

EXTRACTION OF OPIATES FROM BLOOD/ PLASMA/SERUM/URINE OR TISSUE USING CLEAN SCREEN XCEL® I COLUMN



UCT Featured Products

CSXCE106
CLEAN SCREEN XCEL® I
130 mg, 6 mL Tube

ASBETA-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

Procedure:

1) Sample Preparation

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) 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 3 mL D.I. H₂O
1 x 3 mL 98% Methanol: 2% Acetic Acid
Dry column thoroughly under full vacuum or positive pressure for a minimum of 5-10 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 µL of mobile phase Inject 10 µL

GC-MS: Derivatize with propionic anhydride: pyridine
Add 200 µL of a 1:1 solution of propionic 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

DERIVATIZE with TMS. Add 50 µL Ethyl Acetate and 50 µL. BSTFA w/ 1% TMCS. Overlay with N₂ and cap. Mix/ vortex. React 30 minutes at 70 °C. Remove from heat source to cool. **NOTE:** Do not evaporate BSTFA solution.



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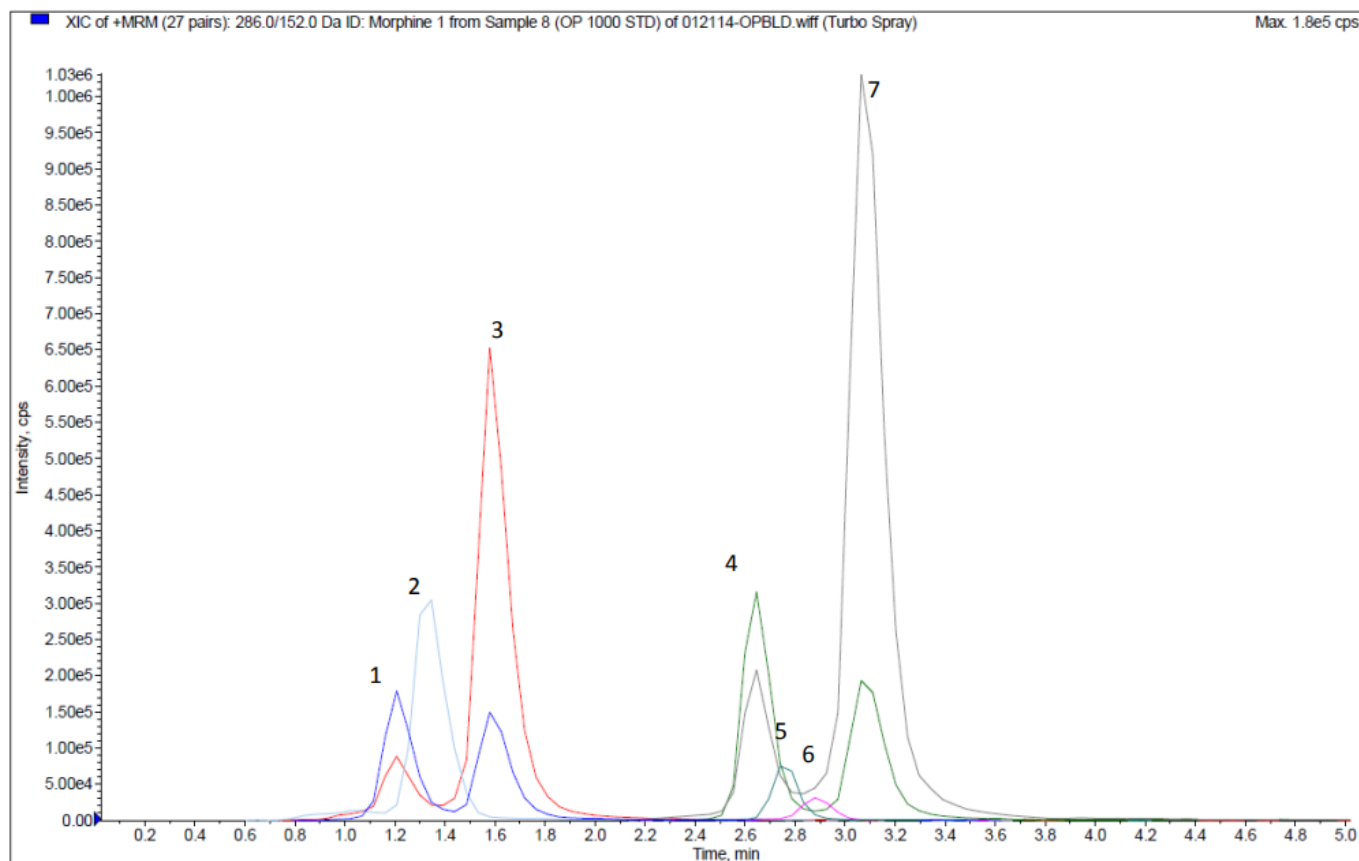
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Notes:

Hydroxylamine can be added to sample within method if oxime derivative is preferred.
 Following hydrolysis, add 200 μ L 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):

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	199.9	3.10



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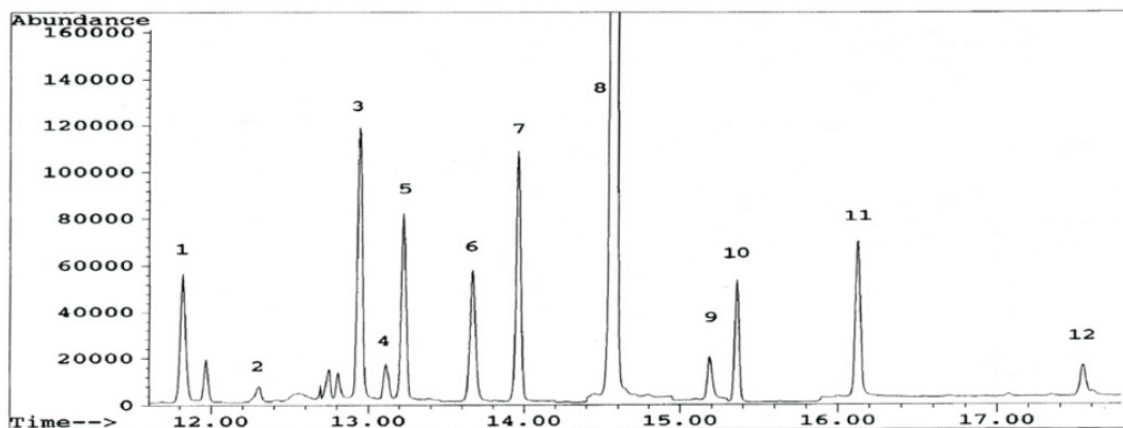


INSTRUMENT CONDITIONS (LC-MS/MS):

LC-MS/MS Parameters		
Instrument	API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC	
Flow Rate	0.6 mL/minute	
Injection Volume	10 µL	
LC Column	Selectra® DA HPLC Column 50 x 2.1 mm 5 µm	
Polarity	Positive	
Mobile Phase A	0.1% Formic Acid in D.I. H ₂ O	
Mobile Phase B	0.1% Formic Acid in Methanol	
Time	%A	%B
0.00	85	15
7.00	40	60
7.01	20	80
8.00	85	15
9.00	STOP	STOP

INSTRUMENT CONDITIONS (GC/MS):

GC/MS 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 °C (1 minute) to 250 °C (25 °C/minute): hold (2 minutes) to 290 °C (10 °C/minute): hold (0.5 minutes) to 325 °C (25 °C/minute): hold (3.1 minutes).
Carrier gas	Helium (1.2 mL/minute)
MSD condition	Aux temperature: 280 °C, MS Source: 350 °C, MS Quad: 150 °C
References	Hackett, J.; Telepchak, M.J.; Coyer, M.J. Automation of solid-phase extraction for urinary opiate analysis. American Laboratory. 2008.



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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
9. Morphine-D ₃	344.3	271.3	400.3
10. Oxymorphone	357.0	300.0	413.0
11. Naloxone	327.1	383.2	328.2
12. Nalorphine	367.2	350.2	294.2
13. Norcodeine	223.1	224.1	236.1

BSTFA-Oxime Derivatives			
Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion
Morphine TMS	429.0	414.0	401.0
Morphine-D3 TMS	432.0	417.0	404.0
Morphine-D6 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-D3 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-D3 TMS	374.0	359.0	346.0
Codeine-D6 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-D3 TMS	477.0	462.0	420.0
Oxycodone Oxime-D6 TMS	480.0	465.0	420.0
Hydrocodone Oxime TMS	386.0	297.0	371.0
Hydrocodone Oxime-D3 TMS	389.0	300.0	374.0
Hydrocodone Oxime-D6 TMS	392.0	303.0	377.0
Meperidine-D4	251.0	222.0	250.0
Meperidine	247.0	218.0	246.0
Normeperidine-D4 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

