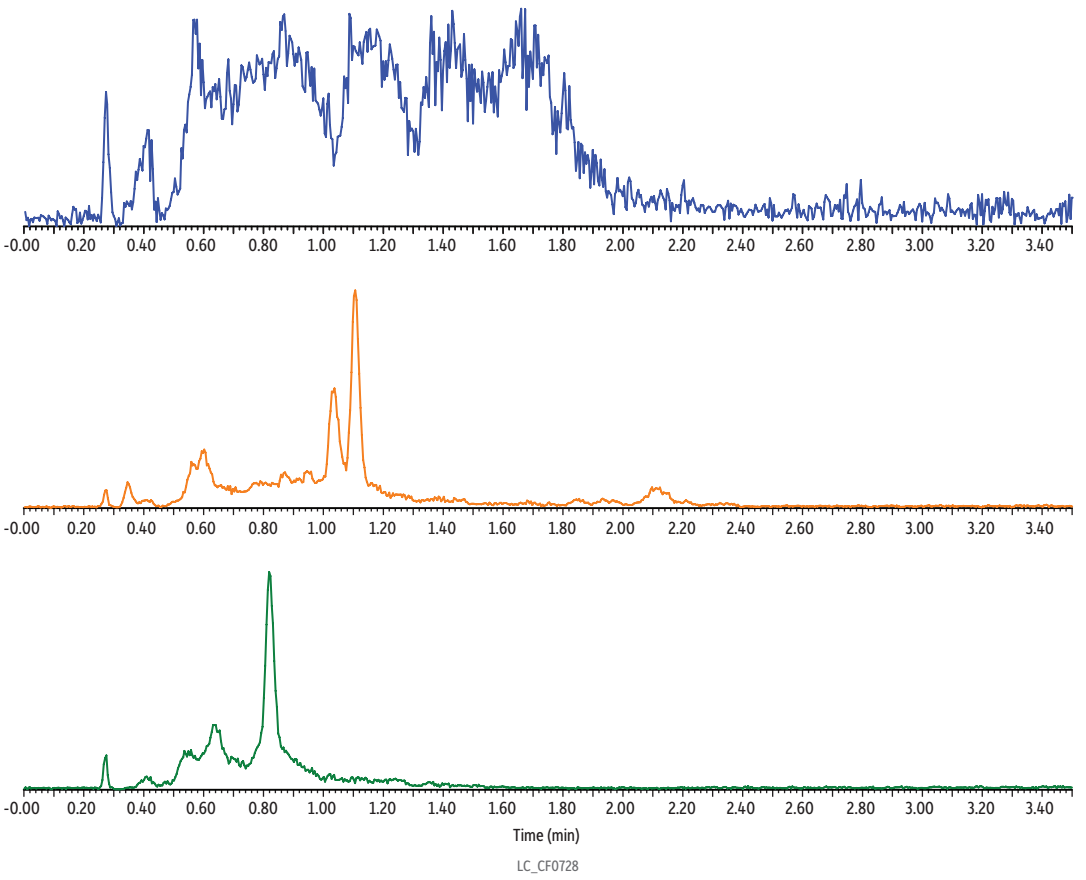
Vitamin K1 and K2 in Blank Artificial Serum on Raptor Biphenyl by LC-MS/MS



Peaks	Precursor Ion	Product Ion
1. Vitamin MK4	445.5	187.2
2. Vitamin K1	451.5	187.2
3. Vitamin MK7	649.7	187.2

Column Raptor Biphenyl (cat.# 9309A52)
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Temp.: 40 °C
Standard/Sample
Diluent: 15:85 Water:methanol
Conc.: Blank SeraFlx BIOMATRIX sample
Inj. Vol.: 5 µL
Mobile Phase
A: Water, 0.1% formic acid, 5 mM ammonium formate
B: Methanol, 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	10	90
1.00	0.4	0	100
3.00	0.4	0	100
3.01	0.4	10	90
4.00	0.4	10	90

Detector MS/MS
Ion Mode: ESI+
Mode: MRM
Instrument UHPLC
Sample Preparation A 500 µL aliquot of blank sample (SeraFlx BIOMATRIX) was mixed with 5 µL of internal standard solution (K1-d7, MK4-d7, and MK7-d7 at 100 ng/mL in methanol) and 1.5 mL of acetonitrile followed by vortexing for 20 seconds at 3000 rpm. After centrifugation at 4300 rpm for 10 minutes, the supernatant was loaded onto a Biotage ISOLUTE PLD+ 96-well plate (50 mg) and vacuum was applied to collect the eluate. The eluate was then evaporated to dryness at 50 °C under a gentle stream of nitrogen. The dried extract was reconstituted with 100 µL of diluent and 5 µL of sample was injected for analysis.