



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

Part #

CSXCE111 – CLEAN SCREEN XCEL[®] I 130 mg, 1 mL Tube

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

SLDA50ID21-5UM - SELECTRA[®] DA HPLC Column 50 x 2.1 mm, 5 μ m

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 \pm 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
Centrifuge for 10 minutes at 2000 rpm and discard pellet

2. APPLY SAMPLE

Load sample directly to column without any preconditioning.
Pull sample through at a rate of 1-2 mL/ minute.
Dry column thoroughly under full vacuum or positive pressure for 1 minute.

3. WASH

1 x 2 mL 50:50 Methanol: 100mM HCl
Dry column thoroughly under full vacuum or positive pressure for a minimum of 5 minutes.

4. ELUTION

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).

5. DRY ELUTE

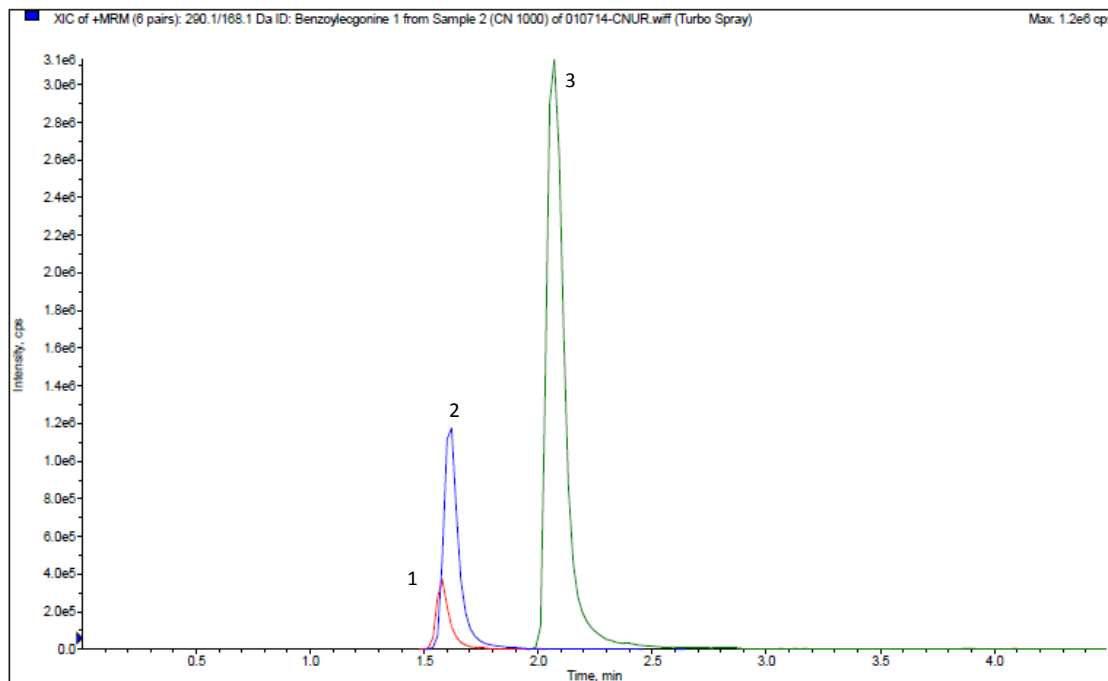
Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

6. RECONSTITUTE / DERIVATIZE

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

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



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

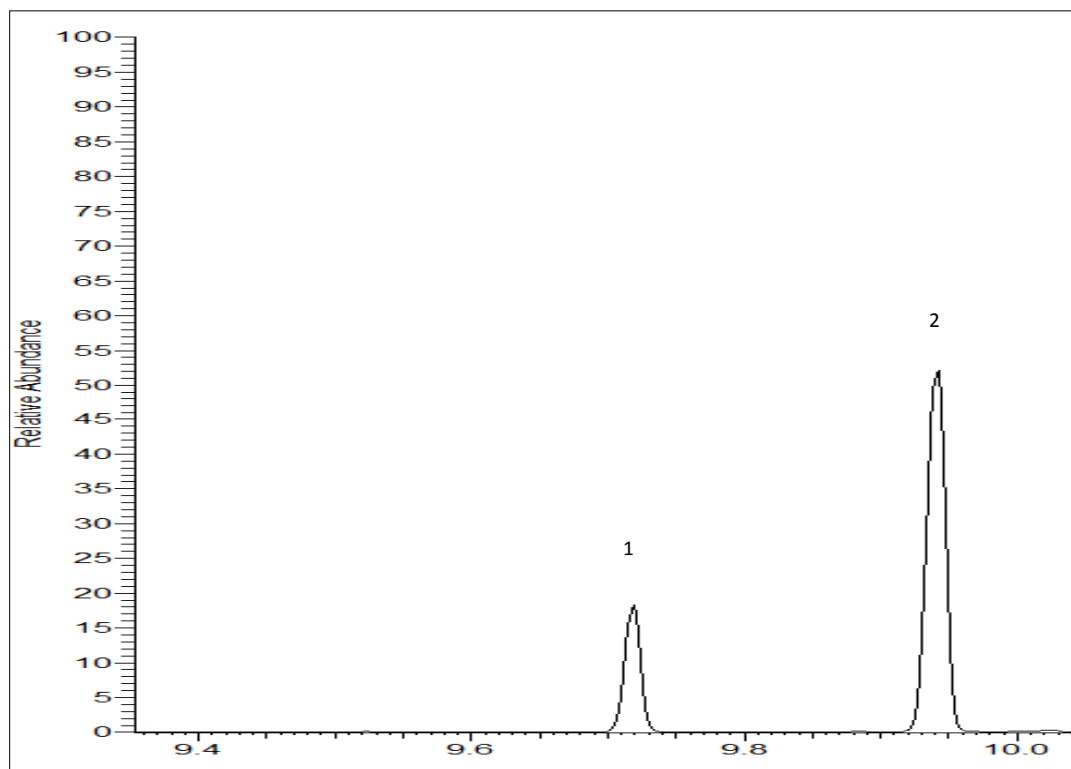
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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):

CHROMATOGRAM



Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Cocaine	182	198	303	9.72
Cocaine D3	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: 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 °C, MS Source: 350 °C, MS Quad: 150 °C