

A Superficially Porous Particle (SPP) C30 Phase Designed for High Speed HPLC Applications

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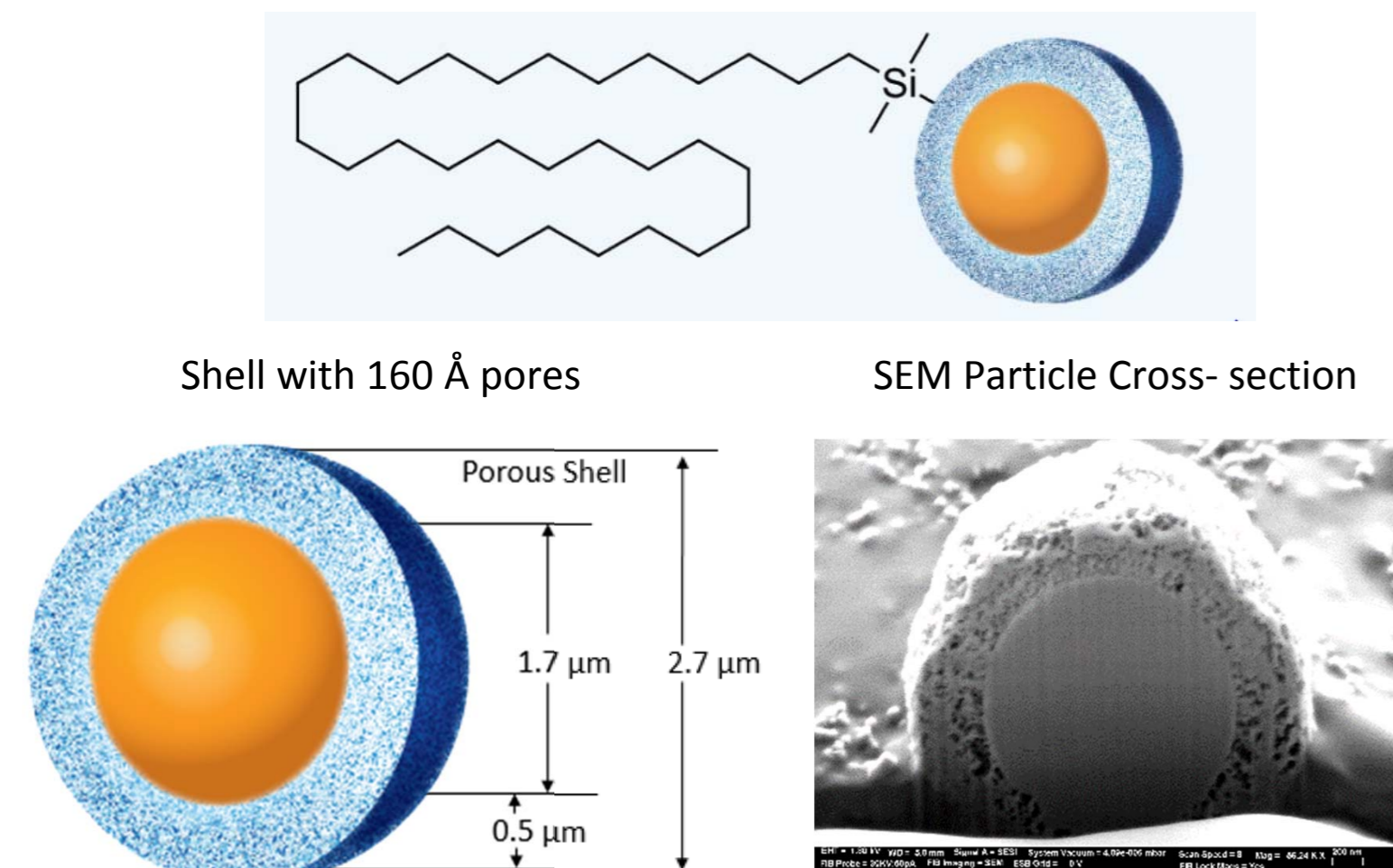
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Introduction

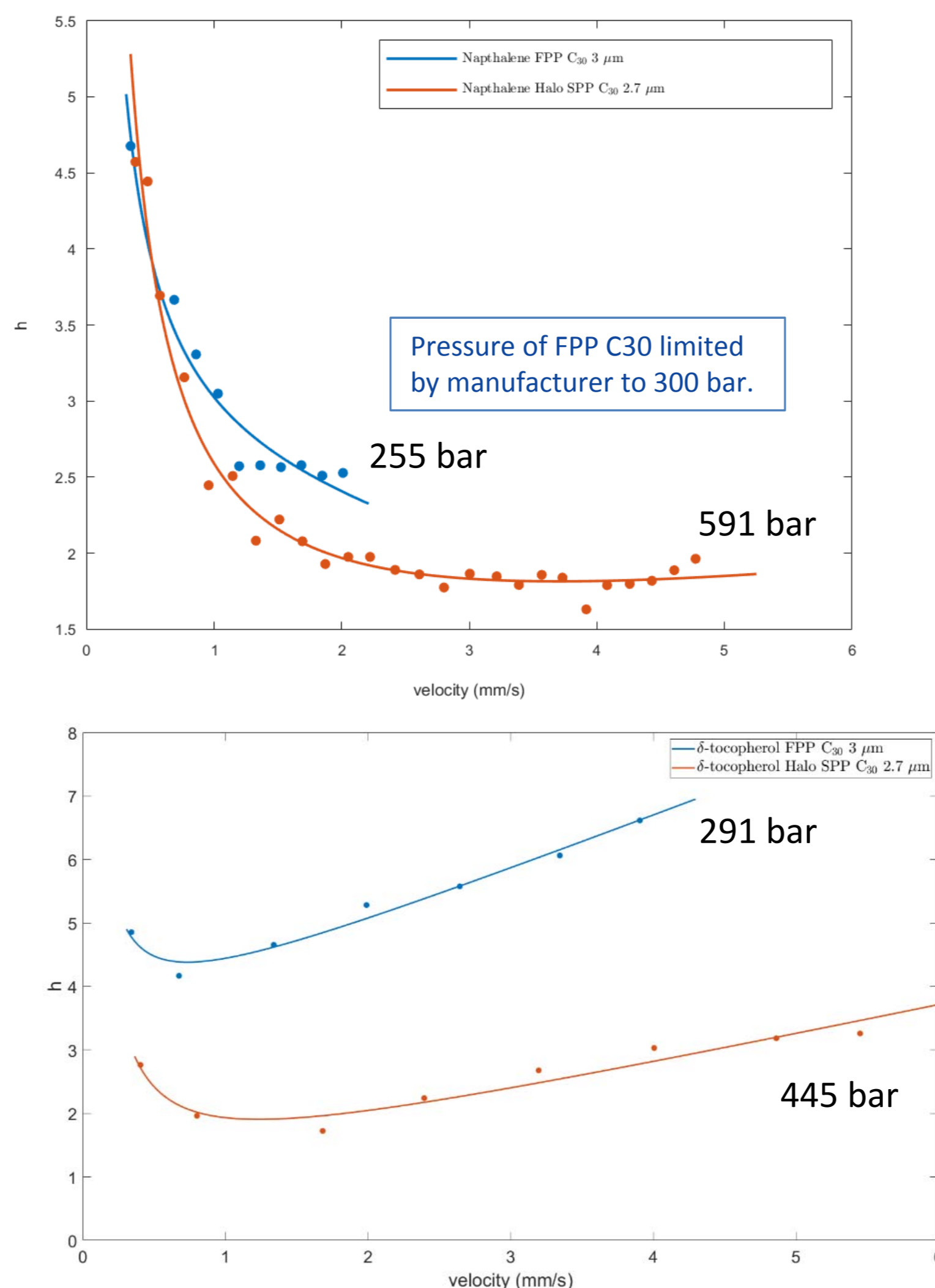
- C30 stationary phases have demonstrated special selectivity for isomers, such as fat-soluble vitamins, over traditional alkyl phases.
- SPP columns can be designed with wide-pore, 160 Å particles that permit separation at high flows without performance and pressure restrictions that limit assay speed and adversely impact resolution.
- Wider pore SPP particles show advantages for attaching larger stationary phases such as C30 while maintaining fast mass transfer and high column efficiency.
- The benefit of 100% aqueous compatibility allows the C30 to be a selectivity option for polar compounds.

Ligand: Tricontyldimethyl USP Designation: L62 Endcapped: Yes
Particle Size: 2.7 µm Carbon Load: 4.5 % Low pH Limit/ Max T: 2/ 60°C
Pore Size: 160 Å Surface Area: 90 m²/g High pH Limit/ Max T: 9/ 40°C



The HALO 160 Å particle is a superficially porous particle made with a 1.7 µm solid core and a 0.5 µm porous shell.

Fig. 4: Lower Reduced Plate Heights for SPP C30



Naphthalene Test Conditions:

Column: HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm
Mobile Phase A: Water
Mobile Phase B: Acetonitrile
Isocratic: 50% B
Temperature: 30 °C
Detection: UV 254 nm, PDA
Injection Volume: 1 µl
Sample Solvent: 50/50 Acetonitrile/ Water
Data Rate: 40 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µl
LC System: Shimadzu Nexera X2

Delta Tocopherol Test Conditions:

Column: HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm
Isocratic: 100% Acetonitrile
Temperature: 30 °C
Detection: UV 280 nm, PDA
Injection Volume: 1 µl
Sample Solvent: Acetonitrile
Data Rate: 40 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µl
LC System: Shimadzu Nexera X2

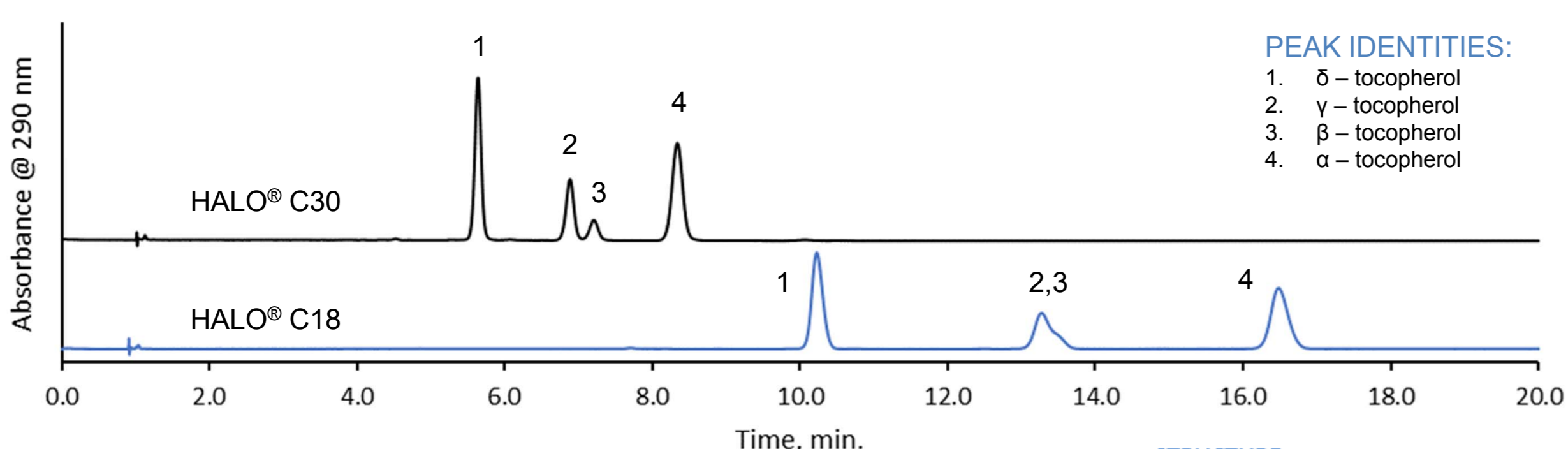
Δ-Tocopherol FPP C30 3 µm (pore size not specified), pmax 300 Bar

Flow	T ₀ (min)	T ₀ (Secs)	velocity mm/sec	Plates	Plate Ht. (mm)	Reduced Plt Ht.	Pressure (Bar)	Void Volume
0.25	7.33	440.02	0.341	10299	0.0146	4.85	23	1.83
0.5	3.70	222.22	0.675	11999	0.0125	4.17	45	1.85
1.00	1.87	112	1.339	10742	0.0140	4.65	88	1.87
1.50	1.26	75.38	1.990	9466	0.0158	5.28	138	1.88
2.00	0.95	56.74	2.644	8966	0.0167	5.58	188	1.89
2.50	0.75	44.84	3.345	8248	0.0182	6.06	244	1.87
2.90	0.64	38.4	3.906	7559	0.0198	6.61	291	1.86

Δ-Tocopherol SPP C30 2.7 µm 160 Å, pmax 600 bar

Flow	T ₀ (min)	T ₀ (Secs)	velocity mm/sec	Plates	Plate Ht. (mm)	Reduced Plt Ht.	Pressure (Bar)	Void Volume
0.25	6.18	370.58	0.405	20103	0.0075	2.49	29	1.54
0.5	3.12	187.32	0.801	28316	0.0053	1.77	57	1.56
1.00	1.49	89.14	1.683	32223	0.0047	1.55	115	1.49
1.50	1.04	62.64	2.395	24793	0.0061	2.02	175	1.57
2.00	0.78	46.92	3.197	20747	0.0072	2.41	240	1.56
2.50	0.62	37.44	4.006	18331	0.0082	2.73	309	1.56
3.00	0.51	30.84	4.864	17448	0.0086	2.87	386	1.54
3.50	0.46	27.52	5.451	17046	0.0088	2.93	445	1.61

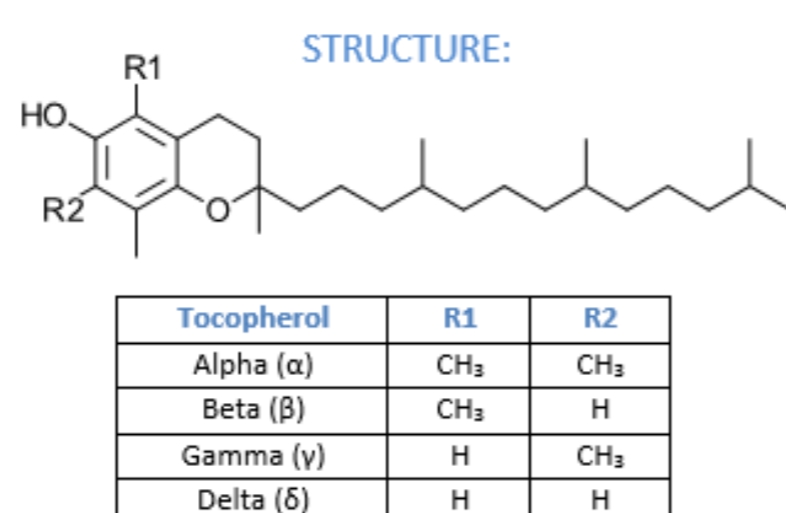
Fig. 1: Vitamin E: Tocopherols



PEAK IDENTITIES:
1. δ - tocopherol
2. γ - tocopherol
3. β - tocopherol
4. α - tocopherol

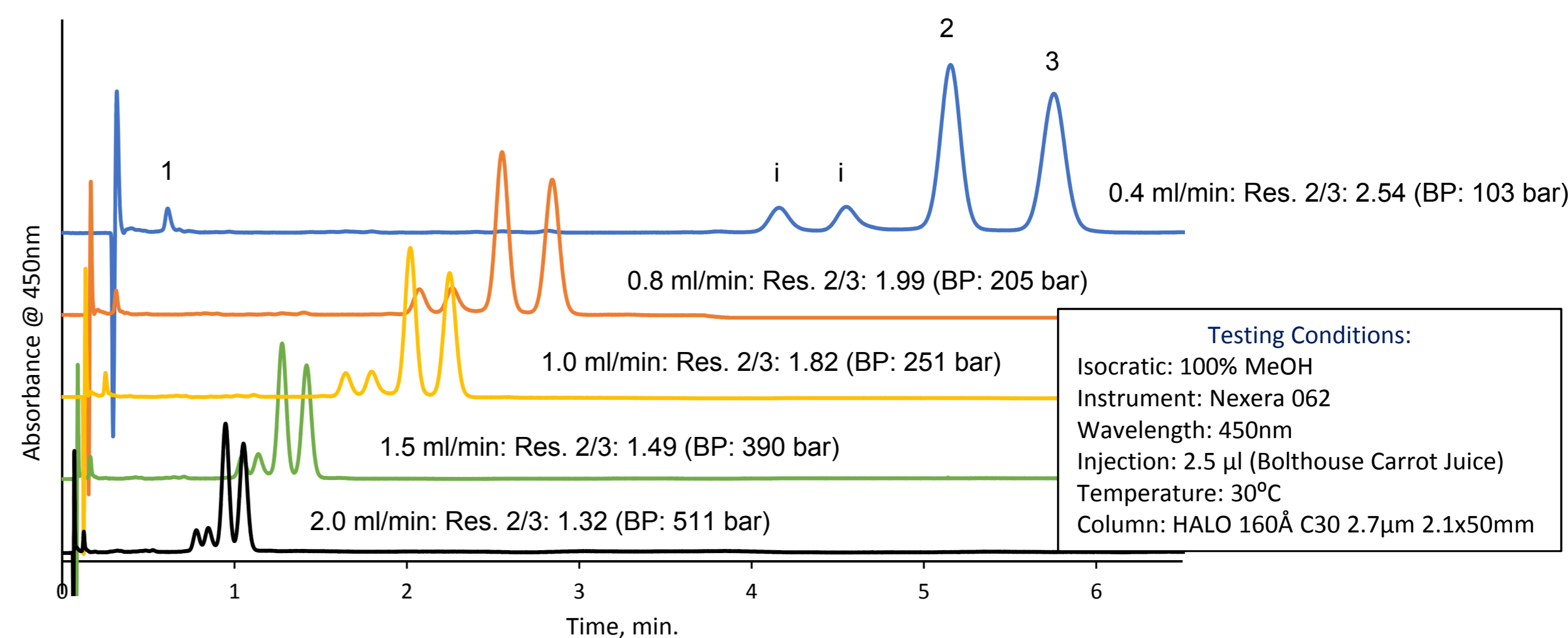
TEST CONDITIONS:

Columns: HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm
HALO 90 Å C18, 2.7 µm, 4.6 x 150 mm
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



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.

Fig. 5: Flat van Deemter Plots Allow Fast Carotenoid Separations



PEAK IDENTITIES:

- Lutein
- α-carotene
- β-carotene
- unidentified isomers

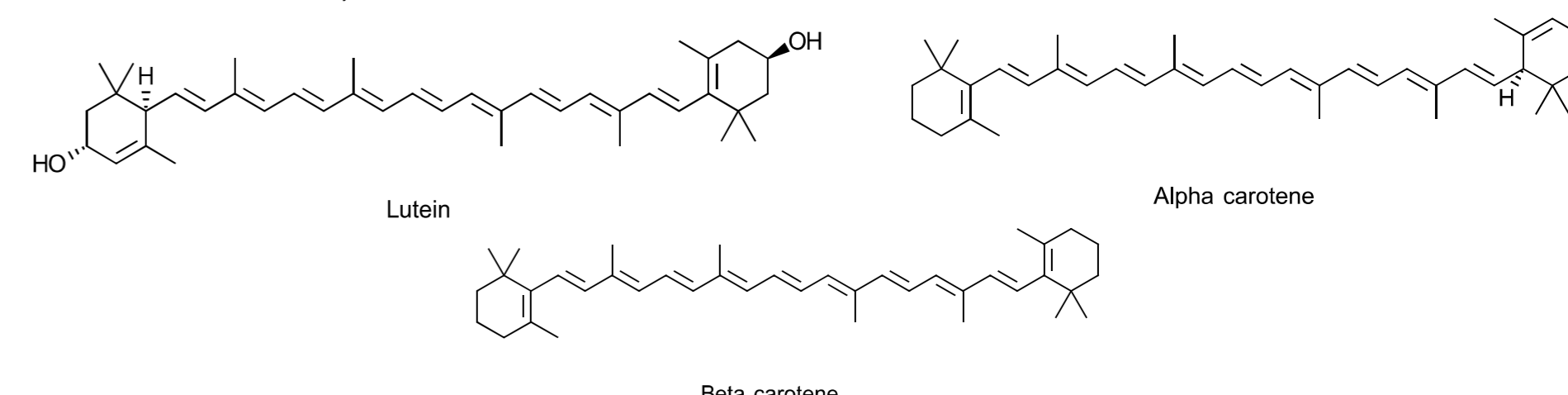
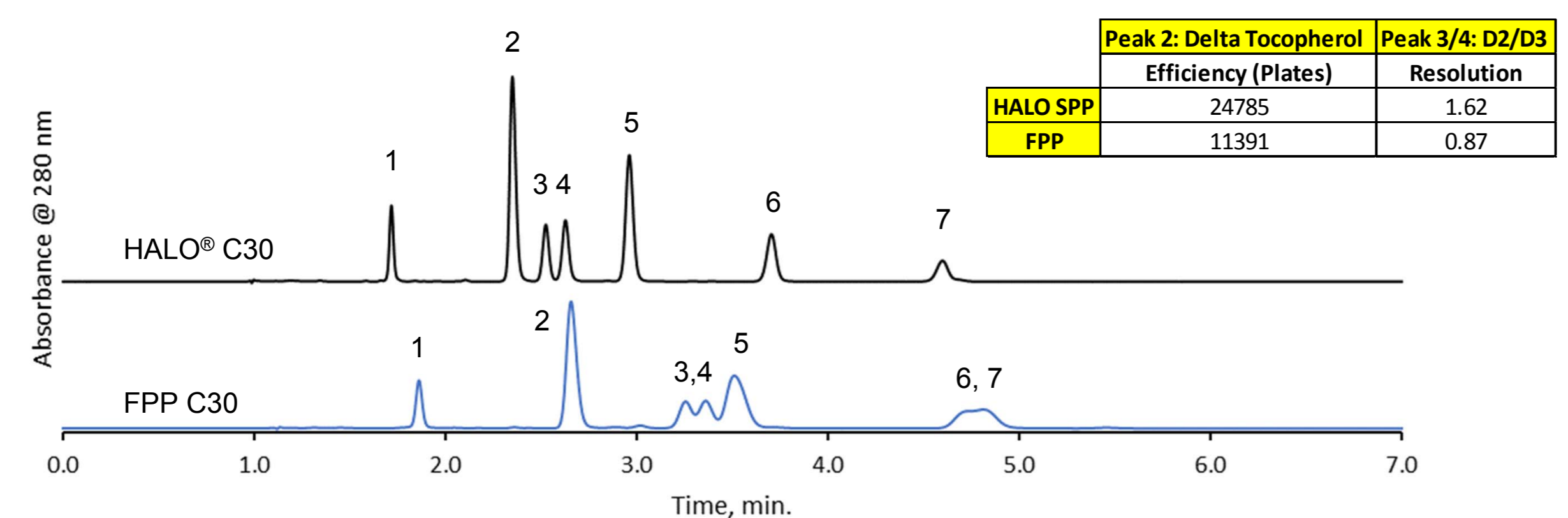


Fig. 2: Fat-soluble Vitamins: SPP vs. FPP



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

TEST CONDITIONS:

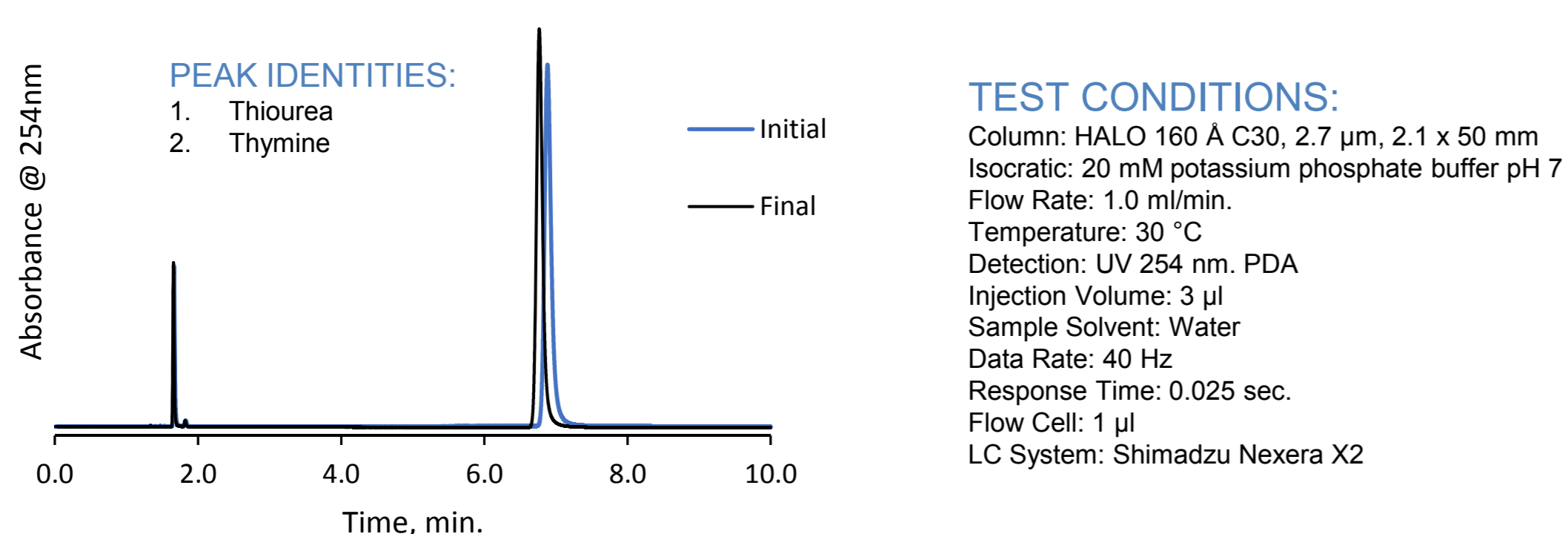
Column: HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm
Isocratic: 100% Methanol
Flow Rate: 1.5 mL/min
Initial HALO Pressure: 262 bar
Initial Competitor Pressure: 207 bar
Temperature: 30 °C
Detection: UV 280 nm, PDA
Injection Volume: 2.0 µl
Sample Solvent: Methanol
Data Rate: 40 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µl
LC System: Shimadzu Nexera X2

PEAK IDENTITIES:

- Retinyl acetate (Vitamin A)
- Δ-tocopherol (Vitamin E)
- Ergocalciferol (Vitamin D2)
- Cholecalciferol (Vitamin D3)
- α-tocopherol (Vitamin E)
- dl-α-tocopherol acetate (Vitamin E)
- 2,3- trans- phyloquinone (Vitamin K)

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 improved when using the SPP technology.

Fig. 3: HALO® C30: 100% Aqueous Compatibility

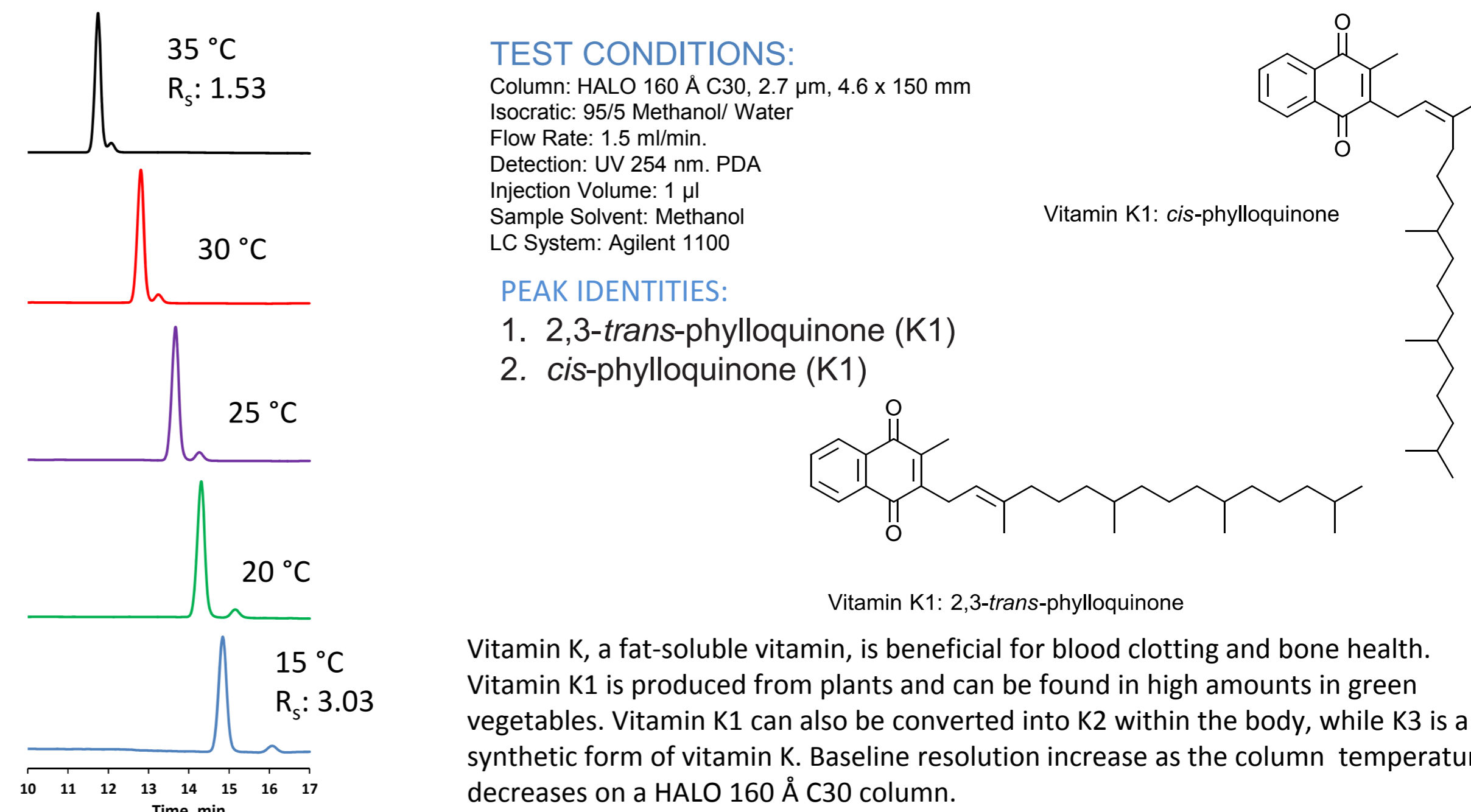


TEST CONDITIONS:

Column: HALO 160 Å C30, 2.7 µm, 2.1 x 50 mm
Isocratic: 20 mM potassium phosphate buffer pH 7
Flow Rate: 1.0 mL/min.
Temperature: 30 °C
Detection: UV 254 nm, PDA
Injection Volume: 3 µl
Sample Solvent: Water
Data Rate: 40 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µl
LC System: Shimadzu Nexera X2

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.

Fig. 6: Effect of Temperature on Resolution of trans and cis Vitamin K1

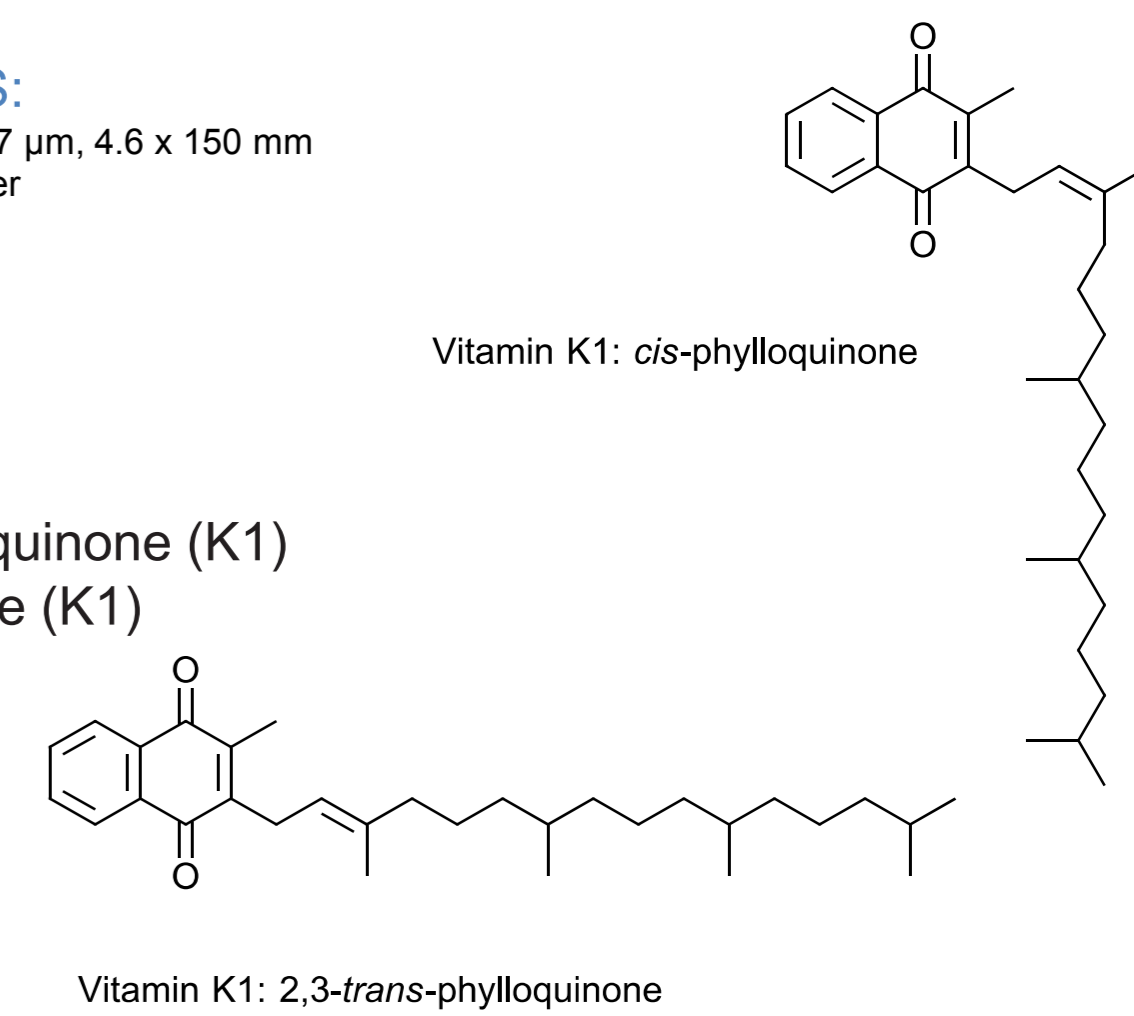


TEST CONDITIONS:

Column: HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm
Isocratic: 95/5 Methanol/ Water
Flow Rate: 1.5 mL/min.
Detection: UV 254 nm, PDA
Injection Volume: 1 µl
Sample Solvent: Methanol
LC System: Agilent 1100

PEAK IDENTITIES:

- 2,3-trans-phyloquinone (K1)
- cis-phyloquinone (K1)



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. Baseline resolution increase as the column temperature decreases on a HALO 160 Å C30 column.

Conclusions

- HALO® columns are rugged and stable for both UHPLC and HPLC use at high velocities.
- HALO® C30 offers shape selectivity which is an advantage when separating positional isomers as shown with vitamins and carotenoids.
- HALO® C30 with 160 Å pores offers the Fused-Core® advantages which include sharper peaks, ability to run at increased flow rates while maintaining high efficiency and resolution, and the added benefit of 100% aqueous mobile phase compatibility.

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