COCAINE, BENZOYLECGONINE, AND COCAETHYLENE IN BLOOD, PLASMA/SERUM, URINE AND TISSUE USING:
200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020

LC-MSMS

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH 6) add internal standard.*

   Add 1 mL of whole blood, serum/ plasma, urine, or 1g tissue homogenate (1:4). Add 2 mL of 100 mM phosphate buffer (pH 6).

   Vortex and centrifuge as appropriate.

2. **CONDITION COLUMN:**
   1 x 3 mL CH_3OH

   1 x 3 mL D.I. H_2O

   1 x 1 mL 100 mM phosphate buffer (pH 6).

   Note: aspirate at < 3 inches Hg to prevent sorbent drying out

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**

   1 x 3 mL DI H_2O

   1 x 3 mL 100 mM hydrochloric acid.

   1 x 3 mL CH_3OH

   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE COCAINE, BENZOYLECGONINE, COCAETHYLENE:**

   1 x 3 mL ethyl acetate; acetonitrile: ammonia (78: 20: 2 v/v)

   Or

   1 x 3 mL CH_2Cl_2/ IPA/ ammonia (78:20:2 v/v)

   Collect eluate at 1-2 mL /minute.

6. EVAPORATION:
Evaporate eluates to dryness under a gentle stream of nitrogen.

7. RECONSTITUTE sample in 50 µL of CH_3OH.
Inject 5 µL.

7. INSTRUMENT CONDITIONS:
Column: 50 x 2.1 mm (3 µm) Selectra® Phenyl (UCT, Inc.,)

MOBILE PHASE:

Time	% Acetonitrile	%0.1% Formic acid
0	33	67
5	33	67

Flowrate: 0.35 mL/ minute

Column Temperature: ambient

Detector: API 2000 MS/MS

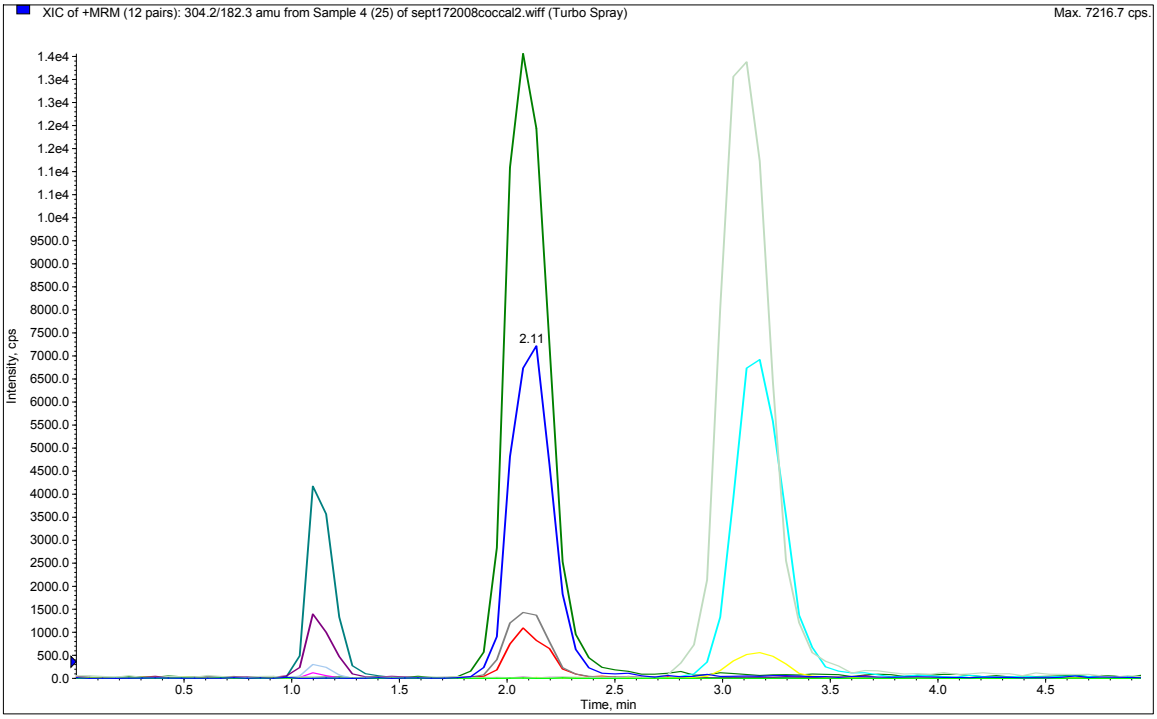
Compound	MRM Transition	Cerilliant #
Cocaine	304.2/ 182.3	C-008
* Cocaine-D3	307.2/ 185.2	C-009
Benzoylecgonine	290.1/168.0	B-004
*Benzoylecgonine-D8	298.2/171.3	B-013
Cocaethylene	318.2/ 196.2	C-010
*Cocaethylene-D8	326.2/204.2	C-024

CHROMATOGRAM SHOWING:

Cocaine/ Cocaine-D3

Benzoylecginine/ Benzoylecgonine-D8

Cocaethylene/ Cocaethylene-D8



Recovery: > 90% (n=10)

LOD: 10 ng/ mL

UCT, Inc. • 2731 Bartram Road • Bristol, PA 19007 • 800.385.3153 • 215.781.9255 • www.unitedchem.com • Email: methods@unitedchem.com
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