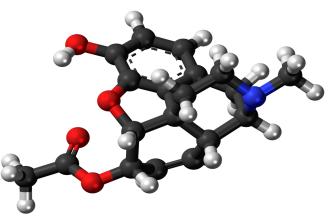
6-Acetylmorphine (6MAM) In Urine/Vitreous Humor GC/MS Confirmations Using: 200 mg Clean Screen® Extraction Column



UCT Part Numbers

Or

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

Procedure:

1. Prepare Sample

- a) To 2 mL of 100 mM phosphate buffer (pH= 6.0), add internal standard* and Mix/vortex.
- b) Add 4 mL of sample
- c) Centrifuge for 10 minutes at 2000 rpm and discard pellet.
- d) Sample pH should be 6.0 \pm 0.5.
- e) Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

2. Condition Clean Screen® Extraction Column

- a) 1 x 3 mL CH₃OH.
- b) 1 x 3 mL D.I. H₂O.
- c) $1 \times 1 \text{ mL} 100 \text{ mM}$ phosphate buffer (pH= 6.0).
- Note: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. Apply Sample

a) Load at 1 mL/minute.

4. Wash Column

- a) 1 x 3 mL D.I. H₂O.
- b) 1 x 2 mL 100 mM acetate buffer (pH 4.5).
- c) 1 x 3 mL CH₃OH.
- d) Dry column (10 minutes at >10 inches Hg).

5. Elute 6-AM

- a) 1 x 3 mL CH₂Cl₂/IPA/NH₄OH (78:20:2);
- b) Collect eluate at 1 to 2 mL/minute.
- Note: Prepare elution solvent daily. Add IPA/NH₄OH, mix, then add CH_2Cl_2 (pH 11-12).

6. Dry Eluate

a) Evaporate to dryness at < 40°C.

7. Derivatize

- a) Add 50 μL ethyl acetate. Vortex mix and add 50 μL BSTFA (with 1% TMCS)***.
- b) Overlay with N₂ and cap. Mix/vortex.
- c) React 45 minutes at 70°C. Remove from heat source to cool.

Note: Do not evaporate BSTFA solution.

8. Quantitate

- a) Inject 1 to 2 μ L onto chromatograph.
- b) Monitor the following ions (GC/MS):





Compound	Primary Ion****	Secondary	Tertiary	Cerilliant #
D ₆ -6-AM-TMS	405	406	343	A-026
6-AM-TMS	399	400	340	A-009

* Suggested internal standard for GC/MS:

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

**** Quantitation ion

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