



OPIATES IN HUMAN URINE- PROPYL DERIVATIVES 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 SAMPLE-ENZYMATIC HYDROLYSIS OF GLUCURONIDES:

To 2 mL of urine, add internal standard(s), and 1 mL of β -Glucuronidase solution. β -Glucuronidase solution contains 5,000 F units/mL *Patella vulgata* in 100 mM acetate buffer (pH 5.0).

Hydrolyze for 3 hours at 60°C.

Centrifuge for 10 minutes at 2000 rpm and discard pellet.

Adjust sample pH to 5 - 6 with 1.0 N NaOH.

2. CONDITION CLEAN SCREEN[®] EXTRACTION COLUMN

1 x 3 mL CH₃OH.

1 x 3 mL D.I. H₂O.

1 x 1 mL 100 mM phosphate buffer (pH 6.0); Aspirate.

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; Aspirate.

1 x 3 mL 100 mM acetate buffer (pH 4.5); Aspirate.

1 x 3 mL CH₃OH; Aspirate.

Dry column (5 minutes at > 10 inches Hg).

5. ELUTE OPIATES

1 x 3 mL ethyl acetate/isopropanol/ammonium hydroxide (84:12:4).

6. DRY ELUANT

Evaporate to dryness at < 40°C.

7. DERIVATIZE

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).

* Suggested internal standard for GC/MS: Codeine-D3 and Morphine-D3

** Quantitation ion

*** Hydrocodone does not derivatize under these conditions.

**** Part # SPIA-0-1,10, 25

***** Part # SPYR-0-50

8. QUANTITATE

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

<u>Compound (Propyl)</u>	<u>Primary Ion**</u>	<u>Secondary Ion</u>	<u>Tertiary Ion</u>	<u>Cerilliant#</u>
Hydrocodone	299	242	214	H-003
Codeine	355	282	229	C-006
*Codeine-D3	358	285	232	C-007
Oxycodone	371	314	298	O-002
Hydromorphone	285	341	228	H-004
6-Acetylmorphine	327	268	383	A-003
Oxymorphone	357	300	413	O-004
Morphine	341	268	397	M-005
Morphine-D3	344	271	400	M-006

SOURCE: UCT Internal Publication working with the Philadelphia Medical Examiner's Office