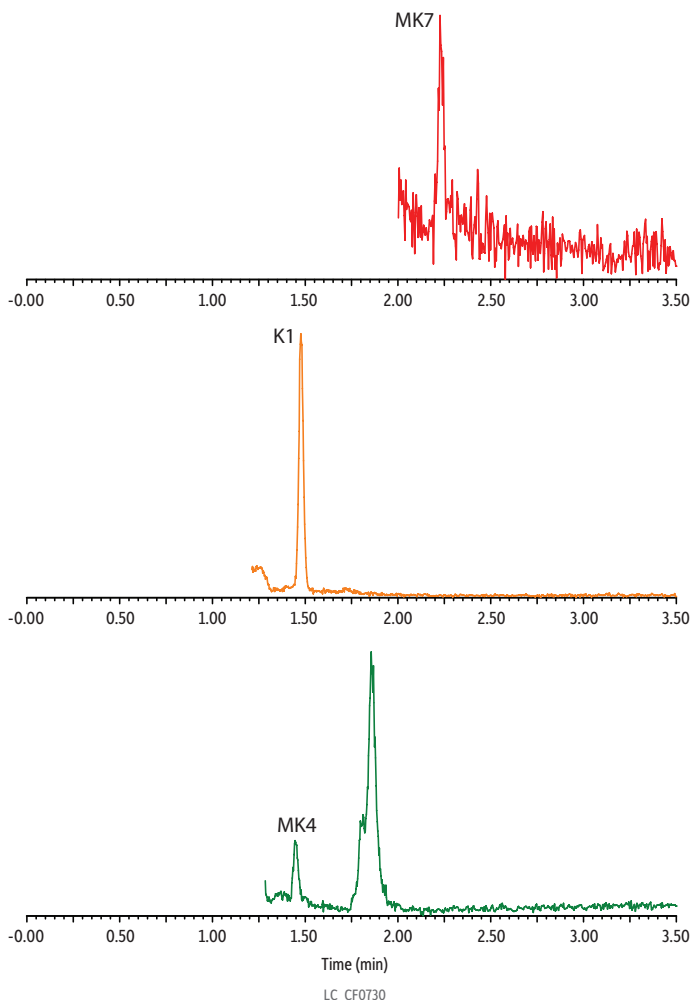


Endogenous Vitamin K1 and K2 in Caucasian Female Plasma (Patient Taking Vitamin K2 Medication) on Raptor Biphenyl

- Complete solution for Vitamin K1 and K2
- Fast, simple Biotage ISOLUTE PLD+ 96-well plate sample preparation removes phospholipids.
- Raptor Biphenyl column provides high sensitivity for trace-level analysis.



Peaks	tr (min)	Precursor Ion	Product Ion
1. Vitamin MK4	1.45	445.5	187.2
2. Vitamin K1	1.48	451.5	187.2
3. Vitamin MK7	2.23	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: 85:15 Methanol:water
 Conc.: Endogenous vitamin K1 and K2
 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 plasma sample 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.