Extraction Methods Guide for Mixed-Mode Drug-Clean™ SPE

(Part No. 8604527)

S*PURE

Complete list of Extraction Methods for Drug-Clean™ SPE

Drug-Clean™ SPE Extraction Methods

COMPOUNDS	MATRIX	ANALYSIS
General Drug Screening	Urine, Serum, Plasma or Whole Blood	GC, GC/MS
Forensic Drug Screening	Urine, Whole Blood, Tissue	GC, GC/MS
Amphetamines	Urine	GC, GC/MS
Anabolic Steroids	Urine	GC, GC/MS
Antidepressants (Tricyclic)	Serum, Plasma, Whole Blood	GC, GC/MS
Antidepressants (Tricyclic)	Serum, Plasma, Whole Blood	HPLC
Basic Drugs	Urine	HPLC
Barbiturates	Urine	GC, GC/MS
Benzodiazepines	Urine	GC, GC/MS
Benzodiazepines	Serum, Plasma	HPLC
Carboxy-THC	Urine	GC, GC/MS
Carboxy-THC and THC	Whole Blood	GC/MS
Cocaine and Benzoylecgonine	Urine, Serum, Plasma or Whole Blood	GC, GC/MS
Cocaine and Benzoylecgonine	Serum, Plasma or Whole Blood	HPLC
Cocaine and Benzoylecgonine	Meconium	GC, GC/MS
Cocaine and Benzoylecgonine	Meconium	HPLC
Fentanyl and Analogs	Urine	GC, GC/MS
Fluoxetine and Norfluoxetine	Serum, Plasma, Whole Blood	GC, GC/MS
LSD	Urine, Serum, Plasma or Whole Blood	GC, GC/MS
Meperidine	Urine	GC, GC/MS
Methadone	Urine	GC, GC/MS
Methaqualone	Urine	GC, GC/MS
6-Monoacetyl Morphine	Urine	GC, GC/MS
Opiates	Urine, Serum, Plasma or Whole Blood	GC, GC/MS
Phencyclidine	Urine	GC, GC/MS
Propoxyphene	Urine	GC, GC/MS
Sertraline and Norsertraline	Serum, Plasma, Whole Blood	HPLC

General Drug Screening for Acidic, Basic and Neutral Drugs (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment Urine:

Urine:

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

Serum, Plasma or Whole Blood:

- To 1mL of sample add internal standard(s) and 4mL DI H2O (pH 5.5-5.7).
  [Whole Blood: Mix/vortex and let stand 5 minutes.]
- Centrifuge for 10 minutes at 2000 rpm and discard pellet.
- Add 2mL 100mM phosphate buffer (pH 6.0). Mix/vortex.
- Sample pH should be 6.0±0.5.
- Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate.

2. Tube Conditioning

- Rinse with 1 x 3mL CH3OH. Aspirate.
- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.
  NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1-2mL/minute.

4. Tube Wash

- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM acetic acid. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).
- Rinse with 1 x 2mL hexane. Aspirate.

5. Elution – Acidic and Neutral Drugs

- Elute with 1 x 3mL hexane/ethyl acetate (50:50). Collect eluate at ≤ 5 mL/minute.
- Evaporate to dryness at <40°C.
- Reconstitute with 100µL ethyl acetate.
- Inject 1-3µL into chromatograph.

6. Second Tube Wash

- Rinse with 1 x 3mL CH3OH. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).

7. Elution - Basic Drugs

- Elute with 1 x 3mL CH2Cl2/IPA /NH4OH (78:20:2).
  NOTE: Prepare elution solvent fresh daily.
  [To 40mL of isopropanol, add 4mL of concentrated ammo
- nium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]
- Collect eluate at 1-2mL/minute.
- Evaporate to dryness at <40° C taking care not to over -heat or over-evaporate.
- Certain compounds are heat labile, such as the amphe -tamines and phencyclidine.
- Reconstitute with 100µL methanol.
- Inject 1-3µL into chromatograph.

Forensic Drug Screening (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Urine:

- To 5mL of urine add 150-300µL of 1.0M acetic acid to adjust sample pH to between 4.8 and 5.5.

Whole Blood:

- To 2mL of blood, add 8mL of DI H2O. Mix/vortex and let stand 5 minutes.
- Add 150-300µL of 1.0M acetic acid to adjust sample pH to between 4.8 and 5.5.
- Centrifuge for 10 minutes at 2000 rpm and discard pellet.

Tissue:

- Homogenize 1 part tissue with 3 parts DI H2O.
- Centrifuge for 10 minutes at 2000 rpm and discard pellet.
- Transfer 10mL of supernatant to a clean tube.
- Add 150-300µL of 1.0M acetic acid to adjust sample pH to between 4.8 and 5.5.

2. Tube Conditioning

- Rinse with 1 x 3mL CH3OH. Aspirate.
- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM acetic acid. Aspirate.

NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1-2mL/minute.

4. Tube Wash

- Rinse with 1 x 3mL 100mM phosphate buffer (pH 6.0). Aspirate.
- Rinse with 1 x 1mL 100mM acetic acid. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).
- Rinse with 1 x 3mL hexane. Aspirate.

5. Elution and Analysis - Acidic and Neutral Drugs (Fraction A)

- Elute with 2 x 2mL CH2Cl2. Collect eluate at 1-2mL/minute.
- Evaporate to dryness at <40°C.
- Add 1mL hexane and 1mL CH3OH/H2O (80:20). Mix/vortex.
- Centrifuge to separate layers. Aspirate and discard hexane (upper) layer.
- Evaporate again to dryness at <40°C.
- Reconstitute with 100µL ethyl acetate and inject 1-3µL into chromatograph.

6. Tube Wash

- Rinse with 1 x 2mL methanol. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).

7. Elution and Analysis - Basic Drugs (Fraction B)

- Elute with 1 x 2mL CH3OH/NH4OH (98:2). Collect eluate at 1-2mL/minute.

  NOTE: Prepare elution solvent daily.

  [To 98mL of methanol, add 2mL concentrated ammonium hydroxide. Mix.]
- Add 3mL DI H2O and 250µL chloroform to eluate. Mix/vortex for 30 seconds.
- Centrifuge to separate phases. Aspirate and discard aqueous (upper) layer.
- Inject 1-3µL of the chloroform layer into chromatograph.

Amphetamines (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Urine:

- To 5mL of urine add internal standard(s)* and 2mL of 100mM phosphate buffer (pH 6.0).
- Mix/vortex. Sample pH should be 6.0±0.5.
- Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate.

2. Tube Conditioning

- Rinse with 1 x 3ml CH_3OH. Aspirate.
- Rinse with 1 x 3ml DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.

NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1-2mL/minute.

4. Tube Wash

- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM acetic acid. Aspirate.
- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).

5. Elution

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

NOTE: Prepare elution solvent fresh daily.

[To 40mL of isopropanol, add 4mL of concentrated ammonium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]

- Add 30µL silylation grade DMF to eluate.
- Evaporate to 30µL at <40°C.
- Add 50µL PFPA (PFAA). Blanket with N2 and cap.
- React 20 minutes at 70°C. Evaporate to dryness at <40°C.
- Reconstitute with 100µL ethyl acetate.

For GC or GC/MS Analysis:

- Inject 1-3µL into chromatograph.
- Monitor the following ions (GC/MS):

Amphetamine: 190**, 91, 118
Amphetamine-d5: 194**, 91, 123
Methamphetamine: 204**, 118 (or 91), 160
Methamphetamine-d5: 204**, 119 (or 92), 163

* Suggested internal standards for GC/MS: amphetamine -d5, methamphetamine-d5

** Quantitation ion

Anabolic Steroids (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Urine:

- To 5mL of urine add internal standard and 2mL of β-glucuronidase.
  [β-Glucuronidase: 5,000 F units/mL patella vulgata in 100mM acetate buffer (pH 5.0).]
- Mix/vortex. Hydrolyze for 3 hours at 65ºC. Cool before proceeding.
- Centrifuge for 10 minutes at 2000 rpm and discard pellet.
- Adjust sample pH to 6.0±0.5 with approximately 700µL of 1.0N NaOH.

2. Tube Conditioning

- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer. Aspirate.
  NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1-2mL/minute.

4. Tube Wash

- Rinse with 1 x 3mL 10% (v/v) CH_3OH in DI H_2O. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).
- Rinse with 1 x 1mL hexane or hexane/ethyl acetate (50:50). Aspirate.

5. Elution (Choose Methods A, B, C or D)

- A. Elute with 1 x 3mL CH_2Cl_2/IPA/NH_4OH (78:20:2). Collect eluate at 1-2mL/minute.
  NOTE: Prepare elution solvent fresh daily.
  [To 40mL of isopropanol, add 4mL of concentrated ammonium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]
- B. Elute with 1 x 3mL CH_2Cl_2/IPA (80:20)
- C. Elute with 1 x 3mL ethyl acetate
- D. Elute with 1 x 3mL CH_3OH
- Evaporate to dryness at <40ºC.

For GC or GC/MS Analysis:

- Add 50µL ethyl acetate and 50µL MSTFA (with 3% trimethylsilyliodide).
- Blanket with N_2 and cap. Mix/vortex.
- React 20 minutes at 70ºC. Remove from heat source to cool.
  NOTE: Do not evaporate MSTFA solution.
- Inject 1-3µL of sample (in MSTFA solution) into chromatograph.
- Monitor the following ions (GC/MS):
  Testosterone-TMS: 432, 301, 209
  11-β-Hydroxyandosterone: 522, 417, 158
  19-Noretiocholanone-TMS: 405, 315, 225
  Methandienone: 409, 313, 281
  Oxymetholone: 640, 552, 462, 370,143
  19-Norandosterone-2TMS: 405, 315, 225
  Dehydroepiandosterone-2TMS: 432, 327, 297
  16-A-Hydroxyetiocholanone-TMS: 504, 417
  10-Nortestosterone-2TMS: 418, 287, 194
  17-A-Epitestosterone-TMS: 432, 341, 327,209
  Oxymetholone metab. #1: 640, 552, 462, 143
  Stanazolol-TMS: 472, 381, 342, 149
  Oxymetholone metab. #2: 625, 462, 370, 143

Tricyclic Antidepressants (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Serum, Plasma, or Whole Blood:

- To 1mL of sample add 4mL DI H2O and internal standard (clomipramine or protriptyline).
- Add 2mL of 100mM 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 100mM monobasic or dibasic sodium phosphate.

2. Tube Conditioning

- Rinse with 1 x 3mL CH2OH. Aspirate.
- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.

  NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1-2mL/minute.

4. Tube Wash

- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM acetic acid. Aspirate.
- Rinse with 1 x 3mL CH3OH. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).

5. Elution

- Elute with 1 x 3mL CH2Cl2/IPA/NH4OH (78:20:2). Collect eluate at 1-2mL/minute.

  NOTE: Prepare elution solvent fresh daily.

  [To 40mL of isopropanol, add 4mL of concentrated ammonium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]
- Evaporate to dryness at <40°C.

For GC or GC/MS Analysis:

Underivatized Analytes:

- Reconstitute with 100µL methanol. Inject 1-3µL into chromatograph.

Derivatized Analytes:

- Reconstitute with 50µL ethyl acetate.
- Add 50µL of PFPA.
- Blanket with N2 and cap. React 20 minutes at 70°C.
- Evaporate to dryness at < 40°C. Reconstitute with 100µL ethyl acetate.
- Inject 1-3µL into chromatograph.

Tricyclic Antidepressants (HPLC)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment Urine:

Serum, Plasma, or Whole Blood:

- To 1mL of sample add internal standard(s)*, 4mL DI H_2O and 2mL of 100mM 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 100mM monobasic or dibasic sodium phosphate.

2. Tube Conditioning

- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.

NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1mL/minute.

4. Tube Wash

- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM acetic acid. Aspirate.
- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Dry tube (5 minutes at ≥ 10 inches Hg).

5. Elution

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

NOTE: Prepare elution solvent fresh daily.

[To 40mL of isopropanol, add 4mL of concentrated ammonium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]

- Evaporate to dryness at <40°C.

For HPLC Analysis:

- Reconstitute with 200µL acetonitrile/DI H_2O (1:3). Mix/vortex vigorously for 30 seconds.
- Inject 100µL into chromatograph.

*Suggested internal standards: Trimipramine and Protriptyline

Basic Drugs (HPLC)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Urine:

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

2. Tube Conditioning

- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.

NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1-2mL/minute.

4. Tube Wash

- Rinse with 1 x 2mL DI H_2O. Aspirate.
- Rinse with 1 x 2mL 100rnM HCl. Aspirate.
- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Dry tube (5 minutes at ≥ 10 inches Hg).

5. Elution

- Elute with 1 x 2mL CH_3OH/NH_4OH (98:2). Collect eluate at 1-2mL/minute.

NOTE: Prepare elution solvent daily.

[To 98mL of methanol, add 2mL concentrated ammonium hydroxide. Mix.]

- To eluate add 2.0mL DI H_2O and 500µL CH_2Cl_2.
- Mix/vortex. Centrifuge.
- Transfer organic (lower) layer to a clean tube.
- Evaporate to dryness at <40°C.

For HPLC Analysis:

- Reconstitute in mobile phase and inject into chromatograph.

Barbiturates (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Urine:

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

2. Tube Conditioning

- Rinse with 1 x 3mLCH3OH. Aspirate.
- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.

NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1-2mL/minute.

4. Tube Wash

- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM acetic acid. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).
- Rinse with 1 x 2mL hexane. Aspirate.

5. Elute Barbiturates

- Elute with 1 x 3mL hexane/ethyl acetate (50:50). Collect eluate at ≤ 5mL/minute.
- Evaporate to dryness at <40°C.
- Reconstitute with 100µL ethyl acetate.

For GC or GC/MS Analysis:

Inject 1-3µL into chromatograph.

- Monitor the following ions (GC/MS):

  Amobarbital: 156**, 141, 157
  Pentobarbital: 156**, 141, 157
  Butabarbital: 156**, 141, 157
  Phenobarbital: 204**, 117, 232
  Butalbital: 168**, 153, 141
  Secobarbital: 168**, 153, 195
  Hexobarbital: 221**, 157, 156

* Suggested internal standard for GC/MS: hexobarbital

** Quantitation ion

Benzodiazepines (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Urine:

- To 5mL of urine add internal standard(s)* and 2mL of β-glucuronidase solution.
  [β-Glucuronidase solution contains 5,000 F units/mL patella vulgata in 100mM acetate buffer (pH 5.0)].
  Mix/vortex. Hydrolyze for 3 hours at 65ºC.
- Centrifuge for 10 minutes at 2000 rpm and discard pellet.
- Cool before proceeding.

2. Tube Conditioning

- Rinse with 1 x 3mL CH3OH. Aspirate.
- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.
  NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1-2mL/minute.

4. Tube Wash

- Rinse with 1 x 2mL DI H2O. Aspirate.
- Rinse with 1 x 2mL 20% acetonitrile in 0.1M phosphate buffer (pH 6.0). Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).
- Rinse with 1 x 2mL hexane. Aspirate.

5. Elution

- Elute with 1 x 3mL ethyl acetate. Collect eluate at 1-2mL /minute.
- Evaporate to dryness at <40º C.

For GC or GC/MS Analysis:

- Add 50µL ethyl acetate and 50µL BSTFA (with 1% TMCS).
- Blanket with N2 and cap. Mix/vortex.
- React 20 minutes at 70º C. Remove from heat source to cool. NOTE: Do not evaporate BSTFA solution.
- Inject 1-3µL of sample into chromatograph.
- Monitor the following ions (GC/MS):
  Alprazolam: 308**, 279, 204
  Temazepam (TMS): 343**, 283, 257
  Clonazepam: 387**, 352, 306
  Chlordiazepoxide: 282**, 283, 284
  Desalkylflurazepam (TMS): 359**, 341, 245
  α–Hydroxytriazolam (TMS): 415**, 417, 430
  Diazepam 256**, 283, 221
  α–Hydroxyalprazolam (TMS):381**, 396, 383
  Halazepam: 324**, 352, 289
  Hydroxyethylflurazepam: 288**, 287, 289
  Lorazepam (TMS): 429**, 430, 347
  Triazolam: 313**, 314, 342
  Nordiazepam (TMS): 341**, 342, 343
  Prazepam: 269**, 241, 324
  Oxazepam (TMS): 429**, 430, 313
  Hydroxydiazepam: 86**, 109, 307

* Suggested internal standards for GC/MS: prazepam, oxazepam-d5

** Quantitation ion

NOTE: Flurazepam does not extract under these conditions; however metabolites such as desalkylflurazepam and hydroxyethylflurazepam will extract with high recovery. A basic wash is necessary in order to recover flurazepam, however this reduces the recovery of other benzodiazepines

Benzodiazepines (HPLC)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Serum or Plasma:

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

2. Tube Conditioning

- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.

NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1-2mL/minute.

4. Tube Wash

- Rinse with 1 x 2mL DI H_2O. Aspirate.
- Rinse with 1 x 2mL 20% acetonitrile in 0.1M phosphate buffer (pH 6.0). Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).
- Rinse with 1 x 2mL hexane. Aspirate.

5. Elution

- Elute with 1 x 3mL ethyl acetate. Collect eluate at 1-2mL/minute.
- Evaporate to dryness at <40° C.

For HPLC Analysis:

- Reconstitute in mobile phase and inject into chromatograph.

Carboxy-THC (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Urine:

- To 5mL of urine add internal standard* and 200µL of 10N NaOH. Mix/vortex.
- Hydrolyze for 20 minutes at 60ºC. Cool before proceeding.
- Adjust sample pH to 3.5±0.5 with 2.0mL of glacial acetic acid.

2. Tube Conditioning

- Rinse with 1 x 3mL CH3OH. Aspirate.
- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM HCl. Aspirate.

NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1mL/minute.

4. Tube Wash

- Rinse with 1 x 2mL DI H2O. Aspirate.
- Rinse with 1 x 2mL 100mM HCl/acetonitrile (70:30). Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).
- Rinse with 1 x 200µL hexane. Aspirate.

5. Elution

- Elute with 1 x 3mL hexane/ethyl acetate (50:50). Collect eluate at 1-2mL/minute.
- Evaporate to dryness at <40ºC.

For GC or GC/MS Analysis:

- Add 50µL ethyl acetate and 50µL BSTFA (with 1% TMCS).
- Blanket with N2 and cap. Mix/vortex.
- React 20 minutes at 70ºC. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA.

- Inject 1-3µL of sample into chromatograph.
- Monitor the following ions (Mass Selective Detection):

Carboxy-Δ9-THC - 371**, 473, 488

Carboxy-Δ9-THC-d3 - 374**, 476, 491

* Suggested internal standard for GC/MS: carboxy-Δ9-THC-d3

** Quantitation ion.

THC and Carboxy-THC (GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Whole Blood:

- To 1mL of whole blood sample, add internal standard(s)* and 1mL of acetonitrile.
- Mix/vortex. Let stand 5 minutes. Vortex.
- Centrifuge for 10 minutes at maximum rpm.
- Decant and add 5mL of 100mM acetate buffer (pH 4.5) to supernatant.
- Mix/vortex. Centrifuge 5 minutes to remove blood fragments or foam.

2. Tube Conditioning

- Rinse with 1 x 3mL hexane/ethyl acetate (75:25). Aspirate.
- Rinse with 1 x 3mL CH3OH. Aspirate.
- Rinse with 1 x 3mL DI H2O. Aspirate.

NOTE: Use gravity flow or minimal vacuum.

- Rinse with 1 x 1mL 100mM HCl. Aspirate.

3. Sample Loading

- Load at 1mL/minute.

NOTE: Use gravity flow or minimal vacuum.

4. Tube Wash

- Rinse with 1 x 2mL DI H2O. Aspirate.
- Rinse with 1 x 2mL 100mM HCl/acetonitrile (70:30). Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).
- Rinse with 1 x 200µL hexane. NOTE: Use gravity flow or minimal vacuum.

5. Elution

- Elute with 1 x 3mL hexane/ethyl acetate (75:25).

NOTE: Use gravity flow or minimal vacuum.

- Evaporate slowly to dryness at <40ºC.

For GC/MS Analysis:

- Add 50µL BSTFA (with 1% TMCS) and 50µL of ethyl acetate.
- Blanket with N2 and cap. Mix/vortex.
- React 30 minutes at 70ºC. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

- Inject 2µL sample into chromatograph.
- Monitor the following ions (GC/MS):

THC - 303**, 315, 386

THC-d3 - 306**, 318, 389

Carboxy-Δ9-THC - 371**, 473, 488

Carboxy-Δ9-THC-d3 - 374*, 476, 491

*Suggested internal standards for GC/MS: THC-d3 and carboxy-Δ9-THC-d3

** Quantitation ion

Cocaine and Benzoylecgonine (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Urine:

- To 5mL of urine add internal standard(s)* and 2mL of 100mM phosphate buffer (pH 6.0).
- Mix/vortex. Sample pH should be 6.0±0.5.
- Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate.

Serum, Plasma or Whole Blood:

- To 1mL of serum or plasma, add internal standard(s)* and 4mL of DI H2O (pH 5.0-7.0).
- Mix/vortex.
  [Whole Blood: Let stand 5 minutes.]
- Centrifuge for 10 minutes at 2000 rpm and discard pellet.
- Add 2mL of 100mM phosphate buffer (pH 6.0). Mix/vortex.
- Sample pH should be 6.0±0.5.
- Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate.

2. Tube Conditioning

- Rinse with 1 x 3mL CH3OH. Aspirate.
- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.
  NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1mL/minute.

4. Tube Wash

- Rinse with 1 x 2mL DI H2O. Aspirate.
- Rinse with 1 x 2mL 100mM HCl. Aspirate.
- Rinse with 1 x 3mL CH3OH. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).

5. Elution

- Elute with 1 x 3mL CH2Cl2/IPA/NH4OH (78:20:2); collect eluate at 1-2mL/minute.
  NOTE: Prepare elution solvent fresh daily.
  [To 40mL of isopropanol, add 4mL of concentrated ammonium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]
- Evaporate to dryness at <40°C.

For GC or GC/MS Analysis:

- Add 50µL ethyl acetate and 50µL BSTFA (with 1% TMCS).
- Blanket with N2 and cap. Mix/vortex.
- React 20 minutes at 70°C. Remove from heat source to cool.
  NOTE: Do not evaporate BSTFA solution.
- Inject 1-3µL of sample (in BSTFA solution) into chromatograph.
- Monitor the following ions:

Cocaine: 182**, 198, 303

Cocaine-d3: 185**, 201, 306

TMS-Benzoylecgonine: 240**, 256, 361

TMS-Benzoylecgonine-d3: 243**, 259, 364

* Suggested internal standards for GC/MS: cocaine-d3, benzoylecgonine-d3

** Quantitation ion

Cocaine and Benzoylecgonine (HPLC)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Serum, Plasma or Whole Blood:

- To 1mL of serum or plasma, add internal standard(s) and 4mL of DI H2O (pH 5.0-7.0).
- Mix/vortex. [Whole Blood: Let stand 5 minutes.]
- Centrifuge for 10 minutes at 2000 rpm and discard pellet.
- Add 2mL of 100mM phosphate buffer (pH 6.0). Mix/vortex.
- Sample pH should be 6.0±0.5.
- Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate.

2. Tube Conditioning

- Rinse with 1 x 3mL CH3OH. Aspirate.
- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.

  NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1-2mL/minute.

4. Tube Wash

- Rinse with 1 x 2mL DI H2O. Aspirate.
- Rinse with 1 x 2mL 100mM HCl. Aspirate.
- Rinse with 1 x 3mL CH3OH. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).

5. Elution (Choose Method A or B)

Method A:

- Elute with 1 x 3mL CH2Cl2/IPA/NH4OH (78:20:2). Collect eluate at 1 2mL/minute.

  NOTE: Prepare elution solvent fresh daily.

  [To 40mL of isopropanol, add 4mL of concentrated ammonium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]

Method B:

- Elute with 1 x 2mL CH3OH/NH4OH (98:2). Collect eluate at 1-2mL/minute.

  NOTE: Prepare elution solvent daily.

  [Add 3 ml DI H2O and 500µl CH2Cl2 to eluate. Mix/vortex 10 seconds. Centrifuge if necessary to separate layers. Aspirate and discard aqueous (upper) layer.]

For HPLC Analysis:

- Evaporate to dryness at <40°C.
- Reconstitute in mobile phase and inject into chromatograph.

Cocaine and Benzoylecgonine (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Meconium:

- To 0.5-1g of meconium add 2mL of CH3OH. Mix/vortex.
- Centrifuge and transfer the supernatant to a clean tube.
- To each tube add 3mL 100mM phosphate buffer (pH 3.0), internal standard and vortex.
- Matrix must be more aqueous than organic for good retention to occur.

2. Tube Conditioning

- Rinse with 2 x 3mL CH3OH. Aspirate.
- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 3mL 100mM phosphate buffer (pH 3.0). Aspirate.

NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

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

4. Tube Wash

- Rinse with 1 x 1mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM HCl. Aspirate.
- Rinse with 1 x 3mL CH3OH. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).

5. Elution

- Elute with 1 x 3mL CH2Cl2/IPA/NH4OH (78:20:2). Collect eluate at 1-2mL/minute.

NOTE: Prepare elution solvent fresh daily.
[To 40mL of isopropanol, add 4mL of concentrated ammonium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]

- Evaporate the elution solvent to dryness without heating.

For GC or GC/MS Analysis:

-- Add 50µL ethyl acetate and 50µL BSTFA (with 1% TMCS).

- Blanket with N2 and cap. Mix/vortex.
- React 20 minutes at 70°C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

- Inject 1-3µL of sample into the chromatograph.
- Monitor the following ions (GC/MS):

Cocaine - 182**, 198, 303

Cocaine-d3 - 185**, 201, 306

TMS-Benzoylecgonine - 240**, 256, 361

TMS-Benzoylecgonine-d3 - 243**, 259, 364

* Suggested internal standards for GC/MS: cocaine-d3 and benzoylecgonine-d3

** Quantitation ion

Cocaine and Benzoylecgonine (HPLC)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Meconium:

- To 0.5-1g of meconium add 2mL of CH_3OH. Mix/vortex.
- Centrifuge and transfer the supernatant to a clean tube.
- Add 3mL 100mM phosphate buffer (pH 3.0), internal standard and vortex.
- Matrix must be more aqueous than organic for good retention to occur.

2. Tube Conditioning

- Rinse with 2 x 3mL CH_3OH. Aspirate.
- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 3mL 100mM phosphate buffer (pH 3.0). Aspirate.

  NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

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

4. Tube Wash

- Rinse with 1 x 1mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM HCl. Aspirate.
- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).

5. Elution

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

  NOTE: Prepare elution solvent fresh daily.

  [To 40mL of isopropanol, add 4mL of concentrated ammonium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]
- Evaporate the elution solvent to dryness without heating.

For HPLC Analysis:

- Reconstitute with 100µL methanol.
- Inject 20µL into chromatograph.

Fentanyl and Analogs (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Urine:

- To 5mL of sample add internal standard* and 2mL of 100mM phosphate buffer (pH 6.0).
- Mix/vortex. Sample pH should be 6.0±0.5.
- Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate.

2. Tube Conditioning

- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.

NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1-2mL/minute.

4. Tube Wash

- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM acetic acid. Aspirate.
- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).

5. Elution

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

NOTE: Prepare elution solvent fresh daily.

[To 40mL of isopropanol, add 4mL of concentrated ammonium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]

- Evaporate to dryness at <40°C.
- Reconstitute with 50µL ethyl acetate.

For GC or GC/MS Analysis:

- Inject 1-3µL of sample into chromatograph.
- Monitor the following ions (GC/MS):

Fentanyl: 245**, 146, 189
Fentanyl-d5: 250**, 151, 194
α-Methylfentanyl: 259**, 203, 146
p-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: fentanyl-d5

** Quantitation ion

Fluoxetine and Norfluoxetine (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Serum, Plasma or Whole Blood:

- To 1mL of sample add internal standard* and 4mL DI H2O.
- Add 2mL of 100mM 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 100mM monobasic or dibasic sodium phosphate.

2. Tube Conditioning

- Rinse with 1 x 3mL CH3OH. Aspirate.
- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.

  NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1-2mL/minute.

4. Tube Wash

- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM acetic acid. Aspirate.
- Rinse with 1 x 3mL CH3OH. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).

5. Elution

- Elute with 1 x 3mL CH2Cl2/IPA/NH4OH (78:20:2). Collect eluate at 1-2mL/minute.

  NOTE: Prepare elution solvent fresh daily.

  [To 40mL of isopropanol, add 4mL of concentrated ammonium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]
- Evaporate to dryness at <40ºC.

For GC or GC/MS Analysis:

- Add 100µL of ethyl acetate and 50µL of PFPA.
- Blanket with N2 and cap. Mix/vortex.
- React for 30 minutes at 90ºC.
- Evaporate to dryness at <40ºC.
- Reconstitute with 200µL of ethyl acetate.
- Inject 2µL into chromatograph.
- Monitor the following ions (GC/MS):

  Fluoxetine: 90**, 117, 294

  Norfluoxetine: 117**, 176, 280

  Protriptyline: 191**, 409

* Suggested internal standard for GC/MS: Protriptyline

** Quantitation ion

LSD (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Urine:

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

Serum, Plasma, or Whole Blood:

- To 1mL of serum, plasma, or whole blood add 4mL DI H2O and internal standard*.
- Mix/vortex and let stand 5 minutes.
- Centrifuge for 10 minutes at 2000 rpm and discard pellet.
- Add 2mL 100mM phosphate buffer (pH 6.0).
- Mix/vortex. Sample pH should be 6.0±0.5.
- Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate.

2. Tube Conditioning

- Rinse with 1 x 3mL CH3OH. Aspirate.
- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.

  NOTE: Aspirate at ≤ 3 inches Hg. to prevent packing bed from drying.

3. Sample Loading

- Load at 1-2mL/minute.

4. Tube Wash

- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM acetic acid. Aspirate.
- Rinse with 1 x 3mL CH3OH. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).

5. Elution

- Elute with 1 x 3mL CH2Cl2/IPA/NH4OH (78:20:2). Collect eluate at 1-2mL/minute.

  NOTE: Prepare elution solvent fresh daily.

  [To 40mL of isopropanol, add 4mL of concentrated ammonium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]
- Evaporate to dryness at <40° C.

For GC or GC/MS Analysis:

- Add 20µL ethyl acetate and 20µL BSTFA (with 1% TMCS).
- Blanket with N2 and cap. Mix/vortex.
- React 20 minutes at 70°C. Remove from heat source to cool.

  NOTE: Do not evaporate BSTFA solution.
- Inject 1-3µL of sample into chromatograph.
- Monitor the following ions (GC/MS):

  LSD: 395**, 293, 268

  LSD-d3: 398**, 296, 271

* Suggested internal standard for GC/MS: LSD-d3

** Quantitation ion

Meperidine (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Urine:

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

2. Tube Conditioning

- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.

  NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1-2mL/minute.

4. Tube Wash

- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM acetic acid. Aspirate.
- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).

5. Elution

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

  NOTE: Prepare elution solvent fresh daily.

  [To 40mL of isopropanol, add 4mL of concentrated ammonium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]
- Evaporate to dryness at <40°C. Remove immediately upon completion.
- Reconstitute with 100µL ethyl acetate.

For GC or GC/MS Analysis:

- Inject 1-3µL into chromatograph.
- Monitor the following ions (GC/MS):

  Meperidine: 247**, 218, 172

  Phenyltoloxamine: 58**

* Suggested internal standard for GC/MS: Phenyltoloxamine

** Quantitation ion

Methadone (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Urine:

- To 5mL of urine add internal standard(s)* and 2mL of 100mM phosphate buffer (pH 6.0).
- Mix/vortex. Sample pH should be 6.0±0.5.
- Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate.

2. Tube Conditioning

- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.

NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1mL/minute.

4. Tube Wash

- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM acetic acid. Aspirate.
- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).

5. Elution

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

NOTE: Prepare elution solvent fresh daily.
[To 40mL of isopropanol, add 4mL of concentrated ammonium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]

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

For GC or GC/MS Analysis:

Inject 1-3µL into chromatograph.

- Monitor the following ions (GCMS):

Methadone: 72**, 91, 165

Methadone-d3: 75**, 94, 168

Phenyltoloxamine-: 58**

* Suggested internal standard for GC/MS: methadone-d3 or phenyltoloxamine

** Quantitation ion

Methaqualone (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Urine:

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

2. Tube Conditioning

- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.

NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1mL/minute.

4. Tube Wash

- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).
- Rinse with 1 x 2mL hexane. Aspirate.

5. Elution

- Elute with 1 x 3mL hexane/ethyl acetate (50:50). Collect eluate at 1-2mL/minute.
- Evaporate to dryness at <40°C.
- Reconstitute with 100µL ethyl acetate.

For GC or GC/MS Analysis:

- Inject 1-3µL into chromatograph.
- Monitor the following ions (GC/MS):

Methaqualone - 235 **, 250, 233

Hexobarbital - 221**, 157, 156

* Suggested internal standard for GC/MS: hexobarbital

** Quantitation ion

6-Monoacetylmorphine (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Urine:

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

2. Tube Conditioning

- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.

NOTE: Aspirate at ≤ 3 inches Hg. to prevent packing bed from drying.

3. Sample Loading

- Load at 1mL/minute.

4. Tube Wash

- Rinse with 1 x 2mL DI H_2O. Aspirate.
- Rinse with 1 x 2mL 100mM acetate buffer (pH 4.5). Aspirate.
- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).

5. Elution

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

NOTE: Prepare elution solvent fresh daily.

[To 40mL of isopropanol, add 4mL of concentrated ammonium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]

- Evaporate to dryness at <40°C.

For GC or GC/MS Analysis:

- Add 50µL ethyl acetate and 50µL BSTFA (with 1% TMCS).
- Blanket 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.

- Inject 1-3µL sample (in BSTFA solution) into chromatograph.
- Monitor the following ions (GC/MS):

TMS-6-Monoacetylmorphine: 399**, 340, 287

TMS-6-Monoacetylmorphine-d3: 402**, 343, 290

* Suggested internal standard for GC/MS: 6-monoacetylmorphine-d3

** Quantitation ion

Opiates (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Urine: (Choose Method A or B)

A. Enzymatic Hydrolysis of Glucuronide:

- To 5mL of urine add internal standard(s)* and 2mL of β-glucuronidase.
  [β-Glucuronidase: 5,000 F units/mL patella vulgata in 1.0M 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± 05 with approximately 700µL of 1.0N NaOH.

B. Acid/Autoclave Hydrolysis of Glucuronide:

- To 5mL of urine add internal standard(s)* and 500µL concentrated HCl. Mix/vortex.
- Autoclave for 20 minutes at 121°C. Cool before proceeding
- Centrifuge for 10 minutes at 2000 rpm and discard pellet. Add 1000µL 7.4M NH4OH.
- Mix/vortex. Adjust sample pH to 6.0± 0.5 with 1-3mL 500mM phosphoric acid.

Serum, Plasma or Whole Blood: [Free (Unbound) Opiates]

- To 1mL of sample add internal standard(s)* and 4mL of DI H2O (pH 5.0-7.0).
  [For whole blood matrix: Mix/vortex and let stand 5 minutes.]
- Centrifuge for 10 minutes at 2000 rpm and discard pellet.
- Add 2mL of 100mM phosphate buffer (pH 6.0). Mix/vortex.
- Sample pH should be 6.0±0.5.
- Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate.

2. Tube Conditioning

- Rinse with 1 x 3mL CH3OH. Aspirate.
- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.

NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load into tube at 1mL/minute.

4. Tube Wash

- Rinse with 1 x 2mL DI H2O. Aspirate.
- Rinse with 1 x 2mL 100mM acetate buffer (pH 4.5). Aspirate.
- Rinse with 1 x 3mL CH3OH. Aspirate.
- Dry tube (5 minutes at ≥ 10 inches Hg).

5. Elution

- Elute with 1 x 3mL CH2Cl2/IPA/NH4OH (78:20:2). Collect eluate at 1-2mL/minute.
  NOTE: Prepare elution solvent fresh daily.
  [To 40mL of isopropanol, add 4mL of concentrated ammonium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]
- Evaporate to dryness at <40°C.

For GC or GC/MS Analysis:

- Add 50µL ethyl acetate and 50µL BSTFA (with 1% TMCS).
- Blanket with N2 and cap. Mix/vortex.
- React 20 minutes at 70°C. Remove from heat source to cool.
  NOTE: Do not evaporate BSTFA solution.
- Inject 1-3µL of the eluate into chromatograph.
- Monitor the following ions (GC/MS):

TMS-Codeine: 371**, 234, 343

TMS-Morphine: 429**, 287, 324

TMS-Codeine-d3: 374**, 237, 346

TMS-Morphine-d3: 432**, 290, 327

* Suggested internal standards: codeine-d3 and morphine-d3

** Quantitation ion

Phencyclidine (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Urine:

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

2. Tube Conditioning

- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.

NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1-2mL/minute.

4. Tube Wash

- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM acetic acid. Aspirate.
- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).

5. Elution

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

NOTE: Prepare elution solvent fresh daily.
[To 40mL of isopropanol, add 4mL of concentrated ammonium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]

- Evaporate to dryness at <40°C. Remove immediately upon completion.
- Reconstitute with 100µL ethyl acetate.

For GC or GC/MS Analysis:

- Inject 1-3µL into chromatograph.
- Monitor the following ions (GC/MS):

Phencyclidine: 200**, 91, 242

Phencyclidine-d5: 205**, 96, 247

* Suggested internal standards: GC/MS: phencyclidine-d5; GC: ketamine

** Quantitation ion

Propoxyphene (GC, GC/MS)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Urine:

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

2. Tube Conditioning

- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.

NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1-2mL/minute.

4. Tube Wash

- Rinse with 1 x 3mL DI H_2O. Aspirate.
- Rinse with 1 x 1mL 100mM acetic acid. Aspirate.
- Rinse with 1 x 3mL CH_3OH. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).

5. Elution

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

NOTE: Prepare elution solvent fresh daily.

[To 40mL of isopropanol, add 4mL of concentrated ammonium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]

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

For GC or GC/MS Analysis:

- Inject 1-3µL into chromatograph.
- Monitor the following ions (GC/MS):

Propoxyphene: 58**, 115, 208

* Suggested internal standard for GC/MS: propoxyphene-d5

** Quantitation ion

Sertraline and Norsertraline (HPLC)

Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk (Part No. 8604527)

1. Sample Pretreatment

Serum, Plasma, or Whole Blood:

- To 1mL of serum add internal standard, 4mL DI H2O and 2mL of 100mM 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 100mM monobasic or dibasic sodium phosphate.

2. Tube Conditioning

- Rinse with 1 x 3mL CH3OH. Aspirate.
- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM phosphate buffer (pH 6.0). Aspirate.

  NOTE: Aspirate at ≤ 3 inches Hg to prevent packing bed from drying.

3. Sample Loading

- Load at 1-2mL/minute.

4. Tube Wash

- Rinse with 1 x 3mL DI H2O. Aspirate.
- Rinse with 1 x 1mL 100mM acetic acid. Aspirate.
- Rinse with 1 x 3mL CH3OH. Aspirate.
- Dry column (5 minutes at ≥ 10 inches Hg).

5. Elution

- Elute with 1 x 3mL CH2Cl2/IPA/NH4OH (78:20:2). Collect eluate at 1-2mL/minute.

  NOTE: Prepare elution solvent fresh daily.

  [To 40mL of isopropanol, add 4mL of concentrated ammonium hydroxide. Mix. Add 156mL of methylene chloride. Mix.]
- Evaporate to dryness at <40°C

For HPLC Analysis:

- Reconstitute with 200µL acetonitrile/DI H2O (1:3).
- Mix/vortex vigorously for 30 seconds.
- Inject 100µL into chromatograph.

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