Effects of Particle Size and Shell Thickness on Fused-Core® Column Performance

S. A. Schuster, J. J. DeStefano, W.L. Johnson, J. M. Lawhorn, J. J. Kirkland

Advanced Materials Technology, Inc.
3521 Silverside Rd., Suite 1-K, Wilmington, DE 19810

EAS 2012

Abstract

Fused-core (superficially porous or porous shell) particles have been shown to have distinct advantages over comparable totally porous particles for separating small molecules. Columns of fused-core particles show higher efficiency than totally porous particles of similar size because of superior eddy diffusion properties (smaller van Deemter A term) resulting from the homogeneous packed beds made possible by the exceptionally narrow particle size distributions of the fused-core particles. The efficiency for columns of 2.7 µm fused-core particles actually rivals that for sub-2 µm totally porous particles with only about one-half the back pressure. Wider-pore fused-core particles now are commercially available for separating larger molecules such as peptides and proteins. These particles show special advantages with these larger molecules for fast separations at high mobile phase velocities because of superior mass transfer (kinetic) properties (smaller van Deemter C term) in the thin porous shells. Fused-core particles with a wide range of particle sizes and porous shell thicknesses have been synthesized to allow the study of the effects of these physical characteristics on chromatographic performance. This report describes the effect of these particle characteristics on several factors of separation importance, including reduced plate height, separation efficiency, and sample loading. Surprisingly, the performance of the larger fused-core particles (5 µm) exceeded expectations, likely because larger particles are easier to pack into homogeneous packed beds. Not surprisingly, thinner shells on fused-core particles provide performance advantages but at the cost of decreased sample retention and loadability. Chromatograms demonstrating the advantages of using fused-core particles over totally porous particles will be shown for a variety of applications.

Report Objectives

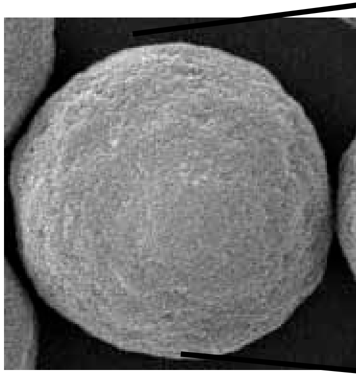
Vary Characteristics of Fused-core particles that are controllable

- **Particle Size – affects efficiency, pressure**
- **Shell Thickness – affects efficiency, sample loading, retention**
- **Pore Size – defines molecular weight range for solutes (not discussed here)**

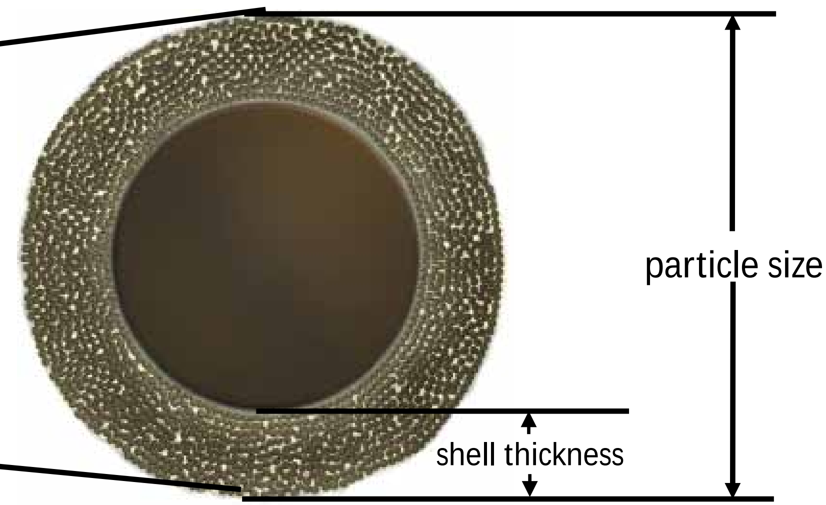
Demonstrate Features and Benefits of Different Fused-core particles

- **Examine performance trade-offs resulting from particle size differences, shell thicknesses**
- **Compare performance of 3 and 5 µm totally porous particles with new 5 µm Fused-core particles**
- **Demonstrate advantages of selecting the characteristics of fused-core particles for high-speed or high-resolution separations.**

HALO® Fused-Core Particles



SEM of HALO Fused-core



Graphical representation of HALO Fused-core

Effect of Particle Size

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

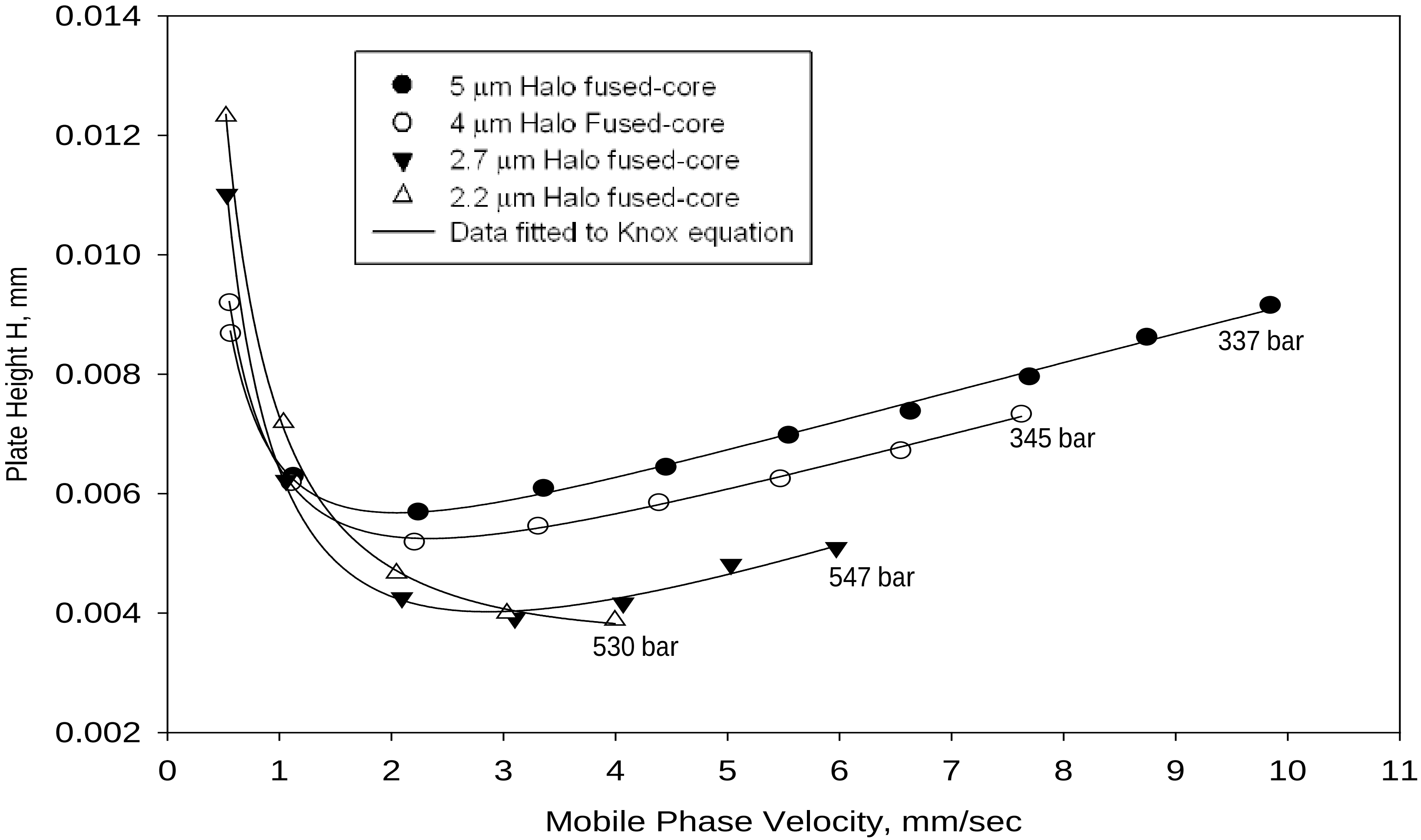
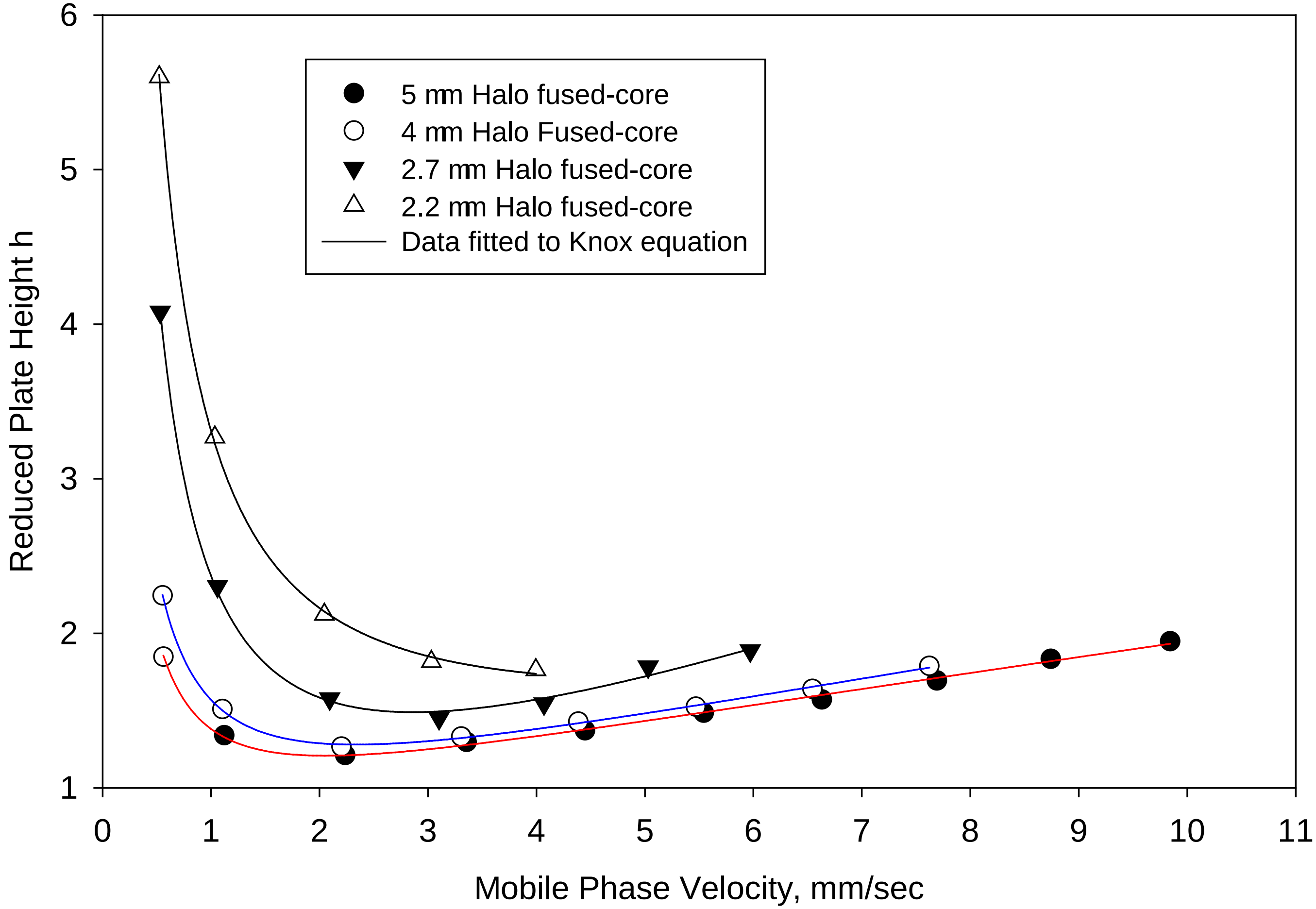


Plate height decreases with decreased particle size, as expected.
2.2, 2.7 µm column limit – 600 bar; 2.2 µm does not reach plate height minimum

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

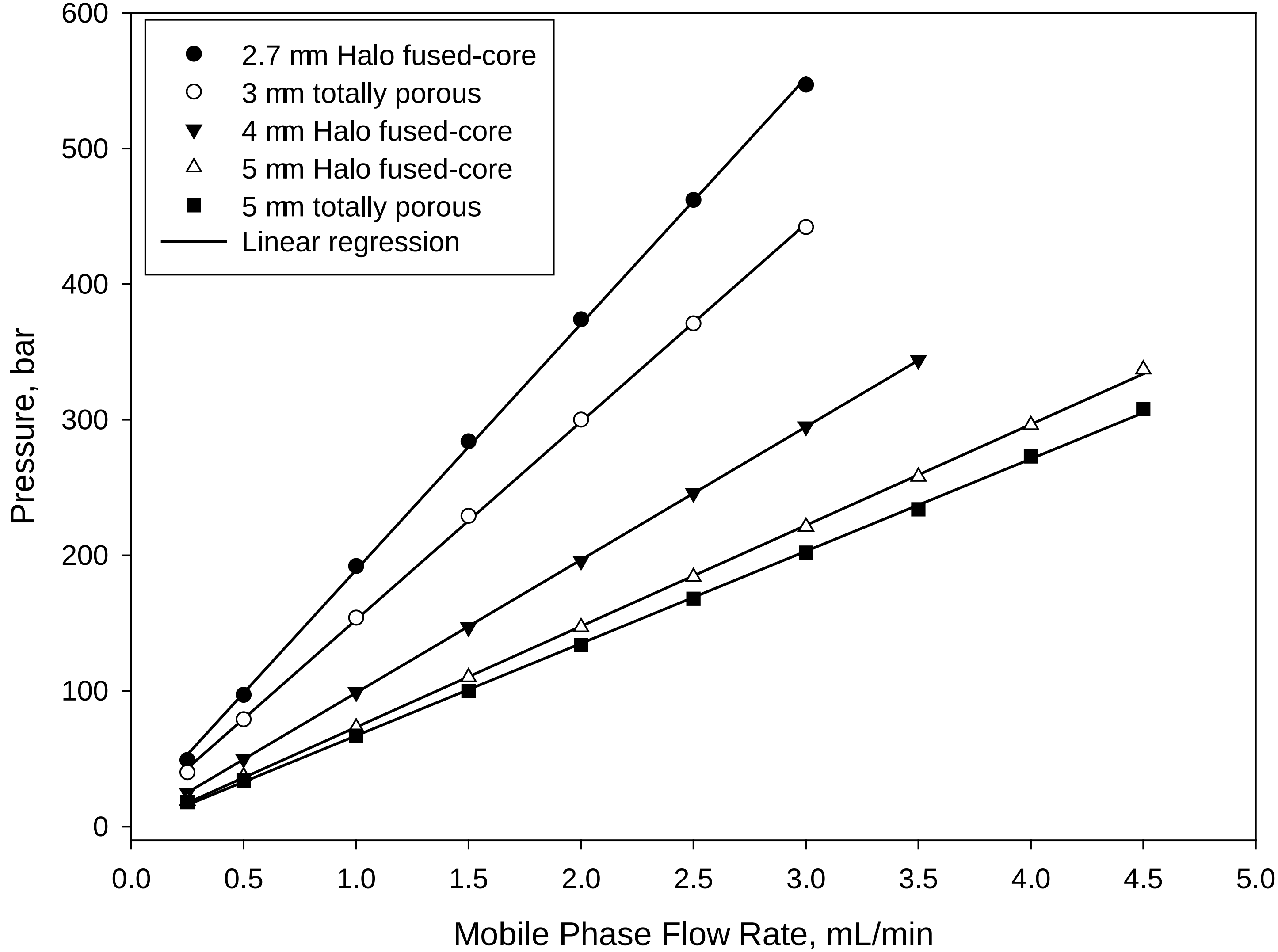


h values lower for 5 µm HALO (h = 1.2) - more homogeneously packed bed structure

Effect of Flow Rate on Column Pressure

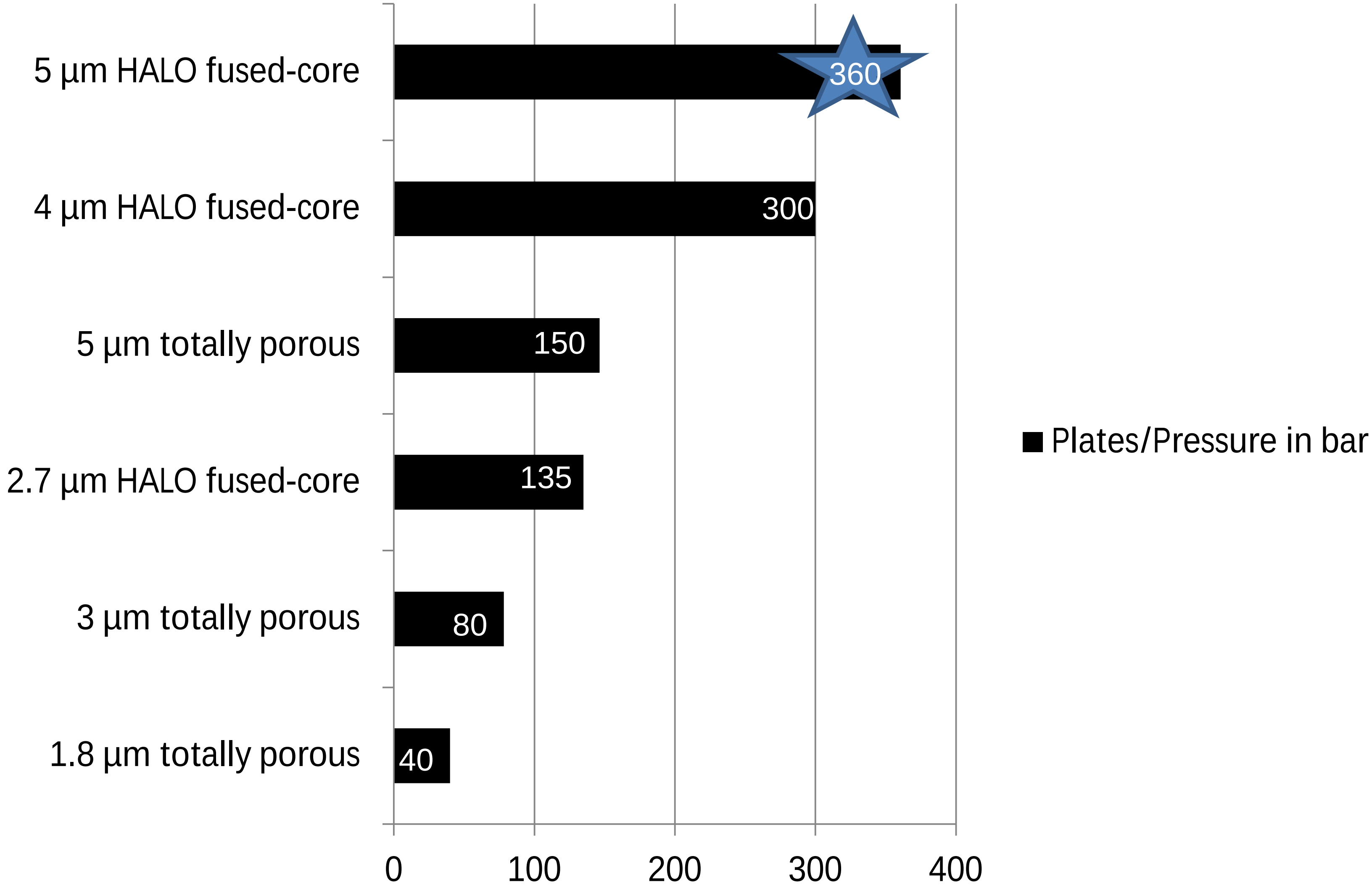
Columns: 4.6 x 150 mm; Temperature: 30 ^{o}C

Mobile phase: 50% acetonitrile/50% water



Pressure increases with $\left(\frac{1}{d_p}\right)^2$; small particles require high pressure

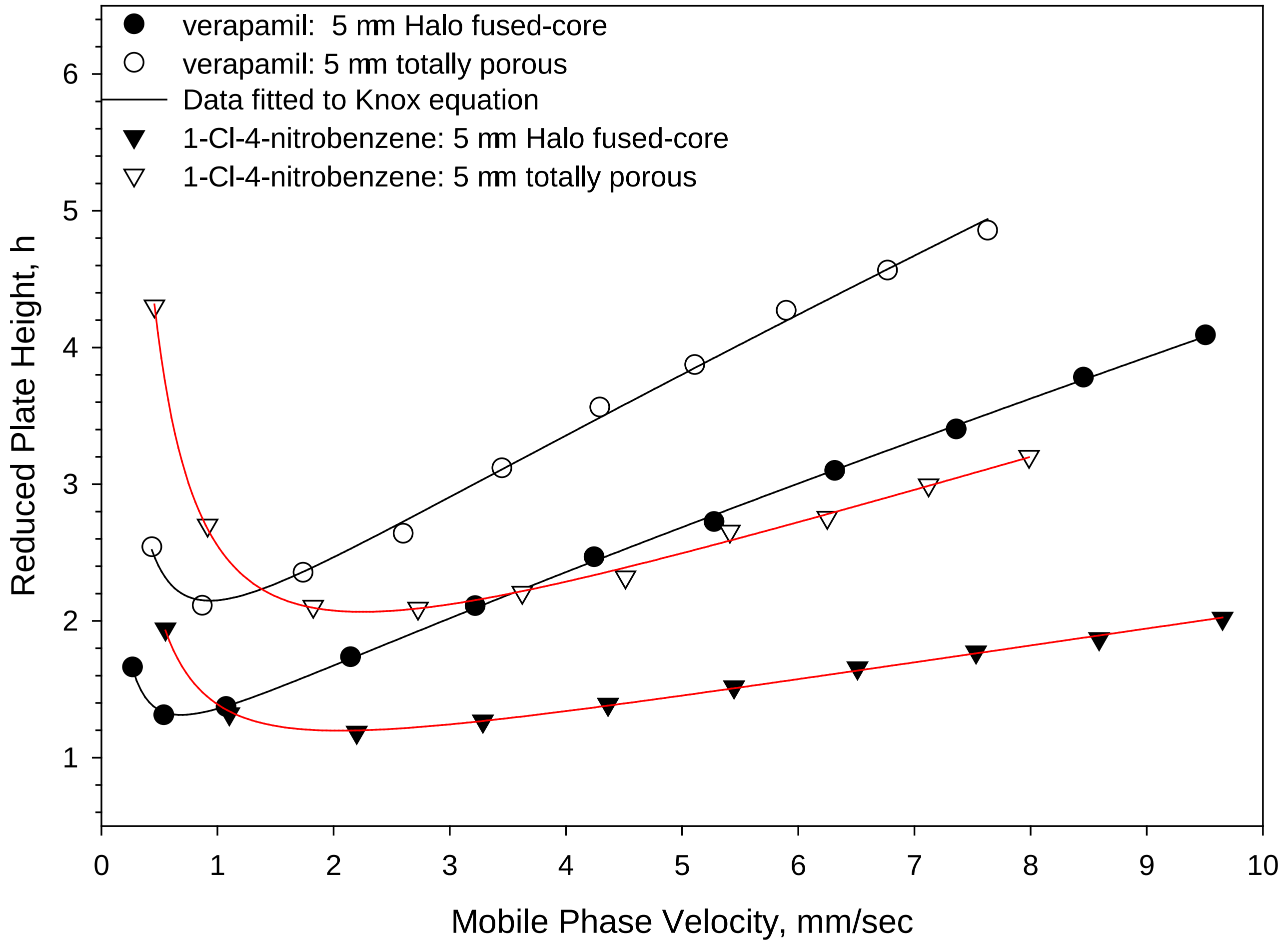
Plates/Pressure for Various Particle Sizes



The 5 µm HALO fused-core particle has more than double the number of plates/pressure of the 5 µm totally porous particles and four times the number of plates/pressure of the 3 µm totally porous particles. Data on 4.6 x 150 mm columns at the plate height minimum, except for 1.8 µm particle (estimated).

Comparison: Fused-core vs. Totally Porous Particles

Columns: 4.6 x 150 mm; Instrument: Agilent 1100, autosampler
Verapamil - Mobile phase: 35% acetonitrile/65% 0.1% aqueous trifluoroacetic acid;
Temperature: 40 °C; fused-core k = 2.8, totally porous k = 6.3
1-Cl-4-Nitrobenzene - Mobile phase: 50% acetonitrile/50% water;
Temperature: 30 °C: fused-core k = 2.7, totally porous k = 4.3



Fused-core particles: reduced plate height = 1.2 (no extra-column band broadening corrections)
: higher efficiency than totally porous particles

Particle Characteristics

Particle Type	Shell thickness (µm)	BET Surface Area (m^2/g)	Average Pore Diameter (Å)	Plates*	Pressure* (bar)
2.7 µm HALO	0.5	135	90	38300	284
5 µm HALO	0.6	90	90	28300	78
3 µm totally porous	N/A	300	100	24200	309
5 µm totally porous A	N/A	300	100	14600	100
5 µm totally porous B	N/A	170	120	14400	63
5 µm totally porous C	N/A	450	100	15300	120

Columns: 4.6 x 150 mm
Mobile Phase: 50/50 ACN/water
Temperature: 30°C
Injection: 1 uL
Instrument: Agilent 1100 or Agilent 1200 with autosampler
***Plates and Pressure: reported for 1-chloro-4-nitrobenzene at the flow rate corresponding to the plate height minimum**

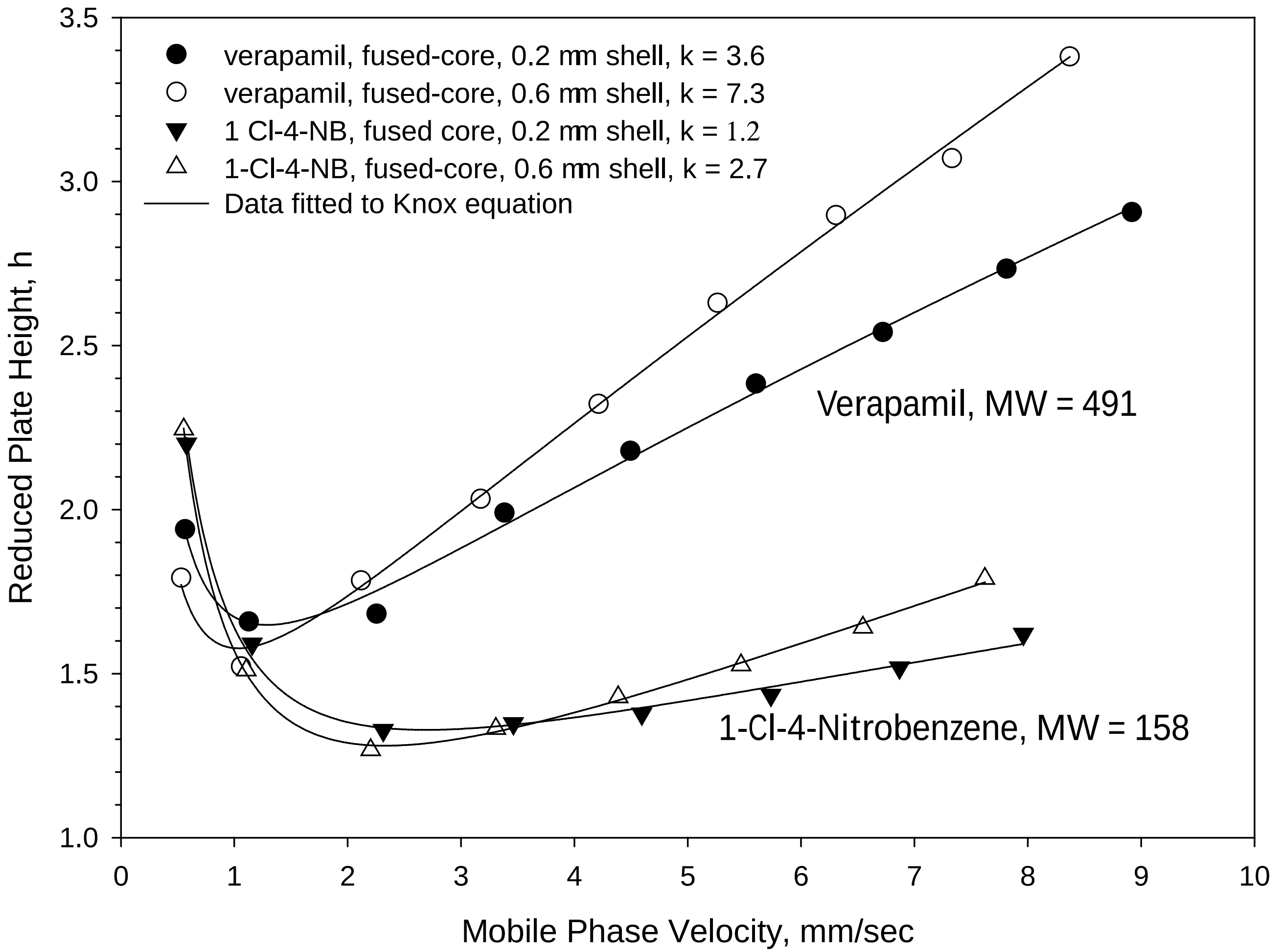
Effect of shell thickness

Effect of Shell Thickness and Solute Size on Particle Efficiency

Columns: 4.6 x 150 mm; Instrument: Agilent 1100 with autosampler

Verapamil - Mobile phase - 30% acetonitrile/70% 0.1% trifluoroacetic acid in water, temperature 40 °C, injection: 0.5 mL

1-Cl-4-Nitrobenzene - 50% acetonitrile/50% water, temperature: 30 °C, Injection: 1.0 mL

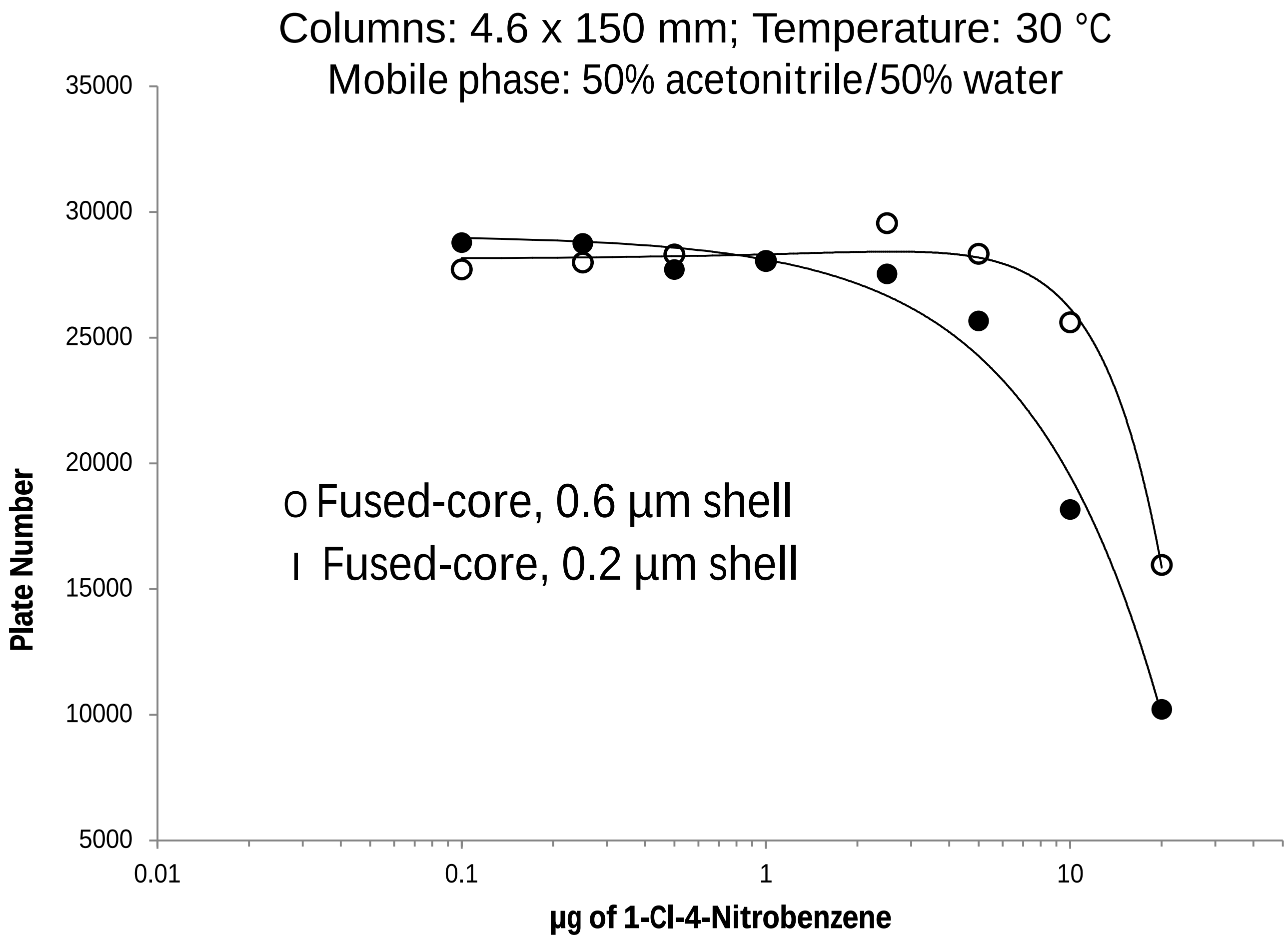


Lower MW solute: small difference in h due to shell thickness

Higher MW solute: larger h with thicker shell; mass transfer poorer

Thinner shell: lower surface area yields reduced retention and solute loading

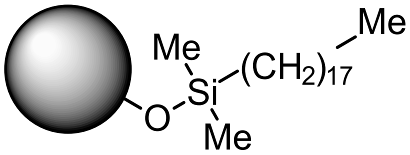
Effect of Shell Thickness on Sample Loading



Greater sample loadability with thicker porous shell.

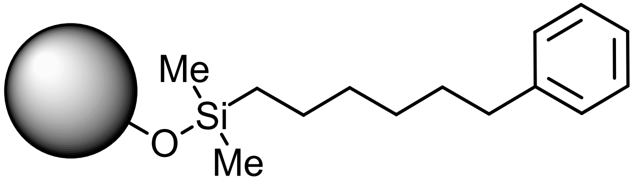
Applications using Fused-Core Columns

HALO-5 Bonded Phases

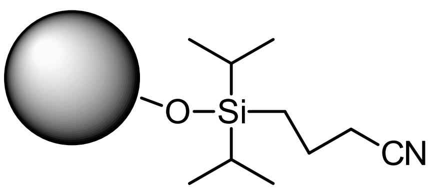


C18 (octadecyl)

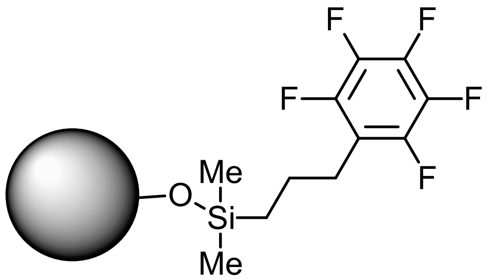
C8 (octyl) [not shown]*



Phenyl-Hexyl*



ES-CN
(extra stable-cyanopropyl)*



PFP
(pentafluorophenylpropyl)

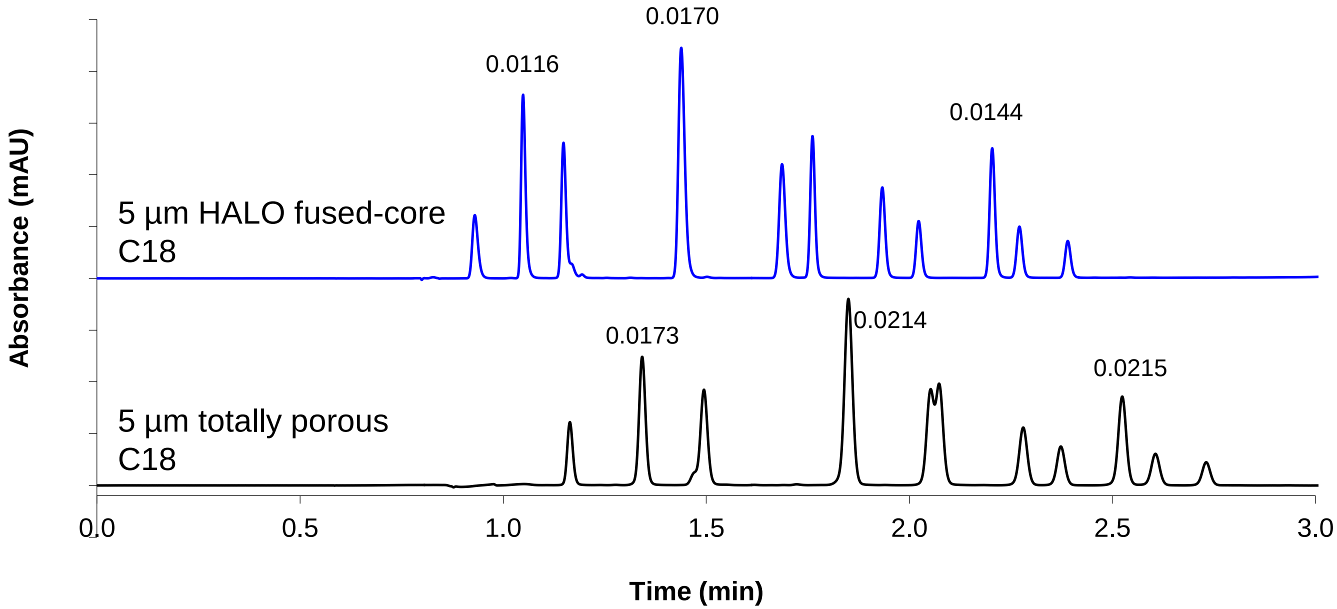
* Introduced at EAS 2012

Available February 2013

- HILIC
- Penta-HILIC

5 µm HALO Fused-core vs. 5 µm Totally Porous: Phenolics Gradient

Columns: 4.6 x 50 mm Instrument: Agilent 1100 Quaternary
Flow rate: 2.0 mL/min, Injection Volume: 4.8 µL,
Injection Delay 0.41 min.; Detection: 275 nm
Mobile Phase: 3–70% ACN/water w/0.1% HCOOH in 2.7 min.
Temperature = 45 °C
Values above peaks are widths at half height

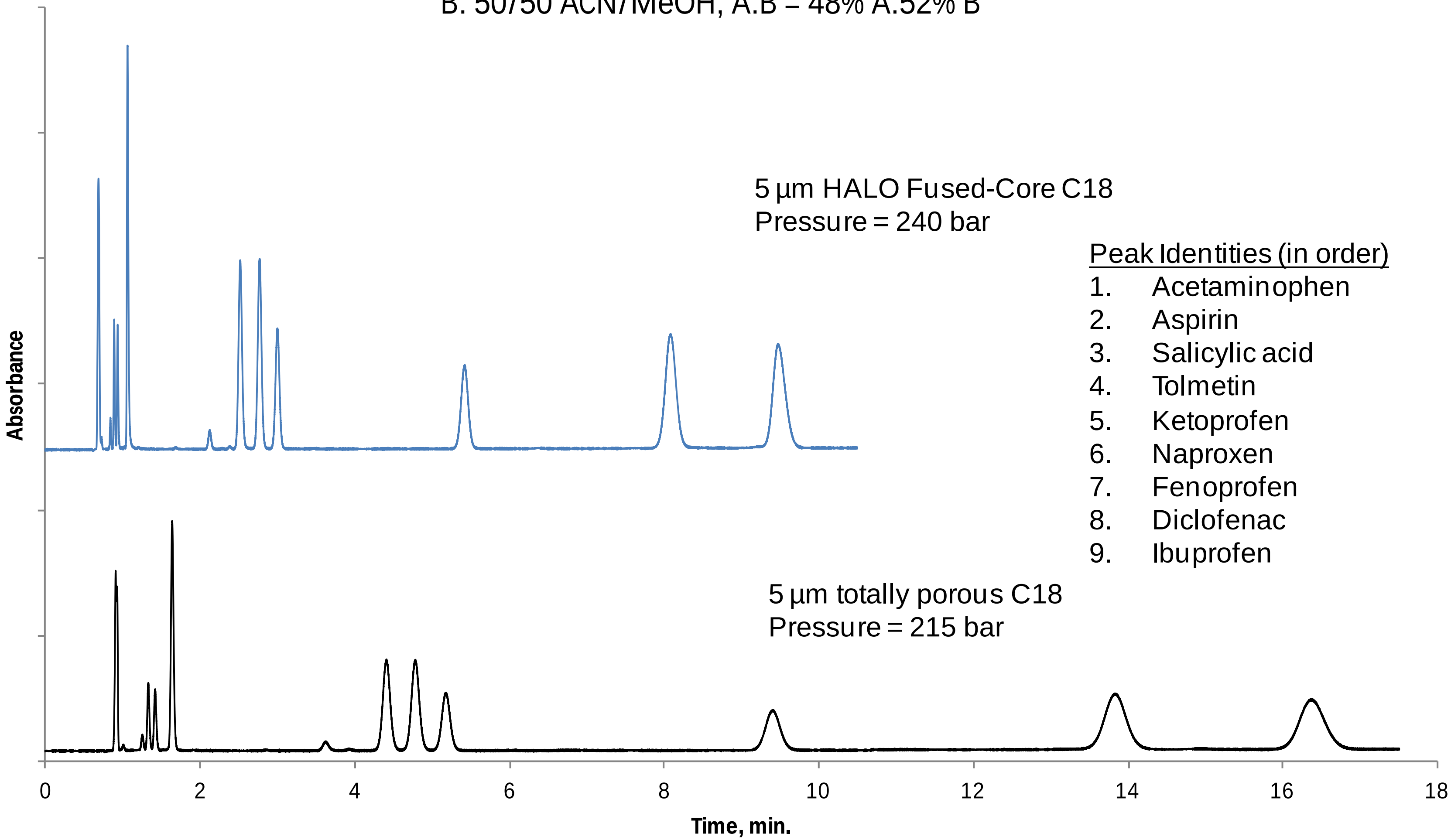


Peak identities (in order): hydroquinone, resorcinol, catechol, phenol, 4-nitrophenol, 4,4'-biphenol, 2-chlorophenol, 4-chlorophenol, 2,2'-biphenol, 2,6 –dichlorophenol, 2,4-dichlorophenol

Faster separation and sharper peaks using fused-core particles.

5 µm HALO Fused-core vs. 5 µm Totally Porous: NSAIDs

Columns: 4.6 x 150 mm Instrument: Shimadzu Prominence UFLC XR
Flow rate: 2.0 mL/min, Injection Volume: 2 µL,
Detection: 254 nm; Temperature = 35 °C
Mobile Phase: A: 20 mM pH 2.5 Potassium Phosphate
B: 50/50 ACN/MeOH; A:B = 48% A:52% B

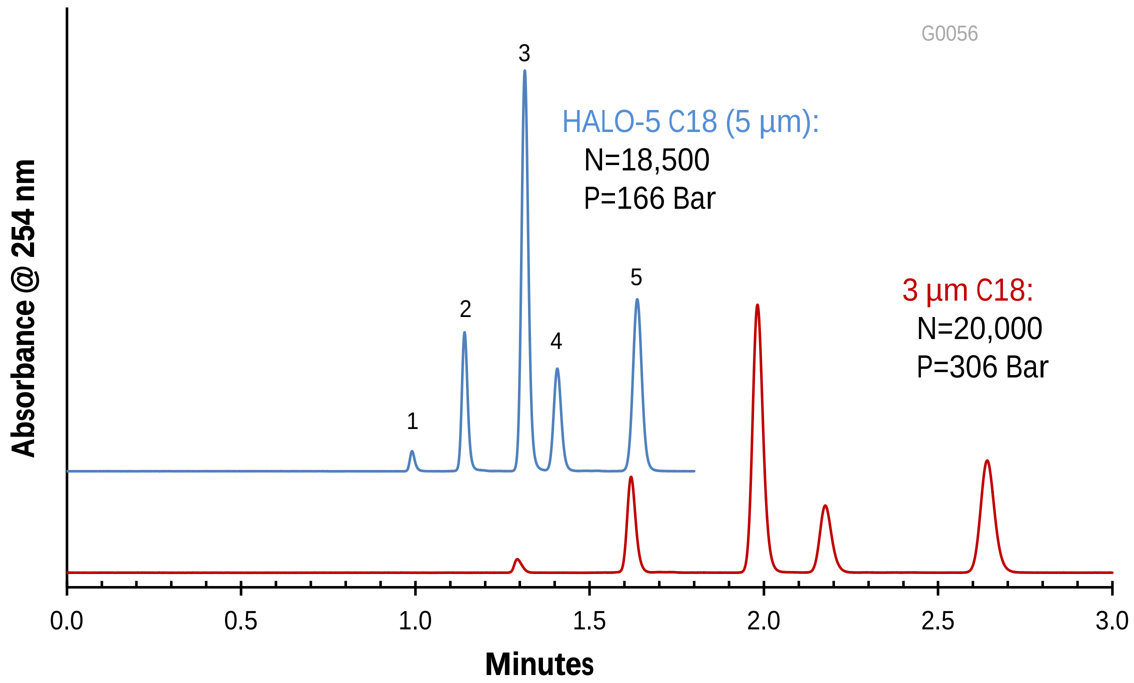


Peak Identities (in order)
1. Acetaminophen
2. Aspirin
3. Salicylic acid
4. Tolmetin
5. Ketoprofen
6. Naproxen
7. Fenoprofen
8. Diclofenac
9. Ibuprofen

Nearly 2X improved efficiency at equivalent pressure.

Obtain 5-Micron Benefits for 3-Micron Methods

Columns: 4.6 x 150 mm HALO-5 C18, 4.6 x 150 mm 3 µm totally porous C18 column
Mobile phase: isocratic: 25/75 A/B; A = 20 mM potassium phosphate, pH 3, B = methanol
Flow rate: 1.3 mL/min; Temperature: 30 ºC; Detection: 254 nm; Injection: 0.5 µL
LC System: Shimadzu Prominence UFLC XR

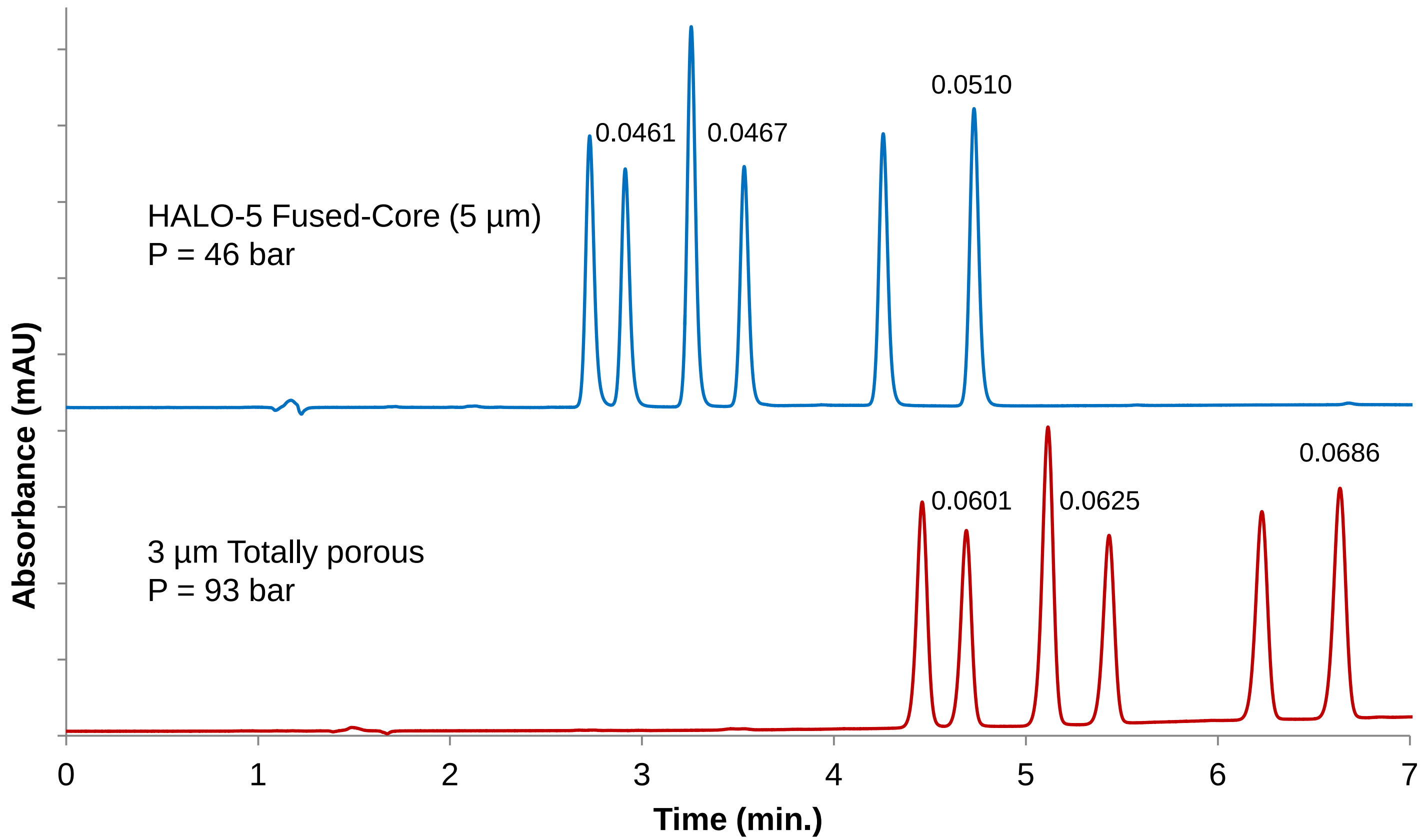


Peak Identities:
1. Uracil (t_0)
2. Fenuron
3. Monuron
4. Fluometuron
5. Diuron

- HALO-5 columns provide the same efficiency as 3-Micron columns at about half the pressure

Faster, More Efficient Separations with HALO-5 PFP

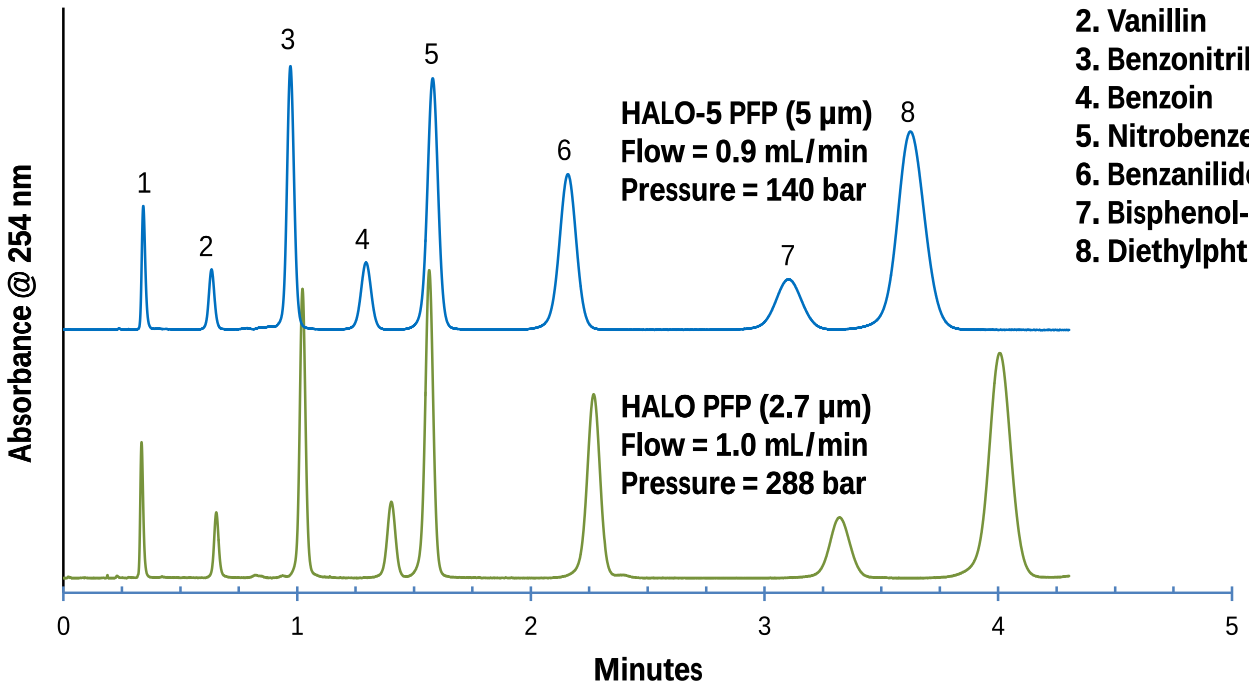
Columns: 4.6 x 100 mm HALO-5 PFP (pentafluorophenylpropyl), 4.6 x 100 mm 3 µm totally porous PFP; Mobile phase: A = 25 mM ammonium acetate, pH 5.5, B = acetonitrile; gradient: 36 – 65% B in 7 min. Flow rate: 0.75 mL/min; Temperature: 35 ^{o}C; Detection: 254 nm.



- Solutes in order of elution: 1) oxazepam, 2) lorazepam, 3) nitrazepam, 4) clonazepam, 5) flunitrazepam, 6) diazepam. Peak widths in minutes above selected peaks.
- Separation using HALO-5 is nearly complete by the time peaks are beginning to elute from the 3 µm totally porous column.

Comparable Selectivity between HALO-5 and 2.7 µm HALO: PFP

Columns: 3.0 x 50 mm HALO-5 PFP (pentafluorophenylpropyl), 3.0 x 50 mm HALO 2.7 µm PFP
Mobile phase: isocratic: 55/45 A/B; A = 20 mM potassium phosphate, pH 3, B = methanol
Flow rate: as indicated; Temperature: 30 ºC; Detection: 254 nm; Injection: 0.5 µL

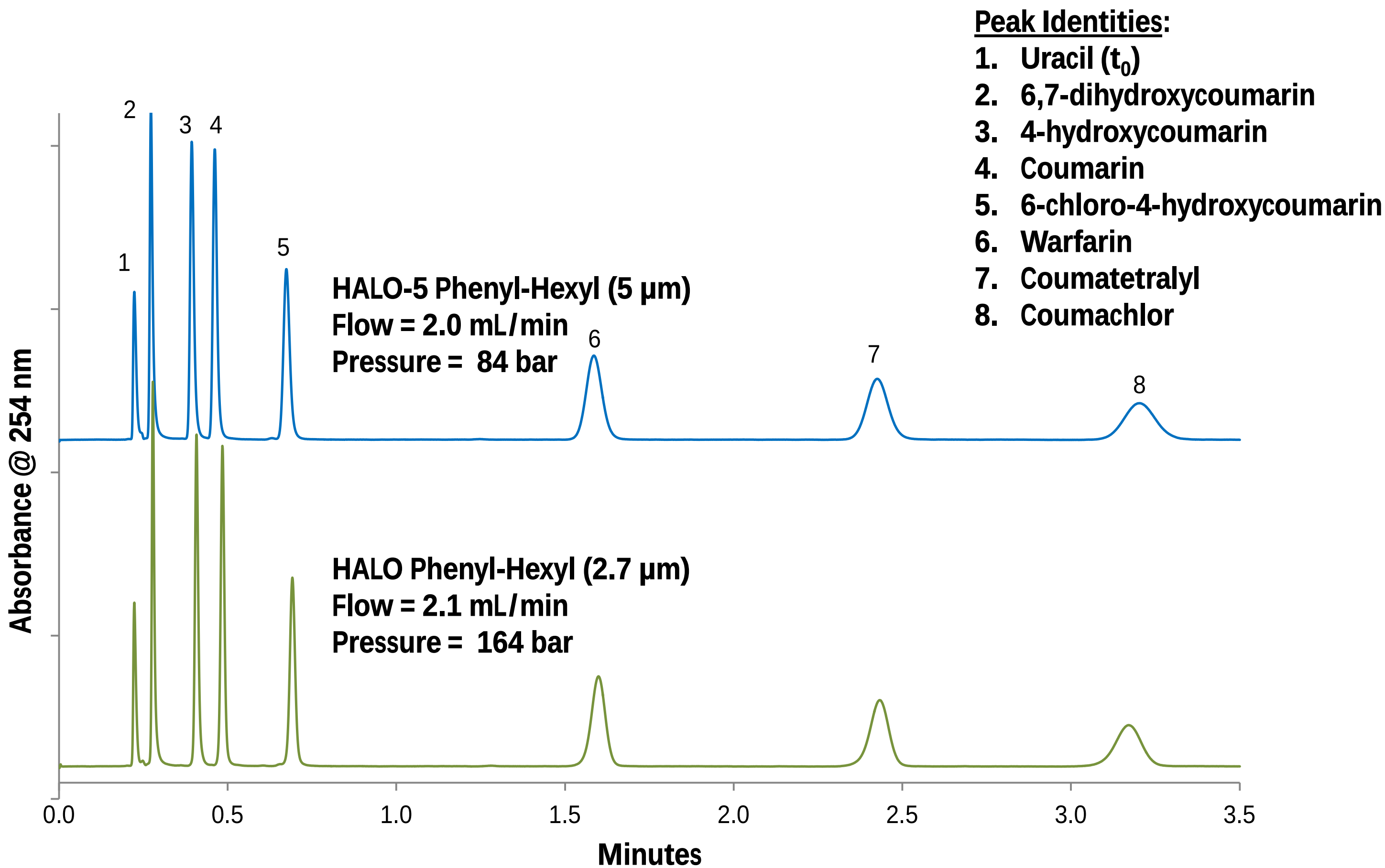


Peak Identities:
1. Resorcinol
2. Vanillin
3. Benzonitrile
4. Benzoin
5. Nitrobenzene
6. Benzanilide
7. Bisphenol-A
8. Diethylphthalate

- Methods are easily transferred from HALO 2.7-micron columns to HALO-5 columns with only minor adjustment to flow rate

Comparable Selectivity between HALO-5 and 2.7 µm HALO: Phenyl-Hexyl

Columns: 4.6 x 50 mm HALO-5 Phenyl-Hexyl, 4.6 x 50 mm HALO 2.7 µm Phenyl-Hexyl
Mobile phase: isocratic: 55/45 A/B; A = water/0.1% formic acid, B = 50/50 acetonitrile/methanol
Flow rate: as indicated; Temperature: 45 °C; Detection: 254 nm; Injection: 2 µL



- Equivalent high efficiency separations are obtained for this sample of anticoagulants on 5 µm and 2.7 µm HALO particles with Phenyl-Hexyl bonded phase

Conclusions

- Reduced plate heights of 5 µm fused-core particles: smaller than smaller fused-core particles.
  - More homogenously packed beds with larger particles?
- Thinner shell for the fused-core particles: flatter van Deemter plot.
  - especially evident for larger molecular weight solutes.
- Sample Loading: thinner shells = reduced surface area and therefore reduced loading and retention
- Plates/pressure: 5 µm fused-core particle has more than double the plates/pressure of 5 µm totally porous particles.
- 5 µm fused-core particles can provide faster, more efficient separations compared to 3 µm and 5 µm totally porous particles.