



COCAINE AND BENZOYLECGONINE FROM MECONIUM BY LC-MS/MS OR GC-MS CLEAN SCREEN[®] DAU EXTRACTION COLUMN

Part #:

ZSDAU020- CLEAN SCREEN[®] DAU 200mg, 10 mL Tube

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

SLDA50ID21-5UM-SELECTRA[®] DA HPLC Column 50 x 2.1MM, 5µm

1. PREPARE SAMPLE

Vortex 0.5 -1 g meconium with 2 mL of Methanol.

Centrifuge and transfer the supernatant to a clean tube.

To each tube add 3 mL 100 mM phosphate buffer (pH 6.0), internal standard and vortex.

Matrix must be more aqueous than organic for good extraction to occur.

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 1 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

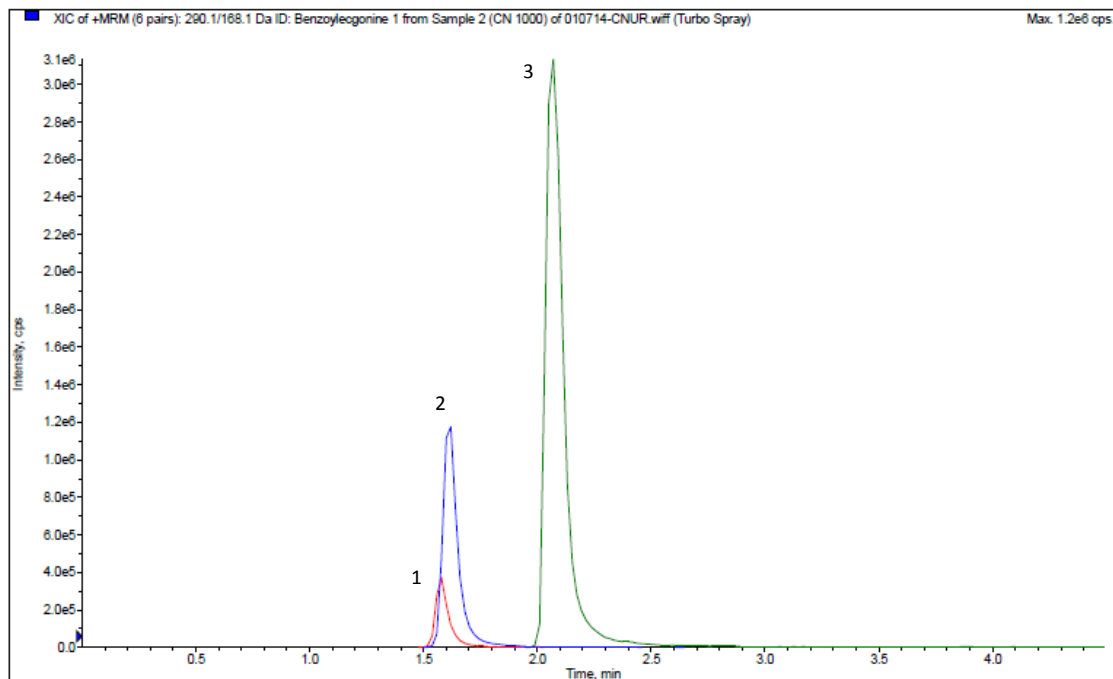
Evaporate to dryness at < 40°C.

7. RECONSTITUTE / DERIVATIZE

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
Inject 10µL.
- **GC-MS:** Dissolve residue in 50 µL of Ethyl Acetate and 50 µL BSTFA (with 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):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q2	
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

Flow Rate: 0.7mL/minute

Reconstitute: 100 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:

Mobile Phase B: 0.1% Formic Acid in Methanol

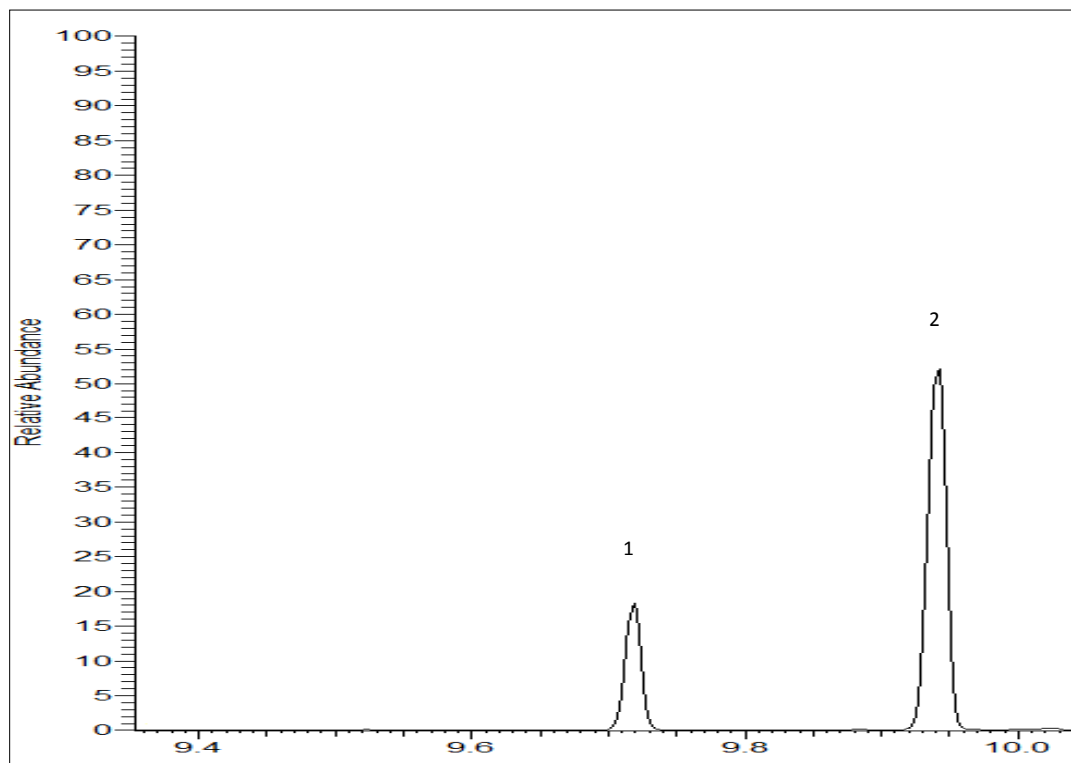
Polarity: Positive

Injection Volume: 10 µL

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)
4. Cocaine	182	198	303	9.72
Cocaine D3	185	201	306	-
5. Benzoylcegonine TMS	240	256	361	9.94
Benzoylcegonine 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