



## PSILOCIN IN URINE

### FOR GC OR 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 5 mL of urine add internal standard and 2 mL of 100 mM phosphate buffer (pH 6.0).

Mix/vortex.

Add 12,500 to 25,000 units of  $\beta$ -Glucuronidase, Mix/Vortex.

Place the sample into a water bath at 45°C for 90 minutes.

Remove from the bath and allow to cool.

Centrifuge at 3,000 rpm for 10 min.

Use the clear filtrate (discard the plug) for SPE.

#### 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 20% Acetonitrile in water.

1 x 1 mL 100 mM Acetic Acid.

Dry column (3 minutes at > 10 inches Hg).

1 x 2 mL Hexane.

1 x 3 mL Hexane/ Ethyl Acetate (50:50).

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

Dry Column (3 min at > 10 inches Hg).

#### 5. ELUTE PSILOCIN

1 x 3 mL Dichloromethane/ Isopropanol/Ammonium Hydroxide (78:20:2).

Collect eluant at 1 mL /min.

NOTE: Prepare elution solvent daily.

#### 6. DRY ELUATE

Evaporate to dryness at < 35°C.

#### 7. DERIVATIZE

Add 50  $\mu$ L of ethyl acetate. Vortex mix.

Add 50  $\mu$ L of MSTFA\*

React for 30 minutes at 70°C.

Remove from heat

**NOTE:** Do not evaporate MSTFA solution

## 8. QUANTITATE

Inject 1 to 2 µL onto chromatograph.

Monitor the following ions (Mass Selective Detection):

| <u>Compound</u>     | <u>Primary**</u> | <u>Secondary</u> | <u>Tertiary</u> | <u>Cerilliant #</u> |
|---------------------|------------------|------------------|-----------------|---------------------|
| PSILOLOCIN – TMS    | 290              | 348              | 73 (291)        | P-048               |
| *PSILOLOCIN-D10-TMS | 300              | 358              | 83(301)         | P-049               |

\* Part # SMSTFA-0-1, 10, 25, 100

\*\*Quantitation Ion

### GC CONDITIONS:

HP Model 5890 GC with a 5970 MSD

COLUMN = DB5 (25 m x 0.32 mm ID x 0.17µm Film Thickness)

CARRIER GAS -Helium (5 psi head pressure)

INJECTION Size= 1 µL

SPLITLESS MODE

Injection Temperature = 275°C

Detector Temperature = 300 °C

**TEMPERATURE PROGRAM:** 70° C hold 1 min then ramp to 240 °C at 20 °C/min hold for 2 minutes

**SOURCE** - The Detection of Psilocin in Human Urine Grieshaber A, Moore K, Levine B and Smith M Presented at the TRI-SERVICES Meeting Nov 1999