifold
    c) With vacuum off add 10 mL of methylene chloride to the cartridge
    d) Let it soak for 1 minute
    e) Turn on vacuum and draw through to waste
    f) Draw vacuum through the cartridge to remove all methylene chloride
    g) Add 10 mL of methanol to the cartridge
    h) Draw the methanol to the level of the frit
    i) Add 10 mL of deionized water to the cartridge
    j) Draw most of the water to waste but do not allow the sorbent to dry

    Note: Do not let the cartridge go completely dry after addition of methanol otherwise repeat starting at step 1.g)
2. **Sample Addition**
    a) Adjust the pH of the sample to 2 or less using 5 mL of 1:1 HCl, shake
    b) Add surrogate, and DRO spiking solution for fortified samples, to 1 mL methanol in a test tube, then transfer the mix to the water sample. Rinse the test tube with 1 mL methanol, transfer the rinse to the sample, and mix well. This helps to improve the recovery of LCS samples, especially for the lightweight hydrocarbons.
    c) Add the sample to the cartridge under vacuum. Draw the sample through the cartridge no faster than 20 – 30 minutes per liter
    d) Allow the cartridge to dry under full vacuum for 5 minutes***

3. **Extract Elution**
    a) Place a collection tube or vial in the vacuum manifold, and add approximately 1 cm of pre-baked sodium sulfate anhydrous to the SPE cartridge
    b) Rinse sample bottle with 10 mL of methylene chloride to remove any analyte from the glass
    c) Add the methylene chloride rinse to the cartridge
    d) Allow to soak for 1-2 minutes then draw through
    e) Repeat this procedure three more times using 10 mL aliquots of methylene chloride
    f) Dry the extract by passing it through about 10-20 grams of methylene chloride pre-rinsed sodium sulfate **ECSS25K**
    g) Thoroughly rinse the collection container with methylene chloride and add this solvent to the sodium sulfate and collect

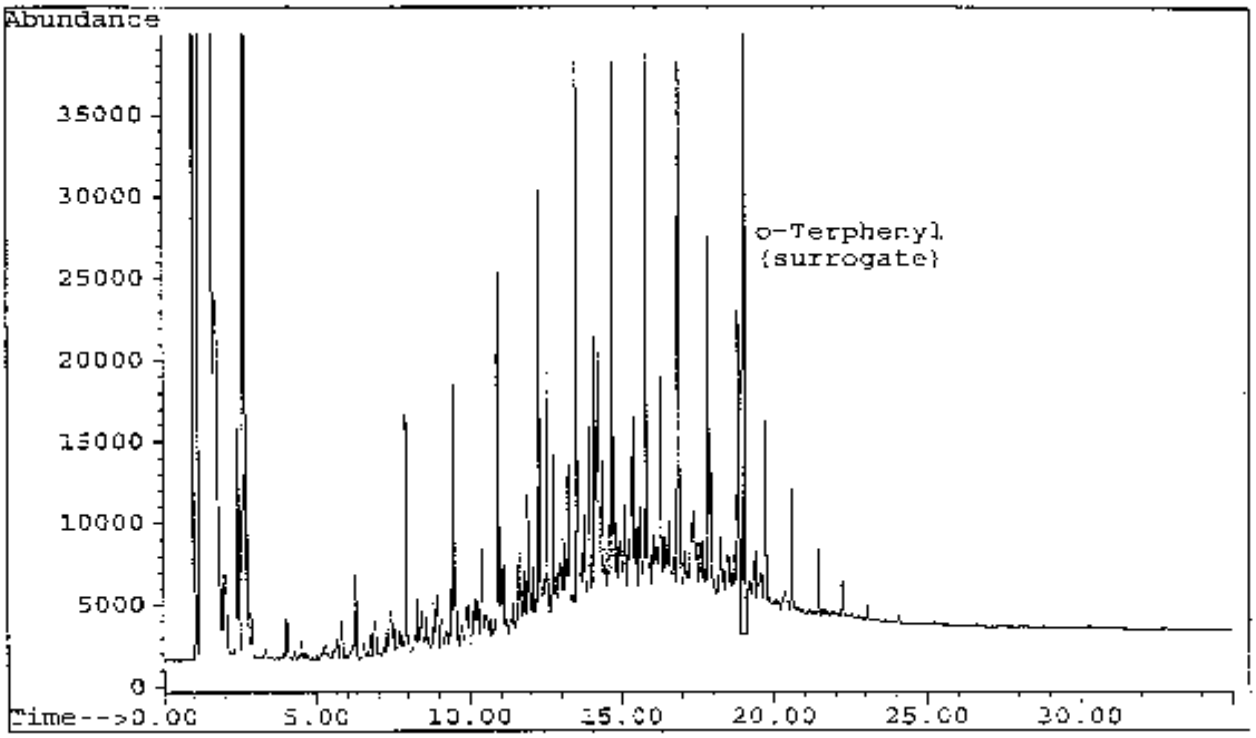
4. **Concentration**

    Option 1: Evaporate the dried extract to 1 mL using Turbovap with a gentle stream of nitrogen (7-8 psi) at 35 ºC. Do not overdry the extract otherwise low recovery will result due to the loss of more volatile hydrocarbons.

    Option 2: Concentration of final extract using a micro Kaderna-Danish flask equipped with a 3-ball Snyder column is recommended to avoid loss of low boiling alkanes. A micro-Snyder column can be used to evaporate the extract to a 1 mL final volume.

    Note: Extracts should not be stored with headspace for extended periods of time as low recoveries will result

Example Chromatogram of a 30 ppm Diesel Standard



Accuracy and Precision of LCS (Spiked at 500 ppm)

Analyte	Ave Recovery%	RSD% (n=3)
Diesel	100.7	1.5

*Summarized from Method 8015D Nonhalogenated Organics Using GC/FID, Revision 4, June 2003, and associated appropriate methods

**(steps 1- 4 added to this method summary for use with UCT PAH/DRO cartridges)

***Faster drying results can be obtained by removing the cartridge during drying and shaking or tapping the excess moisture from the bottom of the cartridge. Drying times are approximate. Do not over dry as low recoveries may result

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EPA Method 8141B Determination of Organophosphorus Pesticides and Triazine Herbicides in Water by Solid Phase Extraction

UCT Part Numbers:
ECHLD156-P – Enviro-Clean® HL DVB 500 mg, 6 mL cartridge, PE Frit
ECSS15M6 - 5 g anhydrous sodium sulfate in 6 mL cartridge
AD0000AS - Cartridge adaptor

This application note describes a solid phase extraction (SPE) method for the determination of EPA method 8141B analytes, including organophosphorus compounds and triazine herbicides in water. Target analytes are extracted onto a polymeric sorbent from water samples at neutral pH. Proper sample pH is critical for good analyte recoveries as organophosphorus esters hydrolyze under acidic or basic conditions. The adsorbed analytes are eluted with acetone and dichloromethane (DCM). A drying cartridge is attached downstream from the SPE cartridge in the elution step, eliminating the need for an additional extract drying step. The eluate is evaporated and exchanged to n-hexane. A gas chromatograph (GC) equipped with a 30-meter capillary column along with either a FPD, NPD or MS detector is employed for analyte separation and detection. Excellent recoveries and relative standard deviations (RSD) were obtained for 13 representative compounds (11 organophosphorus pesticides and 2 triazine herbicides).

Experimental:

SPE procedure:

1. To 1 L* portions of neutral water samples add appropriate amounts of surrogates. Surrogates and target analytes are spiked into fortified samples.
2. Attach the SPE cartridges to a glass block manifold.
3. Cartridge Conditioning
   a) Wash the SPE cartridges with 5 mL DCM, pass 1/3 through to wet the sorbent, soak 1 min before draw the remaining through, turn full vacuum on for 1 min. Repeat the wash with additional 2 aliquots of 5 mL DCM.

b) Condition the cartridges with 2 aliquots of 5 mL methanol. Do not allow the sorbent go dry unless instructed so in the cartridge drying step.

c) Equilibrate the cartridges with 2 aliquots of 5 mL DI water. Leave about 4 mL of water in the cartridge, and connect sample transfer tubes to the SPE cartridges (**ECHLD156-P**).

4. Sample Loading

a) Insert the stainless steel ends of the sample transfer tubes into the sample containers and completely draw the samples through the SPE cartridges in a fast drop-wise fashion (10-15 mL/min).

b) Remove the transfer tubes from the SPE cartridges and dry the SPE cartridges under full vacuum for 10 min.

5. Eluate Drying and Elution

a) Attach the drying cartridges (**ECSS15M6**) to the bottom of the SPE cartridges with the cartridge adaptors (**AD0000AS**).

b) Insert a collection tube or vial into the manifold underneath each SPE cartridge.

c) Rinse each sample bottle with 5 mL acetone, and pull the rinsates through the SPE cartridges slowly using the transfer tubes. Turn full vacuum on for 1 min to pull all of the elution solvent into the collection container.

d) Repeat Step 5c. with 10 mL DCM.

e) Remove the transfer tubes from the SPE cartridges. Add 5 mL DCM into the SPE cartridges, pass 1/3 though, soak 1 min and draw the remaining through in a slow drop- wise fashion.

6. Eluate Concentration

a) Concentrate the eluates to about 0.5 mL under a gentle stream of nitrogen at 40 ºC. b. Rinse the wall of the eluate containers with 3 mL of n-hexane, and continue concentrating to about 2 mL.

b) Transfer the extracts to 2-mL auto-sampler vials, and adjust the final volume to 2 mL with n-hexane.

c) Add internal standard, the samples are ready for GC analysis.

**Use of a smaller sample volume is permitted if method sensitivity is not an issue.*

GC/MS method:

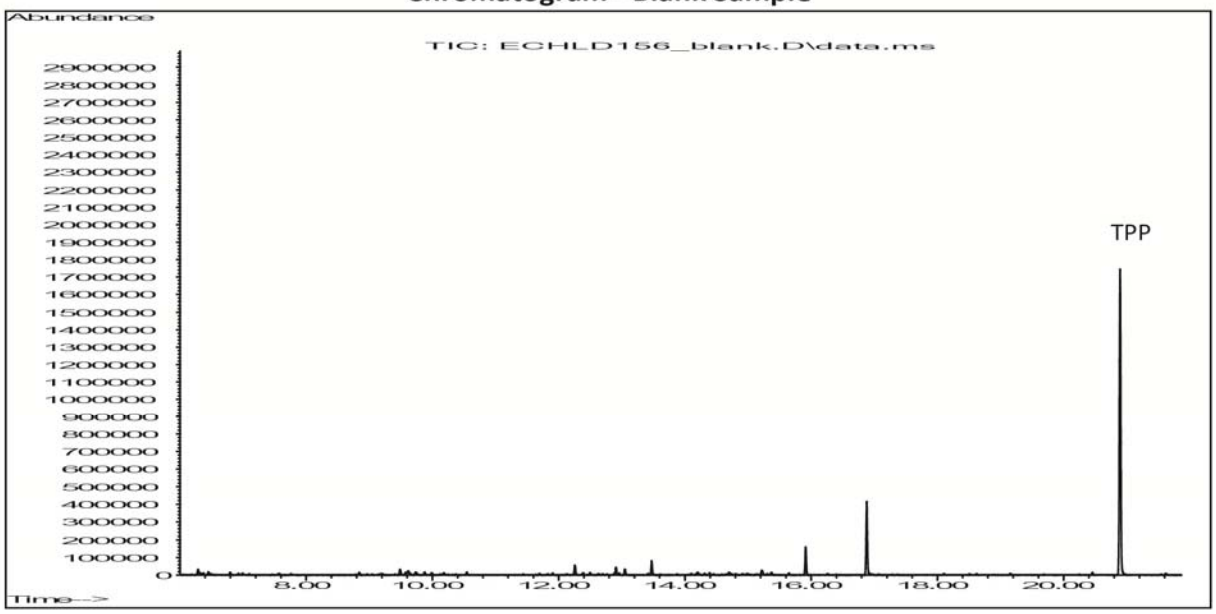
GC/MS	Agilent 6890N GC coupled with 5975C MSD
Injector Temp	250 ºC
Injection Volume	2 µL splitless
GC column	Restek Rxi®-5sil MS 30mx0.25mm, 0.25µm with 10m guard column
Carrier gas	Helium at a constant flow of 1.2 mL/min
Oven	Initial temperature at 60 ºC, hold for 1 min; ramp at 10 ºC/min to 300 ºC, hold for 2 min.
Transfer line	280 ºC
Tune	dftpp.u
Full Scan	45-450 amu

Results:

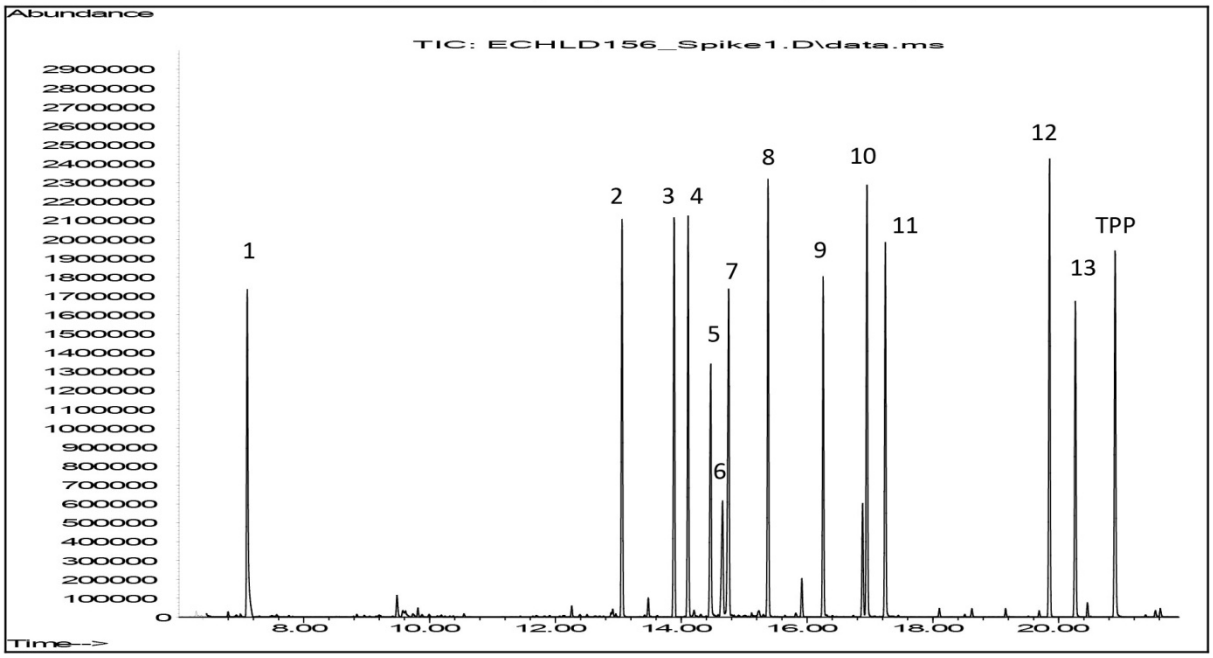
Accuracy and Precision Data

Compound	Class	Spiked (µg/L)	Recovery%	RSD% (n=5)
o,o,o-Triethyl phosphorothioat	Organophosphorus	10	**90.7**	1.8
Thionazin	Organophosphorus	10	**98.3**	1.8
Sulfotep	Organophosphorus	10	**100.5**	1.0
Phorate	Organophosphorus	10	**94.2**	1.2
Dimethoate	Organophosphorus	10	**80.4**	7.4
Simazine	Triazine	10	**100.1**	2.1
Atrazine	Triazine	10	**103.5**	1.1
Disulfoton	Organophosphorus	10	**88.1**	1.7
Methyl	Organophosphorus	10	**105.9**	1.8
Malathion	Organophosphorus	10	**109.4**	1.3
Parathion	Organophosphorus	10	**106.0**	1.3
Ethion	Organophosphorus	10	**108.0**	0.7
Famphur	Organophosphorus	10	**105.0**	1.9
		Overall mean	**99.2**	**1.9**

Chromatogram - Blank Sample



Chromatogram - Sample spiked with 10 µg/L analytes



Peak list: 1. o,o,o-Triethyl phosphorothioate; 2. Thionazin; 3. Sulfotep; 4. Phorate; 5. Dimethoate; 6. Simazine; 7. Atrazine; 8. Disulfoton; 9. Methyl parathion; 10. Malathion; 11. Parathion; 12. Ethion; 13. Famphur.

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EPA Method 8270: Determination of Semi-volatile Organic Compounds in Water using Solid Phase Extraction and GC/MS

UCT Part Numbers:
EC82701M15 - 1000 mg 8270 sorbent/15 mL cartridge (≤ 500 mL sample)
EU52112M6 - 2000 mg activated carbon/6 mL cartridge (≤ 500 mL sample)
EC82702M15 - 2000 mg 8270 sorbent/15 mL cartridge (> 500 mL sample)
EU52113M6 - 3000 mg activated carbon/6 mL cartridge (> 500 mL sample)
AD0000AS - Cartridge adaptor
VMFSTFR12 - Large volume sample transfer tubes
VMF016GL - 16 position glass block manifold
VMF02125 - 12 position large volume collection rack
RFV1F15P - 15 mL reservoirs with 1 frit, 10 micron porosity
ECSS25K - Sodium sulfate, anhydrous, ACS grade, granular, 60 mesh
GCLGN4MM-5 - GC liner, 4 mm splitless gooseneck

EPA method 8270 allows the use of liquid-liquid extraction (LLE) and solid phase extraction (SPE) to extract semi-volatile organic compounds (SVOCs) in aqueous samples and TCLP leachates. LLE requires multiple extractions at two different pH values, consumes large amounts of organic solvents, and causes emulsion when real world dirty samples are extracted.

This application note outlines a reliable, efficient, and cost-effective SPE method utilizing two stacked SPE cartridges, UCT's EC8270 and activated carbon cartridges for the extraction of SVOCs in water and TCLP samples. Prior to extraction, samples are dechlorinated and adjusted to pH < 2, then passed through the SPE system, the 8270 SPE cartridge retains the majority of the target analytes including acids, bases, and neutrals with mid to high hydrophobicity, while the carbon cartridge connected downstream will capture a few very polar compounds*, such as *1,4-dioxane*, n-nitrosodimethylamine, n-nitrosomethylethylamine, methyl methanesulfonate, ethyl methanesulfonate, and1-Nitrosopyrrolidine. High sample throughout can be achieved by extracting multiple samples simultaneously using a multi-port SPE manifold.

**: Carbon cartridge is NOT needed if none of the very polar analytes is being analyzed, such as for the TCLP SVOCs list.*

SPE Procedure:

1. Sample Pretreatment

a) Dechlorinate sample with 80 mg/L of sodium thiosulfate if free chlorine presents.
b) Adjust sample pH to < 2 using 6N HCl.
c) Spike with surrogates, and target analytes for fortified samples.

Tip 1: The spiking solutions should be prepared in water miscible solvents, such as methanol, acetonitrile, or acetone.

2. SPE System Setup

a) Connect the carbon cartridge (**EU52112M6** or **EU52113M6** depending on sample volume) to the end of the 8270 cartridge (**EC82701M15** or **EC82702M15** depending on sample volume) using a cartridge adaptor (**AD0000AS**).
b) Insert a loose plug of deactivated glass wool into the 8270 cartridge to prevent sorbent clogging caused by samples with high particulate content.
c) Attach the connected SPE cartridges to the SPE manifold (**VMF016GL**).

Tip 2: The carbon cartridge is not needed if very polar analytes, such as 1,4-dioxane, n-nitrosodimethylamine, n-nitrosomethylethylamine, methyl methanesulfonate, ethyl methanesulfonate, and 1-Nitrosopyrrolidine are not being analyzed.

3. Cartridge Conditioning

a) Wash the SPE cartridges with 15 mL of dichloromethane (DCM), soak 1 min, and apply full vacuum for 1 min.
b) Condition the SPE cartridges with 10 mL of methanol. Draw most of the way through the column leaving a thin layer (about 0.5 cm) of solvent above the frit. Do not allow cartridges to go dry from this step until instructed to do so in the cartridge drying step.
c) Equilibrate the cartridges with 10 mL of reagent water and 10 mL of 0.05N HCl.

4. Sample Loading

a) Attach the large volume sample delivery tube (**VMFSTFR12**) to the top of the 8270 cartridge, and insert the stainless steel end of the tube into the sample bottle.

b) Adjust vacuum for a fast dropwise sample flow (about 10-15 mL/min), and draw the entire sample through.

5. Washing and Drying

a) Rinse the sample bottle with 10 mL of reagent water, and apply the rinsate to the SPE cartridges.

b) Disassemble the transfer tube and the connected SPE cartridges. Dry the 8270 cartridge under full vacuum for 10 min, and the carbon cartridge for 15 min.

Tip 3: Remove as much water as possible, wet sorbents result in low analyte recovery.

6. Analyte Elution

a) Insert the collection rack (**VMF02125**) with 40-60 mL glass vials into the manifold.

b) Elute the SPE 8270 and carbon cartridges separately. Apply elution solvent to the SPE cartridges, draw 1/3 through, soak 1-2 min, and then draw the remaining solvent through the cartridge in a slow dropwise fashion. Leave full vacuum on for 1 min after each elution.

8270 Cartridge	10 mL 1:1 acetone:n-hexane (bottle rinse added to 8270 cartridge using transfer tube)
	10 mL DCM
	3 - 4 mL ammonium hydroxide (28-30%), drain to waste
	3 x 7 mL DCM
Carbon Cartridge	5 x 3 mL DCM

Tip 4: Bottle rinse is critical for good recovery of PAHs, which tend to adsorb on the glass wall.

7. Eluate Drying

a) Dry the eluates using a 15-mL reservoir (or a glass funnel stopped with glass wool) holding about 15-20 g of anhydrous Na_2SO_4, pre-rinse the Na_2SO_4 with 10 mL of DCM.

b) Insert the collection rack with 40-60 mL glass vials into the manifold to collect the dried eluates.

c) Pass the eluates through the Na_2SO_4 bed.

d) Rinse the eluate vials with 2 x 5 mL of DCM, transfer the rinses to the Na_2SO_4 bed.

Tip 5: If Na_2SO_4 appears greenish, rinse with more solvent until it turns white.

8. Concentration

a) Concentrate the eluates to 0.7-0.9 mL under a gentle stream of N_2 at 40 ºC.

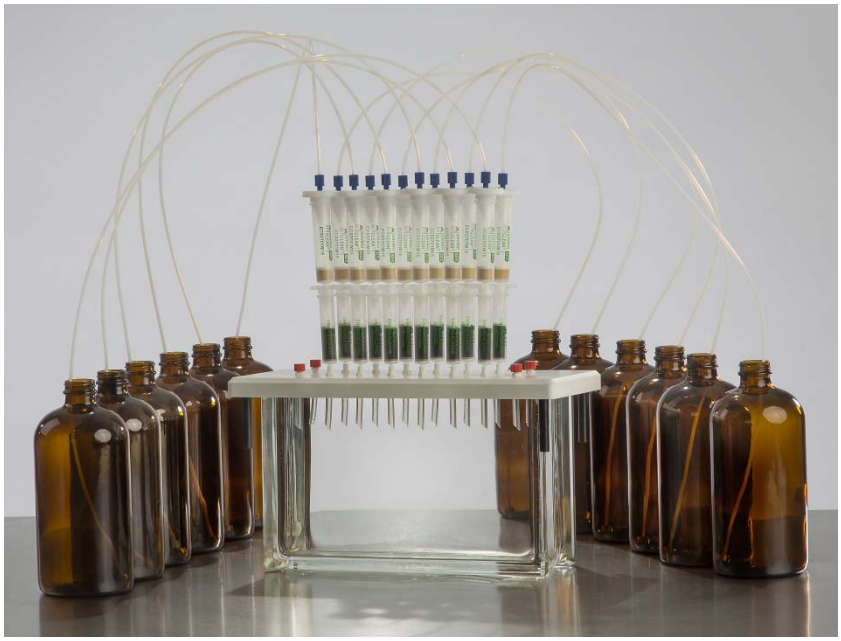
b) Add internal standards, transfer the extract to a 2-mL auto-sampler vial, and adjust the final volume to 1 mL.

c) The samples are ready for GC/MS analysis.

GC/MS Method

GC/MS	Agilent 6890N GC coupled to a 5975C MSD
Injection	1 µL splitless injection at 250 ºC, split vent of 30 mL/min at 1 min
GC Liner	4 mm splitless gooseneck (GCLGN4MM-5), packed with deactivated glass wool
GC Column	Restek Rxi®-5sil MS 30m x 0.25mm, 0.25µm with 10m integrated guard column
Carrier Gas	Ultra high purity helium at a constant flow of 1.5 mL/min
Oven Temp. Program	Initial temperature at 40 ºC, hold for 3 min; ramp at 15 ºC/min to 240 ºC; ramp at 6 ºC/min to 310 ºC; and hold for 2 min
MSD Temp.	Transfer line 280 ºC; Source 250 ºC; Quadrupole 150 ºC
Full Scan Range	35 - 500 amu

SPE Setup



Recovery and RSD in Laboratory Fortified Blanks

(500 mL sample fortified with 40 µg/L of 139 analytes and 6 surrogates)

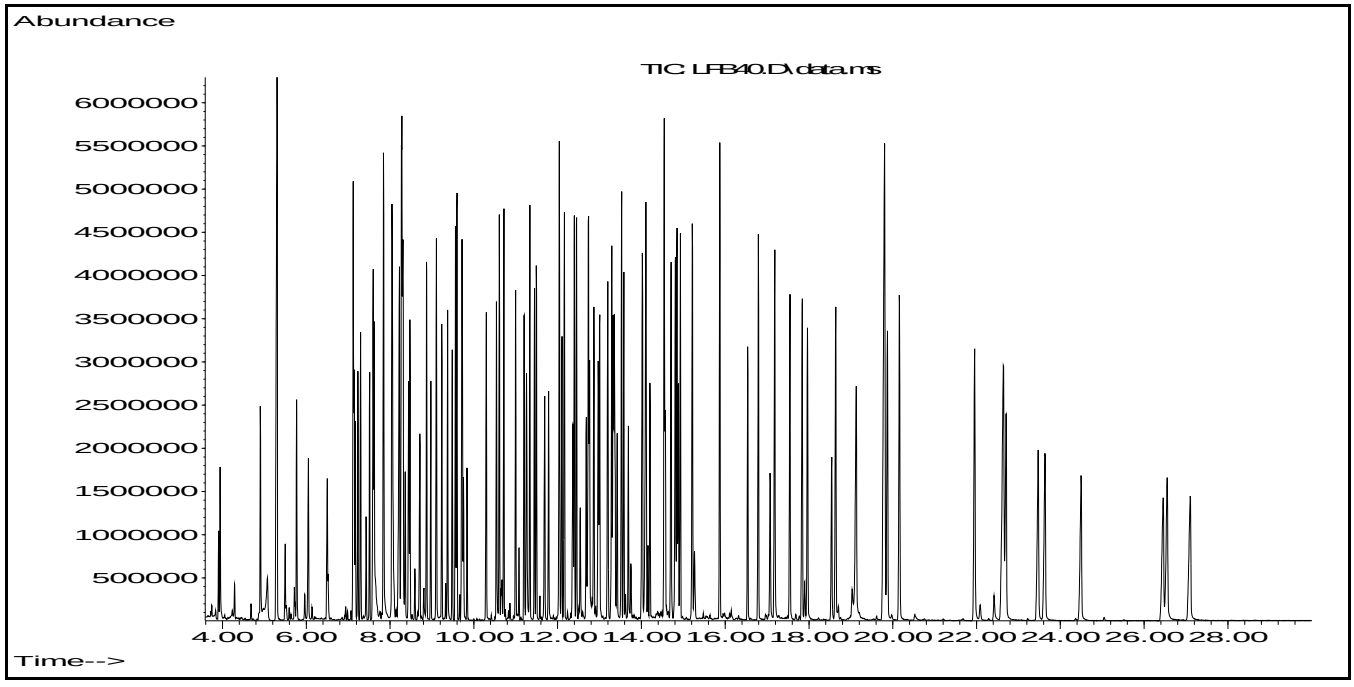
Compound	Avg Recovery%	RSD% (n=4)
1,2,4,5-Tetrachlorobenzene	**99.5**	4.1
1,2,4-Trichlorobenzene	**88.5**	5.7
1,2-Dichlorobenzene	**90.3**	3.9
1,3,5-Trinitrobenzne	**124.4**	2.8
1,3-Dichlorobenzene	**85.8**	2.8
1,4-Dichlorobenzene	**89.1**	1.1
1,4-Naphthalenedione	**95.3**	4.3
1-Chloronaphthalene	**112.2**	2.7
1-Methyl fluorene	**86.9**	0.9
1-Methyl phenanthrene	**89.8**	1.3
1-Methylnaphthalene	**102.1**	2.7
1-Naphthalenamine	**112.3**	4.7
1-Nitrosopiperidine	**88.9**	5.8
1-Nitrosopyrrolidine	**91.8**	7.2
2,3,4,6-Tetrachlorophenol	**103.2**	0.9
2,3-Dichloroaniline	**91.4**	0.6
2,4,5-Trichlorophenol	**123.5**	4.7
2,4,6-Trichlorophenol	**106.5**	3.6
2,4-Dichlorophenol	**97.3**	6.5
2,4-Dimethylphenol	**99.0**	6.4
2,4-Dinitrophenol	**122.4**	2.0
2,4-Dinitrotulene	**112.0**	1.7
2,6-Dichlorophenol	**113.3**	0.7

2,6-Dinitrotoluene	**106.3**	2.3
2-Acetylaminofluorene	**109.0**	6.5
2-Chloronaphthalene	**96.9**	2.8
2-Chlorophenol	**99.4**	2.9
2-Isopropyl naphthalene	**73.1**	0.1
2-Methylnaphthalene	**101.2**	4.9
2-Methylphenol	**97.6**	6.7
2-Naphthalenamine	**130.5**	2.7
2-Nitroaniline	**107.5**	3.6
2-Nitrophenol	**98.2**	5.9
2-Picoline	**74.4**	5.0
3&4-Methylphenol	**104.2**	6.6
3,3'-Dichlorobenzidine	**72.3**	11.4
3,6-Dimethyl phenanthrene	**90.6**	0.9
3-Methylcholanthrene	**106.5**	1.4
3-Nitroaniline	**100.4**	4.9
3-Nitrophenol	**99.5**	8.2
4,4'-DDD	**94.4**	0.8
4,4'-DDE	**91.8**	0.4
4,4'-DDT	**94.0**	0.3
4,6-Dinitro-2-methylphenol	**116.8**	4.5
4-Aminobiphenyl	**103.8**	13.5
4-Chloro-3-methylphenol	**111.7**	6.3
4-Chloroaniline	**105.0**	3.9
4-Chlorophenylphenylether	**99.5**	3.0
4-Nitroaniline	**114.9**	4.6
4-Nitrophenol	**97.2**	3.0
5-Nitro-o-toluidine	**94.7**	4.0
7,12-Dimethyl benz[a]anthracene	**99.9**	6.1
Acenaphthene	**100.1**	1.3
Acenaphthylene	**102.6**	0.6
Acetophenone	**101.8**	7.4
Aldrin	**89.5**	0.8
alpha lindane	**90.1**	0.2
Aniline	**90.0**	3.2
Anthracene	**109.7**	1.1
Azobenzene	**105.5**	5.2
Benz[a]anthracene	**103.3**	6.2
Benzidine	**66.8**	14.0
Benzo[a]pyrene	**99.3**	2.1
Benzo[b]fluoranthene	**99.4**	7.0
Benzo[ghi]perylene	**104.2**	1.1
Benzo[k]fluoranthene	**108.1**	5.4
Benzoic acid	**115.0**	4.7
Benzyl alcohol	**72.9**	12.9
Benzyl butyl phthalate	**111.8**	6.0
beta lindane	**95.2**	1.1
Bis(2-ethylhexyl) phthalate	**113.2**	2.0
Bis[2-chloroethoxy]methane	**91.0**	7.8
Bis[2-chloroethyl]ether	**88.5**	3.0

Bis[2-chloroisopropyl]ether	**87.3**	4.5
Bromophenoxybenzene	**99.6**	4.8
Carbazole	**109.6**	3.3
Chlorobenzilate	**116.3**	9.4
Chrysene	**103.3**	1.2
delta lindane	**95.2**	0.8
Diallate (cis & trans)	**104.7**	4.5
Dibenz[ah]anthracene	**108.8**	2.5
Dibenzofuran	**102.0**	0.6
Dibutyl phthalate	**114.6**	6.2
Dieldrin	**94.5**	0.7
Diethyl phthalate	**110.4**	1.2
Dimethoate	**96.6**	0.7
Dimethyl phthalate	**110.3**	1.3
Di-n-octyl phthalate	**116.6**	5.9
Dinoseb	**121.9**	1.7
Diphenylamine	**109.9**	4.8
Disulfoton	**87.0**	0.6
Endosulfan I	**93.8**	0.7
Endosulfan II	**96.5**	0.5
Endosulfan sulfate	**96.2**	0.7
Endrin	**97.4**	1.0
Endrin aldehyde	**93.4**	0.5
Ethyl methanesulfonate	**92.5**	3.7
Famphur	**109.3**	1.2
Fluoranthene	**105.8**	6.1
Fluorene	**103.7**	2.6
gamma lindane	**93.1**	1.3
Heptachlor	**88.1**	1.0
Heptachlor epoxide	**93.4**	0.9
Hexachlorobenzene	**101.3**	6.1
Hexachlorobutadiene	**85.0**	1.0
Hexachloroethane	**92.6**	6.0
Hexachloropropene	**72.1**	1.1
Hexachorocyclopentadiene	**85.9**	3.1
Indeno[123-cd]pyrene	**103.2**	2.5
Isodrin	**105.1**	7.2
Isophorone	**91.0**	6.8
Isosafrole (cis & trans)	**102.9**	6.1
Methyl methanesulfonate	**70.8**	3.5
Methyl parathion	**96.6**	0.4
Naphthalene	**97.2**	2.3
Nitrobenzene	**94.0**	7.2
N-nitro-di-n-propylamine	**99.3**	6.3
N-nitroso di-n-butylamine	**99.9**	4.7
N-nitrosodiethylamine	**89.4**	3.7
N-nitrosodimethylamine	**68.8**	3.0
N-nitrosomethylethylamine	**87.4**	2.5
o,o,o-Triethylphosphorothioate	**90.8**	0.4
o-Toluidine	**91.4**	9.7

Parathion	**95.8**	0.7
p-Dimethylaminoazobenzene	**91.5**	10.5
Pentachlorobenzene	**90.9**	1.0
Pentachloroethane	**86.0**	3.8
Pentachloronitrobenzene	**104.3**	4.2
Pentachlorophenol	**109.3**	3.3
Phenacetin	**116.4**	3.9
Phenanthrene	**108.0**	0.4
Phenol	**56.2**	4.2
Phorate	**86.7**	0.1
Pronamide	**111.2**	5.2
Pyrene	**109.1**	8.5
Pyridine	**46.1**	8.0
Safrole	**90.7**	4.3
Sulfotep	**92.5**	0.8
Thionazin	**95.1**	0.7
Surrogates		
2-Fluorophenol (S)	**87.2**	0.6
Phenol d6 (S)	**59.1**	0.4
Nitrobenzene d5 (S)	**94.3**	1.0
2-Fluorobiphenyl (S)	**81.5**	0.5
2,4,6-Tribromophenol (S)	**95.4**	0.2
p-Terphenyl d14 (S)	**97.5**	1.0

Chromatogram of an LFB Fortified at 40 µg/L



5108-04-01

Determination of Carbonyl Compounds In Water by Dinitrophenylhydrazine Derivatization and HPLC/UV*

EPA Method 8315A

UCT Part Number:

EUC1812M15 (Unendcapped C18 - 2000 mg/15 mL cartridge)

Method Summary

This method provides procedures for the determination of free carbonyl compounds and aldehydes in water. A measured volume of sample is buffered to pH 3 and the analytes derivatized at 40°C for one hour using 2,4-dinitrophenylhydrazine (DNPH) then extracted through SPE cartridges containing 2000 mg of C18. The cartridge(s) are eluted with 10 mL of ethanol and the derivatives are determined by absorbance at 360 nm after separation by HPLC.

The following compounds can be determined using this method:

Analyte	CASRN
2,5-Dimethylbenzaldehyde	5779-94-2
Acetaldehyde	75-07-0
Acetone	67-64-1
Acrolein	107-02-8
Benzaldehyde	100-52-7
Butanal	123-72-8
Crotonaldehyde	123-73-9
Cyclohexanone	108-94-1
Decanal	112-31-2
Formaldehyde	50-00-0
Heptanal	111-71-7
Hexanal (hexaldehyde)	66-25-1
Hexanal	66-25-1
Isovaleraldehyde	590-86-3
m-Tolualdehyde	620-23-5
Nonanal	124-19-6
Octanal	124-13-0
o-Tolualdehyde	529-20-4
Pentanal (propionaldehyde)	110-62-3
Propanal	123-38-6
p-Tolualdehyde	104-87-0

Note: Do not rinse glassware with acetone or methanol. These solvents react with DNPH to form interferences

Stock Standard Solutions
Stock standard formaldehyde solution approximately 1 mg/mL—

- Prepare by diluting 265 µL of formalin to 100 mL with reagent water

Standardization of formaldehyde stock solution –

- Transfer 25 mL of a 0.1M Na_2SO_3 solution to a beaker
- Record the pH
- Add a 25.0 mL aliquot of the formaldehyde stock solution and record the pH
- Titrate this mixture back to the original pH using 0.1 N HCl
- Calculate the formaldehyde concentration using the following equation:
  Concentration (mg/mL) = 30.03 x (N HCl) x (mL HCl) / 25.0 where:
  N HCl = Normality of HCl solution used

mL HCl = mL of standardized HCl solution used

30.03 = MW of formaldehyde

Note: The pH value of the 0.1 Na_2SO_3 should be 10.5 ±0.2 when the stock formaldehyde solution and the 0.1 M Na_2SO_3 solution are mixed together. The pH should be 11.5 ±0.2. It is recommended that new solutions be prepared if the pH deviates from this value

Stock aldehyde(s) and ketone(s) –

- Prepare by adding an appropriate amount of the analyte to 90 mL of methanol
- Dilute to 100 mL to give a final concentration of 1.0 mg/mL. Replace after six weeks, or sooner, if comparison with check standards indicates a problem

Reaction Solutions

2,4-Dinitrophenylhydrazine (DNPH $[2,4\text{-}(O_2N)_2\ C_6\ H_3\ ]NHNH_2$ **) (3.00 g/L) –**

- Dissolve 428.7 mg of 70% (w/w) reagent in 100 mL absolute ethanol. Sonication may be needed

Note: If the DHPH does not completely dissolve, filter the solution

Citrate buffer pH 3 (1 M) –

- Prepare by adding 80 mL of 1 M citric acid solution to 20 mL 1 M sodium citrate solution. Mix thoroughly. Adjust pH with 6N NaOH or 6N HCl

Sodium Chloride Solution (saturated) –

- Prepare by mixing an excess of the reagent grade solid with reagent water

Reducing agent, ammonium chloride (100 mg/L) –

- Add to all samples containing residual chlorine. The ammonium chloride may be added as a solid with stirring until dissolved, to each volume of water
- Sodium thiosulfate is not recommended because it may produce a residue of elemental sulfur that can interfere with some analytes

Procedure

1. Derivatization Procedure

a) Quantitatively transfer 100 mL of sample into a 250 mL Florence or Erlenmeyer flask

Note: Other volumes may be used depending on the expected concentration of the analytes. If less than 100 mL is used adjust volume to 100 mL using reagent water

b) Add 4 mL acetate buffer and adjust pH to 5.0 ± 0.1 with 6M HCl or 6M NaOH (if only formaldehyde is being analyzed)

c) Add 4 mL of citrate buffer and adjust the pH to 3.0 ± 0.1 with 6M HCL or 6M NaOH (if other aldehydes are being analyzed)

d) Add 6 mL of DNPH reagent, seal the flask and place in a heated orbital shaker at 40° C for 1 hour

e) Adjust the agitation to produce a gentle swirling of the reaction solution

2. Cartridge Conditioning

a) Assemble a vacuum manifold

b) Place **EUC1812M15** cartridge(s) on manifold

c) Condition the cartridge by adding 15 mL of acetonitrile (ACN)

d) Draw through then rinse using a 15 mL portion of reagent water

e) While cartridge is still wet add 10 mL of dilute citrate buffer (10 mL of 1M citrate buffer dissolved in 250 mL of water)

f) Remove the reaction vessel from the shaker after 1 hour and add 10 mL of saturated NaCl solution to the flask

g) Quantitatively transfer the reaction solution to the SPE cartridge and apply a vacuum to draw the solution through at 3-5 mL/min

h) Continue to apply vacuum for about 1 minute after the liquid sample has passed through the cartridges

3. **Cartridge Elution**

a) Turn off vacuum and place 10 mL volumetric flasks under cartridges

b) Turn on vacuum and elute the cartridges with approximately 9 mL of acetonitrile directly into flasks

c) Bring the eluate to 10 mL volume with ACN, mix thoroughly, then transfer aliquot to analysis vial and seal

Note: Because this method uses an excess of DNPH, the cartridges will retain a yellow color after this step. The color does not indicate incomplete recovery of the analyte derivatives

HPLC Analysis (suggested)

- **HPLC Column:** C18, 250 mm x 4.6 mm i.d., 5 µm particle size
- **Mobile Phase:** 70/30 methanol/water (v/v)
- **Injection Vol:** 20µL
- **Flow Rate:** 1.2 mL/min
- **UV Detector:** 360 nm
- **Flow Program:**
    - 70/30 methanol/water (v/v) for 20 min,
    - to 100% acetonitrile in 15 minutes
    - hold at 100% acetonitrile for 10 minutes

Representative Retention Times in Reagent Water Using Specified Analysis Conditions

Analyte	Retention Time *(min)*
Formaldehyde	5.3
Acetaldehyde	7.4
Propanal	11.7
Crotonaldehyde	16.1
Butanal	18.1
Cyclohexanone	27.6
Pentanal	28.4
Hexanal	34.1
Heptanal	35.0
Octanal	40.1
Nonanal	40.4
Decanal	44.1

*Adapted from Method 8315a, Determination Of Carbonyl Compounds By High Performance Liquid Chromatography (HPLC), and EPA Method 554, Revision 1.0, James W. Eichelberger, W.J. Bashe (Technology Applications, Incorporated), Environmental Monitoring Systems Laboratory, Office Of Research And Development, U. S. Environmental Protection Agency, Cincinnati, Ohio 45268

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Determination of Phenoxyacid Herbicides in Water by Solid Phase Extraction and LC-MS/MS Detection

UCT Part Numbers:
ECHLD156-P (Enviro Clean® HL DVB 500mg/6mL, PE Frits)
VMFSTFR12 (Sample Transfer Tubes)

EPA Method 8321B*

Procedure:

1. **Sample Pretreatment**
   a) Adjust sample pH to <1 with 1:1 sulfuric acid in water, low pH is critical to obtain high recoveries.

2. **Cartridge Conditioning**
   a) Attach sample transfer tubes (**VMFSTFR12**) to the top of the SPE cartridges (**ECHLD156-P**), and attach the SPE cartridges to an SPE manifold.
   b) Wash the SPE cartridges (with transfer tubes connected) using 10 mL methylene chloride, let solvent soak sorbent for 2 min before drawing to waste, leave full vacuum on for 1 min.
   c) Condition the SPE cartridges with 10 mL methanol, leave a thin layer above the frit.
   d) Equilibrate the SPE cartridges with 15 mL DI water, leave a thin layer above the frit.

3. **Sample Loading**
   a) Insert the stainless steel ends of the sample transfer tubes into sample bottles, adjust vacuum for a fast dropwise sample flow (about 20-25 mL/min).
   b) After all sample is passed through, dry the SPE cartridges under full vacuum for 10 min.

4. Analyte Elution

a) Insert the collection vials to the manifold.

b) Rinse the sample bottles with 5 mL acetonitrile, apply the rinse to the SPE cartridges. Let the elution solvent soak the sorbent for 1-2 min before drawing through slowly.

c) Repeat the elution (step 9) with 2 additional aliquots of 5 mL acetonitrile.

Instrumental Analysis

Analyze the eluate directly by LC-MS/MS, or concentrate to 1 mL and analyze by HPLC.

Note: Use acid washed sodium sulfate and glassware if SPE eluates need be dried and concentrated.

Results

Compound	LCS1 Recovery%	LCS2 Recovery%	RPD%
2,4-D	96.6	96.2	0.4
MCPA	95.0	94.4	0.6
Dichlorprop	93.6	92.7	1.0
Mecoprop	94.4	93.4	1.1
2,4,5-T	94.2	93.0	1.3
Dichlorobenzoic acid	89.5	87.6	2.1
2,4-DB	85.1	83.7	1.7
Acifluorfen	108.6	88.4	20.5
Silvex	103.1	86.0	18.1
Bentazone	89.8	89.7	0.1

*EPA Method 8321B SOLVENT-EXTRACTABLE NONVOLATILE COMPOUNDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY/THERMOSPRAY/MASS SPECTROMETRY (HPLC/TS/MS) OR ULTRAVIOLET (UV) DETECTION

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Solid-Phase Extraction and Determination of Organotin by Micro-Liquid Chromatography Electrospray Ion Trap MS
(Part A Water Samples)

UCT Part Number:
ECUNIC18 (C18 endcapped, 1100 mg/83 mL cartridge)

EPA Method 8323

Analytes Determined Using This Method

Analyte	CAS No	LOD
Tributyltin chloride	1461-22-9	780 pg
Dibutyltin dichloride	683-18-1	970 pg
Monobutyltin trichloride	1118-46-3	1 ng
Triphenyltin chloride	668-34-8	NA
Diphenyltin dichloride	1135-99-5	920 pg
Monophenyltin trichloride	1124-19-2	NA

Note: Organotins can bond to glass surfaces, glassware must be specially treated. All glassware used in the extraction and analysis of organotins must be acid washed using the following procedure.

- Wash glassware in hot soapy water then rinse with DI water
- Prepare a pH 2 acid bath using 12 N HCl and soak glassware in acid for 24 hours
- Remove glassware from bath, then rinse with DI water followed by a methanol rinse
- Place in a 60°F oven until dry

Procedure

1. Initial Preparation

a) Fill a 2 liter volumetric flask with sample water

b) Adjust pH to 2.5 by adding about 600 µL of 12N HCl

c) Stopper flask and invert several times to mix acid

2. Cartridge Conditioning

a) Add 10 mL of methanol to the cartridge to activate

b) Briefly turn on vacuum to draw through a small amount to top of frit

c) Wait 1-2 minutes

d) Add 10 mL of a methanol/1% acetic acid solution

e) Draw about 2 mL through the cartridge then turn off vacuum

f) Let solution sit for 1-2 minutes then draw through

g) Add 10 mL of reagent water to cartridge and partially draw through

Note: Do not let the cartridge dry out after start of activation otherwise start over at step 2. a)

3. Sample Extraction

a) Add the 2 liter sample to the cartridge and draw through at approximately 50 mL/minute (fast drip)

b) Rinse volumetric flask and cartridge sides with 100 mL of reagent water and draw through

4. Elution

a) Dry cartridge by drawing full vacuum for 10 minutes

b) Place a clean, treated collection tube in the manifold

c) Add a **first** portion of 10 mL of methanol/1% acetic acid solution to the cartridge, rinsing the sides during addition, then slowly draw through cartridge

d) Add a **second** 10 mL portion of methanol/1% acetic acid solution to the cartridge, then slowly draw through

e) Add a **third** 10 mL portion of methanol/1% acetic acid solution to the cartridge, then slowly draw through

Micro-concentration by TurboVap® Nitrogen Evaporation

1. Place the concentrator tube in the TurboVap® or other analytical evaporator in a lukewarm water bath at 30° C
2. Evaporate the solvent volume to 0.5 mL using a gentle stream of clean, dry N_2
3. The internal wall of the tube must be rinsed down several times with the final solvent (methanol/1% acetic acid) during the evaporation
4. Do not allow the extract to become dry
5. Transfer the extract to a 2 mL glass vial with a PTFE-lined screw-cap or crimp-top vial and store refrigerated at 4° C
6. Sample is ready for µ-LC-ES-ITMS analysis

Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC) Aqueous Matrices

UCT Part Number:
ECDVB156P- Enviro-Clean® DVB 500 mg, 6 mL cartridge, PE Frit
ECHLD156-P - Enviro-Clean® HL DVB 500 mg, 6 mL cartridge, PE Frit
VMFSTFR12 - Sample Transfer Tubes

EPA Method 8330B

RCRA Compounds Using This Method

Table 1

Analyte	CAS	Abbreviation	% Recovery n=3
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	2691-41-0	HMX	100
Hexahydro-1,3,5-trinitro-1,3,5-triazine	121-82-4	RDX	110
1,3,5-Trinitrobenzene	99-35-4	1,3,5-TNB	100
1,3-Dinitrobenzene	99-65-0	1,3-DNB	100
1,4-Dinitrobenzene	10025-4	1,4-DNB	97
Methy-2,4,6-trinitrophenylnitramine	47945-8	Tetryl	85
Nitrobenzene	98-95-3	NB	100
2,4,6-Trinitrotoluene	118-96-7	TNT	94
4-Amino-2,6-dinitrotoluene	19406-51-0	4-Am-DNT	120
2-Amino-2,6-dinitrotoluene	35572-78-2	2-Am-DNT	110
2,4-Dinitrotoluene	121-14-2	2,4-DNT	98
2,6-Dinitrotoluene	606-20-2	2,6-DNT	110
2-Nitrotoluene	88-72-2	2-NT	90
3-Nitrotoluene	99-08-1	3-NT	91
4-Nitrotoluene	99-99-0	4-NT	92
Nitroglycerin	55-63-0	NG	100
Pentaerythritol tetranitrate	78-11-5	PETN	100
3,5-Dinitroaniline	618-87-1	3,5-DNA	68
1-Nitronaphthalene	86-57-7	NN	97
o-Dinitrobenzene	528-29-0	o-NB	100

Safety

- Extra caution that should be taken when handling the analytical standard neat material to prevent detonation

Interferences

- 2, 4-DNT and 2, 6-DNT elute at similar retention times on C18 columns using method separation conditions. If it is not apparent that both isomers are present or are not detected an isomeric mixture should be reported
- Tetryl is thermally labile (decomposed with heat at temperature above room temperature) and decomposes in methanol/water solutions. All aqueous samples expected to contain tetryl should be diluted with acetonitrile and acidified with sodium bisulfate to pH <3 prior to filtration
- Degradation products of tetryl appear as a shoulder on the 2,4,6-TNT peak when using C18 columns

Note: All samples should be stored at 2° to 4° C prior to extraction and should be extracted within 14 days of collection

Sample Preparation--Aqueous matrices, (e.g. water)

(from Method 3535)

Important Notes:

- Any particulate matter in the original sample must be included in the sample aliquot that is extracted.
- The sample container must be rinsed with solvent as the majority of organic analytes are hydrophobic and may adhere to the sample container surfaces.
- Do not concentrate explosives residue to dryness as they may DETONATE
- For explosives and nitramines or nitroaromatics the extraction pH should be as received in the sample
- Using a graduated cylinder, measure 1 liter of sample water. A smaller sample size may be used when analytical sensitivity is not a concern
- Add methanol (ACN if tetryl is being analyzed) so that the sample is 0.5% v/v. Add surrogate standards to all samples and blanks
- Add matrix spikes standards to representative sample replicates
- Adjustment of sample pH may result in precipitation or flocculation reactions and potentially remove analytes from the aqueous portion. Transfer the precipitate with rinses to the SPE extraction cartridge

Do not let the cartridge dry out after cartridge conditioning with acetonitrile (ACN)

Procedures:

1. Glass Apparatus and SPE Cartridge Washing

a) Attach the sample transfer tubes to the top of the SPE cartridges, place onto the SPE manifold, and wash with the solvents in the table below

Analyte	1st solvent wash	2nd solvent wash	3rd solvent wash
Explosives	5 mL acetone	15 mL isopropanol	15 mL methanol
Nitramines, Nitroaromatics	5 mL ACN	15 L ACN	

b) Let solvents soak the SPE sorbents for 2-3 min, then draw solvents through under full vacuum

2. Cartridge Conditioning

a) Follow the conditioning steps in the table below:

Analyte	Condition Step 1	Step 2	Step 3	Step 4
Explosives	20 mL ACN, 3 min*	20 mL ACN	50 mL DI water	50 mL DI water
Nitramines, Nitroaromatics	15 mL ACN, 3 min*	30 mL DI water	----	----

b) Draw solvents through the cartridge under low vacuum. Do not let the cartridge dry out once cartridge is conditioned, which will affect analyte recovery

3. Sample Extraction

a) Draw sample from the sample bottle to the SPE cartridge using the sample transfer tube

b) Adjust vacuum to obtain a flow rate of about 10-20 mL/min

c) After all the sample is drawn through, remove the transfer tube, and dry the SPE cartridge for 15 minutes. Do not dry for longer than 20 minutes as lower recovery may result

4. Cartridge Elution

a) Insert a collection tube in the base of the vacuum manifold
b) Add 5 mL of ACN and soak for 3 min
c) Draw the ACN through with a low vacuum into the collection tube, and turn full vacuum on for 1 min
d) Adjust the extract volume to 5 mL with ACN, add 5 mL DI water with 0.1% formic acid
e) Add internal standard (5 µg/mL in the extract), mix well and analyze by HPLC

HPLC Columns for the Analysis of Explosive Residues	
Primary Columns	C18 RP HPLC column, 25-cm x 4.6-mm, 5 µm C8 RP HPLC column, 15-cm x 3.9-mm, 4 µm
Secondary Columns	CN RP HPLC column, 25-cm x 4.6-mm, 5 µm Luna Phenyl-Hexyl RP HPLC column, 25-cm x 3.0-mm, 5 µm

Injection volume: 100 µL

UV Detector: Dual 254 & 210 nm or Photodiode Array

Mobile phase: For C18 & CN column, 50:50 Methanol:D.I. Water

Flow Rate: 1.5 mL/minute

Retention Times

Analyte	LC-18 (min)	LC-CN (min)
HMX	2.44	8.35
RDX	3.78	6.15
1,3,5-TNB	5.11	4.05
1,3-DNB	6.16	4.18
3,5-DNA	6.90	NA
Tetryl	6.93	7.36
NB	7.23	3.81
NG	7.74	6.00
2,4,6-TNT	8.42	5.00
4-Am-DNT	8.88	5.10
2-Am-DNT	9.12	5.65
2,6-DNT	9.82	4.61
2,4-DNT	10.05	4.87
2-NT	12.26	4.37
4-NT	13.26	4.41
PETN	14.10	10.10
3-NT	14.23	4.45

*For complete details on Method 8330 “Nitroaromatics and Nitramines by High performance Liquid Chromatography” Revision 2 October 2006, the analyst is referred to: National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268 and Method 3550 Revision 0, December 1996

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Other Water Analyses

Determination of Hormones in Water by Solid-Phase Extraction (SPE) and Liquid Chromatography – Tandem Mass Spectrometry (LC-MS/MS)*

UCT Part Number:
EUC18156 (500 mg unendcapped C18, 6 mL)

Analytes Determined Using This Method

Analyte	CASRN
Estriol 16α-Hydroxyestradiol	50-27-1
17β-Estradiol	50-28-2
17α-Ethynylestradiol	57-63-6
Testosterone	58-22-0
Estrone 3-hydroxy-13-methyl-6,7,8,9,11,12,13,14,15,16-decahydrocyclopenta[a]phenanthren-17-one	53-16-7
4-Androstene-3,17-dione	63-05-8
Equilin 3-hydroxyestra-1,3,5,7-tetraen-17-one	474-86-2

Method Summary

Water samples are dechlorinated with sodium thiosulfate and protected from microbial degradation with 2-mercaptopyridine-1-oxide sodium salt during collection. Samples are fortified with surrogates and extracted using C18 cartridges before elution with methanol. The extract is concentrated to dryness with N_2 before adjusting to a 1 mL volume with 50:50 methanol:water. An aliquot is injected into an LC equipped with a C18 column interfaced to a MS/MS. Detection limits of 0.02-0.37 ng/L can be obtained using this application from samples fortified at 0.25-0.875 ng/mL.

Safety

- The toxicity and carcinogenicity of each reagent has not been defined
- Each chemical should be treated as a potential health hazard and exposure minimized by the use of PPE (personal protective equipment) such as gloves, respirators and other personal safety equipment
- Pure standard materials and stock standard solutions of the method compounds should be handled with suitable protection
- Ammonium hydroxide, used during method development as a pH modifier for the HPLC mobile phase, should be fresh* and handled in a fume hood

*NH_4OH loses its strength after opening due to evaporation. A fresh bottle should be used

Sample Collection, Preservation, and Storage

- Use one-liter amber glass bottles with PTFE-lined screw caps
- If smaller sample sizes are used, adjust the amount of preservatives and surrogate/analyte fortification levels according to the sample size
- Fill sample bottles taking care not to flush out the preservatives. Analytes are not volatile so bottles need not be headspace-free
- When sampling from a cold water tap, remove the aerator and allow the system to flush until the water temperature has stabilized. Invert the bottles several times to mix the sample with the preservation reagents
- Samples should be chilled during shipment and should not exceed 10° C during the first 48 hours after collection
- In the laboratory, store samples at or below 6° C and protect from light until analysis. **Do not freeze unprocessed samples**
- All compounds listed in the method have adequate stability for 28 days when collected, preserved, shipped and stored as described
- Samples should be extracted as soon as possible for best results
- After sample preparation, extracted samples should be stored at 0° C or less and analyzed within 28 days after extraction

Sample Preservatives

Compound	Amount	Purpose
Sodium thiosulfate	80 mg/L	Removes free chlorine
2-mercaptopyridine-1-oxide sodium salt	65 mg/L	Microbial inhibitor

Note: Preservation reagents listed above should be added to each sample bottle prior to shipment to the field or prior to sample collection

Interferences

- Matrix interferences may be caused by contaminants that are co-extracted from the sample and will vary from source to source
- Humic and/or fulvic material in environmental samples may be co-extracted during SPE and can cause enhancement and/or suppression in the electrospray ionization source. Total organic carbon (TOC) is an indicator of the humic content of a sample
- Use only high purity analogs. Depending on the source and purity, labeled analogs used as internal standards may contain a small percentage of the corresponding native analyte which may be significant when attempting to determine LCMRLs and DLs
- Nitrile gloves should be worn at all times. Handling clean glassware may be a potential source of interference

- It may be appropriate to include a Field Blank with the sampling bottles depending on the sampling site. The Field Blank is analyzed along with the samples to ensure that no human hormones were introduced into the samples during the collection and handling process

Internal Standards

Internal Standard	CASRN	Neat Material Cat #	Solution Standard Cat #
16α-Hydroxyestradiol-*d*2 (Estriol-*d*2)	53866-32-3	C/D/N Isotopes Cat. No. D-5279	N/A
^{13}C6-Estradiol	None	None	Cambridge Isotope Labs 100 µg/mL in Methanol Cat. No. CLM-7936-1.2
^{13}C2-Ethynylestradiol	None	None	Cambridge Isotope Labs 100 µg/mL in Acetonitrile Cat. No. CLM-3375-1.2
Testosterone-*d*3	77546-39-5	None	Sigma Drug Std., 100 µg/mL in dimethoxyethane Cat. No. T5536

Internal Standard Stock Standards

ISSSS 500 µg/mL - Weigh 5 mg of α-hydroxyestradiol-d_2 (estriol-d_2) into a tared 10 mL volumetric flask and dilute to volume with methanol. The remaining internal standards can be purchased as100 µg/mL solutions

Internal Standard Primary Dilution Standard (IS-PDS)

IS PDS 1.0 – 4.0 µg/mL: The table below can be used as a guide for preparing the IS PDS. The IS PDS is prepared in acetonitrile and is stable for about six months if stored at a temperature < 6° C. Use 5 µL of the 1.0 – 4.0 µg/mL IS PDS to fortify the final 1 mL extracts. This will yield a final concentration of 5.0 – 20 ng/mL of each IS.

Internal Standard Concentrations

Internal Standard	Conc IS Stock µg/mL	Volume of IS Stock, µL	Final Volume of IS PDS,	Final Conc. of IS PDS (µg/mL)
16α-Hydroxyestradiol-*d*2 (Estriol-*d*2)	500	40	10	2.0
^{13}C6-Estradiol	100	400	10	4.0
^{13}C2-Ethynylestradiol	100	400	10	4.0
Testosterone-*d*3	100	100	10	1.0

Internal Standard Fortification Standard

5 µL of the IS PDS is difficult to accurately and reproducibly transfer into HPLC vials. Therefore, a 1-in-10 dilution of the IS PDS is prepared by transferring 1 mL of the 1.0 – 4.0 µg/mL IS PDS into a 10 mL volumetric flasks and diluting to volume with methanol. This results in a 0.1-0.4 µg/mL IS solution

50 µL of the prepared ISPDS is added to the samples prior to analysis

Surrogate Analytes

Two isotopically labeled surrogates are listed below. Select the surrogate that performs best under the LC-MS/MS conditions employed for the analysis

For this application, Bisphenol A-d$_{16}$ was chosen as surrogate analyte

Surrogate Analytes

Surrogate Analyte	CASRN	Neat Materials Catalog No.
Ethynylestradiol-d_4	350820-06-3	C/D/N Isotopes, Cat. No. D-4319
Bisphenol A-d_{16}	96210-87-6	Sigma, Cat. No. 451835

Surrogate Stock Standards

1000 µg/mL - Prepare individual solutions of the surrogate standards by weighing 10 mg of the solid material into tared 10 mL volumetric flasks and diluting to volume with methanol

Surrogate Analyte Primary Dilution Standard

SUR PDS 2.5 – 7.0 µg/mL
Use the table below as a guide for preparation of the **SUR PDS** in methanol.

Use 10 µL of **SUR PDS** to fortify 1-L samples yielding a final concentration of 70 ng/mL ethynylestradiol-d_4 or 25 ng/mL bisphenol A-d_{16} in the 1 mL extracts

Surrogate Analyte Solutions

Surrogate Analyte	Conc of SUR Stock (µg/mL)	Volume of SUR Stock (µg/mL)	Final Volume of SUR PDS (mL)	Final Conc. Of SUR PDS (µ g/mL)
Ethynylestradiol-d_4	1000	70	10	7.0
Bisphenol A-d_{16}	1000	25	10	2.5

Surrogate Fortification Standard (SUR PDS)

10 µL of the **SUR PDS** is difficult to accurately and reproducibly transfer into samples. Therefore, a 1-in-10 dilution of the **SUR PDS** is prepared by transferring 1 mL of the 2.5 µg/mL IS PDS into a 10 mL volumetric flask and diluting to volume with methanol, resulting in a 0.25 µg/mL IS solution. Use 100 µL of this fortification standard to fortify 1-L samples yielding a final concentration of 25 ng/mL bisphenol A-d_{16} in the 1 mL extracts.

Analyte Stock Standard Solution

Each concentration equals 1000 µg/mL - Obtain the analytes listed in Table 1 above as standard solutions or as neat materials. Prepare stock standards individually by weighing 10 mg of the solid standards into tared 10 mL volumetric flasks and diluting to volume with methanol.

Analyte Primary Dilution Standard

Prepare concentrations of the stock standard solutions between 1.0 – 3.5 µg/mL – Dilute the Analyte Stock Standard solutions into 50% methanol in reagent water. The concentrations vary based on the instrumental sensitivity. The Analyte PDS is used to prepare calibration standards, and to fortify LFBs, LFSMs, and LFSMDs with the method analyte.

Analyte Stock	Stock Concentration µg/mL	Stock Volume µL	Final Volume (ml, 50% MeOH)	Analyte PDS Concentration µg/L
Estriol, 16α- Hydroxyestradiol	1000	20	10	2.0
Estrone	1000	20		2.0
17β-Estradiol	1000	25		2.5
17α-Ethynylestradiol	1000	35		3.5
Equilin	1000	20		2.0
Androstenedione	1000	10		1.0
Testosterone	1000	10		1.0

Analyte Fortification Standard

10 µL of the Analyte PDS is difficult to accurately and reproducibly pipette into samples. Therefore, a 1-in-10 dilution of the Analyte PDS is prepared by transferring 1 mL of the 1.0 – 3.5 µg/mL Analyte PDS into a 10 mL volumetric flask and diluting to volume with 50% methanol, resulting in a 0.1 – 0.35 µg/mL solution. Use 100 µL of this fortification standard to fortify 1-L samples, yielding a final concentration of 10 – 35 ng/mL in the 1 mL extracts.

An additional 1-in-10 dilution of the 0.1 – 0.35 µg/mL fortification solution is prepared in a 10 mL volumetric flask using 50% methanol. This yields a 0.01 – 0.035 µg/mL solution that is used to fortify 1-L samples yielding a final concentration of 1 – 3.5 ng/mL in the 1 mL extracts.

Procedure

1. **Sample Preparation**
   a) Add a 100-µL aliquot of SUR Fortification Standard to each 1-L sample for a final concentration of 25 ng/L bisphenol A-d_{16}
   b) Fortify LFBs, LFSMs, or LFSMDs with an appropriate volume of Analyte Fortification Standard
   c) Cap and invert each sample several times to mix
2. **Cartridge Preparation**
   a) Assemble a vacuum manifold. Automated extraction equipment may also be used
   b) Place **EUC18156** cartridge(s) on the manifold
   c) Add a 10 mL aliquot of methanol to the cartridge and draw through the cartridge until dry
   d) Add another 5 mL aliquot of methanol and draw through the cartridge until dry

   Note: Do Not Let the Cartridge Go Dry after Starting the Following Steps
   e) Add approximately 10 mL of methanol to each cartridge
   f) Draw about 1 mL of solvent through the cartridge and turn off the vacuum temporarily
   g) Let the cartridge soak for about one minute then draw most of the remaining solvent through the cartridge leaving a thin layer of methanol on the surface of the cartridge
   h) Add 10 mL of reagent water to each cartridge and draw through leaving a thin layer of liquid on the surface of the cartridge
   i) Add another 10 mL aliquot of reagent water
   j) Draw the water through the cartridge keeping the water level above the cartridge surface
   k) Turn off the vacuum
3. **Sample Extraction**
   a) Add the sample to the extraction reservoir containing the conditioned cartridge and turn on the vacuum (approximately 10 to 15 in. Hg). Flow of sample through the cartridge should be a fast drip. Adjust vacuum if necessary
   b) Do not let the cartridge go dry before the entire sample volume is extracted

c) After the entire sample has been drawn through the cartridge, add a 10 mL aliquot of 15% methanol to the sample container and wash the cartridge with the rinsate from the container

d) Using full vacuum, draw air through the cartridge by maintaining full vacuum for 10–15 minutes

e) After drying, turn off and release the vacuum

4. **Sorbent Elution**

a) Insert collection tubes into the manifold to collect the cartridge extracts. The collection tube should fit around the drip tip of the base to ensure collection of all the eluent

b) Add 5 mL of methanol to the cartridge and draw the methanol into the cartridge to soak the sorbent

c) Allow the cartridge to soak for about one minute

d) Using vacuum, draw the remaining methanol slowly through the cartridge into the collection tube

e) Elute with an additional 2 x 5 mL aliquots of methanol

Note: The methanol can be eluted directly into a 15 mL tube (or larger). Otherwise 5 mL MeOH can be eluted into a 5 mL culture tube and evaporated to near dryness prior to adding the second 5 mL eluate. This process is repeated until all 15 mL MeOH has been added to the 5 mL culture tube.

5. **Extract Concentration**

a) Concentrate the extract to dryness under a gentle stream of N_2 in a warm water bath (~45° C)

b) Rinse the collection tube with 950 µL of 50% methanol, vortex for 2 min and transfer the rinse into a HPLC vial

c) Add 50 µL IS Fortification Standard (ISFS) and vortex for an additional 1 min

6. **Sample Filtration**

a) It is highly recommended that extracts be filtered with at least a 0.45 micron syringe filter prior to analysis to remove the particulates in a sample.

b) If filtering is incorporated as part of the sample preparation, the first lot of syringe filters should be included in the procedure to document the potential interferences that are introduced or analytes are retained on the filter.

c) Subsequent lots of syringe filters can be verified by examining CAL standards.

Sample Analysis

HPLC Conditions

HPLC instrument: Thermo Scientific Dionex UltiMate 3000 System**		
Column: Thermo Scientific Accucore C18, 100 x 2.1 mm, 2.6 µm with 10 mm guard column		
Column Temperature: 35° C		
Column Flow Rate: 0.200 mL/min		
Injection Volume: 20 µL		
Gradient		
Time (min)	**Moblie phase A H_2O + 0.02% NH4OH**	**Moblie phase B MeOH +0.02% NH4OH**
0	70	30
1	35	65
9	35	65
9.1	15	85
11	15	85
11.1	70	30
15	70	30

ESI-MS/MS Method Conditions

MS Parameters	
MS instrument	Thermo Scientific TSQ Vantage**
Polarity	$HESI^+$ & $HESI^-$
Spray Voltage V	+4500 / -3500 V
Vaporizer Temperature	350°C
Ion Transfer Capillary Temperature	300 °C
Sheath Gas Pressure	45 arbitrary units
Auxiliary Gas Pressure	40 arbitrary units
Q1 and Q3 Peak Width (FWHM)	0.4 and 0.7 Da
Collision Gas and Pressure	Ar at 1.5 mTorr
Scan Type	SRM
Cycle Time	0.75 Sec

**Alternative LC-MS/MS systems may be used

LC-ESI-MS/MS Analyte Retention Times, Precursor and Product Ions, S-lens, and Collision Energy

Analyte	Ret. Time (min.)	ESI Mode	Precursor Ion	Product Ion	S-lens V	Collision Energy eV	Internal Standard
Estriol	4.99	ESI-	286.75	144.96	100	39	Estriol-d_2
Bisphenol A-d_{16}	6.20	ESI-	240.91	223.02	60	18	$^{13}C_6$-Estradiol
Equilin	7.03	ESI-	266.80	142.97	67	37	$^{13}C_6$-Estradiol
17β-Estradiol	7.14	ESI-	270.93	144.98	67	40	$^{13}C_6$-Estradiol
Androstenedione	7.18	ESI+	286.87	96.88	76	20	Testosterone-d_3
17α-Ethynylestradiol	7.19	ESI-	294.79	244.97	80	39	$^{13}C_2$-Ethynylestradiol
Estrone	7.24	ESI-	268.78	144.97	109	39	$^{13}C_2$-Ethynylestradiol
Testosterone	7.76	ESI+	288.96	96.88	76	20	Testosterone-d_3

LC-ESI-MS/MS Internal Standard Retention Times, Precursor and Product Ions, S-lens, and Collision Energy

Internal Standard	Ret. Time (m	Precursor Ion	Product Ion	S-Lens V	Collision Energy eV
Estriol-*d*2	5.00	288.75	146.96	100	39
$^{13}C_6$-Estradiol	7.13	276.93	146.98	67	40
$^{13}C_2$-Ethynylestradiol	7.16	296.80	144.97	80	39
Testosterone-*d*3	7.73	291.96	96.88	70	20

Analyte Recovery

n = 5	Estriol	Equilin	Estradiol	Androstenedione	Testosterone	Ethynylestradiol	Estrone
Fortified Conc (ng/mL)	20	20	25	10	20	10	35
Mean	97.61	82.81	96.98	92.88	90.76	96.49	93.95
SD	6.45	5.90	5.17	2.55	3.47	1.31	2.02
RSD	6.60	7.13	5.33	2.75	3.82	1.36	2.15

n = 5	Estriol	Equilin	Estradiol	Androstenedione	Testosterone	Ethynylestradiol	Estrone
Fortified Conc (ng/mL)	2	2	2.5	1	1	3.5	2
Mean	83.05	96.34	93.68	98.31	104.63	109.45	106.10
SD	4.02	8.92	7.02	3.90	4.95	6.96	6.19
RSD	4.84	9.26	7.49	3.96	4.73	6.36	5.84

*Based on EPA Method 539, Version 1.0, November 2010, Glynda A. Smith (U.S. EPA, Office of Ground Water and Drinking Water) Alan D. Zaffiro, (Shaw Environmental, Inc.) M. L. Zimmerman (Shaw Environmental, Inc.) D. J. Munch (U.S. EPA, Office Of Ground Water And Drinking Water), Technical Support Center , Standards And Risk Management Division, Office Of Ground Water And Drinking Water, U. S. Environmental Protection Agency, Cincinnati, Ohio 45268

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Polycyclic Aromatic Hydrocarbons (PAH) from a Water Matrix

UCT Part Numbers:
ECUNIPAH (2000 mg, unendcapped C18, 83 mL cartridge)
Or
EUC1812M15 (2000 mg, unencapped C18, 15 mL cartridge)
CUC181M6 (1000 mg, unencapped C18, 6 mL cartridge)

ECSS25K (anhydrous sodium sulfate)

Polynuclear Aromatic Compounds Recovered in this Method

PAH	CASRN	PAH	CASRN
Acenaphthene	83-32-9	Chrysene	218-01-9
Acenaphthylene	208-96-8	Dibenzo(a,h)anthracene	53-70-3
Anthracene	120-12-7	Fluoranthene	206-44-0
Benzo(a)anthracene	56-55-3	Fluorene	86-73-7
Benzo(a)pyrene	50-32-8	Indeno(1,2,3-cd)pyrene	193-39-5
Benzo(b)fluoranthene	205-99-2	Naphthalene	91-20-3
Benzo(g,h,i)perylene	191-24-2	Phenanthrene	85-01-8
Benzo(k)fluoranthene	207-08-9	Pyrene	129-00-0

1. Condition Cartridge

a) Place **ECUNIPAH** cartridge(s) on the vacuum manifold*
b) With vacuum turn off add 10 mL of methylene chloride to the cartridge
c) Let it soak for 1 minute
d) Turn on vacuum and draw through to waste
e) Add 10 mL of methanol to the cartridge
f) Let it soak for 1 minute
g) Draw the methanol to the level of the frit
h) Add 10 mL of deionized water to the cartridge
i) Let it soak for 1 minute
j) Draw most of the water to waste but do not allow the sorbent to dry

Note: Do not let the cartridge go dry after addition of methanol otherwise repeat starting at step 1. e)

2. Sample Addition

a) Add surrogates to the sample

b) Shake

c) Add the sample to the cartridge under vacuum. Draw the sample through the cartridge in 20 – 30 minutes (30 – 50 ml/min)

d) Allow the cartridge to dry under full vacuum for 10 minutes**

Optional: Before proceeding to the drying step use UCT Zero-Blank™ Filter (**ECBLANK**) to reduce potential background contamination

3. Extract Elution

a) Place a collection tube or vial in the vacuum manifold

b) Rinse sample bottle with 10 mL of methylene chloride

c) Add the methylene chloride rinse to the cartridge

d) Allow to soak for 1 minute then draw through

e) Repeat this procedure 3 more times using 10 mL aliquots of methylene chloride

f) Dry the extract by passing it through 10-20 grams of **ECSS25K** anhydrous sodium sulfatepre-rinsed with methylene chloride

g) Thoroughly rinse the collection device with methylene chloride

h) Add the methylene chloride rinse to the sodium sulfate and collect

4. Concentration and Analysis

a) Using a standard analytical evaporator with gentle N_2 flow and low temperature (40° C) carefully concentrate the extract to a final volume for GC/MS analysis.

b) Solvent exchange into acetonitrile and bring to a 1 ml final volume for HPLC analysis

c) Sample is now ready for analysis

Note: Most analysis errors are caused by poor concentration technique. Do not concentrate below 0.5 mL as low recoveries may result

*****The ENVIRO-CLEAN® Universal PAH cartridge can be used on standard disk manifolds (#ECUCTVAC6) (with adapter part # ECUCTADP). The cartridge is designed to fit Horizon 4790 automated extraction systems or J.T. Baker manifold with the use of an adaptor (ECBMADP)

******Faster drying results can be obtained by removing the cartridge during drying and shaking or tapping the excess moisture from the bottom of the cartridge. Drying times are approximate. Do not over dry as low recoveries may result

Organochlorine Pesticides and Polychlorinated Biphenyls by Solid-Phase Extraction

UCT Part Numbers:
EEC181M6 (1000 mg C18, 6 mL Cartridge)
or
ECUNIC18 (1100mg C18, Universal Cartridge)

Compounds Recovered Using This Method

Analyte	CASRN
1,2-Dibromo-3-chloropropane (DBCP)	96-12-8
4,4'-DDD	72-54-8
4,4'-DDE	72-55-9
4,4'-DDT	50-29-3
Alachlor	15972-60-8
Aldrin	309-00-2
Captafol	2425-06-1
Carbophenthion	786-19-6
Chlordane - not otherwise specified (n.o.s.)	57-74-9
Chlorobenzilate	510-15-6
Chloroneb	2675-77-6
Chloropropylate	5836-10-2
Chlorothalonil	1897-45-6
Dacthal (DCPA)	1861-32-1
Diallate	2303-16-4
Dichlone	117-80-6
Dichloran	99-30-9
Dicofol	115-32-2
Dieldrin	60-57-1
Endosulfan I	959-98-8
Endosulfan II	33213-65-9
Endosulfan sulfate	1031-07-8
Endrin	72-20-8
Endrin Aldehyde	7421-93-4
Endrin Ketone	53494-70-5
Etridiazole	2593-15-9
Halowax-1000	58718-66-4
Halowax-1001	58718-67-5
Halowax-1013	12616-35-2
Halowax-1014	12616-36-3
Halowax-1051	2234-13-1
Halowax-1099	39450-05-0
Heptachlor	76-44-8
Heptachlor epoxide	1024-57-3
Hexachlorobenzene	118-74-1
Hexachlorocyclopentadiene	77-47-4
Isodrin	465-73-6
Methoxychlor	72-43-5
Mirex	2385-85-5
Nitrofen	1836-75-5

Pentachloronitrobenzene (PCNB)	82-68-8
Permethrin (*cis* + *trans*)	52645-53-1
Perthane	72.56-0
Propachlor	1918-16-7
Strobane	8001-50-1
Toxaphene	8001-35-2
Trans-Nonachlor	39765-80-5
Trifluralin	1582-09-8
α-BHC	319-84-6
α-chlordane	5103-71-9
β-BHC	319-85-7
γ-BHC (Lindane)	58-89-9
γ-chlordane	5103-74-2
δ-BHC	319-86-8

PCB's Recovered Using This Method

Compound	CASRN	IUPAC #
Aroclor 1016	12674-11-2	-
Aroclor 1221	11104-28-2	-
Aroclor 1232	11141-16-5	-
Aroclor 1242	53469-21-9	-
Aroclor 1248	12672-29-6	-
Aroclor 1254	11097-69-1	-
Aroclor 1260	11096-82-5	-
2-Chlorobiphenyl	2051-60-7	1
2,3-Dichlorobiphenyl	16605-91-7	5
2,2',5-Trichlorobiphenyl	37680-65-2	18
2,4',5-Trichlorobiphenyl	16606-02-3	31
2,2',3,5'-Tetrachlorobiphenyl	41464-39-5	44
2,2',5,5'-Tetrachlorobiphenyl	35693-99-3	52
2,3',4,4'-Tetrachlorobiphenyl	32598-10-0	66
2,2',3,4,5'-Pentachlorobiphenyl	38380-02-8	87
2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	101
2,3,3',4',6-Pentachlorobiphenyl	38380-03-9	110
2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2	138
2,2',3,4,5,5'-Hexachlorobiphenyl	52712-04-6	141
2,2',3,5,5',6-Hexachlorobiphenyl	52663-63-5	151
2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	153
2,2',3,3',4,4',5-Heptachlorobiphenyl	35065-30-6	170
2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3	180
2,2',3,4,4',5',6-Heptachlorobiphenyl	52663-69-1	183
2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0	187
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	40186-72-9	206

Procedure

1. Condition Cartridge

a) Assemble a suitable vacuum manifold system

b) Place cartridge(s) in the bulkhead fittings or cartridge adapters of the vacuum manifold.

c) Attach sample transfer tube (VMFSTFR12) to the cartridge (s) if nescessary

d) Rinse cartridge with 10 mL of methylene chloride ($MeCl_2$)

e) Let the $MeCl_2$ soak for 2 min.

f) Using vacuum draw the $MeCl_2$ to waste

g) Add 10 mL of acetone. Let the acetone soak for 2 minutes

h) Draw the acetone to waste

i) Dry the cartridge using full vacuum for a few seconds

j) Add10 ml of methanol and allow the methanol to soak for 1 min.

Note: Do not allow the cartridge to go dry otherwise repeat starting with step 1) j)

k) Draw some of the methanol through leaving a layer just covering the frit

l) Add 20 mL of DI water. Draw most of the water through to waste but do not allow the sorbent to completely dry

2. Sample Addition

a) Adjust sample pH to ≤ 2 using 1:1 sulfuric acid

b) Mix thoroughly

c) Start vacuum and add the sample. Draw sample through the cartridge at a rate ≤ 50 mL/minute (1 L should pass through in 20 minutes or longer)

d) Allow the cartridge to dry under full vacuum for 10 min**

3. Extract Elution

a) Place a collection tube or vial under the cartridge

b) Add 5 mL of acetone to the sample bottle and swirl to remove any residue

c) Add the acetone to the cartridge. Allow the solvent to soak for 1 minute then draw into collection vial

d) Repeat this procedure 3 more times using a 10 mL portions of $MeCl_2$

e) Prepare a 10-15 gram bed of sodium sulfate anhydrous in a glass funnel using glass wool

f) Dry the extract by passing it through the funnel of sodium sulfate anhydrous

g) Carefully rinse the collection vial with $MeCl_2$, then add to the sodium sulfate, rinsing the sodium sulfate and collect

4. Concentration and Analysis

a) Carefully concentrate the extract. Solvent exchange if necessary

Note: Most extraction errors are caused by poor concentration technique

K-D Concentration Technique

Sample extracts may be concentrated to the final volume necessary by using the K-D technique or nitrogen evaporation.

a) Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10 mL concentrator tube to an evaporation flask

b) Collect the dried extract in the K-D concentrator

c) Rinse the collection tube and drying funnel then quantitatively transfer into the K-D flask with an additional 20 mL portion of solvent

d) Add boiling chips to the flask then attach a three-ball Snyder column

e) Attach the solvent vapor recovery glassware (condenser and collection device to the Snyder column of the K-D apparatus)

f) Pre-wet the Snyder column by adding about 1 mL of methylene chloride or acetone

g) Place the K-D apparatus on a hot water bath (15 - 20°C) above the boiling point of the solvent) so that the concentrator tube is partially immersed in the hot water

h) Adjust the vertical position of the apparatus and the water temperature as necessary to complete the concentration in 10 - 20 min. At the proper rate of distillation the boiling chips of the column will actively chatter, but the chambers should not flood

i) When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 min.

j) If a solvent exchange is needed quickly remove the Snyder column, add 50 mL of the exchange solvent and a new boiling chip

k) Reattach the Snyder column. Concentrate the extract increasing the temperature of the water bath to maintain a proper distillation rate

l) Remove the Snyder column. Rinse the K-D flask and the lower joints of the Snyder column into the concentrator tube with 1 - 2 mL of solvent

m) Adjusted to a final volume of 5.0 - 10.0 mL using an appropriate solvent

Note: If further concentration is necessary, use either the micro-Snyder column technique or a N_2 evaporation technique described below

Micro-Snyder Column Technique

a) Add a fresh clean boiling chip to the concentrator tube and attach a two-ball micro-Snyder column directly to the concentrator tube

b) Attach the solvent vapor recovery glassware (condenser and collection device) to the micro-Snyder column of the K-D apparatus, following the manufacturer's instructions

c) Pre-wet the Snyder column by adding 0.5 mL of methylene chloride or the exchange solvent

d) Place the micro-concentration apparatus in a hot water bath so that the concentrator tube is partially immersed in the hot water

e) Adjust the vertical position of the apparatus and water temperature, as necessary, to complete the concentration in 5 - 10 min. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood

f) When the apparent volume of liquid reaches 0.5 mL, remove the apparatus from the water bath and allow it to drain and cool for at least 10 min.

g) Remove the Snyder column and rinse its lower joints into the concentrator tube with 0.2 mL of solvent

h) Adjust the final extract volume to 1.0 - 2.0 mL

Nitrogen Evaporation Technique

a) Place the concentrator tube in a warm bath (30° C) and evaporate the solvent to 0.5 mL using a gentle stream of clean, dry N_2 (filtered through a column of activated carbon)

Note: New plastic tubing must not be used between the carbon trap and the sample as phthalate interferences may be introduced

b) Rinse down the internal wall of the concentrator tube several times with solvent during the concentration

c) Position the concentrator tube to avoid condensing water into the extract

d) Do not allow extract to become dry. If the volume of solvent is reduced below 1 mL, some analytes may be lost

e) The extract may now be cleaned up or analyzed for analytes using the appropriate technique(s)

f) If the sample is not analyzed immediately, cover the concentrator tube and store in a refrigerator. If the extract will be stored longer than 2 days, transfer to a vial with a PTFE-lined screw-cap and store in a refrigerator

**Faster drying results can be obtained by removing the cartridge during drying and shaking or tapping the excess moisture from the bottom of the cartridge. Drying times are approximate. Do not over dry. Low recoveries could result

*For complete details on Method 8081B "Organochlorine pesticides by Gas Chromatography/Mass Spectrometry," December 1996,and 8082A, Polychlorinated Biphenyls by Gas Chromatography," the analyst is referred to Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268

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

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

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

Procedure

9. Cartridge Preparation

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

10. Extraction

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

11. Elution

a) Insert test tubes in the manifold then elute cartridges using 5 mL ethyl acetate dropwise followed by 5 mL of DCM dropwise
b) Dry extracts by passing through anhydrous Na_2SO_4

c) Rinse test tubes with DCM and add to Na_2SO_4

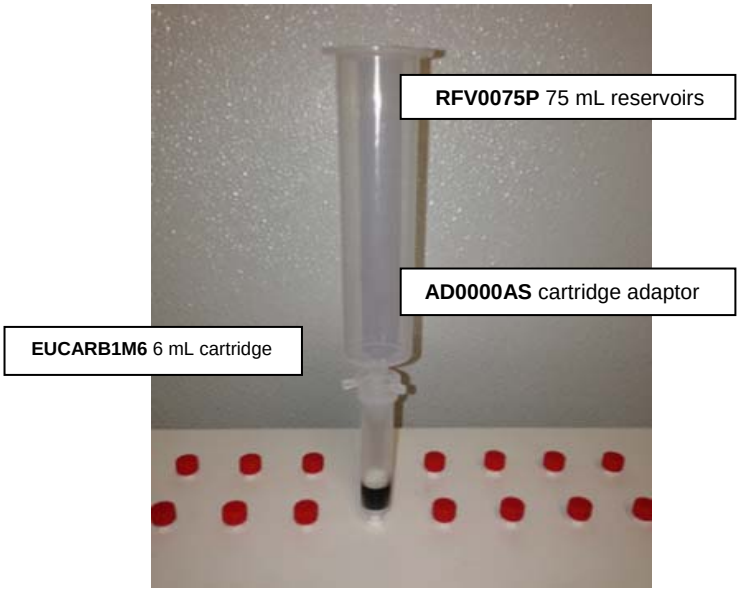
d) Concentrate extracts to 1 mL using a gentle stream of N_2 at 35 °C

e) Add IS prior to GC/MS analysis

12. Analysis

Parameters

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



Detail of Reservoir, Adaptor, and Cartridge Setup

Accuracy and Precision Data

Compound	Intra-day (n=4)		Inter-day (n=17)	
	Rec%	RSD	Rec%	RSD
alpha Lindane	93	2.1	89	9.3
beta Lindane	96	1.9	91	8.8
gamma Lindane	93	1.7	92	8.3
delta Lindane	95	3.3	89	11.7
Heptachlor	97	3.2	91	11.1
Aldrin	95	1.5	84	12.9
Heptachlor epoxide	102	2.4	97	12.0
trans-Chlordane	93	3.8	90	8.8
Endosulfan I	94	5.0	91	8.4
cis-Chlordane	96	3.3	91	9.7
p,p'-DDE	91	3.5	89	8.8
Dieldrin	98	1.9	93	9.4
Endrin	100	2.1	95	11.8
Endosulfan II	105	1.4	97	10.3
p,p'-DDD	98	2.2	92	9.8
Endrin aldehyde	95	5.4	92	9.3
Endosulfan sulfate	102	3.8	97	10.2
p,p'-DDT	99	3.0	94	9.6
Endrin ketone	106	2.1	99	10.9
Methoxychlor	105	2.7	99	10.5
Dichlofluanid	107	2.8	98	10.8
Dicofol	95	0.7	86	11.6
Tolyfluanide	106	3.1	98	11.6
Captan	119	4.2	105	13.4
Folpet	107	3.9	95	10.0
Overall average	**99**	**2.8**	**93**	**10.4**

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Extraction of Metals

UCT Part Number:
EUTAX15Z (Tri-acetic Acid 500 mg/10 mL)

Metals: Tin, Nickel, Mercury, Copper, Chromium, Ruthenium

Matrix: Water, Blood, Biological Fluids, Organic Solvents & Tissue Homogenates

1. Sample Pre-treatment

- It is important when using ion exchangers to adjust the pH of both the sorbent and analytes of interest so that they are totally ionized. Information about the analyte pKa is important

Aqueous or Organic Solvent Samples:

- Adjust the sample to pH 7 with 100 mM dibasic sodium phosphate buffer or ammonium hydroxide then vortex

Whole Blood, Serum or Plasma:

a) To 1 ml of sample add 4 ml of D.I. H20 and vortex

b) Let stand 5 minutes and centrifuge for 10 minutes at 2000 rpm and discard pellet

c) Adjust to pH 9.0 with 100 mM dibasic sodium phosphate buffer or ammonium hydroxide

2. Column Conditioning

a) Add 3 ml of methanol and draw to waste

b) Add 3 ml of water and draw to waste

c) Add 3 ml of buffer pH 7.0 and draw to waste leaving sorbent wet

3. Sample Application

a) Apply the sample to the column at a rate of 1 ml per minute. A faster rate of application may exceed the rate of ion-exchange

4. Analyte Purification

a) Wash the column with 2 ml of pH 7.0 buffer used in column equilibration.

5. Elution

a) Elute with 3 ml of acidic methanol (2% HCl, pH 2.0).

Alternative eluants:

- Elute with 3 ml of acidic methanol (Formic acid to pH 2.0).
- Elute with 3 ml of 0.1 M Nitric Acid (pH 2.0).

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Ion Exchange Sorbents for Metals Extraction-Analysis & Sorbent Use Selection Guide

UCT Part Number:
UCT ENVIRO-CLEAN® (Ion-Exchange Cartridges)*

The determination of trace metals in aqueous environmental samples or other matrices often require sample pretreatment and clean-up procedures prior to analysis by using specific ion-exchange sorbents. The sorbents are used to eliminate matrix interferences and achieve high concentrations of metal ions for good analytical accuracy. They are important when using such techniques as AA, IES and ICP-AES.

The use of ion-exchange sorbents for the preconcentration, separation, and determination of metal ions for trace analysis is well established in the literature. Selection of an appropriate sorbent ensures both high efficiency in metal chelating while minimizing the mass of sorbent required for a particular analytical task. A high efficiency sorbent means that a smaller bed mass may be used thereby reducing the quantity of solvent required for elution yielding greater analytical sensitivity.

Recommendations in this application note include the following metal ions:

Zinc (II)	**Arsenic (V)**	**Tin (IV)**	**Selenous IV)**
Mercury (II)	**Chromium (III)**	**Copper (II)**	**Platinum (0)**

Other metal ions may be extracted by the use of these ion-exchange sorbents

Sorbent Selection for Metals Extraction

Solid-phase sorbents have differing capacity and selectivity for various metal ions due to the specific nature of the ion-exchange functional group, the metal species and the valence state of the metal of interest. Depending on the specific metal ion of interest, elution of the cartridge may be most efficient using both the acid followed by the base elution procedure. This can be determined by looking at the following Extraction Protocol Tables. For example, when eluting Hg(II) from PSA the highest recovery is obtained using acid elution (green box) followed by base elution (yellow box).

Sample Analysis

1. Sample Extraction

a) Place a UCT ion exchange cartridge on a SPE manifold

Note: Cartridge selection will depend on the volume of sample or the concentration of metal to be extracted

b) Condition 1 mL cartridge by adding 3 mL of methanol. (Larger cartridges will require a larger volume of solvent and water wash volume in steps c) and d))

c) Add 3 mL of reagent water and allow to drip through the cartridge

Note: Do not allow the cartridge to dry out after addition of water, otherwise repeat step d)

d) Add 10-50 mL of sample water to the cartridge. A larger sample volume may be used depending on metal concentration or suspended solids content

e) Adjust vacuum setting so that the water flows at 1-3 mL/minute until sample has passed completely through the cartridge

f) Allow the cartridge to air dry for about 1 minute under full vacuum

2. Elution--<u>Acid</u>

a) Prepare a 100 mM nitric acid elution solution

b) Place a collection vial in the vacuum manifold

c) Add 3 mL of the nitric acid solution to the cartridge

d) Adjust flow rate for a flow of 1-3 mL/minute

e) Dilute eluant to an appropriate volume for detection using reagent water

f) Sample is ready for analysis

3. Elution--<u>Base</u>

a) Prepare a 100 mM triethylamine elution solution

b) Place a collection vial in the vacuum manifold

c) Add 3 mL of the triethylamine solution to the cartridge

d) Adjust flow rate for a flow of 1-3 mL/minute

e) Dilute eluant to an appropriate volume for detection using reagent water

f) Sample is ready for analysis

4. Analysis

a) Prepare calibration curves for use with atomic absorption (AA) or Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) using appropriate metallic ion standards

Extraction Protocol Tables

How to use these tables: When choosing an ion-exchange sorbent to capture arsenic (V) for example, all ion-exchange sorbents will capture a small quantity of metal ions, however, only a base extraction would elute metal ions from these sorbents. For extraction of zinc ions, all sorbents would have moderate to high capacity but elution could only occur from the sorbent using acidic elution conditions.

Acid Extraction Protocol

Sorbent	Cu (II)	Zn (II)	As (V)	Sn (IV)	Se (IV)	Hg (II)	Cr (III)	Pt (0)
PSA								
BCX-HL								
CCX								
TAX								
THX								
NAX								

Base Extraction Protocol

Sorbent	Cu (II)	Zn (II)	As (V)	Sn (IV)	Se (IV)	Hg (II)	Cr (III)	Pt (0)
PSA								
BCX-HL								
CCX								
TAX								
THX								
NAX								

Good to High Capacity
Moderate Capacity
Little or No Capacity

Ion-Exchange Sorbent Key

PSA	Primary secondary amine
BCX-HL	Benzene sulfonic acid –high load
CCX	Carboxylic acid
TAX	Triacetic acid
THX	Sulfhydryl (thiopropyl)
NAX	Aminopropyl

Primary Secondary Amine (PSA)

The PSA ion-exchange sorbent has a significant capacity for Hg(II) Se(IV) followed by a lesser capacity for Sn(IV), Cu(II), Zn(II) and Cr(III). Metal ions are readily eluted from PSA by the use of weak acid solutions such as 100 mM nitric acid solution. Additional recovery for selenium can be obtained by following the acid elution by the use of 100 mM triethylamine solution

Benzenesulfonic Acid-High Load (BCX-HL)

The BCX-HL ion-exchange sorbent is the least selective of all ion-exchange sorbents and has significant capacity for Hg(II) and Sn(IV) thus ensuring high extraction efficiency for trace analysis. It is also a strong sorbent for Cu(II), Zn(II), Cr(III) and small amounts of Pt. In most cases, metal ions are readily eluted from BCX-HL by the use of 100 mM nitric acid solution. Improvement in Hg(II) recovery yield and Sn(IV) can be achieved when eluting with 100 mM triethylamine solution.

Carboxylic Acid (CCX)

The CCX sorbents have high selectivity for Sn(IV) and Hg(II). Metal ions are readily eluted from CCX by the use of weak acid solutions such as 100 mM nitric acid solution. Sn(IV) is eluted using 100 mM triethylamine solution. Additional Hg(II) is released under basic elution.

Triacetic Acid (TAX)

TAX sorbents have the highest affinity for Sn(IV) and Hg(II) followed by lesser amounts of Cu(II) and Zn(II). Metal ions are readily eluted from TAX by the use of weak acid solutions such as 100 mM nitric acid solution. Sn(IV) is eluted using 100 mM triethylamine solution. Additional Hg(II) is released under basic elution.

Sulfhydryl THX (thiopropyl)

THX sorbents have the highest affinity for Hg(II) and Sn(IV), and approximately equal weights of Sn(IV) and Cu(II). Metal ions are readily eluted from THX by the use of weak acid solutions such as 100 mM nitric acid solution. Sn(IV), Se(IV) and Hg(II) are eluted using 100 mM triethylamine solution.

Aminopropyl (NAX)

The NAX ion-exchange sorbent has a significant capacity for Hg(II) followed by Se(IV). Metal ions are readily eluted from NAX by the use of weak acid solutions such as 100 mM nitric acid solution. Additional Hg(II) is released under basic elution.

*UCT ENVIRO-CLEAN® Ion-exchange cartridges are available in a variety of cartridge sizes, sorbent mass and particle size to most analytical requirements. For further information, contact UCT.

Determination of Diquat and Paraquat in Drinking Water by Solid Phase Extraction and LC-MS/MS Detection

UCT Part Numbers:
Enviro-Clean® RFV0050CT (50 mL centrifuge tubes)
Enviro-Clean® SPE cartridge: EUCCX11Z (Carboxylic acid 100 mg/10 mL)

Summary:

Diquat and paraquat are fast-acting, non-selective herbicides used widely as desiccants and defoliants. They are quaternary amines that are highly water soluble. Their toxicity and presence in bodies of water have negative effects on aquatic life and human health. Therefore, it is important to determine their levels in drinking water samples.

The traditional drinking water method for diquat and paraquat analysis is EPA method 549.2. This method employs an ion-paring reverse phase (C8) solid phase extraction (SPE) followed by ion-pairing HPLC with UV or photodiode array detection. The traditional method is time-consuming (extracting 250 mL sample), needs ion-pairing reagents, and is less sensitive than alternative extraction and analysis options.

This application outlines a novel weak cation exchange SPE method with LC-MS/MS detection for diquate and paraquat. The method is fast and sensitive using only 10 mL of water sample. In addition there is no need for ion-pairing. Moreover, quaternary amines are retained onto the sorbent by a cation exchange mechanism; washing the sorbent with organic solvents after extraction will not wash off the retained amines, however will ultimately provide a much cleaner extract than using the traditional reverse phase C8 sorbent.

Notes: Diquat and paraquat cations tend to be adsorbed onto glass surfaces; therefore plastic labware was used for the entire procedure.

Deuterated diquat and paraquat are not stable in aqueous solutions, thus were added to the final extracts as instrumental internal standards.

Preparation of buffers, elution solvent, and mobile phase:

A. 400 mM phosphate buffer (pH 7)

Dissolve 20.9 g of potassium phosphate dibasic and 10.9 g of potassium phosphate monobasic in 500 mL reagent water. Adjust pH to 7 with diluted potassium hydroxide or phosphoric acid.

B. 25 mM phosphate buffer (pH 7)

Mix 50 mL of solution **A.** with 750 mL reagent water.

C. 25 mM ammonium formate buffer (pH 8)

Weigh 1.6 g of ammonium formate to a 1-L volumetric flask, add 950 mL reagent water and 1.4 mL of ammonium hydroxide and mix well. Adjust pH to 8 with diluted formic acid or ammonium hydroxide. Dilute to mark with reagent water.

D. Elution solvent: 10% formic acid in acetonitrile

Add 10 mL of formic acid to 90 mL of acetonitrile (MeCN), and mix well.

E. Mobile phase buffer: 100 mM ammonium acetate buffer (pH 5)

Weigh 7.78 g of ammonium acetate and 2 g of glacial acetic acid into a 1-L mobile phase reservoir, and add 998 mL of reagent water. Sonicate for 30 min to dissolve the salt and acid, and remove the dissolved gases.

Sample pretreatment:

Transfer 10 mL of water sample to a 50 mL centrifuge tube (**RFV0050CT**), add 25 µL of 400 mM phosphate buffer (pH7), and spike with appropriate amounts of diquat and paraquat standards for fortified samples, cap and mix well.

SPE Procedure:

1. Place the labeled SPE cartridges (**EUCCX11Z**) onto the glass block manifold lid.
2. Condition the cartridges with 3 mL of methanol (MeOH), and 3 mL of 25 mM phosphate buffer (pH 7).
3. Load the pretreated water samples onto the SPE cartridges, and apply a low vacuum for a slow dropwise flow (about 2-3 mL/min).
4. Wash the 50 mL centrifuge tubes with 3 mL of 25 mM ammonium formate buffer (pH 8), and apply the rinsate to the cartridges. Repeat with 3 mL of MeOH.

5. Dry the cartridges by applying full vacuum for 3 min.
6. Insert labeled 12*75 mm polypropylene test tubes into the manifold.
7. Elute with 3*1 mL of 10% formic acid in MeCN, pass 1/3 through, soak for 1 min, and draw the remaining through slowly.
8. Evaporate the eluates to dryness under a stream of nitrogen in a 45 ºC water bath.
9. Reconstitute with 900 µL of the mobile phase (100 mM ammonium acetate buffer (pH5): MeCN, 30:70, v/v), add 100 µL of 1 ppm IS mix, vortex and transfer 200 µL to 250-µL polypropylene inserts held in 2-mL vials.
10. Extracts are ready for analysis.

LC-MS/MS method:

HPLC: Thermo Scientific Dionex UltiMate 3000® LC System	
Column: Thermo Scientific, Acclaim® Trinity™ Q1, 50 x 2.1 mm, 3 µm	
Guard Column: Thermo Scientific, Acclaim® Trinity™ Q1,10 x 2.1 mm, 3 µm	
Column Temperature: 25 °C	
Column Flow Rate: 0.300 mL/min	
Auto-sampler Temperature: 10 °C	
Injection Volume: 5 µL	
Mobile phase (isocratic): 30% of 100 mM ammonium acetate buffer (pH 5) and 70% of MeCN	
MS parameters	
Polarity	ESI +
Spray voltage V	3500 V
Vaporizer Temperature	400 °C
Ion transfer capillary temperature	350 °C
Sheath gas pressure	30 arbitrary units
Auxiliary gas pressure	15 arbitrary units
Q1 and Q3 peak width (FWHM)	0.4 and 0.7 Da
Collision gas and pressure	Ar at 2.3 mTorr
Scan type	SRM
Cycle time	1 sec
Acquisition method	EZ Method

SRM transitions

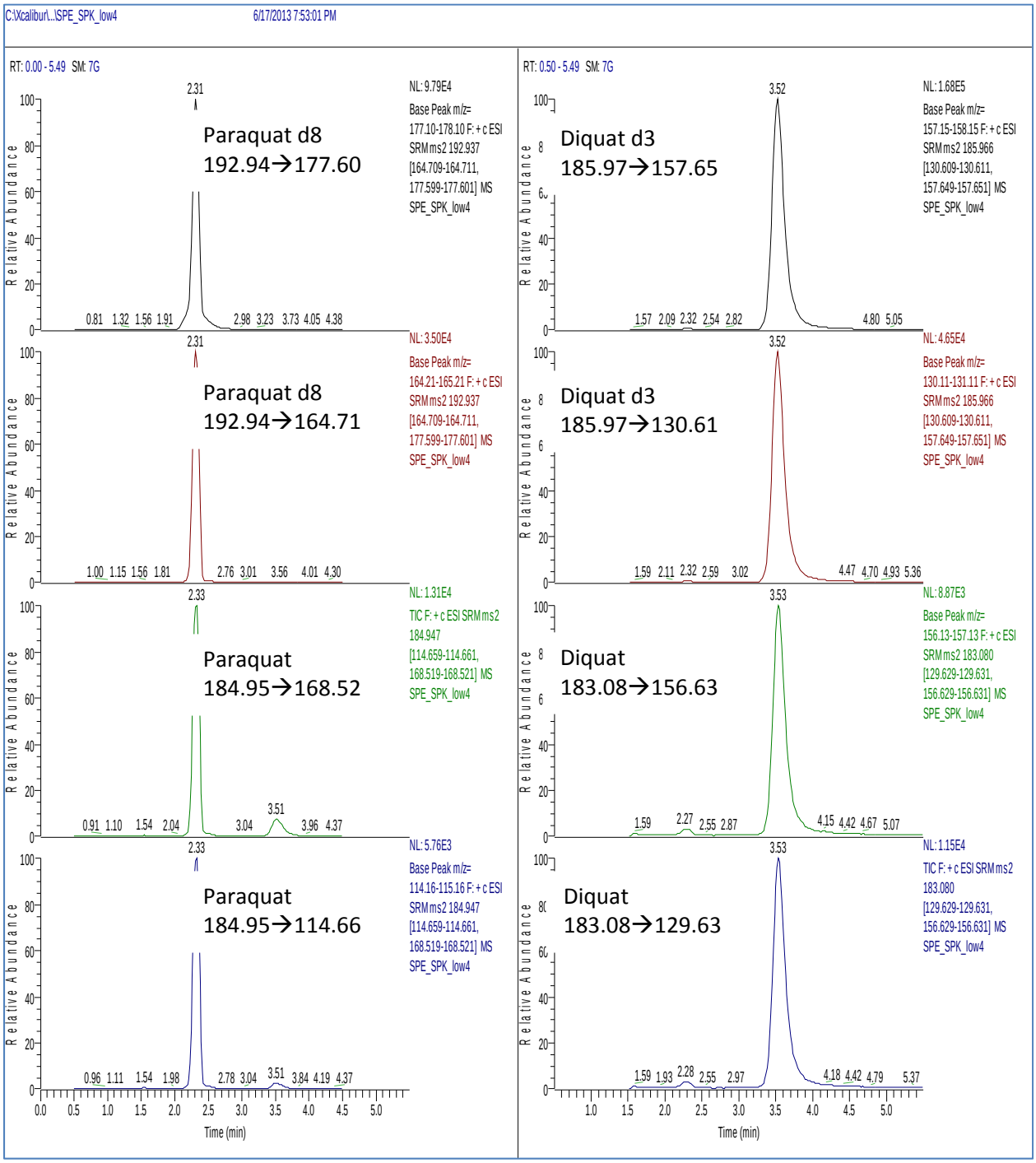
Compound	Rt (min)	Precursor ion	Product ion 1	CE 1	Product ion 2	CE 2	S-lens (V)
Paraquat d8	2.31	192.94	177.60	24	164.71	30	53
Paraquat	2.33	184.95	168.52	17	114.66	23	59
Diquat d3	3.52	185.97	157.65	22	130.61	34	55
Diquat	3.53	183.08	156.63	22	129.63	33	55

Results:

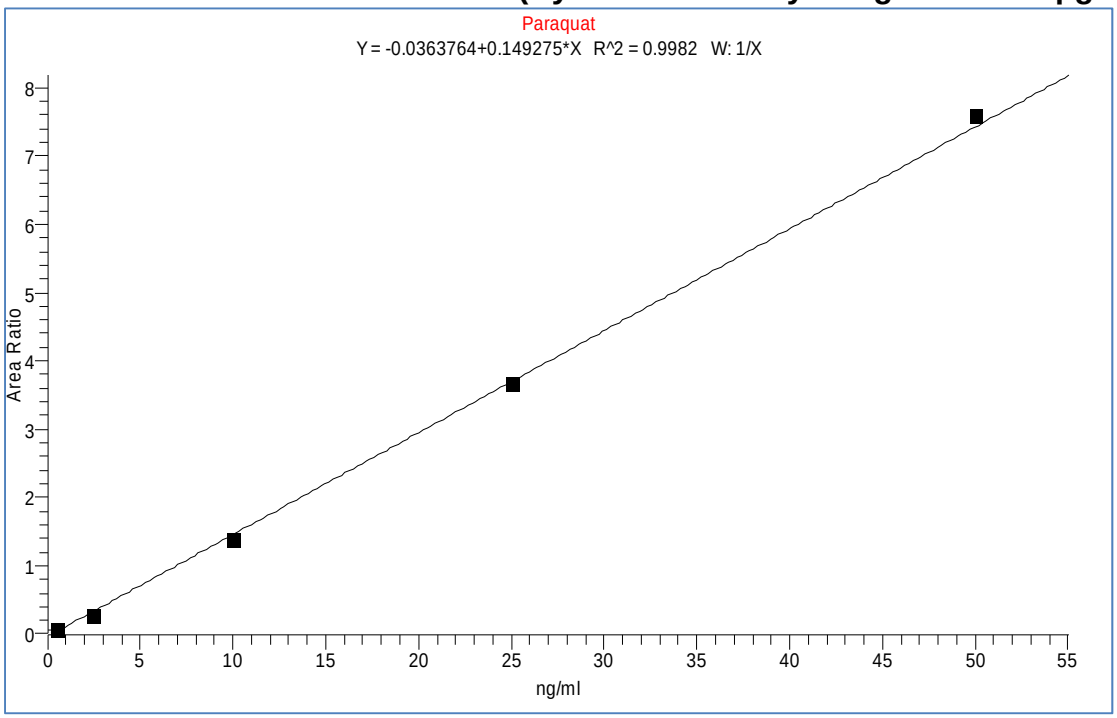
Recovery and RSD% Obtained from 6 Replicated Fortified Water Samples

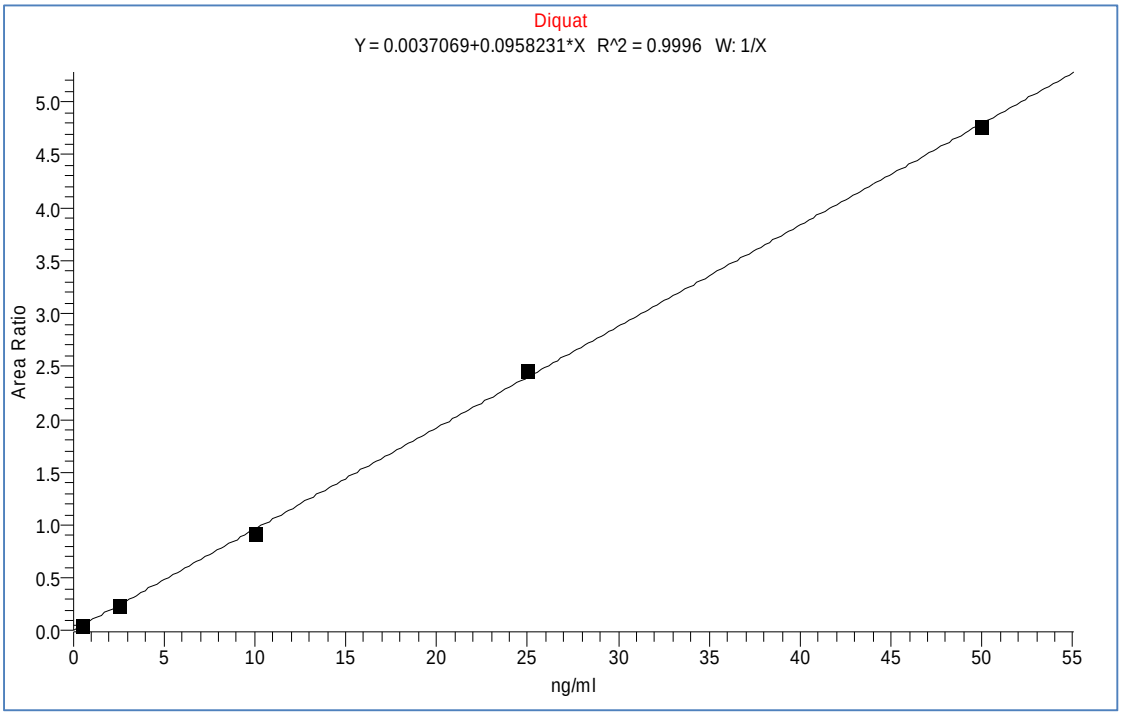
Compound	Spiked at 0.5 µg/L		Spiked at 25 µg/L	
	Recovery%	RSD% (n=6)	Recovery%	RSD% (n=6)
Paraquat	**96.1**	7.1	**97.9**	5.2
Diquat	**89.2**	7.0	**87.9**	7.1

Chromatogram of a Water Sample Fortified with 0.5 µg/L of Diquat and Paraquat



Matrix Matched Calibration Curves (Dynamic Linearity Range: 0.5 – 50 µg/L)





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A Novel, Versatile Polymeric Sorbent for Solid Phase Extraction of Various Organic Compounds in Water

UCT Part Numbers:
ECHLD156-P - 500 mg Enviro-Clean® HL DVB in 6 mL cartridge
VMFSTFR12 - Sample transfer tubes
ECSS25K - 25 kg sodium sulfate, anhydrous, ACS grade, granular 60 mesh
RFV1F15P - 15 mL reservoirs with 1 frit, 10 micron porosity
ECSS15M6 - 5 g anhydrous sodium sulfate in 6 mL cartridge
AD0000AS - Cartridge adaptors

Introduction

This application describes the use of a novel, versatile polymeric sorbent for the solid phase extraction (SPE) of a range of organic compounds. Depending on the extraction protocol used analysts are able to extract acids, bases, and neutrals including non-polar and even some mid-polar to polar compounds. Several of the compounds extracted are typically not well retained by silica-based sorbents, such as C8 and C18. The analytical performance of this new sorbent is demonstrated by selectively extracting acidic compounds (phenols), neutrals (explosives, organophosphorus pesticides, triazine herbicides, and other polar/non-polar compounds), and bases.

The SPE methods are simple and easy to use. It involves cartridge washing and conditioning, sample loading, cartridge drying and analyte elution with proper organic solvents. To ensure good extraction efficiency, the water sample must be de-chlorinated and the sample pH adjusted so that the target analytes are in their uncharged molecular forms. It is only in the neutral form that the analytes are retained by reverse phase functionality on the polymeric sorbent. For acidic compounds, sample pH should be adjusted to 2 units below the lowest pKa of the compounds to be extracted. For basic compounds, sample pH should be 2 units above the highest pKa of the compounds. For neutral compounds sample pH is not as critical as with acids or bases and can be extracted as received, unless one or more compounds to be extracted are sensitive under certain pH. Sample pH should be adjusted to the value at which any sensitive compounds are most stable to avoid low recovery caused by analyte hydrolysis or degradation. For example, captan and flumioxazin degrade much faster under alkaline conditions; therefore the sample should be adjusted to acidic conditions to avoid analyte degradation. Some compounds hydrolyze under both alkaline and acidic condition (e.g. organophosphorus esters), thus the optimum sample pH is neutral. Knowing the chemical and physical properties of the target analytes helps reduce overall method development time.

UCT has created a new sample transfer tube (**VMFSTFR12**) that fits SPE cartridges of varying sizes (1, 3, 6, 10, 15, and 25 mL). These transfer tubes allow analysts to load large sample volumes onto SPE cartridges with limited attendance. Simply connect the transfer tube to the SPE cartridge and drop the opposite end, outfitted with a SS sinker into the sample container.

Another time and effort saving option is utilized by connecting a drying cartridge (**ECSS15M6**) directly to the end of the SPE cartridge (**ECHLD156-P**) using a cartridge adaptor (**AD0000AS**) during the elution step. This eliminates the need for an additional eluate drying step. This procedure can be used when the elution solvents are more non-polar, such as ethyl acetate or dichloromethane. Another drying option is available when eluting using more polar solvents, such as methanol or acetone. Pack 15 gm of bulk sodium sulfate (**ECSS25K**) into a 15-mL fritted reservoir (**RFV1F15P**) and place in-line at the end of the SPE cartridge. Polar solvents elute more water residue from the SPE cartridges than non-polar solvents. The use of the 15mL reservoir with 15 gm of bulk sodium sulfate ensures plenty of drying capacity.

SPE applications of various organic compounds in water

1: Acidic compounds

SPE procedure	
Sample pretreatment	1 L water sample, pH adjusted to < 2
Cartridge washing	3 x 3 mL dichloromethane (DCM)
Cartridge conditioning	3 x 3 mL methanol (MeOH); 4 x 3 mL 0.05 N HCl
Sample loading	15 mL/min
Cartridge drying	15 min under full vacuum
Elution with in-line drying	10 mL DCM bottle rinse; 3 mL DCM to cartridge
Eluate evaporation	Nitrogen at 40 ºC to 1 mL

Analyte	Single-lot results		Multiple-lot results	
	Recovery%	RSD% (n=5)	Recovery%	RSD% (n=35)
Phenol	**88.2**	2.2	**86.4**	4.0
2-chlorophenol d4 (Surr)	**88.7**	1.6	**87.3**	4.7
2-chlorophenol	**87.4**	1.3	**85.3**	3.5
2-methylphenol	**88.6**	1.5	**86.8**	3.6
2-nitrophenol	**85.6**	0.8	**85.5**	3.8
2,4-dimethylphenol d3 (Surr)	**88.5**	1.4	**86.9**	6.6
2,4-dimethylphenol	**88.4**	1.1	**85.1**	6.5
2,4-dichlorophenol	**87.4**	1.3	**86.5**	3.8
4-chloro-3-methylphenol	**90.4**	1.0	**89.5**	2.9
2,4,6-trichlorophenol	**88.3**	0.6	**87.8**	3.2
2,4-dinitrophenol	**103.2**	7.6	**108.4**	5.6
4-nitrophenol	**96.5**	1.2	**97.4**	4.2
2-methyl-4,6-dinitrophenol	**92.9**	2.5	**97.9**	6.7
2,4,6-tribromophenol (Surr)	**88.7**	0.9	**89.5**	4.3
Pentachlorophenol	**94.3**	1.1	**95.8**	4.7

2: Neutral compounds

2a. Explosives

SPE procedure	
Sample pretreatment	1 L water sample, pH as received
Cartridge washing	3 x 5 mL ethyl acetate (EtOAc)
Cartridge conditioning	3 x 5 mL MeOH; 2 x 10 mL DI water
Sample loading	15 mL/min
Cartridge drying	10 min under full vacuum
Elution with in-line drying	5 mL EtOAc bottle rinse; 5 mL EtOAc to cartridge
Eluate evaporation	Nitrogen at 40 ºC to 1 mL

Analyte	Single-lot results		Multiple-lot results	
	Recovery%	RSD% (n=5)	Recovery%	RSD% (n=25)
Nitrobenzene d5 Surr	**92.4**	3.5	**88.9**	4.3
Nitrobenzene	**86.8**	2.7	**88.8**	4.6
2-Nitrotoluene	**87.6**	3.6	**89.1**	4.7
3-Nitrotoluene	**86.6**	3.6	**87.7**	4.6
4-Nitrotoluene	**84.4**	3.3	**87.2**	4.9
1,3-Dinitrobenzene	**102.4**	5.3	**99.7**	4.2
2,6-Dinitrotoluene	**98.2**	5.7	**97.3**	4.8
2,4-Dinitrotoluene	**91.2**	5.3	**92.9**	4.2
1,3,5-Trinitrobenzene	**100.0**	9.1	**100.4**	5.5
2,4,6-Trinitrotoluene	**103.0**	6.3	**100.9**	5.3
RDX	**107.0**	1.7	**111.1**	5.8
4-Amino-2,6-Dinitrotoluene	**100.1**	7.5	**99.6**	5.8
3,5-Dinitroaniline	**104.3**	5.6	**103.6**	6.3
2-Amino-4,6-Dinitrotoluene	**103.3**	5.2	**105.7**	5.0
Tetryl	**102.2**	3.7	**105.4**	4.7

2b. Organophosphorus pesticides and triazine herbicides

SPE procedure	
Sample pretreatment	1 L water sample, at neutral pH
Cartridge washing	3 x 5 mL DCM
Cartridge conditioning	2 x 5 mL MeOH; 2 x 5 mL DI water
Sample loading	15 mL/min
Cartridge drying	10 min under full vacuum
Elution	5 mL acetone bottle rinse; 5 mL DCM bottle rinse; 5 mL DCM to cartridge
Eluate drying	15 g sodium sulfate in 15 mL fritted reservoir
Eluate evaporation	Nitrogen at 40 ºC to 0.5 mL
Solvent exchange	Solvent exchange to n-hexane

Analyte	Class	Recovery%	RSD% (n=5)
o,o,o-Triethyl phosphorothioate	Organophosphorus	**87.8**	1.8
Thionazin	Organophosphorus	**100.9**	1.8
Sulfotep	Organophosphorus	**96.2**	0.9
Phorate	Organophosphorus	**93.0**	1.2
Dimethoate	Organophosphorus	**108.7**	7.3
Simazine	Triazine	**104.2**	2.1
Atrazine	Triazine	**101.5**	1.2
Disulfoton	Organophosphorus	**85.5**	1.7
Methyl parathion	Organophosphorus	**112.4**	1.8
Malathion	Organophosphorus	**110.7**	1.3
Parathion	Organophosphorus	**106.8**	1.3
Ethion	Organophosphorus	**107.8**	0.7
Famphur	Organophosphorus	**120.0**	1.8

2c. Other polar and non-polar neutral compounds

SPE procedure	
Sample pretreatment	1 L water sample, pH adjusted to <5
Cartridge washing	3 x 5 mL DCM
Cartridge conditioning	3 x 5 mL MeOH; 10 mL DI water
Sample loading	15 mL/min
Cartridge drying	10 min under full vacuum
Elution with in-line drying	2 x 5 mL DCM
Eluate evaporation	Nitrogen at 40 ºC to 1 mL

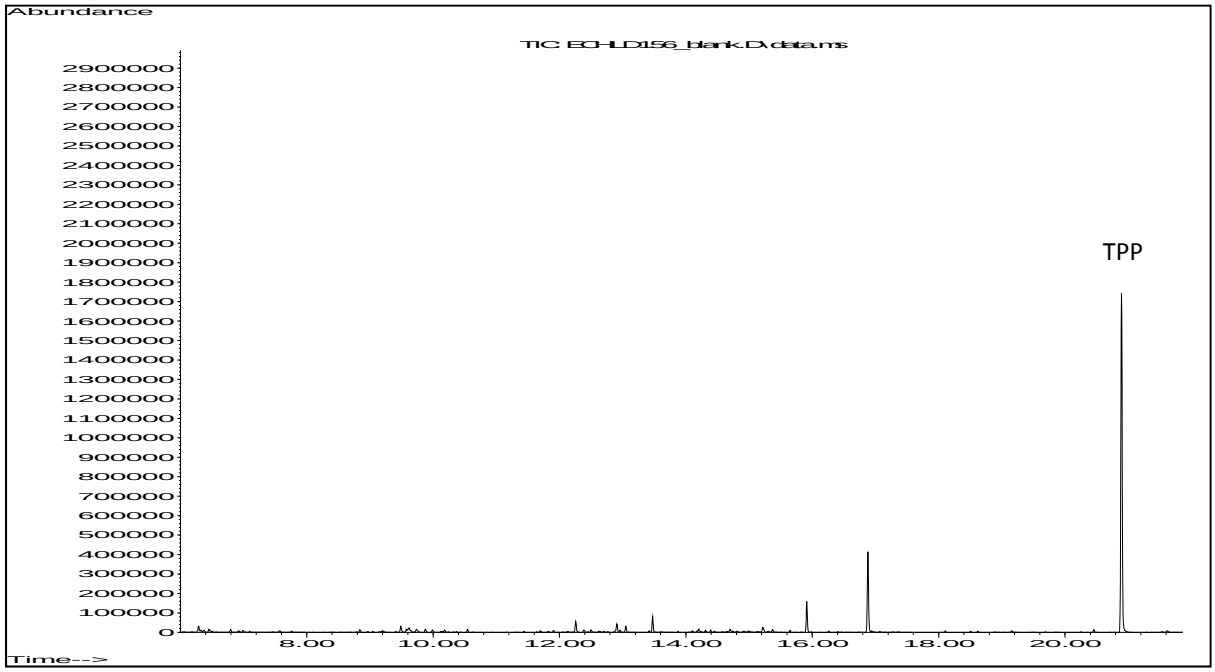
Analyte	Chemical properties	Recovery%	RSD% (n=4)
Dicrotophos	Polar, LogP -0.24	**96.0**	2.0
Dimethipin	Polar, LogP -1.53	**105.3**	3.1
Butylated hydroxyanisole	Non-polar, LogP 3.06	**102.5**	0.5
Diethyl phthalate	Non-polar, LogP 2.42	**96.7**	0.6
Methyl parathion	Non-polar, LogP 2.8	**97.4**	2.2
Carbaryl	Non-polar, LogP 2.36	**101.3**	3.5
Malathion	Non-polar, LogP 2.36	**101.9**	1.0
Captan	Non-polar, LogP 2.35	**100.6**	1.8
Nitrofen	Non-polar, LogP 4.62	**93.7**	1.9
Ethion	Non-polar, LogP 3.93	**99.9**	1.4
4,4'-DDT	Non-polar, LogP 6.46	**96.1**	1.8
Captafol	Non-polar, LogP 3.95	**103.2**	3.2
Bis(2-ethylhexyl) phthalate	Non-polar, LogP 4.89	**96.5**	1.7

3: Basic compounds

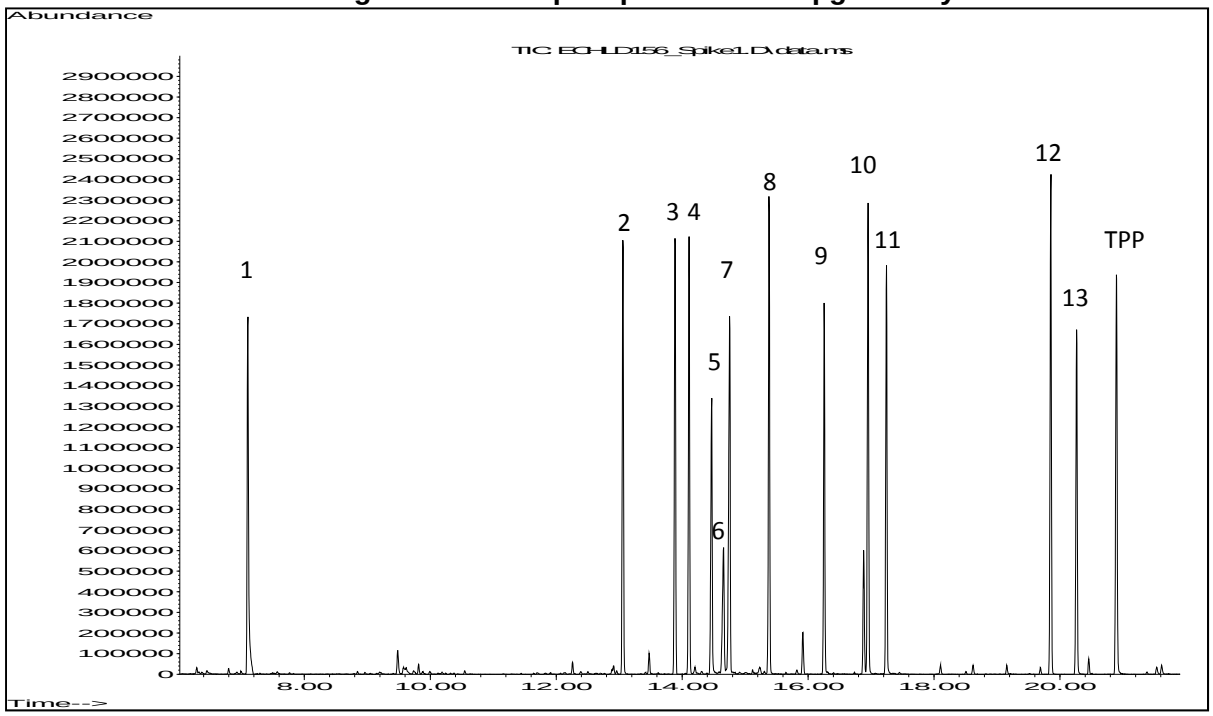
SPE procedure	
Sample pretreatment	1 L water sample, pH adjusted to > 7
Cartridge washing	3 x 5 mL DCM
Cartridge conditioning	3 x 5 mL MeOH; 10 mL DI water
Sample loading	15 mL/min
Cartridge drying	10 min under full vacuum
Elution with in-line drying	2 x 5 mL DCM
Eluate evaporation	Nitrogen at 40 ºC to 1 mL

Analyte	Chemical properties	Recovery%	RSD% (n=4)
o-Toluidine	Basic, pKa 4.48	**94.8**	4.2
Quinoline	Basic, pKa 4.50	**112.1**	2.1

Chromatogram of blank sample



Chromatogram of a sample spiked with 10 µg/L analytes



Peak list: 1. o,o,o-Triethyl phosphorothioate; 2. Thionazin; 3. Sulfotep; 4. Phorate; 5. Dimethoate; 6. Simazine; 7. Atrazine; 8. Disulfoton; 9. Methyl parathion; 10. Malathion; 11. Parathion; 12. Ethion; 13. Famphur.

Conclusions

- A novel and versatile polymeric sorbent effectively retains a variety range of organic compounds, including acids, neutrals (polar and non-polar compounds), and bases
- Cross-linked polymeric sorbent with > 5 times greater capacity than silica-based sorbents
- Stable in samples with pH ranged from 0 to 14
- Straightforward SPE extraction protocols with excellent precision and accuracy
- Unique polymeric structure contributed to consistent analytical performance with minimum lot-to-lot variations

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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)

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

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Agricultural and Food Safety

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)

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

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QuEChERS Extraction and Clean-Up of Pesticides from Olive Oil

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

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

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

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

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

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

Procedure

1. Extraction

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

2. Quick QuEChERS Clean-up

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

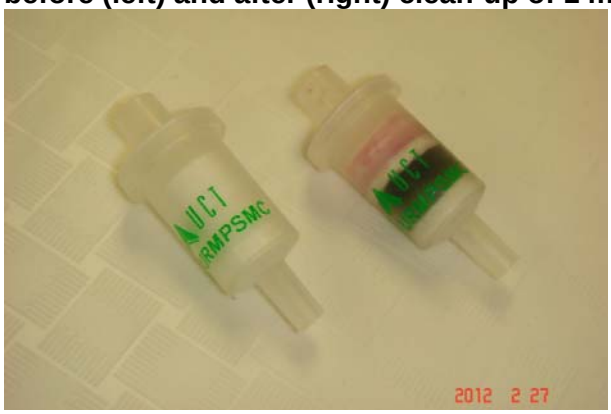
c) Collect 0.5 mL of the cleaned extract into a 2 mL auto-sampler vial

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

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

Clean-up red wine extract with Quick QuEChERS

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



3. LC/MS/MS Detection

LC: Thermo Accela 1250 pump with PAL auto-sampler

LC Conditions

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

LC Gradient Program

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

MS/MS: Thermo TSQ Vantage tandem MS

MS Conditions

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

SRM transitions

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

Matrix matched calibration, LOD and LOQ

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

Accuracy and Precision Data

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

Pesticides detected in red wine samples (ng/mL)

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

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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)

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"

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Acrylamide by QuEChERS Extraction with LC/MS/MS Detection

UCT Part Numbers:
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)

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

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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

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

*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

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

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

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

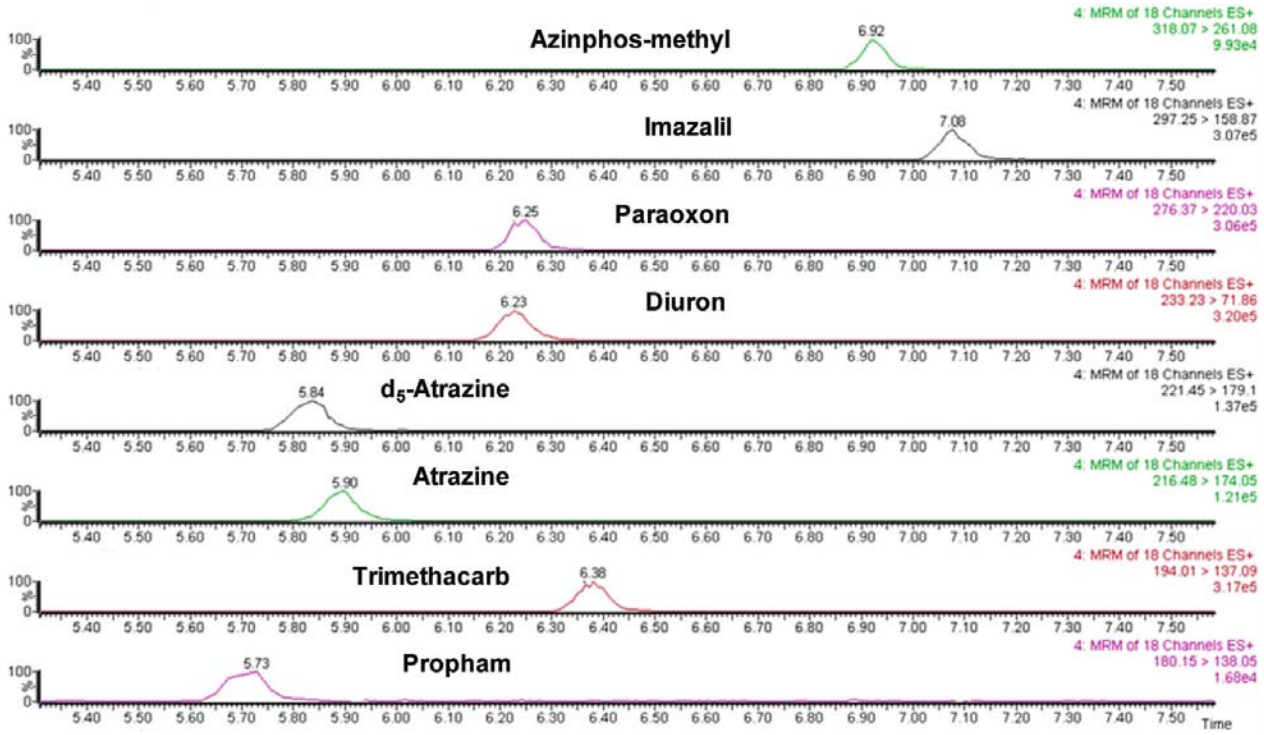
Automated DSI Injection:

- 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:

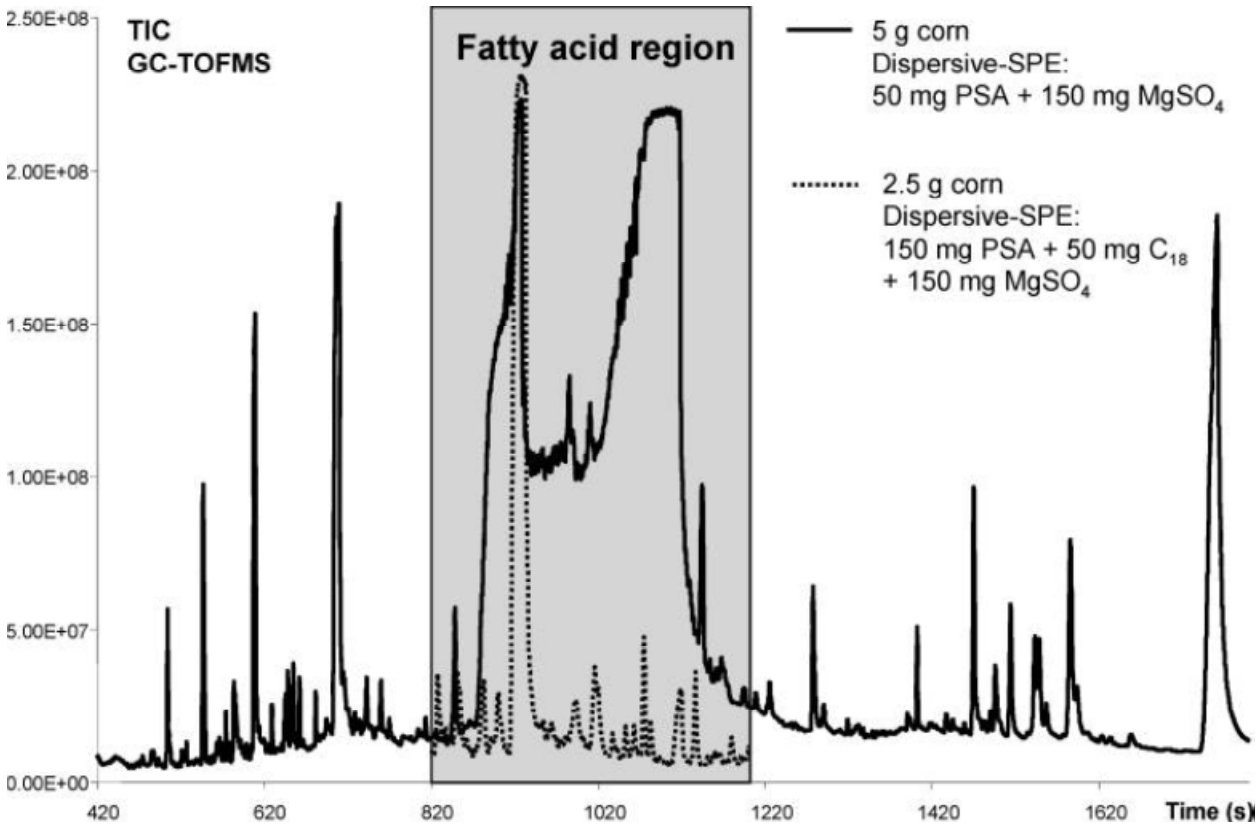
- Solvent vent at an injector temperature of 100° C for 4.5 minutes
- Initial column flow of 0.8 mL/minutes and a split flow of 50 mL/minutes,
- Follow by a splitless transfer of analytes for 4 minutes. The injector temperature was ramped to 280° C (at 16° C/s) Column flow changed to 1.5 mL/minutes (kept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

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Extraction of Pesticides from Tomato Using the QuEChERS Approach

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

UCT Part Numbers:
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)

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

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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)

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

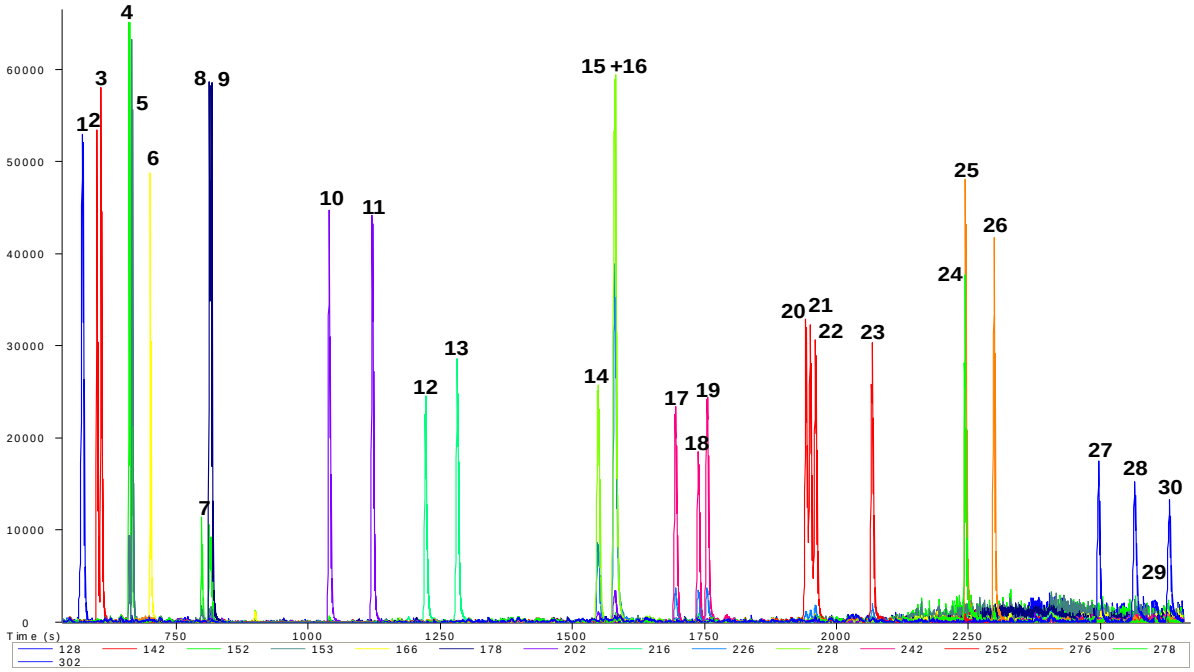
Analyte PAH's	Abbreviation	Confirmation Ions *(m/z)*	Quantification Ions *(m/z)*
Anthracene	Ant	177	178
Benz[*a*]anthracene	BaA	226	228
Benzo[*a*] pyrene	BaP	253	252
Benzo[*b*]fluoranthene	BpF	253	252
Benzo[*k*]fluoranthene	BkF	253	252
Benzo[*g,h,i*]perylene	BghiP	277	276
Chrysene	Chr	226	228
Dibenz[*a,h*]anthracene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

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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)

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

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

UCT Part Numbers:
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)

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

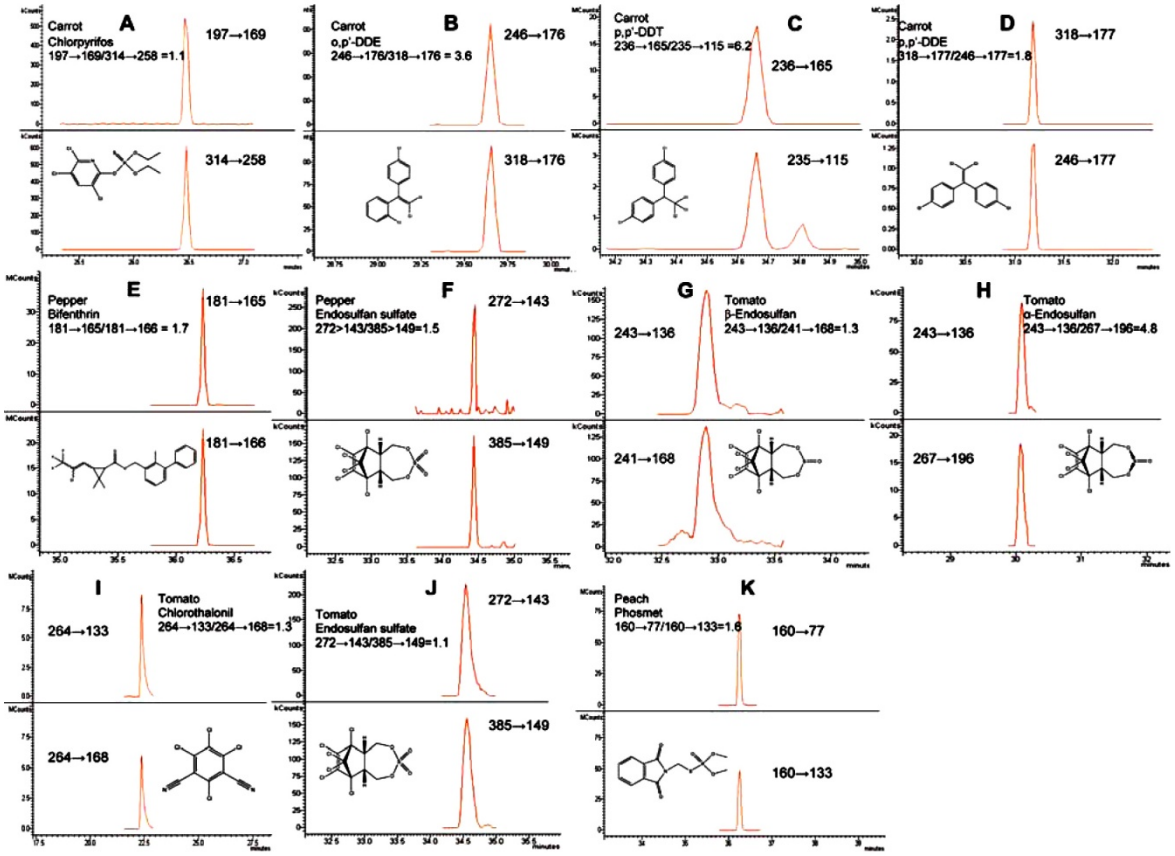
Analytes		
acenaphthene-d_{10}	Diamidafos (nellite)	*p,p'*-methoxychlor
acrinathrin	Diazinon	metolachlor
akton	Dibutyl chlorenate	mevinphos
alachlor	Dicapthon	mirex
aldrin	Dichlobenil	naphthalene-d_8
allethrin	Dichlofenthion	*cis*-nonachlor
atrazine	Dichlofluanid	*trans*-nonachlor
azamethiophos	3,4'-dichloroaniline	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 Part Numbers:
ECMSSA50CT-MP (6 g anhydrous $MgSO_4$ and 1.5 g Na Acetate)
EEC18156 (500 mg endcapped C18, 6 mL cartridge)

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 (C18, 5 µm, 2.1 x 7.5 mm)
Analytical column (C18, 5 µm, 2.1 x 250 mm)
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

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

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

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 Part Numbers:
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)*

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

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

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	
Mobile 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

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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 Part 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)

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

- Aqueous C18, 3 µm, 100 x 2.1 mm with 10 x 2.1 mm guard column

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

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.	49	22	9	356.	119.1	49	46	8
Pirimicarb	239.2	72.1	64	35	10	239.	182.1	64	23	10
Prochloraz	376	308	45	17	10	376	70	45	44	12
Promecarb	208.1	109	37	23	8	208.	151	37	13	10
Propamocarb	189.2	102	60	25	8	189.	144	61	19	13
Propargite+NH4	368.2	231.	46	15	13	368.	175.1	46	23	12
Propiconazole	342.1	159	62	40	9	342.	69	62	36	10
Propoxur	210.1	111	39	19	6	210.	168.1	39	11	10
Pymetrozine	218	105	71	27	5	218	78	71	47	5
Pyracarbolid	218.1	125	59	27	8	218.	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.	90	32	12	395.	192.1	90	34	10
Siduron	233.3	137.	66	21	5	233.	94	66	31	5
Spinetoram A	748.5	142.	86	45	8	748.	98.1	86	109	18
Spinetoram B	760.5	142.	96	41	10	760.	98.1	96	101	18
SpinosynA	732.5	142.	111	43	10	732.	98.1	111	103	16
Spirodiclofen	411.3	313.	72	23	8	411.	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

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

UCT Part Numbers:

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)

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)

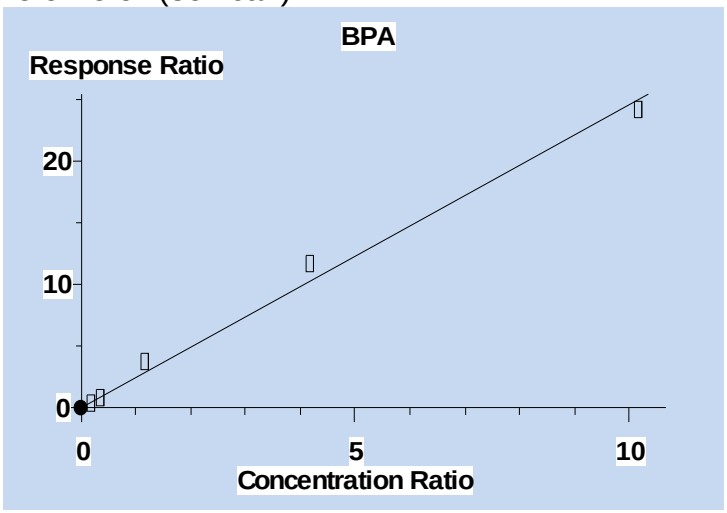
Dwell time: 100 ms for all ions
Matrix matched curves are generated with the adjustment of the concentrations in the blank and baby food sample

Experimental Data

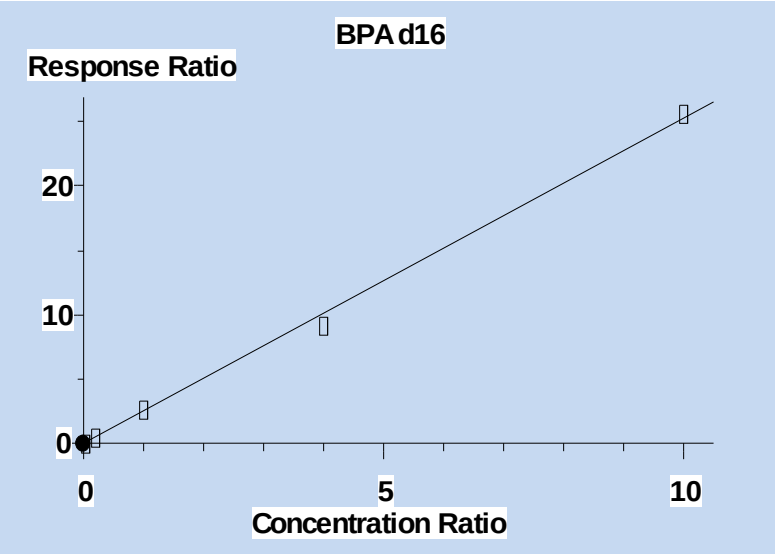
Calculations

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

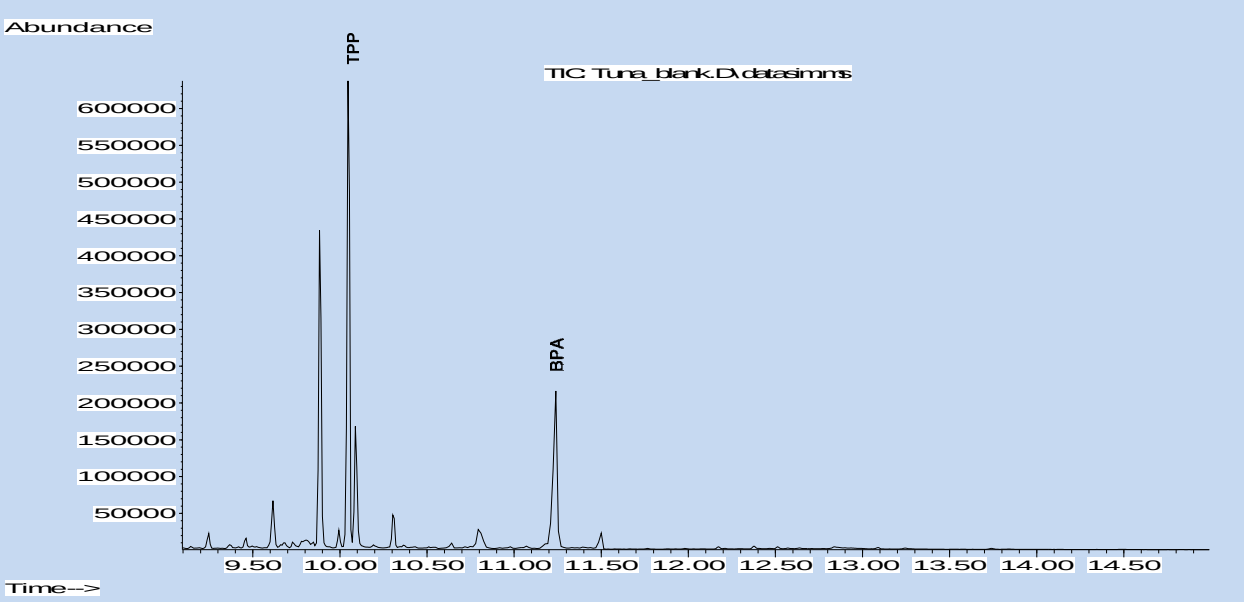


BPA d16: Linear dynamic range: 1-500 ng/mL (conc. per 1 mL extract) R^2=0.9983

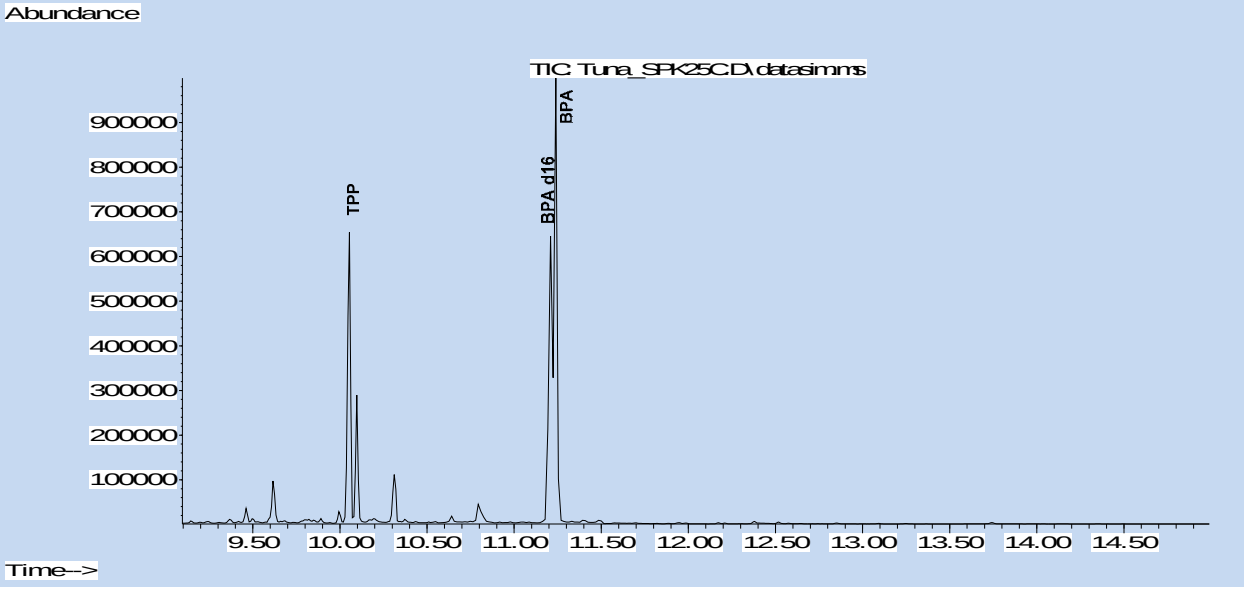
Bisphenol A in Canned Tuna

	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

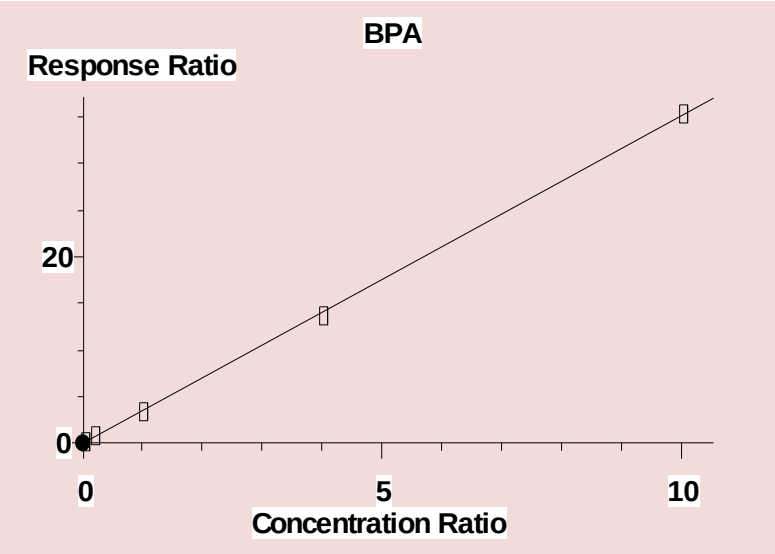
Chromatogram of Canned Tuna



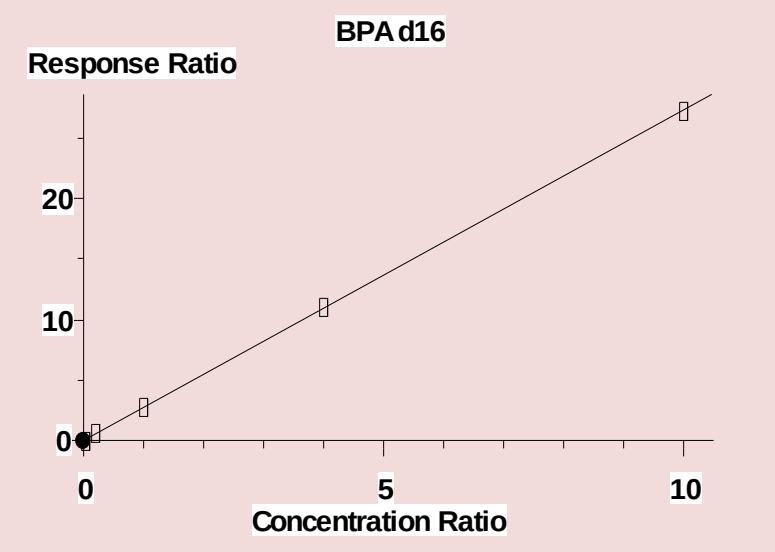
Chromatogram of canned tuna sample fortified with 25 ng/g BPA d16 and BPA



Baby Food: Peaches, Stage 2



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

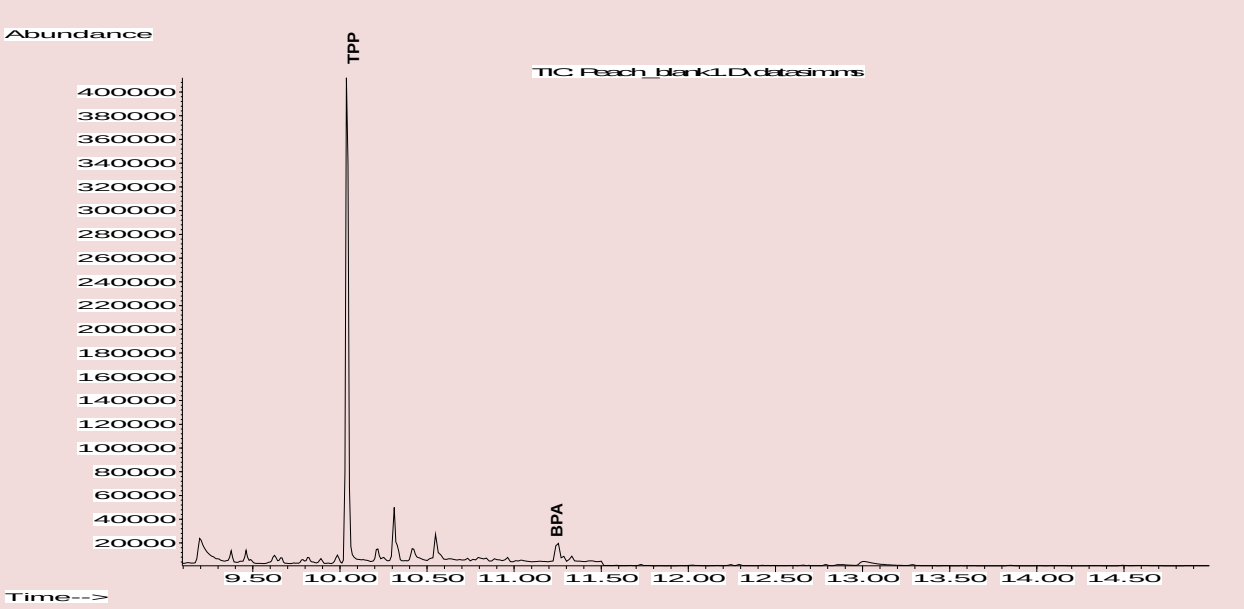


BPA d16: Linear dynamic range: 1-500 ng/mL (conc. per 1 mL extract) R^2=0.9999

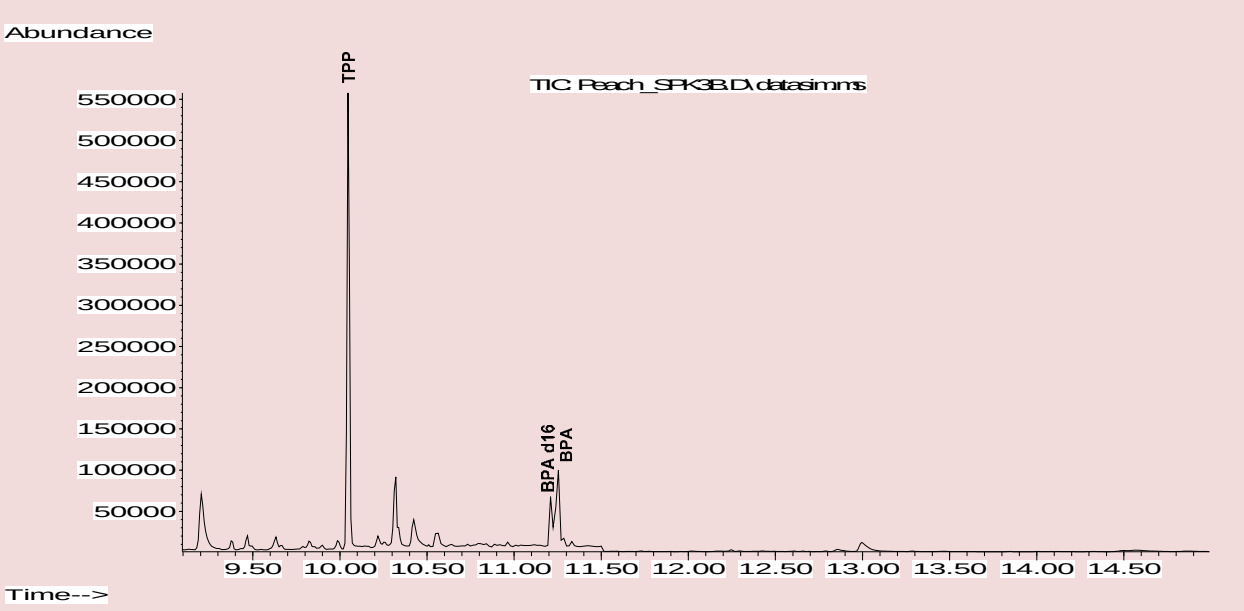
Bisphenol A in Baby Food

	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

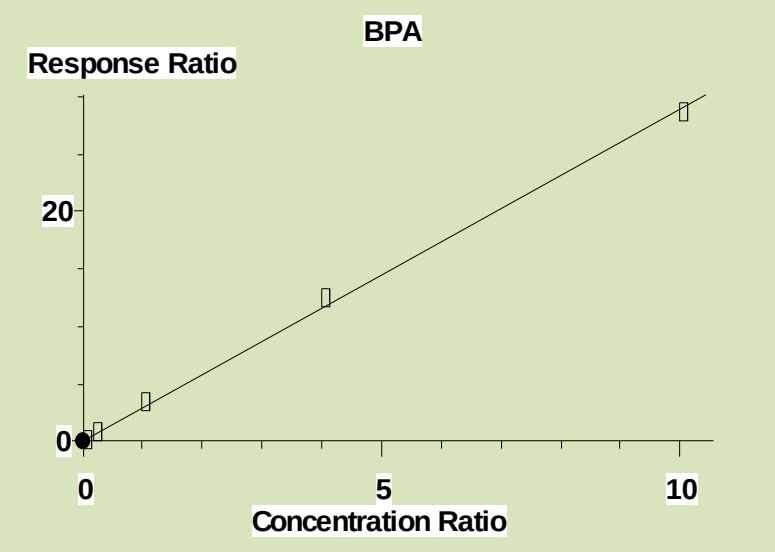
Chromatogram of Baby Food



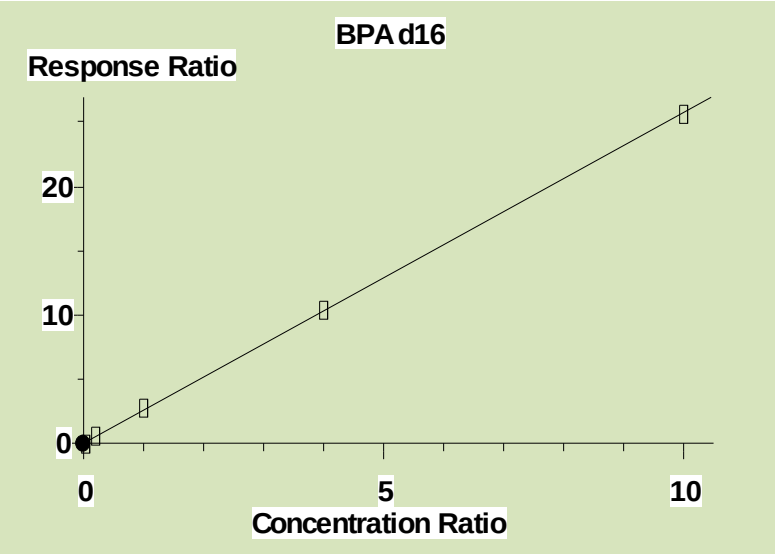
Chromatogram of baby food sample fortified with 3 ng/g BPA d16 and BPA



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

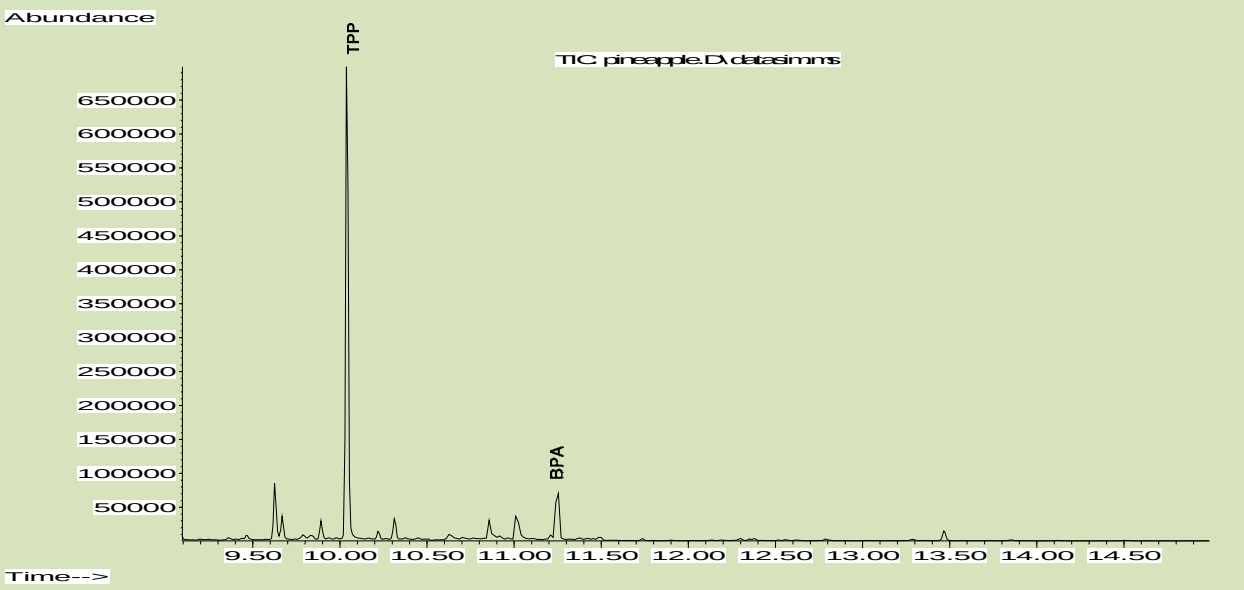


BPA d16: Linear dynamic range: 1-500 ng/mL (conc. per 1 mL extract) R^2=0.9999

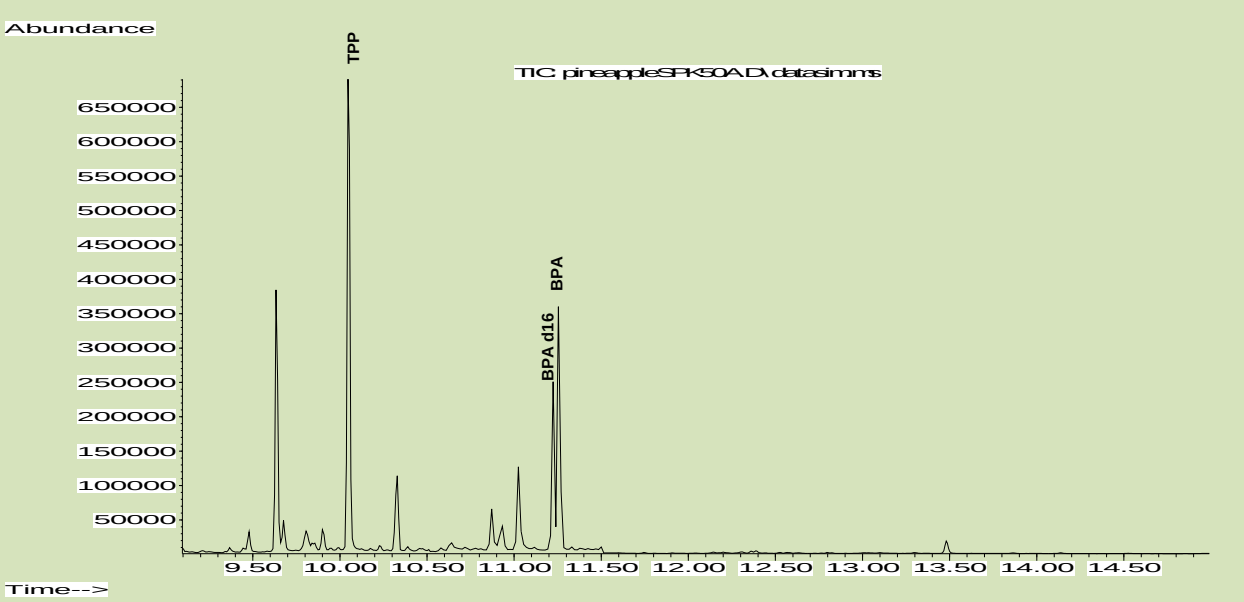
Bisphenol A in Canned Pineapple

	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

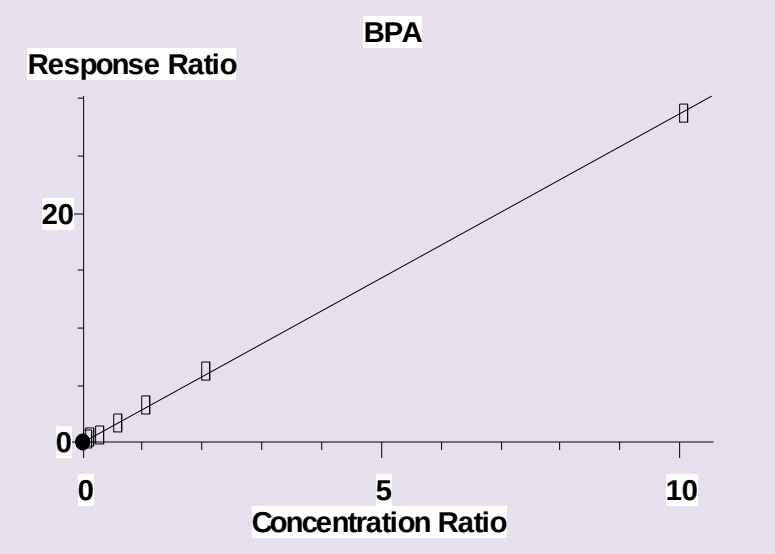
Chromatogram of Canned Pineapple



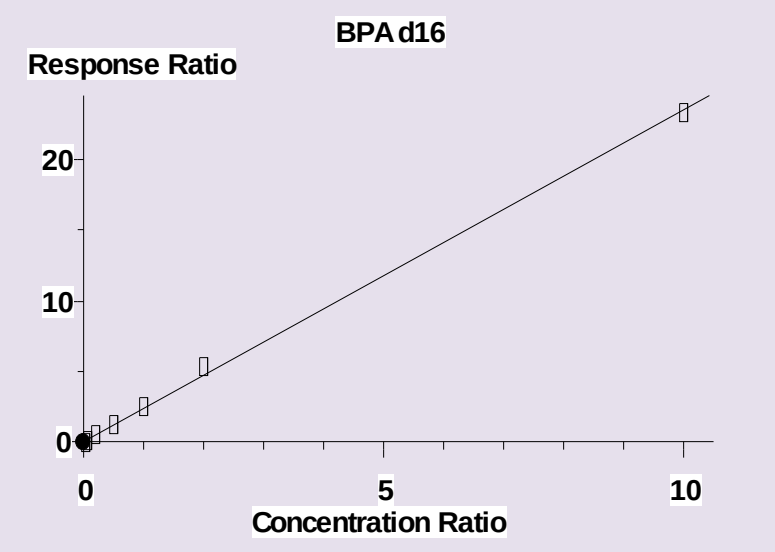
Chromatogram of canned pineapple sample fortified with 5 ng/g BPA d16 and BPA



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

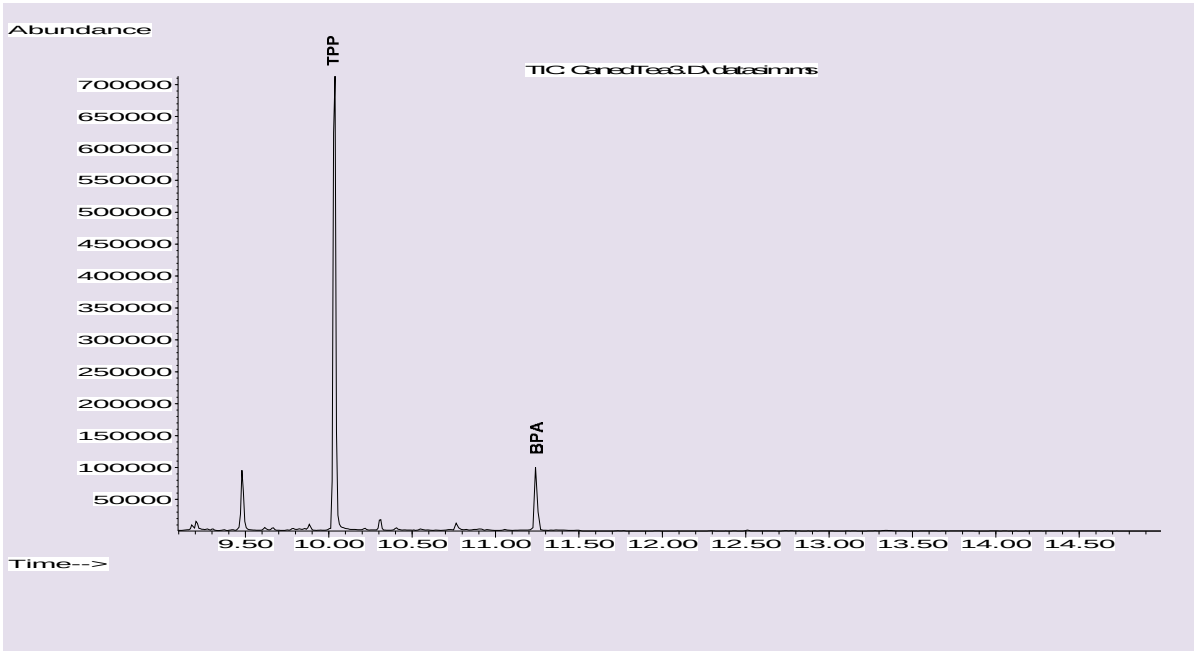


BPA d16: Linear dynamic range: 1-500 ng/mL (conc. per 1 mL extract) R^2=0.9993

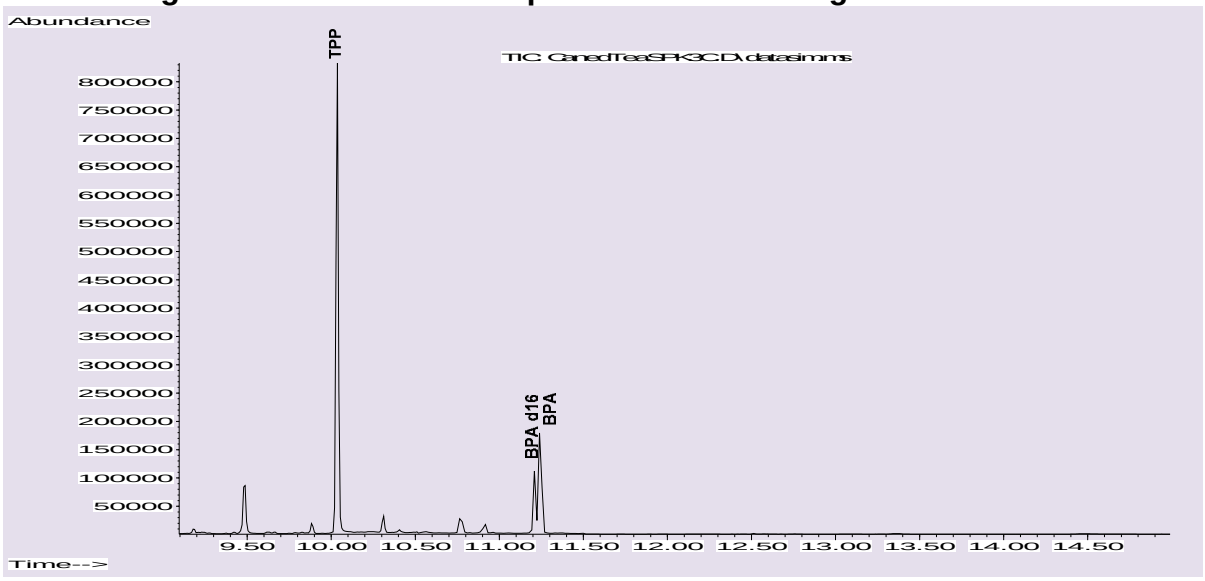
Bisphenol A in Canned Tea

	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



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

UCT Part Numbers:
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)

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

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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)

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)

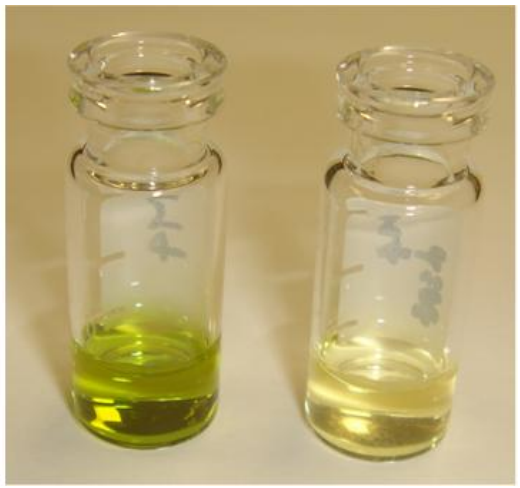

Figure 1b Marijuana (Sample #4) extracts before (left) and after dSPE clean-up (right)

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

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

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

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

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

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	Cimicifugaracemosa	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®)

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

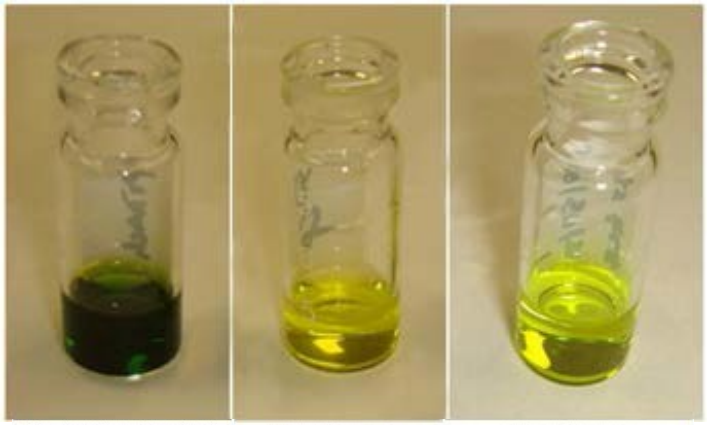
a) Transfer 1 mL of the extract to the 2 mL **CUMPSGGC182CT** (ChloroFiltr) dSPE micro centrifuge tube
b) Shake for 30 sec
c) Centrifuge at 3,000 rpm for 5 min
d) Transfer 0.4 mL of the supernatant to a 2 mL autosampler vial
e) Sample is ready for LC/MS/MS analysis

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



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)

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: Aqueous C18 column (3 µm, 100 x 2.1 mm)
Guard Column: (10 x 2.1 mm)
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

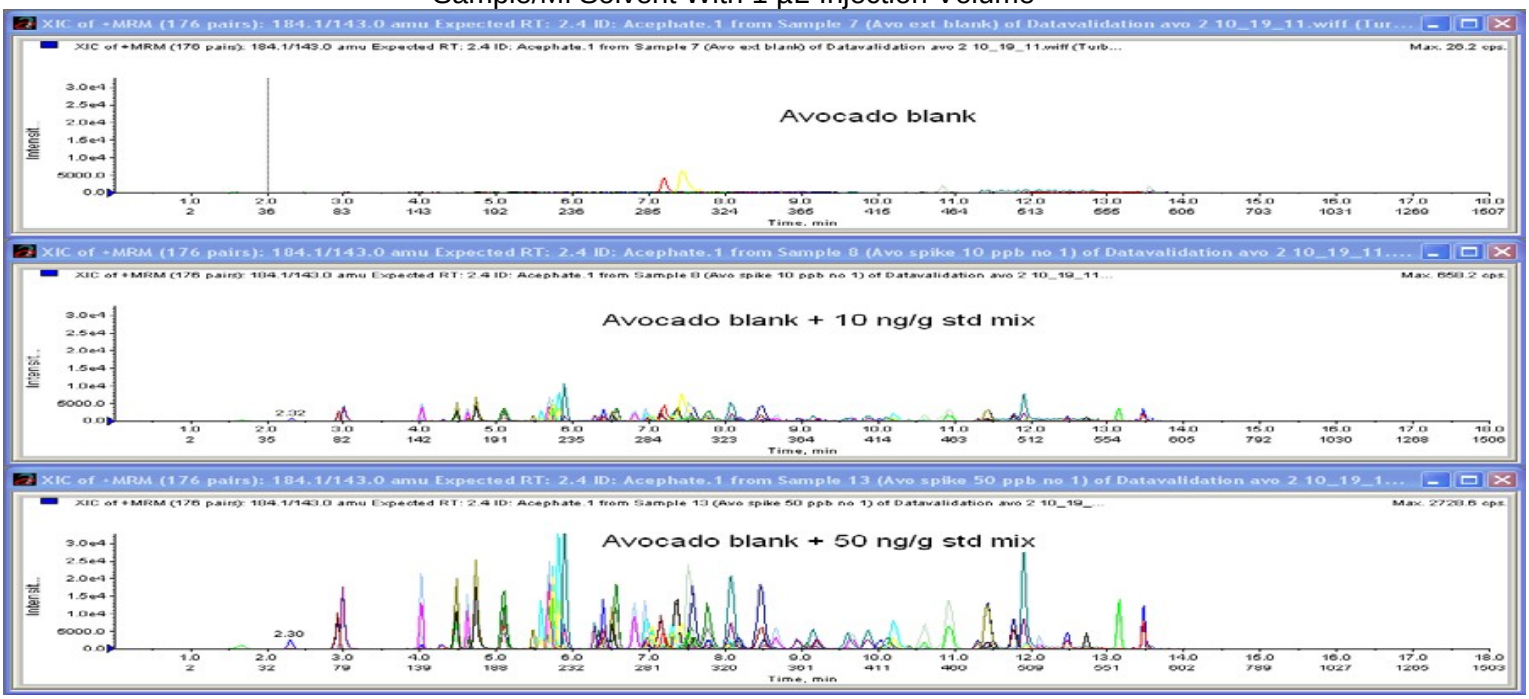
Tolyfluanid	129.6	4.2	86.7	9.6	89.8	6.0
Triadimefon	95.9	16.4	86.8	7.0	99.9	6.1
Triazophos	102.9	25.3	89.1	7.7	102.9	6.6
Tricyclazole	104.3	4.5	84.0	9.1	93.3	4.2
Trifloxystrobin	96.8	4.2	82.7	10.6	90.9	5.9
Triflumizole	101.5	5.7	84.0	11.1	92.1	4.7
Average	**107.1**		**86.1**		**94.8**	
Std. Dev	**9.9**		**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]
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Determination of Chlorophenoxyacetic Acid and Other Acidic Herbicides Using a QuEChERS Sample Preparation Approach and LC-Ms/MS Analysis

UCT Part Numbers:

ECQUEU750CT-MP - Mylar pouch containing extraction salts (4000 mg $MgSO_4$, 1000 mg NaCl, 500 mg sodium citrate dibasic sesquihydrate, 1000 mg sodium citrate tribasic dihydrate)

CUMC182CT (2mL centrifuge tube containing 150 mg $MgSO_4$ and 50 mg C18)

Chlorophenoxyacetic acid herbicides, such as 2,4-dichlorophenoxyacetic acid (2,4-D; Agent Orange), along with other acidic pesticides can be extracted and analyzed using the QuEChERS approach outlined in this application note. LC-MS/MS is used for detection and quantitation.

Prior to extraction, samples need to undergo base hydrolysis to release any bound herbicide residues (in the form of esters). Hydrolysis is conducted in 30 min using a NaOH solution. The samples are subsequently neutralized using sulfuric acid, and are now ready to undergo QuEChERS extraction.

The citrate-buffered QuEChERS salts are used to lower the sample pH during extraction in order to ensure that the acidic herbicides are in their neutral state (i.e. protonated) and effectively partition into the acetonitrile layer. Only C18 sorbent is used for dSPE cleanup as the acidic compounds are otherwise retained on PSA sorbent.

Compounds included in method	Abbreviation
Picloram	-
Nitrophenol	-
Bentazon	-
2,4-dichlorophenoxyacetic acid	2,4-D
2,4-dichlorophenoxybutyric acid	2,4-DB
2-methyl-4-chlorophenoxyacetic acid	MCPA
Dichlorprop	-
2,4,5-trichlorophenoxyacetic acid	2,4,5-T
Mecoprop (MCPP)	-
Dichlorobenzoic acid	-
Silvex (fenoprop)	-
Acifluorfen	-
Thiabendazole-d_5 (IS)	TBZ-d_5

Procedure

1. Alkaline Hydrolysis

a) Weigh 10 g of thoroughly homogenized sample into a 50 mL centrifuge tube.
b) Add internal standard.
c) Add an appropriate volume of 5M NaOH in order to adjust the sample to pH 12 - this volume depends on the type and pH of the sample (e.g. 1000 µL for apples and 500 µL for tomatoes).
d) Cap tube, shake briefly, and let sit for 30 min at room temperature.
e) Neutralize the sample with the addition of 2.5M H_2SO_4 – it should use about the same volume as NaOH added (e.g. 500 µL for apples and 250 µL for tomatoes).

2. Sample Extraction

a) Add 10 mL of acetonitrile to each sample.
b) Add contents of the **ECQUEU750CT-MP** pouch to each centrifuge tube.
c) Immediately vortex or shake (manually or mechanically) for at least 1 min. For our process, samples were shaken for 1 min on a Geno/Grinder at 1500 rpm.
d) Centrifuge for 5 min at ≥3000 rcf.

3. Sample Cleanup

a) Transfer 1 mL aliquot of supernatant to 2mL **CUMC182CT** dSPE tube.
b) Vortex (or shake) for 0.5 - 1 min.
c) Centrifuge for 2 min at high rcf (e.g. ≥ 5000).
d) Filter purified supernatant through a 0.2 µm syringe filter directly into a sample vial.
e) Analyze by LC-MS/MS.

HPLC Conditions:

Instrumentation	Thermo Scientific™ Dionex™ Ultimate™ 3000 LC system
Column	Selectra® C18, 100 x 2.1 mm, 3 µm (p/n SLC-18100ID21-3UM)
Guard cartridge	Selectra®C18, 10 x 2.1 mm, 3 µm (p/n SLC-18GDC20-3UM)
Run time	16 min (including re-equilibration time)
Column temperature	40°C
Injection volume	5 µL
Autosampler temperature	10°C
Wash solvent	MeOH : ultrapure water (1:1, v/v)
Flow rate	400 µL/min
Waste divert	The mobile phase was diverted to waste from 0 - 1.4 min and 12.2 - 16 min to prevent ion source contamination.

Mobile phase:		
A	0.1% formic acid in ultrapure water	
B	0.1% formic acid in MeOH	
LC gradient:		
Time (min)	**A (%)**	**B (%)**
0.0	95	5
1.0	50	50
6.0	50	50
10.0	30	70
12.2	30	70
12.5	95	5
18.0	95	5

MS Conditions:

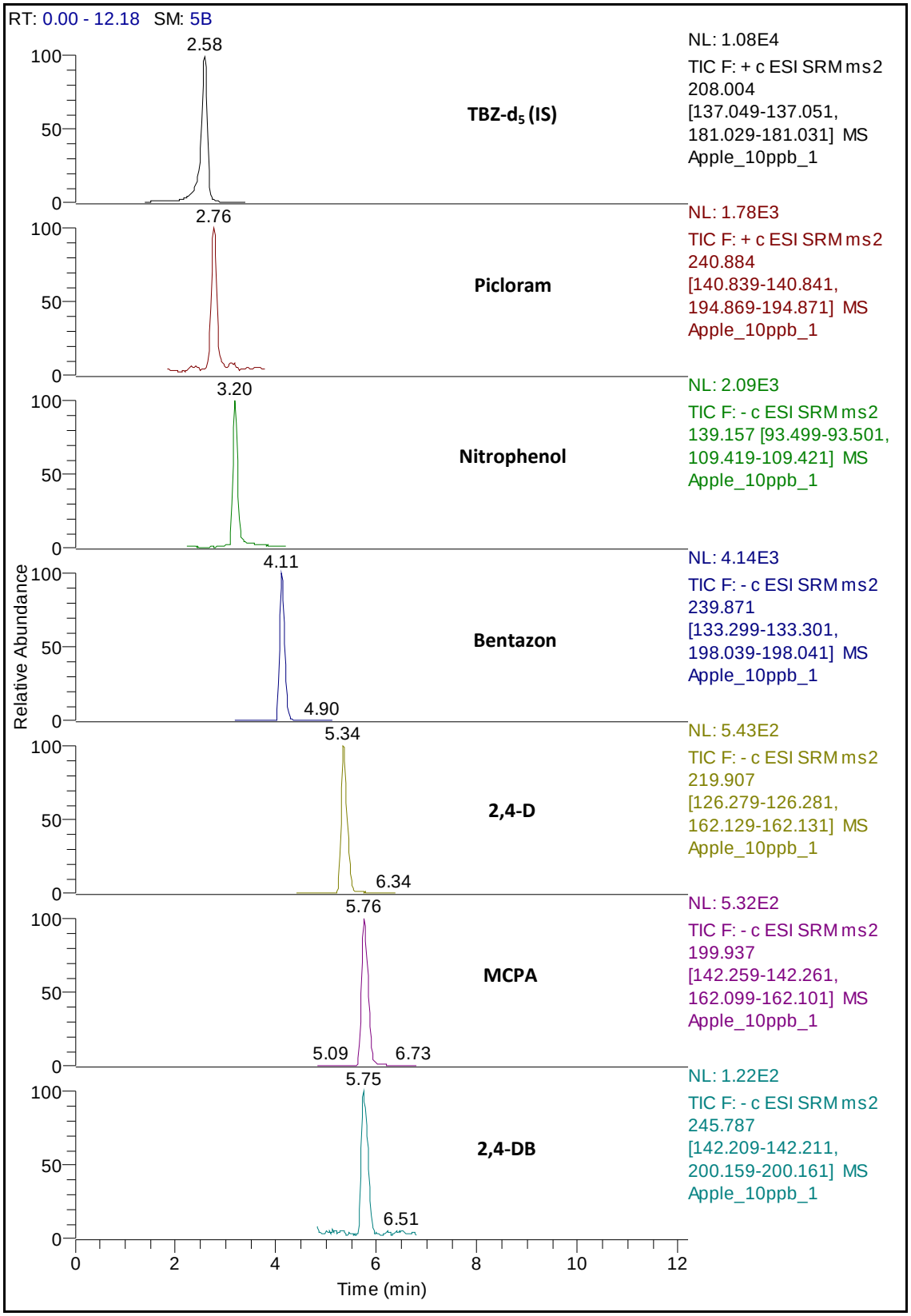
Instrumentation	Thermo Scientific™ TSQ Vantage™ tandem mass spectrometer
Ionization mode	ESI^+ & ESI^- with fast polarity switching
Spray voltage	5000V (ESI^+) and 4000V (ESI^-)
Vaporizer temperature	400 °C
Capillary temperature	250 °C
Sheath gas pressure	60 arbitrary units
Auxiliary gas pressure	45 arbitrary units
Ion sweep gas	0 arbitrary units
Declustering potential	0 V
Q1 and Q3 peak width	0.2 and 0.7 Da
Collision gas	argon
Collision gas pressure	1.1 mTorr
Acquisition method	EZ method (SRM)
Cycle time	1 sec
Software for data processing	TraceFinder™ version 3.0
Weighting factor applied to calibration curves	1/X

SRM Transitions							
Analyte	**t_R (min)**	**Precursor ion**	**Product ion 1**	**CE 1**	**Product ion 2**	**CE 2**	**S-lens (V)**
ESI^+							
TBZ-d_5 (IS)	2.58	208.0	181.0	25	137.0	31	43
Picloram	2.76	240.9	194.9	22	140.8	39	72
ESI^-							
Nitrophenol	3.17	139.2	109.4	18	93.5	24	68
Bentazon	4.11	239.9	133.3	27	198.0	21	86
2,4-D	5.34	219.9	162.1	17	126.3	30	50
2,4-DB	5.74	245.8	142.2	22	200.2	11	20
MCPA	5.74	199.9	142.3	17	162.1	20	50
Dichlorprop	7.56	233.8	162.1	17	126.3	31	50
2,4,5-T	7.95	253.7	196.0	16	160.1	28	53
Mecoprop	7.99	213.9	142.3	17	106.5	27	52
Dichlorobenzoic	8.68	189.9	146.2	13	36.8	20	54
Silvex	10.31	267.6	196.0	17	160.1	31	56
Acifluorfen	10.52	360.4	196.0	29	286.7	16	50
Dinoseb	11.13	239.8	195.1	23	135.3	47	85

Accuracy and precision data for the chlorophenoxyacetic acid herbicides at 2 concentrations.

Apple	10 ppb (n = 6)		100 ppb (n = 6)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Picloram	87.3	3.1	90.6	3.7
Nitrophenol	104.2	4.2	103.2	5.0
Bentazone	86.6	2.7	91.4	4.0
2,4-D	89.6	3.9	96.4	3.4
MCPA	90.8	3.7	100.8	5.2
2,4-DB	90.3	9.9	99.4	3.7
Dichlorprop	95.0	2.9	96.3	4.8
2,4,5-T	87.3	5.0	97.1	2.7
Mecoprop	84.0	1.4	94.7	0.7
Dichlorobenzoic acid	86.6	4.6	89.6	0.7
Silvex	101.6	6.9	102.7	6.3
Acifluorfen	88.5	3.2	94.8	3.0
Overall average	***91.0***	***4.3***	***96.4***	***3.6***

Tomato	10 ppb (n = 6)		100 ppb (n = 6)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Picloram	90.3	2.1	88.7	3.1
Nitrophenol	93.2	8.0	97.3	3.2
Bentazone	85.5	3.2	92.4	1.8
2,4-D	91.5	3.3	97.9	1.9
MCPA	94.3	4.8	105.2	3.5
2,4-DB	101.5	4.3	100.8	2.9
Dichlorprop	98.4	5.6	102.2	5.0
2,4,5-T	95.4	4.7	104.2	3.7
Mecoprop	88.2	1.6	97.1	2.2
Dichlorobenzoic acid	97.4	2.6	98.2	1.3
Silvex	101.8	8.5	111.0	6.7
Acifluorfen	101.2	7.3	99.8	2.9
Overall average	***94.9***	***4.7***	***99.6***	***3.2***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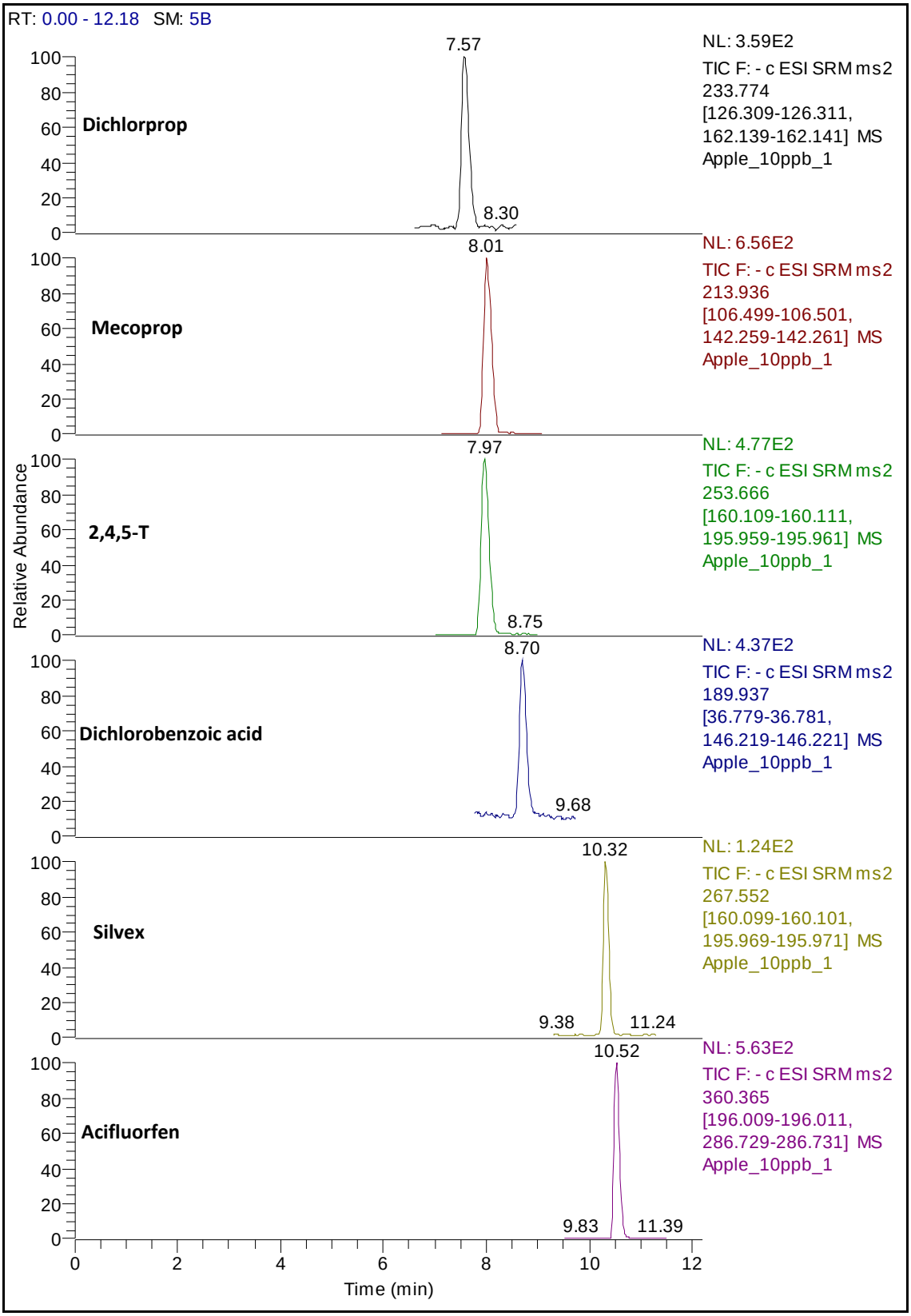
2.58
TBZ-d5 (IS)
NL: 1.08E4
TIC F: + c ESI SRM ms2 208.004 [137.049-137.051, 181.029-181.031] MS Apple_10ppb_1
2.76
Picloram
NL: 1.78E3
TIC F: + c ESI SRM ms2 240.884 [140.839-140.841, 194.869-194.871] MS Apple_10ppb_1
3.20
Nitrophenol
NL: 2.09E3
TIC F: - c ESI SRM ms2 139.157 [93.499-93.501, 109.419-109.421] MS Apple_10ppb_1
4.11
4.90
Bentazon
NL: 4.14E3
TIC F: - c ESI SRM ms2 239.871 [133.299-133.301, 198.039-198.041] MS Apple_10ppb_1
5.34
6.34
2,4-D
NL: 5.43E2
TIC F: - c ESI SRM ms2 219.907 [126.279-126.281, 162.129-162.131] MS Apple_10ppb_1
5.76
5.09
6.73
MCPA
NL: 5.32E2
TIC F: - c ESI SRM ms2 199.937 [142.259-142.261, 162.099-162.101] MS Apple_10ppb_1
5.75
6.51
2,4-DB
NL: 1.22E2
TIC F: - c ESI SRM ms2 245.787 [142.209-142.211, 200.159-200.161] MS Apple_10ppb_1
Relative Abundance
Time (min)



Chromatogram: Apple sample spiked with 10 ng/g chlorophenoxyacetic acid herbicides.

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

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

Introduction

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

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

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

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

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

QuEChERS procedure

Sample Extraction

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

Sample Cleanup

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

Analytical Procedure

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

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

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

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

Accuracy & Precision Data (cont)				
Analyte	**20 ng/g (n=6)**		**100 ng/g (n=6)**	
	Mean (%)	**RSD (%)**	**Mean (%)**	**RSD (%)**
Malathion	52.3	9.29	78.1	1.78
Profenofos	79.5	8.76	88.6	2.75
Pyrazophos	80.5	8.01	93.9	2.63
Pyrimethanil	90.2	4.88	92.2	2.36
Simazine	92.4	7.74	98.9	2.77
Tebuconazole	88.5	6.69	93.1	3.08
Tebuthiuron	100.7	7.39	101.1	2.14
Thiabendazole	52.8	5.61	63.1	6.80
Zoxamide	92.4	7.92	99.4	2.11

Note: TPP was used as an internal standard. Matrix-matched calibration curves were used for quantification.

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

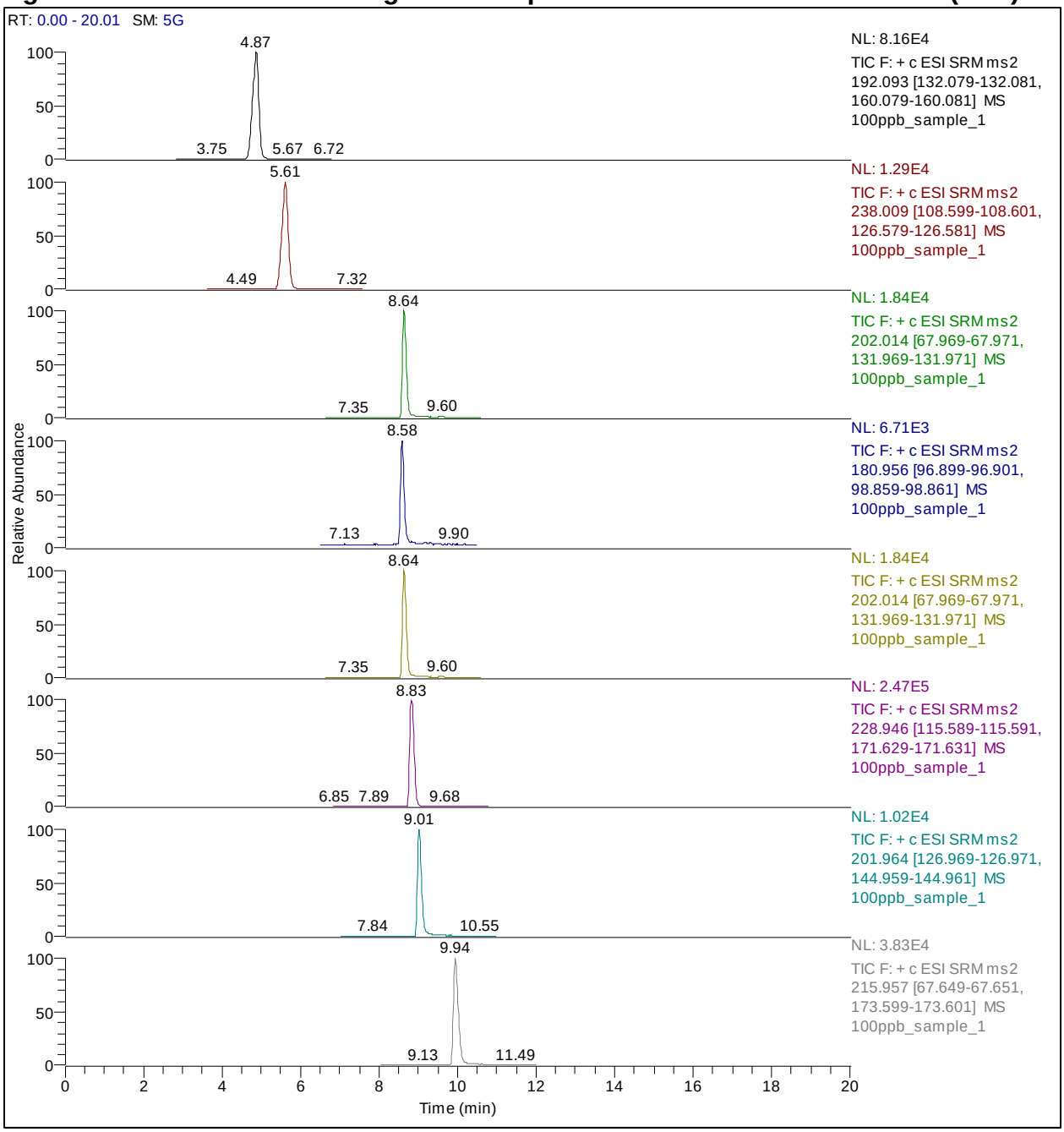


Figure 1 continued.

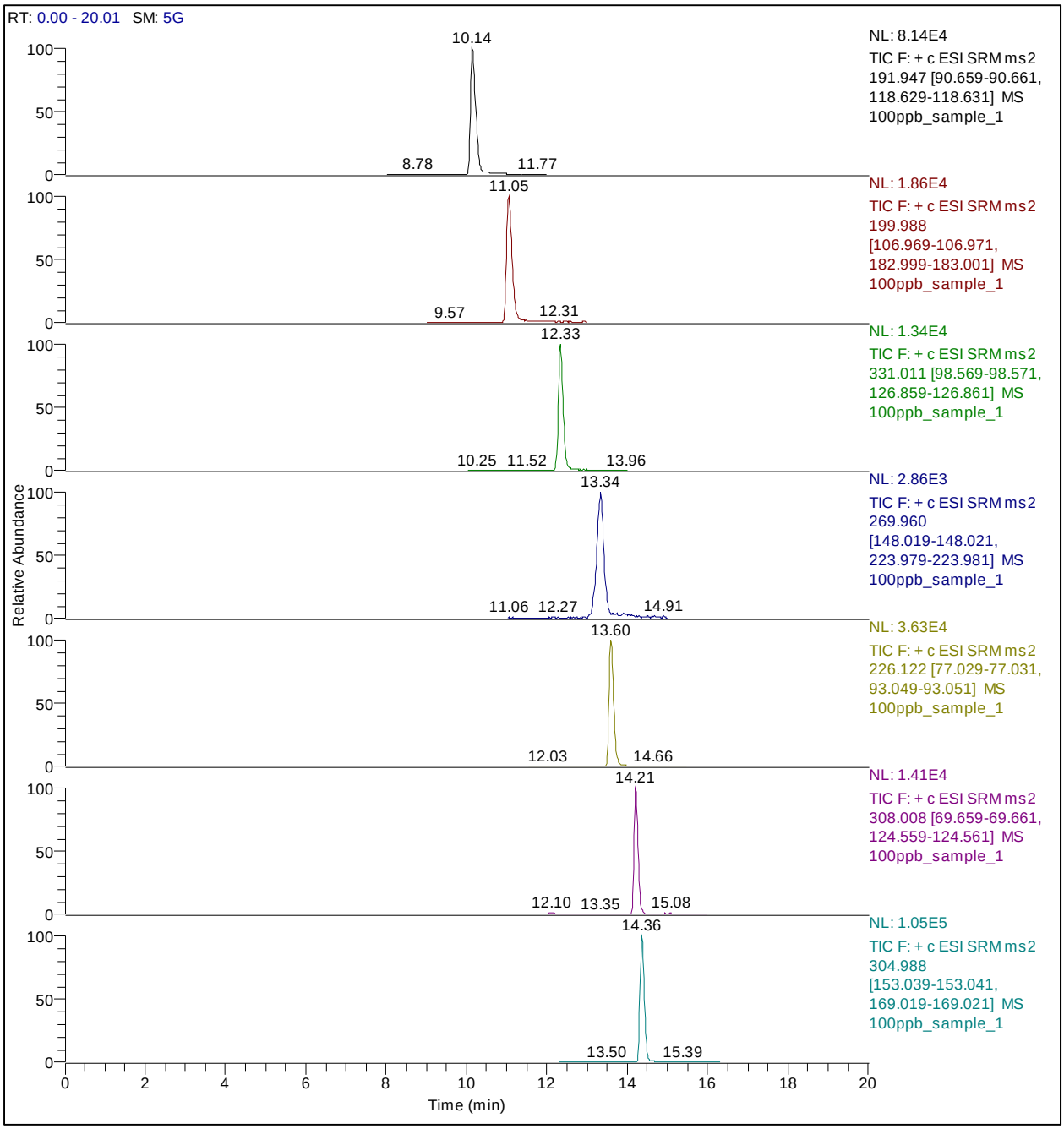
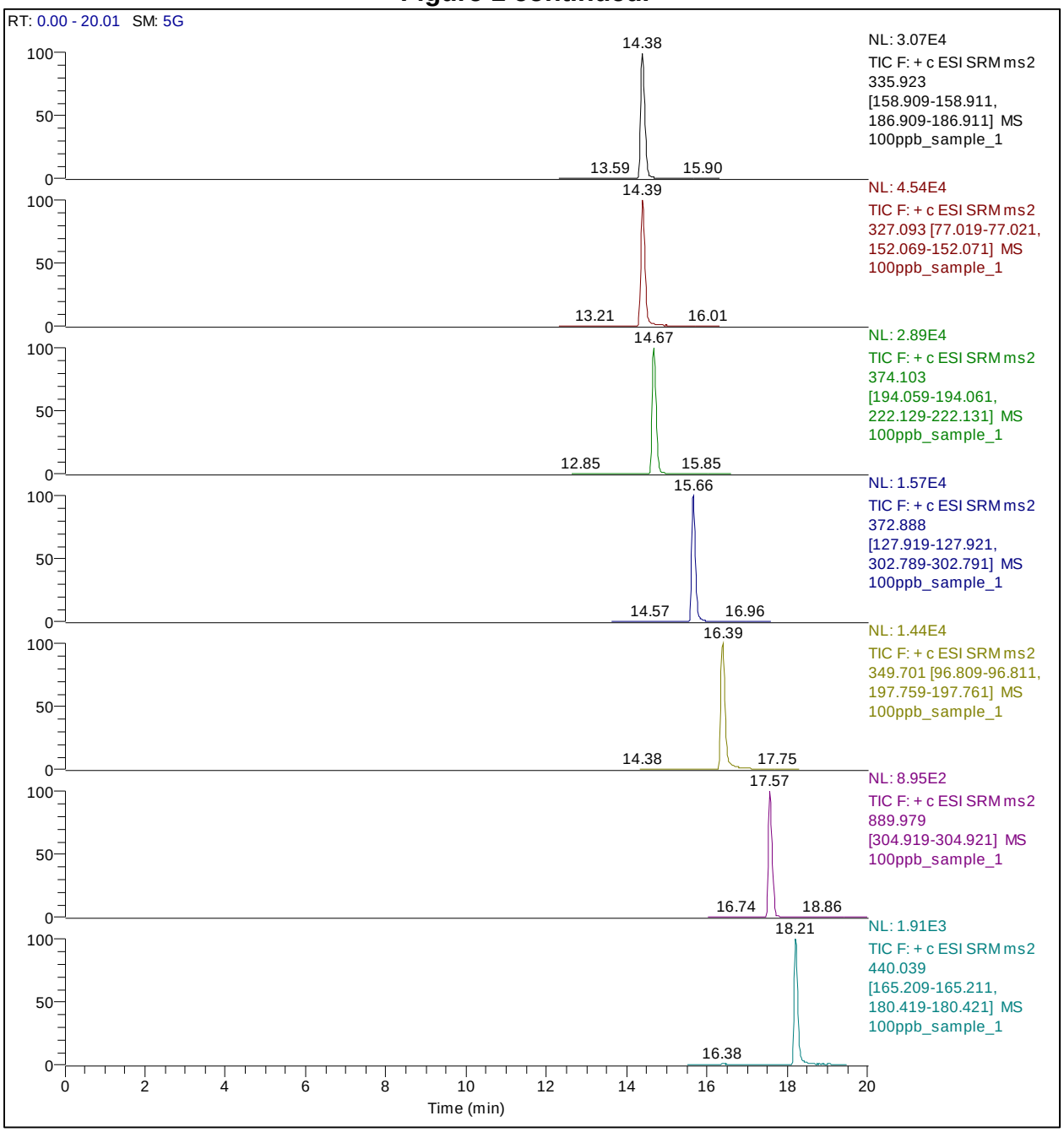


Figure 1 continued.



Results & Discussion

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

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

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

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

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Determination of Pesticide Residues in Tea: An AOAC Collaborative Study

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

Summary:

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

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

Procedure:

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

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

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

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

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

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

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

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

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

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

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

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

GS/MS method:

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

Retention times, quantifying and qualifying ions with ion ratios

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

Results:

Linearity parameters of Green and Oolong tea

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

Recovery and RSDs obtained from the spiked Green tea

Pesticide	Spiked (µg/kg)	Rec% 1	Rec% 2	Rec% 3	Rec% 4	Rec% 5	Ave	RSD% (n=5)
Trifluralin	200	91.5	91.5	88.5	91.5	88.5	**90.3**	1.8
Tefluthrin	100	93.1	93.1	93.1	93.1	93.1	**93.1**	0.0
Pyrimethanil	100	90.1	90.1	87.1	90.1	90.1	**89.5**	1.5
Propyzamide	100	99.1	99.1	99.1	99.1	99.1	**99.1**	0.0
Pirimicarb	100	114.1	105.1	120.1	120.1	120.1	**116**	5.7
Fenchlorphos	200	93.0	94.5	91.5	93.0	91.5	**92.7**	1.4
Dimethenamid	40	97.7	97.7	90.2	97.7	90.2	**94.7**	4.3
Tolclofos-methyl	100	93.1	93.1	90.1	93.1	93.1	**92.5**	1.5
Pirimiphos-methyl	100	93.1	96.1	90.1	93.1	93.1	**93.1**	2.3

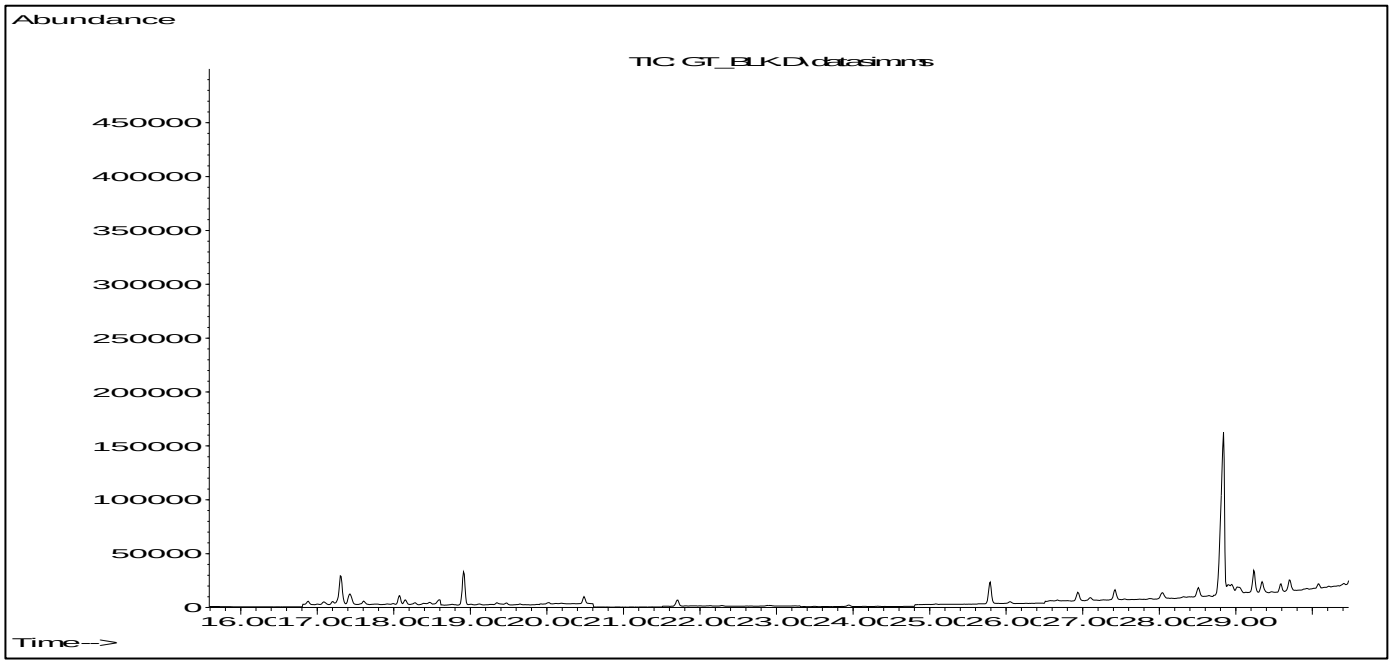
2,4'-DDE	400	93.0	94.5	90.8	93.0	91.5	**92.6**	1.6
Bromophos-ethyl	100	93.1	96.1	93.1	93.1	93.1	**93.7**	1.4
4,4'-DDE	400	93.0	94.5	92.3	93.0	92.3	**93.0**	1.0
Procymidone	100	96.1	96.1	93.1	96.1	93.1	**94.9**	1.7
Picoxystrobin	200	94.5	96.0	93.0	94.5	94.5	**94.5**	1.1
Quinoxyfen	100	90.1	90.1	90.1	93.1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

Recovery and RSDs obtained from the spiked Oolong tea

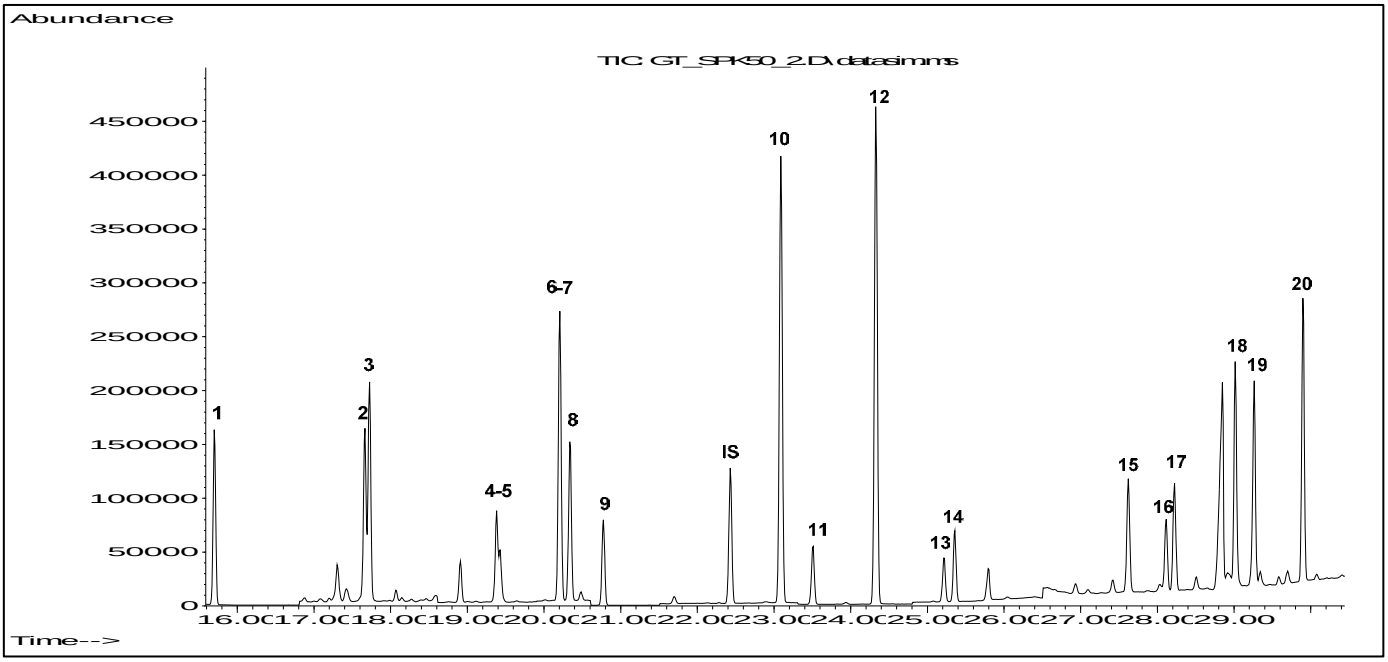
Pesticide	**Spiked** (μg/kg)	**Rec% 1**	**Rec% 2**	**Rec% 3**	**Rec% 4**	**Rec% 5**	**Ave**	**RSD%** (n=5)
Trifluralin	200	84.0	91.5	87.0	85.5	87.0	**87.0**	3.2
Tefluthrin	100	81.1	87.1	87.1	84.1	81.1	**84.1**	3.6
Pyrimethanil	100	78.1	81.1	81.1	78.1	78.1	**79.3**	2.1
Propyzamide	100	81.1	87.1	84.1	84.1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

Chromatograms:

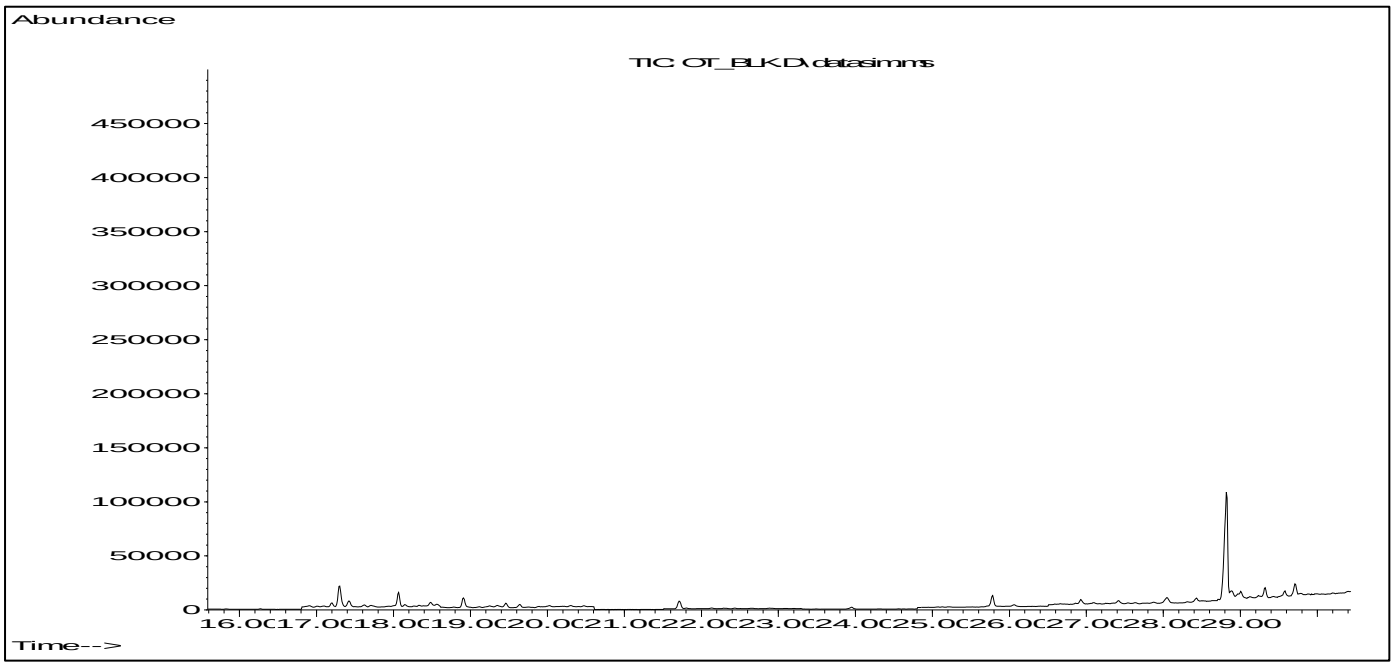
(a) Chromatogram of blank Green tea



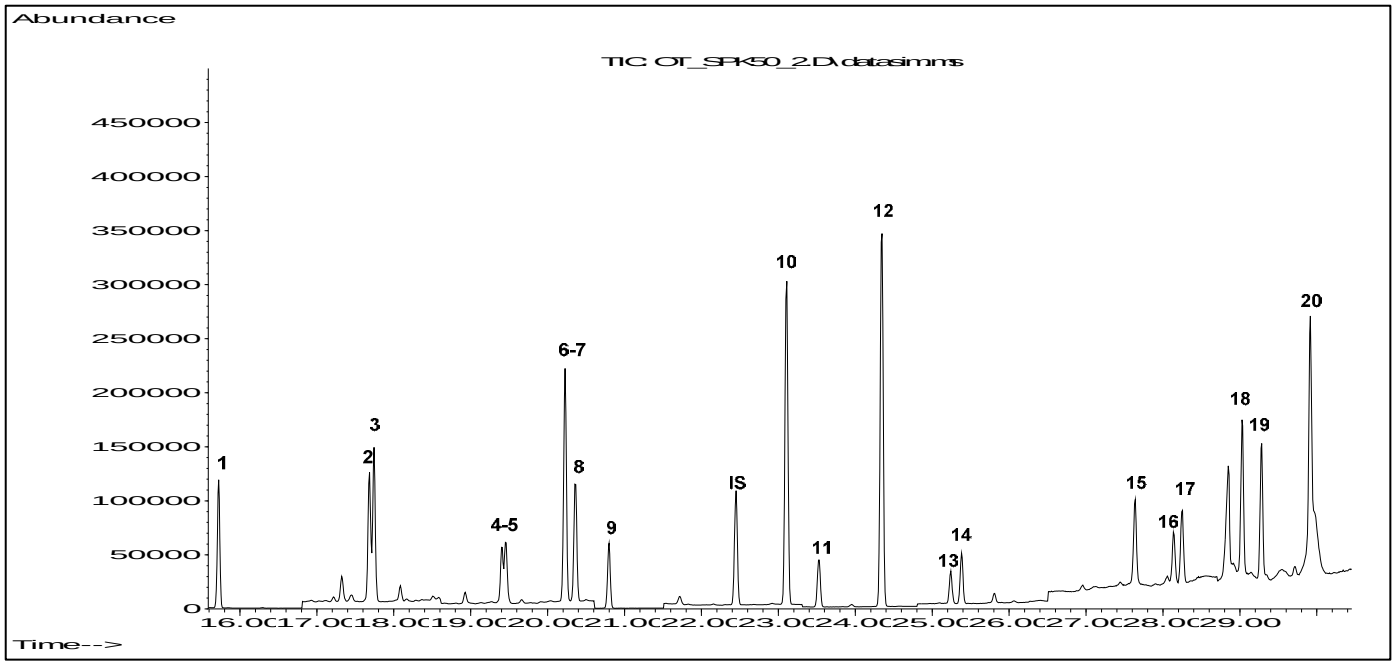
(b) Chromatogram of spiked Green tea



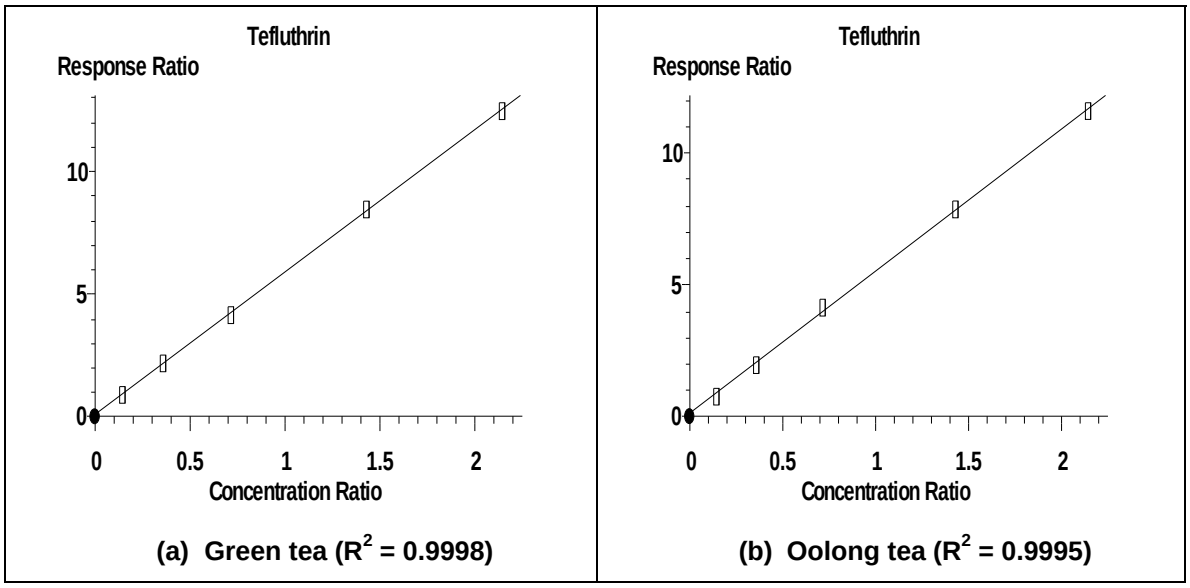
(c) Chromatogram of blank Oolong tea



(d) Chromatogram of spiked Oolong tea



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



Example calibration curves of Tefluthrin in Green and Oolong teas

References:

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

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

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

Summary:

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

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

Procedure:

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

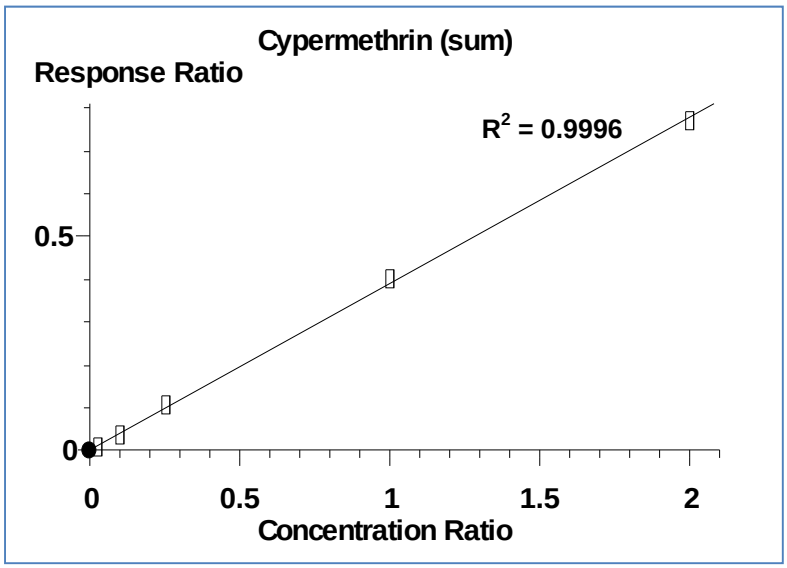
QuEChERS extraction

Silica gel SPE cleanup

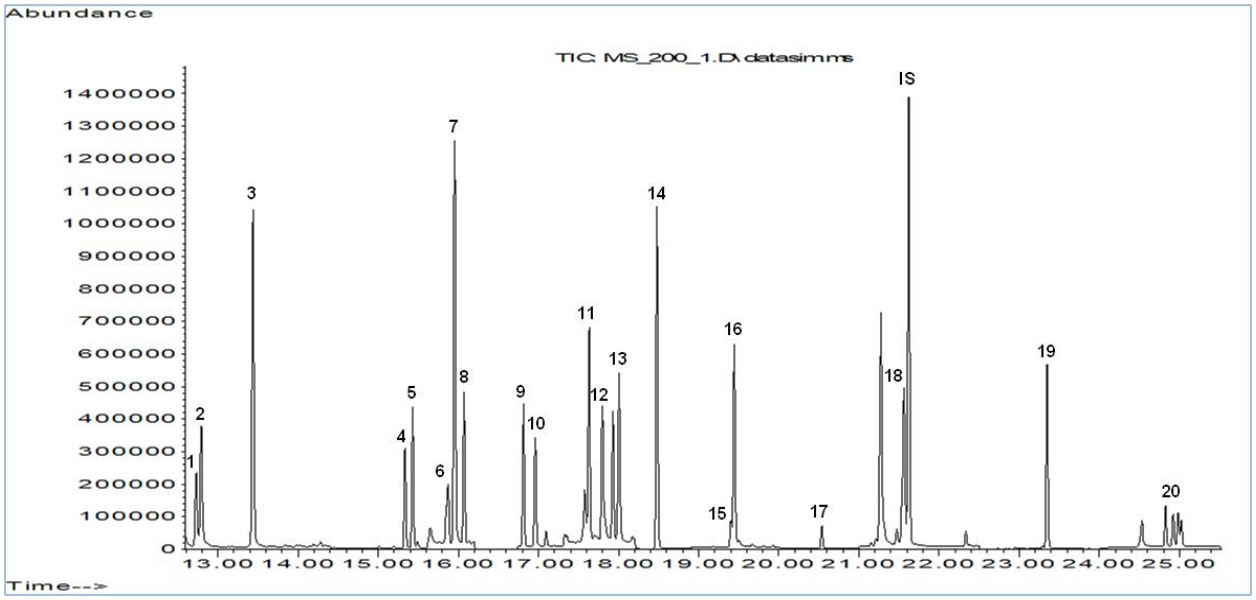
GC-MS Conditions	
Instrumentation	Agilent 6890N GC coupled to a 5975C MSD
Column	Restek Rxi®-5Sil MS (30m × 0.25mm × 0.25µm)
Carrier gas	Helium (1.2 mL/min)
GC inlet temp.	250°C
Injection volume	1 µL (splitless)
Temp gradient	60°C for 1 min, 10°C/min to 310°C, hold for 2 min; 28 min total
Transfer line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

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

Results:



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



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

Recovery and RSD% from Spiked Coffee Samples

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

References:

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

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Analysis of Neonicotinoids in Honey by QuEChERS and UHPLC-MS/MS

UCT part numbers:
ECQUEU7-MP – Mylar pouch containing 4 g $MgSO_4$, 1 g NaCl, 0.5 g sodium citrate dibasic sesquihydrate and 1 g sodium citrate tribasic dehydrate
CUMPSC18CT – 150 mg $MgSO_4$, 50 mg PSA and 50 mg endcapped C18; 2 mL dSPE tube
SLDA50ID21-18UM – Selectra® DA, 50 × 2.1 mm, 1.8 µm UHPLC column
SLDAGDC20-18UM – Selectra® DA, 10 × 2.0 mm, 1.8 µm guard cartridge
SLGRDHLDR – Guard cartridge holder

INTRODUCTION:

Neonicotinoids are a relatively new class of insecticide that were introduced as an alternative to organophosphate, carbamate and pyrethroid insecticides. Their novel mode of action works by irreversibly binding to nicotinic acetylcholine receptors, resulting in paralysis and death of insects. Since their introduction in the 1990s the neonicotinoids have been used extensively in crop protection. However, they have recently come under increasing scrutiny over their environmental and ecological impact, especially their role in bee deaths and colony collapse disorder (CCD)[1]. It has been reported that neonicotinoid residues can accumulate in the pollen and nectar of treated plants and poses a potential risk to honey bees[2]. In addition, neonicotinoid residues can be transferred to products derived from bees, including the popular food source honey[3]. Due to their potential negative impact, the European Union recently restricted the use of three neonicotinoids (clothianidin, thiamethoxam, and imidacloprid) for a period of 2 years[4].

This application note outlines a simple, fast and cost-effective method for the determination of 7 neonicotinoid pesticides in honey. Honey is dissolved in water and extracted using a citrate-buffered QuEChERS procedure. The sample extract then undergoes cleanup by dispersive-SPE with PSA/C18 sorbent to remove unwanted waxes, pigments and carbohydrates that are present. Analysis is performed by UHPLC/MS-MS using a Selectra® DA UHPLC column. Recovery studies were carried out by spiking raw and processed honey at two concentration levels (10 and 50 ng/g). Matrix-matched calibration curves, ranging from 1-250 ng/g, were used for quantitation. The mean recovery was found to be in the range of 82 to 113%, while repeatability was less than 10%.

PROCEDURE:

Sample extraction

1. Weigh 10 g of honey sample into a 50 mL polypropylene centrifuge tube.
2. Add internal standard (*optional*).
3. Add 10 mL of deionized water and shake/vortex until the honey is dissolved.
4. Add 10 mL of acetonitrile.
5. Add the contents of the **ECQUEU7-MP** Mylar pouch and shake either by hand or mechanically for at least 1 min. For this study a SPEX® SamplePrep® 2010 Geno/Grinder® was used.
6. Centrifuge the samples at greater than 3000×g for 5 min.

Sample clean-up

1. Transfer 1ml of supernatant into a **CUMPSC18CT** dSPE tube.
2. Vortex the samples for 30 sec.
3. Centrifuge the samples at greater than 3000×g for 2 min.
4. Transfer 500-600 µL of purified supernatant into an autosampler vial.

INSTRUMENTAL:

HPLC Conditions	
Instrumentation	Thermo Scientific™ Dionex™ Ultimate™ 3000
HPLC column	UCT Selectra® DA, 50 × 2.1 mm, 1.8 µm (p/n:
Guard column	UCT Selectra® DA, 10 × 2.0 mm, 1.8 µm (p/n:
Guard column	p/n: SLGRDHLDR
Column temp.	40°C
Flow rate	300 µL/min
Injection volume	2 µL
Autosampler	10°C
Wash solvent	methanol: water (1:1, v/v)

Time (min)	Mobile phase A water + 0.1% formic acid	Mobile phase B methanol + 0.1% formic acid
0	95%	5%
1	0%	100%
4.5	0%	100%
4.6	95%	5%
7.5	95%	5%

MS Conditions	
Instrumentation	Thermo Scientific™ TSQ
Ionization mode	ESI^+
Spray voltage	5000 V
Vaporizer	400°C
Capillary	350°C
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	5 arbitrary units
Ion sweep gas	0 arbitrary units
Declustering	0 V
Q1 and Q3 peak	0.2 and 0.7 Da
Collision gas	argon
Collision gas pressure	1.5 mTorr
Acquisition method	EZ method (scheduled SRM)
Cycle time	0.6 sec
Software	Xcalibur™ version 2.2

SRM Transitions							
Analyte	**t_R (min)**	**Precursor ion**	**Product ion 1**	**CE 1**	**Product ion 2**	**CE 2**	**S-lens (V)**
Dinotefuran	2.78	203.08	114.09	12	100.08	15	50
Nitenpyram	2.82	271.03	196.01	15	99.04	15	68
Clothianidin	3.07	249.97	169.01	10	131.94	15	56
Clothianidin-D_3	3.07	253.01	172.06	11	131.95	16	64
Thiamethoxam	3.14	291.97	211.02	10	181.01	18	59
Imidacloprid	3.33	256.02	209.04	16	175.08	16	69
Acetamiprid	3.45	223.01	125.95	20	89.98	33	68
Thiacloprid	3.62	252.99	125.99	19	90.02	32	83

RESULTS:

Accuracy & Precision Data for Processed Honey				
	10 ng/g (n=5)		50 ng/g (n=5)	
Analyte	Mean Recovery (%)	RSD (%)	Mean Recovery (%)	RSD (%)
Dinotefuran	106.3	2.6	113.4	3.6
Nitenpyram	92.3	2.4	99.6	2.6
Clothianidin	105.0	2.0	113.4	3.8
Thiamethoxam	107.5	1.2	110.1	4.3
Imidacloprid	102.0	2.5	109.7	5.4
Acetamiprid	103.4	3.0	113.6	4.6
Thiacloprid	105.8	1.4	112.9	4.8

Accuracy & Precision Data for Raw Honey				
Analyte	10 ng/g (n=5)		50 ng/g (n=5)	
	Mean Recovery (%)	RSD (%)	Mean Recovery (%)	RSD (%)
Dinotefuran	100.1	5.4	93.6	2.3
Nitenpyram	91.9	5.3	95.9	4.6
Clothianidin	87.5	4.6	82.2	2.6
Thiamethoxam	87.7	5.7	85.8	4.7
Imidacloprid	101.4	4.3	98.4	3.3
Acetamiprid	87.1	8.3	91.9	7.4
Thiacloprid	87.3	1.8	89.2	9.9

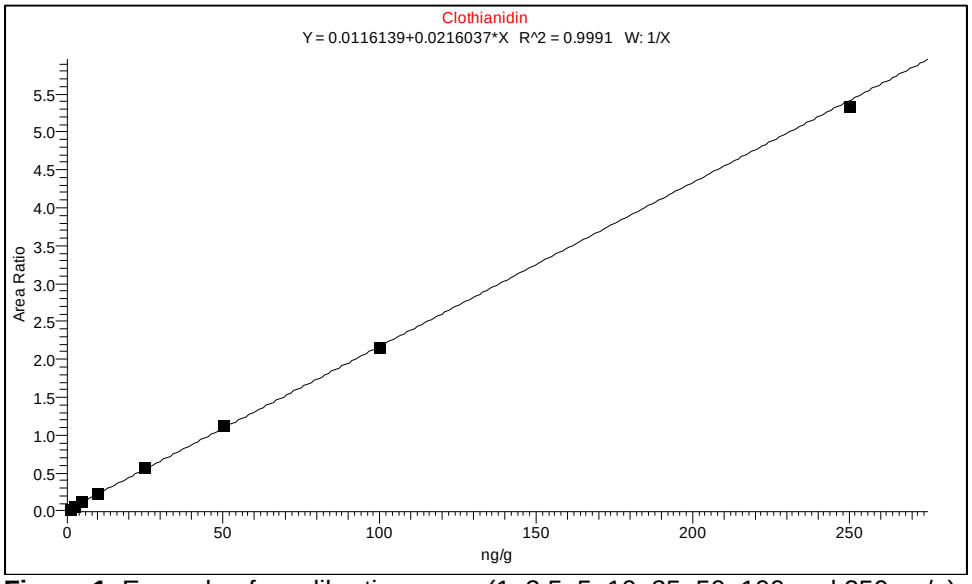

Figure 1. Example of a calibration curve (1, 2.5, 5, 10, 25, 50, 100 and 250 ng/g).

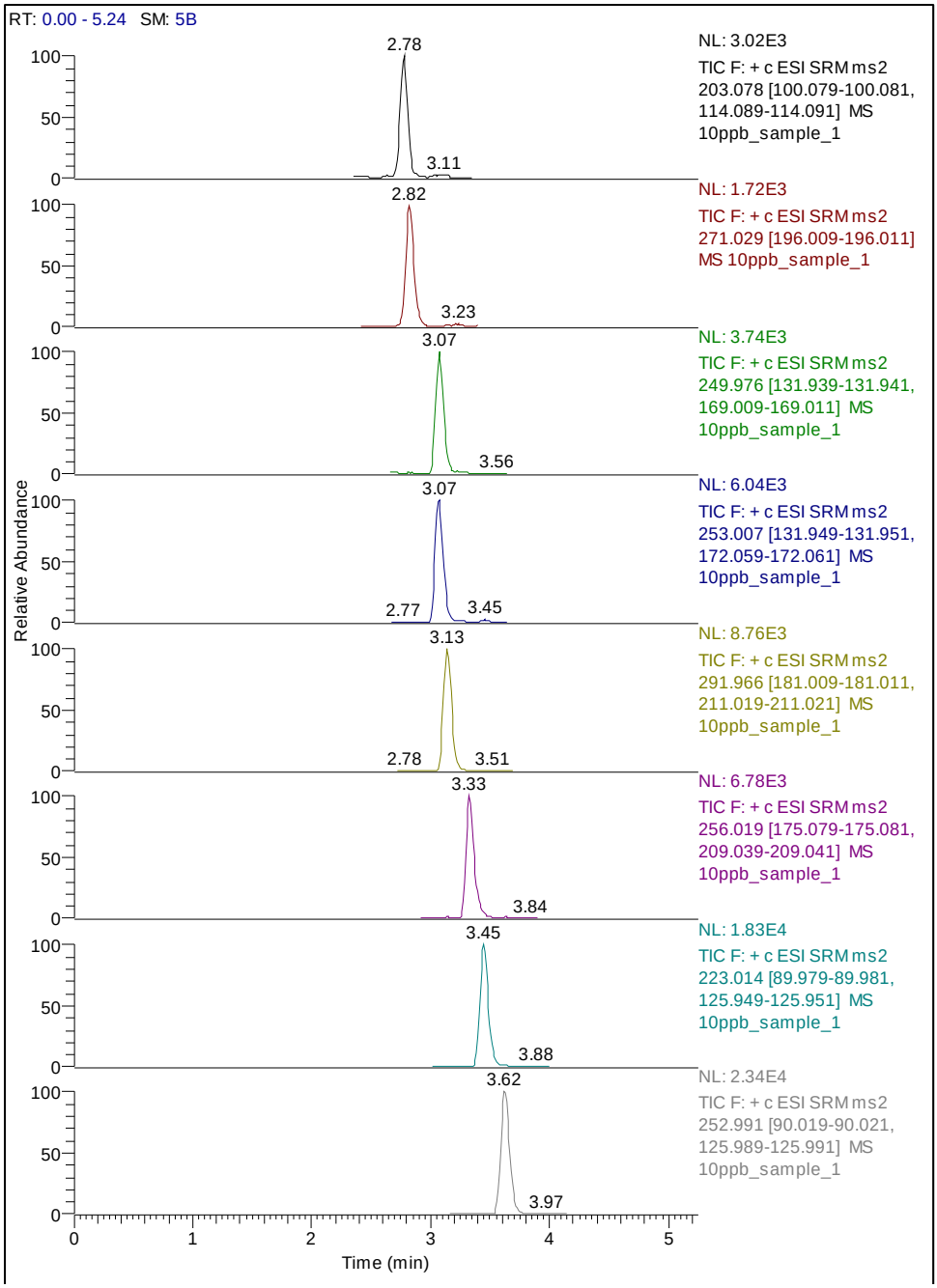

Figure 2. Chromatogram of an extracted raw honey sample fortified at 10 ng/g.

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[1] C. Lu, K. M. Warchol, R. A. Callahan, Bulletin of Insectology, 67,125-130, 2014.
[2] T. Iwasa, N. Motoyama, J. T. Ambrose, R. M. Roe, Crop Protection, 23, 371–378, 2004.
[3] M. P. Galeano, M.Scordino, L. Sabatino, *et al.,* International Journal of Food Science, vol. 2013, Article ID 863904, 7 pages, 2013.
[4] Commission Regulation (EU) No 485/2013, Official Journal of the European Union, L 139, 12-26, 2013.

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Determination of Pesticides in Bananas by QuEChERS and LC-MS/MS

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

Summary:

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

Matrix matched calibration curves were constructed for pesticide quantification. The responses for all 24 pesticides were linear with R^2 ranging from 0.9939 to 0.9998 over the concentration range of 2 to 400 ng/g. Recoveries were excellent with an average recovery of 97% and an average relative standard deviation of about 6.25% using the data from spiked matrix samples with concentrations of 10ng/g and 50ng/g. The results from this application indicate this pesticide method is suitable in bananas especially when pymetrozine is required to be analyzed.

Procedure:

1. QuEChERS extraction

a) Weigh 15 ± 0.15 g of peeled and homogenized banana sample into a 50-mL centrifuge tube (**RFV0050CT**). Prepare 5 fortified samples, each at two spiking levels.

b) Add 5 mL of reagent water to each tube (to increase the water content in banana from 74% to > 80%).

c) Add 30 µL of 50-ppm triphenyl phosphate (TPP) internal standard (IS) solution to all samples, and appropriate amounts of 2-ppm pesticide working solution to fortified samples.

d) Add 15 mL of MeCN with 1%(v/v) HAc. Cap and shake for 1 min at 1000 strokes/min using a Spex 2010 Geno-Grinder.

e) Add salts (6 g $MgSO_4$ and 1.5 g NaOAc) from pouch (**ECMSSA50CT-MP**), and vortex for 10 sec to break up any salt agglomerates.

f) Shake for 1 min at 1000 strokes/min using Spex 2010 Geno-Grinder.

g) Centrifuge at 5000 rpm (or 3830 rcf) for 5 min.

2. dSPE cleanup

g) Transfer 1 mL of the supernatant to 2 mL dSPE tube (**CUMPSC18CT**).

h) Shake for 2 min at 1000 strokes/min using Spex 2010 Geno-Grinder.

i) Centrifuge at 10,000 rpm (or 15,300 rcf) for 5 min.

j) Transfer 0.3 mL of the cleaned extract into a 2-mL auto-sampler vial, add 0.3 mL of reagent water, and vortex for 30 sec.

k) The samples are ready for LC-MS/MS analysis.

LC-MS/MS method:

HPLC: Thermo Scientific Dionex UltiMate 3000® LC System
Column: Thermo Scientific, Accucore aQ®, 100 x 2.1 mm, 2.6 µm
Guard Column: Thermo Scientific, Accucore aQ®, 10 x 2.1 mm, 2.6 µm
Column Temperature: 40 °C
Column Flow Rate: 0.200 mL/min
Auto-sampler Temperature: 10 °C
Injection Volume: 10 µL
Gradient Program: **Mobile Phase A:** 0.3 % formic acid and 0.1 % ammonia formate in water **Mobile Phase B:** 0.1 % formic acid in MeOH

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	99	1
1.5	99	1
3.5	20	80
10	10	90
12	0	100
15	0	100
15.2	99	1
20	99	1

Divert mobile phase to waste from 0 - 0.5 and 15 - 20 min to prevent ion source contamination.

MS parameters	
Polarity	ESI +
Spray voltage V	4000 V
Vaporizer Temperature	300 °C
Ion transfer capillary	200 °C
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	25 arbitrary units
Q1 and Q3 peak width	0.2 and 0.7 Da
Collision gas and pressure	Ar at 1.5 mTorr
Scan type	SRM
Cycle time	1 sec
Acquisition method	EZ Method

SRM transitions

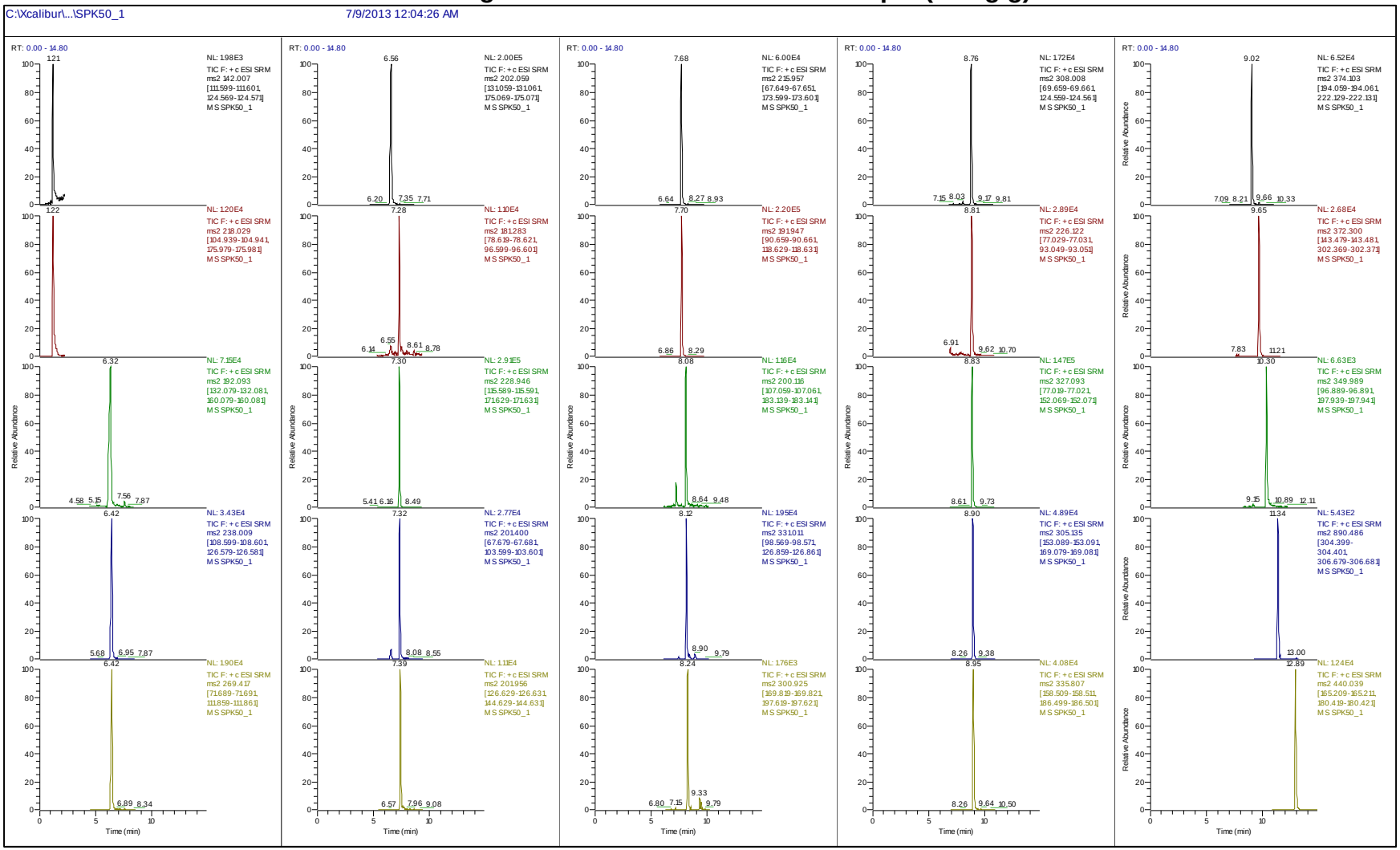
Name	Rt (min)	Precursor ion	Product ion 1	CE 1	Product ion 2	CE 2	S-lens (V)
Methamidophos	1.21	142.007	124.57	14	111.6	5	60
Pymetrozine	1.22	218.029	104.94	18	175.98	16	70
Carbendazim	6.32	192.093	160.08	17	132.08	29	81
Dicrotophos	6.42	238.009	126.58	17	108.60	33	73
Acetachlor	6.42	269.417	111.86	15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

Results:

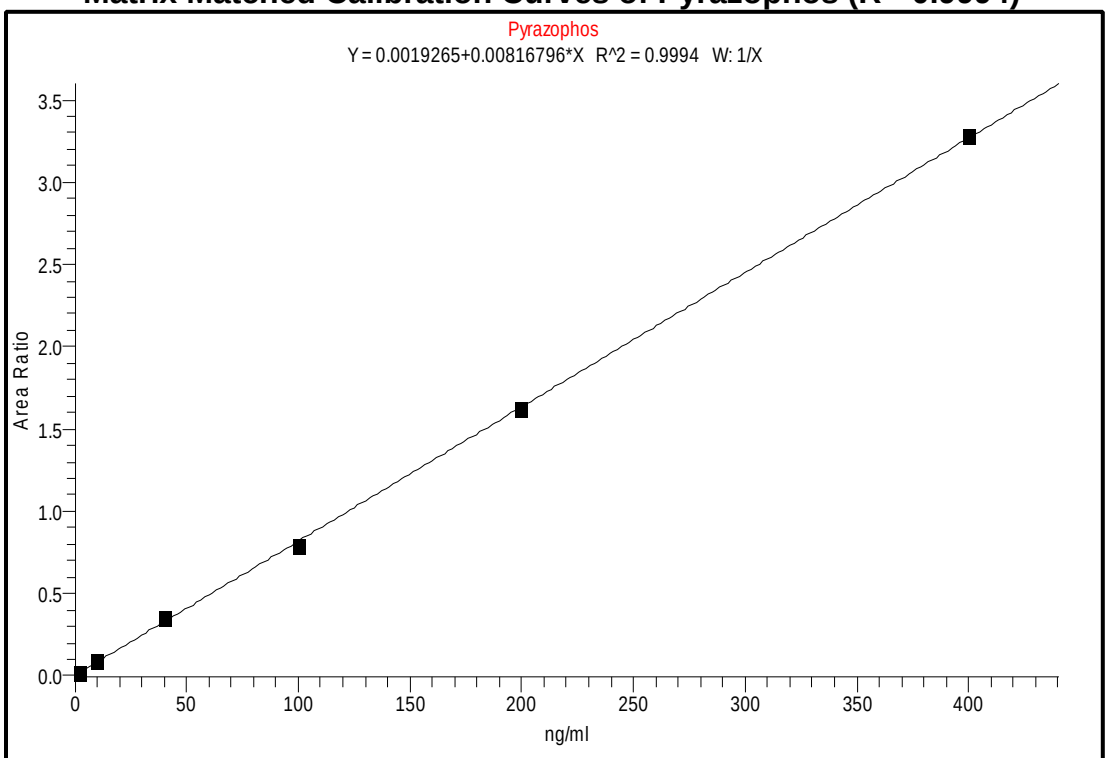
Recovery and RSD% Data Obtained from Fortified Banana Samples

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

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



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



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

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

Summary:

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

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

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

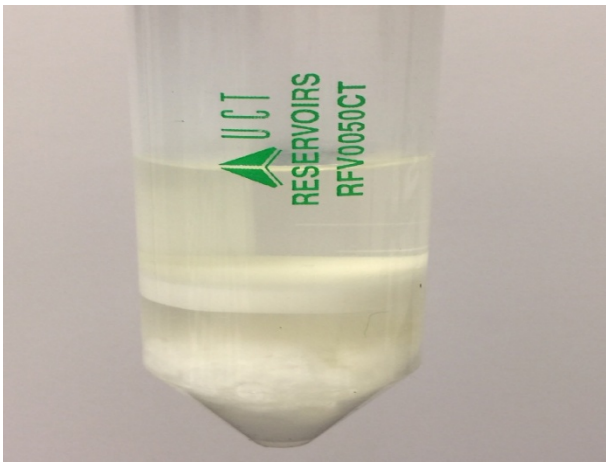
Procedure:

1. QuEChERS extraction

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

2. dSPE cleanup

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



Whole Milk Samples Extracted by the AOAC QuEChERS Procedure

LC-MS/MS method:

HPLC: Thermo Scientific Dionex UltiMate 3000® LC System
Column: Thermo Scientific, Accucore aQ®, 100 x 2.1 mm, 2.6 µm
Guard Column: Thermo Scientific, Accucore aQ®, 10 x 2.1 mm, 2.6 µm
Column Temperature: 40 °C
Column Flow Rate: 0.200 mL/min
Auto-sampler Temperature: 10 °C
Injection Volume: 10 µL
Gradient Program: **Mobile Phase A:** 0.3 % formic acid and 0.1 % ammonia formate in water **Mobile Phase B:** 0.1 % formic acid in MeOH

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	99	1
1.5	99	1
3.5	20	80
10	10	90
12	0	100
15	0	100
15.2	99	1
20	99	1

Divert mobile phase to waste from 0 - 0.5 and 15 - 20 min to prevent ion source contamination.

MS parameters	
Polarity	ESI +
Spray voltage V	4000 V
Vaporizer Temperature	300 °C
Ion transfer capillary temperature	200 °C
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	25 arbitrary units
Q1 and Q3 peak width (FWHM)	0.2 and 0.7 Da
Collision gas and pressure	Ar at 1.5 mTorr
Scan type	SRM
Cycle time	1 sec
Acquisition method	EZ Method

SRM transitions

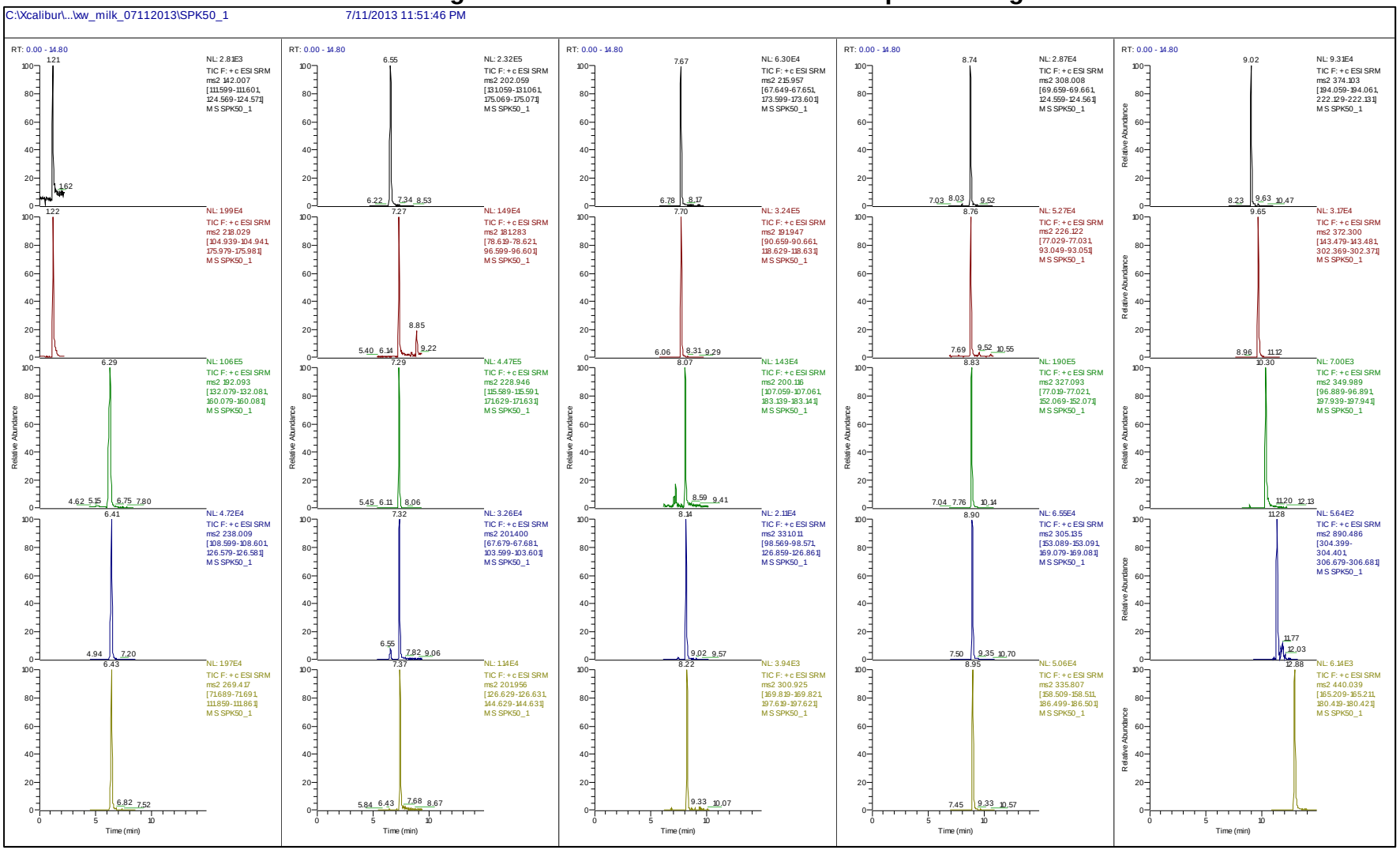
Name	Rt (min)	Precursor ion	Product ion 1	CE 1	Product ion 2	CE 2	S-lens (V)
Methamidophos	1.21	142.007	124.57	14	111.6	5	60
Pymetrozine	1.22	218.029	104.94	18	175.98	16	70
Carbendazim	6.29	192.093	160.08	17	132.08	29	81
Dicrotophos	6.41	238.009	126.58	17	108.60	33	73
Acetachlor	6.43	269.417	111.86	15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

Results:

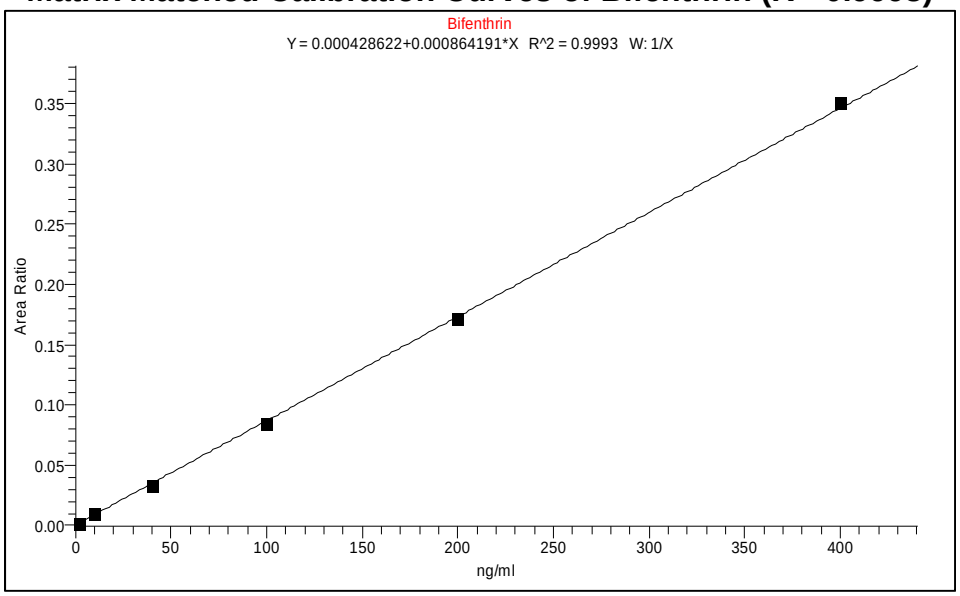
Recovery and RSD% Obtained from 5 Replicated Fortified Milk Samples

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

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



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



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

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

Summary:

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

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

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

Procedure:

2. QuEChERS extraction

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

3. dSPE cleanup

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

LC-MS/MS Method:

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

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

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

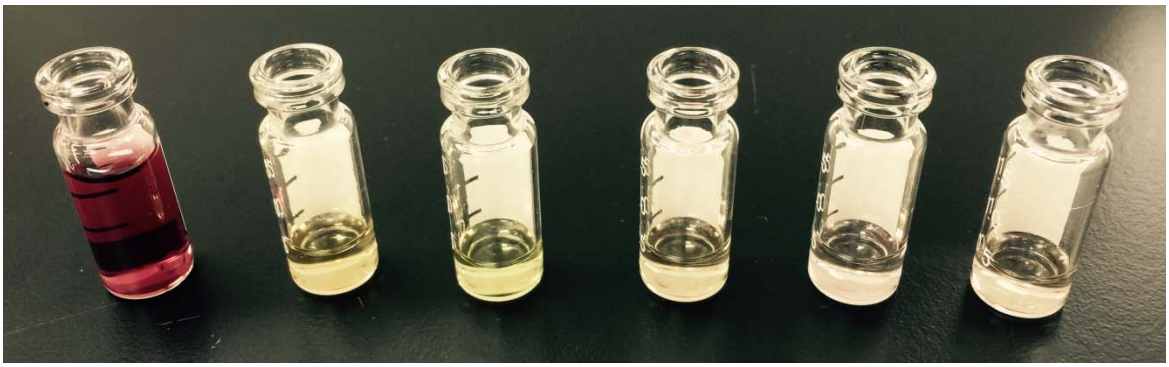
SRM Table						
Compound	**Precursor**	**Product 1**	**CE1**	**Product 2**	**CE2**	**S-lens RF**
Metamidophos	142.0	94.1	14	125.0	13	50
Acephate	184.0	143.0	6	95.0	25	33
Aldicarb sulfoxide	207.1	89.1	13	69.1	16	32
Oxydemeton methyl	247.0	169.0	13	109.0	27	57
Pymetrozine	218.1	105.1	20	176.1	17	63
Dichrotophos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

Results:

Selection of dSPE cleanup sorbents:

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

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



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

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

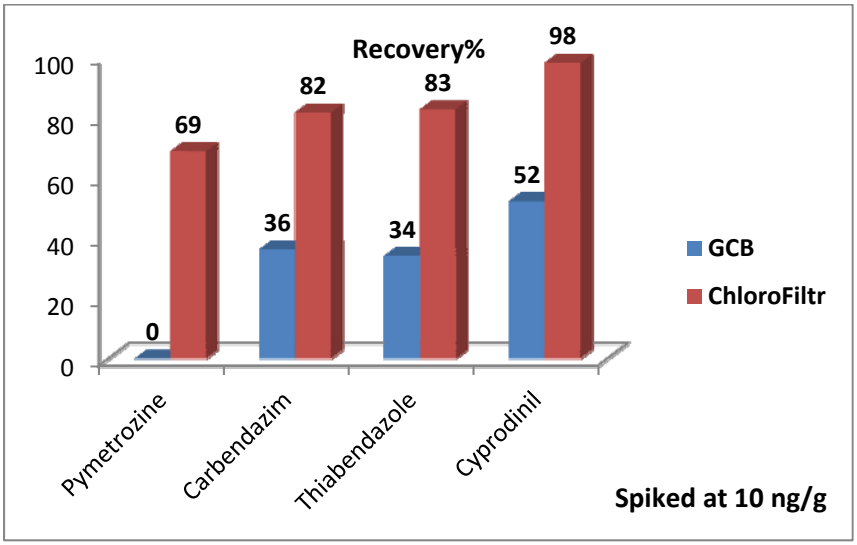
Recovery study:

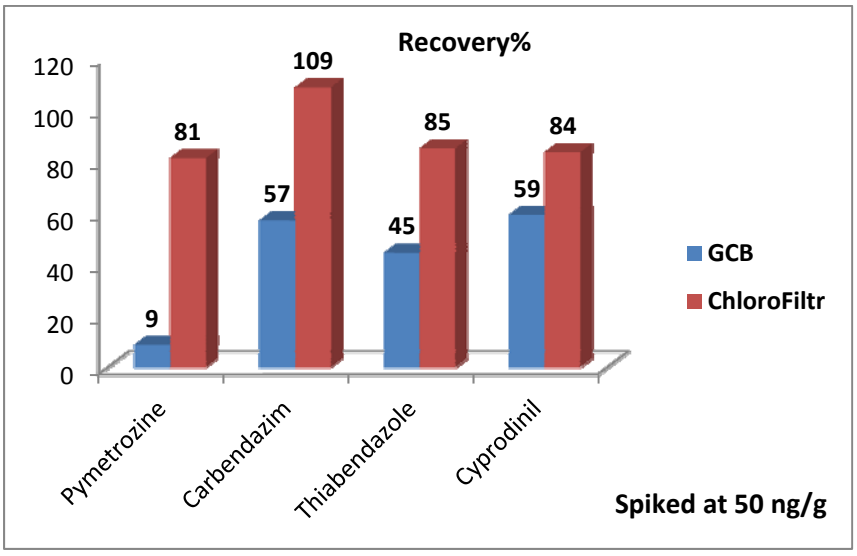
Blueberry samples were fortified with 10 ng/g and 50 ng/g of pesticides, and underwent the AOAC QuEChERS extraction and dSPE cleanup with 150 mg MgSO4, 50 mg PSA, 50 mg C18, and 7.5 mg GCB (D) or 50 mg ChloroFiltr® (E) as described above. The mean recoveries and RSD% of 6 replicated samples are listed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

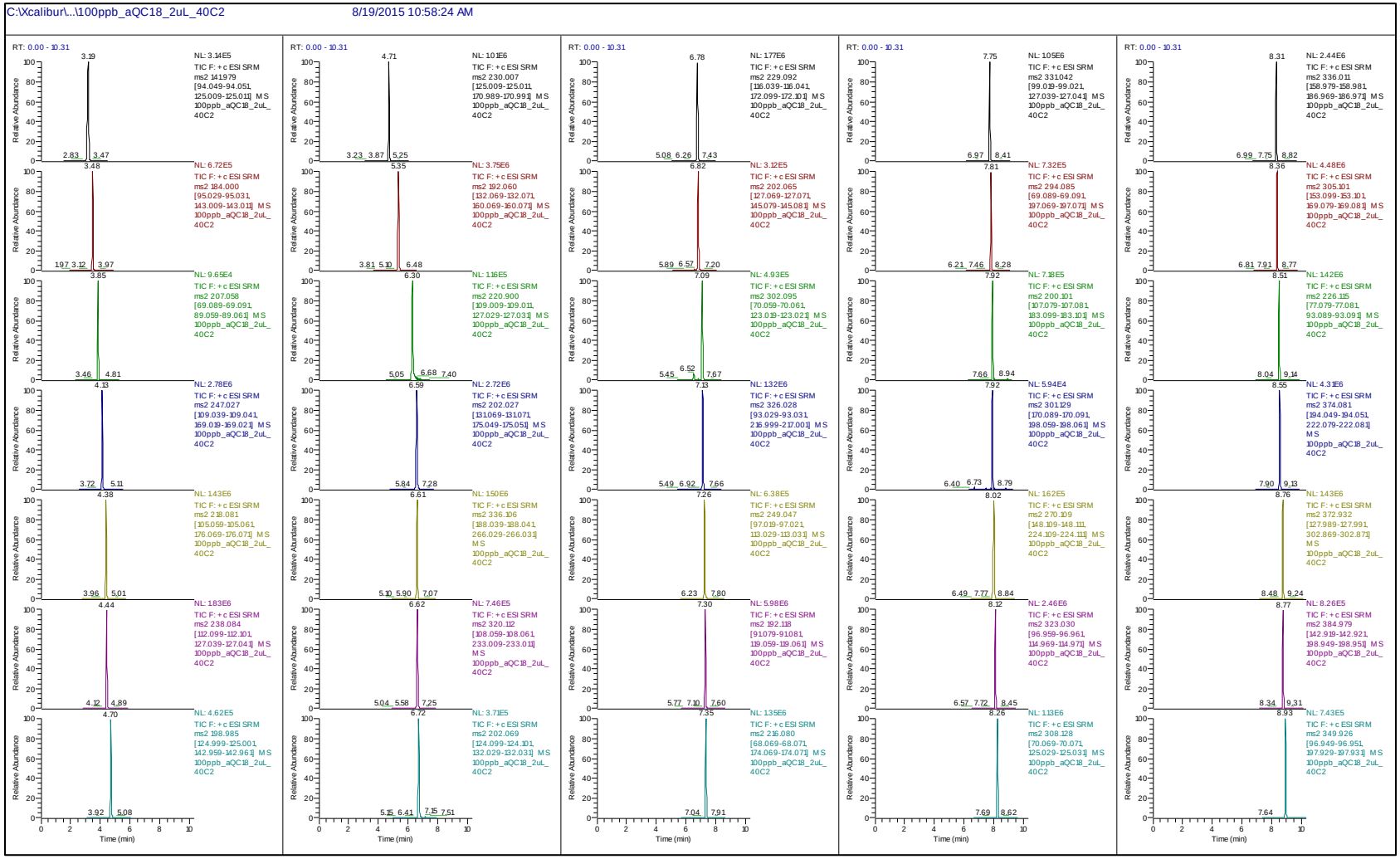
Accuracy and Precision of Pesticides in Spiked Blueberries

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

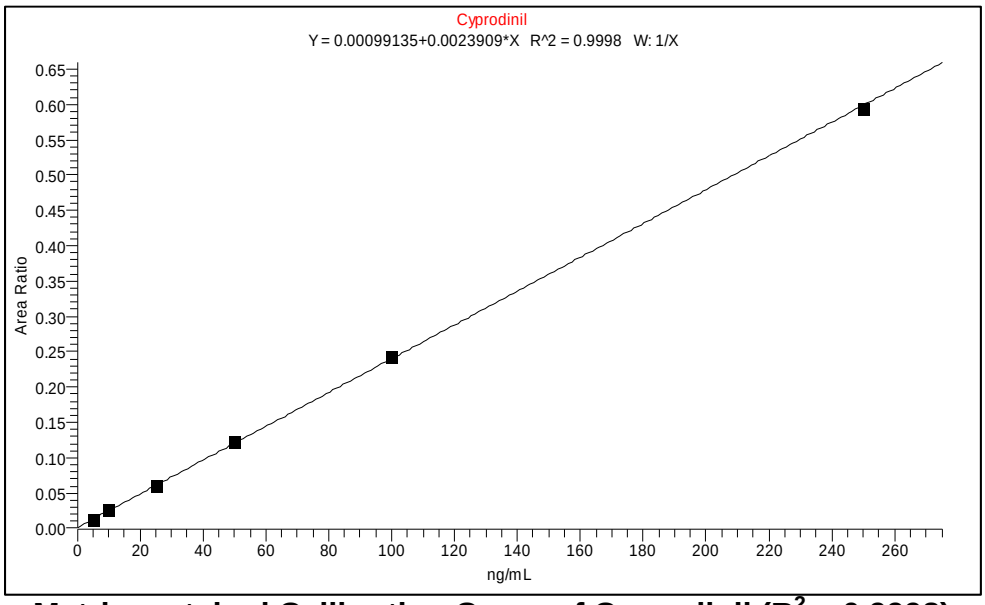
The recoveries of several pesticides such as pymetrozine, carbendazim, thiabendazole, and cyprodinil, were found be adversely affected by GCB, but unaffected when ChloroFiltr® was used; therefore, this sorbent combination was selected for blueberry extract cleanup in the final optimized procedure. The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







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



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

References:

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

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Analysis of Tobacco Alkaloids

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

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

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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 $MgSO_4$)

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

Procedure

1. Extraction

b) Weigh 2 g of homogenized tissue sample into a 50 mL centrifuge tube

c) Add 10 mL of MeCN/water (4/1 v/v)

d) Shake or vortex for 5 min

e) Centrifuge for 5 min at >3700 rcf

f) 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.

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Determination of Phthalates Leached from Toys into Artificial Saliva

UCT Part Numbers:
ECPAHFR50CT-50 mL polypropylene centrifuge tubes
ECQUUS2-MP - Mylar pouch with 4000 mg $MgSO_4$ and 2000 mg NaCl

Phthalates are plasticizers added to polymers to make them softer and more flexible. They are commonly used in the manufacturing of toys. Since phthalates are not permanently bonded in polymers, they may be released through touching, licking, or chewing. This new method artificially reproduces the licking and chewing actions of children by shaking cut toys in artificial saliva. Following extraction, phthalate levels are determined by GC/MS.

Preparation of Artificial Saliva (AS)[1]

AS was prepared by mixing 0.18 g xanthan gum, 1.2 g potassium chloride, 0.85 g sodium chloride, 0.05 g magnesium chloride, 0.13 g calcium chloride, and 0.13 g di-potassium hydrogen orthophosphate with 1 L of reagent water (1) and stirred for 4 hours.

Procedure

1. Cut plastic toys into small pieces
2. Weigh 1 to 2 g of toy samples to a 50 mL centrifuge tube **ECPAHFR50CT**
3. Add 10 mL of **AS** and two stir bars to the 50 mL tube
4. Shake for 1 hour using a horizontal shaker
5. Transfer the **AS** to a new 50 mL tube, add 10 mL of ethyl acetate and shake for 1 min
6. Add salts from pouch **ECQUUS2-MP** and shake vigorously for 1 min
7. Centrifuge at 5000 rpm for 5 min
8. Transfer 1 mL of the supernatant into a 2 mL auto-sampler vial
9. Add 10 µL of 50 ppm triphenyl phosphate (TPP) as internal standard
10. The sample is ready for GC/MS analysis

INSTRUMENT CONDITIONS:

GC/MS: Agilent 6890N GC coupled with 5975C MSD, equipped with 7683 auto sampler
Injector: 1µL splitless injection at 250 ºC, 30 mL/min split vent at 1 min
Liner: 4 mm splitless gooseneck, 4mmID*6.5mmOD*78.5mm (UCT cat#: GCLGN4MM)
GC capillary column: Restek Rxi-5sil MS 30m*0.25mm*0.25µm integrated with 10m guard column
Oven temperature program: Initial temperature of 70 ºC, hold for 1 min; ramp at 20 ºC/min to 315 ºC, hold for 4.75 min. Acquire data from 6 to 14 min.
Carrier gas: He at a constant flow of 1.2 mL/min.
MSD: Transfer line: 280 ºC; MS Source (EI): 250 ºC; MS Quad: 150 ºC
Simultaneous Scan/SIM: Scan range: 55-350 amu

SIM Table

Compound	Abbreviation	Rt (min)	Group #	Start (min)	Dwell time (ms)	Quantify ion	Qualifier ion 1	Qualifier ion 2
Dimethyl phthalate	DMP	6.989	1	6	25	163	194	133
Diethyl phthalate	DEP	7.858	2	7.5	25	149	177	105
Dibutyl phthalate	DBP	9.865	3	9	25	149	223	150
Benzyl butyl phthalate	BBP	11.716	4	11	25	149	91	206
Triphenyl phosphate	TPP	11.964			25	326	325	
Bis(2-ethylhexyl) phthalate	DEHP	12.432	5	12.3	25	149	167	279
Di-n-octyl phthalate	DOP	13.138			25	149	279	150

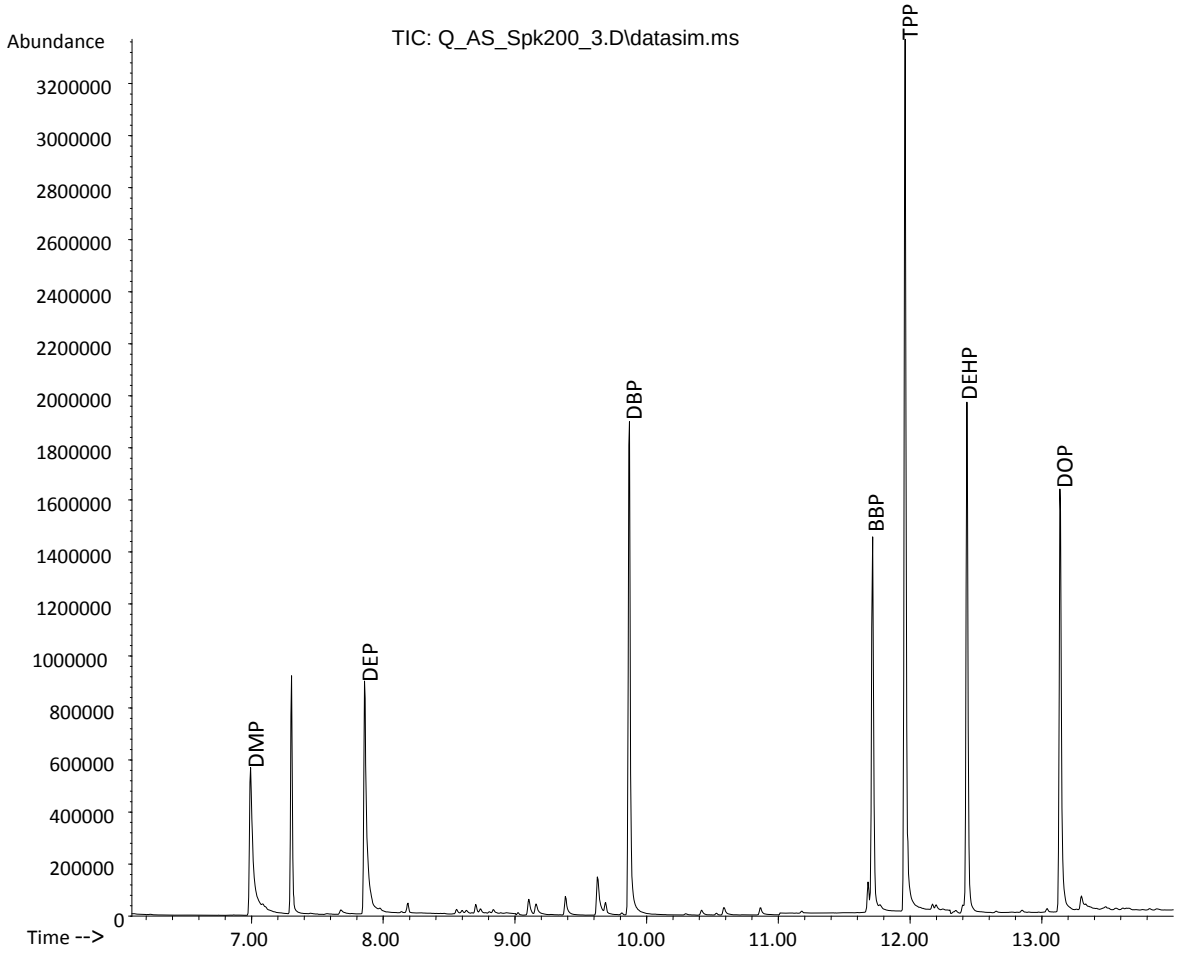
Calibration Curves

Matrix matched calibration curves are constructed by analyzing matrix matched standards (0-1000 ng/mL). The responses were linear with correlation coefficient higher than 0.9973. The limit of quantification of this method is 25 ng/mL.

Accuracy and Precision Data (n=4)*		
Analyte	**Fortified 200 ng/mL**	**Fortified 500 ng/mL**
	Recovery ± RSD %	**Recovery ± RSD %**
Dimethyl phthalate	90.2 ± 2.6	94.6 ± 2.1
Diethyl Phthalate	91.1 ± 1.4	95.6 ± 2.0
Dibutyl phthalate	90.6 ± 3.8	97.0 ± 2.2
Benzyl butyl phthalate	85.5 ± 1.2	92.1 ± 2.5
Bis(2-ethylhexyl) phthalate	93.2 ± 2.2	92.7 ± 2.6
Di-n-octyl phthalate	88.8 ± 3.1	92.8 ± 1.3

*The control sample did not contain phthalates

Chromatogram of AS fortified with 200 ng/mL phthalates



Results

Diethyl phthalate was found leaching from toy samples into artificial saliva at a concentration of 285 ng/g (RSD=5.9%, n=3).

References

(1) A. Preetha and R. Banerjee, Comparison of Artificial Saliva Substitutes, *Trends Biomater. Artif. Organs* **18(2)**,178-186 (2005).

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Simultaneous Determination of 2- and 4-methylimidazoles in Beverages using a Simple Filter and Shoot (FASt) Method

UCT Part Numbers:
CSFAS203 (Clean Screen FASt 200mg/3mL)

Summary:

2-methylimidazole (2-MeI) and 4-methylimidazole (4-MeI) are byproducts generated from manufacturing of caramel color additives used in beverages, soy sauces, baked foods, etc. The International Agency of Research of Cancer classified these two compounds as “possibly carcinogenic to humans”, and proposed the no significant risk level (NSRL) to be 29 µg/day for 4-MeI, while California listed 4-MeI as a probable carcinogen and proposed a 16 µg/day NSRL. However, the European Food Safety Authority (EFSA) considered 4-MeI safe and established a maximum level of 250 mg/kg in caramels.

Traditional analytical methods for 2-MeI and 4-MeI involve tedious ion-paring extraction and derivatization with GC or GC/MS detections, or solid phase extraction (SPE) with HPLC or LC/MS-MS detections. This application offers a simple, fast, and cost effective method to determine 2-MeI and 4-MeI in beverages simultaneously. Beverage samples were degassed, diluted by 10 times with acetonitrile (MeCN), and filtered through a SPE cartridge with 200 mg of the novel FASt sorbent, onto which the undesired matrix components, such as sugars and organic acids were retained, resulted in cleaned samples for LC/MS-MS analysis. 2-MeI and 4-MeI are isomers with identical MS/MS transitions, making the separation and quantification of such compounds very difficult. A new HILIC HPLC method has been developed with baseline separation achieved in an 8-min run.

Experimental:

Sample pretreatment:

Pour the entire bottle or can of beverage samples into 500-mL beakers, and degas the samples by stirring at high speed for 2 hr.

Filter and Shoot (FASt) procedure:

a) Transfer 0.1 mL of the degassed samples into test tubes or glass vials, dilute with 0.9 mL of MeCN. Add 10 µL of a 10-ppm imidazole solution as internal standard (IS), and appropriate amounts of target analytes to fortified samples. Vortex for 10 sec.
b) Attach the FASt cartridges (**CSFAS203**) to a glass block or positive pressure manifold, insert test tubes or 2-mL auto-sampler vials into the manifold.
c) Transfer the diluted samples into the cartridges, apply low vacuum or positive pressure and collect the filtrates.
d) The samples are ready for LC-MS/MS analysis.

LC-MS/MS method:

HPLC: Thermo Scientific, Dionex UltiMate 3000® LC System
Column: Thermo Scientific, Accucore HILIC, 100 x 2.1 mm, 2.6 µm
Guard Column: Thermo Scientific, Accucore HILIC, 10 x 2.1 mm, 2.6 µm
Column Temperature: 40 °C
Column Flow Rate: 0.400 mL/min
Auto-sampler Temperature: 10 °C
Injection Volume: 10 µL
Mobile Phase (Isocratic 8 min): 5% of 50 mM ammonium formate in water and 95% of MeCN
Divert mobile phase to waste from 0 - 1 min to prevent ion source contamination.

MS parameters	
Polarity	ESI +
Spray voltage V	5000 V
Vaporizer Temperature	242 °C
Ion transfer capillary temperature	398 °C
Sheath gas pressure	60 arbitrary units
Auxiliary gas pressure	20 arbitrary units
Q1 and Q3 peak width (FWHM)	0.4 and 0.7 Da
Collision gas and pressure	Ar at 0.8 mTorr
Scan type	SRM
Cycle time	0.75 sec
Acquisition method	EZ Method

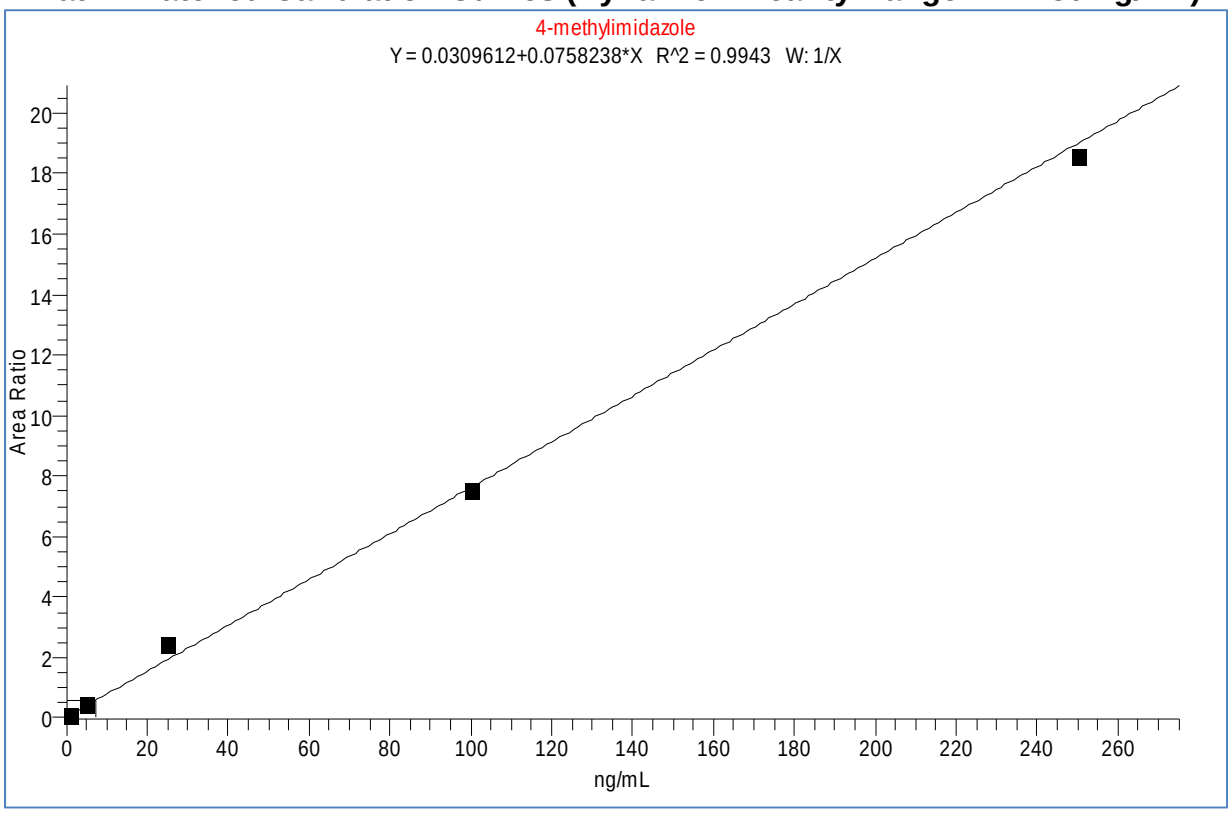
SRM transitions							
Compound	**Rt (min)**	**Precursor ion**	**Product ion 1**	**CE 1**	**Product ion 2**	**CE 2**	**S-lens (V)**
Imidazole (IS)	1.96	69.07	42.01	21	28.08	74	65
4-MeI	3.18	83.08	56.05	17	42.00	27	45
2-MeI	5.72	83.07	42.04	20	56.05	19	48

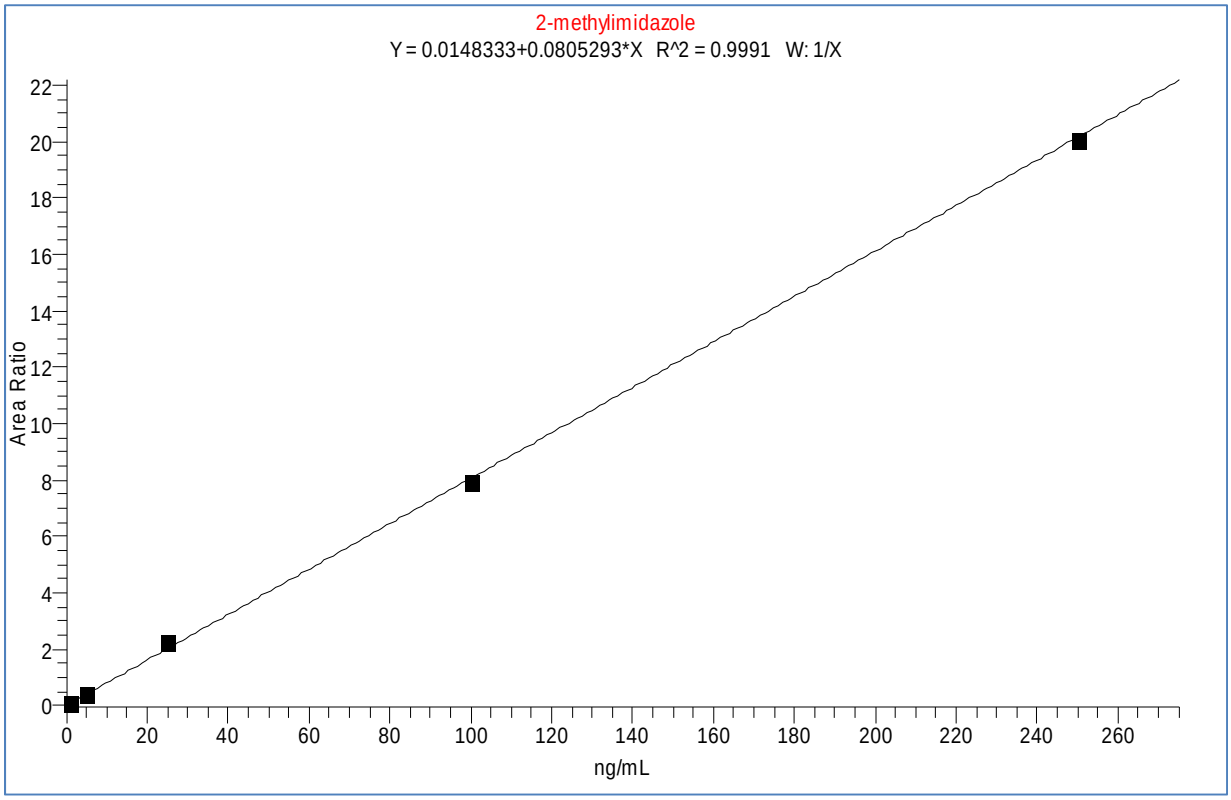
Results:

Recovery and RSD Obtained from a Negative Beverage Sample Fortified with 100 ng/mL of 2-MeI and 4-MeI

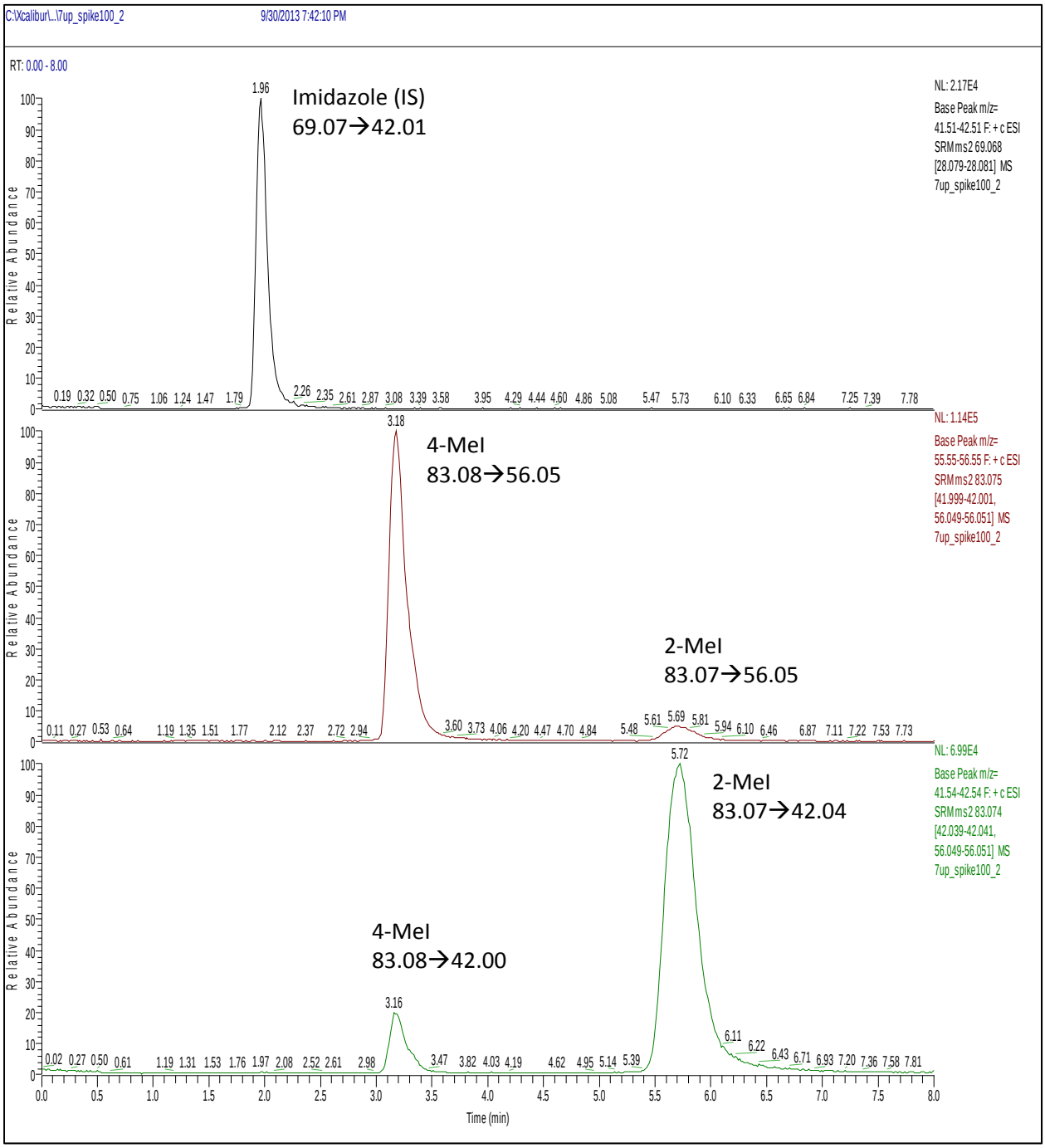
Compound	Recovery%	RSD% (n=6)
4-MeI	**103.6**	4.1
2-MeI	**102.9**	1.6

Matrix Matched Calibration Curves (Dynamic Linearity Range: 1 – 250 ng/mL)





Chromatogram of a Negative Beverage Sample Fortified with 100 ng/mL of 2-MeI and 4-MeI



Results of Real Sample Analysis

Beverages	Detected 4-MeI* (ng/mL)	
	Conc. in the diluted sample	Conc. in the original sample
Colorless soda	< 1	**< 10**
Root beer	7.4	**74**
Sweet tea	12.8	**128**
Coke_AZ	84.0	**840**
Coke _CO	28.3	**283**
Coke _LA	43.9	**439**
Coke _MD	46.4	**464**
Coke _MS	59.2	**592**
Coke _OR	10.7	**107**
Coke _TN	58.6	**586**
Coke _TX	59.2	**592**
Coke_WA	10.1	**101**

*: 2-MeI was not detected in any samples tested in this study.

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Determination of Mycotoxin Residues by LC-MS/MS Featuring Two Alternate Sample Extraction Procedures

UCT Part Numbers:
ECHLD126-P - EnviroClean® HL DVB, 200 mg/6 mL cartridge
ECMSSC-MP - Mylar pouch containing 4000mg $MgSO_4$ and 1000mg NaCl
CUMPSC18CT - 2mL centrifuge tube containing 150mg anhydrous $MgSO_4$, 50mg PSA and 50mg endcapped C18
SLDA100ID21-3UM - Selectra® DA, 100 × 2.1 mm, 3 µm
SLDAGDC21-3UM - Selectra® DA, 10 × 2.1 mm guard cartridge
SLGRDHLDR – Guard cartridge holder

Introduction:

Mycotoxins are toxic natural secondary metabolites produced by several species of fungi on agricultural commodities in the field or during storage. To date more than 300 mycotoxins, possessing varying degrees of toxicity, have been identified, although only a relatively few of these are widely accepted as presenting a significant food or animal feed safety risk [1]. Mycotoxins are chemically stable and cannot be destroyed during food processing and heat treatment, thus, monitoring these compounds in food is an important health, agricultural production, food processing and trade concern. The analysis of mycotoxins is challenging due to the large number of compounds to be detected and the wide physicochemical properties they possess. Additionally, typical food commodity matrices are complex in nature and often contaminated with several mycotoxins at low concentrations.

Sample preparation approaches for mycotoxin analysis include solid–liquid extraction, liquid–liquid extraction, matrix solid-phase dispersion, QuEChERS, immunoaffinity chromatography and solid-phase extraction (SPE). All approaches are complicated by the considerably different polarity and solubility of the mycotoxins, in particular the polar trichothecenes. Due to limited sample cleanup that can be incorporated into a method, sample extracts may still contain large amounts of matrix components that can negatively affect the detection system. To overcome some of the limitations of existing methods, there is a need to further develop extraction and clean-up methods for the simultaneous determination of several mycotoxins with high recoveries of the polar trichothecenes and minimizing sample matrix effects.

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is the most widely used detection system for mycotoxin analysis. Advantages of using LC-MS/MS include selectivity, sensitivity and the ability to cover a wide range of mycotoxins. However, challenges still remain, including finding conditions that are suitable for all mycotoxins included in a method, adequate LC retention of polar trichothecenes, and matrix effects. The latter issue is particularly relevant to trichothecene mycotoxins as their ionization efficiency can be affected by the presence of co-eluting matrix interferences leading to signal suppression or enhancement [2]. This can be compensated by using isotopically labeled internal standards and matrix-matched standards [3]. Numerous LC-MS/MS conditions have been reported for the analysis of mycotoxins [4-6]. There doesn't appear to be universal LC-MS/MS conditions that work for all the mycotoxins, and the choice of ion source and mobile phase is dependent on the compounds included in a method. Electrospray ionization (ESI) is the most commonly used ion source in mycotoxins analysis, although atmospheric pressure chemical ionization (APCI) is also employed, particularly for the analysis of trichothecene mycotoxins [7, 8].

This application note details two sample preparation approaches (SPE and QuEChERS) that can be used for the extraction and clean-up of mycotoxins from grain-based food. LC-MS/MS was used for the accurate detection and quantification of the mycotoxins in both methods. HPLC separation of the 16 mycotoxins and 3 internal standards included in the study was successfully conducted within 16 min using a Selectra® DA column, a polyaromatic phase capable of greater retention of the polar trichothecenes compared to a standard C18 stationary phase. The compounds included in this method are representative of a wide range of mycotoxins, including type A- and B-trichothecenes, ochratoxin A, alternariol, zearalenone, α- & β-zearalanol and aflatoxins (B1, B2, G1, G2).

Experimental (SPE):

1. **Sample Preparation**
   a) Weigh 2 g of sample into a 50 mL polypropylene centrifuge tube.
   b) Add 2 mL water and briefly vortex.
   c) Allow samples to hydrate for ≥15 min.
   d) Add 10 mL MeCN.
   e) Shake or vortex samples for 10-15 min to extract the mycotoxins.
      Note: For this study a SPEX® SamplePrep® GenoGrinder® was used.
   f) Centrifuge the samples for 10 min at ≥3000 × g (4°C).
   g) Transfer the supernatant to a clean polypropylene or glass tube and evaporate to dryness at 50°C under a gentle stream of nitrogen.
   h) Add 10 mL of water to each sample and vortex for 5 min to ensure the sample is fully dissolved.
      Note: Alternatively, sonicate the samples for 5 min.

2. **SPE Extraction**
   a) Condition SPE cartridges with 3 mL MeOH and 3 mL water.
   b) Load supernatant from step 1h).
   c) Allow the sample to percolate through the cartridge under gravity.
      Note: If necessary, apply a low vacuum to pull the sample through the cartridge dropwise.

3. **Wash Cartridge**
   a) Add 3 mL of water and slowly draw through.
   b) Add 3 mL of 10% MeOH and slowly draw through.
   c) Dry cartridges under vacuum (≥10 inHg) for 10 minutes.
   d) Add 3 mL of hexane and slowly draw through.
   e) Dry cartridges under vacuum (≥10 inHg) for 5 minutes.

4. **Elute Cartridge**
   a) Elute the mycotoxins using 4 mL MeCN.
   b) Evaporate the samples to dryness at 40-50°C under a gentle stream of nitrogen.
   c) Reconstitute in 1 mL of MeOH:H_2O (50:50, v/v).

Experimental (QuEChERS):

1. **Sample Extraction**
   a) Weigh 5 g of thoroughly homogenized sample into a 50 mL centrifuge tube.
   b) Add 10 mL water and briefly vortex.
   c) Allow samples to hydrate for ≥15min.
   d) Add internal standard.
   e) Add 10 mL MeCN containing 2% formic acid (or 10 mL MeCN and 200 µL formic acid).
   f) Vortex/shake samples for 5-10 min to extract the mycotoxins.
   g) Add contents of the **ECMSSC-MP** Mylar pouch to each centrifuge tube.
   h) Immediately shake (manually or mechanically) for 1 min.
      Note: For this study a SPEX® SamplePrep® GenoGrinder® was used.
   i) Centrifuge for 5 min at ≥3000 × g (4°C).

2. **Sample Cleanup**
   a) Transfer a 1 mL aliquot of supernatant to a **CUMPSC18CT** dSPE tube.
   b) Vortex for 30 sec.
   c) Centrifuge for 5 min at ≥3000 × g (4°C).
   d) Transfer 500 µL of purified supernatant to a 5 mL test tube and solvent-exchange the sample into MeOH:H_2O (50:50, v/v) for optimum LC-MS/MS performance.

For improved sensitivity at low concentrations, the dSPE step can be scaled-up and a concentration step included in the method. Use product **CUMPSC1815CT2** (15mL centrifuge tube with 1200mg $MgSO_4$, 400mg PSA and 400mg endcapped C18):

   a) Transfer 8ml of supernatant to a **CUMPSC1815CT2** dSPE tube.
   b) Vortex for 30 sec.
   c) Centrifuge for 5 min at ≥3000 × g (4°C).
   d) Transfer 5 mL of supernatant to a glass tube.
   e) Evaporate the sample to dryness at 40-50°C under a gentle stream of nitrogen.
   f) Reconstitute samples in 1 mL MeOH:H_2O (50:50, v/v).

LC-MS/MS Conditions:

MS Conditions	
Instrumentation	Thermo Scientific™ TSQ Vantage™ tandem
Ionization mode	$APCI^+$ & $APCI^-$
Discharge current	5 ($APCI^+$) & 20 ($APCI^-$) µA
Vaporizer temperature	250°C
Capillary temperature	250°C
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	15 arbitrary units
Ion sweep gas	0 arbitrary units
Declustering potential	0 V
Q1 and Q3 peak width	0.2 and 0.7 Da
Collision gas	argon
Collision gas pressure	1.5 mTorr
Acquisition method	EZ method (scheduled SRM)
Cycle time	0.7 sec
Software for data processing	Xcalibur™ version 2.2
Weighting factor applied to	1/X

SRM Transitions								
Analyte	t_R (min)	Precursor ion		Product ion 1	CE 1	Product ion 2	CE 2	S-lens (V)
Nivalenol	3.63	357.	[M+HC	281.91	16	311.79	15	65
Deoxynivaleno	4.52	341.	[M+HC	265.87	13	295.96	16	63
Fusarenon X	5.35	354.	$[M+H]^+$	136.97	31	174.96	19	76
Neosolaniol	6.03	399.	$[M+NH_4$	184.99	20	215.03	16	81
AcDON	6.79	338.	$[M+H]^+$	231.10	13	90.98	48	74
AcDON-D_3 (IS)	6.76	341.	$[M+H]^+$	230.99	14	213.04	15	80
Thiabendazole-	8.35	207.	$[M+H]^+$	181.02	25	137.04	32	123
Diacetoxyscirp	9.55	383.	$[M+NH_4$	247.06	13	229.08	16	82
Alternariol	9.76	257.	$[M-H]^-$	214.03	23	216.01	26	113
Ochratoxin A	10.44	403.	$[M+H]^+$	238.93	23	220.90	36	101
-zearalanol	10.40	321.	$[M-H]^-$	277.94	24	303.86	24	125
-zearalanol	11.56	321.	$[M-H]^-$	277.94	24	303.86	24	125
Gemfibrozil-D_6	11.66	255.	$[M-H]^-$	122.41	21	-	-	60
T-2 toxin	11.76	483.	$[M+NH_4$	185.02	21	214.99	17	84
Aflatoxin G2	13.53	330.	$[M+H]^+$	189.02	36	245.05	27	137
Zearalenone	13.57	317.	$[M-H]^-$	176.13	27	273.95	22	110
Aflatoxin G1	14.07	328.	$[M+H]^+$	199.02	41	200.03	36	143
Aflatoxin B2	14.39	314.	$[M+H]^+$	287.06	23	259.01	27	129
Aflatoxin B1	14.75	312.	$[M+H]^+$	241.02	36	285.05	22	121

Note: CE = collision energy. AcDON = 3-acetyldeoxynivalenol.

HPLC Conditions	
Instrumentation	Thermo Scientific™ Dionex™ Ultimate™ 3000 LC system
HPLC column	UCT Selectra® DA, 100 × 2.1 mm, 3 µm (p/n: SLDA100ID21-3UM)
Guard column	UCT Selectra® DA, 10 × 2.1 mm, 3 µm, (p/n: SLDAGDC21-3UM)
Guard column	p/n: SLGRDHLDR
Mobile phase A	10 mM ammonium formate
Mobile phase B	MeOH
Flow rate	300 µL/min
Column temp.	45°C
Run time	21 min (including 5 min equilibration)
Injection volume	10 µL
Autosampler	10°C
Wash solvent	MeOH: H_2O (50:50, v/v)
Divert valve	mobile phase was sent to waste for 0-2 & 16-21 min to reduce ion

LC gradient		
Time (min)	A (%)	B (%)
0.0	98	2
2.0	60	40
5.0	60	40
6.0	40	60
10.0	40	60
12.0	0	100
16.0	0	100
16.2	98	2
21.0	98	2

Results and Discussion:

1. SPE Sample Preparation Procedure

The first step in the sample preparation process is to find a suitable extraction solvent. It has been reported that 100% organic solvent is not a suitable extraction solution in multi-class mycotoxin methods as it does not sufficiently extract all residues, particularly the polar trichothecenes [7,9,10]. MeCN:water, usually in the ratio 84:16 (v/v), is the most commonly used extraction solvent in mycotoxin analysis. Other extraction solvents that have been reported in the literature for

single- and multi-class methods include MeOH:water, MeCN, MeOH, acetone, ethyl acetate, dichloromethane and aqueous buffers [9,10]. The extraction solvent used in this application was MeCN:water (83:17, v/v).

After extracting the mycotoxin residues from the sample, the sample extract undergoes a solvent exchange to 100% water prior to application to the SPE cartridge. Applying an aqueous solution onto the SPE cartridge ensures optimum retention of the mycotoxins on the sorbent and reduces any chance of analyte breakthrough. The solvent exchange step requires the inclusion of an evaporation step in the method, which makes it desirable to limit the water content of the extraction solvent in order to speed up the evaporation process. With this in mind, an attempt was made to use 100% MeCN as the extraction solvent, but as reported elsewhere it did not sufficiently extract all of the mycotoxins, namely the polar trichothecenes. Ultimately, adding water to the extraction solvent improved the extraction of the polar trichothecenes, while keeping the water content low ensured the evaporation step was relatively straightforward using the conditions described in the experimental procedure. It was subsequently found that hydrating the sample with water prior to adding MeCN gave greater extraction efficiency than adding aqueous MeCN directly to dry samples. This is probably due to greater access of the MeCN to the solvated matrix.

Rinsing the SPE sorbent to remove matrix components is limited when analyzing for multiple mycotoxins due to the potential loss of analytes. In this study, the SPE cartridges were rinsed with water to remove very polar matrix components and hexane to remove very hydrophobic matrix components. In addition, a 10% MeOH solution was used to remove additional matrix components without eluting any of the mycotoxins. Further increasing the MeOH content of the wash solution increases the risk of washing some of the mycotoxins off the sorbent, leading to reduced recovery.

Lastly, the elution solvent had to be optimized; MeOH, a commonly used elution solvent in SPE, was too weak to fully elute all the mycotoxins from the divinylbenzene sorbent (particularly zearalenone, - & -zearalanol, alternariol and the aflatoxins). Ethyl acetate, acetone and MeCN were evaluated as alternative elution solvents. Ultimately, MeCN was found to give the best results and was chosen as the final elution solvent.

To generate accuracy and precision data, recovery experiments were carried out using cereal (composed of various grains and nuts) as a representative sample matrix. Samples were fortified at two concentrations (n = 6 for each concentration) in order to obtain the necessary data. The cereal samples were fortified at 10 and 100 ng/g and prepared according to the experimental procedure described above. As outlined in table 1, the majority of results were found to be within an acceptable recovery range of 80-120 % with RSD values 10 %, demonstrating that the developed SPE method is suitable for the analysis of mycotoxins in grain-based foods. Deoxynivalenol and Fusarenon X were not included in the results due to elevated recoveries at the 10 ng/g level. Fusarenon X also had somewhat elevated recovery at the 100 ng/g level, although the result was reproducible (3% RSD). The elevated recovery was possibly caused by co-eluting matrix components leading to signal enhancement. The inclusion of isotopically labeled internal standards for these compounds would help to address this issue.

Table 1. Accuracy and precision data obtained for the SPE sample preparation method.

Analyte	10 ng/g		100 ng/g	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Nivalenol	87.3	3.4	87.2	1.6
Deoxynivalenol	*	-	102.0	3.7
AcDON	90.2	4.3	100.0	0.5
Fusarenon X	*	-	144.3	3.1
Neosolaniol	92.7	1.1	98.5	1.4
Diacetoxyscirpenol	86.8	3.4	86.2	2.1
Alternariol	95.0	4.3	97.9	2.1
-zearalanol	77.0	2.6	80.0	4.0
-zearalanol	78.6	1.8	78.8	3.6
Zearalenone	112.3	1.9	100.1	2.3
Ochratoxin A	88.7	3.5	117.6	5.0
T-2 toxin	79.0	4.8	82.0	3.1
Aflatoxin B1	107.2	6.3	98.8	1.9
Aflatoxin B2	98.3	3.1	97.0	2.1
Aflatoxin G1	83.1	6.4	86.1	2.7
Aflatoxin G2	111.4	8.5	101.2	2.3

*Recovery values obtained were not included due to elevated recovery values high.

2. QuEChERS Sample Preparation Procedure

The QuEChERS procedure is a popular sample preparation approach for the analysis of mycotoxin residues due its simplicity, speed and cost. MeCN is the preferred extraction solvent as it extracts the widest range of mycotoxins and least amount of matrix components. To efficiently extract acidic mycotoxins (ochratoxins and fumonisins), the sample pH needs to be lowered so that the analytes are in their neutral state (i.e. protonated) and effectively partition into the MeCN layer. This is achieved by incorporating acid in the extraction solvent or using buffered QuEChERS extraction salts. Cleanup of the sample extract is carried out by dispersive-SPE (dSPE) using primary secondary amine (PSA) and/or C18 sorbent. PSA effectively removes organic acids, carbohydrates and polar matrix components, while C18 removes fats and other lipophilic matrix components. For acidic analytes, the sample pH needs to be sufficiently low to ensure the acidic mycotoxins do not get retained on the PSA sorbent. Nivalenol, a

very polar compound, is the only mycotoxin reported to not give high recoveries using the QuEChERS approach. This is caused by the incomplete partitioning of nivalenol into the organic phase during the extraction/partitioning step. Reported recovery is typically still 60% and the reproducibility acceptable.

In this study, MeCN containing 2% formic acid was used as the extraction solvent. Unbuffered extraction salts were used to maintain a low sample pH. Using buffered extraction salts (acetate or citrate) would raise the pH and lead to lower recovery of ochratoxin A or require the use of higher amounts of acid to maintain a low sample pH. dSPE cleanup of the sample extracts was successfully carried out using PSA/C18 sorbent. Using 2% formic acid in the extraction solvent was necessary to prevent the retention of ochratoxin A on the PSA sorbent. Using smaller amounts of acid lead to lower recovery of ochratoxin A. Other dSPE sorbents evaluated included PSA on its own and PSA/C18/GCB (graphitized carbon black). GCB is a typically used for highly pigmented samples (e.g. chlorophyll and sterols) and yields very clean extracts. However, it can also retain analytes of interest, leading to reduced recovery. This was found to occur for several of the mycotoxins included in this method (e.g. ochratoxin A, alternatiol, zearalenone, aflatoxins). No major variation in recovery was observed between PSA and PSA/C18. However, the combination of PSA/C18 yielded cleaner extracts and was therefore used in the final method.

Cereal, consisting of various grains and nuts, was used as the representative sample matrix for recovery experiments. Samples were fortified at three concentrations (n = 6 for each concentration) in order to obtain accuracy and precision data. The cereal samples were fortified at 20, 40 and 100 ng/g and prepared according to the experimental procedure described above. As outlined in table 2, the majority of results were found to be within an acceptable recovery range of 80-120 % with RSD values ≤ 10 %, demonstrating that the developed QuEChERS method is suitable for the analysis of mycotoxins in grain-based foods. Nivalenol, fortified at 20ng/g, gave a mean recovery value of 45% and was the only compound with a result outside the acceptable limits

(70-120%). This is probably due to the reduced MS sensitivity of the analyte at that particular concentration. As already mentioned, it is known that nivalenol does not get as efficiently extracted as the other mycotoxins using the QuEChERS approach. However, samples fortified at higher concentrations gave satisfactory recoveries (≈80%). 20ng/g was chosen as the lowest fortification level because at lower concentrations the sensitivity of some of the mycotoxins, primarily the trichothecenes, becomes more challenging. If lower concentrations are desired, it is suggested to scale-up the dSPE step and include a concentration step in the method. However, some mycotoxins (e.g. aflatoxins, zearalenone, diacetoxyscirpenol) can be readily detected at concentrations ≤20ng/g without using a concentration step.

Table 2. Accuracy and precision data obtained for the QuEChERS sample preparation method.

	20 ng/g		40 ng/g		100 ng/g	
Analyte	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Nivalenol	44.6	4.1	80.9	3.0	79.1	3.7
Deoxynivalenol	97.8	8.5	89.2	8.6	85.0	7.6
AcDON	94.1	4.7	105.0	3.1	95.8	3.0
Fusarenon X	97.8	9.3	105.2	3.3	104.6	2.4
Neosolaniol	89.3	4.6	104.3	4.8	98.2	1.7
Diacetoxyscirpenol	91.5	1.8	102.2	2.6	96.8	2.1
Alternariol	79.7	3.5	104.4	2.4	94.2	4.3
-zearalanol	85.6	4.7	109.4	3.6	100.8	1.6
-zearalanol	90.6	3.1	102.8	2.5	97.9	0.9
Zearalenone	75.8	2.9	107.5	4.5	98.4	2.8
Ochratoxin A	81.3	8.8	99.9	2.6	82.5	1.9
T-2 toxin	91.0	4.6	102.1	2.6	99.7	1.5
Aflatoxin B1	92.1	3.4	98.1	2.6	95.5	3.4
Aflatoxin B2	102.2	3.0	98.5	1.9	97.3	1.7
Aflatoxin G1	75.7	4.0	101.0	4.1	96.9	1.9
Aflatoxin G2	103.7	11.2	99.0	1.5	87.5	2.7

LC-MS/MS analysis

Prior to developing the sample preparation procedures, a LC-MS/MS method was developed for the simultaneous determination of the 16 mycotoxins and 3 internal standards included in the study. However, developing an LC-MS/MS method to simultaneously detect all the mycotoxins poses a challenge due to the different physicochemical properties that they possess. It has been reported that some mycotoxins produce better results using an APCI source, although ESI has been shown to produce higher sensitivity for the majority of mycotoxins and is therefore used most frequently [8]. A problem encountered with using ESI is that the trichothecene mycotoxins are prone to adduct formation, including sodium $[M+Na]^+$ and potassium $[M+K]^+$ adducts. Most trichothecenes do not form protonated molecular ions $[M+H]^+$ or produce very weak signal rsponse. For the type-A trichothecenes (neosolaniol, diacetoxyscirpenol and T-2 toxin), incorporating an ammonium containing buffer into the mobile phase results in the formation of $[M+NH_4]^+$ adducts that exhibit good MS response. In contrast, the type-B trichothecenes (nivalenol, deoxynivalenol, acetyldeoxynivalenol and fusarenon X) do not form $[M+NH_4]^+$ adducts. However, the type-B trichothecenes are capable of forming acetate $[M+CH_3COO]^-$ and formate $[M+HCOO]^-$ adducts with adequate signal response. Alternatively, the formation of $[M+Na]^+$ and $[M+K]^+$ adducts can be avoided by using APCI instead of ESI.

Initially a variety of mobile phase additives were evaluated in ESI mode, including formic acid, acetic acid, ammonium formate, ammonium acetate, ammonium hydroxide and ammonium bicarbonate. However, none were found to be suitable for all of the mycotoxins. Some of the additives produced poor peak shapes for certain compounds, while the signal intensity obtained by ESI was still rather poor for several trichothecenes (particularly nivalenol, deoxynivalenol and fusarenon X). To improve the response of the trichothecenes, APCI was evaluated as an alternative to ESI. Using ammonium formate as the mobile phase additive, APCI was found to produce better signal intensity for the problematic

trichothecenes. Nivalenol and deoxynivalenol were detected as $[M+HCOO]^-$ adducts and fusarenon X as $[M+H]^+$ ion. In the end, while ESI gave better signal response for some compounds (e.g. aflatoxins), APCI was chosen for use as it provided the best overall results. Both acetonitrile (MeCN) and methanol (MeOH) were evaluated for use as organic eluent in the mobile phase. MeOH was found to give superior peak shape for the trichothecene mycotoxins compared to MeCN. In addition, the use of MeOH also improved the signal response, while the use of acetonitrile led to much lower signals. Similar observations have been previously reported [5]. Ultimately, 10mM ammonium formate, MeOH and APCI were found to be the best compromise and included in the final LC-MS/MS method.

Owing to their polarity, the type-B trichothecenes usually elute early in the chromatographic run, and are known to be prone to matrix effects in the ion source [2, 3]. To reduce the possibility of matrix effects they should be sufficiently retained on the LC column so that they do not co-elute with polar matrix components. The Selectra® DA column contains a polyaromatic phase that is capable of greater retention of the polar trichothecenes compared to a standard C18 stationary phase. Using this column, the first compound (nivalenol) does not elute until 3.65 min and using 40% organic solvent. In addition, the first 2 min of flow is diverted to waste, which minimizes ion source contamination. In the final method, separation of the mycotoxins, including - and -zearalanol, was achieved within 16 min on the Selectra® DA column. The use of rapid polarity switching allows all target analytes to be detected in a single run.

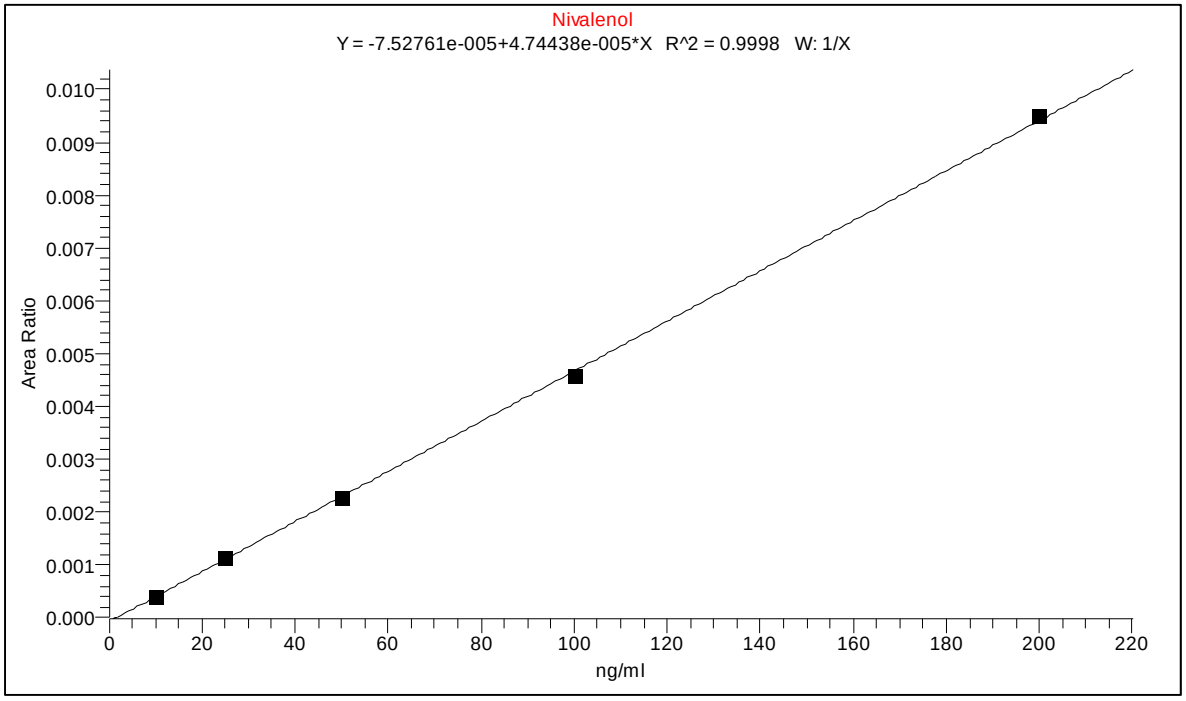

Figure 1. Example of a matrix-matched calibration curve (calibration curve for nivalenol was used to quantify results of the QuEChERS procedure).

Table 3. Linearity expressed as correlation coefficient, R^2 (values obtained were from the calibration curves used to quantify results of the QuEChERS procedure).

Analyte	R^2
Nivalenol	0.9998
Deoxynivalenol	0.9967
AcDON	0.9993
Fusarenon X	0.9984
Neosolaniol	0.9986
Diacetoxyscirpenol	0.9994
Alternariol	0.9988
β-zearalanol	0.9957
α-zearalanol	0.9983
Zearalenone	0.9990
Ochratoxin A	0.9994
T-2 toxin	0.9952
Aflatoxin B1	0.9966
Aflatoxin B2	0.9968
Aflatoxin G1	0.9950
Aflatoxin G2	0.9964

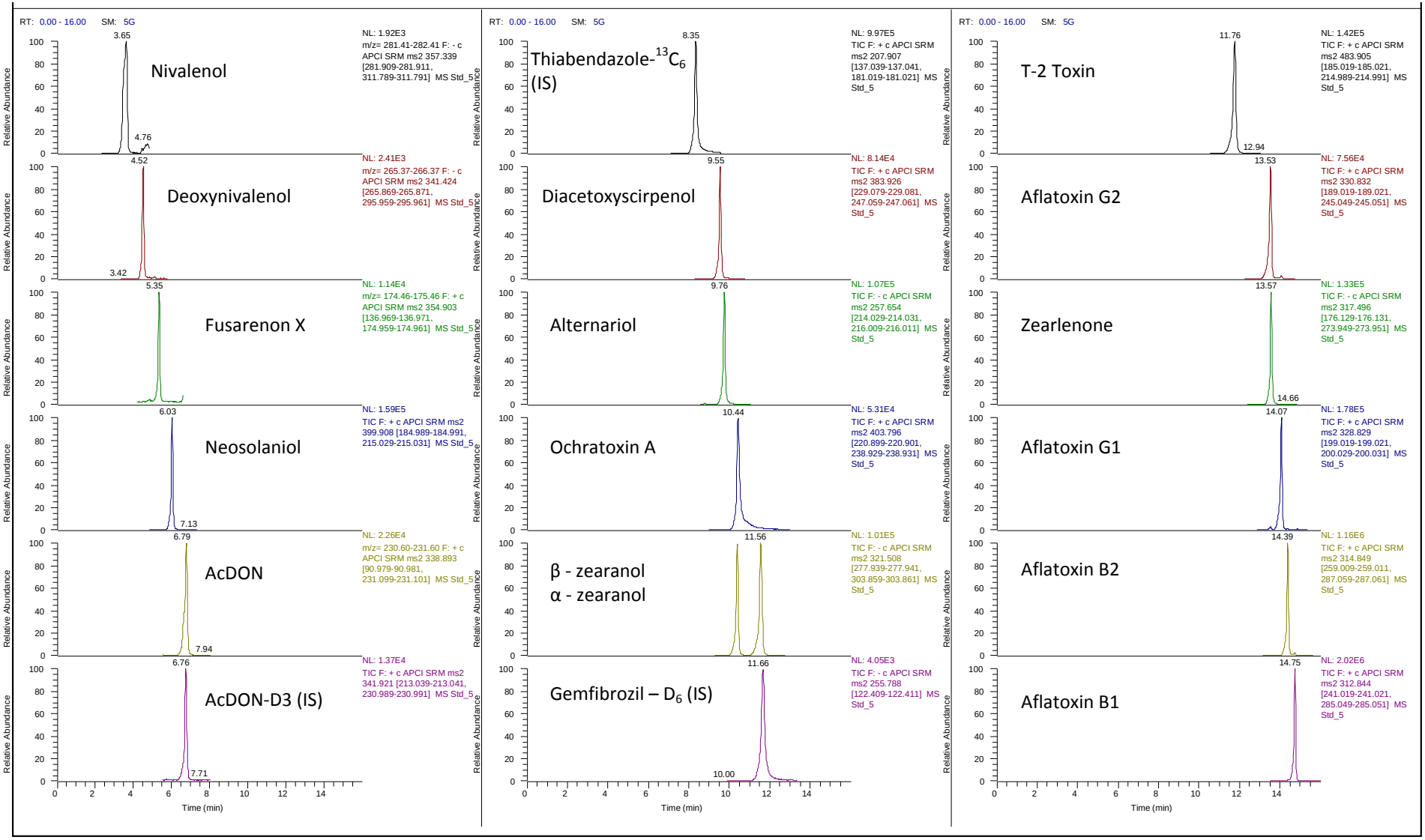

Figure 2. Example of a chromatogram containing the 16 mycotoxins and 3 internal standards included in the method.

Conclusions:

- Two sample preparation methods have been successfully developed for the extraction and cleanup of 16 representative mycotoxins in grain-based food.
- The SPE sample preparation procedure uses a hypercrosslinked divinylbenzene sorbent to effectively retain all the mycotoxins, including the polar trichothecene mycotoxins.
- The SPE wash step was optimized to remove matrix interferences without losing any analytes of interest.
- The QuEChERS sample preparation procedure uses acidified MeCN and un-buffered salts for extraction, and PSA/C18 for dSPE cleanup.
- An optimized LC-MS/MS method was developed for the accurate detection and quantification of the mycotoxin residues.
- APCI ionization was chosen over ESI as it provides better overall results, including better signal response for problematic trichothecenes.
- The use of rapid polarity switching allows all target analytes to be detected in a single run.
- Separation of the mycotoxins, including baseline resolution of - and - zearalanol, was achieved within 16 min on a Selectra® DA column.
- Overall, good accuracy and precision were obtained for these difficult compounds.
- For best results, it is recommended to use matrix-matched calibration curves and include isotopically internal standards (particularly for the type-B trichothecenes).

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Detection of Aflatoxins in Milk at Picogram Levels Using SPE and LC-MS/MS

UCT Part Numbers:
ECHLD126-P – EnviroClean® HL DVB , 200 mg/6 mL SPE cartridge
SLC-18100ID21-3UM – Selectra® C18, 100 × 2.1 mm, 3 µm HPLC column
SLC-18GDC20-3UM – Selectra® C18, 10 × 2.0 mm, 3 µm guard cartridge
SLGRDHLDR – Guard cartridge holder

Summary:

Aflatoxins are naturally occurring mycotoxins that are produced by several species of fungi (*Aspergillus flavus* and *Aspergillus parasiticus*). They are classified by the International Agency for Research on Cancer as group 1 carcinogens (compounds known to be carcinogenic in humans) [1]. Aflatoxins can occur in food products as a result of fungal contamination of crops (prior to harvest or during storage). There are approximately 20 related aflatoxin metabolites, although only B1, B2, G1 and G2 are normally found in food [2]. Of these, aflatoxin B1 is the most biologically active and most commonly encountered [3].

Aflatoxins can occur in milk as a result of dairy animals consuming contaminated feed. The main residue of concern in milk is aflatoxin M1, the major metabolite of B1. The intake of contaminated milk, even at low concentrations, is a significant threat to human health, especially to children who are a major consumer of dairy products. Therefore, the US Food and Drug Administration has established a tolerance of 0.50 µg/kg for aflatoxin M1 in milk [4], while the European Union has imposed more stringent limits - 0.050 µg/kg in raw milk and 0.025 µg/kg in infant formula [5]. No limits have been established for aflatoxin B1, B2, G1 and G2 in milk.

This application note outlines a method for the low level determination of aflatoxins in milk using a polymeric solid-phase extraction (SPE) cartridge. Analysis is performed by LC-MS/MS using a Selectra® DA HPLC column. The method was optimized to allow the detection of aflatoxins at the low regulatory concentrations required. Recovery studies were carried out by spiking whole milk at two concentration levels (0.025 and 0.5 µg/kg). Matrix-matched calibration curves, ranging from 0.01-2 µg/kg, were used for quantitation. The mean recovery was found to be in the range of 84 to 100%, and repeatability was ≤7%.

Procedure:

Aflatoxins are relatively unstable in light and air, particularly in polar solvents or when exposed to oxidizing agents, ultraviolet light, or solutions with a pH below 3 or above 10. They should be protected from ultraviolet light as much as possible.

1. **Sample extraction 1 (aqueous extraction)**
   a) Weigh 20 g of milk into a 50 mL polypropylene centrifuge tube.
   b) Add 200 µL of glacial acetic acid.
   c) Vortex for 5 minutes to deproteinize the milk.
   d) Centrifuge for 5 minutes at ≥ 4000 *g*.

2. **SPE extraction**
   a) Condition SPE cartridge with:
      a) 1 × 3 mL methanol.
      b) 1 × 3 mL ultrapure water.
   b) Apply the supernatant to the SPE cartridge, taking care to avoid any transfer of the lipid layer. If required, use a low vacuum to draw the sample through (≤5 mL/min).

3. **Sample extraction 2 (solvent extraction)**
   a) Add 10 mL acetone to any residual milk solids from the aqueous extraction.
   b) Vortex for 2 minutes to extract the aflatoxins.
   c) Centrifuge for 5 minutes at ≥ 4000 *g*.
   d) Transfer the supernatant to a clean polypropylene tube and evaporate to ≤0.5 mL at 50°C under a gentle stream of nitrogen.
   e) Add 10 mL ultrapure water and vortex briefly.
   f) Apply sample to the SPE cartridge (same cartridge as step B).

4. **Wash cartridge**
   a) 1 × 3 mL ultrapure water.
   b) 1 × 3 mL 50% methanol.
   c) Dry cartridge under vacuum (≥10 inHg) for 5-10 minutes to remove residual water.
   d) 1 × 3 mL hexane.
   e) Dry cartridge under vacuum (≥10 inHg) for 1 minute to remove residual hexane.

5. **Elution**
   a) Elute the aflatoxins with 4 mL acetone.
   b) Evaporate the sample to dryness at 50°C under a gentle stream of nitrogen.
   c) Reconstitute in 1 mL of methanol:water (50:50, v/v).
   d) Filter extract with a 0.22 µm nylon (or other suitable membrane) syringe filter into an autosampler vial.

LC-MS/MS Conditions:

HPLC Conditions	
Instrumentation	Thermo Scientific™ Dionex™ Ultimate™ 3000
HPLC column	UCT Selectra® C18, 100 × 2.1 mm, 3 µm **(p/n: SLC-18100ID21-3UM)**
Guard column	UCT Selectra® C18, 10 × 2.0 mm, 3 µm **(p/n: SLC-18GDC20-3UM)**
Guard column holder	**p/n: SLGRDHLDR**
Column temp.	40°C
Mobile phase A	Water + 0.1% formic acid
Mobile phase B	Acetonitrile + 0.1% formic acid
Flow rate	300 µL/min
Gradient	0 min (5% B), 5-6 min (hold 100% B), 6.1-11 min (equilibrate 5% B)
Injection volume	20 µL
Autosampler temp.	10°C
Wash solvent	Methanol
Divert valve	Divert to waste at 0-4 and 6-11 min to reduce ion source contamination

MS Conditions	
Instrumentation	Thermo Scientific™ TSQ
Ionization mode	ESI^+
Spray voltage	3500 V
Vaporizer	400°C
Capillary	350°C
Sheath gas	55 arbitrary units
Auxiliary gas	45 arbitrary units
Ion sweep gas	0 arbitrary units
Declustering	0 V
Q1 and Q3 peak	0.2 and 0.7 Da
Collision gas	Argon
Collision gas	2.2 mTorr
Acquisition	EZ method (scheduled
Cycle time	0.6 sec
Software	Xcalibur™ version 2.2

SRM Transitions							
Analyte	**t_R (min)**	**Precursor ion**	**Product ion 1**	**CE 1**	**Product ion 2**	**CE 2**	**S-lens (V)**
Aflatoxin	4.7	329.0	273.0	21	259.0	23	120
Aflatoxin	4.9	331.0	245.0	27	189.0	38	115
Aflatoxin	5.0	329.0	243.0	25	199.0	46	117
Aflatoxin	5.0	315.0	287.0	24	259.0	27	111
Aflatoxin	5.2	313.0	241.0	35	285.0	21	111

Results and Discussion:

Accuracy & Precision Data for Whole Milk				
	0.025 µg/kg (n=5)		0.5 µg/kg (n=5)	
	Mean Recovery (%)	RSD (%)	Mean Recovery (%)	RSD (%)
Aflatoxin M1	94.43	3.54	90.91	4.31
Aflatoxin B1	89.82	4.19	84.34	4.31
Aflatoxin B2	93.27	3.76	88.27	7.51
Aflatoxin G1	92.51	5.48	89.28	7.06
Aflatoxin G2	100.05	1.71	93.51	6.83

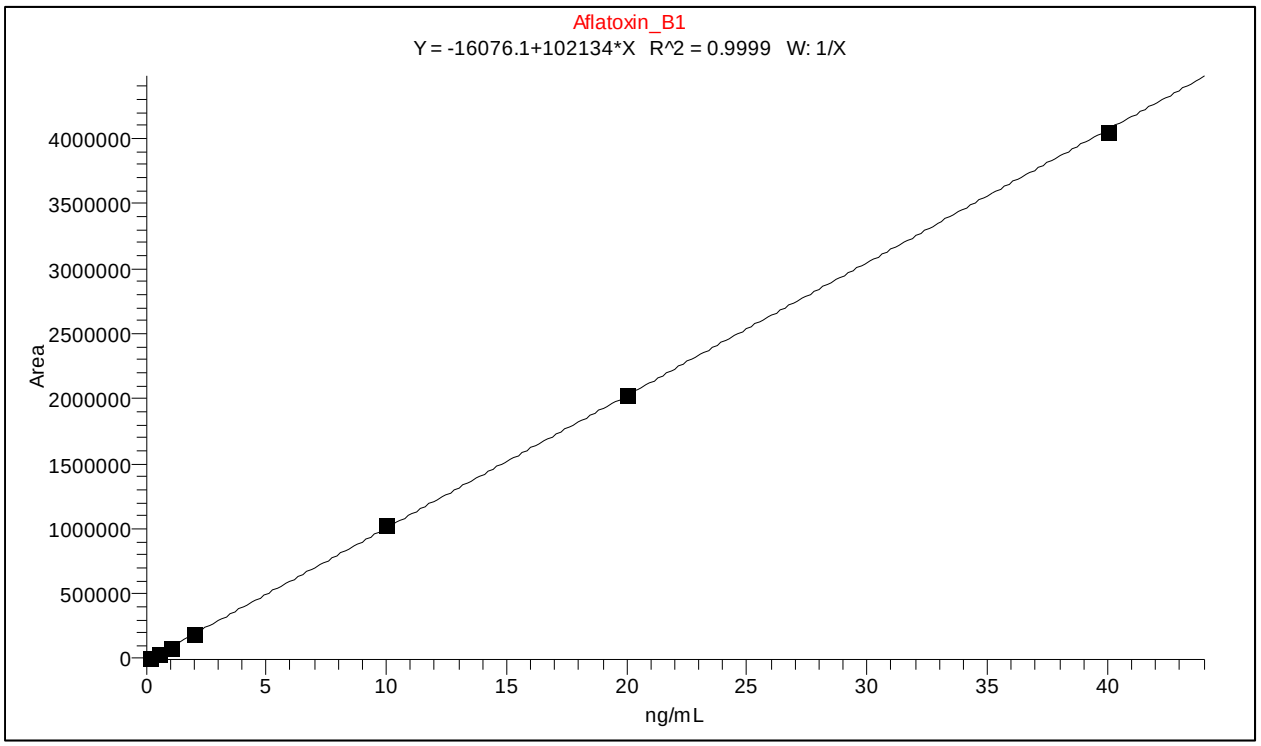

Figure 1. Example of a seven point matrix-matched calibration curve (0.2, 0.5, 1, 2, 10, 20 and 40 ng/mL; equivalent to 0.01, 0.025, 0.05, 0.1, 0.5, 1 and 2 µg/kg in milk).

Chromatograms

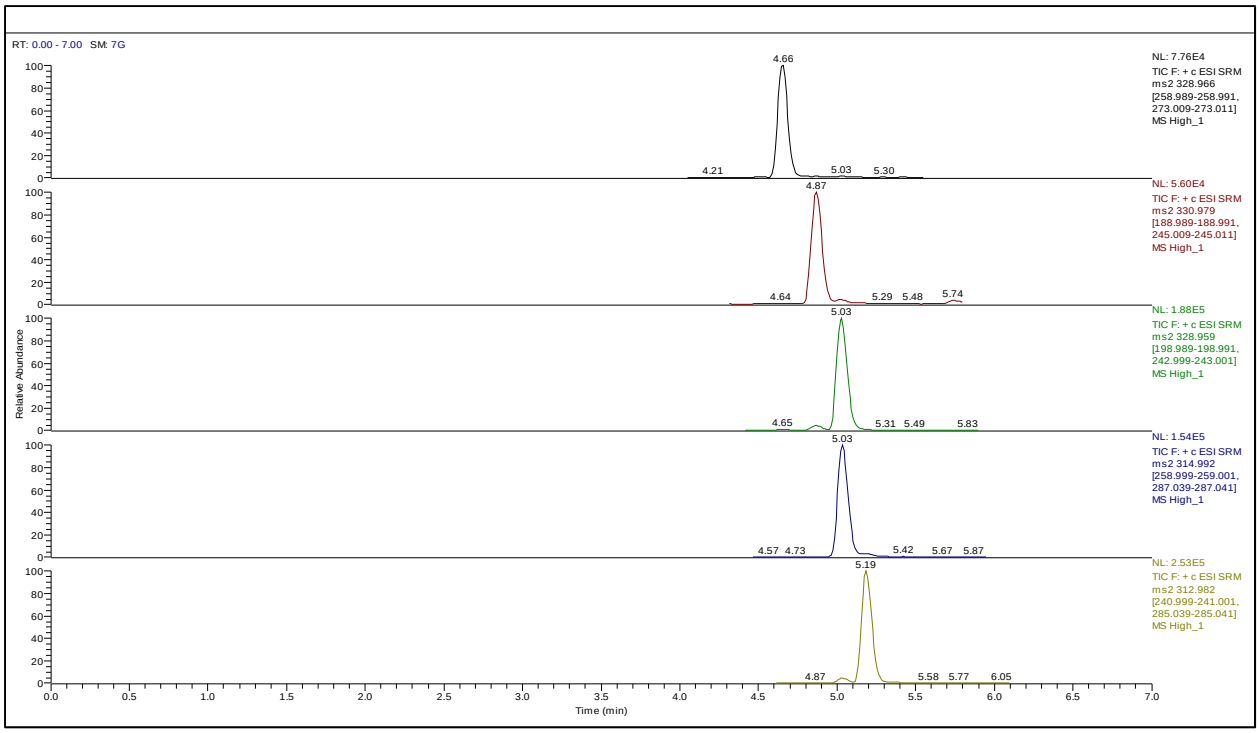

Figure 2. Chromatogram of an extracted milk sample fortified at 0.5 µg/kg.

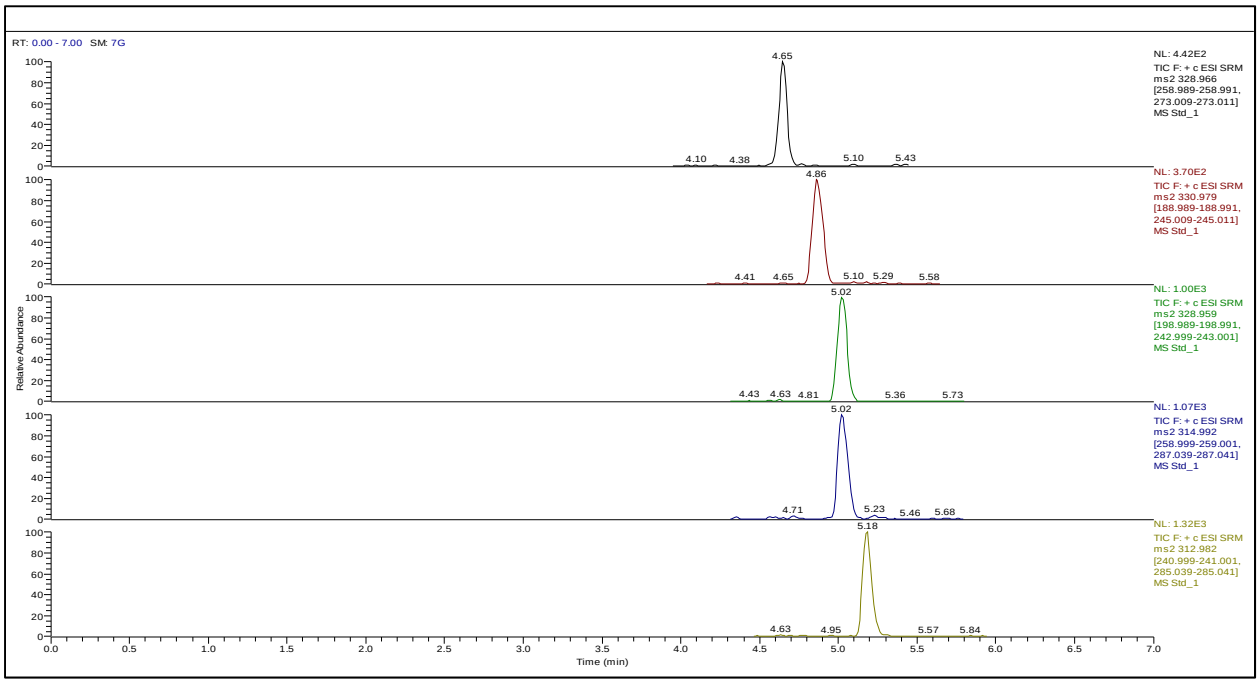

Figure 3. Chromatogram of the lowest matrix-matched calibration point (0.01 µg/kg).

Prior to instrumental analysis, a sample pre-treatment step is required to concentrate the analyte(s) of interest and eliminate non-desirable matrix components. This is particularly important for the analysis of aflatoxins in milk because of the low regulatory limits established for aflatoxin M1 and the larger sample size required to obtain the necessary method sensitivity. SPE is ideally suited to achieving these objectives. One of the biggest difficulties in milk analysis is the high fat and protein content that can often interfere with instrumental analysis. The sample preparation procedure was therefore optimized to remove as much co-extracted matrix components as possible.

Initially, a simple deproteinization step using acetic acid followed by centrifugation was executed to separate the proteins and lipids prior to SPE extraction. However, the recoveries were found to be low (<40%) using this approach, which is most likely caused by the adsorption of the aflatoxins onto proteins or lipids in the milk. It was determined that a solvent extraction step was necessary to adequately extract the aflatoxin residues from milk prior to SPE cleanup. Due to the high water content of milk, direct extraction with an organic solvent would result in a large volume of supernatant that could not be directly applied to the SPE cartridge (organic content too high) or require a time consuming evaporation step to remove the solvent. As a result, a two-step extraction procedure incorporating an initial aqueous extraction step was included in the final method. This simple step removes most of the water from the sample prior to a second extraction with acetone, a volatile organic solvent that is readily removed by evaporation. This extract is then reconstituted in water prior to application to the SPE cartridge. The SPE sorbent was washed with 50% methanol to remove medium to highly polar matrix components and hexane to remove lipophilic compounds. Acetone was used as the SPE elution solvent as it was found to be more effective than methanol and very easy to remove by evaporation. Filtration of the final sample extract prior to LC-MS/MS analysis and the use of matrix-matched calibration curves and/or isotopically labeled internal standards are recommended to obtain optimal results.

References:

1. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, International Agency for Research on Cancer (IARC) website, http://monographs.iarc.fr/ENG/Classification/index.php (accessed June 2015).
2. European Mycotoxins Awareness Network website, http://services.leatherheadfood.com/eman/FactSheet.aspx?ID=6 (accessed June 2015).
3. European Food Safety Authority website, http://www.efsa.europa.eu/en/topics/topic/aflatoxins.htm (accessed June 2015)
4. FDA Compliance Policy Guide, Sec. 527.400 Whole Milk, Lowfat Milk, Skim Milk - Aflatoxin M1.
5. Commission Regulation (EU) No 165/2010. Amending Regulation (EC) No 1881/2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs as Regards Aflatoxins. *Official Journal of the European Union*, Feb 26, 2010, pp L 50/8 – L 50/12.

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Determination of Ephedra Alkaloids & Synephrine by Strong Cation Exchange SPE and LC-MS/MS Detection Using a Pentaflurophenylpropyl HPLC Column

UCT Part Numbers:
CUBCX1HL56 – High-Load Benzenesulfonic Acid , 500 mg/6 mL SPE cartridge
SLPFPP100ID21-3UM – Selectra® PFPP, 100 × 2.1 mm, 3 µm HPLC column
SLPFPPGDC20-3UM – Selectra® CPFPP, 10 × 2.0 mm, 3 µm guard cartridge
SLGRDHLDR – Guard cartridge holder

Introduction

Ephedra alkaloids are phenethylamines that occur naturally in plants, including the herb *Ma Huang* used in traditional Chinese medicine. Ephedra alkaloids are potent CNS stimulants and also have a sympathomimetic effect on the peripheral nervous system. Some active ingredients of these plants are used as ingredients in cold remedies (e.g. pseudoephedrine). The ephedra alkaloids are also incorporated into dietary supplements to promote weight loss or to increase alertness and physical activity (e.g. body building). However, severe contraindications have been reported for individuals with hypertension or other cardiovascular diseases, particularly when used in combination with caffeine [1]. Products containing ephedrine were popular dietary supplements until the FDA banned their use in 2004 [2]. Since then the active ingredient in dietary supplements has largely been replaced by synephrine, a naturally occurring alkaloid found in plants such *Citrus* fruits; it is similar in structure to ephedrine.

The ephedra alkaloids are small, hydrophilic, basic analytes that are difficult to retain and separate on traditional HPLC columns using alkyl-bonded stationary phases. They are capable of strongly interacting with free silanols on the surface, which leads to peak tailing of the analytes and affects the resolution and quantification. Current methodologies used for the separation of ephedra alkaloids use reversed- phase columns with ion-pairing reagents, time-consuming derivatization procedures, or use strong cation-exchange phases. However, these approaches are not very amenable to LC-MS/MS analysis. An alternative approach

to traditional alkyl phases is the use of a fluorinated stationary phase. In addition to dispersive interactions available on traditional alkyl phases, pentafluorophenylpropyl phases can undergo dipole–dipole, and pi–pi interactions. This imparts unique selectivity to the column that can sufficiently resolve the ephedra alkaloids.

The aim of this study was to develop a multi-analyte procedure for the extraction, cleanup, and quantification of the ephedra alkaloids in functional foods and natural products. High capacity strong cation-exchange SPE cartridges were used for the isolation of the phenethylamines from dietary supplements. HPLC separation, including separation of the stereoisomers, was carried out using a UCT Selectra® PFPP column prior to detection by LC-MS/MS.

Sample Preparation Procedure

1. Sample Extraction

a) Weigh 1 ± 0.1g of sample into a 15 mL polypropylene centrifuge tube.
   - For this study a dietary supplement (1 g tablet) for weight loss was used.

b) Add 10 mL of 1% formic acid to each sample.

c) Shake or vortex sample for 15 minutes to fully extract the ephedra alkaloids. Ensure tablet samples are fully dissolved.
   - For this study a SPEX® SamplePrep® GenoGrinder® was used.

d) Centrifuge the sample for 10 min at 3000 *x* g and 4°C.

2. Condition Cartridge

a) Add 2 ×4 mL of methanol to **CUBCX1HL56** SPE cartridge.

b) Add 4 mL of ultrapure water.

c) Add 4 mL of 1% formic acid

Note: Do not let the cartridge go dry otherwise repeat steps a) through c).

3. SPE Extraction

a) Load supernatant from step 1d).

b) Allow sample to percolate through the cartridge or apply a vacuum if necessary (adjust vacuum for flow of 1–3 mL per minute).

4. Second extraction (Optional)

a) Add 5 mL of 1% formic acid to each sample.

b) Shake or vortex sample for 5 minutes.

c) Centrifuge the sample for 10 min at 3000 $\times$ g and 4°C.

d) Apply supernatant to the SPE cartridge.

5. Wash Cartridge

a) Add 2 × 4 mL of 0.1% formic acid and slowly draw through.

b) Add 2 × 4 mL methanol and slowly draw through.

c) Dry under vacuum for 30 sec to remove excess solvent.

6. Elute Cartridge

a) Elute the ephedra alkaloids using 8 mL of methanol containing 2% ammonium hydroxide.

b) Evaporate off the methanol solvent at 40°C under a gentle stream of nitrogen until it reaches a volume of 1 mL.

c) Add 1 mL of aqueous mobile phase (10mM ammonium acetate).

d) Evaporate off any remaining methanol.

- Ephedra alkaloids are similar to amphetamines, which are known to be volatile compounds. Therefore, extra care was taken during the evaporation step to avoid any potential loss in recovery that may occur during this step.

e) Vortex the samples for 1 min and filter through a 0.2 µM syringe filter directly into a HPLC vial.

LC-MS/MS Conditions:

HPLC Conditions	
Instrumentation	Thermo Scientific™ Dionex™ Ultimate™ 3000 LC system
HPLC column	UCT Selectra® PFPP, 100 × 2.1 mm, 3 µm (p/n: SLPFPP100ID21-3UM)
Guard column	UCT Selectra® PFPP, 10 × 2.1 mm, 3 µm, (p/n: SLPFPPGDC20-3UM)
Guard column	p/n: SLGRDHLDR
Mobile phase A	10 mM ammonium acetate
Mobile phase B	methanol
Isocratic elution	85:15 (A:B, v:v)
Flow rate	500 µL/min
Column temp.	50°C
Run time	20 min
Injection volume	5 µL
Autosampler temp.	10°C
Wash solvent	methanol: water (1:1, v/v)
Divert valve	mobile phase was sent to waste for 1.5 min to reduce ion source

MS Conditions	
Instrumentation	Thermo Scientific™ TSQ Vantage™ tandem mass
Ionization mode	ESI^+
Spray voltage	4500 V
Vaporizer temperature	400°C
Capillary temperature	300°C
Sheath gas pressure	60 arbitrary units
Auxiliary gas pressure	55 arbitrary units
Ion sweep gas	0 arbitrary units
Declustering potential	2 V
Q1 and Q3 peak width	0.2 and 0.7 Da
Collision gas	argon
Collision gas pressure	1.8 mTorr
Acquisition method	EZ method (scheduled SRM)
Cycle time	2 sec
Software for data processing	TraceFinderTM version 3.0
Weighting factor applied to calibration	1/X

SRM Transitions							
Analyte	t_R (min)	Precursor ion	Product ion 1	CE 1	Product ion 2	CE 2	S-lens (V)
Ephedrine	9.9	166.17	91.0	29	115.02	22	46
Pseudoephedrine	11.4	166.17	91.0	29	115.02	22	46
Norephedrine	5.6	152.141	91.0	31	115.02	22	38
Norpseudoephedrine	6.7	152.141	91.0	31	115.02	22	38
Methylephedrine	14.7	180.17	90.99	31	147.04	15	52
Synephrine	2.1	168.10	90.9	19	106.97	29	37
Ephedrine-d_3 (IS)	9.9	169.17	90.9	31	115.00	24	49
Pseudoephedrine-d_3	11.4	169.17	90.9	31	115.00	24	49

Results

Accuracy & precision Data for 6 the Ephedra Alkaloids at 100 ppb (n=5)						
	Ephedrine	Pseudoephedrin	Norephedrine	Norpseudoephedrin	Methylephedrin	Synephrine
Sample 1	94.13	93.07	80.22	108.30	79.8	47.35
Sample 2	91.62	94.28	48.49	88.11	79.9	38.14
Sample 3	92.62	92.74	63.24	89.02	89.7	52.41
Sample 4	92.60	93.56	61.63	75.05	79.4	45.43
Sample 5	93.39	93.56	49.86	70.75	83.2	51.85
Mean	**92.87**	**93.44**	**60.69**	**86.25**	**82.44**	**47.04**
RSD	**1.02**	**0.62**	**21.08**	**17.03**	**5.27**	**12.30**

*__Note:__ Synephrine is more polar than the ephedra alkaloids and is not as well retained on the sorbent. It is recommended to include an isotopically labeled internal standard in order to achieve the best results.

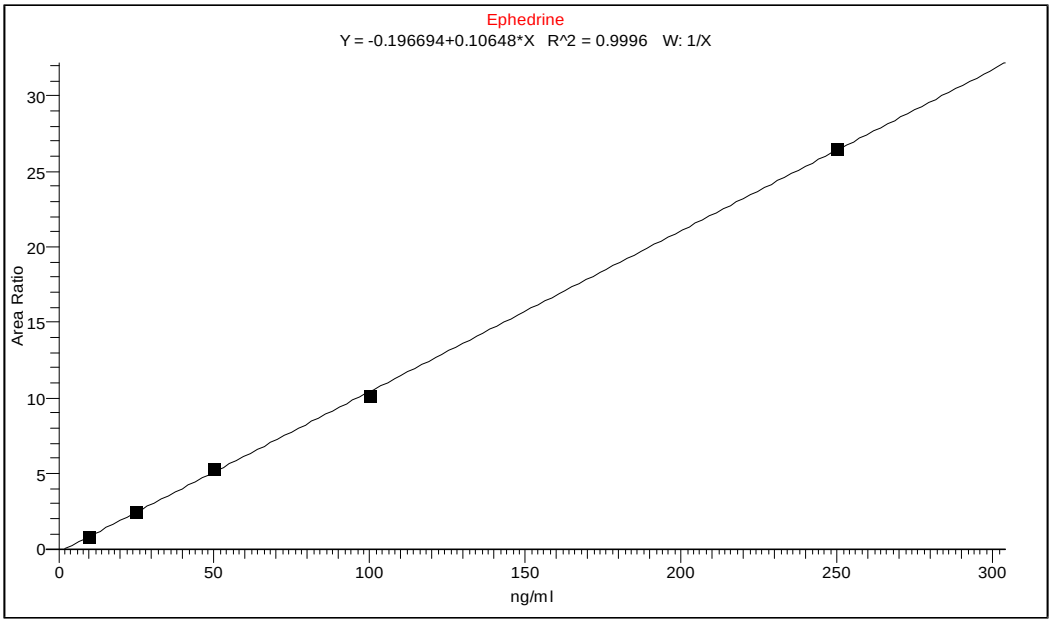

Figure 1. Example calibration curve (ephedrine) over a 10-250 ng/mL concentration range.

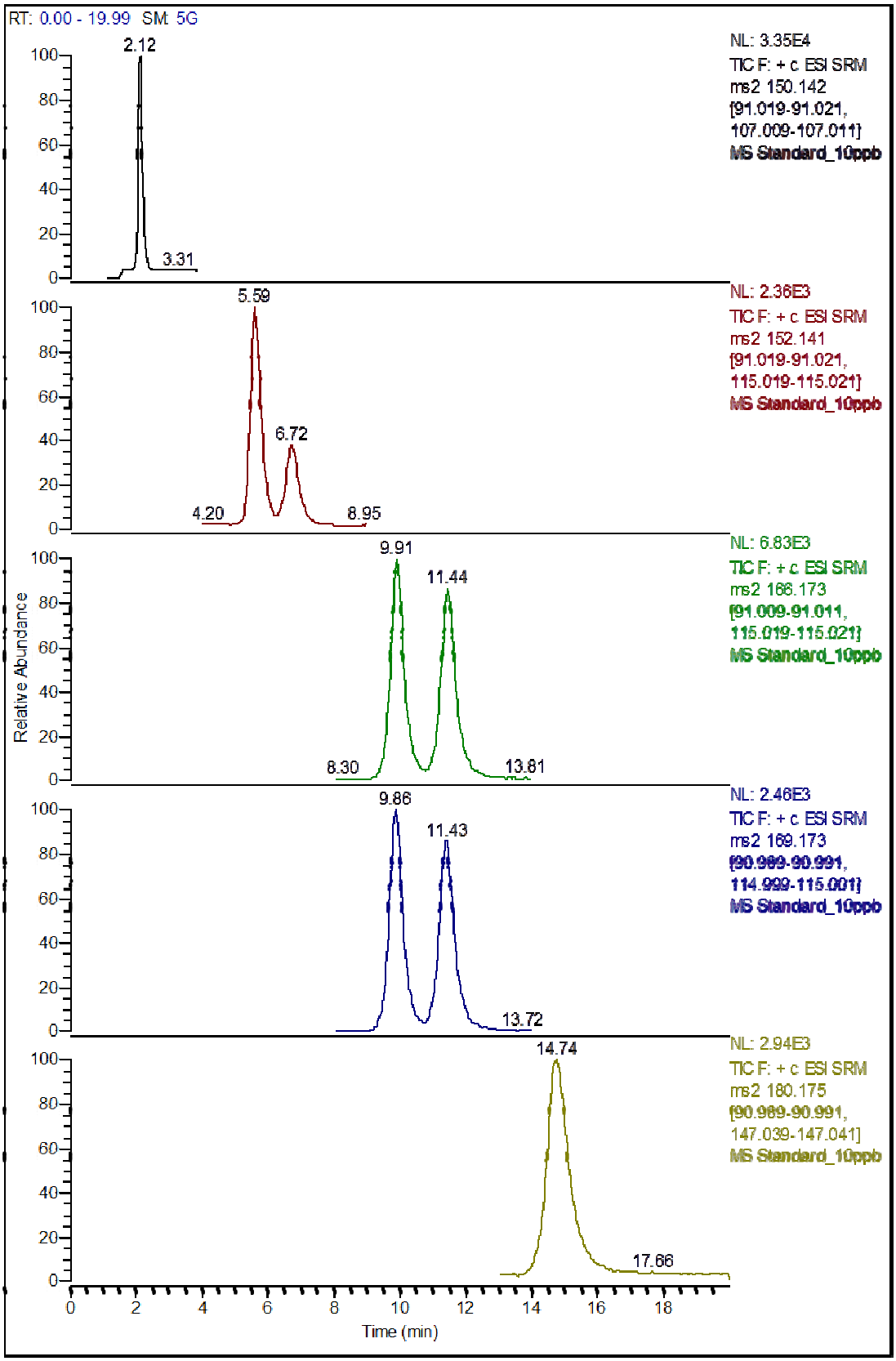

Figure 2. Chromatogram of a 10 ng/mL standard.

Conclusions:

- A method was successfully developed for the extraction, cleanup, and quantification of the ephedra alkaloids and synephrine in functional foods and natural products.
- Strong cation-exchange SPE was used to isolate the phenethylamines from the complex sample matrix, which consisted of 9 herbal extracts containing a high concentration of calcium, caffeine and additional excipients.
- A high capacity SPE sorbent was used as it offers better retention than standard SCX sorbent.
- It is recommended to include isotopically labeled internal standards into the method, particularly for the hydrophilic synephrine which is not as efficiently retained by SPE as the ephedra alkaloids.
- HPLC separation of the 5 ephedra alkaloids and synephrine was successfully conducted on UCT's Selectra® PFPP column, including baseline resolution of the 2 sets of stereoisomers included in the method (ephedrine / pseudoephedrine and norephedrine / norpseudoephedrine).
- Good LC-MS/MS sensitivity was observed for all compounds (< 10 ng/mL).

References

[1] Journal of Chromatography A, 1161 (2007) 71–88

[2] Food and Drug Administration, Federal Registry 69 (2004) 6787

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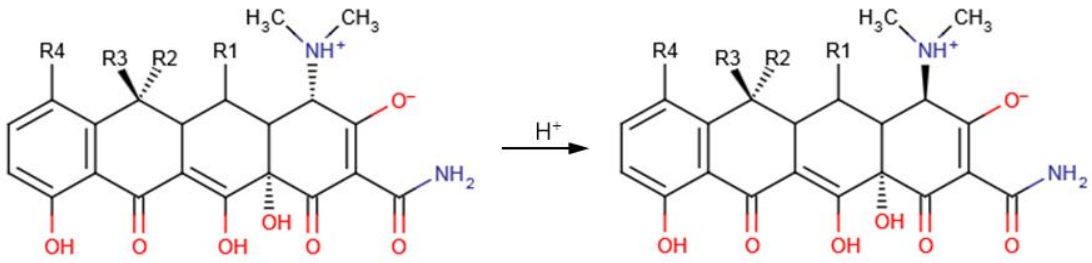
Determination of Tetracycline Antibiotics in Milk Using a Simple Strong Cation-Exchange SPE Cleanup Procedure and LC-MS/MS Analysis

UCT Product Numbers:
CSDAU206: 200mg / 6mL SPE cartridge
SLDA100ID21-3UM: Selectra® DA HPLC column, 100 x 2.1 mm, 3 µm
SLDAGDC21-3UM: Selectra® DA guard cartridge, 10 x 2.0 mm, 3 µm
SLGRDHLDR: Guard cartridge holder

Introduction

Tetracyclines (TC's) are broad spectrum antibiotics that are widely used in animal husbandry for the prevention, control and treatment of bacterial infections [1]. They are amphoteric molecules that always carry a charge and only achieve a "neutral" state as zwitterions. As a result they are highly polar molecules that are only soluble in polar organic solvents (e.g. alcohols) and acidic/basic solutions. TC's are prone to degradation under strongly acidic and alkaline conditions where they form anhydro-, iso- or epi-analogues [2]. Chlortetracycline (CTC), in particular, is vulnerable to alkaline decomposition and forms iso-CTC at high pH. Under mildly acidic aqueous conditions (pH 2–6) the TC's readily undergo epimerization at the C-4 position (amine). In addition to pH instability, TC's are also prone to degradation under certain light and redox conditions [2] (Figure 1.). All of the aforementioned degradation products can undergo additional epimerization or form alternative degradation products [3]. Under certain conditions TC's are capable of undergoing intramolecular H-bonding, while keto-enol tautomerization may also occur but appears to be temperature dependent [4].



Compound	R_1	R_2	R_3	R_4
Tetracycline	H	CH_3	OH	H
Oxytetracycline	OH	CH_3	OH	H
Chlortetracycline	H	CH_3	OH	Cl
Demeclocycline	H	H	OH	Cl
Doxycycline	OH	CH_3	H	H
Minocycline	H	H	H	$N(CH_3)_2$

Figure 1. Structure of tetracycline antibiotics and the acid-catalyzed epimerization at C-4 position.

To ensure food safety and prevent the unnecessary exposure of antibiotic drugs to consumers, TC's are typically included in national chemical residue surveillance plans. The EU has established a maximum residue limit (MRL) for tetracycline, oxytetracycline and chlortetracycline at 100 µg/kg in the muscle and milk of all food producing species [5]. An MRL of 100 µg/kg has also been established for doxycycline in muscle, but it is not allowed for use in animals from which milk is produced for human consumption. The MRL's are based on the sum of the parent compound and its 4-epimer. In the US, tolerances are established for the sum of tetracycline residues (including chlortetracycline, oxytetracycline and tetracycline) in muscle and milk at 2000 and 300 µg/kg, respectively [6].

TC's are difficult to analyze due to their instability, their tendency to form chelation complexes with multivalent cations (i.e. metals), their ability to bind with proteins, and their ability to interact with charged silanol groups on silica-based sorbents [7]. As such, it is important to take all these issues into account when developing a method or when doing routine analysis of these compounds. Most reported methods for TC analysis use lengthy sample preparation procedures or do little to no sample cleanup. As a result, there is a need for a simple method for the analysis of TC antibiotics in foods of animal origin. Liquid-liquid extraction/partitioning is difficult to perform due to TC's charge and low affinity for organic solvents. Therefore, solid-phase extraction (SPE) combined with LC–MS/MS analysis is the most widely used method for the determination of TC residues. Aqueous-based extraction is the primary extraction mechanism. EDTA-McIlvaine's buffer (pH 4) is the most frequently used extraction solvent. At this pH TC's exist as zwitterions and are in their most stable state. In addition, at this pH it is possible to sufficiently deproteinize biological samples prior to SPE cleanup. However, deproteinization can also be carried out under mildly acidic conditions using trichloroacetic acid (TCA), hydrochloric acid (HCl) or phosphoric acid [8]. The inclusion of EDTA in the extraction solvent minimizes the interaction of TC's with chelating complexes present in the sample [8] (Figure 2.). This is particularly important when extracting milk which contains a large amount of calcium.

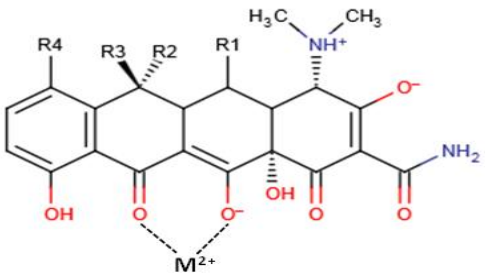


Figure 2. Chelation of divalent metal ions at the C-11 and C-12 position.

TC's chemical complexity and instability also causes problems during HPLC analysis, which often results in broad or tailing peaks and poor resolution. In addition, the difficulty in controlling or preventing epimer formation (at the C-4 position) during the LC process can result in poor peak resolution between the parent TC and its 4-epimer, which can lead to peaks of varying intensity and affect quantification. To overcome peak tailing it is common to prewash an LC column with EDTA prior to use or to use oxalic acid (a dicarboxylic acid with chelating properties) as mobile phase additive [8].

The aim of this study was to develop a simple but efficient procedure for the extraction, cleanup, and quantification of TC antibiotics in milk. Mixed-mode cartridges with a strong cation-exchange functionality were used for the isolation of the TC's from milk samples. HPLC separation was carried out using a Selectra® DA HPLC column prior to detection by mass spectrometry. This simple method allows for the rapid analysis of TC's in milk while achieving good accuracy, precision, and sensitivity without an evaporation step.

Procedure

1. Sample Extraction

a) Weigh 2 ± 0.1g of sample into a 15mL polypropylene centrifuge tube.
b) Add 10 mL of extraction buffer (50mM acetic acid + 10mM EDTA, pH 3.6) to each sample.
c) Shake or vortex samples for 15 minutes to deproteinize the sample and extract the tetracycline antibiotics.
    - For this work a SPEX® SamplePrep® GenoGrinder® was used (operated at 1500 rpm).
d) Centrifuge the samples for 10 min at 3000 rcf and 4°C.

2. Condition Cartridge

a) Add 3 mL of methanol to cartridge CSDAU206.
b) Add 3 mL of buffer (50mM acetic acid + 10mM EDTA, pH 3.6).

Note: Do not let the cartridge go dry otherwise repeat steps 2.a) and 2.b).

3. SPE Extraction

a) Load supernatant from step 1d).
b) Adjust vacuum for flow of 1–3 mL per minute.

4. Wash Cartridge

a) Add 3 mL of ultrapure H_2O and slowly draw through.
b) Add 3 mL of MeOH and slowly draw through.
c) Dry under vacuum for 1 minute to remove excess solvent.

5. Elute Cartridge

a) Elute the tetracyclines from the SPE cartridge using 3mL elution solvent (1M oxalic acid + 2% TEA in MeOH).
b) Vortex the samples for 2 min and transfer a 1 mL aliquot to an autosampler vial for analysis.

LC-MS/MS Conditions:

HPLC Conditions	
HPLC column	UCT Selectra® DA, 100 x 2.1 mm, 3 µm (p/n: SLDA100ID21-3UM)
Guard cartridge	UCT Selectra® DA, 10 x 2.1 mm, 3 µm (p/n: SLDAGDC21-3UM)
Guard Cartridge	p/n: SLGRDHLDR
Column temp.	40°C
Injection volume	5 µL
Flow rate	300 µL/min
Mobile Phase:	A: 1mM oxalic acid in ultrapure H_2O
	B: 1mM oxalic acid in MeOH

Gradient		
Time (min)	**A (%)**	**B (%)**
0.0	95	5
1.0	95	5
5.0	60	40
10.0	60	40
12.0	0	100
16.0	0	100
16.2	95	5
21.0	95	5

MS Conditions	
Instrumentation	Thermo Scientific™ TSQ Vantage™ tandem mass
Ionization mode	ESI^+
Spray voltage	5000 V
Vaporizer temperature	350°C
Capillary temperature	350°C
Sheath gas pressure	60 arbitrary units
Auxiliary gas pressure	10 arbitrary units
Ion sweep gas	2 arbitrary units
Declustering potential	2 V
Q1 and Q3 peak width	0.2 and 0.7 Da
Collision gas	Argon
Collision gas pressure	2.0 mTorr
Cycle time	2 sec

SRM Transitions							
Analyte	t_R (min)	Precursor ion	Product ion 1	CE 1	Product ion 2	CE 2	S-lens (V)
Thiabendazole-	8.6	208.00	137.05	31	181.03	25	43
Oxytetracycline	8.4	461.13	426.07	17	200.96	32	95
Tetracycline	9.0	445.10	410.04	18	153.91	24	79
Minocycline	10.0	458.10	441.09	16	282.98	41	136
Demeclocycline	10.3	465.10	448.04	16	430.02	19	99
Chlortetracycline	12.5	479.10	462.05	11	444.02	18	102
Doxycycline	13.2	445.08	428.08	16	321.01	28	90
Anydrotetracycline	14.0	427.15	410.01	16	153.98	24	105

Results and Discussion

Polymeric SPE cartridges, particularly those containing polar modified polymeric sorbent, have been used to extract TC's from food samples. A major drawback of using these cartridges is the inability to rinse the sorbent with an organic solvent, which leads to "dirty" sample extracts containing matrix co-extractives. An alternative approach is to use ion-exchange SPE to retain the TC residues on the sorbent, which can then be rinsed with 100% organic solvent. This is very effective at removing matrix interferences and results in a clean sample extract. Due to the instability and complex physicochemical properties of TC's, the SPE procedure needs to be carefully optimized in order to achieve optimal results.

Polymeric cation-exchange sorbents (strong and weak) were evaluated using a variety of different loading conditions (buffer type, pH and ionic strength) but the TC's were found to be inadequately retained. Ultimately, a silica-based sorbent containing a strong cation exchange component was found to be the best choice. Several different SPE elution solvents were evaluated but were not found to be effective at eluting the TC's from the SCX sorbent:

- 2% NH_4OH in MeOH – this is a commonly used elution solvent for eluting basic compounds from SCX sorbent. The high pH neutralizes amino functional groups on the analyte(s) which allows them to be eluted from the sorbent. However, it was not effective at eluting the strongly retained TC's. In addition, NH_4OH is not recommended for use with TC's due to potential alkaline degradation.
- 2% TEA in MeOH – also provides a high pH to neutralize amino functional groups, but does not contain OH^- ions.
- Triethylammonium formate (1% TEA + 0.5% formic acid in MeOH) – At a low pH, the TEA is fully ionized and forms a protonated tertiary amine, which is capable of acting as a counter-ion to displace positively charged analytes from the SCX sorbent.

Accuracy & Precision Data for Tetracycline Antibiotics in Milk (100ppb, n=5)						
	Tetracycline	Oxytetracycline	Demeclocycline	Chlortetracycline	Doxycycline	Minocycline
Sample 1	74.7	53.4	86.7	87.6	88.8	129.2
Sample 2	70.8	52.0	87.7	85.4	91.4	128.9
Sample 3	75.5	51.9	88.7	85.7	86.4	126.6
Sample 4	87.4	58.1	80.8	87.1	99.7	136.3
Sample 5	84.4	66.9	74.6	83.8	91.5	153.4
Mean	**78.6**	**56.5**	**83.7**	**85.9**	**91.5**	**134.9**
RSD	**9.0**	**11.3**	**7.1**	**1.7**	**5.5**	**8.13**

An alternative elution approach to pH manipulation is the use of a high salt concentration (1 M) to disrupt the ionic interaction between the analyte(s) and ion-exchange sorbent. It was determined that MeOH containing 1M oxalic acid was required to elute the TC's from the ion-exchange sorbent. Oxalic acid was used because it is a good metal chelating agent, which is necessary for the elution of TC's from silica-base sorbent. Lower concentrations of oxalic acid were not as effective, which is similar to the results reported by Pena *et al.* [9]. Minocycline, containing an additional amino functional group, did not elute with 1M oxalic acid. Therefore, 2% TEA was incorporated into the extraction solvent to act as a counter-ion and displace the minocycline from the sorbent. The eluted extracts can be analyzed directly by LC-MS/MS. If lower sensitivity is required (low ppb range), an evaporation step could be included although great care should be taken (low temperature and N_2 flow).

Oxalic acid is used as a mobile phase additive in TC analysis due to its good chelating properties, which significantly improves peak shape over traditional mobile phase additives (e.g. formic acid or acetate buffer). Oxalic acid also gives a suitable pH (2) for efficient ESI^+ ionization of the TC's. A low amount (1mM) of oxalic acid was used as mobile phase additive because oxalic acid is not as volatile as alternative buffers. However, oxalic acid is widely used in TC analysis and can be employed as long as the HPLC column is sufficiently rinsed after each run, no oxalic acid is allowed to remain stagnant in the HPLC column for long periods of time, and the MS source is periodically cleaned (i.e. routine maintenance). The inclusion of TEA in the final sample extract improved peak shape by acting as an ion-pairing agent and reducing peak tailing of the TC residues.

Other problems faced during LC-MS/MS analysis of TCs include isobaric interference and the formation of epimers and/or degradation products. Isobaric interference is observed between tetracycline and doxycycline as they have similar *m/z* values and common fragment ions that cannot be distinguished by a triple quadrupole mass spectrometer. Therefore, sufficient LC separation (4 min) was obtained between the two compounds to overcome this problem (not obvious in Figure 4 due to the use of time-segmented acquisition). Isobaric interference and degradation products can also be generated from other TCs (e.g. tetracycline and anhydrotetracycline, and CTC and demeclocycline). As can be seen in Figure 4, chlortetracycline and demeclocycline have 2 peaks each. For CTC there is a peak at 12.4 min corresponding to the analyte and a secondary peak at 10.36 min, which could correlate to domeclocycline (10.29 min), an epimer or an alternative degradation product. For demeclocycline, there is a peak at 10.3 min corresponding to the analyte and a secondary peak at 8.9 min (epimer or degradation product).

Epimerization can potentially create difficulties in accurate quantification as the epimer peaks can be equal or larger than the parent TCs. Epimers have the same *m/z* values (parent and product ions), similar abundances and usually elute close to the parent TCs. It is often not possible to separate the epimers from the parent TCs and some analysts will use a fast gradient so that the epimers co-elute with the TCs. The Selectra DA® column is capable of separating out the epimers. However, epimerization was not a major problem in this work. Some minor peaks can be observed in the chromatograms but they do not affect the quantification. Lastly, if the final sample extracts (and calibration curve/QC standards) are prepared in an aqueous solution, particularly acidic solutions, epimerization is readily observed. However, if the final sample extracts are prepared in organic solvent the epimerization is drastically reduced, which limits the formation of a large epimer peaks and simplifies quantification.

Anhydrotetracycline was initially included in the method but was found to degrade rapidly on contact with the extraction buffer. The analyte is displayed in the chromatogram for informational purposes only and was not included in the final quantitative method. Thiabendazole-$^{13}C_6$ was included as an internal standard in the method but
there was no improvement in results when it was incorporated into the calculations. Therefore, it was excluded from the calculations and only used as a QC standard. If an isotopically labeled internal standard for one (or more) of the TC's is available, its inclusion in the method would be an obvious advantage. However, at the time of this research no appropriate isotopically labeled internal standard could be commercially sourced.

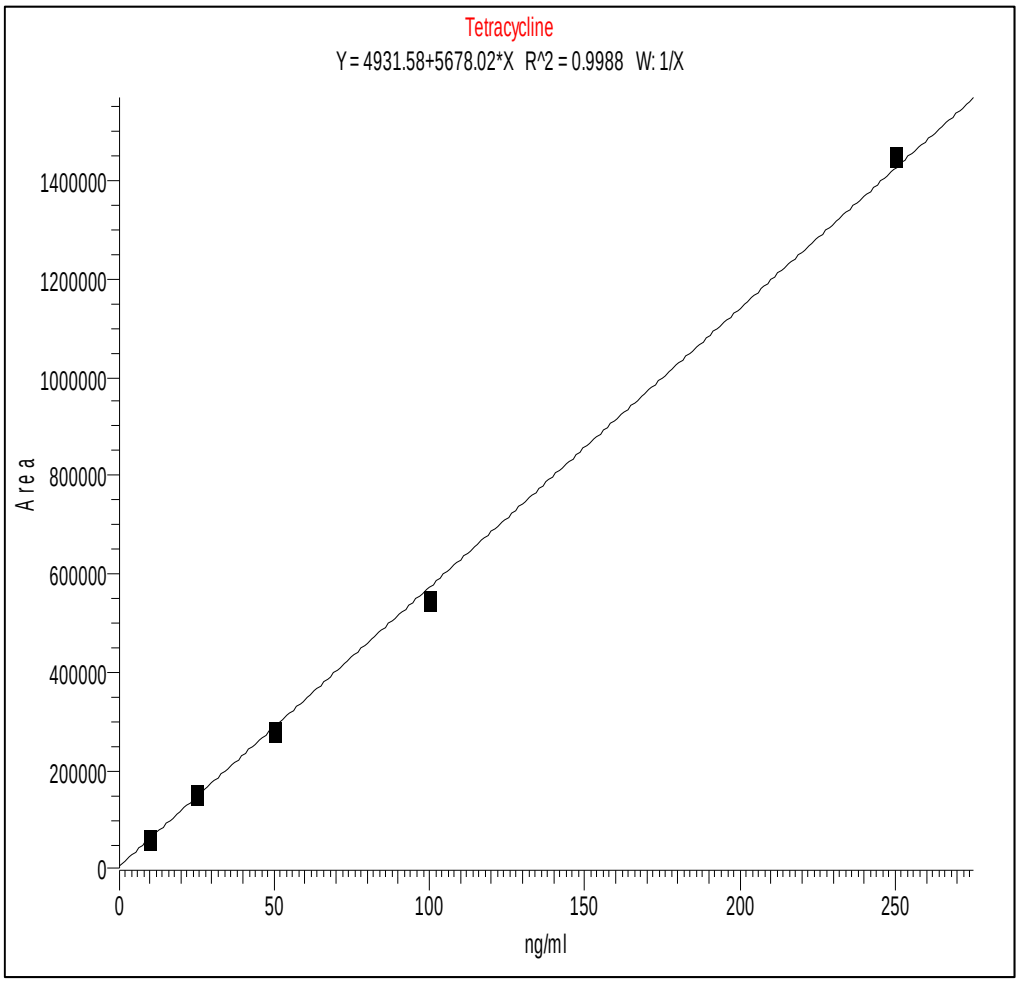


Figure 3. Calibration curve example (tetracycline).

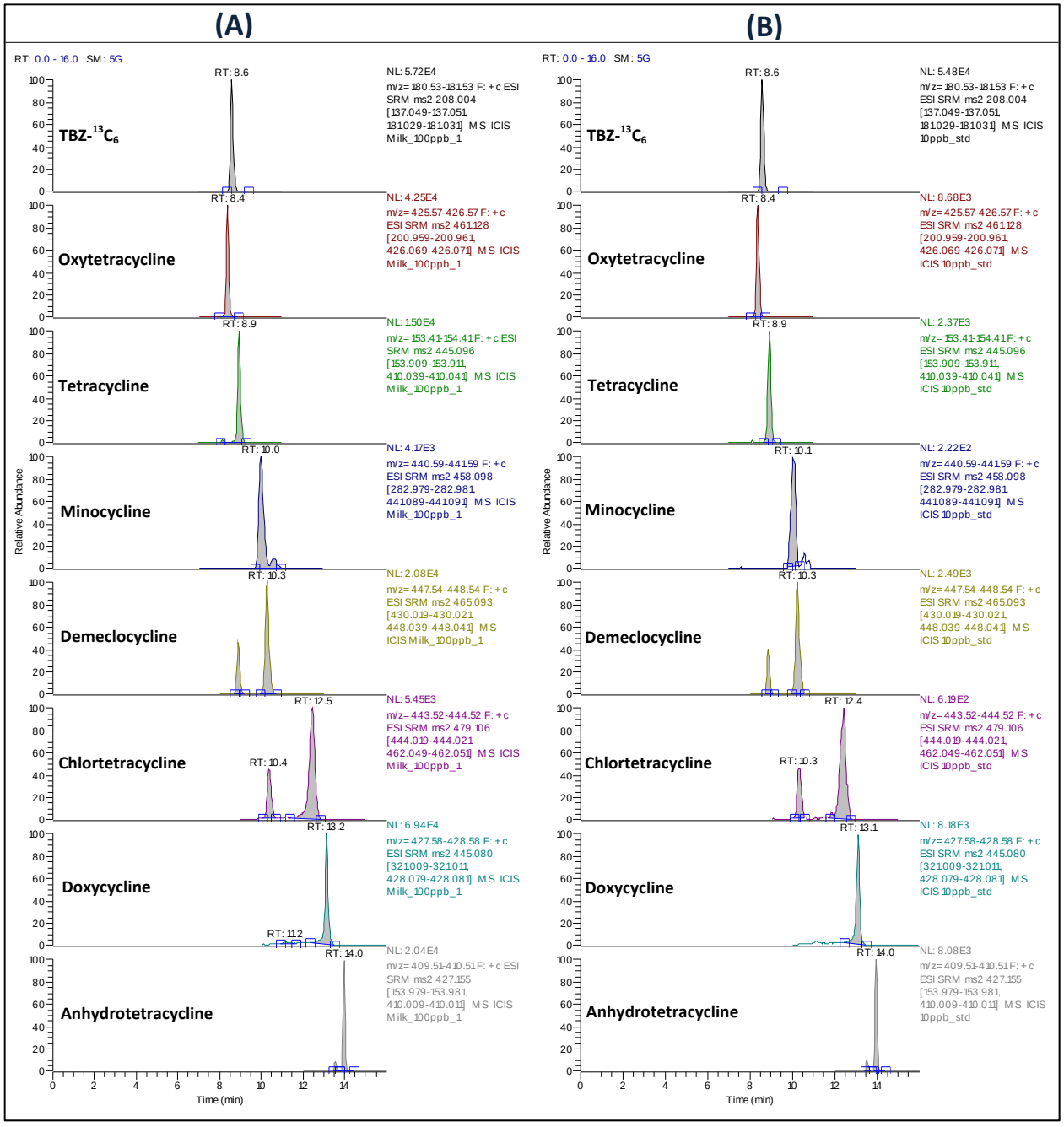


Figure 4. Chromatogram of **(A)** a milk sample fortified with tetracycline antibiotics at 100 ng/g (I.S. at 25 ng/g) and **(B)** a neat standard equivalent to 10 ng/g (I.S. at 25 ng/g).

Conclusions

A simple, fast, and cost effective SPE and LC-MS/MS method was developed for the quantitative detection of six tetracycline antibiotics (TC's) in milk. Samples were extracted with pH 3.6 acetate buffer containing EDTA, and SPE cleanup was carried out using mixed-mode cartridges containing a strong cation exchange component. Separation of the TC's was achieved within 16 min on a Selectra® DA HPLC column. The use of LC-MS/MS detection provided sufficient selectivity and sensitivity for the identification and quantification of the TC's. Good accuracy and precision were obtained for these difficult compounds. No evaporation step is included in the method which avoids any potential loss during this step. Based the lowest calibration point used, the method is capable of detecting TC residues at <10 ng/g. The accuracy and precision of the method could be further improved by incorporating an isotopically labeled internal standard for one of the TC's (if available). The method outlined here provides an attractive alternative to currently used methods for TC analysis.

References:

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A Determination of Veterinary Drug Residues in Milk Using Polymeric SPE and UHPLC-MS/MS Analysis

UCT Part Numbers:
ECHLD126-P – EnviroClean® HLDVB , 200 mg/6mL SPE cartridge, PE Frit
SLDA100ID21-18UM – Selectra® DA, 100 × 2.1 mm, 1.8 µm HPLC column
SLDAGDC20-18UM – Selectra® DA, 10 × 2.0 mm, 1.8 µm guard cartridge
SLGRDHLDR – Guard cartridge holder

Summary:

This application note outlines a multi-class, multi-reside method for the determination of 49 representative veterinary drugs in milk using a simple, solid-phase extraction (SPE) procedure and analysis by UHPLC-MS/MS. To achieve fast and simultaneous extraction of the various drug residues, a generic liquid extraction procedure using EDTA/acetic acid buffer is conducted prior to extraction on a polymeric SPE cartridge. UHPLC separation is carried out with a Selectra® DA column, which exhibits alternative selectivity to a C18 phase and is capable of enhanced retention for the more polar drugs. The method was evaluated for each compound at three varying concentrations (1, 10 and 100 g/kg). For most compounds, recoveries were between 70% and 120% and reproducibility was <20%. In addition, the majority of compounds could be accurately detected at a concentration of 1 g/kg, demonstrating that the presented method is sufficient to monitor a wide range of veterinary drugs in milk. The drugs investigated belonged to several different classes, including -agonists, macrolides, amphenicols, sulfonamides, tetracyclines and quinolones.

Introduction:

Veterinary drugs are frequently administered to food-producing animals, including dairy cows, to treat and prevent disease and/or increase growth rates. The inappropriate or illegal use of these drugs can result in the presence of their residues in food of animal origin which could pose a potential threat to human health. Milk is an important food commodity that is consumed by a large portion of the population, including infants. To ensure food safety and prevent the unnecessary exposure of consumers to veterinary drugs, it is vital to test milk for drug residues. The United States, European Union (EU), CODEX and other international organizations have established maximum residue limits

(MRLs) for veterinary drugs in a variety of biological matrices, including milk [1-3]. The MRLs for milk are typically lower than those set for other
biological matrices (muscle, liver and kidney) and span a wide concentration range (low µg/kg to >1000 µg/kg). In addition, a number of drugs are prohibited for use in food producing animals or are unauthorized for use in lactating animals and require very low detection limits (2 g/kg).

Milk is a complex matrix containing dissolved fats, carbohydrates, proteins and minerals (including calcium), which can complicate the development of a fast, easy and reliable analytical method for the identification and quantification of veterinary drug residues. Development of a multi-class, multi-residue (MMR) method can be challenging not only due to the inclusion of a large number of drugs with diverse physicochemical properties, but also on account of the complex sample matrix and the instability of certain drug classes (e.g. -lactams, tetracyclines and macrolides). A MMR method should ideally be capable of extracting a wide range of drugs, reduce major matrix interferences, obtain good analyte recovery, be reproducible and achieve adequate limits of detection (LOD's). The use of a generic sample preparation procedure, such as SPE using a polymeric sorbent, is a suitable approach for achieving these goals. Ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) is the detection system of choice for veterinary drugs as it allows rapid detection of trace-level residues in complex matrices. However, the diverse physicochemical properties of the veterinary drugs still pose challenges and analytical conditions must be optimized to obtain adequate sensitivity of all the compounds as well as good retention and peak shape of problematic compounds.

Sample Preparation Procedure:

A) Sample extraction

1. Weigh 5 g of milk into a 15 mL polypropylene centrifuge tube.
2. Add 5 mL of 0.1M EDTA-Na_2 + 2% acetic acid.
3. Vortex for 5 minutes to de-proteinize the milk.
4. Centrifuge for 5 minutes at 3500 *g*.

Note: A larger volume of extraction solvent or a second extraction of the milk sample

(5mL buffer) can be carried out if deemed necessary.

B) SPE extraction

1. Condition SPE cartridge with:

c) 1 × 3 mL methanol

d) 1 × 3 mL ultrapure water

2. Apply the supernatant to the SPE cartridge, taking care to avoid any transfer of the lipid layer. If required, use a low vacuum to draw the sample through (5 mL/min).

C) Wash cartridge

1. 1 × 3 mL ultrapure water.
2. 1 × 3 mL 10% methanol.
3. Dry cartridge under vacuum (10 inHg) for 5-10 minutes to remove residual water.
4. 1 × 3 mL hexane.
5. Dry cartridge under vacuum (10 inHg) for 1 minute to remove residual hexane.

D) Elution

1. Elute with 3 mL acetone.
2. Evaporate the sample to dryness at 35-40°C under a gentle stream of nitrogen.
3. Reconstitute in 1 mL of methanol:water (50:50, v/v).
4. Filter extract with a 0.22 µm nylon (or other suitable membrane) syringe filter into an autosampler vial.

LC-MS/MS Conditions:

HPLC Conditions	
Instrumentation	Thermo Scientific™ Dionex™ Ultimate™ 3000
HPLC column	UCT Selectra® DA, 100 × 2.1 mm, 1.8 µm (p/n: SLDA100ID21-18UM)
Guard column	UCT Selectra® DA, 10 × 2.1 mm, 1.8 µm (p/n: SLDAGDC20-18UM)
Guard column	p/n: SLGRDHLDR
Column temp.	60°C
Flow rate	400 µL/min
Injection volume	5 µL
Autosampler temp.	10°C
Wash solvent	Methanol
Divert valve	Divert to waste at 0-1.5 and 12-16.5 min to reduce ion source contamination

Time (min)	Mobile phase A Water + 0.1% formic acid	Mobile phase B Methanol + 0.1% formic acid
0.0	95%	5%
0.5	70%	30%
4.0	70%	30%
5.0	40%	60%
8.0	40%	60%
8.5	0%	100%
12.0	0%	100%
12.1	95%	5%
16.5	95%	5%

MS Conditions	
Instrumentation	Thermo Scientific™ TSQ Vantage™
Ionization mode	ESI^+ & ESI^-
Spray voltage	4000 V
Vaporizer	450°C
Capillary temperature	350°C
Sheath gas pressure	55 arbitrary units
Auxiliary gas	45 arbitrary units
Ion sweep gas	0 arbitrary units
Declustering potential	0 V
Q1 and Q3 peak width	0.7 Da
Collision gas	Argon
Collision gas	1.7 mTorr
Acquisition method	EZ method (scheduled SRM)
Cycle time	0.5 sec
Software	Xcalibur™ version 2.2

SRM Transitions							
Analyte	t_R (min)	Precursor ion	Product ion 1	CE 1 (V)	Product ion 2	CE 2 (V)	S-lens (V)
Sulfanilamide	2.14	156.00	92.11	12	108.10	10	45
Albuterol	2.55	240.12	121.07	28	148.07	18	51
Albuterol-D_3 (IS)	2.55	243.13	124.15	28	151.15	17	50
Lincomycin	3.13	407.16	126.11	29	359.23	17	93
Ampicillin	3.82	350.08	106.07	17	192.03	14	70
Trimethoprim	3.90	291.10	123.06	24	230.11	22	88
Trimethoprim-$^{13}C_3$ (IS)	3.90	294.09	233.19	22	264.16	25	70
Thiamphenicol(ESI^-)	4.10	353.92	121.08	54	184.99	21	70
Sulfadiazine	4.15	251.02	92.10	25	156.02	15	66
Sulfathiazole	4.30	255.98	92.08	27	156.01	13	66
Norfloxacin	4.35	320.09	233.08	24	276.11	16	82
Ormetoprim	4.53	275.11	123.08	26	259.12	26	90
Thiabendazole	4.57	202.01	131.07	32	175.05	25	75
Thiabendazole-D_6 (IS)	4.57	208.02	137.14	33	181.10	25	70
Oxytetracycline	4.60	461.08	337.09	28	426.14	18	82
Cefalexin	4.85	348.04	157.98	6	174.00	14	55
Ofloxacin	5.06	362.10	261.09	26	318.15	17	92
Ciprofloxacin	5.25	332.09	231.05	34	288.15	17	82
Ciprofloxacin-^{15}N-$^{13}C_3$ (IS)	5.25	336.09	235.10	36	291.21	16	80
Tetracycline	5.30	445.08	154.04	15	410.18	17	76
Sulfamethoxazole	5.38	254.09	92.07	26	148.08	17	55
Sulfamethoxazole-$^{13}C_6$ (IS)	5.38	260.03	98.15	26	162.07	15	61
Sulfamerazine	5.41	265.03	92.09	28	155.96	16	72
Lomefloxacin	6.04	352.09	265.08	22	308.16	16	78
Sulfamethizole	6.12	270.99	92.08	26	156.01	13	62
Chloramphenicol (ESI^-)	6.43	320.93	121.04	35	152.01	19	70
Cefotaxime	6.50	455.99	124.97	43	166.96	19	74

Enrofloxacin	6.51	360.12	245.12	24	316.19	17	86
Demeclocycline	6.55	465.03	430.12	19	448.13	14	92
Sulfachloropyridazine	6.76	284.98	92.10	27	156.01	14	70
Sulfamethazine	6.80	279.05	124.09	25	186.03	16	68
Sulfamethazine-$^{13}C_6$ (IS)	6.80	285.06	124.17	24	186.07	16	76
Azithromycin	6.85	749.11	116.00	38	591.45	24	128
Sarafloxacin	6.88	386.07	299.06	27	342.17	17	90
Clindamycin	6.96	425.10	126.10	29	377.19	17	95
Chlortetracycline	7.05	479.04	153.99	27	444.13	19	95
Cefazolin	7.15	454.97	111.92	31	155.97	14	68
Doxycycline	7.24	445.08	321.08	28	428.18	16	79
Diphenhydramine	7.34	256.12	115.07	65	165.05	62	45
Carbadox	7.40	263.04	129.06	30	231.06	12	69
Sulfadimethoxine	7.60	311.03	108.05	29	156.04	19	87
Erythromycin	7.91	734.37	157.99	28	576.41	16	107
Erythromycin-$^{13}C_2$ (IS)	7.91	736.36	160.07	28	578.41	13	108
Cephalothin	8.10	418.95	204.01	16	359.05	10	55
Penicillin G	8.15	367.09	114.04	31	160.02	14	71
Anhydroeythromycin	8.23	716.36	158.01	27	558.36	15	112
Clarithromycin	8.53	748.38	157.99	27	590.41	15	120
Ceftiofur	8.73	523.95	124.92	54	240.96	14	100
Penicillin V	8.83	383.09	114.04	32	160.03	15	70
Tylosin	8.97	916.42	173.92	34	772.47	23	173
Roxithromycin	9.30	837.43	157.95	31	679.47	16	120
Oxolinic acid	9.39	262.03	160.05	36	216.04	29	66
Oxacillin	9.52	434.08	144.01	31	160.00	15	75
Cloxacillin	10.10	468.04	160.00	16	177.96	31	85
Flumequine	10.40	262.04	126.05	48	202.03	32	70
Virginiamycin	10.80	526.19	337.08	19	355.08	16	83

Results and Discussion:

Summary of the recovery, reproducibility and method performance data generated (n=12 each).

	1 µg/kg		10 µg/kg		100 µg/kg			
Analyte	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	LCL (µg/kg)	Linearity (R^2)
Sulfanilamide	87.1[a]	1.6	82.6	12.2	75.2	8.2	0.5	0.9993
Albuterol	100.1	6.5	106.6	4.4	105.9	1.7	0.5	0.9996
Lincomycin	109.3	5.8	87.4	9.1	94.0	4.6	0.5	0.9974
Ampicillin	109.8	10.4	92.3	7.8	96.3	3.6	0.5	0.9983
Trimethoprim	94.8	5.6	105.6	3.6	103.1	1.8	0.5	0.9992
Thiamphenicol	81.3	23.3	103.3	7.7	80.2	17.2	0.5	0.9962
Sulfadiazine	88.7	10.1	89.7	7.8	86.7	6.1	0.5	0.9985
Sulfathiazole	90.0	8.9	92.4	12.8	98.4	4.4	0.5	0.9990
Norfloxacin	96.4	4.7	98.0	5.5	98.1	3.3	0.5	0.9992
Ormetoprim	95.3	6.1	101.6	10.1	98.0	4.2	0.5	0.9974
Thiabendazole	96.5	5.7	102.3	4.5	101.6	1.5	0.5	0.9997
Oxytetracycline	98.8	11.6	90.9	13.8	96.2	4.2	0.5	0.9984
Cefalexin	69.3[a]	7.8	78.4	25.9	82.1	21.6	1.0	0.9981
Ofloxacin	101.2[a]	4.9	83.7	9.1	100.1	7.3	0.5	0.9988
Ciprofloxacin	95.0	3.5	99.4	4.2	100.9	1.8	0.5	0.9988
Tetracycline	108.8	10.0	99.0	5.2	99.4	7.7	0.5	0.9993
Sulfamethoxazole	89.0	9.2	101.6	3.6	101.7	2.5	0.5	0.9994
Sulfamerazine	87.7	10.7	88.9	10.8	100.1	12.4	0.5	0.9975
Lomefloxacin	107.1	4.4	111.0	4.6	108.5	1.9	0.5	0.9989
Sulfamethizole	91.0	13.5	95.2	12.2	98.8	5.0	0.5	0.9990
Enrofloxacin	96.4	4.2	84.4	5.6	99.5	4.8	0.5	0.9985
Chloramphenicol	98.0	12.2	110.3	7.4	101.5	4.1	0.5	0.9959
Cefotaxime	96.6	11.3	90.7	6.2	92.1	3.2	0.5	0.9979
Demeclocycline	100.3	11.1	95.2	5.8	95.9	7.3	0.5	0.9991
Sulfachloropyridazine	85.6	9.0	82.7	15.1	88.7	14.3	0.5	0.9994
Sulfamethazine	97.7	9.1	99.4	4.3	99.7	2.4	0.5	0.9996
Azithromycin	71.0	7.7	83.4	8.0	82.8	6.2	0.5	0.9981
Sarafloxacin	79.0	16.2	102.4	11.5	98.5	6.1	0.5	0.9994

Clindamycin	82.4	19.2	81.0	12.3	86.2	19.0	0.5	0.9978
Chlortetracycline	108.1	4.7	89.7	8.6	92.2	5.4	0.5	0.9999
Cefazolin	96.1	1.9	97.3	7.2	101.7	3.8	1.0	0.9980
Doxycycline	102.9	4.3	87.2	6.1	92.7	5.3	0.5	0.9988
Diphenhydramine	92.3	8.2	96.6	6.0	97.8	8.0	0.5	0.9999
Carbadox	82.3	11.4	95.3	12.2	95.9	6.5	0.5	0.9962
Sulfadimethoxine	74.4	14.0	74.6	7.4	81.4	5.5	0.5	0.9991
Erythromycin	97.1	8.0	102.6	3.8	100.1	1.4	0.5	0.9970
Cephalothin	105.4	5.4	96.5	11.1	99.8	6.0	0.5	0.9997
Penicillin G	103.2	5.4	98.0	7.1	99.9	2.5	0.5	0.9995
Anhydroeythromycin	112.7	9.8	107.8	6.7	100.2	4.4	0.5	0.9971
Clarithromycin	104.5	7.1	99.3	7.3	100.8	4.9	0.5	0.9986
Ceftiofur	57.1	7.7	67.8	20.3	66.2	25.9	0.5	0.9982
Penicillin V	101.7	10.1	88.9	6.2	96.7	5.9	0.5	0.9990
Tylosin	82.5	7.4	71.4	5.2	79.4	6.1	0.5	0.9995
Roxithromycin	97.2	9.1	92.4	10.5	95.6	4.2	0.5	0.9986
Oxolinic acid	101.0	5.4	97.5	7.9	99.5	3.9	0.5	0.9986
Oxacillin	92.8	10.5	83.4	5.8	88.6	6.6	0.5	0.9990
Cloxacillin	87.6	11.5	79.0	6.7	84.2	3.5	0.5	0.9982
Flumequine	80.8	13.9	93.0	6.8	91.8	10.5	0.5	0.9992
Virginiamycin	89.3	16.1	91.4	9.3	92.7	10.0	0.5	0.9979

[a]n=6.

Chromatographic separation

The unique chemistry of the Selectra® DA column, which contains a polyaromatic stationary phase, provides orthogonal selectivity to a traditional C18 column and offers a high degree of retention and selectivity for aromatic compounds. The stationary phase is capable of retaining analytes through hydrophobic (dispersive) interactions as well as through pi-pi (-) interactions which exhibit a substantial increase in retention for dipolar, unsaturated or conjugated analytes. The Selectra® DA column is ideally suited for the analysis of veterinary drug residues, as most compounds (and metabolites) possess aromatic functionality.

In the final UHPLC-MS/MS method, methanol was chosen as the organic mobile phase solvent, as it was found to give better overall peak shape than acetonitrile, particularly for the tetracycline and fluoroquinolone antibiotics. A hold was included in the

gradient to improve chromatographic separation and all compounds were successfully eluted in <12 min. The enhanced retention of the Selectra® DA column ensured that the most polar compound included in the method, sulfanilamide, didn't elute until >2 minutes (30% methanol). Although it was possible to start the gradient at a higher percentage of organic solvent (20%) and reduce the overall run time, this required the use of a smaller injection volume (2 L) which negatively affected the method sensitivity. Ultimately, the best sensitivity was obtained by starting the gradient at 5% methanol and using a 5 L injection volume.

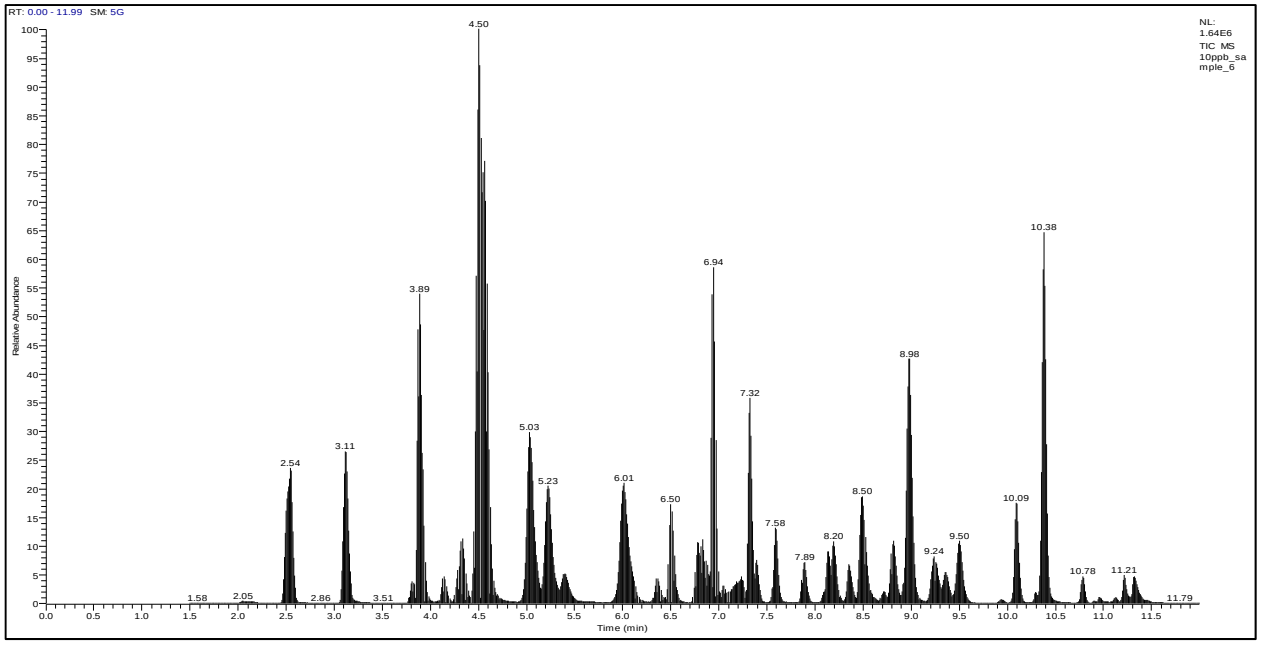

Figure 1. TIC chromatogram of an extracted milk sample (10 g/kg) containing the 49 veterinary drugs and 7 internal standards.

Sample preparation procedure:

Prior to instrumental analysis, a sample pre-treatment step is required to concentrate the analytes of interest and eliminate non-desirable matrix components. This is particularly important for the analysis of veterinary drugs in milk because of their low regulatory limits and the larger sample size required to obtain necessary method sensitivity. One of the biggest difficulties in milk analysis is the high fat, protein, and calcium content that can often interfere with instrumental analysis. The instability of certain drug classes, namely -lactams, tetracyclines and macrolides, causes additional complications by limiting the conditions that can be used for sample extraction and cleanup. Therefore, the sample preparation procedure was optimized to remove as much

co-extracted matrix components as possible while minimizing any loss of the veterinary drug residues.

A simple deproteinization procedure using an EDTA/acetic acid buffer (sample pH should be 4-4.5) followed by centrifugation to separate the proteins and lipids was carried out prior to SPE extraction and cleanup. The inclusion of EDTA in the extraction buffer prevents the complexation of drugs with metal ions (e.g. calcium), particularly the tetracyclines and fluoroquinolones. After application of the sample supernatant to the SPE cartridge, the sorbent was washed with 10% methanol to remove polar matrix components and hexane to remove lipophilic compounds. Acetone was used as the SPE elution solvent as it was found to be more effective than methanol, particularly for hydrophobic compounds that contain multiple aromatic functional groups and are strongly retained on the DVB sorbent. Furthermore, acetone is a volatile organic solvent that is readily removed by evaporation under mild conditions (35-40°C). Filtration of the sample extract prior to LC-MS/MS analysis and the use of isotopically labeled internal standards and matrix-matched calibration curves are recommended in order to obtain the best possible results.

For most compounds, the recovery was between 70% and 120% and the reproducibility <20%. Only a a small number of compounds gave results outside of the acceptable limits, which was due to analyte instability (cefalexin and ceftiofur) or inadequate sensitivity at the lowest concentration level (sulfanilamide and thiamphenicol). In addition, all compounds could be accurately detected at a concentration of 10 g/kg and the vast majority of compounds at 1 g/kg, demonstrating that the presented method is suitable for monitoring a wide range of veterinary drug residues in milk.

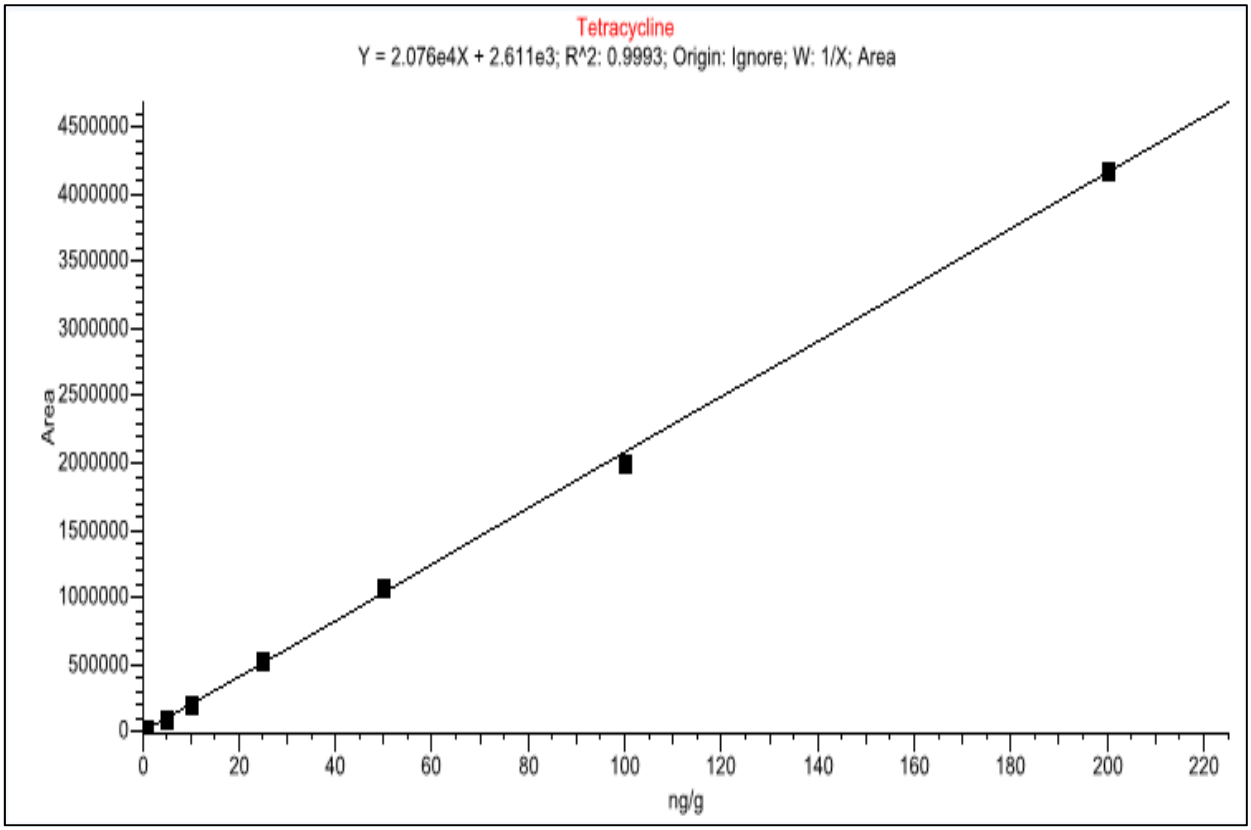

Figure 2. Example of an eight-point matrix-matched calibration curve (0.5-200 µg/kg, equivalent to 2.5-1000 ng/mL in final extract).

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Clean-Up and Fractionation

Clean-Up of Organochlorine Pesticides and PCB Extracts Using FLORISIL®

UCT Part Numbers:
EUFLSA1M6 (1000 mg small particle Grade A Florisil® for slower gravity flow)
EUFLS1M6 (1000 mg regular particle PR Grade Florisil® for more viscous samples)

This Florisil® is a magnesium silicate with basic properties. It is used to remove polar interferences from organochlorine pesticide and PCB extracts in hexane prior to chromatographic analysis. Other analytes may potentially be cleaned up using this method provided that adequate performance is demonstrated.

Procedure

1. Pre-rinse a column with 9 mL of 90:10 hexane/acetone by gravity flow
   (A low vacuum may be required to start flow)
2. Discard solvent
3. Add a collection tube under the column
4. Add a 2 mL aliquot of the sample extract (in hexane) to the column
5. Collect extract by gravity
6. Add 9 mL of 90:10 hexane/acetone to the column
7. Continue to collect by gravity
8. Gently evaporate the extract to a volume of 1 mL
9. Bring to a final volume of 2 mL with hexane

Florisil® is a registered trademark of U.S. Silica Co.

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Fractionation of Aliphatic And Aromatic Hydrocarbons Using ENVIRO-CLEAN® EPH SILICA

(Developed in cooperation with Lancaster Laboratories, Inc.)

UCT Part Numbers:

XRSIHT13M15 (Enviro-Clean® EPH Fractionation 3000 mg/15 mL)

The composition of petroleum is a complex mixture of hundreds of different hydrocarbon compounds. The resultant makeup of hydrocarbons released into the environment is variable and dependent on the conditions to which it is subsequently exposed. While in the environment, petroleum composition is influenced by a number of factors including volatilization, leaching, and/or biological degradation. These environmental effects yield a mixture whose toxicological properties can be vastly different than the parent product. Based on the known toxicological properties of petroleum products we can assume that:

- aromatic compounds are more toxic than aliphatic compounds
- the toxicity of aliphatic compounds is dependent upon their molecular weight with low molecular weight compounds showing relatively higher toxicity

The fractionation of the total petroleum hydrocarbon extract is necessary to determine the concentration of the aliphatic versus aromatic compounds. The Massachusetts Department of Environmental Protection (MADEP) has taken the approach of fractionating the C9-C18 aliphatics (n-nonane to n-octadecane), C19-C36 aliphatics (n-nonadecane to hexatriacontane), and the C11-C22 aromatics (naphthalene to benzo (g,h,i)perylene). These compounds are associated with the release of hydrocarbons in the environment. The aromatics are considered the most toxic form of hydrocarbon.

Procedure

1. Prepare Extract

a) Solvent exchange the hydrocarbon extract from methylene chloride to hexane using a K-D apparatus

2. Prepare Cartridge

a) Thoroughly rinse cartridge with two, 10 mL aliquots of pentane

b) Add 1 mL of the extract to the cartridge

c) Elute aliphatic fraction with pentane by gravity and collect everything in an ampoule. About 10 mL* should be collected

d) Place a fresh ampoule under the cartridge and elute the aromatic fraction with methylene chloride by gravity. About 10 mL** should be collected

e) Concentrate each fraction separately to a final volume on a steam bath using an ampoule and micro-Snyder column combination. Other techniques may be used but the loss of C9-C18 hydrocarbons may result

It is very important to keep the silica cartridges dry and away from room air prior to use. Moisture and contaminants in the air will reduce the effectiveness of the silica and may cause contamination of the extract. Pre-rinsing the cartridges with acetone may reduce this problem.

Results

Classification	Range	Percent Recovery
Aromatics	**C11-C22**	**88**
surrogates	2-fluorobiphenyl	**123**
surrogates	o-terphenyl	**100**
Aliphatics	**C9-C18**	**85**
	C19-C36	**89**
surrogates	1-chlorooctadecane	**58**

MA EPH DATA from Lancaster Labs

*Pentane volume should be optimized with aliphatics being eluted only.

** Methylene Chloride volume should be optimized for aromatics elution.

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Removal of Sulfur from Environmental Samples Using Copper Beads

UCT Part Numbers:
ECCU01K (Copper granules 99.5% 30 mesh - 1kg)

1. Post Sample Extraction

a) Place 4 g of copper beads in a glass vial

b) Add 2 mL of liquid sample extract to the vial

2. Sulfur Removal

a) Seal the glass vial and mix sample with copper beads for 2 minutes

b) Allow to stand for approximately 10 minutes

c) If sample contains high levels of sulfur, repeat process with 4 g of fresh copper beads

Note: For the analysis of PCB type analytes, copper may reside in the extract; Copper cleanup may result in low recoveries of several pesticides.

3. Analysis by GC/MS or GC/ECD

a) Transfer clean extract to autosampler vial

b) Inject 1-2 µL for GC

c) Inject 5-10 µL for LC

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