# COCAINE AND BENZOYLECGONINE IN URINE BY LC-MS/MS OR GC-MS STYRE SCREEN® DBX EXTRACTION COLUMN

Part #

SSDBX033 – STYRE SCREEN® DBX 30 mg, 3 mL Tube

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

SLDA50ID21-5UM – SELECTRA® DA HPLC Column 50 x 2.1 mm, 5µm

**1. PREPARE SAMPLE:**

To 1 mL of urine add internal standard(s) and 300 µL 100mM HCl.
Mix/Vortex.

**2. APPLY SAMPLE:**

Load at 1 to 2 mL/minute.

**3. WASH COLUMN:**

1 x 1 mL D.I. $H_2O$.
1 x 1 mL 100 mM HCl
1 x 1 mL $CH_3OH$.
Dry column (10 minutes at full vacuum or pressure).

**4. ELUTE COCAINE/METABOLITE:**

2 x 0.5 mL $CH_2Cl_2$/ IPA /$NH_4OH$ (78:20:2)
Collect eluate at 1 to 2 mL/minute.

**NOTE:** Prepare elution solvent daily.
Add IPA /$NH_4OH$, mix, then add $CH_2Cl_2$ (pH 11-12).

**5. DRY ELUATE:**

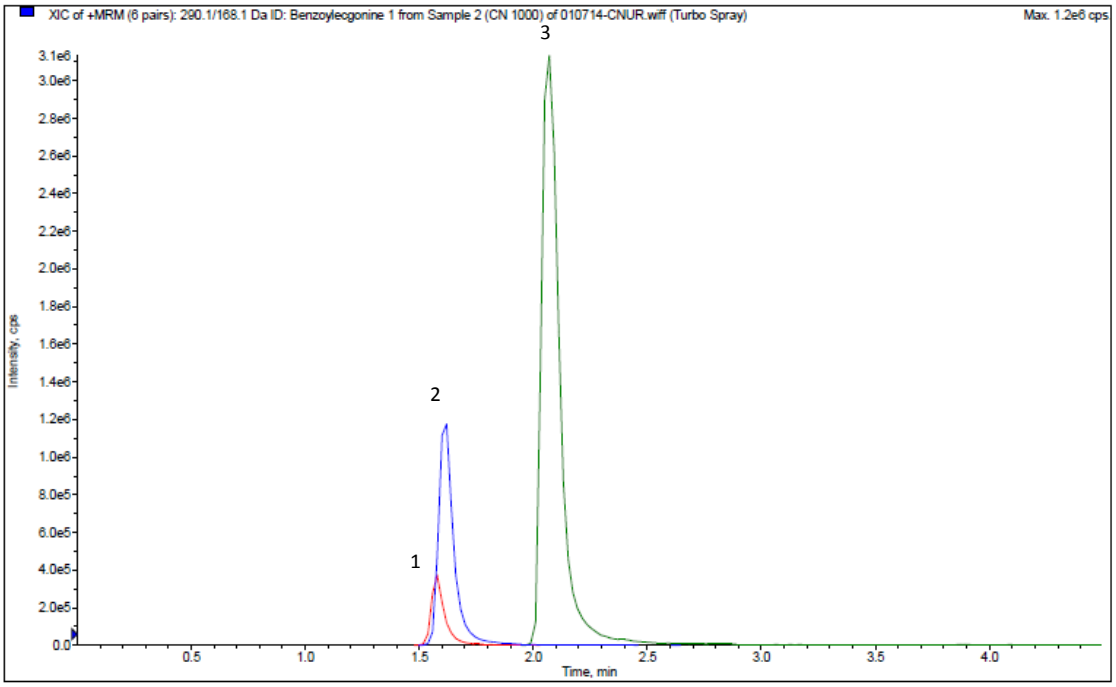
Evaporate to dryness at < 40 °C.

**6. 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 $N_2$ and cap. Mix/vortex
  React 30 minutes at 70°C; Cool and inject 1 µL

## INSTRUMENT CONDITIONS (LC-MS/MS):

### CHROMATOGRAM




| Analyte | MRM Transitions | | Relative Retention Time (min) |
|---|---|---|---|
| | Q1 | Q3 | |
| **1. Benzolyecgonine $D_8$** | **298.1** | **171.1** | **1.58** |
| **2. Benzolyecgonine** | **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. $H_2O$

**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

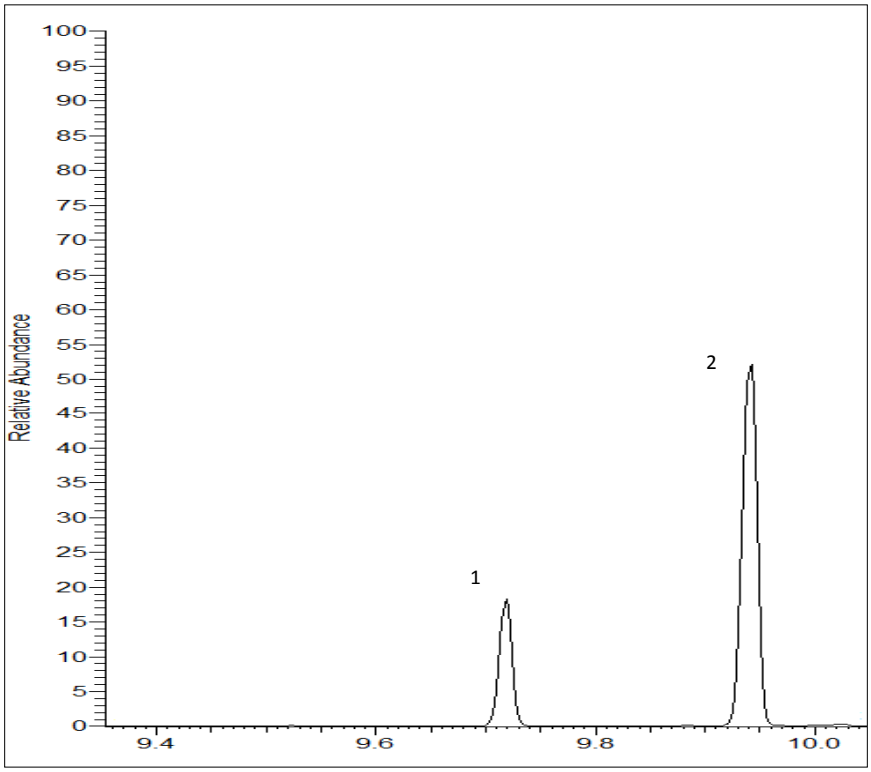
**Instrument:** API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

**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):

### CHROMATOGRAM




| Analyte | Quantify Ion | Qualifier Ion 1 | Qualifier Ion 2 | Relative Retention Time (min) |
|---|---|---|---|---|
| **6. Cocaine** | **182** | **198** | **303** | **9.72** |
| **Cocaine D3** | **185** | **201** | **306** | **-** |
| **7. Benzolyecgonine TMS** | **240** | **256** | **361** | **9.94** |
| **Benzolyecgonine 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:** 1 µL 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 ºC, MS Source: 350 ºC, MS Quad: 150 ºC