FREE AND CONJUGATED PSILOCIN IN URINE By LC-MS/MS Using an 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part Number: ZSDAU020

September 1, 2009

1. PREPARE SAMPLE:

To 1 mL of 100 mM phosphate buffer (pH 6) add internal standard.*

Add 1 mL of urine sample.

Add 2 mL of 100 mM phosphate buffer (pH 6).

Vortex and centrifuge as appropriate.

URINE HYDROLYSIS:

To 1 mL of urine add internal standard* and 1 mL of ß-glucuronidase solution.

(ß-glucuronidase solution contains: 5,000 F units/mL Patella vulgata in 100 mM acetate buffer (pH=5.0)).

Mix/vortex.

Hydrolyze for 3 hours at 65°C.

Centrifuge for 10 minutes at 2000 rpm and discard pellet.

2. CONDITION COLUMN:

1 x 3 mL CH_3OH

1 x 3 mL D.I. H_2O

1 x 1 mL 100 mM phosphate buffer (pH 6).

Note: aspirate at < 3 inches Hg to prevent sorbent drying out

3. APPLY SAMPLE:

Load sample at 1-2 mL / minute.

4. WASH COLUMN:

1 x 3 mL DI H_2O

1 x 3 mL 100 mM acetic acid

1 x 3 mL CH_3OH

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

5. **ELUTE PSILOCIN**:
   1 x 3 mL ethyl acetate; acetonitrile: ammonia (78: 20: 2 v/v)

   Or

   1 x 3 mL CH_2Cl_2/ IPA/ ammonia (78:20:2 v/v)

   Collect eluate at 1-2 mL /minute.

6. **EVAPORATION:**
   Evaporate eluates to dryness under a gentle stream of nitrogen.

7. **RECONSTITUTE** sample in 50 µL of CH_3OH.
   Inject 5 µL.

INSTRUMENT CONDITIONS:

Column: 50 x 2.1 mm (3 µm) SELECTRA® Phenyl (UCT, LLC)

MOBILE PHASE:

Time	% Acetonitrile	%0.1% Formic acid
0	20	80
5	20	80

Flowrate: 0.20 mL/ minute

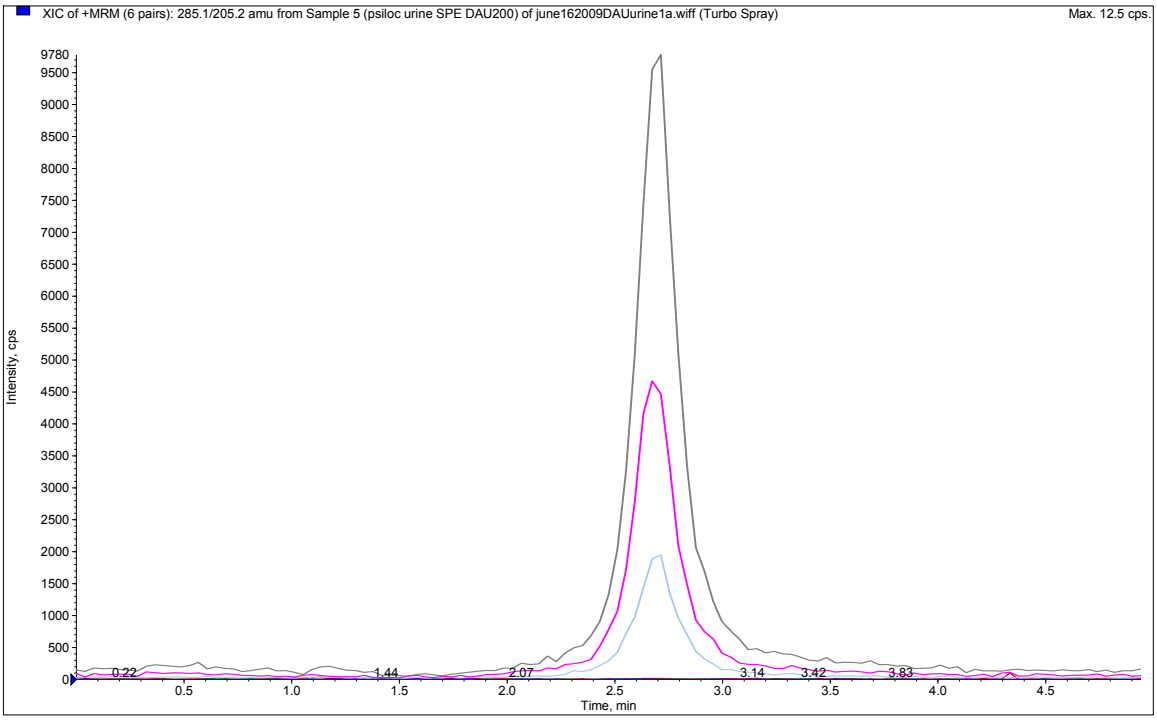
Column Temperature: ambient

Detector: API 2000 MS/MS

Compound	MRM Transition
Psilocin	205.2/58.2
*Psilocin-D10	215.2/68.2

CHROMATOGRAM SHOWING:

Psilocin extracted from Urine



Recovery > 90% (n=10)

LOD: 10 ng/ mL

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