UCT

SOLID PHASE EXTRACTION APPLICATIONS MANUAL

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 ($NaH_2PO_4 \cdot H_2O$): 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 2.7 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 BENZODIAZEPINES 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	

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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°.

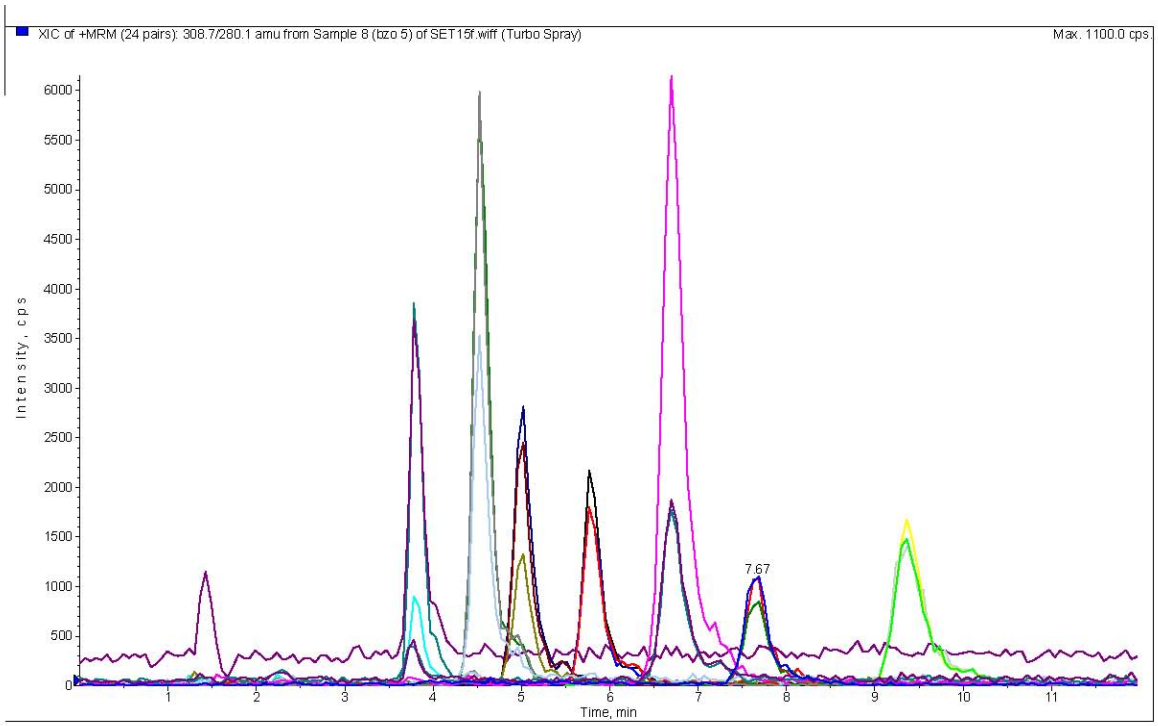
7. Reconsitute sample in 50 µL of 0.02% formic acid (aqueous).

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

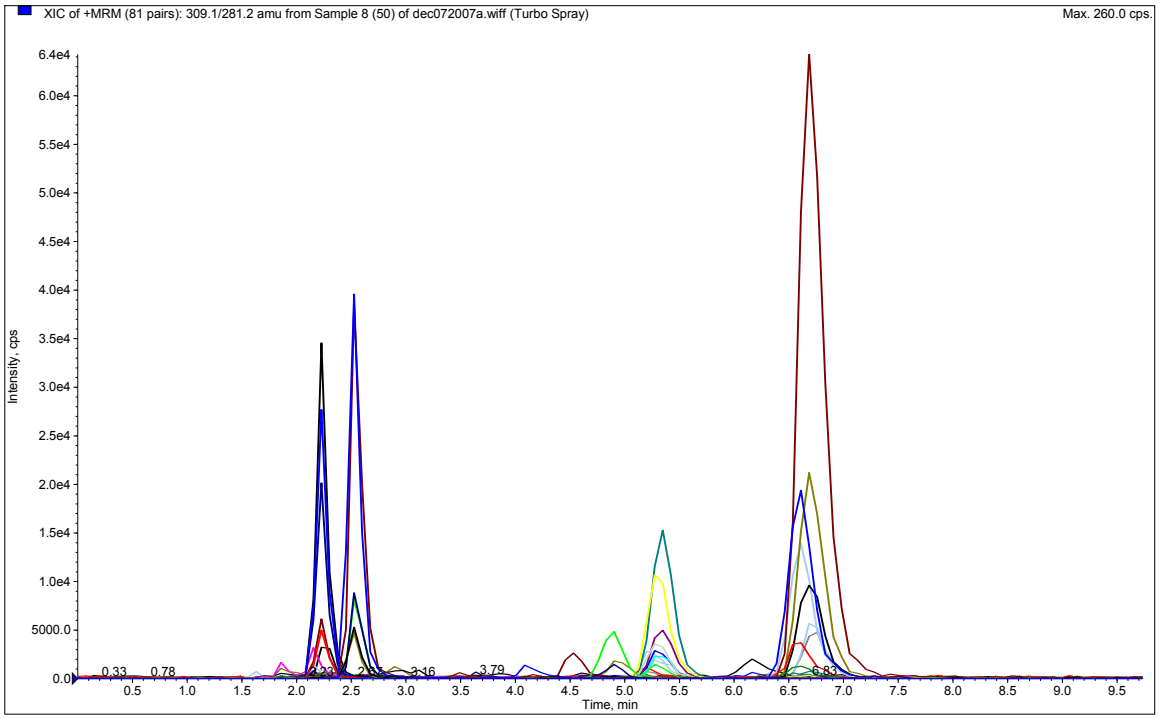
Chromatogram of:

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

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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-D5l	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.

7. **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)

DCN-903020-47

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

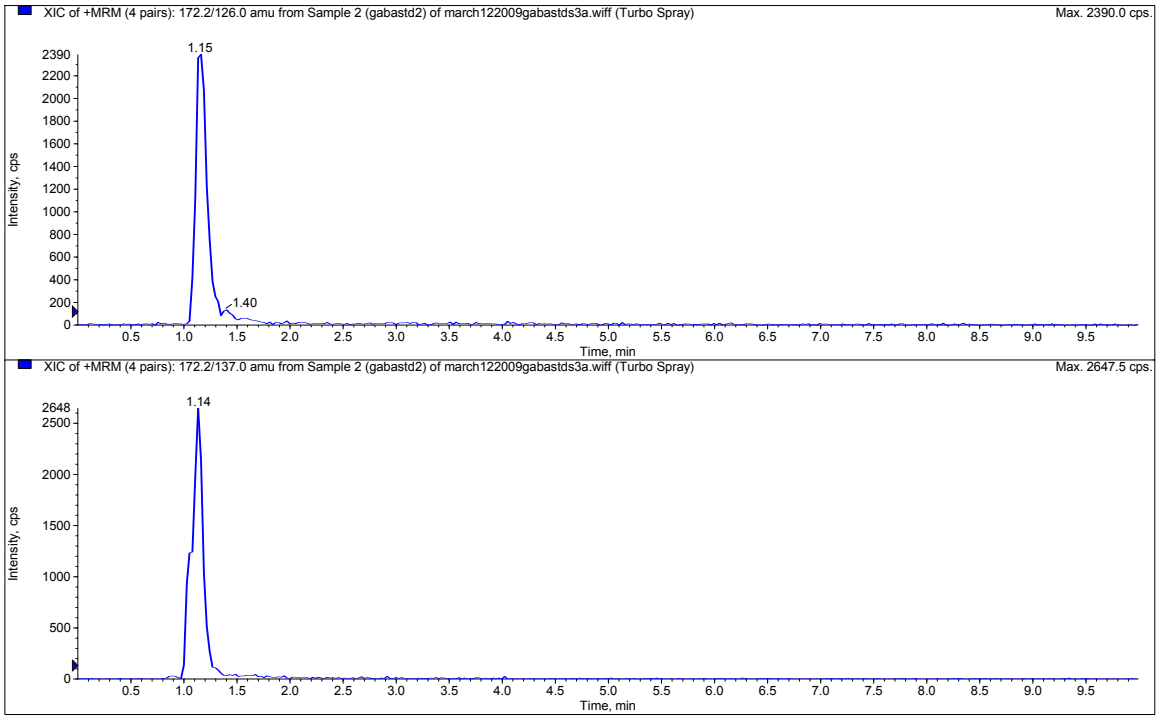
Flowrate: 0.2 mL/ minute

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

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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

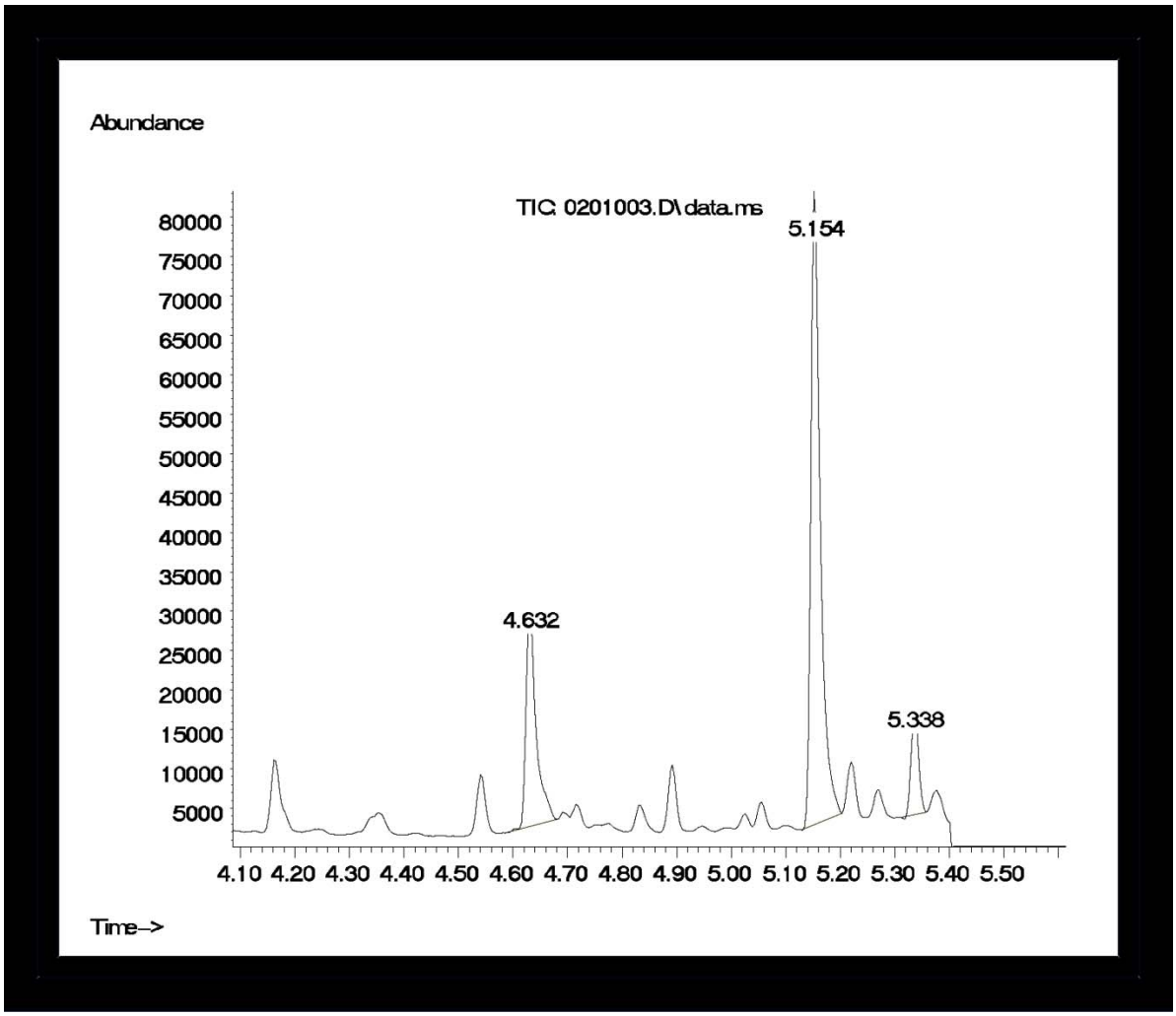
7. Dry samples

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).

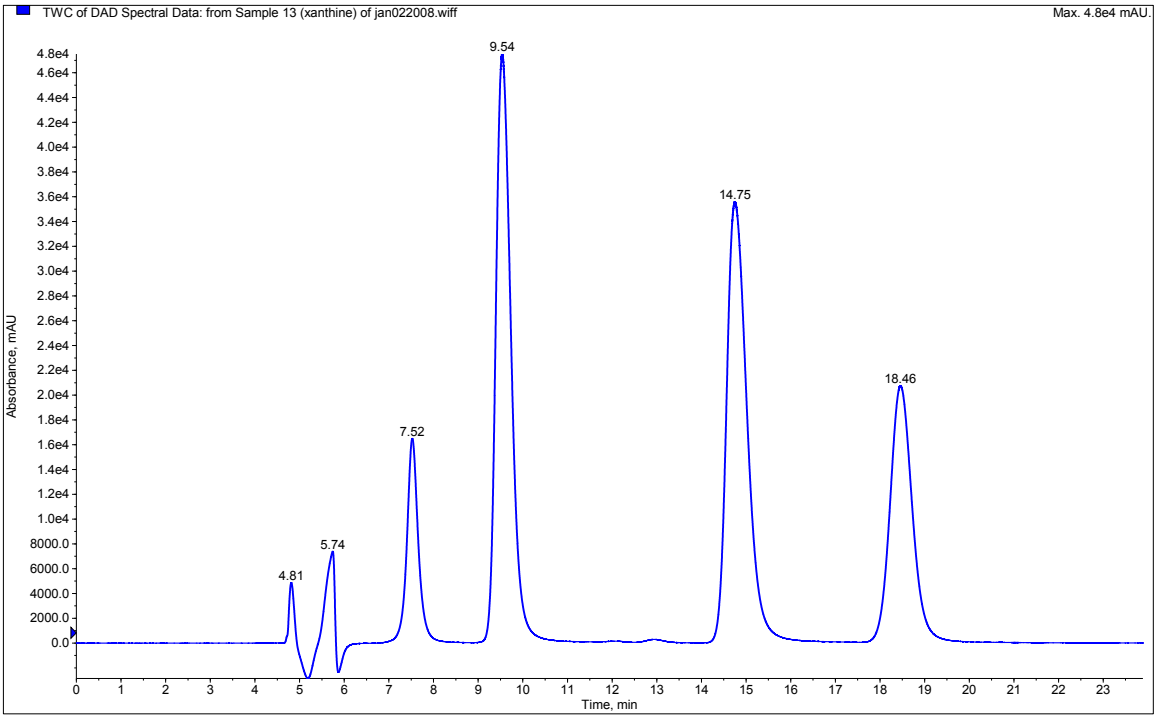
Flowrate: 0.1 mL/ minute

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.

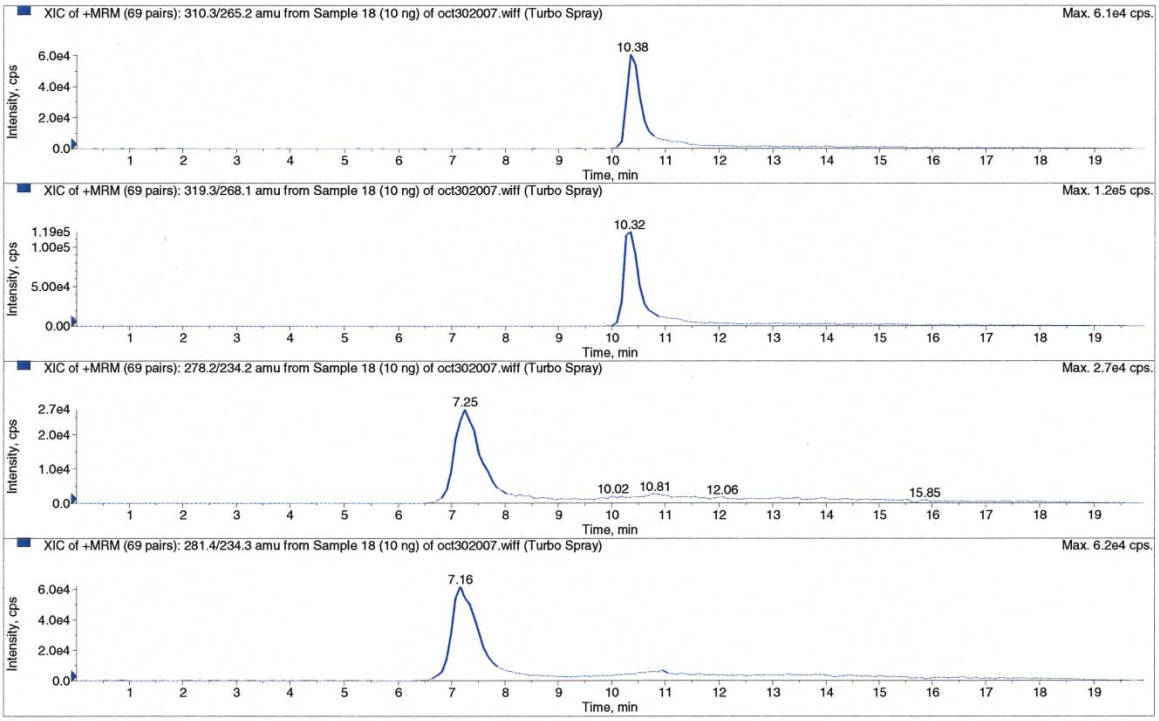
INSTRUMENT CONDITIONS:

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

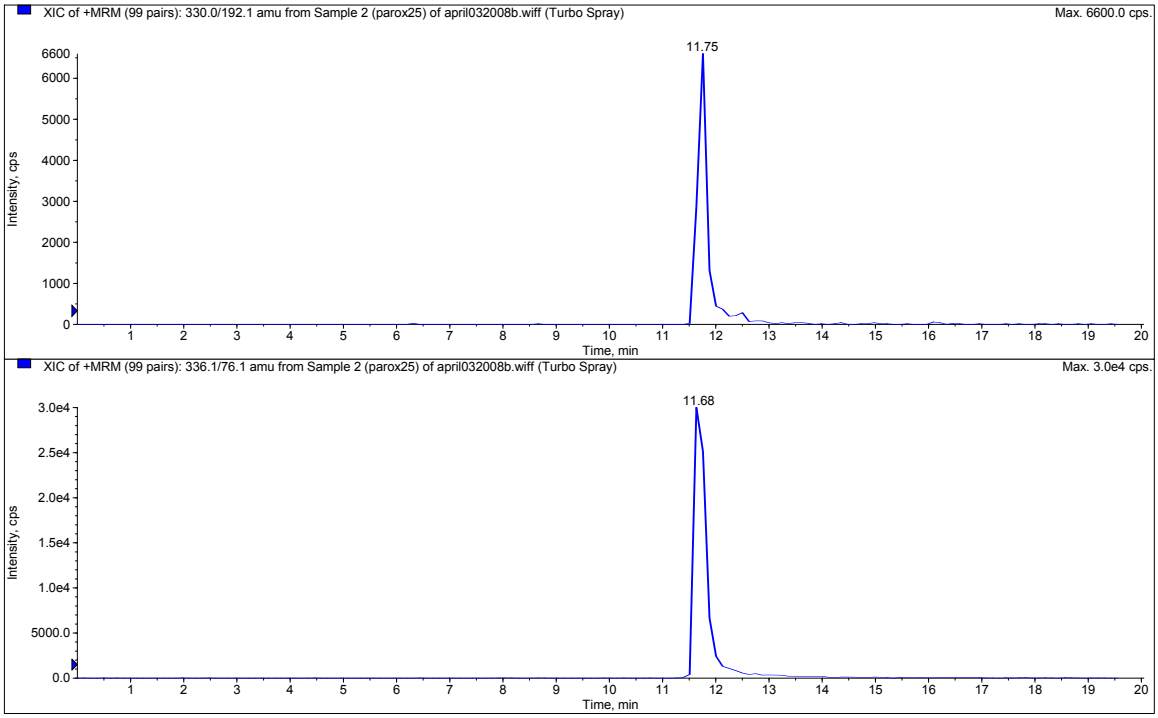
Flow rate: 0.35 mL/ minute
Injection Volume: 5 µL
Column Temperature: ambient
Detector: API 2000 MS/MS.

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:

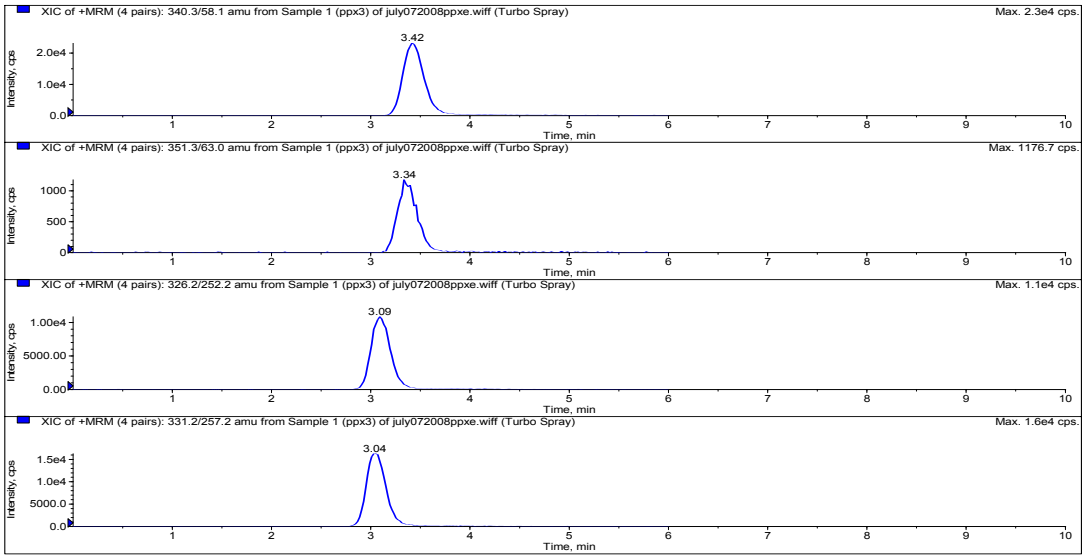
Time	% Acetonitrile	%0.1% Formic acid
0	30	70
10	30	70

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.

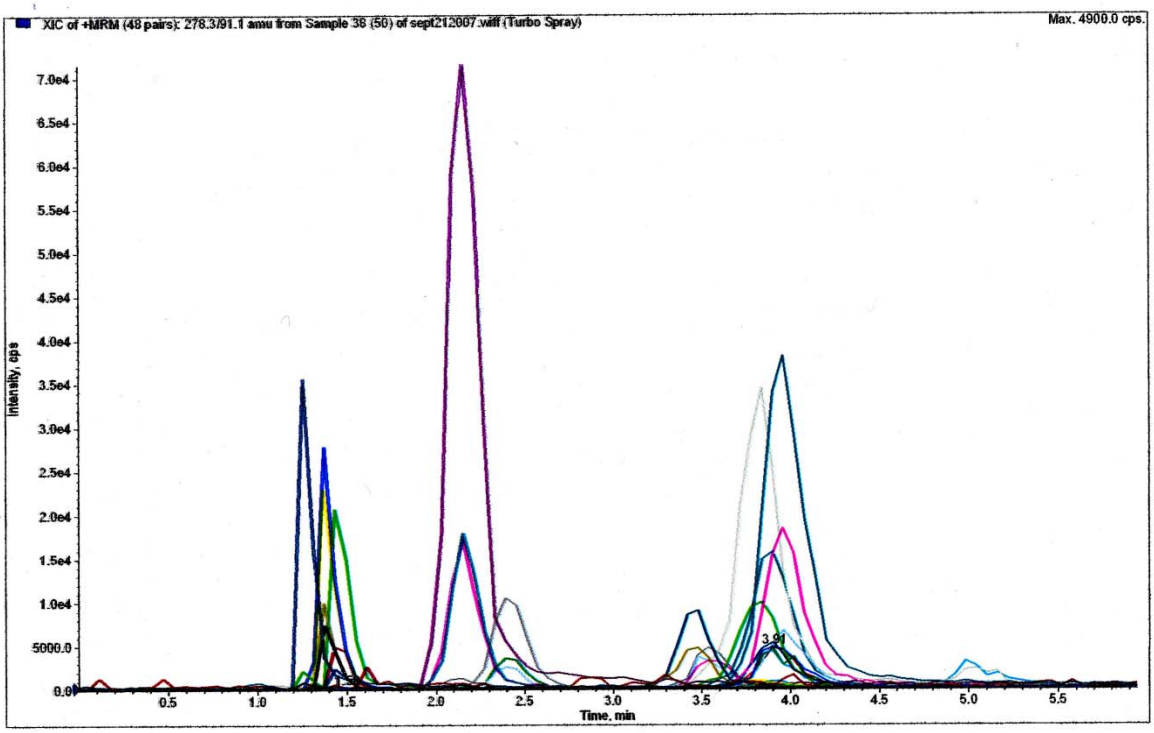
7. **RECONSTITUTE sample in 100 µL of methanol.**
   Inject 5 µL.

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.

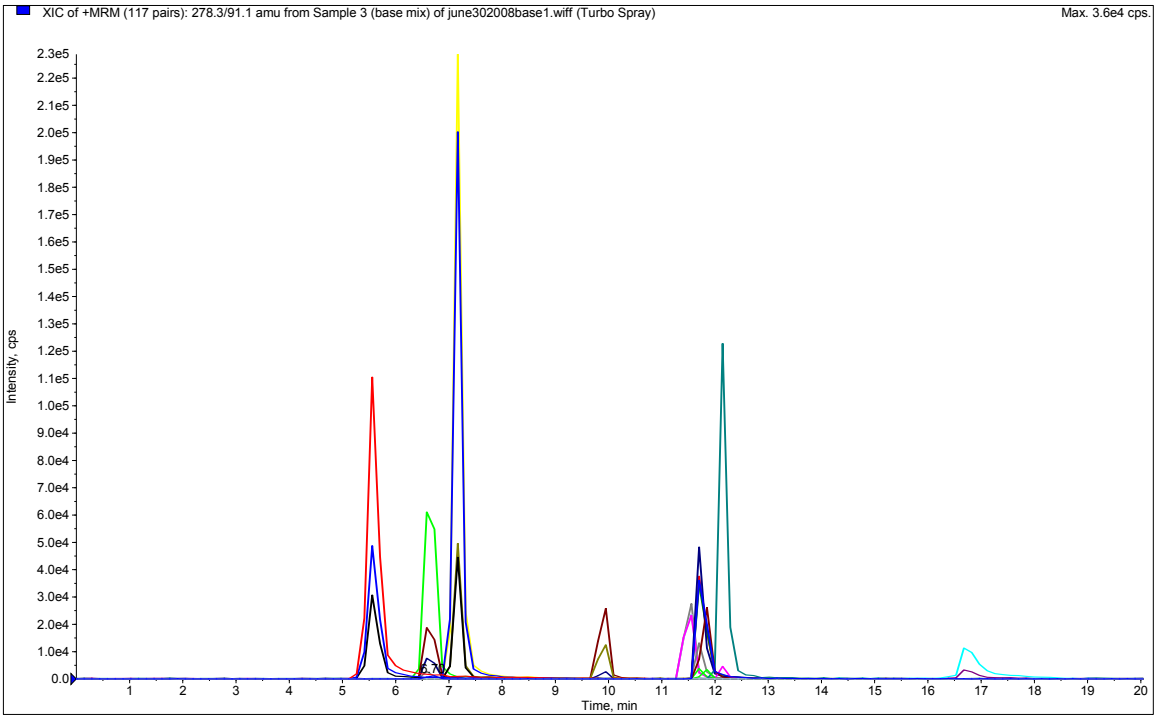
INSTRUMENT CONDITIONS:

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.

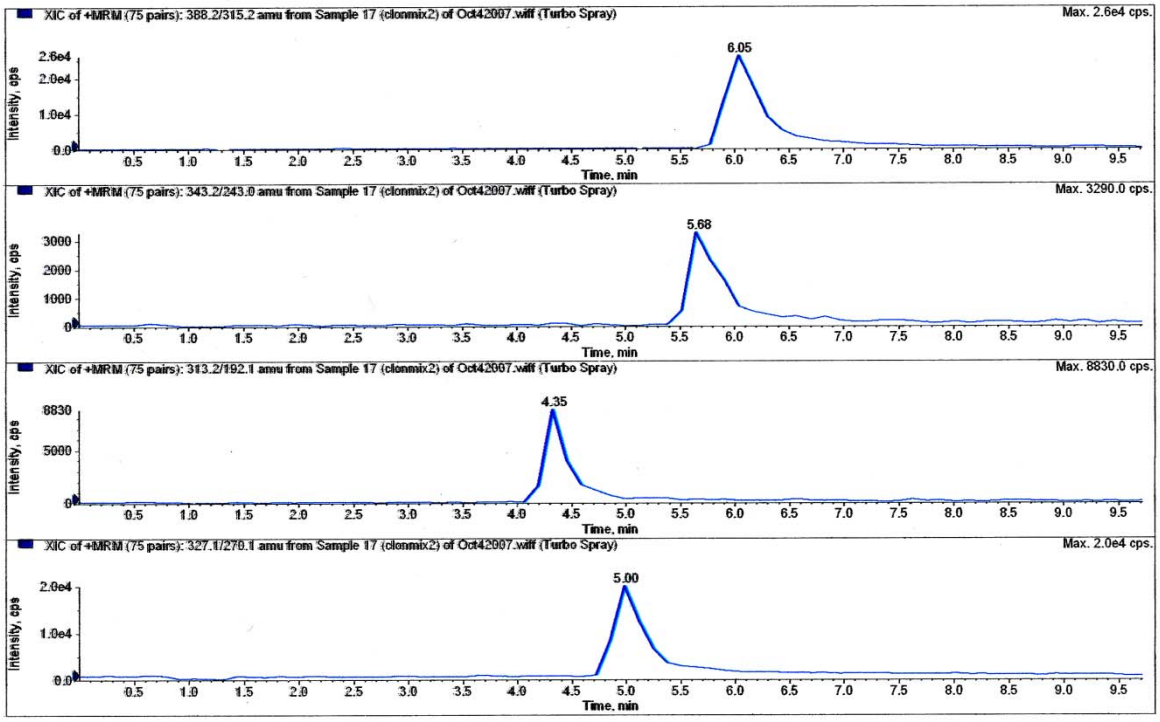
4. **WASH COLUMN**
   1 x 1 mL D.I. Water
   1 x 0.5 mL 1% ammonium hydroxide in D.I. water.
   Dry column (5 minutes at > 10 inches Hg).

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.

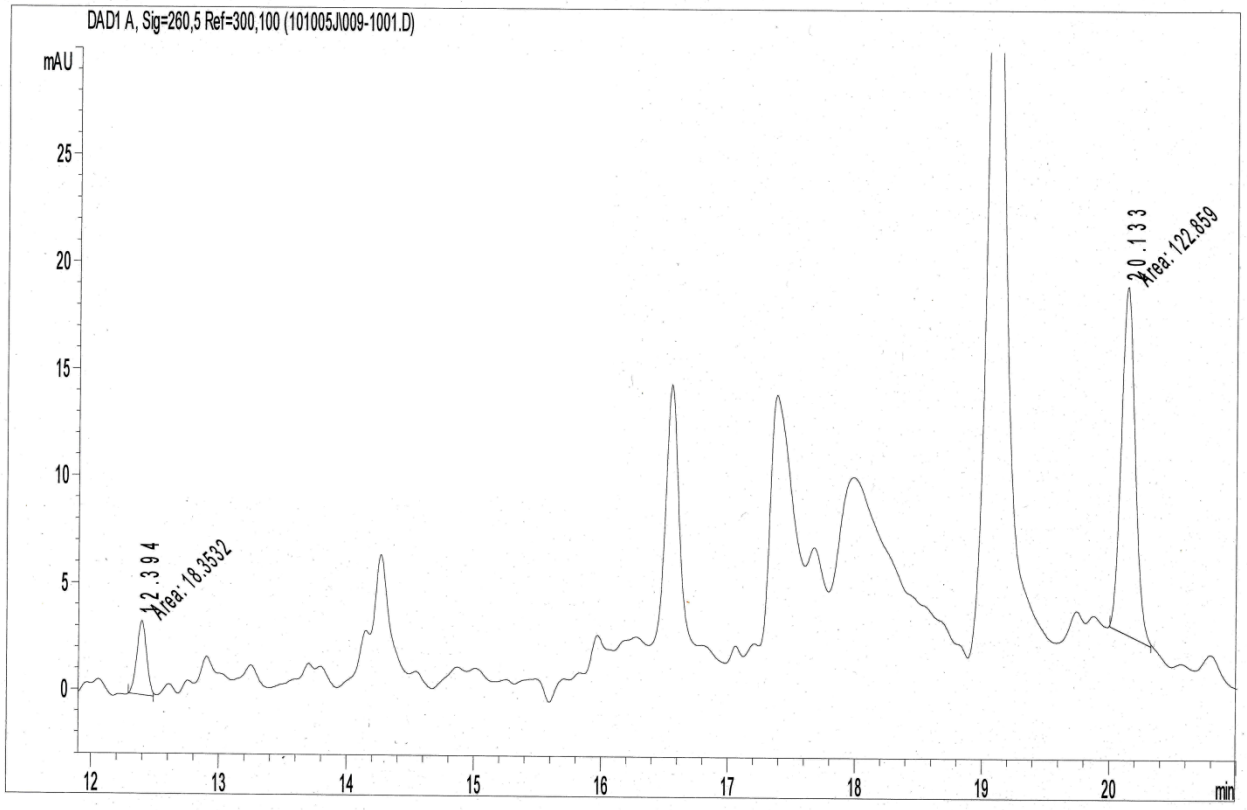
INSTRUMENT CONDITIONS:

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



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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

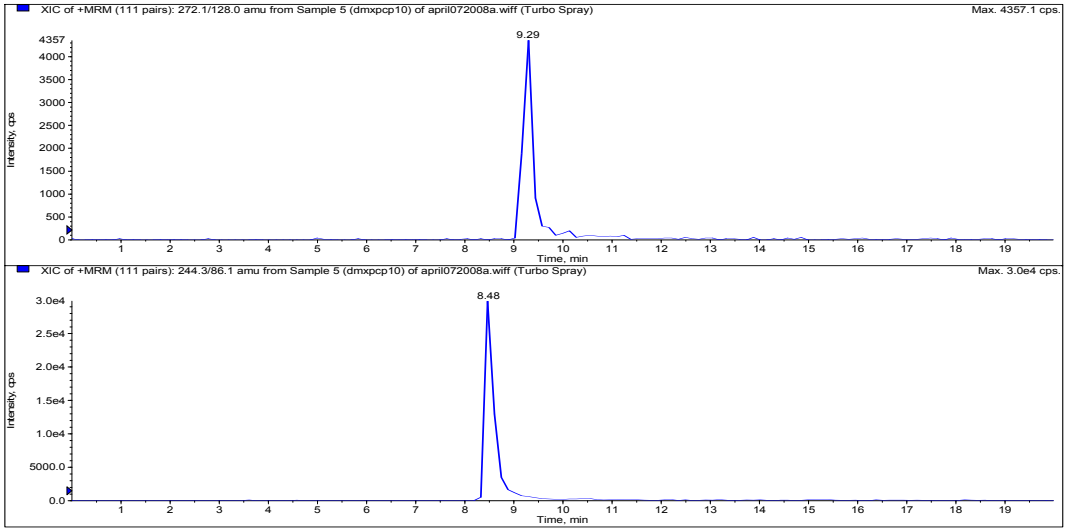
Flowrate: 0.35 mL/ minute

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:

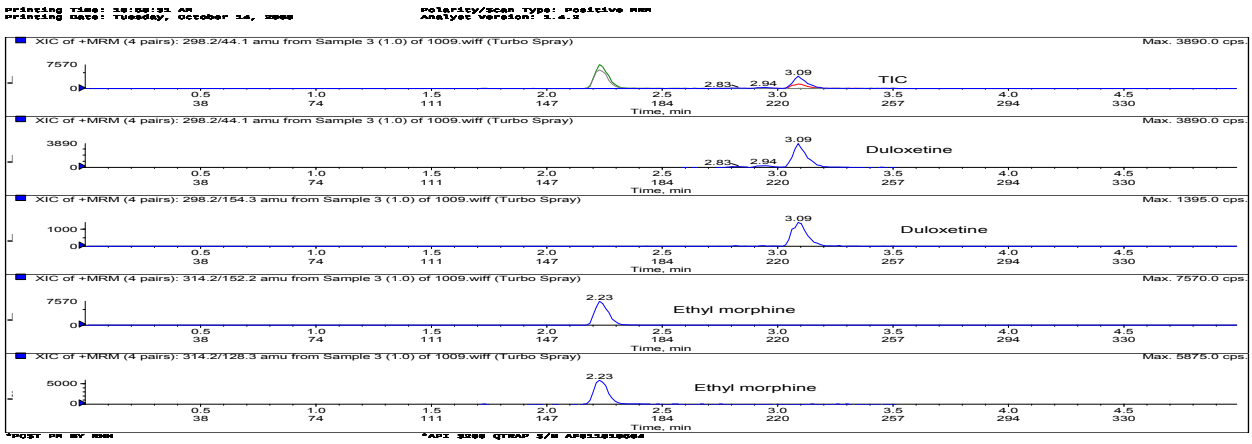
Column: 50 x 2.1 mm (5µm) C_{18}
Mobile phase:

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

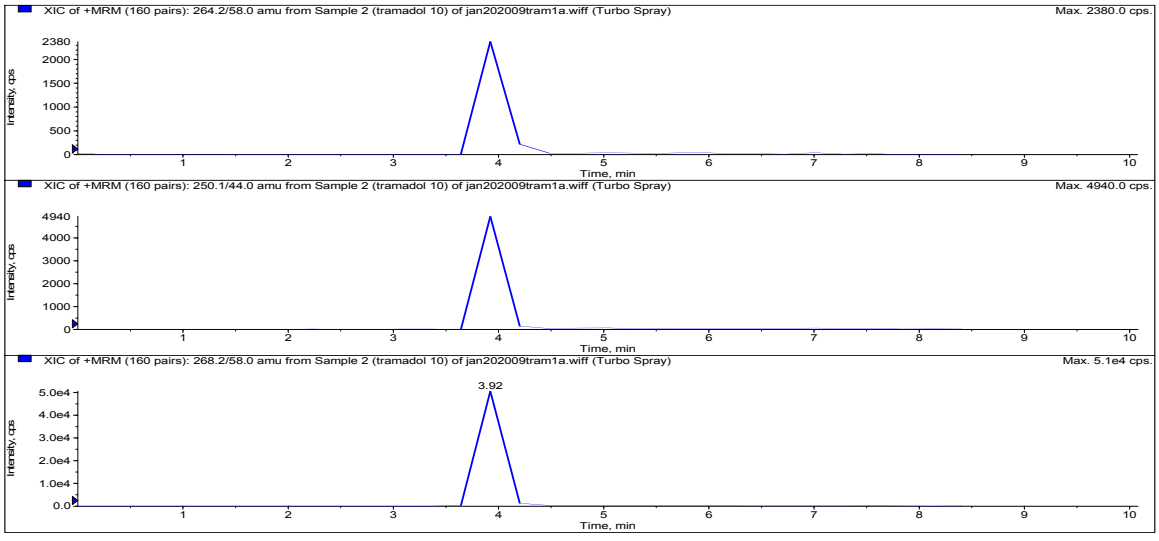
Column Temperature: ambient

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.

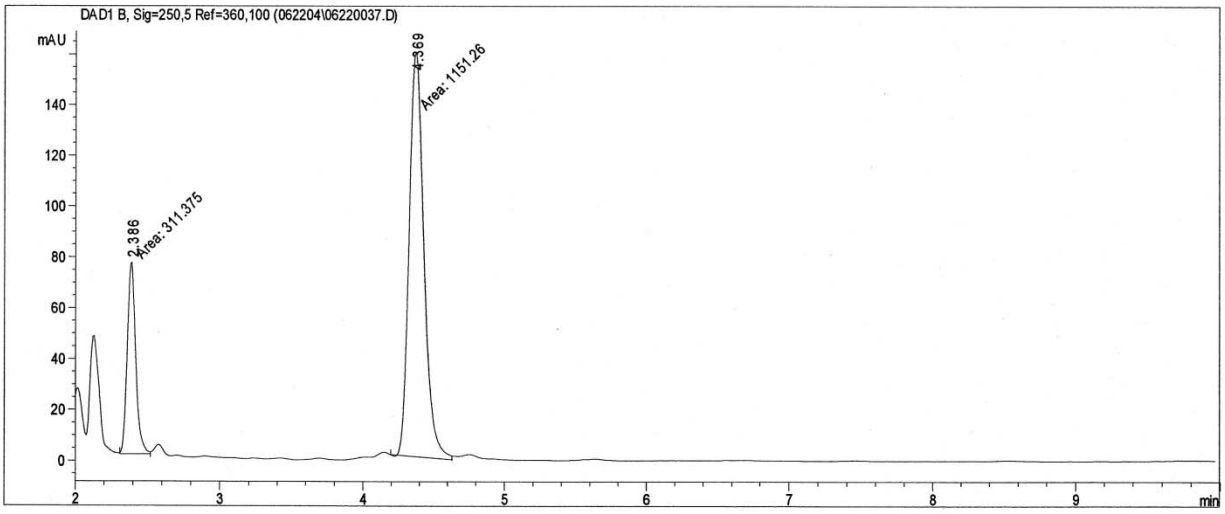
INSTRUMENT CONDITIONS:

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

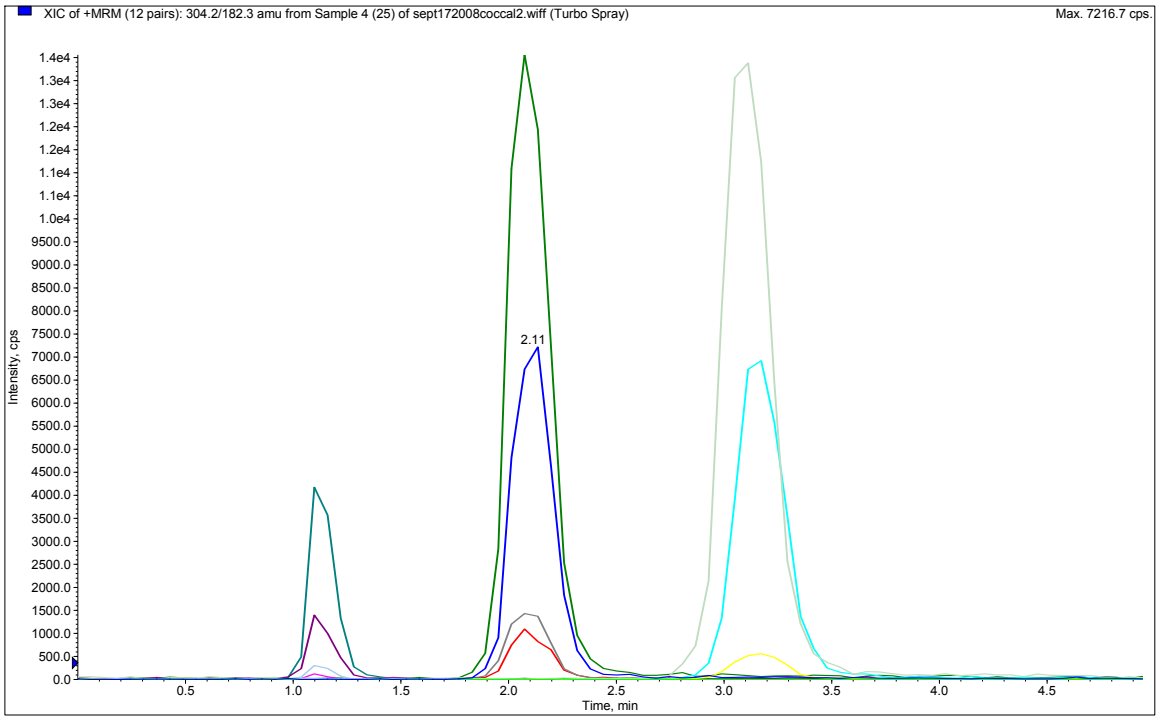
Compound	MRM Transition	Cerilliant #
Cocaine	304.2/ 182.3	C-008
* Cocaine-D3	307.2/ 185.2	C-009
Benzoylecgonine	290.1/ 168.0	B-004
*Benzoylecgonine-D8	298.2/ 171.3	B-013
Cocaethylene	318.2/ 196.2	C-010
*Cocaethylene-D8	326.2/ 204.2	C-024

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

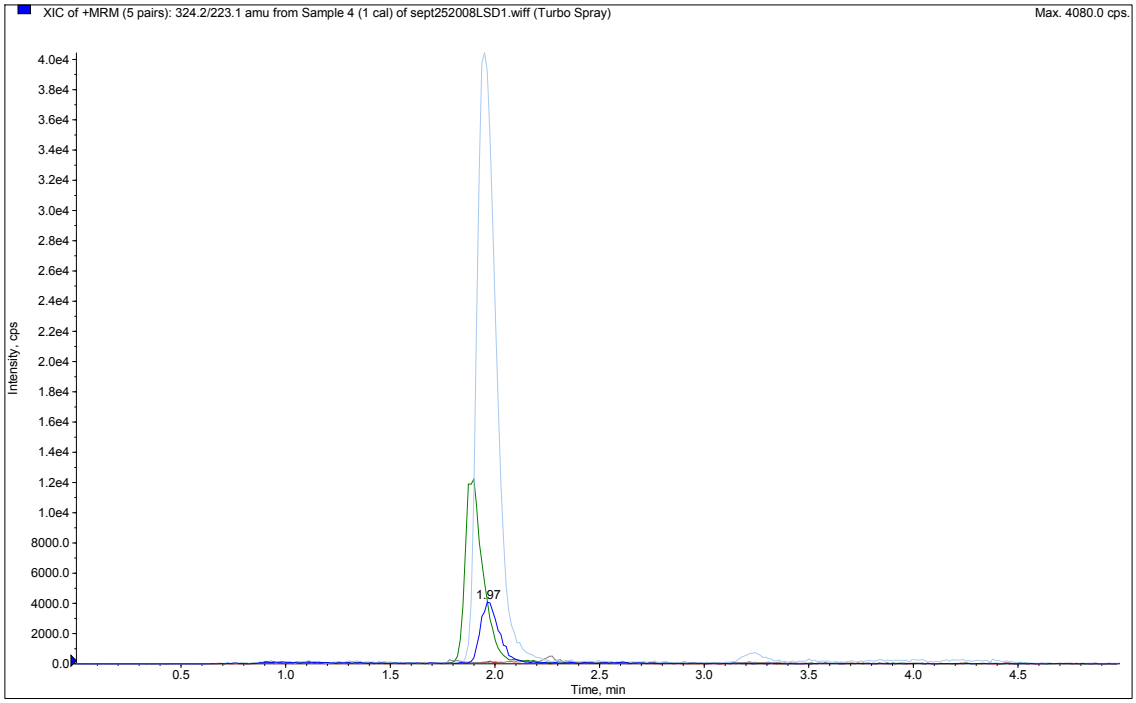
Flowrate: 0.5 mL/ minute

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

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.

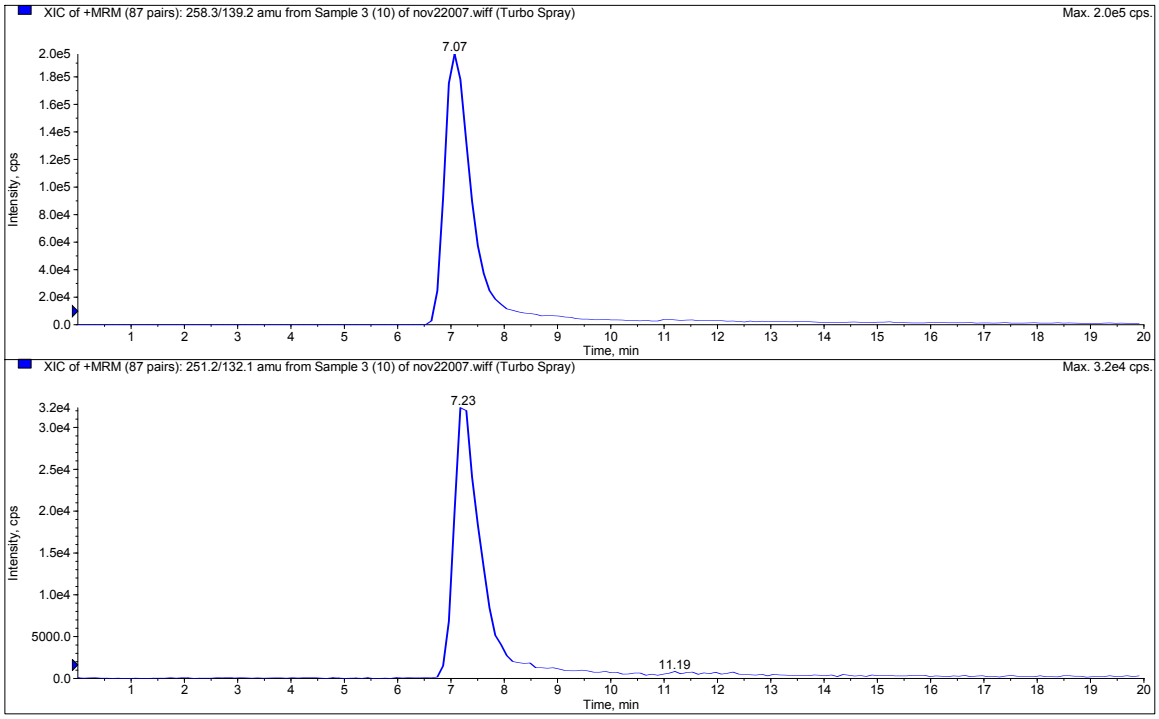
6. **EVAPORATION:**
   Evaporate eluates under a gentle stream of nitrogen < 40°.

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

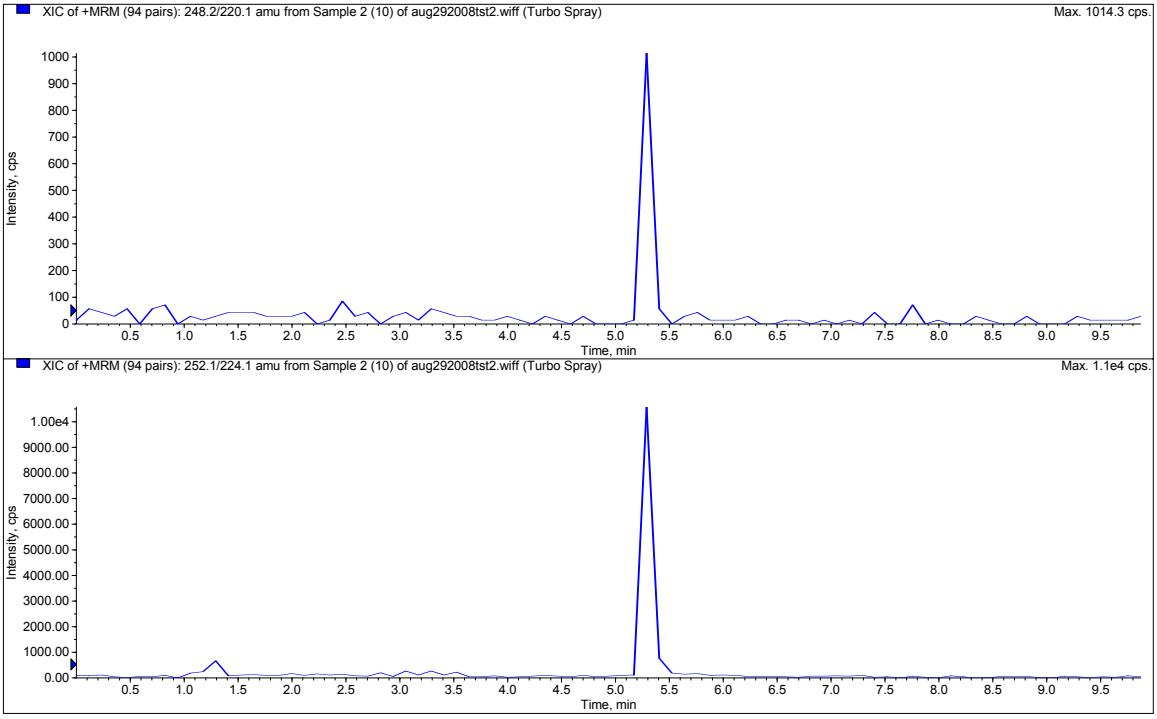
Detector: API 2000 MS/MS

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

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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

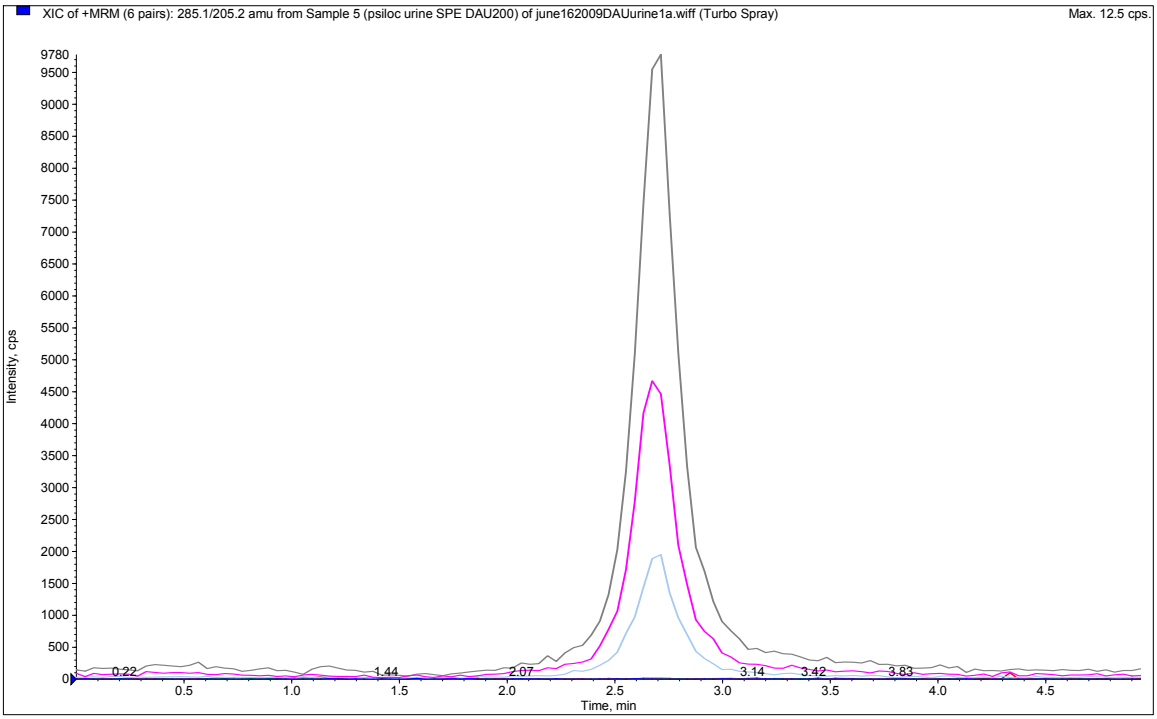
Column Temperature: ambient

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

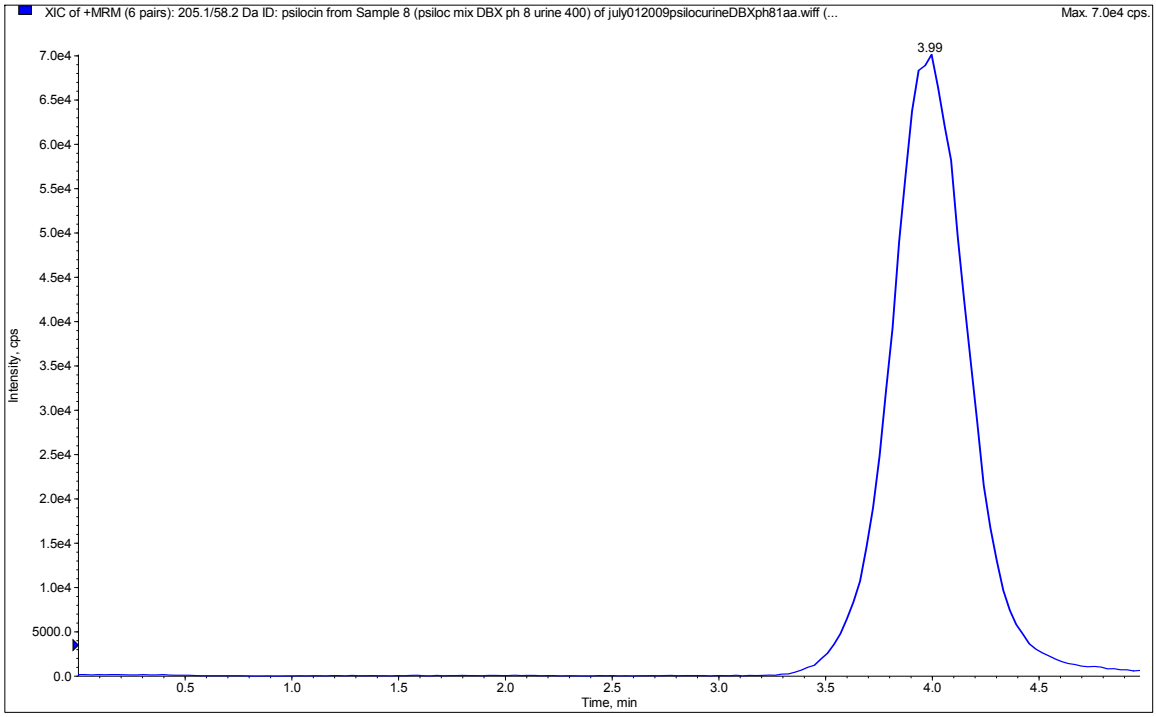
Flowrate: 0.3 mL/ minute

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

DCN-903020-112

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.

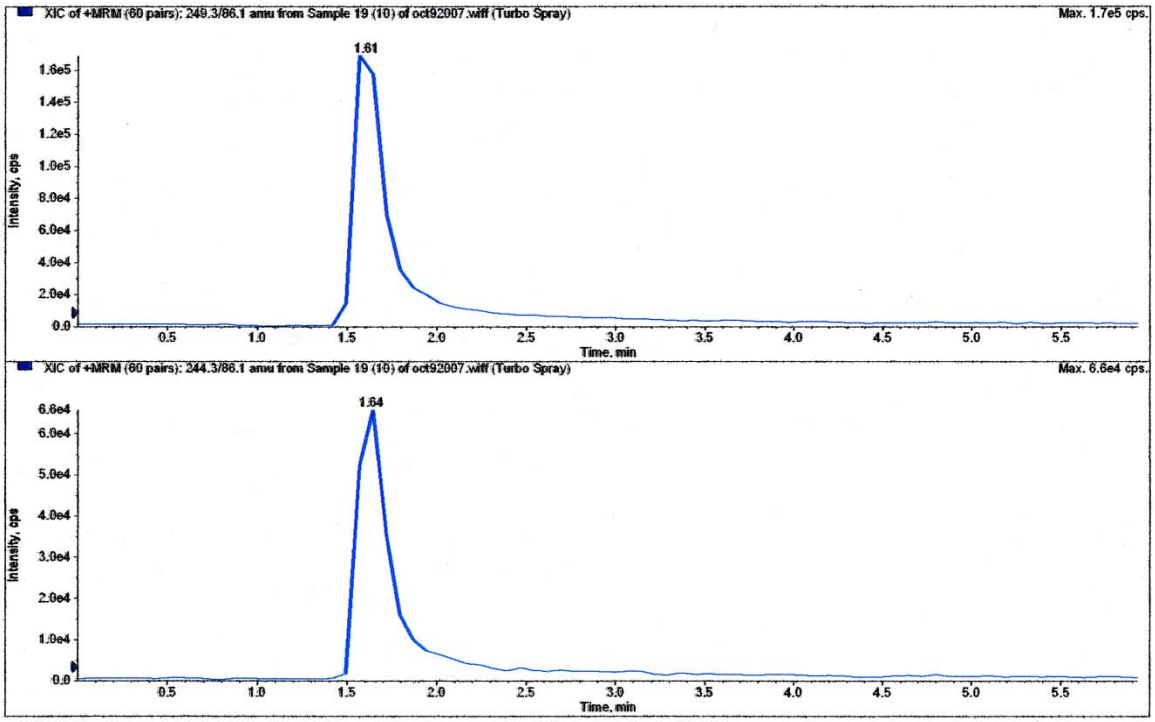
7. **RECONSTITUTE sample in 100 µL of CH3OH.**

   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.

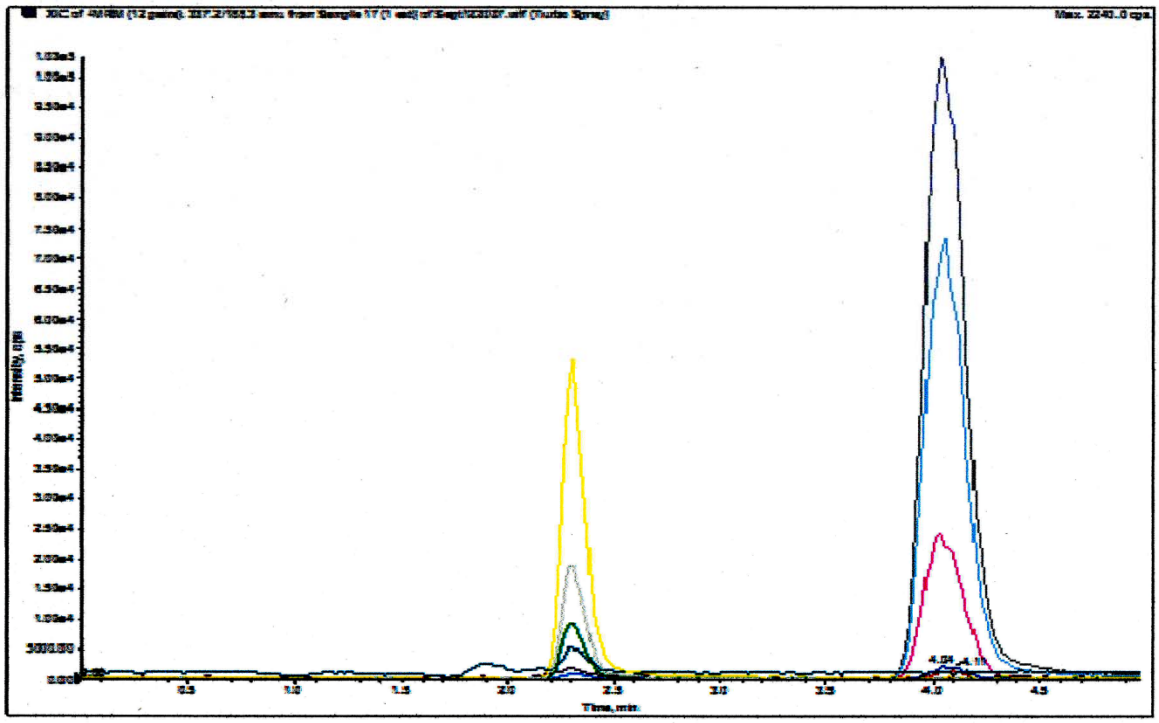
7. RECONSTITUTE SAMPLE IN 100 µL OF METHANOL.

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



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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.

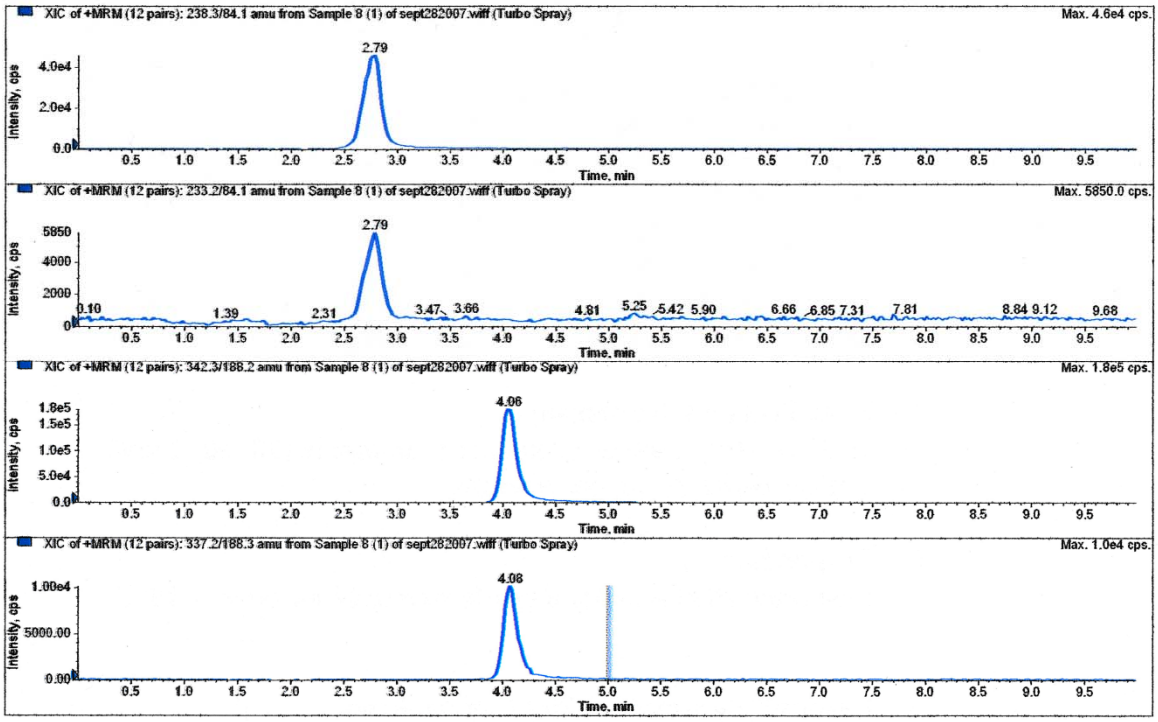
7. **RECONSTITUTE sample in 20 µL of methanol.**

   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

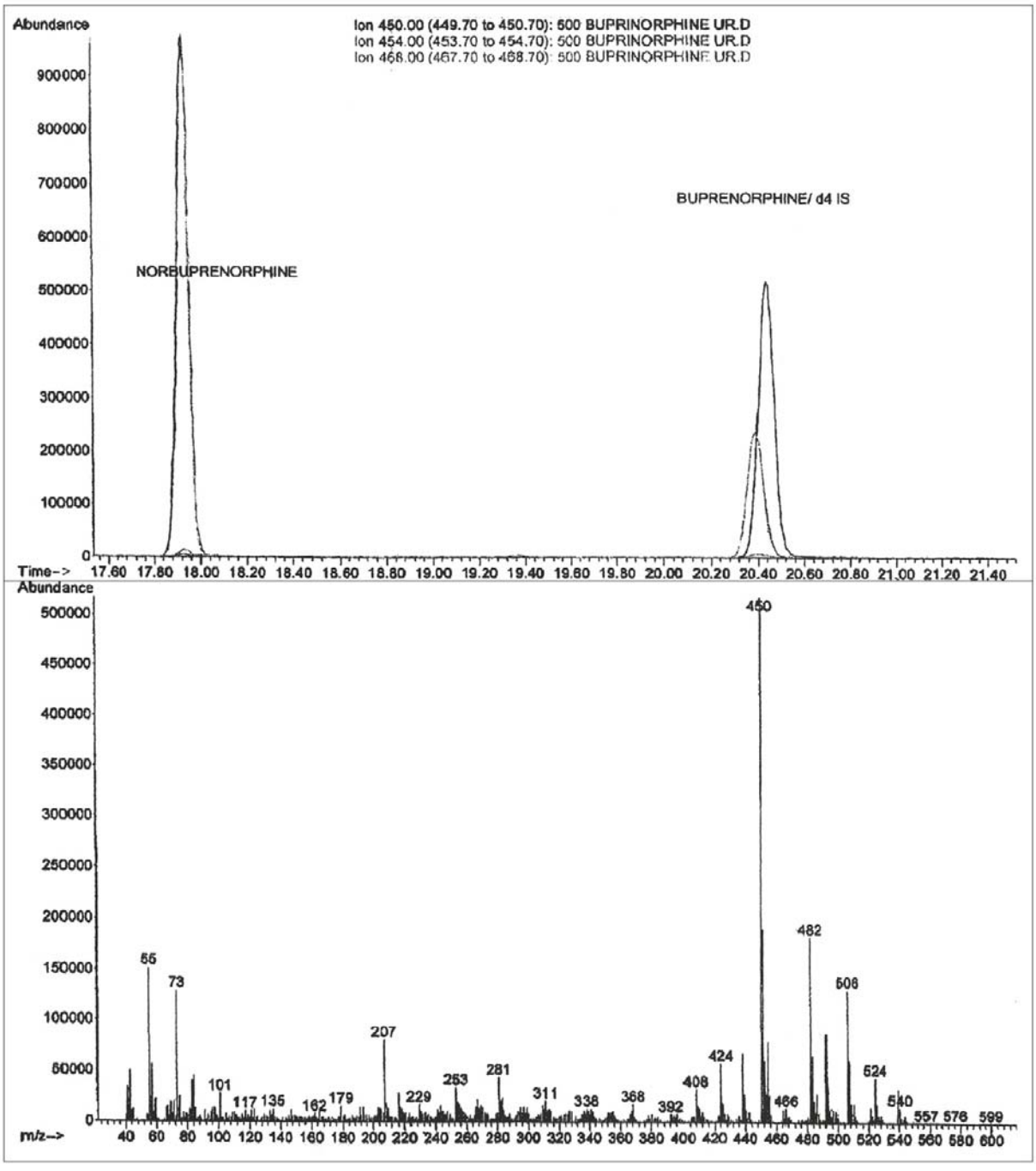
Add 50 µL ethyl acetate and 50 µL BSTFA (with 1 % TMCS).
React 20 minutes at 70°C.
Remove from heat source to cool.
NOTE: Do not evaporate BSTFA

8. QUANTITATE

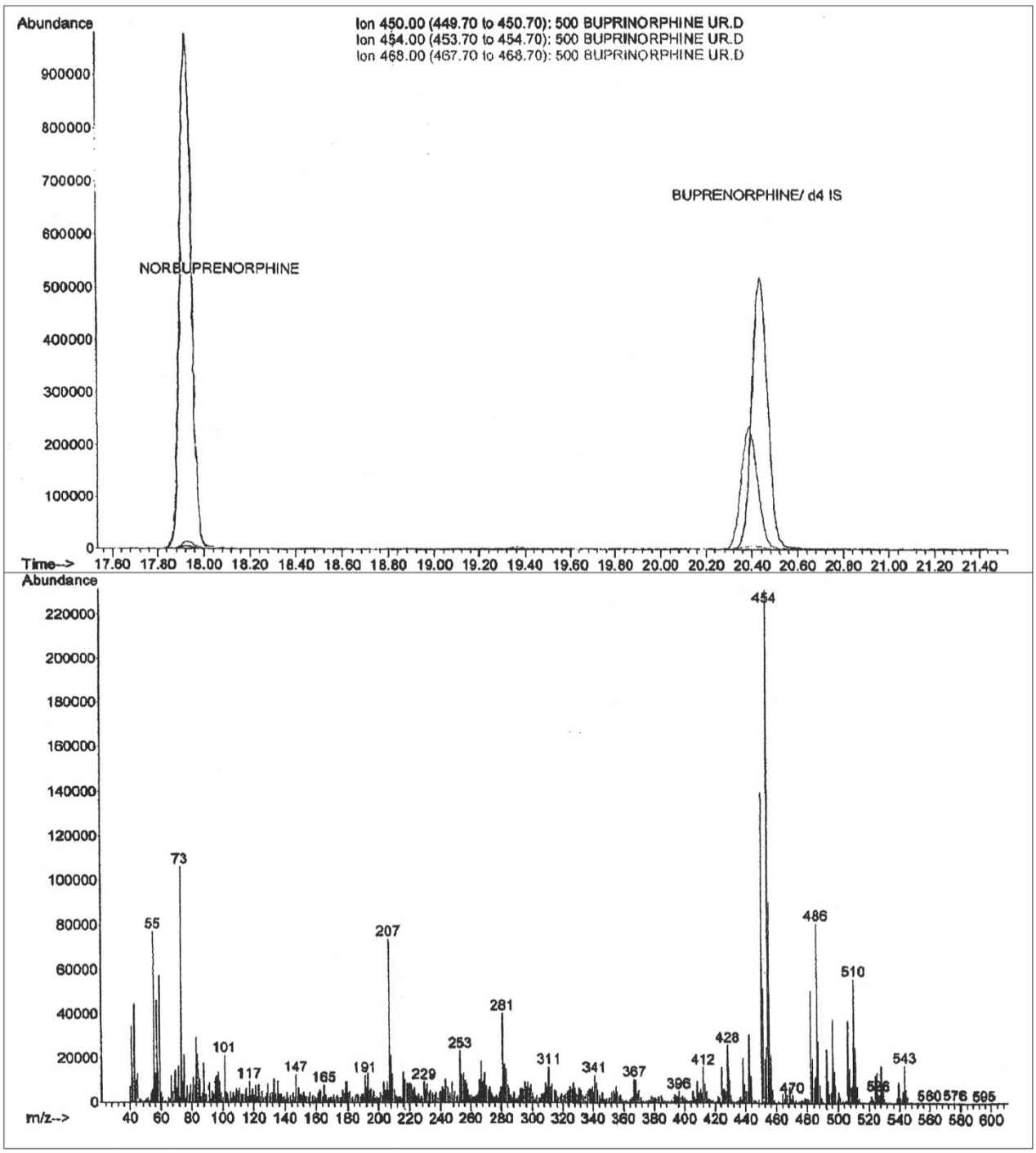
Inject 1 to 2 µL onto gas chromatograph/mass spectrometer
For MSD monitor the following ions:

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

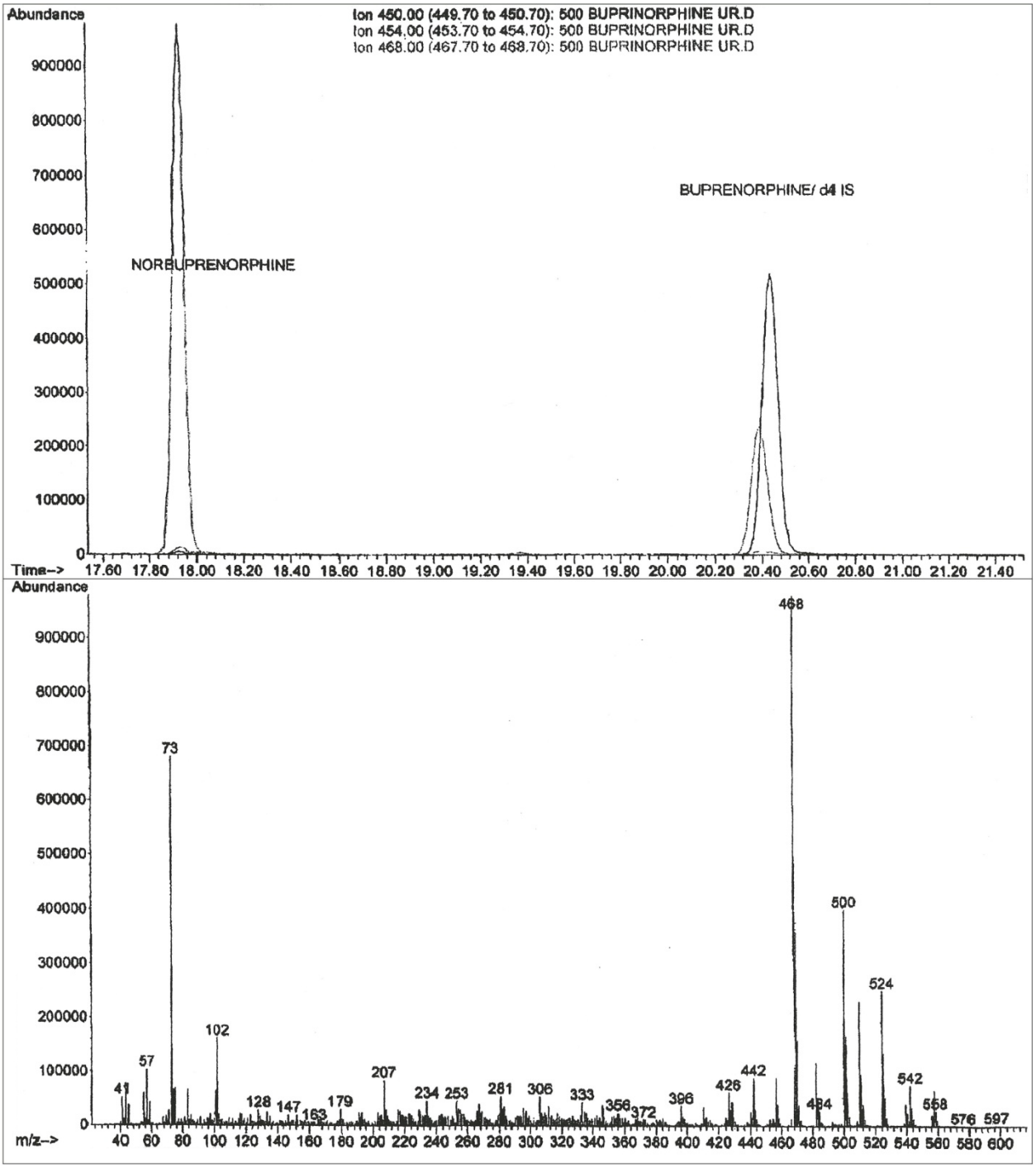
BUPRENORPHINE AND NORBUPRENORPHINE
CHROMATOGRAM I



BUPRENORPHINE AND NORBUPRENORPHINE
CHROMATOGRAM II



BUPRENORPHINE AND NORBUPRENORPHINE
CHROMATOGRAM III



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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.)

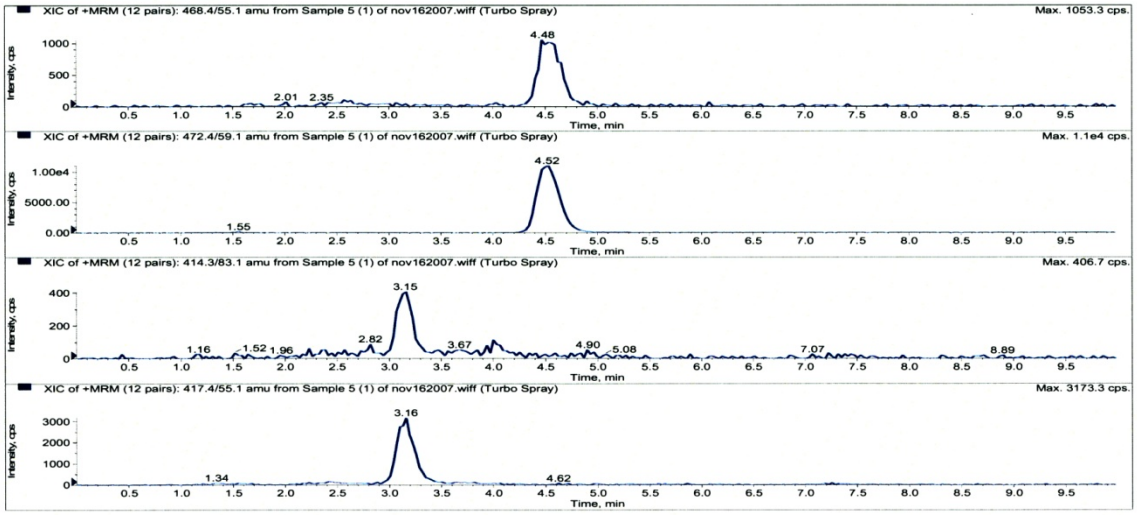
Mobile phase:	Acetonitrile:	0.1% Formic acid
	50	50

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

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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.

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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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PHARMACEUTICAL METHODS

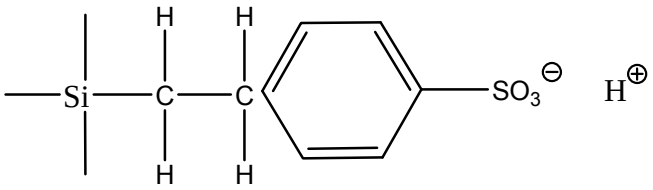
PURIFICATION OF SMALL MOLECULE LIBRARIES BY PHARMA-SIL® ION EXCHANGE SPE

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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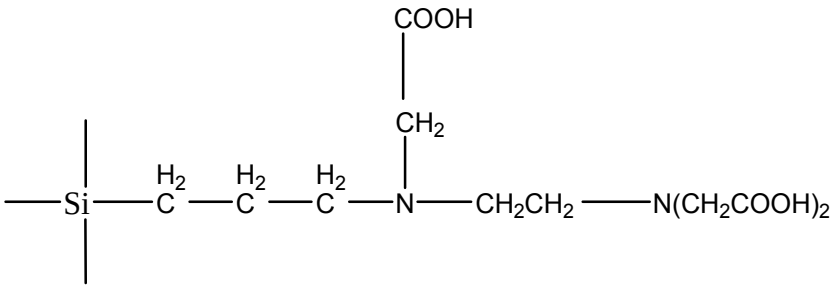
PURIFICATION OF SMALL MOLECULE LIBRARIES
TIN (Sn) REMOVAL BY PHARMA-SIL® ION EXCHANGE SPE

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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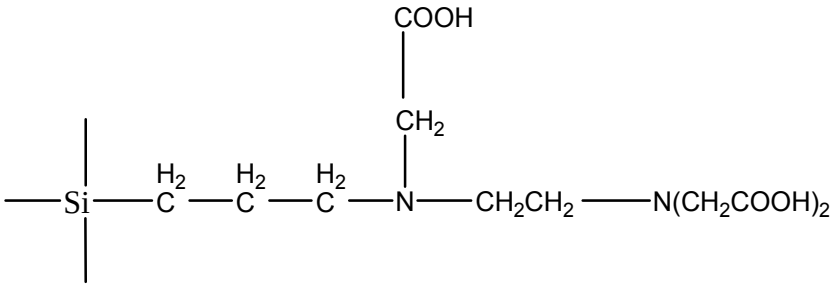
PURIFICATION OF SMALL MOLECULE LIBRARIES
Palladium (Pd) REMOVAL BY PHARMA-SIL® ION EXCHANGE SPE

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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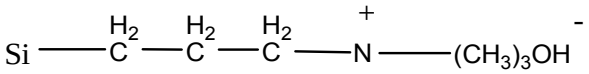
PURIFICATION OF SMALL MOLECULE LIBRARIES
TFAA REMOVAL BY PHARMA-SIL® ION EXCHANGE SPE

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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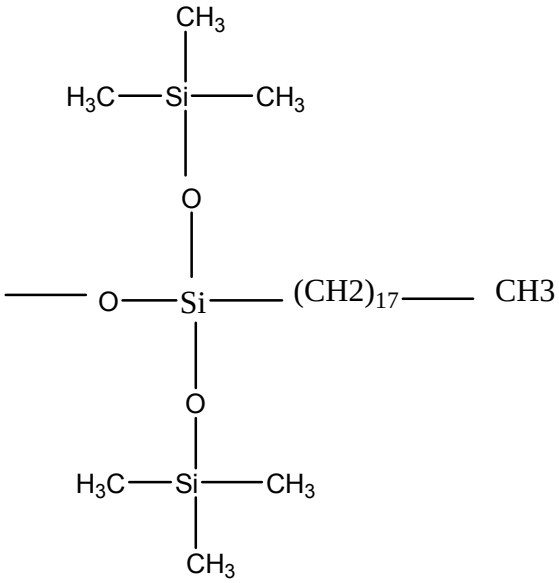
PURIFICATION OF SMALL MOLECULE LIBRARIES
DESALTING SAMPLES USING PHARMA-SIL® REVERSE PHASE SPE

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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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/MSanalysis.

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

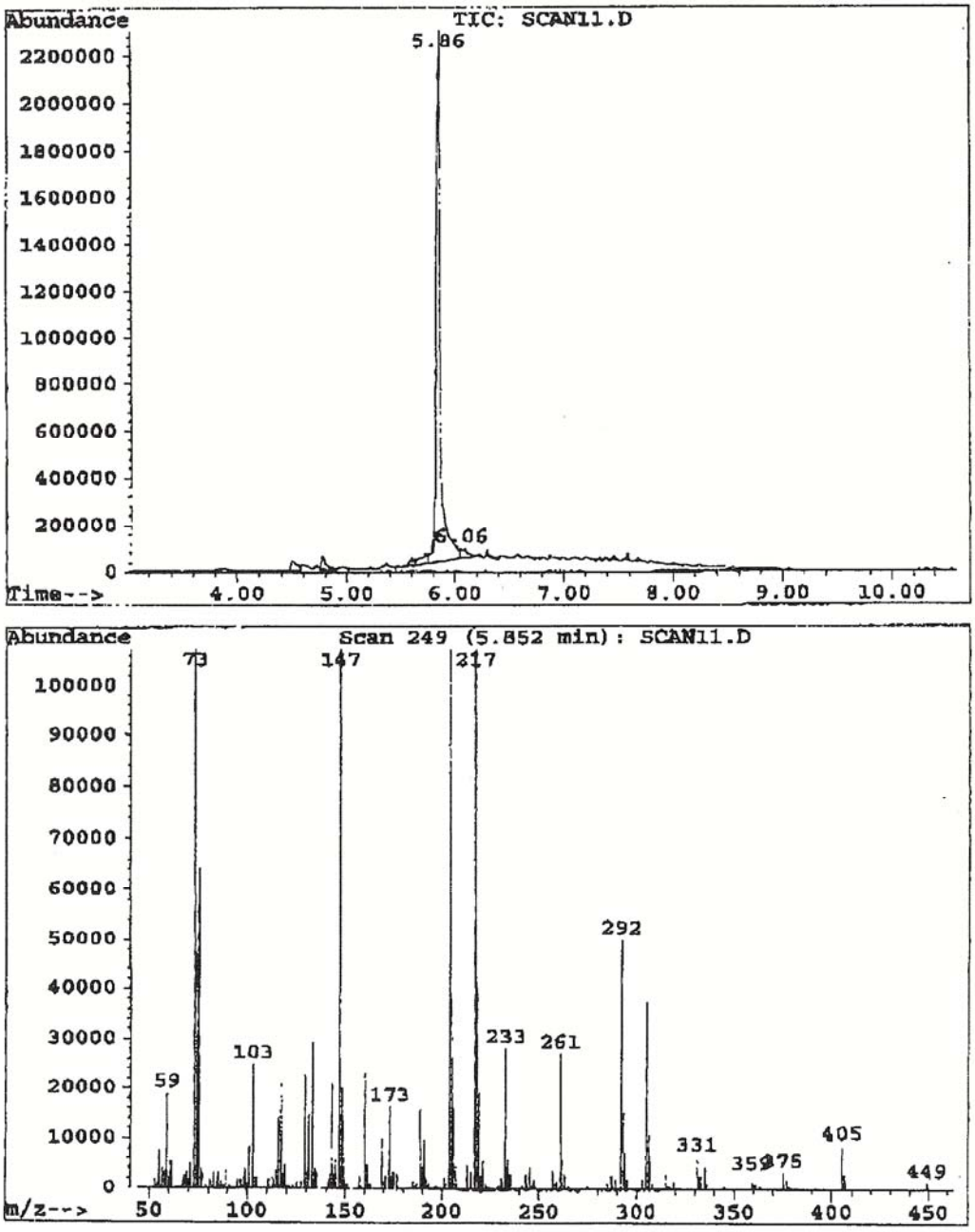
3. SUGGESTED LC/MS/MS PARAMETERS:

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)

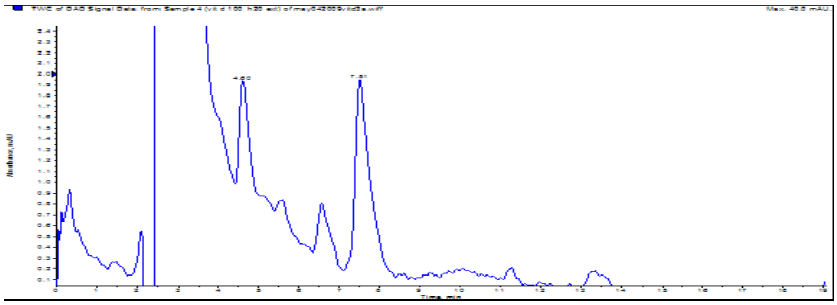
Mobile phase: 90: 10 CH_3OH (containing 0.1% Formic acid): 0.1% Formic acid aqueous

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