OPIATES IN BLOOD, PLASMA/SERUM, URINE, OR TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #

ZSDAU020 – CLEAN SCREEN® DAU 200 mg, 10 mL Tube
BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
SPIA-0-1– SELECTRA-SIL® PIA (propionic anhydride)
SPYR-0-50– SELECTRA-SIL® Pyridine
SLDA50ID21-5UM – Selectra® DA HPLC Column, 50 x 2.1 mm, 5 µm

1. **PREPARE SAMPLE:**

Blood: To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards.
Add 1-2 mL of blood, plasma/ serum, 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 ± 0.5.
Centrifuge for 10 minutes at 2000 rpm and discard pellet

Urine: PREPARE SAMPLE FOR ENZYME HYDROLYSIS OF GLUCURONIDES:
To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Selectrazyme® β-glucuronidase.
Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase.
Vortex and heat for 1-2 hours at 65 °C.
Allow sample to cool
Do not adjust pH~ sample is ready to be added to the extraction column.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
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. H_2O.
1 x 3 mL 100 mM acetate buffer (pH 4.5).
1 x 3 mL CH_3OH.
Dry column (5 minutes at full vacuum or pressure).

5. **ELUTE OPIATES:**
1 x 3 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).

6. **DRY ELUATE:**
Evaporate to dryness at < 40 °C.

7. RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 10 µL

- **GC-MS**: **Derivatize with proprionic anhydride: pyridine**
  Add 200 µL of a 1:1 solution of proprionic anhydride: pyridine
  Make this solution fresh daily.
  Mix/vortex.
  React for 60 minutes at 60 °C in a heater block.
  Remove from heat source to cool.
  Evaporate to dryness at < 40 °C.
  Reconstitute the residue with 50 µL of Ethyl Acetate / Methanol (70:30)

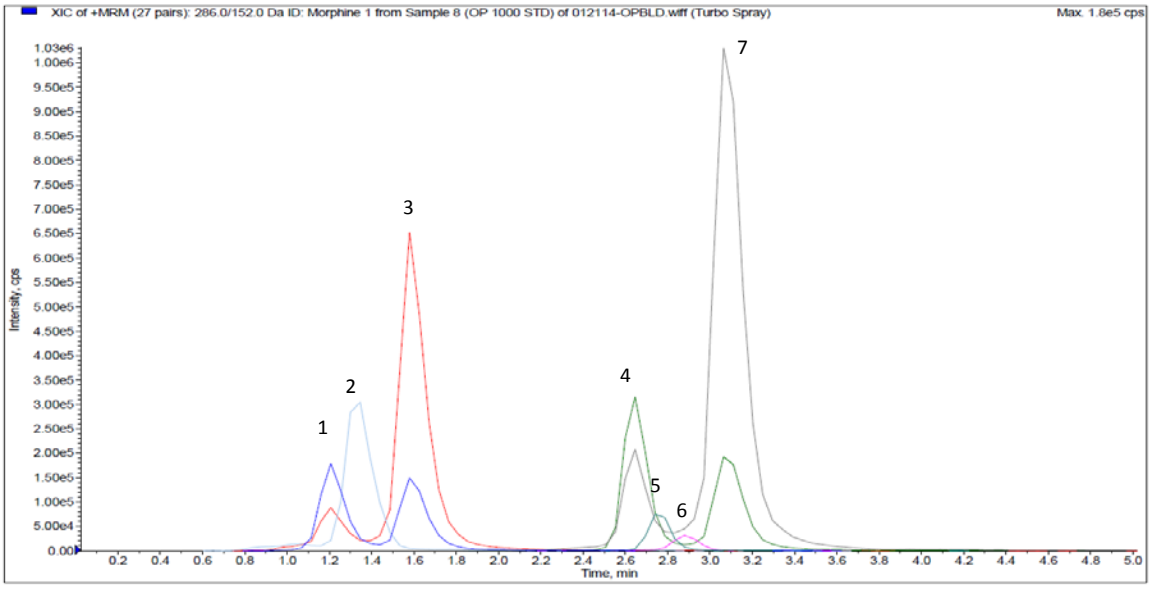
Alternate Derivatization

1. **DERIVATIZE with TMS**
   Add 50 µL Ethyl Acetate and 50 µL BSTFA w/ 1% TMCS
   Overlay with N_2 and cap. Mix/vortex.
   React 30 minutes at 70 °C. Remove from heat source to cool.
   NOTE: Do not evaporate BSTFA solution

Note: *Hydroxylamine can be added to sample within method if oxime derivative is preferred.*
Following hydrolysis, add 200 µL 10% Hydroxylamine solution.
Heat to 60 °C for 30 min in a heating block.
Allow sample to cool then adjust pH back to 5 with 1.0 N NaOH.
Centrifuge for 10 minutes at 2000 rpm and discard pellet
Sample is now ready to be added to the extraction column

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Morphine	286.0	152.0	1.21
2. Oxymorphone	302.0	227.0	1.30
3. Hydromorphone	286.0	185.0	1.60
4. Codeine	300.0	152.0	2.65
5. 6-MAM	328.0	165.1	2.75
6. Oxycodone	316.0	240.0	2.85
7. Hydrocodone	300.0	199.0	3.10

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.6 mL/minute

Polarity: Positive

Injection Volume: 10 µL

LC Column: Selectra® DA HPLC Column 50 x 2.1mm 5 µm

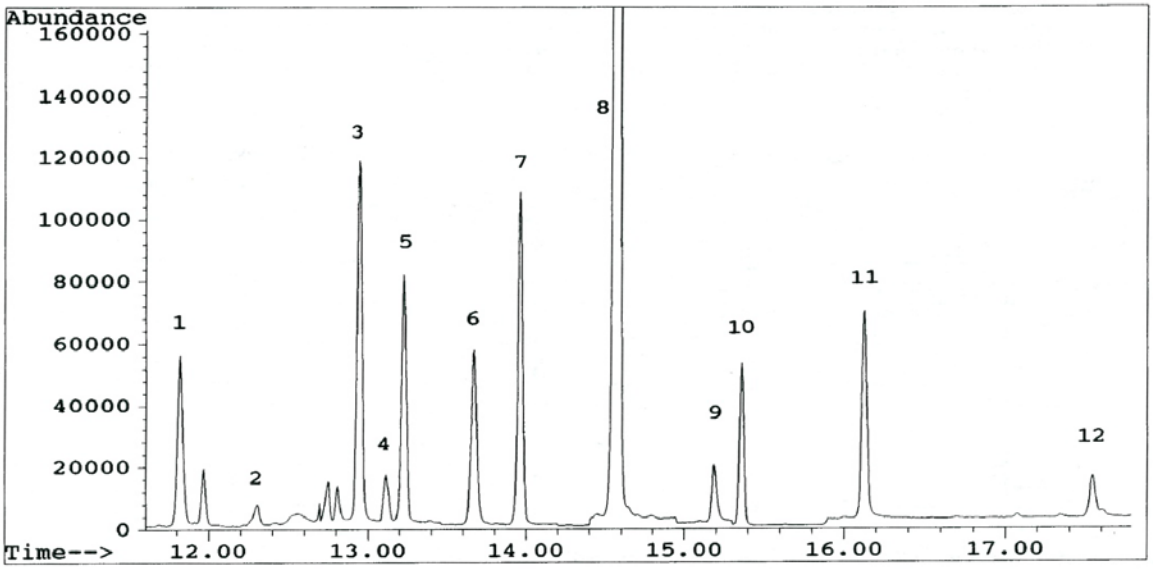
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	85	15
7.00	40	60
7.01	20	80
8.00	85	15
9.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



PROPYL DERIVATIVES

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion
1. Hydrocodone	299.0	242.0	214.0
2. Thebaine	311.2	296.2	312.2
3. Codeine	355.0	282.0	229.0
4. Oxycodone	371.0	314.0	298.0
5. Heroin	327.2	369.2	268.2
6. Hydromorphone	285.0	341.0	228.0
7. 6-Mam	327.2	268.0	383.2
8. Morphine	341.0	268.0	397.0
- Morphine-D_3	344.3	271.3	400.3
9. Oxymorphone	357.0	300.0	413.0
10. Naloxone	327.1	383.2	328.2
11. Nalorphine	367.2	350.2	294.2
12. Norcodeine	223.1	224.1	236.1

BSTFA-OXIME DERIVATIVES

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2
Morphine TMS	429.0	414.0	401.0
Morphine-D_3 TMS	432.0	417.0	404.0
Morphine-D_6 TMS	435.0	420.0	404.0
Normorphine TMS	487.0	472.0	414.0
Diacetylmorphine	369.0	327.0	268.0
Oxymorphone Oxime TMS	532.0	517.0	287.0
Oxymorphone Oxime-D_3 TMS	535.0	520.0	290.0
Hydromorphone Oxime TMS	444.0	429.0	355.0
Hydromorphone Oxime-D_3 TMS	447.0	432.0	358.0
Codeine TMS	371.0	356.0	343.0
Codeine-D_3 TMS	374.0	359.0	346.0
Codeine-D_6 TMS	377.0	349.0	316.0
Dihydrocodeine TMS	373.0	315.0	358.0
Norcodeine TMS	429.0	414.0	356.0
6-MAM TMS	399.0	400.0	340.0
Oxycodone Oxime TMS	474.0	459.0	417.0
Oxycodone Oxime-D_3 TMS	477.0	462.0	420.0
Oxycodone Oxime-D_6 TMS	480.0	465.0	420.0
Hydrocodone Oxime TMS	386.0	297.0	371.0
Hydrocodone Oxime-D_3 TMS	389.0	300.0	374.0
Hydrocodone Oxime-D_6 TMS	392.0	303.0	377.0
Meperidine-D_4	251.0	222.0	250.0
Meperidine	247.0	218.0	246.0
Normeperidine-D_4 TMS	308.0	280.0	309.0
Normeperidine TMS	305.0	276.0	304.0
Tramadol TMS	335.0	245.0	290.0
O-Desmethyltramadol TMS	393.0	378.0	303.0
N-Desmethyltramadol TMS	393.0	378.0	116.0
Pentazocine TMS	357.0	342.0	289.0

PARAMETERS

GC/MS: Hewlett Packard 5971A/ 5890 GCMS System with 7673 ALS

GC capillary column: Rtx-5 30 m x 0.25 mm, 0.25 µm

Injector: 2 µL Splitless, 250 °C

Oven temperature program: 100 ºC (1 minute) to 250 ºC (25 ºC/minute): hold (2 minutes) to 290 ºC (10 ºC/minute): hold (0.5 minutes) to 325 ºC (25 ºC/minute): hold (3.1 minutes).

Carrier gas: Helium (1.2 mL/minute)

MSD condition: Aux temperature: 280 ºC, MS Source: 350 ºC, MS Quad: 150 ºC

References: Hackett,J.; Telepchak, M.J.; Coyer, M.J. Automation of solid-phase extraction for urinary opiate analysis. American Laboratory. 2008.