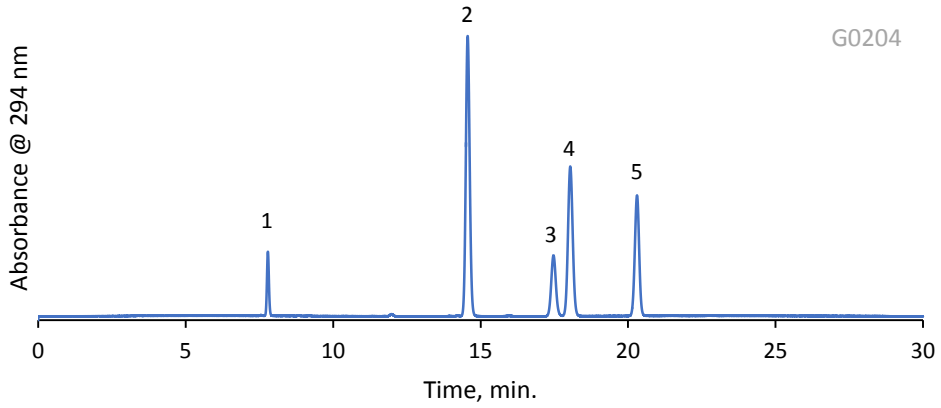
Application Note: 210-V

Analysis of Vitamin A and Vitamin E Isomers using GB Method



PEAK IDENTITIES:

1. Retinyl Acetate
2. δ- tocopherol
3. γ- tocopherol
4. β- tocopherol
5. α- tocopherol

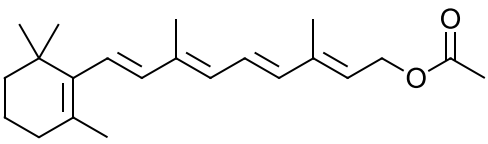
TEST CONDITIONS:

Column: HALO 160 Å C30, 2.7 µm, 4.6 x 250mm
Part Number: 92114-930
Mobile Phase A: Water
Mobile Phase B: Methanol
Gradient:

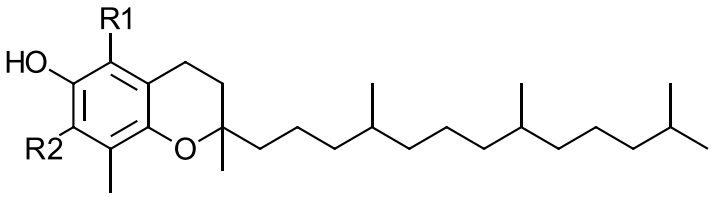
Time	%B
0.0	96
13.0	96
20.0	100
24.0	100
24.5	96
30.0	96

Flow Rate: 0.8 mL/min
Initial Pressure: 237 bar
Temperature: 20°C
Detection: 294 nm, PDA
Injection Volume: 10 µL
Sample Solvent: Methanol/ Ethanol
Data Rate: 14 Hz
Response Time: 0.12 sec.
Flow Cell: 5 µL semi-micro
LC System: Agilent 1100

STRUCTURES



Retinyl acetate

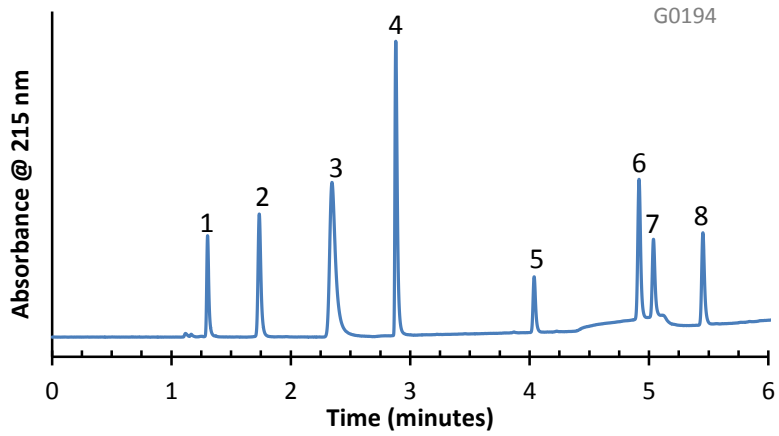


Tocopherol	R1	R2
Alpha (α)	CH_3	CH_3
Beta (β)	CH_3	H
Gamma (γ)	H	CH_3
Delta (δ)	H	H

The 2.7µm HALO® C30 is an ideal choice for the separation of vitamin A and the isomers of vitamin E using the official GB method. The shape selectivity of C30 allows for baseline resolution of gamma and beta tocopherol, which typically coelute on other bonded phases.

Application Note: 200-V

Separation of Water-Soluble Vitamins on HALO AQ-C18



PEAK IDENTITIES:

1. Thiamine (B1)
2. Ascorbic acid (C)
3. Nicotinamide (B3)
4. Pyridoxine (B6)
5. Pantothenic acid (B5)
6. Cyanocobalamin (B12)
7. Folic acid (B9)
8. Riboflavin (B2)

TEST CONDITIONS:

Column: HALO 90 Å AQ-C18, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-722
Mobile Phase: A/B
A= 0.025 M, potassium phosphate in water, pH=2.5
B= Methanol

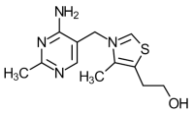
Gradient:

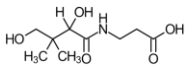
Time (min.)	%B
0.0	0
1.0	0
6.0	70
10.0	70

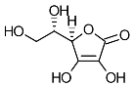
Flow Rate: 1.2 mL/min.
Initial pressure: 243 bar
Temperature: 30°C
Injection Volume: 2.0 µL
Sample Solvent: water
Detection: 215 nm, VWD
Response Time: 0.02 sec.
Data rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
ECV: ~14 µL

HALO AQ-C18 columns can be used with totally or mostly aqueous mobile phases. In this application, eight water-soluble vitamins are well-separated using this phase in under six minutes using a gradient from 0–70% methanol, with a 1-minute initial hold.

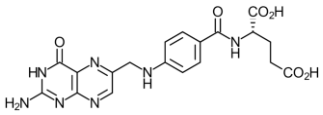
STRUCTURES:

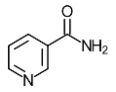

Thiamine

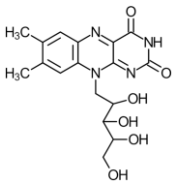

Pantothenic acid

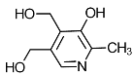

Ascorbic acid

Cyanocobalamin
(structure not included to space constraints)


Folic Acid


Nicotinamide

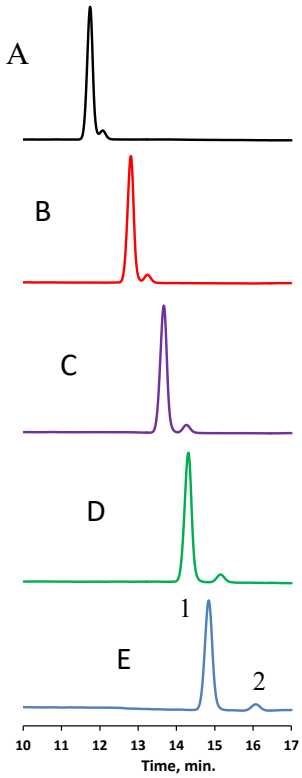

Riboflavin


Pyridoxine

FOR MORE INFORMATION OR TO PLACE AN ORDER, CONTACT:

Application Note: 197-V

Vitamin K1 Analysis: Temperature vs. Resolution



PEAK IDENTITIES:

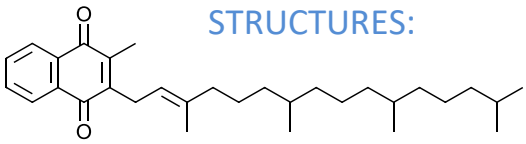
1. 2,3-*trans*-phylloquinone (K1)
2. *cis*-phylloquinone (K1)

	Resolution	Temperature
A	1.53	35°C
B	1.58	30°C
C	1.78	25°C
D	2.2	20°C
E	3.03	15°C

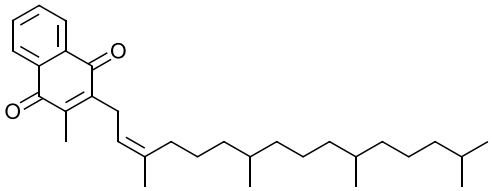
TEST CONDITIONS:

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
Back Pressure: 341 bar
Detection: 280 nm, PDA
Injection Volume: 1.0 µL
Sample Solvent: Methanol
Response Time: 0.12 sec.
Flow Cell: 5 µL Semi-Micro
LC System: Agilent 1100 Series

STRUCTURES:



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



Vitamin K1: *cis*-phylloquinone

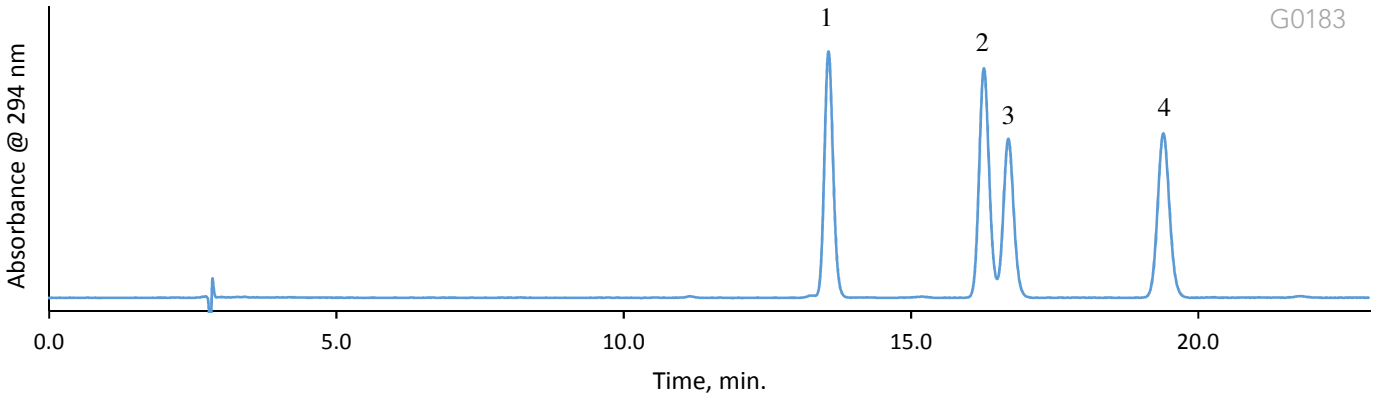
Vitamin K, a fat-soluble vitamin, is beneficial for blood clotting and bone health. Vitamin K1 is produced from plants and can be found in high amounts in green vegetables. Baseline resolution of the vitamin K1 isomers is increased as the temperature of the column decreases.

FOR MORE INFORMATION OR TO PLACE AN ORDER, CONTACT:

HALO® | Fused-Core® Particle Technology

Application Note: 189-V

Separation of Tocopherols on HALO® C30 based on GB (Chinese Standards)



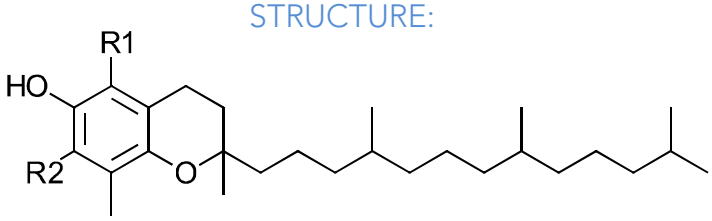
TEST CONDITIONS:

Columns: HALO 160 Å C30, 2.7 µm, 4.6 x 250mm
Part Number: 92114-930
Mobile Phase A: Water
Mobile Phase B: Methanol
Isocratic: 95% B
Flow Rate: 0.9 mL/min
Initial Pressure: 240 bar
Temperature: 30°C
Detection: UV 294 nm, PDA
Injection Volume: 20 µL
Sample Solvent: Methanol
Data Rate: 20 Hz
Response Time: 2 sec.
Flow Cell: 13 µL
LC System: Agilent 1100
Data Courtesy of Beijing Institute for Drug Control

PEAK IDENTITIES:

1. δ-tocopherol
2. γ- tocopherol
3. β- tocopherol
4. α- tocopherol

STRUCTURE:



Tocopherol	R1	R2
Alpha (α)	CH_3	CH_3
Beta (β)	CH_3	H
Gamma (γ)	H	CH_3
Delta (δ)	H	H

Tocopherols are forms of vitamin E (fat-soluble) that have antioxidant properties in both the human body and in food. They are also used for cosmetics and many personal care products. Here, tocopherols are separated on a 250 mm 160 Å pore size HALO® C30 column using a GB (Chinese standard) method. Due to the shape selectivity of the C30 phase, separation of the four isomers is achieved.

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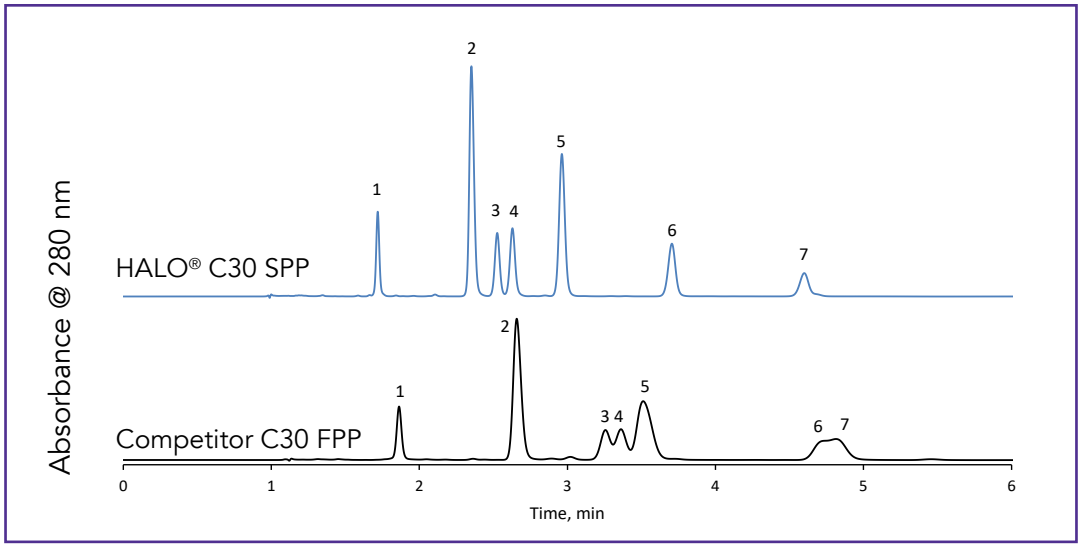
FOR MORE INFORMATION OR TO PLACE AN ORDER, CONTACT:

HALO®

VITAMINS

Separation of Fat Soluble Vitamins: SPP vs. FPP

187-V



TEST CONDITIONS:

Column: HALO® C30, 2.7 µm, 4.6 x 150 mm
Part Number: 92114-730
Competitor: FPP C30, 3.0 µm, 4.6 x 150 mm
Isocratic: 100% Methanol
Flow Rate: 1.5 mL/min
Initial HALO® Pressure: 262 bar
Initial Competitor FPP Pressure: 207 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 (Vitamin A)
2. Delta tocopherol (Vitamin E)
3. Ergocalciferol (Vitamin D2)
4. Cholecalciferol (Vitamin D3)
5. Alpha tocopherol (Vitamin E)
6. dl-Alpha-tocopherol acetate (Vitamin E)
7. 2,3-trans-phylloquinone (Vitamin K1)

	Peak 2: Delta Tocopherol	Peak 3/4: D2/ D3
	Efficiency	Resolution
HALO SPP	24785	1.62
FPP	11391	0.87

A mixture of fat-soluble vitamins is separated using a superficially porous particle HALO® C30 column and a competitor's fully porous (FPP) C30 column. Efficiency, peak tailing, and peak width are all improved when using the SPP technology along with better resolution.

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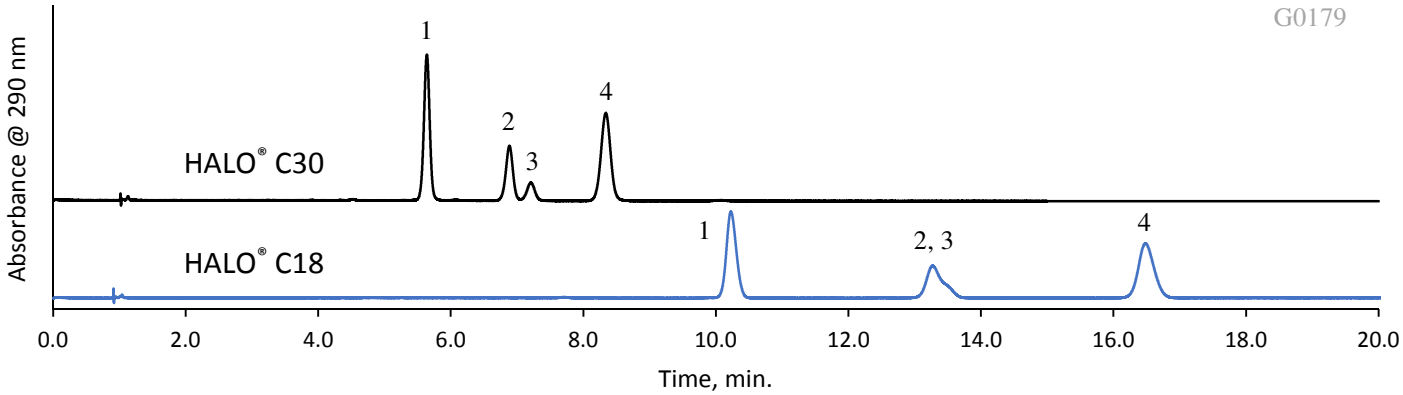
PERFORMANCE YOU CAN RELY ON

HALO® | Fused-Core® Particle Technology

Application Note: 185-V

Separation of Tocopherols on HALO® C30



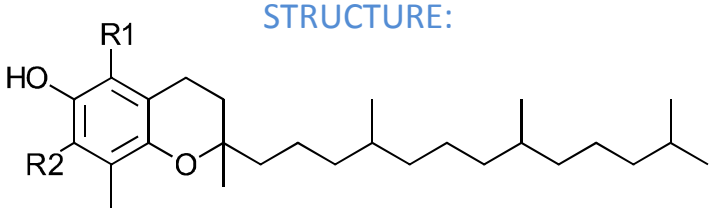
TEST CONDITIONS:

Columns: HALO 160 Å C30, 2.7 µm, 4.6 x 150mm
Part Number: 92114-730
HALO 90 Å C18, 2.7 µm, 4.6 x 150mm
Part Number: 92814-702
Mobile Phase A: Water
Mobile Phase B: Methanol
Isocratic: 95% B
Flow Rate: 1.5 mL/min
C30 Pressure: 337 bar
C18 Pressure: 348 bar
Temperature: 10°C
Detection: UV 290 nm, PDA
Injection Volume: 1.5 µL
Sample Solvent: Ethanol/ Methanol
Data Rate: 80 Hz
Response Time: 0.02 sec
Flow Cell: 2 µL
LC System: Agilent 1200 SL

PEAK IDENTITIES:

1. δ-tocopherol
2. γ- tocopherol
3. β- tocopherol
4. α- tocopherol

STRUCTURE:



Tocopherol	R1	R2
Alpha (α)	CH_3	CH_3
Beta (β)	CH_3	H
Gamma (γ)	H	CH_3
Delta (δ)	H	H

Tocopherols are a form of vitamin E (fat-soluble) that have antioxidant properties in both the body and in food. They are also used for cosmetics and many personal care products. Here, tocopherols are separated on a 160 Å pore size HALO® C30 column with baseline resolution between the beta and gamma isomers compared to a 90 Å HALO® C18 column. While the HALO® C18 has more surface area (135 m²/g vs. 90 m²/g) and exhibits twice the retention, it produces a coelution of the isomers. Due to the C30's shape selectivity, complete separation of the isomers is achieved.

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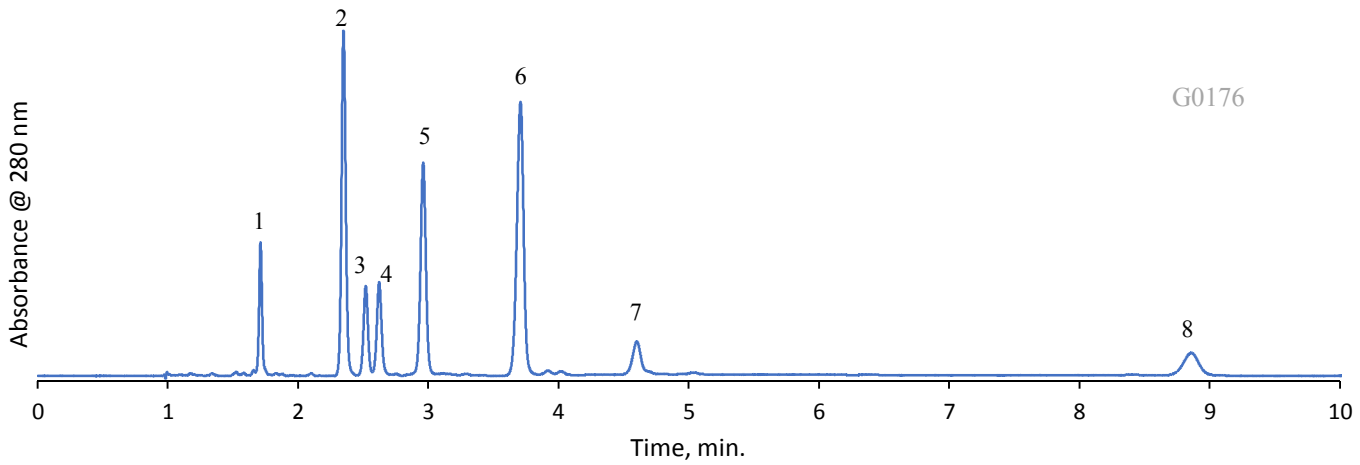
FOR MORE INFORMATION OR TO PLACE AN ORDER, CONTACT:

Application Note: 182-V

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

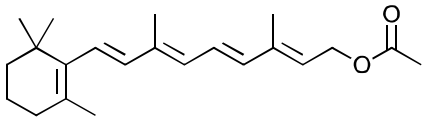
PEAK IDENTITIES:	CONCENTRATION:
1. Retinyl acetate (A)	0.15 mg/mL
2. Delta tocopherol (E)	0.08 mg/mL
3. Ergocalciferol (D2)	0.08 mg/mL
4. Cholecalciferol (D3)	0.08 mg/mL
5. Alpha tocopherol (E)	0.08 mg/mL
6. DL-alpha-tocopherol acetate (E)	0.08 mg/mL
7. 2,3-*trans*-phylloquinone (K)	0.31 mg/mL
8. Retinyl palmitate (A)	0.15 mg/mL

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.

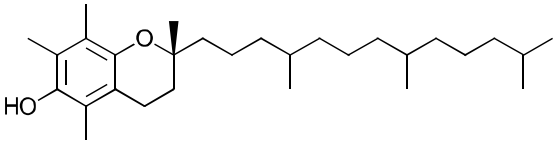
FOR MORE INFORMATION OR TO PLACE AN ORDER, CONTACT:

Application Note: 182-V

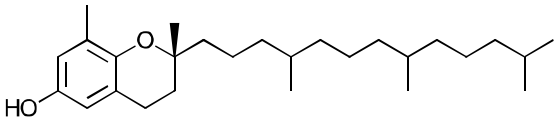
Fat Soluble Vitamin Structures



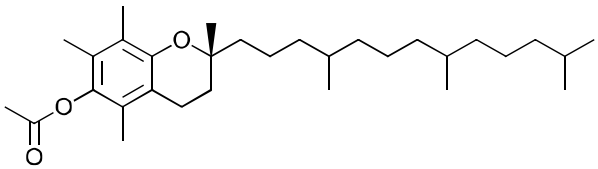
Retinyl acetate (A)



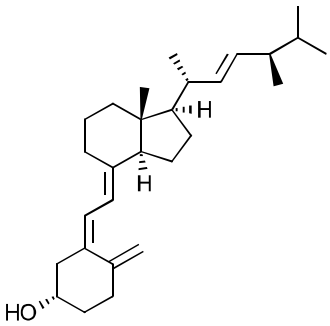
Alpha tocopherol (E)



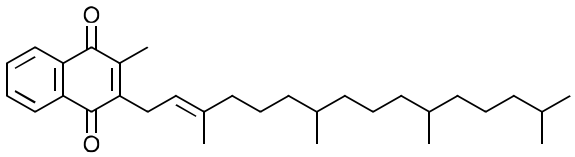
Delta tocopherol (E)



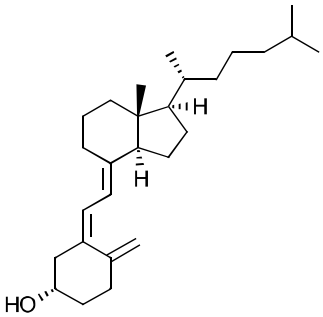
DL-alpha-tocopherol acetate (E)



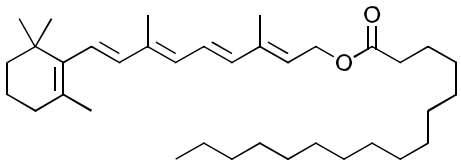
Ergocalciferol (D2)



2,3-*trans*-phylloquinone (K)



Cholecalciferol (D3)

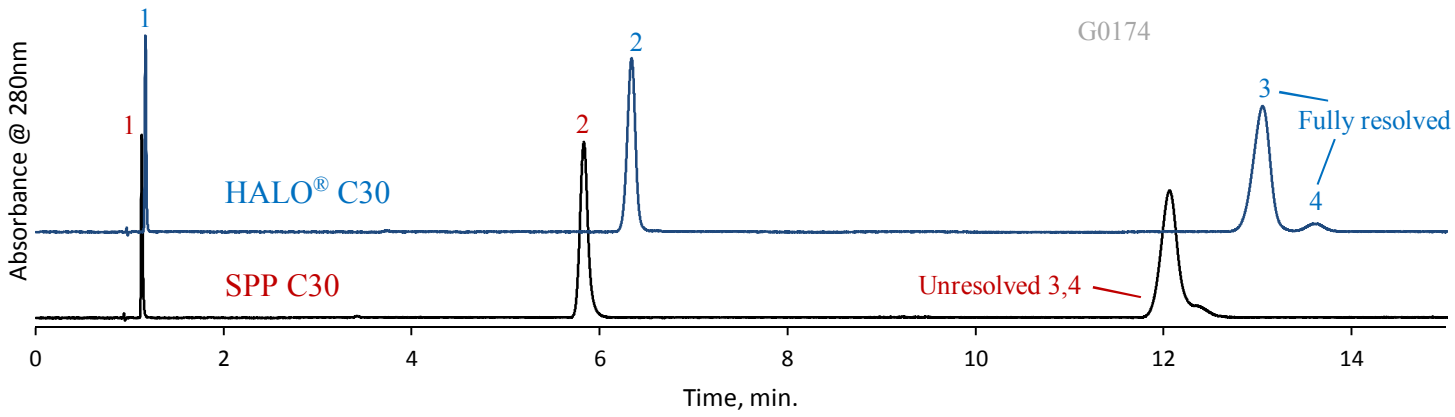


Retinyl palmitate (A)

HALO® | Fused-Core® Particle Technology

Application Note: 180-V

Vitamin K1 Isomer Analysis on HALO® C30



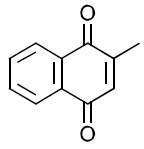
PEAK IDENTITIES:

1. Menadione (K3)
2. Menaquinone 4 (K2)
3. 2,3-*trans*-phylloquinone (K1)
4. *cis*-phylloquinone (K1)

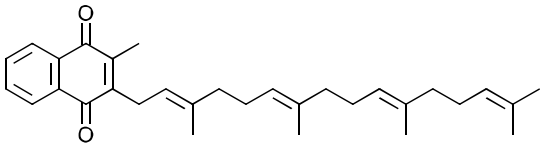
TEST CONDITIONS:

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

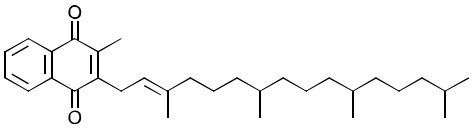
STRUCTURES:



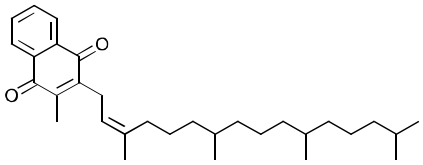
Vitamin K3: Menadione



Vitamin K2: Menaquinone 4



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



Vitamin K1: *cis*-phylloquinone

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.

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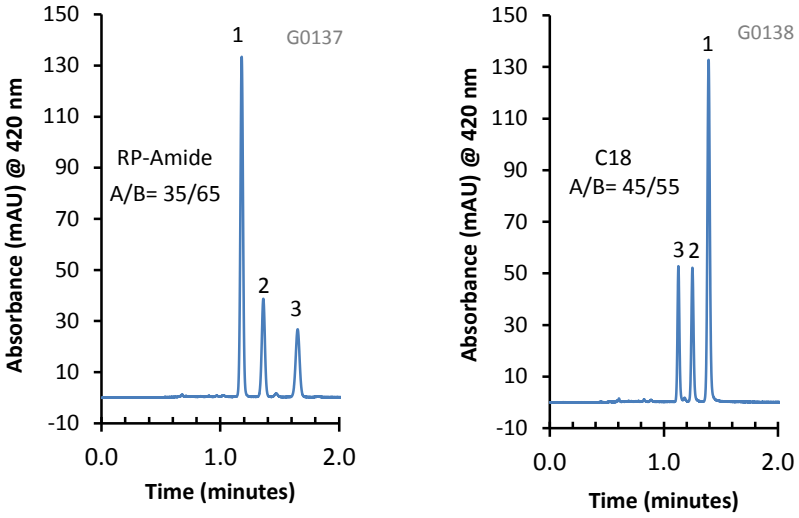
FOR MORE INFORMATION OR TO PLACE AN ORDER, CONTACT:

Application Note: 148-F

Analysis of Curcumins on HALO RP-Amide and HALO C18



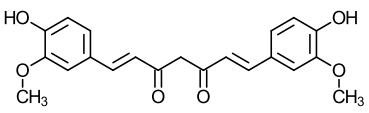
PEAK IDENTITIES:

1 Curcumin
2. Desmethoxycurcumin
3. *bis*-Desmethoxycurcumin

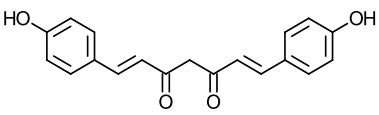
TEST CONDITIONS:

Column: 4.6 x 100 mm, HALO, 2.7 µm
Part Number: 92814-602
Part Number: 92814-607
Mobile Phase: A/B: See chromatograms
A= 0.025M phosphate buffer in water, pH=3
B= Acetonitrile
Flow Rate: 1.8 mL/min.
Pressure: 215 bar
Temperature: 35°C
Detection: UV 420 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: methanol
Response Time: 0.02 sec.
Data rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
ECV: ~14 µL

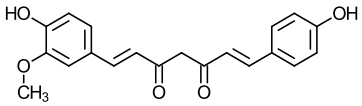
STRUCTURES:



Curcumin



bis-Desmethoxycurcumin



Desmethoxycurcumin

Curcumin isomers were extracted from commercial turmeric spice by adding 0.42 g of as-received turmeric to 20 mL of methanol in a vial. The mixture was vortexed and then sonicated for 5 minutes and allowed to stand overnight. After vortexing and settling, an aliquot of the supernate was filtered through a 0.2 µm porosity Teflon syringe filter. A sample of this clear orange liquid was diluted 1:4 with methanol for injection. The chromatograms show a very different selectivity for the curcumin compounds on the two phases. This difference in selectivity for hydroxy-substituted compounds can be exploited, especially using mobile phases containing acetonitrile.

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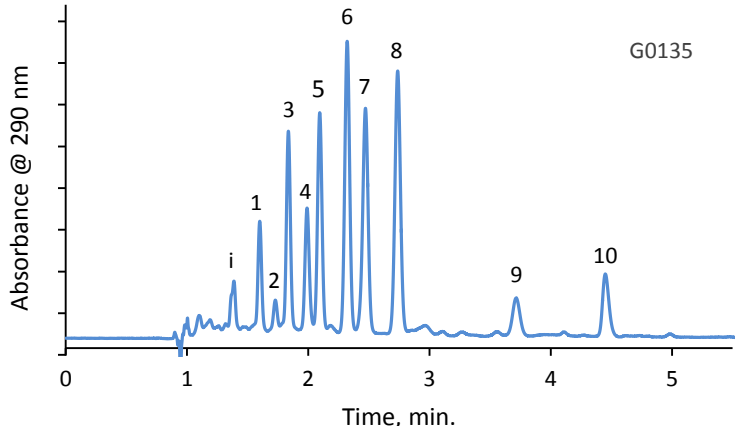
FOR MORE INFORMATION OR TO PLACE AN ORDER, CONTACT:

Application Note: 146-V

Rapid Separation of Vitamin E Congeners on HALO PFP



PEAK IDENTITIES:

1. δ-Tocotrienol
2. β-Tocotrienol
3. γ-Tocotrienol
4. α-Tocotrienol
5. δ-Tocopherol
6. β-Tocopherol
7. γ-Tocopherol
8. α-Tocopherol
9. α-Tocopherol acetate
10. α-Tocopherol nicotinate

i = impurity

TEST CONDITIONS:

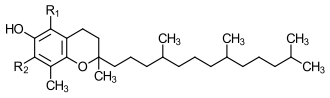
Column: HALO PFP, 4.6 x 150 mm, 2.7 µm
Part Number: 92814-709
A= Water
B= Methanol
Gradient:

Time (min.)	%B
0.00	92
2.75	92
3.00	95
5.00	95

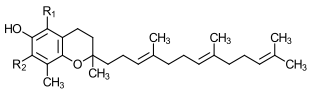
Flow Rate: 1.5 mL/min.
Pressure: 380 bar
Temperature: 25 °C
Injection Volume: 5 µL
Sample Solvent: Ethanol
Detection: UV 290 nm, PDA
Data Rate: 40 Hz
Response Time: 0.05 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

STRUCTURES:

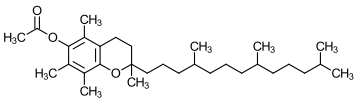
Tocopherol/Tocotrienol	R_1	R_2
Alpha (α)	CH_3	CH_3
Beta (β)	CH_3	H
Gamma (γ)	H	CH_3
Delta (δ)	H	H



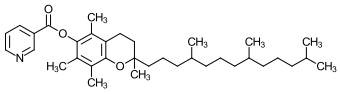
Tocopherol



Tocotrienol



α-Tocopherol acetate



α-Tocopherol nicotinate

Vitamin E capsules can contain up to eight related, but different constituents, including up to four tocopherols and four tocotrienols. Ester derivatives of Vitamin E are made to increase the stability of the compound. Vitamin E is important for its antioxidant properties in both the body and in food and cosmetics.
The sample used for analysis was combination of standards and a vitamin supplement purchased locally. The soft gel vitamin supplement contained the four tocotrienols and α-tocopherol. Only the liquid in the soft gel was used for the analysis. The four tocopherols, α-tocopherol acetate, and α-tocopherol nicotinate were standards obtained from Sigma-Aldrich. The small, unidentified peaks are unknown materials from the soft gel capsule.

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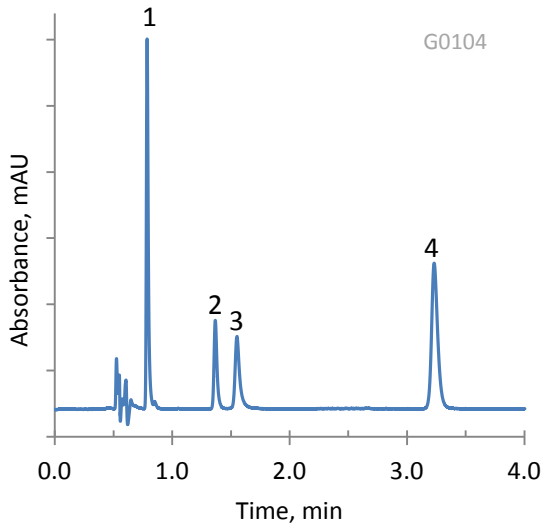
FOR MORE INFORMATION OR TO PLACE AN ORDER, CONTACT:

Application Note: 120-F

Separation of Water Soluble Vitamins on HALO 2 HILIC



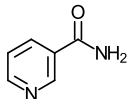
PEAK IDENTITIES:

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

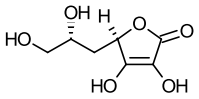
TEST CONDITIONS:

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

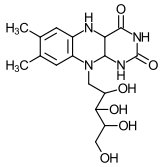
STRUCTURES:



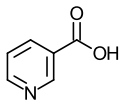
Nicotinamide



Ascorbic Acid



Riboflavin



Nicotinic Acid

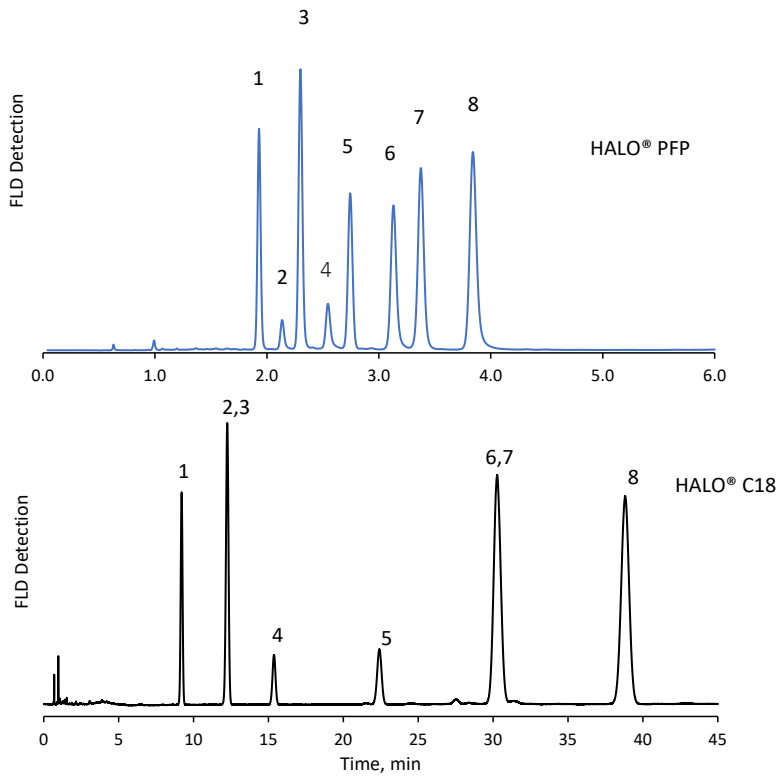
A fast separation of water soluble vitamins is accomplished with a HALO 2 HILIC column.

HALO®

VITAMINS

Phase Comparison for Tocopherols and Tocotrienols

242-V



PEAK IDENTITIES

1. δ-tocotrienol
2. β-tocotrienol
3. γ-tocotrienol
4. α-tocotrienol

TEST CONDITIONS:

Column: HALO 90 Å PFP, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-709
Column: HALO 90 Å C18, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-702
Mobile Phase A: Water
B: Methanol
Isocratic: 90 %B
Flow Rate: 1.5 mL/min
Initial Back Pressure: 383 bar
Temperature: 25 °C
Detection: FLD: Ex: 296/ Em: 325
Injection Volume: 1.0 µL
Sample Solvent: Methanol
Data Rate: 100 Hz
LC System: Shimadzu Nexera X2

Tocopherols and tocotrienols are a form of Vitamin E (fat-soluble) that have antioxidant properties in both the body and in food. They are also used for cosmetics and many personal care products. A separation of tocopherols and tocotrienols is performed on a HALO® PFP and C18 column. The PFP column shows 10x faster run times along with baseline resolution compared to the C18 column under the same testing conditions.

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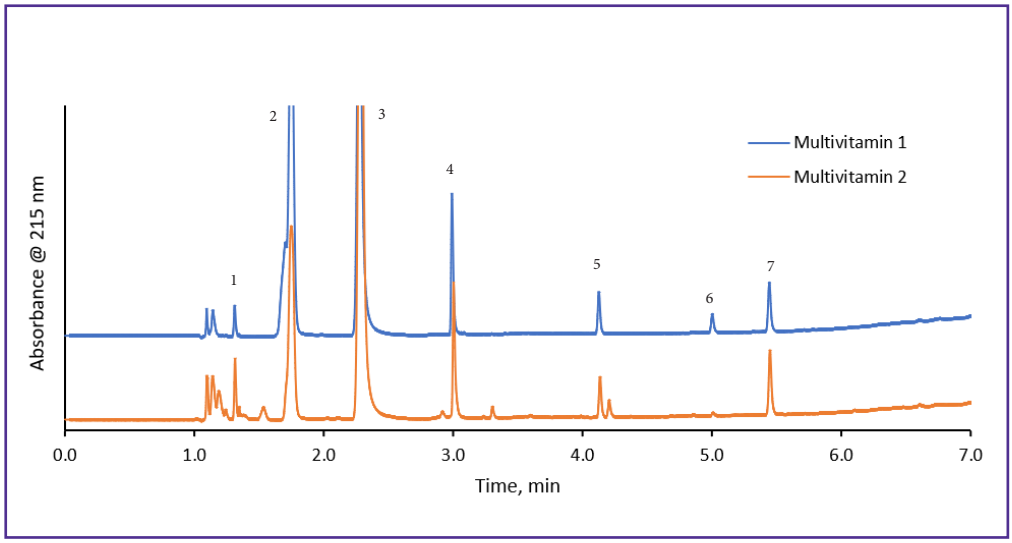
VITAMINS

Separation of Water Soluble Vitamins Found in Multivitamins

253-V



PEAK IDENTITIES

1. Thiamine (B1)
2. Ascorbic acid (C)
3. Nicotinamide (B3)
4. Pyridoxine (B6)
5. Pantothenic acid (B5)
6. Folic acid (B9)
7. Riboflavin (B2)

TEST CONDITIONS:

Column: HALO 90 Å AQ-C18, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-722
Mobile Phase A: 25mM Potassium Phosphate, pH: 2.5
Mobile Phase B: Methanol
Gradient:

Time (min)	%B
0.0	0
1.0	0
6.0	70
10.0	70

Flow Rate: 1.2 mL/min
Initial Back Pressure: 243 bar
Temperature: 30 °C
Detection: UV 215 nm, PDA
Injection Volume: 2.0 µL
Sample Solvent: Water
Data Rate: 100 Hz
LC System: Shimadzu Nexera X2

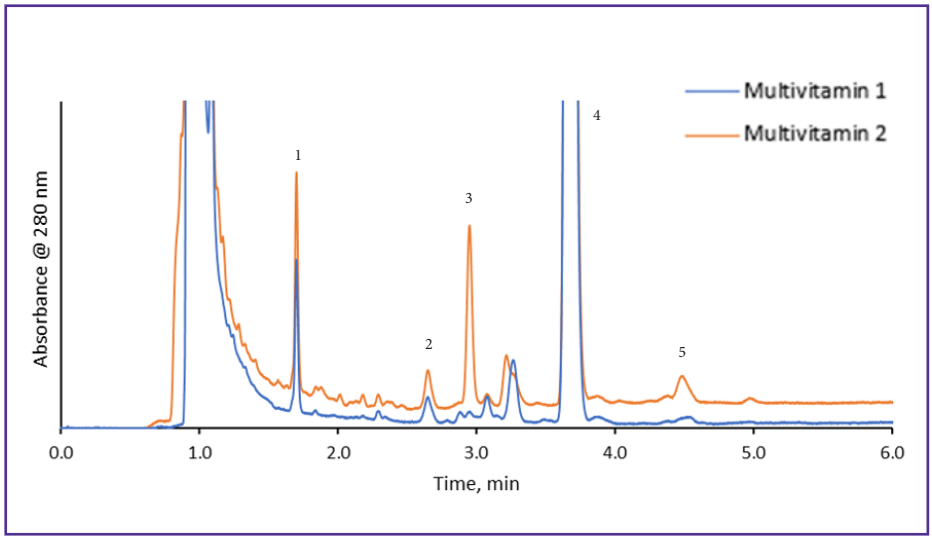
HALO® AQ-C18 columns can be used with high or completely aqueous mobile phases making the column an ideal candidate for separating water-soluble vitamins. Seven water-soluble multivitamins are well-separated from multivitamin tablets in under six minutes using a 100% aqueous isocratic hold. Minor differences are seen between the two samples, varying in each component's concentration.

HALO®

VITAMINS

Separation of Fat Soluble Vitamins Found in Multivitamins

254-V



PEAK IDENTITIES

1. Retinyl acetate (A)
2. Cholecalciferol (D3)
3. Alpha tocopherol (E)
4. DL-alpha tocopherol acetate (E)
5. 2,3-trans-phylloquinone (K)

TEST CONDITIONS:

Column: HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm
Part Number: 92114-730
Isocratic: Methanol
Flow Rate: 1.5 mL/min
Initial Back Pressure: 262 bar
Temperature: 30 °C
Detection: UV 280 nm, PDA
Injection Volume: 2.0 µL
Sample Solvent: Methanol
Data Rate: 100 Hz
LC System: Shimadzu Nexera X2

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. HALO® C30 enables a fast, efficient separation of fat soluble vitamins in two different multivitamin tablets. The column is capable of identifying differences between the two tablets, which at first glance may seem similar due to the solvent front and the high abundance of DL-alpha tocopherol acetate (E). Upon closer inspection, differences in the concentrations of the relatively minor peaks, particularly for alpha-tocopherol are clearly evident. Such capabilities are vital to confirm the food label content information. Also, in some extreme cases, it could be crucial to verify the identity of a multi-vitamin e.g. fradulent re-labelling of cheaper tablets as higher priced products.

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