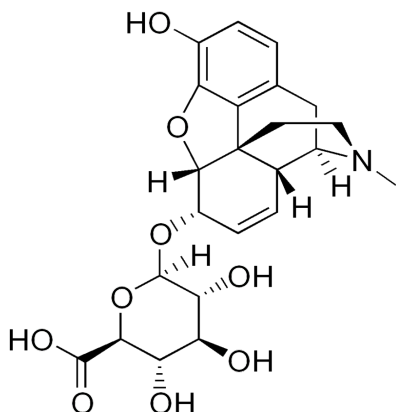


Free Opiates and Glucuronides in Urine Extracted By Clean Screen® DAU and Analyzed by LC-MS/MS



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

6. Dry Eluate

- Evaporate to dryness under nitrogen < 35°C.

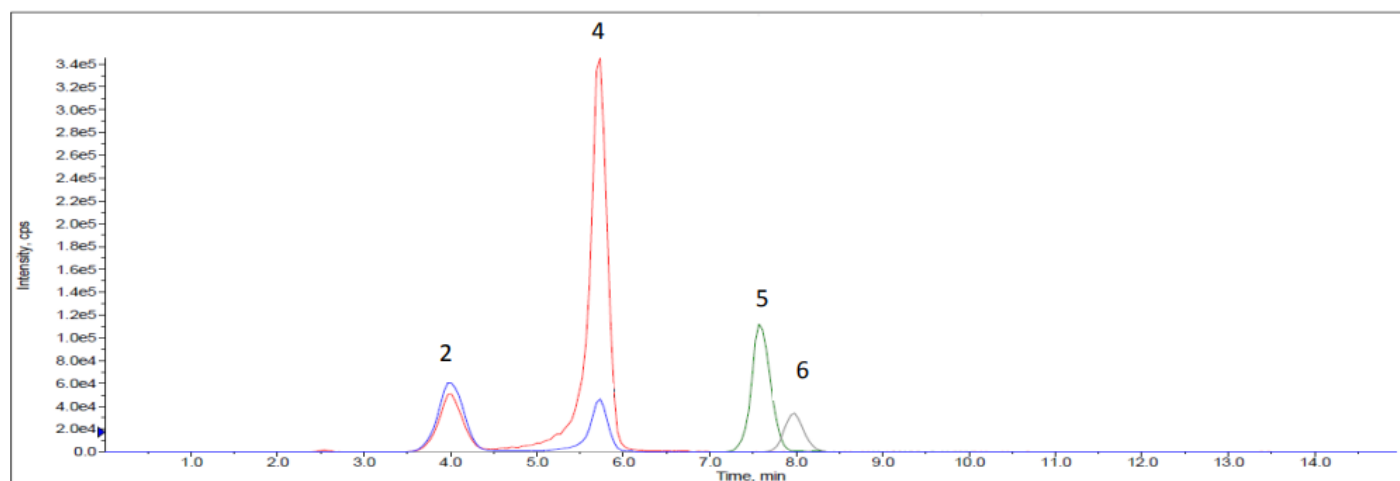
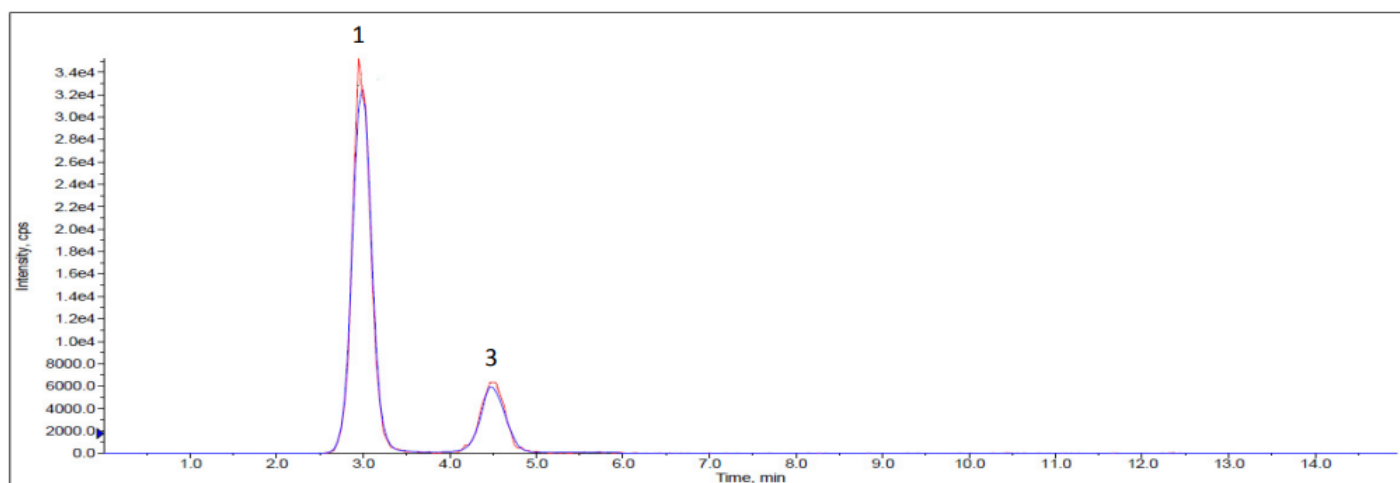
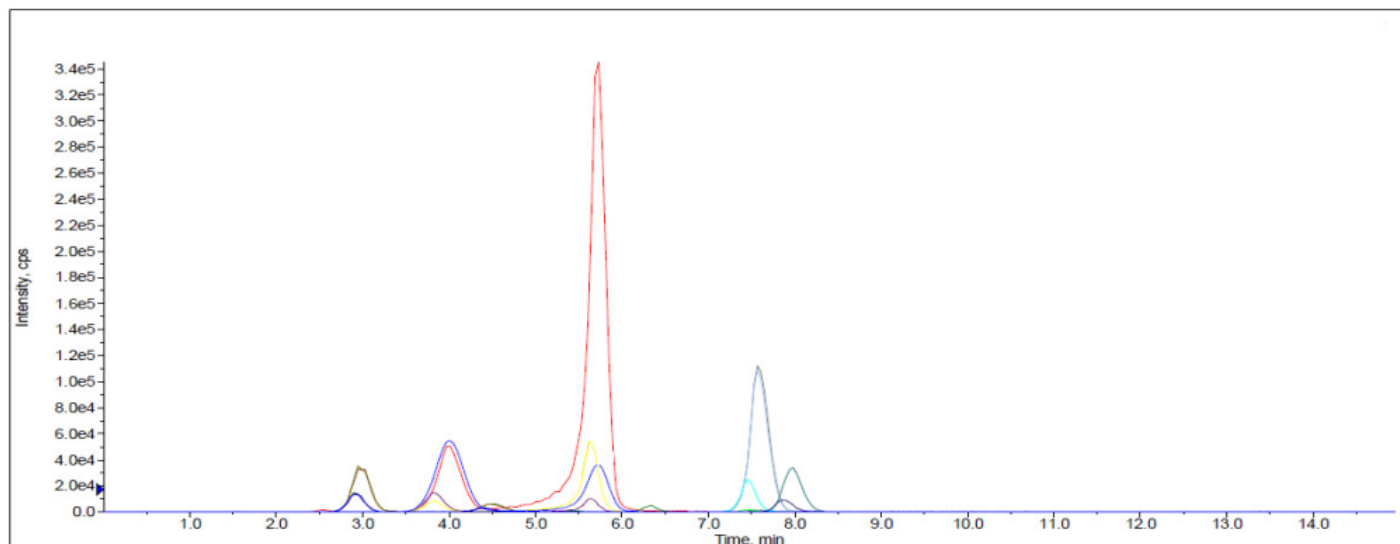
7. Reconstitute

- LC-MS/MS: Reconstitute sample in 100 µL of mobile phase and vortex mix.
- Inject 10µL.



Instrument Conditions (LC-MS/MS):

Chromatograms:



MRM Transitions

Analyte	Q1	Q3	Relative Retention Time (minutes)
Morphine-3-Glucuronide	462.4	286.0	2.99
Morphine	286.0	152.0	3.98
Morphine-6-Glucuronide	462.4	286.0	4.48
Hydromorphone	286.0	185.0	5.72
Codeine	300.0	152.0	7.58
6-MAM	328.0	165.1	7.97

Parameters

Instrument	API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL
LC Column	Selectra® DA HPLC Column; 100 x 2.1mm 5µm
Injection Volume	10 µl
Flow Rate	0.6 mL/minute
Mobile Phase A	0.1% Formic Acid in H ₂ O
Mobile Phase B	0.1% Formic Acid in MeOH
Polarity	Positive

Gradient Program

Time (min)	% Mobile Phase A:	% Mobile Phase 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	STOP



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