United Chemical Technologies, Inc.
Solid Phase Extraction

APPLICATIONS MANUAL

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(ENVIRO-CLEAN® Universal Cartridges)

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(Removal by **PHARMA-SIL™** Ion Exchange SPE **)**

Miscellaneous

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 World-wide Monitoring® 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)

United Chemical Technologies 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 (HCI): concentrated (12.1 M)
N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS)
Pentafluoropropionic Acid Anhydride (PFAA or PFPA)
Phosphoric Acid (H_3PO_4): concentrated (14.7 M)
Sodium Acetate Trihydrate ($NaCH_3COO\text{-}3H_2O$): F.W. 136.08
Sodium Borate Decahydrate ($Na_2B_4O_7\text{-}10\ H_2O$): F.W. 381.37
Sodium Hydroxide, (NaOH): F.W. 40.00
Sodium Phosphate Dibasic, Anhydrous (Na_2HPO_4): F.W. 141.96
Sodium Phosphate Monobasic, Monohydrate ($NaH_2PO_4 \bullet H_2O$): F.W. 137.99

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

HOW TO PREPARE SOLUTIONS AND BUFFERS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

100 mM Sodium Borate:
Dissolve 3.81 g $Na_2B_4O_7$•10 H_2O in 90 mL D.I. H_2O. Dilute to 100 mL with D.I. H_2O. Mix. Storage: 25°C in glass or plastic. Stability: 6 months.

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

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

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

Extraction Hints

- Verify sample application pH. Analytes that are not in their proper form (i.e., neutral or charged), will not effectively bind to the sorbent and may result in erratic recoveries.

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

- Prior to elution, fully dried cartridges will ensure optimal analyte recovery. To confirm column dryness, press the sides of the cartridge at the sorbent level at full vacuum. Columns should feel ambient temperature, not cool. If the column feels cool, water is probably present. Dry the column further.

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

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

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

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

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

SYMPATHOMIMETIC AMINES 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)

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 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 (pH 11-12).

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 mcL of 5% formic acid 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
D_5-Amphetamine*	194	92	123
Amphetamine	190	91	118
D_5-Methamphetamine*	208	92	163
Methamphetamine	204	91	160
Pseudoephedrine	204	160	119
Ephedrine	204	160	119
Phenylephrine	190	119	267
Methylenedioxyamphetamine	135	162	325
Methylenedioxymethamphetamine	204	162	339

* 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
D_5-Amphetamine*	120	197	92
D_6-Amphetamine*	120	198	93
D_{10}-Amphetamine*	120	202	97
D_{11}-Amphetamine*	120	203	98
Amphetamine	116	192	91
D_5-Methamphetamine*	134	211	92
D_8-Methamphetamine*	137	214	92
D_9-Methamphetamine*	137	215	93
Methamphetamine	130	206	91
Pseudoephedrine	130	147	294
Ephedrine	130	147	294
Methylenedioxyamphetamine	116	236	135
Methylenedioxymethamphetamine	130	250	131
Para-Methoxamphetamine	116	222	121

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 (4-CB)	Primary Ion***	Secondary	Tertiary
D_5-Amphetamine****	298	270	399
Amphetamine	294	266	248
D_5-Methamphetamine****	312	284	266
Methamphetamine	308	280	262
D_5-Methylenedioxyamphetamine****	136	434	270
Methylenedioxyamphetamine	162	429	266
D_5-Methylenedioxymethamphetamine****	312	284	266
Methylenedioxymethamphetamine	308	280	262
D_6-Methylenedioxyethylamphetamine****	328	165	300
Methylenedioxyethylamphetamine	322	162	294

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

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)

1. PREPARE SAMPLE
To 2 mL of urine add internal standard(s)*, 1 mL of 100 mM phosphate buffer (pH 6.0), 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).

6. CONCENTRATE ELUATE
Add 30 µL silylation grade DMF to eluate.
Evaporate to 30 µL 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
D_5-Amphetamine*	194	92	123
Amphetamine	190	91	118
D_5-Methamphetamine*	208	92	163
Methamphetamine	204	91	160

* 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

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)

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).

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):

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

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

SOURCE- UCT Internal Publication

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)

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 2mL / 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:

Drug	Primary Ion**	Secondary Ion	Tertiary Ion
Butalbital	168	167	181
Amobarbital	156	141	157
Pentobarbital	156	141	197
Butabarbital	156	141	157
Secobarbital	168	167	195
Hexobarbital*	221	157	236
Phenobarbital	204	232	117
Thiopental	172	157	173

DERIVATIZED

Drug	Primary Ion**	Secondary Ion	Tertiary Ion
D_5-Butalbital m/z:	**201**	214	
Butalbital m/z:	**196**	195	209
Amobarbital	**169**	184	185
Pentobarbital	**169**	184	112
$^{13}C_4$-Secobarbital m/z:	**200**	185	
Secobarbital m/z:	**196**	195	181
D_5-Phenobarbital m/z:	**237**	151	
Phenobarbital m/z:	**232**	146	175

**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

BARBITURATES IN URINE FOR GC OR GCMS CONFIRMATION USING A 80 mg CLEAN SCREEN® REDUCED SOLVENT VOLUME EXTRACTION COLUMN

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

1. **PREPARE SAMPLE**
   To 1 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 0.5 mL CH_3OH.
   1 x 0.5 mL D.I. H_2O.
   1 x 0.25 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 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).

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
Amobarbital:	156	141	157
Butabarbital:	156	141	157
Butalbital:	168	167	181
Hexobarbital*	221	157	236
Pentobarbital	156	141	197
Phenobarbital	204	232	117
Secobarbital	168	167	195
Thiopental:	172	157	173

DERIVATIZED

Drug	Primary Ion**	Secondary Ion	Tertiary Ion
D_5-Butalbital	**201**	214	
Butalbital	**196**	195	209
Amobarbital	**169**	184	185
Pentobarbital	**169**	184	112
$^{13}C_4$-Secobarbital	**200**	185	
Secobarbital	**196**	195	181
D_5-Phenobarbital	**237**	151	
Phenobarbital	**232**	146	175

* 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

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

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

1. **PREPARE SAMPLE**
   To 5 mL of urine add internal standard(s) and 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 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

BENZODIAZEPINES 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)

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; 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 D_5-Oxazepam
**** 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
Alprazolam	Xanax®	308	279	204
a-Hydroxyalprazolam-TMS		381	396	383
Chlordiazepoxide	Librium®	282	283	284
Clonazepam	Clonopin®	387	352	306
Diazepam	Valium®	256	283	221
Desalkylflurazepam-TMS		359	341	245
Hydroxyethylflurazepam-TMS		288	360	389
Lorazepam-TMS	Ativan®	429	430	347
Nordiazepam-TMS		341	342	343
Oxazepam-TMS	Serax®	429	430	313
Prazepam*		269	241	324
Temazepam-TMS	Restoril®	343	283	257
Triazolam	Halcion®	313	314	342
a-Hydroxytriazolam-TMS		415	417	430

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
D_5-Nordiazepam-TBDMS		332	334	333
Nordiazepam-TBDMS		327	328	329
D_5-Oxazepam-TBDMS		462	519	462
Oxazepam-TBDMS	Serax®	457	513	459
D_5-Temazepam-TBDMS		362	390	288
Temazepam-TBDMS	Restoril®	357	359	385
Lorazepam-TBDMS	Ativan®	491	513	493
Clonazepam	Clonopin®	372	374	326
7-Aminoclonazepam		456	458	513
Diazepam	Valium®	256	283	221
Desalkylflurazepam-TBDMS		345	347/402	
Prazepam*		269	241	324
α-Hydroxymidazolam-TBDMS	Versid	398	400/440	
Desmethylflunitrazepam-TBDMS			356	357/310
7-Aminoflunitrazepam-TBDMS			397	324/398
Alprazolam	Xanax®	308	279	204
D_5-α-Hydroxyalprazolam-TBDMS			386	388/387
α-Hydroxyalprazolam-TBDMS			381	383/384
Triazolam	Halcion®	313	314	342
α-Hydroxytriazolam-TBDMS		415	417/190	

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

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

1. **PREPARE SAMPLE**
   To 1 mL of serum add internal standard and 1.0 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 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; 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

BENZODIAZEPINES (SERUM) BY HPLC 100 mg CLEAN-UP® C2 EXTRACTION COLUMN

(Part # CEC02111)

I. REAGENTS:

1. **SATURATED SODIUM BORATE BUFFER, pH 9.5**
   Dissolve 10 grams of sodium borate in 950 mL of D.I. H_2O.
   Adjust pH to 9.5 with 10 N sodium hydroxide. q.s. to 1000 mL.

2. **0.01 M POTASSIUM PHOSPHATE BUFFER (pH 6.0)**
   Dissolve 1.36 grams of monobasic potassium phosphate in 950 mL of D.I. H_2O. Adjust pH to 6.0 with 1 N sodium hydroxide. q.s. to 1000 mL.

3. **MOBILE PHASE:**
   18.5% acetonitrile.
   26.5% methanol.
   55.0% 10 mM potassium phosphate buffer (pH 6.0).

II. EXTRACTION METHOD:

1. **PREPARE SAMPLE:**
   Add 200 µL of sodium borate buffer to 0.5 mL of serum. Vortex.

2. **CONDITIONING CLEAN-UP® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   (vacuum settings should be 3-5 inches of Hg.)

3. **APPLY SAMPLE TO COLUMN:**
   Add buffered sample to top of column.
   Pull through at a flow of 1-2 mL/minute.

4. **WASH COLUMN:**
   1 x 1 mL D.I. H_2O. Dry column 2-3 minutes under vacuum (15-20 inches of Hg).

5. **ELUTE BENZODIAZEPINES:**
   2 x 0.5 mL CH_3OH.

6. **DRY ELUATE:**
   Dry eluate under nitrogen and minimal heat.
   Reconstitute with 100 µL of mobile phase.

7. **QUANTITATE**
   Inject 15-20 µL onto HPLC.

Reference - UCT Internal Publication

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

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

1. 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.

2. APPLY SAMPLE
Take one mL of urine and add 2 mL of 100 mM Acetate Buffer (pH 4.5).
Load at 1 to 2 mL/ minute.

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

4. 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).

5. DRY ELUATE
Evaporate to dryness at < 40°C.

6. 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.

7. 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)

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)

1. 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.

2. APPLY SAMPLE
Take one mL of urine and add 2 mL of 100 mM Acetate Buffer (pH 4.5)
Load at 1 to 2 mL/ minute.

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

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

5. DRY ELUATE
Evaporate to dryness at < 40°C.

6. 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.

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

CARBOXY- delta$_9$-THC (pKa = 4.5) IN URINE FOR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

(Part # ZSTHC020 or ZSDAU206)

1. PREPARE SAMPLE - BASE HYDROLYSIS OF GLUCURONIDES

To 2 mL of urine add internal standard* and 100 µL of 10 N 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
D_3-Carboxy-delta $_9$-THC*	374	476	491
D_9-Carboxy-delta $_9$-THC*	380	479	497
Carboxy-delta $_9$-THC	371	473	488

* Suggested internal standard for GC/MS: D9-Carboxy-delta $_9$-THC

CARISOPRODOL AND MEPROBAMATE FOR GC OR GC/MS CONFORMATIONS USING A 200 mg CLEAN SCREEN® EXTRACTION COLUMN

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

1. **PREPARE SAMPLE**
   To 0.5 mL of urine sample add internal standard* and 2 mL of 100 mM phosphate buffer (pH 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 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
Carisoprodol	158	104	245
Meprobamate	83	114	144
Hexobarbital	221	157	81

*Suggested internal standard for GC/MS: Hexobarbital
***Quantitation ion

CLONAZEPAM & 7-AMINOCLONAZEPAM IN URINE FOR GC/MS CONFIRMATIONS USING 200 mg CLEAN SCREEN® EXTRACTION COLUMN

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

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 SAMPLE**
   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 (TBDMS)	Primary Ion**	Secondary	Tertiary
Clonazepam	372	374	326
7-Aminoclonazepam	342	344	399
D_5-Oxazepam*	462	464	463

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

COCAINE AND BENZOYLECGONINE 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)

1. PREPARE SAMPLE
To 2 mL 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 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
D_3-Cocaine*	185	201	306
Cocaine	182	198	303
D_3-Benzoylecgonine-TMS*	243	259	364
Benzoylecgonine-TMS	240	256	361

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

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

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

1. PREPARE SAMPLE
To 1 mL of sample (Serum, Plasma or Whole Blood) add internal standard* and 4 mL of D.I. H_2O.
Mix/vortex and let stand 5 minutes.
Centrifuge for 10 minutes at 2,000 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).

5. 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).

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

7. DERIVATIZE
Add 50 µL ethyl acetate and 50 mL 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
D_3-Cocaine*	185	201	306
Cocaine	182	198	303
D_3-Benzoylecgonine-TMS*	243	259	364
Benzoylecgonine-TMS	240	256	361

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

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)

1. PREPARE SAMPLE
To 1 mL of sample (Serum, Plasma or Whole Blood) add internal standard(s) and 4 mL of D.I. H_2O.
Whole Blood: 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

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

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

1. PREPARE SAMPLE
Vortex 0.5 -1 g meconium and 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
D_3-Cocaine*	185	201	306
Cocaine	182	198	303
D_3-Benzoylecgonine-TMS*	243	259	364
Benzoylecgonine-TMS	240	256	361

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

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)

1. PREPARE SAMPLE
Pipette five 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.

6. ENZYMATIC HYDROLYSIS
Dry eluate under a stream of nitrogen; Add 2 mL of 0.2 M phosphate buffer (pH 7.0) and 250 units of ß-glucuronidase 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

FENTANYL AND ANALOGUES 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)

1. PREPARE SAMPLE
To 5 mL of sample add internal standard* and 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 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
Fentanyl	245	146	189
D_5-Fentanyl*	250	151	194
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

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)

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
Flunitrazepam	312	286	266
7-Aminoflunitrazepam	283	255	254
Desmethylflunitrazepam	356	357	310
D_5-Oxazepam (ISTD)	462	464	463

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

GC CONDITIONS
Column-DB-5 or equivalent capillary column (15 meters x 0.25.mm ID x 0.25 µm film);
Injector Temperature = 250 °C; Splitless mode;
Oven Temperature Program: 180 °C to 275 °C at 10 °C/min then 275 °C to 300 °C at 25 °C/min

Reference- UCT Internal Publication

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

(Part # CUC18111)

1. **PREPARE SAMPLE**
   500 µL of sample, calibrator, or control was placed into a disposable glass test tube and 25 µL of internal standard* (5.0 mg/L) was added. Vortex tube. Add 500 µL of 20% acetic acid and vortex tube again.

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 HCL.

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 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.

* INTERNAL STANDARD: 1-aminomethyl-1-cycloheptyl acetic acid
** 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)

A Solid Phase method for Gamma-Hydroxybutyrate (GHB) In Urine without conversion to Gamma-Butrylactone (GBL)

(Part # ZSGHB020)

Developed by: United Chemical Technologies, Inc.,
2731 Bartram Road Bristol, Pennsylvania 19007

1. **PREPARE SAMPLE**
   To 200 µL of urine add internal standard* (D_6-GHB) 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. **APPLY 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:

D_6-GHB-di-TMS*	239****, 240, 241
GHB-di-TMS	233****, 234, 235

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

Blood GHB Extraction Procedure

(Part # ZSGHB020)

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.

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

A Solid Phase method for Gamma-Hydroxybutyrate (GHB) In Blood, Urine, Vitreous or Tissue without conversion to Gamma-Butrylactone (GBL)

(Part # ZSGHB020)

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 Radian stock 1 mg/mL.

D_6-GHB working internal standard; 100 µg/mL; use as supplied Radian 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 70ºC for 30 minutes.

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

D_6-GHB-di-TMS*	**239**, 240, 241
GHB-di-TMS	**233**, 234, 235

* 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.

GLYCOPYRROLATE (ROBINUL) FROM EQUINE URINE BY LC/MS/MS USING 500 mg CLEAN UP® CCX2 EXTRACTION COLUMN

(Part # CUCCX25Z)

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.

KETAMINE 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)

1. PREPARE SAMPLE
To 2 mL of urine add internal standard* 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. 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 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
D_4-Ketamine*	184	213	156
Ketamine	180	209	152

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

SOURCE- UCT Internal Publication

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

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

1. **PREPARE SAMPLE**
   To 1 mL of sample (serum, plasma, or whole blood) add 4 mL deionized water and internal standard.*
   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:

D_3-LSD-TMS*	298****, 296, 271
LSD-TMS	395****, 293, 268

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

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)

1. **PREPARE SAMPLE**
   To 5 mL of Urine add internal standard and 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 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:

D_3-LSD-TMS*	298****, 296, 271
LSD-TMS	395****, 293, 268

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

MANUAL METHOD FOR IMMUNOASSAY: PRELIMINARY SCREENING IN WHOLE BLOOD USING A CLEAN SCREEN® EXTRACTION COLUMN

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

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

METHAQUALONE 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)

1. PREPARE SAMPLE
To 2 mL of urine add internal standard* 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 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
Methaqualone	235	250	233
Hexobarbital*	221	157	156

* Suggested internal standard for GC/MS: Hexobarbital
*** Quantitation ion

SOURCE: UCT Internal Publication

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)

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
D_9-Methadone*	78	226	303
Methadone	72	223	294

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

SOURCE: UCT Internal Publication

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

(Part # CUQAX15Z)

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

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)

1. PREPARE SAMPLE
To 2 mL of urine or serum add internal standard(s)* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex.

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**	Secondary	Tertiary
Nicotine	84	133	162
Cotinine	98	119	176

* D_3-Cotinine and D_4-Nicotine are available as deuterated internal standards.
** Quantitation Ion

SOURCE- UCT Internal Publication

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)

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.
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
D_4-Meperidine	251	222	250
Meperidine	247	218	246
D_4-Normeperidine TMS*	308	280	309
Normeperidine TMS*	305	276	304
Tramadol TMS	335	245	290
O-Desmethyltramadol TMS	393	378	303
N-Desmethyltramadol TMS	393	378	116
Pentazocine TMS	357	342	289
D_3-Codeine TMS*	374	359	346
D_6-Codeine TMS*	377	349	316
Codeine TMS	371	356	343
Norcodeine TMS	429	414	356
Dihydrocodeine TMS	373	315	358
D_3-Morphine TMS*	432	417	404
D_6-Morphine TMS*	435	420	404
Morphine TMS	429	414	401
Normorphine TMS	487	472	414
Diacetylmorphine	369	327	268
D_3-Hydrocodone Oxime TMS	389	300	374
D_6-Hydrocodone Oxime TMS	392	303	377
Hydrocodone Oxime TMS	386	297	371
D_3-Hydromorphone Oxime TMS	447	432	358
Hydromorphone Oxime TMS	444	429	355
D_3-Oxycodone Oxime TMS	477	462	420
D_6-Oxycodone Oxime TMS	480	465	420
Oxycodone Oxime TMS	474	459	417
D_3-Oxymorphone Oxime TMS	535	520	290
Oxymorphone Oxime TMS	532	517	287

*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

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)

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.
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).

8. QUANTITATE

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

Compound (Propyl)	Primary Ion**	Secondary Ion	Tertiary Ion
Hydrocodone***	299	242	214
Codeine	355	282	229
D_3-Codeine*	358	285	232
Oxycodone	371	314	298
Hydromorphone	285	341	228
6-Acetylmorphine	327	268	383
Oxymorphone	357	300	413
Morphine	341	268	397
D_3-Morphine*	344	271	400

SOURCE: UCT Internal Publication working with the Philadelphia Medical Examiner's Office

* Suggested internal standard for GC/MS: D_3-Codeine and D_3-Morphine
** Quantitation ion
*** Hydrocodone does not derivatize under these conditions.
**** Part # SPIA-0-1,10, 25
***** Part # SPYR-0-50

FREE (UNBOUND) OPIATES IN SERUM, PLASMA OR WHOLE BLOOD FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

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

1. **PREPARE SAMPLE**
   To 1 mL of sample (serum, plasma or whole blood) add internal standard(s)* and 4 mL of D.I. H_2O.
   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 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 Ion	Tertiary Ion
D_3-Codeine-TMS*	374	237	346
Codeine-TMS:	371	234	343
D_3-Morphine-TMS*	432	290	327
Morphine-TMS	429	287	324

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

6-ACETYLMORPHINE (MAM) IN URINE GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

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

1. **PREPARE SAMPLE**
   To 4 mL of sample, add internal standard* and 2 mL of 100 mM phosphate buffer (pH 6.0).
   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 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 and 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
D_6-6-AM-TMS*	405	406	343
6-AM-TMS:	399	400	340

* Suggested internal standard for GC/MS: D_3-Codeine, D_6-Morphine
*** Part # SBSTFA-1-1,10,25,100
**** Quantitation ion

PHENCYCLIDINE 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)

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 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, then add CH_2Cl_2 (pH 11-12).

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
D_5-Phencyclidine*	205	96	247
Phencyclidine	200	91	242

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

REFERENCE- UCT Internal Publication

PROPOXYPHENE 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)

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 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, then add CH_2Cl_2 (pH 11-12).

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
D_5-Propoxyphene*	63	120	213	255, 270
Propoxyphene	58	115	208	250, 265

* 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)

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)

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
Reconstitute with 50 µL MSTFA*. Cap the sample tube and place it into a heater block at 70°C for 30 minutes.

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

Compound	Primary**	Secondary	Tertiary
PSILOCIN – TMS	290	348	73 (291)

* 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

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

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

1. PREPARE SAMPLE
To 1 mL of sample (serum, plasma or whole blood) add internal standard, 4 mL D.I. H_2O and 2 mL of 100 mM phosphate buffer (pH 6.0).
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, 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 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.

TACROLIMUS, CYCLOSPORIN AND RAPAMYCIN IN WHOLE BLOOD 30 mg STYRE SCREEN® EXTRACTION COLUMN

(Part # SSDVB031)

1. PREPARE SAMPLE
Add 50 mcL whole blood and 50 mcL of 0.1 M ZnSO4 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

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 ZSDAU206)

1. PREPARE SAMPLE
To 1-2 mL of whole blood add internal standard(s)* and 4 mL of D.I. water.
Mix/vortex.
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

D_3-Carboxy- delta 9-THC* 374 476 491	D_3-Hyroxy- delta 9-THC* 374 462 477	D_3-delta 9-THC* 374 389
D_9-Carboxy- delta 9-THC* 380 479 497	Hyroxy- delta 9-THC 371 459 474	delta 9-THC 371 386
Carboxy-delta 9-THC 371 473 488		(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.

THERAPEUTIC AND ABUSED DRUGS IN 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)

1. PREPARE SAMPLE

Urine

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.

Serum, Plasma or Whole Blood

To 1 mL of sample add internal standard(s) and 4 mL D.I. H_2O (5.5 pH 5.7).
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 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

CLEAN SCREEN® DAU Forensic Applications

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) 823-7464

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

United Chemical Technologies, Inc.
2731 Bartram Road
Bristol, Pennsylvania 19007

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)

1. PREPARE SAMPLE
To 1 mL of serum or plasma add internal standard* and 2 mL of 100 mM phosphate buffer (pH 6.0).
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
Trimipramine ISTD*	2.048	100.0%	5.53%
Doxepin	3.048	96.5%	8.04%
Amitriptyline	3.433	98.9%	5.64%
Imipramine	3.865	97.2%	6.09%
Nortriptyline	5.349	88.9%	9.49%
Nordoxepin	5.788	85.0%	5.29%
Desipramine	6.067	85.3%	5.04%
Protriptyline ISTD*	6.476	86.3%	5.39%

* 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.

AMPHETAMINES, OPIATES, & PHENCYCLIDINE IN ORAL FLUID FOR GC/MS ANALYSIS USING: 50 mg CLEAN SCREEN® DAU EXTRACTION COLUMN

(Part # ZSDAU005)

1. PREPARE SAMPLE
Add 100 - 500 µL of neat 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.

COCAINE & BENZOYLECGONINE IN ORAL FLUID FOR GC/MS ANALYSIS USING: 50 mg CLEAN SCREEN® DAU EXTRACTION COLUMN

(Part # ZSDAU005)

1. PREPARE SAMPLE
Add 100 - 500 µL of neat 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 0.1 N 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 0.1 N 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.

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

*Alternate derivatizations may be used.

THC FROM ORAL FLUIDS FOR GC/MS ANALYSIS USING 200 mg CLEAN SCREEN® DAU EXTRACTION COLUMN

(Part # ZSDAU020)

1. PREPARE SAMPLE
To 1 mL of 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
THC-	371(Q),	386	387
THCA D9 (Internal Standard)-	380(Q),	479	

Contributed by: Janet Putnam, Assistant Laboratory Director/RP
Advanced Toxicology Network, Memphis, TN

THC IN ORAL FLUID FOR GC/MS ANALYSIS USING: CLEAN SCREEN® DAU 50 mg EXTRACTION COLUMN

(Part # ZSDAU005)

1. PREPARE SAMPLE*
Add 100 - 500 µL of neat 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 N_2.

7. DERIVATIZE**
Add 25 µL BSTFA (with 1% TMCS), and 25 µL ethyl acetate.
Overlay with N_2 and cap.
Vortex.
React 30 minutes at 70°C.

8. QUANTITATE
Inject 2 µL onto gas chromatograph.

* 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.

AMPHETAMINES IN URINE FOR GC/MS CONFIRMATIONS USING: STYRE SCREEN® DBX 30 MG EXTRACTION COLUMN

(Part # SSDBX033 without Tips or SCDBX033 with CLEAN-THRU® Tips)

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
Amphetamine	140	91, 118
Amphetamine-d11*	144	98, 128
Methamphetamine	154	110, 118
Mehtamphetamine-d11*	160	113, 126

*Suggested internal standards

COCAINE/BENZOYLECGONINE IN URINE FOR GC/MS CONFIRMATIONS USING: STYRE SCREEN® DBX 30 MG EXTRACTION COLUMN

(Part # SSDBX033 without Tips or SCDBX033 with CLEAN-THRU® Tips)

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 w/ 1% TMCS, then cap, mix/vortex.
Heat for 20 mins at 70°C, allow to cool.

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

Analyte	Target (Quantitation) Ion	Qualifier Ions
Cocaine	182	198, 303
Cocaine-d_3*	185	201, 306
Benzoylecgonine (TMS)	240	256, 361
Benzoylecgonine-d8 (TMS)*	243	259, 369

*Suggested internal standards

OPIATES IN URINE FOR GC/MS CONFIRMATIONS USING: STYRE SCREEN® DBX 30MG EXTRACTION COLUMN

(Part # SSDBX033 without Tips or SCDBX033 with CLEAN-THRU® Tips)

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 w/ 1% TMCS, then cap, mix/vortex.
   Heat for 20 mins at 70°C, allow to cool.

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

Analyte (TMS)	Target (Quantitation) Ion	Qualifier Ions
Codeine	371	234, 343
Codeine-d6*	377	237, 349
Morphine	429	401, 414
Morphine-d6*	435	404, 420
6-Acetylmorphine	399	287, 340

*Suggested internal standards

PHENCYCLIDINE IN URINE FOR GC/MS CONFIRMATIONS USING: STYRE SCREEN® DBX 30MG EXTRACTION COLUMN

(Part # SSDBX033 without Tips or SCDBX033 with CLEAN-THRU® Tips)

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
Phencyclidine	200	91, 242
Phencyclidine-d5*	205	96, 247

*Suggested internal standards

CARBOXY-THC IN URINE FOR GC/MS CONFIRMATIONS USING: STYRE SCREEN® DBX 30MG EXTRACTION COLUMN

(Part # SSDBX033 without Tips or SCDBX033 with CLEAN-THRU® Tips)

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 and 50 µL BSTFA w/ 1% TMCS, then cap, mix/vortex.
Heat for 20 mins at 70°C, allow to cool.

6. ANALYZE

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

Analyte (TMS)	Target (Quantitation) Ion	Qualifier Ions
Carboxy-THC	371	473, 488
Carboxy-THC-d3*	374	476, 491

*Suggested internal standards

United Chemical Technologies, Inc.
Solid Phase Extraction

ENVIRONMENTAL APPLICATION NOTES

UNITED CHEMICAL TECHNOLOGIES, INC.

Extraction of Organic Compounds in Drinking Water By Method 525.2

(UCT part # ECUNI525)*

Reagents:

- Methylene Chloride
- Ethyl Acetate
- Methanol
- 6N HCl
- Anhydrous Sodium Sulfate (# ECSS50K)
- Enviro-Clean® Universal 525 cartridge (# ECUNI525)

Condition Cartridge

Rinse cartridge with 10 ml of methylene chloride and let the methylene chloride soak on the cartridge for approximately 1.5 min. Pull the methylene chloride through the cartridge to waste. Add 10 ml of ethyl acetate to the cartridge and let the ethyl acetate soak for approximately 1.5 minutes. Pull the ethyl acetate to waste and air dry the cartridge with full vacuum for a few seconds. Add approx. 10 ml of methanol to the cartridge and allow the methanol to soak for approx. 1.5 min. From this point until sample addition the cartridge must not go dry. Pull some of the methanol through the cartridge leaving a layer just covering the frit. Add approx 20 ml of deionized water to the cartridge and pull most of the water through the cartridge to waste but do not allow the sorbent to dry.

Sample Addition

Adjust the pH of the sample to <2 with 6N HCl. Add 5 ml of methanol and any surrogates to the sample. Mix. Add the sample to the cartridge under vacuum. Ideally, the sample should pass through the cartridge in approximately 15 – 20 minutes. Allow the cartridge to dry under vacuum for 10 minutes.**

Extract Elution

Place a collection tube or vial under the cartridge. Add 10 ml of ethyl acetate to the sample bottle to remove any residue. Add the ethyl acetate to the cartridge. Allow the solvent to soak for 1 minute and pull the ethyl acetate into the collection device. Repeat this procedure 2 more times using 10 ml of 1:1 methylene chloride: ethyl acetate. Dry the extract by passing it through anhydrous sodium sulfate. Carefully rinse the collection device with 1:1 methylene chloride: ethyl acetate and add the solvent to the sodium sulfate.

Concentration and Analysis

Carefully concentrate the extract to a final volume. A micro-KD followed by micro-Snyder column concentration or gentle TurboVap concentration is recommended. Note: Most extraction errors are caused by poor concentration technique. Do not concentrate below 0.5 ml. or low recoveries will result.

- The Enviro-Clean® Universal 525 cartridge can be used on standard vacuum manifolds (# VMFF016GL), standard disk manifolds (#ECUCTVAC6) (with adapter part # ECUCTADP). The cartridge is specifically designed to fit the Horizon SPE DEX 4790® made by Horizon Technologies, Inc.

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

UNITED CHEMICAL TECHNOLOGIES, INC.

Extraction of Diesel Range Organics

(UCT part # ECUNIPAH)*

Reagents:

Methylene Chloride
Acetone
Methanol
1:1 HCl
Anhydrous Sodium Sulfate (# ECSS50K)
Enviro-Clean® Universal PAH/DRO cartridge (# ECUNIPAH)

Condition Cartridge

Rinse cartridge with 10 ml of methylene chloride and let the methylene chloride soak on the cartridge for approximately 1.5 min. Pull the methylene chloride through the cartridge to waste. Add 10 ml of acetone to the cartridge and let the acetone soak for approximately 1.5 minutes. Pull the acetone to waste and air dry the cartridge with full vacuum for a few seconds. Add approx. 10 ml of methanol to the cartridge and allow the methanol to soak for approx. 1.5 min. From this point until sample addition the cartridge must not go dry. Pull some of the methanol through the cartridge leaving a layer just covering the frit. Add approx 20 ml of deionized water to the cartridge and pull most of the water through the cartridge to waste but do not allow the sorbent to dry.

Sample Addition

Add 5 ml of 1:1 HCl to the sample. Add 5 ml of methanol (optional) and any surrogates to the sample. Mix. Add the sample to the cartridge under vacuum. Ideally, the sample should pass through the cartridge in approximately 15 – 20 minutes. Allow the cartridge to dry under vacuum for 10 minutes.**

Extract Elution

Place a collection tube or vial under the cartridge. Add 10 ml of acetone to the sample bottle to remove any residue. Add the acetone to the cartridge. Allow the solvent to soak for 1 minute and pull the acetone into the collection device. Repeat this procedure 2 more times using methylene chloride. Dry the extract by passing it through anhydrous sodium sulfate. Carefully rinse the collection device with methylene chloride and add the solvent to the sodium sulfate.

Concentration and Analysis

Carefully concentrate the extract to a final volume. A micro-KD followed by micro-Snyder column concentration is recommended. Note: Most extraction errors are caused by poor concentration technique. Do not concentrate below 0.5 ml. or low recoveries will result.

- The Enviro-Clean® Universal PAH/DRO cartridge can be used on standard vacuum manifolds (# VMFF016GL), standard disk manifolds (#ECUCTVAC6) (with adapter part # ECUCTADP). The cartridge is specifically designed to fit the Horizon SPE DEX 4790® made by Horizon Technologies, Inc.

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

UNITED CHEMICAL TECHNOLOGIES, INC.

Extraction of Polycyclic Aromatic Hydrocarbons from a Water Matrix

(UCT part # ECUNIPAH)*

Reagents:

Methanol
Acetonitrile**
Methylene Chloride
Anhydrous Sodium Sulfate (# ECSS50K)
Enviro-Clean® Universal PAH cartridge (# ECUNIPAH)

Condition Cartridge

Add 10 ml of methylene chloride to the cartridge and let it soak for 1 minute. Pull through to waste. Add 10 ml of acetonitrile to the cartridge and let it soak for 1 minute. Pull through to waste. Add 10 ml of methanol to the cartridge and let it soak for 1 minute. Pull most of the methanol to waste but do not allow the sorbent to dry. Add 10 ml of deionized water to the cartridge and let it soak for 1 minute. Pull most of the water to waste but do not allow the sorbent to dry.

Sample Addition

Add 5 ml of methanol (optional) and any surrogates to the sample. Mix. Add the sample to the cartridge under vacuum. Ideally, the sample should pass through the cartridge in approximately 15 – 20 minutes. Allow the cartridge to dry under vacuum for 10 minutes.***

Extract Elution

Place a collection tube or vial under the cartridge. Add 10 ml of acetonitrile to the sample bottle to remove any sample residue. Add the acetonitrile to the cartridge. Allow to soak for 1 minute and pull the solvent into the collection device. Repeat this procedure two more times using methylene chloride. Dry the extract by passing it through anhydrous sodium sulfate. Thoroughly rinse the collection device with methylene chloride and add this solvent to the sodium sulfate.

Concentration and Analysis

Carefully concentrate the extract to a final volume. Note: Most extraction errors are caused by poor concentration technique. Do not concentrate below 0.5 ml. or low recoveries will result.

- The Enviro-Clean® Universal PAH cartridge can be used on standard vacuum manifolds (# VMFF016GL), standard disk manifolds (#ECUCTVAC6) (with adapter part # ECUCTADP). The cartridge is specifically designed to fit the Horizon SPE DEX 4790® made by Horizon Technologies, Inc.

** Acetone, methanol, or ethyl acetate may be substituted for acetonitrile.

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

UNITED CHEMICAL TECHNOLOGIES, INC.

Oil and Grease Analysis for EPA #1664

(Part # ECUNIOAG)*

1. **Prepare Sample**
   Add 5 ml of methanol to the sample. Adjust the pH of the sample to 2 using 6N HCl or H_2SO_4. If deionized water is used for the LCS, confirm that the pH is 2 using a pH meter. Using pH paper could provide a false reading. (Allow suspended solids to settle so that the liquid portion of the sample may be decanted onto the cartridge first.)

2. **Condition Enviro-Clean® Universal Oil and Grease cartridge**
   Wash the cartridge twice with 10 ml of hexane by pulling the hexane through the cartridge with vacuum. Discard the hexane. Pull full vacuum through the cartridge for 1 minute to remove the hexane. Add 15 ml of methanol to the cartridge and slowly pull through the cartridge, but do not let the sorbent dry. Stop elution when the meniscus enters the top frit. Soak for one minute. Add 30 ml of deionized water to the cartridge and stop when the meniscus enters the top frit. Do not allow the cartridge to dry out prior to adding the sample.

3. **Sample addition**
   Add the sample to the cartridge under vacuum. Ideally, the sample should pass through the cartridge in approximately 15 – 20 minutes. (Decant liquids before solids.) If the top frits clog, you may puncture or remove the top frits. Rinse the sample container with deionized water and add to the cartridge. Allow the cartridge to dry under full vacuum for 10 minutes. Faster drying results can be obtained by removing the cartridge during drying and shaking or tapping the excess moisture from the bottom of the cartridge. Drying times are approximate. Do not over dry. Low recoveries could result.

4. **Elution**
   Place a sample collection vial or flask beneath the cartridge. Pour a small layer of sodium sulfate anhydrous on top of the cartridge. Rinse the sample container with 10 ml of acetone. Add the acetone to the cartridge, rinsing the sides of the cartridge during addition. Also, rinse the bottle/pour adapter if used. Soak for 2 minutes. Pull the acetone through the cartridge and into the collection vessel. Repeat using 10 ml of hexane. Add another 10 ml aliquot of hexane to the cartridge. Soak for 2 minutes. Elute.

5. **Dry the extract**
   Decant the extract through a sodium sulfate funnel containing approximately 5 to 10 g of anhydrous sodium sulfate and collect in a clean, tared vessel. Rinse the collection vessel with hexane and add to the sodium sulfate. Rinse the sodium sulfate with an additional aliquot of hexane and collect.

6. **Gravimetric analysis**
   Carefully evaporate the hexane until a constant mass is obtained. Record this mass as the mass per unit volume of oil and grease.

* This cartridge was specifically designed to fit the Horizon SPE-DEX® 4790 automated extraction system. This cartridge will also fit a standard 3 (# ECUCTVAC3) or 6 station (#ECUCTVAC6) disk manifold with our optional adapter (#ECUCTADP). The cartridge will also fit a standard vacuum manifold (#VMF016GL).

UNITED CHEMICAL TECHNOLOGIES, INC.

EPA Method 1664,
UCT Universal Oil and Grease Cartridge
Precision and Recovery Data

10 mg/L stearic acid hexadecane standard

n	% Recovery	Standard Deviation
1	107	3.25
2	104	3.25
3	101	3.25
4	107	3.25

Lubricating Oil

n	Mass (g.)	% Recovery	Standard Deviation
1	0.5	105	4.15
2	1.0	100	4.15
3	2.0	108	4.15
4	3.0	98	4.15

UNITED CHEMICAL TECHNOLOGIES, INC.

Extraction of Organochlorine Pesticides

(UCT part # ECUNIC18)*

Reagents:

Methylene Chloride
Acetone
Methanol
Anhydrous Sodium Sulfate (# ECSS50K)
Enviro-Clean® Universal C18 cartridge (# ECUNIC18)

Condition Cartridge

Rinse cartridge with 10 ml of methylene chloride and let the methylene chloride soak on the cartridge for approximately 1.5 min. Pull the methylene chloride through the cartridge to waste. Add 10 ml of acetone to the cartridge and let the acetone soak for approximately 1.5 minutes. Pull the acetone to waste and air dry the cartridge with full vacuum for a few seconds. Add approx. 10 ml of methanol to the cartridge and allow the methanol to soak for approx. 1.5 min. From this point until sample addition the cartridge must not go dry. Pull some of the methanol through the cartridge leaving a layer just covering the frit. Add approx 20 ml of deionized water to the cartridge and pull most of the water through the cartridge to waste but do not allow the sorbent to dry.

Sample Addition

Adjust the pH of the sample to 2 using concentrated sulfuric acid (approx. 30 drops per liter). Do not use pH paper to test the pH of deionized water as poor recoveries may result. Add 5 ml of methanol (optional) and any surrogates to the sample. Mix. Add the sample to the cartridge under vacuum. Ideally, the sample should pass through the cartridge in approximately 15 – 20 minutes. Allow the cartridge to dry under full vacuum for 10 minutes.**

Extract Elution

Place a collection tube or vial under the cartridge. Add 10 ml of acetone to the sample bottle to remove any residue. Add the acetone to the cartridge. Allow the solvent to soak for 1 minute and pull the acetone into the collection device. Repeat this procedure 2 more times using methylene chloride. Dry the extract by passing it through anhydrous sodium sulfate. Carefully rinse the collection device with methylene chloride and add the solvent to the sodium sulfate.

Concentration and Analysis

Carefully concentrate the extract. Solvent exchange if necessary. Note: Most extraction errors are caused by poor concentration technique.

- The Enviro-Clean® Universal C18 cartridge can be used on standard vacuum manifolds (# VMFF016GL), standard disk manifolds (#ECUCTVAC6) (with adapter part # ECUCTADP). The cartridge is specifically designed to fit the Horizon SPE DEX 4790® made by Horizon Technologies, Inc.

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

UNITED CHEMICAL TECHNOLOGIES, INC.

Fractionation of Aliphatic and Aromatic Hydrocarbons Using Enviro-Clean® TPH Silica

(developed with Lancaster Laboratories, Inc.)

Part number XRSIHT13M15
3000 mg in 15 ml cartridge

Because petroleum is a mixture of hundreds of different hydrocarbon compounds, the composition of petroleum products released into the environment is variable. While in the environment, petroleum composition is further influenced by volatilization, leaching and biological degradation. As a result, the toxicological properties of the weathered petroleum product can be vastly different from the parent product. Based on the known toxicological properties of petroleum products we can assume that:

1. aromatic compounds are more toxic than aliphatic compounds
2. the toxicity of aliphatic compounds is dependent on their molecular weight

The fractionation of the total petroleum hydrocarbon extract is necessary to determine the concentration of the aliphatic versus aromatic compounds. The Massachusetts Department of Environmental Protection (MADEP) has taken the approach of fractionating the C9-C18 aliphatics, C19-C36 aliphatics and the C11-C22 aromatics.

Lancaster Laboratories, Inc., in cooperation with United Chemical Technologies, Inc., has developed a fractionation product and technique that provides consistent, accurate and uncontaminated results.

PROCEDURE

1. **Prepare Extract**
   Solvent exchange the hydrocarbon extract from methylene chloride to hexane using a K-D* apparatus.

2. **Prepare Cartridge**
   Thoroughly rinse cartridge with 2, 10 ml aliquots of pentane.

3. Add 1 ml of the extract to the cartridge.

4. Elute aliphatic fraction with pentane by gravity and collect everything in an ampoule. A total of 10 ml should be collected.

5. Place a fresh ampoule under the cartridge and elute the aromatic fraction with methylene chloride by gravity. A total of 10 ml should be collected.

6. Concentrate each fraction separately to a final volume on a steam bath using an ampoule and micro-Snyder column combination*.

- Other techniques may be used but the loss of C9-C18 hydrocarbons may result.

It is very important to keep the silica cartridges dry and away from room air prior to use. Moisture and contaminants in the air will reduce the effectiveness of the silica and cause a contaminated extract. Prerinsing the cartridges with acetone may reduce this problem.

ANALYSIS OF TOBACCO ALKALOIDS
Using: CLEAN UP® Extraction Column

(Part # CUBCX1HL2Z)

SAMPLE PREP

1. To 0.1 gram tobacco, add 6 mL 0.1M sodium acetate buffer (pH 4.5) and 100 µL internal standard (d4-nornicotine, 1 µg/µL).

2. Mix on rotating shaker for 10 minutes, then filter extract through 20 micron frit filter column.

3. Add 300 µL glacial acetic acid, mix.

5. Condition SPE column, **part# CUBCX1HL2Z** with 3 mL of MeOH:1.0M acetic acid (80:20).

6. Pour sample onto column, aspirate at 1-2 mL/min by vacuum.

7. Wash column with 3 mL of MeOH:1.0M acetic acid (80:20).

8. Dry column for 5-10 min with full vacuum.

9. Elute alkaloids with 3 mL CH_2Cl_2/isopropanol/NH_4OH (70:26:4) by gravity.

10. Evaporate eluant to dryness with nitrogen and low heat (< 40° C).

11. Reconstitute with 200 µL ethyl acetate.

12. Analyze on GC/FID/NPD or GC/MSD.

United Chemical Technologies, Inc.
Solid Phase Extraction

PHARMACEUTICAL APPLICATION NOTES

Purification of Small Molecule Libraries by Pharmasil™ Ion Exchange SPE

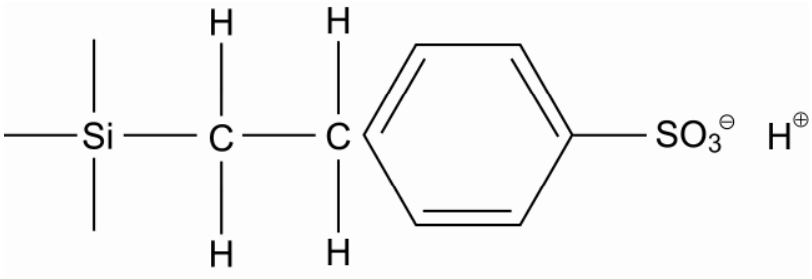
Principle:

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 Pharmasil™ 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 selectively retain the basic compounds from the reaction mixture. The sorbent can also be used as a scavenger in the synthesis of ureas.

Chemistry of Pharmasil™ BCX1HL Sorbent



Advantages of Pharmasil™ Based Sorbents

- Clean background
- High recoveries
- High levels of purification of anaytes
- Applicable to a broad range of compounds
- Simple to develop methods

Purification Profile

This profile is based on the use of a Pharmasil™ BCX1HL 500 mg column (columns are available with varying volumes). This column is capable of purification of up to 50mg 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.

Purification of Small Molecule Libraries
TIN (Sn) Removal by Pharmasil™ Ion Exchange SPE

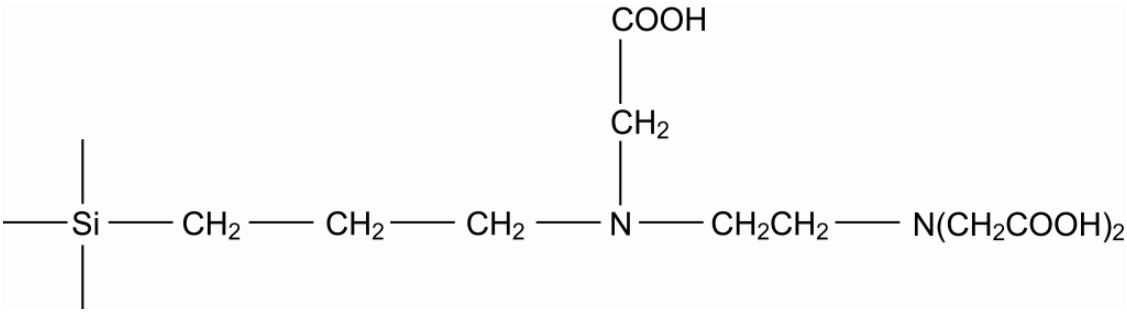
Principle:

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 Pharmasil™ 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 Pharmasil™ TAX Sorbent



Advantages of Pharmasil™ 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 to develop methods

Purification Profile

This profile is based on the use of a Pharmasil™ TAX 500 mg column (columns are available with varying volumes). This column is capable of removal of up to 50mg 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 with 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 1ml of buffer used in column equilibration.

Product Elution

Elute compound of interest with 1ml of methanol.

Technical Document P-103

Purification of Small Molecule Libraries
Palladium (Pd) Removal by Pharmasil™ Ion Exchange SPE

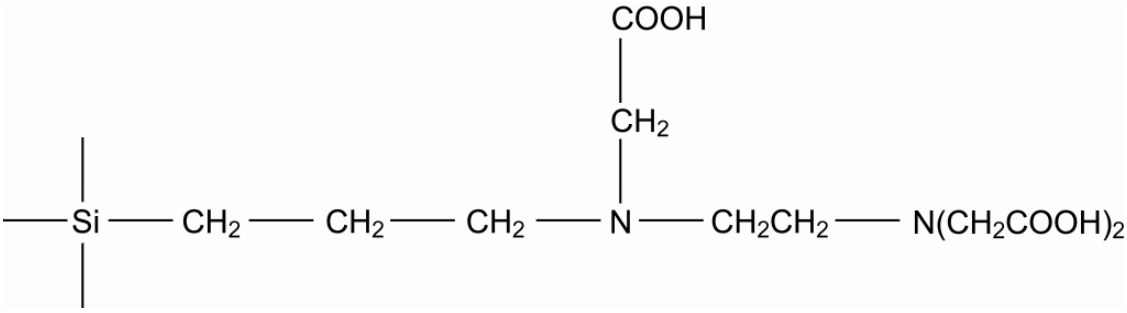
Principle:

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 Pharmasil™ 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 Pharmasil™ TAX Sorbent



Advantages of Pharmasil™ 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 to develop methods

Purification Profile

This profile is based on the use of a Pharmasil™ TAX 500 mg column (columns are available with varying volumes). This column is capable of removal of up to 50mg 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 with 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 1ml of methanol.

Purification of Small Molecule Libraries
TFAA Removal by Pharmasil™ Ion Exchange SPE

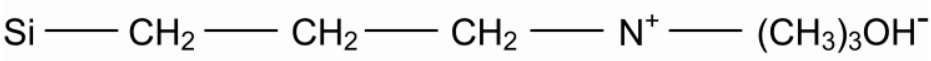
Principle:

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 Pharmasil™ 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 Pharmasil™ CHQAX Sorbent



Advantages of Pharmasil™ 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 to develop methods

Purification Profile

This profile is based on the use of a Pharmasil™ 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.

Technical Document P-105

Purification of Small Molecule Libraries
Desalting Samples Using Pharmasil™ Reverse Phase SPE

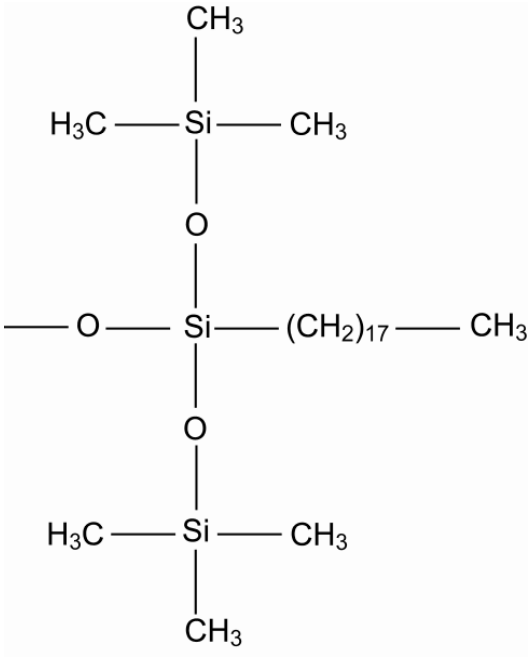
Principle:

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. Pharmasil ™ Reverse Phase SPE can be used to desalt these libraries.

Application:

This application details the use of Pharmasil™ 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 Pharmasil™ CEC18 Sorbent



Advantages of Pharmasil™ Based Sorbents

• Complete removal of salts
• Clean background
• High recoveries
• High levels of purification of anaytes
• Applicable to a broad range of compounds
• Simple to develop methods

Purification Profile

This profile is based on the use of a Pharmasil™ 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 with 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.

United Chemical Technologies, Inc.
Solid Phase Extraction

MISCELLANEOUS APPLICATION NOTES

ABUSED DRUGS IN CANINE OR EQUINE URINE USING: 500 mg XtrackT® EXTRACTION COLUMN

(Part # XRDAH515)

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** I
   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

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)

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

Solid Phase Extraction
APPLICATIONS MANUAL

DCN48-0506