Vitamin K Isomers by Reverse Phase

Phytonadione separation by shape selectivity

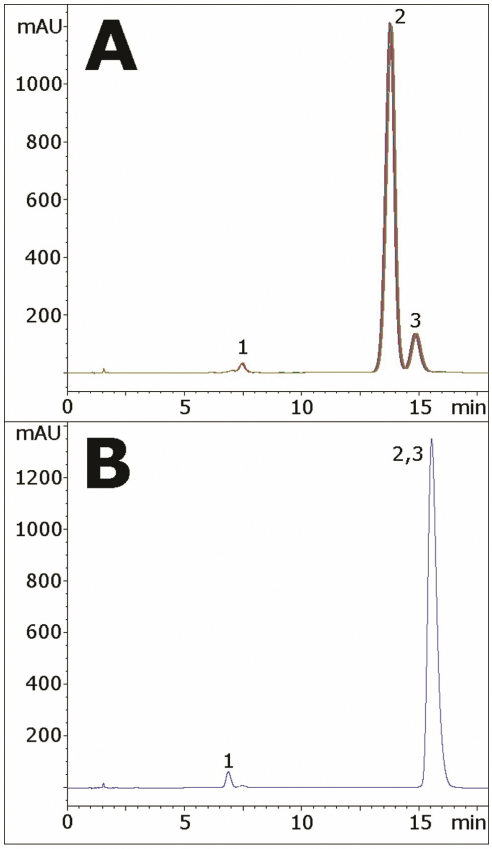
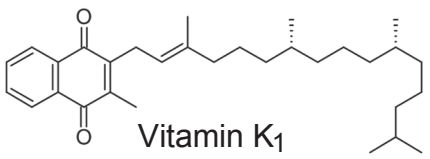


Fig. A: UDC-Cholesterol column, 5-run overlay
Fig. B: Ordinary C8 column



Note: Phytonadione (a.k.a. Phylloquinone, Vitamin K_1) is a lipophilic vitamin that can be obtained in the diet from leafy green vegetables. It plays an essential role in blood clotting by acting as a cofactor for formation of coagulation factors II, VII, IX, and X. The letter designation for Vitamin K was based on the first letter of "Koagulationsvitamin" (coagulation vitamin), which is from the German journal that first published its identification by Danish biochemist Henrik Dam.

Method Conditions

Column: **Cogent UDC-Cholesterol™**, 4µm, 100Å

Catalog No.: 69069-15P

Dimensions: 4.6 x 150 mm

Solvents: A: 50% DI H_2O / 50% MeOH / 0.1% formic acid
B: 97% acetonitrile / 3% DI H_2O / 0.1% formic acid

Gradient:

time (min.)	%B
0	80
15	92
16	80

Temperature: 12˚C

Post time: 2 min

Flow rate: 1.5 mL/min

Detection: UV 254 nm

Sample: **Stock Solution:** 10µL/mL phytonadione in acetonitrile diluent. (The solution was vortexed for 10 minutes.)
Working Solution: Stock solution was diluted 1:10 with acetonitrile.

Peaks: 1. Impurity
2. Phytonadione (E isomer)
3. Phytonadione (Z isomer)

t_0: 1.0 min

Discussion

Using the USP methodology, assay of Vitamin K_1 (phytonadione) is done in organic normal phase (ONP) with a bare silica column. This can be inconvenient for many laboratories which may not have a dedicated ONP instrument and therefore must spend both time and solvents to convert their reverse phase system to ONP for the analysis. In reverse phase, adequate separation between the E and Z isomers of phytonadione may not be obtained using conventional alkyl chain-based stationary phases (as Figure B illustrates). However, a resolution of 1.5 was obtained between isomers using the Cogent UDC-Cholesterol column (shown in Figure A), which meets the USP requirement for resolution. The Cogent UDC-Cholesterol is able to separate the two isomers on the basis of shape selectivity.

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