



COCAINE AND BENZOYLECGONINE IN BLOOD, PLASMA/SERUM, URINE, TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #

ZSDAU020 – CLEAN SCREEN® DAU 200 mg, 10 mL Tube

SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS

SLDA501D21-5UM - SELECTRA® DA HPLC Column 50 x 2.1 mm, 5µm

1. PREPARE SAMPLE:

To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards

Add 1 -2 mL of blood, plasma/ serum, urine, or 1 g (1:4) tissue homogenate

Mix/vortex and let stand for 5 minutes

Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex

Sample pH should be 6.0 ± 0.5 .

Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

Centrifuge for 10 minutes at 2000 rpm and discard pellet

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN:

1 x 3 mL CH₃OH.

1 x 3 mL D.I. H₂O.

1 x 3 mL 100 mM phosphate buffer (pH 6.0).

NOTE: Aspirate at full vacuum or pressure

3. APPLY SAMPLE:

Load at 1 to 2 mL/minute.

4. WASH COLUMN:

1 x 3 mL D.I. H₂O.

1 x 3 mL 100 mM HCl

1 x 3 mL CH₃OH.

Dry column (10 minutes at full vacuum or pressure).

5. ELUTE COCAINE/METABOLITES:

1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2)

Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12).

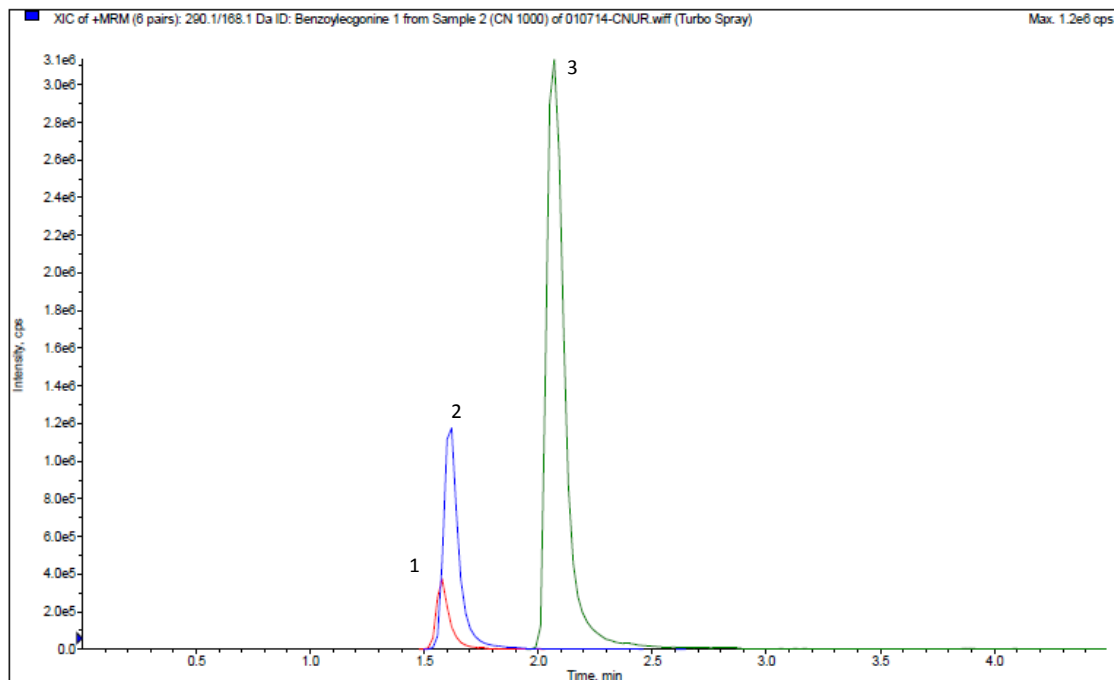
6. DRY ELUATE:

Evaporate to dryness at < 40 °C.

7. RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
Inject 10 µL.
- **GC-MS:** Dissolve residue in 50 µL of Ethyl Acetate and 50 µL BSTFA w/
1%TMCS
Overlay with N₂ and cap. Mix/vortex
React 30 minutes at 70 °C; Cool and inject 1 µL

INSTRUMENT CONDITIONS (LC-MS/MS):



| Analyte | MRM Transitions | | Relative Retention Time (min) |
|-----------------------------------|-----------------|-------|-------------------------------|
| | Q1 | Q3 | |
| 1. Benzoylcegonine D ₈ | 298.1 | 171.1 | 1.58 |
| 2. Benzoylcegonine | 290.1 | 168.1 | 1.60 |
| 3. Cocaine | 304.1 | 182.1 | 2.10 |

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H₂O

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.7 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

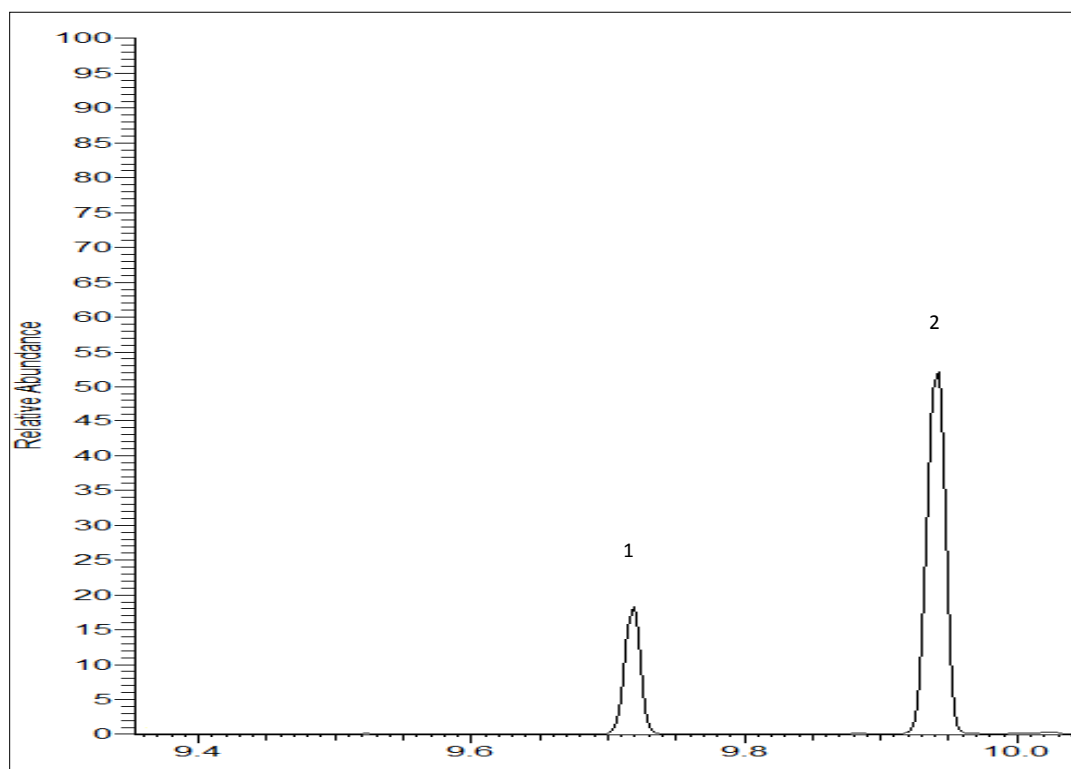
LC Column: Selectra[®] DA HPLC Column 50 x 2.1 mm 5 µm

Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

| Time | %A | %B |
|------|------|----|
| 0.00 | 75 | 25 |
| 3.00 | 50 | 50 |
| 3.01 | 10 | 90 |
| 4.00 | 75 | 25 |
| 5.50 | STOP | |

INSTRUMENT CONDITIONS (GC-MS):



| Analyte | Quantify Ion | Qualifier Ion 1 | Qualifier Ion 2 | Relative Retention Time (min) |
|-----------------------------------|--------------|-----------------|-----------------|-------------------------------|
| 1. Cocaine | 182 | 198 | 303 | 9.72 |
| Cocaine D ₃ | 185 | 201 | 306 | - |
| 2. Benzoyecgonine TMS | 240 | 256 | 361 | 9.94 |
| Benzoyecgonine TMS D ₃ | 243 | 259 | 369 | - |

PARAMETERS

GC/MS: Thermo ISQ Trace 1300

GC capillary column: 30 m x 0.25 mm (0.25 µm) TG-1MS

Injector: 1µL Splitless, 250 °C

Oven temperature program: 70 °C (0.5) to 320 °C (25 °C/ minute): hold (2 minutes)

Carrier gas: Helium (1.2 mL/ minute)

MSD condition: Aux temperature: 280 °C, MS Source: 350 °C, MS Quad: 150 °C