PSILOCIN AND PSILOCYBIN IN URINE BY LC/MS/MS CLEAN SCREEN® EXTRACTION COLUMN

Part #
CSDAU206 – CLEAN SCREEN® DAU, 200 mg, 6 mL Tube
BETA-GLUC-10 - Selectrazyme® Beta-glucuronidase

1. PREPARE SAMPLE:

To 1-2 mL of urine sample, add 500 µL of acetate buffer (pH 5.0) containing 5,000 units/mL Selectrazyme® β-glucuronidase.
Optionally, add 500 µL of acetate buffer and 25 µL of concentrated β-glucuronidase. Vortex and heat for 1-2 hours at 65 °C.
Allow sample to cool
Do not adjust pH~ sample is ready to be added to the extraction plate.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN:

1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 3 mL 100 mM phosphate buffer (pH 6.0).

NOTE: Aspirate at full vacuum or pressure

3. APPLY SAMPLE:

Load at 1 to 2 mL/minute.

4. WASH COLUMN:

1 x 3 mL D.I. H_2O
1 x 3 mL of CH_3OH

Aspirate at full vacuum or pressure for 5 minutes

5. ELUTE PSILOCIN:

1 x 3 mL Ethyl Acetate containing 2% NH_4OH
Collect eluate at 1-2 mL /minute

5b. ELUTE PSILOCYBIN:

1 x 3 mL CH_3OH containing 2% NH_4OH
Collect eluate at 1-2 mL /minute

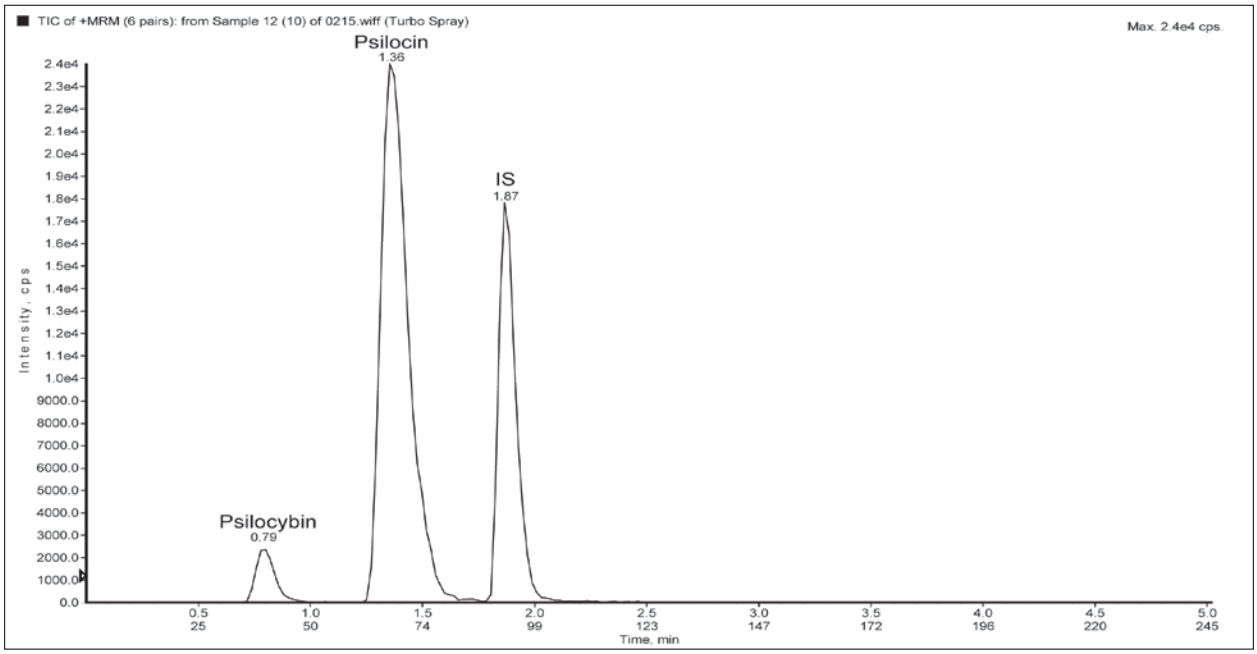
6. DRY ELUATE:

Evaporate to dryness at < 40 °C.

7. RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100 µL of Methanol
  Inject 10 µL.

CHROMATOGRAM



PARAMETERS

*Time = Dwell Time: *DP= Declustering Potential; *EP= Exit Potential: *CXP= Collision Cell Exit Potential: * CE=Collision Energy

Compound	Q1	Q2	Time/ms	DP/volts	SP/volts	CXP/volts	CE/volts
Psilocybin (1)	284.97	205.2	200	36	4.5	16	23
Psilocybin (2)	284.97	240.0	200	36	4.5	16	25
Psilocin (1)	205.081	58.1	200	26	8.5	14	23
Psilocin (2)	205.081	160.2	200	26	8.5	14	23
Ethyl morphine (1)	314.203	152.2	200	51	4	14	85
Ethylmorphine (2)	314.203	128.3	200	51	4	14	81

Mobile Phase A: 1% Formic Acid in D.I. H_2O **Mobile Phase B:** 1% Formic Acid in Acetonitrile
Instrument: API 3200 QTrap MS/MS Compound MRM Transition
LC Column: 50 x 2.0 mm (3 µm) C_{18}
Flow Rate: 0.5 mL/minute **Injection Volume**: 10 µL

Gradient:

Time	%A	%B
0	95	5
4	5	95
5.1	5	95