



**6-ACETYLMORPHINE (6MAM) IN URINE/ VITREOUS HUMOR  
GC/MS CONFIRMATIONS USING:  
200 mg CLEAN SCREEN<sup>®</sup> EXTRACTION COLUMN**  
Part # ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU<sup>®</sup> Tips

**1. PREPARE SAMPLE**

To 2 mL of 100 mM phosphate buffer (pH 6.0), add internal standard\* and Mix/vortex.

Add 4 mL of sample

Centrifuge for 10 minutes at 2000 rpm and discard pellet.

Sample pH should be 6.0 ± 0.5.

Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

**2. CONDITION CLEAN SCREEN<sup>®</sup> EXTRACTION COLUMN**

1 x 3 mL CH<sub>3</sub>OH.

1 x 3 mL D.I. H<sub>2</sub>O.

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

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

**3. APPLY SAMPLE**

Load at 1 mL/minute.

**4. WASH COLUMN**

1 x 3 mL D.I. H<sub>2</sub>O.

1 x 2 mL 100 mM acetate buffer (pH 4.5).

1 x 3 mL CH<sub>3</sub>OH.

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

**5. ELUTE 6-AM**

1 x 3 mL CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2);

Collect eluate at 1 to 2 mL/minute.

**NOTE:** Prepare elution solvent daily.

Add IPA/NH<sub>4</sub>OH, mix, then add CH<sub>2</sub>Cl<sub>2</sub> (pH 11-12).

**6. DRY ELUATE**

Evaporate to dryness at < 40°C.

**7. DERIVATIZE**

Add 50 µL ethyl acetate. Vortex mix and add 50 µL BSTFA (with 1% TMCS)<sup>\*\*\*</sup>.

Overlay with N<sub>2</sub> and cap. Mix/vortex.

React 45 minutes at 70°C. Remove from heat source to cool.

**NOTE:** Do not evaporate BSTFA solution.

## 8. QUANTITATE

Inject 1 to 2  $\mu$ L onto gas chromatograph.

For MSD monitor the following ions:

<u>Compound</u>	<u>Primary Ion****</u>	<u>Secondary Ion</u>	<u>Tertiary Ion</u>	<u>Cerilliant #</u>
6-Acetylmorphine- D6 -TMS*	405	406	343	A-026
6-Acetylmorphine-TMS	399	400	340	A-009

\* Suggested internal standard for GC/MS:

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

\*\*\*\* Quantitation ion