HPLC Application
ID No.: 19316

25-OH Vitamin D2 and D3 Control from Serum on Fully Porous 5µm C18 after PPT

Column: Luna® 5 µm C18(2) 100 Å, LC Column 50 x 2 mm, Ea
Dimensions: 50 x 2 mm ID
Order No: 008-4252-B0
Elution Type: Gradient
Eluent A: 0.05 % formic acid
Eluent B: 5 mM ammonium acetate + 0.1 % formic acid in methanol
Gradient

<table>
<thead>
<tr>
<th>Step No.</th>
<th>Time (min)</th>
<th>Pct A</th>
<th>Pct B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>205</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>290</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>360</td>
<td>92</td>
<td>8</td>
</tr>
</tbody>
</table>

Flow Rate: 1 mL/min
Col. Temp.: 35 °C
Detection: Mass Spectrometer (MS) @ amu (ambient)
Detector Info: SCIEX
Analyst Note:
- SecurityGuard™ Guard Cartridge System extends column lifetime.
  - SecurityGuard Cartridges, C18 4 x 2.0mm ID, 10/Pk Part No.: AJ0-4286
  - Holder Part No.: KJ0-4282

ANALYTES:

1. 25-Hydroxy vitamin D2 (25-OH D2) (MRM: 383.2 / 257.2)
   Retention Time: 0.83 min

2. 25-Hydroxy vitamin D3-d6 (25-OH D3-d6) (MRM: 389.3 / 263.3)
   Retention Time: 0.83 min

3. 25-Hydroxy vitamin D2 (25-OH D2) (MRM: 395.3 / 209.3)
   Retention Time: 0.86 min
Sample Preparation Details
for HPLC  Application ID No.: 19316

25-OH Vitamin D2 and D3 Control from Serum on Fully Porous 5µm C18 after PPT

PRODUCT DESCRIPTION:
Strata™-X 33 µm Polymeric Reversed Phase, 30 mg / 3 mL, Tubes, 50/Pk

Order No.: 8B-S100-TBJ

SOLID PHASE EXTRACTION (SPE) PROCEDURE:
Note: The solvent volumes shown below are for a 30 mg bed mass.
The solvent volumes will need to be adjusted for a smaller or larger bed mass.

Condition:
Load:
Wash:
Dry:
Elute:

Final Prep and Analysis:
Note: Avoid over drying eluates to avoid loss of analytes and/or internal standard, which will impact both precision and accuracy of data
Inject: 50 µL on HPLC Mass Spectrometer (MS) @ amu (ambient)

ANALYTES:

<table>
<thead>
<tr>
<th>Spiked Conc.</th>
<th>Log P</th>
<th>pKa</th>
<th>% Rec</th>
<th>%RSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>(ng/mL)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1 25-Hydroxy vitamin D2 (25-OH D2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 25-Hydroxy vitamin D3-d6 (25-OH D3-d6)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 25-Hydroxy vitamin D2 (25-OH D2)</td>
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</tbody>
</table>

Note: This method is designed as a convenient starting point for further investigation and can be tailored to meet your extraction goals.
Call your local Phenomenex Representative for assistance in method development and optimization techniques.

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