



Superficially Porous Particles with C30 Stationary Phase for High Resolution Separations

Stephanie A. Schuster

Conner McHale, Andrew Harron

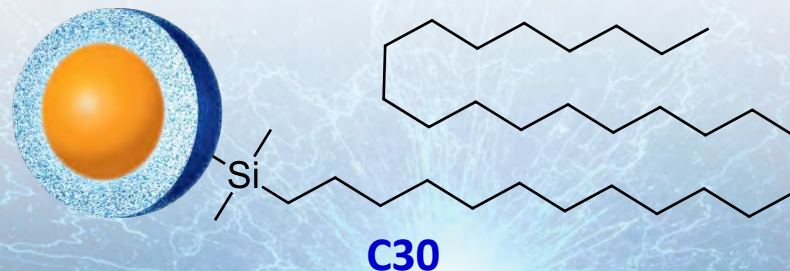
Advanced Materials Technology, Inc., Wilmington, DE 19810

Eastern Analytical Symposium

November 19, 2019

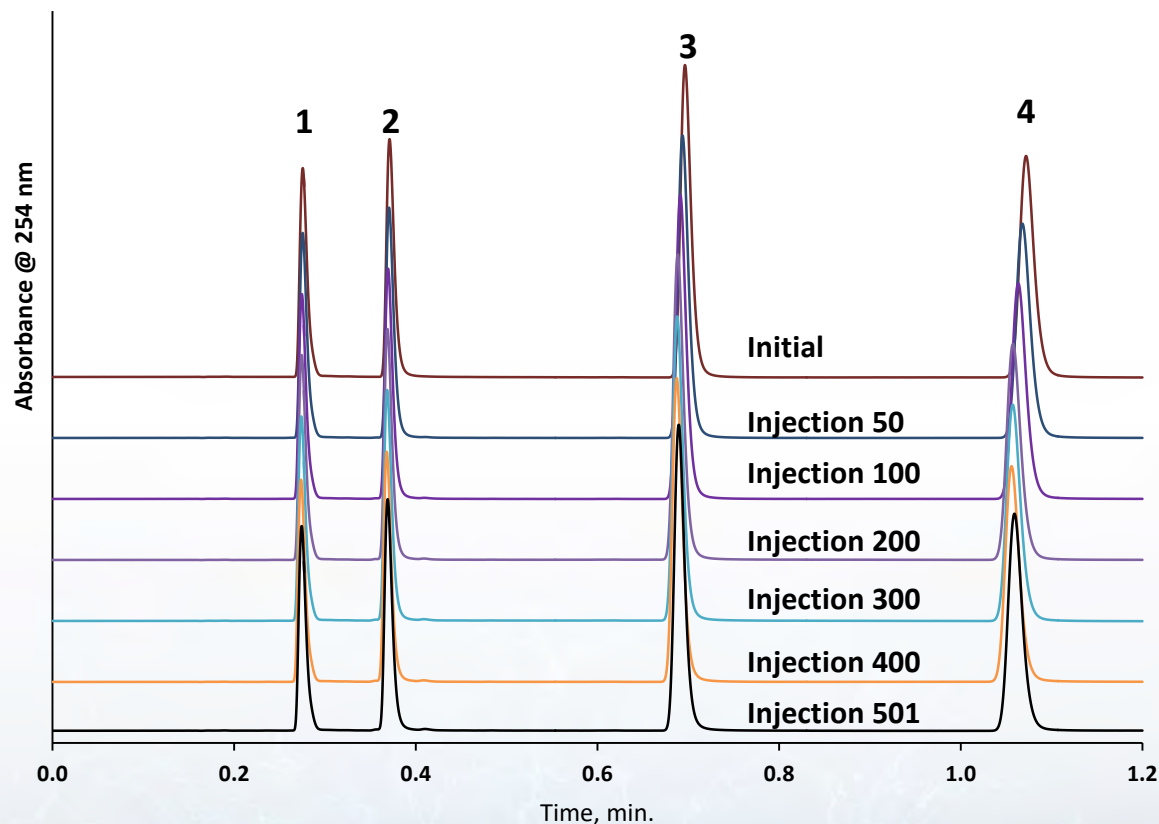
HALO[®] C30: Properties

- High shape selectivity for hydrophobic, long-chain structurally related isomers
- Endcapped phase with 4.5% carbon
- Low pH Limit /Max T: 2/60°C
- High pH Limit/Max T: 9/40°C
- Surface area: 90 m²/g
- 100% aqueous compatible
- USP designation L62



Stability at High Temperature and High Pressure

Excellent stability at 600 bar and 60 °C



Column: HALO 160 Å C30, 2.7 µm 2.1 x 50 mm

Isocratic: 50/50 ACN/H₂O

Flow Rate: 1.1 mL/min

Back Pressure: 602 bar

Temperature: 60 °C

Injection Volume: 1 µL

Instrument: Shimadzu Nexera X2

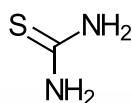
Detection: UV 254 nm, PDA

PEAK IDENTITIES:

1. Uracil
2. Phenol
3. 1-chloro-4-Nitrobenzene
4. Naphthalene

HALO® C30: 100% Aqueous Compatibility

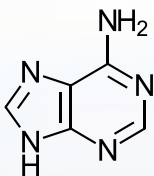
1. Thiourea
2. 5-Fluorocytosine
3. Adenine
4. Thymine



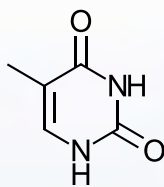
Thiourea



5-Fluorocytosine

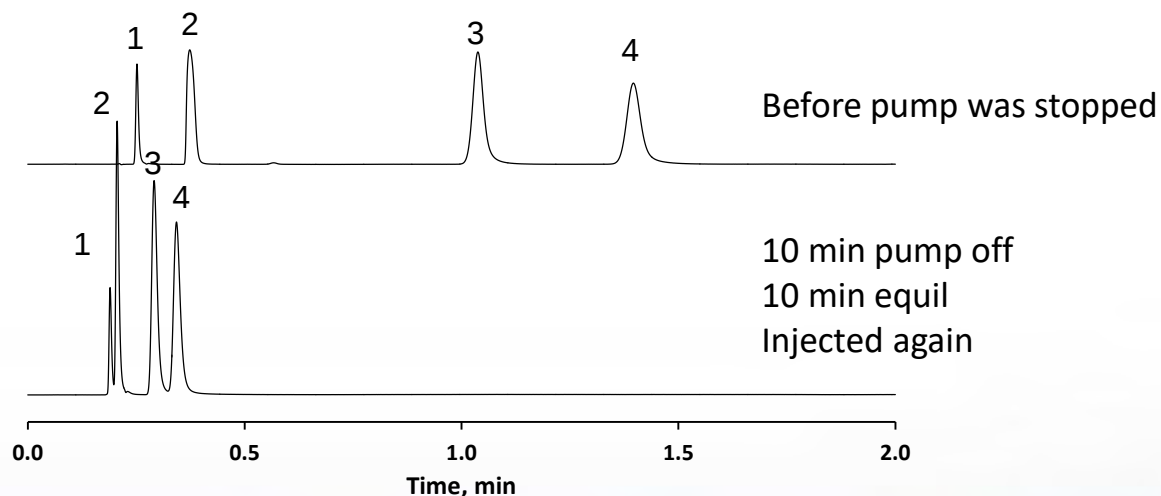


Adenine



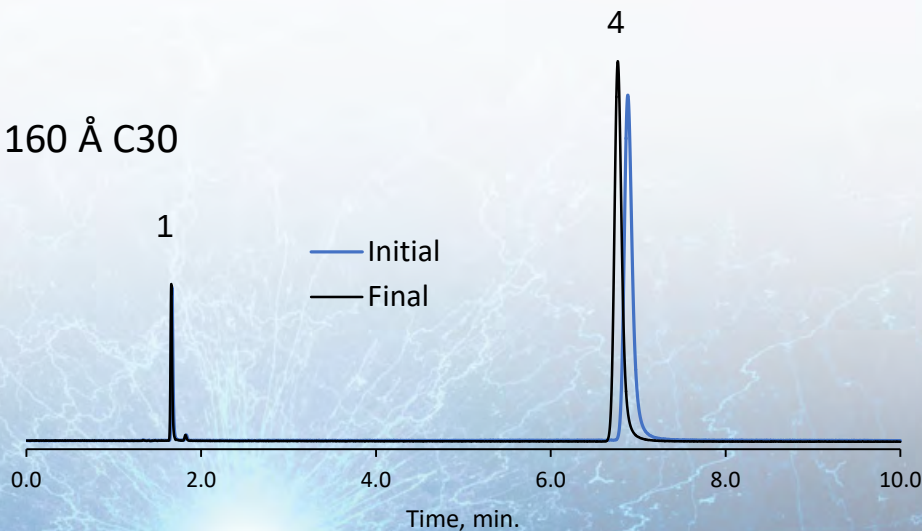
Thymine

HALO 90 Å C18



HALO® C30 does not exhibit dewetting

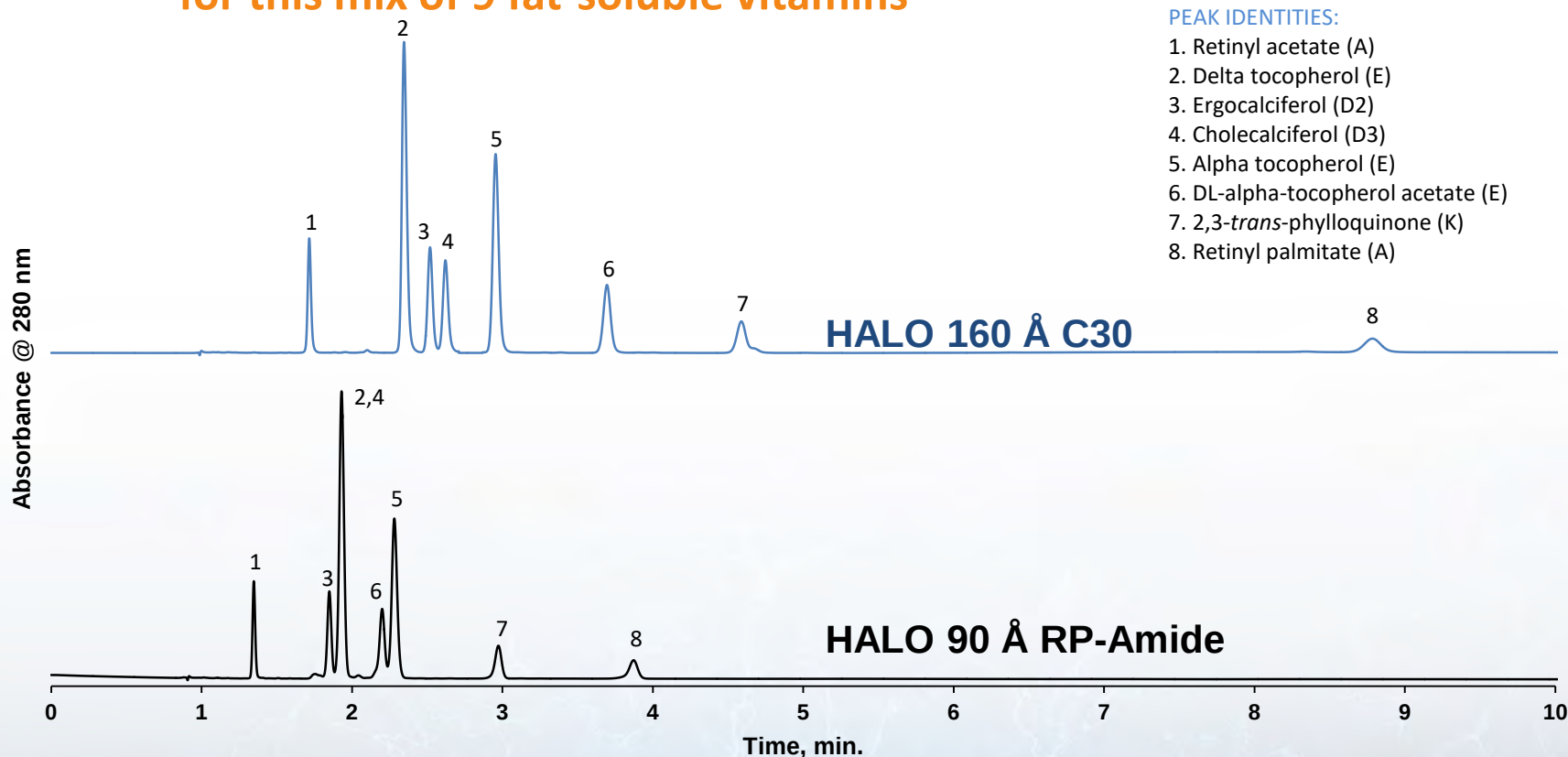
HALO 160 Å C30



Columns: HALO 90Å, C18, 2.7 μ m, 4.6 x 50 mm
 HALO 160 Å C30, 2.7 μ m, 2.1 x 50 mm
 Isocratic: 20 mM Phosphate buffer, pH 7
 Flow Rate: 2.0 mL/min (C18) / 1.0 mL/min (C30)
 Temperature: 30 °C
 Detection: UV 254 nm, PDA

Fat-Soluble Vitamins: C30 vs. RP-Amide Selectivity

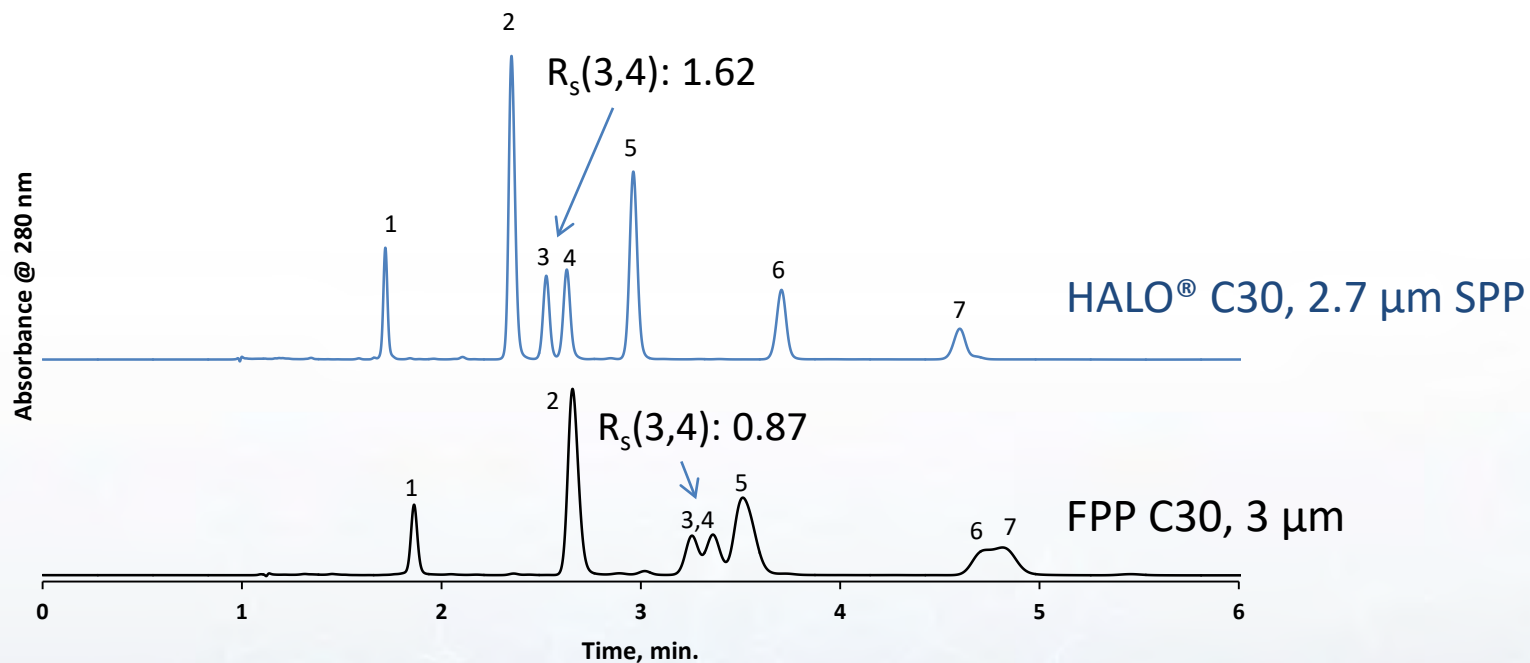
HALO® C30 exhibits different selectivity from HALO® RP-Amide for this mix of 9 fat-soluble vitamins



Isocratic: 100% Methanol
Instrument: Nexera X2
Wavelength: 280 nm
Injection: 2 µL
Temperature: 30 °C
Flow Rate: 1.5 mL/min
Columns: 4.6 x 150 mm, 2.7 µm

Fat-Soluble Vitamins: HALO[®] C30 compared to FPP C30

Sharper peaks and increased resolution with the HALO[®] C30 column!



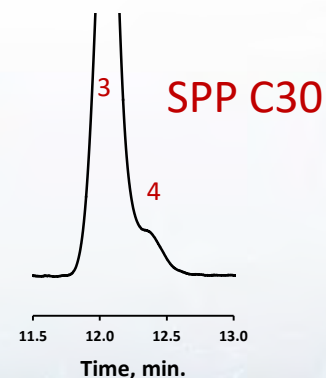
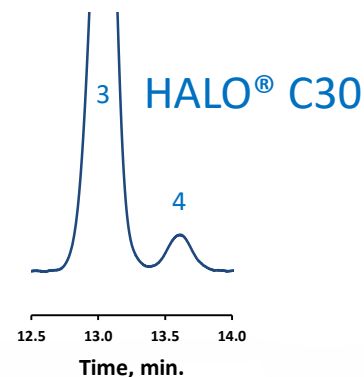
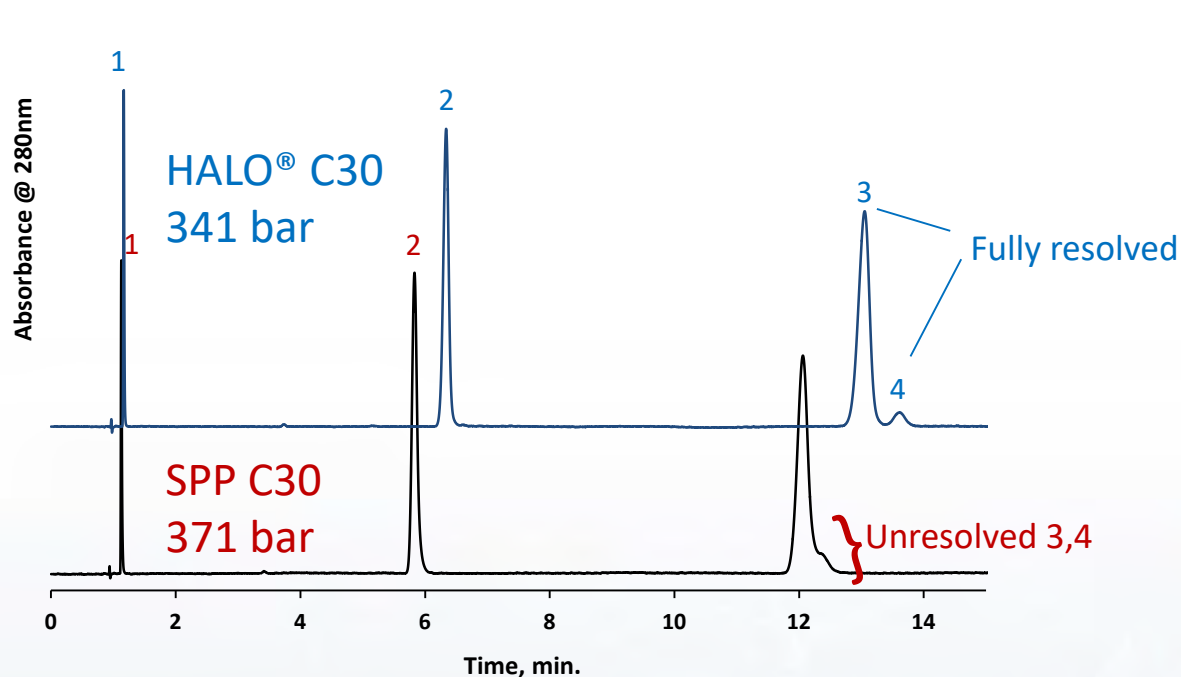
Isocratic: 100% Methanol
Wavelength: 280nm
Injection: 2 μL
Temperature: 30 °C
Flow Rate: 1.5 mL/min
Columns: 4.6 x 150 mm

PEAK IDENTITIES:

1. Retinyl acetate (A)
2. Delta tocopherol (E)
3. Ergocalciferol (D2)
4. Cholecalciferol (D3)
5. Alpha tocopherol (E)
6. DL-alpha-tocopherol acetate (E)
7. 2,3-*trans*-phyllloquinone (K)

Vitamin K: HALO® C30 compared to SPP C30

HALO® C30 shows increased retention and resolution compared to SPP C30



Test Conditions:

Columns: HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm

SPP 150 Å C30, 2.6 µm, 4.6 x 150 mm

Isocratic: 5/95 water/methanol

Flow Rate: 1.5 mL/min

Temperature: 25 °C

Injection Volume: 1 µL

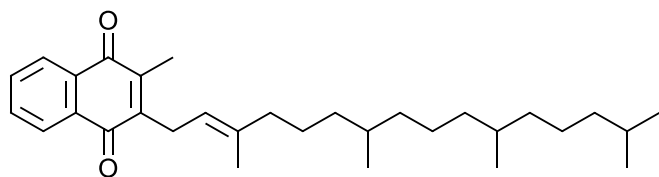
Instrument: Shimadzu Nexera

Detection: PDA at 280 nm

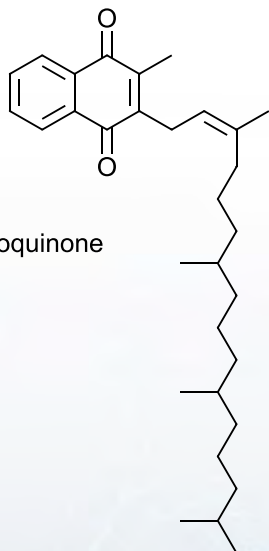
PEAK IDENTITIES:

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

Effect of Temperature on Resolution of *trans* and *cis* Vitamin K1

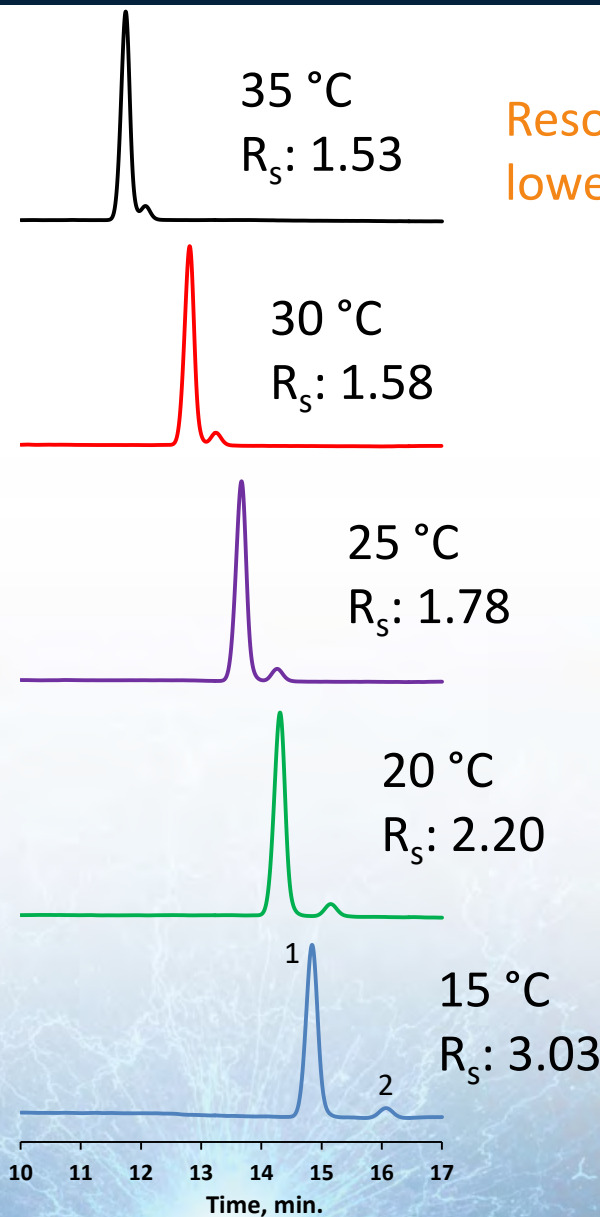


Vitamin K1: 2,3-*trans*-phyllloquinone



Vitamin K1: *cis*-phyllloquinone

Column: HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm
Isocratic: 5/95 water/methanol
Flow Rate: 1.5 mL/min
Wavelength: 254 nm
Injection: 1 µL of 1 mg/mL Vitamin K1
Temperature: as indicated
Instrument: Agilent 1100



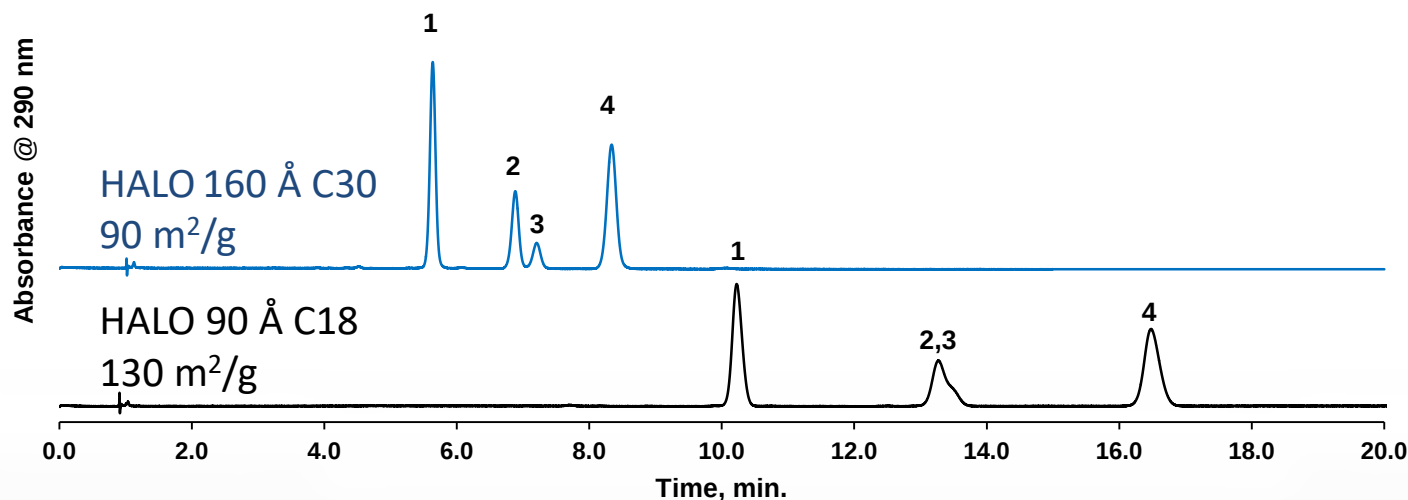
Resolution increases with lower temperatures

PEAK IDENTITIES:

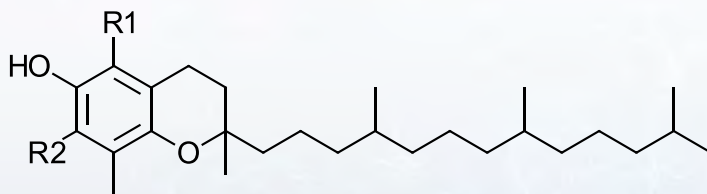
1. 2,3-*trans*-phyllloquinone (K1)
2. *cis*-phyllloquinone (K1)

Vitamin E: Tocopherol Separation

Baseline resolution with HALO® C30 compared to HALO® C18



Generic 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

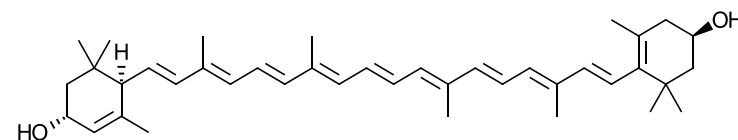
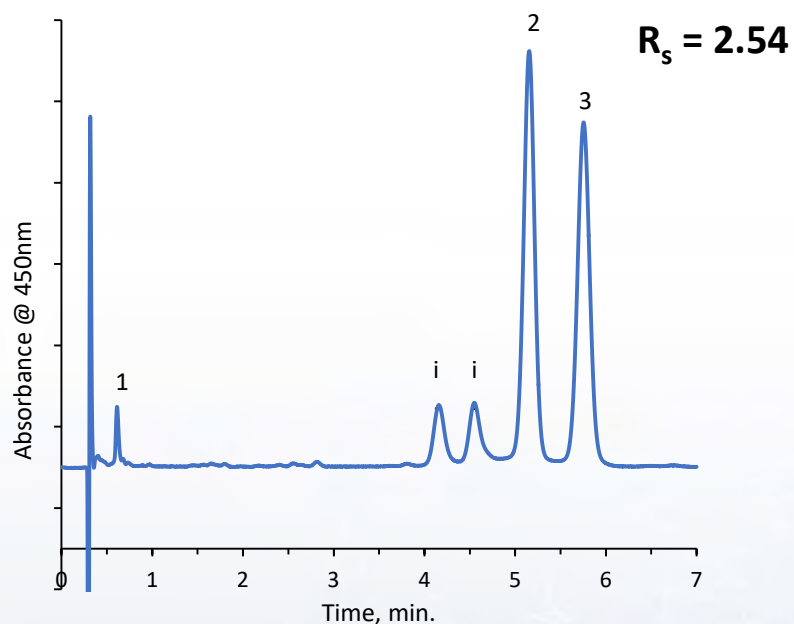
Columns: HALO 160 Å C30 and HALO 90 Å C18, 2.7 µm, 4.6 x 150 mm
Isocratic: 5/95 water/methanol
Flow Rate: 1.5 mL/min
Temperature: 10 °C
Injection Volume: 1.5 µL
LC System: Agilent 1200 SL
Detection: UV 290 nm, PDA

PEAK IDENTITIES:

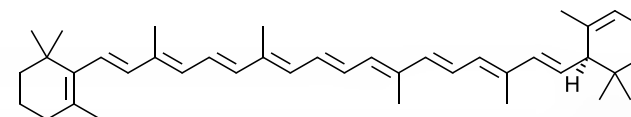
1. δ-tocopherol
2. γ-tocopherol
3. β-tocopherol
4. α-tocopherol

Carotenoids Extracted from Carrot Juice

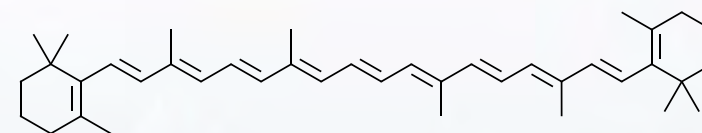
Baseline separation of α and β -carotene with HALO[®] C30 in less than 6.5 min



Lutein



α -carotene



β-carotene

Column: HALO 160 Å C30, 2.7 μm, 2.1 x 50 mm

Isocratic: 100% methanol

Flow Rate: 0.4 mL/min

Wavelength: UV 450 nm, PDA

Injection: 2.5 μ L

Temperature: 30 °C

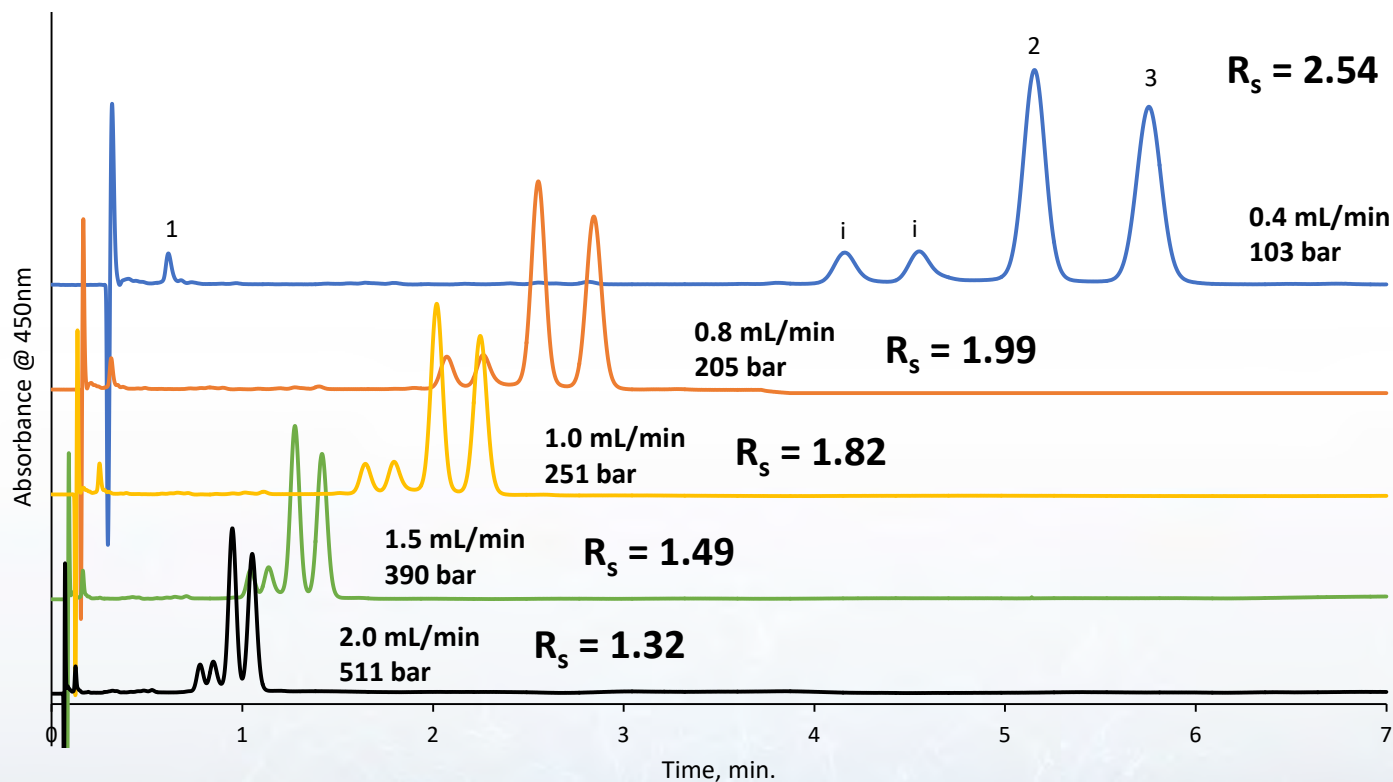
Instrument: Shimadzu Nexera X2

PEAK IDENTITIES:

1. Lutein
2. α -carotene
3. β -carotene

i = unidentified carotenoid

Fast Carotenoid Separations



Column: HALO 160 Å C30, 2.7 μ m, 2.1 x 50 mm
Isocratic: 100% methanol
Flow Rate: as indicated
Wavelength: UV 450 nm, PDA
Injection: 2.5 μ L
Temperature: 30 °C
Instrument: Shimadzu Nexera X2

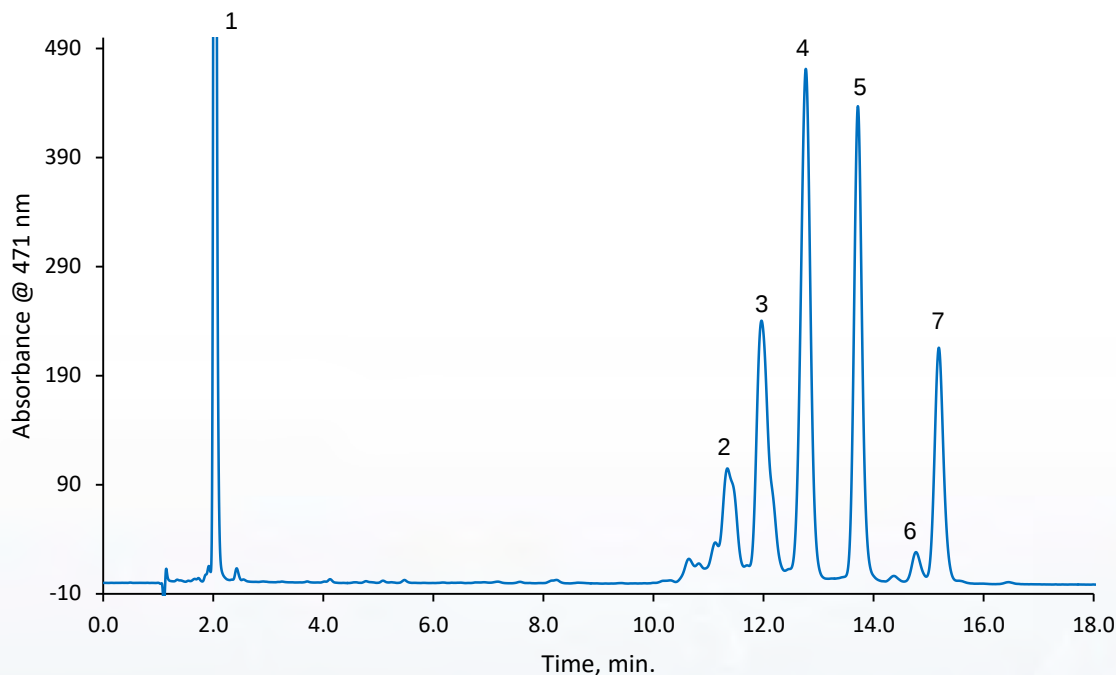
PEAK IDENTITIES:

1. Lutein
2. α -carotene
3. β -carotene

i = unidentified carotenoid

Fast Separation of Carotenoids

HALO® C30 separates 7 carotenoids in less than 16 minutes with high resolution



PEAK IDENTITIES:

1. Lutein
2. cis-carotenoid 1
3. cis-carotenoid 2
4. α -Carotene
5. β -Carotene
6. cis-Lycopene
7. Lycopene

Column: HALO 160 Å C30, 2.7 μ m, 3.0 x 150 mm

Mobile Phase A: Methanol

Mobile Phase B: Ethanol

Gradient: 0-40% B in 20 min

Flow Rate: 0.65 mL/min

Temperature: 38 °C

Injection Volume: 0.6 μ L

LC System: Agilent 1100

Detection: UV 471 nm, PDA

Data courtesy of Nature's Sunshine Products.

Summary and Conclusions

- HALO® C30 columns are stable and rugged
- HALO® C30 offers shape selectivity which is an advantage when separating positional isomers, such as vitamins and carotenoids
- HALO® C30 offers Fused-Core® advantages which includes narrow peaks and the ability to run at increased flow rates while maintaining resolution

Acknowledgements

- Advanced Materials Technology, Inc.
 - Joe DeStefano
 - Tim Langlois
 - Barry Boyes
 - Stephanie Rosenberg
 - Bill Johnson
 - Harry Ritchie
 - Mark Schure
 - Brian Wagner
 - Robert Moran
 - Will Miles
 - Justin Godinho
 - Ben Libert
 - Matt Jackson
- Mac-Mod Analytical, Inc.
 - Tom Waeghe

For Your Information

- Mac-Mod Analytical (mac-mod.com)
 - Exclusive distributor of HALO® columns in the US
 - Booth L10
- fused-core.com
- Stephanie Schuster
 - sschuster@advanced-materials-tech.com

HALO and Fused-Core are registered trademarks of Advanced Materials Technology, Inc.