



OPIATES IN URINE-OXIME TMS PROCEDURE FOR GC OR GC/MS CONFIRMATIONS USING:

200 mg CLEAN SCREEN[®] EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU[®] Tips

1. PREPARE SAMPLE ACID HYDROLYSIS OF GLUCURONIDES:

To 2 mL of urine add internal standard(s)* and 400 μ L concentrated HCl.

Add 200 μ L 10% Hydroxylamine solution in DI H₂O.

Mix/vortex.

Heat to 90°C for 40 min in a heating block, or an autoclave for 15 minutes on a liquid cycle.

Cool before proceeding.

Centrifuge for 10 minutes at 2000 rpm and discard pellet.

Add 500 μ L 50% Ammonium Hydroxide. Mix/vortex.

Adjust sample pH 5 - 6 by drop wise addition with 50% Ammonium Hydroxide.

PREPARE ENZYME HYDROLYSIS OF GLUCURONIDES:

To 2 mL of urine add internal standard(s)* and enzyme preparation in buffer

Mix/vortex.

Heat to 60 °C for sufficient time in a heating block (depends on analytes and enzyme)

Add 200 μ L 10% Hydroxylamine solution.

Heat to 60°C for 30 min in a heating block.

Adjust pH to 5 - 6

Centrifuge for 10 minutes at 2000 rpm and discard pellet.

2. CONDITION CLEAN SCREEN[®] EXTRACTION 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 < 3 inches Hg to prevent sorbent drying.

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 > 10 inches Hg).

5. ELUTE OPIATES

1 x 3 mL CH₂Cl₂/IPA/NH₄OH (76:20:4)

Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

Add IPA/NH₄OH, mix, then add CH₂Cl₂ (pH 11-12).

6. DRY ELUATE

Evaporate to dryness at < 40°C.

7. DERIVATIZE

Add 100 µL ethyl acetate and 100 µL BSTFA (with 1% TMCS)***.

Overlay with N₂ and cap. Mix/vortex.

React 45 minutes at 70°C. in a heat block.

Remove from heat source to cool

NOTE: Do not evaporate BSTFA solution.

8. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph. For MSD monitor the following ions:

<u>Compound</u>	<u>Quant Ion**</u>	<u>Secondary</u>	<u>Tertiary</u>	<u>Cerilliant #</u>
Meperidine-D4	251	222	250	M-036
Meperidine	247	218	246	M-035
Normeperidine-D4 TMS*	308	280	309	N-020
Normeperidine TMS*	305	276	304	N-017
Tramadol TMS	335	245	290	T-027
O-Desmethyltramadol TMS	393	378	303	T-035
N-Desmethyltramadol TMS	393	378	116	D-023
Pentazocine TMS	357	342	289	P-039
Codeine-D3 TMS*	374	359	346	C-039
Codeine-D6 TMS*	377	349	316	C-040
Codeine TMS	371	356	343	C-006
Norcodeine TMS	429	414	356	N-005
Dihydrocodeine TMS	373	315	358	D-019
Morphine-D3 TMS*	432	417	404	M-099
Morphine-D6 TMS*	435	420	404	M-085
Morphine TMS	429	414	401	M-005
Normorphine TMS	487	472	414	N-006
Diacetylmorphine	369	327	268	H-038
Hydrocodone Oxime-D3 TMS	389	300	374	H-008
Hydrocodone Oxime-D6 TMS	392	303	377	H-047
Hydrocodone Oxime TMS	386	297	371	H-003
Hydromorphone Oxime-D3 TMS	447	432	358	H-010
Hydromorphone Oxime TMS	444	429	355	H-004
Oxycodone Oxime-D3 TMS	477	462	420	O-006
Oxycodone Oxime-D6 TMS	480	465	420	O-008
Oxycodone Oxime TMS	474	459	417	O-002
Oxymorphone Oxime-D3 TMS	535	520	290	O-019
Oxymorphone Oxime TMS	532	517	287	O-004

*Suggested internal standards for GC/MS: D₄-Meperidine,
D₄-Normeperidine, D₃-Codeine, D₃-Morphine D₆-Hydrocodone
D₆-Oxycodone

*Suggest trying D₆-Codeine, and D₆-Morphine for lowest LOD/LOQ

*** Part # SBSTFA-1-1, 10, 25, 100