



***DULOXETINE IN BLOOD AND URINE USING:
200 mg CLEAN SCREEN[®] EXTRACTION COLUMN**

Part # ZSDAU020

LC-MSMS

1. PREPARE SAMPLE

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

Add 1 mL of blood or urine. Add 2 mL of 100 phosphate buffer (pH= 6). Mix/vortex.

Sample pH should be 6.0 ± 0.5 .

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

Mix/vortex.

Centrifuge as appropriate.

2. CONDITION CLEAN SCREEN[®] EXTRACTION COLUMN

1 x 3 mL CH₃OH.

1 x 3 mL DI H₂O.

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 H₂O.

1 x 3 mL 100 mM acetic acid.

1 x 3 mL CH₃OH.

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

5. ELUTE DULOXETINE:

1 x 3 mL dichloromethane/ isopropanol/ ammonia (78: 20: 2 v/v).

Collect eluate at 1-2 mL /minute.

6. EVAPORATION:

Evaporate eluate under a gentle stream of nitrogen < 40°C.

7. RECONSTITUTE sample in 200 µL of 0.1% Formic acid.

Inject 5 µL.

INSTRUMENT CONDITIONS:

Column: 50 x 2.1 mm (5 μ m) C₁₈

Mobile phase:

<u>Time/ min</u>	<u>% Acetonitrile</u>	<u>% 0.1 % Formic Acid</u>
0	5	95
4	90	10
4.1	5	95
5	5	95

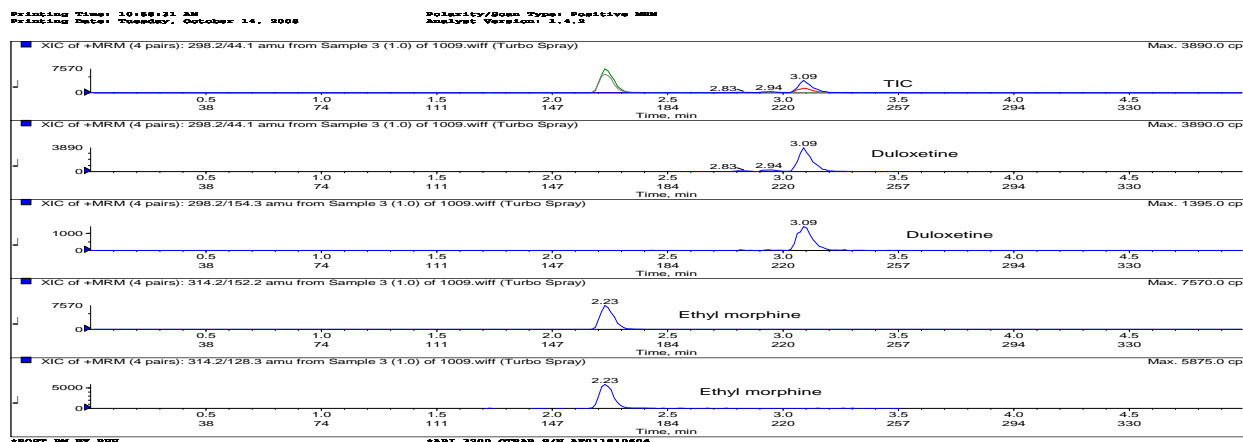
Flowrate: 0.5 mL/minute.

Column Temperature: ambient.

Detector: API 3200 Q-Trap MS/MS.

<u>Compound</u>	<u>MRM Transition</u>	<u>Cerilliant #</u>
* Ethyl Morphine	314.2/ 152.2	E-052
Duloxetine	298.1/44.1	D-004

Chromatogram of Ethyl Morphine and Duloxetine



Recovery > 90%

*Presented at SOFT annual meeting 2008 by A.A. Elian