



Column: [Kinetex™ 2.6 μm C18 100 Å LC Column 50 x 4.6 mm](#)

Part No: [00B-4462-E0](#)

Dimensions: 50 x 4.6 mm ID

Elution Type: Gradient

Elution A: 0.05 % Formic acid

Elution B: 5 mM Ammonium acetate + 0.1 % Formic acid in methanol

Gradient Profile:

| Step No. | Time (min) | Pct A | Pct B |
|----------|------------|-------|-------|
| 1 | 0 | 92 | 8 |
| 2 | 5 | 92 | 8 |
| 3 | 205 | 0 | 100 |
| 4 | 290 | 0 | 100 |
| 5 | 360 | 92 | 8 |

Flow Rate: 1 mL/min

Col. Temp: 35 °C

Detection: Mass Spectrometer (MS) @ amu (450°C)

Compounds:

- 1- 25-hydroxyvitamin D3 (25-OH D3)
- 2- 25-hydroxyvitamin D3-d6 (25-OH D3-d6)
- 3- 25-hydroxyvitamin D2 (25-OH D2)