UCT

SOLID PHASE EXTRACTION APPLICATIONS MANUAL

ENVIRO

FORENSICS

PHARMA

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SOLVENTS

Acetone; HPLC Grade Acetonitrile (CH_3CN); HPLC Grade Chloroform ($CHCl_3$); HPLC Grade Distilled or Deionized Water (D.I. H_2O, 5 < pH < 7); Ethyl Acetate (EtAc); HPLC Grade Hexane; HPLC Grade Isopropyl Alcohol (IPA); HPLC Grade Methanol (CH_3OH): HPLC Grade Methylene Chloride (CH_2Cl_2): HPLC Grade

SOLVENT MIXTURES

Acetone / Hexane (1:99)
Acetonitrile / D.I. H_2O (20:80)
Ethyl Acetate / IPA (75:25)
Ethyl Acetate / Hexane (50:50), (75:25)
Methanol / D.I. H_2O (80:20)
Methanol / D.I. H_2O (70:30)
Methanol / D.I. H_2O (10:90)

USE OF NON-CHLORINATED ELUTION SOLVENTS

In response to environmental concerns over the use of chlorinated compounds in the laboratory, UCT offers these suggested non-chlorinated elution solvents. The recommended parameters have been used successfully on UCT columns by our customers throughout the world and may be routinely used as an alternative to chlorinated elution solvents. You may however see subtle differences on certain compounds due to solubility effects.

Assay	Chlorinated	Non-chlorinated
Opiates	CH_2Cl_2 / IPA/NH_4OH(78:20:2)	EtAc / IPA/NH_4OH (90:6:4)
Propoxyphene	CH_2Cl_2 / IPA/NH_4OH(78:20:2)	EtAc / IPA/NH_4OH (90:6:4)
Cocaine / BE	CH_2Cl_2 / IPA/NH_4OH(78:20:2)	EtAc / CH_3OH/NH_4OH(68:28:4)
Amphetamines	CH_2Cl_2 / IPA/NH_4OH(78:20:2)	EtAc / IPA/NH_4OH (90:6:4)

UCT would like to thank Dr. Leon Glass for his efforts in developing these non chlorinated mixtures.

REAGENTS

Acetic Acid, Glacial (CH_3COOH):17.4 M
Ammonium Hydroxide (NH_4OH): concentrated (14.8 M)
ß-Glucuronidase: lyophilized powder from limpets (Patella vulgata)
Dimethylformamide (DMF): silylation grade
Hydrochloric Acid (HCl): concentrated (12.1 M)
N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS)
Pentafluoropropionic Acid Anhydride (PFAA or PFPA)
Phosphoric Acid (H_3PO_4): concentrated (14.7 M)
Sodium Acetate Trihydrate ($NaCH_3COO\text{-}3H_2O$): F.W. 136.08
Sodium Borate Decahydrate ($Na_2B_4O_7\text{-}10\ H_2O$): F.W. 381.37
Sodium Hydroxide, (NaOH): F.W. 40.00
Sodium Phosphate Dibasic, Anhydrous (Na_2HPO_4): F.W. 141.96
Sodium Phosphate Monobasic, Monohydrate ($NaH_2PO_4 \bullet H_2O$): F.W. 137.99

NOTES:

Storage of organics in some plastic containers may lead to plasticizer contamination of the solvent or solvent mixture, this may interfere with analyte quantitation. Good laboratory practice dictates all who handle or are potentially exposed to reagents, solvents and solutions used or stored in the laboratory should familiarize themselves with manufacturer's recommendations for chemical storage, use and handling, and should also familiarize themselves with an appropriate Material Safety Data Sheet (MSDS).

HOW TO PREPARE SOLUTIONS AND BUFFERS

1.0 M Acetic Acid:
To 400 mL D.I. H_2O add 28.6 mL glacial acetic acid. Dilute to 500 mL with D.I. H_2O. Storage: 25°C in glass or plastic. Stability: 6 months

100 mM Acetic Acid:
Dilute 40 mL 1.0 M acetic acid to 400 mL with D.I. H_2O. Mix. Storage: 25°C in glass or plastic. Stability: 6 months

100 mM Acetate Buffer (pH 4.5):
Dissolve 2.93 g sodium acetate trihydrate in 400 mL D.I. H_2O; Add 1.62 mL glacial acetic acid. Dilute to 500 mL with D.I. H_2O. Mix. Adjust pH to 4.5 ± 0.1 with 100 mM sodium acetate or 100 mM acetic acid. Storage: 25°C in glass or plastic. Stability: 6 months; Inspect daily for contamination.

1.0 M Acetate Buffer (pH 5.0):
Dissolve 42.9 g sodium acetate trihydrate in 400 mL D.I. H_2O; Add 10.4 mL glacial acetic acid. Dilute to 500 mL with D.I. H_2O. Mix. Adjust pH to 5.0 ± 0.1 with 1.0 M sodium acetate or 1.0 M acetic acid. Storage: 25°C in glass or plastic. Stability: 6 months; Inspect daily for contamination.

100 mM Acetate Buffer (pH 5.0):
Dilute 40 mL 1.0 M acetate buffer to 400 mL with D.I. H_2O. Mix. Storage: 25°C in glass or plastic. Stability: 6 months

7.4 M Ammonium Hydroxide:
To 50 mL D.I. H_2O add 50 mL concentrated NH_4OH. Mix. Storage: 25°C in glass or fluoropolymer plastic. Stability: Storage condition dependent.

ß-Glucuronidase, Patella vulgata, 5,000 Fishman units/mL:
Dissolve 100,000 Fishman units lyophilized powder with 20 mL 100 mM acetate buffer (pH 5.0). Storage: 5°C in plastic. Stability: Several days; Prepare daily for best results.

100 mM Hydrochloric Acid:
To 400 mL D.I. H_2O add 4.2 mL concentrated HCl. Dilute to 500 mL with D.I. H_2O. Mix. Storage: 25°C in glass or plastic. Stability: 6 months

Methanol /Ammonium Hydroxide (98:2):
To 98 mL CH_3OH add 2 mL concentrated NH_4OH. Mix. Storage: 25°C in glass or fluoropolymer plastic. Stability: 1 day.

0.35 M Sodium Periodate:
Add 37.5 g sodium periodate to a 500 mL volumetric flask, q.s. to volume with D.I. H_2O. Mix. Stability: 2 mos. at room temperature.

Methylene Chloride / Isopropanol / Ammonium Hydroxide (78:20:2):
To 40 mL IPA, add 4 mL concentrated NH_4OH. Mix. Add 156 mL CH_2Cl_2. Mix. Storage: 25°C in glass or fluoropolymer plastic. Stability: 1 day

100 mM Phosphate Buffer (pH 6.0):
Dissolve 1.70 g Na_2HPO_4 and 12.14 g NaH_2P0_4 H_2O in 800 mL D.I. H_2O. Dilute to 1000 mL using D.I. H_2O. Mix. Adjust pH to 6.0 ± 0.1 with 100 mM monobasic sodium phosphate (lowers pH) or 100 mM dibasic sodium phosphate (raises pH). Storage: 5°C in glass. Stability: 1 month; Inspect daily for contamination.

500 mM Phosphoric Acid:
To 400 mL D.I. H_2O add 17.0 mL concentrated phosphoric acid. Dilute to 500 mL with D.I. H_2O. Mix. Storage: 25°C in glass or plastic. Stability: 6 months

1.0 M Sodium Acetate:
Dissolve 13.6 g sodium acetate trihydrate in 90 mL D.I. H_2O. Dilute to 100 mL with D.I. H_2O. Mix. Storage: 25 °C in glass or plastic. Stability: 6 months

100 mM Sodium Acetate:
Dilute 10 mL 1.0 M sodium acetate to 100 mL with D.I. H_2O. Mix. Storage: 25°C in glass or plastic. Stability: 6 months

100 mM Sodium Borate:
Dissolve 3.81 g Na2B4O7•10 H_2O in 90 mL D.I. H_2O. Dilute to 100 mL with D.I. H_2O. Mix. Storage: 25°C in glass or plastic. Stability: 6 months.

100 mM Sodium Phosphate Dibasic:
Dissolve 2.84 g Na_2HPO_4 in 160 mL D.I. H_2O. Dilute to 200 mL using D.I. H_2O. Mix. Storage: 5°C in glass. Stability: 1 month; Inspect daily for contamination.

100 mM Sodium Phosphate, Monobasic:
Dissolve 2.76 g NaH_2PO_4-H_2O in 160 mL D.I. H_2O. Dilute to 200 mL with D.I. H_2O. Mix. Storage: 5°C in glass. Stability: 1 month. Inspect daily for contamination.

100 mM Sulfuric Acid:
To 400 mL D.I. H_2O add 5.6 mL concentrated H_2SO_4. Dilute to 500 mL with D.I. H_2O. Mix. Storage: 25°C in glass or plastic. Stability: 6 months

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 erratic recoveries.

- Do not allow the sorbent to dry between conditioning steps or before sample application. To insure properly solvated columns, 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 column dryness, press the sides of the cartridge at the sorbent level at full vacuum. Columns should feel ambient temperature, not cool. If the column feels cool, water is probably present. Dry the column further.

- Always use fresh NH_4OH when preparing basic elution solvents. Proper elution pH (11-12) is critical to achieving optimal recovery of basic drugs with high pKa's (i.e., amphetamines, some tricyclics, morphine). NH_4OH rapidly loses its strength when exposed to air. Weak NH_4OH may lead to erratic recoveries.

- NH_4OH is more soluble in IPA than CH_2Cl_2. To ensure complete mixing of eluate solvents, add NH_4OH and IPA, then add CH_2Cl_2.

- Some drugs are heat labile and will degrade if overheated. Closely monitor elution dry down to prevent loss of analyte.

- Always condition the column with the strongest solution the column will see to ensure the cleanest extraction of your eluate.

- Solvent quantities for RSV methods are suggested and might be further reduced to meet particular laboratory needs.

UCT

FORENSIC METHODS

SYMPATHOMIMETIC AMINES IN BLOOD, PLASMA/SERUM URINE AND TISSUE FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. **PREPARE SAMPLE**
   To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standard(s).* Add 1 mL of blood, plasma/serum, urine or 1g of (1:4) tissue homogenate. Mix/vortex
   Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex.
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
   Centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**
   Load at 1 to 2 mL/minute.

4. **WASH COLUMN**
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM acetic acid.
   1 x 3 mL CH_3OH.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE SMA**
   1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2); Collect eluate at
   1 to 2 mL/minutes.
   NOTE: Prepare elution solvent daily. Add IPA/NH_4OH, mix, then add CH_2Cl_2.

6. **CONCENTRATE ELUATE**
   Add 30 µL silylation grade DMF to eluate.
   Evaporate to 30 µL at < 40°C.

ALTERNATE DRYING PROCEDURE
   Evaporate for 4 min.
   Add 100 µL of 1% HCl in methanol.
   Evaporate to dryness.

7. **FLUOROACYLATE WITH PFPA (PFAA)*****
   Add 50 µL PFPA (PFAA). Overlay with N_2 and cap.
   Improved derivatization by addition of 50 µL PFPOH.****
   React 20 minutes at 70°C. Evaporate to dryness at < 40°C.
   Reconstitute with 100 µL ethyl acetate.

8. **QUANTITATE**
   Inject 1 to 2 µL onto gas chromatograph.
   For MSD monitor the following ions:

ANALYTE (PFPA)	Primary Ion**	Secondary	Tertiary	Cerilliant#
Amphetamine-D5*	194	92	123	A-005
Amphetamine	190	91	118	A-007
Methamphetamine-D5*	208	92	163	M-004
Methamphetamine	204	91	160	M-009
Pseudoephedrine	204	160	119	P-035
Ephedrine	204	160	119	E-011
Phenylephrine	190	119	267	
Methylenedioxyamphetamine	135	162	325	M-012
Methylenedioxymethamphetamine	204	162	339	M-013

* Suggested internal standards for GC/MS: D_5-Amphetamine and D_5-Methamphetamine
** Quantification Ion
*** Part # SPFAA-0-1, 10, 25,100
**** Part # SPFPOH-1, 10, 25,100

ALTERNATE DERIVATIZATION

7. **Form TMS derivatives:**
   Add 50 µL BSTFA with 1% TMCS and 50 µL of ethyl acetate.
   React 45 minutes at 70°C.

8. **QUANTITATE**
   Inject 1 to 2 µL onto gas chromatograph.
   For MSD monitor the following ions:

ANALYTE (TMS)	Primary Ion**	Secondary	Tertiary	Cerillinat#
Amphetamine-D5*	120	197	92	A-005
Amphetamine-D6*	120	198	93	A-044
Amphetamine-D10*	120	202	97	A-038
Amphetamine-D12*	120	203	98	A-019
Amphetamine	116	192	91	A-007
Methamphetamine-D5*	134	211	92	M-023
Methamphetamine-D8*	137	214	92	
Methamphetamine-D9*	137	215	93	M-091
Methamphetamine	130	206	91	M-004
Pseudoephedrine	130	147	294	P-035
Ephedrine	130	147	294	E-011
Methylenedioxyamphetamine	116	236	135	M-012
Methylenedioxymethamphetamine	130	250	131	M-013
Para-Methoxamphetamine	116	222	121	NMID1908

ALTERNATE DERIVATIZATION

7. **Form 4-CB** (4-Carbethoxyhexafluorobutyrl chloride)*
   derivatives: Add 20 µL 4-CB* and 100 µL of ethyl acetate.
   React 45 minutes at 70°C.

8. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

ANALYTE (TMS)	Primary Ion**	Secondary	Tertiary	Cerillinat#
Amphetamine-D5****	298	270	399	A-005
Amphetamine	294	266	248	A-007
Methamphetamine-D5****	312	284	266	M-023
Methamphetamine	308	280	262	M-004
Methylenedioxyamphetamine-D5****	136	434	270	M-010
Methylenedioxyamphetamine	162	429	266	M-012
Methylenedioxymethamphetamine-D5****	312	284	266	M-011
Methylenedioxymethamphetamine	308	280	262	M-013
Methylenedioxyethylamphetamine-D6****	328	165	300	
Methylenedioxyethylamphetamine	322	162	294	M-065

* Part # S4CB-0-10
*** Quantification Ion
**** Suggested internal standards for GC/MS: D_5-Amphetamine and D_5-Methamphetamine

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AMPHETAMINES IN URINE, OXIDATION WITH PERIODATE FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. **PREPARE SAMPLE**
   To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standard(s)*. Add 2 mL of urine add, and 1 mL 0.35 M sodium periodate.
   Mix/vortex. Incubate at room temp. for 20 min.
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH 6.0).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**
   Load at 1 to 2 mL/minute.

4. **WASH COLUMN**
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM acetic acid.
   1 x 3 mL CH_3OH.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE AMPHETAMINES**
   1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2);
   Collect eluate at 1 to 2 mL/minutes.
   NOTE: Prepare elution solvent daily. Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

* Suggested internal standards for GC/MS: Amphetamine-D5 and Methamphetamine-D_5
** Quantification Ion
*** Part # SPFAA-0-1, 10, 25,100
**** Part # SPFPOH-1, 10, 25,100

6. **CONCENTRATE ELUATE**
   Add 30 µL silylation grade DMF to eluate.
   Evaporate to 30 µL at < 40°C.
   Alternative: Add 100 µL of 1% HCl in methanol
   Evaporate to dryness at < 40°C.

7. **FLUOROACYLATE WITH PFPA** (PFAA)***
   Add 50 µL PFPA (PFAA). Overlay with N_2 and cap.
   Improved derivatization by addition of 50 µL PFPOH.****
   React 20 minutes at 70°C. Evaporate to dryness at < 40°C.
   Reconstitute with 100 µL ethyl acetate.

8. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.

For MSD monitor the following ions:

ANALYTE (TMS)	Primary Ion**	Secondary	Tertiary	Cerilliant #
Amphetamine-D5*	194	92	123	A-002/ A-005
Amphetamine	190	91	118	A-007
Methamphetamine-D5*	208	92	163	A-004
Methamphetamine	204	91	160	A-009

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EXTRACTION OF BENZODIAEPINES FROM URINE USING SPE CARTRIDGES

130mg CLEAN SCREEN XCEL I Column
(Part #: CSXCE106 6 mL - 130 mg Cartridge)
(Part #: CSXCE103 3 mL - 130 mg Cartridge)
September 9, 2009

1. Sample Preparation

(**Hydrolysis Step)**
To 1-5 mL urine sample add 1-2 mL of 0.1M acetate buffer (pH= 5.0) containing 5,000 units/mL β-glucuronidase. **Optionally**, add 1-2 mL of acetate buffer and 25 µL of concentrated β-glucuronidase.
Add appropriate volume and concentration internal standards.
Vortex and heat for 1-2 hours at 65 °C.
Allow sample to cool.

Do not adjust pH- sample is ready to be added to extraction column.

2. Applying Sample to Column

Load sample directly to column without any preconditioning.
Pull sample through at a rate of 1-2 mL/ minute.
Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi) for 1 minute.

3. *Wash*

Wash sample with 1 mL of methylene chloride.
Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi) for a minimum of 5-10 minutes.

NOTE 2: *(It is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.)*

4. Elution

Elute samples with 1 mL ethyl acetate/ ammonium hydroxide (98/2)
Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

GC/MS Analysis

It is recommended to add 50 µL of Ethyl Acetate to 50 µL of derivatization agent and react at ~70 °C for 15 minutes. Inject 1-2 µL of cooled (50:50) solution in the GC/MS system for analysis.

LC/MS Analysis

Reconstitute in methanol or appropriate mobile phase.

Benzodiazepine Analytes Extracted

Diazepam	Clonazepam	Alprazolam	Midazolam
Nordiazepam	7-aminoclonazepam	α-OH Alprazolam	α-OH Midazolam
Temazepam	Oxazepam	Lorazepam	Flurazepam

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EXTRACTION OF BENZODIAZEPINES FROM URINE

(Part # WSH96XCE11 - 130 mg 96 well plate)
(Part # WSH48XCE11 - 130 mg 48 well plate)
August 5, 2009

CLEAN SCREEN XCEL I

Sample Preparation

(**Hydrolysis Step)**
To 1-2 mL urine sample add 500 µL of acetate buffer (pH= 5.0) containing 5,000 units/mL β-glucuronidase. **Optionally**, add 500µL of acetate buffer and 25 µL of concentrated β-glucuronidase.
Add appropriate volume and concentration internal standards.
Vortex and heat for 1-2 hours at 65 °C.
Allow sample to cool.

Do not adjust pH- sample is ready to be added to extraction column.

Applying Sample to Column

Load sample directly to column without any preconditioning.
Pull sample through at a rate of 1-2 mL/ minute.
Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi) for 1 minute.

Wash

Wash sample with 1 mL of methylene chloride.
Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi)
for a minimum of 5-10 minutes.

NOTE 2: *(It is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.)*

Elution

Elute samples with 1 mL ethyl acetate/ ammonium hydroxide (98/2)
Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

GC/MS Analysis

It is recommended to add 50 µL of Ethyl Acetate to 50 µL of derivatization agent and react at ~70 °C for 15 minutes. Inject 1-2 µL of cooled (50:50) solution in the GC/MS system for analysis.

LC/MS Analysis

Reconstitute in methanol or appropriate mobile phase.

Benzodiazepine Analytes Extracted

Diazepam	Clonazepam	Alprazolam	Midazolam
Nordiazepam	7-aminoclonazepam	α-OH Alprazolam	α-OH Midazolam
Temazepam	Oxazepam	Lorazepam	

DCN-900170-168

BENZODIAZEPINES IN WHOLE BLOOD FOR GC OR GC/MS CONFIRMATIONS USING:
300 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSBNZ030 without Tips or ZCBNZ030 with CLEAN-THRU® Tips

February 3, 2009

1. PREPARE SAMPLE

To 1 mL of pH 6 buffer add Internal standards*, add 2 mL of whole blood and vortex mix. Add 5 mL of pH 6 buffer Sonicate with a probe sonifier for ~10 seconds and centrifuge at ~2700rpm for 15 minutes.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL Ethyl Acetate.
1 x 3 mL MeOH.
1 x 3 mL DI H_2O.
1 x 3 mL 0.1M phosphate buffer (pH 6.0).

3. APPLY SAMPLE

Load sample by gravity.

4. WASH SAMPLE

1 x 3 mL DI H_2O.
1 x 3 mL 5% acetonitrile in 0.1M phosphate buffer (pH 6.0).
Dry columns 5 minutes at full vacuum or > 10 inches Hg.
1 x 3 mL Hexane.

5. ELUTE BENZODIAZEPINES

2 x 3 mL Ethyl Acetate.

6. DRY ELUATE

Evaporate to dryness under nitrogen at ~ 55° C.
Add external standards.*

7. DERIVATIZE

Add 100µL acetonitrile and 100µL MTBSTFA w/1% t-BDMCS.
Heat for 30 minutes at 70° C.
Remove from heat source to cool.
Inject 1µL into GC/MS-NCI.
Note: Do not evaporate MTBSTFA solution.

GC/MS Conditions:

Agilent 6890/5975 inert MSD
Inlet 260°, Split injection 2:1
Column: ZB-5MS, 15m, 0.25mm ID, 0.25µm film
Temperature program: 70° hold 0.25 min, ramp 20° per min to 300°, hold 4 min.
NCI parameters: Source 150°, Quad 106°, Methane as collision gas

Suggested Internal standards : Diazepam-D5 and Lorazepam-D4.

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1. PREPARE SAMPLE- ß-GLUCURONIDASE HYDROLYSIS

To 2 mL of urine add internal standard(s)*** and 1 mL of ß-glucuronidase solution.
ß-glucuronidase solution contains: 5,000 F units/mL Patella vulgata in 100 mM acetate buffer (pH=5.0).
Mix/vortex.
Hydrolyze for 3 hours at 65°C.
Centrifuge for 10 minutes at 2000 rpm and discard pellet.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 mL/minute.

4. WASH COLUMN

1 x 2 mL D.I. H_2O.
1 x 2 mL 20% acetonitrile in 100 mM phosphate buffer (pH= 6.0).
Dry column (5 minutes at > 10 inches Hg).
1 x 2 mL hexane.

5. ELUTE BENZODIAZEPINES

1 x 5 mL ethyl acetate containing 4% ammonium hydroxide
collect eluate at 1 to 2 mL/minute.

6. DRY ELUATE

Evaporate to dryness at < 40°C.

7. DERIVATIZE

Add 50 µL ethyl acetate and 50 µL BSTFA* (with 1% TMCS)*.
Overlay with Nitrogen and cap. Mix/vortex.
React 20 minutes at 70°C. Remove from heat source to cool.
NOTE: Do not evaporate BSTFA solution.

* Part # SBSTFA-1-1,10, 25,100
*** Suggested internal standard for GC/MS: Prazepam or Oxazepam-D5
**** Quantitation ion
***** Part # SMTBSTFA-1-1,10, 25,100

8. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

Generic Name	Trade Name	Primary Ion****	Secondary	Tertiary	Cerilliant#
Alprazolam	*Xanax®*	*308*	*279*	*204*	A- 907
α-Hydroxyalprazolam-TMS		381	396	383	A-903
Chlordiazepoxide	Librium®	282	283	284	C-022
Clonazepam	Clonopin®	387	352	306	C-907
Diazepam	Valium®	256	283	221	A-907
Desalkylflurazepam-TMS		359	341	245	D-915
Hydroxyethylflurazepam-TMS		288	360	389	F-902
Lorazepam-TMS	Ativan®	429	430	347	L-901
Nordiazepam-TMS		341	342	343	N-905
Oxazepam-TMS	Serax®	429	430	313	O-902
Prazepam*		269	241	324	P-906
Temazepam-TMS	Restoril®	343	283	257	T-907
Triazolam	Halcion®	313	314	342	T-910
α-Hydroxytriazolam-TMS		15	417	430	T-911

NOTE: Flurazepam does not extract under these conditions; However metabolites such as desalkyflurazepam and hydroxyethylflurazepam will extract with high recovery.

ALTERNATE DERIVATIZATION

7. DERIVATIZE

Add 50 µL ethyl acetate and 50 µL MTBSTFA***** (with 1% MTBDMCS).
Overlay with Nitrogen and cap. Mix/vortex.
React 20 minutes at 70°C. Remove from heat source to cool.
NOTE: Do not evaporate MTBSTFA solution.

8. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

Generic Name	Trade Name	Primary Ion****	Secondary	Tertiary	Cerilliant#
Nordiazepam -D5-TBDMS		332	334	333	N-903
Nordiazepam-TBDMS		327	328	329	N-902
Oxazepam-D5-TBDMS		462	519	462	O-901
Oxazepam-TBDMS	Serax®	457	513	459	O-902
Temazepam-D5-TBDMS		362	390	288	T-903
Temazepam-TBDMS	Restoril®	357	359	385	T-903
Lorazepam-TBDMS	Ativan®	491	513	493	L-901
Clonazepam	Clonopin®	372	374	326	C-907
7-Aminoclonazepam -TBMS		456	458	513	A-915
Diazepam	Valium®	256	283	221	A-907
Desalkylflurazepam-TBDMS		345	347	402	D-915
Prazepam*		269	241	324	P-906
α-Hydroxymidazolam-TBDMS	Versid®	398	400	440	H-902
Desmethylflunitrazepam-TBDMS		357	310	356	D-919
7-Aminoflunitrazepam-TBDMS		397	324	398	D-912
Alprazolam	Xanax®	308	279	204	A-907
α-Hydroxyalprazolam-D5-TBDMS		386	388	387	A-904
α-Hydroxyalprazolam-TBDMS		383	384	381	A-903
Triazolam	Halcion®	313	314	342	T-910
α-Hydroxytriazolam-TBDMS		415	417	190	T-9111

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1. **PREPARE SAMPLE**

   To 1 mL of 100 mM phosphate buffer (pH= 6) add internal standards.*
   Add 1 mL blood/ Urine or 1g of (1:4) tissue homogenate. Mix/ vortex. Add 3 mL of 100 mM phosphate buffer (pH= 6).
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate
   Vortex mix.
   Centrifuge as appropriate.

   PROCEDURE FOR URINE:
   To 1 mL of Acetate buffer (pH=5.0) containing 5000 F units/ mL β-Glucuronidase. Add internal standards*.
   To this solution add 1 mL of urine.
   Mix/ Vortex .
   Hydrolyze for 3 Hrs at 65˚C.
   Allow to cool.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet.
   Add 3 mL of 100 mM phosphate buffer (pH 6.0) and mix.

2. **CONDITION CLEAN SCREEN® CSBNZ EXTRACTION COLUMN**

   1 x 3 mL MeOH
   1 x 3 mL 100 mM phosphate buffer (pH= 6).
   NOTE: aspirate at < 3 inches Hg to prevent sorbent drying out.

3. **APPLY SAMPLE:**

   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**

   1 x 3 mL of 5 % (v/v) acetonitrile in 100 mM phosphate buffer (pH6).
   Dry column (5 minutes at > 10 inches Hg).
   1 x 3 mL of hexane.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE BENZODIAZEPINES**

   1 x 3 mL ethyl acetate; ammonia (98:2 v/v).
   Collect eluate at 1-2 mL /minute.

6. **EVAPORATION**

   Evaporate eluates under a gentle stream of nitrogen < 40°.

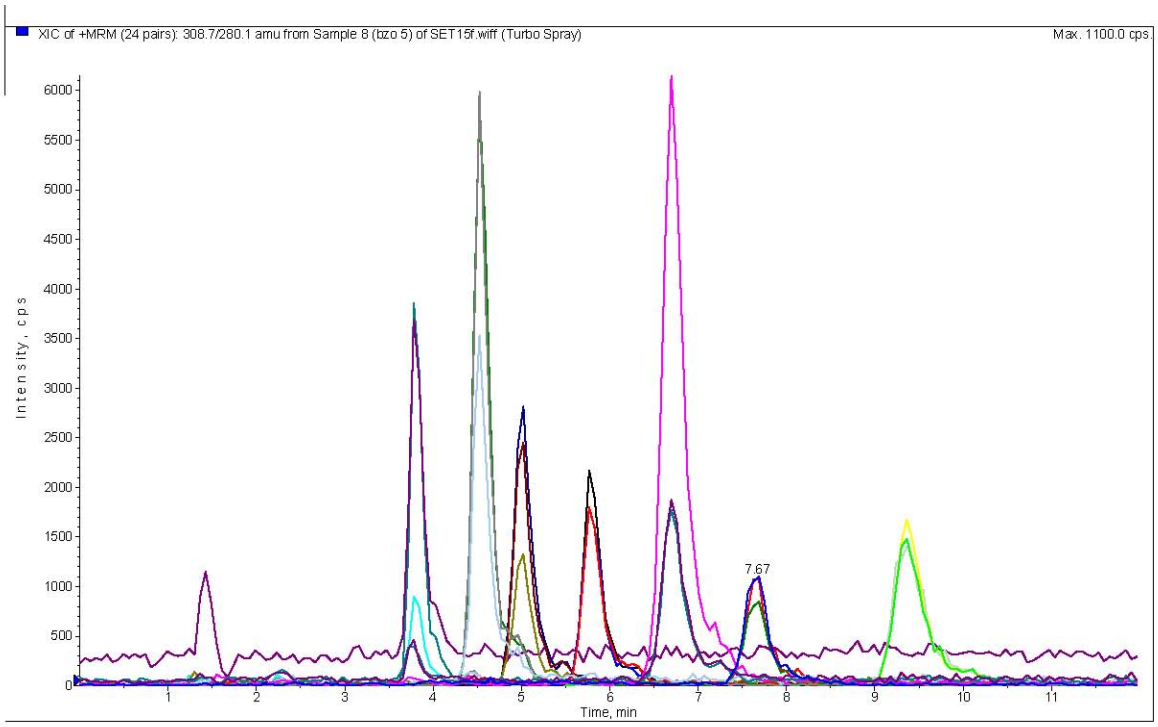
7. Reconsitute sample in 50 µL of 0.02% formic acid (aqueous).

INSTRUMENTAL CONDITIONS: LC-MSMS

Column:	150 x 2.1 mm (3µm) Gold PFP (Thermo Fisher)
Mobile phase	30: 70 (Acetonitrile: 0.02% aq. Formic acid)
Flow rate:	0.35 mL/ min
Column Temp:	Ambient
Injection Volume:	5 µL
Mass Spectrometer:	Applied Biosystem API2000

Compound	MRM Transition	Cerilliant#
Alprazolam	309.1/281.2	A-903
*Alprazolam-D5	314.1/286.2	A-910
Alphahydroxyalaprazolam	325.1/242.9	A-907
*Alphahydroxyalprazolam-D5	330.1/302.2	A-902
Chlordiazepoxide	300.1/227.0	C-022
Diazepam	285.5/192.5	D-907
*Diazepam	292.2/198.2	D-902
Lorazepam	321.1/275.1	L-901
*Lorazepam-D4	325.1/279.0	L-902
Nordiazepam	271.1/140.1	N-905
*Nordiazepam-D5	275.6/140.1	N-903
Oxazepam	287.1/241.1	O-902
*Oxazepam-D5	290.2/198.2	O-901
Temazepam	301.1/255.1	T-907
*Temazepam-D5	306.1//260.1	T-902

Chromatogram of 5 ng Benzodiazepines (without IS)



DCN-903020-34

BENZODIAZEPINE SCREEN: BLOOD, SERUM, URINE AND TISSUE FOR GC-GC/MS USING: 300 mg CLEAN SCREEN® CSBNZ EXTRACTION COLUMN

Part #: CSBNZ203

February 3, 2009

1. **PREPARE SAMPLE**

   To 1 mL of 100 mM phosphate buffer (pH= 6) add internal standards.*
   Add 1 mL blood/ Urine or 1g of (1:4) tissue homogenate.
   Vortex mix. Add 3 mL of 100 mM phosphate buffer (pH= 6).
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate
   Vortex and centrifuge as appropriate.

   PROCEDURE FOR URINE

   To 1 mL of Acetate buffer (pH 5.0) containing 5000 F units/ mL β-Glucuronidase. Add internal standards*.
   To this solution add 1 mL of urine.
   Mix/ Vortex .
   Hydrolyze for 3 Hrs at 65˚C.
   Allow to cool.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet.
   Add 3 mL of 100 mM phosphate buffer (pH 6.0) and mix.

2. **CONDITION CLEAN SCREEN® CSBNZ EXTRACTION COLUMN**

   1 x 3 mL MeOH
   1 x 3 mL 100 mM phosphate buffer (pH 6).
   NOTE: aspirate at < 3 inches Hg to prevent sorbent drying out.

3. **APPLY SAMPLE**

   Load sample at 1-2 mL / minute.

4. **WASH COLUMN**

   1 x 3 mL of 5 % (v/v) acetonitrile in 100 mM phosphate buffer (pH6).
   Dry column (5 minutes at > 10 inches Hg).
   1 x 3 mL of hexane.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE BENZODIAZEPINES**

   1 x 3 mL ethyl acetate; ammonia (98:2 v/v).
   Collect eluate at 1-2 mL /minute.

6. **EVAPORATION**

   Evaporate eluates under a gentle stream of nitrogen < 40°.

7. **DERIVATIZE**

   Add 50 µL acetonitrile and 50µL BSTFA with 1% TCMS
   Heat for 30 minutes at 70° C.
   Remove from heat source to cool.
   Inject 1µL into GC/MS

Compound	Primary ion	Secondary	Tertiary	Cerilliant#
Alprazolam	308,	279	204	A-903
*Alprazolam-D	513	284		A-910
Alphahydroxyalprazolam	318	396	383	A-907
*Alphahydroxyalprazolam-D5	386	401		A-902
Diazepam	256	283	284	D-907
*Diazepam-D5	287	289		D-902
Lorazepam	429	430	347	L-901
*Lorazepam-D4	433	435		L-902
Nordiazepam	34	342	343	N-905
*Nordiazepam-D5	345	347		N-903
Oxazepam	429	313	430	O-902
*Oxazepam-D5	435	433		O-901
Temazepam	343	257	283	T-907
*Temazepam-D5	348	262		T-902

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BENZODIAZEPINES IN SERUM OR PLASMA FOR HPLC ANALYSIS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. **PREPARE SAMPLE**
   To 1 mL 1.0 mL of 100 mM phosphate buffer (pH 6.0) add internal standard(s)*.
   Add 1 mL of serum or plasma
   Mix/vortex.
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
   Centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**
   Load at 1 mL/minute.

4. **WASH COLUMN**
   1 x 2 mL D.I. H_2O.
   1 x 2 mL 20% acetonitrile in 100 mM phosphate buffer (pH 6.0).
   Dry column (10 minutes at > 10 inches Hg).
   1 x 2 mL hexane.

5. **ELUTE BENZODIAZEPINES**
   1 x 5 mL ethyl acetate containing 2 % ammonium hydroxide.
   collect eluate at 1 to 2 mL/minute.

6. **DRY ELUATE**
   Evaporate to dryness at < 40°C.

7. **RECONSTITUTE**
   Reconstitute in mobile phase.

8. **QUANTITATE**
   Inject sample onto HPLC.

Reference - UCT Internal Publication

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1. **PREPARE SAMPLE: ß-GLUCURONIDASE HYDROLYSIS**.
   To 2 mL of urine, add internal standard(s)* and 1 mL of ß-Glucuronidase solution.
   ß-Glucuronidase solution contains 5,000 F units/mL Patella vulgata in 100 mM acetate buffer (pH 5.0).
   Mix/vortex.
   Hydrolyze for 3 hours at 65°C.
   Cool before proceeding.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL deionized water.
   1 x 1 mL 100 mM phosphate buffer (pH 6.0).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMP**
   Load at 1 to 2 mL/ minute.

4. **WASH COLUMN**
   1 x 2 mL deionized water.
   1 x 2 mL 20% acetonitrile in 100 mM phosphate buffer (pH 6.0).
   Dry column (5 minutes at > 10 inches Hg).
   1 x 2 mL hexane.

5. **ELUTE CLONAZEPAM / 7-AMINOCLONAZEPAM**
   1 x 3 mL ethyl acetate with 2% NH_4OH:
   Collect eluate at 1 to 2 mL/minute.
   NOTE: Prepare fresh daily.

6. **DRY ELUATE**
   Evaporate to dryness at < 40°C.

7. **DERIVATIZE**
   Add 50 µL ethyl acetate and 50 µL MTBSTFA (with 1% TBDMCS)****.
   Mix/vortex.
   React 20 minutes at 90°C.
   Remove from heat source to cool.
   NOTE: Do not evaporate MTBSTFA solution.

8. **ANALYSIS**
   Inject 1 to 2 µL sample.
   For MSD monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary	Cerilliant#
Clonazepam-TBDMS	372	374	326	C-907
7-Aminoclonazepam-TBDMS	342	344	399	A-915
Clonazepam-D4-TBDMS	376	378	377	C-905
7-Aminoclonazepam-D4-TBDMS	346	348	403	A-917

*Suggested internal standard for GC/MS: Clonazepam-D4, 7-aminoclonazepam-D4.
**Quantitation ion
****Part # SMTBSTFA-1-1, 10, 25, 100

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FLUNITRAZEPAM AND METABOLITES IN URINE FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. **PREPARE SAMPLE- ß-GLUCURONIDASE HYDROLYSIS**
   To 2 mL of urine add internal standard(s)* and 1 mL of ß-glucuronidase solution.
   ß-glucuronidase solution contains 5,000 F units/mL Patella vulgata in 100 mM acetate buffer (pH=5.0).
   Mix/vortex.
   Hydrolyze for 3 hours at 65°C.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**
   Load at 1 mL/minute.

4. **WASH COLUMN**
   1 x 3 mL D.I. H_2O.
   1 x 2 mL 20% acetonitrile in 100 mM phosphate buffer (pH 6.0).
   Dry column (5 minutes at > 10 inches Hg).
   1 x 2 mL hexane.

5. **ELUTE FLUNITRAZEPAM, 7-AMINOFLUNITRAZEPAM AND DESMETHYLFLUNITRAZEPAM**
   1 x 3 mL ethyl acetate with 2% NH_4OH;
   Collect eluate at 1 to 2 mL/minute.
   Prepare fresh daily.

6. **DRY ELUATE**
   Evaporate to dryness at < 40°C.

7. **DERIVATIZE**
   Add 50 µL ethyl acetate and 50 µL MTBSTFA (with 1% TBDMCS)***.
   Overlay with N_2 and cap. Mix/vortex.
   React 20 minutes at 70°C. Remove from heat source to cool.
   NOTE: Do not evaporate MTBSTFA solution.

8. **QUANTITATE**
   Inject 1 to 2 µL onto gas chromatograph.
   For MSD monitor the following ions:

Compound	Primary Ion****	Secondary	Tertiary	Cerilliant #
Flunitrazepam-TBDMS	312	286	266	F-907
7-Aminoflunitrazepam-TBDMS	283	255	254	A-911
Desmethylflunitrazepam-TBDMS	356	357	310	D-918
*Oxazepam-D5 -TBDMS	462	464	463	O-901

* Suggested internal standard for GC/MS: D_5-Oxazepam
*** Part # SMTBSTFA-1-1,10,25,100
**** Quantitation ion

Reference - UCT Internal Publication

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CLONAZEPAM / FLUNITRAZEPAM & METABOLITES IN BLOOD, PLASMA / SERUM AND URINE FOR LC/MS/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® CSBNZ EXTRACTION COLUMN

Part #: CSBNZ203

February 3, 2009

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH 6) add internal standards*.
   Add 1 mL whole blood, Serum/Plasma or Urine. Add 3 mL of 100 mM phosphate buffer (pH 6).
   Vortex and centrifuge as appropriate.

2. **CONDITION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL 100 mM phosphate buffer (pH 6).
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out.

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**
   1 x 3 mL of 5 % (v/v) acetonitrile in 100 mM phosphate buffer (pH6).
   Dry column (5 minutes at > 10 inches Hg).
   1 x 3 mL of hexane.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE CLONAZEPAM/ 7-AMINOCLONAZEPAM- FLUNITRAZEPAM/7-AMINOFLUNITRAZEPAM:**
   2 x 3 mL ethyl acetate / ammonium hydroxide (96: 4).
   Collect eluate at 1-2 mL /minute.

6. **EVAPORATION:**
   Evaporate eluates under a gentle stream of nitrogen < 40°.
   Dissolve residue in 50 µL CH_3OH.

INSTRUMENT CONDITIONS:

Column: 150 x 2.1 mm (3 µm) Gold PFP (Thermofisher).

Mobile phase: Acetonitrile: 0.1% Formic acid (33: 67)

Flowrate: 0.35 mL / minute

Injection Volume: 5 µL

Column Temperature: ambient

Detector: API 2000 MS/MS.

Compound	MRM Transition	Cerilliant #
7-Aminoclonazepam	286.1/121.1	A-915
*7-Aminoclonazepam-D4	290.2/121.1	A-917
Clonazepam	316.0/270.2	C-907
*Clonazepam-D4	320.1/270.4	C-905
7-Aminoflunitrazepam	284.2/135.0	A-912
*7-Aminoflunitrazepam-D7	291.3/138.2	A-917
Flunitrazepam	314.1/268.2	F-907
*Flunitrazepam-D7	321.1/275.2	F-915

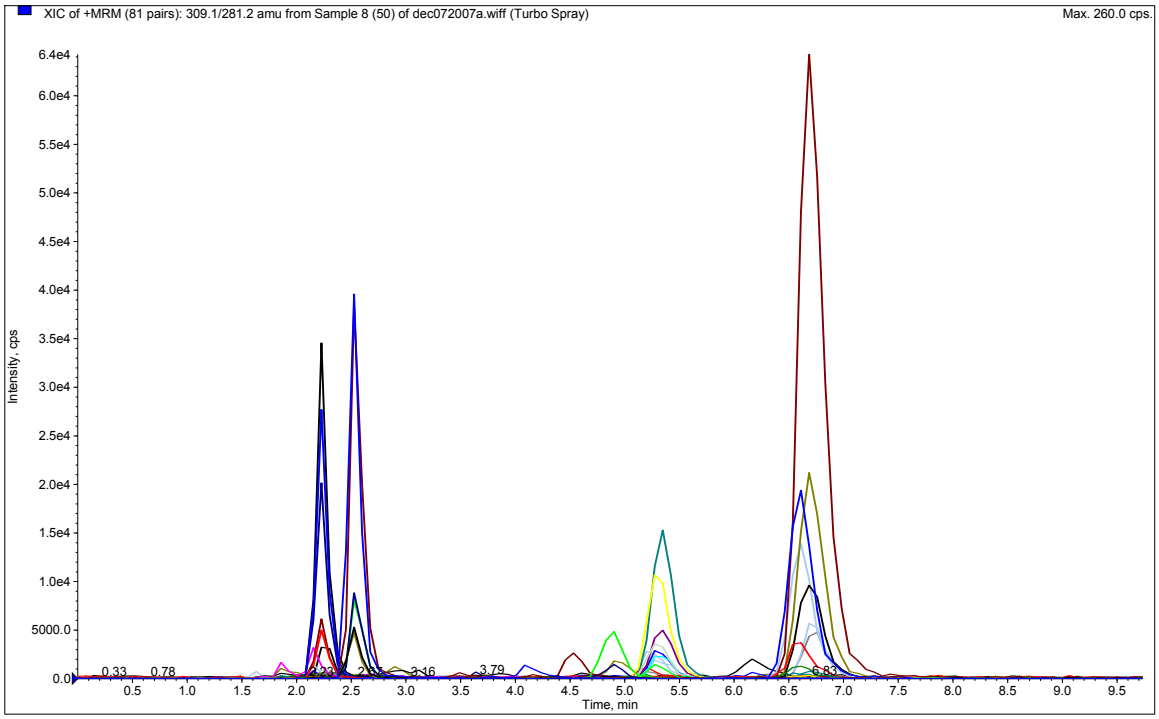
Chromatogram of:

7- aminoclonazepam/ 7-aminoclonazepam-D4

7-aminoflunitrazepam/ 7-aminoflunitrazepam-D7

Clonazepam/ Clonazepam-D4

Flunitrazepam/ Flunitrazepam-D7



Recovery: > 90% (N=20)
LOD: 1 ng/ mL

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Sample Preparation

Hydrolysis of Urine Sample for THC-delta-9-COOH

To 2 mL urine add appropriate internal standards prepared.
Add 50 µL of 10 N NaOH. Heat for 15 minutes at 60-70 °C
Add 50 µL 1:1 acetic acid: DI water. (pH should be 7.0±1.0)
Add 200 µL pH 7.0 0.1M Phosphate buffer)
(The sample is ready to be extracted.)

Applying Sample to Column

Load sample directly to column without any preconditioning.
Pull sample through at a rate of 1-2 mL/ minute.
Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi) for 2 minutes.

Wash

Wash sample with 1-2 mL of Hexane. (Be sure no sample droplets remain on sides of column.)
Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi)
for a minimum of 10 minutes.

NOTE 1: *(It is important to dry the column thoroughly before elution to achieve the highest recovery of THC-delta-9-COOH. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.)*

Elution

Elute samples with 1 mL ethyl acetate/ hexane/ acetic acid (49/49/2)
Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

GC/MS Analysis

It is recommended to add 50 µL of Ethyl Acetate to 50 µL of derivatization agent and react at ~70 °C for 15 minutes. Inject 1-2 µL of cooled solution in the GC/MS system for analysis.

LC/MS Analysis

Reconstitute in methanol or appropriate mobile phase.

GC/MS Derivatization Ions

Derivatizing Agent	THC-delta-9-COOH	(Cerilliant Part #T-006)
	(D9 THC-delta-9-COOH)	(Cerilliant Part #T-007)
BSTFA	371, 473, 488	(380, 479, 497)
MTBSTFA	413, 515, 572	(422, 524, 581)

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CARBOXY- delta 9-THC (pKa = 4.5) IN URINE FOR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSTHC020 or CSDAU206

February 3, 2009

1. **PREPARE SAMPLE - BASE HYDROLYSIS OF GLUCURONIDES**
   To 2 mL of urine add internal standard* and 100 µL of 10 M NaOH.
   Mix/vortex.
   Hydrolyze for 20 minutes at 60°C. Cool before proceeding.
   Adjust sample pH to 3.0 with approx. 1.0 mL of glacial acetic acid. Check pH to insure that the pH value is ~ 3.0.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL Acetate buffer (pH=3.0)
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**
   Load at 1 to 2 mL/minute.

4. **WASH COLUMN**
   1 x 2 mL D.I. H_2O.
   1 x 2 mL 100 mM HCl/acetonitrile (95:5).
   Dry column (5-10 minutes at greater than 10 inches Hg/ Full Flow for Positive Pressure manifold).
   1 x 200 µL hexane; Aspirate. (Additional step to remove any residual moisture.)

5. **ELUTE CARBOXY THC**
   1 x 3 mL hexane/ethyl acetate (50:50).
   Collect eluate at 1 to 2 mL/minute.

   NOTE: Before proceeding, insure there are no water droplets at the bottom of the collection tube. This may increase drying time and decrease BSTFA derivatizing efficiency.

6. **DRY ELUATE**
   Evaporate to dryness at < 40°C.

7. **DERIVATIZE**
   Add 50 µL ethyl acetate and 50 µL BSTFA (with 1% TMCS).
   Mix/vortex.
   React 20 minutes at 70°C.
   Remove from heat source to cool.
   NOTE: Do not evaporate BSTFA.

8. **QUANTITATE**
   Inject 1 to 2 µL onto gas chromatograph.
   For MSD monitor the following ions:

ANALYTE (TMS)	Primary Ion**	Secondary	Tertiary	Cerilliant #
Carboxy-delta 9-THC-D3*	374	476	491	T-008
Carboxy-delta 9-THC-D9*	380	479	497	T-007
Carboxy-delta 9-THC	371	473	488	T-019

* Suggested internal standard for GC/MS: -Carboxy-delta 9-THC-D9

DCN-903020-40

delta 9-THC (parent), delta 9-HYDROXY THC, CARBOXY- delta 9-THC IN WHOLE BLOOD FOR GC/MS CONFIRMATIONS USING:
200 mg CLEAN SCREEN® EXTRACTION COLUMN
Part #: ZSTHC020 or CSDAU206
February 3, 2009

1. **PREPARE SAMPLE**
   To 1-2 mL of whole blood add internal standards*.
   Mix/vortex.
   Add dopwise whilst vortexing, 1 mL of *Ice Cold* acetonitrile.
   Centrifuge and transfer acetonitrile to a clean tube.
   Adjust sample pH to 3.0 ± 0.5 with approx. 2.0 mL of 100mM Sodium Acetate buffer.
   (Check pH of buffer to insure that the pH value is ~ 3.0.)

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL Acetate buffer (pH=3.0)
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**
   Load at 1 to 2 mL/minute.

4. **WASH COLUMN**
   1 x 2 mL D.I. H_2O.
   1 x 2 mL 100 mM HCl/acetonitrile (95:5).
   Dry column (5-10 minutes at greater than 10 inches Hg/ Full Flow for Positive Pressure manifold).
   1 x 200 µL hexane; Aspirate. (Additional step to remove any residual moisture. Could substitute 200 µL MeOH for hexane.)
   Optional: Dry column (5 minutes at greater than 10 inches Hg/ Full Flow for Positive Pressure manifold).

   NOTE: The delta-9-THC (parent) will elute in hexane so special attention must be paid to not use more than 200 µL hexane in the wash/ dry step. The 200 µL hexane wash step can be eliminated if the column is allowed to dry longer under vacuum or by positive pressure gas flow.

5. **ELUTE THC (metabolites)**
   1 x 2 ml hexane (optional, contains delta-9-THC)
   1 x 3 mL hexane/ethyl acetate (50:50).
   Collect eluate at 1 to 2 mL/minute.

   NOTE: Before proceeding, insure there are no water droplets at the bottom of the collection tube. This may increase drying time and decrease BSTFA derivatizing agent efficiency.

6. DRY ELUATE

Evaporate to dryness at < 40°C.

7. DERIVATIZE

Add 50 µL ethyl acetate and 50 µL BSTFA (with 1% TMCS).
Mix/vortex.
React 20 minutes at 70°C.
Remove from heat source to cool.
NOTE: Do not evaporate BSTFA.

8. QUANTITATE

Inject 2 µL onto gas chromatograph.
For MSD monitor the following ions:

ANALYTE (TMS) Primary Ion / Secondary / Tertiary

Compound	Primary ion	Secondary	Tertiary	Cerilliant#
Carboxy- delta 9-THC-D3 TMS*	374	476	491	T-008
Carboxy- delta 9-THC-D9-TMS*	380	479	497	T-007
Carboxy-delta 9-THC	371	473	488	T-018
delta 9-THC-D3-TMS*	374	389		T-003
delta 9-THC-TMS	371	386		T-005
Hydroxy- delta 9-THC-D3-TMS*	374	462	477	H-041
Hydroxy- delta 9-THC-TMS	371	459	474	H-027

(303, 315, 330, 343)**

* Suggested internal standard for GC/MS: D9-Carboxy-delta $_{9}$-THC, D$_{3}$-Hyroxy- delta $_{9}$-THC, D$_{3}$-delta $_{9}$-THC
** Ions common to deuterated delta-9 THC and non-deuterated compounds.

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1. PREPARE SAMPLE:

To 1-2 mL whole blood add appropriate internal standards prepared in alcohol.

Add drop-wise 2 mL ice cold acetonitrile.*

Mix thoroughly and centrifuge.

Decant acetonitrile into a clean tube. Evaporate acetonitrile under a stream or air or nitrogen to ~ 200uL.

Add 2 mL distilled water (pH~6.0-7.0)

(The sample is ready to be extracted.)

***NOTE:** The acetonitrile should be cold (recommended storage in freezer at <0 C just prior to use) and it should be added very slowly to ensure proper mixing of organic phase with the whole blood. If added too quickly, the blood could precipitate to fast possibly resulting in lower recoveries.*

2. APPLY SAMPLE:

Load sample directly to column without any preconditioning.

3. WASH COLUMN:

Wash with 1 mL (84/15/1) Water/ Acetonitrile/ NH_4OH.

Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi) for 10-15 minutes.

***NOTE:** (It is important to dry the column properly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization. Also, any residual moisture could reduce the reactivity of the derivatizing agent.)*

4. ELUTE THC, THC-OH, THC-COOH

1 x 3 mL Hexane/ Ethyl Acetate/ Glacial Acetic Acid (49: 49:2)

Collect at 1-2 mL/ minute.

5. DRY ELUATE:

Evaporate fraction(s) to complete dryness under stream of dry air or nitrogen at <40°C

6. DERIVITIZE

Add 50 µL ethyl acetate, vortex mix
Add 50 µL BSTFA (with 1% TMCS).
or
Add 50 µL MTBSTFA (with 1% TBMCS).
Mix/vortex.
React 20 minutes at 70°C.
Remove from heat source to cool.
NOTE: Do not evaporate BSTFA.

7. QUANTITATE

Inject 2 µL onto gas chromatograph.
For MSD monitor the following ions:

DERIVATIZATION PROCEDURE

Derivatizing Agent	THC {T-005}** (D3 THC) {T-003}**	THC-OH {H-041}** (D3 THC-OH) {H-027}**	THC-COOH {T-006}** (D9 THC-COOH) {T-007}**
BSTFA	371, 343, 386 (374, 346, 389)	371, 459, 474 (374, 462, 477)	371, 473, 488 (380, 479, 497)
MTBSTFA	371, 428, 345 (374, 431, 348)	413, 369, 501 (416, 372, 504)	413, 515, 572 (422, 524, 581)

* Suggested internal standard for GC/MS: D9-Carboxy-delta ^{9}-THC, D$_3$-Hyroxy- delta ^{9}-THC, D$_3$-delta ^{9}-THC
** Ions common to deuterated delta-9 THC and non-deuterated compounds.

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Sample Preparation

To 1-2 mL urine add 0.5-1.0 mL of 0.1M Phosphate buffer (pH=6.0 ± 0.5).
Add appropriate volume and concentration internal standards.

Applying Sample to Column

Load *sample directly to column without any pre-conditioning.*

- Pull sample through at a rate of 1-2 mL/ minute.
- Apply pressure to column for **~1 minute** *[vacuum (10 mm Hg) or positive pressure (~ 80-100 psi)]* to make certain the entire sample and any residual is pulled through to waste.

Wash 1 (Acidic/ Neutral - Fraction -1)

Wash sample with 1 mL of **0.1M Acetic Acid**.

- Apply pressure to column for **~1 minute** *[vacuum (10 mm Hg) or positive pressure (~ 80-100 psi)]* to make certain the entire sample and any residual is pulled through to waste.

Wash sample with 1-2 mL **Hexane** to remove residual aqueous phase.

- **Dry** column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi) for a minimum of **~5 minutes**.

Elution 1 (Acidic/ Neutral Compounds – Fraction -1)

Elute samples with 1 mL **Hexane: Ethyl Acetate (50/50).**

- **Acid/ Neutral Fraction 1** can be analyzed separately or combined with **Basic Fraction 2** depending on analysis

Wash 2 (Basic Compounds – Fraction -2)

Wash sample with 1 mL of **2% acetic acid/ 98% methanol**. (Note: As an option, the **Wash 2** step can be omitted and proceed directly to Elution 2.)

- **Dry** column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi) for a minimum of **~5 minutes**.

NOTE 1: *(If analyzing by GC/MS, it is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.)*

Elution 2 (Basic Compound Fraction -2)

Elute samples with 1 mL **Methylene Chloride/ Iso-propanol/ Ammonium Hydroxide (78/20/2).**
Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 30-40 °C.

GC/MS Analysis

It is recommended to add 50 µL of Ethyl Acetate to 50 µL of derivatization agent and react at ~70 °C for 30 minutes. Inject 1-2 µL of cooled (50:50) solution in the GC/MS system for analysis.

LC/MS Analysis

Reconstitute in methanol or appropriate mobile phase.

Representative ACID/ NEUTRAL and BASIC Analytes Extracted**

Amphetamine/ Sympaths (12)	Opiates (12)*	Methadone/EDDP	Meperidine/Normeperidine
Tramadol/Nortramadol	Cocaine/BE/EME	TCA's(7)	Fentanyl/ Norfentanyl
Carisoprodol/ Meprobamate	Barbiturates (7)	Carbemazepine	Ibuprofen

*Sample must be hydrolyzed prior to extraction to determine total opiates.

** To extract the benzodiazepine group at higher recovery: a.) The **Wash -2** step (i.e. 2% Acetic acid/ 98 % Methanol, must be eliminated and b.) The **Elution 2** solvent must be changed to ***98% Ethyl Acetate/ 2% Ammonium Hydroxide***.

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BARBITURATES IN URINE FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. **PREPARE SAMPLE**
   To 2 mL of urine add internal standard(s)* and 1 mL of 100 mM phosphate buffer (pH 5.0).
   Mix/vortex.
   Sample pH should be 5.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH 5.0).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**
   Load at 1 to 2 mL/minute.

4. **WASH COLUMN**
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM acetic acid.
   Dry column (5 minutes at > 10 inches Hg).
   1 x 2 mL hexane.

5. **ELUTE BARBITURATES**
   1 x 3 mL hexane/ethyl acetate (50:50); Collect eluate at 1 to 2 mL / minute.

6. **DRY ELUATE**
   Evaporate to dryness at < 40°C.
   Reconstitute with 100 µL ethyl acetate.
   OPTIONAL DERIVATIZATION
   Add 25-50 µL of 0.2 M TMPAH****,
   Reaction occurs in injection port

7. **QUANTITATE**
   Inject 1 to 2 µL onto gas chromatograph.
   For MSD monitor the following ions:

UNDERIVATIZED

Drug	Primary Ion**	Secondary Ion	Tertiary Ion	Cerilliant #
Amobarbital:	156	141	157	A-020
Butabarbital:	156	141	157	B-006
Butalbital:	168	167	181	B-024
Hexobarbital*	221	157	236	H-013
Pentobarbital	156	141	197	P-010
Phenobarbital	204	232	117	P-008
Secobarbital	168	167	195	S-002
Thiopental:	172	157	173	

DERIVATIZED

Drug	Primary Ion**	Secondary Ion	Tertiary Ion	Cerilliant #
Butalbital-D5	201	214		B-005
Butalbital	196	195	209	B-024
Amobarbital	169	184	185	A-020
Pentobarbital	169	184	112	P-010
$^{13}C_4$-Secobarbital	200	185		
Secobarbital	196	195	181	S-002
Phenobarbital-D5I	237	151		P-017/P-018
Phenobarbital	232	146	175	P-008

**Target ions in bold

* Suggested internal standard for GC/MS: Hexobarbital or a Deuterated Barbiturate analog.
** Quantitation Ion
**** Part # STMPAH-0-1 1, 10, 25, 100

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BARBITURATES IN BLOOD, PLASMA/SERUM, URINE, AND TISSUE FOR GC OR GCMS
CONFIRMATION USING: 80 mg CLEAN SCREEN®
REDUCED SOLVENT VOLUME EXTRACTION COLUMN

Part #: ZSDAUA08 without Tips or ZCDAUA08 with CLEAN-THRU® Tips

February 3, 2009

1. **PREPARE SAMPLE**

   To 1 mL of 100 mM phosphate buffer (pH =6.0) add internal standards*. Mix/vortex and add 1 mL of blood, plasma/serum, urine or 1 g (1:4) tissue homogenate.
   Mix/vortex.
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
   Centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**

   1 x 0.5 mL CH_3OH.
   1 x 0.5 mL D.I. H_2O.
   1 x 0.25 mL 100 mM phosphate buffer (pH= 6.0).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**

   Load at 1 to 2 mL/minute.

4. **WASH COLUMN**

   1 x 0.5 mL D.I. H_2O; Aspirate.
   1 x 0.5 mL 100 mM acetic acid; Aspirate.
   Dry column (5 minutes at > 10 inches Hg).
   1 x 0.1 mL hexane; Aspirate.

5. **ELUTE BARBITURATES**

   1.5 mL hexane/ethyl acetate (50:50).
   Collect at 1-2 mL/ minute

6. **DRY ELUATE**

   Evaporate to dryness at < 40°C.
   Reconstitute with 100 µL ethyl acetate.
   OPTIONAL DERIVATIZATION
   Add 25 µL of 0.2 M TMPAH****,
   Reaction occurs in injection port

7. **QUANTITATE**

   Inject 1 to 2 µL onto gas chromatograph.
   For MSD monitor the following ions:

UNDERIVATIZED

Drug	Primary Ion**	Secondary Ion	Tertiary Ion	Cerilliant #
Amobarbital:	156	141	157	A-020
Butabarbital:	156	141	157	B-006
Butalbital:	168	167	181	B-024
Hexobarbital*	221	157	236	H-013
Pentobarbital	156	141	197	P-010
Phenobarbital	204	232	117	P-008
Secobarbital	168	167	195	S-002
Thiopental:	172	157	173	

DERIVATIZED

Drug	Primary Ion**	Secondary Ion	Tertiary Ion	Cerilliant #
Butalbital-D5	201	214		B-005
Butalbital	196	195	209	B-024
Amobarbital	169	184	185	A-020
Pentobarbital	169	184	112	P-010
$^{13}C_4$-Secobarbital	200	185		
Secobarbital	196	195	181	S-002
Phenobarbital-D5I	237	151		P-017/P-018
Phenobarbital	232	146	175	P-008

* Suggested internal standard for GC/MS: Hexobarbital or a Deuterated Barbiturate analog.
** Quantitation Ion Target ions in bold
**** Part # STMPAH-0-1, 10, 25, 100

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BETA AGONISTS IN URINE FOR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. **PREPARE SAMPLE**:
   To 1 mL of 100 mM Acetate Buffer (pH 4.5) add 1 mL of Urine. Add 2 mL of of 100 mM Acetate Buffer (pH 4.5).
   Mix/ vortex.
   Centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 3 mL 100 mM Acetate Buffer (pH 4.7).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**
   Load at 1 to 2 mL/ minute.

4. **WASH COLUMN**
   2 X 1 mL Acetone/ Methanol (1:1) aspirate.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE BETA AGONISTS**
   1 x 1 mL Dichloromethane/ Isopropanol and Ammonium Hydroxide (78:20:2).
   Collect the eluate at 1-2 mL/ minute (or gravity).
   NOTE: Prepare elution solvent fresh daily. Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

6. **DRY ELUATE**
   Evaporate to dryness at < 40°C.

7. **DERIVATIZE**
   Derivatization Solution: Methaneboronic acid at 5 mg/mL prepared in dry ethyl acetate (use molecular sieve).
   Store this solution at –20°C (freezer conditions) until use.

   Reaction Mixture:
   Add 100 µL of the Methaneboronic acid solution (see above).
   Mix/vortex.
   React 15 minutes at 70°C. Remove from heat source to cool.

   NOTE: Do not evaporate this solution.

8. **ANALYSIS**
   Inject 1 to 2 µL sample (derivatized solution).

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CARISOPRODOL AND MEPROBAMATE IN BLOOD, PLASMA/SERUM, URINE, TISSUE FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. PREPARE SAMPLE

To 1 mL of 100 mM phosphate buffer (pH= 3) add internal standard*. Add 1 mL blood, plasma serum, urine, or 1 g (1:4) tissue homogenate. Add 2 mL of 100 mM phosphate buffer (pH= 3). Mix/ vortex
Centrifuge as appropriate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM phosphate buffer (pH= 3.0).
NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 to 2 mL/ minute.

4. WASH COLUMN

1 x 4 mL deionized water.
1 x 2 mL 100 mM HCl.
Dry column (5 minutes at > 10 inches Hg).
1 x 3 mL hexane.

5. ELUTE CARISOPRODOL / MEPROBAMATE

1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2);
Collect eluate at 1 to 2 mL/minute.

6. DRY ELUATE

Evaporate to dryness at < 40°C.

7. QUANTITATE

Reconstitute with 100 µL ethyl acetate.
Inject 1 to 2 µL sample on gas chromatograph.
For MSD monitor the following ions:

Compound	Primary Ion***	Secondary	Tertiary	Cerilliant #
Carisoprodol	158	104	245	C-007
Meprobamate	83	114	144	M-039
*Hexobarbital	221	157	81	H-013
*Meprobamate-D7	90	121	151	M-131

*Suggested internal standard for GC/MS: Hexobarbital, or Meprobamate-D7
***Quantitation ion

DCN-903020-46

GABAPENTIN IN BLOOD, PLASMA/ SERUM FOR GC OR GC/MS ANALYSIS USING: 100 mg CLEAN-UP® C18 EXTRACTION COLUMN

Part #: CUC18111

February 3, 2009

1. **PREPARE SAMPLE**
   To 500 µL of 20% acetic acid add internal standard*. Mix/vortex. Add 500 µL of blood, plasma/ serum.
   Mix/ vortex.
   Centrifuge as appriate

2. **CONDITION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM HCL.

NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**
   Load at 1 to mL/minute.

4. **WASH COLUMN**
   1 x 3 mL D.I. H_2O.
   1 x 3 mL ethyl acetate.
   1 x 3 mL hexane.
   Dry column (5 minutes at > 10 inches Hg) or until column is dry.

5. **ELUTION**
   1 x 1 mL 2% NH_4OH in CH_3OH.

6. **DRY ELUATE**
   Evaporate to dryness at < 40°C.

1. **DERIVATIZATION**
   Add 50 µL of ethyl acetate and 50 µL of BSTFA (1 % TCMS)
   or
   Add 50 µL of MTBSTFA + 1 % t-BDMCS** and 50 µL ethyl acetate.
   Cap and heat at 70°C for 30 minutes.
   Remove and allow to cool.

8. **QUANTITATE**
   Inject 1 to 2 µL onto gas chromatograph.

Compound	Primary	Secondary	Tertiary	Ceriliant #
Gabapentin-TMS	210	225	182	G-007
*Gabapentin-D10-TMS	220	235	192	B-130315-10

*** INTERNAL STANDARD:**
1-aminomethyl-1-cycloheptyl acetic acid (FID): Gabapentin-D10 (GC-MS)
** Part # SMTBSTFA-1-1,10, 25,100

Reference:

Carl E. Wolf II, Joseph Sady, and Alphonse Pokalis Determination of Gabapentin in Serum using Solid Phase Extraction and Gas-Liquid Chromatography. Journal of Analytical Toxicology 20:498-501 (October 1996)

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1. **PREPARE SAMPLE:**

   To 0.2-0.5 mL of sample add 1 mL of acetone (dropwise) whilst vortexing

   Add internal standard*

   Vortex mix and centrifuge as appropriate

   Transfer organic phase to clean tube

   Evaporate to dryness

   Add 3 mL of 100 mM HCl

   Vortex mix and centrifuge as appropriate

2. **CONDITION COLUMN:**

   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 mL 100 mM HCl
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out.

3. **APPLY SAMPLE:**

   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**

   1 x 3 mL D.I. H_2O
   1 x 3 mL ethyl acetate
   1 x 3 mL hexane
   Dry column (10 minutes at > 10 inches Hg).

5. **ELUTE GABAPENTIN**

   1 x 3 mL CH_3OH containing 2% NH_4OH
   Collect eluate at 1-2 mL /minute.

6. EVAPORATION:

Evaporate eluates under a gentle stream of nitrogen < 40°.
Dissolve residue in 100 µL CH_3OH.

INSTRUMENT CONDITIONS:

Column: 50 x 2.1 mm (3 µm) Selectra® Phenyl (UCT, LLC)

Mobile phase:	Time	Acetonitrile	0.1% Formic Acid
	0	10	90
	5	90	10
	5.5	10	90
	10	10	90

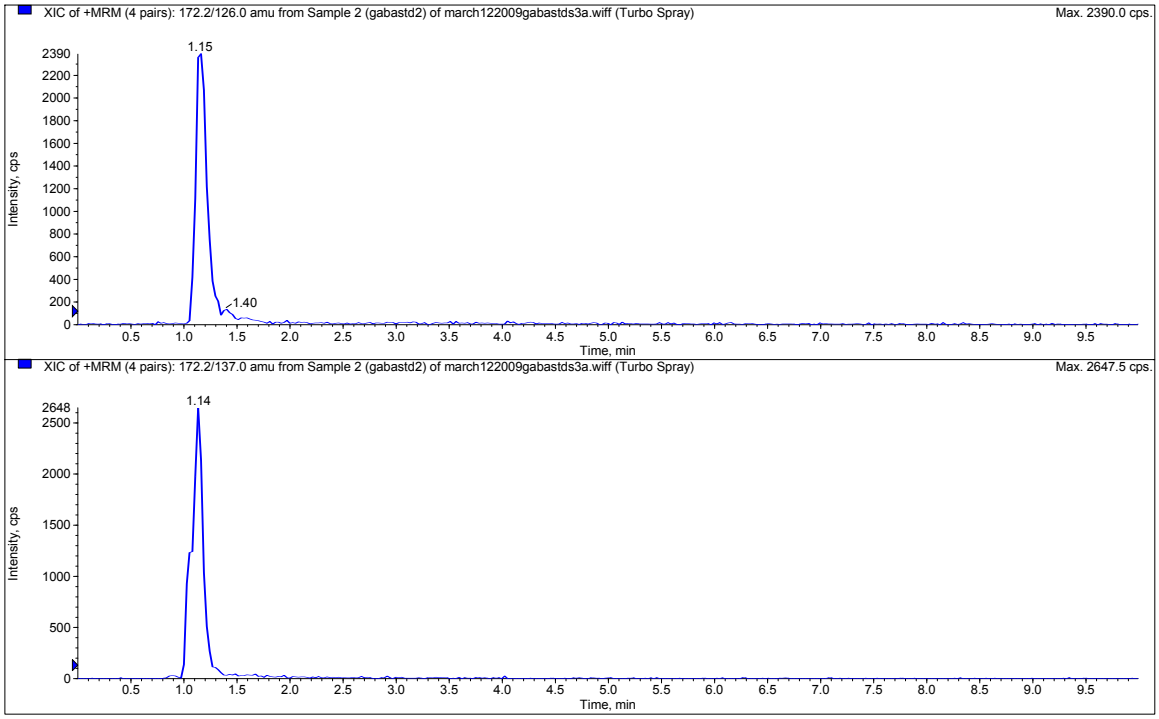
Flowrate: 0.2 mL/ minute

Injection Volume: 5 µL

Column Temperature: ambient

Detector: API 2000 MS/MS

Chromatogram of Gabapentin (top) and Aminocyclohexanepropionic acid (lower)



Compound	MRM Transition
Gabapentin	172.2/ 137
*Gabapentin-D10	182.2/147
* Aminocyclohexane-propionic acid	172.2/ 126

Recovery (approx 70%)

LOD= 50 ng/ mL

DCN-900330-145

METHYLMALONIC ACID FROM SERUM OR PLASMA FOR GC/MS ANALYSIS USING: 500 mg CLEAN-UP® QAX EXTRACTION COLUMN

Part #: CUQAX15Z

February 3, 2009

1. PREPARE SAMPLE

Add 100 µL of internal standard D_3-MMA and 1 mL of acetonitrile to 1 mL of plasma or serum.
Vortex for 20 sec.
Centrifuge for 5 min at 2000 rpm.

2. CONDITION CLEAN UP® EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.

3. APPLY SAMPLE

Decant supernatant onto SPE column.

4. WASH COLUMN

1 x 10 mL of D.I. H_2O.
Dry with vacuum for 3 min.
1 x 5 mL of CH_3OH.
Dry with vacuum for 3 min.
1 x 2 mL of MTBE*.
Dry with vacuum for 3 min.

5. ELUTE METHYLMALONIC ACID

1 x 5 mL of 3% formic acid in MTBE, collect at 1 to 2 mL/min.

6. DRY ELUATE

Dry under a stream of nitrogen at < 35°C.

7. DERIVATIZE

Reconstitute with 25 µL of MSTFA + 1% TMCS** and 25 µL ethyl acetate.
Heat for 20 min at 60°C.

8. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.

* MTBE: methyl-tert-butyl ether
** Part # SMSTFA-1-1, 10, 25,100

Compliments of
Mark M. Kusmin and Gabor Kormaromy-Hiller ARUP LABORATORIES

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NICOTINE AND COTININE IN URINE OR SERUM FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. PREPARE SAMPLE

To 2 mL of 100 mM phosphate buffer (pH =6.0) add internal standards*. Add 2 mL of urine or serum..
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphateMix/vortex.
Centrifuge as appropriate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM phosphate buffer (pH 6.0).
NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 mL/minute.

4. WASH COLUMN

1 x 3 mL D.I. H_2O.
1 x 2 mL 200 mM HCl.
Dry column (5 minutes at > 10 inches Hg).
1 x 2 mL Hexane.

5. WASH COLUMN

Remove rack of collection tubes to rewash columns.
1 x 3 mL CH_3OH.
Dry column, (5 minutes at > 10 inches Hg).

6. ELUTE COTININE AND NICOTINE

Replace rack of collection tubes.
1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2); Collect eluate at 1 mL/minute.
NOTE: Prepare elution solvent daily. Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

7. CONCENTRATE

Evaporate to dryness at < 40 °C.
Take care not to over-heat or over evaporate.
Reconstitute with 100 µL ethyl acetate.

8. QUANTITATE

Inject 1 to 2 µL onto chromatograph.
Monitor the following ions (GC/MS):

Compound	Primary ion**	Secondary	Tertiary	Cerilliant#
Nicotine	84	133	162	N-008
*Nicotine-D4	88	137	166	N-048
Cotinine	98	119	176	C-016
*Cotine-D3	101	122	179	C-017

** Quantitation Ion

SOURCE - UCT Internal Publication

DCN-903020-49

THERAPEUTIC AND ABUSED DRUGS IN BLOOD, PLASMA/SERUM AND URINE FOR ACID/NEUTRAL AND BASIC DRUGS FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. **PREPARE SAMPLE**

Urine

To 1 mL of 100 mM phosphate buffer (pH=6.0) add internal standards*. Add 2 mL of urine.
Mix/vortex.
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate
Centrifuge as appropriate.

Blood, Plasma or Serum

To 1 mL of 100 mM phosphate buffer (pH =6.0) add internal standards* Add 1 mL of sample and 4 mL D.I. H_2O .
Mix/vortex and let stand 5 minutes.
Centrifuge for 10 minutes at 2000 rpm and discard pellet.
Add 2 mL 100 mM phosphate buffer (pH 6.0). Mix/vortex.
Sample pH should be 6.0 ± 0.5. Adjust pH accordingly with100 mM monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**

Load at 1 to 2 mL/minute.

4. **WASH COLUMN**

1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM acetic acid.
Dry column (5 minutes at > 10 inches Hg).
1 x 2 mL hexane.

5. **ELUTE ACIDIC AND NEUTRAL DRUGS (FRACTION 1)**

1 x 3 mL hexane/ethyl acetate (50:50);
Collect eluate at < 2 mL/minute.

6. **DRY ELUATE**

Evaporate to dryness at < 40°C.
Reconstitute with 100 µL ethyl acetate.

7. **QUANTITATE ACIDIC AND NEUTRAL DRUGS**

Inject 1 to 2 µL onto gas chromatograph.

8. **WASH COLUMN**

1 x 3 mL CH_3OH; Aspirate.
Dry column (5 minutes at > 10 inches Hg).

9. ELUTE BASIC DRUGS (FRACTION 2)

1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2).

Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent fresh daily.

Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

10. DRY ELUATE

Evaporate to dryness at < 40°C using a TurboVap® or equivalent evaporator. Take care not to overheat or over evaporate. Certain compounds are heat labile, such as the amphetamines and phencyclidine. Reconstitute with 100 µL ethyl acetate.

11. QUANTITATE Basic Drugs

Inject 1 to 2 µL onto gas chromatograph.

NOTES:

(1) Fraction 1 (Acid Neutrals) and Fraction 2 (Bases) can be combined together.

(2) A keeper solvent such as DMF can be used to prevent the volatilization of amphetamines and phencyclidine. Use 30-50 µL of high purity DMF in the sample (Fraction 2) before evaporation.

(3) A 1% HCl in CH_3OH solution has been used to prevent volatization by the formation of the hydrochloric salt of the drugs. Evaporate fraction 2 to approximately 100 µL, then add 1 drop of the solution. Continue to evaporate to dryness.

SOURCE: UCT Internal Publication

Data Provided By:
City of Philadelphia,
Department of Public Health Office of the Medical Examiner
321 University Avenue Philadelphia, Pennsylvania 19104
Contact: Frank Caputo, Analytical Chemist II (215) 8237464

The following are some of the many compounds that have been extracted from forensic samples with the CLEAN SCREEN® DAU bonded silica extraction cartridge (Part #: CSDAU303):

I. ACIDIC / NEUTRAL DRUG FRACTION (A)

Acetaminophen
Barbiturates
Benzoic acid
Caffeine
Carbamazepine
Carisoprodol
Chlorpropamide
Clonazepam
Cotinine
Diazepam
Glutethimide and metabolite
Ibuprofen
Meprobamate
Methyl salicylate
Nordiazepam
Phenytoin
Primidone
Salicylic acid
Theophylline
Thiopental

II. BASIC DRUG FRACTION (B)

Amantadine
Amitriptyline and metabolite
Amphetamine
Benzocaine
Benzoylecgonine
Benztropine
Bromodiphenhydramine
Chlordiazepoxide
Chloroquine
Chlorpheniramine
Chlorpromazine
Cocaine and metabolite
Codeine
Cresol
Dextromethorphan
Dextrorphan
Dihydrocodeine
Dihehydramine
Doxepin and metabolite
Ephedrine
Fluoxetine
Imipramine and metabolite
Ketamine
Lidapine
Loxapine
Meperidine
Methadone and metabolite
Methamphetamine
Methyl ρ-aminobenzoate
Methyl benzoate
Methyl ecgonine
Methylparaben
Methylphenidate
Methyprylon and metabolites
Morphine
Nicotine
Oxycodone
Pentazocine
Phencyclidine
Phenethylamine
Phentermine
Phenylpropanolamine
Procaine
Propoxyphene and metabolite
Propylparaben
Tranylcypromine
Trifluoperazine
Trimipramine
Thioridazine
Trazodone

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WARFARIN IN WHOLE BLOOD:
MANUAL METHOD FOR GC-MS OR LC CONFIRMATIONS
USING: 200 mg CLEAN-UP® C-30 EXTRACTION COLUMN

Part #: CEC30203

February 3, 2009

1. PREPARE SAMPLE

To 9 mL of 100 mM phosphate buffer (pH= 6.0) add internal standard. Add 1mL of whole blood) and Mix/vortex.
Sample pH should be 6.0 + 0.5.
Adjust pH accordingly with 0.1 M monobasic or dibasic sodium phosphate.
Centrifuge as appropriate

2. CONDITION EXTRACTION COLUMN

1 x 3 mL CH_3OH
1 x 3 mL DI H_2
1 x 3 mL 100 mM phosphate buffer, (pH=6.0) aspirate.
NOTE: Aspirate at < 3 inches. Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1-2 mL/min.

4. WASH COLUMN

Add 1 x 3 mL of phosphate buffer (0.1 M pH 6)
Dry under full vacuum for 10 mins
Add 1 x 3 mL of Hexane
Dry under full vacuum for 10 mins

5. ELUTE WARFARIN:

Add 2 x 3 mL of methanol/ ethyl acetate (12:88)
Note: Prepare elution solvent daily.

6. Collect eluates at approx 1-2 mL/minute

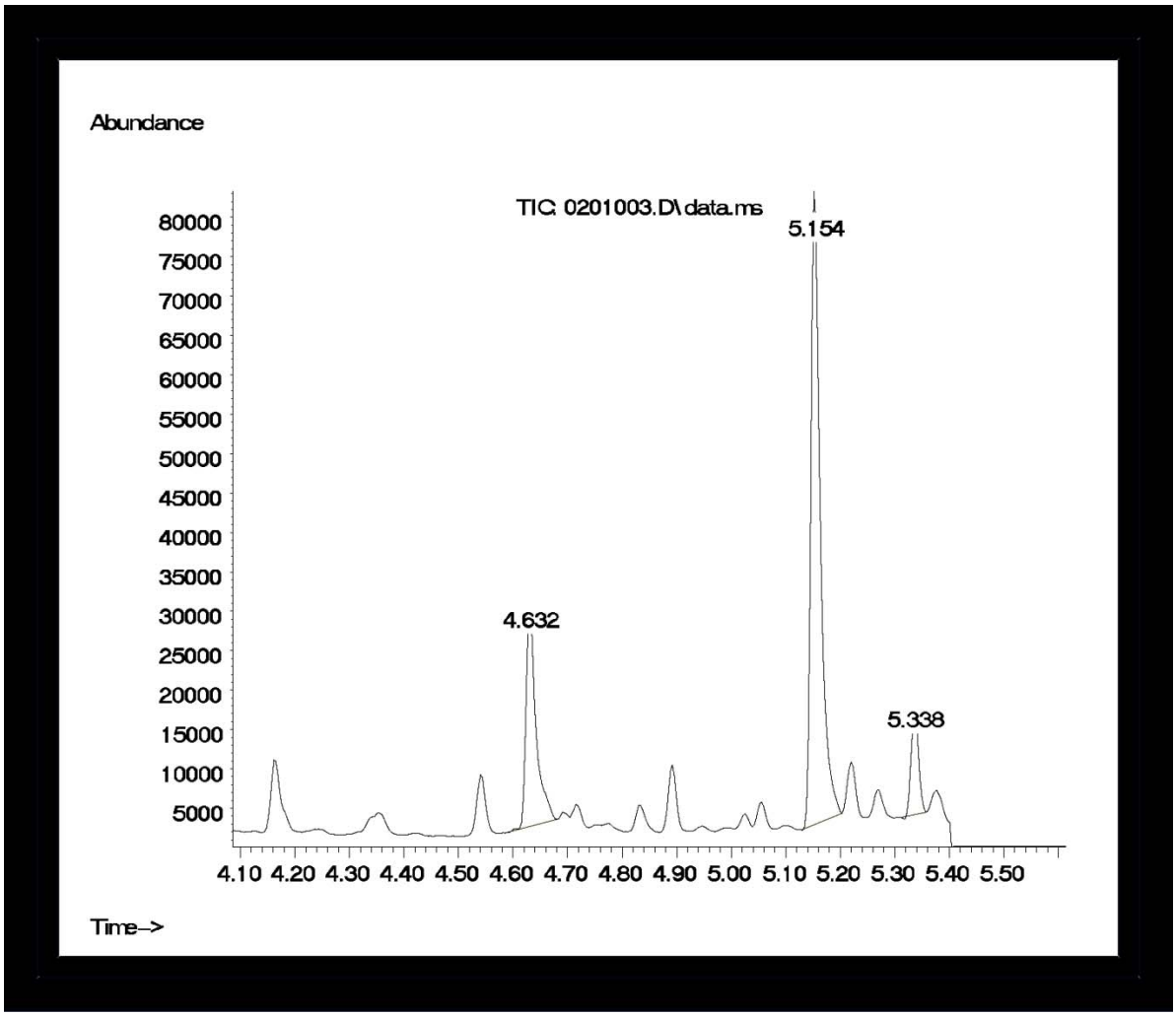
7. Dry samples

Evaporate to dryness at <40°C
Add 50 µL of ethyl acetate.
Add 50 µL of TMAH, and vortex.
React at for 1 hour at 70˚C.
Cool and inject 1-2 µL onto GC-MS
Monitor the following ions:

Compound	Primary	Secondary	Tertiary	Cerilliant #
Warfarin	279	322	280	W-003
*p-chlorowarfarin	313	315	356	

WARFARIN CHROMATOGRAM

GC-MS (methylation)



DCN-903020-51

CAFFEINE, THEOPHYLLINE AND THEOBROMINE IN BLOOD, PLASMA/SERUM, AND URINE USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020

LC-PDA

February 3, 2009

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM acetic acid add internal standard.*
   Add 1 mL Blood, Serum/ Plasma, or Urine. Add 2 mL of 100 mM acetic acid.
   Vortex and centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 mL 100 mM acetic acid.
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**
   1 x 3 mL DI H_2O
   1 x 3 mL 100 mM acetic acid.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE CAFFEINE/THEOBROMINE/THEOPHYLLINE:**
   1 x 3 mL ethyl acetate : methanol (90: 10)
   Collect eluate at 1-2 mL / minute.

6. **EVAPORATION:**
   Combine eluates
   Evaporate eluates under a gentle stream of nitrogen < 40°C.

7. **RECONSITITUE sample in 1000 µL of 0.1 % Formic acid (aq).**
   Inject 20 µL.

INSTRUMENT CONDITIONS:

Column: 150 x 2.1 mm (3 µm) Gold C_{18} (Thermofisher)

Mobile phase: Acetonitrile: 0.1% Formic acid aqueous (10:90).

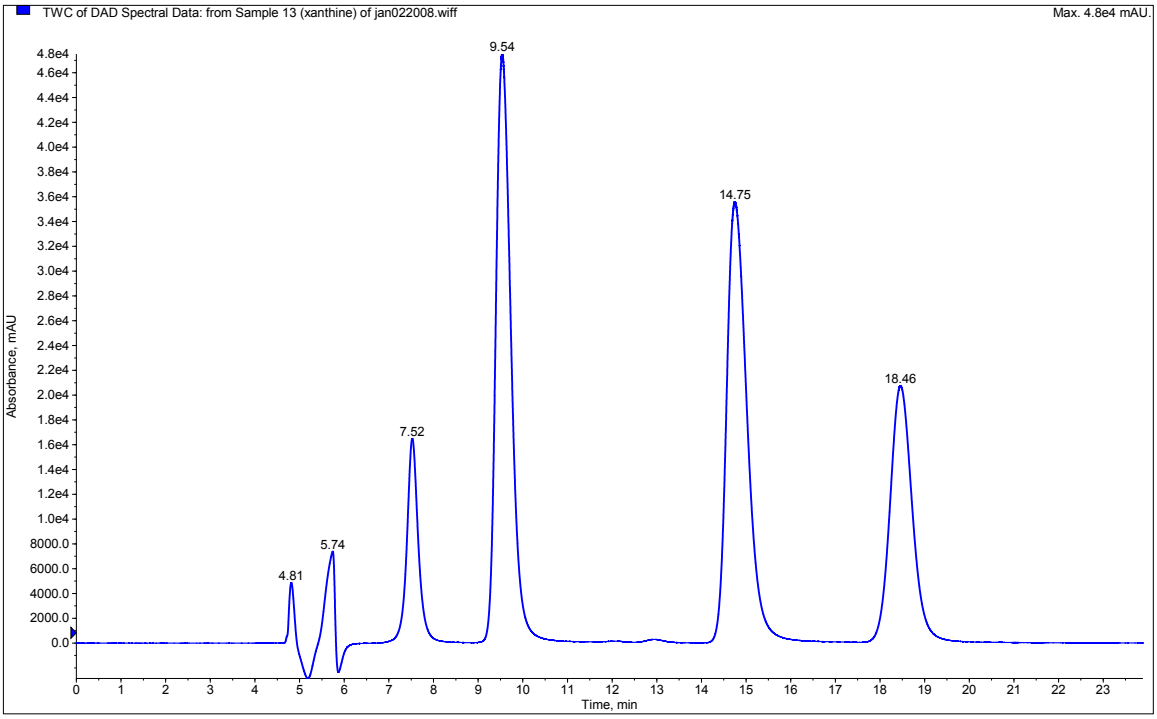
Flowrate: 0.1 mL/ minute

Column Temperature: ambient

Detector: Diode Array (200-350 nm)

CHROMATOGRAM OF SHOWING:

Compound		**Cerilliant #**
Theobromine :	7.5 minutes	T--013
Theophylline :	9.5 minutes	IMPC-051-01
Caffeine:	14.5 minutes	C-0151
*8-Chlorotheophylline:	18.0 minutes	



Recovery: > 90% (N=10)

LOD: 1 µg/ mL

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EXTRACTION OF BASIC DRUGS AND METABOLITES FROM URINE SPE CARTRIDGES

100mg Clean Screen Xcel I Column
Part #: CSXCE106 6 mL - 100 mg Cartridge
Part #: CSXCE103 3 mL - 100 mg Cartridge
September 9, 2009

SAMPLE PREPARATION

To 1-5 mL urine add 1-2 mL of 0.1M Phosphate buffer (pH=6.0 + 0.5). Add appropriate volume and concentration internal standards. **Note 1:** See alternate hydrolysis step for opiate samples.

(Alternate Hydrolysis Step)

To 1-5 mL urine sample 1-2 mL of acetate buffer (pH= 5.0) containing 5,000 units/mL ß-glucuronidase. **Optionally,** add 500 µL of acetate buffer and 25 µL of concentrated ß-glucuronidase. Vortex and heat for 1-2 hours at 65 °C. (Hydroxylamine can be added to sample if oxime derivative is preferred.) Allow sample to cool.
Do not adjust pH- sample is ready to be added to extraction column.

APPLYING SAMPLE TO COLUMN

Load sample directly to column without any preconditioning.Pull sample through at a rate of 1-2 mL/ minute.
Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi) for 1 minute.

WASH

Wash sample with 1-2 mL of 2% acetic acid/ 98% methanol. Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi) for a minimum of 5 minutes.

NOTE 2: (It is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.)

ELUTION

Elute samples with 1-2 mL Methylene Chloride/ Iso-propanol/ Ammonium Hydroxide (78/20/2).
Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 30-40 °C.

GC/MS Analysis

It is recommended to add 50 µL of Ethyl Acetate to 50 µL of derivatization agent and react at ~70 °C for 30 minutes. Inject 1-2 µL of cooled (50:50) solution in the GC/MS system for analysis.

LC/MS Analysis

Reconstitute in methanol or appropriate mobile phase.

Representative Analytes Extracted

Amph/Methamph	MDMA/MDA/MDEA	Opiates (7)	Methadone/EDDP
Sympathomimetics	Meperidine/Normeperidine	Phencyclidine	Cocaine/Benzoylecgonine
Tricyclic Antidepressants (7)	Cyclobenzaprine	Fentanyl/ Norfent	Sertraline
Tramadol/Nortramodol	Diphenhydramine	Citalopram	Clonidine

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EXTRACTION OF BASIC DRUGS AND METABOLITES FROM URINE USING SPE WELL PLATES

(Part #: WSH96XCE11 - 130 mg 96 well plate)
(Part #: WSH48XCE11 - 130 mg 48 well plate)
July 1, 2009

Sample Preparation

To 1-2 mL urine add 500 µL of pH=6.0 ± 0.5 0.1M Phosphate buffer.
Add appropriate volume and concentration internal standards.
(***Note 1:*** *See alternate hydrolysis step for opiate samples*).

(**Alternate Hydrolysis Step)**
To 1-2 mL urine sample add 500 µL of acetate buffer (pH= 5.0) containing 5,000 units/mL β-glucuronidase. **Optionally**, add 500 µL of acetate buffer and 25 uL of concentrated β-glucuronidase.
Vortex and heat for 1-2 hours at 65 °C.
(Hydroxylamine can be added to sample if oxime derivative is preferred.)
Allow sample to cool.

Do not adjust pH- sample is ready to be added to extraction column.

Applying Sample to Column

Load sample directly to column without any preconditioning.
Pull sample through at a rate of 1-2 mL/ minute.
Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi) for 1 minute.

Wash

Wash sample with 1 mL of 2% acetic acid/ 98% methanol.
Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi)
for a minimum of 5 minutes.

NOTE 2: *(It is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.)*

Elution

Elute samples with 1 mL Methylene Chloride/ Iso-propanol/ Ammonium Hydroxide (78/20/2)
Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 30-40 °C.

GC/MS Analysis

It is recommended to add 50 µL of Ethyl Acetate to 50 µL of derivatization agent and react at ~70 °C for 30 minutes. Inject 1-2 µL of cooled (50:50) solution in the GC/MS system for analysis.

LC/MS Analysis

Reconstitute in methanol or appropriate mobile phase.

Representative Analytes Extracted

Amph/Methamph	MDMA/MDA/MDEA	Opiates (7)	Methadone/EDDP
Sympathomimetics	Meperidine/Normeperidine	Phencyclidine	Cocaine/Benzoylecgonine
Tricyclic Antidepressants (7)	Cyclobenzaprine	Fentanyl/ Norfent	Sertraline
Tramadol/Nortramodol	Diphenhydramine	Citalopram	Clonidine

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BASIC DRUGS FOR HPLC ANALYSIS USING:
200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. **PREPARE SAMPLE**
   To 1 m of 100 mM phosphate buffer (pH= 6.0) add internal standards*. Mix/vortex. Add 1-5 mL of urine or 1 mL of blood, plasma/serum or 1g (1: 4) tissue homogenate.
   Mix/vortex.
   Add 2 mL of 100 mM phosphate buffer (pH 6.0)
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
   Centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**
   Load at 1 to 2 mL/minute.

4. **WASH COLUMN**
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM acetic acid.
   1 x 3 mL methanol.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE BASES**
   1 x 2 mL CH_3OH/NH_4OH (98:2).
   Collect eluate at 1 to 2 mL/minute.
   NOTE: Prepare elution solvent daily.

6. **EXTRACT**
   To eluate add 2.0 mL D.I. H_2O and 500 µL methylene chloride.
   Mix/vortex.
   Centrifuge at 2,000 RPM for 10 minutes.
   Transfer organic lower layer to a clean test tube.

7. **EVAPORATE**
   Evaporate to dryness at < 40°C.

8. **QUANTITATE**
   Reconstitute in mobile phase and inject onto the HPLC.

SOURCE: UCT Internal Publication

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BETA BLOCKERS IN BLOOD, URINE FOR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. PREPARE SAMPLE

To 1 mL of Acetate buffer (pH=4.5) add 1 mL of blood or urine. Add 2 mL of Acetate buffer (pH 4.5).
Mix/vortex
Centrifuge as appropriate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 3 mL 100 mM Acetate Buffer (pH= 4.5).
NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 to 2 mL/ minute.

4. WASH COLUMN

2 x 1 mL Acetone/ Methanol (1:1) aspirate.
Dry column (5 minutes at > 10 inches Hg).

5. ELUTE BETA BLOCKERS

1 x 1 mL Dichloromethane/ Isopropanol/Ammonium Hydroxide (78:20:2).
Collect the eluate by gravity.
NOTE: Prepare elution solvent fresh daily. Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

6. DRY ELUATE

Evaporate to dryness at < 40°C.

7. DERIVATIZE

Derivatization Solution: Methaneboronic acid at 5 mg/mL prepared in dry ethyl acetate (use molecular sieve).
Store this solution at –20°C (freezer conditions) until use.

Reaction Mixture

Add 100 µL of the Methaneboronic acid solution (see above).
Mix/vortex.
React 15 minutes at 70°C. Remove from heat source to cool.

NOTE: Do not evaporate this solution.

8. ANALYSIS

Inject 1 to 2 µL sample.

Reference:

Branum G, Sweeney S, Palmeri A, Haines L and Huber C
The Feasibility of the Detection and Quantitation of ß Adrenergic Blockers By Solid Phase Extraction and Subsequent Derivatization with Methaneboronic Acid. Journal of Analytical Toxicology 22: 135-141 (1998

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TRICYCLIC ANTIDEPRESSANTS IN SERUM AND PLASMA FOR HPLC USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. **PREPARE SAMPLE**

   To 2 mL of 100 mM phosphate buffer (pH= 6.0) add internal standard*. Add 1 mL of plasma/serum.
   Mix/vortex.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet
   Sample pH should be 6.0 ± 0.5. Adjust pH accordingly with
   100 mM monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**

   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**

   Load at 1 mL/minute.

4. **WASH COLUMN**

   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM acetic acid.
   1 x 3 mL CH_3OH.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE**

   1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2). Collect eluate at
   1 mL/minute or use gravity flow.
   NOTE: Prepare elution solvent fresh daily. Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

6. **DRY ELUATE**

   Evaporate to dryness at < 40°C.

7. **QUANTITATE**

   Reconstitute with 200 µL ethyl acetate/D.I. H_2O (1:3).
   Mix/vortex vigorously for 30 seconds.
   Inject 100 µL onto HPLC.

HPLC CONDITIONS

HPLC COLUMN – Propylcyano, Endcapped
4.6 mm x 150 mm, 5 µm particle size
COLUMN TEMPERATURE = 30°C
MOBILE PHASE- Acetonitrile/ Buffer/ Methanol (60:25:15),
Buffer = 0.01 M K_2HPO_4 adjusted to pH 7.0 with H_3PO_4
FLOW RATE = 1.75 mL/min.

ANALYTES AND EXTRACTION EFFICIENCY

COMPOUND	Retention Time (min)	% Recovery	%RSD	Cerilliant #
Trimipramine ISTD*	2.048	100.0%	5.53%	T-904
Doxepin	3.048	96.5%	8.04%	D-905
Amitriptyline	3.433	98.9%	5.64%	A-923
Imipramine	3.865	97.2%	6.09%	I-902
Nortriptyline	5.349	88.9%	9.49%	N-907
Nordoxepin	5.788	85.0%	5.29%	D-007
Desipramine	6.067	85.3%	5.04%	D-906
Protriptyline ISTD*	6.476	86.3%	5.39%	P-903

* Internal Standards

HINTS:

(1) Silica Based HPLC columns are sensitive to pH. To prevent dissolution of the packing especially at the head of the column, it is best to place a silica column before the injector. This will saturate the mobile phase with silica.

(2) Secondary Amines bind to glass and polyethylene. It is recommended to silylate all surfaces that come in contact with the sample. Immersion into 5% DMCS in toluene or vapor deposition will deactivate the surface by silylation.

(3) To ensure the proper strength of elution solvent measure the apparent pH of the elution solvent. It should be pH 10 or higher. Add 1-2% of Ammonium Hydroxide and check again.

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METHADONE IN URINE FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. PREPARE SAMPLE

To 2 mL of urine add internal standard(s)* and 1 mL of 100 mM phosphate buffer (pH 6.0).
Mix/vortex. Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 2 mL 100 mM phosphate buffer (pH 6.0).
NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 to 2 mL / minute.

4. WASH COLUMN

1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM acetic acid.
1 x 3 mL CH_3OH.
Dry column (5 minutes at > 10 inches Hg).

5. ELUTE METHADONE

1 x 3 mL CH_2Cl_2 / IPA/NH_4OH (78:20:2); Collect eluate at
1 to 2 mL / minute.
NOTE: Prepare elution solvent daily. Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

6. CONCENTRATE

Evaporate to dryness at < 40°C.
Reconstitute with 100 µL acetonitrile**.

7. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

Compound	Primary***	Secondary	Tertiary	Cerilliant #
*Methadone-D9	78	226	303	M-008
Methadone	72	223	294	M-007

* Suggested internal standard for GC/MS: D_9-Methadone
** Part # SACN-0-50
*** Quantitation ion

DCN-903020-56

METHADONE / EDDP IN WHOLE BLOOD, PLASMA / SERUM, TISSUE AND URINE USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

PART #: ZSDAU020

LC-MSMS

February 3, 2009

1. PREPARE SAMPLE

To 1 mL of 100 mM phosphate buffer (pH= 6) add internal standard.*
Add 1 mL of whole blood, serum/ plasma urine or tissue (1 g of 1:4 homogenate).
Add 2 mL of 100 mM phosphate buffer (pH= 6).
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate
Mix/vortex.
Centrifuge as appropriate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH
1 x 3 mL D.I. H_2O
1 x 1 mL 100 mM phosphate buffer (pH= 6).
Note: aspirate at < 3 inches Hg to prevent sorbent drying out

3. APPLY SAMPLE

Load sample at 1-2 mL / minute.

4. WASH COLUMN

1 x 3 mL DI H_2O
1 x 3 mL 100 mM acetic acid.
1 x 3 mL CH_3OH
Dry column (5 minutes at > 10 inches Hg).

5. ELUTE METHADONE/EDDP

1 x 3 mL ethyl acetate; acetonitrile: ammonia (78: 20: 2 v/v)
Collect eluate at 1-2 mL /minute.
NOTE: Prepare elution solvent daily

6. EVAPORATION

Evaporate eluates under a gentle stream of nitrogen < 40°C.

7. Reconsititue sample in 100 µL of CH_3OH.

Inject 5 µL.

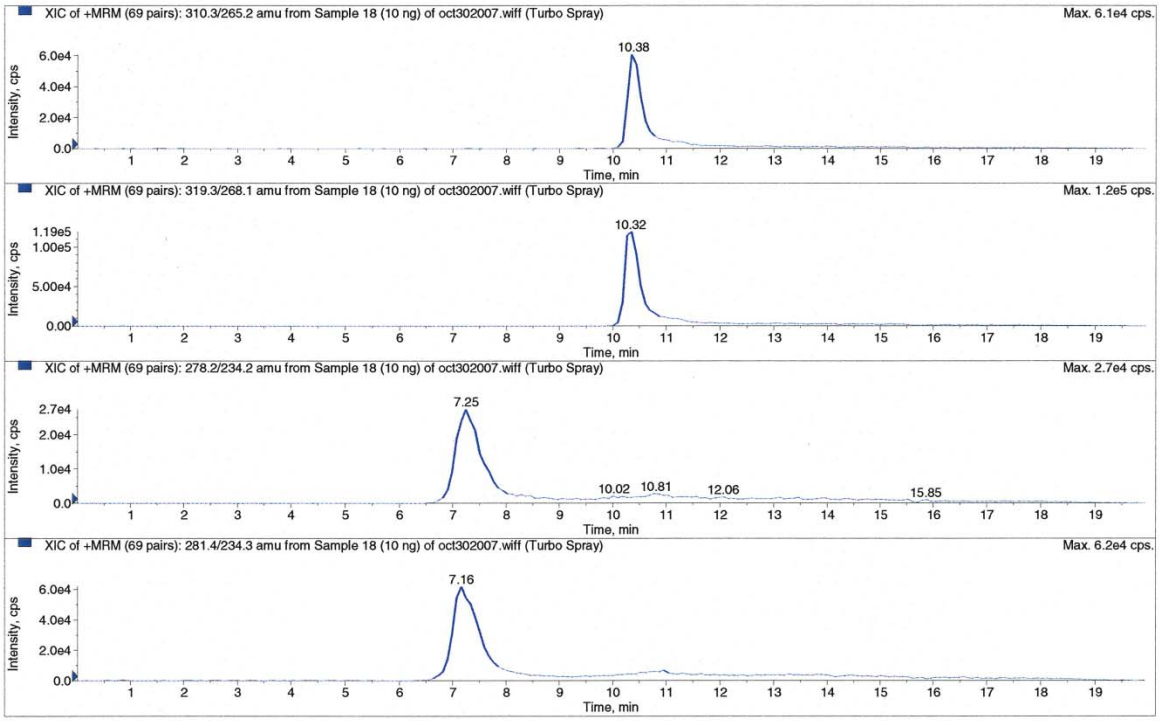
INSTRUMENT CONDITIONS:

Column: 150 x 2.1 mm (3 µm) SB-Aq (Agilent Technologies)
Mobile phase:

Time	% Acetonitrile	%0.1% Formic acid
0	25	75
5	25	75
14	90	10
15	25	75
20	25	75

Flowrate: 0.35 mL/ minute
Column Temperature: ambient
Detector: API 2000 MS/MS

Compound	MRM Transistion	Cerilliant #
Methadone	310.2/105.1	M-007
*Methadone-D9	319.2/268.3	M-088
EDDP	278.2/234.2	E-022
*EDDP-D3	281.4/234.3	E-021



DCN-903020-57

PAROXETINE IN BLOOD, PLASMA/ SERUM AND URINE. LC/MS/MS CONFIRMATIONS USING: 200 mg CSDAU EXTRACTION COLUMN

Part #: ZSDAU020

February 3, 2009

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH= 6) add internal standards*.
   Add 1 mL whole blood, Serum/Plasma or Urine. Add 2 mL of 100 mM phosphate buffer (pH= 6).
   Vortex and centrifuge as appropriate.

2. **CONDITION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 3 mL 100 mM phosphate buffer (pH= 6).
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out.

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**
   1 x 3 mL D.I. H_2O
   1 x 3 mL 100 mM acetic acid
   1 x 3 mL CH_3OH
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE PAROXETINE:**
   1 x 3 mL Ethyl acetate: acetonitrile: ammonium hydroxide (78:20:2)
   Collect eluate at 1-2 mL / minute.

6. **EVAPORATION:**
   Evaporate eluates under a gentle stream of nitrogen < 40°.
   Dissolve residue in 100 µL CH_3OH.

INSTRUMENT CONDITIONS:

Column: 50 x 2.1 mm (3 µm) Selectra® Phenyl (UCT, LLC)

Mobile phase: Time	Acetonitrile	0.1% Formic Acid aq
0	10	90
15	50	50
16	10	90
20	10	10

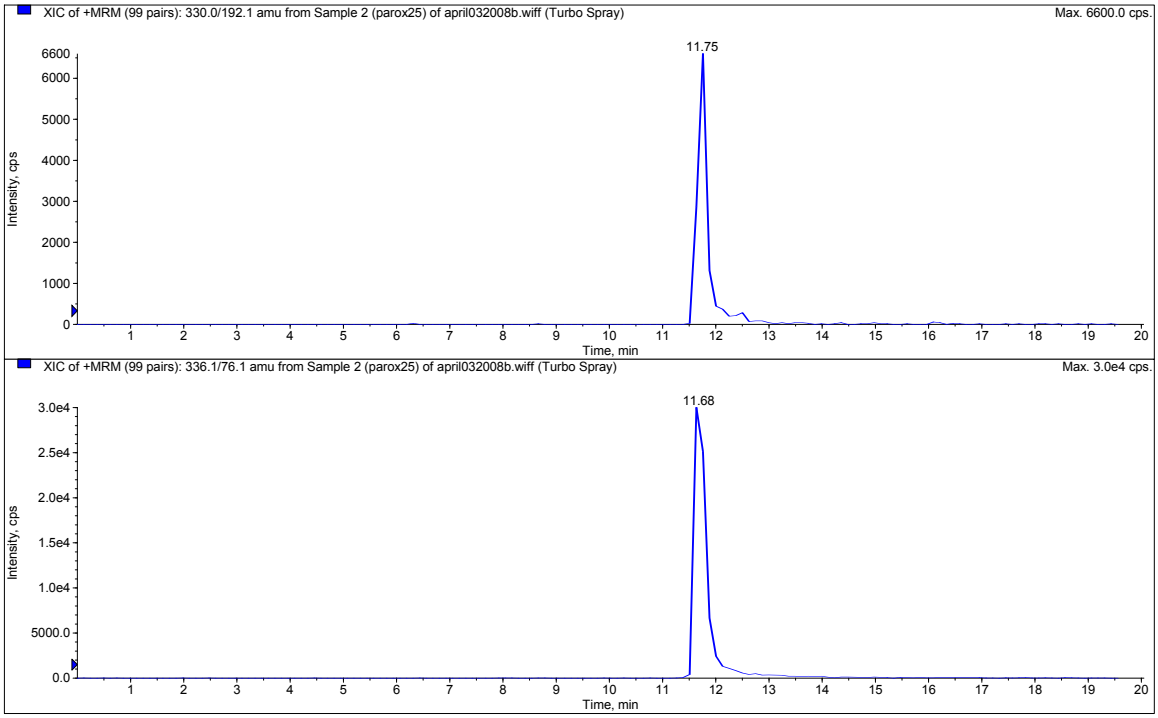
Flow rate: 0.35 mL/ minute
Injection Volume: 5 µL
Column Temperature: ambient
Detector: API 2000 MS/MS.

Compound	MRM Transition	Cerilliant #
Paroxetine	330.0/190.1	P-915
Paroxetine-D6	336.0/ 76.1	A-916

CHROMATOGRAM OF:

Paroxetine

Paroxetine-D6



Recovery: > 90% (N=20)

LOD: 1 ng/ mL

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PROPOXYPHENE IN BLOOD, PLASMA/SERUM, URINE AND TISSUE FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. PREPARE SAMPLE

To 1 mL of 100 mM phosphate buffer (pH =6.0) add internal standard*. Add 1 mL of blood, plasma/ serum or 1 g (1:4) tissue homogenate or 2 mL of urine. Mix/vortex.

Sample pH should be 6.0 ± 0.5.

Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

Centrifuge as appropriate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.

1 x 3 mL D.I. H_2O.

1 x 1 mL 100 mM phosphate buffer (pH= 6.0).

NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 mL/minute.

4. WASH COLUMN

1 x 3 mL D.I. H_2O.

1 x 1 mL 100 mM acetic acid.

1 x 3 mL CH_3OH.

Dry column (5 minutes at > 10 inches Hg).

5. ELUTE PROPOXYPHENE

1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2); Collect eluate at 1 mL/minute.

NOTE: Prepare elution solvent daily. Add IPA/NH_4OH, mix, and then add CH_2Cl_2

6. CONCENTRATE

Evaporate to dryness at < 40°C.

Reconstitute with 100 µL ethyl acetate.

7. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

Compound	Primary***	Secondary	Tertiary	OTHER	Cerilliant #
Propoxyphene-D5*	63	120	213	255, 270	P-901
Propoxyphene	58	115	208	250, 265	P-011

* Internal Standard
*** Quantitation Ion

NOTE: To improve the analysis for Norpropoxyphene, the primary metabolite of Dextropropoxyphene, add 1 drop of 35% sodium hydroxide solution to the urine sample and then after mixing bring the pH to 6 for SPE extraction. This step converts the Norpropoxyphene to Norpropoxyphene amide, a more stable compound.

For more information see the following reference:

Amalfitano G, Bessard J, Vincent F, Esseric H and Bessard G Gas Chromatographic Quantitation of Dextropropoxyphene and Norpropoxyphene in Urine after Sold Phase Extraction Journal Analytical Toxicology 20:547-554 (1996)

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6. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH= 6) add internal standard.*
   Add 1 mL of whole blood, serum/ plasma, urine or tissue (1 g of 1:4 homogenate).
   Add 2 mL of 100 mM phosphate buffer (pH= 6).
   Vortex and centrifuge as appropriate.

7. **CONDITION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 mL 100 mM phosphate buffer (pH 6).

   Note: aspirate at < 3 inches Hg to prevent sorbent drying out

8. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute.

9. **WASH COLUMN:**
   1 x 3 mL DI H_2O
   1 x 3 mL 100 mM acetic acid.
   1 x 3 mL CH_3OH

   Dry column (5 minutes at > 10 inches Hg).

10. **ELUTE PROPOXYPHENE/ NORPROPOXYPHENE:**
    1 x 3 mL ethyl acetate; acetonitrile: ammonia (78: 20: 2 v/v)
    Or
    1 x 3 mL CH_2Cl_2/ IPA/ ammonia (78:20:2 v/v)
    Collect eluate at 1-2 mL /minute.

6. **EVAPORATION:**
   Evaporate eluates under a gentle stream of nitrogen < 40°C to half volume.
   Add 100 µL of 0.1% HCl in CH_3OH.
   Vortex mix.
   Continue evaporation to dryness <40 ˚C.

7. **Reconstitute** sample in 100 µL of CH_3OH.
   Inject 5 µL.

INSTRUMENT CONDITIONS:

Column: 50 x 2.1 mm (3 µm) Selectra® Phenyl (UCT, LLC)

Mobile phase:

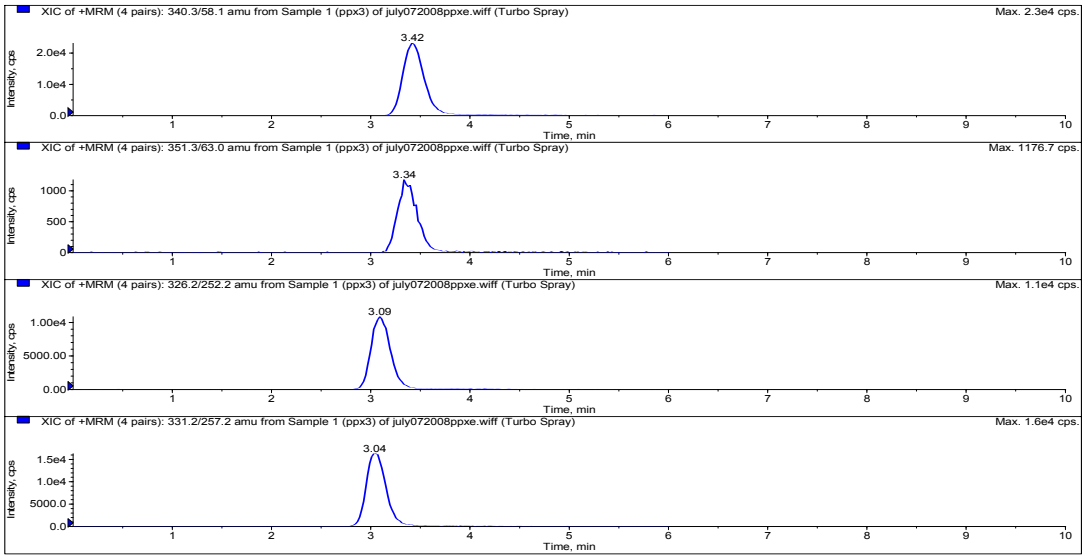
Time	% Acetonitrile	%0.1% Formic acid
0	30	70
10	30	70

Flowrate: 0.35 mL/ minute
Column Temperature: ambient
Detector: API 2000 MS/MS

Compound	MRM Transition	Cerilliant #
Propoxyphene	340.0/ 58.0	P-011
*Propoxyphene-D11	351.2/ 64.0	P-013
Norpropoyphene	326.0/ 252.0	N-013
*Norpropoxyphene-D5	331.0/257.0	N-904

Chromatogram of:

Propoxyphene (top)
Propoxyphene-D11
Norpropoxyphene
Norpropoxyphene-D5 (lower)



DCN-903020-61

SERTRALINE AND DESMETHYLSERTRALINE IN BLOOD, PLASMA / SERUM FOR HPLC ANALYSIS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. PREPARE SAMPLE

To 4 mL D.I. H_2O add 2 mL of 100 mM phosphate buffer (pH= 6.0). To this add internal standard*
Add 1 mL of blood, plasma/serum or urine.Mix/vortex.
Centrifuge for 10 minutes at 2000 rpm and discard pellet.
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 mL/minute.

4. WASH COLUMN

1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM acetic acid.
1 x 3 mL CH_3OH.
Dry column (5 minutes at > 10 inches Hg).

5. ELUTE

1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2); Collect eluate at 1 mL/minute.
NOTE: Prepare elution solvent fresh daily. Add IPA/NH_4OH, mix, and then add CH_2Cl_2

6. DRY ELUATE

Evaporate to dryness at < 40°C.

7. QUANTITATE

Reconstitute with 200 µL acetonitrile: D.I. H_2O (1 :3).
Mix/vortex vigorously for 30 seconds.
Inject 100 µL onto chromatograph at wavelength 235 nm.
Mobile phase = 0.25 M potassium phosphate (pH 2.7).
Containing 30% CH_3CN.
Flow rate = 2 mL/minute.

HPLC SYSTEM:

Isocratic HPLC using a Pump thru a C8 HPLC Column
(LC-8 or equivalent HPLC Column) 15 cm x 4.6 mm ID
Coupled to a UV detector set at 235 nm.

Compound	**Cerillant #**
Sertraline	S-006
**Desmethylsertraline	N-049

** Norsertraline= Desmethylsertraline

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ANTIDEPRESSANTS / PAINKILLERS IN BLOOD AND URINE USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

PART #: ZSDAU020

LC/MS/MS

February 3, 2009

1. **PREPARE SAMPLE**
   To 1 mL of 100 mM prosphate buffer (pH= 6) add internal standard.*
   Add 1 mL of blood or urine. Add 2 mL of 100 phosphate buffer (pH= 6). Mix/vortex.
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
   Mix/vortex.
   Centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL MeOH.
   1 x 3 mL H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH= 6).
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out.

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**
   1 x 3 mL DI Water.
   1 x 3 mL 1% acetic acid.
   1 x 3 mL Methanol.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE ANTIDEPRESSANTS/PAINKILLERS:**
   1 x 3 mL ethyl acetate; acetonitrile: ammonia (78: 20: 2 v/v).
   Collect eluate at 1-2 mL /minute.

6. **EVAPORATION:**
   Evaporate eluate under a gentle stream of nitrogen < 40ºC.

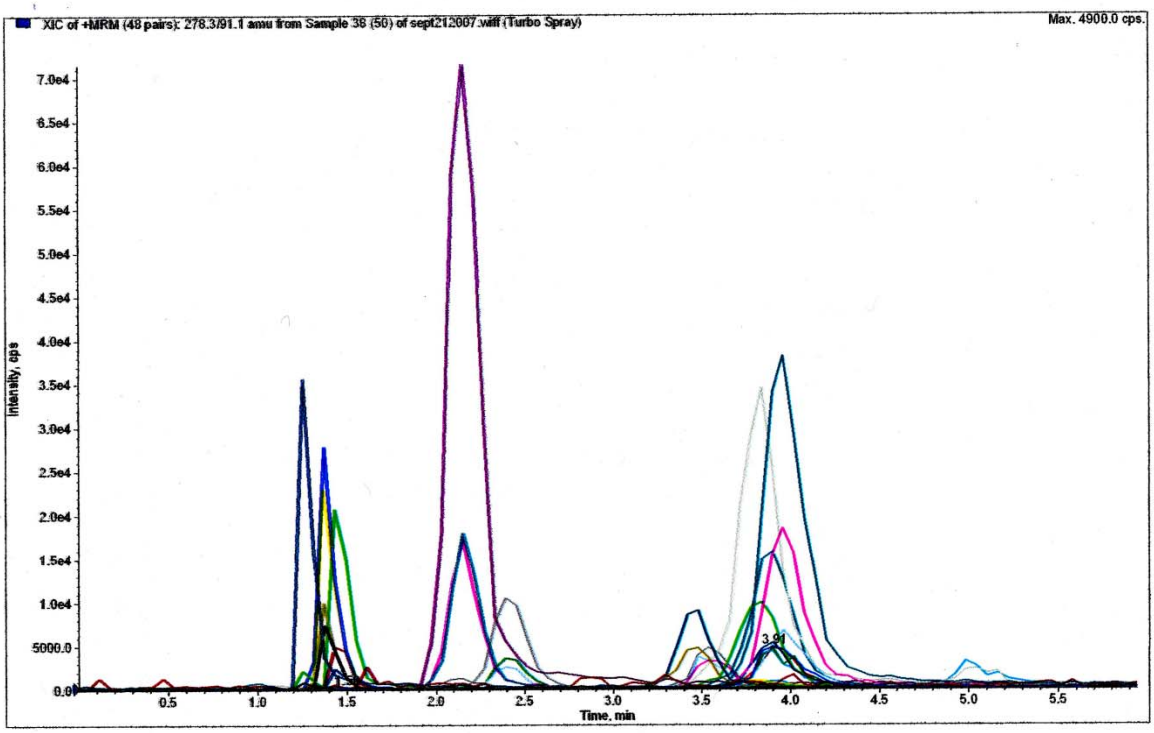
7. **RECONSTITUTE sample in 100 µL of methanol.**
   Inject 5 µL.

INSTRUMENT CONDITIONS:

Column: 150 x 2.1 mm (3.5 µm) Zorbax: Agilent Technologies.
Mobile phase: Acetonitrile: 0.1% Formic acid (33:67).
Flowrate: 0.35 mL/minite.
Column Temperature: ambient.
Detector: API 2000 MS/MS.

Compound	MRM Transistion	Cerilliant #
Amitriptyline	278.8/91.1	A-923
*Amitriptyline-D3	281.2/91.2	B-130284 N-10
Diphenhydramine	256.2/167.1	D-015
*Diphenhydramine-D3	259.2/167.1	D-017
Doxepin	280.2/107.1	D-005
EDDP	278.2/234.2	E-022
*EDDP-D3	281.4/234.3	E-021
Methadone	310.2/105.1	M-007
*Methadone-D9	319.2/268.3	M-088
Nortriptyline	264.2/91.1	N-907
Norpropoxyphene	326.2/44.1	N-913
*Norpropoxyphene-D5	331.1/267.1	N-904
Propoxyphene	340.2/58.1	P-011
*Propoxyphene-D11	351.3/64.0	P-013
Sertraline	308.1/161.0	S-006
Tramadol	264.2/58.1	T-027
*Tramadol-D3	268.2/58.0	T-029
Venlafaxine	278.2/58.2	V-004
Zolpidem	308.2/235	Z-901

ANTIDEPRESSANTS / PAINKILLERS IN BLOOD AND URINE
CHROMATOGRAM



Recovery: > 90% (N=100)
LOD: 1 ng/mL

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ANTIDEPRESSANTS IN BLOOD, SERUM/PLASMA, AND URINE USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

PART #: CSDAU020

LC/MS/MS

June 29, 2009

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM prosphate buffer (pH= 6) add internal standard.
   Add 1 mL of blood or urine. Add 2 mL of 100 phosphate buffer (pH= 6). Mix/vortex.

   Sample pH should be 6.0 ± 0.5.

   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

   Mix/vortex.

   Centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH
   1 x 3 mL DI H_2O
   1 x 1 mL 100 mM phosphate buffer (pH= 6)
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out.

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**
   1 x 3 mL DI H_2O
   1 x 3 mL 100 mM acetic acid.
   1 x 3 mL CH_3OH.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE ANTIDEPRESSANTS**
   1 x 3 mL ethyl acetate; acetonitrile: ammonium hydroxide (78: 20: 2 v/v).

   OR

   1 x 3 mL dichloromethane: isopropanol/ ammonium hydroxide (78:20:2 v/v)

   Collect eluate at 1-2 mL /minute.

6. **EVAPORATION:**
   Evaporate eluate under a gentle stream of nitrogen < 40ºC.

7. **RECONSTITUTE** sample in 100 µL of methanol**.**
   Inject 5 µL.

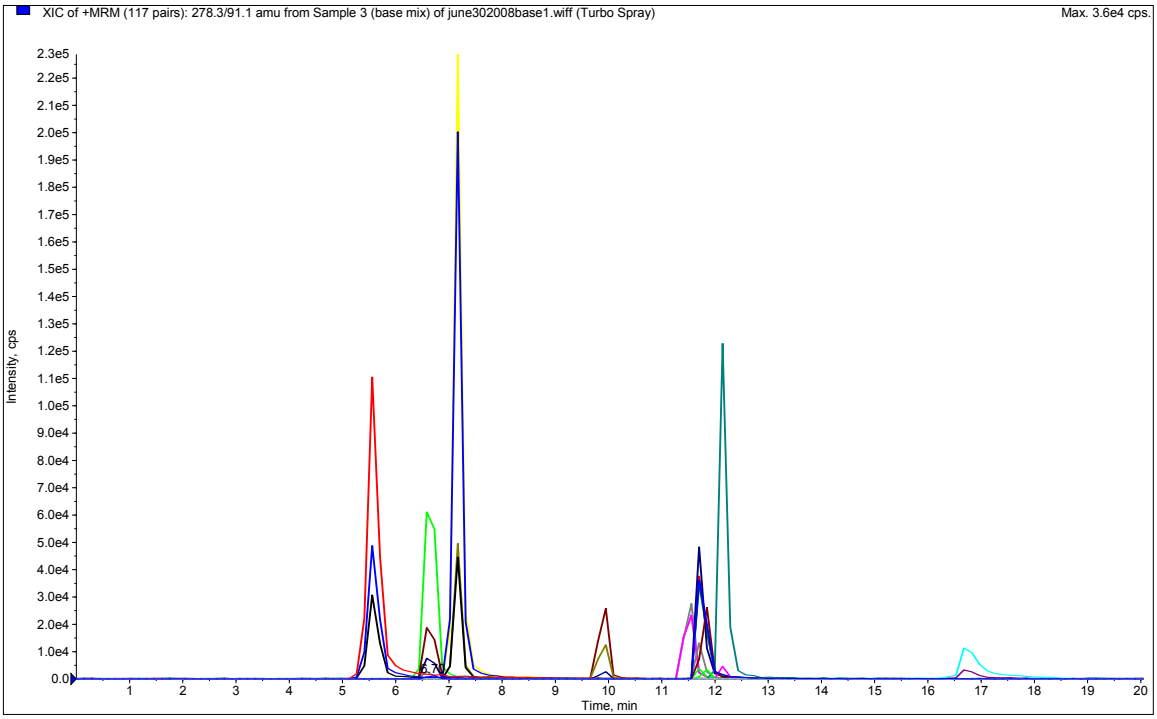
INSTRUMENT CONDITIONS:

Column: 50 x 2.1 mm (3µm) SELECTRA® Phenyl UCT, LLC.,
Mobile phase:

Time/ min	% Acetonitrile	% 0.1 % Formic Acid
0	10	90
15	50	50
16	10	90
20	10	90

Flowrate: 0.35 mL/minute
Column Temperature: ambient.
Detector: API 2000 MS/MS.

Chromatogram of drugs extracted from whole blood (1 mL)



COMPOUND	MRM TRANSITIONS	CERILLIANT#
Amitriptyline	278.2/233.1	A-923
Buproprion	240.1/184.1	B-034
Citalopram	325.2/109.0	C-057
Fluoxetine	310.1/44.0	F-918
Norfluoxetine	296.2/134.2	N-923
Nortriptyline	264.2/233.1	N-907
Venlafaxine	278.2/58.0	V-004
Zolpidem	308.2/235.0	Z-901

Recovery: > 90%

LOD : 10 ng/ mL (n=10)

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CLOZAPINE AND METABOLITES IN WHOLE BLOOD, SERUM/PLASMA AND URINE USING: 300 mg CLEAN-UP® EXTRACTION COLUMN

PART #: CECN41L3

LC/MS/MS / LC-UV

February 3, 2009

1. PREPARE SAMPLE

To 1 mL of 100 mM prosphate buffer (pH= 6) add internal standard.*
Add 1 mL of blood, serum/plasma or urine. Add 2 mL of 100 mM phosphate buffer (pH=6).
Mix/vortex Centrifuge as appropriate.

2. CONDITION CLEAN SCREEN® CECN41L# EXTRACTION COLUMN

1 x 1 mL MeOH.
1 x 1 mL H_2O.
1 x 0.5 mL 100 mM phosphate buffer (pH= 6).
Note: aspirate at < 3 inches Hg to prevent sorbent drying out.

3. APPLY SAMPLE

Load sample at 1-2 mL / minute.

4. WASH COLUMN

1 x 1 mL D.I. Water
1 x 0.5 mL 1% ammonium hydroxide in D.I. water.
Dry column (5 minutes at > 10 inches Hg).

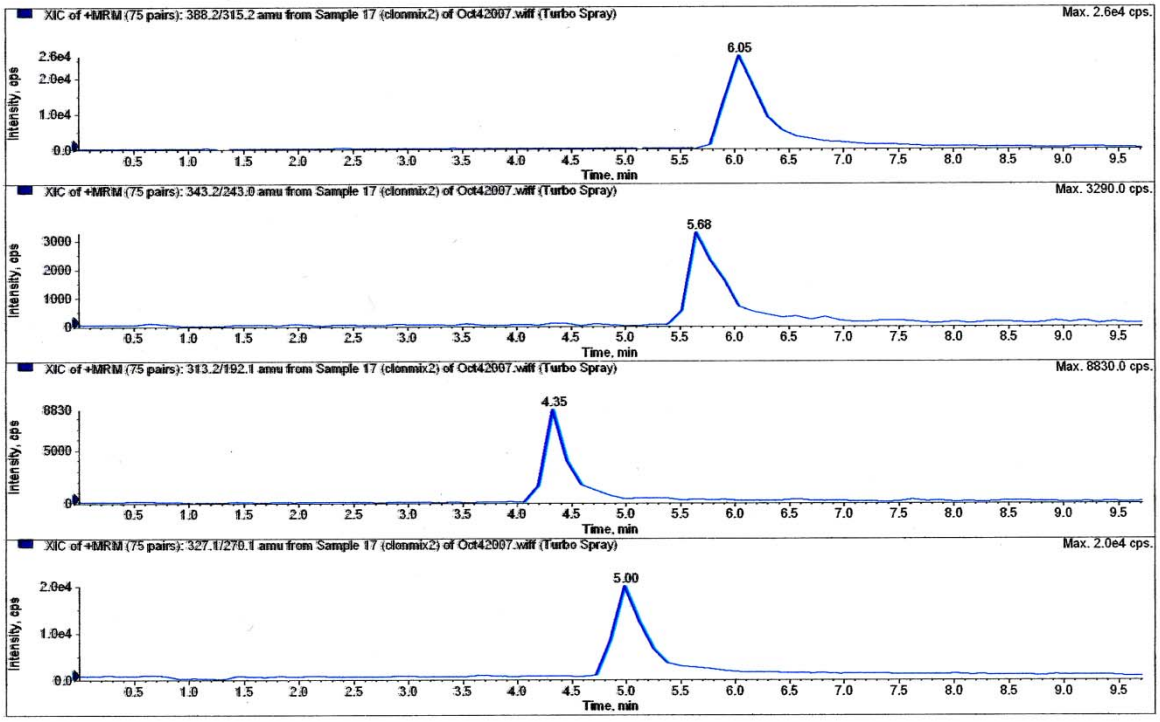
5. ELUTE CLOZAPINE

1 x 0.2 mL 1% ammonium hydroxide in methanol.
Collect eluate at 1-2 mL /minute.
Inject 5 µL (LCMSMS).
Inject 20 µL (LC-UV).

INSTRUMENT CONDITIONS

Column: 150 x 2.1 mm (3 µm) Zorbax: Agilent Technologies.
Mobile phase: Acetonitrile: 0.1% Formic acid (33:67).
Flowrate: 0.35 mL/minite.
Column Temperature: ambient.
Detector: API 2000 MS/MS.
HP1100 Diode-Array (230 nm).

Compound	MRM Transistion	Cerilliant #
Clozapine	327.1/270.1	C-059
Desmethylclozapine	313.1/192.1	
Clozapine-N-oxide	343.1/243.1	
*Flurazepam	388.1/315.2	F-003



DCN-903020-64

OLANZAPINE IN WHOLE BLOOD USING: 300 mg CLEAN-UP® EXTRACTION COLUMN

PART #: CECN4123

LC-UV

February 3, 2009

1. **PREPARE SAMPLE**
   To 1 mL of D.I. H_2O add internal standard.*
   Add 1 mL blood. Add 8 mL of D,I. H_2O.
   Vortex and centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® CECN4123 EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out.

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**
   1 x 3 mL 1 % acetic acid (aq).
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE OLANZAPINE:**
   2 x 3 mL 1% acetic acid in CH_3OH.
   Collect eluate at 1-2 mL /minute.
   NOTE: Prepare elution solvent daily

6. **EVAPORATION:**
   Evaporate eluates under a gentle stream of nitrogen < 40°C.

6. **RECONSITITUE sample in 100 µL 0.1% trifluoroacetic acid (aq).**
   Inject 50 µL.

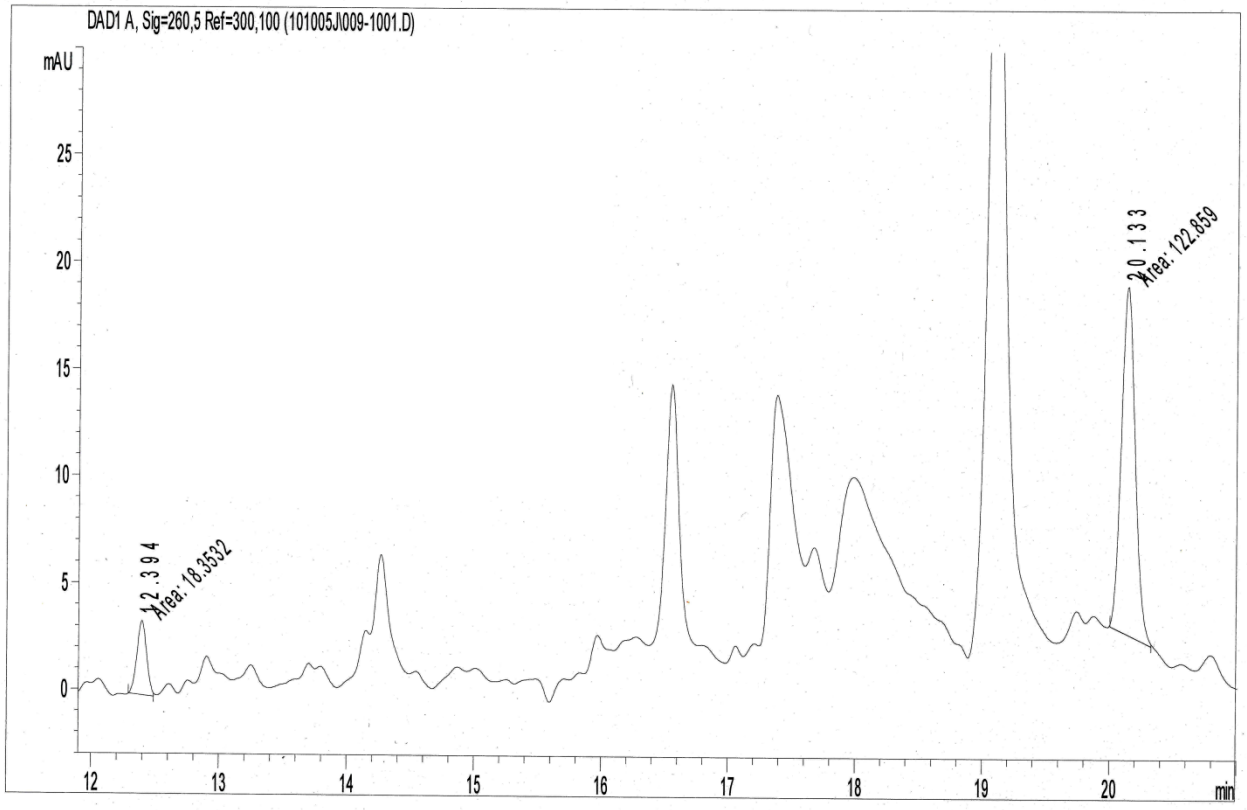
INSTRUMENT CONDITIONS:

Column: Gold C18 150 x 2.1 mm (3 µm) Thermofisher.
Mobile phase:

Time (minutes):	% Acetonitrile:	%0.1% TFA
0	5	95
5	5	95
14	67	33
15	5	95
25	5	95

Flowrate: 0.15 mL/ min
Column Temperature: 40˚ C
Detector: Diode Array (260 nm)

Compound	Cerilliant Part #
Olanzapine	O-024
*Prazepam	P-906



1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH 6) add internal standard.*
   Add 1 mL blood, urine. Add 2 mL of 100 mM phosphate buffer (pH 6).
   Vortex and centrifuge as appropriate.

2. **CONDITION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 mL 100 mM phosphate buffer (pH 6).
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out.

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**
   1 x 3 mL D.I. H_2O
   1 x 3 mL 100 mM acetic acid
   1 x 3 mL CH_3OH
   Dry column (10 minutes at > 10 inches Hg).

5. **ELUTE DEXTROMETHORPHAN / PHENCYCLIDINE**
   1 x 3 mL ethyl acetate: acetonitrile: ammonium hydroxide (78:20:2)
   Collect eluate at 1-2 mL / minute.

6. EVAPORATION:

Evaporate eluates under a gentle stream of nitrogen < 40°.

Dissolve residue in 50 µL CH_3OH.

INSTRUMENT CONDITIONS:

Column: 50 x 2.1 mm (3 µm) Selectra® Phenyl (UCT, LLC)

Mobile phase:	**Time**	**Acetonitrile**	**0.1% Formic Acid**
	0	10	90
	15	90	10
	16	10	90
	20	10	90

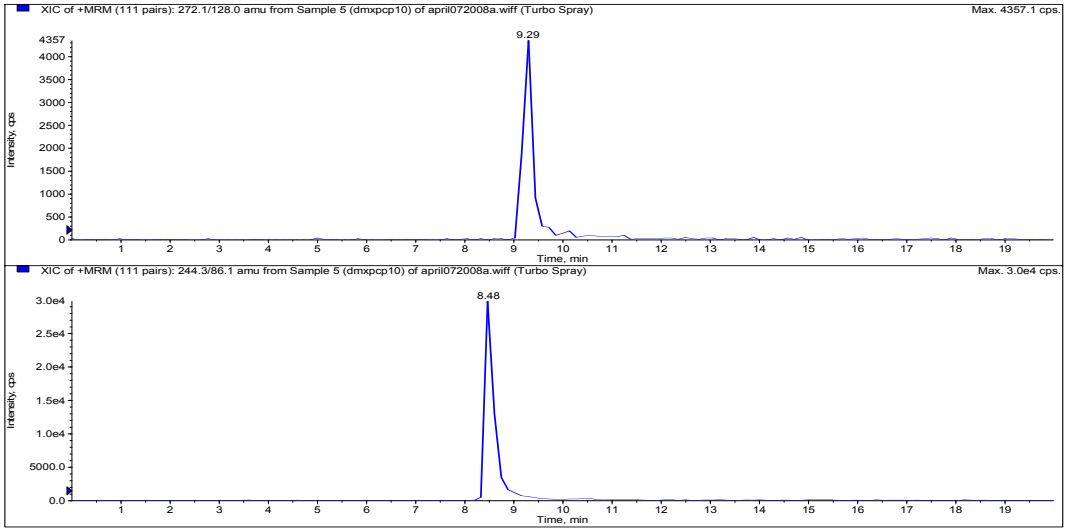
Flowrate: 0.35 mL/ minute

Injection Volume: 5 µL

Column Temperature: ambient

Detector: API 2000 MS/MS.

CHROMATOGRAM OF DEXTROMETHORPHAN / PHENCYCLIDINE



Compound	MRM Transition	Cerilliant #
PCP	244.3/ 159.2	P-007
PCP-D5	249.3/264.1	P-003
Dextromethorphan	272.1/128.1	D-013
Dextromethorphan-D3	275.1/ 131.0	D-041

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*DULOXETINE IN BLOOD AND URINE USING:
200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part # ZSDAU020

LC/MS/MS

March 9, 2009

1. **PREPARE SAMPLE**
   To 1 mL of 100 mM prosphate buffer (pH= 6) add internal standard.*
   Add 1 mL of blood or urine. Add 2 mL of 100 phosphate buffer (pH= 6). Mix/vortex.

   Sample pH should be 6.0 ± 0.5.

   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

   Mix/vortex.

   Centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL DI H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH= 6).
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out.

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**
   1 x 3 mL DI H_2O.
   1 x 3 mL 100 mM acetic acid.
   1 x 3 mL CH_3OH.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE DULOXETINE:**
   1 x 3 mL dichloromethane/ isopropanol/ ammonia (78: 20: 2 v/v).
   Collect eluate at 1-2 mL /minute.

6. **EVAPORATION:**
   Evaporate eluate under a gentle stream of nitrogen < 40ºC.

7. **RECONSTITUTE sample in 200 µL of 0.1% Formic acid.**
   Inject 5 µL.

INSTRUMENT CONDITIONS:

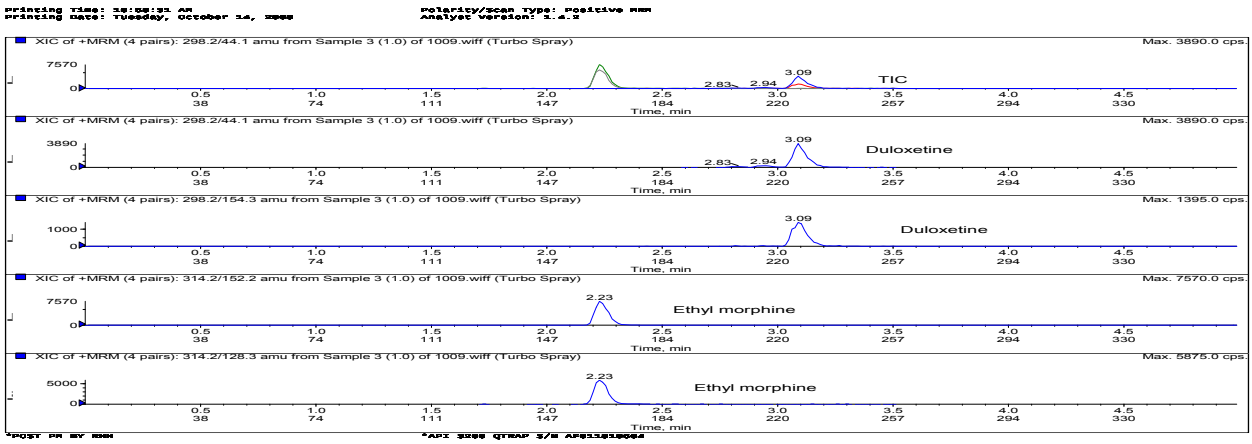
Column: 50 x 2.1 mm (5µm) C_{18}
Mobile phase:

Time/ min	% Acetonitrile	% 0.1 % Formic Acid
0	5	95
4	90	10
4.1	5	95
5	5	95

Flowrate: 0.5 mL/minite.
Column Temperature: ambient.
Detector: API 3200 Q-Trap MS/MS.

Compound	MRM Transistion	Cerilliant #
* Ethyl Morphine	314.2/ 152.2	E-052
Duloxetine	298.1/44.1	D-004

Chromatogram of Ethyl Morphine and Duloxetine



Recovery > 90%

*Presented at SOFT annual meeting 2008 by A.A. Elian

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TRAMADOL AND DESMETHYLTRAMADOL IN BLOOD, PLASMA/SERUM, URINE AND TISSUE USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020

LC/MS/MS

January 30, 2009

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH 6) add internal standard.*

   Add 1 mL of whole blood, serum/ plasma, urine, or 1g tissue homogenate (1:4). Add 2 mL of 100 mM phosphate buffer (pH 6).

   Vortex and centrifuge as appropriate.

2. **CONDITION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 mL 100 mM phosphate buffer (pH 6).
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**
   1 x 3 mL DI H_2O
   1 x 3 mL 100 mM acetic acid.
   1 x 3 mL CH_3OH
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE TRAMADOL AND DESMETHYLTRAMADOL**:
   1 x 3 mL ethyl acetate; acetonitrile: ammonia (78: 20: 2 v/v)

   Or

   1 x 3 mL CH_2Cl_2/ IPA/ ammonia (78:20:2 v/v)
   Collect eluate at 1-2 mL /minute.

6. **EVAPORATION:**
   Evaporate eluates to dryness under a gentle stream of nitrogen.

7. **RECONSTITUTE** sample in 50 µL of CH_3OH.
   Inject 5 µL.

INSTRUMENT CONDITIONS:
Column: 50 x 2.1 mm (3 µm) Selectra® Phenyl (UCT, LLC)

MOBILE PHASE:

Time	% Acetonitrile	%0.1% Formic acid
0	10	90
5	90	10
5.5	10	90
10	10	90

Flowrate: 0.35 mL/ minute

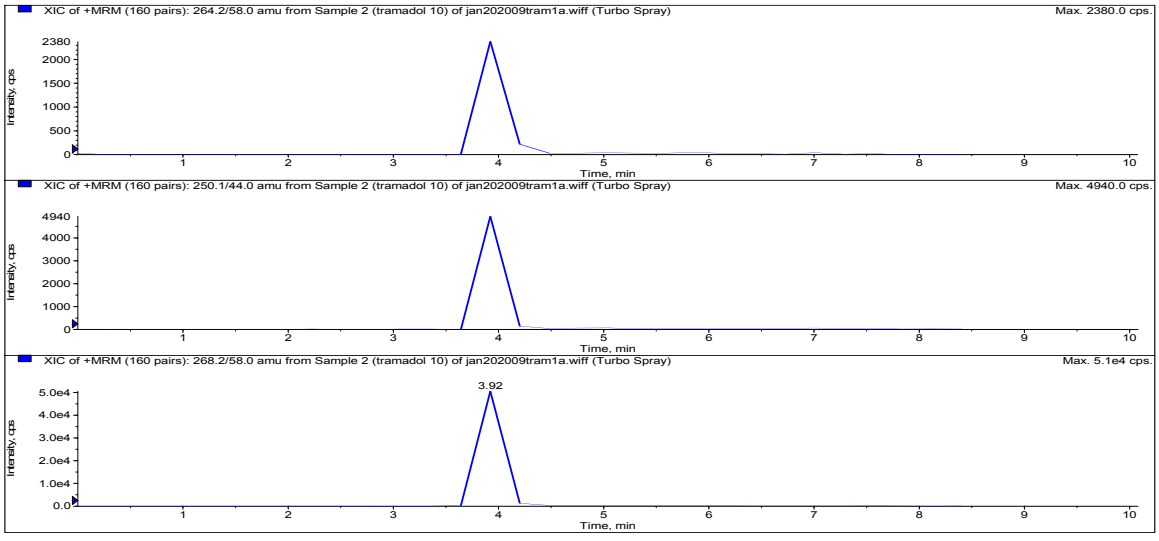
Column Temperature: ambient

Detector: API 2000 MS/MS

Compound	MRM Transition	Cerilliant #
Tramadol	264.2/58	T-027
Desmethyltramadol	250.2/44	D-023/T-035
*Tramdol-C^{13}-D3	268.2/58	T-029

CHROMATOGRAM SHOWING:

Tramadol/ Desmethyltramadol/ Tramdol-C^{13}-D3



Recovery: > 90% (n=10)
LOD: 10 ng/ mL

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QUETIAPINE IN BLOOD, PLASMA/SERUM, URINE AND TISSUE USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

PART #: ZSDAU020

February 3, 2009

1. PREPARE SAMPLE:

To 1 mL of 100 mM phosphate buffer (pH= 6) add internal standard.* Add 1 mL blood, plasma/serum, urine or 1 g (1:4) tissue homogenate
Add 2 mL of 100 mM phosphate buffer (pH= 6).
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
Centrifuge as appropriate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 3 mL 100 mM phosphate buffer (pH= 6).
Note: aspirate at < 3 inches Hg to prevent sorbent drying out.

3. APPLY SAMPLE:

Load sample at 1-2 mL / minute.

4. WASH COLUMN:

1 x 3 mL 100 mM phosphate buffer (pH 6).
1 x 3 mL 1.0 M acetic acid.
1 x 3 mL CH_3OH.
Dry column (5 minutes at > 10 inches Hg).
1 x 3 mL of hexane.
Dry column (5 minutes at > 10 inches Hg).

5. ELUTE QUETIAPINE:

1 x 3 mL ethyl acetate; acetonitrile: ammonia (78: 20: 2 v/v).
Collect eluate at 1-2 mL /minute.
NOTE: Prepare elution solvent daily

6. EVAPORATION:

Evaporate eluates under a gentle stream of nitrogen < 40°C.

6. Reconsititue sample in 100 µL 0.1% trifluoroacetic acid (aq).

Inject 50 µL.

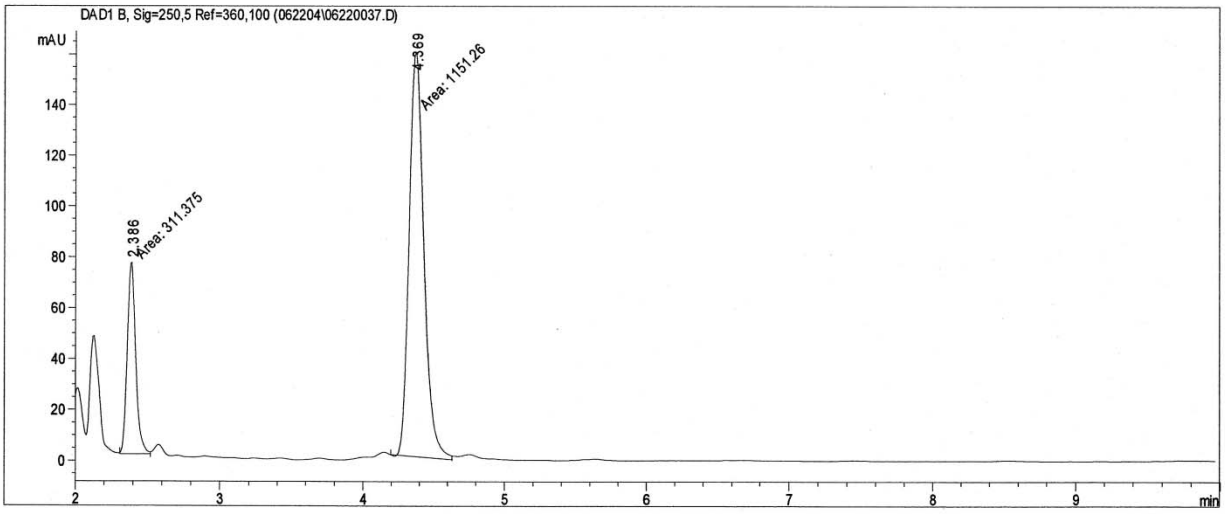
INSTRUMENT CONDITIONS:

Column: C_{18} 150 x 4.6 mm (3 µm) Zorbax (Agilent Technologies).
Mobile phase: Acetonitrile: 0.1% Trifluoroacetic acid (25: 75).
Flowrate: 1 mL / min.
Column Temperature: 35˚ C.
Detector: Diode Array (250 nm).

Chromatogram :

	Cerilliant Part #
Quetiapine	Q001

*Quinidine



COCAINE AND BENZOYLECGONINE IN SERUM, PLASMA, OR WHOLE BLOOD FOR HPLC USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. PREPARE SAMPLE

To 4 mL of D.I. H_2O add internal standards. 1 mL of sample (Serum, Plasma or Whole Blood) add internal standard(s)
Mix/vortex and let stand 5 minutes.
Centrifuge for 10 minutes at 2000 rpm and discard pellet.
Add 2 mL of 100 mM phosphate buffer (pH= 6.0).
Mix/vortex.
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM phosphate buffer (pH 6.0).
NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 to 2 mL/minute.

4. WASH COLUMN

1 x 3 mL D.I. H_2O.
1 x 2 mL 100 mM HCl.
1 x 3 mL CH_3OH.
Dry column (5 minutes at > 10 inches Hg).

5A*. ELUTE COCAINE AND BENZOYLECGONINE

1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2); collect eluate at 1 to 2 mL/minute.
NOTE: Prepare elution solvent daily.
Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

5B*. ELUTE COCAINE AND BENZOYLECGONINE

1 x 2 mL CH_3OH/NH_4OH (98:2); collect eluate at
1 to 2 mL/minute.
NOTE: Prepare elution solvent daily.

Add 3 mL D.I. H_2O and 500 µL CH_2Cl_2 to eluate.
Mix / vortex 10 seconds. Centrifuge if necessary to separate layers.
Aspirate and discard aqueous (upper) layer.

6. CONCENTRATE

Evaporate to dryness at < 40°C.
Reconstitute in mobile phase for injection into HPLC.

* Choose either 5A or 5B

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COCAINE AND ITS METABOLITES FROM MECONIUM FOR GC OR GC/MS ANALYSIS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. PREPARE SAMPLE

Vortex 0.5 -1 g meconium with 2 mL of CH_3OH.
Centrifuge and transfer the supernatant to a clean tube.
To each tube add 3 mL 100 mM phosphate buffer (pH 6.0), internal standard and vortex.
Matrix must be more aqueous than organic for good extraction to occur.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 3 mL 100 mM phosphate buffer (pH= 6.0).
NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 to 2 mL/minute. Allow to dry.

4. WASH COLUMN

1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM HCl.
1 x 3 mL CH_3OH.
Dry column (5 minutes at > 10 inches Hg).

5. ELUTE COCAINE AND METABOLITES

1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2); Collect eluate at
1 to 2 mL/minute.
NOTE: Prepare elution solvent daily. Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

6. EVAPORATE

Evaporate the elution solvent to dryness without heating.

7. DERIVATIZE

Add 50 µL ethyl acetate and 50 µL BSTFA (with 1%TMCS)***.
Overlay with N_2 and cap. Mix/vortex.
React 20 minutes at 70°C. Remove from heat source to cool.
NOTE: Do not evaporate BSTFA solution.

8. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

Compound	Primary Ion****	Secondary	Tertiary	Cerilliant #
Cocaine-D3*	185	201	306	C-004
Cocaine	182	198	303	C-008
Benzoylecgonine-D3-TMS*	243	259	364	B-008
Benzoylecgonine-TMS	240	256	361	B-007

* Suggested internal standards for GC/MS
*** Part # SBSTFA-1-1, 10, 25, 100
**** Quantitation ion

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COCAINE AND BENZOYLECGONINE IN BLOOD, PLASMA/ SERUM, URINE AND TISSUE FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. PREPARE SAMPLE

To 1 mL of of 100 mM phosphate buffer (pH= 6.0) add internal standards*. Add 2 mL of blood, plasma/ serum, urine or 1 g (1:4) tissue homogenate. Mix/vortex. Add 2 mL of 100 mM phosphate buffer (pH= 6). Mix/vortex

Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
Centrifuge as appropriate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 to 2 mL/minute.

4. WASH COLUMN

1 x 3 mL D.I. H_2O.
1 x 2 mL 100 mM HCl.
1 x 3 mL CH_3OH.
Dry column (5 minutes at > 10 inches Hg).

5. ELUTE COCAINE AND BENZOYLECGONINE

1 x 3 mL Methylene Chloride/Isopropanol/
Ammonium Hydroxide (78:20:2).
Collect eluate at 1 to 2 mL/minute.
NOTE: Prepare elution solvent daily. Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

6. DRY ELUATE

Evaporate to dryness at < 40°C.

7. DERIVATIZE

Add 50 µL ethyl acetate and 50 µL BSTFA (with 1% TMCS)***.
Overlay with N_2 and cap. Mix/vortex.
React 20 minutes at 70°C.
Remove from heat source to cool.
NOTE: Do not evaporate BSTFA solution.

8. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

Compound	Primary Ion****	Secondary	Tertiary	Cerilliant #
Cocaine-D3*	185	201	306	C-004
Cocaine	182	198	303	C-008
Benzoylecgonine-D3-TMS*	243	259	364	B-008
Benzoylecgonine-TMS	240	256	361	B-007

* Suggested internal standards for GC/MS:
D_3-Cocaine, D_3-Benzoylecgonine
*** Part # SBSTFA-1-1, 10, 25, 100
**** Quantitation Ion

DCN-903020-69

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COCAINE, BENZOYLECGONINE, AND COCAETHYLENE IN BLOOD, PLASMA/SERUM, URINE AND TISSUE USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020

LC/MS/MS

February 3, 2009

1. PREPARE SAMPLE:

To 1 mL of 100 mM phosphate buffer (pH 6) add internal standard.*

Add 1 mL of whole blood, serum/ plasma, urine, or 1g tissue homogenate (1:4). Add 2 mL of 100 mM phosphate buffer (pH 6).

Vortex and centrifuge as appropriate.

2 CONDITION COLUMN:

1 x 3 mL CH_3OH

1 x 3 mL D.I. H_2O

1 x 1 mL 100 mM phosphate buffer (pH 6).

Note: aspirate at < 3 inches Hg to prevent sorbent drying out

3. APPLY SAMPLE:

Load sample at 1-2 mL / minute.

4. WASH COLUMN:

1 x 3 mL DI H_2O

1 x 3 mL 100 mM hydrochloric acid.

1 x 3 mL CH_3OH

Dry column (5 minutes at > 10 inches Hg).

5. ELUTE COCAINE, BENZOYLECGONINE, COCAETHYLENE:

1 x 3 mL ethyl acetate; acetonitrile: ammonia (78: 20: 2 v/v)

Or

1 x 3 mL CH_2Cl_2/ IPA/ ammonia (78:20:2 v/v)

Collect eluate at 1-2 mL /minute.

6. EVAPORATION:
Evaporate eluates to dryness under a gentle stream of nitrogen.

7. RECONSTITUTE sample in 50 µL of CH_3OH.
Inject 5 µL.

8. INSTRUMENT CONDITIONS:
Column: 50 x 2.1 mm (3 µm) Selectra® Phenyl (UCT, Inc.,)

MOBILE PHASE:

Time	% Acetonitrile	%0.1% Formic acid
0	33	67
5	33	67

Flowrate: 0.35 mL/ minute

Column Temperature: ambient

Detector: API 2000 MS/MS

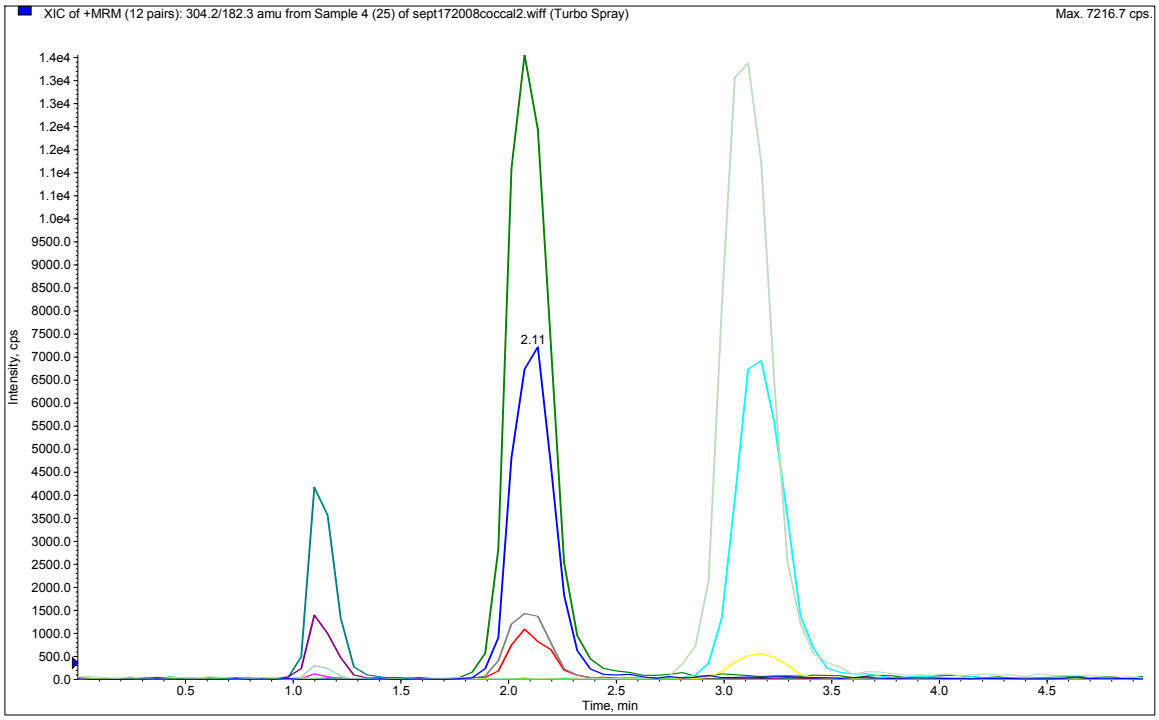
Compound	MRM Transition	Cerilliant #
Cocaine	304.2/ 182.3	C-008
* Cocaine-D3	307.2/ 185.2	C-009
Benzoylecgonine	290.1/ 168.0	B-004
*Benzoylecgonine-D8	298.2/ 171.3	B-013
Cocaethylene	318.2/ 196.2	C-010
*Cocaethylene-D8	326.2/ 204.2	C-024

CHROMATOGRAM SHOWING:

Cocaine/ Cocaine-D3

Benzoylecginine/ Benzoylecgonine-D8

Cocaethylene/ Cocaethylene-D8



Recovery: > 90% (n=10)

LOD: 10 ng/ mL

DCN-903020-129

A SOLID PHASE METHOD FOR GAMMA-HYDROXYBUTYRATE (GHB) IN URINE WITHOUT CONVERSION TO GAMMA-BUTRYLACTONE (GBL)

Part #: ZSGHB020

February 3, 2009

Developed by: UCT, LLC
2731 Bartram Road Bristol, Pennsylvania 19007

1. **PREPARE SAMPLE**
   To 200 µL of urine add internal standard* and 100 µL of 100 mM phosphate buffer (pH 6.0). Mix/vortex.

2. **CONDITION CLEAN SCREEN® GHB EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
   NOTE: Aspirate at < 3 inches of Hg to prevent sorbent drying.

3. **LOAD SAMPLE**
   Place test tubes into vacuum manifold for collection.
   The sample loading and wash are both collected.
   Decant sample onto column. Aspirate at ~1 inch Hg.

4. **WASH COLUMN**
   Add 1 mL of CH_3OH /NH_4OH (99:1) to original sample test tube; Vortex.
   Decant wash onto column.
   Note: Aspirate at ~1 inch of Hg.

5. **CONCENTRATE**
   Evaporate to dryness at 60°C using a stream of air or N_2.

6. **SAMPLE CLEAN UP**
   Add 200 µL of dimethylformamide.
   Add 1 mL of hexane saturated with dimethylformamide.
   Mix by inversion for 5 minutes.
   Centrifuge at 3000 rpm for 5 minutes.
   Transfer lower dimethylformamide layer to a clean test tube.

7. **CONCENTRATE**
   Evaporate to dryness at < 50°C using a stream of air or N_2.

8. **DERIVATIZE**
   Add 100 µL ethyl acetate and 100 µL BSTFA (with 1% TMCS)***.
   Mix/vortex.

9. **QUANTITATE**
   Inject 1 to 2 µL onto gas chromatograph.
   For MSD monitor the following ions:

Compound	Primary Ion *	Secondary	Tertiary	Cerilliant #
GHB-D6-di-TMS	239,	240,	241	G-006
GHB-di-TMS	233,	234,	235	G-001

* Suggested internal standard for GC/MS: GHB-D6
*** Part # SBSTFA-1-1, 10, 25, 100
**** Quantitation ion

DCN-903020-70

BLOOD GHB EXTRACTION PROCEDURE

Part #: ZSGHB020

February 3, 2009

By: Mr. Jim Oeldrich,
Wisconsin State Crime Lab, Milwaukee, WI

1. PREPARE SAMPLE

To 1 mL blood sample add internal standard and 0.5 mL of 100 mM phosphate buffer (pH 6.0).
Mix/vortex.
Rock for 10 minutes.
Centrifuge for 10 minutes at 2700 rpm.

2. CONDITION CLEAN SCREEN® GHB EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM phosphate buffer (pH 6.0).
NOTE: Aspirate at less than 3 inches of Hg to prevent sorbent drying.

3. APPLY SAMPLE

Place centrifuge tubes into vacuum manifold for collection.
The sample loading is collected.
Decant sample onto column. Aspirate at about 1 inch Hg.
After the sample is off the columns apply full vacuum for about 15 seconds to remove any residual blood.

4. ELUTE GHB

Remove centrifuge tubes, set aside.
Place clean centrifuge tubes into vacuum manifold for collection.
1 x 2 mL of CH_3OH /NH_4OH (99:1).
Aspirate at about 1 inch of Hg.

5. CONCENTRATE

Remove test tubes from vacuum manifold.
Vortex the sample prior to concentrating.
Evaporate to dryness at 60°C using a stream of nitrogen.

6. SAMPLE CLEAN UP

Add 200 µL of dimethylformamide.
Add 1 mL of hexane saturated with dimethylformamide.
Rock for 5 minutes.
Centrifuge at 5 minutes at 2700 rpm.
Transfer lower dimethylformamide layer to a clean test tube.
(If necessary transfer all liquid to a clean tube and allow to separate, then proceed to extract the lower layer)

7. CONCENTRATE

Evaporate to dryness at 50°C using a stream of air or nitrogen.

8. DERIVATIZE

Add 25 µL ethyl acetate and 25 µL BSTFA w 1% TMCS**.
Mix/vortex.
Heat at 70°C for 30 minutes.

9. QUANTITATE

Inject a 1 to 2 µL of the sample onto GC/MS.

Compound	Primary Ion *	Secondary	Tertiary	Cerilliant #
GHB-D6-di-TMS	239,	240,	241	G-006
GHB-di-TMS	233,	234,	235	G-001

** Part # SBSTFA-1-1,10, 25,100

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A SOLID PHASE METHOD FOR GAMMA-HYDROXYBUTYRATE (GHB) IN BLOOD, URINE, VITREOUS OR TISSUE WITHOUT CONVERSION TO GAMMA-BUTRYLACTONE (GBL)

Part #: ZSGHB020

February 3, 2009

Developed by: Mr. Joseph A. Crifasi, M.A., M.T., (ASCP) Certified Toxicology Specialist, ABFT; Saint Louis University Health Sciences Center,
Saint Louis University Medical School Forensic Toxicology,
6030 Helen Ave. St.
Louis, MO 63134
314-522-6410 ext. 6517, 314-522-0955 fax

GHB working standard; 200 µg/mL in H_2O; prepared from Cerilliant stock 1 mg/mL.

GHB –D6working internal standard; 100 µg/mL; use as supplied Cerilliant stock (0.1 mg/mL).

Working Standard	Whole Blood	Concentration
10 µL	200 µL	10 µg/mL
25 µL	200 µL	25 µg/mL
50 µL	200 µL	50 µg/mL
100 µL	200 µL	100 µg/mL

1. Make calibration standards and pipet 200 µL of QC and unknown bloods* into appropriately labeled 1.5 mL plastic centrifuge tubes.

***ALL SAMPLES INCLUDING URINE, VITREOUS OR HOMOGENIZED TISSUES (1:4)**

2. Add 25 µL of internal standard.

3. Add 1 mL of acetone; Vortex 15 seconds.

4. Centrifuge; Transfer acetone layer to culture tubes.

5. Evaporate extracts @ 70°C w/nitrogen.

6. Reconstitute the dried extracts with 200 µL of 100 mM Phosphate Buffer (pH 6.0); Vortex 15 seconds.

7. **CONDITION CLEAN SCREEN® GHB EXTRACTION COLUMN:**
   1 x 3 mL of CH_3OH.
   1 x 3 mL of D.I. H_2O.
   1 x 1 mL of 100 mM Phosphate Buffer (pH 6.0).

NOTE: Aspirate at 3 inches of Hg or less to prevent sorbent drying.

8. **APPLY SAMPLE**
   Add sample with Eppendorf pipette.
   Aspirate at ~1 inch Hg.

9. ELUTE GHB

Place clean test tubes into vacuum manifold
Add 1 mL of CH_3OH/NH_4OH (99:1) to original sample test tube; Vortex.
Decant onto column and collect extract.
Aspirate ~1 inch Hg.

10. CONCENTRATE

Remove test tube from Vacuum Manifold.
Evaporate to dryness at 70°C using a steam of nitrogen or air.

11. DERIVATIZE

Add 100 µL of ethyl acetate and 100 µL of BSTFA with 1% TCMS**. Mix/Vortex.
Heat at 70°C for 30 minutes.

12. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

Compound	Primary Ion *	Secondary	Tertiary	Cerilliant #
GHB-D6-di-TMS	239,	240,	241	G-006
GHB-di-TMS	233,	234,	235	G-001

* Suggested internal standard for GC/MS: D_6-GHB.
** Part # SBSTFA-1-1, 10, 25, 100

Quantitation ion

Quality Control NOTE:
Quality control samples were prepared using drug free blood and 1 mg/mL in house stock standard prepared using GHB stock from Sigma (#H-3635). A negative, low and high QC sample was prepared and stored frozen in 0.5-mL aliquots until use.

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KETAMINE IN BLOOD, PLASMA/SERUM AND URINE FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. PREPARE SAMPLE

To 1 m L of 100 mM phosphate buffer (pH= 6.0) add internal standard*. Add 1-2 mL of blood, plasma/serum or urine.
Mix/vortex. Add 2 mL 100 mM phosphate buffer (pH= 6.0)
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
Centrifuge as appropriate. 1 mL of 100 mM phosphate buffer (pH= 6.0)

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 mL/minute.

4. WASH COLUMN

1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM Acetic Acid.
1 x 3 mL CH_3OH.
Dry column (5 minutes at > 10 inches Hg).

5. ELUTE KETAMINE

1 x 3 mL Dichloromethane/ Isopropanol/ Ammonium Hydroxide (78:20:2).
Collect eluants at 1-2 mL/min using minimal vacuum.
NOTE: Make the elution solvent fresh daily.
Add IPA/NH_4OH, mix then add CH_2Cl_2 (pH 11-12).

6. DRY ELUATE

Evaporate to dryness at < 40°C.
Reconstitute with 100 µL ethyl acetate.

7. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

Compound	Primary Ion***	Secondary	Tertiary	Cerilliant #
Ketamine-D4*	184	213	156	K-003
Ketamine	180	209	152	K-002

* Suggested internal standard for GC/MS: D_4-Ketamine
*** Quantitation ion

SOURCE - UCT Internal Publication.

DCN-903020-73

LYSERGIC ACID DIETHYLAMIDE (LSD) IN BLOOD AND PLASMA/ SERUM FOR GC OR GC/MS CONFIRMATIONS
USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. **PREPARE SAMPLE**
   To 4 mL of D.I. H_2O internal standard* add 1 mL of blood or plasma/serum
   Mix/vortex and let stand 5 minutes.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet.
   Add 2 mL 100 mM phosphate buffer (pH =6.0).
   Mix/vortex. Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
   NOTE: Aspirate at < 3 inches Hg. to prevent sorbent drying.

3. **APPLY SAMPLE**
   Load at 1 mL/minute.

4. **WASH COLUMN**
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM acetic acid.
   1 x 3 mL CH_3OH.
   Dry column (5 minutes at > 10" Hg).

5. **ELUTE LSD**
   1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2);
   Collect eluate at 1 mL/minute.
   NOTE: Prepare elution solvent daily. Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

6. **DRY ELUATE**
   Evaporate to dryness at < 40°C.

7. **DERIVATIZE**
   Add 20 µL ethyl acetate and 20 µL BSTFA (with 1% TMCS)***.
   Overlay with N_2 and cap. Mix/vortex.
   React 30 minutes at 70°C. Remove from heat source to cool.
   NOTE: Do not evaporate BSTFA solution.

8. **QUANTITATE**
   Inject 1 to 2 µL onto gas chromatograph.
   For MSD monitor the following ions:

Compound	Primary Ion***	Secondary	Tertiary	Cerilliant #
LSD-D3-TMS*	298,	296	271	L-006
LSD-TMS	395	293,	268	L-005

* Suggested internal standard for GC/MS: D_3 -LSD
*** Part # SBSTFA-1-1, 10, 25, 100
**** Quantitation ion

DCN-903020-74

LYSERGIC ACID DIETHYLAMIDE (LSD) IN URINE FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. PREPARE SAMPLE

To 2 mL 100 mM phosphate buffer (pH= 6.0) add internal standard. Add 5 mL of urine.
Mix/vortex.
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
Centrifuge as appropriate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
NOTE: Aspirate at < 3 inches Hg. to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 mL/minute.

4. WASH COLUMN

1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM acetic acid.
1 x 3 mL CH_3OH.
Dry column (5 minutes at > 10 inches Hg).

5. ELUTE LSD

1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2);
Collect eluate at 1 to 2 mL/minute.
NOTE: Prepare elution solvent daily. Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

6. DRY ELUATE

Evaporate to dryness at < 40°C.

7. DERIVATIZE

Add 20 µL ethyl acetate and 20 µL BSTFA (with 1% TMCS)***.
Overlay with N_2 and cap. Mix/vortex.
React 20 minutes at 70°C. Remove from heat source to cool.
NOTE: Do not evaporate BSTFA solution.

8. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

Compound	Primary Ion***	Secondary	Tertiary	Cerilliant #
LSD-D3-TMS*	298,	296	271	L-006
LSD-TMS	395	293,	268	L-005

* Suggested internal standard for GC/MS: D_3-LSD
*** Part # SBSTFA-1-1,10,25,100
**** Quantitation ion

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1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH 6) add internal standards.*
   Add 1 mL of whole blood, serum/ plasma, urine. Add 2 mL of 100 mM phosphate buffer (pH 6). Vortex and centrifuge as appropriate.

2. **CONDITION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 mL 100 mM phosphate buffer (pH 6).
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**
   1 x 3 mL DI H_2O
   1 x 3 mL 100 mM acetic acid.
   1 x 3 mL CH_3OH
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE LSD AND METABOLITES**
   1 x 3 mL ethyl acetate; acetonitrile: ammonia (78: 20: 2 v/v)
   Or
   1 x 3 mL CH_2Cl_2/ IPA/ ammonia (78:20:2 v/v)
   Collect eluate at 1-2 mL /minute.

6. **EVAPORATION:**
   Evaporate eluates to dryness under a gentle stream of nitrogen.

7. **RECONSTITUTE** sample in 50 µL of CH_3OH.
   Inject 5 µL.

INSTRUMENT CONDITIONS:

Column: 100 x 2.1 mm (3 µm) Selectra® Phenyl (UCT, LLC)

Mobile Phase:

Time%	Acetonitrile%	0.1% Formic acid
0	30	70
3.0	90	10
3.1	30	70
5.0	30	70

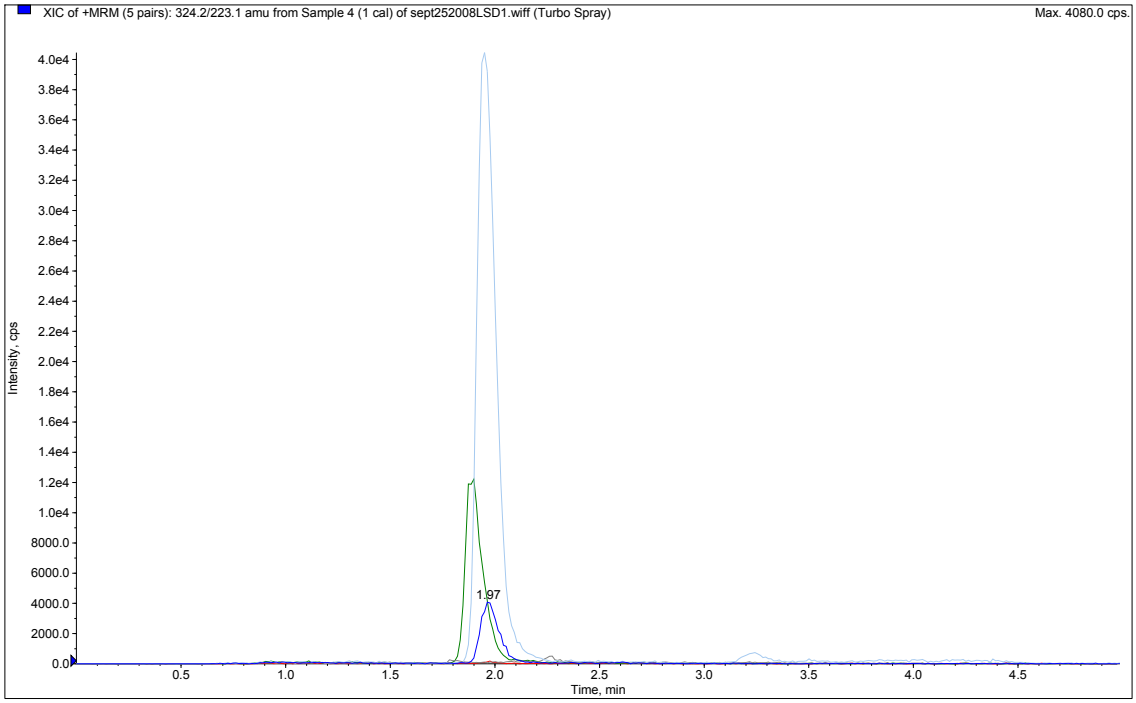
Flowrate: 0.5 mL/ minute

Column Temperature: ambient

Detector: API 3200 QTRAP MS/MS

Compound	MRM Transistion	Cerilliant #
LSD	324.2/ 223.1	-005
Iso-LSD	324.2/281 (223.1)	I-010
Nor-LSD	310.2/209.1	L-017
OH-LSD	356.2/338.1	O-013
*LSD-D3	327.2/226.1	L-002

CHROMATOGRAM OF LSD AND METABOLITES



Recovery: > 90% (N=10)

LOD: 0.1 ng/ mL

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METHAQUALONE IN BLOOD, PLASMA/SERUM AND URINE FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. PREPARE SAMPLE

To 1 mL of 100 mM phosphate buffer (pH= 6.0) and add internal standard*. Add 2 mL of blood, plasma/serum or urine.
Add 2 mL of 100 mM phosphate buffer (pH= 6.0). Mix/vortex.
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
Centrifuge as appropriate

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 mL/minute.

4. WASH COLUMN

1 x 3 mL D.I. H_2O.
Dry column (5 minutes at > 10 inches Hg).
1 x 2 mL hexane.

5. ELUTE METHAQUALONE

1 x 3 mL hexane/ethyl acetate (50:50); Collect eluate.

6. DRY ELUATE

Evaporate to dryness at < 40°C.
Reconstitute with 100 µL ethyl acetate.

7. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

Compound	Primary***	Secondary	Tertiary	Cerilliant #
Methaqualone	235	250	233	M-015
Hexobarbital*	221	157	156	H-013
Methaqualone-D7	240	257	240	M-014

* Suggested internal standard (s) for GC/MS: Hexobarbital, Methaqualone-D7
*** Quantitation ion

SOURCE - UCT Internal Publication

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METHAQUALONE IN BLOOD, PLASMA/SERUM, AND URINE USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part # ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH= 6) add internal standard.*
   Add 1 mL of whole blood, serum/plasma or urine. Add 2 mL of 100 mM phosphate buffer (pH= 6).
   Vortex and centrifuge as appropriate.

2. **CONDITION COLUMN:**
   1 x 1 mL CH_3OH.
   1 x 1 mL D.I. H_2O.
   1 x 0.5 mL 100 mM phosphate buffer (pH=6).
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out.

3. **APPLY SAMPLE**:
   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**
   1 x 3 mL D.I. H_2O
   Dry column (5 minutes at > 10 inches Hg).
   1 x 3 mL hexane
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE METHAQUALONE:**
   1 x 3 mL hexane/ ethyl acetate (50:50).
   Collect eluate at 1-2 mL /minute.

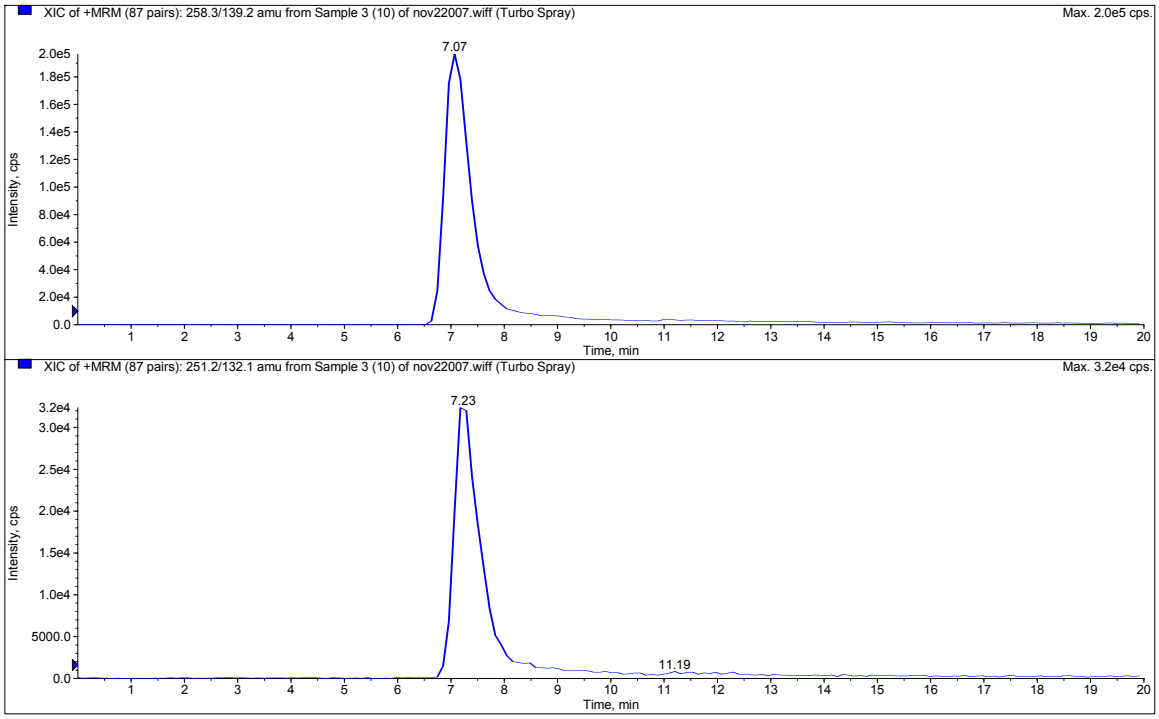
6. **EVAPORATION:**
   Evaporate eluates under a gentle stream of nitrogen < 40°.

7. **Reconstitute the residue in 100 µL of CH_3OH.**
   Inject 5 µL.

INSTRUMENT CONDITIONS:
Column: 150 x 2.1 mm (3 µm) SB-Aq (Agilent Technologies)
Mobile phase: Acetonitrile: 0.1% Formic acid (30: 70)
Flow rate: 0.35 mL/ minute
Column Temperature: ambient
Detector: API 2000 MS/MS

Compound	MRM Transistion	Cerilliant #
Methaqualone	251.2/ 132.1	M-015
*Methaqualone-D7	258.2/138.2	M-014

Chromatogram of Methaqualone/ Methaqualone-D7 (10 ng/ mL)



Recovery: > 90% (N=10)

LOD: 1.0 ng/ mL

DCN-903020-77

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH 6) add internal standard.*

   Add 1 mL of whole blood, serum/ plasma, urine. Add 2 mL of 100 mM phosphate buffer (pH 6).

   Vortex and centrifuge as appropriate.

2. **CONDITION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 mL 100 mM phosphate buffer (pH 6).
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute

4. **WASH COLUMN:**
   1 x 3 mL DI H_2O
   1 x 3 mL 100 mM acetic acid
   1 x 3 mL CH_3OH
   Dry column (5 minutes at > 10 inches Hg)

5. **ELUTE MEPERIDINE/ NORMEPERDINE:**
   1 x 3 mL ethyl acetate; acetonitrile: ammonia (78: 20: 2 v/v)

   Or

   1 x 3 mL CH_2Cl_2/ IPA/ ammonia (78:20:2 v/v)
   Collect eluate at 1-2 mL /minute

6. **EVAPORATION:**
   Evaporate eluates to dryness under a gentle stream of nitrogen

8. **RECONSTITUTE** sample in 50 µL of CH_3OH.
   Inject 5 µL.

INSTRUMENT CONDITIONS:

Column: Silica Hydride (150 x2.0 4µm) MicroSolv Corp.

Mobile phase:

Time	% Acetonitrile	%0.1% Formic acid
0	90	10
5	30	70
6	90	10
10	90	10

Flowrate: 0.35 mL/ minute

Column Temperature: ambient

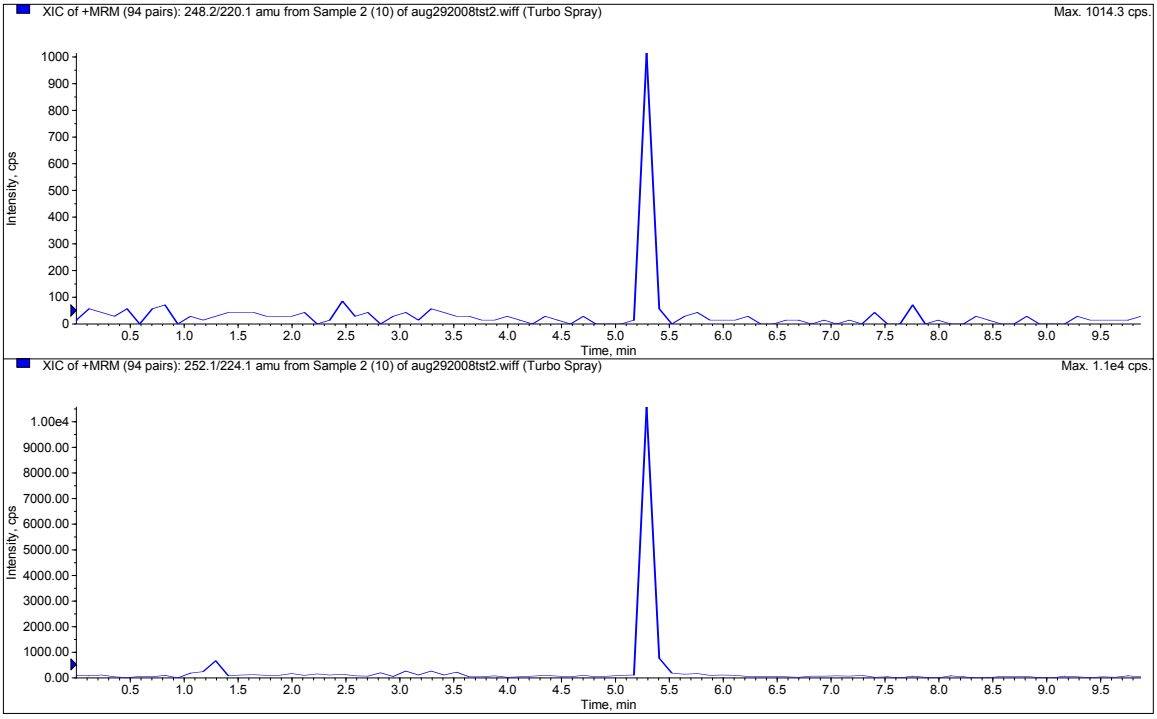
Detector: API 2000 MS/MS

Compound	MRM Transition	Cerilliant #
Meperidine	248.2/ 220.0	M-035
*Meperidine-D4	252.2/224.1	M-036
Normeperdidine	234.1/160.0	N-017
*Normeperidine-D4	238.1/164.0	N-021

CHROMATOGRAM SHOWING:

Meperidine

Meperdine-D4



Recovery: > 90% (n=10)

LOD: 10 ng/ mL

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PSILOCIN IN URINE
FOR GC OR GC/MS CONFIRMATIONS USING:
200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. PREPARE SAMPLE

To 5 mL of urine add internal standard and 2 mL of 100 mM phosphate buffer (pH 6.0).
Mix/vortex.
Add 12,500 to 25,000 units of ß-Glucuronidase, Mix/Vortex.
Place the sample into a water bath at 45°C for 90 minutes.
Remove from the bath and allow to cool.
Centrifuge at 3,000 rpm for 10 min.
Use the clear filtrate (discard the plug) for SPE.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM phosphate buffer (pH 6.0).
NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 mL/minute.

4. WASH COLUMN

1 x 3 mL D.I. H_2O.
1 x 2 mL 20% Acetonitrile in water.
1 x 1 mL 100 mM Acetic Acid.
Dry column (3 minutes at > 10 inches Hg).
1 x 2 mL Hexane.
1 x 3 mL Hexane/ Ethyl Acetate (50:50).
1 x 3 mL CH_3OH.
Dry Column (3 min at > 10 inches Hg).

5. ELUTE PSILOCIN

1 x 3 mL Dichloromethane/ Isopropanol/Ammonium Hydroxide (78:20:2).
Collect eluant at 1 mL /min.
NOTE: Prepare elution solvent daily.

6. DRY ELUATE

Evaporate to dryness at < 35°C.

7. DERIVATIZE

Add 50 µL of ethyl acetate. Vortex mix.
Add 50 µL of MSTFA*
React for 30 minutes at 70˚C.
Remove from heat

NOTE: Do not evaporate MSTFA solution

8. QUANTITATE

Inject 1 to 2 µL onto chromatograph.
Monitor the following ions (Mass Selective Detection):

Compound	Primary**	Secondary	Tertiary	Cerilliant #
PSILOCIN-TMS	290	348	73 (291)	P-048
*PSILOCIN-D10-TMS	300	358	83(301)	P-049

* Part # SMSTFA-0-1, 10, 25, 100
**Quantitation Ion

GC CONDITIONS:
HP Model 5890 GC with a 5970 MSD
COLUMN = DB5 (25 m x 0.32 mm ID x 0.17µm Film Thickness
CARRIER GAS -Helium (5 psi head pressure)

INJECTION Size = 1 µL
SPLITLESS MODE

Injection Temperature = 275°C
Detector Temperature = 300 °C
TEMPERATURE PROGRAM: 70° C hold 1 min then ramp to 240 °C at 20 °C/min hold for 2 minutes

SOURCE - The Detection of Psilocin in Human Urine Grieshaber A, Moore K, Levine B and Smith M Presented at the TRI-SERVICES Meeting Nov 1999

6. PREPARE SAMPLE:

To 1 mL of 100 mM phosphate buffer (pH 6) add internal standard.*

Add 1 mL of urine sample.

Add 2 mL of 100 mM phosphate buffer (pH 6).

Vortex and centrifuge as appropriate.

URINE HYDROLYSIS:

To 1 mL of urine add internal standard* and 1 mL of ß-glucuronidase solution.

(ß-glucuronidase solution contains: 5,000 F units/mL Patella vulgata in 100 mM acetate buffer (pH=5.0)).

Mix/vortex.

Hydrolyze for 3 hours at 65°C.

Centrifuge for 10 minutes at 2000 rpm and discard pellet.

7. CONDITION COLUMN:

1 x 3 mL CH_3OH

1 x 3 mL D.I. H_2O

1 x 1 mL 100 mM phosphate buffer (pH 6).

Note: aspirate at < 3 inches Hg to prevent sorbent drying out

8. APPLY SAMPLE:

Load sample at 1-2 mL / minute.

9. WASH COLUMN:

1 x 3 mL DI H_2O

1 x 3 mL 100 mM acetic acid

1 x 3 mL CH_3OH

Dry column (5 minutes at > 10 inches Hg).

10. ELUTE PSILOCIN:
1 x 3 mL ethyl acetate; acetonitrile: ammonia (78: 20: 2 v/v)

Or

1 x 3 mL CH_2Cl_2/ IPA/ ammonia (78:20:2 v/v)

Collect eluate at 1-2 mL /minute.

6. EVAPORATION:
Evaporate eluates to dryness under a gentle stream of nitrogen.

9. RECONSTITUTE sample in 50 µL of CH_3OH.
Inject 5 µL.

INSTRUMENT CONDITIONS:

Column: 50 x 2.1 mm (3 µm) SELECTRA® Phenyl (UCT, LLC)

MOBILE PHASE:

Time	% Acetonitrile	%0.1% Formic acid
0	20	80
5	20	80

Flowrate: 0.20 mL/ minute

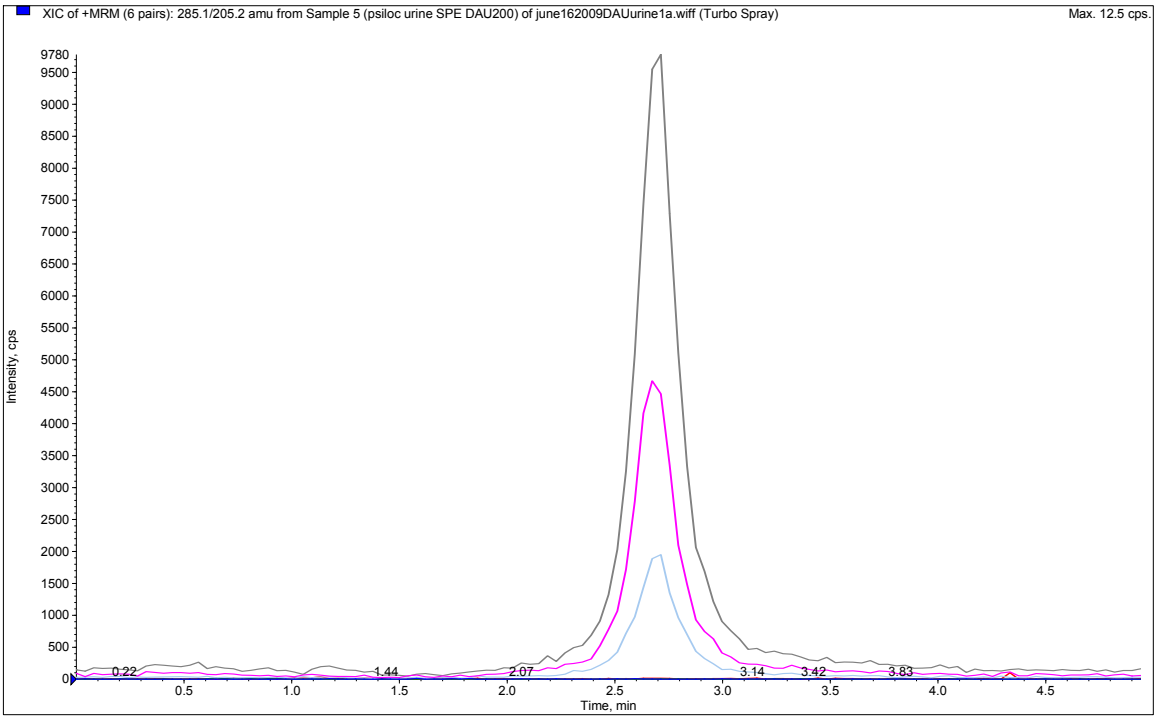
Column Temperature: ambient

Detector: API 2000 MS/MS

Compound	MRM Transition
Psilocin	205.2/58.2
*Psilocin-D10	215.2/68.2

CHROMATOGRAM SHOWING:

Psilocin extracted from Urine



Recovery > 90% (n=10)

LOD: 10 ng/ mL

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PSILOCIN IN BLOOD, PLASMA/SERUM, URINE USING: 200 g STYRE SCREEN® EXTRACTION COLUMN

Part #: SSDBX0206

LC-MSMS

September 1, 2009

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH 6) add internal standard.*

   Add 1 mL of whole blood, serum/ plasma, or urine and vortex mix.

   Add 3 mL of 100 mM phosphate buffer (pH 6).

   Vortex and centrifuge as appropriate.

2. **CONDITION COLUMN:**
   Not required for this SPE

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**
   1 x 3 mL DI H_2O

   1 x 3 mL 100 mM acetic acid

   1 x 3 mL CH_3OH

   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE PSILOCIN**:
   1 x 3 mL ethyl acetate; acetonitrile: ammonia (78: 20: 2 v/v)

   Or

   1 x 3 mL CH_2Cl_2/ IPA/ ammonia (78:20:2 v/v)

   Collect eluate at 1-2 mL /minute.

6. EVAPORATION:
Evaporate eluates to dryness under a gentle stream of nitrogen.

10. RECONSTITUTE sample in 50 µL of CH_3OH.
Inject 5 µL.

INSTRUMENT CONDITIONS:

Column: 50 x 2.1 mm (3 µm) SELECTRA ® Phenyl (UCT, LLC)

MOBILE PHASE:

Time	% Acetontrile (0.1% Formic acid)	%0.1% Formic acid
0	80	20
5	80	20

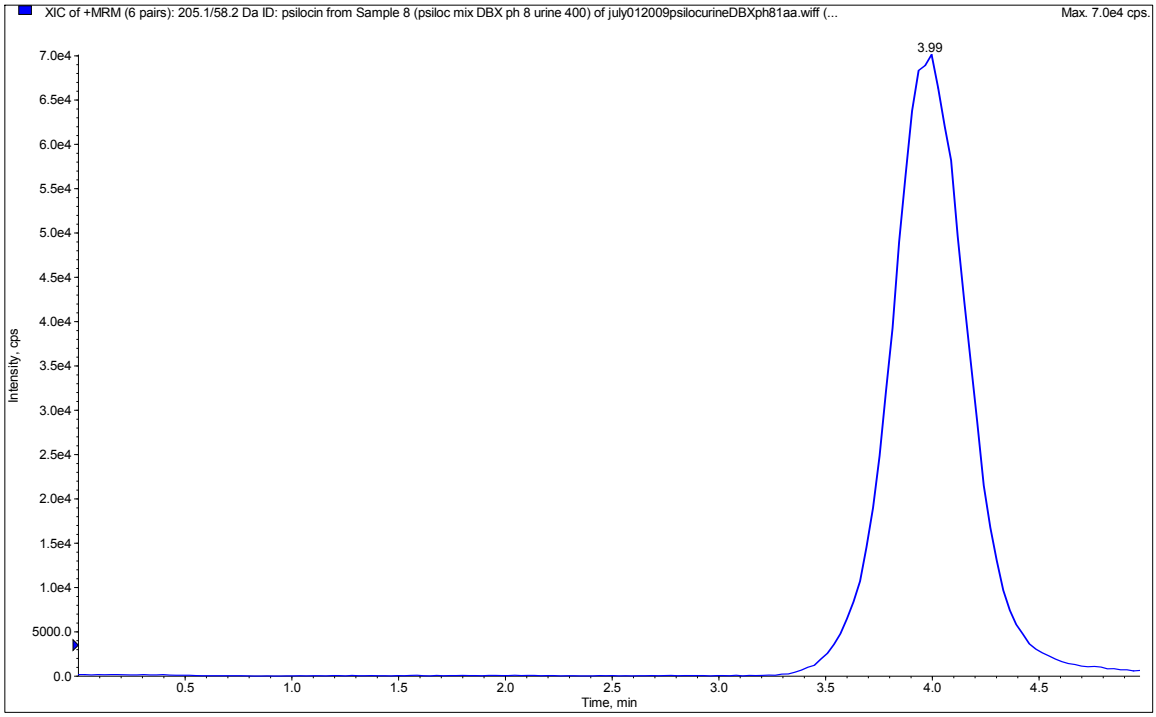
Flowrate: 0.3 mL/ minute

Column : 40 ˚C

Detector: API 3200 QTrap MS/MS

Compound	MRM Transition
Psilocin	205.2/58.2
*Psilocin-D10	215.2/68.2

CHROMATOGRAM SHOWING: PSILOCIN



Recovery > 90% (n=10)

LOD: 10 ng/ mL

PHENCYCLIDINE IN BLOOD, PLASMA/SERUM URINE AND TISSUE FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. **PREPARE SAMPLE**
   To 1 mL of 100 mM phosphate buffer (pH= 6.0) add internal standard(s)*. Add 1 mL blood, plasma/serum, urine or 1 g (1:4) tissue homogenate. Mix/vortex.
   Add 2 mL of100 mM phosphate buffer (pH= 6.0).
   Mix / vortex.
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
   Centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**
   Load at 1 to 2 mL/minute.

4. **WASH COLUMN**
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM acetic acid.
   1 x 3 mL CH_3OH.
   Dry column (5 minutes at > 10 inch Hg).

5. **ELUTE PHENCYCLIDINE**
   1 x 3 mL Methylene Chloride/Isopropanol/ Ammonium Hydroxide (78:20:2).
   NOTE: Prepare elution solvent daily. Add IPA/ NH_4OH, mix, and then add CH_2Cl_2.

6. **DRY ELUATE**
   Evaporate to dryness at < 40°C.
   Remove immediately upon completion.
   Reconstitute with 100 µL ethyl acetate.

7. **QUANTITATE**
   Inject 1 to 2 µL onto gas chromatograph.
   For MSD monitor the following ions:

Compound	Primary***	Secondary	Tertiary	Cerilliant #
Phencyclidine-D5*	205	96	247	P-003
Phencyclidine	200	91	242	P-007

* Suggested internal standard for GC/MS: D_5-Phencyclidine
*** Quantitation Ion

REFERENCE - UCT Internal Publication

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PHENCYCLIDINE IN WHOLE BLOOD, SERUM / PLASMA AND URINE USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

PART #: ZSDAU020

LC/MS/MS

February 3, 2009

1. **PREPARE SAMPLE**

   To 1 mL of 100 mM prosphate buffer (pH= 6) add internal standard*. Add 1 mL of blood, serum/plasma or urine. Add 2 mL of 100 mM phosphate buffer (pH= 6). Mix/vortex

   Sample pH should be 6.0 ± 0.5.

   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate

   Mix/vortex.

   Centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**

   1 x 3 mL CH_3OH

   1 x 3 mL D.I. H_2O

   1 x 1 mL 100 mM phosphate buffer (pH= 6)

   Note: aspirate at < 3 inches Hg to prevent sorbent drying out

3. **APPLY SAMPLE:**

   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**

   1 x 3 mL D.I. Water

   1 x 3 mL 100 mM acetic acid

   1 x 3 mL CH_3OH

   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE PHENCYCLIDINE:**

   1 x 3 mL ethyl acetate; acetonitrile: ammonia (78: 20: 2 v/v)

   Collect eluate at 1-2 mL / minute.

   Note: Prepare elution solvent daily.

6. **EVAPORATION:**

   Evaporate eluates under a gentle stream of nitrogen < 40ºC.

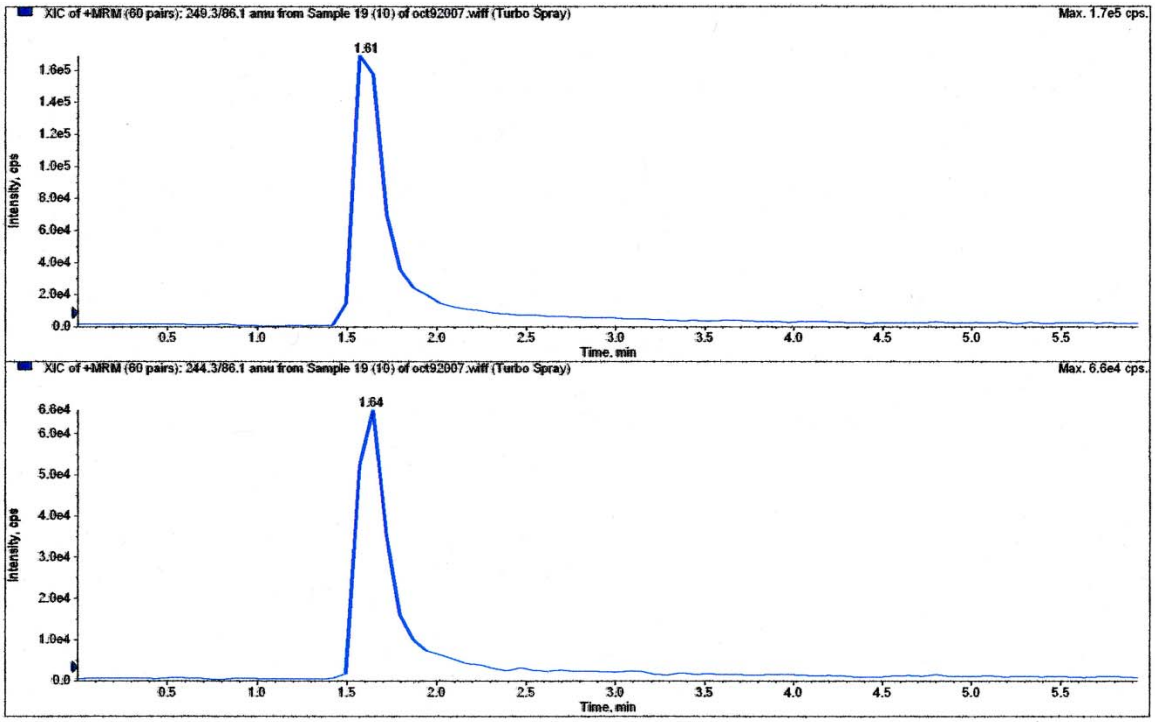
7. **RECONSTITUTE sample in 100 µL of CH3OH.**

   Inject 5 µL.

INSTRUMENT CONDITIONS:

Column: 150 x 2.1 mm (3 µm) Zorbax: Agilent Technologies
Mobile phase: Acetonitrile: 0.1% Formic acid (33:67)
Flowrate: 0.35 mL/minite
Column Temperature: ambient
Detector: API 2000 MS/MS

Compound	MRM Transistion	Cerilliant
Phencyclidine	244.3/86.1	P-007
* Phencyclidine-D5	249.3/86.1	P-003



Recovery: > 90% (N=10)
LOD: 1 ng/mL

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FENTANYL / NORFENTANYL IN WHOLE BLOOD, SERUM / PLASMA AND URINE USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

PART #: ZSDAU020

LC/MS/MS

February 3, 2009

1. PREPARE SAMPLE

To 1 mL of 100 mM phosphate buffer (pH= 6) add internal standard.*

Add 1 mL whole blood, plasma/ serum or urine. Add 2 mL of 100 mM phosphate buffer (pH= 6).

Mix/vortex.

Centrifuge as appropriate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH

1 x 3 mL D.I. H_2O

1 x 1 mL 100 mM phosphate buffer (pH 6)

Note: aspirate at < 3 inches Hg to prevent sorbent drying out

3. APPLY SAMPLE

Load sample at 1 ~2 mL / minute.

4 . WASH COLUMN:

1 x 3 mL D.I .H_2O

1 x 3 mL 100 mM acetic acid

1 x 3 mL CH_3OH

Dry column (5 minutes at > 10 inches Hg).

5. ELUTE FENTANYL / NORFENTANYL:

1 x 3 mL ethyl acetate; acetonitrile: ammonia (78: 20: 2 v/v)

Collect eluate at 1-2 mL / minute.

NOTE: Prepare elution solvent daily

6. EVAPORATION:

Evaporate eluates under a gentle stream of nitrogen < 40°C.

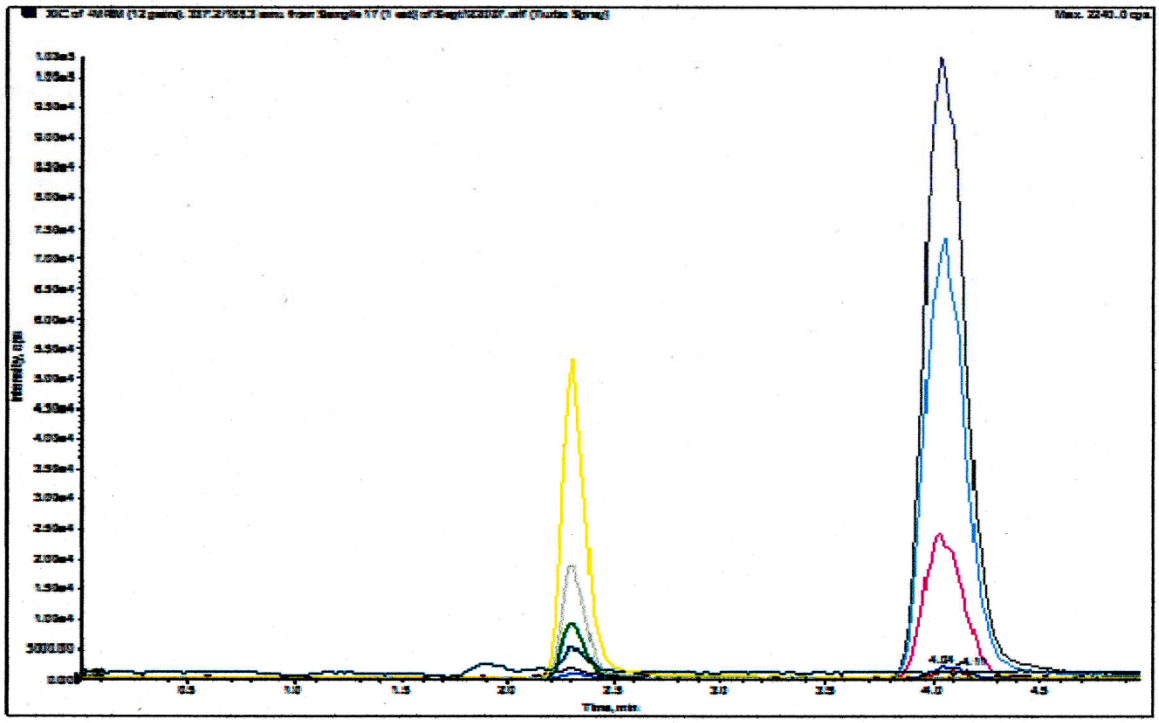
7. RECONSTITUTE SAMPLE IN 100 µL OF METHANOL.

Inject 5 µL.

INSTRUMENT CONDITIONS:

Column: 150 x 2.1 mm (3 µm) PFP Gold Thermofisher
Mobile phase: Acetonitrile: 0.1 % Formic acid (50: 50)
Flowrate: 035 mL/ minute
Column Temperature: ambient
Detector: API 2000 MS/MS

Compound	MRM Transistion	Cerilliant #
Fentanyl	333.2/188.3	F-002
*Fentanyl-D5	342.3/188.2	F-001
Norfentanyl	233.2/84.1	N-031
*Norfentanyl-D5	238.3/84.1	N-030



DCN-903020-81

FENTANYL AND ANALOGUES IN BLOOD, PLASMA/SERUM, URINE AND TISSUE FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. PREPARE SAMPLE

To 1 mL of 100 mM phosphate buffer (pH= 6) add internal standards*. Add 1- 5 mLof blood, plasmas/ serum, urine or 1 g (1: 4) tissue homogenate. Mix/ vortex.

Add 2 mL of 100 mMphosphate buffer (pH 6.0).

Mix/vortex.

Sample pH should be 6.0 ± 0.5.

Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

Centrifuge as appropriate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.

1 x 3 mL D.I. H_2O.

1 x 1 mL 100 mM phosphate buffer (pH 6.0).

NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 mL/minute.

4. WASH COLUMN

1 x 3 mL D.I. H_2O.

1 x 1 mL 100 mM acetic acid.

1 x 3 mL CH_3OH.

Dry column (5 minutes at > 10 inches Hg).

5. ELUTE FENTANYLS

1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2); Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

6. CONCENTRATE

Evaporate to dryness at < 40°C.

Reconstitute with 50 µL ethyl acetate.

7. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

Compound	Primary Ion***	Secondary	Tertiary	Ceriliant #
Fentanyl	245	146	189	F-002
Fentany-D5l*	250	151	194	F-001
α-Methylfentanyl	259	203	146	
Para-Fluorofentanyl	263	164	207	
3-Methylfentanyl	259	160	203	
Thienfentanyl	245	146	189	
Sufentanil	289	140		
Carfentanil	303	187		
Lofentanil	317	201	289	
Alfentanil	289	268	194	

* Suggested internal standard for GC/MS: D_5-Fentanyl

*** Quantitation ion

SOURCE - UCT Internal Publication working with the Philadelphia Medical Examiner's Office

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FENTANYL / NORFENTANYL ON ORAL SWABS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

PART #: ZSDAU020

LC/MS/MS

February 3, 2009

1. **PREPARE SAMPLE**

   Preparation of Standards:

   To separate tube add 0, 1, 5, 10 ng of Fentanyl / Norfentanyl in methanol. Evaporate off the solvent. Add 100 µL of drug free oral fluid. Vortex mix and allow to stand for 30 minutes. Take clean, dry (drug free) swab and swab up the oral fluid and allow standing for 15 minutes. Remove oral swab.

   SAMPLE PRE TREATMENT:

   To 200 µL of methanol (pH 6) add internal standard.*
   Insert oral swab into methanol and mix for 1 minute, add a further 100 µL of methanol, allow to stand for 10 minutes. Remove swab and 3 mL of 100 mM phosphate buffer (pH 6). Vortex and centrifuge as appropriate.

2. **CONDITION COLUMN:**

   1 x 3 mL MeOH
   1 x 3 mL H_2O
   1 x 1 mL 100 mM phosphate buffer (pH 6)
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out

3. **APPLY SAMPLE:**

   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**

   1 x 3 mL DI Water
   1 x 3 mL 1% actate acid
   1 x 3 mL Methanol
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE FENTANYL/ NORFENTANYL:**

   1 x 3 mL ethyl acetate; acetonitrile: ammonia (78: 20: 2 v/v)
   Collect eluate at 1-2 mL /minute.

6. **EVAPORATION:**

   Evaporate eluates under a gentle stream of nitrogen < 40ºC.

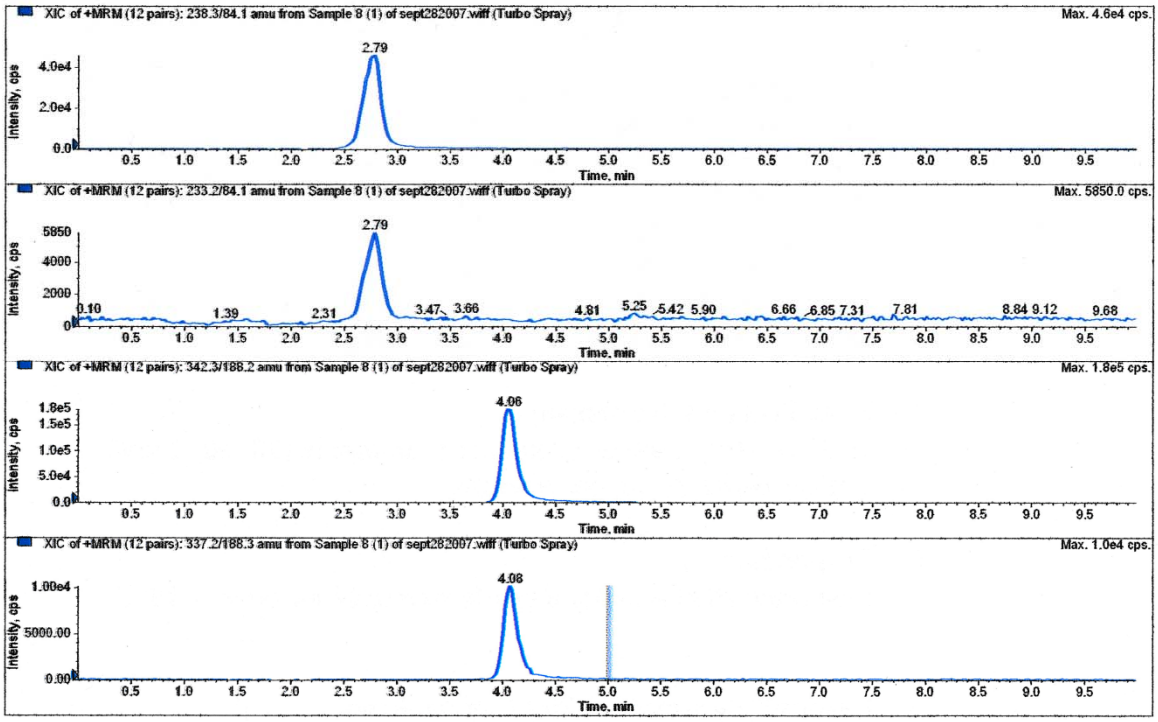
7. **RECONSTITUTE sample in 20 µL of methanol.**

   Inject 5 µL.

INSTRUMENT CONDITIONS:

Column: 150 x 2.1 mm (3 µm) PFP Thermofisher
Mobile phase: Acetonitrile: 0.1% Formic acid (60:40)
Flowrate: 0.35 mL/minite
Column Temperature: ambient
Detector: API 2000 MS/MS

Compound	MRM Transistion	Cerilliant #
Fentanyl	333.2/188.3	F-002
*Fentanyl-D5	342.2/188.2	F-001
Norfentanyl	233.2/84.1	N-031
*Norfentanyl-D5	238.3/84.1	N-030



Recovery: > 90% (N=100)
LOD: 1 ng/mL

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OPIATES IN URINE-OXIME TMS PROCEDURE FOR GC OR GC/MS CONFIRMATIONS USING:
200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. PREPARE SAMPLE ACID HYDROLYSIS OF GLUCURONIDES:

To 2 mL of urine add internal standard(s)* and *400 µL concentrated HCl*.
Add 200 µL 10% Hydroxylamine solution in DI H_20.
Mix/vortex.
Heat to 90°C for 40 min in a heating block, or an autoclave for 15 minutes on a liquid cycle.
Cool before proceeding.
Centrifuge for 10 minutes at 2000 rpm and discard pellet.
Add 500 µL 50% Ammonium Hydroxide. Mix/vortex.
Adjust sample pH 5 - 6 by drop wise addition with 50% Ammonium Hydroxide.

PREPARE ENZYME HYDROLYSIS OF GLUCURONIDES:

To 2 mL of urine add internal standard(s)* and enzyme preparation in buffer
Mix/vortex.
Heat to 60 °C for sufficient time in a heating block (depends on analytes and enzyme)
Add 200 µL 10% Hydroxylamine solution.
Heat to 60°C for 30 min in a heating block.
Adjust pH to 5 - 6
Centrifuge for 10 minutes at 2000 rpm and discard pellet.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 3 mL 100 mM phosphate buffer (pH 6.0).
NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 to 2 mL/minute.

4. WASH COLUMN

1 x 3 mL D.I. H_2O.
1 x 3 mL 100 mM acetate buffer (pH 4.5).
1 x 3 mL CH_3OH.
Dry column (5 minutes at > 10 inches Hg).

5. ELUTE OPIATES

1 x 3 mL CH_2Cl_2/IPA/NH_4OH (76:20:4)
Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.
Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

6. DRY ELUATE

Evaporate to dryness at < 40°C.

7. DERIVATIZE

Add 100 µL ethyl acetate and 100 µL BSTFA (with 1% TMCS)***.
Overlay with N_2 and cap. Mix/vortex.
React 45 minutes at 70°C. in a heat block.
Remove from heat source to cool

NOTE: Do not evaporate BSTFA solution.

8. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph. For MSD monitor the following ions:

Compound	Quant Ion**	Secondary	Tertiary	Cerilliant #
Meperidine-D4	251	222	250	M-036
Meperidine	247	218	246	M-035
Normeperidine-D4 TMS*	308	280	309	N-020
Normeperidine TMS*	305	276	304	N-017
Tramadol TMS	335	245	290	T-027
O-Desmethyltramadol TMS	393	378	303	T-035
N-Desmethyltramadol TMS	393	378	116	D-023
Pentazocine TMS	357	342	289	P-039
Codeine-D3 TMS*	374	359	346	C-039
Codeine-D6 TMS*	377	349	316	C-040
Codeine TMS	371	356	343	C-006
Norcodeine TMS	429	414	356	N-005
Dihydrocodeine TMS	373	315	358	D-019
Morphine-D3 TMS*	432	417	404	M-099
Morphine-D6 TMS*	435	420	404	M-085
Morphine TMS	429	414	401	M-005
Normorphine TMS	487	472	414	N-006
Diacetylmorphine	369	327	268	H-038
Hydrocodone Oxime-D3 TMS	389	300	374	H-008
Hydrocodone Oxime-D6 TMS	392	303	377	H-047
Hydrocodone Oxime TMS	386	297	371	H-003
Hydromorphone Oxime-D3 TMS	447	432	358	H-010
Hydromorphone Oxime TMS	444	429	355	H-004
Oxycodone Oxime-D3 TMS	477	462	420	O-006
Oxycodone Oxime-D6 TMS	480	465	420	O-008
Oxycodone Oxime TMS	474	459	417	O-002
Oxymorphone Oxime-D3 TMS	535	520	290	O-019
Oxymorphone Oxime TMS	532	517	287	O-004

*Suggested internal standards for GC/MS: D_4-Meperidine, D_4-Normeperidine, D_3-Codeine, D_3-Morphine D_6-Hydrocodone D_6-Oxycodone

*Suggest trying D_6-Codeine, and D_6-Morphine for lowest LOD/LOQ

*** Part # SBSTFA-1-1, 10, 25, 100

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OPIATES IN HUMAN URINE- PROPYL DERIVATIVES FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part # ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. **PREPARE SAMPLE ACID HYDROLYSIS OF GLUCURONIDES**:
   To 2 mL of urine add internal standard(s)* and 400 µL concentrated HCl.
   Add 200 µL 10% Hydroxylamine solution in DI H_20.
   Mix/vortex.
   Heat to 90°C for 40 min in a heating block, or an autoclave for 15 minutes on a liquid cycle.
   Cool before proceeding.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet.
   Add 500 µL 50% Ammonium Hydroxide. Mix/vortex.
   Adjust sample pH 5 - 6 by drop wise addition with 50% Ammonium Hydroxide.

   PREPARE SAMPLE-ENZYMATIC HYDROLYSIS OF GLUCURONIDES:
   To 2 mL of urine, add internal standard(s), and 1 mL of ß-Glucuronidase solution. ß-Glucuronidase solution contains 5,000 F units/mL Patella vulgata in 100 mM acetate buffer (pH 5.0).
   Hydrolyze for 3 hours at 60°C.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet.
   Adjust sample pH to 5 - 6 with 1.0 N NaOH.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH 6.0); Aspirate.
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**
   Load at 1 to 2 mL/minute.

4. **WASH COLUMN**
   1 x 3 mL D.I. H_2O; Aspirate.
   1 x 3 mL 100 mM acetate buffer (pH 4.5); Aspirate.
   1 x 3 mL CH_3OH; Aspirate.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE OPIATES**
   1 x 3 mL ethyl acetate/isopropanol/ammonium hydroxide (84:12:4).

6. **DRY ELUANT**
   Evaporate to dryness at < 40°C.

7. DERIVATIZE

Add 200 µL of a 1:1 solution of proprionic anhydride****
pyridine.*****
Make this solution fresh daily.
Mix/vortex.
React for 60 minutes at 60°C in a heater block.
Remove from heat source to cool.
Evaporate to dryness at < 40°C.
Reconstitute the residue with 50 µL of ethyl acetate / methanol (70:30).

* Suggested internal standard for GC/MS: Codeine-D3 and Morphine-D3
** Quantitation ion
*** Hydrocodone does not derivatize under these conditions.
**** Part # SPIA-0-1,10, 25
***** Part # SPYR-0-50

8. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph. For MSD monitor the following ions:

Compound (Propyl)	Primary Ion**	Secondary Ion	Tertiary Ion	Cerilliant#
Hydrocodone	299	242	214	H-003
Codeine	355	282	229	C-006
*Codeine-D3	358	285	232	C-007
Oxycodone	371	314	298	O-002
Hydromorphone	285	341	228	H-004
6-Acetylmorphine	327	268	383	A-003
Oxymorphone	357	300	413	O-004
Morphine	341	268	397	M-005
Morphine-D3	344	271	400	M-006

SOURCE: UCT Internal Publication working with the Philadelphia Medical Examiner's Office

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FREE (UNBOUND) OPIATES IN BLOOD, PLASMA/SERUM, TISSUE FOR GC OR GC/MS CONFIRMATIONS
USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part # ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. **PREPARE SAMPLE**

   To 1 mL of 100 mM phosphate buffer (pH=6) add internal standards*. Add 1 mL of blood, plasma/ serum, or 1 g (1:4) tissue homogenate.
   Mix/vortex and let stand 5 minutes
   Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex

.

   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**

   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**

   Load at 1 mL/minute.

4. **WASH COLUMN**

   1 x 2 mL D.I. H_2O.
   1 x 2 mL 100 mM acetate buffer (pH= 4.5).
   1 x 3 mL CH_3OH.
   Dry column (5 minutes at >10 inches Hg).

5. **ELUTE OPIATES**

   1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2);
   Collect eluate at 1 to 2 mL/minute.
   NOTE: Prepare elution solvent daily.
   Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

6. **DRY ELUATE**

   Evaporate to dryness at < 40°C.

7. **DERIVATIZE**

   Add 50 µL ethyl acetate and 50 µL BSTFA (with 1% TMCS)***.
   Overlay with N_2 and cap. Mix/vortex.
   React 30 minutes at 70°C. Remove from heat source to cool.
   NOTE: Do not evaporate BSTFA solution.

8. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.

For MSD monitor the following ions:

Compound	Primary Ion****	Secondary	Tertiary	Cerilliant#
Codeine- D3-TMS*	374	237	346	C-039
Codeine-TMS:	371	234	343	C-015
Morphine-D3-TMS*	432	290	327	M-003
Morphine-TMS	429	287	324	M-005

* Suggested internal standard for GC/MS: Codeine-D3, Morphine-D3

*** Part # SBSTFA-1-1,10, 25, 100

**** Quantitation ion

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6-ACETYLMORPHINE (6MAM) IN URINE/ VITREOUS HUMOR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part # ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. **PREPARE SAMPLE**
   To 2 mL of 100 mM phosphate buffer (pH 6.0), add internal standard* and Mix/vortex.
   Add 4 mL of sample
   Centrifuge for 10 minutes at 2000 rpm and discard pellet.
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH=6.0).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**
   Load at 1 mL/minute.

4. **WASH COLUMN**
   1 x 3 mL D.I. H_2O.
   1 x 2 mL 100 mM acetate buffer (pH 4.5).
   1 x 3 mL CH_3OH.
   Dry column (10 minutes at >10 inches Hg).

5. **ELUTE 6-AM**
   1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2);
   Collect eluate at 1 to 2 mL/minute.
   NOTE: Prepare elution solvent daily.
   Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

6. **DRY ELUATE**
   Evaporate to dryness at < 40°C.

7. **DERIVATIZE**
   Add 50 µL ethyl acetate. Vortex mix and add 50 µL BSTFA (with 1% TMCS)***.
   Overlay with N_2 and cap. Mix/vortex.
   React 45 minutes at 70°C. Remove from heat source to cool.
   NOTE: Do not evaporate BSTFA solution.

8. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

Compound	Primary Ion****	Secondary Ion	Tertiary Ion	Cerilliant #
6-Acetylmorphine- D6 -TMS*	405	406	343	A-026
6-Acetylmorphine-TMS	399	400	340	A-009

* Suggested internal standard for GC/MS:
*** Part # SBSTFA-1-1,10,25,100
**** Quantitation ion

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BUPRENORPHINE AND NORBUPRENORPHINE IN BLOOD, URINE FOR GC/MS CONFIRMATIONS USING:

200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: CSDAU206

February 3, 2009

1. PREPARE SAMPLE

To 1 mL of 100 mM Acetate buffer (pH= 5) add internal standard*. Mix/ vortex and add 1 mL of blood, plasma/ serum. Add 2 mL of 100 mM Acetate buffer (pH= 5) and mix/ vortex.

Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate
Centrifuge as appropriate.

ENZYME HYDROLYSIS OF GLUCURONIDES

To 1 mL of 100 mM Acetate buffer add internal standard*. Add 1-5 mL of blood or urine. Mix/ vortex. Add 2 mL of 100 mM Acetate buffer (pH= 5).

Hydrolyze with Helix Pomatia (5,000 units/mL), heat for 3 hours at 60°C Cool before proceeding.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH
1 x 3 mL D.I.H_2O.
1 x 1 mL 100 mM Acetate buffer (pH=5.0)
NOTE: Aspirate at < 3 Inches Hg to prevent sorbent drying

3. APPLY SAMPLE

Load at 1 to 2 mL/minute

4. WASH COLUMN

1 x 2 mL D.I. H_2O.
1 x 3 mL 100 mM acetate buffer (pH=5.0)
1 x 3 mL Methanol
Dry column (5-10 minutes at greater than 10 inches *Hg* / Full flow for Positive Pressure manifold).

5. ELUTE Buprenorphine / Norbuprenorphine

1 x 3 mL methylene chloride / iso-propano / ammonium hydroxide (78/20/12). (Make elution solvent fresh).
Collect eluate at 1 to 2 mL/minute

NOTE: Before proceeding, insure there are no water droplets at the bottom of the collection tube. This may increase drying time and decrease BSTFA derivitizing efficiency.

6. DRY ELUATE

Evaporate to dryness at < 40°C.

7. DERIVATIZE

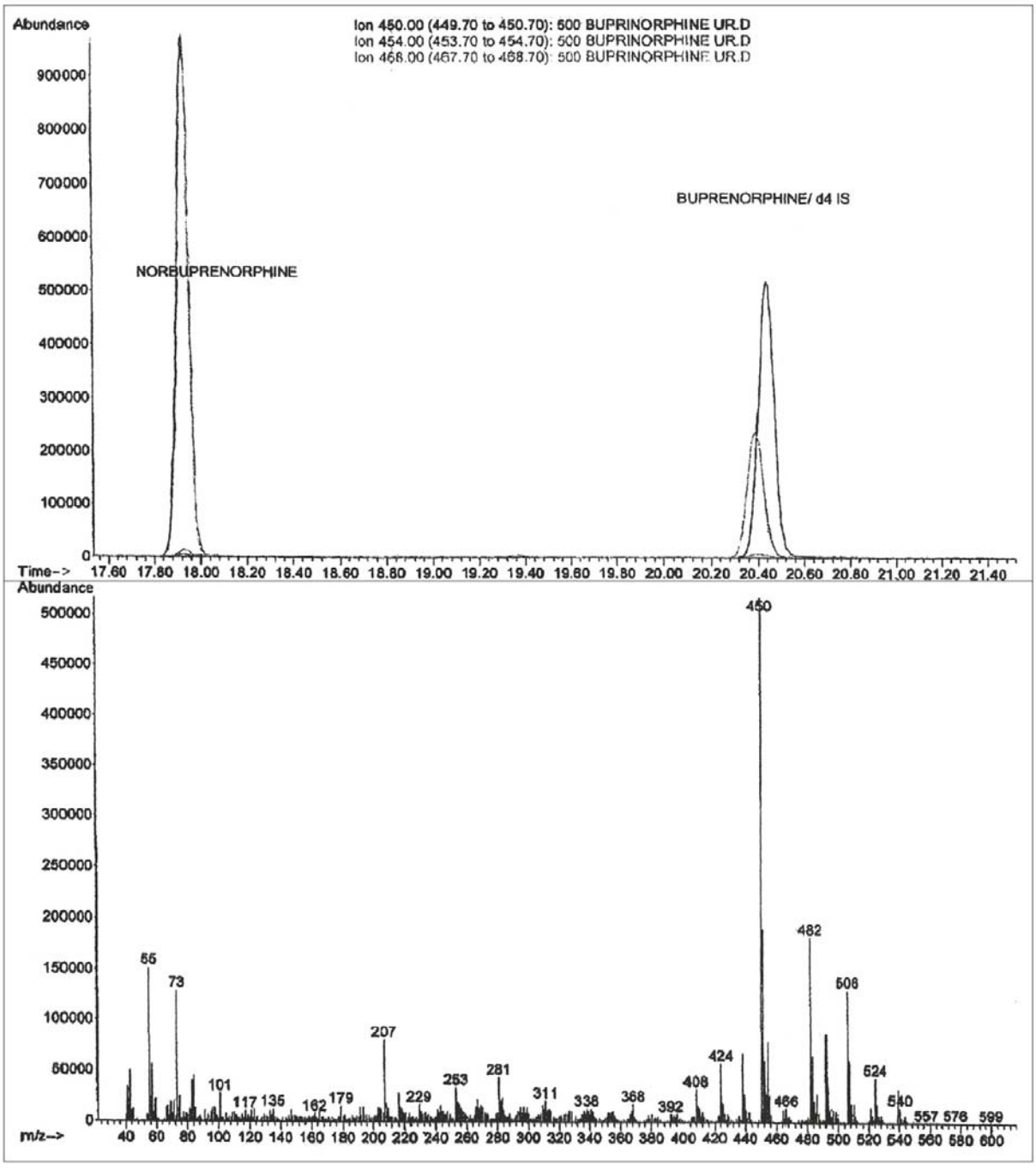
Add 50 µL ethyl acetate and 50 µL BSTFA (with 1 % TMCS).
React 20 minutes at 70°C.
Remove from heat source to cool.
NOTE: Do not evaporate BSTFA

8. QUANTITATE

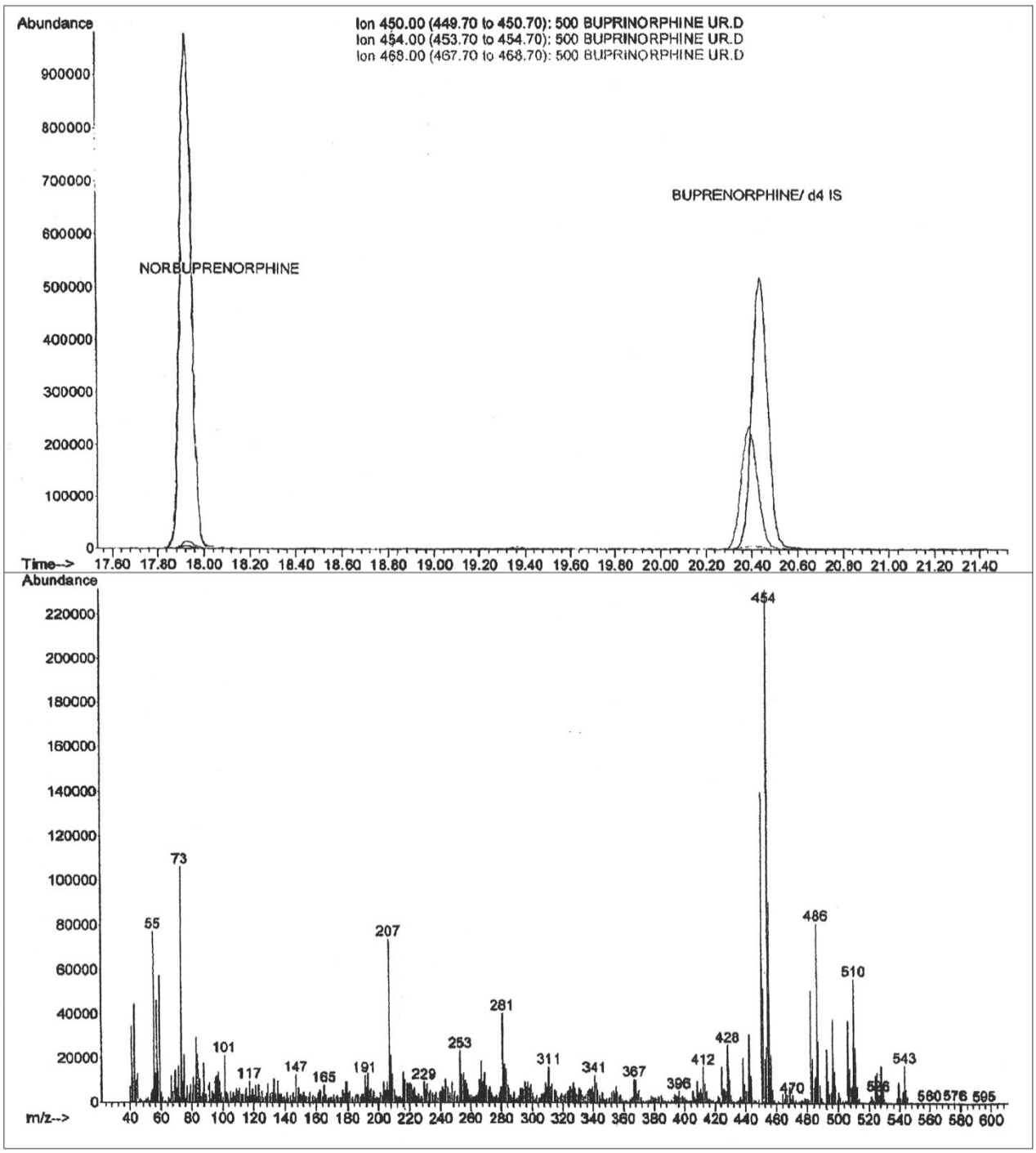
Inject 1 to 2 µL onto gas chromatograph/mass spectrometer
For MSD monitor the following ions:

ANALYTE	Primary Ion	Secondary	Tertiary	Cerilliant #
*Buprenorphine-D4-TMS	454	486	510	B-901
Buprenorphine-TMS	450	482	506	B-902
Norbuprenorphine-TMS	468	500	524	N-912
* Norbuprenorphine-D3-TMS	471	503	527	N-920

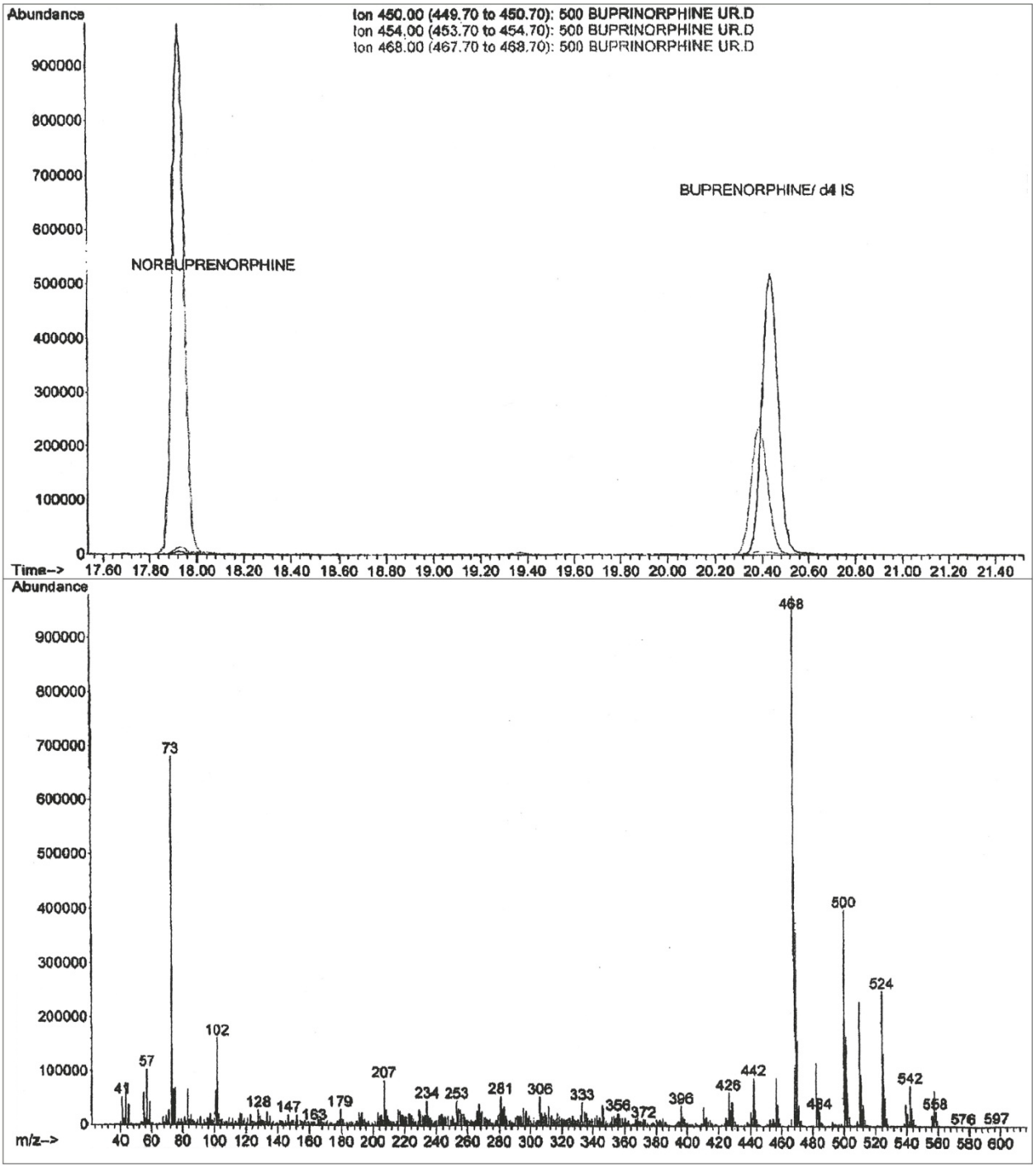
BUPRENORPHINE AND NORBUPRENORPHINE
CHROMATOGRAM I



BUPRENORPHINE AND NORBUPRENORPHINE
CHROMATOGRAM II



BUPRENORPHINE AND NORBUPRENORPHINE
CHROMATOGRAM III



BUPRENORPHINE AND NORBUPRENORPHINE IN BLOOD, PLASMA/SERUM, AND URINE USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020

LC/MS/MS

February 3, 2009

1. **PREPARE SAMPLE (FREE BUPRENORPHINE/ NORBUPRENORPHINE):**
   To 1 mL of 100 mM phosphate buffer (pH= 6) add internal standards.*
   Add 1 mL of whole blood, serum/ plasma, urine.
   Add 2 mL of 100 mM phosphate buffer (pH= 6).
   Vortex and centrifuge as appropriate.

   TOTAL (FREE AND CONJUGATED) BUPRENORPHINE/ NORBUPRENORPHINE:
   To 1 mL of Acetate buffer (pH= 5) containing 5000 F units/ mL β-Glucuronidase.
   Add internal standards*.
   To this solution add 1 mL of whole blood or urine.
   Mix/ Vortex .
   Hydrolyze for 3 Hrs at 65˚C.
   Allow to cool.
   Add 3 mL of 100 mM phosphate buffer (pH= 6) and mix.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet.

2. **CONDITION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 mL 100 mM phosphate buffer (pH= 6).
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**
   1 x 3 mL DI H_2O
   1 x 3 mL 100 mM acetic acid.
   1 x 3 mL CH_3OH
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE BUPRENORPHINE/NORBUPRENORPHINE:**
   1 x3 mL ethyl acetate; acetonitrile: ammonia (78: 20: 2 v/v)
   Or
   1 x 3 mL CH_2Cl_2/ IPA/ ammonia (78:20:2 v/v)
   Collect eluate at 1-2 mL /minute.

6. **EVAPORATION:**
   Evaporate eluates to dryness under a gentle stream of nitrogen.

7. **Reconstitute sample in 50 µL of CH_3OH.**
   Inject 5 µL.

INSTRUMENT CONDITIONS:

Column: 50 x 2.1 mm (3µm) Selectra® Phenyl (UCT Inc.)

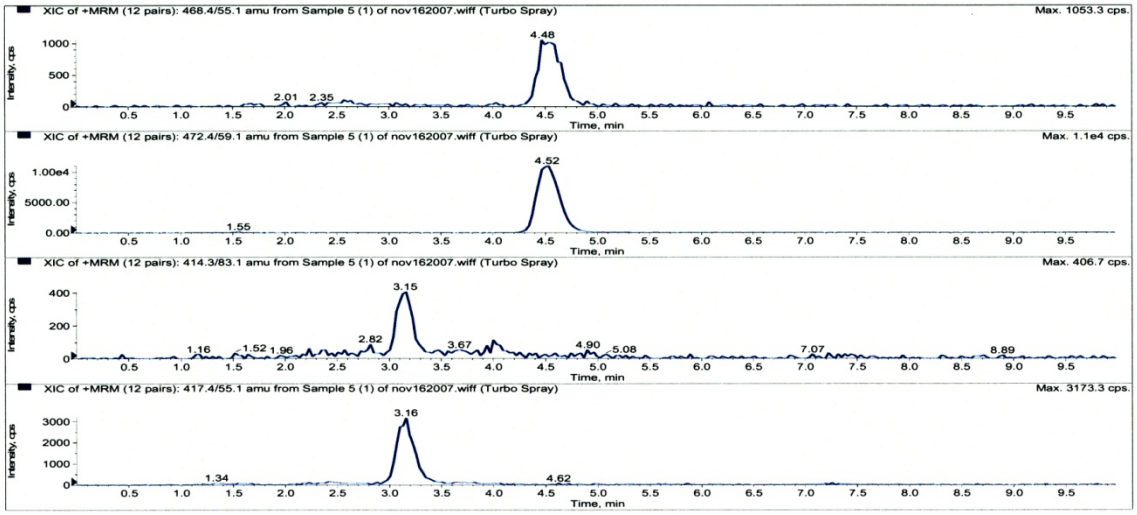
Mobile phase:	Acetonitrile:	0.1% Formic acid
	50	50

Flowrate: 0.35 mL/ minute
Column Temperature: ambient
Detector: API 2000 MS/ MS

Compound	**MRM Transistion**	**Cerilliant #**
Buprenorphine	468.4/55.1	B-902
*Buprenorphine-D4	472.4/59.1	B-901
Norbuprenorphine	414.3/83.1	N-912
*Norbuprenorphine-D3	417.4/55.1	N-920

Chromatogram of Buprenorphine/ Norbuprenorphine (1 ng/ mL)

Buprenorphine Norbuprenorphine
Buprenorphine-D4 Norbuprenorphine-D3



Recovery: > 90% (N=10)
LOD: 0.5 ng/ mL

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AMPHETAMINES, OPIATES, & PHENCYCLIDINE IN ORAL FLUID FOR GC/MS ANALYSIS USING: 50 mg CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #: ZSDAU005

February 3, 2009

1. **PREPARE SAMPLE**
   Add 100 - 500 µL of neat oral fluid sample to a clean tube.
   Add internal standard(s) and let sit for 10 minutes at room temperature.
   Add 800 µL of 100 mM phosphate buffer (pH= 6.0).
   Mix/vortex for 10 seconds. Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 200 µL CH_3OH.
   1 x 200 µL D.I. H_2O.
   1 x 200 µL 100 mM phosphate buffer (pH=6.0).

3. **APPLY SAMPLE**
   Do not exceed 1 mL/minute.

4. **WASH COLUMN**
   1 x 500 µL D.I. H_2O.
   1 x 500 µL 100 mM acetic acid.
   1 x 500 µL CH_3OH.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTION**
   1 x 800 µL CH_2Cl_2/IPA/NH_4OH (70:26:4).
   Do not exceed 1 mL/minute.
   NOTE: Prepare elution solvent daily.
   Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

6. **DRY ELUATE**
   For amphetamines and PCP, add 100 µL of 5% trifluoroacetic acid in methanol after 5 min. drying.
   (5 min drying removes ammonia, addition of acid ionizes volatile analytes preventing loss)
   Evaporate to full dryness at < 40°C under a stream of N_2.

7. **DERIVATIZE**

   For Amphetamines*: Add 50 µL PFPA (PFAA).
   Vortex. Overlay with N_2 and cap.
   React 20 minutes at 70°C.
   Evaporate to dryness at < 40°C.
   Reconstitute with 50 µL ethyl acetate.

   For Opiates*: Add 200 µL of a 1:1 solution of propionic anhydride/pyridine.
   Make fresh daily.
   Vortex.
   React 60 minutes at 40°C.
   Evaporate to dryness at < 40°C.
   Reconstitute with 50 µL ethyl acetate.

8. **QUANTITATE**
   Inject 2 µL onto gas chromatograph.

*Alternate derivatizations may be used.
Phencyclidine does not derivatize.

DCN-903020-90

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COCAINE & BENZOYLECGONINE IN ORAL FLUID FOR GC/MS ANALYSIS USING: 50 mg CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #: ZSDAU005

February 3, 2009

1. PREPARE SAMPLE

Add 100 - 500 µL of neat oral fluid sample to a clean tube.

Add internal standard(s)* and let sit for 10 minutes at room temperature.

Add 800 µL of 100 mM phosphate buffer (pH= 6.0).

Mix/vortex for 10 seconds. Sample pH should be 6.0 ± 0.5.

Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 200 µL CH_3OH.

1 x 200 µL D.I. H_2O.

1 x 200 µL 100 mM HCl.

3. APPLY SAMPLE

Do not exceed 1 mL/minute.

4. WASH COLUMN

1 x 500 µL D.I. H_2O.

1 x 500 µL 100 mM HCl acid.

1 x 500 µL CH_3OH/D.I. H_2O (50:50).

Dry column (5 minutes at > 10 inches Hg).

5. ELUTION

1 x 800 µL CH_2Cl_2/IPA/NH_4OH (70:26:4).

Do not exceed 1 mL/minute.

NOTE: Prepare elution solvent daily. Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

6. CONCENTRATE ELUATE

Evaporate at < 40°C under a stream of N_2

7. DERIVATIZE*

Fluoroalkylate: Add 100 µL PFPA (PFAA) or HFIP.
Overlay with N_2 and cap.
React 20 minutes at 70°C.
Evaporate to dryness at < 40°C.
Reconstitute with 50 µL ethyl acetate.

TMS: Add 25 µL BSTFA (w. 1% TMCS) and
25 µL ethyl acetate.
Overlay with N_2 and cap.
Mix/vortex
React 30 minutes at 70°C.
Remove from heat and allow to cool.
NOTE: Do not evaporate BSTFA solution

8. QUANTITATE

Inject 2 µL onto gas chromatograph.
For MSD monitor the following ions:

Analyte	Target (Quantitation) Ion	Qualifier Ions	Cerilliant #
Cocaine	182	198, 303	C-008
*Cocaine-D3	185	201, 306	C-004
Benzoylecgonine-TMS	240	256, 361	B-007
*Benzoylecgonine-D8-TMS	243	259, 369	B-013

*Alternate derivatizations may be used.

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THC FROM ORAL FLUIDS FOR GC/MS ANALYSIS USING: 200 mg CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #: ZSDAU020

February 3, 2009

1. PREPARE SAMPLE

To 1 mL of oral fluid specimen add 50 ng/mL internal standard (THCA D-9) and let sit for 10 minutes at room temperature.
Vortex for 10 seconds.
Add .5 mL of glacial acetic acid and vortex for 10 seconds.

2. CONDITION CLEAN UP® EXTRACTION COLUMN

Wash with 3 mL MeOH.
Wash with 3 mL DI H_2O.
Wash with 1 mL of 0.1 N HCl.

3. APPLY SAMPLE

Pour sample into extraction column and pull though.
Do not exceed 1 mL/min.

4. WASH COLUMN

Wash with 2 mL DI H_2O.
Wash with 2 mL of 70/30 (0.1 N HCl/Acetonitrile)
Dry with vacuum for 5 minutes or until dry.
Add 200 µL of Hexane.

5. ELUTION

2 mL of Hexane/Ethyl Acetate (50.50) Do not exceed 1 mL/min.

6. DRY ELUATE

Dry under a stream of nitrogen at < 40°C.

7. DERIVATIZE

Add 50 µL MSTFA.
Vortex for 10 seconds.
Heat for 20 minutes at 60°C.
Vortex for 10 seconds while hot.
Reconstitute in 50 µL of Ethyl Acetate.

8. QUANTITATE

Inject 2 µL onto gas chromatograph.

The Oral Fluid THC ions monitored are the following on Agilient 5973

ANALYTE	Primary Ion	Secondary	Tertiary	Cerilliant #
THCA-TMS	371(Q),	386	387	T-005
THCA D9-TMS (Internal Standard)-	380(Q),	479		T-019

Contributed by:

Janet Putnam, Assistant Laboratory Director/RP Advanced Toxicology Network, Memphis, TN

DCN-903020-92A

THC IN ORAL FLUID FOR GC/MS ANALYSIS USING: 50 mg CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #: ZSDAU005

February 3, 2009

1. PREPARE SAMPLE*

Add 100 - 500 µL of neat oral fluid sample to a clean tube.
Add internal standard. Vortex and let sit for 10 minutes at room temperature.
Add 500 µL of glacial acetic acid.
Mix/vortex for 10 seconds.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 200 µL CH_3OH.
1 x 200 µL D.I. H_2O.
1 x 200 µL 100 mM HCl.

3. APPLY SAMPLE

Do not exceed 1 mL/minute.

4. WASH COLUMN

1 x 500 µL D.I. H_2O.
1 x 500 µL 0.2 N HCl.
1 x 500 µL 100 mM HCl/Acetonitrile (70:30).
Dry column (1 minute at > 10 inches Hg).

5. ELUTION

1 x 800 µL Ethyl Acetate/Hexane (25:75).
Do not exceed 1 mL/minute.

6. DRY ELUATE

Evaporate at < 40°C under a stream of N2.

7. DERIVATIZE**

Add 25 µL BSTFA (with 1% TMCS), and 25 µL ethyl acetate.
Overlay with N2 and cap.
Vortex.
React 30 minutes at 70°C.
Remove from hear and allow to cool
Note: Do not evaporate BSTFA solution

8. QUANTITATE

Inject 2 µL onto gas chromatograph.

Monitor the following ions:

	Primary ion	Secondary	Tertiary	Cerilliant #
THC-TMS	371	386	303	T-005
THC-D3-TMS	374	389	318	T-003

* Sample is from either a neat sample capillary tube collection, or eluted off the cotton pad of a swab collection device with Oral Fluid THC buffer.

**Alternate derivatizations may be used.

DCN-903020-92B

ANABOLIC STEROIDS IN URINE FOR GC OR GC/MS CONFIRMATIONS USING:
200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. PREPARE SAMPLE - ß-GLUCURONIDASE HYDROLYSIS

To 5 mL of urine add internal standard(s)* and 2 mL of ß-Glucuronidase.
ß-Glucuronidase: 5,000 F units/mL Patella vulgata in 100 mM acetate buffer (pH 5.0).
Mix/vortex.
Hydrolyze for 3 hours at 65°C. Cool before proceeding.
Centrifuge for 10 minutes at 2000 rpm and discard pellet.
Adjust sample pH to 6.0 ± 0.5 with approximately 700 µL of 1.0 N NaOH.

2. PREPARE CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 to 2 mL/minute.

4. WASH COLUMN

1 x 3 mL 10% (v/v) CH_3OH in D.I. H_2O.
Dry column (5 minutes at > 10 inches Hg).
1 x 1 mL hexane or hexane/ethyl acetate (50:50).

** Part # SMSTFA-0-1,10, 25, 100

5. ELUTE ANABOLIC STEROIDS (Choose a, b, c or d)

a. 1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2); Collect eluate at 1 to 2 mL/minute.
NOTE: Prepare elution solvent daily. Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).
b. 1 x 3 mL CH_2Cl_2/IPA (80:20).
c. 1 x 3 mL ethyl acetate.
d. 1 x 3 mL CH_3OH.

6. DRY ELUATE

Evaporate to dryness at < 40°C.

7. DERIVATIZE

Add 50 µL ethyl acetate and 50 µL MSTFA** (with 3% trimethylsilyliodide).
Over layer with N_2 and cap.
Mix/vortex.
React 20 minutes at 70°C.
Remove from heat source to cool.

NOTE: Do not evaporate MSTFA solution.

8. QUANTITATE

Inject 1 to 2 µL onto chromatograph.
Monitor the following ions (GC/MS):

Compound	Primary*	Secondary	Tertiary	OTHER
Testosterone-TMS	432	301	209	
19-Noretiocholanone-TMS	405	315	225	
Oxymethalone	640	52	462	370,143
Dehydroepiandosterone-2TMS	432	327	297	
10-Nortestosterone-2TMS	418	287	194	
Oxymethalone Metabolite #1	640	52	462	143
Oxymethalone Metabolite #2	625	462	370	143
11-ß-Hydroxyandosterone	522	417	158	
Methandienone	409	313	281	
19-Norandosterone-2TMS	405	315	225	
Alpha-Hydroxyetiocholanone	504	417		
17-α-Epitestosterone-TMS	432	341	327	209
Stanazolol	472	381	342	149

*Quantitation Ion

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DHEA, TESTOSTERONE, AND EPITESTOSTERONE IN URINE FOR GC OR GC/MS ANALYSIS USING:
200 mg CLEAN THRU® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. PREPARE SAMPLE

Pipette 5 mL of urine into borosilicate glass test tubes.
Add internal standard*, adjust sample pH to 5.5 - 6.5 using concentrated sodium phosphate monobasic or dibasic.
Mix sample.
Centrifuge samples at 3000 rpm for 5 min.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM phosphate buffer (pH 6.0).

3. APPLY SAMPLE

Pour supernatant onto column. Allow to flow via gravity.

4. WASH COLUMN

1 x 3 mL D.I. H_2O.
Dry column (10 minutes at > 10 mm Hg).

5. ELUTE STEROIDS

1 x 3 mL of CH_3OH.
Collect at 1 -2 mL/ minute.

6. ENZYMATIC HYDROLYSIS

Dry eluate under a stream of nitrogen; Add 2 mL of 200 mM phosphate buffer (pH 7.0) and 250 units of ßglucuronidase Mix Vortex and allow to incubate at 50°C for 1 hour. Cool sample, cap and adjust the pH to 10-11 using a 1:1 mixture of $NaHCO_3/Na_2CO_3$.

7. ADDITIONAL CLEAN-UP®

Add 5 mL of n-butyl chloride to each sample. The tubes and shake vigorously for 10 minutes and then centrifuge at 3000 rpm for 5 min. Transfer the organic layer to clean test tubes and dry under a stream of nitrogen. Place dried sample in a desicator and further dry under vacuum for 30 minutes.

8. DERIVATIZE

Add 50 µL of MSTFA**/NH_4I/dithioerythritol. (1000:2:5, V/W/W) and incubate at 70°C for 20 min.
Centrifuge sample at 3000 rpm for 1 min. and transfer directly to GC injector vials.

9. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

Compound	Primary Ion***	Secondary
Testosterone	432	417
Epitestosterone	432	417
DHEA	432	417
16 α Hydroxytestosterone*	520	259

* Suggested internal standard at 20 ng/mL
** Part # SMSTFA-0-1, 10, 25, 100
*** Quantitation ion

SOURCE - UCT Internal Publication

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AMPHETAMINES IN URINE FOR GC/MS CONFIRMATIONS USING: 30 mg STYRE SCREEN® DBX EXTRACTION COLUMN

Part #: SSDBX033 without Tips or SCDBX033 with CLEAN-THRU® Tips

February 3, 2009

1. SAMPLE PREPERATION

To 1 mL of urine add internal standard(s) and 1 mL 100mM phosphate buffer (pH 6.0).
Mix/Vortex.

2. APPLY SAMPLE TO DBX COLUMN

Load at a rate of 1 to 2 mL/min.

3. WASH COLUMN

1 x 1 mL DI H_2O.
1 x 1 mL 100mM acetic acid.
1 x 1 mL MeOH.

Dry column (3 mins at > 10 inches Hg).

4. ELUTE AMPHETAMINES

2 x 0.5 mL CH_2Cl_2/IPA/NH_4OH (78/20/2), collect eluate at 1 to 2 mL/min.

5. CONCENTRATE ELUATE

Add 1 drop 1% HCl in MeOH to eluate before evaporating.
Evaporate to dryness at < 40°C.

6. DERIVATIZATION

Add 50 uL ethyl acetate and 50 uL TFA (trifluoroacetic acid anhydride) then cap, mix/vortex.
Heat for 15 mins at 70°C, allow to cool, then evaporate to dryness at < 40°C.
Reconstitute with 100 µL ethyl acetate.

7. ANALYZE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

Analyte (TFA)	Target (Quantitation) Ion	Qualifier Ions	Cerilliant #
Amphetamine	140	91, 118	A-049
Amphetamine-D11	144	98, 128	A-016
Methamphetamine	154	110, 118	M-009
Methamphetamine-D11	160	113, 126	M-059

*Suggested internal standards

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CARBOXY-THC IN URINE FOR GC/MS CONFIRMATIONS USING: 30 mg STYRE SCREEN® DBX EXTRACTION COLUMN

Part #: SSDBX033 without Tips or SCDBX033 with CLEAN-THRU® Tips

February 3, 2009

1. SAMPLE PREPERATION (BASE HYDROLYSIS)

To 2 mL of urine add internal standard and 100 µL 10N NaOH.
Mix/vortex. Hydrolyze for 20 mins at 60°C. Cool before proceeding.
Adjust sample pH to 3.5 ± 0.5 with 1.0 mL glacial acetic acid.

2. APPLY SAMPLE TO DBX COLUMN

Load at a rate of 1 to 2 mL/min.

3. WASH COLUMN

1 x 1 mL DI H_2O.
1 x 1 mL 0.1M HCl/acetonitrile (70/30).
Dry column (3 mins at > 10 inches Hg).
1 x 200 µL hexane.

4. ELUTE CARBOXY-THC

2 x 0.5 mL hexane/ethyl acetate (50:50); Collect eluate at 1 to 2 mL/min.

Evaporate eluate to dryness at < 40°C.

5. DERIVATIZATION

Add 50 µL ethyl acetate
Vortex mix
Add 50 µL BSTFA (1% TMCS,) then cap, mix/vortex.
Heat for 20 minutes at 70°C, allow to cool.
Note: Do not evaporate BSTFA solution.

6. ANALYZE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

Analyte (TMS)	Target (Quantitation) Ion	Qualifier Ions	Cerilliant #
Carboxy-THC-TMS	371	473, 488	T-018
*Carboxy-THC-D3-TMS	374	476, 491	T-004

*Suggested internal standards

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COCAINE / BENZOYLECGONINE IN URINE
FOR GC/MS CONFIRMATIONS USING: 30 mg STYRE SCREEN® DBX EXTRACTION COLUMN

Part #: SSDBX033 without Tips or SCDBX033 with CLEAN-THRU® Tip

February 3, 2009

1. SAMPLE PREPERATION

To 1 mL of urine add internal standard(s) and 300 µL.
100mM HCl.
Mix/Vortex.

2. APPLY SAMPLE TO DBX COLUMN

Load at a rate of 1 to 2 mL/min.

3. WASH COLUMN

1 x 1 mL DI H_2O.
1 x 1 mL 100mM HCl.
1 x 1 mL MeOH.

Dry column (3 mins at > 10 inches Hg).

4. ELUTE COCAINE/BENZOYLECGONINE

2 x 0.5 mL CH_2Cl_2/IPA/NH_4OH (78/20/2), Collect eluate at
1 to 2 mL/min.

5. CONCENTRATE ELUATE

Evaporate to dryness at < 40°C.

6. DERIVATIZATION

Add 50 µL ethyl acetate and 50 µL BSTFA (1% TMCS) then cap, mix/vortex.
Heat for 20 mins at 70°C, allow to cool.
NOTE: Do not evaporate BSTFA solution.

7. ANALYZE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

Analyte	Target (Quantitation) Ion	Qualifier Ions	Cerilliant #
Cocaine	182	198, 303	C-008
*Cocaine-D3	185	201, 306	C-004
Benzoylecgonine-TMS	240	256, 361	B-007
*Benzoylecgonine-D8-TMS	243	259, 369	B-013

*Suggested internal standards

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OPIATES IN URINE FOR GC/MS CONFIRMATIONS USING: 30 mg STYRE SCREEN® DBX EXTRACTION COLUMN

Part #: SSDBX033 without Tips or SCDBX033 with CLEAN-THRU® Tips

February 3, 2009

1. SAMPLE PREPERATION (ENZYMATIC HYDROLYSIS)

To 1 mL of urine add internal standard(s) and 1.0 mL ß-Glucuronidase solution. (ß-Glucuronidase solution contains 5000 Funits/mL Patella Vulgata in 100mM acetate buffer, pH 5.0). Hydrolyze for 3 hours at 60°C. Cool, then centrifuge for 10 minutes at high speed and discard pellet. Adjust pH to 6.0 ± 0.5 with 1.0N NaOH.

NOTE: For unconjugated (free) opiates; to 1 mL urine, add internal standard(s) and 1 mL 100mM phosphate buffer (pH 6.0). Proceed to Step #2.

2. APPLY SAMPLE TO DBX COLUMN

Load at a rate of 1 to 2 mL/min.

3. WASH COLUMN

1 x 1 mL DI H_2O.
1 x 1 mL 100mM acetate buffer (pH 4.5).
1 x 1 mL MeOH.
Dry column (3 mins at > 10 inches Hg).

4. ELUTE OPIATES

2 x 0.5 mL CH_2Cl_2/IPA/NH_4OH (78/20/2), collect eluate at 1 to 2 mL/min.
Evaporate eluate to dryness at < 40°C.

5. DERIVATIZATION

Add 50 uL ethyl acetate and 50 µL BSTFA (1% TMCS), then cap, mix/vortex.
React for 20 mins at 70°C, allow to cool.
NOTE: Do not evaporate BSTFA solution.

6. ANALYZE

Inject 1 to 2 µL onto gas chromatograph: For MSD monitor the following ions:

Analyte (TMS)	Target (Quantitation) Ion	Qualifier Ions	Cerilliant #
Codeine-TMS	371	234, 343	C-006
*Codeine-D6-TMS	377	237, 349	C-040
Morphine-TMS	429	401, 414	M-005
*Morphine-D6-TMS	435	404, 420	M-085
6-Acetylmorphine-TMS	399	400, 340	A-009
6-Acetylmorphine-D6-TMS	405	406, 343	A-026

*Suggested internal standards

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PHENCYCLIDINE IN URINE
FOR GC/MS CONFIRMATIONS USING: 30 mg STYRE SCREEN® DBX EXTRACTION COLUMN

Part #: SSDBX033 without Tips or SCDBX033 with CLEAN-THRU® Tips

February 3, 2009

1. SAMPLE PREPERATION

To 1 mL of urine add internal standard and 1 mL 100mM phosphate buffer (pH 6.0).
Mix/Vortex.

2. APPLY SAMPLE TO DBX COLUMN

Load at a rate of 1 to 2 mL/min.

3. WASH COLUMN

1 x 1 mL DI H_2O.
1 x 1 mL 100mM acetic acid.
1 x 1 mL MeOH.

Dry column (3 mins at > 10 inches Hg).

4. ELUTE PHENCYCLIDINE

2 x 0.5 mL CH_2Cl_2/IPA/NH_4OH (78/20/2), Collect eluate at 1 to 2 mL/min.

5. CONCENTRATE ELUATE

Add 1 drop 1% HCl in MeOH to eluate before evaporating.
Evaporate to dryness at < 40°C.
Reconstitute with 100 µL ethyl acetate.

6. ANALYZE

Inject 1 to 2 µL onto gas chromatograph.
For MSD monitor the following ions:

Analyte	Target (Quantitation) Ion	Qualifier Ions	Cerilliant #
Phencyclidine	200	91, 242	P-007
*Phencyclidine-D5	205	96, 247	P-003

*Suggested internal standard

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UCT

PHARMACEUTICAL METHODS

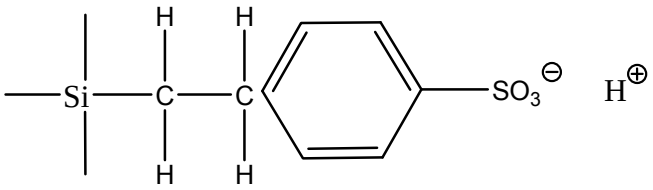
PURIFICATION OF SMALL MOLECULE LIBRARIES BY PHARMA-SIL® ION EXCHANGE SPE

February 3, 2009

Principle: The generation of small molecule libraries for screening against biological targets has emerged as an area of intense interest in the pharmaceutical industry. Ion exchange chromatography has been demonstrated to expedite work up and purification of organic molec**ules** synthesized in solution, and in the automated construction of small molecule libraries. The advantage of ion exchange chromatography over more traditional small molecule purification modes such as flash chromatography or HPLC is that one can reliably predict the elution characteristics of a broad range of molecules soley by the presence or absence of an ionizable site on the molecule.

Application: This application details the use of PHARMA-SIL® BCX1HL, a highly loaded strong cation exchange sorbent, for the purification of amine compounds from organic synthesis mixtures. In combinatorial chemistry and organic synthesis, reactions are often carried out in solvents such as DMSO or DMF in $MeCl_2$. Once the reaction is complete, it is usually necessary to separate the products of the reaction from excess reagents and by-products. This can be done using a highly loaded. Strong cation exchanger to selectivelyretain the basic compounds from the reaction mixture. The sorbent can also be used as scavengers in the synthesis of ureas.

Chemistry of PHARMA-SIL® BCX1HL Sorbent



Advantages of PHARMA-SIL® Based Sorbents

- Clean background
- High recoveries
- High levels of purification of anaytes
- Applicable to a broad range of compounds
- Simple easy to develop methods

Purification Profile

This profile is based on the use of a PHARMA-SIL® BCX1HL 500 mg column (columns are available with varying volumes). This column is capable of purification of up to 50 mg of basic product with a molecular weight of < 300amu. The method can be scaled up as necessary by using columns of higher bed mass of sorbent and increasing the solvent volumes proportionately**. The following profile is meant to be a guideline for these types of purifications. Each drug class has its own specific requirements based on solubility, stability, and pKa and may require slight adjustments in methodology. Therefore think of the following profile as a beginning rather than a final method.**

Sample Pre-treatment

Samples may or may not require pretreatment before addition. The primary concern using ion exchangers is to adjust the pH of the compound of interest so that it is totally ionized. This may require the addition of an acid or buffer. Ion exchange can be done out of organic solvents such as methanol or ethyl acetate as long as the compound of interest is ionized.

Column Conditioning

Condition the column with the appropriate solvents. (ethylacetate/hexane, methanol/ethylacetate, methanol, often times the elution solvent makes an excellent conditioning solvent).

Column Equilibration

Equilibrate the column with the same solvent you pretreat the sample with.(buffer, ethylacetate/hexane, etc.)

Sample Application

Apply the sample to the column under gravity. Positive pressure or vacuum can also be used just be certain the application rate does not exceed 1-2 mL per min. The volume of the sample is not important and will probably be dictated by the equipment you use. The critical factor is concentration and capacity of the sorbent. If the concentration of the compound of interest exceeds the capacity of the sorbent you will not get the highest recovery of your compound. If you think this is a problem use a larger bed mass.

Product Purification

Elute neutral and polar reagents and byproducts with ethyl acetate, 25% methanol/ethylacetate, or buffers**. (Caution: when using buffer washes be sure the pH of the buffer remains 2 pH units below the pKa of the compounds of interest you want to retain on the column)**

Product Elution

Elute compound of interest with ethylacetate/ammonium hydroxide, ethylacetate/triethylamine, or ethylacetate/methanol/ammonium hydroxide.(the important factor is to be sure the pH of the elution solvent is 2 pH units above the pKa of your compound of interest. These solutions can be easily dried down to remove unwanted solvents before analysis.

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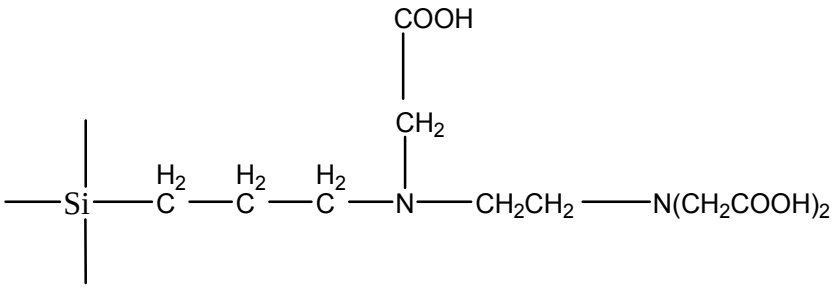
PURIFICATION OF SMALL MOLECULE LIBRARIES
TIN (Sn) REMOVAL BY PHARMA-SIL® ION EXCHANGE SPE

February 3, 2009

Principle: The generation of small molecule libraries for screening against biological targets has emerged as an area of intense interest in the pharmaceutical industry. Ion exchange chromatography has been demonstrated to expedite work up and purification of organic molecules synthesized in solution, and in the automated construction of small molecule libraries. The advantage of ion exchange chromatography over more traditional small molecule purification modes such as flash chromatography or HPLC is that one can reliably predict the elution characteristics of a broad range of molecules solely by the presence or absence of an ionizable site on the molecule.

Application: This application details the use of PHARMA-SIL® TAX, a highly loaded weak cation exchange sorbent, for the removal of tin catalysts from organic synthesis mixtures. In combinatorial chemistry and organic synthesis tin compounds are common catalysts. Once the reaction is complete, it is usually necessary to separate the products of the reaction from the catalysts. If the catalyst is not removed it can interfere with further testing as well as ruin expensive analytical equipment. This can be done using a highly loaded weak cation exchanger to selectively remove the tin catalyst from the reaction mixture.

Chemistry of PHARMA-SIL® TAX Sorbent



Advantages of PHARMA-SIL® Based Sorbents

- Complete removal of tin catalyst
- Clean background
- High recoveries
- High levels of purification of anaytes
- Applicable to a broad range of compounds
- Simple easy to develop methods

Purification Profile

This profile is based on the use of a PHARMA-SIL® TAX 500 mg column (columns are available with varying volumes). This column is capable of removal of up to50mg of tin. The method can be scaled up as necessary by using columns of higher bed mass of sorbent and increasing the solvent volumes proportionately

The following profile is meant to be a guideline for these types of purifications. Each drug class has its own specific requirements based on solubility, stability, and pKa and may require slight adjustments in methodology. Therefore think of the following profile as a beginning rather than a final method.

Sample Pre-treatment

Samples may or may not require pretreatment before addition. The primary concern using ion exchangers is to adjust the pH of the compound of interest so that it is totally ionized. This may require the addition of an acid or buffer. Ion exchange can be done out of organic solvents such as methanol or ethyl acetate as long as the compound of interest is ionized**...** Tin catalysts are strong cations and are charged across the complete pH range.

Column Conditioning

Condition the column 1 mL of Methanol followed by 1 mL of water.

Column Equilibration

Condition the column with buffer: If sample is a base, you want the pH at 7-8.

If sample is an acid, you want the pH at 3-4.

Sample Application

Apply the sample to the column under gravity. The tin will stick to the column. The volume of the sample is not important and will probably be dictated by the equipment you use. The critical factor is concentration and capacity of the sorbent. If the concentration of the tin of exceeds the capacity of the sorbent you will not get the highest removal of tin. If you think this is a problem use a larger bed mass.

Product Purification

Wash the column with 1 mL of buffer used in column equilibration.

Product Elution

Elute compound of interest with 1 mL of methanol.

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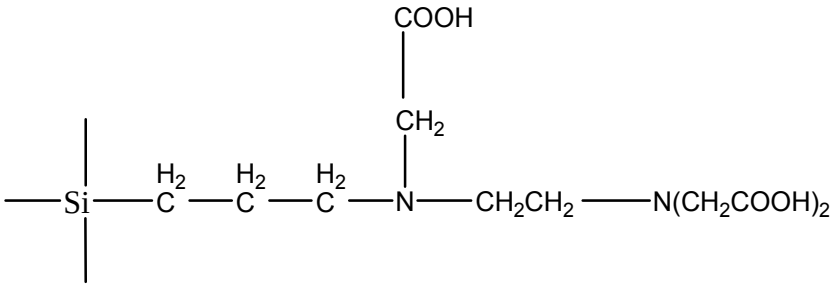
PURIFICATION OF SMALL MOLECULE LIBRARIES
Palladium (Pd) REMOVAL BY PHARMA-SIL® ION EXCHANGE SPE

February 3, 2009

Principle: The generation of small molecule libraries for screening against biological targets has emerged as an area of intense interest in the pharmaceutical industry. Ion exchange chromatography has been demonstrated to expedite work up and purification of organic molecules synthesized in solution, and in the automated construction of small molecule libraries. The advantage of ion exchange chromatography over more traditional small molecule purification modes such as flash chromatography or HPLC is that one can reliably predict the elution characteristics of a broad range of molecules solely by the presence or absence of an ionizable site on the molecule.

Application: This application details the use of PHARMA-SIL® TAX, a highly loaded weak cation exchange sorbent, for the removal of palladium catalysts from organic synthesis mixtures. In combinatorial chemistry and organic synthesis palladium compounds are common catalysts. Once the reaction is complete, it is usually necessary to separate the products of the reaction from the catalysts. If the catalyst is not removed it can interfere with further testing as well as ruin expensive analytical equipment. This can be done using a highly loaded weak cation exchanger to selectively remove the tin catalyst from the reaction mixture.

Chemistry of PHARMA-SIL® TAX Sorbent



Advantages of PHARMA-SIL® Based Sorbents

- Complete removal of palladium catalyst
- Clean background
- High recoveries
- High levels of purification of anaytes
- Applicable to a broad range of compounds
- Simple easy to develop methods

Purification Profile

This profile is based on the use of a PHARMA-SIL® TAX 500 mg column (columns are available with varying volumes). This column is capable of removal of up to50mg of palladium. The method can be scaled up as necessary by using columns of higher bed mass of sorbent and increasing the solvent volumes proportionately

The following profile is meant to be a guideline for these types of purifications. Each drug class has its own specific requirements based on solubility, stability, and pKa and may require slight adjustments in methodology. Therefore think of the following profile as a beginning rather than a final method.

Sample Pre-treatment

Samples may or may not require pretreatment before addition. The primary concern using ion exchangers is to adjust the pH of the compound of interest so that it is totally ionized. This may require the addition of an acid or buffer. Ion exchange can be done out of organic solvents such as methanol or ethyl acetate as long as the compound of interest is ionized... Palladium catalysts are strong cations and are charged across the complete pH range. Adjust the sample to pH 9 with buffer or ammonium hydroxide.

Column Conditioning

Condition the column 1 mL of Methanol followed by 1 mL of water.

Column Equilibration

Condition the column with buffer of pH 9.

Sample Application

Apply the sample to the column under gravity. The palladium will stick to the column. The volume of the sample is not important and will probably be dictated by the equipment you use. The critical factor is concentration and capacity of the sorbent. If the concentration of the palladium exceeds the capacity of the sorbent you will not get the highest removal of palladium. If you think this is a problem use a larger bed mass.

Product Purification

Wash the column with 1mL of buffer used in column equilibration.

Product Elution

Elute compound of interest with 1mLl of methanol.

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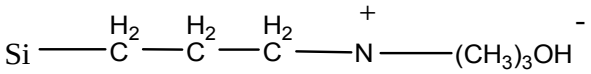
PURIFICATION OF SMALL MOLECULE LIBRARIES
TFAA REMOVAL BY PHARMA-SIL® ION EXCHANGE SPE

February 3, 2009

Principle: The generation of small molecule libraries for screening against biological targets has emerged as an area of intense interest in the pharmaceutical industry. Ion exchange chromatography has been demonstrated to expedite work up and purification of organic molecules synthesized in solution, and in the automated construction of small molecule libraries. The advantage of ion exchange chromatography over more traditional small molecule purification modes such as flash chromatography or HPLC is that one can reliably predict the elution characteristics of a broad range of molecules solely by the presence or absence of an ionizable site on the molecule.

Application: This application details the use of PHARMA-SIL® CHQAX, a highly loaded quaternary amine exchange sorbent, for the removal of acid catalysts from organic synthesis mixtures. In combinatorial chemistry and organic synthesis TFAA is a common catalyst. Once the reaction is complete, it is usually necessary to separate the products of the reaction from the catalyst. If the catalyst is not removed it can interfere with further testing as well as ruin expensive analytical equipment. This can be done using a highly loaded quaternary amine exchanger to selectively remove the acid catalyst from the reaction mixture.

Chemistry of PHARMA-SIL® CHQAX Sorbent



Advantages of PHARMA-SIL® Based Sorbents

- Complete removal of acid catalyst
- Clean background
- High recoveries
- High levels of purification of anaytes
- Applicable to a broad range of compounds
- Simple easy to develop methods

Purification Profile

This profile is based on the use of a PHARMA-SIL® CHQAX 500 mg column (columns are available with varying volumes). This column is capable of removal of up to 50mg of TFAA. The method can be scaled up as necessary by using columns of higher bed mass of sorbent and increasing the solvent volumes proportionately.

The following profile is meant to be a guideline for these types of purifications. Each drug class has its own specific requirements based on solubility, stability, and pKa and may require slight adjustments in methodology. Therefore think of the following profile as a beginning rather than a final method.

Sample Pre-treatment

Samples may or may not require pretreatment before addition. The primary concern using ion exchangers is to adjust the pH of the compound of interest so that it is totally ionized. This may require the addition of a pH 7 buffer. Ion exchange can be done out of organic solvents such as methanol or ethyl acetate as long as the compound of interest is ionized**...** acid catalysts are strong anions and are charged across the complete pH range.

Column Conditioning

Condition the column with 1 mL of methanol followed by 1 mL of DI water.

Column Equilibration

Condition the column with pH 7 buffer.

Application

Apply the sample to the column under gravity. The TFAA will stick to the column. The volume of the sample is not important and will probably be dictated by the equipment you use. The critical factor is concentration and capacity of the sorbent. If the concentration of the TFAA exceeds the capacity of the sorbent you will not get the highest removal of TFAA. If you think this is a problem use a larger bed mass.

Product Purification

Wash the column with 1ml of buffer used in column equilibration.

Product Elution

Elute compound of interest with 1ml of methanol.

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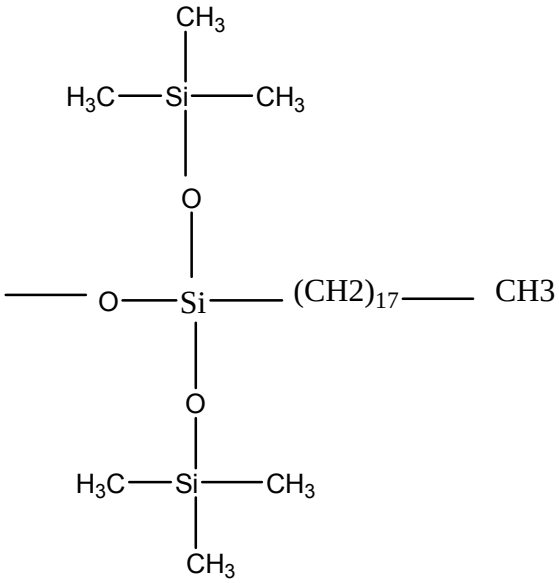
PURIFICATION OF SMALL MOLECULE LIBRARIES
DESALTING SAMPLES USING PHARMA-SIL® REVERSE PHASE SPE

February 3, 2009

Principle: The generation of small molecule libraries for screening against biological targets has emerged as an area of intense interest in the pharmaceutical industry. SPE has been demonstrated to expedite work up and purification of organic molecules synthesized in solution, and in the automated construction of small molecule libraries. Samples that have been synthesized in aqueous salt, buffer solutions, or low polarity organic solvents containing salts may require the removal of those salts prior to analysis. PHARMA-SIL® Reverse Phase SPE can be used to desalt these libraries.

Application: This application details the use of PHARMA-SIL® CEC18, a highly loaded reverse phase sorbent, for desalting synthetic mixtures. In combinatorial chemistry and organic synthesis salts are sometimes present in the reaction mixtures. Once the reaction is complete, it is usually necessary to separate the products of the reaction from the salts. If the salt is not removed it can interfere with further testing as well as ruin expensive analytical equipment. This can be done using a highly loaded reverse phase SPE column to selectively remove the salt from the reaction mixture.

Chemistry of PHARMA-SIL® CEC18 Sorbent



Advantages of PHARMA-SIL® Based Sorbents

- Complete removal of salts
- Clean background
- High recoveries
- High levels of purification of anaytes
- Applicable to a broad range of compounds
- Simple easy to develop methods

Purification Profile

This profile is based on the use of a PHARMA-SIL® CEC18 500 mg column (columns are available with varying volumes). This column is capable of removal of salts. The method can be scaled up as necessary by using columns of higher bed mass of sorbent and increasing the solvent volumes proportionately

The following profile is meant to be a guideline for these types of purifications. Each drug class has its own specific requirements based on solubility, stability, and pKa and may require slight adjustments in methodology. Therefore think of the following profile as a beginning rather than a final method.

Sample Pre-treatment

Samples may or may not require pretreatment before addition. The primary concern using desalting columns is to adjust the pH of the compound of interest so that it is totally molecular. This may require the addition of an acid or base. Desalting can be done out of low polarity organic solvents such as hexane or methylene chloride as long as the compound of interest is protonated.

Column Conditioning

Condition the column 1 mL of Methanol followed by 1 ml of water.

Column Equilibration

Condition the column with buffer: If sample is a base, you want the pH to be >9

If sample is an acid, you want the pH to be<2.5

Apply the sample to the column under gravity. The salts will flow through the column and the sample will stick to the column. The volume of the sample is not important and will probably be dictated by the equipment you use. The critical factor is concentration and capacity of the sorbent. If the concentration of the compound exceeds the capacity of the sorbent you will not get the highest recovery. If you think this is a problem use a larger bed mass.

Product Purification

Wash the column with 1mL of DI water or hexane. .

Product Elution

Elute compound of interest with 1mL of methanol, ethyl acetate, or the organic solvent of your choice.

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UCT

ENVIRONMENTAL METHODS

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

April 8, 2009

Method 506 Revison 1.1*

Background

Method 506 is an EPA analysis procedure for the determination of certain **phthalate and adipate esters.** The method can be applied to drinking water and ground water. Phthalate compounds efficiently partition from water using a C18 bonded silica solid-phase sorbent packed into cartridges. The following compounds determined by this method employ capillary column gas chromatography with a photoionization detector.

Analyte	Common Abreviation	CAS	GC Elution Order[1]
Bis(2-ethylhexyl) Phthalate	DEHP	117-81-7	6
Butyl benzyl Phthalate	BBP	85-68-7	4
Di-n-butyl Phthalate	DBP	84-74-2	3
Diethyl Phthalate	DEP	84-66-2	2
Dimethyl Phthalate	DMP	131-11-3	1
Bis(2-ethylhexyl) Adipate	DEHA	103-23-1	5
Di-n-octyl Phthalate	DnOP	117-84-0	7

[1]DB-5 fused silica capillary, 30mx0.32mm, 0.25 micron film

UCT Products Required:

ENVIRO-CLEAN® Universal C18 cartridge ECUNIC18

FLORISIL® Cleanup Cartridge (for phthalate esters, optional for dirty water)

Alumina Cleanup Cartridge (for phthalate esters, optional for dirty water)

Summary of Method

A 1-liter sample of water is extracted using an 83 mL C18 cartridge UCT ECUNIC18 then eluted with methylene chloride. The eluant is concentrated using a gentle stream of N_2 gas to reduce the volume to 1.0 mL. The concentration of analytes in the extract is determined with capillary column GC using photoionization detection.

Interferences

Care must be exercised to avoid sample contamination, as phthalate compounds are ubiquitous in the environment. Phthalate are used as plasticizers in PVC tubing and other common plastics found throughout the laboratory therefore the use of plastics must be avoided. Exhaustive clean up of reagents and glassware is required to eliminate background artifacts and prevent elevated GC baselines.

- Clean all glassware as soon as possible after use
- Rinse thoroughly with the last solvent used
- Heat glassware (except volumetric flasks) in a muffle oven at 400^{O}C for 1 hour
- Seal glassware with aluminum foil and store in a clean environment
- Use of high purity solvents will minimize interference problems

1. Sample Collection

a) Dechlorinate sample by adding 80 mg of sodium thiosulfate per liter. Mix until dissolved.
b) Samples must be refrigerated at 4^{O}C in a dark environment until analysis
c) Analyte stability may be effected by the matrix components

2. Cartridge Activation Procedure

a) Set-up a vacuum manifold system and mount cartridge on the glass adaptor. Various automated extraction systems may also be used.
b) Add a 10 mL aliquot of methylene chloride (CH_2Cl_2) and slowly draw through the cartridge
c) Repeat with a second 10 mL aliquot of methylene chloride. Hold for 1 minute.
d) Dry cartridge by drawing air through at full vacuum for 2-3 minutes

Note: The cartridge must not be allowed to go dry after the following steps, otherwise repeat

e) Add 10 mL of methanol to the cartridge and draw through until meniscus touches the surface of the cartridge frit
g) Add a second 10 mL portion of methanol and wait 1-2 minutes to activate sorbent
h) Add 10 mL reagent water to the cartridge and draw through until meniscus reaches the top of the frit
i) Cartridge is now ready for sample extraction

3. Sample Extraction

a) Add 5 mL of methanol to the 1-liter sample and mix well
b) Add the water sample to the cartridge and draw through over a period of about 20-30 minutes
c) Add 5 mL of acetonitrile (CH_3CN) to the sample bottle, shake then add to cartridge
d) After extraction, use full vacuum to dry the cartridge for 10 minutes
e) Place an eluate collection vial in the vacuum manifold
f) Add 5 mL of methylene chloride to the sample bottle and rinse
g) Using a disposable pipette transfer the methylene chloride to the cartridge and rinse the sides while adding
h) Repeat this procedure using another 5 mL aliquot of methylene chloride

4. Eluate Drying and Concentration

a) Pour eluant over a 3 gram bed of anhydrous sodium sulfate
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) Place extract in a 28^{O}C heated evaporator and pass a stream of N_2 over solvent to evaporate
e) If sample is clean proceed to GC analysis

5. Florisil Column Cleanup for Phthalate Esters (if required)

Clean-up procedures are not required for clean drinking water. Under certain circumstances for dirty water, a Florisil or Alumina cleanup may be needed. If necessary, the following steps are used:

a) Add a 1 cm layer of anhydrous sodium sulfate to the top of a UCT Florisil cartridge
b) Flush cartridge with 40 mL of hexane, then discard but leave enough to cover frit
c) Add sample extract to the cartridge then rinse vial with 2 mL of hexane
d) Add 40 mL of hexane to the cartridge and elute. Discard this hexane solution
e) Elute using 100 mL of 20% diethyl ether in hexane (v/v) into a 500 mL K-D flask equipped with a 10 mL concentrator tube. Elute at a rate of about 2 mL/minute
f) No solvent exchange is required
g) Concentrate eluate in hot water bath at 85^{O}C

6. Alumina Column Cleanup for Phthalate Esters (if required)

a) Add a 1 cm layer of anhydrous sodium sulfate to the top of a UCT Alumina cartridge
b) Flush cartridge with 40 mL of hexane, then discard but leave enough to cover frit
c) Add sample extract to the cartridge then rinse vial with 2 mL of hexane
d) Add 35 mL of hexane to the cartridge and elute. Discard this hexane solution
e) Elute using 140 mL of 20% diethyl ether in hexane (v/v) into a 500 mL K-D flask equipped with a 10 mL concentrator tube. Elute at a rate of about 2 mL/minute
f) No solvent exchange is required
g) Concentrate eluate in hot water bath at 85^{O}C

7. Sample is now ready for GC analysis

Chromatographic Conditions

- Inject: 1-2 µL of sample extract or standard
- Injector: 295^{O}C
- Detector: 295^{O}C
- Program: 1 minute hold @ 60^{O}C
- 6^{O}C/minute to 260^{O}C, hold 10 minutes
- Splitless injection with 45-second delay
- Photoionization detection @ 10 eV

*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

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ENVIRO-CLEAN® Cartridges

Part Number: ECUNIC18

EPA Method 508.1 Rev 2.0

August 25, 2009

The UCT ECUNIC18 Universal cartridge is designed to provide a new level of performance for the solid-phase extraction (SPE) for the analysis of **chlorinated pesticides, herbicides and organohalides** using EPA method 508.1. With the cartridge's high capture efficiency, fast flow and excellent dry times, laboratory throughput is improved reducing sample preparation time.

Product Benefits

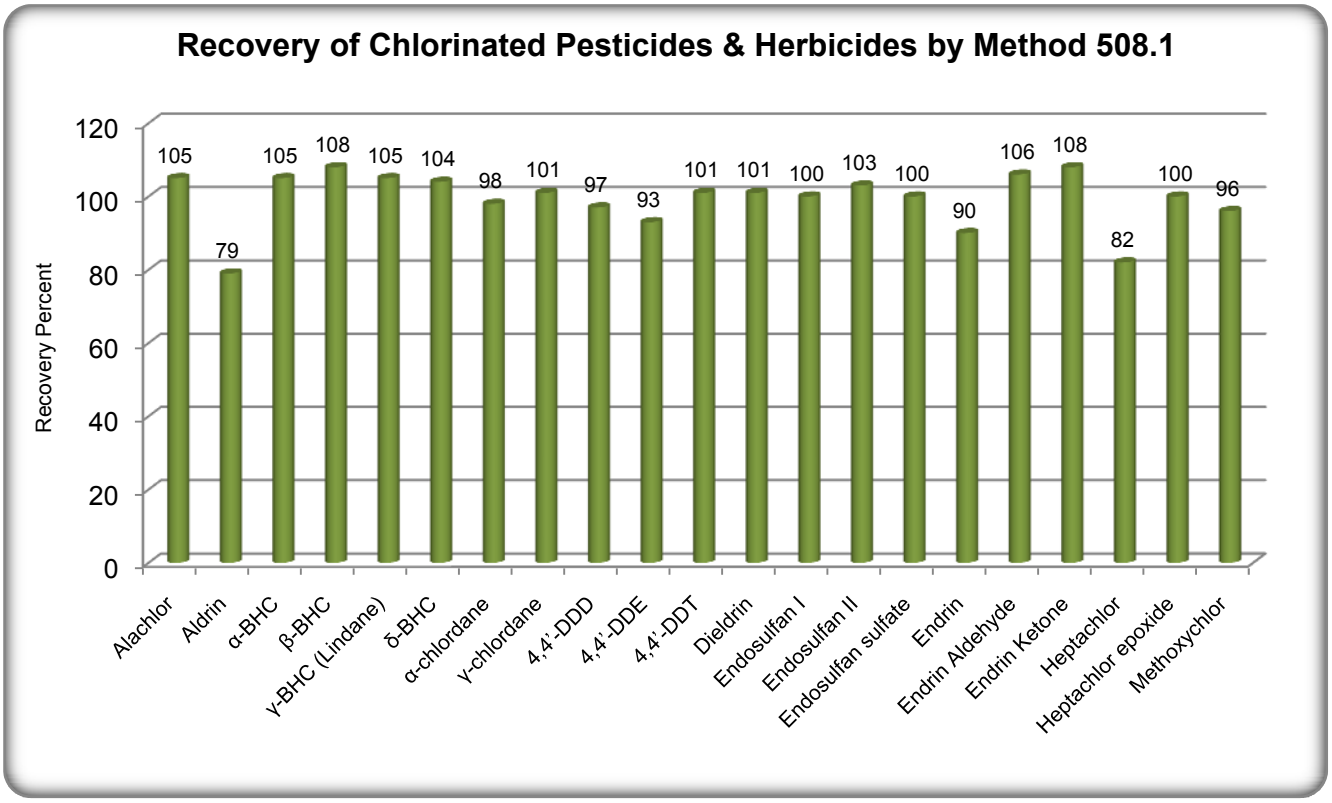
- SPE cartridge containing bonded C18 phase
- Excellent pH stability
- Fast flow rates for rapid analyte capture
- Works well at all levels of analyte loading
- Consistent results for excellent reproducibility
- PTFE frits eliminate potential contamination yielding clean extracts
- Packaged in metalized bags to maintain product cleanliness

Product Features

- Cartridges manufactured from special proprietary polypropylene
- Each cartridge contains 1100 mg endcapped C18 bonded ultra-clean silica sorbent
- Can be used on manual single or multi-station vacuum manifold systems
- Can be used with automated extraction systems

UCT Cartridge ECUNIC18 Shows Excellent Recovery with Replicate Samples of Laboratory Fortified Blanks (LFB)

Analyte	CAS	Retention Time[1]	Amt Spiked µg/L	Average Recovery %	Stdev
Alachlor	15972-60-8	22.86	0.1032	105	NA
Aldrin	309-00-2	24.81	0.0999	79	2.08
α-BHC	319-84-6	NA	0.1032	105	0.96
β-BHC	319-85-7	NA	0.1043	108	1.50
γ-BHC (Lindane)	58-89-9	NA	0.1038	105	0.82
δ-BHC	319-86-8	NA	0.1040	104	1.26
α-chlordane	5103-71-9	29.58	0.0969	98	0.50
γ-chlordane	5103-74-2	28.65	0.0969	101	1.71
4,4'-DDD	72-54-8	33.38	0.2056	97	2.08
4,4'-DDE	72-55-9	31.97	0.2006	93	2.38
4,4'-DDT	50-29-3	35.80	0.2014	101	0.96
Dieldrin	60-57-1	30.95	0.2046	101	0.82
Endosulfan I	959-98-8	29.36	0.1028	100	0.82
Endosulfan II	33213-65-9	32.81	0.2054	103	0.96
Endosulfan sulfate	1031-07-8	35.38	0.2124	100	2.06
Endrin	72-20-8	32.24	0.2016	90	7.33
Endrin Aldehyde	7421-93-4	33.96	0.2012	106	9.54
Endrin Ketone	53494-70-5	NA	0.2068	108	1.73
Heptachlor	76-44-8	NA	0.1032	82	5.29
Heptachlor epoxide	1024-57-3	27.20	0.1034	100	0.96
Methoxychlor	72-43-5	25.02	1.0016	96	1.71



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

Method 508.1 Rev 2.0

Method Summary*

A 1 liter sample of water is extracted by drawing through a **UCT C18 Universal cartridge ECUNIC18**. The analytes captured on the solid-phase are eluted from the cartridge using a small volume of ethyl acetate (EtAc) and methylene chloride ($MeCl_2$). The extract is concentrated by evaporation before analysis by injection into a gas chromatograph/electron capture system (GC/ECD) fitted with a high resolution fused silica capillary column

Sample Collection, Preservation and Storage

- Collect samples in glass containers
- Do not prerinse the container with the sample water

Preserve the sample by adding mercuric chloride ($MgCl_2$) to achieve a concentration of 10 mg/L. Other preservatives may be used if shown to be effective

Interferences

- Interfering contamination may occur when a sample of low concentration is analyzed immediately after a sample of high concentration. A laboratory blank should be inserted between low and high concentration samples to minimize this problem
- Other interferences may be caused by contaminants in solvents, reagents and sample processing apparatus that lead to anomalous GC peaks or elevated baselines.

1) Condition Cartridge

a) Insert a cartridge into the glass vacuum manifold or automated extraction system
b) Wash the cartridge with 5mL of a 1:1 mixture of ethyl acetate (EtAc) and methylene chloride ($MeCl_2$)
c) Draw the solvent through the cartridge with a low vacuum setting so that the solvent slowly drips through
d) Add 10 mL of methanol (MeOH) to the cartridge then slowly draw some of it through
e) Rinse the cartridge with 10 mL of reagent water and draw most of it through leaving a thin layer on the top of the sorbent

Do not let the cartridge go dry after addition of methanol otherwise repeat the steps d) and e)

2) Sample Addition

a) Add 5 mL of methanol to the 1 liter sample and mix well
b) Add 50 µL of the surrogate compound to the water sample and shake well
c) Draw the water sample through the cartridge under sufficient vacuum to require about 20 minutes or more for extraction
d) Dry the cartridge by drawing air or nitrogen through for about 10 minutes

3) Extract Elution

a) Insert an eluate collection tube into the vacuum manifold
b) Rinse the inside walls of the sample bottle using 10 mL EtAc then transfer solvent to the cartridge using a disposable pipette
c) Rinse the inside walls of the sample bottle using 10 mL of $MeCl_2$ then transfer to the cartridge using a disposable pipette
d) Using a disposable pipette rinse the cartridge and filter reservoir with two 3 mL portions of 1:1 EtAc:$MeCl_2$

e)

4) Sample Drying

a) Pour the combined elutes together through a drying tube (**UCT ECSS15M6**) which contains 5 grams anhydrous sodium sulfate
b) Rinse the drying tube with two 3 mL portions of 1:1 EtAc:$MeCl_2$
c) Concentrate the extract to about 0.8 mL under a gentle stream of nitrogen while heating in a water bath
d) Rinse the inside walls of the concentrator tube two or three times with EtAc during the evaporation
e) Add IS
f) Adjust the final volume of the extract to 1.0 mL

5) Analysis

a) Inject a 1-2 µL aliquot into a GC
b) Identify the analytes in the sample by comparison of the retention time to known reference chromatograms

6) GC Analysis Conditions

Retention time was determined with the following GC conditions:

- Injector temperature -- 250°C
- Detector temperature -- 320°C
- Injection volume -- 2 µL, splitless for 45 seconds
- Temperature program -- Inject at 60°C and hold one minute

-- program at 20°C/min. to 160°C hold three minutes
-- program at 3°C/min. to 275°C with no hold
-- program at 20°C/min. to 310°C with no hold

The IS retention time using these conditions is 21.15 minutes. The SUR retention time using these conditions is 28.18 minutes.

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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EPA Method 509

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

UCT Product CLEAN-ELUTE™ 25,000 mg diatomatious earth, 200 mL cartridge

October 1, 2009

Background

Method 509 is a gas chromatographic method for the determination of ethylene thiourea (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

Analysis Procedure

1) Sample Extraction

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 a 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

5) GC Analysis Conditions

Primary:

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; H2 @ 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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ENVIRO-CLEAN® Carbon Cartridges

Part Number: EU52112M6

EPA Method 521

August 25, 2009

The UCT activated carbon cartridge is designed to provide a high level of performance in solid-phase extraction for the analysis of **nitrosamines** in finished drinking water.

Product Benefits

- Designed to meet US EPA specifications
- Free from interferences that cause false positives
- Double ringed to prevent fines
- No Lot to Lot variability
- Excellent analytical reproducibility
- Packaged in metalized, sealed pouches to maintain product cleanliness

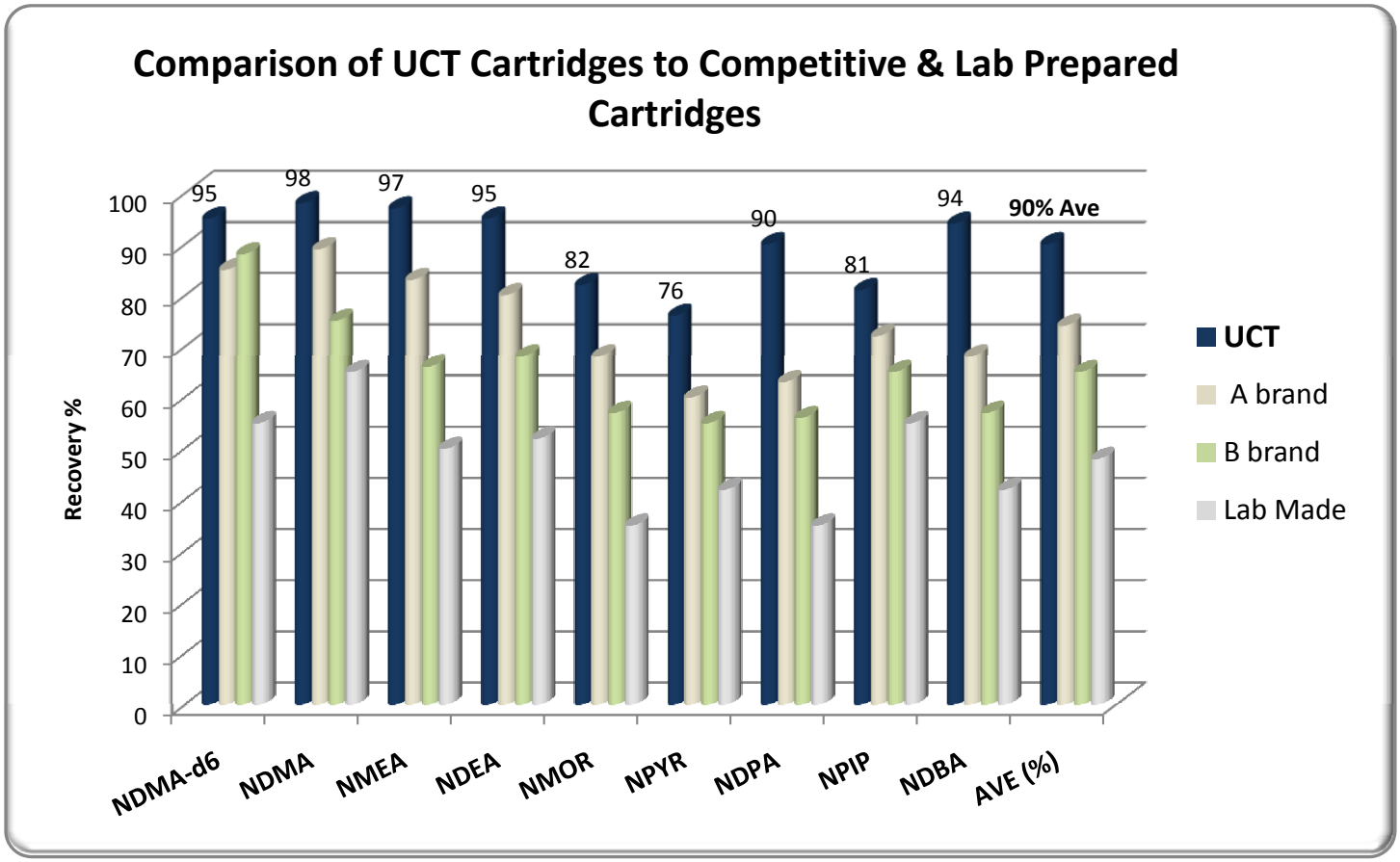
Product Features

- Each 6 mL cartridge contains 2000 mg of activated coconut carbon sorbent
- Can be used on manual single or multi-station manifold systems
- Cartridges may be used with automated extraction systems

Nitroaromatics, Nitramines and Nitrate Ester Analytes with CAS

UCT Product Number: ENVIRO-CLEAN® EU52112M6

Analyte	Abbreviation	CAS	% Recovery n=3
N-Nitrosodimethylamine	NDMA	62-75-9	95
N-Nitrosomethyldiethylamine	NMEA	10595-95-6	98
N-Nitrosodiethylamine	NDEA	55-18-5	95
N-Nitrosodi-n-propylamine	NDPA	621-64-7	90
N-Nitrosodi-n-butylamine	NDBA	924-16-3	94
N-Nitrosopyrollidine	NPYR	930-55-2	76
N-Nitrosopiperidine	NPIP	100-75-4	81



Data indicate the performance of **UCT** brand cartridges exceeds competitive brands **A** and **B,** and cartridges prepared in the laboratory

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EPA Method 521* Summary

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)

Scope and Application

Method 521 is a procedure using activated carbon for the determination of various nitrosamines in finished drinking water. The method can also be used for untreated source waters but has not been evaluated for these sources. Nitrosamines are sufficiently thermally stable and volatile for direct analysis by gas chromatography. Single laboratory LCMRL for the analytes in this method range from 1.2-2.1 ng/L.

Method Summary

Analytes and surrogates are readily extracted when a 500 mL water sample is drawn through a solid-phase extraction cartridge containing 2 grams of coconut activated carbon. The organic compounds eluted from the solid-phase with a small quantity of methylene chloride. The solvent is concentrated and an internal standard added. The sample components are identified after injection on a fused silica capillary column of a GC/MS/MS equipped with a large volume injection injector.

Interferences

- Major contaminant sources are reagents and water
- Nitrosamines may be present in trace amount in rubber products such as gloves and water systems. NDMA can leach from rubber products. These products must be avoided in the reagent water system. Analysis of a laboratory blank can provide information about the source of contamination
- Water stored in glass bottles with PTFE caps is recommended
- Rubber coated septa on injection vial may also introduce method analytes into the sample extracts giving false high readings

Sample Collection

- Filed sampling equipment must be free of plastic or rubber tubing
- All field samples must be dechlorinated with 80-100 mg of sodium thiosulfate per liter at time of collection
- Samples must iced during shipment and not exceed 10 °C
- Sample stored in the lab must be held at 6ºC
- Analyze with 14 days after collection
- Sample extracts can be stored up to 28 days in amber vials at -15°C or less and protected from light

Safety

The analytes in this method are classified as known human and mammalian carcinogens. Standard and stock solutions should be handled using suitable protection to skin and eyes.

Notes

- GC systems must be capable of temperature programming
- Deactivated post liners should be used
- Tandem mass spectrometers may be either triple quadrupole or ion trap

Procedure (manual or automated)

1) **Cartridge Conditioning**
   a) Add 3 mL of methylene chloride to the cartridge, then turn on the vacuum and slowly draw completely through the cartridge
   b) Add 3mL 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 layer of carbon.

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

   d) Add 3 mL of reagent water to the cartridge 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 step 3)

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 then turn vacuum off and allow cartridge to soak for 1 minute
d) Draw the remaining methylene chloride through in dropwise fashion
e) Continue to add methylene chloride to the cartridge as it is being drawn through until a total of 12-13 mL have been added

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 **UCT 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.

f) 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

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

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 internal std	14.63	113	81(81)
NDPA-d6 internal std	22.07	145	97(97)

Retention times were obtained on a Varian Saturn 4 GC/MS/MS using the following conditions:

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

*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

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1,4-dioxane (CAS 123-91-1), often referred to as dioxane, is a highly soluble non-biodegradable ether. This compound is used as solvent stabilizer to prevent the breakdown of chlorinated solvents.

1) Sample Preparation:

a) To dechlorinate the water sample add 25 mg of sodium sulfite, **then** acidify with 0.5 g of sodium bisulfate and swirl until dissolved.

2) Condition Cartridge:

a) Place a EU52112M6 cartridge on a single or multi-station vacuum manifold or automated extraction system
b) Add 3 mL of dichloromethane (methylene chloride) to the cartridge and draw through
c) Add 3 mL of methanol and draw through
d) Repeat step c)
e) Add 3 mL of DI water and draw through
f) Repeat step e) 4 times

3) Load Sample:

a) Add Internal standard * to the sample
b) Add sample to the cartridge. Adjust for a flow rate of 5 - 10 mL/minute
c) Dry cartridge by drawing air at full vacuum for 10 minutes

4) Elution:

a) Place a clean collection vial in the vacuum manifold
b) Add 3 mL of dichloromethane to the cartridge. Slowly draw through
c) Add a second 6 mL aliquot of dichloromethane and draw through
d) Add surrogate** (0.5 ppm) to the extract and bring to 10 mL final volume with dichloromethane
e) Dry extract by adding 2 g anhydrous sodium sulfate (Na_2SO_4) and vortex to mix the extract

5) Quantitate:

a) GC-MS-SIM (electron ionization)

6) Instrument & Conditions:

Column: Varian CP-Select 624 CB (6% cyanopropyl phenyl, 94& PDMS, 30 m 0.25mm x 1.4 um column) or equivalent
Injector: 200°C (Splitless mode)
Injector Volume: 1 µL
Flow: 1 mL minute
Oven: 30°C for 1 minute, 90°C at 7°C/minute, 200°C at 20°C/minute for 3 minutes

MS: quadrupole MS

SIM MODE

Dwell time 100 µs

Emission current 100 µA

***** Quantitation ions**

Segment 1 (0-8 minutes)	****THF-d_8**	**m/z 40***, 78, 80**
Segment 2 (8-16.5 minutes)	**1, 4 dioxane**	**m/z 58, 88*****
	***1, 4 dioxane-d_8**	**m/z 62, 64, 96*****

R 1.0 20090114

*Grinmmett, Paul E., Munch, Jean W., Method Development for the Analysis of 1,4-Dioxane in Drinking Water Using Solid-Phase Extraction and Gas Chromatography-Mass Spectrometry, Journal of Chromatographic Science, Vol. 47, January 2009. Office of Research and Development, National Exposure Laboratory, Cincinnati, OH.

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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.

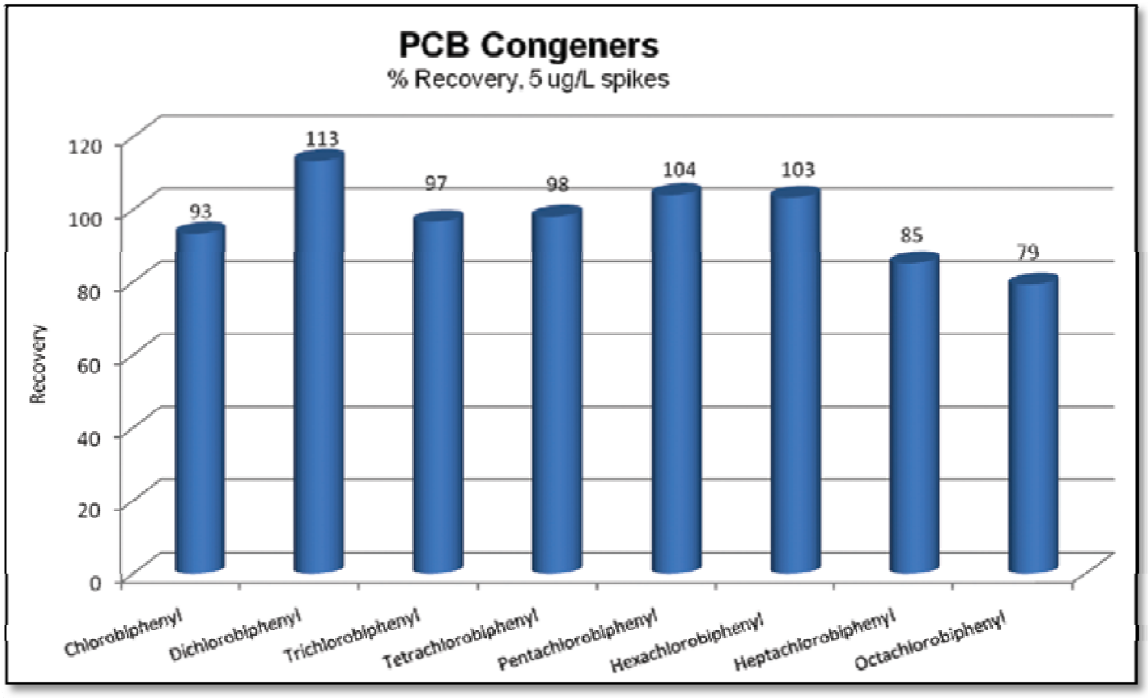
The validation data presented herein were determined on independent lots of UCT ENVIRO-CLEAN® Universal Cartridges. MDLs were not determined on all analytes as part of this validation. In addition to the listed method analytes, recovery data for an extended list of analytes is also included.

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

Analyte	Average 3 Replicates % Recovery	Std Dev
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



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

Method Summary

A one liter water sample is adjusted to pH <2 using 6N HCL before passing through an 83 mL ENVIRO-CLEAN® Universal 525 UCT cartridge (ECUNI525). Analytes are eluted from the cartridge with ethyl acetate and methylene chloride. The extract is reduced in volume to 1.0 mL and analyzed by GC/MS.

Method

1. Assemble an all glass filtration apparatus using an 83 mL UCT Universal 525 cartridge.

a) Use of a vacuum manifold for multiple extractions or automated extractions is acceptable

2. Wash the extraction apparatus and cartridge

a) Add 10 mL of a 1: 1 mixture of ethyl acetate:methylene chloride (EtAc: $MeCl_2$) 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 one minute
d) Draw the remaining solvent through the cartridge to waste
e) Allow the cartridge to dry for 3 minutes under full vacuum

3. Condition the 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 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

5. Remove cartridge assembly

a) Insert a suitable sample tube for eluate collection
b) Add 10 mL of EtAc 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 EtAc:$MeCl_2$
h) Using a disposable pipette, rinse down the sides of the cartridge and bottle holder with another 10 mL aliquot of 1: 1 EtAc:$MeCl_2$
i) Add the rinse to the cartridge, then draw through

10. Dry the combined eluant

a) Use granular anhydrous sodium sulfate
b) Rinse the collection tube and sodium sulfate with two x 3 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

12. Analyze by GC/MS

Revison 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

DETERMINATION OF PHENOLS IN DRINKING WATER BY SOLID-PHASE EXTRACTION (SPE) AND CAPILLARY COLUMN GAS CHROMATOGRAPHY (GC/MS)

Part #: ECDVB156

February 3, 2009

Method 528 Revision 1.0

Reagents

Methylene Chloride

Methanol

Anhydrous Sodium Sulfate 5 g in a 6 mL cartridge ECSS25K

Enviro-Clean® 500mg in a 6 mL cartridge ECDVB156

Method Summary

A one liter sample of drinking water is extracted by drawing through a UCT Enviro-Clean® 6 mL cartridge containing 500 mg of polystyrene divinyl benzene copolymer. The phenolic compounds are eluted from the solid-phase using a small quantity of methylene chloride. An aliquot of the concentrated extract is injected into a high resolution fused silica capillary column of a GC/MS system. Respective phenols are identified by comparing their mass spectra and retention times by comparison to standards.

Condition Cartridge

- Adjust the pH of the water sample to 2 or less by the addition of 6N HCl acid
- Rinse the cartridge with three, 3 mL aliquots of methylene chloride, then draw to waste
- Rinse the cartridge with three, 3 mL aliquots of methanol then draw to waste. After the third rinse, leave enough methanol in the cartridge to cover the frit. **Do not let the cartridge dry out at this point.**
- Rinse the cartridge with three, 3 mL aliquots of 0.05N HCl. **Turn off the vacuum before the HCl solution drops below the level of the frit**

Sample Addition

- Add the water sample to the cartridge and adjust the vacuum such that the flow rate is about 20 mL/minute (50 minutes for a 1 liter sample). Allow the cartridge to dry for at least 10-15 minutes before proceeding to the next step. A dry cartridge is important for good recoveries.

Extract Elution

- Rinse the inside of the sample bottle with a 10 mL portion of methylene chloride. Add this to the cartridge and draw this through to the collection tube in a dropwise fashion.
- Add 2-3 mL of methylene chloride to the cartridge then slowly draw this through to the collection tube in a dropwise fashion.

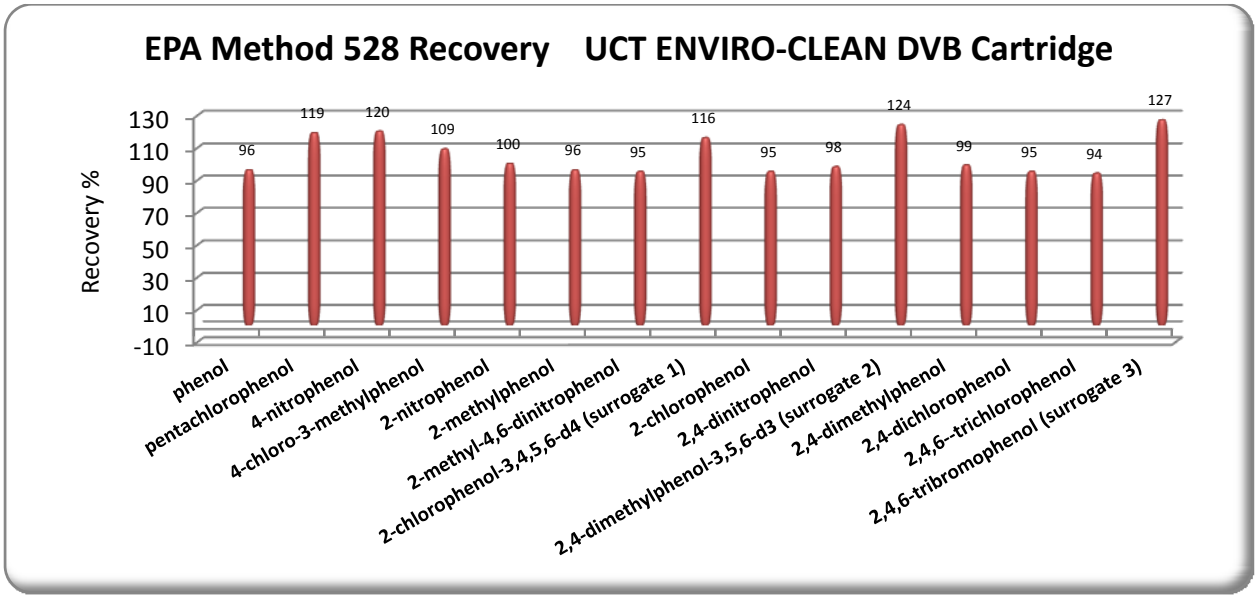
Eluate Drying

- Dry the eluate by passing through prerinsed anhydrous sodium sulfate column UCT Part number ECSS15M6 and collect eluate in a clean tube
- Rinse the sodium sulfate column with two, 3 mL aliquots of methylene chloride and collect in the tube
- Concentrate the extract to about 0.9 mL in a warm water bath (40^{O}C) under a gentle stream of nitrogen
- Adjust final volume to 1.0 mL with methylene chloride

Analysis

- Analyze the extract with GC/MS

Phenolic Analyte	CAS NUMBER
phenol	108-95-2
pentachlorophenol	87-86-5
4-nitrophenol	93951-79-2
4-chloro-3-methylphenol	59-50-7
2-nitrophenol	88-75-5
2-methylphenol (o-cresol)	95-48-7
2-methyl-4,6-dinitrophenol	534-52-1
2-chlorophenol-3,4,5,6-d4 (surrogate 1)	
2-chlorophenol	95-57-8
2,4-dinitrophenol	51-28-5
2,4-dimethylphenol-3,5,6-d3 (surrogate 2)	
2,4-dimethylphenol	105-67-9
2,4-dichlorophenol	120-83-2
2,4,6--trichlorophenol	88-06-2
2,4,6-tribromophenol (surrogate 3)	



UCT Product ENVIRO-CLEAN® ECDVB156

Results show that the UCT Product ENVIRO-CLEAN® ECDVB156

styrene divinyl benzene cartridge yields excellent recoveries of phenolic compounds

J. W. Munch, April 2000, National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268

DCN-903020-123

ENVIRO-CLEAN®
EPA METHOD 535 GRAPHITIZED CARBON (90 m^2) CARTRIDGE

Part #: EC535156

February 3, 2009

The UCT EC535156 cartridge has been designed to provide a new level of performance in solid-phase extraction for the analysis of acetamide and acetanilide compounds. With its high capture efficiency, fast flow and excellent dry times, laboratory throughput can be significantly improved

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

Method Summary

A 250 mL water sample is drawn through and captured on a **UCT cartridge EC535156** 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.

Condition Cartridge

- Rinse the **UCT EC535156** cartridge with 20 mL of 10 mM ammonium acetate/methanol solution
- Rinse cartridge with 30 mL of reagent water. Do not let water drop below level of cartridge packing
- Add about 3 mL of reagent water to the top of the cartridge

Do not let the cartridge go dry during any step otherwise the conditioning process should be started over

Sample Addition

- Add sample water to the cartridge and adjust vacuum so the flow is about 10-15 mL/minute
- Rinse cartridge with 5 mL of reagent water
- Draw air or N_2 through the cartridge at high vacuum (10-15 in/Hg) for 3 minutes

Extract Elution

All glassware must be meticulously washed to avoid contamination

- Insert a clean collection tube into the extraction manifold
- 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 drop wise fashion at this vacuum setting

Eluate Drying

- Concentrate the extract to dryness under a gentle stream of N_2 in a heated water bath at 60^{O}-70^{O}C to remove all of the ammonium acetate/methanol
- Reconstitute the dried eluate by adding 1 mL of 5 mM ammonium acetate/methanol solution

Extract Analysis

- 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
- 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

*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

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

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UCT ENVIRO-CLEAN® Cartridges
EPA Method 548.1 Endothall

Part Number: EC548006

September 28, 2009

The UCT EC548006 cartridge has been designed to provide a new level of performance for endothall (CAS 145-73-3) analysis using ion-exchange solid-phase extraction. Endothall can be easily captured without difficult derivatization techniques for faster analysis. With the cartridge's high capture efficiency, fast flow and excellent dry times, laboratory throughput can be significantly improved.

Product Benefits

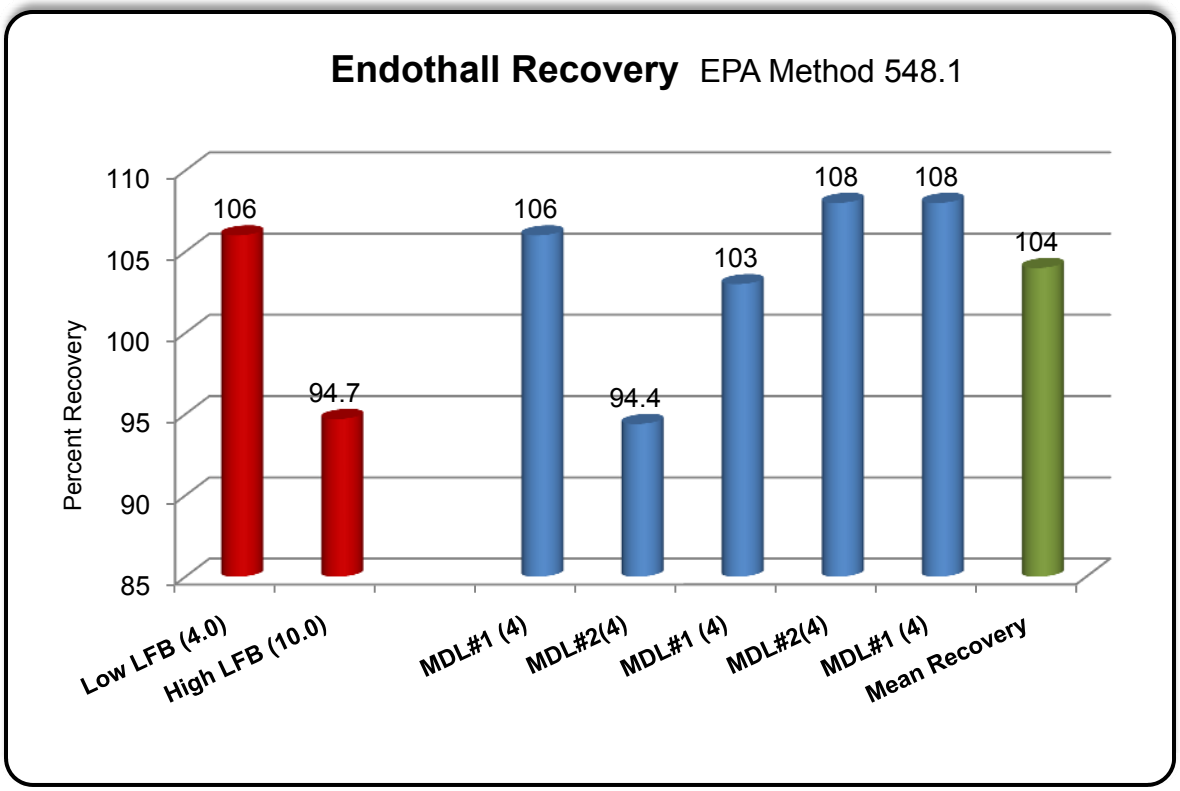
- Pre-packed SPE columns using anion capture resin
- No sample channeling due to improper cartridge packing
- Fast flow rates for rapid analyte capture
- Polypropylene frits eliminate potential contamination yielding clean extracts
- Works well at all levels of analyte loading
- Consistent results for excellent reproducibility
- Cartridges sealed at both ends to maintain product integrity
- Packaged in polypropylene bags to maintain product cleanliness
- Cartridge provides an effective sample clean-up for potential organic matrix interferences

Product Features

- 6 mL cartridges manufactured from special proprietary polypropylene
- Each cartridge contains the EPA prescribed 3.5 ± 0.1 cm bed of slurry packed sorbent
- Anion resin fully compliant with Method 548.1
- Can be used on manual single or multi-station manifold systems
- Can be used with automated extraction systems

Pre-packed UCT column is sealed at both ends to assure product integrity

The UCT column is fully compliant with EPA Method 548.1



UCT Cartridge EC548006 Shows Excellent Recovery with Laboratory Fortified Blanks (LFB) and Replicate Samples

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

Method 548.1 Revision 1.0

Method Summary*

The endothall molecule contains two dissociable carboxylic acid functional groups. Endothall is captured using liquid-solid cartridges containing a primary tertiary amine anion exchange resin. A 100 mL water sample is passed through the cartridge and the analyte is eluted with 8 mL of acidic methanol. After a small amount of methylene chloride is added as co-solvent to the extract and heated for 60 minutes at 50°C, the dimethyl ester of endothall is formed. The ester is partitioned into 8-10 mL of methylene chloride by the addition of salted water. The extract is reduced to 1 mL volume with nitrogen purge for a concentration factor of 100. The extract is analyzed by GC/MS or GC/FID using a megabore capillary column.

Interferences

- Glassware must be scrupulously clean by rinsing with the last solvent used in it
- Bake all glassware except volumetric flasks at 400°C for several hour prior to use
- The use of high purity solvents is essential
- 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)

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

1) Condition Cartridge

a) Remove the seal caps on each end of the cartridge and place it on the vacuum manifold system
b) Leave a 1 cm layer of reagent water over the resin bed between each liquid addition
c) Add 10 mL of methanol and draw through
d) Add 10 mL of reagent water and draw through
e) Add 10 mL of 10% H_2SO_4 in methanol and draw through
f) Add 10 mL of reagent water and draw through
g) Add 20 mL 0f 1 N NaOH and draw through
h) Add 20 mL of reagent water and draw through
i) Draw each reagent through the cartridge at a rate of 10 mL/minute

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

2) Sample Addition

a) Fill the reservoir with 60 mL of sample and adjust sample flow rate to 3 mL/minute. Add the remaining sample to keep the reservoir from going dry
b) After the sample has been drawn through the cartridge add 10 mL of methanol and draw through
c) Dry cartridge for 5 minutes under 10-20 in Hg vacuum
d) Place a culture tube inside the manifold to collect the eluant

3) Extract Elution

- Elute the cartridge with 8 mL of 10% H_2SO_4 in methanol followed by 6 mL of methylene chloride (CH_2Cl_2) over a 1 minute period

4) Sample Derivatization and Partition

- Place the cap on the culture tube and hold for 1 hour at 50°C
- 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. Add the rinse to the separatory funnel
- Add 20 mL of 10% $NaSO_4$ in reagent water to the separatory funnel. Vigorously shake the separatory funnel three times venting the funnel each time
- Allow the phases to separate then drain the organic layer into a 15 mL graduated centrifuge tube
- Repeat the above extraction with two additional 2 mL aliquots of methylene chloride adding the methylene chloride to the organic phase in the centrifuge tube

5) Analysis

a) Analyze the extract by injecting 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 g/L spike**	**FID**
Endothall	16.02	19.85	18.32	1.79	**µg/L**	0.7
D10-Acenapththene	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.

*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

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UCT ENVIRO-CLEAN® CARTRIDGES
EPA METHOD 549.2

Part #: EEC08156

February 3, 2009

The UCT cartridge (EEC08156) for Diquat / Paraquat Analysis has been designed to provide a new level of performance in solid-phase extraction. With its high capture efficiency, fast flow and excellent dry times, laboratory throughput can be significantly increased.

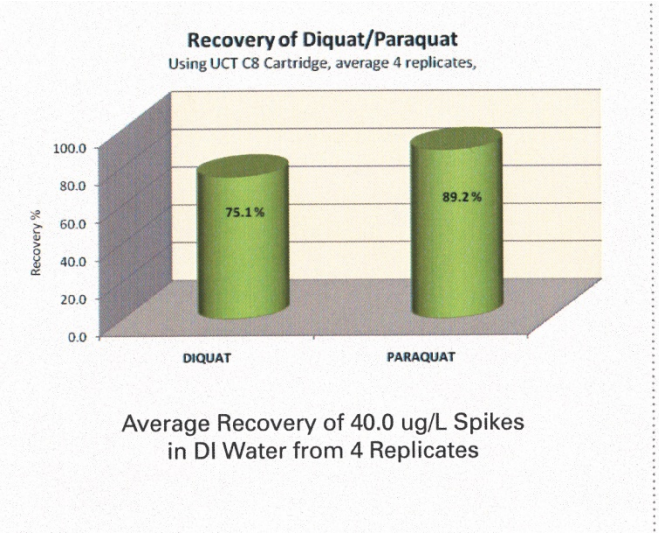
Product Benefits

- Fast flow rates for rapid analyte capture
- SPE using bonded endcapped C8
- Excellent pH stability (1-14)
- Teflon frits eliminate potential contamination
- Works well at all levels of analyte loading
- No lot to lot variability (EPA has noted significant variability with some brands)*
- Excellent reproducibility (MDL diquat 1.6 µg/L, paraquat 1.7 µg/L)
- Specially packaged to maintain product cleanliness

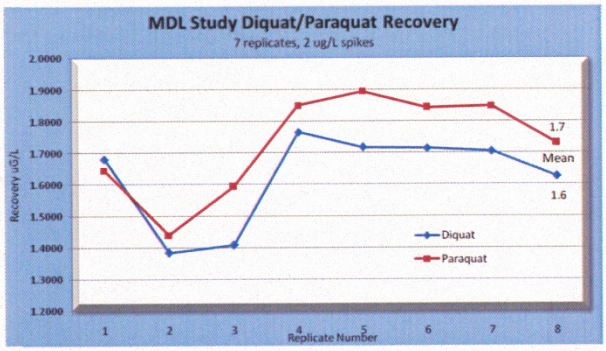
Product Features

- 6 mL polypropylene cartridge packed with 500 mg of endcapped C8
- Can be used on manual single or multi-station manifolds system
- Can be used with automated extraction systems
- Not all bonded sorbents are capable of achieving acceptable recoveries using method 549.1

The C8 used in UCT cartridges has been tested for both diquat and paraquat recoveries.



Average Recovery of 40.0 ug/L Spikes in DI Water from 4 Replicates



Results of an MDL Study Using 7 Replicates
2.0 ug/L spikes
- Mean Recovery = Diquat 1.6255 µg/L and Paraquat 1.7310 µg/L
- Standard Dev = Diquat 0.1587 µg/L and Paraquat 0.1725 µg/L

EPA Method 549.2
Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography with Ultraviolet Detection

Method Summary

This method determines diquat (1,1'-ethylene-2,2'-bipyridilium dibromide salt) and paraquat (1,1'-dimethyl-4,4'-bipyridilium dichloride salt) in drinking water and drinking water sources using HPLC with photodiode array UV detection. The analytes are extracted from 250 mL of water adjusted to pH 7.0 to 9.0 using 6 mL C8 solid phase extraction cartridges with ion-pairing. Using an acidic aqueous solvent, analytes are eluted from the cartridge and detected at 308nm and 257nm, respectively.

This summary highlights major steps in the 549.2 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

- Mean Recovery = Diquat 1.6255 µg/L and Paraquat 1.7310 µg/L
- Standard Dev = Diquat 0.1587 µg/L and Paraquat 0.1725 µg/L

Spike level for both compounds 2.0 µg/L; n=7

Preparation

- Since diquat and paraquat are ionic, all glassware should be silanized prior to use to deactivate the glass surface. The use of plastic lab ware is preferable.[1]
- Adjust a 250 mL of sample to pH 7.0 to 9.0 with 10 % aqueous sodium hydroxide or 10 % aqueous hydrochloric acid solution
- Assemble a C8 bonded silica extraction cartridges in an all-glass or plastic filtration apparatus.

Condition the Cartridge

- Add 3 mL of methanol to the cartridge soaking for about one minute. Apply vacuum to pull most of the methanol through the cartridge leaving a 3 to 5 mm layer of methanol on top of the cartridge
- Add 3 mL reagent water to the cartridge. Using vacuum, draw most of the water through the cartridge leaving 3 to 5 mm of water on the surface of the cartridge.
- Apply 5 mL of conditioning **Solution A**[2] to the cartridge. Using vacuum draw a small amount through then allow the cartridge to soak for one minute. After one minute, draw most of the remaining solution through the cartridge leaving 3-5 mm layer on top.
- Using two 10 mL aliquots of reagent grade water, rinse Solution A from the cartridge. Allow 3-5 mm of water to remain on the cartridge surface.
- Repeat the above procedure a second time using conditioning **Solution B.**[3] Return solution in cartridge.
- Cartridges may be prepared in advance and stored up to 48 hour prior to use if capped and stored at 40^{O}C. Do not let the cartridge dry out.

Sample Extraction

- Add the water sample to the reservoir and start the vacuum. Draw the sample through the cartridge. Drain as much of the water from the sample bottle as possible.
- Rinse the cartridge with 5 mL methanol.
- Remove the filtration assembly and insert a silanized 5 mL volumetric flask for collection of the eluate.

Cartridge Elution

- Add 4 mL of the cartridge eluting solution[4] to the cartridge and allow to soak for one minute. Draw any remaining solution through the cartridge, leaving 3 to 5 mm on the cartridge. The cartridge eluting solution contains acid and diethylamine which disrupts the ion-pair interactions releasing the analytes.
- Add another 4 mL of cartridge eluting solution to the remaining solution on the cartridge, and draw it completely through.
- Using cartridge **ion-pair solution,**[5] bring the eluate to a known volume. The extract is now ready for HPLC analysis.

Notes and Working Solutions

1. Since diquat and paraquat tend to adsorb onto glass surfaces, all glassware that comes into contact with samples, sample extracts, or standard solutions should be deactivated by silanization. The use of PVC lab ware circumvents this step.

2. Solution A contains cetyl trimethyl ammonium bromide to deactivate residual silanol groups on the C8

3. Solution B contains 1-hexanesulfonic acid which adsorbs to C8 forming a cation exchange sorbent.

4. The aqueous elution solution contains orthophosphoric acid and diethylamine in DI water

5. Ion-pair concentrate contains hexanesulfonic acid

* EPA Method 549.2 Revision 1.0 Issued June, 1997 as a part of "Methods for the Determination of Organic Compounds in Drinking Water," Hodgeson, J.W., Bashe, W.J., (Technology Applications, Inc.) and Eichelberger, J., Environmental Monitoring Systems Laboratory, Office of Research and Development, US Environmental Protection Agency, Cincinnati, Ohio 45268

DCN-903020-116

Bisphenol A Analysis in Water by GC/MS Using an ENVIRO-CLEAN® 200 mg C18 Extraction Cartridge

Part #: EEC1812Z

February 5, 2009

1. **Prepare Sample**
   a) Using 100 mL of sample water, adjust the pH to 7 or less using 100mM acetic acid
   b) Add internal standard to water sample

Note: Bisphenol A has a pKa value of approximately 9.5

2. **Condition ENVIRO-CLEAN® Extraction Cartridge**
   a) Place a cartridge(s) on a multistation vacuum manifold or automated extraction system
   b) Condition the cartridge by adding 3 mL of methanol
   c) Partially draw the methanol through until the surface of the liquid reaches the top of the cartridge frit
   d) Wait 1 minute then add 3 mL of DI water to the cartridge.
   e) Add 1 mL of 100 mM acetic acid
   f) Draw liquid through until it touches the top of the frit
   g) Cartridge is now ready for sample extraction

Note: Do not allow the sorbent to completely dry out after the addition of methanol, otherwise repeat procedure.

3. **Apply Sample**
   a) Add sample to cartridge at a rate of approximately 5 mL/minute by adjusting vacuum

4. **Wash Cartridge**
   a) Wash by drawing through 5 mL of deionized water
   b) Dry sorbent (5 minutes at > 10 inches Hg)

5. **Elute**
   a) Insert a collection vial in the vacuum manifold
   b) Rinse sample bottle with 3 mL of methanol
   c) Add the methanol to the cartridge
   d) Elute at 5 mL per minute
   e) Add 3 mL of methanol to the cartridge
   f) Elute at 5 mL per minute

6. **Evaporate**
   1. Evaporate methanol eluate using gentle N_2 (< 40°C) to dryness
   2. Add 50 µL of ethyl acetate to dissolve
   3. Add 50 µL of reagent MTBSTFA* or BSTFA** to derivatize. Vortex
   4. Heat mixture for 20-30 minutes @ 70°C
   5. Cool. Sample is now ready for GC injection

*MTBSTFA-- N-(t-butyldimethylsilyl)-N-methyltrifluoroacetamide
**BSTFA-- *N,O* -Bis(trimethylsilyl)trifluoroacetamide

7. **Instrument Conditions**

- **Column:** Rtx-5MS, 30m, 0.25 mm ID, 0.50 µm df (5% diphenyl/95% dimethyl polysiloxane)
- **Injector Temperature:** 250°C
- **Detector Temperature:** 250° C
- **Oven Program:** Initial 70°C, ramp @ 20°C/minute to 320°C, hold 3.0 min
- **Purge Flow:** Initially Off. On at 0.75 minutes
- **Split Flow:** 30 mL/minute
- **Inject:** 2 µL

8. **Quantitate**

MS in EI (+) mode:

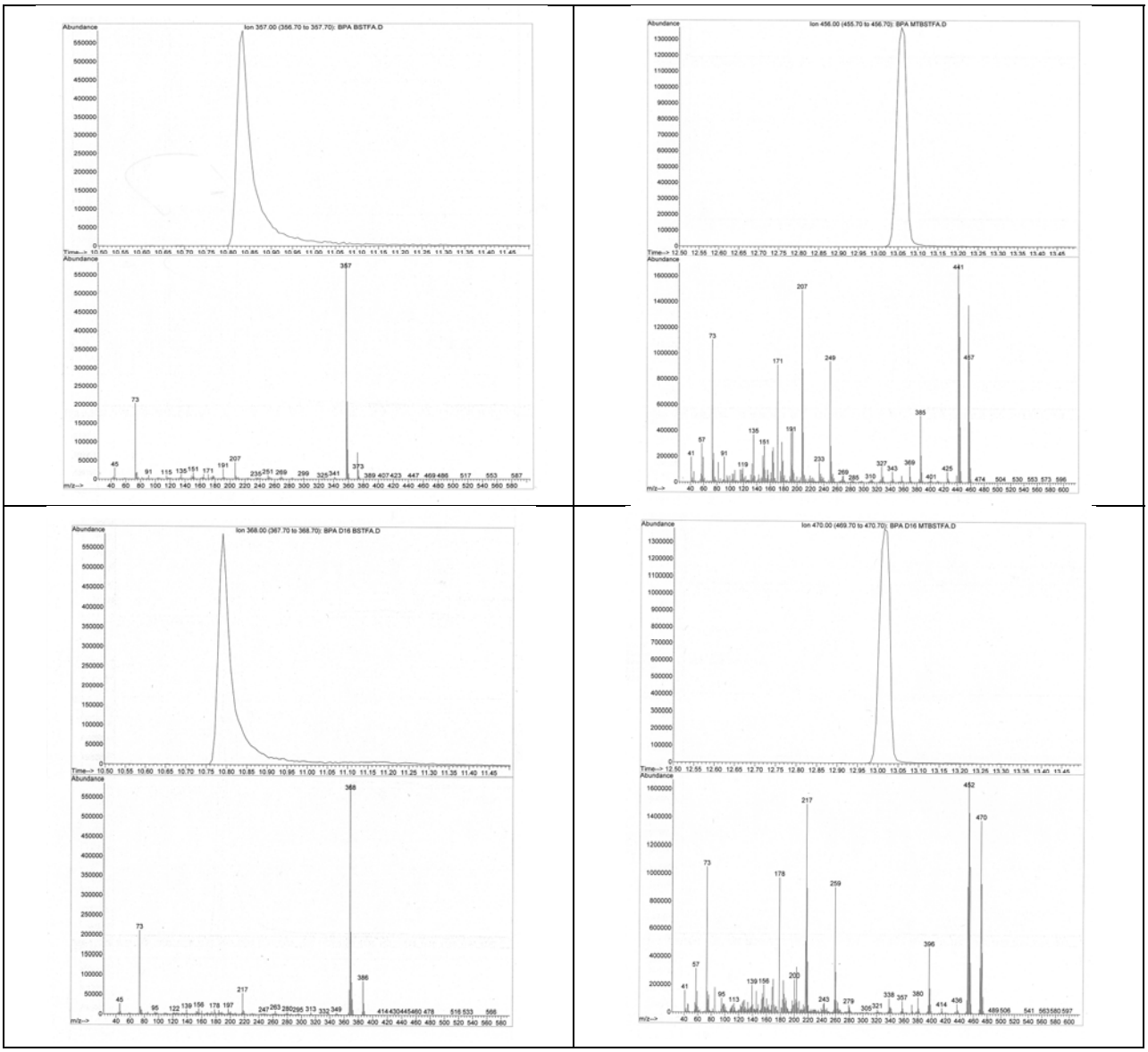
BSTFA

	Primary ion	Secondary ion	Tertiary ion
BPA	357	373	207
BPA-D16	368	386	217

MTBSTFA

BPA	441	457	207
BPA-D16	452	470	217

Chromatogram Showing Retention Time for Bisphenol A in Water



Spike Concentration: 0.15 µg/L
Calc Concentration: 0.157 µg/L
Recovery: 105%

DCN-905020-132

Bisphenol A Analysis in Water by LC/MS/MS Using an ENVIRO-CLEAN® 200 mg C18 Extraction Cartridge

Part #: EEC1812Z

February 5, 2009

1. **Prepare Sample**
   a) Using 100 mL of sample water, adjust the pH to 7 or less using 100mM acetic acid
   b) Add internal standard to water sample

   Note: Bisphenol A has a pKa value of approximately 9.5

2. **Condition ENVIRO-CLEAN® Extraction Cartridge**
   a) Place a cartridge(s) on a multistation vacuum manifold or automated extraction system
   b) Condition the cartridge by adding 3 mL of methanol
   c) Partially draw the methanol through until the surface of the liquid reaches the top of the cartridge frit
   d) Wait 1 minute then add 3 mL of DI water to the cartridge.
   e) Add 1 mL of 100 mM acetic acid
   f) Draw liquid through until it touches the top of the frit
   g) Cartridge is now ready for sample extraction

   Note: Do not allow the sorbent to completely dry out after the addition of methanol, otherwise repeat procedure.

3. **Apply Sample**
   a) Add sample to cartridge at a rate of approximately 5 mL/minute by adjusting vacuum

4. **Wash Cartridge**
   a) Wash by drawing through 5 mL of deionized water
   b) Dry sorbent (5 minutes at > 10 inches Hg)

5. **Elute**
   a) Insert a collection vial in the vacuum manifold
   b) Rinse sample bottle with 3 mL of methanol
   c) Add the methanol to the cartridge
   d) Elute at 5 mL per minute
   e) Add 3 mL of methanol to the cartridge
   f) Elute at 5 mL per minute

6. **Evaporate**
   a) Evaporate methanol eluate using gentle N_2 (< 40°C) to less than 500 µL
   b) Bring sample volume to 500 µL
   c) Sample is ready for injection

7. **Quantitate**
   a. LC-MS/MS MRM transition **(**negative ion mode)

Instrumental & Conditions:

Column: 100 x 2.1 (3 µm) Selectra® Phenyl, UCT, LLC
Instrument: Applied Biosytems Triplequad LC/MS/MS (other systems may be used)

- Detector: API3200 QTrap
- bisphenol A Precursor ion mass 226.9, product ion mass 109.1
- *bisphenol A standard deuterated A-D16. Precursor ion mass 241.1, Product ion mass 223.1

Inject: 5-10 µL

Mobile Phase: acetonitrile/0.1% formic acid

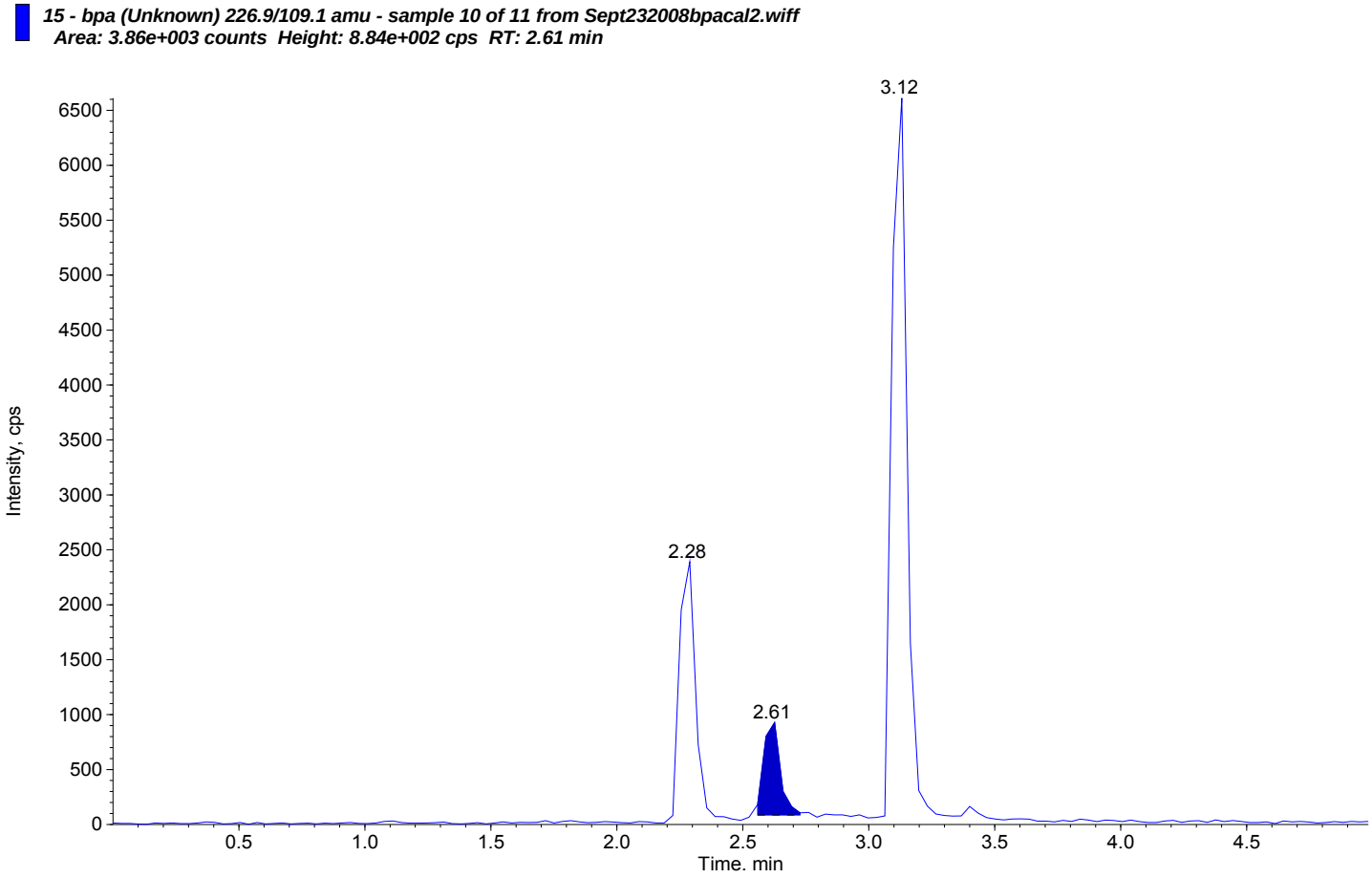
Flowrate: 0.5 mL/ minute

Flow Program

Time in minutes	**% Acetonitrile**	**% 0.1% Formic Acid**
0	30	70
3.0	90	10
3.5	30	70
5.0	30	70

Chromatogram Showing Retention Time for Bisphenol A in Water

Spike concentration: 15 µg/L
Calc Recovery: 15.70 µg/L
Recovery: 105%



DCN-905020-131

Extraction of Diesel Range Organics

Part Number: ENVIRO-CLEAN® Universal DRO cartridge (# ECUNIPAH)*
Anhydrous Sodium Sulfate (# ECSS50K)
August 25, 2009

1. Condition Cartridge

a) Add 10 mL of methylene chloride ($MeCl_2$) to the cartridge. Let the methylene chloride soak on the cartridge for about 1-2 minutes
b) Draw the methylene chloride through the cartridge to waste
c) Add 10 mL of acetone to the cartridge. Let the acetone soak for about 2 minutes
d) Draw the acetone to waste
e) Air dry the cartridge with full vacuum for a few seconds
f) Add 10 mL of methanol to the cartridge.
g) Draw some of the methanol through the cartridge leaving a layer just covering the frit
h) Allow the methanol to soak for about 2 minutes

Note: Do not allow the cartridge to dry after addition of methanol

i) Add 20 mL of deionized water to the cartridge. Draw most of the water through the cartridge to waste

2. Sample Addition

a) Add 5 mL of 1:1 HCl to the sample
b) Add 5 mL of methanol (optional) and any surrogates to the sample. Mix.
c) Add the sample to the cartridge using vacuum
d) Draw the sample through the cartridge in 15 – 20 minutes
e) Allow the cartridge to dry under vacuum for 10 minutes**

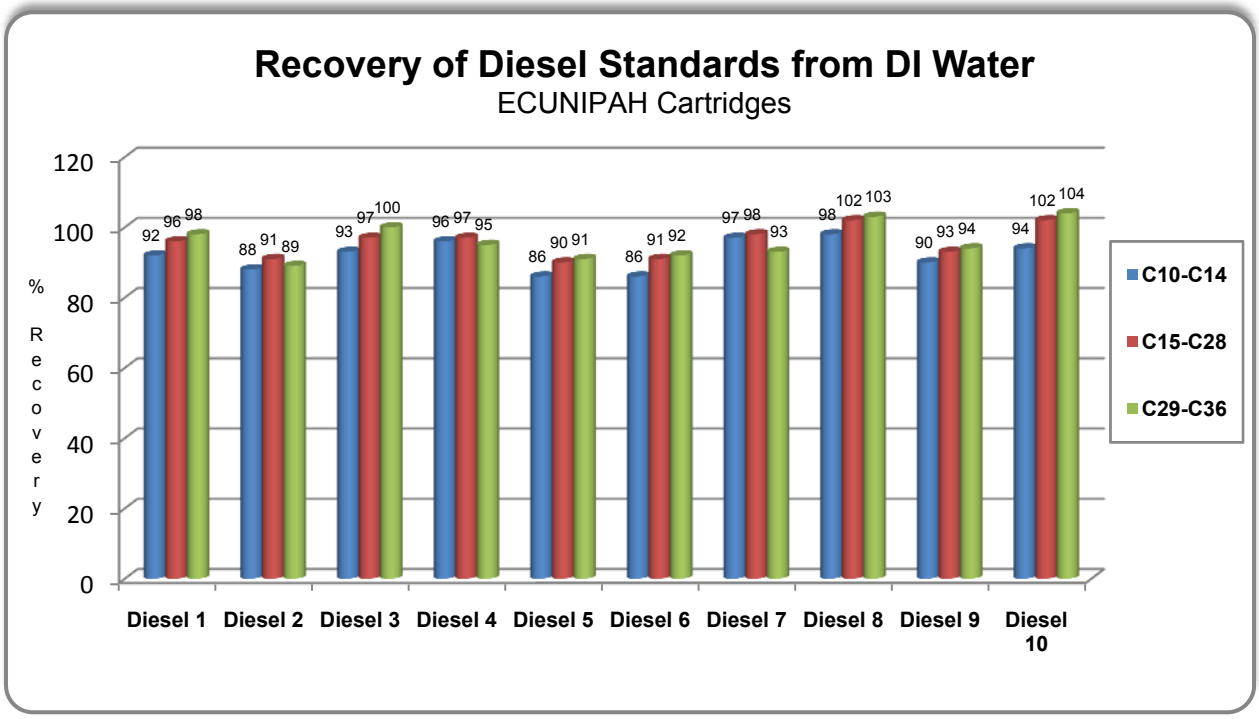
3. Extract Elution

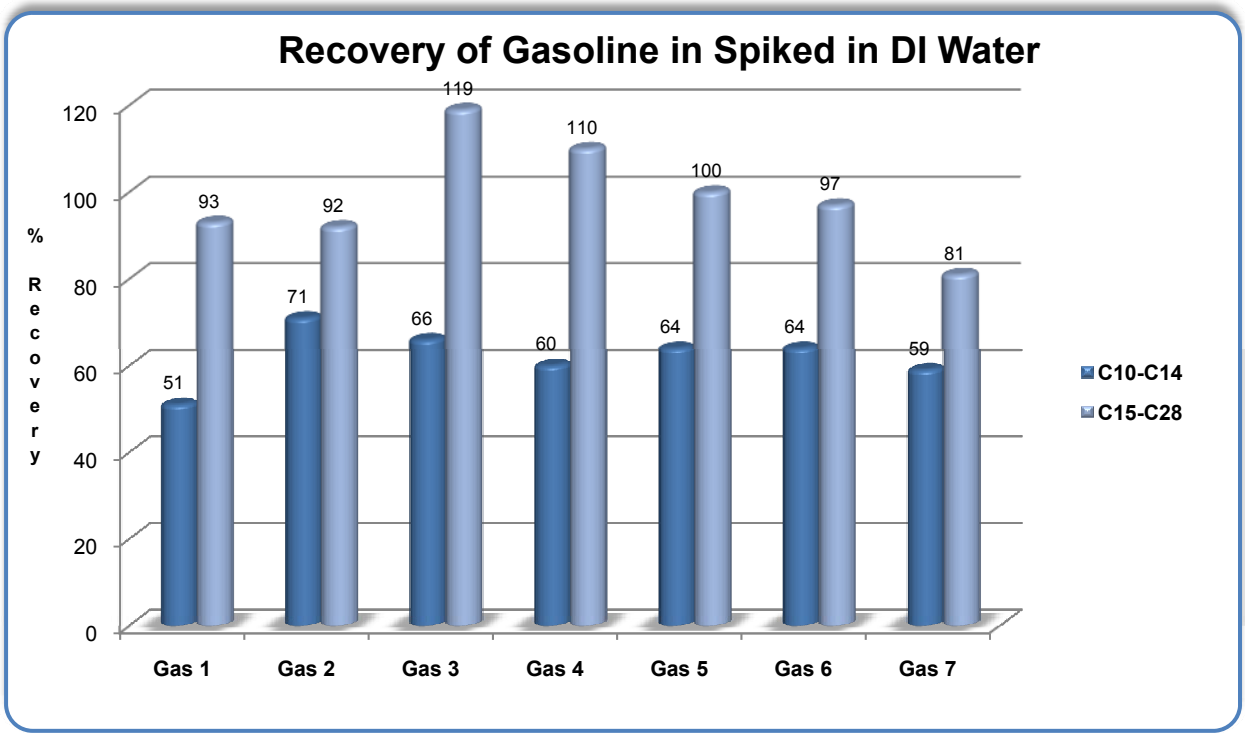
a) Place a collection tube or vial under the cartridge
b) Add 5 mL of acetone to the sample bottle to remove any residue
c) Add 5 mL of acetone to the cartridge. Allow the solvent to soak for 1 minute and draw the acetone into the collection vial
d) Repeat this procedure 3 times using 10 mL aliquots of methylene chloride
e) Dry the extract by passing through anhydrous sodium sulfate
f) Carefully rinse the collection vial with methylene chloride and add the solvent to the sodium sulfate

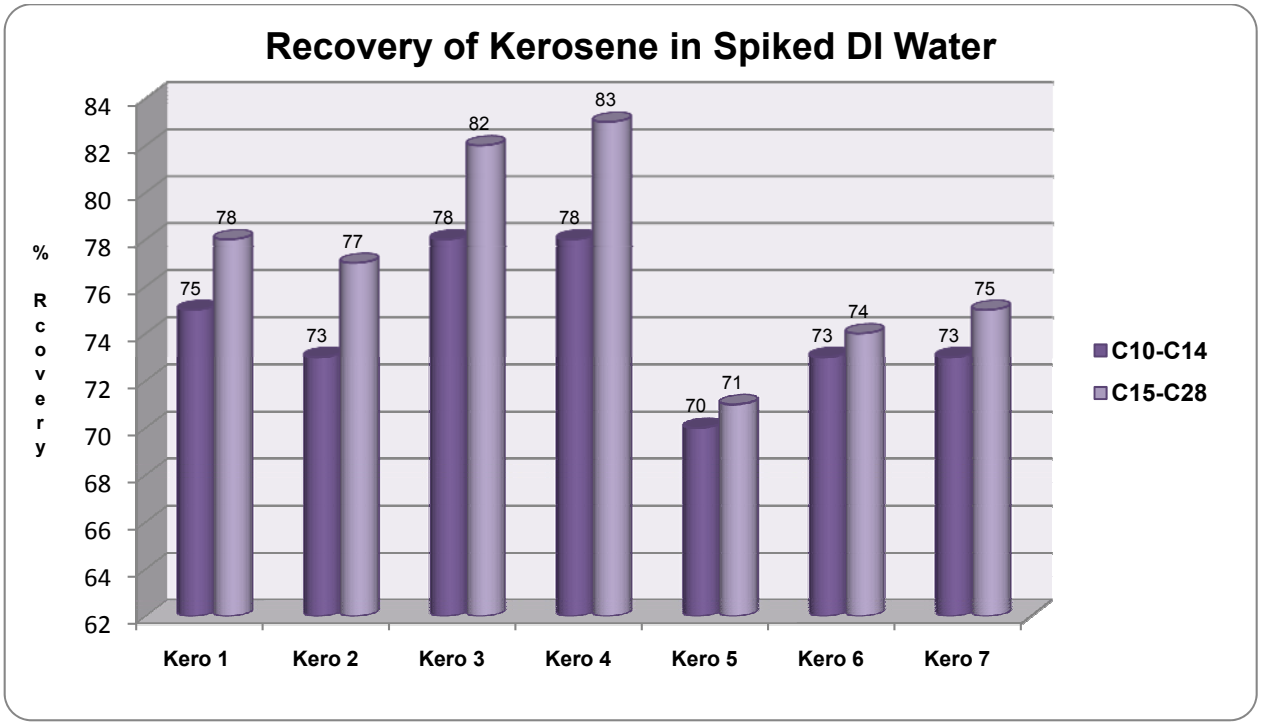
4. Concentration and Analysis

a) Carefully concentrate the extract to a final volume. A micro-KD followed by micro-Snyder column concentration is recommended

Note: Most extraction errors are caused by poor concentration technique. Do not concentrate below 0.5 ml. or low recoveries will result







*The ENVIRO-CLEAN® Universal DRO cartridge may be used on standard vacuum manifolds (# VMFF016GL), standard disk manifolds (#ECUCTVAC6) (with adapter part # ECUCTADP). The cartridge is specifically designed to fit the Horizon SPE DEX 4790® made by Horizon Technologies, Inc.

**Faster drying results are 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.

DCN-905290-121

POLYCYCLIC AROMATIC HYDROCARBONS FROM A WATER MATRIX

Part #: ECUNIPAH

February 3, 2009

Reagents:
Methanol
Acetonitrile**
Methylene Chloride**
Anhydrous Sodium Sulfate (# ECSS25K)
ENVIRO-CLEAN® Universal PAH cartridge (# ECUNIPAH)

Condition Cartridge
Add 10 mL of methylene chloride to the cartridge and let it soak for 1 minute. Draw through to waste. Add 10 mL of acetone to the cartridge and let it soak for 1 minute. Draw through to waste. Add 10 mL of methanol to the cartridge and let it soak for 1 minute. Pull most of the methanol to waste but do not allow the sorbent to dry. Add 10 mL of deionized water to the cartridge and let it soak for 1 minute. Draw most of the water to waste but do not allow the sorbent to dry.

Sample Addition
Add 5 mL of methanol (optional) and any surrogates to the sample. Mix. Add the sample to the cartridge under vacuum. Ideally, the sample should pass through the cartridge in approximately 15 – 20 minutes. Allow the cartridge to dry under vacuum for 10 minutes.***

Extract Elution
Place a collection tube or vial under the cartridge. Add 5 mL of acetone to the sample bottle to remove any sample residue. Add the acetone to the cartridge. Allow to soak for 1 minute and draw the solvent into the collection device. Repeat this procedure three more times using 10 mL aliquots of methylene chloride. Dry the extract by passing it through anhydrous sodium sulfate. Thoroughly rinse the collection device with methylene chloride and add this solvent to the sodium sulfate.

Concentration and Analysis
Carefully concentrate the extract to a final volume. **Note:** Most extraction errors are caused by poor concentration technique. Do not concentrate below 0.5 mL or low recoveries will result.

• The ENVIRO-CLEAN® Universal PAH cartridge can be used on standard vacuum manifolds (# VMFF016GL), standard disk manifolds (#ECUCTVAC6) (with adapter part # ECUCTADP). The cartridge is specifically designed to fit the Horizon SPE DEX 4790® made by Horizon Technologies, Inc.

** Acetonitrile may be substituted for acetone and methylene chloride.
***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.

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EXTRACTION OF ORGANOCHLORINE PESTICIDES AND PCBs

Part #: ECUNIC18

February 3, 2009

Reagents:

Methylene Chloride
Acetone
Methanol
Anhydrous Sodium Sulfate (# ECSS25K)
ENVIRO-CLEAN® Universal C18 cartridge (# ECUNIC18)

Condition Cartridge

Rinse cartridge with 10 mL of methylene chloride and let the methylene chloride soak on the cartridge for approximately 1.5 min. Pull the methylene chloride through the cartridge to waste. Add 10 mL of acetone to the cartridge and let the acetone soak for approximately 1.5 minutes. Pull the acetone to waste and air dry the cartridge with full vacuum for a few seconds. Add approximately 10 ml of methanol to the cartridge and allow the methanol to soak for approximately 1.5 min. From this point until sample addition the cartridge must not go dry. Pull some of the methanol through the cartridge leaving a layer just covering the frit. Add approximately 20 mL of deionized water to the cartridge and pull most of the water through the cartridge to waste but do not allow the sorbent to dry.

Sample Addition

Adjust the pH of the sample to 2 using sulfuric acid. Do not use pH paper to test the pH of deionized water as poor recoveries may result. Add 5 mL of methanol (optional) and any surrogates to the sample. Mix. Add the sample to the cartridge under vacuum. Ideally, the sample should pass through the cartridge in approximately 15 – 20 minutes. Allow the cartridge to dry under full vacuum for 10 minutes.**

Extract Elution

Place a collection tube or vial under the cartridge. Add 5 mL of acetone to the sample bottle to remove any residue. Add the acetone to the cartridge. Allow the solvent to soak for 1 minute and pull the acetone into the collection device. Repeat this procedure 3 more times using a 10 mL aliquot of methylene chloride. Dry the extract by passing it through anhydrous sodium sulfate. Carefully rinse the collection device with methylene chloride and add the solvent to the sodium sulfate.

Concentration and Analysis

Carefully concentrate the extract. Solvent exchange if necessary. Note: Most extraction errors are caused by poor concentration technique.

• The ENVIRO-CLEAN® Universal C18 cartridge can be used on standard vacuum manifolds (# VMF016GL), standard disk manifolds (# ECUCTVAC6) (with adapter part # ECUCTADP). The cartridge is specifically designed to fit the Horizon SPE DEX 4790® made by Horizon Technologies, Inc.

**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.

Results:

spikes in DI water

Compound	Amount Spiked	% Recovery Ave n=4	STDEV
4,4'-DDD	0.2056	**97.0**	2.08
4,4'-DDE	0.2006	**93.0**	2.38
4,4'-DDT	0.2014	**101.0**	0.96
Aldrin	0.0999	**79.0**	5.10
Dieldrin	0.2046	**101.0**	0.82
Endosulfan I	0.1028	**100.0**	0.82
Endosulfan II	0.2054	**103.0**	0.96
Endosulfan sulfate	0.2124	**100.0**	2.06
Endrin	0.2016	**90.0**	7.33
Endrin aldehyde	0.2012	**106.0**	9.54
Endrin ketone	0.2068	**108.0**	1.73
Heptachlor	0.1032	**82.0**	5.29
Heptachlor epoxide	0.1034	**100.0**	0.96
Methoxychlor	1.0016	**96.0**	1.71
alpha-BHC	0.1032	**105.0**	0.96
alpha-Chlordane	0.099	**98.0**	0.50
beta-BHC	0.1043	**108.0**	1.50
delta-BHC	0.104	**104.0**	1.26
gamma-BHC (Lindane)	0.1038	**105.0**	0.82
gamma-Chlordane	0.0969	101.0	1.71

The recovery data shows excellent analyte recovery using UCT cartridge product ECUNIC18

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EPA Method 1664, Revision A
N-Hexane Extractable Material (HEM) (Oil & Grease) by
Solid-Phase Extraction & Gravimetry

ENVIRO-CLEAN® Universal Oil and Grease Cartridges
Part Number ECUNIOGXF
August 3, 2009

Method Summary

A sample of water, pH adjusted to <2, is extracted using a UCT Universal Oil and Grease Cartridge containing the solid-phase sorbent C18.

1) **Sample Collection**
   a) All samples must be acidified prior to analysis
   b) Adjust the pH of a 1-liter sample to 2 or lower by adding 5 mL of 6N HCl or 2.5 mL of concentrated H_2SO_4. A smaller volume of sample may be used provided all quality control requirements are met
   c) If the acid was added to the sample in the field, it is not necessary to repeat this step unless the pH has increased during storage
   d) Refrigerate sample to 0-6ºC if analysis is delayed more than 4 hours from collection

2) **Assemble**
   a) Assemble the UCT cartridge adapter(s) on a vacuum manifold
   b) Add the **ENVIRO-CLEAN® Universal Oil and Grease Cartridge** (Note 1)
   c) Place a cartridge in an automated extraction system if used in instead of a vacuum manifold
   d) Connect the vacuum manifold to a suitable trap and attach to a vacuum system capable of attaining a minimum of 25” Hg (635 mm) of vacuum

3) **Sample Spikes**
   a) Prepare a matrix spike by adding 40 mg/L of a PAR (precision and recovery) standard (Note 2)
   b) A concentration of 20 mg/L may also be used as long as the spike concentration is higher than the background concentration

4) **Condition the Cartridge**
   a) Wash the cartridge, including the sides, with 10 mL of hexane
   b) Allow to soak for 1 minute
   c) Draw the hexane through the cartridge to waste using vacuum. Discard the hexane
   d) Draw full vacuum through the cartridge for 2 minutes to remove the hexane
   e) Add 10 mL of methanol to the cartridge and slowly draw the methanol through the cartridge leaving a layer on the surface of the cartridge.
      Do not let the sorbent dry out.
   f) Soak for one minute then add 30 mL of DI water to the cartridge
   g) Draw the water through the cartridge to waste
   h) Do not allow the sorbent to completely dry to a powder before adding the sample, otherwise repeat this step starting with the addition of methanol

5) Sample Addition

a) Add the water sample directly to the cartridge and draw through under vacuum. This may take several minutes for complete flow through the cartridge depending upon the level of solids in the sample. Do not allow the flow rate to exceed 500 mL per minute. Note 3
b) Remove the cartridge before drying and tap the excess water from the bottom of the cartridge
c) Allow the cartridge to dry under full vacuum for 10 minutes to remove any residual water

6) Elution

a) Remove any water remaining in the bottom support of the cartridge with a paper towel
b) Place an extract collection vial in the manifold. Note 4
c) Rinse the sample bottle with 10 mL of hexane. Add the hexane rinse to the cartridge, washing the sides of the cartridge and the bottle holder if used. Soak for 1 minute. **Do not use any other solvents except hexane.**
d) Slowly draw the hexane through the cartridge and into the collection vial.
e) Do not allow the vacuum to blow air over the extract
f) Immediately, repeat this procedure 2 additional times using 10 mL of hexane for each rinse
g) While collecting the hexane do not allow it to splash out of the collection vial
h) Add another 10 mL of hexane to the cartridge, rinsing the bottle holder. Soak for 2 minutes. Draw the hexane through the cartridge and collect

7) Dry the Extract

a) Remove the collection vial from the manifold and cover with a screw cap
b) Shake the extract to form a water/hexane emulsion and immediately pour the extract through a sodium sulfate funnel or column containing approximately 40 g of anhydrous sodium sulfate. Do not use filter paper to hold the sodium sulfate. Glass wool is acceptable.
c) Collect the extract in a clean, tared vessel
d) Rinse the collection vial with 5 mL of hexane and add it to the sodium sulfate. Note 5

8) Gravimetric Analysis

a) Carefully evaporate the hexane using an analytical evaporator or similar device at $40^{O}C$ until a constant weight is obtained
b) Alternate concentration techniques and containers such as glass beakers or aluminum pans may be used
c) Do not dry on a hot plate or in an oven
d) Allow to cool in a desiccator before weighing
e) Record this weight as the mass per unit volume of oil and grease and report HEM as mg/L. Note 6

Notes

1) This cartridge is designed to fit the Horizon SPE-DEX® 4790 automated extraction system. The cartridge will also fit a standard 3 (# ECUCTVAC3) or 6 station (#ECUCTVAC6) disk manifold with our optional adapter (#ECUCTADP). The cartridge also fits a standard vacuum manifold (#VMF016GL).

2) PAR standards may be prepared by dissolving 20 mg of stearic acid and 20 mg of hexadecane in 5 mL of acetone.

3) To achieve good flow if very high solids are present, add glass wool to the cartridge prior to extraction to prevent clogging. The glass wool must be thoroughly rinsed with hexane as part of the cartridge during the elution step.

4) Procedures for drying the hexane extract:
   - Place a plug of glass wool in the bottom of a small funnel, then add 3-5 grams of sodium sulfate to the top.
   - Record the weight of a clean vial or weigh pan and place under the funnel
   - Pour the hexane from the eluate onto the bed of sodium sulfate
   - Rinse the sides of the vial and the sodium sulfate bed with clean hexane and collect in the weighed vial
   - Evaporate the hexane and report the results as mg/L HEM

5) Gloves are recommended when handling the vial as skin oils may affect the actual sample weight.

6) It is important that the extract not be over dried or dried at high temperatures as low recoveries may result by evaporation of volatile oils.

For Method 1664 updates see: http://www.epa.gov/waterscience/methods/

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ENVIRO-CLEAN® DVB Cartridge

Part Number: **ECDVB156**

EPA Method 8330B

September 16, 2009

The UCT ECDVB156 styrene divinylbenzene cartridge is designed to provide a new level of performance in solid-phase extraction for the analysis of **nitroaromatics, nitramines and nitrate ester compounds--explosive and explosive residue compounds**. With its high capture efficiency, fast flow and excellent dry times, laboratory throughput can be significantly improved using this solid-phase product

Product Benefits

- SPE using styrene divinylbenzene polymeric gel
- SPE has been shown to provide equal or superior results as compared to liquid-liquid extraction LLE*
- No hydrolysis of the solid-phase DVB
- Fast flow rates for rapid analyte capture
- Teflon frits in the cartridge eliminate particle fines yielding clean extracts
- Works well at all levels of analyte loading
- No Lot to Lot variability
- Excellent analytical reproducibility
- Packaged in metalized, sealed pouches to maintain product purity

Product Features

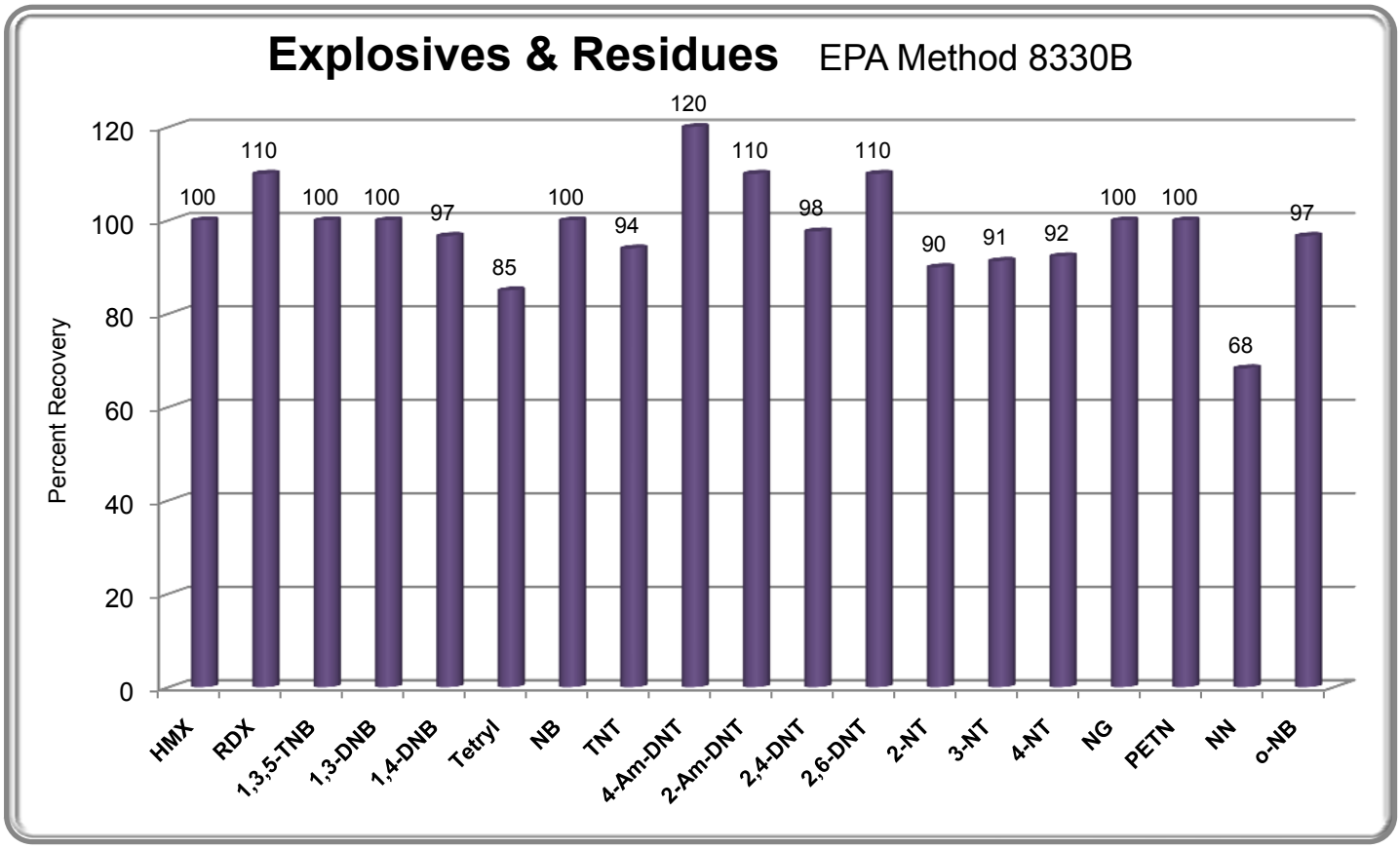
- 6 mL cartridge body manufactured from special polypropylene
- Each cartridge contains 500 mg of styrene divinylbenzene DVB sorbent
- Can be used on manual single or multi-station manifold systems
- Cartridges may be used with automated extraction systems

Nitroaromatics, Nitramines and Nitrate Ester Analytes and CAS Number

The following RCRA compounds have been determined by this method in water, soil & sediment matrices

Analyte	Abbreviation	CAS	% Recovery n=3
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX	2691-41-0	100
Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	121-82-4	110
1,3,5-Trinitrobenzene	1,3,5-TNB	99-35-4	100
1,3-Dinitrobenzene	1,3-DNB	99-65-0	100
1,4-Dinitrobenzene	1,4-DNB	10025-4	97
Methy-2,4,6-trinitrophenylnitramine	Tetryl	47945-8	85
Nitrobenzene	NB	98-95-3	100
2,4,6-Trinitrotoluene	TNT	118-96-7	94
4-Amino-2,6-dinitrotoluene	4-Am-DNT	19406-51-0	120
2-Amino-2,6-dinitrotoluene	2-Am-DNT	35572-78-2	110
2,4-Dinitrotoluene	2,4-DNT	121-14-2	98
2,6-Dinitrotoluene	2,6-DNT	606-20-2	110
2-Nitrotoluene	2-NT	88-72-2	90
3-Nitrotoluene	3-NT	99-08-1	91
4-Nitrotoluene	4-NT	99-99-0	92
Nitroglycerin	NG	55-63-0	100
Pentaerythritol tetranitrate	PETN	78-11-5	100
3,5-Dinitroaniline	3,5-DNA	618-87-1	68
1-Nitronaphthalene	NN	86-57-7	97
o-Dinitrobenzene	o-NB	528-29-0	100

Method 8330B Recovery Values



The UCT ECDVB156 styrene divinyl benzene cartridge for EPA Method 8330B shows excellent recovery of explosive and explosive residue analytes

Nitroaromatics, Nitramines, and Nitrate Esters by High Performance Liquid Chromatography (HPLC)

Method 8330B

Method Summary

This method is used for the trace analysis (ppb) of explosive and propellant residues in water, soil and sediment matrices using high performance liquid chromatography (HPLC) and a dual wavelength UV or diode array detector. It is an updated method from 8330 promulgated September 1996. In this method aqueous samples are preconcentrated using the UCT styrene divinylbenzene SPE sorbent cartridge ECDVB156 as described in Method 3535 then eluted with acetonitrile or other appropriate solvent. The final extract is diluted with water as appropriate to bring the concentration into an analytical range suitable for HPLC analysis.

Interferences

- Solvents, reagents, glassware and other sample processing hardware may show interferences in sample analysis. All material must be demonstrated to be free from interferences under conditions of the analysis by analyzing method blanks

- 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

I Sample Preparation--Solid Matrices, (e.g. soil) (from Method 3535)

A soil sample is placed in a glass vial and dried with sodium sulfate. Acetonitrile (ACN) is added to the sample then mixed by vortex to suspend the soil in the solvent. The sample vial is then placed in a chilled ultrasonic bath for sonication. After 18 hours of sonication the sample is centrifuged for 15 to 20 minutes and the ACN solvent portion is removed from the vial. The volume of the removed aliquot is doubled by adding an equal volume of calcium chloride solution. The extract is then filtered through 1 μm Teflon filters.

1) Procedure (Ultrasonic Preparation)

a) Weigh 2.0 ± .04 g of solid sample into a 25 mL glass vial
b) Add 2 g of sodium sulfate and mix
c) Add 0.1-mL explosives soil surrogate to all samples, blanks, and spikes
d) Using a syringe or pipette, add 0.5 mL explosives spike to the LCS, LCSD if applicable, matrix spike, and matrix spike duplicate samples
e) Using a graduated cylinder, add 10 mL of ACN and vortex swirl the sample for approximately 1 minute to suspend the soil in the ACN
    a. Place the sample in a cooled ultrasonic bath (<10°C). Make sure the water level in the sonicator is at least as deep as the level of solvent in the vial. Sonicate for 18 hours
    b. After sonication, centrifuge the sample for 15 to 20 minutes to separate the solids from the solvent

2) Final Preparation

a) Using a pipette, add 5 mL of a (5.0 gram/L) calcium chloride ($CaCl_2$) solution to a 10 mL volumetric flask
b) Using a disposable pipette, bring to volume using the solvent layer of the centrifuged sample
c) Mix thoroughly then allow the mixture to stand 15 minutes
d) Filter the sample through 1 μm Teflon filters using a disposable syringe
e) Discard the first 3 mL and retain the remainder in an appropriately labeled 12 mL vial
f) Store in a refrigerator until analysis

II Sample Preparation--Aqueous matrices, (e.g. water) (from Method 3535)

A measured volume of the aqueous sample is adjusted to a specified pH then extracted using the UCT styrene divinylbenzene SPE sorbent cartridge **ECDVB156**. Two challenges are noted for aqueous sample preparation. First, any particulate matter in the original sample must be included in the sample aliquot that is extracted. Second, the sample container must be rinsed with solvent as the majority of organic analytes are hydrophobic and may adhere to the sample container surfaces.

Note

- 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 5.0 mL of methanol and surrogate standards to all samples and blanks
- Add matrix spikes standards to representative sample replicates

Note: Adjustment of sample pH may result in precipitation or flocculation reactions and potentially remove analytes from the aqueous portion. The analyst should note the formation of such precipitates or floc and transfer any such material with rinses to the SPE extraction cartridge. Do not let the cartridge dry out after cartridge conditioning with acetonitrile (ACN)

A. Glass Apparatus Washing:

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 mL ACN	

Draw solvents through the cartridge under low vacuum

B. Cartridge Conditioning:

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		

*Soak time

Draw solvents through the cartridge under low vacuum

1) **Initial Preparation**
   a) Assemble a DVB extraction cartridge **UCT ECDVB156** in an all-glass manifold.
   b) Use of a manifold for multiple extractions or automated extraction equipment is acceptable

2) **Cartridge Conditioning**
   a) Follow the 4 steps in Table **Cartridge Conditioning for** solvent quantities

Do not let the cartridge dry out once the cartridge is conditioned as this may affect analyte recovery

b) **Sample Extraction**
   a) Add the contents of the sample bottle to the cartridge
   b) Adjust vacuum to about 10-15 mm Hg to obtain a uniform flow rate of approximately 10 ml per minute. This will require about 1 hour for sample extraction
   c) After all the sample is drawn through, draw air through the cartridge for 15 minutes to dry it
   d) Do not dry for longer than 20 minutes as lower recovery may result

c) **Cartridge Elution**
   a) Insert a collection tube in the base of the vacuum manifold

Explosives

   b) Add 4 mL of ACN and soak for 3 minutes
   c) Draw through using gravity flow or very low vacuum into a collection tube
   d) Store extract in freezer until analysis

Nitramines and Nitroaromatics

   b) Add 5 mL ACN, soak for 3 minutes
   c) Draw through using a gravity flow or very low vacuum into a collection tube
   d) Store extract in freezer until analysis

d) **Extract Concentration**
   a) Concentrate the extract to 0.7 mL under a gentle stream of nitrogen in a warm bath at 40^{O} C
   b) Transfer the extract to a 1 mL volumetric flask
   c) Add internal standard for a extract concentration of 5 µg/mL
   d) Extract is now ready for analysis by HPLC

RP-HPLC Columns for the Analysis of Explosive Residues	
Primary Columns	C-18 reversed-phase HPLC column, 25-cm x 4.6-mm, 5 µm C8 reversed-phase HPLC column, 15-cm x 3.9-mm, 4 µm
Secondary Columns	CN reversed-phase HPLC column, 25-cm x 4.6-mm, 5 µm Luna Phenyl-Hexyl reversed-phase HPLC column, 25-cm x 3.0-mm, 5 µm

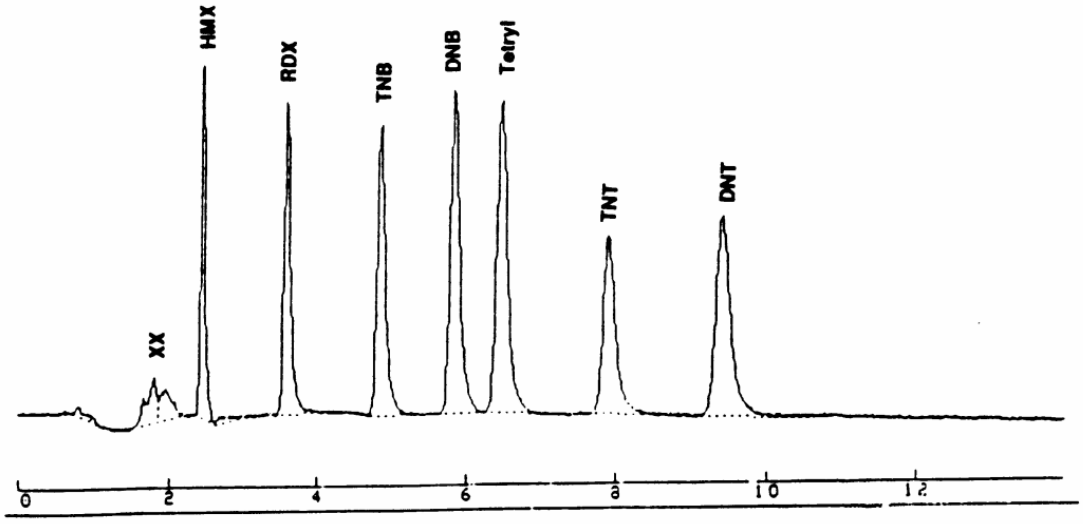
Injection volume: 100 µL

UV Detector: Dual 254nm & 210nm or Photodiode Array

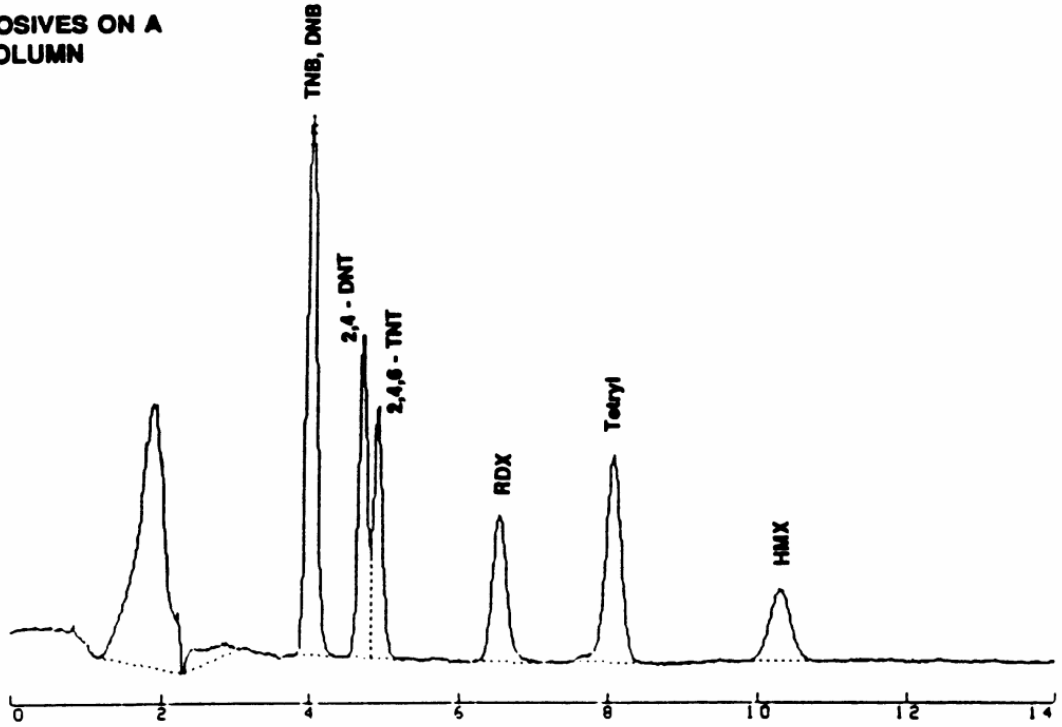
Mobile phase: For C18 & CN column, 50:50 methanol:water

Energetic Compounds Currently Not Target Analytes of Method 8330	
Compound	**Symbol**
picric acid (PA)/Ammonium picrate	AP
2,4-diamino-6-nitrotoluene	
2,6-diamino-4-nitrotoluene	
hexanitro-hexaazaisowurtzitane	CL-20
1,3,3-trinitroazetidine	TNAZ
hexahydro-1-nitroso-3,5-dinitro-1,3,5- triazine	MNX
hexahydro-1,3,5-trinitroso-1,3,5-triazine	TNX
nitrocellulose	NC
nitroguanidine	NQ
diphenylamine	DPA
n-nitroso-diphenylamine	NDPA
2-nitrodiphenylamine	
4-nitrodiphenylamine	
2,4-dinitrodiphenylamine	

EXPLOSIVES ON A C18 COLUMN



EXPLOSIVES ON A CN COLUMN



RETENTION TIMES AND CAPACITY FACTORS ON LC-18 AND LC-CN COLUMNS

|Analyte	LC-18 RT minutes	LC-CN RT minutes
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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Recovery of Various Herbicides in Water Using UCT ENVIRO-CLEAN® Universal C18 Solid-Phase Extraction Cartridges

Part #: ENVIRO-CLEAN® Universal C18 cartridge ECUNIC18

April 8, 2009

The GC analysis of select phenoxy acid based herbicides found in various surface waters may be easily determined by the use of this procedure. Herbicides DCPA (Dacthal), MCPP (Mecoprop), Dicamba, MCPA, Dichloroprop, 2,4-D and 2,4,5-TP (Silvex), 2,4,5-T, Dinoseb, 2,4-DB, common household weed killers are evaluated using this method.

1. **Vacuum Manifold Set-Up**
   a) Assemble a disk manifold vacuum extraction apparatus
   b) Install an 83 mL C18 ENVIRO-CLEAN® Universal cartridge

   Note: All glassware must be acid rinsed with dilute sulfuric acid

2. **Sample Preparation**
   a) Adjust the pH of a 500-1000 mL water sample to 2.0 ±0.5 with 6N HCl
   Use of a pH meter is highly recommended over pH paper

3. **Cartridge Conditioning**
   a) Add 5 mLs of methanol to the cartridge
   b) Draw a few drops through until methanol touches top of frit
   c) Hold for 1 minute
   d) Rinse cartridge with 15 mLs of water adjusted to pH 2 with 6N HCl

Note: Do not let the cartridge dry out at this point otherwise repeat conditioning step

4. **Sample Extraction**
   a) Adjusting the vacuum setting such that flow is about 25 mLs per minute
   b) Draw the water sample through the cartridge
   c) Draw air through the cartridge under full vacuum for 5 minutes to dry the cartridge
   d) Tap the cartridge to help remove excess water. **DO NOT OVERDRY**

5. **Elution**
   a) Insert a collection tube containing 2 grams of acidified sodium sulfate
   b) Add 10 mLs of methanol to the sample bottle and swirl
   c) Add to cartridge

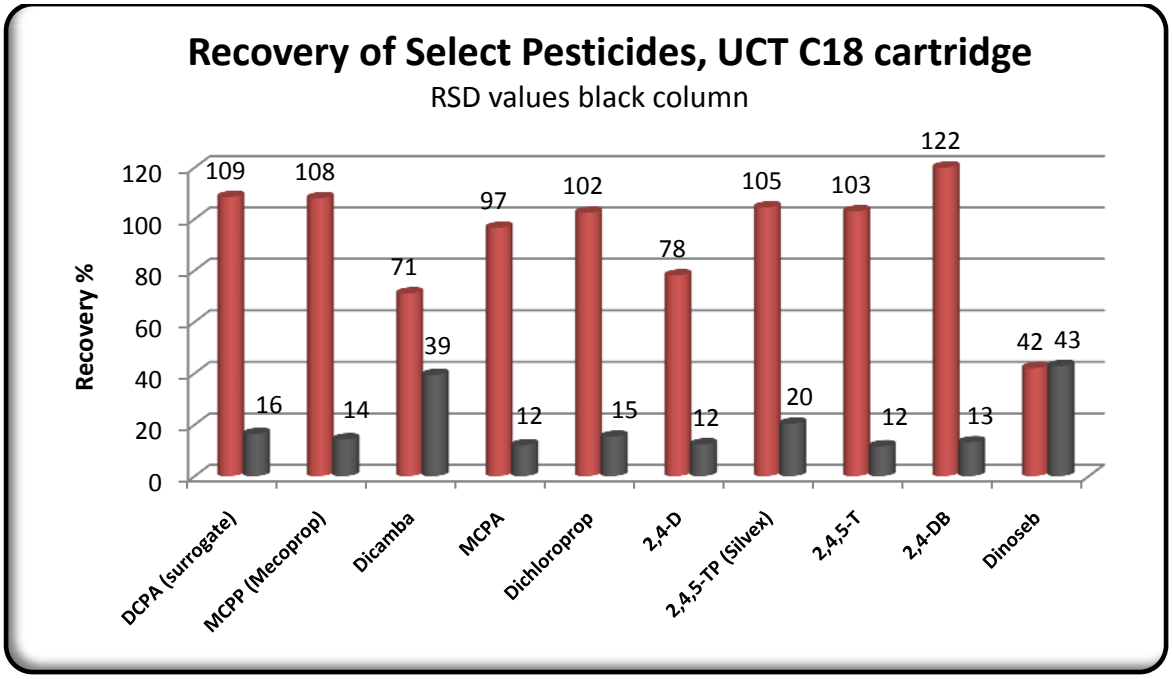
6. **Analyte Elution**
   a) Elute cartridge using 8 mL of methanol
   b) Repeat with a second aliquot of methanol
   c) Add 5 mLs of methylene chloride to the sample bottle and rinse
   d) Add to cartridge
   e) Elute cartridge using 5 mL of methylene chloride (CH_2Cl_2)
   f) Repeat with a second aliquot of methylene chloride and add to e)

7. **Concentration and Exchange**
   a) Derivitize 1 mL from step 6.f) with diazomethane
   b) Exchange into 1 mL hexane

Methylation is detailed in EPA Method 8151 or 515. Extract must be very dry otherwise methylation may be incomplete

Herbicide Data Table

Pesticide	CAS	UCT C18	% SD
DCPA (surrogate)	1861-32-1	109	16
MCPP (Mecoprop)	93-65-2	108	14
Dicamba	62610-39-3	71	39
MCPA	94-74-5	97	12
Dichloroprop	120-36-5	102	15
2,4-D (amine salt)	2008-39-1	78	12
2,4,5-TP (Silvex)	93-72-1	105	20
2,4,5-T	93-76-5	103	12
2,4-DB	94-82-6	122	13
Dinoseb	88-85-7	42	43



* **Procedure for Preparing Acidified Sodium Sulfate Anhydrous from EPA Method 8151A 5.10**,
Product Number: UCT brand sodium sulfate ECSS25K

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A Method for the Extraction of Imidachloprid from Pond Water

Part #: EUBCX1M6 1000 mg of Benzenesulfonic acid in 6 mL cartridge

April 8, 2009

1. **Prepare Sample**
   a) Adjust the sample pH to less than 7 using 0.1N HCl
   b) If solids are present, settle by centrifugation. Do not use filter paper

2. **Condition ENVIRO-CLEAN® Extraction Cartridge**
   a) Wash the BCX cartridge with 5 mL of methanol and 5 mL of deionized water.
   Do not allow the sorbent to dry out

3. **Add Sample**
   a) Add water sample to the BCX cartridge
   b) Adjust vacuum for a flow rate of 1-3 mL per minute. One drop every 3 seconds is ideal
   c) Wash cartridge using deionized water or other pH neutral solvent through the BCX cartridge at high vacuum
   d) Dry cartridge under full vacuum for 10 minutes

4. **Sample Elution**
   a) Add a collection tube to the vacuum manifold
   b) Elute the cartridge with 6 mL of 4% ammonium hydroxide in methanol at a rate of 1 mL per minute*

5. **Evaporate**
   a) Concentrate under gentle N_2

6. **Analyze by LC**

*Only use fresh ammonium hydroxide. The 4% ammonium hydroxide in methanol must be fresh daily.

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EPA Method 608 ATP*

Part #: ECUNIC18

August 25, 2009

The EPA has accepted the use of C18 bonded phases in packed cartridge format expanding the method from a disk only approach. This method is used in place of liquid-liquid extraction. The UCT ECUNIC18 Universal cartridge has been designed to provide a high level of performance for the solid-phase extraction (SPE) and analysis of certain **organochlorine pesticides and PCB's** in municipal and industrial discharges. With the cartridge's high capture efficiency, fast flow and rapid dry times, laboratory throughput can be significantly improved and sample preparation time reduced.

Product Benefits

- SPE cartridge containing bonded C18 phase
- Excellent pH stability under acidic conditions
- Fast flow rates for rapid analyte capture
- Works well at all levels of analyte loading
- Consistent results for excellent reproducibility
- PTFE frits eliminate potential contamination yielding clean extracts
- Packaged in metalized bags to maintain product cleanliness

Product Features

- Cartridges manufactured from clean proprietary polypropylene
- Each cartridge contains 1100 mg endcapped C18 bonded ultra-clean silica sorbent
- Can be used on manual single or multi-station vacuum manifold systems
- Can be used with a variety of automated extraction systems

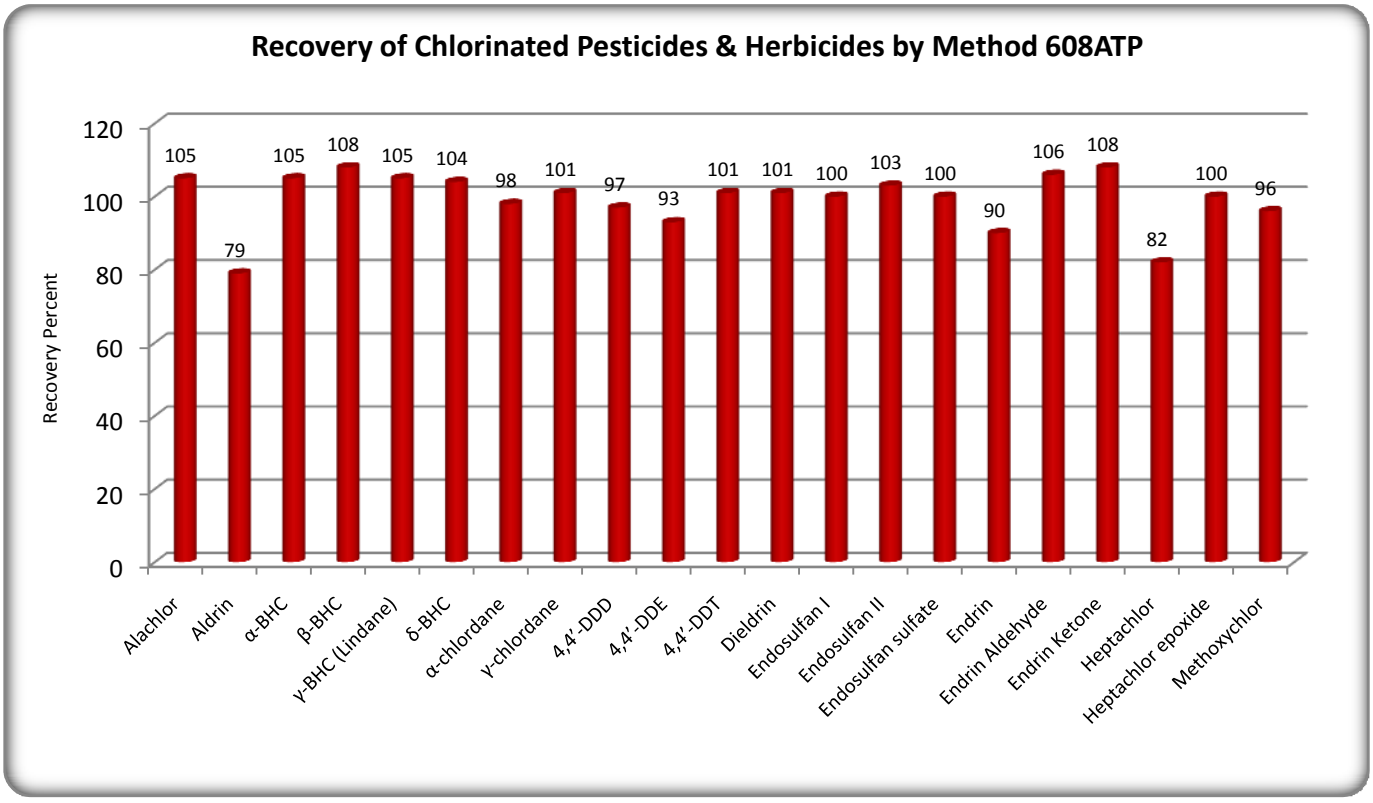
UCT Products Required:

1) Universal Cartridge ECUNIC18
2) Florisil PR® column cleanup (optional) **EUFLS1M6**
3) $NaSO_4$ Drying Tube UCT ECSS15M6

Recovery of Method 608ATP Analytes

Analyte	CAS	Amt Spiked µg/L	Average Recovery %	Stdev
Aldrin	309-00-2	0.0999	79	2.08
α-BHC	319-84-6	0.1032	105	0.96
β-BHC	319-85-7	0.1043	108	1.50
γ-BHC (Lindane)	58-89-9	0.1038	105	0.82
δ-BHC	319-86-8	0.1040	104	1.26
α-chlordane	5103-71-9	0.0969	98	0.50
γ-chlordane	5103-74-2	0.0969	101	1.71
4,4'-DDD	72-54-8	0.2056	97	2.08
4,4'-DDE	72-55-9	0.2006	93	2.38
4,4'-DDT	50-29-3	0.2014	101	0.96
Dieldrin	60-57-1	0.2046	101	0.82
Endosulfan I	959-98-8	0.1028	100	0.82
Endosulfan II	33213-65-9	0.2054	103	0.96
Endosulfan sulfate	1031-07-8	0.2124	100	2.06
Endrin	72-20-8	0.2016	90	7.33
Endrin Aldehyde	7421-93-4	0.2012	106	9.54
Endrin Ketone	53494-70-5	0.2068	108	1.73
Heptachlor	76-44-8	0.1032	82	5.29
Heptachlor epoxide	1024-57-3	0.1034	100	0.96
Methoxychlor	72-43-5	1.0016	96	1.71

UCT Cartridge ECUNIC18 Shows Excellent Recovery with Laboratory Fortified Blanks (LFB)



An Alternative Test Procedure for the Measurement of Organochlorine Pesticides and Polychlorinated Biphenyls in Waste Water

Method 608ATP

Federal Register Vol. 60, # 148, August 2, 1995

Method Summary*

A 1-liter sample of water is extracted by drawing through a **UCT C18 Universal cartridge ECUNIC18**. The analytes captured on the solid-phase are eluted from the cartridge using a small volume of acetone followed by methylene chloride ($MeCl_2$). The eluant is dried and exchanged into hexane for analysis by injection into a gas chromatograph with electron capture detection system (GC/ECD) fitted with a high-resolution fused silica capillary column.

Interferences

- Interferences may generally be attributed to contamination from solvents, glassware or other laboratory equipment leading to anomalous GC peaks. Glassware must be scrupulously cleaned and high purity solvents used.
- Interfering contamination may occur when a sample of low concentration is analyzed immediately after a sample of high concentration. A laboratory blank should be inserted between low and high concentration samples to minimize this potential problem
- Phthalate esters may pose a problem in pesticide analysis when using electron capture detection. This results from contact with common laboratory plastics such as PVC

Sample Collection

- Samples must be collected in glass containers following conventional practices **except** the bottle must not be prerinsed with sample before collection
- Samples must be refrigerated at 4°C from collection to analysis and extracted within 72 hours of collection
- If samples are to be held longer than 72 hours the pH must be adjusted to 5.0-9.0 with sodium hydroxide or sulfuric acid depending upon initial pH

Procedure

1) Condition Cartridge

a) Insert a cartridge into the glass vacuum manifold or automated extraction system
b) Wash the cartridge with 10 mL of methylene chloride ($MeCl_2$). Apply solvent for 1 minute then draw through to waste
c) Draw air under full vacuum to completely dry cartridge
d) Add 10 mL of methanol (MeOH) to the cartridge then slowly draw some of it through
e) Allow the cartridge to soak for 1 minute in methanol
f) Do not let the cartridge go dry after addition of methanol otherwise repeat the addition of methanol addition step
g) Rinse the cartridge with 10 mL of reagent water and draw most of it through leaving a thin layer on the top of the sorbent

2) Sample Addition

a) Adjust sample pH to < 2 using sulfuric acid
b) Adjust the vacuum and draw the sample water through the cartridge over a 20-30 minute time period
c) 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
d) Dry the cartridge by drawing air through for about 5-10 minutes

3) Extract Elution

a) Insert an eluate 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 20 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 tube (**UCT ECSS15M6**) which contains 5 grams anhydrous sodium sulfate. Alternatively, use 5 grams of sodium sulfate over a bed of glass wool in a funnel
b) Rinse the drying tube or sodium sulfate bed with 2 x 3 mL portions of 1 methylene chloride
c) Concentrate sample using a Kuderna-Danish (KD) concentrator while performing solvent exchange into hexane. Other drying techniques may be used
d) Concentrate sample under a gentle stream of N_2 while gently heating in a water bath
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® Clean-up (if needed)

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

UCT EUFLSA1M6 – 1000 mg small particle Grade A Florisil® for slower gravity flow

UCT EUFLS1M6 – 1000 mg regular particle PR Grade Florisil® for more viscous samples

1) Procedure

a) Place a cartridge in a vacuum manifold

b) Prerinse 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 column

f) Collect extract by gravity

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

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

Sulfur Clean-up (if needed)

UCT ECCU01K – 1 kG copper granules

1) Procedure

a) Post Sample Extraction

a) Place 4 grams of copper bead in a glass vial
b) Add 2 mL of liquid sample extract to the vial

b) 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 4grams of fresh copper beads

Note: For the analysis of PCB type analytes, copper may reside in the extract

c) Analysis, GC/MS or LC/MS

a) Transfer clean extract to autosample vial
b) Inject 1-2 µL for GC
c) Inject 5-10 µL for LC

d) Sample is now ready for 6081tp analysis

Sample Analysis by 608ATP

5) Analysis

a) Inject a 1-2 µL aliquot into a GC
b) Identify the analytes in the sample by comparison of the retention time to known reference chromatograms

*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

Florisil® is a registered trademark of U.S. Silica

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EXTRACTION OF METALS

Part #: EUTAX15Z

February 3, 2009

Metals: Tin, Nickel, Mercury, Copper, Chromium, Ruthenium

Matrix: Water, Blood, Biological Fluids, Organic Solvents & Tissue Homogenates

1) **Sample Pre-treatment**

The primary concern using ion exchangers is to adjust the pH of the compound of interest so that it is totally ionized. Adjust the sample to pH 7 with buffer or ammonium hydroxide.

Aqueous or Organic Solvent Samples:

Adjust sample to pH 7.0 with 100 mM dibasic sodium phosphate buffer and vortex.

Whole Blood, Serum or Plasma:

To 1 ml of sample add 4 ml of D.I. H20 and vortex. Let stand 5 minutes and centrifuge for 10 minutes at 2000 rpm and discard pellet. Adjust to pH 9.0 with 100 mM dibasic sodium phosphate buffer or ammonium hydroxide.

2) **Column Conditioning**

Add 3 ml of methanol followed by
Add 3 ml of water.
Add 3 ml of buffer pH 7.0

3) **Sample Application**

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**

Wash the column with 2 ml of pH 7.0 buffer used in column equilibration.

5) **Elution**

Elute with 3 ml of acidic methanol (2% HCl, pH 2.0).

Alternative elutions:

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).

DCN-903020-119

Ion Exchange Sorbents for Metals Extraction-Analysis & Sorbent Use Selection Guide

UCT ENVIRO-CLEAN® (Ion-Exchange Cartridges)*

January 5, 2010

The determination of trace metals in aqueous environmental samples or other matrices often require sample pretreatment and cleanup 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) Assemble an all glass extraction apparatus

b) Place a UCT ENVIRO-CLEAN® cartridge on the apparatus

Note: Cartridge selection will depend on the volume of sample or the concentration of metal to be extracted

c) Condition 1mL 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))

d) 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)

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

a) Add 3 mL of the triethylamine solution to the cartridge

b) Adjust flow rate for a flow of 1-3 mL/minute

c) Dilute eluant to an appropriate volume for detection using reagent water

d) 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 metals 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 100mM 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 100mM 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 100mM 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 100mM 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 100mM nitric acid solution. Additional Hg(II) is released under basic elution.

For further data and specific information and discussion of each sorbent see separate UCT publications: **Topics in Solid-Phase Extraction: Metals Analysis.**

*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.

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PESTICIDES IN FATTY MATRICES EXTRACTION PROCEDURE REAGENTS:

February 3, 2009

Reagents:

Acetone
Toluene
Acetonitrile
Ethyl acetate
Magnesium sulfate anhydrous (UCT #ECMAG00D)
Sodium chloride (UCT #ECNACL05K)
UCT QuEChERS product (CUMPSC1815CT)
UCT product (ECPSAC1856)

1) Weigh 20.0 ± 0.10 grams (g) of homogenized sample into a 250 mL plastic centrifuge bottle that has been tared on a weigh balance capable of weighing to 0.01 grams.

2) Fortify each sample with process control spiking (PCS) solution.

3) Add 50 mLs of Ethyl Acetate (EtAc) to each tube containing a sample.

4) Fortify each sample with internal standard (ISTD) spiking solution.

5) Reduce sample material particle size by using a high speed disperser for approximately 1 minute.

6) Add 2 g of anhydrous magnesium sulfate (MgSO4) **(#ECMAG00D)** and 0.5 g anhydrous sodium chloride (NaCl) **(#ECNACL05K)**. {Note: Do not get the powders in the threads or rims of the tubes.}

7) Seal the tube and shake vigorously for approximately 1 minute mechanically or by hand, making sure that the solvent interacts well with the entire sample and that the crystalline agglomerates are broken up sufficiently.

8) Cool the sample in a -20 ºC freezer for approximately 30 minutes.

9) Centrifuge at 10,000 RCF for 5 minutes.

10) Decant > 50 mL of the EtAc 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 to 50 mL with a Pasteur pipette.

11) Concentrate the extract under a stream of nitrogen with a 70 ºC water bath until the volume remains constant (this will be ~ 3 mLs and will take ~ 1 hour).

12) Dilute to 20 mLs with acetonitrile (MeCN) and cap with a glass stopper, vortex for 1 minute, and freeze at -70 ºC for 30 minutes.

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

14) Directly after centrifugation in step 13, filter > 15 mLs of the MeCN layer of the extract with a 0.45 µm syringe filter into a 15 mL glass centrifuge tube.

15) 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.

16) For LC/MS/MS analysis, transfer 1 mL of extract to a 2 mL mini-centrifuge tube that contains 0.05 g PSA, 0.05 g C18, and 0.15 g MgSO4. **(#CUMPSC18CT)**

17) Vortex for 1 minute and centrifuge.

18) Transfer to auto sampler vial and analyze by LC/MS/MS.

19) For GC analyses, use the dual layer cartridge **(#ECPSAC1856)** containing 500 mg of C18 sorbent and 500 mg of primary secondary amine (PSA) sorbent with approximately 0.75 – 0.80 grams (~ 0.6 cm = 0.25 inches) of anhydrous MgSO4 added to the top of the cartridge.

20) Condition the SPE cartridge by adding one cartridge volume (4.0 mLs) of acetone/toluene (3:1; v/v) using a UCT positive pressure SPE manifold and eluting to waste.

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

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

23) Elute SPE cartridge in a drop wise manner (Regulated Flow Pressure = 35 psi) into a properly labeled 15 mL graduated glass centrifuge tube with acetone/toluene (3:1; v/v), collecting the eluate while washing the SPE cartridge **three times** with **4 mLs of eluant**. Do not allow the cartridge to go dry until step 24.

24) 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.

25) Using an N-Evap with the water bath set at 50°C and nitrogen flow set at <10 liters per minute (LPM) {typical setting in 2 – 6 LPM}, evaporate the sample to approximately 0.5 mL.

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

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

28) Bring the volume to 1.0 mL with toluene and Vortex to mix solvent into sample.

29) Analyze by GCMS-EI and GCMS-NCI.

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ANALYSIS OF TOBACCO ALKALOIDS

Part #: EUBCX1H2Z

February 3, 2009

A strong cation exchange column (benzene sulfonic acid) is used to capture tobacco alkaloids which have been ionized by the use of acid. Non-polar and other extraneous compounds are removed from the extract yielding cleaner chromatograms without loss of target alkaloids.

Sample Preparation

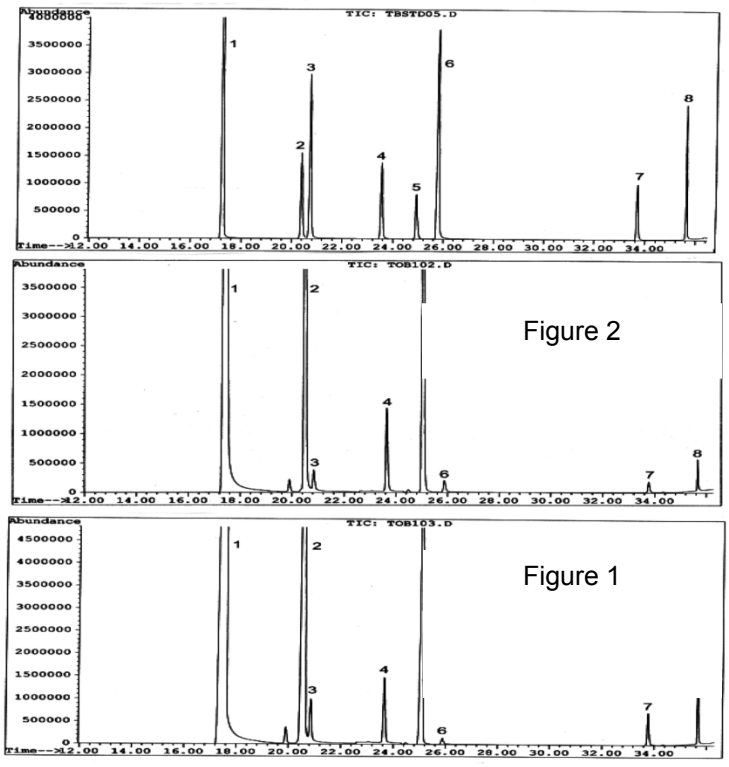
- Weigh 100 mg of fine ground tobacco in a screw cap vial, add 6 mL 0.1M sodium acetate buffer (pH 4.5) and 100 µL internal standard (d4-nornicotine, 1 µg/µL).
- Mix on rotating shaker for 10 minutes, then filter extract through 20 micron frit filter column.
- Add 300 µL glacial acetic acid and mix.
- Condition SPE column, UCT Part #: EUBCX1H2Z (200 mg benzene sulfonic acid sorbent) with 3 mL of MeOH:1.0M acetic acid (80:20).
- Pour sample onto column, aspirate for 1-2 mL/min by vacuum.
- Wash column with 3 mL of MeOH: 1.0M acetic acid (80:20).
- Dry column for 5-10 min with full vacuum.
- Elute alkaloids with 3 mL CH_2Cl_2/isopropanol/NH_4OH (70:26:4) by gravity.
- Evaporate eluant to dryness with nitrogen and low heat (< 40° C).
- Reconstitute with 200 µL ethyl acetate.
- Analyze on GC/FID/NPD or GC/MSD.

Results:

Alkaloid	Pka	Flue Cured Tobacco	Burkey 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

Results show the average of 15 separate runs and indicate that reproducibility is excellent

Sample Chromatogram



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

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

DCN-903020-60

SAMPLE PREPARATION OF GLYPHOSATE (N-PHOSPHONOMETHYL GLYCINE) AND GLUFOSINATE (RS)-2-AMINO-4-(HYDROXYL-METHYL-PHOSPHORYL) BUTANOIC ACID BY SOLID-PHASE ANION EXCHANGE EXTRACTION

Part #: EUQAX2M6

February 3, 2009

Glyphosate, (CAS 1071-83-6) known principally as Roundup®, and glyfosinate (CAS 51276-47-2) (Basta®, Challenge®) are known as broad spectrum, nonselective systemic herbicides that are absorbed through the leaves of plants. It is used in many countries throughout the world because of its effectiveness at killing grass, broadleaf and woody plants. Sample preparation can be achieved using the UCT Enviro-Clean® anion exchange cartridge EUQAX2M6. For this procedure, a water sample is raised to pH 6 or more to ionize the analyte. The sample is drawn through the cartridge followed by elution with acidified methanol.

Sample Conditioning

- Adjust water sample pH to 6 or higher.

Cartridge Conditioning

- Add 5 mL of methanol to the cartridge
- Draw methanol through with vacuum leaving enough to cover surface of sorbent
- Rinse the cartridge using 10 mL of reagent water

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

Extraction Protocol

- Draw a known volume of pH adjusted sample water through the cartridge

Note: Sample volume is determined by the quantitation limit

- Adjust vacuum so that flow is approximately 1 - 3 mL per minute
- Rinse cartridge using 10 ml of reagent water
- Dry the cartridge by drawing full vacuum for 10 minutes

Analyte Elution

- Elute using 4 mL of 1 mol/L HCl/methanol solution (4/1)
- Add eluant to the cartridge then draw through at 1 mL/minute
- Evaporate to dryness with N_2 flow in a water bath heated to 50^{O}C

Analysis

Analysis of glyphosate is conducted using GC/MS

- Add 50 µL of N-methyl-N-(tert-butyldimethylsilyl) triflouroacetamide (MTBSTFA) and 50 µL of dimethylformamide
- Sonicate at room temperature for 2 minutes
- Transfer to GC vial insert and cap
- Heat to 80^{O}C for 30 minutes
- Cool to room temperature and analyze by GC/MS

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 • 800.385.3153 • 215.781.9255 • www.unitedchem.com • Email: methods@unitedchem.com

QuEChERS

Quick, **E**asy, **Ch**eap, **E**ffective, **R**ugged and **S**afe

September 3, 2009

Solid-Phase Method for Extraction of Pesticide Residues

QuEChERS, the Multiresidue Method of Choice

QuEChERS (pronounced *Catchers*), an acronym for **Q**uick, **E**asy, **C**heap, **E**ffective, **R**ugged and **S**afe, is a sample preparation and clean-up technique for the analysis of multiple pesticide residues in high moisture food samples. Since the development and publication of the method by Anastassiades and Lehotay, et al in 2003, **QuEChERS** has been gaining significant popularity. It is the method of choice for food analysis because it combines several steps and extends the range of pesticides recovered over older, more tedious extraction methods. The method has undergone various modifications and enhancements over the years since its first introduction. These have been designed to improve recovery for specific types of pesticides. Although Schenck and Vega published a clean-up method in 2001 prior to the introduction of **QuEChERS,** its techniques may be incorporated in the current method enhancing **QuEChERS** utility.

While primarily used for the analysis of fruits and vegetables, the **QuEChERS** method is also finding utility as the sample preparation method of choice for a full range of food products such as honey, nut meats, soybeans, animal feeds, foliage and other foods as well. Organic acids, plant pigments and other potential contaminants are removed during the cleanup process yielding cleaner chromatograms. The method offers the advantages of high recoveries, accurate results, high sample throughput and low non-chlorinated solvent usage. This reduces reagent costs and staff exposure to hazardous solvents. Additionally, glassware usage and labor costs are reduced since sample requirements are small and less bench space is required. The broad utility and ease of use makes the method an excellent choice for residue analysis.

The Need for QuEChERS

Consensus has been growing within the scientific community that small doses of pesticides and other chemicals can have adverse health effect on humans and animals. In the last few years, pesticide residues in foods have become a major consumer safety issue since application of chemical pesticides for food products is widely used. Also, as large quantities of fruits and vegetables are now imported, concerns have arisen as to their safety versus those grown domestically. To address these concerns, regulatory agencies have resorted to the use of various analytical methods to monitor these food stocks increasing both the scope of residue analysis and the number of samples analyzed.

The analysis of pesticide residues in food and environmental samples has been practiced for over 40 years by laboratories throughout the world. The method of extracting pesticide residues from food samples and preparing them for analysis is a time consuming, expensive, and labor intensive process. To address this problem, new multiresidue methods such as the **QuEChERS** method have been developed to accommodate the increase in

sample loads. This new multiresidue method has yielded an increase in laboratory throughput while also improving analytical sensitivity. Improved throughput has been accomplished primarily by enhanced sample cleanup products that reduce potential interferences to yield cleaner chromatograms and reduced potential instrument downtime.

Multiresidue methods cover a broad scope of pesticides (see Appendix I) and offer the advantages of being cost-effective, rapid, sensitive, and sufficiently accurate for regulatory purposes. The **QuEChERS** method streamlines analysis and makes it easier and less expensive for analytical chemists to examine high moisture foods where water content may present problems with extraction of pesticides. Even dried vegetation can be rehydrated prior to extraction to facilitate the use of the **QuEChERS** method.

How Does QuEChERS Work?

QuEChERS is known as a multiclass, multiresidue method (MRM) for analysis of pesticides from high water content (80-95%) matrices. Multi-residue pesticide analysis of food and environmental samples can be problematic due to the wide range of chemical properties encountered with pesticide residues. Also, the complex sample matrix may contain abundant quantities of chlorophyll, lipids, sterols and other components that can interfere with good sample analysis. Use of the **QuEChERS** method reduces these problems.

The **QuEChERS** method now published as AOAC method **2007.01 "Determination of Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate"** consists essentially of a liquid-liquid micro extraction. This is followed by sample clean-up to remove unwanted matrix materials that interfere with chromatographic analysis. After shaking a sample with acetonitrile buffered with sodium acetate, partitioning is aided by the addition of $MgSO_4$. The preferred solvent is acetonitrile because it has been shown to provide extraction of the broadest range of organic compounds without co-extraction of large amounts of lipophilic material. Although other solvents can be employed depending upon the residues to be extracted, acetonitrile is highly compatible with GC/MS and LC/MS applications showing the fewest interferences.

Some modifications to the original **QuEChERS** method have been introduced to ensure efficient extraction of pH dependent compounds (e.g. phenoxyalcanoic acids), to minimize degradation of susceptible compounds (e.g. base and acid labile pesticides) and to expand the spectrum of food matrices amenable by the method. Buffering with citrate salts has been introduced in the first extraction/partitioning step to adjust the pH to a compromise value of 5 to 5.5, where most acid and base labile pesticides are sufficiently stabilized. To improve stability of base-labile compounds in the sample extracts, a small amount of formic acid is added to the final extract after cleanup using a primary-secondary amine (PSA) sorbent. Acidic pesticides are directly analyzed from the raw extract before PSA cleanup. In another modification introduced by Schenck, graphitized carbon black (GCB) is used to remove plant pigments.
Currently there are three variations of the **QuEChERS** method being used in the United States

1) **The original QuEChERS method.** Introduced in 2003, this method used sodium chloride to enhance extraction.

2) **Dispersive AOAC 2007.01.** Uses sodium acetate as a buffer replacing sodium chloride.

3) **The dual phase column:** This method variation introduces the use of PSA and GCB to remove high levels of chlorophyll and plant sterols in the final extract without the loss of planar pesticides (polar aromatics) using an acetone:toluene solvent blend (3:1).

Dry commodities such as cereals, dried fruits, tobacco or teas require the addition of water prior to extraction to weaken interactions of pesticides with the matrix and to ensure adequate partitioning. Even commodities with high lipid content such as avocados or other high oil load plants can be analyzed by this method. However, due to a partitioning to the lipid phase, highly non-polar pesticides may give relatively low yet consistent recoveries of up to 70%. These co-extracted lipids in the can be reduced by a freezing-out step or the use of a C18 cleanup step.

The **QuEChERS** method gives at least fourfold lower material costs with significantly greater sample throughput per analyst than traditional methods. By combination of several different steps there is less chance for the introduction of error at each step. A polypropylene (PP) tube is the only consumable item required eliminating all glassware used in conventional methods. Furthermore, less than 10 mL of solvent waste is generated, much less than the 75-450 mL generated by other methods. Key to the new approach is the re-discovery of a rapid procedure called dispersive solid-phase extraction. This technique quickly removes residual moisture with magnesium sulfate. Other potential interferences are reduced by employing a primary-secondary amine sorbent to capture acidic components.

How is QuEChERS Conducted?

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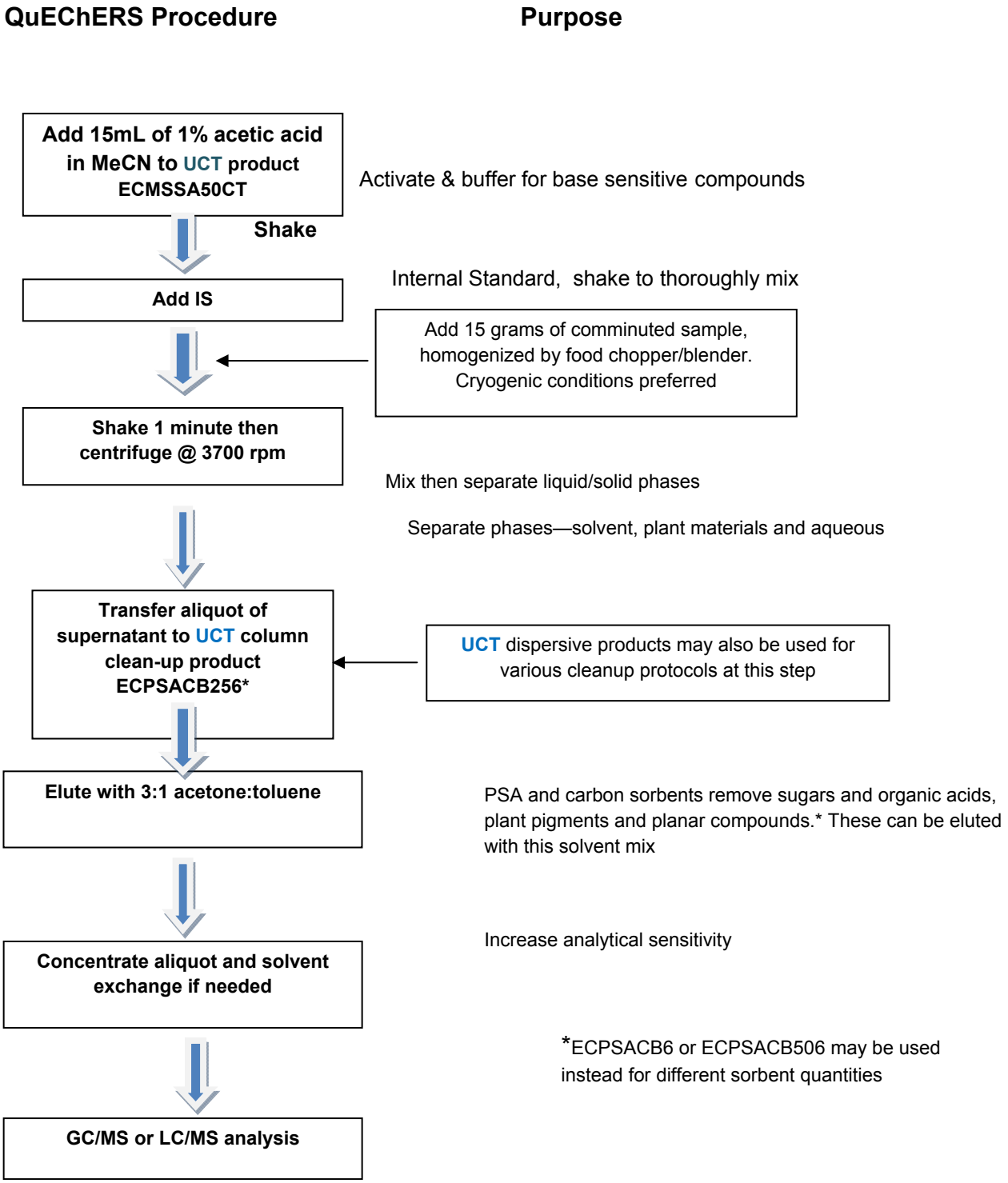
Procedural steps in the QuEChERS analysis for base sensitive compounds can be outlined in the schematic representation below:



QuEChERS Methods

Dispersive Methods

AOAC 2007.01 in brief (if base sensitive compounds are present)

1. To product ECMSSA50CT containing 6 grams anhydrous magnesium sulfate and 1.5 grams of anhydrous sodium acetate in a 50 mL polypropylene centrifuge tube, add 15 ml of 1% acetic acid in acetonitrile
2. Shake to mix contents
3. Add surrogate or internal standards if desired
4. Add 15 grams homogenized hydrated sample to the centrifuge tube
5. Shake for I minute
6. Centrifuge for 1 minute at 3700 rpm
7. Add an aliquot of the supernatant to the appropriate dispersive clean-up product: **UCT** CUMPSCB2CT, CUMPS2CT, CUMPSC18CT, ECMPSCB15CT, or ECMPSC1815CT
8. Shake for 1 minute
9. Centrifuge for 1 minute at 3700 rpm
10. Analyze extract

For compounds that are not base sensitive the following procedure provides a cleaner extract. This procedure is also necessary for acid labile compounds.

1. To product ECMSSC50CT containing 6 grams anhydrous magnesium sulfate and 1.0 gram of sodium chloride in a 50 mL polypropylene centrifuge tube, add 15 ml of acetonitrile
2. Shake to mix contents
3. Add surrogate or internal standards if desired
4. Add 15 grams of homogenized hydrated sample to the centrifuge tube
5. Shake for 1 minute
6. Centrifuge for 1 minute at 3700 rpm
7. Add a aliquot of the supernatant to the appropriate dispersive solid-phase cleanup tube: **UCT** CUMPSCB2CT, CUMPS2CT, CUMPSC18CT, ECMPSCB15CT, or ECMPSC1815CT (See Product List and Use Description below)
8. Shake for 1 minute
9. Centrifuge for 1 minute at 3700 rpm
10. Analyze extract

Matrix plant pigments often interfere with analysis. To reduce these interferences, graphitized carbon can be added to the dispersive solid-phase clean-up tubes. However, the use of carbon may result in a loss of planar (polar aromatic) pesticides. Cleanup of plant pigments without loss of planar pesticides can be accomplished by using the UCT Dual-Phase Cartridge Clean-Up Procedure.

Dual Phase Cartridge Clean-Up Procedure (elution for planar [polar aromatic] compounds)

1. Pre-rinse cartridge with 5 mL of toluene
2. Add an aliquot of the supernatant to the cartridge
3. Start collection
4. Elute with 6-12 mL of 3:1 acetone:toluene
5. Concentrate for GC/MS analysis or
6. Concentrate to dryness and reconstitute in mobile phase for LC analysis

Cartridge product selection used for this analysis: UCT ECPSACB6, ECPSACB256 or ECPSACB506 depending upon sorbent mass required.

Effect of Solvent Volume on Extraction

In a study designed to evaluate the effect of sample mass to solvent ratio (1:1 & 2:1MeCN) on recovery in a spiked fruit sample, Table 1 shows that the most polar pesticides did not partition into the MeCN phase as readily with the use of a lower solvent volume. However recovery still remained above 75% which is suitable for most analytical purposes. This indicates that the use of a minimum quantity of solvent for increased sensitivity will still yield good recovery values.

Average Recovery (%) of Selected Pesticides from a 10 gram Fruit Sample

Table 1

Pesticide	MeCN, 5 mL	MeCN, 10 mL
Dichlorvos	95	96
Methamidophos[b]	76	95
Mevinphos	96	100
Acephate[b]	84	99
o-Phenylphenol	94	94
Omethoate[b]	85	100
Diazinon	95	99
Chlorothalonil	94	95
Metalaxyl	94	100
Carbaryl	93	99
Dichlofluanid	97	97
Captan	97	100
Thiobendazole[b]	88	99
Folpet	92	94
Imazalil	92	102

[b] most polar Anastassiades, M. & Lehotay, S. J of AOAC International, Vol., 86, 2003

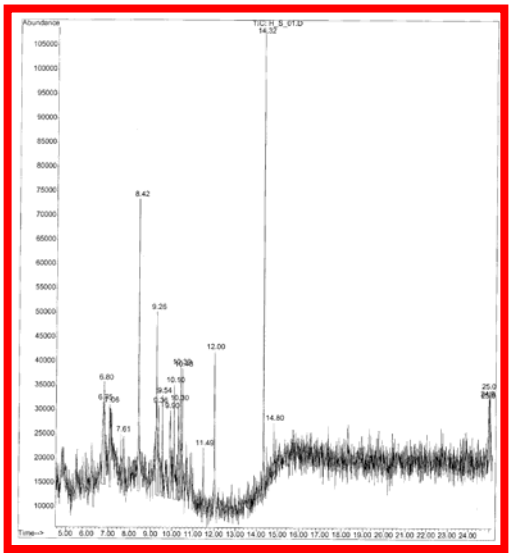
UCT Products Reduce Contamination Often Found in Laboratory Preparations

Many laboratories assemble their own clean-up products for the **QuEChERS** analysis. However contamination may be inadvertently introduced in the final extract complicating analyte peak identification. In a study conducted at the USDA ARS Eastern Regional Research Center, commercially prepared **QuEChERS** products were compared to those products prepared in the USDA lab. Bulk anhydrous magnesium sulfate, PSA and endcapped C18 sorbents provided by **UCT**, were assembled in the USDA lab then compared to **UCT** manufactured products using the same lot of bulk sorbents. The ratio of magnesium sulfate, PSA and C18 was 3:1:1 for this test. The clean-up products were evaluated on extracts of milk, honey and soybean and the efficacy of clean-up was determined by GC/MS analysis. Comparison of the extracts was made by counting the number of GC peaks above the threshold. Results clearly showed that the commercially prepared product provided superior clean-up to the product prepared in the lab. This result was confirmed in all three matrices. The extra peaks observed in the lab prepared product were probably caused by contamination from within the lab environment. The **UCT** assembled products were prepared under controlled manufacturing conditions that eliminated potential contamination typically encountered in lab environments. These results, coupled with obvious time and labor savings for assembly, indicate that **QuEChERS** products preassembled at **UCT** are preferable to products made "in-house".

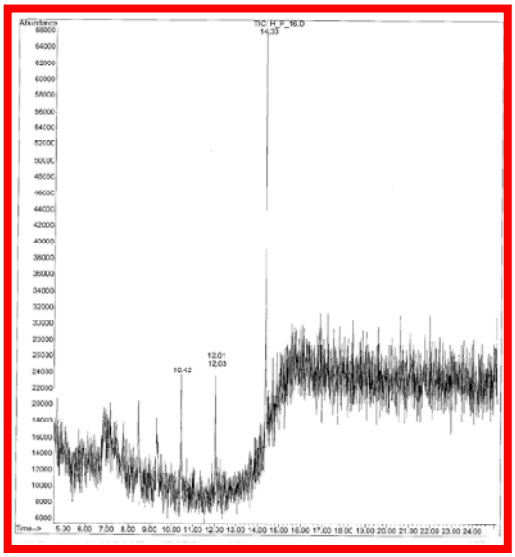
The results from these tests are summarized below for in tabular and graphic format for soybeans, honey and milk products. Chromatograms are shown for honey.

Chromatograms

Honey Extracted with "In-House" Product



Honey Extract Cleaned with UCT Products



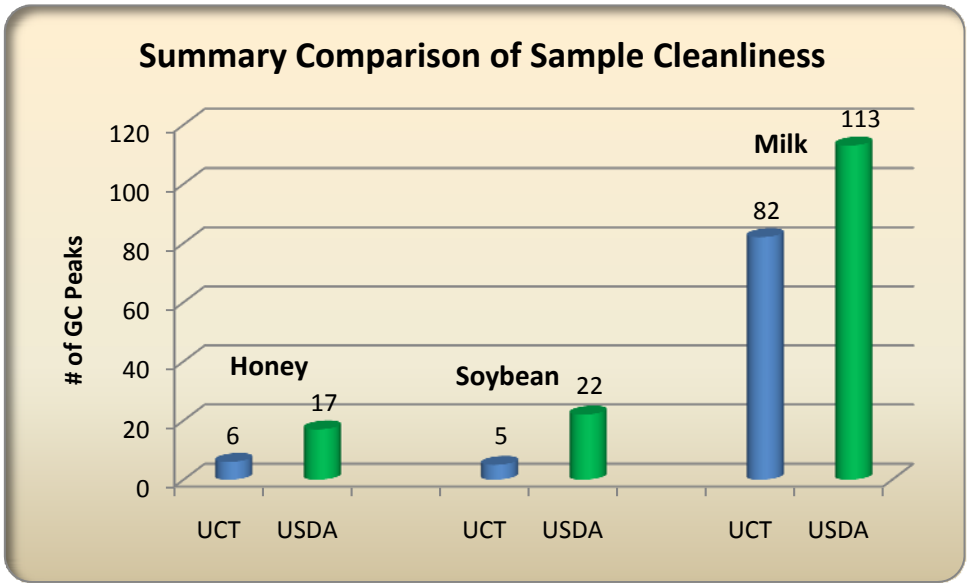
The chromatogram on the left is representative of those obtained when clean-up materials were prepared in-house. The chromatogram on the right was obtained using **UCT** prepackaged **QuEChERS** products and shows a significant reduction in background interferences. This difference in background interference is thought to be due to sorption of contaminants from the laboratory environment

Chromatograms for soybean and milk products showed similar improved clean-up when using UCT manufactured vs. "in-house" prepared products.

Table Showing the Total Number of Peaks as Seen in a GC Chromatogram

Data indicate that the use of UCT prepared products results in cleaner chromatograms

Matrix	HONEY		SOYBEAN		MILK	
	# of peaks	# of peaks	# of peaks	# of peaks	# of peaks	# of peaks
Replicate	UCT	USDA	UCT	USDA	UCT	USDA
1	7	20	7	17	43	91
2	9	12	8	15	49	103
3	7	21	5	20	52	108
4	8	24	2	12	43	121
5	5	18	6	8	46	117
6	5	22	2	13	45	104
7	8	8	7	11	49	89
8	4	13	4	10	103	117
9	5	18	4	7	107	127
10	8	12	3	9	106	127
11	6	15	2	31	116	120
12	6	12	8	28	126	118
13	6	19	6	35	104	119
14	6	21	4	51	106	108
15	5	20	4	43	100	118
16	4	14	7	43	109	113
Average	**6**	**17**	**5**	**22**	**81**	**113**



Why use UCT SPE QuEChERS products?

- Save valuable laboratory time in preparation
- Reduced variability due to consistent product and rigorous quality control
- Cleaner extracts from cleaner products
- Extraneous GC peak counts are significantly lower using **UCT** prepared **QuEChERS** product
- Variability of GC peak counts on replicate samples were significantly lower using **UCT QuEChERS** products
- Dual layer columns are packaged in Mylar to eliminate potential sorbent contamination
- $MgSO_4$ is specially treated in a muffle furnace to remove organic contaminates typically encountered with in-house preparation

UCT provides a variety of solid-phase QuEChERS clean-up products that contain the proper sorbents for optimum extraction, clean-up and separation of analytes from complex matrices

UCT Products Used in the Micro Extraction Step

Cartridge product *ECMSSA50CT* is a 50 mL extraction tube that contains 6 grams of anhydrous magnesium sulfate and 1.5 grams of anhydrous sodium acetate. It complies with AOAC Method 2007.01. It is designed to allow the extraction of "base sensitive" compounds such as chlorothalonil, dichlofluanid, tolyfluanid, folpet, captafol, captan from non-acidic matrices.

Cartridge product *ECMSSC50CT* is a 50 mL extraction tube contains 4 grams of anhydrous magnesium sulfate and 1 gram of sodium chloride. It is designed for use where base sensitive compounds are not present or are not of analytical interest. Eliminating the buffer allows a cleaner extract, and the sodium chloride aids in the extraction of the analytes.

Cartridge product EUMIV50CT is a 50 mL extraction tube that contains 6 grams anhydrous magnesium sulfate, 1.5 grams of sodium chloride and 0.75 grams of sodium citrate sesquihydrate.

UCT Cartridge Products Used for Sample Clean-Up

Several cartridge products are offered for use in sample clean-up. **UCT** provides a variety of **QuEChERS** products in SPE cartridge format which include PSA and GCB. These sorbents are used to remove various polar organic acids, polar pigments, some sugars and fatty acid co-extractables from **QuEChERS** extracts. These sorbents may be additionally combined with C18 for the removal of fatty plant lipids and sterols. Graphitized carbon black is used to remove sterols and pigments such as chlorophyll. Magnesium sulfate or other salts are used to enhance extraction as well as the removal of water and the partitioning of residues into the solvent phase. Because carbon has a strong affinity to retain planar molecules, Schenck et al have reported that the use of a 3:1 acetone:toluene solvent blend performed well at eluting these compounds from carbon sorbents. Bulk sorbents are also available from the **UCT** catalog.

Products List and Use Description

Part Number	Contents and Use Description	
ChloroFiltr® Dispersive Products		
	ChloroFiltr® is a revolutionary new QuEChERS sorbent for the removal of chlorophyll without the loss of analytes.	
CUMPSGG2CT	2mL micro-centrifuge tubes with 150mg magnesium sulfate, 50mg PSA and 50mg ChloroFiltr® Designed to clean-up a 1 mL aliquot of supernatant.	100
ECMPSGG15CT	15mL centrifuge tube with 900mg magnesium sulfate, 300mg PSA and 150mg ChloroFiltr® Designed to clean-up a 3 mL aliquot of supernatant.	50
Dispersive Products		
ECMPSA50CT	50mL centrifuge tube with 1200mg magnesium sulfate, 200mg PSA	100
CUMPSCB2CT	2mL micro-centrifuge tubes with 150mg Anhydrous Magnesium Sulfate, 50mg PSA, 50mg Carbon *A dispersive SPE product for removing polar organic acids, some sugars and lipids which may cause some loss of planar pesticides. Designed for use with a 2mL aliquot of supernatant.*	100
CUMPS2CT	2mL micro-centrifuge tubes with 150mg Anhydrous Magnesium Sulfate, 50mg PSA *A dispersive SPE product for removing polar organic acids, some sugars and lipids. Designed for use with a 2mL aliquot of supernatant.*	100
CUMPSC18CT	2mL micro-centrifuge tubes with 150mg Anhydrous Magnesium Sulfate, 50mg PSA, 50mg endcapped C18 *A dispersive SPE product for removing polar organic acids, sterols, some sugars and lipids. Designed for use with a 2mL aliquot of supernatant.*	100
CUMPS15C18CT	2mL micro-centrifuge tubes with 150mg Anhydrous Magnesium Sulfate, 150mg PSA & 50mg endcapped C18	100
CUMPSC1875CB2CT	2mL centrifuge tube with 150mg magnesium sulfate, 50mg PSA, 50mg endcapped C18 and 7.5mg GCB	100
ECMPSCB15CT	15mL centrifuge tubes with 900mg Anhydrous Magnesium Sulfate, 300mg PSA, 150mg carbon *A dispersive SPE product for removing polar organic acids, some sugars and lipids. This product will cause the loss of planar pesticides. Designed for use with a 10mL aliquot of supernatant.*	50
ECMPSC1815CT	15mL centrifuge tubes with 900mg Anhydrous Magnesium Sulfate, 300mg PSA, 150mg endcapped C18 *A dispersive SPE product for removing polar organic acids, sterols, some sugars and lipids from a 10mL aliquot.*	50
ECMS12CPSA415CT	15mL centrifuge tube with 1200mg magnesium sulfate, 400mg PSA	50
ECMNAX15CT	15mL centrifuge tube with 900mg magnesium sulfate, 150mg of aminopropyl	50
	Florida-Modified QuEChERS for State Program Fruits and Vegetables	
CUMPSC1815CT2	15mL centrifuge tube with 1200mg $MgSO_4$, 400mg PSA and 400mg endcapped C18	50
ECQUUS215CT	15mL centrifuge tube with 1200mg $MgSO_4$, 400mg PSA, 400mg GCB and 400mg endcapped C18	50
ECQUEU1115CT	15mL centrifuge tube with 1200mg $MgSO_4$, 400mg PSA and 400mg GCB	50

Cartridge Products

Dual phase cartridges are available as an alternative to traditional QuEChERS dSPE clean-up.

ECPSACB6	6mL cartridges with 200mg Graphitized Carbon on top, 400mg PSA on bottom, separated by a Teflon frit *Used in the Schenck variation of QuEChERS, this product removes pigments, polar organic acids, some sugars and lipids from an aliquot of extract.*	30
ECPSACB256	6mL cartridges with 250mg Graphitized Carbon on top, 500mg PSA on the bottom, separated with a Teflon frit *Used for the same application as ECPSACB6 but with a different quantity of sorbents. When in doubt use ECPSACB256.*	30
ECPSACB506	6mL cartridges with 500mg Graphitized Carbon on top, 500mg PSA on the bottom, separated with a Teflon frit *Used for the same application as ECPSACB6 but with a different quantity of sorbents. When in doubt use ECPSACB256.*	30
ECNAXCB506	6mL cartridges with 500mg Aminopropyl and 500mg Graphitized Carbon	30

Products available with Polyethylene or Teflon frits. Your choice will depend on your application and price requirements.

European QuEChERS Method EN 15662

ECQUEU12CT	2mL centrifuge tube with 150mg magnesium sulfate, 25mg PSA	100
ECQUEU22CT	2mL centrifuge tube with 150mg magnesium sulfate, 25mg C18, 25mg PSA	100
ECQUEU32CT	2mL centrifuge tube with 150mg magnesium sulfate, 25mg PSA, 2.5mg GCB	100
ECQUEU42CT	2mL centrifuge tube with 150mg magnesium sulfate, 25mg PSA, 7.5mg GCB	100
ECQUEU415CT	15mL centrifuge tube with 4000mg magnesium sulfate, 1000mg NaCl, 500mg sodium citrate dibasic sesquihydrate, 1000mg sodium citrate tribasic dihydrate	50
ECQUEU215CT	15mL centrifuge tube with 6000mg magnesium sulfate, 1500mg sodium acetate	50
ECMPS15CT	15mL centrifuge tube with 900mg magnesium sulfate, 150mg PSA	50
ECQUEU315CT	15mL centrifuge tube with 900mg magnesium sulfate, 150mg PSA, 150mg C18	50
ECQUEU515CT	15mL centrifuge tube with 900mg magnesium sulfate, 150mg PSA, 15mg GCB	50
ECQUEU615CT	15mL centrifuge tube with 900mg magnesium sulfate, 150mg PSA, 45mg GCB	50
ECQUEU750CT	50mL centrifuge tube with 4000mg magnesium sulfate, 1000mg NaCl, 500mg sodium citrate dibasic sesquihydrate, 1000mg sodium citrate tribasic dihydrate	50

QuEChERS Multi-Packs

QuEChERS extraction reagents for all of the popular QuEChERS methods are available in individual metalized pouches for your convenience. Each pack of 50 pouches comes with racks of 50 empty centrifuge tubes with plug seal caps.

EC4MSSA50CT-MP	Each pouch will contain 4000 mg magnesium sulfate and 1g of Sodium Acetate	**50**
ECMSNA50CT-MP	Each pouch will contain 8000 mg magnesium sulfate and 3500mg of sodium chloride	**50**
EUMIV50CT-MP	Each pouch will contain 6000 mg magnesium sulfate, 1500 mg sodium chloride, 1500mg of sodium citrate dihydrate, 750mg of disodium citrate sesquihydrate	**50**
ECMSSA50CT-MP	Each pouch will contain 6000 mg of magnesium sulfate and 1500 mg of sodium acetate	**50**
ECMSSC50CT-MP	Each pouch will contain 4000 mg of magnesium sulfate and 1000 mg of sodium chloride	**50**
ECMSSC50CTFS-MP	Each pouch will contain 6000 mg of magnesium sulfate and 1500 mg of sodium chloride	**50**
ECQUVIN50CT-MP	Each pouch will contain 8000 mg of magnesium sulfate and 2000 mg of sodium chloride	**50**
ECQUEU750CT-MP	Each pouch will contain 4000 mg of magnesium sulfate, 1000 mg of sodium chloride, 500 mg of sodium citrate dibasic sesquihydrate, and 1000mg sodium citrate tribasic dihydrate	**50**

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Appendix I

List of possible pesticide analytes that have been shown to yield >90% (or >70 %*) recoveries using the QuEChERS method. GC-amenable pesticides are capitalized; those preferentially analyzed by LC/MS-MS are not capitalized; those that can be analyzed by either technique are underlined**

Pesticide Analyte

acephate*	acetamiprid	Acrinathrin	aldicarb	aldicarb sulfone
aldicarb sulfoxide	Aldrin	azaconazole	azamethiphos	azinphos-methyl
azoxystrobin	Bifenthrin	bitertanol	Bromopropylate	bromuconazole
Bupirimate	buprofezin	butocarboxim	butocarboxim sulfone	butocarboxim sulfoxide
Cadusafos	carbaryl	carbendazim	carbofuran	3-hydroxy-carbofuran
chlorbromuron	(α-, γ-)Chlordane	(α-,β-Chlorfenvinphos	Chlorpropham	Chlorpyrifos
Chlorpyrifos-methyl	Chlorthaldimethyl	Chlorothalonil*	Chlozolinate	clofentezine
Coumaphos	cycloxydim*	(λ-)Cyhalothrin	cymoxanil	Cypermethrin
cyproconazole	cyprodinil	(2,4'-4,4'-)DDE	(2,4'-4,4'-)DDT	Deltamethrin
demeton	demeton-O-sulfoxide	demeton-S-methyl	demeton-S-methyl sulfone	desmedipham
Diazinon	dichlofluanid*	Dichlorobenzophenone	dichlorvos	diclobutrazole
Dicloran	dicrotophos	Dieldrin	Diethofencarb	difenoconazole
Diflufenican	dimethoate	dimethomorph	diniconazole	Diphenyl
Diphenylamine	disulfoton	disulfoton sulfone	diuron	dmsa
dmst	dodemorph	α- Endosulfan	β-Endosulfan	Endosulfan sulfate
EPN	epoxiconazole	Esfenvalerate	etaconazole	ethiofencarb sulfone
ethiofencarb sulfoxide	Ethion	ethirimol	Ethoprophos	etofenprox
Etridiazole	Famoxadone	fenamiphos	fenamiphos sulfone	Fenarimol
Fenazaquin	fenbuconazole	fenhexamid*	Fenithrothion	fenoxycarb
Fenpiclonil	Fenpropathrin	Fenpropidine	fenpropimorph	fenpyroximate
Fenthion	fenthion sulfoxide	Fenvalerate	florasulam*	Flucythrinate I & II
Fludioxonil	flufenacet	Flufenconazole	flusilazole	Flutolanil
Fluvalinate	Fonophos	fosthiazate	Furalaxyl	furathiocarb
furmecyclox	Heptachlor	Heptachlor epoxide	Heptenophos	Hexachlorobenzene
hexaconazole	hexythiazox	imazalil	imidacloprid	Iprodione
iprovalicarb	isoprothiolane	isoxathion	kresoxim-methyl	Lindane
linuron	Malathion	malathion oxon	Mecarbam	mephosfolan
Mepronil	Metalaxyl	metconazole	methamidophos*	Methidathion
methiocarb	methiocarb sulfone*	methiocarb sulfoxide	methomyl	methomyl-oxime

metobromuron	metoxuron	Mepanipyrim	Mevinphos	monocrotophos
monolinuron	myclobutanil	nuarimol	Ofurace	omethoate
oxadixyl	oxamyl	oxamyl-oxime	oxydemeton-methyl	paclobutrazole
Parathion	Parathion-methyl	penconazole	pencycuron	*cis*- Permethrin
***trans*-Permethrin**	phenmedipham	*o*-Phenylphenol	Phorate	phorate sulfone
Phosalone	Phosmet	Phosmet-oxon	phosphamidon	Phthalimide
picoxystrobin	Piperonyl butoxide	pirimicarb	pirimicarb-desmethyl	Pirimiphos-methyl
prochloraz	Procymidone	profenofos	Prometryn	Propargite
Propham	propiconazole	propoxur	Propyzamide	Prothiofos
pymetrozine	Pyrazophos	pyridaben	pyridaphenthion	pyrifenox
pyrimethanil	Pyriproxyfen	Quinalphos	Quinoxyfen	Quintozene
sethoxydim	spinosad	spiroxamine	tebuconazole	tebufenozide
Tebufenpyrad	tetraconazole	Tetradifon	Tetrahydrophthalimide	Terbufos
Terbufos sulfone	thiabendazole	thiacloprid	thiamethoxam	thiodicarb
thiofanox	thiofanox sulfone	thiofanox sulfoxide	thiometon	thiometon sulfone
thiometon sulfoxide	thiophanate-methyl	Tolclofos-methyl	tolylfluanid	triadimefon
triadimenol	Triazophos	trichlorfon	tricyclazole	tridemorph
trifloxystrobin	trifluminazole	Trifluralin	Triphenylphosphate	vamidothion
vamidothion sulfone	vamidothion sulfoxide	Vinclozolin		

**from “Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) Approach for Determining Pesticide Residues”, Steven J. Lehotay, U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center; 600 East Mermaid Lane; Wyndmoor, Pennsylvania 19038; USA

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1. Sample Pretreatment

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

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

2. Condition C18 SPE Cartridge

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

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

3. Add Sample

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

4. Dry Cartridge

b) Dry cartridge for 10 minutes at full vacuum

5. Elute Phenoxyacetic acid Herbicides

a) Place a clean collection vial in manifold
b) Add 5 mL of CH_3OH and wait 1 minute
c) Add a second 5 mL volume of CH_3OH
d) Adjust vacuum and collect at 1-2 mL/ minute

6. Dry Eluate

a) Evaporate to dryness at < 40˚C using N_2
b) Reconstitute in 100 µL of mobile phase for **LC-MS/MS**
- Inject 10-100 µL

HPLC Analysis and Instrumentation Requirements

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

Analytical Column:

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

HPLC/MS Interface:

- Micromixer 10-µL interface HPLC column system with HPLC post-column addition solvent

Interface:

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

Mass Spectrometer System:

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

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

Recommended HPLC Chromatographic Conditions

Chlorinated Phenoxyacid Compounds

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

A=0.1 M ammonium acetate/methanol

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

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

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1. Sample Preparation

a) Weigh out 1 g of homogenized beef kidney sample, kidney juice or serum in a 50 mL FEP (fluorinated ethylene propylene) centrifuge tube. A disposable polypropylene 50 mL centrifuge tube can also be used

b) Add 100 μL of 1 μg/ml internal standard consisting of ^{13}C-sulfamethazine, penicillin-V and cefadroxil in water. Penicillin and cephalosporin are used for method performance control

c) Add 2 mL water and 8 mL acetonitrile

d) Vortex briefly, then shake for 5 minutes

e) Centrifuge at 3450 rpm for 5 minutes

f) Decant the supernatant into a 50 mL centrifuge tube containing 500 mg of UCT EEC1800X sorbent

g) Vortex briefly, then shake for 30 seconds

h) Centrifuge at 3450 rpm for 1 minute

i) Transfer 5 mL aliquot of the supernatant into a graduated tube

2. Evaporate

a) Reduce extract volume to < 1 mL using a stream of N_2

b) Readjust extract volume to 1 mL with DI water

c) Filter the extract through a 0.45μm PVDF syringe filter into a clean vial

3. Analysis

a) Extract is now ready for analysis using LC-MS/MS

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1. **Sample Preparation**
   a) Weigh 1-5 grams of sample in a clean beaker
   b) Add 1-2 volumes of 5% methanol/water
   c) Stir for 15 minutes on a magnetic stir plate
   d) Adjust sample pH to 2 using 50% aqueous sulfuric acid (H_2SO_4)
   e) Continue extraction for a additional 15 minutes
   f) Adjust pH if necessary to maintain pH 2
   g) Filter or centrifuge sample
   h) Decant supernatant or collect filtrate
   i) Add appropriate internal standards and surrogates

2. **Condition Cartridge**
   Add 10 mL methanol and wet sorbent
   Add 20 mL DI water and draw through cartridge to remove methanol

   Note: Do not allow the cartridge to dry out otherwise repeat steps a) and b)

3. **Extract Sample**
   Draw sample through cartridge at 10 mL/ minute.

4. **Dry Cartridge**
   Dry cartridge for 10 minutes at full vacuum

5. **Elute Brodificoum, Daphacinone, and Hydramethylnon**
   Add 10 mL of ethyl acetate to sample bottle and shake
   Add to cartridge
   Adjust vacuum and collect eluate at 1-2 mL/ minute flowrate
   Add 10 mL of methylene chloride (CH_2Cl_2) to sample bottle
   Add to cartridge
   Collect eluate at 1-2 mL/ minute flowrate
   Pass combined organic phases through anhydrous sodium sulfate tube

6. **Dry Eluate**
   Using N_2, evaporate sample to less than 0.5 mL
   Bring to 1.0 mL with ethyl acetate in a volumetric flask

7. **Analyze Sample**
   Inject 1-2 µL onto GC, GC/MS

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Analysis of Malachite Green and Metabolite Leucomalachite Green by HPLC

Part #: EUBCX256

April 8, 2009

Malachite Green CAS 569-64-2

1. Sample Preparation

a) Add 1 g of sample to a 50 mL centrifuge tube
b) Add 50uL TMPD* solution at 1mg/mL
c) Spike using malachite green and leucomalachite green at 0.1 µg/mL
d) Add internal standard
e) Allow to sit for 10 minutes
f) Add 10 mL of Mcilvaines** Buffer/Methanol 1:1
g) Shake for 1 minute
h) Centrifuge at 5000 rpm for 10 minutes
i) Collect the supernatant
j) Repeat steps f) through i) until a volume of 20 mL of supernatant is obtained

2. Condition Cartridge

a) Add 5 mL of methanol to cartridge EUBCX256 and soak for 1 minute
b) Add 5 mL of water and draw through
c) Add 5 mL of Mcilvaines buffer

Note: Do not let the cartridge go dry otherwise repeat steps a) through c)

3. Extraction

a) Load supernatant from step 1j
b) Adjust vacuum for flow of 1–3 mL per minute

4. Wash Cartridge

a) Add 5 mL 0.1N HCl and slowly draw through
b) Add 10 mL water and draw through
c) Add 5 mL 1:1 MeOH: water and draw through
d) Add 10 mL hexane and draw through
e) Dry under vacuum for 10 minutes

5. **Elute cartridge**
   a) Elute at 1–3 ml per minute using a solution of 50% ethyl acetate, 45% methanol, 5% ammonium hydroxide
   b) Carefully evaporate to dryness at 50°C using N_2
      a) Reconstitute with LC mobile phase (50% acetonitrile and water containing 0.05 M p-toluene sulfonic acid (TSA) as a counter ion

6. **Analyze by LC/MS**
   a) Use a reverse-phase C18 analytical column

Solutions:

* TMPD – N,N,N',N'-tetramethyl-1,4-phenylenediamine dihydrochloride, CAS 637-01-4

**Mcilvaines buffer pH 2.6 – mix equal parts of 0.1M citric acid monohydrate with 0.2M disodium hydrogen phosphate dihydrate (Na_2HPO_4)•H_2O

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Extraction of Bentazone and Acifluorfen from Solid Matrices Using C_{18} SPE with GC/MS Analysis

Part #: ECUNIC18 or EEC181M6

April 8, 2009

1. Sample Pretreatment

Homogenize 5-10 grams of solid sample using 3-4 volumes of 5% aqueous methanol (CH_3OH)
Centrifuge sample
Transfer supernatant to appropriate sized sample bottles
Note: Adjust methanol to pH 2 using 0.1N HCl

8. Condition C_{18} Cartridge

Add 5 mL CH_3OH
Add 15 mL DI H_2O adjusted to pH2 using 0.1N HCl
Note: Do not let the cartridge dry out otherwise repeat steps a) and b)

9. Extract Sample

Adjust vacuum and draw water sample at 25 mL/ minute

10. Dry C_{18} SPE

Dry column for 10 minutes at full vacuum

11. Elute Bentazone/ Acifluorfen

a) Prepare a clean test tube by adding 2g of acidified sodium sulfate*
b) Add 10 mL of ethyl acetate to sample bottle and shake
c) Add to C_{18} cartridge
d) Soak for 1 minute
e) Adjust vacuum and collect eluate in the tube containing sodium sulfate
f) Add 10 mL of methylene chloride (CH_2Cl_2) to the sample bottle and swirl
g) Add to C_{18} column
h) Soak for 1 minute and collect
i) Repeat using another 10 mL of aliquot of CH_2Cl_2
j) Adjust vacuum and collect at 1-2 mL/ minute

12. Dry Eluate

Pass extract through 10 g of acidified sodium sulfate
Collect sample in a clean vial (Do not use soda lime glass)
Add appropriate internal standard

13. Evaporate

Concentrate to desired final volume

14. Derivatize sample

Use trimethylsilyldiazomethane (TMSD) by EPA Method 515 or diazomethane by EPA Method 8151.
Note: The extract must be completely dry or incomplete methylation will occur.

15. Analysis

Inject 1-2 µL onto a GC/MS

*See Procedure for Preparing Acidified Sodium Sulfate Anhydrous, UCT, Inc., Revision 1.1, EPA Method 8151A, 5.10

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QuEChERS Extraction and Clean-Up of Pesticides from Olive Oil

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

April 8, 2009

1. Sample Extraction

a) In a suitable vial, add 1.5mL 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 UCT product 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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QuEChERS Multiresidue Pesticide Method for The Determination of Multiple Pesticides in Wines*

Part Number:

ECQUVIN50CT (50 mL centrifuge tube, 8.0 grams anhydrous $MgSO_4$ & 2 grams NaCl)

ECMPSCB15CT (900 mg anhydrous $MgSO_4$, 300 mg PSA &150 mg GCB)

February 11, 2010

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

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

1. Sample Preparation

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

2. UPLC/MS/MS Analysis

UPLC Conditions:

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

Flowrate: 0.2 mL/minute

Injection volume: 3 µL

Analytical Standards: Matrix Matched

Gradient Program:

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

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

Capillary Voltage: 1.5 kV

Source Temperature: 120 °C

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

Collision Gas: Argon

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

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

Summary of MS/MS Conditions

Pesticide	Molecular Weight	CV (V)	Quantification Transition
Acephate	183.17	20	184.0→143.0
Acetamiprid	222.67	30	223.4→126.1
Acibenzolar S-methyl	210.27	35	211.1→136.0
Aldicarb	190.27	12	208.1→116.0
Aldicarb sulfone	222.27	15	240.0→222.9
Aldicarb sulfoxide	206.26	15	224.2→206.9
Atrazine	215.69	35	215.9→173.85
Avermectin B_{1b}	873.09	20	876.6→553.4
Avermectin B_{1a}	873.09	20	890.7→567.5
Azoxystrobin	403.30	25	404.0→372.1
Benalaxyl	325.41	26	326.1→148.1
Benfuracarb	410.53	20	411.2→190.0
Benzanilide	197.24	30	198.1→105.1
Bifenazate	300.35	20	301.3→170.2
Bitertanol	337.42	20	338.2→ 99.1
Buprofezin	305.44	25	306.3→201.2
Carbaryl	201.22	22	202.1→145.1
Carbendazim	191.19	30	192.0→160.0
Carbofuran	221.26	26	222.1→123.1
Chloroxuron	290.75	35	291.0→ 72.2
Cyprodinil	225.29	45	226.1→ 93.0
Cyromazine	166.19	25	167.2→ 85.1
Diclobutrazol	328.24	30	328.1→70.2
Dimethoate	229.26	20	230.1→199.0
Dimethomorph	387.86	35	388.0→301.1
Dimoxystrobin	326.39	20	327.1→206
Dinotefuran	202.20	20	203.5→14.0
Diuron	233.10	30	233.0→72.1
Ethofumesate	286.35	30	286.9→258.9
Famoxadone	374.39	-32	373.2→ 282
Fenamidone	311.40	25	312.2→236.2
Fenbuconazole	336.82	35	337.1→125.0
Fenhexamid	302.20	65	301.9→261.9
Fenpropimorph	304.49	40	304.4→147.1
Fluconazole	306.27	30	307.2→220
Fludioxinil	248.19	-45	247.0→180.0
Furathiocarb	382.48	30	383.2→195.1
Hexaconazole	314.21	35	314.0→ 70.2
Imazalil	297.18	35	297.1→159.0
Imidacloprid	255.65	25	256.1→175.0
Ipconazole	333.86	35	334.1→70.2
Iprovalicarb	320.43	24	321.2→119.0
Kresoxim-methyl	313.35	20	314.1→116.0
Mepanipyrim	223.28	30	224.4→ 77.3
Metalaxyl	279.34	25	280.1→220.1
Methamidophos	141.13	22	142.0→ 94.0

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

Schematic Diagram of Sample Preparation Steps

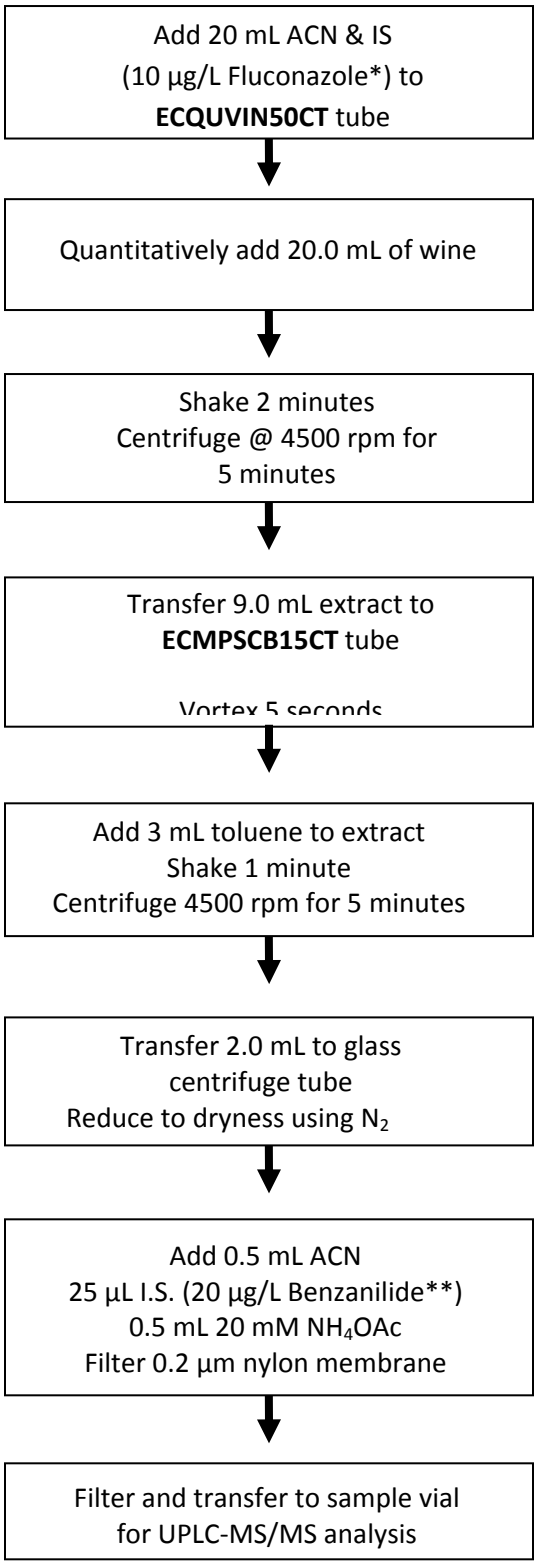

Table of Average Pesticide Recoveries at 100 g/L Spike

(average values with SD, n=4)

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

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

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

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

1) Extraction

a) To 10 g of homogenized/hydrated sample in a 50 mL centrifuge tube add 10 mL acetonitrile
b) Add internal standard (Cyprodinil + 2,4D)
c) Shake for 1 minute
d) Add contents of ECMSSC50CT-MP pouch (4 g of anhydrous magnesium sulfate and 1 g 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 an aliquot of supernatant from (step 1 f) to product ECMSC1850CT (50 mL centrifuge tube containing 1500 mg anhydrous magnesium sulfate and 500 mg C18)
b) Shake for 1 minute
c) Centrifuge for 1 minute at 3450 rcf

3) Analysis

a) Place 0.5 mL into auto sampler vial
b) Add QC spike (TPP)
c) Inject onto LC-MS/MS
d) Use mode ESI + or ESI- depending upon specific analyte of interest

39 Flukicides/Anthelmintics

ESI+		ESI-
IS Triphenylphosphate	**QC Spike** Cyprodinil	**IS** 2,4D
Abamectin	Albendazole	Bithionol
Doramectin	Albendazole sulfoxide	Clorsulon
Emamectin	Albendazole sulfone	Closantel
Eprinomectin	Albendazole amino sufone	Niclosamide
Moxidectin	Cambendazole	Nitroxynil
Lyermectin	Flubendazole	Oxyclozanide
Selemectin	Flubendazole, amino	Rafoxanide
Diclorvos	Flubendazole, hydroxy	Triclabendazole
Coumaphos	Fenbendazole	
Coumaphos-Oxon	Fenbendazole sulfone	
Haloxon	Oxfendazole	
Morantel	Mebendazole	
Levemisole	Mebendazole, amino	
	Mebendazole, hydroxy	
	Oxibendazole	
	Thiabendazole	
	Thiabendazole, 5-hydroxy	
	Triclabendazole	
	Triacleabendazole suldxide	

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

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Antibiotics in Beef or Serum by QuEChERS

Part Number: ENVIRO-CLEAN® ECMSC1850CT

November 23, 2009

This is a streamlined sample preparation method for the analysis of several classes of antibiotics in beef, kidney juice or serum

1) Extraction

a) Weigh 1 g of homogenized beef kidney sample, kidney juice or serum in a 50 mL FEP (fluorinated ethylene propylene) tube or disposable polypropylene tube

b) Add 100 µL of 1 µg/mL composite internal standard solution of ^{13}C-sulfamethazine (to compensate for volume change), penicillin-V and cefadroxil (for method performance) in water

c) Add 2 mL water

d) Add 8 mL acetonitrile

e) Shake for 5 minutes

f) Centrifuge at 3450 rcf for 5 minutes

2) Clean-Up

d) Transfer the supernatant into a 50 mL tube with 500 mg C18 (ECMSC1850CT) (50 mL centrifuge tube containing 1500 mg anhydrous magnesium sulfate and 500 mg C18)

e) Shake for 30 seconds

f) Centrifuge at 3450 rcf for 1 minute

g) Place 5 mL aliquot of the supernatant into a graduated tube

h) Evaporate down to < 1 mL

i) Bring volume to 1 mL with reagent water

j) Transfer the extract into vials by filtering through PVDF 0.45 m membrane filter syringes

k) Sample is now ready for analysis by LC-MS/MS

Table of some antibiotics that were analyzed using this procedure

Sulfonamides	Macrolides	Fluoroquinolones	Tetracyclines
sulfathiazole	erythromycin	ciprofloxacin	oxytetracycline
sulfamethazine	lincomycin	danofloflaxin	tetracycline
sulfachloropyridazine	tytosin	difloxacin	
sulfadoxine		orbifloxacin	
sulfamethazole		sarafloxacin	
sulfadimethoxine			

B-Lactams			
amoxicillin	ampicillian	cefadroxil	cefezolin
cloxacillin	DCCD	dicloxacillin	oxacillin
nafcillin	Penicillin G	Penicillin V	

CCD desfuroylcenftiofur cysteine disulfide

* adapted from work done by Kate Mastovska at USDA

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Optimized QuEChERS Method For Acrylamide Analysis*

UCT Products: ECMSSC50CT-MP
CUMPS2CT

October 21, 2009

1) Extraction

a) Add 1.0 gram of sample to a 50 mL centrifuge tube

b) Add contents of ECMSSC50CT-MP pouch (4g $MgSO_4$ and 1g NaCl)

c) Add 500 ng/g d_3-acrylamide to the tube

d) Add 5 mL of hexane

e) Vortex for 1 minute

f) Add 10 mL of reagent water and 10 mL of acetonitrile

g) Shake vigorously for 1 minute

h) Centrifuge for 5 minutes at 3450 rcf

2) Clean-Up

a) Discard the hexane top layer

b) Add 1 mL of the acetonitrile layer to a CUMPS2CT tube (150 mg $MgSO_4$, 50 mg PSA)

c) Mix for 30 seconds

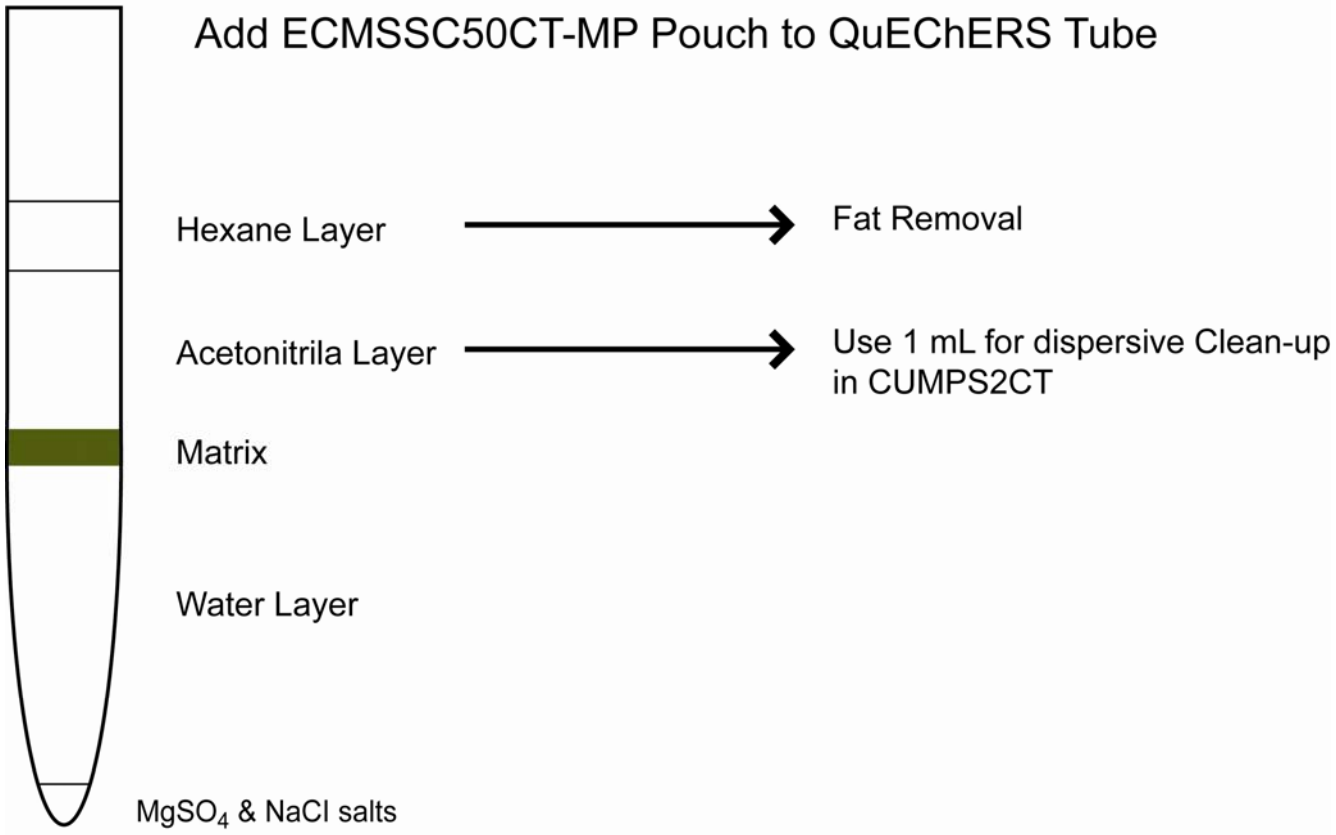
d) Centrifuge at 3450 rcf for 1 minute

e) Transfer liquid portion to an injection vial

3) Analysis

a) Inject 5-10 µL into an LC/MS/MS

Acrylamide Extraction Step



*Adapted from Kate Mastovska, USDA-ARS

DCN-901210-175

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

Part Number:
ECMSSC50CT-MP (50 mL centrifuge tube, 4 g anhydrous magnesium sulfate, 1 g NaCl)
CUMPS2CT (150 mg anhydrous magnesium sulfate and 50 mg PSA
January 22, 2010

Introduction

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). The analysis has been optimized using a modified QuEChERS approach. 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.

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 Cleanup

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

3) Analysis

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

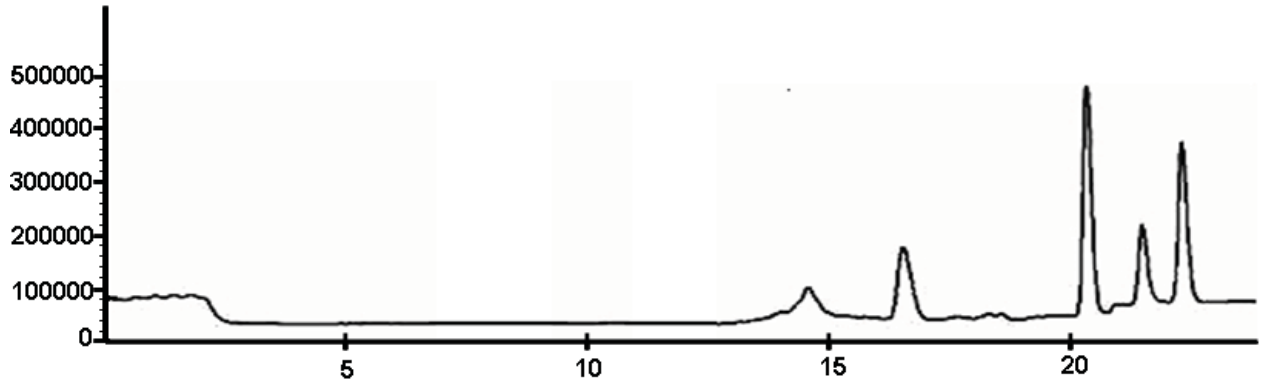
b) Analytical column: Luna C18 (250mm x 4.6 mm x 5 µm) or equivalent may be used but may change elution times

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

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

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

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



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

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

Part Number:
ECMSSC50CT-MP (50 mL centrifuge tube, 4 g anhydrous. magnesium sulfate, 1 g NaCl)
CUMPS15C18CT (150 mg anhydrous magnesium sulfate, 150 mg PSA and 50 mg C18)
February 24, 2010

Introduction

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
  See http://forums.unitedchem.com/ for complete pesticide list and mixtures

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

Prepare at 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

4) Sample Preparation

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

5) Sample Cleanup

f) Transfer a 1 mL aliquot to a 2 mL **CUMPS15C18CT** tube
g) Vortex for 30 seconds
h) Centrifuge for 5 minutes
i) 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*
j) Mix thoroughly
k) 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)
l) Press the 0.2 µm polyvinylidine fluoride (PVDF) filter of the Mini-UniPrep to filter the extract for the UPLC-MS/MS analysis
m) Add 30 µL of QC standard solution
n) Sample is now ready for analysis

6) Analysis UPLC-MS/MS

e) Acquity UPLC interfaced to a Quattro Premier triple-quad mass spectrometer (Water's Corp.) MassLynx software v 4.1 or equivalent
f) **Column:** Acquity UPLC BEH C18 (50 x 2.1 mm, 1.7 µm particle size, 130 Å pore size) or equivalent
g) **Temperature:** 40°C
h) **Injection Volume:** 2 µL

Binary Mobile Phase:

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

j) **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 (N_2) flow of 600 L/h
- cone gas (N_2) 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

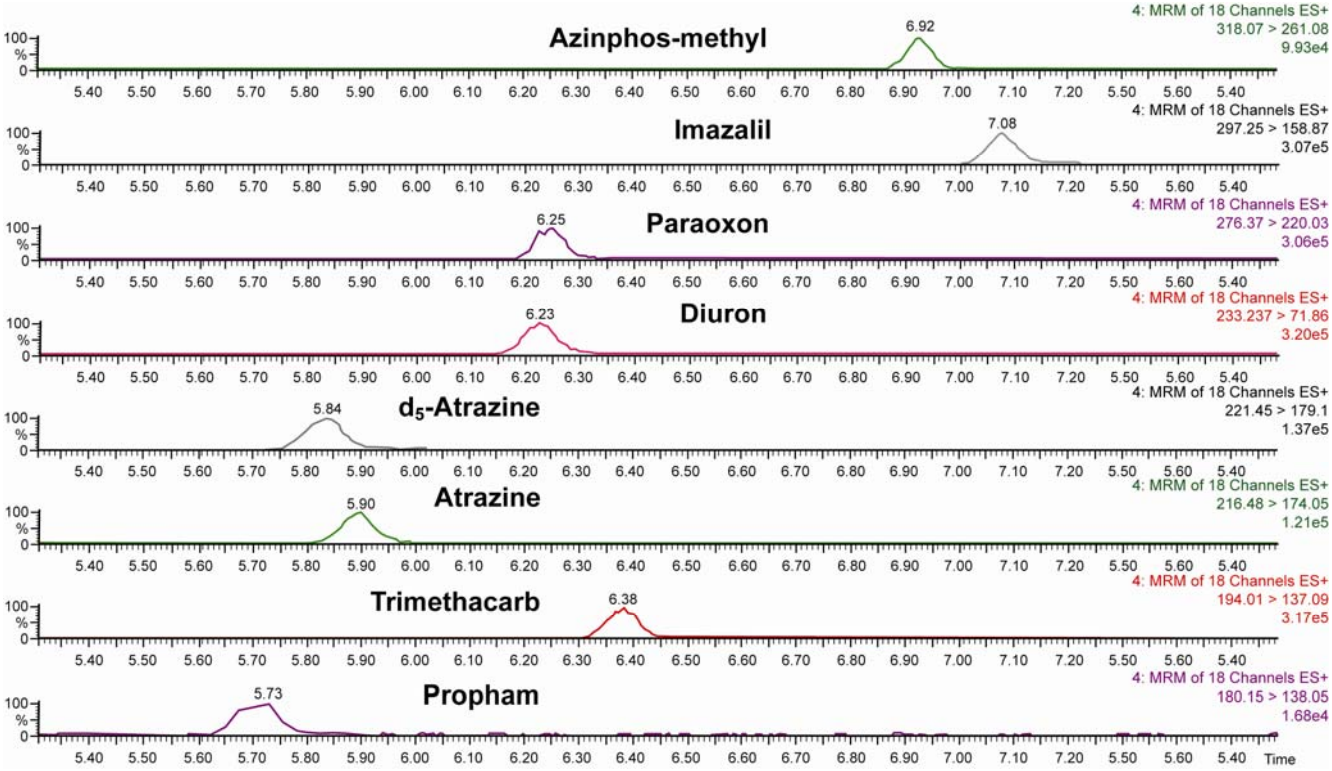
Automated DSI Injection:

- 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:

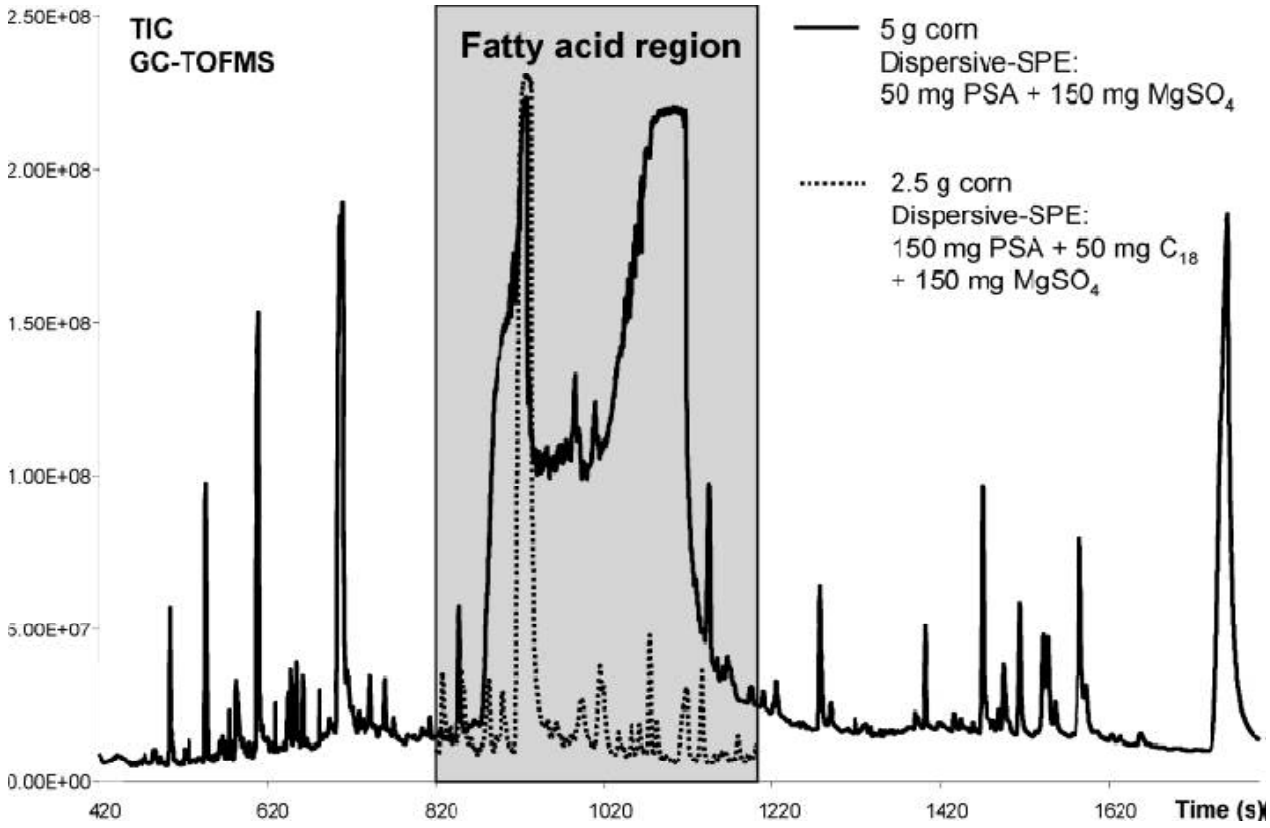
- Solvent vent at an injector temperature of 100° C for 4.5 minutes
- Initial column flow of 0.8 mL/minutes and a split flow of 50 mL/minutes,
- Follow by a splitless transfer of analytes for 4 minutes. The injector temperature was ramped to 280° C (at 16° C/s) Column flow changed to 1.5 mL/minutes (kept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

DCN-014202-183

Extraction of Pesticides from Tomato Using the QuEChERS Approach

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

March 17, 2010

UCT Products:

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 (GCB)

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 (GCB)

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 AOAC Method 2007.01

*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

www.quechers.com

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Pesticide and PAH Extraction of Grass and Other Leafy Vegetation by QuEChERS Using ChloroFiltr® Clean-Up

March 10, 2010

ChloroFiltr®

UCT Products:

ECQUEU750CT-MP 4000 mg mag. Sulfate, 1000 mg sodium chloride, 500 mg sodium citrate dibasic sesquihydrate, 1000 mg sodium citrate tribasic dihydrate

CUMPSGG2CT 2 mL micro-centrifuge tube containing 150 mg magnesium sulfate anhydrous,
50 mg primary secondary amine (PSA) and 50 mg **ChloroFiltr®**

ECMPSGG15CT 15 mL centrifuge tube with 900 mg magnesium sulfate anhydrous, 300 mg primary secondary amine (PSA) and 150 mg **ChloroFiltr®**

1. Sample Preparation

a) Add 15g of homogenized and hydrated sample (> 80% moisture) to a centrifuge tube
b) Add 15 mL acetonitrile including internal standard
c) Shake or vortex for 30 seconds
d) Add the contents of package **ECQUEU750CT-MP** to centrifuge tube
e) Immediately shake vigorously for 2 minutes
f) Centrifuge for 2 minutes at 3450 rcf
g) Draw 1 mL or 3-8 mL of supernatant for clean-up

2. Clean-Up

a) For 1 mL of supernatant, use product **CUMPSGG2CT**
b) For 3 mL of supernatant use product **ECMPSGG15CT**
c) Add supernatant to centrifuge tube and shake vigorously for 1 minute
d) Centrifuge for 2 minutes at 3450 rcf

Notes:

a) Triphenyl phosphate may be used as a surrogate
b) Calibrate instruments using matrix matched standards

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CLEAN-UP OF ORGANOCHLORINE PESTICIDES AND PCB EXTRACTS USING FLORISIL®

Part #: EUFLSA1M6 or EUFLS1M6

February 3, 2009

This application is designed to remove polar interferences from organochlorine pesticide and PCB extracts in hexane prior to analysis.

REAGENTS:

Hexane
Acetone

Product Description:

EUFLSA1M6 – 1000 mg small particle Grade A Florisil® for slower gravity flow.
EUFLS1M6 – 1000 mg regular particle PR Grade Florisil® for more viscous samples.

PROCEDURE:

1. Prerinse a column with 9 mL of 90:10 hexane/acetone by gravity. (A low vacuum may be necessary 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.

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FRACTIONATION OF ALIPHATIC AND AROMATIC HYDROCARBONS USING ENVIRO-CLEAN® TPH SILICA

(Developed in cooperation with Lancaster Laboratories, Inc.)

Part #: XRSIHT13M15

February 3, 2009

Background:

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:

Prepare Extract

1. Solvent exchange the hydrocarbon extract from methylene chloride to hexane using a K-D apparatus.

Prepare Cartridge

2. Thoroughly rinse cartridge with two, 10 mL aliquots of pentane.

3. Add 1 mL of the extract to the cartridge.

4. Elute aliphatic fraction with pentane by gravity and collect everything in an ampoule. A total of 10 mL should be collected.

5. Place a fresh ampoule under the cartridge and elute the aromatic fraction with methylene chloride by gravity. A total of 10 mL should be collected.

6. 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

UCT in cooperation with Lancaster Laboratories, Inc., has developed a fractionation product that provides consistent and accurate results free from contamination

DCN-903020-124

1. **Post Sample Extraction**
   c) Place 4 grams of copper beads in a glass vial
   d) Add 2 mL of liquid sample extract to the vial

2. **Sulfur Removal**
   d) Seal the glass vial and mix sample with copper beads for 2 minutes
   e) Allow to stand for approximately 10 minutes
   f) If sample contains high levels of sulfur, repeat process with 4 grams of fresh copper beads

   Note: For the analysis of PCB type analytes, copper may reside in the extract

3. **Analysis, GC/MS or LC/MS**
   d) Transfer clean extract to autosampler vial
   e) Inject 1-2 µL for GC
   f) Inject 5-10 µL for LC

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UCT

MISCELLANEOUS METHODS

ABUSED DRUGS IN CANINE OR EQUINE URINE USING: 500 mg XTRACKT® EXTRACTION COLUMN

Part #: XRDAH515

February 3, 2009

1. **a. PREPARE SAMPLE-ENZYMATIC HYDROLYSIS OF GLUCURONIDES**
   To 5 mL of urine add internal standard(s) and 2 mL of ß-Glucuronidase 5,000 F units/mL Patella vulgata in 100 mM Acetate Buffer (pH 5.0).
   Mix/vortex. Hydrolyze at 65°C for 3 hours.
   Centrifuge for 10 min. at 2000 rpm, discard pellet.

   b. BASE HYDROLYSIS OF GLUCURONIDES
   To 2 mL of urine add internal standard(s) and 100 µL of 10 N NaOH.
   Mix/vortex. Hydrolyze at 60 °C for 20 minutes.
   Centrifuge for 10 min. at 2000 rpm, discard pellet.

   COMBINE HYDROLYSATES
   Combine both hydrolysis products with 5 mL of 100 mM phosphate buffer (pH 6.0).
   Adjust sample pH = 6.0 ± 0.5 with 0.5 M Phosphoric acid.

2. **CONDITION XtrackT® EXTRACTION COLUMN**
   1 x 5 mL CH_3OH.
   1 x 5 mL D.I. H_2O.
   1 x 3 mL 100 mM phosphate buffer (pH 6.0).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**
   Load at 1 to 2 mL / minute.

4. **WASH COLUMN**
   1 x 3 mL 100 mM phosphate buffer (pH 6.0).
   1 x 2 mL 1.0 M acetic acid.
   Dry column (5 minutes at > 10 inches Hg).
   1 x 2 mL hexane.

5. **ELUTE ACIDIC AND NEUTRAL DRUGS**
   1 x 4 mL methylene chloride; collect eluate at < 5 mL / minute.

6. **ELUTE STEROIDS**
   2 x 4 mL ethyl acetate; collect eluate at < 5 mL / minute.

7. **WASH COLUMN**
   1 x 5 mL CH_3OH; aspirate.

8. **ELUTE BASIC DRUGS**
   1 x 5 mL methylene chloride / isopropanol / ammonium hydroxide (78:20:2).
   NOTE: Prepare elution solvent fresh daily.

9. **DRY ELUATE**
   Evaporate to dryness at < 40°C.
   Reconstitute with 100 µL ethyl acetate.

10. **QUANTITATE**
    Spot onto TLC plate or inject 1 to 2 µL onto chromatograph

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EXTRACTION OF TEAR GAS
Chloroacetophenone (CS), o-Chlorobenzylidenemalononitrile (CN), and trans-8-methyl-N-vanillyl-6-nonenamide (OC) From Cloth for GC/MS Analysis Using: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. **PREPARE SAMPLE:**
   If suspected tear gas is on clothing cut out a portion of the sprayed area and a"negative" control sample. Extract each into hexane. For canisters of suspected tear gas, spray onto a Kimwipe® and extract the sprayed area and a negative control into hexane.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH 6.0).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE:**
   Load at 1 mL/minute.

4. **WASH COLUMN:**
   1 x 3 mL D.I. H_2O.
   1 x 3 mL Hexane.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE ANALYTE:**
   1 x 1 mL CH_3OH.

6. **DRY ELUATE:**
   Evaporate to dryness at < 40°C.

7. **RECONSTITUTE**
   Add 200 µL CH_3OH. Mix/vortex. Transfer to GC/MS vial and cap.

8. **QUANTITATE**
   Inject 1-2 µL sample onto GC/MS.

GC/MS Conditions: Column: HP Ultra 1, Crosslink Methyl Silicone 12 m x 0.2 mm I.D. x 0.33 µm film thickness

GC Oven:
Initial Temp. = 100° C Initial Time = 3.00 min.
Ramp = 17°C/min.
Final Temp. = 305°C.
Final Time = 3.0 min.
Injection Port Temp. = 250°C.
Transfer line Temp. = 280°C.

SCAN Acquisition = 41 amu to 400 amu: Start time = 2.00 min.
Retention times:

Compound	CN	CS	OC
RT (min.)	@4.9	@7.4	@13.4

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GLYCOPYRROLATE (ROBINUL) FROM EQUINE URINE BY LC-MSMS USING: 500 mg CLEAN UP® CCX2 EXTRACTION COLUMN

Part #: CUCCX25Z

February 3, 2009

1. SAMPLE PREPARATION

Buffer 5 mL of urine to pH 7.0 by adding 3 mL of 100 mM phosphate buffer (pH 7.0).

Add (12.5 ng) of mepenzolate (internal standard).

Add 5 mL of water to the sample.

Vortex or shake thoroughly.

Centrifuge for 5 min at 800 rpm.

2. CONDITION CLEAN UP® EXTRACTION COLUMN

1 x 3 mL CH_3OH.

1 x 3 mL D.I. H_2O.

1 x 1 mL 100 mM phosphate buffer (pH 7.0).

3. APPLY SAMPLE

Decant supernatant onto SPE column.

Load at 1 to 2 mL / min.

4. WASH COLUMN

5 mL of CH_3OH.

5 mL of D.I. H_2O.

Dry column (5 min > 10 inches Hg).

5. ELUTE GLYCOPYRROLATE

1 x 4 mL CH_3OH/0.5 M NH_4OAC buffer, pH 3.0 (95:5).

6. DRY ELUTE

Evaporate to dryness at 60°C.

Reconstitute with 100 µL CH_3OH.

7. QUANTITATE

Inject 10 µL onto HPLC.

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LC/MS METHOD FOR EXTRACTING ETHYL GLUCURONIDES FROM URINE USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: CSETG203

February 3, 2009

1. PREPARE SAMPLE:

Add 50 µL of formic acid to 1 mL of urine. (Internal standard EtG –d5 at 200 ng/mL.)
Centrifuge for 10 minutes at 3000 rpm.
Decant solution onto SPE cartridge previously conditioned with 2 mL of 1% formic acid.
Wash sample column with 2 mL DI water and dry at 10 mm Hg for 10 minutes.
Elute the EtG with 2 mL of 1% formic acid/ Methanol solution.
Evaporate to dryness under stream of nitrogen.
Reconstitute with 1 mL of Methanol. The solution should be filtered through a 0.2 µm filter for LC/MS analysis.

2. SUGGESTED LC/MS PROCEDURE:

Prepare 1.0 M ammonium acetate buffer by weighing 3.8 g ammonium acetate and dilute to 5L. (Option: 0.77 g diluted to 1L DI water). This solution should be filtered through 0.2 µm filer for LC use. LC Mobile Phase –ammonium acetate/ acetonitrile (10/90) at a flow rate of 0.2 mL/minute.
Injection Volume – 10 mL.
Detection Limit – 10 ng/mL

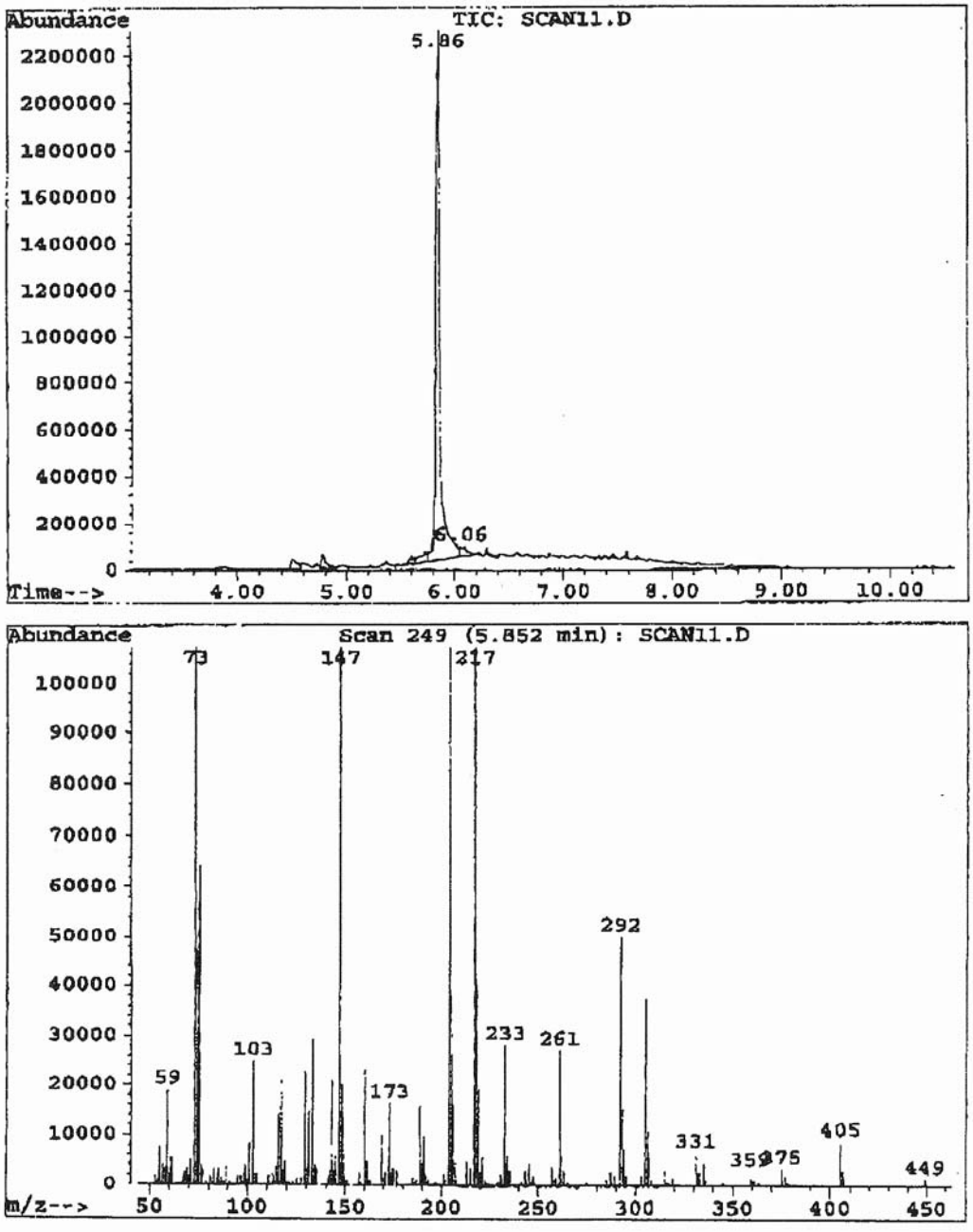
3. SUGGESTED LC/MS/MS PARAMETERS:

Tuning the MS:
Tune MS using PPG
Tune MS using 500 ng/mL EtG, mobile phase 0.2 mL/min, EtG solution 1 µL/min. Optimize ion source and mass analyzer to signal 221 m/z. Determine the collision voltage for ion 75 m/z and reference ions 85 and 113 m/z. Tune file uses scan rate of 0.3 s; acquisition time 6 minutes. Quantifier ion is 75 and qualifier ions are 85 and 113. Collision voltage 75(16), 85(16) / and 113 (14.5).

NOTES:

The prepared buffer should be filtered 30-45 minutes (equilibrated) before analysis for constant results.
After sample elution from the column, the LC must be programmed to flush the column using an acetonitrile / DI water gradient (50/50 to 90/10) to avoid carryover from previous specimen.

ETHYL GLUCURONIDES
CHROMATOGRAM



DCN-903020-108

TACROLIMUS, CYCLOSPORIN AND RAPAMYCIN IN WHOLE BLOOD USING: 30 mg STYRE SCREEN® EXTRACTION COLUMN

Part #: SSDVB031

February 3, 2009

1. PREPARE SAMPLE

Add 50 mcL whole blood and 50 mcL of 0.1 M $ZnSO_4$ to a centrifuge tube. Vortex.

Add 500 mcL methanol and internal

standards. Vortex. Centrifuge. Transfer supernate to a clean tube,

add 500 mcL D.I. water. Vortex.

2. CONDITION CLEAN UP® EXTRACTION COLUMN

1 x 2 mL CH_3OH.

1 x 2 mL D.I. H_2O.

NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Decant the sample onto the column. Load at 1 to 2 mL/minute.

4. WASH COLUMN

1 x 2 mL D.I. H_2O.

Dry column (20 minutes at > 10 inches Hg).

5. ELUTE ANALYTES

Add 750 mcL of ethyl acetate.

Collect eluate at 1 to 2 mL / minute.

7. ANALYSIS

The sample may be injected as is for HPLC analysis.

NOTES:

Suggested internal standards:

Cyclosporin	Cyclosporin-D
Tacrolimus	Ascomycin
Rapamycin	Desmethoxyrapamycin

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MANUAL METHOD FOR IMMUNOASSAY: PRELIMINARY SCREENING IN WHOLE BLOOD USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

February 3, 2009

1. **PREPARE SAMPLE**
   To 1 mL of blood add 4 mL of H_2O (5 < pH< 7).
   Mix/vortex. Let stand for 5 minutes to lyse red blood cells.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet.
   Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex.
   Sample pH should be 6.0 ± 0.5. Adjust pH with 100 mM monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH 6.0).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE**
   Load at 1 to 2 mL/minute.

4. **WASH COLUMN**
   1 x 3 mL D.I. H_2O
   1 x 1 mL 100 mM acetic acid.
   Dry column (5 minutes at > 10 inches Hg).
   1 x 2 mL hexane.

5. **ELUTE ACIDIC AND NEUTRAL DRUGS**
   1 x 3 mL hexane/ethyl acetate (50:50).
   Collect eluate at < 5 mL/minute.
   Remove collection tubes.

6. **WASH COLUMN**
   1 x 3 mL CH_3OH.
   Dry column (5 minutes at > 10 inches Hg).

7. **ELUTE BASIC DRUGS**
   Replace collection tubes from step 5
   1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2);
   Collect eluate at 1 to 2 mL/minute.

NOTE: Elute into tubes containing the acidic and neutral drugs.
Prepare elution solvent daily.
Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

8. **DRY ELUATE-COMBINE ELUATES (STEP 5 & 7)**
   Evaporate to a volume 100 µL at < 40°C.

9. **RECONSTITUTE**
   Add 900 µL of normal saline
   (Sample volume is now its original 1.0 mL).

10. **ANALYZE BY EMIT**
    Process according to urine drug screening protocols provided by immunoassay manufacturer.

SOURCE - UCT Internal Publication DCN-903020-110

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VITAMIN D AND METABOLITES EXTRACTION USING: 200 mg CLEAN SCREEN® VITAMIN D COLUMN

Part #: CEC30126

LC-UV (PDA), LC-MS/MS

May 7, 2009

1. **PREPARE SAMPLE:**
   To 0.2 to 0.5 mL of sample add an equal volume of deproteinating agent+ (containing internal standard)**

   Vortex mix and centrifuge as appropriate.

   Transfer organic phase to clean, dry glass tube.

   Evaporate to approximately 100 µL at < 40 ˚C.

   Dilute with 3 mL of DI H_2O.

   Vortex mix.

2. **CONDITION COLUMN:**
   1 x 3 mL CH_3OH

   1 x 3 mL D.I. H_2O

   Note: aspirate at < 3 inches Hg to prevent sorbent drying out

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**
   1 x 3 mL DI H_2O

   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE VITAMIN D AND METABOLITES:**
   1 x 3 mL CH_3OH

6. **EVAPORATION:**
   Evaporate eluates to dryness at < 40 ˚C under a gentle stream of nitrogen

7. LC-MS/MS

Reconstitute sample in 50 µL of CH_3OH.
Inject 10 µL.

LC-UV (includes PDA)

Reconstitute sample in 100 µL of DI H_2O
Inject 50 µL

INSTRUMENTAL CONDITIONS:

Column: 150 x 2.1 mm (3µm) SB-aq (Agilent Technologies)

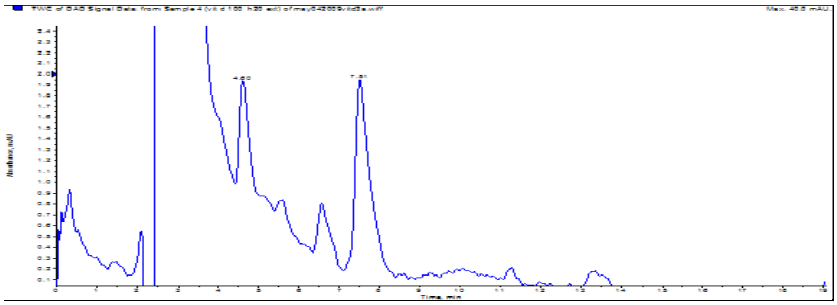
Mobile phase: 90: 10 CH_3OH (containing 0.1% Formic acid): 0.1% Formic acid aqueous

Flowrate: 0.2 mL/ minute

UV (PDA): 260 nm

MS/MS	**MRM Transition (M-H_2O)**
Vitamin D3	386.5/256.7
25 Hydroxy Vitamin D3	383.1/211.1
25 Hydroxy Vitamin D2	395.4/209.1

PDA Chromatogram of Vitamin D (R_t=7.3 min)



* Deproteinating agents include acetonitrile, acetone, or methanol.

** Internal Standard: 25 Hydroxy Vitamin D3-D6: MRM Transition: 389.1/211.1

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SAMPLE PREPARATION FOR

Whole Blood, Serum, Plasma, Urine and Tissues

Sample Preparation: Whole Blood/ Serum/ Plasma

The following methods may be employed with whole blood, serum or plasma samples to disrupt protein binding to drug materials.

pH modification:

1. pH adjustment: Extreme pH values i.e. greater than 9 or less than 3. In these cases, buffer strengths greater than or equal to 0.1 M should be used.
2. pH adjustment in stages: i.e. buffering first to pH 5 then to pH 3.

Precipitation:

1. Employing a polar solvent e.g. Acetonitrile, Acetone, or Methanol (in general, two parts organic solvent to one part biological sample), mix/ vortex then centrifuge down the sample leaving the precipitate at the base of the sample vessel. The supernatant can be removed by decantation.
2. Inorganic salts such as ammonium sulfate or zinc sulfate may also be employed to disrupt protein binding and precipitate the proteins.

Acid Treatment:

1. In this process, biological samples may be treated with Formic, Perchloric, or Trichloroacetic acids e.g 50 µL of 0.1 M perchloric acid per 500 µL of plasma or 1:1 dilution of the sample with 10% w/v trichloroacetic acid.
   Disruption of the protein binding probably occurs through the formation of the acid salt.

Sonication:

1. The addition of an appropriate buffer e.g phosphate or acetate may be followed by sonication of the sample for a period of approximately 15 minutes. The sample is then vortex mixed and centrifuged. The supernatant may be decanted off and the pellet discarded.

Enzymic Hydrolyis:

In this procedure, a proteolytic enzyme such as β- Glucuronidase is used to disrupt the protein binding and cleave the drug.

1. To the sample is added 1 mL of an appropriate buffer for the enzyme e.g. 1 mL of 1.0 mL of acetate buffer (pH 5.0) with 100 µL of β- Glucuronidase. The sample is then heated at 60˚C for approximately 60 minutes. The sample is cooled to room temperature, vortex mixed and may be centrifuged,

Sample Preparation: Urine

The following methods may be employed with urine samples.

pH modification:

1. pH adjustment: Extreme pH values i.e. greater than 9 or less than 3. In these cases, buffer strengths greater than or equal to 0.1 M should be used.
2. pH adjustment in stages: i.e. buffering first to pH 5 then to pH 3.

Precipitation:

1. Employing a polar solvent e.g. Acetonitrile, Acetone, or Methanol (in general, two parts organic solvent to one part biological sample), mix/ vortex then centrifuge down the sample leaving the precipitate at the base of the sample vessel. The supernatant can be removed by decantation.
2. Inorganic salts such as ammonium sulfate or zinc sulfate may also be employed to precipitate the proteins.

Acid Treatment:

1. In this process, biological samples may be treated with Formic, Perchloric, or Trichloroacetic acids e.g 1:1 dilution of the sample with 10% w/v trichloroacetic acid. The samples are vortex mixed and centrifuged. The supernatant can be decanted off.

Filtration:

1. The sample may be simply filtered by passing it through a micro porous filter e.g 45 µm nylon .

Enzymic Hydrolyis:

In this procedure, a proteolytic enzyme such as β- Glucuronidase is used to disrupt the protein binding and cleave the drug.

1 To the sample is added 1 mL of an appropriate buffer for the enzyme e.g. 1 mL of 1.0 mL of acetate buffer (pH 5.0) with 100 µL of β- Glucuronidase. The sample is then heated at 60˚C for approximately 60 minutes. The sample is cooled to room temperature, vortex mixed and may be centrifuged.

Sample Preparation: Tissues

The following methods may be employed with tissues sample to disrupt protein binding to drug materials.

Tissue should be thawed, if frozen. The tissue sample can then be transferred onto a clean disposable weighing boat, avoiding transfer of blood/liquid as much as possible. Cut tissue into small pieces with scalpel, avoiding vessels or other tough membranes/sections.Tare a clean blender cup on the balance and add tissue pieces to desired weight (i.e. ~10g).Add the appropriate amount of DI water to the blender cup to create a 1:4 dilution (e.g. 10g tissue + 30g DI water). Homogenize sample.Transfer homogenized tissue to a suitable labelled container (e.g. 50-mL plastic screw-cap).

pH modification:

1 pH adjustment: Extreme pH values i.e. greater than 9 or less than 3. In these cases, buffer strengths greater than or equal to 0.1 M should be used.
2 pH adjustment in stages: i.e. buffering first to pH 5 then to pH 3.

Precipitation:

2 Employing a polar solvent e.g. Acetonitrile, Acetone, or Methanol (in general, two parts organic solvent to one part biological sample), mix/ vortex then centrifuge down the sample leaving the precipitate at the base of the sample vessel. The supernatant can be removed by decantation.
2 Inorganic salts such as ammonium sulfate or zinc sulfate may also be employed to precipitate the proteins.

Acid Treatment:

1 In this process, biological samples may be treated with Formic, Perchloric, or Trichloroacetic acids e.g 1:1 dilution of the sample with 10% w/v trichloroacetic acid. The samples are vortex mixed and centrifuged. The supernatant can be decanted off.

Enzymic Hydrolyis:

In this procedure, a proteolytic enzyme such as β- Glucuronidase is used to disrupt the protein binding and cleave the drug.

1 To the sample is added 1 mL of an appropriate buffer for the enzyme e.g. 1 mL of 1.0 mL of acetate buffer (pH 5.0) with 100 µL of β- Glucuronidase. The sample is then heated at 60˚C for approximately 60 minutes. The sample is cooled to room temperature, vortex mixed and may be centrifuged.

UCT

SELECTRA-SIL®
DERIVATIZING REAGENTS

SELECTRA-SIL®
Derivatizing Reagents

These reagents are manufactured by UCT to exact standards of purity and consistency.

Benefits of Derivatization:

- Improved chromatographic resolution - increased volatility - reduced intermolecular hydrogen bonding - separation of structurally similar compounds.
- Improved mass spectral characteristics - higher mass fragments - greater S/N (signal to noise ratio) - more unique masses- increased abundance/sensitivity of molecular ions.
- Improved thermal stability of some compounds - reduced thermal degradation – higher temperatures to speed analysis.
- Increased instrument and lab productivity - fewer reinjections or repeats due to peak tailing - high confidence in analyte identification and quantitation - easy to perform - inert by-products of derivatization will not degrade capillary column performance.

SELECTRA-SIL®
Derivatizing Reagents

Silylation Reagents

Silyl derivatives are the most widely used chemical derivatization reagents, especially for gas chromatography. Silyl derivatization requires an "Active" hydrogen as seen in acids, alcohols, thiols, amines, amides, enolizable ketones and aldehydes to be replaced by a trimethysilyl group or tertiary butyl dimethylsily.
These reagents must be protected from moisture.

BSTFA and BSTFA with TMCS (1% or 10%)

(N,O-bis(trimethylsilyltrifluoroacetamide) with/without Trimethylchlorosilane)

- Trimethylsilyl donor strength equal to BSA
- Reacts with the same classes of compounds as BSA producing the same derivatives.
- TMCS (Trimethylchlorosilane) added to derivatize amides, many secondary amines and hindered hydroxyls that are not reactive to BSTFA alone.
- Increased volatility of the reaction by-products over the non-fluorinated derivatives of BSA

MSTFA and MSTFA with 1% TMCS

(N-methyl-N-trimethylsilyltrifluoroacetamide) with/without Trimethylchlorosilane)

- A trimethylsilyl adduct with donor strength equal to BSA and BSTFA
- Most volatile of the TMS derivatives often elutes at the solvent front of the GC.
- Addition of TMCS aids in the derivatization of amides, secondary amines and hindered hydroxy groups.

MTBSTFA and MTBSTFA with 1% TBDMCS

(N-methyl-N-(t-butyldimethysilyl)-trifluoroacetamide with/without T-butyldimethychlorosilane)

- Derivatizes hydroxyl, carboxyl, thiol and primary and secondary amines
- Addition of TBDMCS (tertiary butyl-dimethylchlorosilane) increases the silylation ability of this reagent to derivatize hindered alcohols and amines
- MTBSTFA derivatives are more stable than TMS derivatives to hydrolysis
- Reaction by-products are neutral and volatile

TMCS

(Trimethylchlorosilane)

- Catalysts used to increase the reactivity of other silylation reagents
- Used to form trimethysilyl esters of organic acids.

Derivatizing Reagents

Acylation Reagents

Acylation is the conversion of compounds with active hydrogens (such as –SH, –OH and –NH) into thioesters, esters and amides respectively by forming a carboxylic acid derivative. The primary use of acylation chemistry is to form compounds that chromatograph better and have a greater detectability than the parent molecule. For example, addition of a perfluoro group will improve the detectability of analytes if an electron capture detector is used.

PERFLUORO ACID ANHYDRIDES

MBTFA
(N-Methyl-bis[Trifloroacetamide])

- This reacts with primary and secondary amines, hydroxyl and thiol groups under mild, nonacidic conditions.
- Produces very volatile derivatives of carbohydrates.
- Can be used to selectively acylate amines in the presence of hydroxyl and carboxyl groups that have been protected by silylation.

HFAA
(Heptafluorobutyric Acid Anhydride, HFBA)

- React readily with alcohols, phenols and amines producing stable volatile derivatives for TCD, FID, ECD and other detectors.
- It is suggested that fluoro alcohols (such as PFPOH an HFIP) be used to react with the acidic by-products of these to drive the reaction to completion.

PFAA
(Pentafluoropropionic Acid Anhydride, PFPA)

- React readily with alcohols, phenols and amines producing stable volatile derivatives for TCD, FID, ECD and other detectors.
- Commonly used in the determination of benzoylecgonine and opiates.
- Acidic by-products of this reaction must be removed before the derivative can be injected onto the GC.

TFAA
(Trifluoroacetic Acid Anhydride)

- React readily with alcohols, phenols and amines producing stable volatile derivatives for TCD, FID, ECD and other detectors.
- Most reactive of all the perfluoroacid anhydrides and frequently used to identify methamphetamine.

TFAI
(Trifluoroacetylimidazole)

- React readily with alcohols, phenols and amines producing stable, volatile derivatives for TCD, FID, ECD and other detectors.
- Offer considerable advantages over the anhydrides for the preparation of perfluoroacyl derivatives; the reactions are smooth, quatitative and produce no acid by-products.
- Principal by-product is imidazole (relatively inert).

SELECTRA-SIL®
Derivatizing Reagents

Alkylation Reagents

Alkylation reactions replace active hydrogens by an aliphatic or aliphatic-aromatic (benzyl) group. The principal use of this mode of derivation is to improve upon the chromatography of compounds such as free organic acids. Alkylation reactions can form ethers, thioethers, thioesters, n-alkylamines, amides and sulfonamides. These derivatives possess excellent stability and can be stored for extended periods of time.

TMPAH
(0.2 M Trimethylanilinium Hydroxide in Methanol)

- Used for the methylation of barbiturates, sedatives, xanthines, and alkaloids for GC.
- The derivatization of these compounds often can be done in the injector of the GC.

4 CB
4-Carbethoxyhexafluorobutyrl Chloride

- Peptides + Propionic anhydride converts N-termini and Lysines to propyl amides. This results in a decrease in net charge of the peptides and increased hydrophobicity.

PIA
Propionic Anhydride

- It hydrates with water producing corrosive propionic acid. It is miscible in most organic solvents and decomposes with alcohol. Propionic anhydride used as an intermediate to produce dyes, pharmaceuticals, agrochemicals and other organic compounds

SELECTRA-SIL®
Derivatizing Reagents

Specialized Reagents

PFPOH (Pentafluoropropanol) or HFIP (Hexafluoro-2-propanol)

- Used in combination with the acid anhydrides to promote reaction and removal of acidic by-products.
- This reaction is especially used for carboxylic acids.
- The addition of fluorine atoms into the molecule greatly adds to the sensitivity of certain detectors (ECD).

SELECTRA-SIL®

Solvents for Derivatizing Reagents

Solvent	Part Number	Size	Units
Acetonitrile (ACN)	SACN-0-50	50 mL vial	1 ·
Acetonitrile is used as a solvent to promote silylation reactions. · It is a polar solvent.			
Pyridine	SPYR-0-50	50 mL vial	1 ·
Good solvent for many organic materials and reactions requiring a nucleophilic environment. · A moderately polar solvent.			

Silylation Reagents

in 10 gram vials.

Part #:	Name	Abbreviation
SBSTFA-0-10	N,O-bis(trimethylsilyltrifluoroacetamide)	BSTFA
SBSTFA-1-10	N,O-bis(trimethylsilyltrifluoroacetamide) w/1% trimethylchlorosilane	BSTFA w/1% TMCS
SBSTFA-10-10	N,O-bis(trimethylsilyltrifluoroacetamide) w/10% trimethylchlorosilane	BSTFA w/10% TMCS
SMSTFA-0-10	N-methyl-N-trimethylsilyltrifluoroacetamide	MSTFA
SMSTFA-1-10	N-methyl-N-trimethylsilyltrifluoroacetamide w/1% Trimethychlorosilane	MSTFA w/1% TMCS
SMSTFA-10-10	N-methyl-N-trimethylsilyltrifluoroacetamide w/10% Trimethychlorosilane	MSTFA w/10% TMCS
SMTBSTFA-0-10	N-methyl-N-(t-butyldimethylsilyl)- trifluoroacetamide	MTBSTFA
SMTBSTFA-1-10	N-methyl-N-(t-butyldimethylsilyl)- trifluoroacetamide w/1% t-butyldimethylchlorosilane	MTBSTFA w/1% TMCS
SMTBSTFA-10-10	N-methyl-N-(t-butyldimethylsilyl)- trifluoroacetamide w/10% t-butyldimethylchlorosilane	MTBSTFA w/10% TMCS
STMCS-0-10	Trimethylchlorosilane	TMCS

UCT offers convenient sizes ranging from one gram vials packaged in units of ten or 10, 25 and 100 gram vials.

Acylation Reagents
in 10 gram vials.

Part #:	Name	Abbreviation
SMBTFA-0-10	N-methyl-bis-(trifluoroacetamide)	MBTFA
SHFAA-0-10	Heptafluorobutyric acid anhydride	HFAA
SPFAA-0-10	Pentafluoropropionic acid anhydride	PFAA
STFAA-0-10	Trifluoroacetic acid anhydride	TFAA
STFAI-0-5*	N-trifluoroacetylimidazole	TFAI

* 5 gram vial

Alkylation Reagents
in 10 gram vials.

Part #:	Name	Abbreviation
S4CB-0-10	4-Carbethoxyhexafluorobutyryl Chloride	4CB
STMPAH-0-10	0.2 M Trimethylanilinium Hydroxide in Methanol	TMPAH
SPIA-0-10	Propionic Anhydride	PIA

Specialized Reagents
in 10 gram vials.

Part #:	Name	Abbreviation
SHFIP-0-10	Hexafluoro-2-propanol	HFIP
SPFPOH-0-10	Pentafluoropropanol	PFPOH