# C30 Modified Superficially Porous Particle Columns for Food and Supplement Applications

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#### Introduction

The benefits of superficially porous particles (SPPs) include highly efficient separations in less time and with reduced backpressure requirements. This particle technology continues to expand as more pore sizes, particle sizes, and bonding chemistries become available. Tuning the packing material's characteristics allows for the user to select the column that is best suited for their application. Often users in food and supplement research need to analyze very hydrophobic samples that have subtle positional isomerism. We have found that traditional reversed phase chemistries are lacking in their ability to resolve some of these analytes. For this reason, we developed a C30 phase that provides both high hydrophobicity as well as a proclivity to separate isomeric forms that are required for routine food analysis.

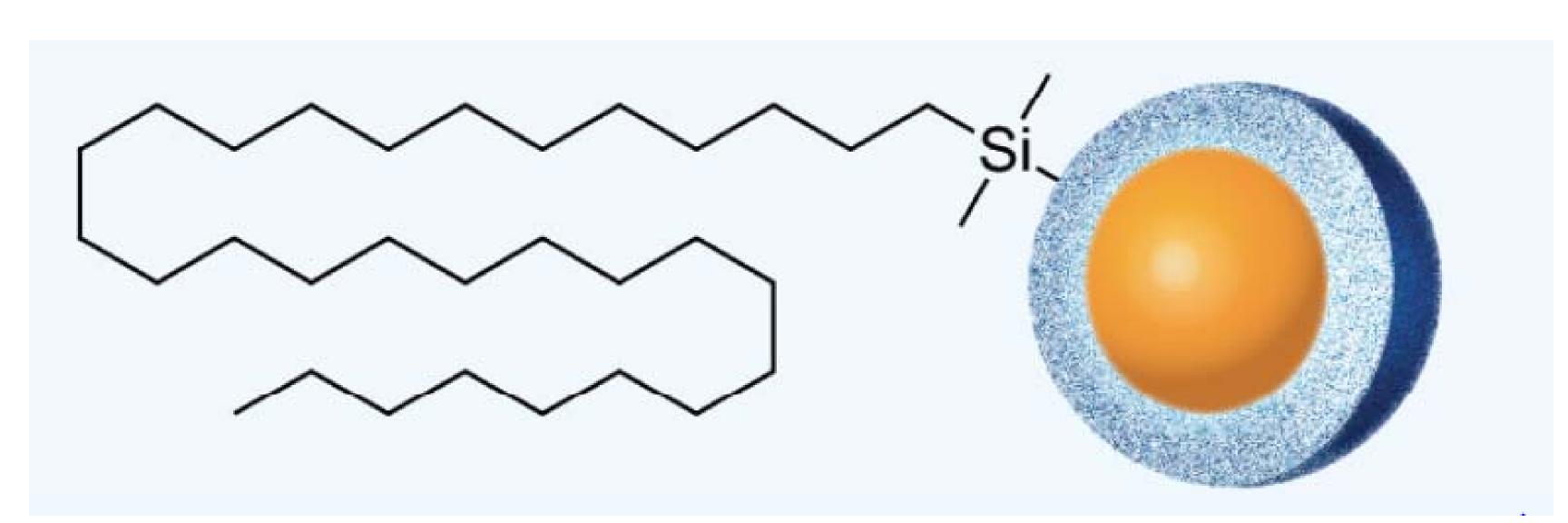
The novel C30 bonded phase on 160 Å, 2.7 μm SPPs has demonstrated high efficiency, excellent stability, and unique selectivity for hydrophobic, long-chained compounds. Analytes, such as vitamins and lipids that contain cis and trans isomers, for example, are better resolved by C30 than traditional alkyl phases (i.e. C8 or C18). Example chromatograms of these types of separations will be shown along with comparisons to similar phases already on the market. We have also found the C30 phase to have some unique features including 100% aqueous compatibility. This expands the phase's utility to gradient applications requiring low organic starting points for separations of complex samples that contain widely varied mixtures of analyte polarities.

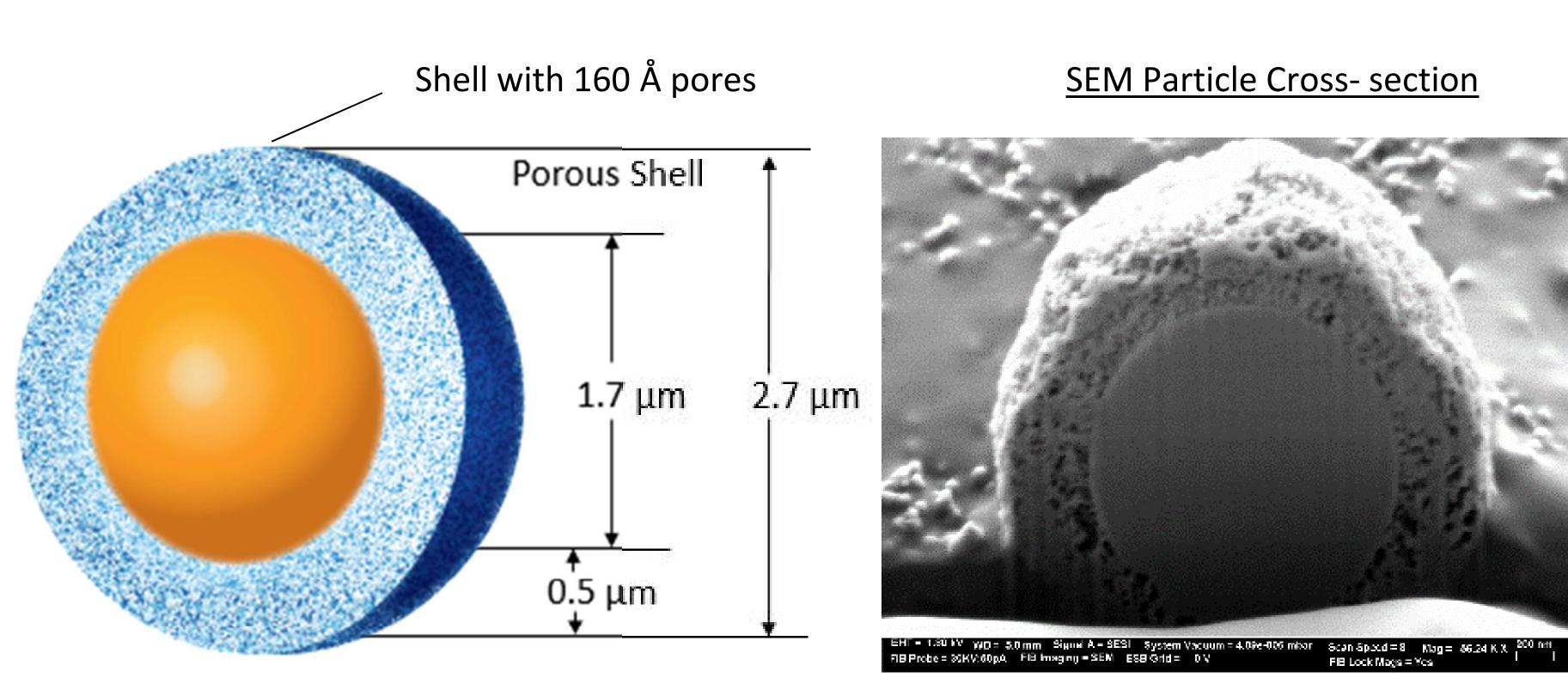
### The Particle

Ligand: Tricontyldimethyl Particle Size: 2.7 μm Pore Size: 160 Å

USP Designation: L62 Carbon Load: 4.5 % Surface Area: 90 m<sup>2</sup>/ g

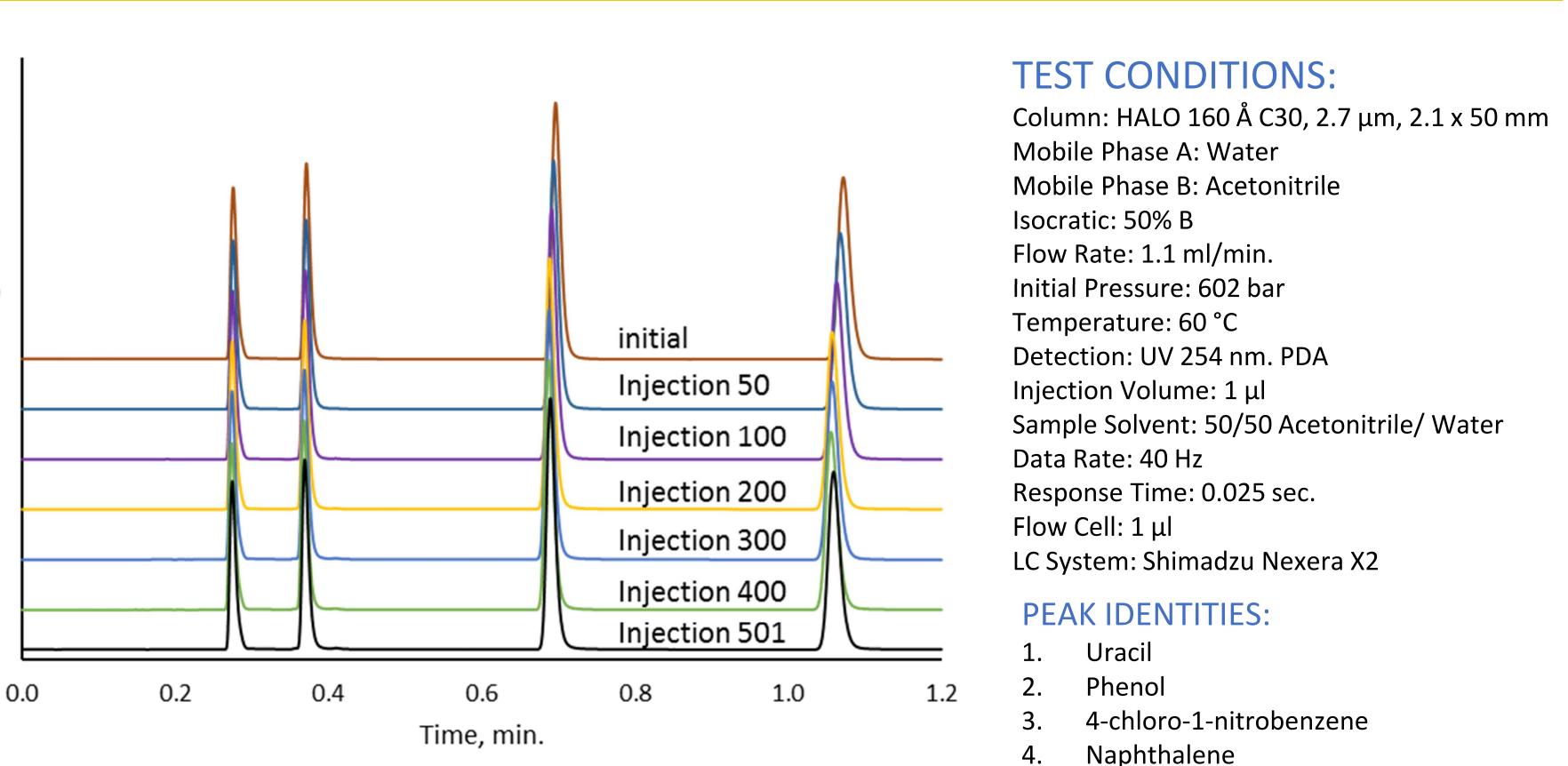
Endcapped: Yes Low pH Limit/ Max T: 2/60°C High pH Limit/ Max T: 9/40°C



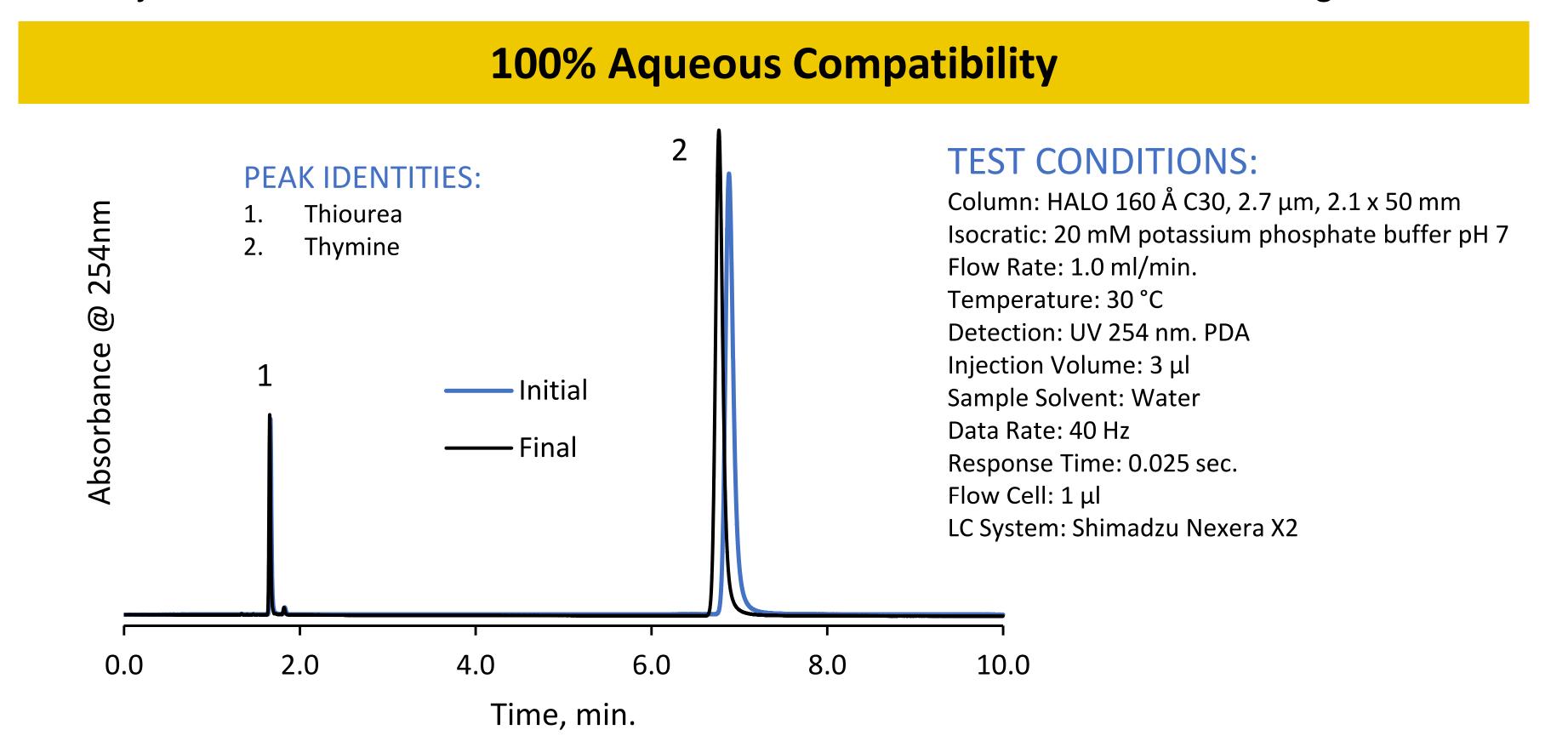


The HALO 160 Å particle is a superficially porous particle made with a 1.7  $\mu$ m solid core and a 0.5  $\mu$ m porous shell. The shell consists of 160 Å pores and shows excellent stability and lower overall back pressure when compared to totally porous particles.

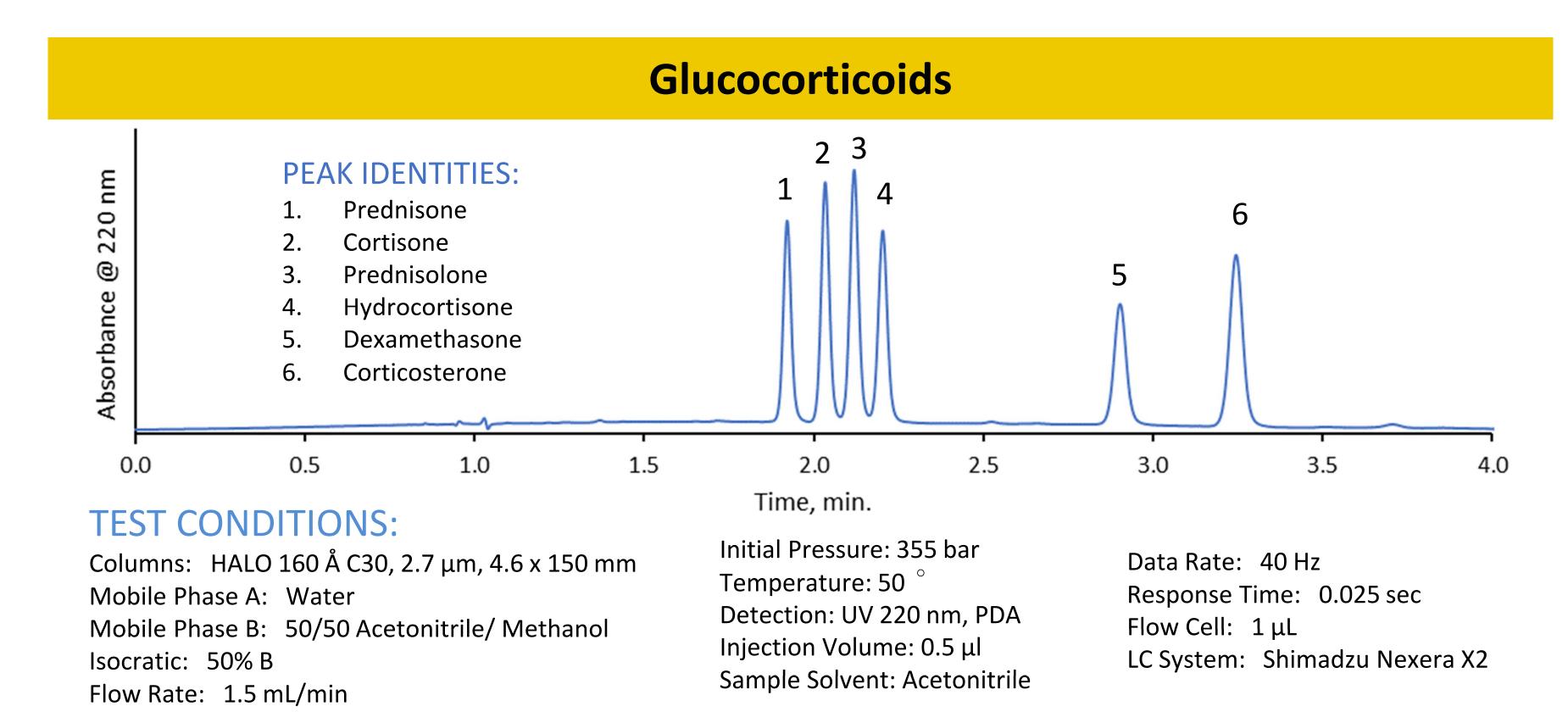
## **High Pressure/ Temperature Stability**



Over 500 injections at over 600 bar at 60°C are observed on a HALO® C30 column showing excellent stability.

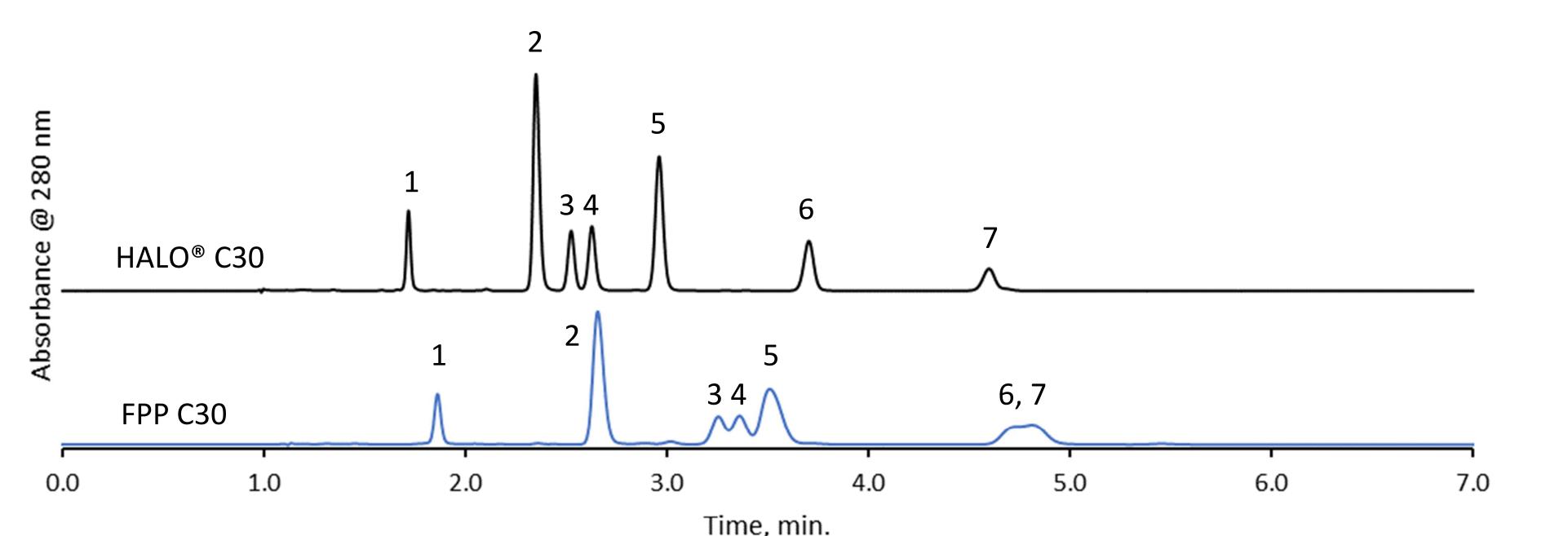


The HALO® C30 phase has 100% aqueous compatibility allowing users to run in high percentages of water/buffers. For example, an injection of thiourea and thymine are done using 100% buffer. The pump is then turned off for 20 minutes and the injection is repeated with only a 1.5% loss in retention.



Glucocorticoids are a class of steroid drugs that have anti-inflammatory and anti-allergy benefits, as well as anti-lymphatic cancer uses. This mixture of six glucocorticoids is separated with high resolution in less than three and a half minutes on a HALO® C30 column.

## Fat-soluble Vitamins: SPP vs. FPP



### **TEST CONDITIONS:** Column: HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm Isocratic: 100% Methanol

Flow Rate: 1.5 ml/min. Initial HALO Pressure: 262 bar Initial Competitor Pressure: 207 bar Temperature: 30 °C Detection: UV 280 nm. PDA Injection Volume: 2.0 µl Sample Solvent: Methanol Data Rate: 40 Hz

improved when using the SPP technology.

Response Time: 0.025 sec.

Flow Cell: 1 µl

Cholecalciferol (Vitamin D3) α- tocopherol (Vitamin E)  $dl - \alpha$  tocopherol acetate (Vitamin E) 2,3- trans- phylloquinone (Vitamin K) Peak 2: Delta Tocopherol | Peak 3/4: D2/D3 Efficiency (Plates) Resolution

**PEAK IDENTITIES:** 

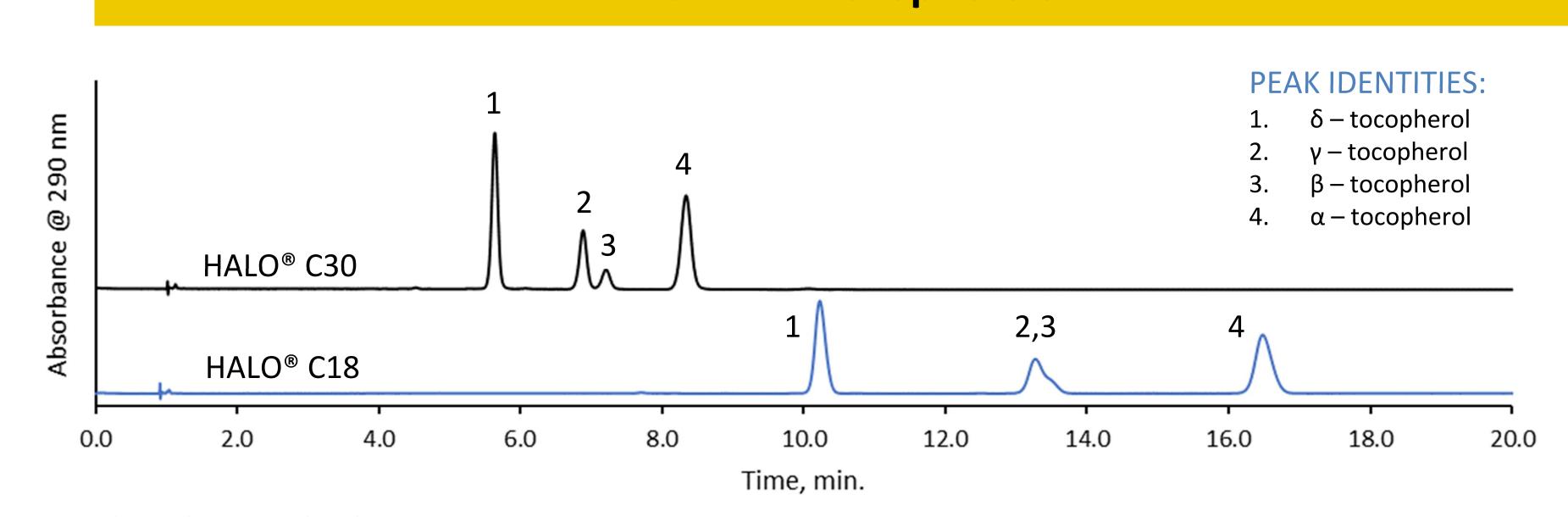
Retinyl acetate (Vitamin A)

Ergocalciferol (Vitamin D2)

Δ- tocopherol (Vitamin E)

0.87 LC System: Shimadzu Nexera X2 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 and resolution are all

#### **Vitamin E: Tocopherols**



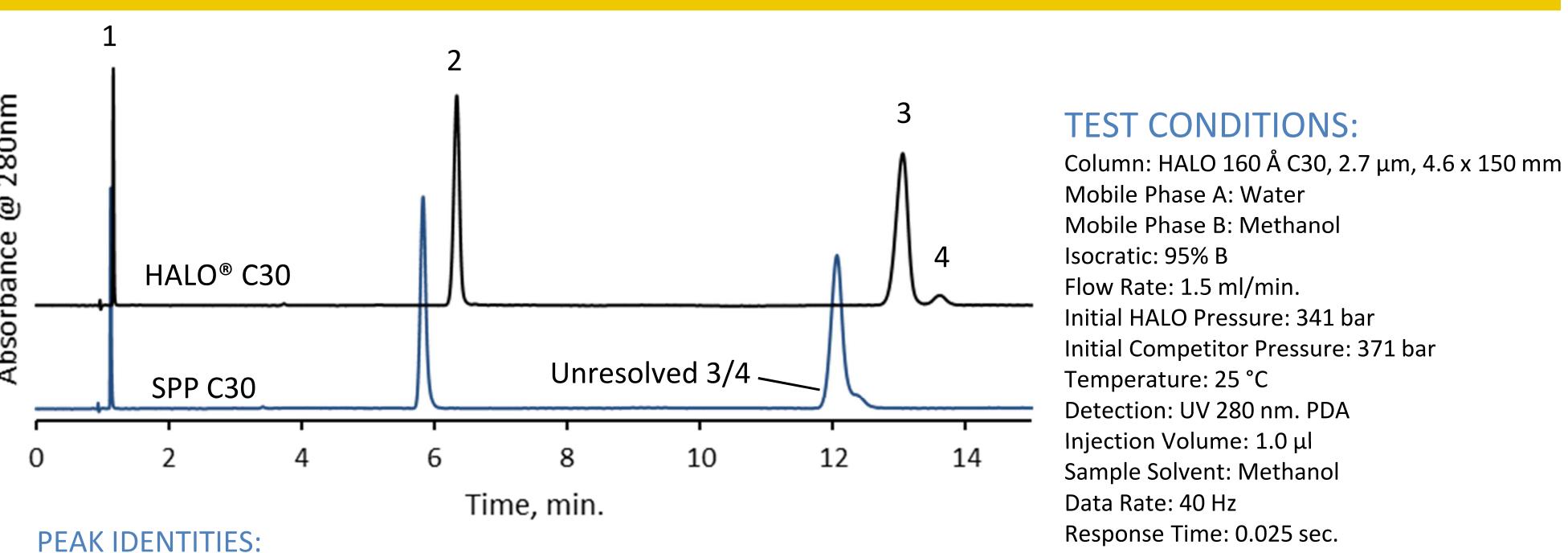
## **TEST CONDITIONS:**

Detection: UV 290 nm, PDA

Columns: HALO 160 Å C30, 2.7 μm, 4.6 x 150 mm Injection Volume: 1.5 μL Sample Solvent: Ethanol/ Methanol HO HALO 90 Å C18, 2.7 μm, 4.6 x 150 mm Data Rate: 80 Hz Mobile Phase A: Water Response Time: 0.02 sec Mobile Phase B: Methanol Flow Cell: 2 μL Isocratic: 95% B LC System: Agilent 1200 SL Flow Rate: 1.5 mL/min C30 Pressure: 337 bar C18 Pressure: 348 bar Temperature:  $10^{\circ}$  C

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 m<sup>2</sup>/g vs. 90 m<sup>2</sup>/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.

### Vitamin K1: cis/ trans isomers



# Initial HALO Pressure: 341 bar

Initial Competitor Pressure: 371 ba 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

**TEST CONDITIONS:** 

Mobile Phase A: Water

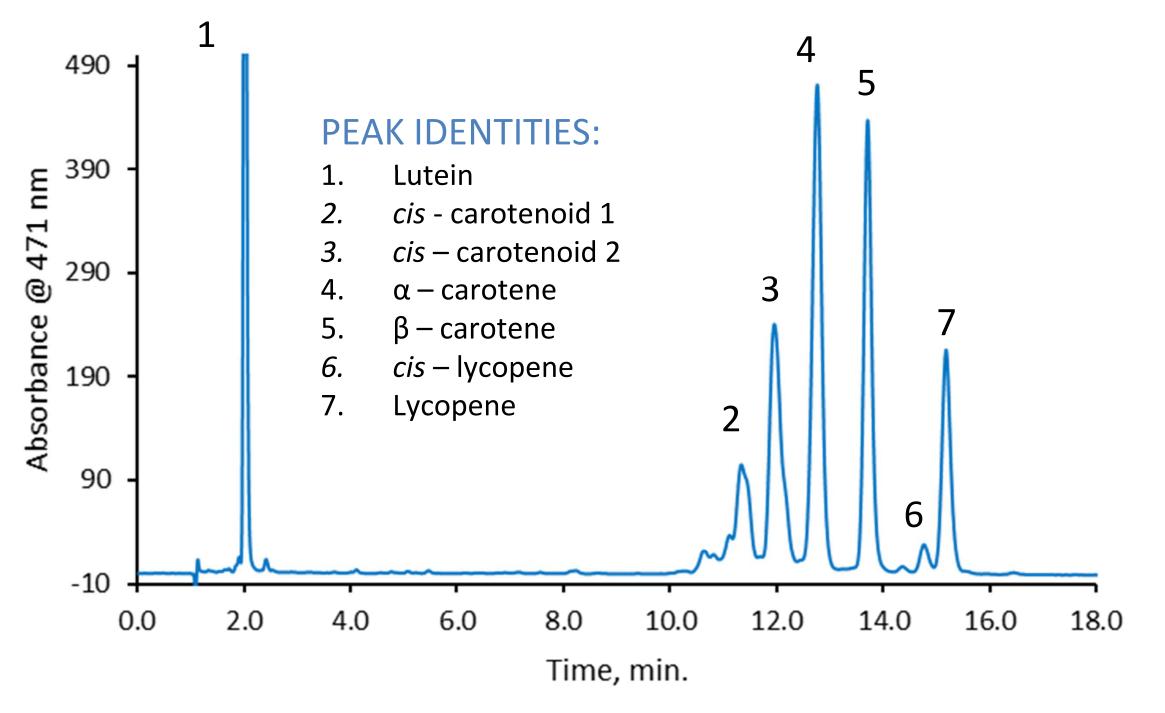
Flow Rate: 1.5 ml/min.

Isocratic: 95% B

Menaguinone 4 (Vitamin K2) 2,3 – *trans* phylloquinone (Vitamin K1) cis – phylloquinone (Vitamin K1)

Vitamin K, a fat-soluble vitamin, is beneficial for blood clot prevention 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. Baseline resolution of K1 isomers is obtained on a HALO C30 column compared to a coelution on a competitor SPP C30 column.

#### Carotenoids



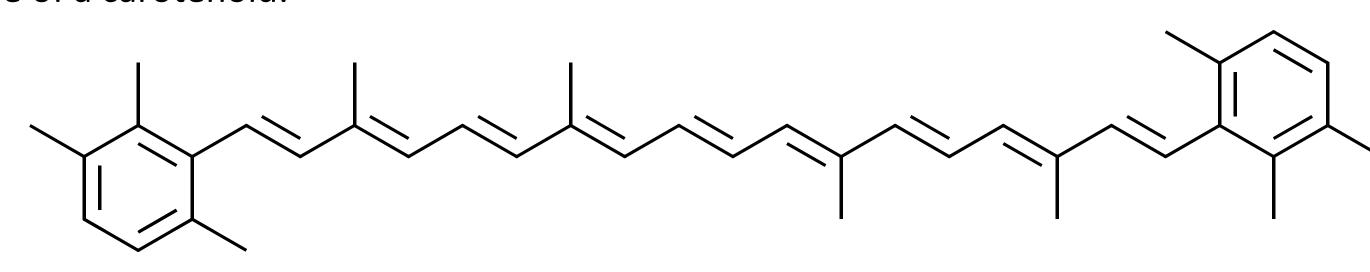
Column: HALO 160 Å C30, 2.7 µm, 3.0 x 150 mm Mobile Phase A: Methanol Mobile Phase B: Ethanol Gradient: 100% A with gradient to 40% B at 20 minutes Flow Rate: 0.65 ml/min. Temperature: 38 °C Model: Agilent 1100 PDA detector using 471 nm

Flow Cell: G1315-60012 (Agilent 10 mm, 13 μl) Data acquisition rate: 2.5 Hz Injection volume: 0.60 μl Instrument: Agilent 1100 Detection: UV 471 nm, PDA

**TEST CONDITIONS:** 

General structure of a carotenoid:

Menadione (Vitamin K3)



Carotenoids can be split into two main classes called xanthophylls and carotenes. They are responsible for absorbing light for photosynthesis and protecting chlorophyll from photodamage. A separation done by Nature's Sunshine Products shows excellent resolution of carotenoids.

#### Conclusions

The HALO® C30 phase is a great column addition to add to your laboratory for multiple reasons. The 100% aqueous compatibility along with its high shape selectivity for hydrophobic, long-chain structurally related isomers gives advantages that other columns do not offer. The column also shows excellent stability at high temperature and pressure and is built on proven Fused-Core® particle technology.

Acknowledgements: William Johnson, Joe DeStefano, Mark Schure <sup>®</sup>HALO and Fused-Core are registered trademarks of Advanced Materials Technology, Inc

