Separation of Vitamin K Compounds by LC-MS/MS Analysis

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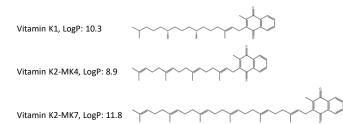
Overview

Vitamin Ks are a group of structurally similar, fat-soluble compounds. There are two natural forms: K1 (Phylloquinone) and K2 (Menaquinone), where the latter has short and longer chain homologues called Vitamin K2-MK4 and Vitamin K2-MK7, respectively. Due to the extremely hydrophobic nature and poor ionization characteristics of these compounds, LC-MS/MS analysis at low concentrations becomes challenging.

The core-shell Kinetex™ EVO C18 LC column, with its unique organo-silica grafting, has been found to be very successful for analytes with high LogP value. The organo-silica chemistry facilitates higher efficiency and sensitive detection capability for desired low level vitamin K analysis. In this application note, the separation of vitamin Ks was performed using the high efficiency Kinetex EVO C18 LC column. The results displayed good peak shape and peak height, resulting in a high signal/noise (S/N) ratio.



Figure 1. Structure and LogP Values for Vitamin K Analytes.



LC Conditions

Column: Kinetex 5 µm EVO C18

Dimensions: 50 x 2.1 mm Part No.: 00B-4633-AN Mobile Phase: A: Water

B: Acetonitrile / Isopropanol (1:1, v/v)

Gradient: Time (min) 70 n 1 70 3.50 3.51 70 70

Flow Rate: 0.6 mL/min hold for 1 min, then to 0.8

mL/min in 0.5 min

Injection Volume: 5 µL Temperature: 25 °C

LC System: Agilent® 1260 Infinity

Detection: MS/MS

Detector: SCIEX® 6500 Triple Quad™

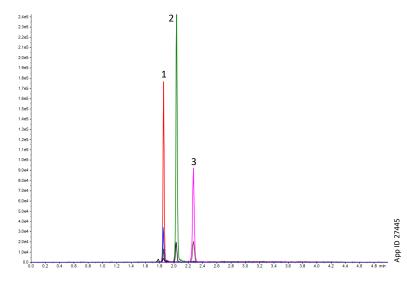
MS/MS Conditions

Ion Source: APCI Polarity: Positive Temperature: 350 °C **GS1**: 40 psi CUR: 25 psi IS: 5500 V

Table 1. MRM Transitions.

Analyte	Q1 (m/z)	Q3 (m/z)		
K2-MK4	445.4	187.1 227.1		
K1	451.3	187.2 199.2		
K2-MK7	649.5	187.0 227.2		

Figure 2. Representative Chromatogram of the Separation of Vitamin Ks.



Peak No.	Analyte	Retention Time (min)	Selectivity		Resolution		Efficiency	S/N
1	K2-MK4	1.9	1.1	-	7.9	-	60492	367
2	K1	2		1.1	7.9	8.7	29407	576
3	K2-MK7	2.3			-		45312	270

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