8.12 COCAINE/COCAINE METABOLITES AND GABAPENTIN PROCEDURE (VIA LC/MS/MS)

8.12.1 Purpose

To qualitatively identify and confirm the presence of cocaine, its metabolites (cocaethylene and benzoylecognine), and gabapentin, in submitted evidence using a solid phase extraction column followed by instrumental analysis with liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS).

8.12.2 Specimen Requirements

Samples for this analysis shall be presumptively positive or be suspected to contain a relevant drug per case circumstances. Acceptable samples for analysis include blood and urine. For additional samples see Alternative Matrices (section 6.6).

Samples to be reported as not detected or that have previously screened positive by ELISA shall only require one sample extraction.

8.12.3 Apparatus and Equipment

UCT Clean Screen DAU solid phase extraction (SPE) columns
Disposable 10 mL culture tubes
Volumetric pipettes and disposable tips
Assorted volumetric glassware
Disposable transfer pipettes
Sample mixer
Centrifuge
Evaporation station
Positive pressure manifold
11 mm autosampler vials, inserts, and caps
11 mm crimper
LC/MS/MS, Analyst and/or Cliquid software, compatible computer, and printer

8.12.4 Reagents and Standards

Negative blood/ urine or other matrix as needed
Cocaine-D3, Gabapentin-D4 certified reference standard (internal standards)
Cocaine, cocaethylene, benzoylecognine, gabapentin certified reference standards
UCT Phosphate Buffer Pouches
100mM Phosphate Buffer
DI Water (H_2O)
Methanol (CH_3OH)
Methanol: water 1:1
Isopropanol (IPA) ($(CH_3)_2CHOH$))
Glacial Acetic Acid (GAA)(CH_3COOH)
Ammonium hydroxide (NH_4OH)

Dichloromethane (Methylene chloride) (CH_2OCl_2)
Acetonitrile (ACN) (CH_3CN)
Hexanes
1 M Ammonium formate (NH_4HCO_2)
Formic acid (CHO_2H)
Elution Solution ($(CH_3)_2CHOH$)/NH_4OH/CH_2Cl_2)
Mobile phase A (99.6% water, 0.2% 1 M ammonium formate, 0.2% formic acid)
Mobile phase B (97.6% HPLC suitable methanol, 2% water, 0.2% 1 M ammonium formate, 0.2% formic acid)
Reconstitution Solution (Mobile Phase A: Mobile Phase B 80:20)

8.12.5 Standard and Reagent Preparation

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

8.12.5.1 Standards

Combined Reference Standard Solution
[1000 ng/mL cocaine, cocaethylene; 2000 ng/mL benzoylecognine]
[100 μg/mL gabapentin]

Pipette the following amounts of [1 mg/mL] certified reference standard

- 50 μL cocaine
- 50 μL cocaethylene
- 100 μL benzoylecognine

Add 5 mL [1 mg/mL] gabapentin certified reference standard and dilute to 50 mL with acetonitrile

Combined Internal Standard Stock Reference Standard Solution
[2000 ng/mL cocaine-d3] [60 μg/mL gabapentin-d4]

Pipette 100 μL cocaine-d3 [1 mg/mL] certified reference standard and 3 mL gabapentin-d4 [1.0 mg/mL] certified reference standard solution and dilute to 50 mL with acetonitrile.

Note: All reference standard solutions expire 6 months from the preparation date and shall be stored in the freezer when not in use.

Working Reference Standard Solutions
To make the working reference standard solutions, add the following amounts and **dilute to 1 mL** with 50:50 negative blood:DI water or negative neat urine (Standards shall be matrix matched)

CONCENTRATION		AMOUNT USED	STD SOLUTION	MATRIX
Cocaine/ cocaethylene Benzoylecognine Gabapentin	10 ng/mL* 20 ng/mL* 1 ug/mL*	10 µL	Combined Stock Reference Solution	990 µL
Cocaine/ cocaethylene Benzoylecognine Gabapentin	100 ng/mL 200 ng/mL 10 ug/mL	100 µL	Combined Stock Reference Solution	900 µL
Cocaine/ cocaethylene Benzoylecognine Gabapentin	250 ng/mL 500 ng/mL 25 ug/mL	250 µL	Combined Stock Reference Solution	750 µL

*Limit of Detection necessary to determine sample result

8.12.5.2 Prepared Reagents

Methanol/Water 1:1 (Needle Rinse)
Add 500 mL of methanol to a volumetric flask and dilute to 1000 mL with H_2O.

1 M Ammonium Formate
Dissolve 63 g of ammonium formate and dilute to 1000 mL with H_2O.

Mobile Phase A
Add 2 mL 1 M ammonium formate and 2 mL formic acid to H_2O and dilute to 1000 mL with H_2O.

Mobile Phase B
Add 2 mL 1 M ammonium formate and 2 mL formic acid to 20 mL H_2O and dilute to 1000 mL with methanol.

100mM Phosphate Buffer
Fill a 1000 mL volumetric flask half full of DI H_2O. Then, mix 1 pouch of UCT Phosphate Buffer into the flask and bring to a final volume of 1000 mL with H_2O.

100mM Acetic Acid Solution
Add 5.71 mL glacial acetic acid to H_2O and dilute to 1000 mL with H_2O.

Elution Solution
First, thoroughly mix 20 mL isopropanol and 2 mL ammonium hydroxide. Then, add 78 mL dimethyl chloride (methylene chloride) for a total volume of 100 mL of solution. Prepare on day of extraction.

Note: If bubbles develop or separate layers form in the solution after adding the dimethyl chloride, dispose of the solution and remake.

Mobile Phase A: Mobile Phase B 80:20 (Reconstitution Solution)
Add 800 mL of Mobile Phase A to a volumetric flask and dilute to 1000 mL with Mobile phase B.

8.12.6 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 matrix matched working reference calibrator and/or control standards from the combined stock reference standard solutions and vortex briefly. See example above.
4. Pipette 1 mL of corresponding 50:50 negative blood:DI water, negative urine, or case sample, into the appropriately labeled 10 mL culture tube.
   Note: Smaller sample volumes down to 500 µL sample and 500 µL of matrix may be analyzed on a case-by-case basis.
5. Pipette 50 µL of combined internal standard stock reference standard solution, for a final concentration of [100 ng/mL cocaine-d3 and 3 µg/mL gabapentin-d4] into each sample and vortex briefly.
6. Add 3 mL of 100 mM phosphate buffer, mix/ vortex for a minimum of 30 seconds and let stand (incubate) for a minimum of 5 minutes.
7. Centrifuge at maximum rpm for 15 minutes.
   Note: Sample may remix if allowed to sit for a length of time. Centrifuge if this occurs.
8. Using the positive pressure manifold, condition labeled UCT Clean Screen DAU SPE Columns by pushing the following through the sorbent bed at full pressure:
   a. 3 mL methanol
   b. 3 mL 100 mM phosphate buffer
9. Decant the supernatant into the appropriately labeled SPE column and push the sample through the sorbent bed at a rate of 1-2 mL per minute (2-4 psi).
10. Wash the SPE columns by pushing the following through the sorbent bed at full pressure (do not allow the sorbent to dry in between):
    a. 3 mL DI H_2O
    b. 3 mL 100mM acetic acid
    c. 2 mL hexane
11. Dry the SPE columns for a minimum of 20 minutes at full pressure.
12. Switch the solid phase extraction apparatus to clean labeled 10 mL culture tubes.
13. Add 2 mL of the elution solution to each SPE column and allow the samples to elute slowly with a flow rate of approximately 1-2 mL/minute (2-4 psi) pressure.
14. Gently evaporate the samples to dryness with heat approximately 40ºC or less and a dry gas (e.g. nitrogen) in an evaporation station (approximately 10-12 minutes). Allow to cool upon complete dryness.
15. Reconstitute the residue with 100 µL of 80:20 Mobile Phase A to Mobile Phase B, mix/vortex, and centrifuge at the maximum rpm level possible for 10 minutes.

16. Transfer to an 11 mm autosampler vial with insert, attempting to avoid transfer any of the particulate matter in the bottom of the tube, and seal with cap.
17. Analyze the samples by LC/MS/MS. Samples are stable for analysis up to 48 hours after extraction.

8.12.7 Reporting

Results can be reported if the following criteria are met:

8.12.7.1 Qualitative

8.12.7.1.1 Retention times of drugs identified and internal standards must fall within ±2% of an analyzed standard.

Note: The examiner may consider both the peak size and shape when determining retention time.

8.12.7.1.2 The ion ratio for both multiple reactions monitoring (MRM) transitions must fall within ± 20% of an analyzed standard. All 3 reference standard ion ratios are required to generate the ion ratio range for each drug in this analysis.

8.12.7.1.3 Drugs in casework may be reported as “Positive” if the drug response ratio (i.e. area of drug/area of internal standard) is equal to or greater than the drug response ratio of the Limit of Detection standard used in the analysis.

Note 1: Gabapentin shall be stated as “Presumptively positive” and “Please contact the crime lab if the confirmation of Gabapentin is necessary” on the case report.

Note 2: Samples presumptively positive for Acid drugs and specifically confirmed for phenobarbital, may show reduced recovery of cocaine and cocaine-d3. This may affect the result obtained for cocaethylene and benzoylecognine. Consult the Technical Leader for reporting on a case by case basis.

Note 3: Drug concentrations of 500 ng/mL for cocaine, cocaethylene, and benzoylecognine and 120 ug/mL for gabapentin produced no carry over using this procedure.

8.12.7.2 Results

8.12.7.2.1 Any qualitative or retention time report not used in a case shall either be lined through and initialed or all the data used shall be highlighted.

8.12.7.2.2 Samples with drug response ratios below the Limit of Detection standard used in the analysis shall be reported as “No cocaine or cocaine metabolites detected”.

Note: Samples analyzed specifically for gabapentin shall be reported as “Gabapentin presumptively not detected”.

8.12.7.2.3 Qualitative results shall be expressed as “positive” and include any clarifying remarks, if applicable.

8.12.7.2.4 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.).

8.12.8 References

See method validation for extensive bibliography.