



PSILOCIN IN BLOOD, PLASMA/SERUM, URINE, OR TISSUE BY LC-MS/MS STYRE SCREEN[®] DBX EXTRACTION COLUMN

Part #

SSDBX206 – STYRE SCREEN[®] DBX, 200 mg, 6 mL Tube

BETA-GLUC-10 – Selectrazyme[®] Beta-glucuronidase

SLDA50ID21-3UM – SELECTRA[®] DA HPLC Column 50 x 2.1mm, 3 μ m

1. PREPARE SAMPLE:

Blood: To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards.

Add 1-2 mL of blood, plasma/ serum, or 1 g (1:4) tissue homogenate.

Mix/vortex and let stand for 5 minutes

Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex

Sample pH should be 6.0 \pm 0.5.

Centrifuge for 10 minutes at 2000 rpm and discard pellet

Urine: PREPARE SAMPLE FOR ENZYME HYDROLYSIS OF GLUCURONIDES:

To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Selectrazyme[®] β -glucuronidase.

Optionally, add 1 mL of acetate buffer and 25-50 μ L 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 column.

2. APPLY SAMPLE:

Load at 1 to 2 mL/minute.

3. WASH COLUMN:

1 x 3 mL D.I. H₂O

1 x 3 mL 100 mM acetic acid

1 x 3 mL of CH₃OH

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

4. ELUTE PSILOCIN:

1 x 3 mL Ethyl Acetate/ Acetonitrile/ NH₄OH (78: 20: 2 v/v)

Or

1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2 v/v)

Collect eluate at 1-2 mL /minute.

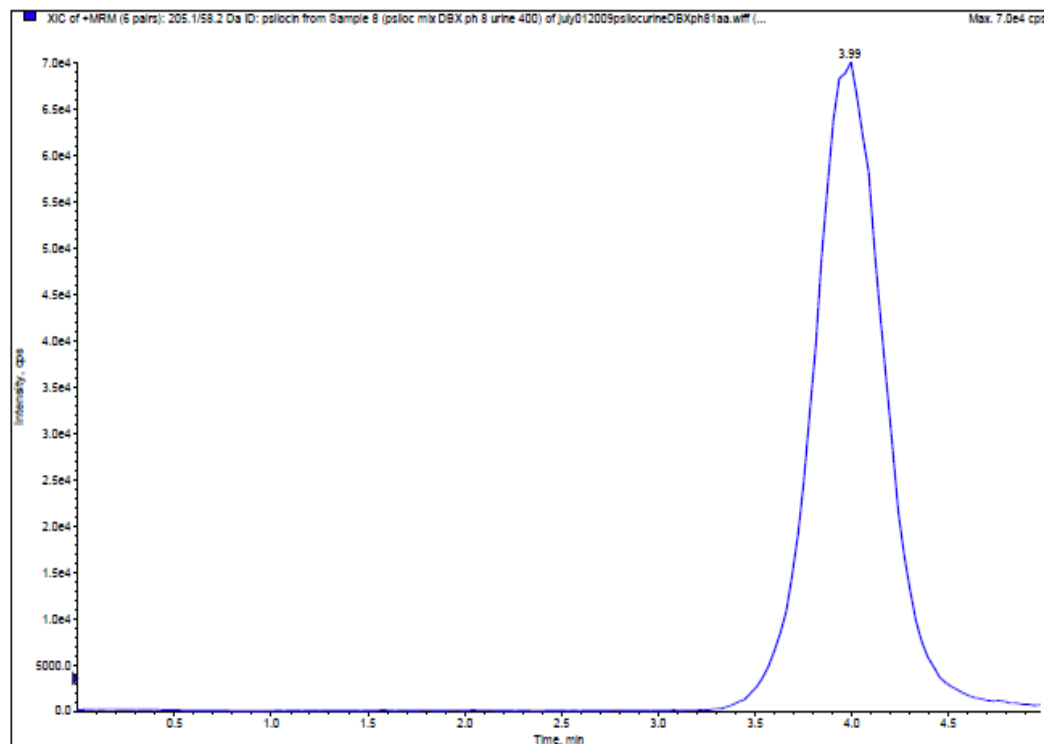
5. DRY ELUATE:

Evaporate to dryness at < 40 °C.

6. RECONSTITUTE:

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

INSTRUMENT CONDITIONS (LC-MS/MS):
CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time
	Q1	Q3	(minutes)
Psilocin	205.2	58.2	3.99
Psilocin-D ₁₀	215.2	68.2	-

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H₂O

Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Flow Rate: 0.3 mL/minute

Polarity: Positive

Injection Volume: 10 µL

LC Column: Selectra[®] DA HPLC Column 50 x 2.1 mm 3 µm

Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Isocratic Flow:

Time	%A	%B
0.00	20	80
5.00	STOP	