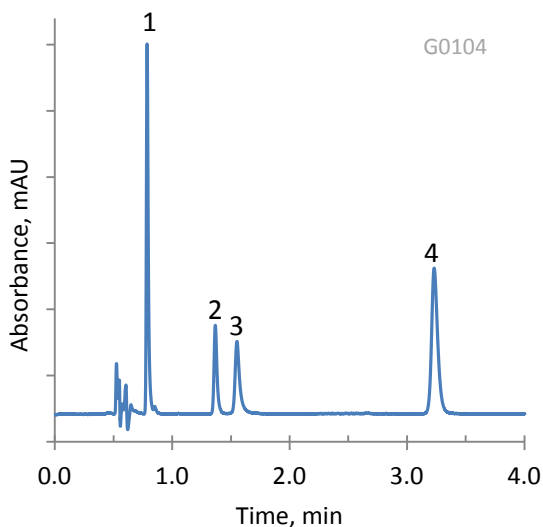


Application Note: 120-F

Separation of Water Soluble Vitamins on HALO 2 HILIC



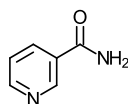
PEAK IDENTITIES:

1. Nicotinamide
2. Riboflavin
3. Ascorbic Acid
4. Nicotinic Acid

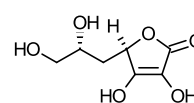
TEST CONDITIONS:

Column: 2.1 x 100 mm, HALO 2 HILIC
Part Number: 91812-601
Isocratic: 92/8: ACN/water with 5 mM Ammonium Formate, pH 3
Flow Rate: 0.5 mL/min.
Pressure: 220 bar
Temperature: 30°C
Detection: UV 265 nm, PDA
Injection Volume: 0.3 µL
Sample Solvent: 75/25: ACN/Methanol with 2% formic acid
Data Rate: 40 Hz
Response Time: 0.1 sec.
Flow Cell: 2.5 µL semi-micro
LC System: Agilent 1200 SL

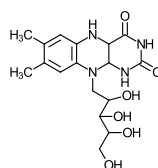
STRUCTURES:



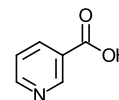
Nicotinamide



Ascorbic Acid



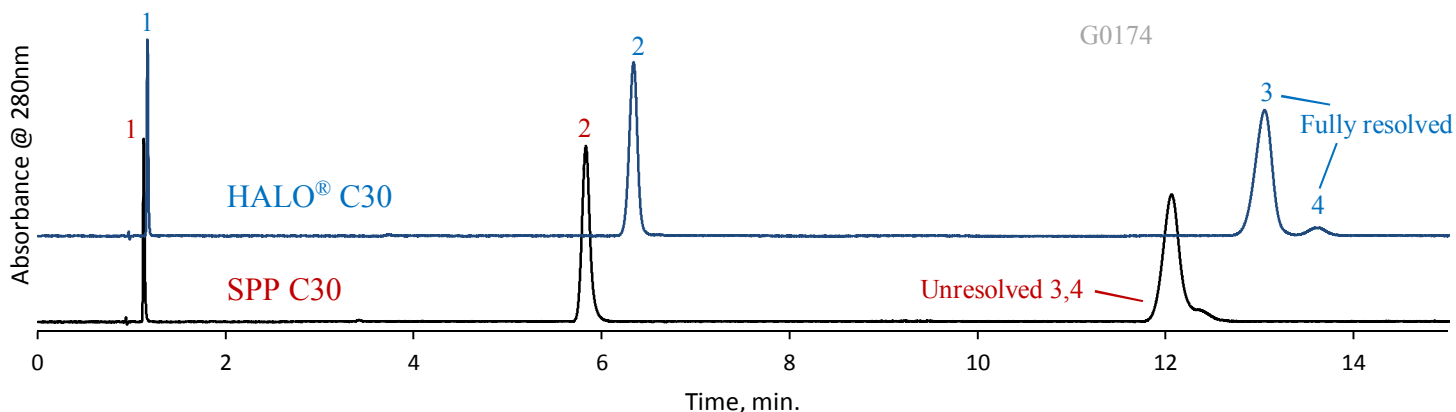
Riboflavin



Nicotinic Acid

A fast separation of water soluble vitamins is accomplished with a HALO 2 HILIC column.

Vitamin K1 Isomer Analysis on HALO® C30



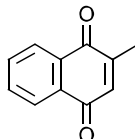
PEAK IDENTITIES:

- | | |
|-----------------------|---|
| 1. Menadione (K3) | 3. 2,3- <i>trans</i> -phyloquinone (K1) |
| 2. Menaquinone 4 (K2) | 4. <i>cis</i> -phyloquinone (K1) |

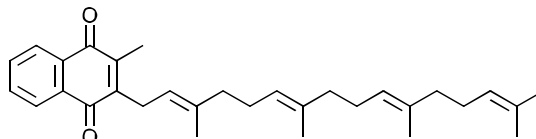
TEST CONDITIONS:

Column: HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm
 Part Number: 92114-730
 Mobile Phase A: Water
 Mobile Phase B: Methanol
 Isocratic: 95% B
 Flow Rate: 1.5 mL/min
 Initial HALO Pressure: 341 bar
 Initial Competitor Pressure: 371 bar
 Temperature: 25°C
 Detection: UV 280 nm, PDA
 Injection Volume: 1.0 µL
 Sample Solvent: Methanol
 Data Rate: 40 Hz
 Response Time: 0.025 sec.
 Flow Cell: 1 µL
 LC System: Shimadzu Nexera X2

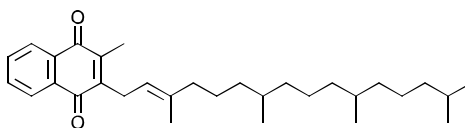
STRUCTURES:



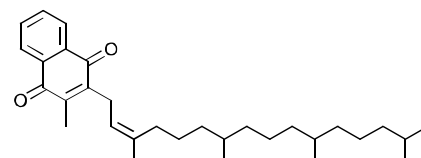
Vitamin K3: Menadione



Vitamin K2: Menaquinone 4



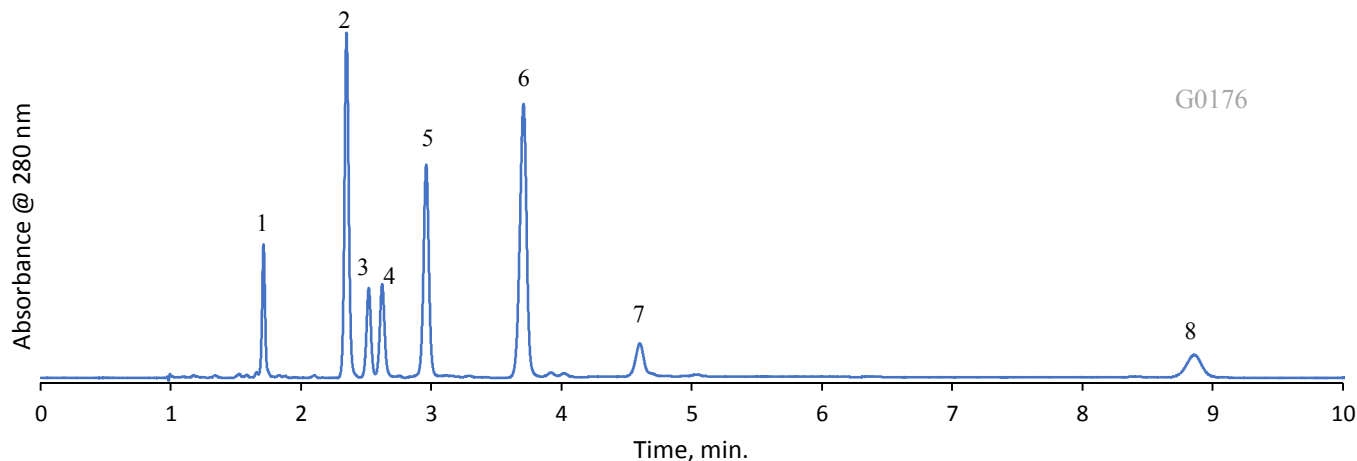
Vitamin K1: 2,3-*trans*-phyloquinone



Vitamin K1: *cis*-phyloquinone

Vitamin K, a fat-soluble vitamin, is beneficial for blood clotting and bone health. Vitamin K1 is produced from plants and can be found in high amounts in green vegetables. Vitamin K1 can also be converted into K2 within the body, while K3 is a synthetic form of vitamin K. The *cis* form of K1 is bio inactive so it is important to monitor how much is present in vitamin supplements. Baseline resolution of K1 isomers is obtained on a HALO® C30 column compared to a coelution on a competitor SPP C30 column.

Separation of Fat Soluble Vitamins on HALO® C30



TEST CONDITIONS:

Column: HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm
Part Number: 92114-730

Isocratic: 100% Methanol

Flow Rate: 1.5 mL/min

Pressure: 262 bar

Temperature: 30°C

Detection: UV 280 nm, PDA

Injection Volume: 2.0 µL

Sample Solvent: Methanol

Data Rate: 40 Hz

Response Time: 0.025 sec.

Flow Cell: 1 µL

LC System: Shimadzu Nexera X2

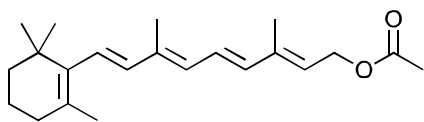
PEAK IDENTITIES:

1.	Retinyl acetate (A)	0.15 mg/mL
2.	Delta tocopherol (E)	0.08 mg/mL
3.	Ergocalciferol (D2)	0.08 mg/mL
4.	Cholecalciferol (D3)	0.08 mg/mL
5.	Alpha tocopherol (E)	0.08 mg/mL
6.	DL-alpha-tocopherol acetate (E)	0.08 mg/mL
7.	2,3- <i>trans</i> -phyloquinone (K)	0.31 mg/mL
8.	Retinyl palmitate (A)	0.15 mg/mL

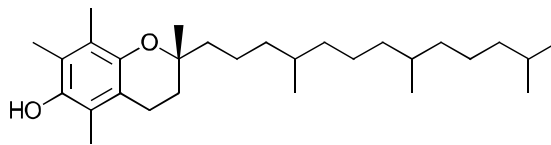
CONCENTRATION:

Fat soluble vitamins are stored in the liver and fatty tissue. These vitamins are essential to good health and contribute to several physiological functions, including bone growth, immune system regulation, cell division, and blood clotting. Vitamin E acts as an antioxidant. HALO® C30 enables a fast, efficient separation of a typical fat soluble vitamin panel in less than 9 minutes, while maintaining baseline resolution between vitamins D2 and D3.

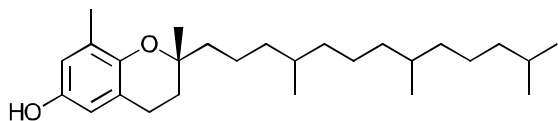
Fat Soluble Vitamin Structures



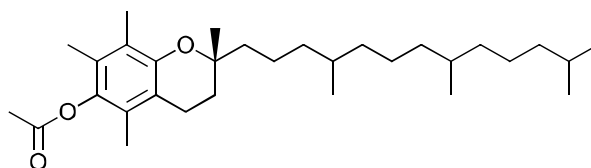
Retinyl acetate (A)



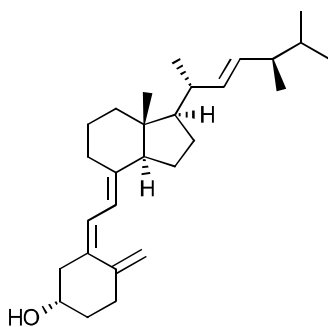
Alpha tocopherol (E)



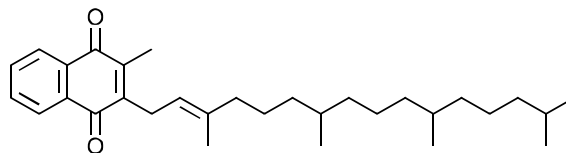
Delta tocopherol (E)



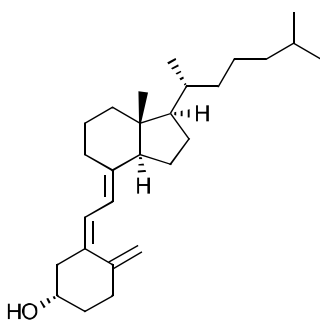
DL-alpha-tocopherol acetate (E)



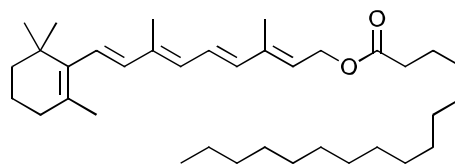
Ergocalciferol (D2)



2,3-trans-phyloquinone (K)



Cholecalciferol (D3)



Retinyl palmitate (A)