Solid Phase Extraction
APPLICATIONS MANUAL

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SOLVENTS

Acetone: HPLC Grade

Acetonitrile (CH_3CN): HPLC Grade

Chloroform ($CHCl_3$): HPLC Grade

Distilled or Deionized Water (DI H_2O): $5 < pH < 7$

Ethyl Acetate (EA): 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/DI H_2O (20/80)

Ethyl Acetate/IPA (75/25)

Hexane/Ethyl Acetate (50/50), (75/25)

Methanol/DI H_2O (80/20)

Methanol/DI H_2O (70/30)

Methanol/DI 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 elusion solvents. The recommended parameters have been used successfully on Worldwide Monitoring® columns by our customers across the country and may be routinely used as an alternative to chlorinated elusion solvents. You may however see subtle differences on certain compounds due to solubility effects.

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

United Chemical Technologies would like to thank Dr. Leon Glass of Lab Specialties 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 Vulgatta)

Dimethylformamide (DMF): silylation grade

Hydrochloric Acid (HCl): concentrated (12.1 M)

N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS)

Pentaflouropropionic Acid Anhydride (PFAA or PFPA)

Phosphoric Acid (H_3PO_4): concentrated (14.7 M)

Sodium Acetate Trihydrate ($NaCH_3COO \cdot 3H_2O$): F.W. 136.08

Sodium Borate Decahydrate ($Na_2B_4O_7 \cdot 10H_2O$): F.W. 381.37

Sodium Hydroxide, (NaOH): F.W. 40.00

Sodium Phosphate Dibasic, Anhydrous (Na_2HPO_4): F.W. 141.96

Sodium Phosphate Monobasic, Monohydrate ($NaH_2PO_4 \cdot H_2O$): F.W. 137.99

NOTES:

Storage of organics in some plastic containers may lead to plasticizer contamination of the solvent or solvent mixture, this may interfering 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).

Instruction Code: SSMRS100491

HOW TO PREPARE SOLUTIONS AND BUFFERS

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

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

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

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

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

Ammonium Hydroxide, 7.4 M:
To 50 mL DI H_2O add 50 mL concentrated NH_4OH. Mix.
Storage: 25º 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 acetate buffer, 100 mM (pH 5.0).

Storage: 5º C in plastic
Stability: Several days; prepare daily for best results.

Hydrochloric Acid, 100 mM:
To 400 mL DI H_2O add 4.2 mL concentrated HCI. Dilute to 500 mL with DI H_2O. Mix.
Storage: 25º 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: 25ºC in glass or fluoropolymer plastic
Stability: 1 day

HOW TO PREPARE SOLUTIONS AND BUFFERS (continued)

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: 25ºC in glass or fluoropolymer plastic
Stability: 1 day

Phosphate Buffer, 100 mM, pH 6.0:
Dissolve 1.70 g Na_2HPO_4 and 12.14 g NaH_2P0_4 H_2O in 800 mL DI H_2O.
Dilute to 1000 mL using DI 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: 5º C in glass
Stability: 1 month; Inspect daily with use for contamination.

Phosphoric Acid, 500 mM:
To 400 mL DI H_2O and 17.0 mL concentrated phosphoric acid. Dilute to 500 mL with DI H_2O. Mix.
Storage: 25º C in glass or plastic
Stability: 6 months

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

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

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

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

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

Sulfuric Acid, 100 mM:
To 400 mL DI H_2O add 5.6 mL concentrated H_2SO_4. Dilute to 500 mL with DI H_2O. Mix.
Storage: 25º 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 insure 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., amphet-amines, 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 insure 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 insure the cleanest extraction of your eluate.

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

AMPHETAMINES 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 0.1M phosphate buffer (pH 6.0).
Mix/vortex.
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 0.1M monobasic or dibasic sodium phosphate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH; aspirate.
1 x 3 mL DI H_2O; aspirate.
1 x 1 mL 0.1 M 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 DI H_2O; aspirate.
1 x 1 mL 100 mM acetic acid; aspirate.
1 x 3 mL CH_3OH; aspirate.
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). Overlayer with N2 and cap.
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 (PFA)	Primary Ion**	Secondary	Tertiary
Amphetamine	**190**	91	118
D_5 Amphetamine*	**194**	91	123
Methamphetamine	**204**	118 or 91	160
D_5 Methamphetamine*	**208**	119 or 92	163

*Suggested internal standards for GC/MS: D_5 Amphetamine and D_5 Methamphetamine
**Quantification Ion

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 0.1 M 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; aspirate.
1 x 3 mL DI H_2O; aspirate.
1 x 1 mL 0.1 M 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 10% (v/v) CH_3OH in DI H_2O; aspirate.
Dry column (5 minutes at > 10 inches Hg).
1 x 1 mL hexane or hexane/ethyl acetate (50/50); aspirate.

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).
Overlayer 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.

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

8. QUANTITATE

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

Compound	Primary*	Secondary	Tertiary	OTHER
Testosterone-TMS	**432**	301	209	
19-Noretiocholanone-TMS	**405**	315	225	
Oxymethalone	**640**	552	462	370,143
Dehydroepiandosterone-2TMS	**432**	327	297	
10-Nortestosterone-2TMS	**418**	287	194	
Oxymethalone Metabolite #1	**640**	552	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-a-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 0.1 M phosphate buffer (pH 6.0).
Mix/vortex. Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 0.1 M monobasic or dibasic sodium phosphate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN
1 x 3 mL CH_3OH; aspirate.
1 x 3 mL DI H_2O; aspirate.
1 x 1 mL 0.1 M 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 DI H_2O; aspirate.
1 x 1 mL 0.1 M acetic acid; aspirate.
Dry column (5 minutes at > 10 inches Hg).
1 x 2 mL hexane; aspirate.

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.

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

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

* Suggested internal standard for GC/MS: Hexobarbital or a Deuterated Barbiturate analog.
** Quantitation Ion

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

(Part # CSDAUA83 without tips or with CLEAN-THRU® Tips CCDAUA83)

1. **PREPARE SAMPLE**
   To 1 mL of urine add internal standard(s)* and 1 mL of 0.1 M phosphate buffer (pH 6.0).
   Mix/vortex. Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 0.1 M monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 0.5 mL CH_3OH; aspirate.
   1 x 0.5 mL DI H_2O; aspirate.
   1 x 0.25 mL 0.1 M 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 0.5 mL DI H_2O; aspirate.
   1 x 0.5 mL 0.1 M acetic acid; aspirate.
   Dry column (5 minutes at > 10 inches Hg).
   1 x 0.1 mL hexane; aspirate.

5. **ELUTE BARBITURATES**
   2 X 0.75 mL hexane/ethyl acetate (50/50)

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:

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

* Suggested internal standard for GC/MS: Hexobarbital or a Deuterated Barbiturate analog.
** Quantitation Ion

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 0.1 M phosphate buffer (pH 6.0).
Mix/vortex. Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 0.1 M monobasic or dibasic sodium phosphate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN
1 x 3 mL CH_3OH; aspirate.
1 x 3 mL DI H_2O; aspirate.
1 x 1 mL 0.1 M 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 DI H_2O; aspirate.
1 x 1 mL 0.1 M acetic acid; aspirate.
1 x 3 mL methanol; aspirate.
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 DI 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 < 40º 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 0.1 M acetate buffer (pH=5.0). Mix/vortex. Hydrolyze for 3 hours at 65° C.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet.
   Cool before proceeding.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 1 mL 0.1 M 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 DI H_2O; aspirate.
   1 x 2 mL 20% acetonitrile in 0.1 M phosphate buffer (pH 6.0); aspirate.
   Dry column (5 minutes at > 10 inches Hg).
   1 x 2 mL hexane; aspirate.

5. **ELUTE BENZODIAZEPINES**
   1 x 3 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).
   Overlayer 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.

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

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

* Suggested internal standard for GC/MS: Prazepam or D_5-Oxazepam
** Quantitation ion

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

Reference- UCT Internal Publication

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 0.1M phosphate buffer (pH 6.0).
   Mix/vortex. Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 0.1M monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 1 mL 0.1M 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 DI H_2O; aspirate.
   1 x 2 mL 20% acetonitrile in 0.1 M phosphate buffer (pH 6.0); aspirate.
   Dry column (5 minutes at > 10 inches Hg).
   1 x 2 mL hexane; aspirate.

5. **ELUTE BENZODIAZEPINES**
   1 x 3 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. POTASSIUM PHOSPHATE BUFFER, pH 6.0 (0.01M)
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% potassium phosphate buffer, pH 6.0 (0.01M)

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
2 x 1 mL methanol
2 x 1 mL D.I. H_2O
DO NOT LET COLUMN DRY OUT
(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 methanol

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 methanol; aspirate.
   1 x 3 mL deionized water; aspirate.
   1 x 3 mL 0.1 M Acetate Buffer (pH 4.7)
   NOTE: Aspirate at $\leq$ 3 inches Hg to prevent sorbent drying.

2. **Apply sample**
   Take one milliter of urine and add 2 mLs of 0.1M Acetate Buffer at pH 4.7
   Load at 1 to 2 mL/ minute.

3. **Wash column**
   2 X 1 mL Acetone/ Methanol (1:1) aspirate.
   Dry column (5 minutes at $\geq$ 10 inches Hg).

4. **Elute Beta Blockers**
   1 x 1 mL Dichloromethane/ Isopropanol and Ammonium Hydroxide (82/16/2)
   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 $\leq$ 40° C.

6. **Derivatize**
   Derivatization Solution- Methaneboronic acid at 5mg/mL was prepared in dry ethyl acetate (use molecular sieve)
   Store this solution at –20ºC (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 methanol; aspirate.
1 x 3 mL deionized water; aspirate.
1 x 3 mL 0.1 M Acetate Buffer (pH 4.7)
NOTE: Aspirate at $\leq$ 3 inches Hg to prevent sorbent drying.

2. Apply sample
Take one milliter of urine and add 2 mLs of 0.1M Acetate Buffer at pH 4.7
Load at 1 to 2 mL/ minute.

3. Wash column
2 X 1 mL Acetone/ Methanol (1:1) aspirate.
Dry column (5 minutes at $\geq$ 10 inches Hg).

4. Elute Beta Blockers
1 x 1 mL Dichloromethane/ Isopropanol and Ammonium Hydroxide (82/16/2)
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 $\leq$ 40° C.

6. Derivatize
Derivatization Solution-
Methaneboronic acid at 5mg/mL was prepared in dry ethyl acetate (use molecular sieve)
Store this solution at –20º C (freezer conditions) until use.
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 THC IN URINE
FOR GC OR GC/MS CONFIRMATIONS USING:
200 mg CLEAN SCREEN® EXTRACTION COLUMN

(Part # ZSTHC020 without Tips or ZCTHC020 with CLEAN-THRU® Tips)

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 3.5 ± 0.5 with 1.0 ml of glacial acetic acid.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH; aspirate.
1 x 3 mL H_2O; aspirate.
1 x 1 mL 0.1M HCl; 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 2 mL DI H_2O; aspirate.
1 x 2 mL 0.1M HCl/acetonitrile (70/30); aspirate.
Dry column (5 minutes at > 10 inches Hg).
1 x 200 µL hexane; aspirate.

5. ELUTE CARBOXY THC

1 x 3 mL hexane/ethyl acetate (50/50) Collect eluate at 1 to 2 ml / minute

6. DRY ELUATE

Evaporate to dryness at < 40° C.

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.

8. QUANTITATE

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

ANALYTE (TMS)	Primary Ion**	Secondary	Tertiary
Carboxy-Δ^9 THC	**371**	473	488
D_3 Carboxy-Δ^9 THC*	**374**	476	491

*Suggested internal standard for GC/MS: D_3Carboxy-Δ^9THC
**Quantitation ion.

Reference- UCT Internal Publication

CARBOXY THC IN URINE MANUAL METHOD FOR GC OR GC/MS CONFIRMATIONS USING: 80 mg CLEAN SCREEN® REDUCED SOLVENT VOLUME EXTRACTION COLUMN

(Part # CSDAUA83 without Tips or CCDAUA83 with CLEAN-THRU® Tips)

1. PREPARE SAMPLE - BASE HYDROLYSIS OF GLUCURONIDES

To 1 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 3.5 ± 0.5 with about 400 µL of glacial acetic acid.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 500 µL Hexane/ Ethyl Acetate (1:1)
1 x 500 µL CH_3OH; aspirate.
1 x 500 µL H_2O; aspirate.
1 x 250 µL 0.1M HCl; aspirate.

NOTE:Use gravity flow or minimal vacuum to condition the column

3. APPLY SAMPLE

Load at 1 to 2 ml/minute.

4. WASH COLUMN

1 x 500 µL DI H_2O; aspirate.
1 x 500 µL 0.1M HCl/acetonitrile (70/30); aspirate.
Dry column (3 minutes at > 10 inches Hg).
1 x 100 µL hexane; aspirate.

5. ELUTE CARBOXY THC

2 x 750 µL hexane/ethyl acetate (50/50).

6. DRY ELUATE

Evaporate to dryness at < 40° C.

7. DERIVATIZE

Add 50 µL ethyl acetate and 50 µL BSTFA (with 1% TMCS).
Overlayer with Nitrogen and cap.
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
Carboxy-Δ^9 THC	**371**	473	488
D_3 Carboxy-Δ^9 THC*	**374**	476	491

*Suggested internal standard for GC/MS: D_3Carboxy-Δ^9 THC
**Quantitation ion.

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 mLs of urine sample add internal standard and 2mLs of 0.1M pH 6.0 Phosphate buffer

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL methanol; aspirate.
   1 x 3 mL deionized water; aspirate.
   1 x 1 mL 0.1 M 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 4 mL deionized water; aspirate.
   1 x 2 mL 0.1M acetic acid; aspirate.
   Dry column (5 minutes at > 10 inches Hg).
   1 x 3 mL hexane; aspirate.

5. **ELUTE CARISOPRODOL/MEPROBAMATE**
   1 x 3 mL hexare/ethyl acetate (50:50)
   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 0.1 M 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 methanol; aspirate.
   1 x 3 mL deionized water; aspirate.
   1 x 1 mL 0.1 M 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; aspirate.
   1 x 2 mL 20% acetonitrile in 0.1 M phosphate buffer (pH 6.0); aspirate.
   Dry column (5 minutes at > 10 inches Hg).
   1 x 2 mL hexane; aspirate.

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
Oxazepam-D_5*	**462**	464	463

*Suggested internal standard for GC/MS: Oxazepam-D_5
**Quantitation ion

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 0.1 M phosphate buffer (pH 6.0).
   Mix/vortex. Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 0.1 M monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 1 mL 0.1 M 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 2 mL DI H_2O; aspirate.
   1 x 2 mL 0.1 M HCl; aspirate.
   1 x 3 mL methanol; aspirate.
   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/minutes.
   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 < 40º C

7. **DERIVATIZE**
   Add 50 µL ethyl acetate and 50 µL BSTFA (with 1% TMCS).
   Overlayer with N_2 and cap. Mix/vortex.
   React 20 minutes at 70º 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**	Secondary	Tertiary
Cocaine	**182**	198	303
D_3-Cocaine*	**185**	201	306
Benzoylecgonine-TMS	**240**	256	361
D_3-Benzoylecgonine-TMS*	**243**	259	364

* Suggested internal standards for GC/MS: D_3-Cocaine, D_3-Benzoylecgonine
**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 DI H_2O.
   Mix/vortex and let stand 5 minutes.
   Centrifuge for 10 minutes at 2,000 rpm and discard pellet.
   Add 2 mL of 0.1 M phosphate buffer (pH 6.0). Mix/vortex.
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 0.1 M monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 1 mL 0.1 M 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 2 mL DI H_2O; aspirate.
   1 x 2 mL 0.1 M HCl; aspirate.
   1 x 3 mL CH3OH; aspirate.
   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 < 40º C.

7. **DERIVATIZE**
   Add 50 µL ethyl acetate and 50 mL BSTFA (with 1% TMCS).
   Overlayer with N_2 and cap. Mix/vortex.
   React 20 minutes at 70º 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**	Secondary	Tertiary
Cocaine	**182**	198	303
D_3-Cocaine*	**185**	201	306
Benzoylecgonine -TMS	**240**	256	361
D_3-Benzoylecgonine -TMS*	**243**	259	364

*Suggested internal standards for GC/MS: D_3-Cocaine, D_3-Benzoylecgonine
** 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 DI 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 0.1M phosphate buffer (pH 6.0). Mix/vortex.

Sample pH should be 6.0 ± 0.5.

Adjust pH accordingly with 0.1M monobasic or dibasic sodium phosphate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 mL CH_3OH; aspirate.

1 x 3 mL DI H_2O; aspirate.

1 x 1 mL 0.1M 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 2 mL DI H_2O; aspirate.

1 x 2 mL 0.1 M HCl; aspirate.

1 x 3 mL CH_3OH; aspirate.

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 Di 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 COLUMNS

(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 0.1M phosphate buffer (pH 6.0), internal standard and vortex.
   Matrix must be more aqueous than organic for good retention to occur.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   2 x 3 mL CH_3OH; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 3 mL 0.1 M 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. Allow to dry

4. **WASH COLUMN**
   1 x 1 mL DI H_2O; aspirate.
   1 x 1 mL 0.1 M HCl; aspirate.
   1 x 3 mL CH_3OH; aspirate.
   Dry column (5 minutes at $>$ 10 inches Hg).

5. **ELUTE COCAINE AND METABOLITES**
   1 x 3 mL CH_2Cl_2/IPA/NH_2OH (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).
   Overlayer 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**	Secondary	Tertiary
Cocaine	**182**	198	303
D_3-Cocaine*	**185**	201	306
Benzoylecgonine-TMS	**240**	256	361
D_3-Benzoylecgonine-TMS*	**243**	259	364

* Suggested internal standards for GC/MS
** 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 milliliters of urine into 16 x 100 mm 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; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 3 mL 0.1M pH 6.0 phosphate.

3. **APPLY SAMPLE**
   Pour supernatant onto column.
   Allow to flow via gravity.

4. **WASH COLUMN**
   1 x 3 mL DI H_2O; aspirate.
   Dry column (10 minutes at > 10 mm Hg).

5. **ELUTE STEROIDS**
   1 x 3 mL of methanol.

6. **ENZYMATIC HYDROLYSIS**
   Eluates were dried under a stream of nitrogen after which 2 mL of 0.2M phosphate buffer (pH 7.0) and 250 units of ß-glucuronidase were added, vortex mixed, and allowed to incubate at 50° C for 1 hour. Samples were then cooled, and the pH was adjusted to 10-11 using a 1:1 mixture of $NaHCO_3/Na_2CO_3$.

7. **ADDITION CLEAN-UP®**
   Add 5 mL of n-butyl chloride to each sample. The tubes were capped and shaken vigorously for 10 minutes and then centrifuged at 3000 rpm for 5 min. the organic layer was transferred to clean test tubes and dried under a stream of nitrogen. Place dried sample in a dessicator 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 Ion
Testosterone	**432**	417
Epitestosterone	**432**	417
DHEA	**432**	417
16 a Hydroxytestosterone*	**520**	

* Suggested internal standard at (20 ng/mL)
** 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 0.1 M phosphate buffer (pH 6.0).
   Mix/vortex. Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 0.1M monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 1 mL 0.1 M phosphate buffer (pH 6.0); aspirate.
   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 DI H_2O; aspirate.
   1 x 1 mL 0.1 M acetic acid; aspirate.
   1 x 3 mL CH_3OH; aspirate.
   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**	Secondary	Tertiary
Fentanyl	**245**	146	189
D_5-Fentanyl*	**250**	151	194
Alpha-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	222

* 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 2mL 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 0.1 M acetate buffer (pH=5.0). Mix/vortex. Hydrolyze for 3 hours at 65° C.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet.
   Cool before proceeding.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 1 mL 0.1 M 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 DI H_2O; aspirate.
   1 x 2 mL 20% acetonitrile in 0.1 M phosphate buffer (pH 6.0); aspirate.
   Dry column (5 minutes at > 10 inches Hg).
   1 x 2 mL hexane; aspirate.

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).
   Overlayer 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.

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)

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
Oxazepam-D_5 ISTD*	**462**	464	463

* Suggested internal standard for GC/MS: Oxazepam-D_5 ISTD
** Quantitation ion

GC CONDITIONS

Column-DB5 or equivalent capillary column (15 meters x 0.25mm ID x 0.25µ Film)
Injector Temperature = 250°C
INJECTOR Splitless mode
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 COLUMNS

(Part # CUC18111)

1. PREPARE SAMPLE

500 µL of sample, calibrator, or control was placed into a 10 x 25 mm disposable glass test tube and 25 µL of internal standard* (5.0mg/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; aspirate
1 x 3 mL DIH_2O; aspirate
1 x 3 mL 0.1N HCL aspirate

3. APPLY SAMPLE

Load at 1 to mL/minute.

4. WASH COLUMN

1 x 3 mL DI H_2O; aspirate
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 MeOH

6. DRY ELUATE

Evaporate to dryness at < 40°C

7. DERIVATIZATION

Add 50 µL of MTBSTFA + 1 % t-BDMCS
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

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, PA 19007
215-781-9255
215-785-1226 fax

1. **PREPARE SAMPLE**
   To 200 µL of urine add internal standard (GHB-D6) and 100 µL of 0.1 M phosphate buffer (pH 6.0). Mix/vortex.

2. **CONDITION CLEAN SCREEN® GHB EXTRACTION COLUMN**
   1 x 3 mL CH_3OH; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 0.5 mL 0.1 M phosphate buffer (pH6.0); aspirate.
   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**
   Remove test tubes from vacuum manifold.
   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:

GHB-diTMS	233**, 234, 235
GHB-D6-diTMS*	239**, 240, 241

* Suggested internal standard for GC/MS: GHB-D6-diTMS
** Quantitation ion

Blood GHB Extraction Procedure
Using:
(United Chemical Technologies, Inc. Worldwide Moritoring® part number 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 0.1 M 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 Methanol; aspirate.
   1 x 3 mL DI Water; aspirate.
   1 x 0.5 mL 0.1 M phosphate buffer (pH 6.0); aspirate.
   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 liguid 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.

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

(Part # ZSGHB020)
1 of 2

Developed by:
Saint Louis University Health Sciences Center
Saint Louis University Medical School
Mr. Joseph A. Crifasi, M.A., M.T., (ASCP)
Certified Toxicology Specialist, ABFT

Forensic Toxicology
6030 Helen Ave.
St. Louis, MO 63134
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GHB working standard; 200 µg/mL in H_2O; prepared from Radian stock 1 mg/mL.

GHB-D_6 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
25µL	200 µL	25 µg/mL
50 µL	200 µL	50µg/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 @ 80°C w/Nitrogen

6) Reconstitute the dried extracts with 200 µL of 0.1 Phosphate Buffer pH 6.0 buffer; vortex 15 seconds.

7) **Prepare CLEAN SCREEN® GHB** (200 mg in a 10 mL tube)
Extraction columns as follows:

1 x 3 mL of CH_3OH; aspirate.
1 x 3 mL of DIH_2O; aspirate.
1 x 3 mL of 0.1 M Phosphate Buffer (pH 6.0) aspirate.
NOTE: Aspirate at 3 inches of Hg or less to prevent sorbent drying.

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

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

9) ELUTE GHB
Place clean test tubes into vaccum 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.
No heating is required.

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

GHB-diTMS	233**, 234, 235
GHB-D6-diTMS*	239**, 240, 241

* Suggested internal standard for GC/MS: GHB-D6-diTMS
** Quantitation ion

Quality Control NOTE:
Quality control samples were prepared using drug free blood and 1mg/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 0.1M 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**
   2 x 2.5 mL MeOH
   2 x 2.5 mL DiH_2O
   2 x 2.5 mL phosphate buffer (0.1M, pH 7.0)

3. **APPLY SAMPLE**
   Decant supernatant onto SPE column
   Load at 1 to 2 mL / min.

4. **WASH COLUMN**
   5 mL of MeOH
   5 mL of H_2O
   Dry column (5 mins > 10 inches Hg)

5. **ELUTE GLYCOPYRROLATE**
   1 x 4 mL MeOH/0.5M NH_4OAC buffer, pH 3.0 (95:5)

6. **DRY ELUTE**
   Evaperate to dryness at 60°C
   Reconstitute with 100 µL MeOH

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 0.1 M phosphate buffer (pH 6.0).
Mix/vortex. Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 0.1 M monobasic or dibasic sodium phosphate.

2. PREPARE CLEAN SCREEN® EXTRACTION COLUMN
1 x 3 mL CH_3OH; aspirate.
1 x 3 mL DI H_2O; aspirate.
1 x 1 mL 0.1 M phosphate buffer (pH 6.0); aspirate.
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 DI H_2O; aspirate.
1 x 1 mL 0.1M Acetic Acid; aspirate
1 x 3 mL Methanol aspirate
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 mLs/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 methanol

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

Compound	Primary**	Secondary	Tertiary
Ketamine	**180**	209	152
D4-Ketamine*	**184**	213	156

* Suggested internal standard for GC/MS: D4-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; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   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 mL/minute.

4. **WASH COLUMN**
   1 x 3 mL DI H_2O; aspirate.
   1 x 1 mL 100 mM acetic acid; aspirate.
   1 x 3 mL CH_3OH; aspirate. Dry column (5 minutes at > 10 inch

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).
   Overlayer 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:

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

* Suggested internal standard for GC/MS: D_3-LSD
** 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; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   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 mL/minute.

4. **WASH COLUMN**
   1 x 3 mL DI H_2O; aspirate.
   1 x 1 mL 100 mM acetic acid; aspirate.
   1 x 3 mL CH_3OH; aspirate.
   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).
   Overlayer 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:

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

* Suggested internal standard for GC/MS: D_3-LSD
** 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 0.1 M phosphate buffer (pH 6.0). Mix/vortex.
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 0.1 M monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL methanol; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 1 mL 0.1 M 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 DI H_2O
   1 x 1 mL 0.1 M acetic acid; aspirate.
   Dry column (5 minutes at > 10 inches Hg).
   1 x 2 mL hexane; aspirate.

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 methanol; aspirate.
   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 DI H_2O (Sample volume is now its original 1.0 mL).

10. **ANALYZE BY EMIT**
    Process according to urine drug screening protocols provided by immunassay 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 0.1 M phosphate buffer (pH 6.0).
   Mix/vortex. Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 0.1 M monobasic or dibasic sodium phosphate.

2. **PREPARE CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 1 mL 0.1 M phosphate buffer (pH 6.0); aspirate.
   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 DI H_2O; aspirate.
   Dry column (5 minutes at > 10 inches Hg).
   1 x 2 mL hexane; aspirate.

5. **ELUTE METHAQUALONE**
   1 x 3 mL hexane/ethyl acetate (50/50); collect eluate a < 5 mL/minute.

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 0.1 M phosphate buffer (pH 6.0).
   Mix/vortex. Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 0.1 M Monobasic or 0.1M Dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 2 mL 0.1 M 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 DI H_2O; aspirate.
   1 x 1 mL 0.1 M acetic acid; aspirate.
   1 x 3 mL CH_3OH; aspirate.
   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 ethyl acetate.

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

Compound	Primary**	Secondary	Tertiary
Methadone	**72**	223	294
Methadone D_9*	**78**	226	303

* Suggested internal standard for GC/MS: Methadone D_9
** 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 d3 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**
   Wash with1 x 3 mL MeOH
   Wash with1 x 5 mL DI H_2O

3. **APPLY SAMPLE**
   Decant supernatant onto SPE column

4. **WASH COLUMN**
   1 x 10 mL of deionized water
   Dry with vacuum for 3 min.
   1 x 5 mL of methanol
   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 acetonitrile
   Heat for 20 mins at 60°C

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

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 0.1M phosphate buffer (pH 6.0).
   Mix/vortex.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 2 mL 0.1M phosphate buffer (pH 6.0); aspirate.
   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 DI H_2O; aspirate.
   1 x 1 mL 0.1 M acetic acid; aspirate.
   Dry column (5 minutes at > 10 inches Hg).
   1 x 2 mL Hexane; apirate

5. **ELUTE COTININE**
   1 x 3 mL Hexane/Ethyl acetate (50:50), collect at 1-2 mL/min.

6. **WASH COLUMN**
   Remove rack of collection tubes to rewash columns
   1 x 3 mL MeOH, aspirate.
   Dry column, 5 minutes at > 10 inches Hq)

7. **ELUTE 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/NO_4OH, mix, then add CH_2Cl_2 (pH 11-12).

8. **CONCENTRATE**
   Evaporate to dryness at < 40° C.
   Take care not to over-heat or over-evaporate
   Reconstitute with 100 µL methanol

9. **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

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

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 of 2

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 20 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 6.0-7.0 by drop wise addition with 50% Ammonium Hydroxide

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL methanol; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 3 mL 0.1 M 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 DI H_2O; aspirate.
   1 x 3 mL 0.1 M acetate buffer (pH 4.5); aspirate.
   1 x 3 mL methanol; aspirate.
   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

OPIATES IN URINE-OXIME TMS PROCEDURE FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

7. **DERIVATIZE**
   Add 100 µL ethyl acetate and 50 µL MSTFA .
   Overlayer with N_2 and cap. Mix/vortex.
   React 20 minutes at 85º C. in a heat block.
   Remove from heat source to cool

   NOTE: Do not evaporate MSTFA solution.

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

Compound	Quant Ion	Secondary	Tertiary
Codeine TMS	**371**	356	343
D_3-Codeine TMS*	**374**	359	346
Morphine TMS	**236**	414	429
D_3-Morphine TMS*	**239**	417	432
Hydrocodone Oxime TMS	**386**	371	329
Hydromorphone Oxime TMS	**355**	429	444
Oxycodone-Oxime TMS	**474**	459	401
Oxmorphone-Oxime TMS	**459**	287	532

*Suggested internal standards for GC/MS: D_3-Codeine, D_3-Morphine

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OPIATES IN HUMAN URINE- PROPYL DERIVATIVES FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

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

1. **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 0.1 M acetate buffer, pH 5.0). Hydrolyze for 3 hours at 60° C. Cool before proceeding. Centrifuge for 10 minutes at 2000 rpm and discard pellet. Adjust sample pH to 6.0 ± 0.5 with 1.0 N NaOH.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 2 mL methanol; aspirate.
   1 x 2 mL DI H_2O; aspirate.
   1 x 2 mL 0.1M 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 DI H_2O; aspirate.
   1 x 3 mL 0.1 M acetate buffer (pH 4.5); aspirate.
   1 x 3 mL methanol; aspirate.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE OPIATES**
   1 x 2 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 propionic anhydride: pyridine
   Make this solution fresh daily.
   Mix/vortex.
   React for 60 minutes at 40° C in a heater block.
   Remove from heat source to cool.
   Evaporate to dryness at < 40° C
   Reconstitute the residue with 50 µL of an ethyl acetate / methanol (70:30).

OPIATES IN HUMAN URINE- PROPYL DERIVATIVES FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

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-Monoacetylmorphine	**327**	268	383
Oxymorphone	**357**	300	413
Morphine	**341**	268	397
d_3-Morphine*	**344**	271	400

* Suggested internal standard for GC/MS: d_3-Codeine and d_3-Morphine
** Quantitation ion
*** Hydrocodone does not derivatize under these conditions.

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

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 DI H_2O
   Mix/vortex and let stand 5 minutes.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet.
   Add 2 mL of 0.1 M phosphate buffer (pH 6.0). Mix/vortex.
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 0.1M monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_2OH; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 1 mL 0.1 M phosphate buffer (pH 6.0); aspirate.
   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 DI H_2O; aspirate.
   1 x 2 mL 0.1M 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 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).
   Overlayer 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 (TMS)	Primary Ion**	Secondary Ion	Tertiary Ion
Codeine:	**371**	234	343
D_3-Codeine*	**374**	237	346
Morphine	**429**	287	324
D_3-Morphine*	**432**	290	327

* Suggested internal standards for GC/MS: D_3-Codeine, D_3-Morphine
** Quantitation ion

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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 0.1 M phosphate buffer (pH 6.0).
   Mix / vortex. Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 0.1 M monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL methanol; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 1 mL 0.1 M 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 DI H_2O; aspirate.
   1 x 1 mL 0.1 M acetic acid; aspirate
   1 x 3 mL methanol; aspirate.
   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/NO_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 Ion**	Secondary	Tertiary
Phencyclidine	**200**	91	242
D_5- Phencyclidine*	**205**	96	247

*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 0.1 M phosphate buffer (pH 6.0).
Mix/vortex. Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 0.1 M monobasic or dibasic sodium phosphate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN
1 x 3 mL CH_3OH; aspirate.
1 x 3 mL DI H_2O; aspirate.
1 x 2 mL 0.1 M phosphate buffer (pH 6.0); aspirate.
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 DI H_2O; aspirate.
1 x 1 mL 0.1 M acetic acid; aspirate.
1 x 3 mL CH_3OH; aspirate.
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/NO_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
Propoxyphene	**58**	115	208	250,265
Propoxyphene D_5 *	**63**	120	213	255,270

* Internal Standard
** Quantitation Ion

NOTE: To improve the analysis for Norpropoxyphene, the primary metabolite of Dextroproxyphene, 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 0.1 M 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; aspirate.
   1 x 2 mL DI H_2O; aspirate.
   1 x 2 mL 0.1 M phosphate buffer (pH 6.0); aspirate.
   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 DI H_2O; aspirate.
   1 x 2 mL 20% Acetonitrile in water; aspirate
   1 x 1 mL 0.1M Acetic Acid; aspirate
   Dry column (3 minutes at $>$ 10 inches Hg).
   1 x 2 mL Hexane; aspirate.
   1 x 3 mL Hexane/ Ethyl Acetate (50:50)
   1 x 3 mL Methanol
   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 1mL /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 20 minutes.

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

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)

* Quantitation Ion

GC CONDITIONS:

HP Model 5890 GC with a 5970 MSD
COLUMN = DB5 (25M x 0.32mmID x 0.17µ Film Thickness
CARRIER GAS -Helium (5psi head pressure)

INJECTION Size=1
l SPLITLESS MODE

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

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

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 DI H_2O and 2 mL of 0.1 M 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 0.1 M monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 1 mL 0.1 M phosphate buffer (pH 6.0); aspirate.
   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 DI H_2O; aspirate.
   1 x 1 mL 0.1 M acetic acid; aspirate.
   1 x 3 mL CH_3OH; aspirate.
   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_3Cl_2 (pH 11-12).

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

7. **QUANTITATE**
   Reconstitute with 200 µl acetonitrile/DI H_2O (1/3). Mix/vortex vigorously for 30 seconds.
   Inject 100 µL onto chromatograph at wavelength 235 nm.
   Mobile phase = 0.25M 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) 15cm X 4.6mm ID
   Coupled to a UV detector set at 235 nm.

THC AND CARBOXY THC IN WHOLE BLOOD FOR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

(Part # ZSTHC020 without Tips or ZCTHC020 with CLEAN-THRU® Tips)
1 of 2

1. **PREPARE SAMPLE**
   To 1 mL of whole blood sample add internal standard(s)* into a silanized screw top test tube.
   Mix/vortex. Let stand up 1 hour.
   Add 2 mL of acetonitrile, Vortex for 30 seconds and allow to sit for 3 minutes.
   Centrifuge for 10 minutes at maximum rpm.
   Decant into another silanized test tube and add 4 mL of 0.1 M acetate buffer (pH 4.5) to supernatant.
   Mix/vortex, centrifuge at maximum rpm for 5 minutes and decant the supernatant into another silanized test tube. This step removes any blood fragments or foam.

2. **PRECONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL hexane/ethyl acetate (75/25); aspirate.

3. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 1 mL 0.1 M HCl; aspirate.
   NOTE: Use gravity flow or minimal vacuum.

4. **APPLY SAMPLE**
   Load at 1 mL/minute.
   NOTE: Use gravity flow or minimal vacuum.

5. **WASH COLUMN**
   1 x 2 mL DI H_2O; aspirate.
   1 x 2 mL 0.1 M HCl/acetonitrile (70/30); aspirate.
   Dry columns (5 minutes at > 10 inches Hg).
   1 x 200 µL hexane.

   NOTE: Use gravity flow or minimal vacuum.

6. **ELUTE THC AND CARBOXY THC**
   1 x 3 mL hexane/ethyl acetate (75/25).
   NOTE: Use gravity flow or minimal vacuum.

7. **DRY ELUATE**
   Evaporate slowly to dryness at < 40º C.

8. **DERIVATIZE**
   Add 50 µL BSTFA (with 1% TMCS) and 50 µL of ethyl acetate
   Overlayer with Nitrogen gas and cap.
   Mix/vortex.
   React 30 minutes at 70° C.
   Remove from heat source to cool.

   NOTE: Do not evaporate BSTFA solution.

THC AND CARBOXY THC IN WHOLE BLOOD FOR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

9. QUANTITATE

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

ANALYTE (TMS)	Primary Ion**	Secondary	Tertiary
Carboxy-Δ^9 THC	**371**	473	488
D_3Carboxy-Δ^9 THC*	**374**	476	491
Tetrahydrocannabinol	**371**	303	386
D_3Tetrahydrocannabinol *	**374**	306	389

*Suggested internal standards for GC/MS: D_3THC and D_3Carboxy-Δ^9 THC
**Quantitation ion

Reference- UCT Internal Publication

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 of 3

1. **PREPARE SAMPLE**

   Urine
   To 2 mL of urine add internal standard(s) and 1 mL of 0.1 M phosphate buffer pH 6.0.
   Mix/vortex. Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 0.1 M monobasic or dibasic sodium phosphate.

   Serum, Plasma or Whole Blood
   To 1 mL of sample add internal standard(s) and 4 mL DI 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 0.1 M phosphate buffer (pH 6.0).
   Mix/vortex.
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 0.1 M monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 1 mL 0.1 M 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 DI H_2O; aspirate.
   1 x 1 mL 0.1 M acetic acid; aspirate.
   Dry column (5 minutes at > 10 inches Hg).
   1 x 2 mL hexane; aspirate.

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 < 40º 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 methanol; aspirate.
   Dry column (5 minutes at > 10 inches Hg).

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

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_3Cl_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 methanol or 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 MeOH 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 metabolit
Methamphetamin
Methyl *p*-aminobenzoate
Methyl benzoate
Methyl ecgonine
Methylparaben
Methylphenidate
Methyprylon and metabol
Morphine
Nicotine
Oxycodone
Pentazocine
Phencyclidine
Phenethylamine
Phentermine
Phenylpropanolamine
Procaine
Propoxyphene and metabolite
Propylparaben
Tranylcypromine
Trifluoperazine
Trimipramine
Thioridazine
Trazodone

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 of 2

1. **PREPARE SAMPLE**
   To 1 mL of serum or plasma add internal standard and 2 mL of 0.1 M 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 0.1 M monobasic or dibasic sodium phosphate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN**
   1 x 3 mL CH_3OH; aspirate.
   1 x 3 mL DI H_2O; aspirate.
   1 x 1 mL 0.1 M phosphate buffer (pH 6.0); aspirate.
   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 DI H_2O; aspirate.
   1 x 1 mL 0.1 M acetic acid; aspirate.
   1 x 3 mL CH_3OH; aspirate.
   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_3Cl_2 (pH 11-12).

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

7. **QUANTITATE**
   Reconstitute with 200 µl acetonitrile/DI H_2O (1/3).
   Mix/vortex vigorously for 30 seconds.
   Inject 100 µL onto HPLC

TRICYCLIC ANTIDEPRESSANTS IN SERUM AND PLASMA FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

HPLC CONDITIONS

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

ANALYTES AND EXTRACTION EFFICIENCY

COMPOUND	Retention Time (min)	% Recovery	%RSD
Trimipramine ISTD*	2.564	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 better. Add 1-2% of Ammonium Hydroxide and check again.

4’4-METHYLENEDIANALINE IN SERUM (ENVIRO-CLEAN® C18 BONDED PHASE)

(Part # CEC18111)

1. **SAMPLE PREPARATION**
   a. none

2. **CONDITION ENVIRO-CLEAN® EXTRACTION COLUMN**
   a. 3 mL Methanol
   b. 3 mL Distilled Water

3. **LOAD 1 mL SERUM SAMPLE**

4. **COLUMN WASH**
   a. 1 mL Distilled Water

6. **ELUTION**
   a. 0.25 mL Methanol containing 1M ammoniun hydroxide

7. **INJECTION / ANALYSIS**
   a. Inject 10 µL onto HPLC system

4’4 Methylenedianaline
Extracted 100 µg/mL sample
Retention time = 3.162 min
Mobile Phase: Methano/H_2O (50:50)
Selectra C18 column
Flow rate = 1.2 mL/m
Inj. volume = 10 µL
wavelength = 254 nm

CHLOROPHENOXY ACID HERBICIDES IN WATER

(ENVIRO-CLEAN® C18 BONDED PHASE)

(Part # CEC181M6)

Chlorophenoxy Acid Herbicides Extracted
2, 4-D Acid
2,4,5-trichloro phenoxy propionic acid (Silvex)
Dicamba
Dinitro-sec-butyl phenol

1. **SAMPLE PREPARATION**
   a. Adjust pH of 1 liter water sample to pH 1.0 with hydrochloric acid

2. **CONDITION ENVIRO-CLEAN® EXTRACTION COLUMN**
   a. 10 mL Hexane / Acetone (50:50)
   b. 10 mL Acidified Methanol (5% HCl in MEOH)
   c. 10 mL Distilled water

3. **LOAD 1 LITER OF PH ADJUSTED WATER SAMPLE (RATE 8-10 ML/MIN)**

4. **COLUMN WASH**
   a. 10 mL Distilled water adjusted to pH 1.0 with HCl

5. **DRY COLUMN FOR 15-30 MINUTES UNDER MAXIMUN VACUUM PRESSURE**

6. **ELUTION**
   a. 10 mL Hexane / Acetone (50:50)

7. **CONCENTRATION / EVAPORATION**
   a. Add 500 µL of a keeper solvent (Methanol, DMF, other). Evaporate to 500 µL under a nitrogen stream at room temperature

8. **INJECTION / ANALYSIS**
   a. Reconstitute with 100 µL TCTEF Inject at 1-2 µL onto GC

PESTICIDES
IN WATER
(ENVIRO-CLEAN® C18 BONDED PHASE)

(Part # CEC181M6)

Pesticides Extracted	
α- hexachlorocychexane	4,4'-DDE
Lindane	Dieldrin
β-hexachlorocychexane	Endrin
Heptechlor	4,4'-DDD
δ-hexachlorocychexane	Endosulffan II
Aldrin	4,4'-DDT
Heptachlor	Endrin Aldehyde
Endosulfan 1	Endosulfan Sulfate

1. **SAMPLE PREPARATION**
   a. None

2. **CONDITION ENVIRO-CLEAN® EXTRACTION COLUMN**
   a. 10 mL Hexane / Acetone (50:50)
   b. 10 mL Methanol
   c. 10 mL Distilled Water

3. **LOAD 1 LITER SAMPLE (RATE 8-10 ML/MIN)**

4. **COLUMN WASH**
   a. 20 mL Distilled Water

5. **DRY COLUMN FOR 15-30 MINUTES UNDER MAXIMUN VACUUM PRESSURE**

6. **ELUTION**
   a. 10 mL Hexane / Acetone (50:50)

7. **CONCENTRATION / EVAPORATION**
   a. Add 500 µL of a keeper solvent (methanol, DMF, other).
   b. Evaporate to 500 µL under a nitrogen stream

Polychlorinated Biphenyls
In Pond Water
(ENVIRO-CLEAN® C18 Bonded Phase)

(Part # CEC182M6)

Polychlorinated Biphenyls Extracted	
Aroclor 1026	Aroclor 1221
Aroclor 1232	Aroclor 1242
Aroclor 1248	Aroclor 1254
Aroclor 1260	

1. **SAMPLE PREPARATION**
   a.Filter water sample through a 0.5 µm filter.
   b. Add 2 mL methanol to 200 mL of filtered water sample.
   c. Mix and degas sample for 2 minutes

2. **CONDITION ENVIRO-CLEAN® EXTRACTION COLUMN**
   a. 15-20 mL Hexane   Dry for 5 minutes
   b. 15-20 mL Methanol
   c. 10 mL Distilled Water

3. **LOAD 200 ML WATER SAMPLE (RATE 8-10 ML/MIN)**

4. **COLUMN WASH**
   a. 20 mL Distilled Water

5. **DRY COLUMN FOR 20-30 MINUTES UNDER MAXIMUN VACUUM PRESSURE**

6. **ELUTION**
   a. 20 mL Hexane

7. **CONCENTRATION / EVAPORATION**
   a. Evaporate to dryness under a nitrogen stream at room temperature

6. **INJECTION / ANALYSIS**
   a. Reconstitute with 100 µL methanol inject at 1-2 µL aliquot onto GC

POLYNUCLEAR AROMATIC HYDROCARBONS IN POND WATER (ENVIRO-CLEAN® C18 BONDED PHASE)

(Part # CUNAX153)

Polynuclear Aromatic Hydrocarbons Extracted	
Naphthalene	Chrysene
Fluorene	B(e)pyrene
Acenaphthene	B(b)fluoranthene
Phenanthrene	B(k)fluoranthene
Anthracene	B(a)pyrene
Fluoranthene	D(a,h)anthracene
Pyrene	B(g,hi)perylene
B(a)anthracene	Indeno(1,2,3,-cd)pyrene

1. **SAMPLE PREPARATION**
   a. Filter water through a 0.5 µm filter.
   b. Add 2 mL of methanol to 200 ml of filtered water sample.
   c. Mix and degas sample for 2 minutes.

2. **PREPARE ENVIRO-CLEAN® EXTRACTION COLUMN**
   a. 15-20 mL Methylene chloride/trichlorotrifluoroethylene (TCTFE)
   b. 15-20 mL TCTFE. Dry for 5 minutes
   c. 15-20 mL Methanol
   d. 20 mL Distilled water

3. **LOAD 200 mL WATER SAMPLE (RATE 8-10 ML/MIN)**

4. **COLUMN WASH**
   a. 20 ml Distilled Water

5. **DRY COLUMN FOR 15-30 MINUTES UNDER MAXIMUN VACUUM PRESSURE**

6. **ELUTION**
   a. 20 mL of TCTFE

7. **CONCENTRATION / EVAPORATION**
   a. Evaporate to dryness under a nitrogen stream at room temperature

8. **INJECTION / ANALYSIS**
   a. Reconstitute with 100 µL TCTEF Inject at 1-2 µL aliquot onto GC

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

(Part # XRDAH515)

1. **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.
   Cool before proceeding.

   BASE HYDROLYSIS OF GLUCURONIDES
   To 2 mL of urine add internal standard(s) and 100 µl of 10N NaOH
   Mix/vortex. Hydrolyze at 60°C for 20 minutes.
   Cool before proceeding.

   COMBINE HYDROLYSATES
   Combine both hydrolysis products with 5 mLs of 0.1M pH 6.0 Phosphate Buffer
   Adjust sample pH = 6.0 ± 0.5 with 0.5M Phosphoric acid

2. **CONDITION XtrackT® EXTRACTION COLUMN**
   1 x 5 mL CH_3OH; aspirate.
   1 x 5 mL DI H_2O; aspirate.
   1 x 3 mL 0.1 M 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 0.1M pH 6.0 Phosphate buffer; aspirate.
   1 x 2 mL 1.0 M acetic acid; aspirate.
   Dry column (5 minutes at > 10 inches Hg).
   1 x 2 mL hexane; aspirate.

5. **ELUTE ACIDIC AND NEUTRAL DRUGS**
   1 x 4 mL methylene chloride; collect eluate at < 5 mL/minute.

6. **ELUTE STEROIDS** 2 x 4 mL ethyl acetate; collect eluate at < 5 mL/minute.

7. **WASH COLUMN** 1 x 5 mL methanol; 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 < 40º 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:
2 x 3 mL CH_3OH; aspirate.
2 x 3 mL DI H_2O; aspirate.
1 x 2 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 ml/minute.

4. WASH COLUMN:
1 x 3 mL DI H_2O; aspirate.
1 x 3 mL Hexane; aspirate.
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 mm x 0.2 x 0.33 µm film thickness
GC Oven: Initial Temp. = 100° C
Initial Time = 3.00 min.

Ramp = 17C/min.
Final Temp. = 305° C
Final Time = 3.00 min.
Injection Port Temp. = 250° C
Transfer line Temp. = 280° C

SCAN Acquisition = 41 amu to 400 amu: Start time = 2.00 mins

Retention times:	Compound	CN	CS	OC
	RT (min.)	@4.9	@7.4	@13.4

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