Determination of Buprenorphine and Norbuprenorphine in Whole Blood Using Polymeric SPE and LC/MS/MS

Irina Dioumaeva1 and John Hughes2; 1Agilent Technologies, Inc., Lake Forest, CA; 2Agilent Technologies, Inc., Pleasanton, CA

Introduction

Determination of buprenorphine and its metabolite norbuprenorphine in whole blood by forensic and pain management laboratories requires an analytical method capable of reliable detection at concentrations below 1 ng/mL. The rigidity of the molecules resulting in the limited availability of collision-induced fragments complicates SRM/MS detection of both compounds. This led some researchers to use a less selective SIM mode, or to employ SIM detecting only a molecular ion to molecular ion transition. With fragmentation applied, most commonly used products are m/z 414.2 and m/z 396.2 for buprenorphine and m/z 382.3 and m/z 101.1 for norbuprenorphine.

In this work we present a new stable isotope fragmentation pattern with abundant fragments, along with a new extraction procedure. Our method allows for a sensitive and specific quantitation of both buprenorphine and norbuprenorphine in whole blood with quantitation limits of 0.2 ng/mL using only 0.5 mL of sample.

Figure 1. Buprenorphine and norbuprenorphine analyses and their structures. Log P – P values are from DGC and PubChem.

Experimental

Materials and instrumentation

• Agilent Bond Elut Plexa PFCX 30 mL cartridges
• Agilent Poroshell 120 EC-C18, 3 x 50 mm, 2.7 µm column
• Agilent AJS electrospray ionization source.

Sample preparation procedure

Spike 8.5 mL blood with STD at 18 ng/mL.

Table 1. MRM transitions (quantifiers shown in bold).

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Precursor Ion</th>
<th>Product Ion</th>
<th>Transition</th>
<th>Quantitation Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>468.3</td>
<td>55.1</td>
<td>230 82</td>
<td></td>
</tr>
<tr>
<td>Norbuprenorphine-D3</td>
<td>417.3</td>
<td>83.1</td>
<td>230 82</td>
<td></td>
</tr>
<tr>
<td>Buprenorphine-D4</td>
<td>472.3</td>
<td>55.1</td>
<td>230 82</td>
<td></td>
</tr>
<tr>
<td>Norbuprenorphine</td>
<td>414.3</td>
<td>55.1</td>
<td>188 50</td>
<td></td>
</tr>
<tr>
<td>Norbuprenorphine-D3</td>
<td>417.3</td>
<td>101.1</td>
<td>188 46</td>
<td></td>
</tr>
</tbody>
</table>

Flow rate: 0.8 mL/min

Max pump pressure:          400 bar

Condition Bond Elut Plexa with 0.5 mL methanol.

Wash 1: 2 x 2 mL of 2% formic acid.

Wash 2: 5 mL of MIC 5% formic acid.

Dry 5-10 min under vacuum (10-15 in Hg).

Pre-run script:                    SCP_MSDiverterValveToWaste

Table 2. Method Validation of MRM transitions.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Accuracy, %</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>99.6</td>
<td>8.0</td>
</tr>
<tr>
<td>Norbuprenorphine-D3</td>
<td>99.6</td>
<td>8.2</td>
</tr>
</tbody>
</table>

Results and Discussion

1. A new SPE –LC/MS/MS method provides reliable quantitation of buprenorphine and norbuprenorphine in whole blood with LODs < 0.1 ng/mL.

2. New ion transitions identified in this study as most abundant and used for quantification are: 414.2 > 83.1 (norbuprenorphine), 468.2 > 55.1 (buprenorphine), 603 > 200 (buprenorphine-D4), 417.3 > 83.1 (norbuprenorphine-D3).

3. With injection of 10 µL of sample extract and preconcentration x5, peak-to-peak signal-to-noise ratio is 641 for 0.2 ng/mL of buprenorphine and 231 for 0.2 ng/mL of norbuprenorphine.

4. Typical calibration curves for buprenorphine and norbuprenorphine in whole blood extract.

Conclusions

Figure 2. Relative responses of buprenorphine and norbuprenorphine MRM signals (MM* = BUP* signal = 100% FR). CE as given in MRM table.

Figure 3. MRM extracted ion chromatograms: a – buprenorphine, b – norbuprenorphine (both at 0.2 ng/mL), c – buprenorphine-D4, d – norbuprenorphine-D3 (both at 18 ng/mL) in whole blood extract processed on Agilent Blood Blue PFCX and an Agilent Poroshell 120 EC-C18 3 x 50 mm, 2.7 µm column. Noise regions are shown in bold.