



**Column:** [Luna® 5 µm C18\(2\) 100 Å LC Column 50 x 2 mm](#)

**Part No:** [00B-4252-B0](#)

**Dimensions:** 50 x 2 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 (22°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)