8.7  BENZOYLECGONINE PROCEDURE

8.7.1  Purpose
To qualitatively and/or quantitatively identify benzoylecgonine (BE) in submitted evidence using solid phase extraction followed by instrumental analysis with gas chromatography/mass spectrometry (GC/MS).

8.7.2  Specimen Requirements
Samples for analysis will be presumptively positive for cocaine metabolite. Acceptable samples for this analysis include blood and urine. For additional samples see Alternative Matrices (section 6.6).

8.7.3  Apparatus and Equipment
Disposable 10 mL culture tubes
Volumetric pipettes and disposable tips
Assorted volumetric glassware
Disposable transfer pipettes
Sample mixer
Centrifuge
Evaporation station
Solid phase columns (Bond Elute SCX)
Solid phase extraction apparatus
11 mm autosampler vials, inserts, and caps
11 mm crimper
GC/MS, ChemStation software, compatible computer, and printer

8.7.4  Reagents and Standards
Negative blood, urine, or other matrix as needed
Benzoylecgonine certified reference standard
Benzoylecgonine-D₃ certified reference standard (internal standard)
Water (H₂O)
5% Tri-chloroacetic acid (TCA)
Methanol (CH₃OH)
12 mM Hydrochloric acid (HCl)
12 mM Phosphoric acid (H₃PO₄)
70 mM Glacial acetic acid (GAA) (CH₃COOH)
Isopropanol (2-propanol) ((CH₃)₂CHOH)
Acetonitrile (ACN) (CH₃CN)
Ammonium hydroxide (NH₄OH)
Elution solution (2% triethylamine, 2% NH₄OH, 20% isopropanol, 76% methylene chloride)
8.7.5 Standard and Reagent Preparation

The following are examples of how to prepare the standards and reagents used in this procedure.

8.7.5.1 Standards

**BE Stock Reference Standard Solution [10,000 ng/mL] (A & B)**
Pipette 500 μL of benzoylcegonine [1 mg/mL] certified reference standard and dilute to 50 mL with H₂O.

**BE Stock Reference Standard Solution [1,000 ng/mL] (A)**
Pipette 10 mL of 10,000 ng/mL BE stock reference standard solution (A) and dilute to 100 mL with H₂O.

**BE-D₃ Reference Standard Solution [10,000 ng/mL] (Internal Standard)**
Pipette 500 μL of benzoylcegonine [1 mg/mL] certified reference standard and dilute to 50 mL with H₂O.

**BE Working Reference Calibrator/Control Standard Solutions**
To make the working reference calibrator/control standard solutions, add the following amounts to a final volume of 1.5 mL with blood or urine.

<table>
<thead>
<tr>
<th>CONCENTRATION</th>
<th>AMOUNT USED</th>
<th>STD SOLUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 ng/mL</td>
<td>75 μL</td>
<td>1,000 ng/mL</td>
</tr>
<tr>
<td>100 ng/mL</td>
<td>150 μL</td>
<td>1,000 ng/mL</td>
</tr>
<tr>
<td>250 ng/mL</td>
<td>375 μL</td>
<td>10,000 ng/mL</td>
</tr>
<tr>
<td>500 ng/mL</td>
<td>75 μL</td>
<td>10,000 ng/mL</td>
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<tr>
<td>1000 ng/mL</td>
<td>150 μL</td>
<td>10,000 ng/mL</td>
</tr>
<tr>
<td>2500 ng/mL</td>
<td>375 μL</td>
<td>10,000 ng/mL</td>
</tr>
<tr>
<td>5000 ng/mL</td>
<td>750 μL</td>
<td>10,000 ng/mL</td>
</tr>
</tbody>
</table>

8.7.5.2 Prepared Reagents

**BE Elution Solution**
Combine 76 mL of methylene chloride, 20 mL isopropanol, 2 mL triethylamine, and 2 mL NH₄OH.
Note: This solution will expire 24 hours after preparation.
5% Tri-chloroacetic acid (TCA)
Dissolve 25 g of TCA and dilute to 500 mL with H₂O (also available in a pre-made 5% solution).

12 mM HCl
Add 983.6 μL HCl to H₂O and dilute to 1000 mL.

12 mM H₃PO₄
Add 807.5 μL H₃PO₄ to H₂O and dilute to 1000 mL.

70 mM GAA
Add 4.02 mL GAA to H₂O and dilute to 1000 mL.

8.7.6 Procedure
1. Allow all reference standards and case samples to equilibrate to room temperature before beginning procedure.
2. Label, check, and load/unload all samples in accordance with the “Sample Pipetting Check List” (see Appendix section).
3. Prepare working reference calibrator and/or control standards from stock reference standards. See example above.
4. Pipette 1.5 mL of corresponding case sample, calibrator, positive control, or negative control into the appropriately labeled 10 mL culture tube. Note: Smaller sample volumes may be analyzed on a case-by-case basis.
5. Pipette 37.5 μL of internal standard into each sample to make a final concentration of 250 ng/mL.
6. Add 4 mL of 5% TCA, mix for approximately 90 seconds, and centrifuge until separated (approximately 20 minutes).
7. Transfer the supernatant into another clean, labeled 10 mL culture tube (optional).
8. Activate the column with the following reagents in the following order:
   3 mL of methanol
   3 mL of 12 mM HCl
9. Add the supernatant to each column slowly (approximately 5-10 psi).
10. Wash the column with the following reagents in order:
    1 mL of 12 mM HCl
    2 mL of 12 mM H₃PO₄
    2 mL of 70 mM GAA
    3 mL isopropanol
11. Allow the column sorbent to dry completely by allowing flow through the solid phase extraction apparatus for approximately 2 minutes at the highest setting or by changing to “full flow/dry” setting (if available).
12. Switch the solid phase extraction apparatus to the collection tubes.
13. Add 2 mL of elution solution and allow samples to elute slowly (gravity may initially be sufficient, but should be followed by vacuum/pressure).
14. Evaporate the sample to dryness with a dry gas (e.g. nitrogen) in an evaporation station without heat.
15. Reconstitute the residue with 50 µL of acetonitrile and 50 µL of BSTFA+TMCS, mix, transfer to an 11 mm autosampler vial with insert, and seal with cap.
16. Derivatize by heating at approximately 90° for approximately 20 minutes.
17. Analyze and quantitate the samples by GC/MS (selected ion monitoring mode).

8.7.7 Reporting

Results can be reported if the following criteria are met:

8.7.7.1 Ions 240, 243, 256, 259, 361, and 364 are present in the sample and used calibration standards and control(s).

8.7.7.2 Ion ratios for the sample must fall within ±20% of those of a calibrator or control standard of similar concentration.

8.7.7.3 The retention times of the 361 (benzoylecgonine) and 364 (benzoylecgonine D₃) ions in the sample are within ±1% of those of a calibrator or control standard of similar concentration.

8.7.7.4 Benzoylecgonine concentrations below 50 ng/mL (the lowest calibration point) shall be reported as “not detected”.

8.7.7.5 Benzoylecgonine concentrations greater than the highest calibration point where the results are necessary for interpretation in the case shall be handled using the following methods:
- Reanalyze using smaller sample amounts (including dilutions).
- Reanalyze using higher standard concentrations.

8.7.7.6 Benzoylecgonine concentrations greater than the highest calibration point where the results are not necessary for interpretation in the case may be reported as “greater than…” the highest calibration point.

8.7.7.7 Results shall be reported in ng/mL and truncated to the whole number.

8.7.7.8 When a definitive conclusion cannot be made, the reason shall be documented on the report (e.g., “insufficient sample for analysis”, “sample unsuitable for analysis”, “results are inconclusive due to sample condition”, etc.).

8.7.8 References