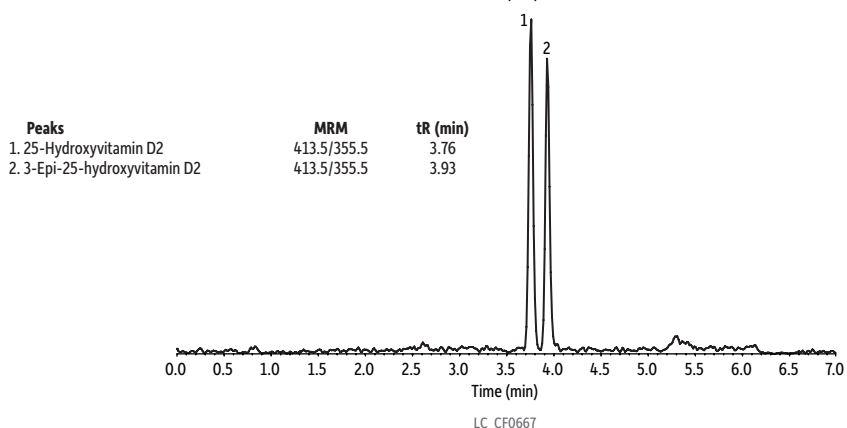
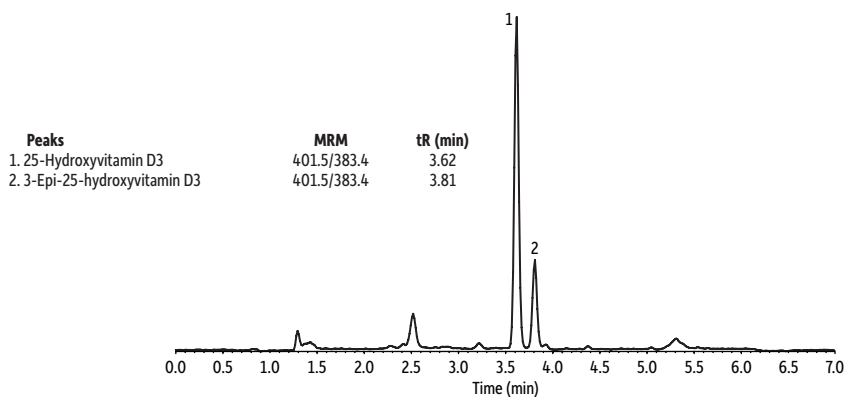


Analysis of C3-Epipimers of 25-Hydroxyvitamin D in 8 ng/mL Fortified Beagle Serum



Column Raptor FluoroPhenyl (cat.# 9319A1E)
Dimensions: 100 mm x 3 mm ID
Particle Size: 2.7 µm
Temp.: 30 °C
Standard/Sample Fortified beagle serum
Diluent: Water:methanol (50:50)
Conc.: 8 ng/mL fortified concentration
Inj. Vol.: 10 µL
Mobile Phase
A: 0.1% Formic acid in water
B: Methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	25	75
4.00	0.6	20	80
4.01	0.6	0	100
5.00	0.6	0	100
5.01	0.6	25	75
7.00	0.6	25	75

Detector MS/MS
Ion Mode: ESI+
Mode: MRM
Instrument UHPLC

Sample Preparation Sample matrix was fortified at the concentrations shown above and then extracted as follows. Fortified matrix (400 µL) was mixed with 0.2 M ZnSO₄ (400 µL) in a 4 mL glass vial. Then, 800 µL of methanol was added and the sample was vortex mixed for 10 seconds. Next, 2 mL of hexane was added and the sample was mixed for 90 seconds, followed by a 10-minute centrifugation at 4300 rpm. The hexane layer was then removed and evaporated to dryness under nitrogen at 55° C. The dried extract was reconstituted with 100 µL of a water:methanol (50:50) solution and injected for analysis.