Speed and Resolution with 5-Micron Superficially Porous Particles

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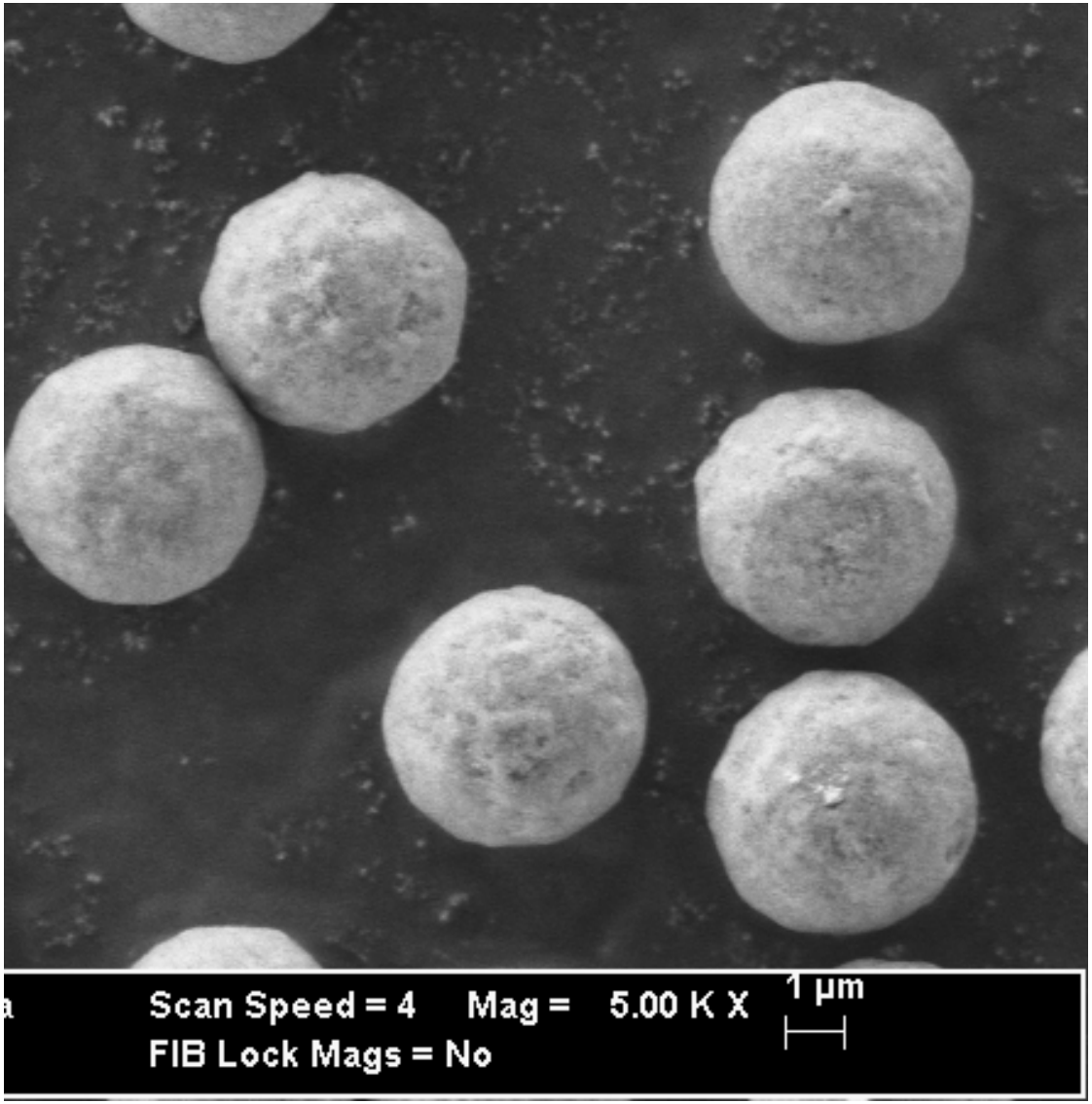
Abstract

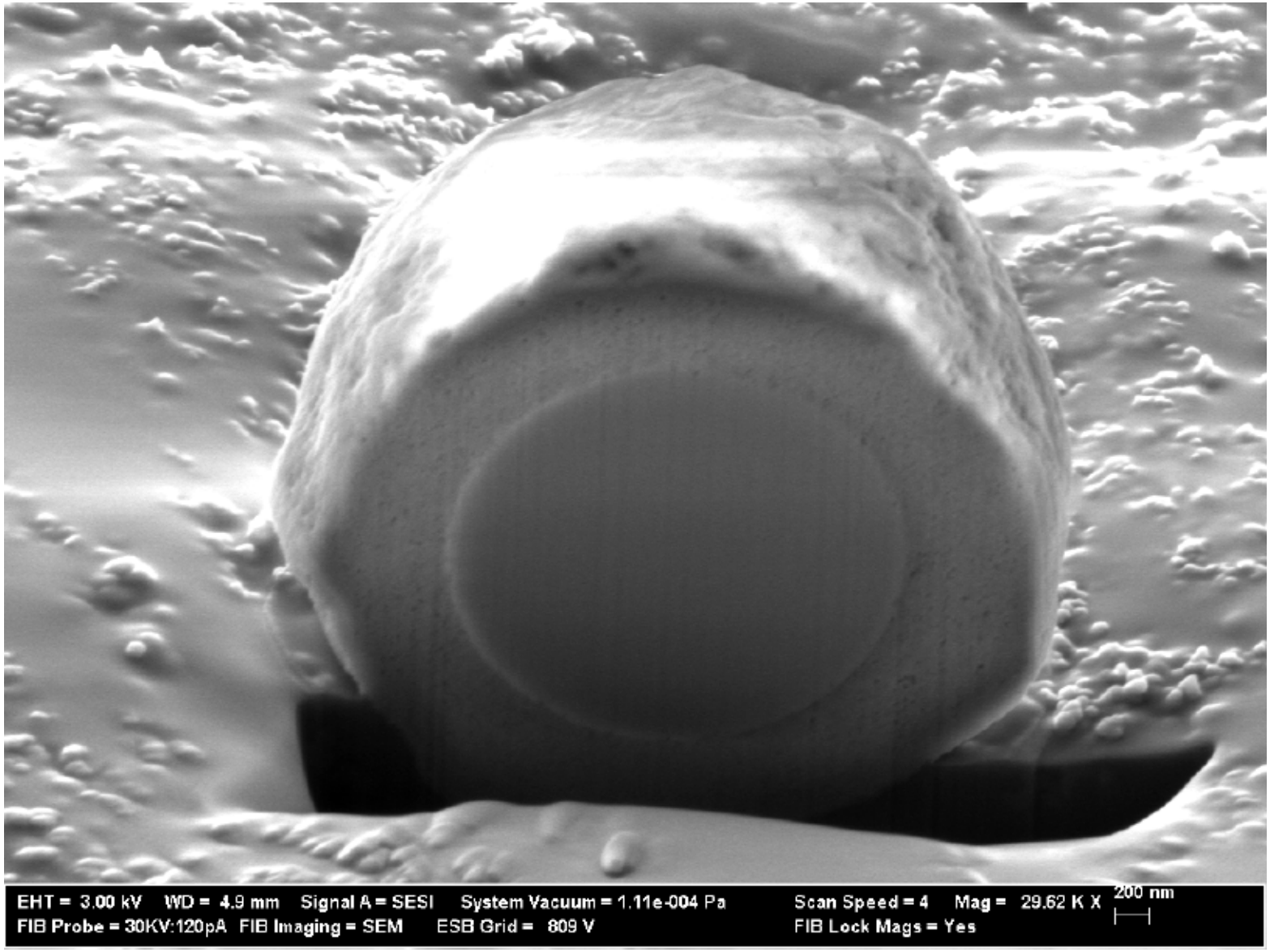
Columns packed with modern superficially porous (core-shell) particles (SPP) exhibit extraordinarily high performance compared to columns packed with totally porous particles of the same size. Many published reports by independent researchers verify that 2.5 – 2.7 micron SPP particles can produce columns with the efficiency of sub-2-micron particle columns with nearly half the back pressure of the smaller particles. The success of the SPP particles has generated interest in the development of SPP particles of other sizes. Sub-2-micron SPP for separating small molecules have recently been introduced. However, columns packed with these particles exhibit the very high back pressures consistent with their very small particle sizes. This pressure requirement limits column lengths and operating flow rates for users with HPLC equipment that cannot operate above 400 - 600 bar. All sub-2-micron particle columns, whether SPP or totally porous, provide high separation efficiencies in short column lengths for high-speed, high-throughput applications, but require ultra high pressure instrumentation for maximum utility with longer columns. Recent introductions of nominally 5-micron SPP particles take the superficially porous particle technology in a different direction. Columns of these larger-particle SPP show significant performance advantages over totally porous particles of similar size. These 5-micron SPP deliver the separation efficiency of 3-micron totally porous particle columns at nearly half the back pressure. This report examines the technology of 5-micron SPP columns with a variety of bonded phases to demonstrate the performance and advantages for larger particle SPP.

SEM Photo of 5-micron HALO-5 Particles

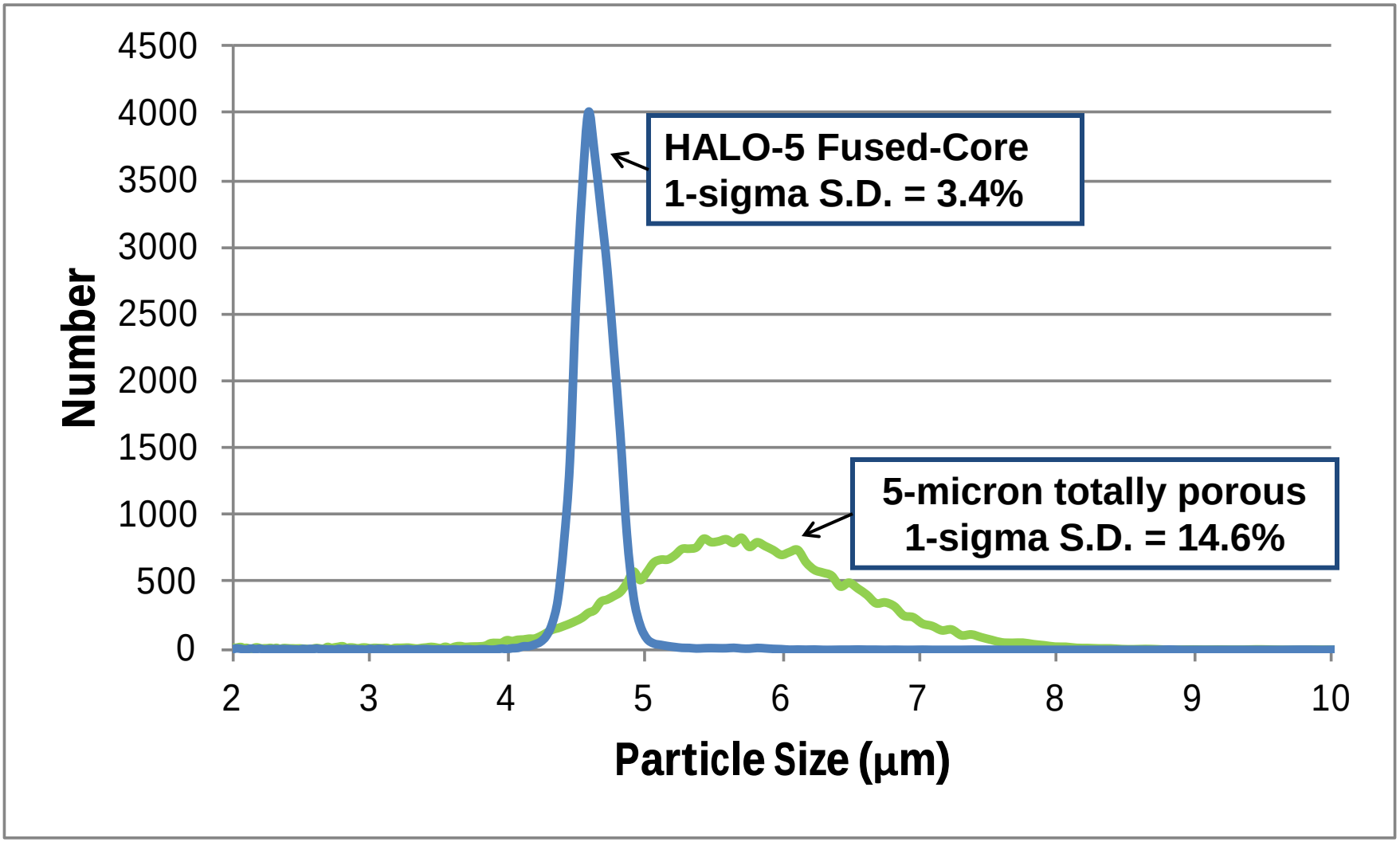


SEM Photo of FIB Sliced HALO-5 Particle

(FIB = Focused Ion Beam)



Improved Size Distributions for 5-Micron Particles



Measured on a Multisizer 3 Coulter Counter

Effect of Particle Size on Plate Height

Columns: 4.6 x 150 mm; Temperature: 30 ^{0}C
Mobile phase: 50% acetonitrile/50% water
Solute: 1-Cl-4-nitrobenzene; Injection: 1 mL
Instruments: <400 bar, Agilent 1100; >400 bar, Agilent 1200

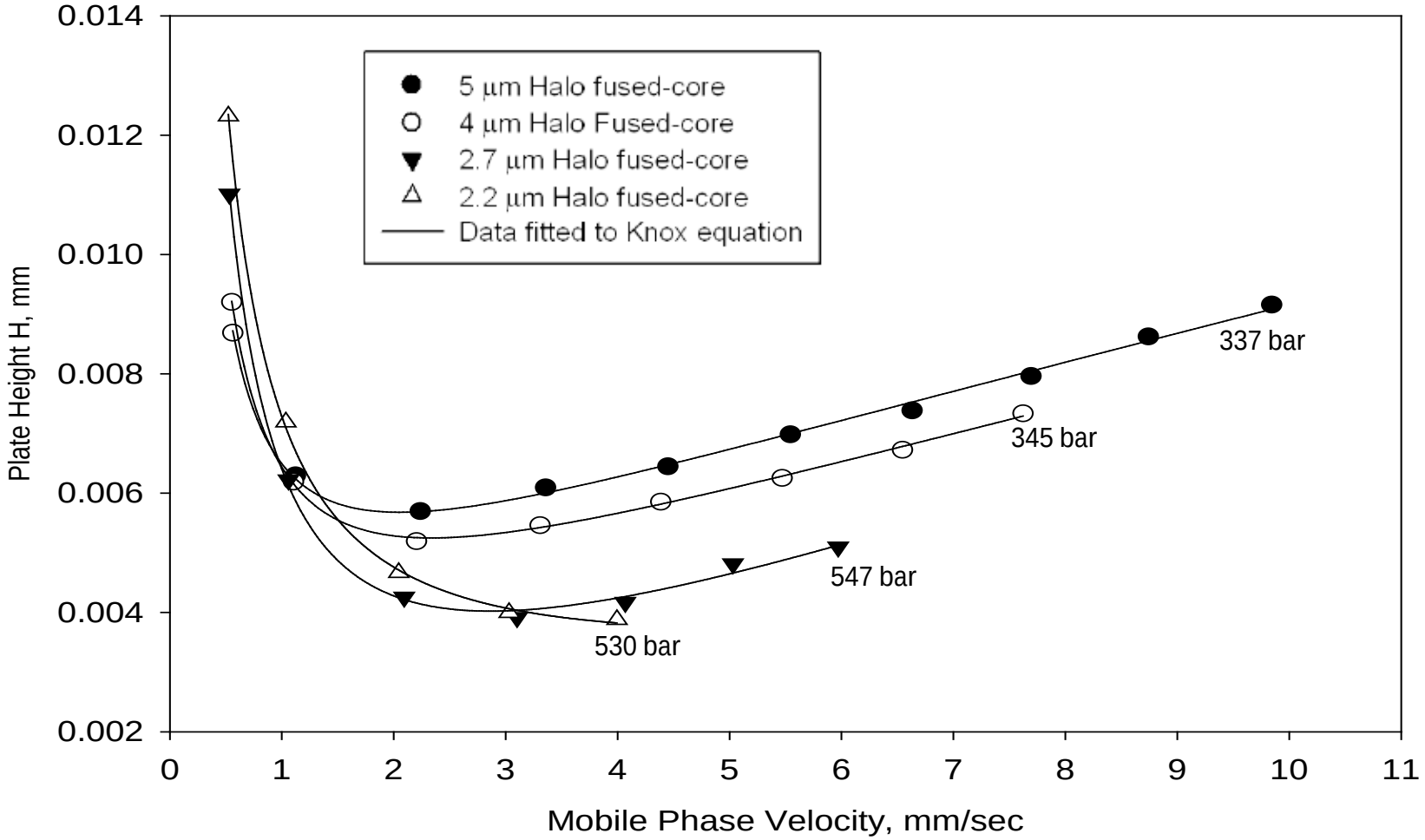
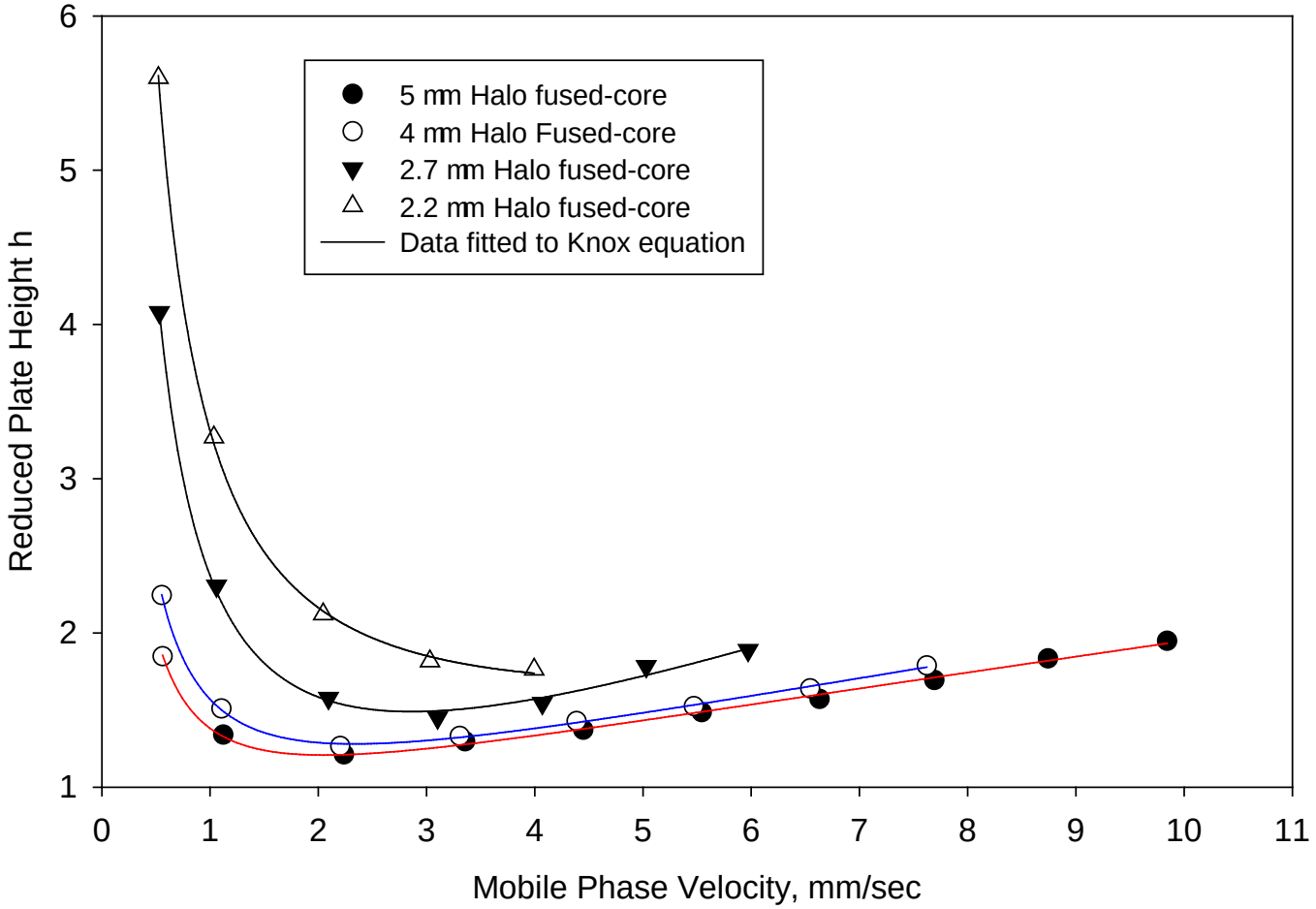


Plate height decreases with decreased particle size, as expected.
2.2, 2.7 µm column limit – 600 bar; 2.2 µm does not reach plate height minimum

Effect of Particle Size on Reduced Plate Height

Columns: 4.6 x 150 mm; Temperature: 30 ^{0}C
Mobile phase: 50% acetonitrile/50% water
Solute: 1-Cl-4-nitrobenzene: Injection: 1 mL
Instruments: <400 bar, Agilent 1100; >600 bar, Agilent 1200



h values lower for 5 µm HALO (h = 1.2) - more homogeneously packed bed structure

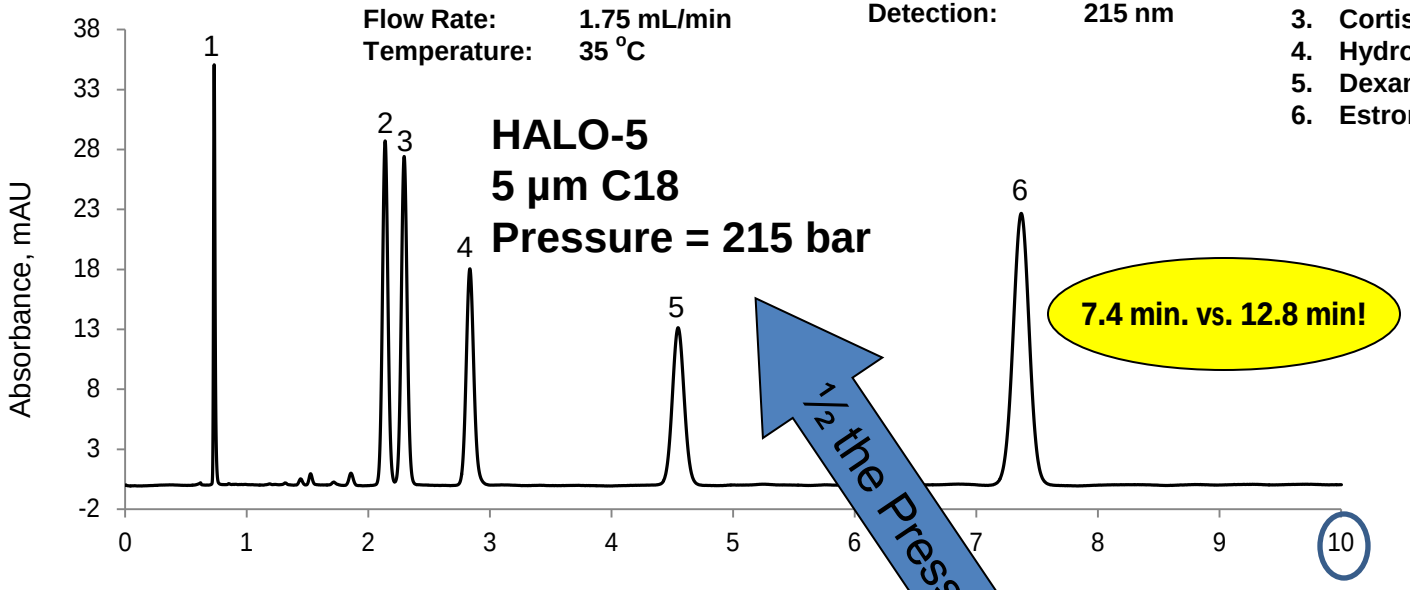
3 µm Performance with 5 µm Pressure

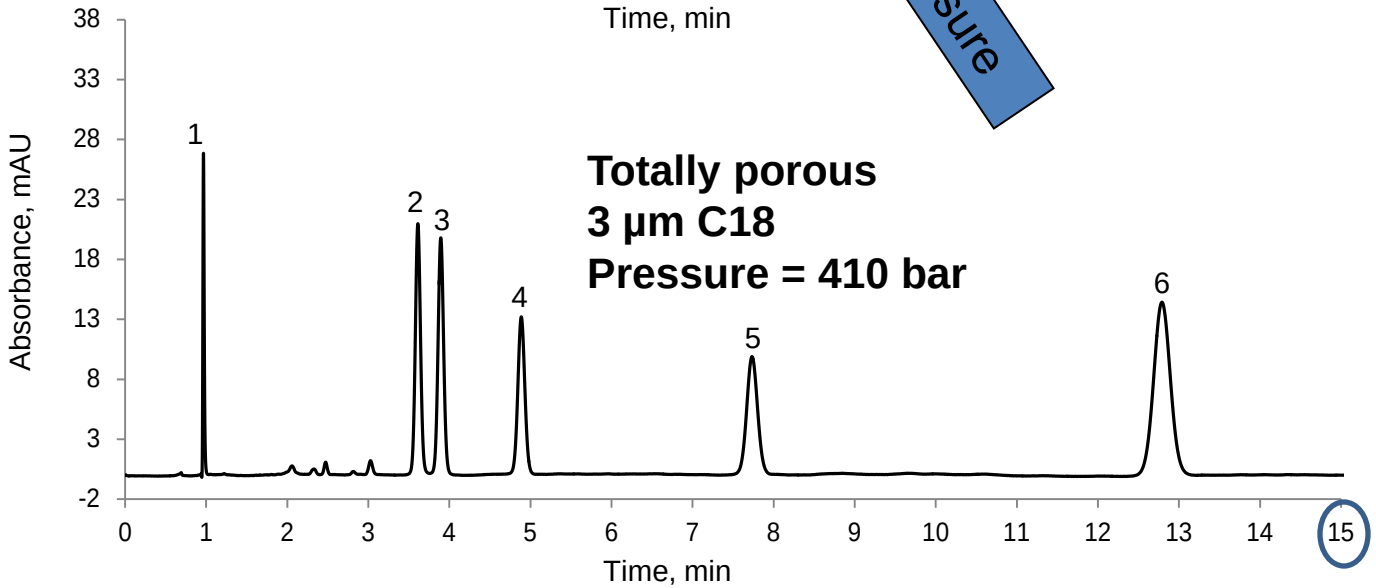
Conditions:

Columns:	**4.6 x 150 mm**	**Injection:**	**5 mL**
Mobile Phase:	**50/50 water/methanol**	**Instrument:**	**Agilent 1200**
Flow Rate:	**1.75 mL/min**	**Detection:**	**215 nm**
Temperature:	**35 °C**		

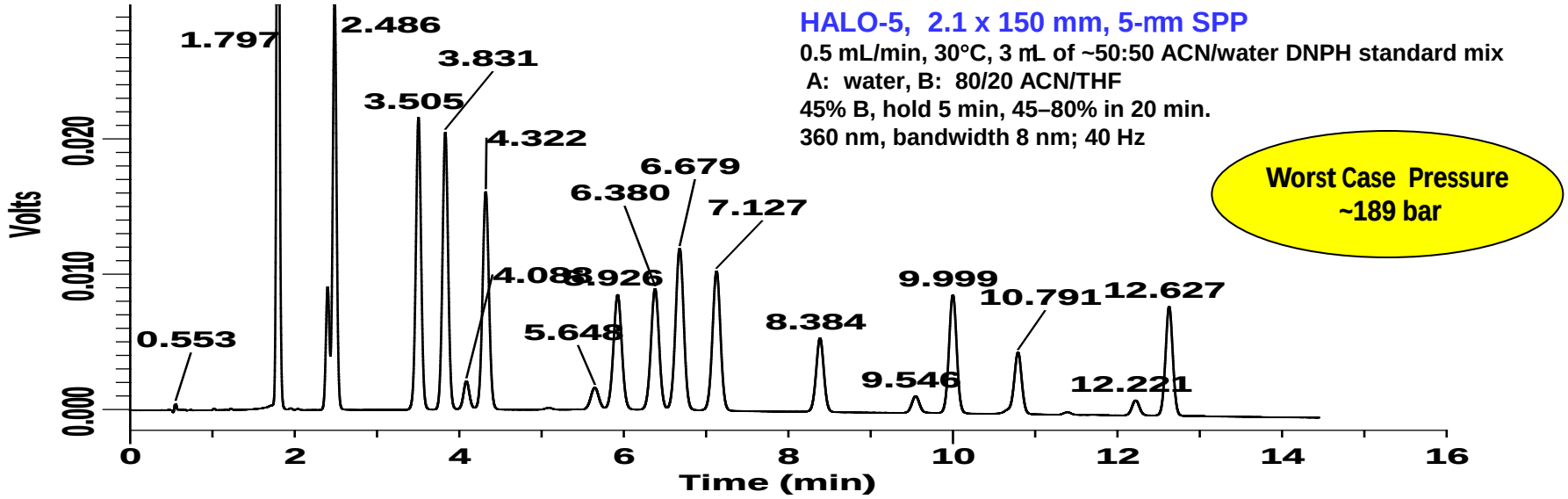
Peak Identities in order:

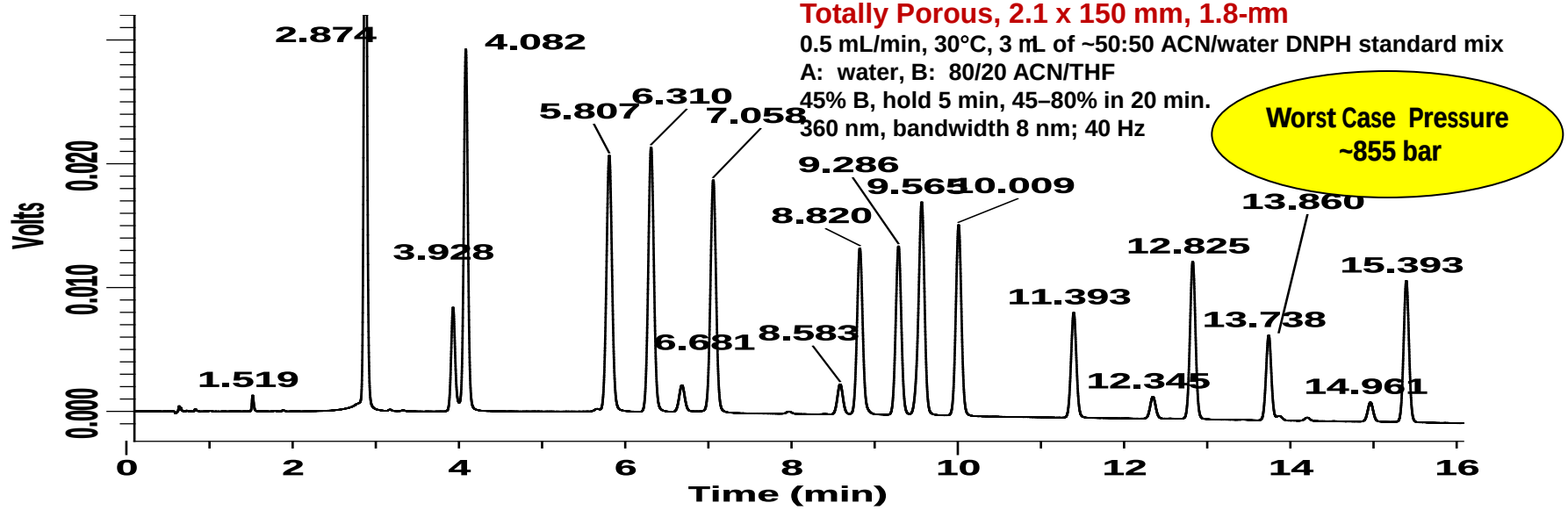
1. **Uracil**
2. **Prednisone**
3. **Cortisone**
4. **Hydrocortisone**
5. **Dexamethasone**
6. **Estrone**





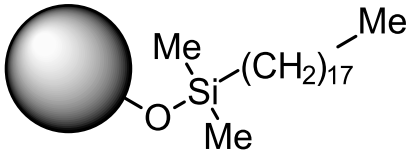
Use 5 µm SPP Columns to Achieve High Resolution at Low Pressure



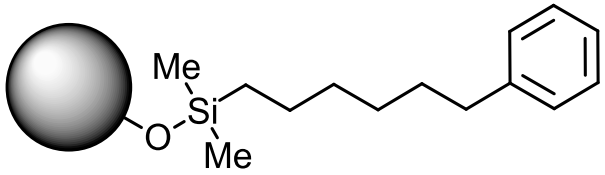


PEAK IDENTITIES (in order): Formaldehyde-2,4-DNPH, Acetaldehyde-2,4-DNPH, Acetone-2,4-DNPH, Acrolein-2,4-DNPH,Propionaldehyde-2,4-DNPH, Crotonaldehyde-2,4-DNPH, 2-Butanone-2,4-DNPH, Methacrolein-2,4-DNPH, Butyraldehyde-2,4-DNPH, Benzaldehyde-2,4-DNPH, Valeraldehyde-2,4-DNPH, m-Tolualdehyde-2,4-DNPH, and Hexaldehyde-2,4-DNPH
Note: Small peaks preceding labeled aliphatic aldehyde peaks are minor geometric isomers (syn/anti).

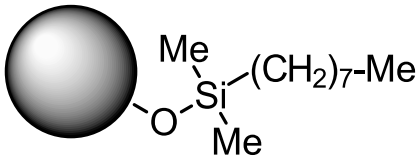
Different Stationary Phases for Modifying Selectivity



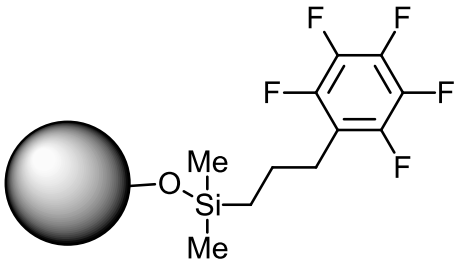
C18 (octadecyl)



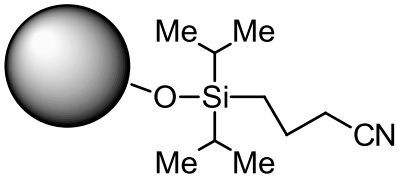
Phenyl-Hexyl



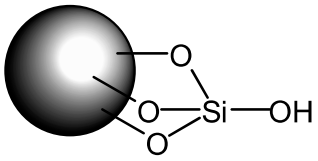
C8 (octyl)



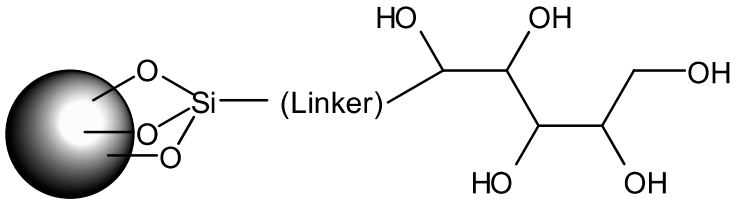
PFP (pentafluorophenylpropyl)



ES-CN

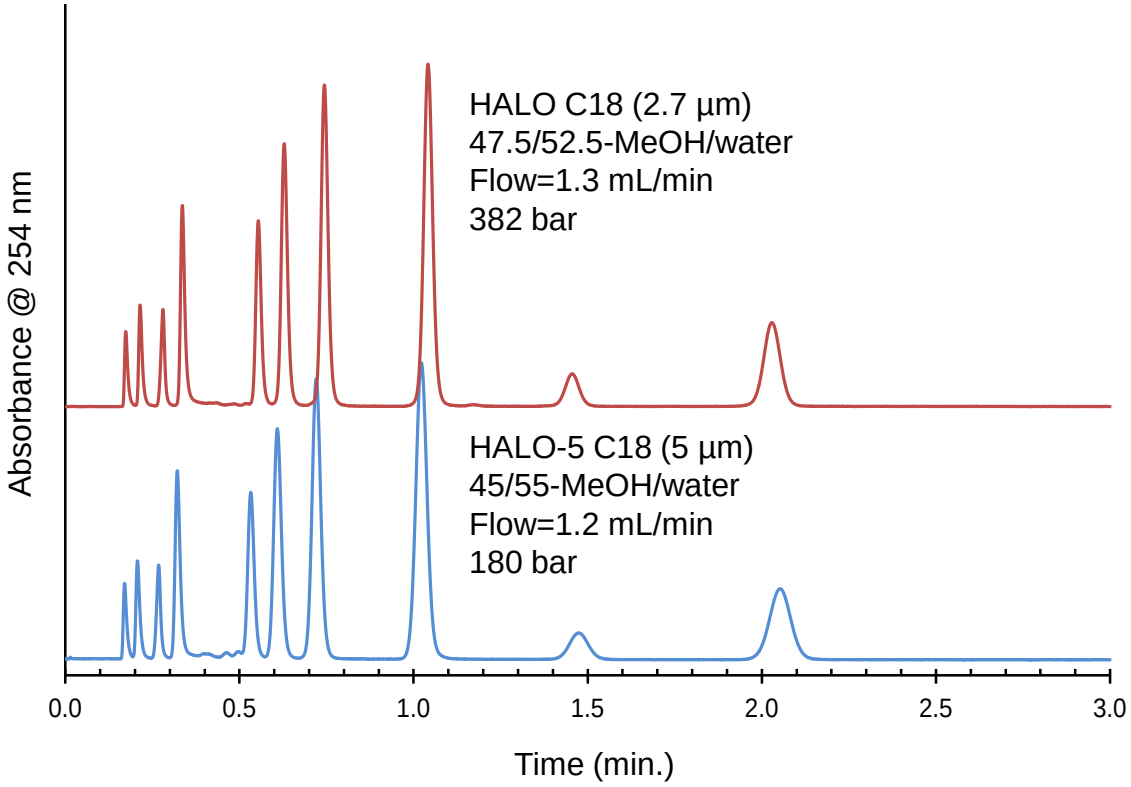


HILIC



Penta-HILIC

Easy Method Transfer from UHPLC to HPLC



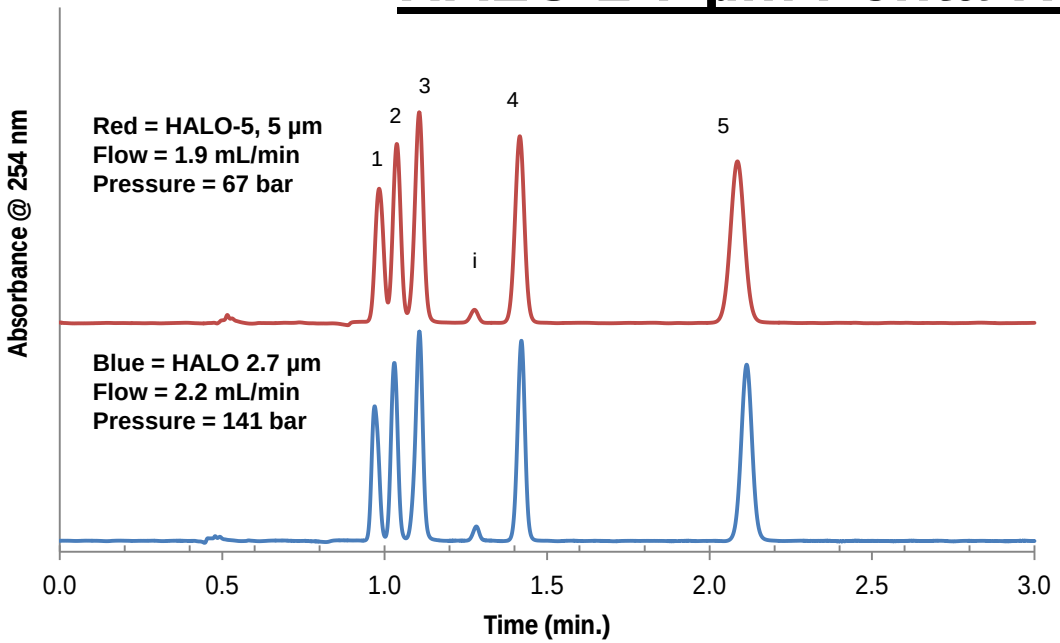
TEST CONDITIONS:

Columns:	**3.0 x 50 mm, HALO**
Mobile Phase:	**See figure.**
Flow Rate:	**See figure.**
Pressure:	**See figure.**
Temperature:	**30°C**
Detection:	**UV 254 nm, VWD**

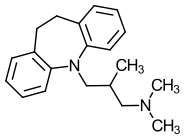
PEAK IDENTITIES (in order):

1. **Uracil**
2. **Resorcinol**
3. **Aniline**
4. **4-Chloroaniline**
5. **Acetoacetanilide**
6. **Dimethylphthalate**
7. **Cinnamyl alcohol**
8. **2,6-Dinitrotoluene**
9. **Tolbutamide**
10. **4-Chloro-3-nitroanisole**

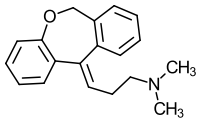
Comparable Selectivity between HALO-5 and HALO 2.7 µm Penta-HILIC Phases



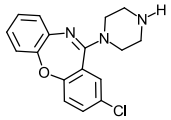
PEAK IDENTITIES:



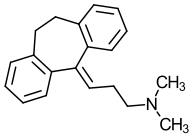
1. Trimipramine



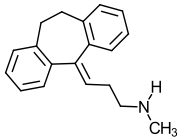
3. Doxepin



5. Amoxapine



2. Amitriptyline



4. Nortriptyline

i = Impurity

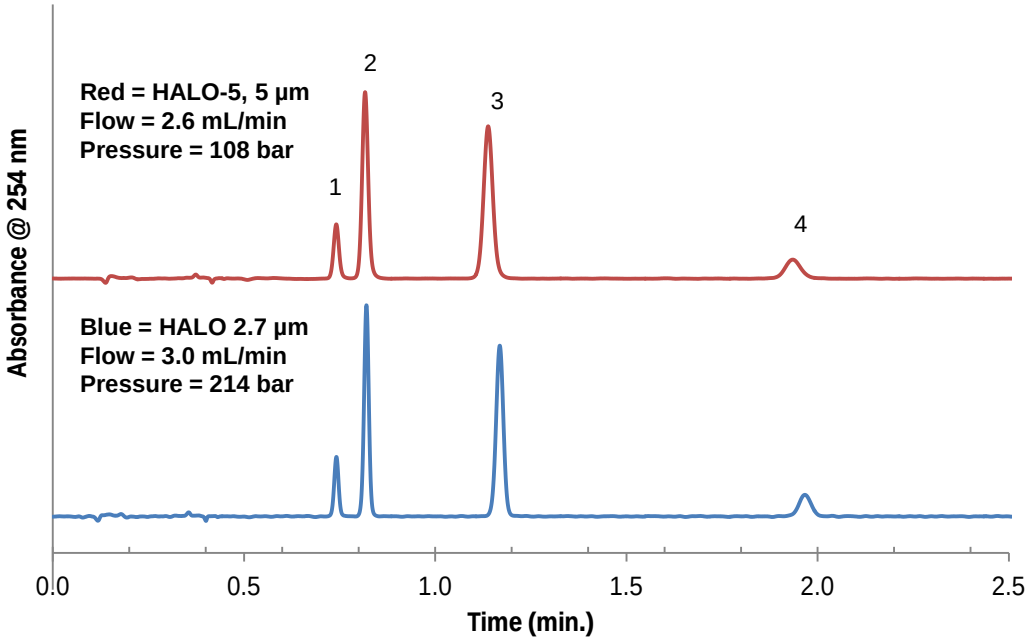
TEST CONDITIONS:

Column 1: 4.6 x 100 mm, HALO 5 µm Penta-HILIC
Part Number: 95814-605
Column 2: 4.6 x 100 mm, HALO 2.7 µm Penta-HILIC
Part Number: 92814-605
Mobile Phase: 5/95: A/B
A= 0.1 M Ammonium formate, pH=3 (adj.)
B= Acetonitrile
Flow Rate: See chart
Pressure: See chart

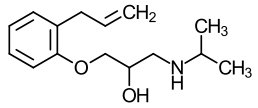
Temperature: 30°C
Detection: UV 254 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: 10/90: Water/Acetonitrile
Response Time: 0.02 sec.
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
ECV: ~14 µL

Similar selectivity is achieved between the 5 µm and 2.7 µm HALO Penta-HILIC particle sizes through a slight flow rate adjustment allowing easy method transfer.

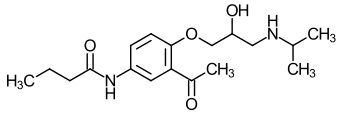
Comparable Selectivity between HALO-5 and HALO 2.7 µm HILIC Phases



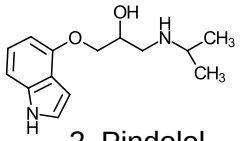
PEAK IDENTITIES:



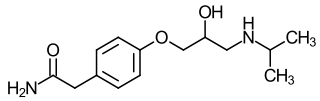
1. Alprenolol



3. Acebutolol



2. Pindolol



4. Atenolol

TEST CONDITIONS:

Column 1: 4.6 x 100 mm, HALO 5 µm HILIC
Part Number: 95814-601
Column 2: 4.6 x 100 mm, HALO 2.7 µm HILIC
Part Number: 92814-601
Mobile Phase: 11/89: A/B
A = 0.1 M Ammonium formate, pH=3 (adj.)
B = Acetonitrile
Flow Rate: See chart
Pressure: See chart

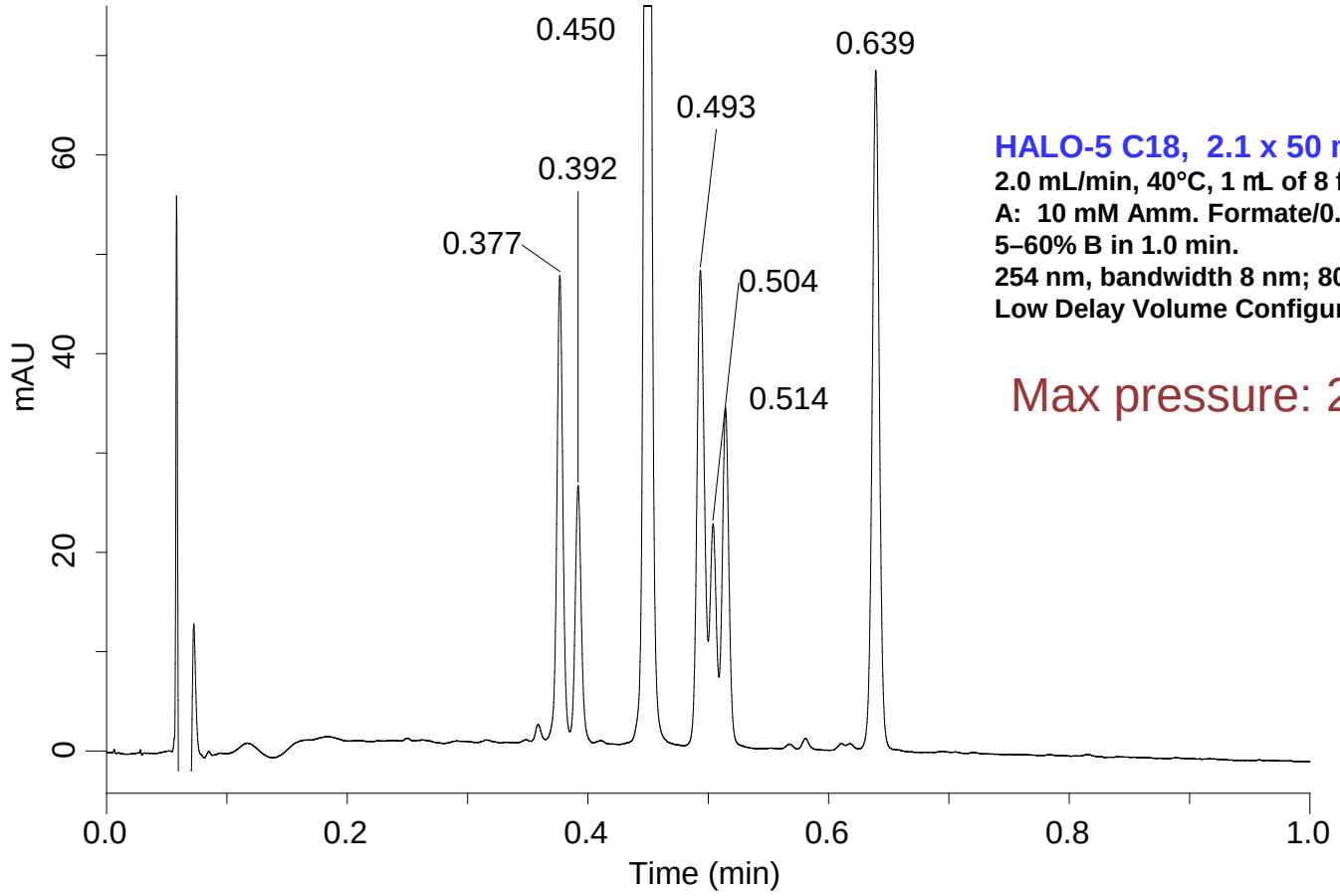
Temperature: 30°C
Detection: UV 254 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: mobile phase
Response Time: 0.02 sec.
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
ECV: ~14 µL

Go Ballistic with 5-µm HPLC Columns

- Ballistic gradients
  - Utilize narrow-bore columns (1.0, 2.1 mm ID)
  - High mobile phase linear velocities (fast flow rates for column diameter)
  - Fast gradient times (1 – 5 minutes) over a wide range of organic modifier (e.g., 5 – 95% or 0 – 100%)

- Useful when high throughput and ruggedness is required
  - Analyzing biological sample extracts (urine, plasma, liposomes, etc.)
  - Assessing identity and purity of compound libraries
  - Screening new product candidates
  - Following reactions
  - Monitoring dissolution experiments

Example Ballistic Gradient Run on HALO-5 Column



HALO-5 C18, 2.1 x 50 mm, 5 ㎜
2.0 mL/min, 40°C, 1 ㎕ of 8 flavonoids in MeOH
A: 10 mM Amm. Formate/0.1% formic acid, B: ACN
5–60% B in 1.0 min.
254 nm, bandwidth 8 nm; 80 Hz; Agilent 1200 SL;
Low Delay Volume Configuration ~ 120 µL; ADVR ON

Max pressure: 210 bar

Analytes, in elution order: hesperidin, myricetin , quercetin, naringenin & apigenin (coeluted), hesperetin, kaempferol, biochanin

Conclusions

- Superficially porous particles (SPP) of nominally 5 µm diameter demonstrate exceptionally high plate numbers for their particle size.
- Reduced plate height values (*h*) for 5 µm SPP columns are even smaller than those found for columns packed with 2.7 µm SPP, likely because it is easier to pack larger particles into homogeneous beds.
- Column efficiencies of 5 µm SPP are comparable to columns packed with 3 µm totally porous particles with about half the operating pressures.
- Narrow-bore 5 µm SPP columns are well-suited for high speed “ballistic gradient” separations.