

Opiates In Human Urine - Propyl Derivatives For GC OR GC/MS Confirmations Using: 200 mg Clean Screen® Extraction Column



UCT Part Numbers

ZSDAU020	Or	ZCDAU020
Clean Screen® DAU 10 mL, 200 mg sorbent Without Tips		Clean Screen® DAU 10 mL, 200 mg sorbent With CLEAN-THRU® Tips

* 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

Procedure:

1. Prepare Sample Acid Hydrolysis of Glucuronides

- a) To 2 mL of urine add internal standard(s)* and 400 µL concentrated HCl.
- b) Add 200 µL 10% Hydroxylamine solution in DI H₂O.
- c) Mix/vortex.
- d) Heat to 90°C for 40 min in a heating block, or an autoclave for 15 minutes on a liquid cycle.
- e) Cool before proceeding.
- f) Centrifuge for 10 minutes at 2000 rpm and discard pellet.
- g) Add 500 µL 50% Ammonium Hydroxide. Mix/vortex.
- h) Adjust sample pH 5 - 6 by drop wise addition with 50% Ammonium Hydroxide.

2. Prepare Sample-Enzymatic Hydrolysis of Glucuronides

- a) 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).
- b) Hydrolyze for 3 hours at 60°C.
- c) Centrifuge for 10 minutes at 2000 rpm and discard pellet.
- d) Adjust sample pH to 5 - 6 with 1.0 N NaOH.

3. Condition Clean Screen® Extraction Column

- a) 1 x 3 mL CH₃OH.
- b) 1 x 3 mL D.I. H₂O.
- c) 1 x 1 mL 100 mM phosphate buffer (pH 6.0); Aspirate.

Note: Aspirate at < 3 inches Hg to prevent sorbent drying.

4. Apply Sample

- a) Load at 1 to 2 mL/minute.

5. Wash Column

- a) 1 x 3 mL D.I. H₂O; Aspirate.
- b) 1 x 3 mL 100 mM acetate buffer (pH 4.5); Aspirate.
- c) 1 x 3 mL CH₃OH; Aspirate.
- d) Dry column (5 minutes at > 10 inches Hg).

6. Elute Opiates

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

7. Dry Eluant

- a) Evaporate to dryness at < 40°C.

8. Derivatize

- a) Add 200 µL of a 1:1 solution of propionic anhydride**** pyridine*****
- b) Make this solution fresh daily. Mix/vortex.
- c) React for 60 minutes at 60°C in a heater block.
- d) Remove from heat source to cool.
- e) Evaporate to dryness at < 40°C.
- f) Reconstitute the residue with 50 µL of ethyl acetate / methanol (70:30).

9. Quantitate

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



Compound (Propyl)	Primary Ion**	Secondary Ion	Tertiary Ion	Cerilliant #
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

References:

[1] UCT Internal Publication working with the Philadelphia Medical Examiner's Office

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