Overlay: Matrix Double Blank Injection with Post-Column EtG Infusion on Raptor EtG/EtS

Column: Raptor EtG/EtS (cat.# 9325A12)
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Guard Column: UltraShield UHPLC precolumn filter, 0.2 µm frit (cat.# 25809)
Temp.: 35 °C

Sample Conc.: 50 µL of urine was diluted with 950 µL of 0.1% formic acid in water. The sample was vortexed at 3500 rpm for 10 seconds to mix. The sample was then centrifuged at 3000 rpm for 5 minutes at 10 °C. The autosampler needle was adjusted to inject from the supernatant. A post-column infusion of 1000 ng/mL EtG was made.

Inj. Vol.: 10 µL

Mobile Phase
A: 0.1% Formic acid in water
B: 0.1% Formic acid in acetonitrile

<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>0.5</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>2.50</td>
<td>0.5</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>2.51</td>
<td>0.5</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>4.00</td>
<td>0.5</td>
<td>95</td>
<td>5</td>
</tr>
</tbody>
</table>

Detector: MS/MS
Ion Mode: ESI-
Mode: MRM
Instrument: HPLC

Peaks
1. Ethyl-β-D-glucuronide

Precursor Ion: 220.8
Product Ion: 84.9
Product Ion: 74.8