



FREE OPIATES AND GLUCURONIDES IN URINE EXTRACTED BY CLEAN SCREEN[®] DAU AND ANALYZED BY LC-MS/MS

UCT Part Numbers:

CSDAU206: Clean Screen DAU, 200mg / 6 mL tube

SPPHO6001-5: Select pH Buffer Pouch, phosphate buffer pH 6

SLDA100ID21-5UM: Selectra[®] DA HPLC Column

SAMPLE EXTRACTION

1. PREPARE SAMPLE

Urine: To 1 mL of 100 mM phosphate buffer (pH=6) add internal standards.

Add 1-2 mL of urine.

Mix/vortex and let stand 5 minutes

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

Sample pH should be 6.0 ± 0.5 .

Centrifuge for 10 minutes at 2000 rpm and discard pellet

2. CONDITION CLEAN SCREEN[®] DAU SPE 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 acetate buffer (pH 4.5).

1 x 3 mL CH₃OH.

Dry column (5 minutes at full vacuum or pressure).

5. ELUTE FREE OPIATES & GLUCURONIDES

1 x 3 mL MEOH containing 4% ammonium hydroxide (maximize recovery of glucuronides with polar elution solvent)

Collect eluate at 1 to 2 mL/minute.

NOTE: NOTE: Prepare a fresh solution daily of the MeOH containing 4% ammonium hydroxide

6. DRY ELUATE

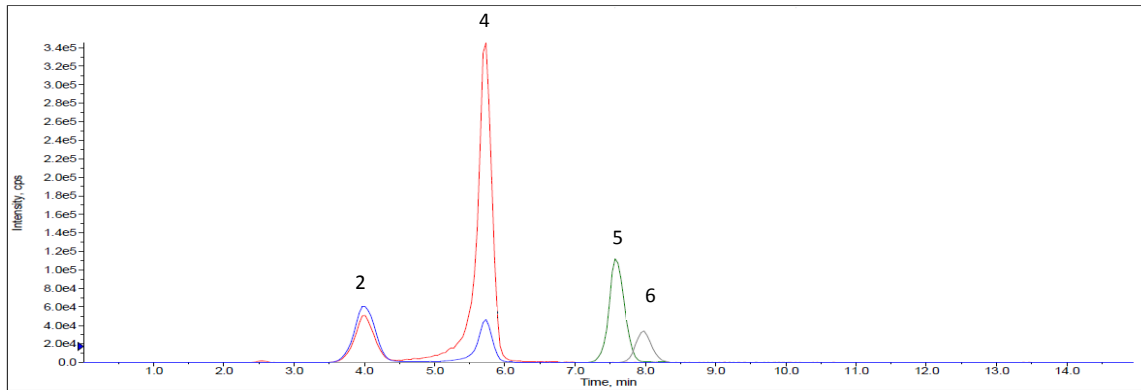
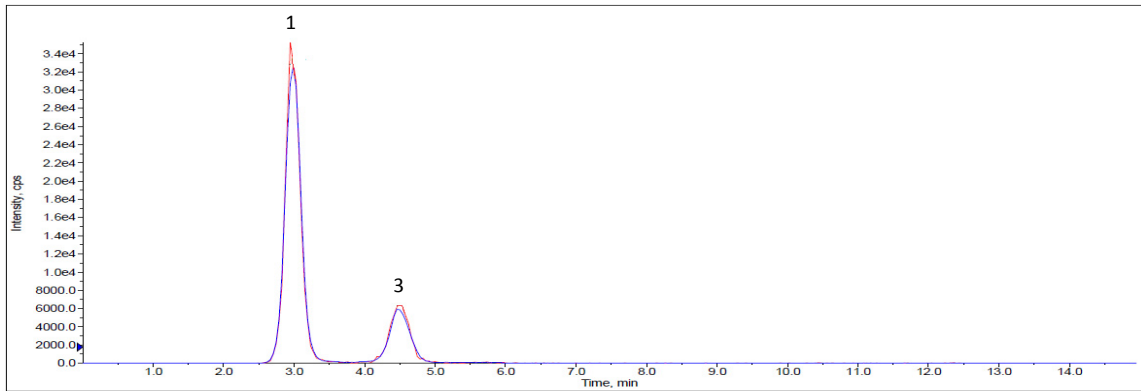
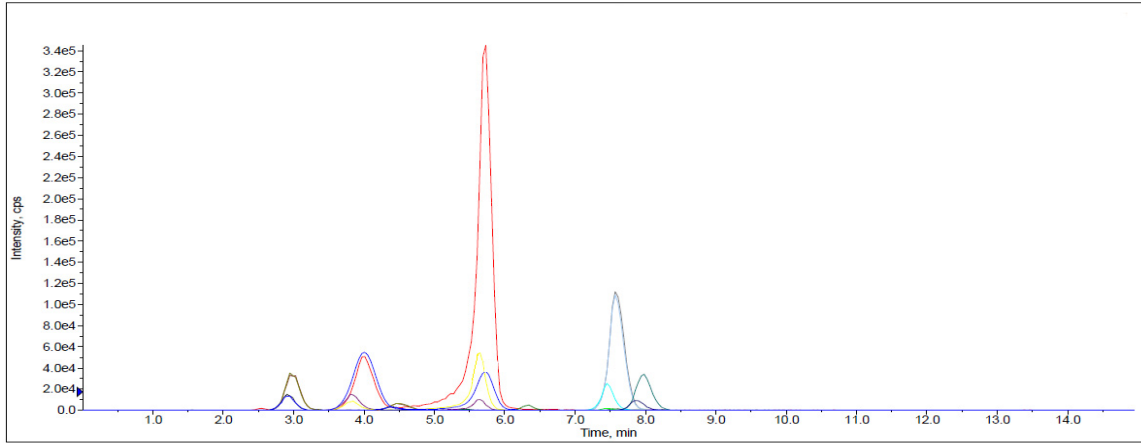
Evaporate to dryness under nitrogen < 35°C.

7. RECONSTITUTE

- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase and vortex mix
Inject 10 μ L

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAMS



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1.Morphine-3-Glucuronide	462.4	286.0	2.99
2. Morphine	286.0	152.0	3.98
3. Morphine-6-Glucuronide	462.4	286.0	4.48
4.Hydromorphone	286.0	185.0	5.72
5.Codeine	300.0	152.0	7.58
6.6-MAM	328.0	165.1	7.97

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in H₂O

Mobile Phase B: 0.1% Formic Acid in MeOH

Flow Rate: 0.6mL/minute

Polarity: Positive

Injection Volume: 10µl

LC Column: Selectra® DA HPLC Column; 100 x 2.1mm 5µm

Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Gradient:

Time	%A	%B
0.00	95	5
3.00	95	5
3.50	80	20
7.00	80	20
9.00	10	90
11.00	10	90
11.20	95	5
15.00	STOP	