BUPRENORPHINE AND NORBUPRENORPHINE IN URINE, BLOOD & PLASMA/SERUM FOR HPLC ANALYSIS USING:
200mg 10mL HyperSep™ Verify™-CX Extraction column
(Part Number: 60108-742)

1. PREPARE SAMPLE
To 1mL of 100mM phosphate buffer (pH 5.0) add internal standards*.
Add 1mL of urine, whole blood, plasma/serum.
Add 2mL of 100mM phosphate buffer (pH 6.0).
Mix / vortex and centrifuge as appropriate

TOTAL (FREE AND CONJUGATED) BUPRENORPHINE / NORBUPRENORPHINE
To 1mL of acetate buffer (pH 5.0) containing 5,000 F units/mL of β-Glucuronidase,
add internal standards*.
To this solution, add 1mL of urine or blood.
Mix / vortex.
Hydrolyze for 3 hours at 65°C.
Allow to cool, then add 3mL of 100mM phosphate buffer (pH 6.0) and mix.
Centrifuge for 10 minutes at 2,000 rpm and discard pellet.

2. CONDITION VERIFY-CX EXTRACTION COLUMN
3mL of CH₃OH then aspirate.
3mL of DI H₂O then aspirate.
1mL of 100mM phosphate buffer (pH 6.0) then aspirate.
NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE
Load sample at 1-2mL/minute.

4. WASH COLUMN
2mL of DI H₂O
3mL of 100mM acetic acid
3mL of methanol
Dry column (5 minutes at > 10 inches Hg / full flow for Positive Pressure Manifold).

5. ELUTE BUPRENORPHINE / NORBUPRENORPHINE
3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2, v/v/v)
Or
3mL of ethyl acetate / acetonitrile / ammonia (78:20:2, v/v/v)
Collect eluate at 1 to 2mL/minute.

6. EVAPORATION
Evaporate to dryness at < 40°C

7. RECONSTITUTION
Add 50µL of methanol
8. QUANTITATE

Inject 5µL of sample onto the LC/MS system.
For MS monitor the following ions:

Recommended Column: Hypersil GOLD Phenyl 3µm 50x2.1mm (Part number 25903-054630)
Mobile phase: acetonitrile: 0.1% formic acid (50:50, v/v)
Flow rate: 0.35mL/min

<table>
<thead>
<tr>
<th>Compound</th>
<th>Detection ions</th>
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</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>468.4/55.1</td>
</tr>
<tr>
<td>*Buprenorphine-D4</td>
<td>472.4/59.1</td>
</tr>
<tr>
<td>Norbuprenorphine</td>
<td>414.3/83.1</td>
</tr>
<tr>
<td>*Norbuprenorphine-D3</td>
<td>417.4/55.1</td>
</tr>
</tbody>
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* Suggested internal standards