**GHB and Related Compounds in Human Blood by LC-MS/MS**

- Simultaneous analysis of GHB, GBL, and 1,4-BD in whole blood.
- Fast 5-minute cycle time.
- Separation of actual GBL and GBL from GHB in-source conversion.
- Sufficient sensitivity to measure endogenous GHB and identify exogenous drug ingestion.

---

**Column**

- Force C18 (cat.# 963431E)
- Dimensions: 100 mm x 3.0 mm ID
- Particle Size: 3 µm
- Pore Size: 100 Å
- Guard Column: Force C18 EXP guard column cartridge 5 mm, 3.0 mm ID, 3 µm (cat.# 96345203)
- Temp.: 30 °C

**Sample**

- Diluent: Water
- Conc.: 500 ng/mL
- Inj. Vol.: 10 µL

**Mobile Phase**

- A: 0.5% Formic acid in water
- B: 0.5% Formic acid in methanol

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow (mL/min)</th>
<th>%A</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.7</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>0.50</td>
<td>0.7</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>3.00</td>
<td>0.7</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>3.01</td>
<td>0.7</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>5.00</td>
<td>0.7</td>
<td>95</td>
<td>5</td>
</tr>
</tbody>
</table>

**Detector**

- MS/MS

**Ion Mode:** ESI+

**Mode:** MRM

**Instrument**

- HPLC

**Notes**

100 µL of whole human blood was fortified at 50 µg/mL with GHB, GBL, 1,4-BD, and GHB-D6 (IS) using 5 µL of 1 mg/mL solutions. The blood was precipitated with 380 µL methanol. The sample was then vortexed at 3000 rpm for 10 seconds and centrifuged at 3000 rpm for 10 minutes at 10 °C. 50 µL of the supernatant was removed and diluted to 1 mL with water. The sample was then vortexed and subjected to LC-MS/MS analysis. (Internal standard not shown on chromatogram.)

---

**Peaks**

<table>
<thead>
<tr>
<th>Peaks</th>
<th>tR (min)</th>
<th>Precursor Ion</th>
<th>Product Ion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. γ-Hydroxybutyric acid (GHB)</td>
<td>1.42</td>
<td>105.2</td>
<td>87.0</td>
</tr>
<tr>
<td>2. 1,4-Butanediol (1,4-BD)</td>
<td>1.42</td>
<td>93.0</td>
<td>55.0</td>
</tr>
<tr>
<td>3. γ-Butyrolactone (GBL)</td>
<td>1.75</td>
<td>87.0</td>
<td>45.0</td>
</tr>
</tbody>
</table>