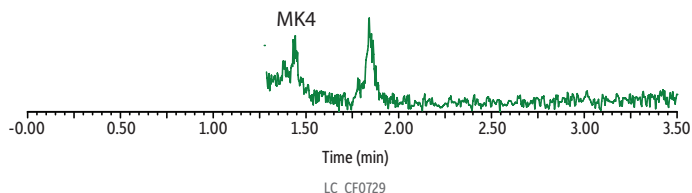
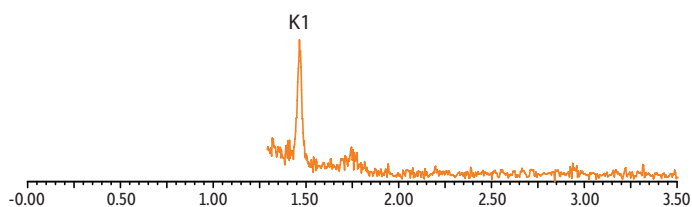
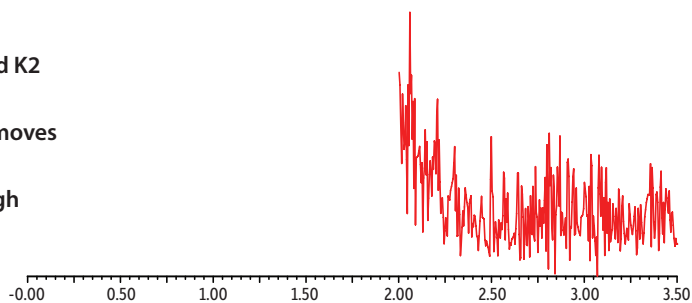


## Endogenous Vitamin K1 and K2 in Pooled Human Plasma on Raptor Biphenyl

- 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	1.44	445.5	187.2
2. Vitamin K1	1.46	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:** 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.