



# Superficially Porous Particles with C30 Stationary Phase for High Resolution Separations

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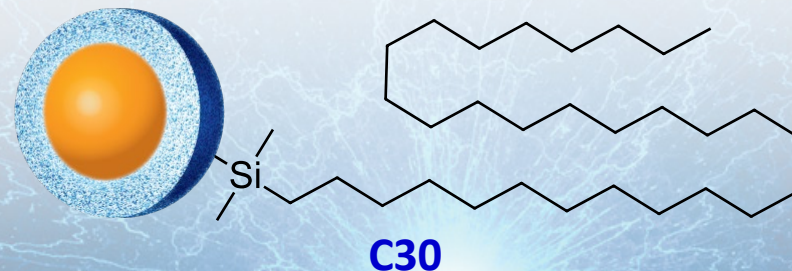
Advanced Materials Technology, Inc., Wilmington, DE 19810

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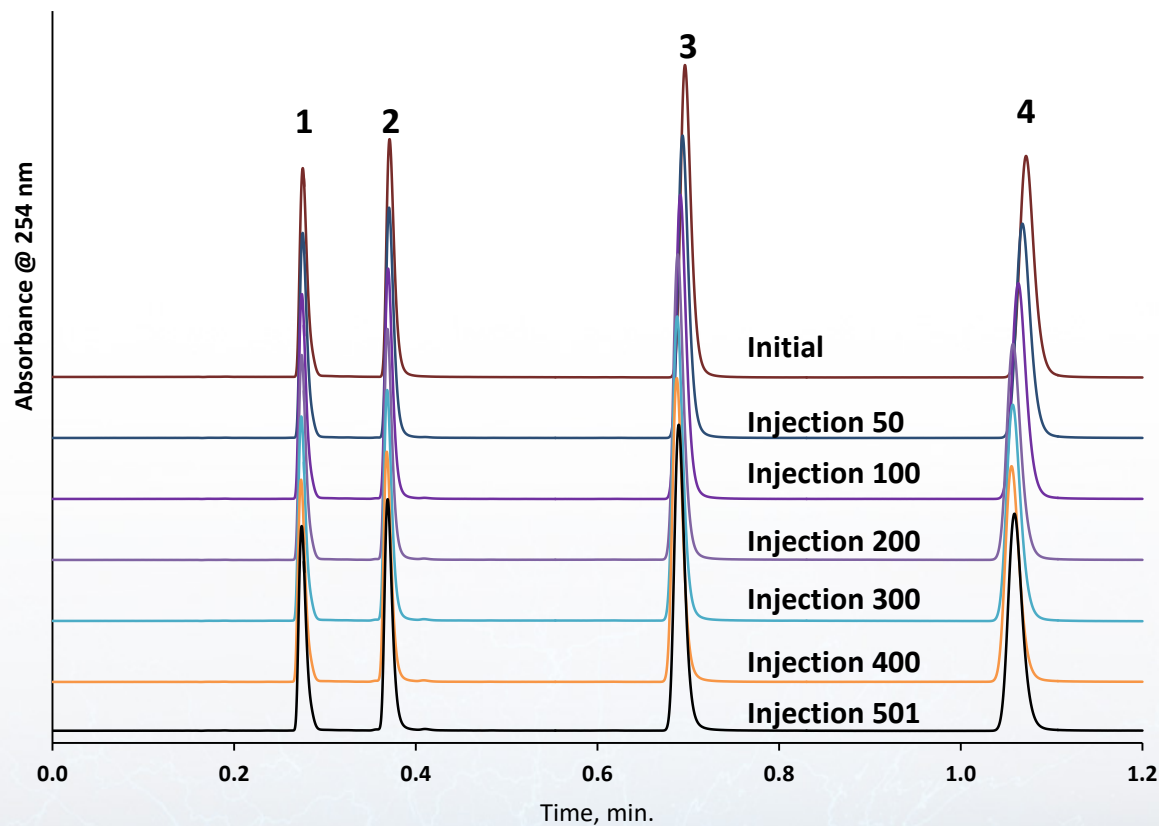
# HALO<sup>®</sup> C30: Properties

- 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 m<sup>2</sup>/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/H<sub>2</sub>O

Flow Rate: 1.1 mL/min

Back Pressure: 602 bar

Temperature: 60 °C

Injection Volume: 1 µL

Instrument: Shimadzu Nexera X2

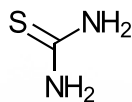
Detection: UV 254 nm, PDA

## PEAK IDENTITIES:

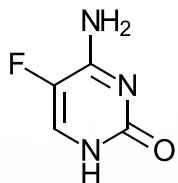
1. Uracil
2. Phenol
3. 1-chloro-4-Nitrobenzene
4. Naphthalene

# HALO<sup>®</sup> C30: 100% Aqueous Compatibility

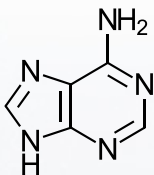
1. Thiourea
2. 5-Fluorocytosine
3. Adenine
4. Thymine



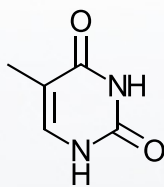
Thiourea



5-Fluorocytosine

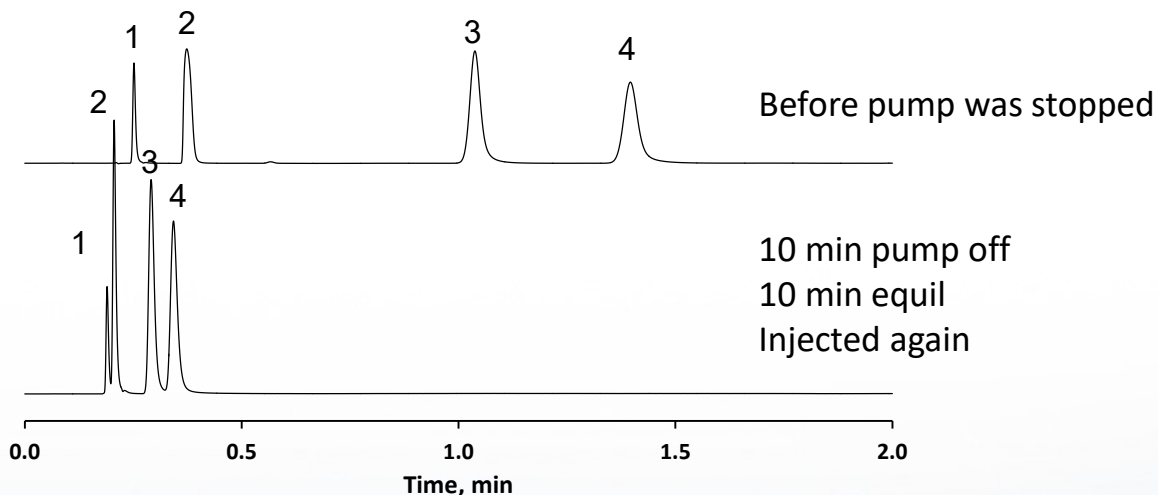


Adenine



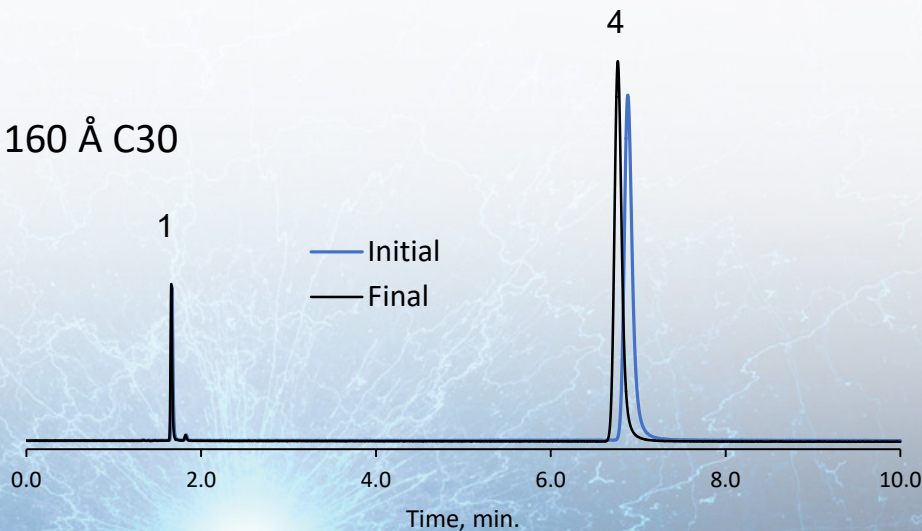
Thymine

HALO 90 Å C18



HALO<sup>®</sup> C30 does not exhibit dewetting

HALO 160 Å C30



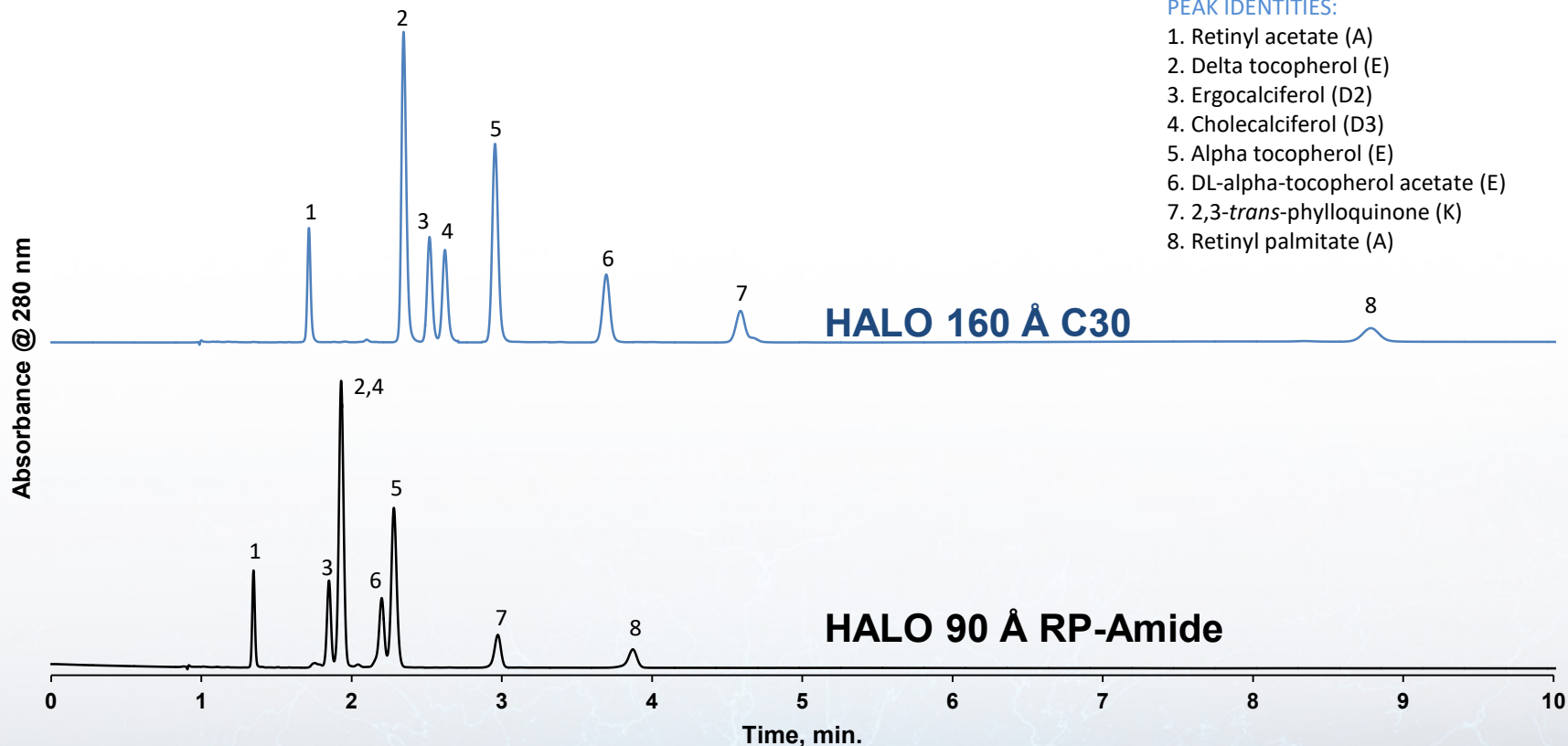
Columns: HALO 90Å, C18, 2.7 μm, 4.6 x 50 mm  
 HALO 160 Å C30, 2.7 μm, 2.1 x 50 mm  
 Isocratic: 20 mM Phosphate buffer, pH 7  
 Flow Rate: 2.0 mL/min (C18) / 1.0 mL/min (C30)  
 Temperature: 30 °C  
 Detection: UV 254 nm, PDA

# 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

PEAK IDENTITIES:

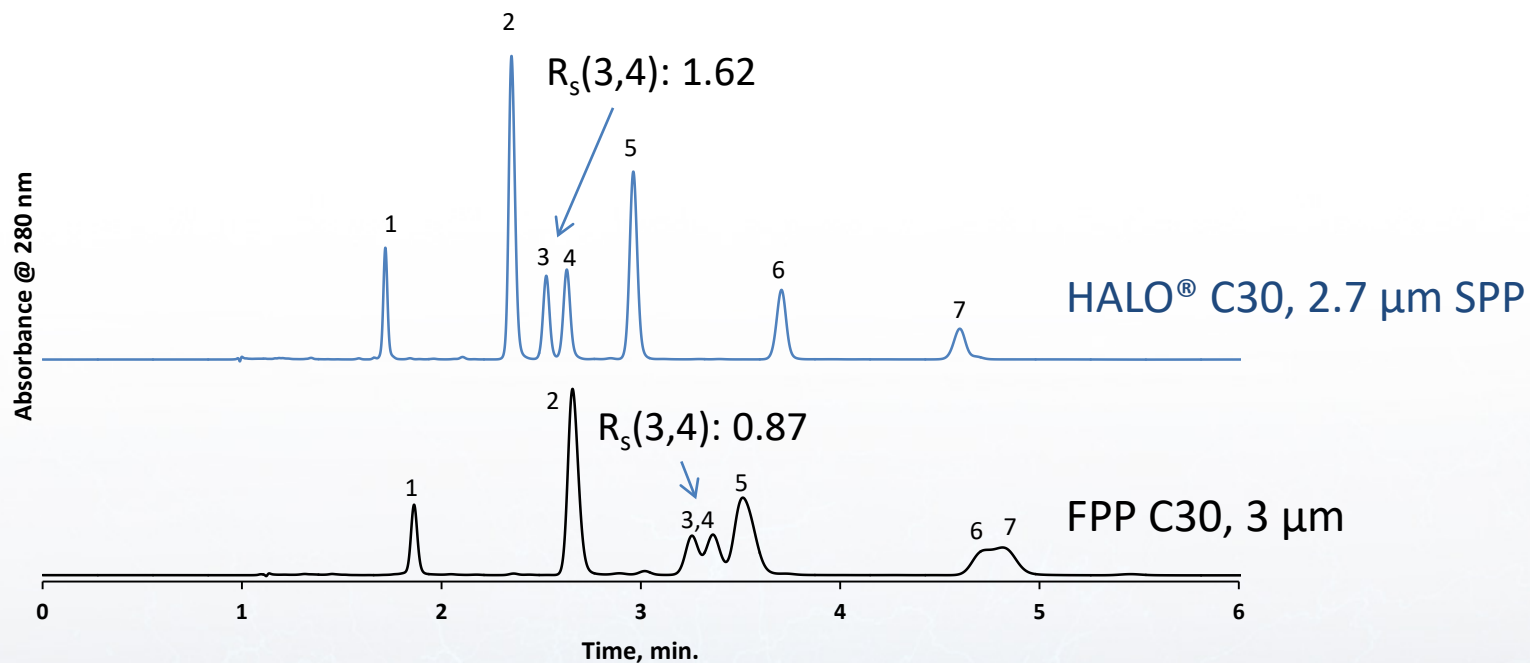
- 1. Retinyl acetate (A)
- 2. Delta tocopherol (E)
- 3. Ergocalciferol (D2)
- 4. Cholecalciferol (D3)
- 5. Alpha tocopherol (E)
- 6. DL-alpha-tocopherol acetate (E)
- 7. 2,3-trans-phyloquinone (K)
- 8. Retinyl palmitate (A)



Isocratic: 100% Methanol  
Instrument: Nexera X2  
Wavelength: 280 nm  
Injection: 2  $\mu$ L  
Temperature: 30  $^{\circ}$ C  
Flow Rate: 1.5 mL/min  
Columns: 4.6 x 150 mm, 2.7  $\mu$ m

# Fat-Soluble Vitamins: HALO<sup>®</sup> C30 compared to FPP C30

Sharper peaks and increased resolution with the HALO<sup>®</sup> C30 column!



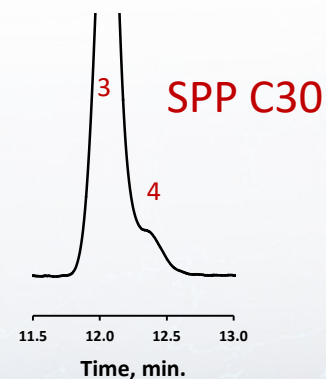
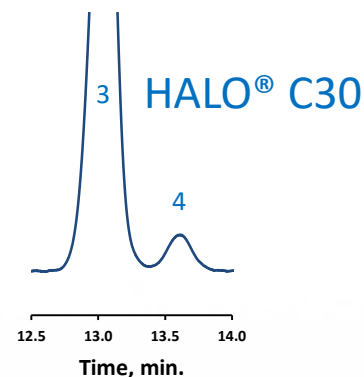
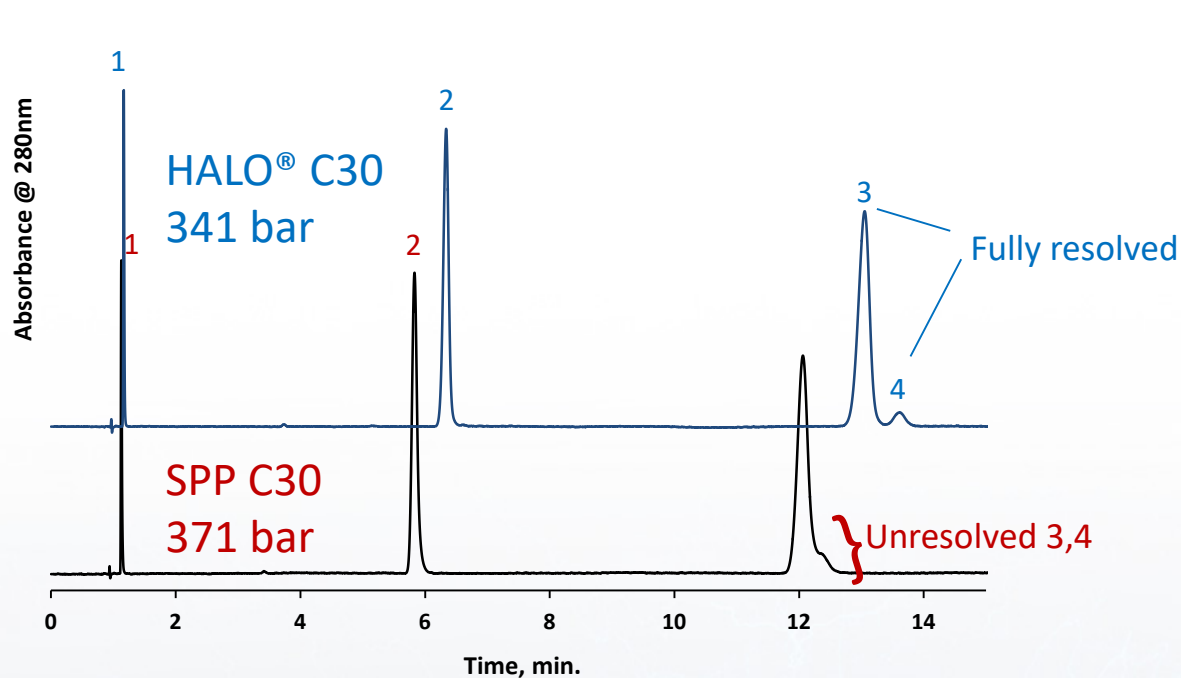
Isocratic: 100% Methanol  
Wavelength: 280nm  
Injection: 2 μL  
Temperature: 30 °C  
Flow Rate: 1.5 mL/min  
Columns: 4.6 x 150 mm

#### 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-phyloquinone (K)

# Vitamin K: HALO<sup>®</sup> C30 compared to SPP C30

HALO<sup>®</sup> 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

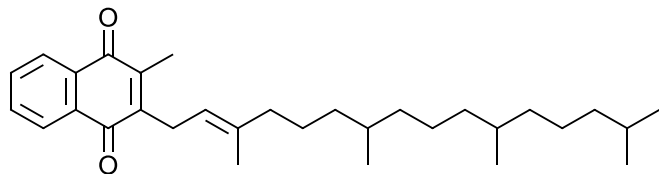
Instrument: Shimadzu Nexera

Detection: PDA at 280 nm

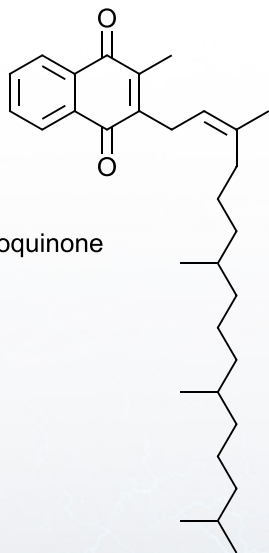
## PEAK IDENTITIES:

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

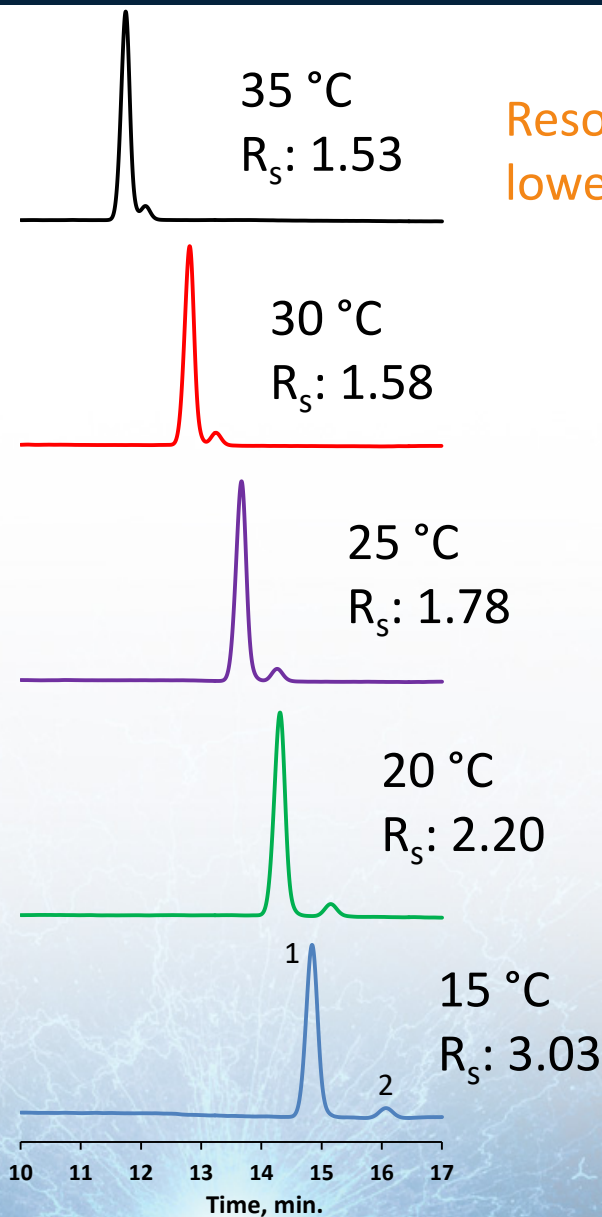


Vitamin K1: 2,3-*trans*-phyloquinone



Vitamin K1: *cis*-phyloquinone

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

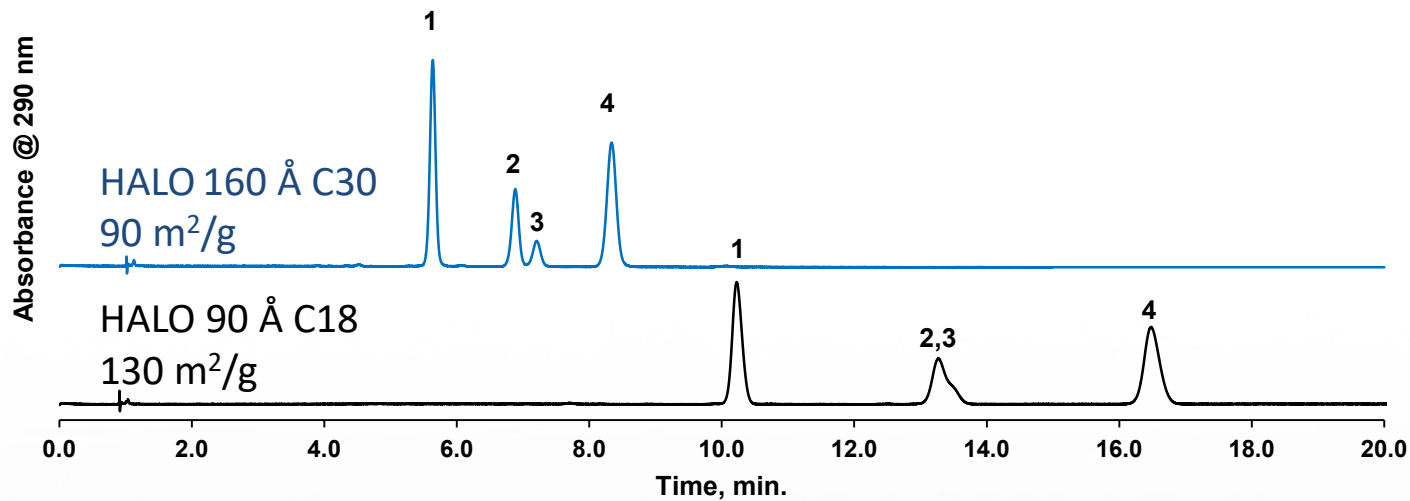
## PEAK IDENTITIES:

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

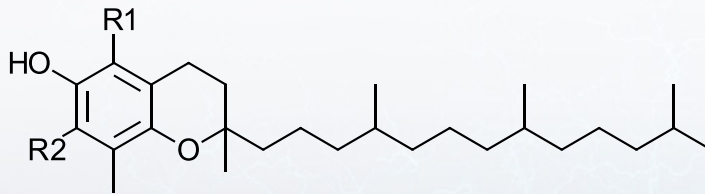


# Vitamin E: Tocopherol Separation

## Baseline resolution with HALO<sup>®</sup> C30 compared to HALO<sup>®</sup> C18



### Generic Tocopherol Structure:



Tocopherol	R1	R2
Alpha ( $\alpha$ )	CH <sub>3</sub>	CH <sub>3</sub>
Beta ( $\beta$ )	CH <sub>3</sub>	H
Gamma ( $\gamma$ )	H	CH <sub>3</sub>
Delta ( $\delta$ )	H	H

Columns: HALO 160 Å C30 and HALO 90 Å C18, 2.7  $\mu$ m, 4.6 x 150 mm

Isocratic: 5/95 water/methanol

Flow Rate: 1.5 mL/min

Temperature: 10 °C

Injection Volume: 1.5  $\mu$ L

LC System: Agilent 1200 SL

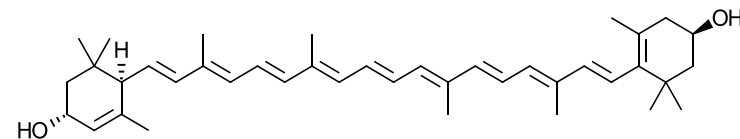
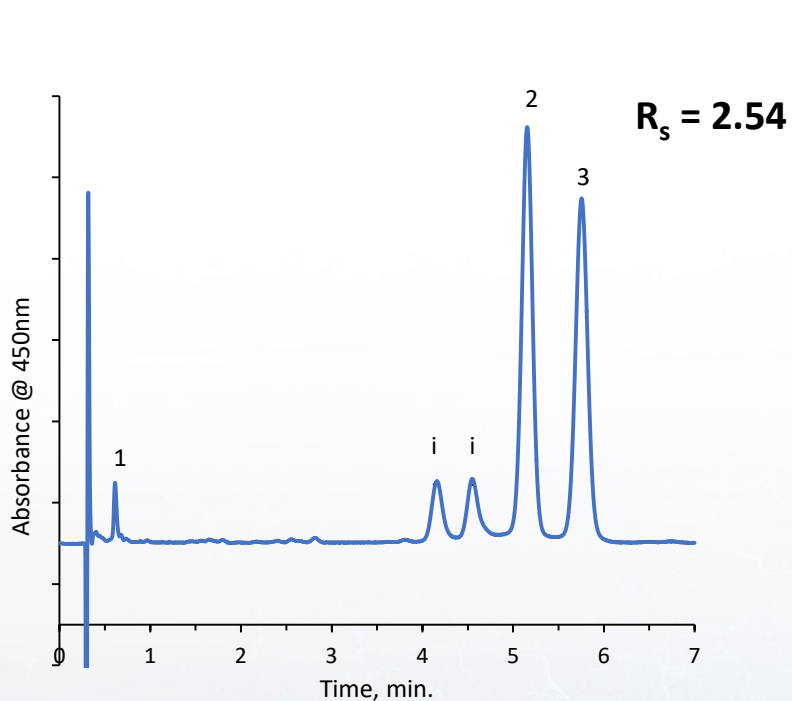
Detection: UV 290 nm, PDA

### PEAK IDENTITIES:

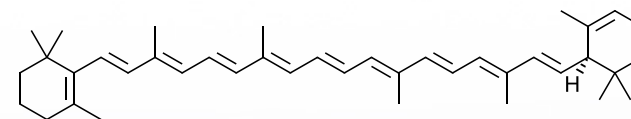
1.  $\delta$ -tocopherol
2.  $\gamma$ -tocopherol
3.  $\beta$ -tocopherol
4.  $\alpha$ -tocopherol

# Carotenoids Extracted from Carrot Juice

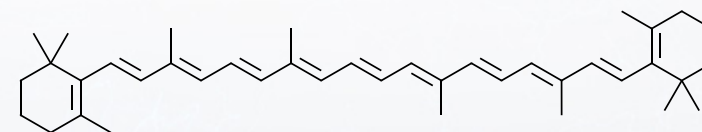
Baseline separation of  $\alpha$  and  $\beta$ -carotene with HALO® C30 in less than 6.5 min



Lutein



$\alpha$ -carotene



$\beta$ -carotene

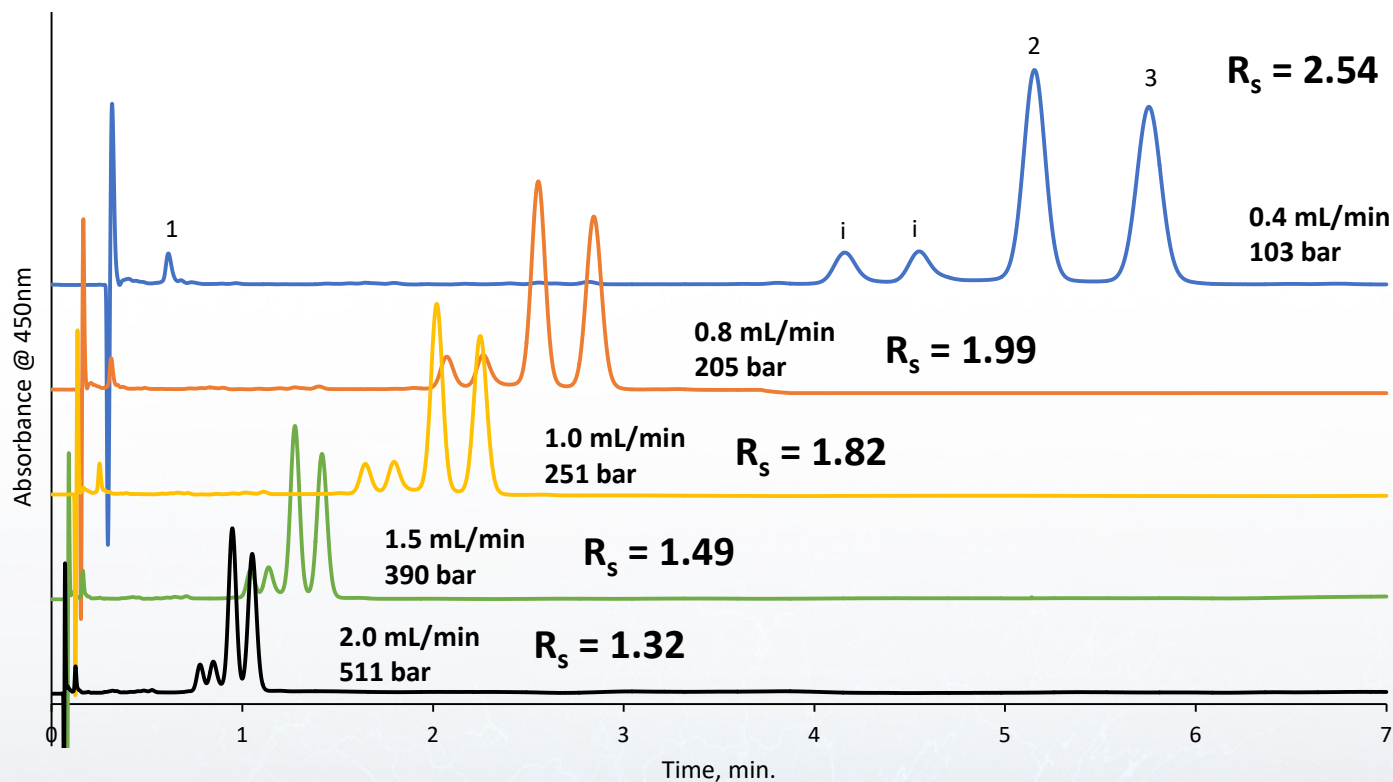
Column: HALO 160 Å C30, 2.7  $\mu$ m, 2.1 x 50 mm  
Isocratic: 100% methanol  
Flow Rate: 0.4 mL/min  
Wavelength: UV 450 nm, PDA  
Injection: 2.5  $\mu$ L  
Temperature: 30 °C  
Instrument: Shimadzu Nexera X2

## PEAK IDENTITIES:

1. Lutein
2.  $\alpha$ -carotene
3.  $\beta$ -carotene

i = unidentified carotenoid

# Fast Carotenoid Separations



Column: HALO 160 Å C30, 2.7  $\mu\text{m}$ , 2.1 x 50 mm  
Isocratic: 100% methanol  
Flow Rate: as indicated  
Wavelength: UV 450 nm, PDA  
Injection: 2.5  $\mu\text{L}$   
Temperature: 30  $^{\circ}\text{C}$   
Instrument: Shimadzu Nexera X2

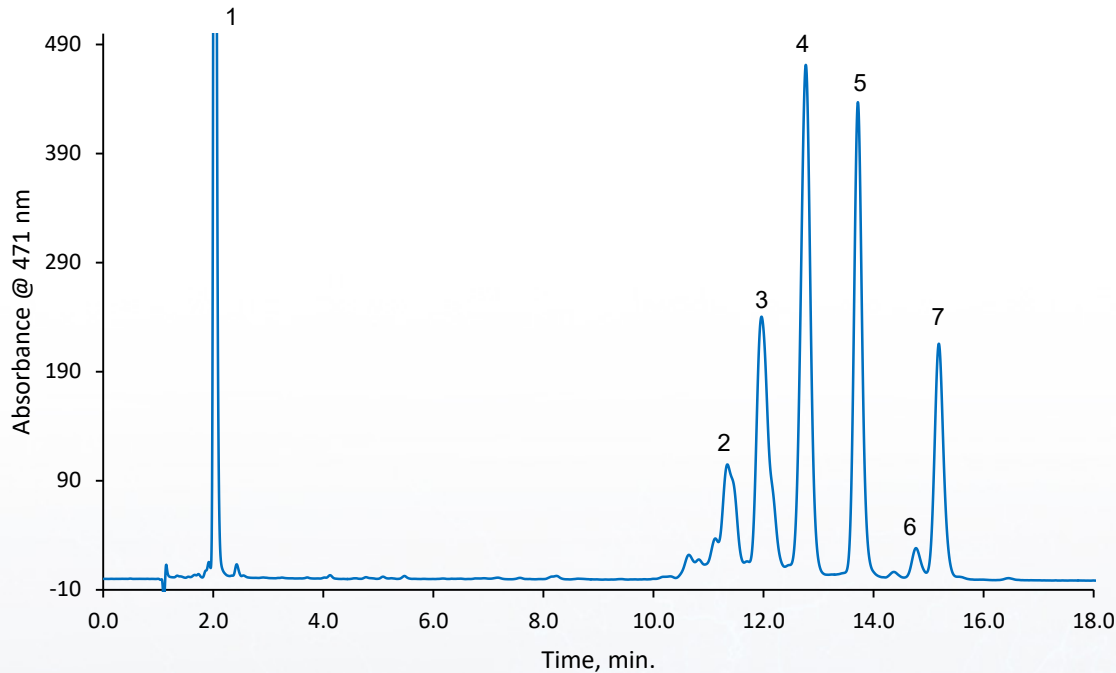
## PEAK IDENTITIES:

1. Lutein
2.  $\alpha$ -carotene
3.  $\beta$ -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.  $\alpha$ -Carotene
5.  $\beta$ -Carotene
6. cis-Lycopene
7. Lycopene

Column: HALO 160 Å C30, 2.7  $\mu$ 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  $\mu$ L

LC System: Agilent 1100

Detection: UV 471 nm, PDA

Data courtesy of Nature's Sunshine Products.



# Summary and Conclusions

- HALO<sup>®</sup> C30 columns are stable and rugged
- HALO<sup>®</sup> C30 offers shape selectivity which is an advantage when separating positional isomers, such as vitamins and carotenoids
- HALO<sup>®</sup> C30 offers Fused-Core<sup>®</sup> 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
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  - 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](http://mac-mod.com))**
  - Exclusive distributor of HALO<sup>®</sup> columns in the US
  - Booth L10
- **[fused-core.com](http://fused-core.com)**
- **Stephanie Schuster**
  - [sschuster@advanced-materials-tech.com](mailto:sschuster@advanced-materials-tech.com)

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