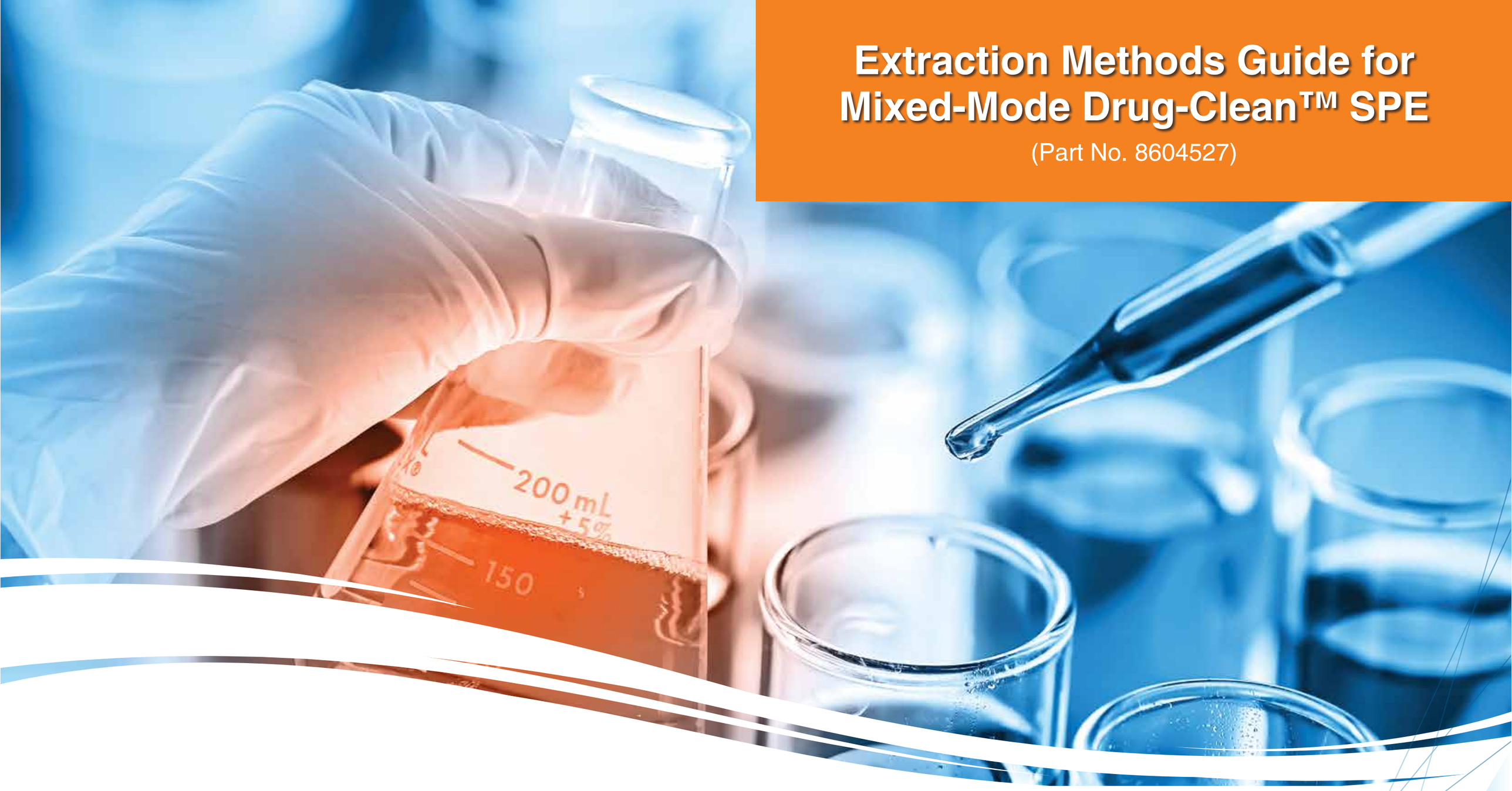
Extraction Methods Guide for Mixed-Mode Drug-Clean™ SPE

(Part No. 8604527)



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Drug-Clean™ SPE Extraction Methods

Ordering Information

Product Code	Description
8604527	Extract-Clean™ SPE Drug-Clean™ C 200mg, 4.0ml. 50pk, P/N: 207010
5122302	Extract-Clean™ SPE Drug-Clean™ C 100mg, 1.5ml. 100pk, P/N: 207015
5122303	Extract-Clean™ SPE Drug-Clean™ C 500mg, 4.0ml. 50pk, P/N: 207017
5122304	Extract-Clean™ SPE Drug-Clean™ C 500mg, 8.0ml. 30pk, P/N: 207019
5122305	Extract-Clean™ SPE Drug-Clean™ A 100mg, 1.5ml. 100pk, P/N: 207030
5122306	Extract-Clean™ SPE Drug-Clean™ A 200mg, 4.0ml. 50pk, P/N: 207034
5122515	Extract-Clean™ SPE Drug-Clean™ PB, 30mg, 1.5ml 100pk, P/N: 250120
5122516	Extract-Clean™ SPE Drug-Clean™ PB, 30mg, 4.0ml 100pk, P/N: 250130
5122517	Extract-Clean™ SPE Drug-Clean™ PB, 50mg, 8.0ml 50pk, P/N: 250140

Other SPE Product Lines

Name	Format	Sizes	Summary
Extract-Clean™ Columns	SPE Columns	1.5, 4, 8, 15, 25, 75ml (the entire tube volume)	In production for over 25 years, with proven consistency, this is our most comprehensive SPE product line. It includes 30 media types in over 10 different bed weights. And with a complete offering of reserved normal, and specialty medias exhibiting unique retention properties, you are sure to find the packing that delivers a cleaner, more concentrated sample
Maxi-Clean™ Catridges	SPE Catridges	300, 600, 900mg (media amount, not device volume)	The Maxi-Clean™ line is offered in many of the same media as the Extract-Clean™ line, but slightly paired down, with over 20 chemistries available. This lure hub catridge devices is not as prevalent in the SPE industry, and while manual processing is most common, this format offers number of other interesting processing option, including multimedia extractions
SEClute™ Columns	SPE Columns	1, 3, 6 and 20ml (the entire tube volume)	SEClute™ offers the best values in our SPE range, with an offering of 15 sorbents in six bed weights. Our latest SEClute™ range includes the HLB & Mixed Mode Polymeric SPE features high surface area and pH stability for reproducible recoveries for a wide range of analyses.
Ultra-Clean™ Columns	SPE Columns	4, 8ml (the entire tube volume)	Choose this ultra-low extractable version for very sensitive applications. Nine selected media are packed into highly inert fluorinted polypropylene tubes with PTFE frits. Less expensive than glass extraction devices, this durable format offers comparable inertness without the added concern of being easily broken.
Vydac® Columns	SPE Columns	1, 3ml (volume above the packing)	Ideal for extraction, concentration and cleanup of biological sample. This 300A silica-based media has the same properties as the industry-leading Vydac® TP HPLC packing. Offered in C18 and C4,use for a variety of protein and peptide applications.

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 <40ºC.
- 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 <40º 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 <40ºC.
- 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 <40ºC.
- 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.

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 <40ºC

For HPLC Analysis:

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

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 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.
- 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

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 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.]

- Add 30µL silylation grade DMF to eluate.
- Evaporate to 30µL at <40ºC.
- Add 50µL PFPA (PFAA). Blanket with N2 and cap.
- React 20 minutes at 70ºC. Evaporate to dryness at <40ºC.
- 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 CH3OH. Aspirate.
- Rinse with 1 x 3mL DI H2O. 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) CH3OH in DI H2O. 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 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.]
- B. Elute with 1 x 3mL CH2Cl2/IPA (80:20)
- C. Elute with 1 x 3mL ethyl acetate
- D. Elute with 1 x 3mL CH3OH
- Evaporate to dryness at <40ºC.

For GC or GC/MS Analysis:

- Add 50µL ethyl acetate and 50µL MSTFA (with 3% trimethylsilyliodide).
- Blanket with N2 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

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 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. 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

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 -glucuronidase.
  [-Glucuronidase: 5,000 F units/mL patella vulgata in 1.0M 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± 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 121ºC. 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 <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 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

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).

- 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:

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 70ºC.
- Evaporate to dryness at < 40ºC. 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 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 1mL/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 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 <40ºC.

For HPLC Analysis:

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

*Suggested internal standards: Trimipramine and Protriptyline

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 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 acetate buffer (pH 4.5). 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 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 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

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 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 3mL DI H2O. 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 <40ºC.
- 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

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 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 100rnM HCl. Aspirate.
- Rinse with 1 x 3mL CH3OH. Aspirate.
- Dry tube (5 minutes at ≥ 10 inches Hg).

5. Elution

- 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.]

- To eluate add 2.0mL DI H2O and 500µL CH2Cl2.
- Mix/vortex. Centrifuge.
- Transfer organic (lower) layer to a clean tube.
- Evaporate to dryness at <40ºC.

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 <40ºC.
- 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

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 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 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.
- 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

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 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. 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

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
β–Hydroxytriazolam (TMS): 415**, 417, 430
Diazepam 256**, 283, 221
β–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 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 HPLC Analysis:

- Reconstitute in mobile phase and inject into chromatograph.

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 <40º 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 70ºC. 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

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

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

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 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.
- 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
-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

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 CH3OH. 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 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 HPLC Analysis:

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

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 <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 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 70ºC. 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

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