



**BUPRENORPHINE AND NORBUPRENORPHINE IN BLOOD,
PLASMA/SERUM, AND URINE USING: 200 mg CLEAN SCREEN®
EXTRACTION COLUMN**

Part #: ZSDAU020

LC-MSMS

1. PREPARE SAMPLE (FREE BUPRENORPHINE/ NORBUPRENORPHINE):

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

Add 1 mL of whole blood, serum/ plasma, urine.

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

Vortex and centrifuge as appropriate.

TOTAL (FREE AND CONJUGATED) BUPRENORPHINE/ NORBUPRENORPHINE:

To 1 mL of Acetate buffer (pH= 5) containing 5000 F units/ mL β -Glucuronidase.

Add internal standards*.

To this solution add 1 mL of whole blood or urine.

Mix/ Vortex .

Hydrolyze for 3 Hrs at 65°C.

Allow to cool.

Add 3 mL of 100 mM phosphate buffer (pH= 6) and mix.

Centrifuge for 10 minutes at 2000 rpm and discard pellet.

2. CONDITION COLUMN:

1 x 3 mL CH₃OH

1 x 3 mL D.I. 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 BUPRENORPHINE/NORBUPRENORPHINE:

1 x3 mL ethyl acetate; acetonitrile: ammonia (78: 20: 2 v/v)

Or

1 x 3 mL CH₂Cl₂/ 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 μ L of CH₃OH.

Inject 5 μ L.

INSTRUMENT CONDITIONS:

Column: 50 x 2.1 mm (3µm) Selectra® Phenyl (UCT Inc.)

Mobile phase: Acetonitrile: 0.1% Formic acid
50 50

Flowrate: 0.35 mL/ minute

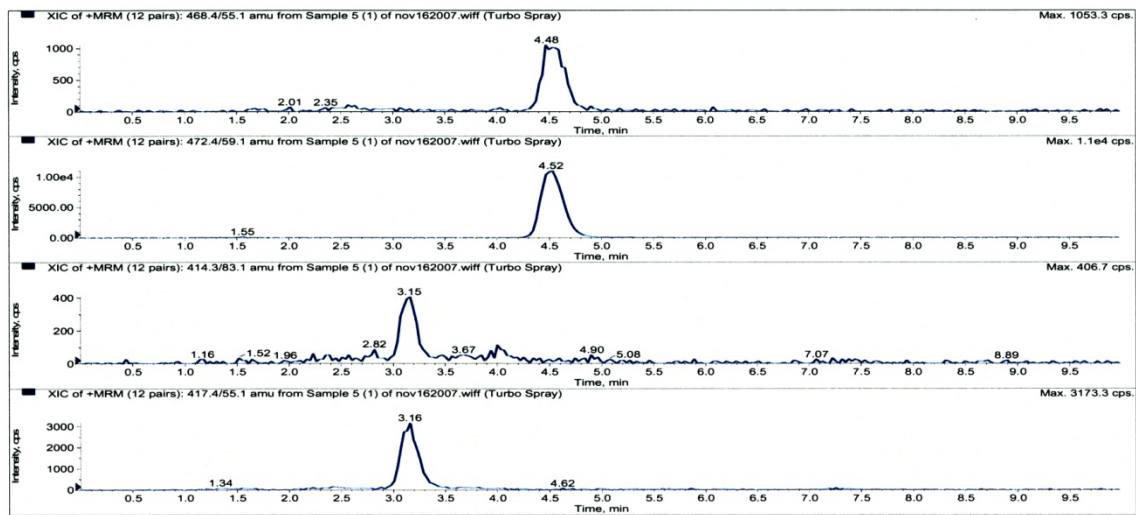
Column Temperature: ambient

Detector: API 2000 MS/ MS

Compound	MRM Transition	Cerilliant #
Buprenorphine	468.4/55.1	B-902
*Buprenorphine-D4	472.4/59.1	B-901
Norbuprenorphine	414.3/83.1	N-912
*Norbuprenorphine-D3	417.4/55.1	N-920

Chromatogram of Buprenorphine/ Norbuprenorphine (1 ng/ mL)

Buprenorphine Norbuprenorphine
Buprenorphine-D4 Norbuprenorphine-D3



Recovery: > 90% (N=10)

LOD: 0.5 ng/ mL