Phosphatidylethanol 16:0/18:1 in Blank Human Blood on Raptor FluoroPhenyl

- Injection of blank immediately following highest standard.
- No carryover or interference.

<table>
<thead>
<tr>
<th>Peaks</th>
<th>Conc.</th>
<th>Precursor Ion</th>
<th>Product Ion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Phosphatidylethanol-d5 (PEth-d5) 16:0/18:1</td>
<td>0 µM</td>
<td>706.57</td>
<td>281.34</td>
</tr>
<tr>
<td>2. Phosphatidylethanol (PEth) 16:0/18:1</td>
<td>0 µM</td>
<td>701.50</td>
<td>255.27</td>
</tr>
</tbody>
</table>

Column: Raptor FluoroPhenyl (cat.# 9319A52)
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 2.7 µm
Temp.: 40 °C
Pore Size: 90 Å

Standard/Sample:
Conc.: Blank blood
Inj. Vol.: 2 µL

Mobile Phase:
A: Water, 5 mM ammonium acetate
B: 9:1 Methanol:2-propanol

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

Instrument: UHPLC

Sample Preparation:
Procedure provided by Redhot Diagnostics:
The blank blood sample (50 µL) was mixed with 50 µL of 2-propanol and 150 µL of 4:1 2-propanol/tetrahydrofuran. The mixture was vortexed for 20 seconds at 3000 rpm and centrifuged for 10 minutes at 4300 rpm. The supernatant (2 µL) was injected for analysis.

Time (min) Flow (mL/min) %A %B
0.00 0.5 30 70
1.00 0.5 20 80
2.00 0.5 20 80
2.50 0.5 0 100
2.51 0.5 30 70
3.50 0.5 30 70