Small Molecule HPLC Method Optimization using Acid, Base, and Neutral Panel and Superficially Porous Particles

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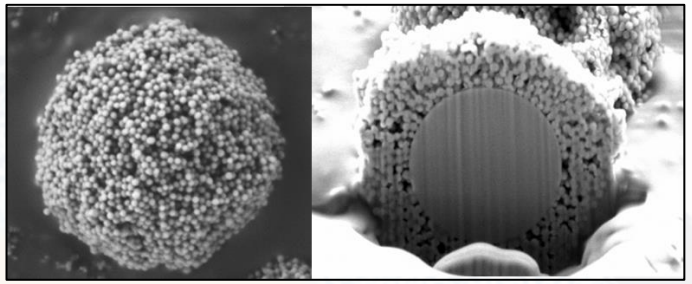
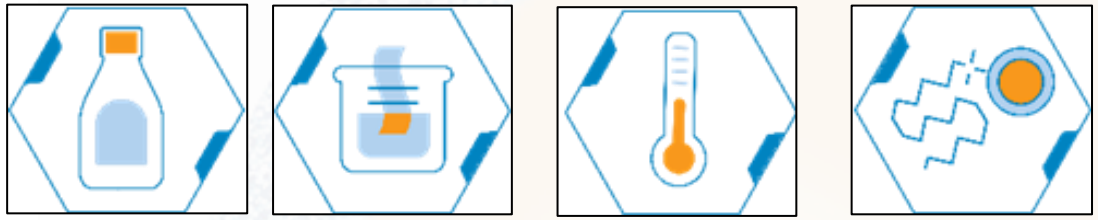
Presentation Outline

- Advanced Materials Technology
  - Superficially Porous Particles (SPP) vs. Fully Porous Particles (FPP)
  - C18 Product Portfolio



- Method Development
  - Gradient vs. Isocratic
  - Phase Selection
  - Mobile Phase Optimization



- HALO 90 Å PCS C18, 2.7 µm
- Column Dimensions
  - HALO® 1.5 mm ID
- Technical Resources/ Support

Founded in 2005 by Tim Langlois and Joe DeStefano

First company to commercially manufacture sub 3 µm superficially porous particles – *Fused-Core®*

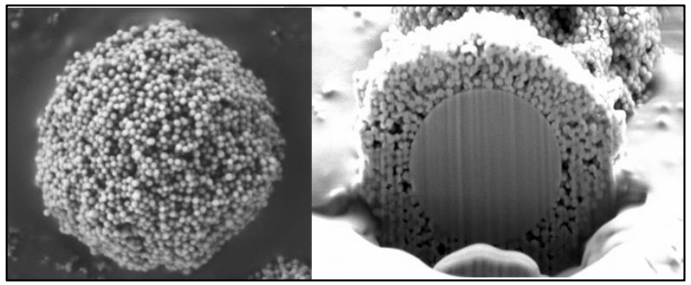
Facility

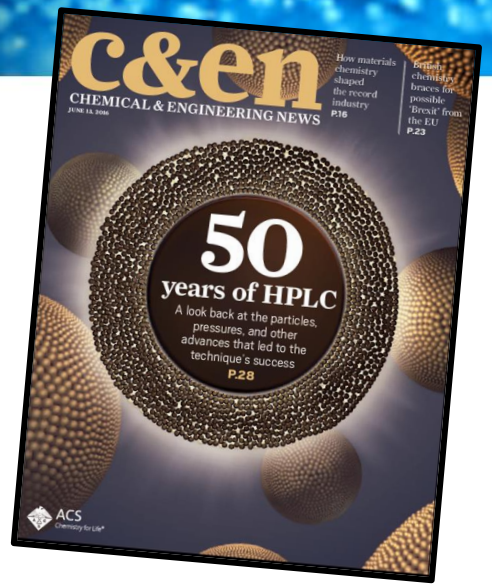
- Fully equipped state of the art laboratories
- All operations handled in Wilmington, DE
  - R&D, Applications, QA/QC, Manufacturing, Sales and Marketing

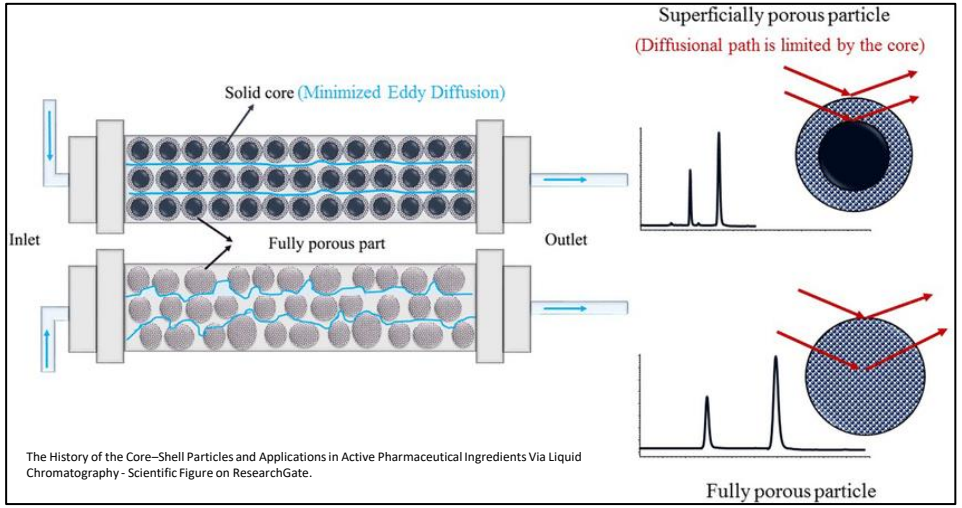
AMT is a company of innovators and continues to grow and deliver enabling materials to market. Our incredible team is our greatest resource.

Superficially Porous Particle Technology (SPP)

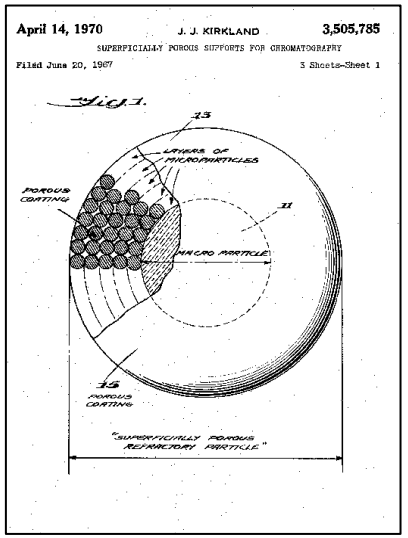
-High Purity Silica Particles (2, 2.7, 3.4, 5 µm)
-Bonded Phase Shell Fused to Solid Core
-Shell Consists of Different Pore Sizes
(90, 160, 400, 1000Å)







The History of the Core–Shell Particles and Applications in Active Pharmaceutical Ingredients Via Liquid Chromatography - Scientific Figure on ResearchGate.

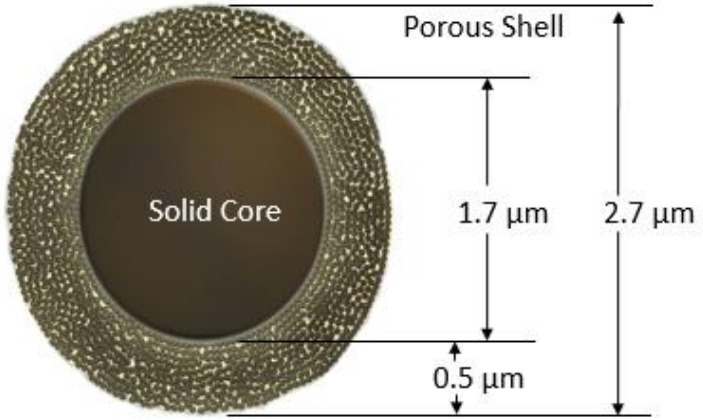

April 14, 1970 J. J. KIRKLAND 3,505,785
SUPERFICIALLY POROUS SUPPORTS FOR CHROMATOGRAPHY
Filed June 20, 1967 3 Sheets-Sheet 1

3,505,785
SUPERFICIALLY POROUS SUPPORTS FOR CHROMATOGRAPHY
Joseph J. Kirkland, Wilmington, Del., assignor to E. I. du Pont de Nemours and Company, Wilmington, Del., a corporation of Delaware
Filed June 20, 1967, Ser. No. 647,506
Int. Cl. B01d *15/08*
U.S. Cl. 55—67 8 Claims

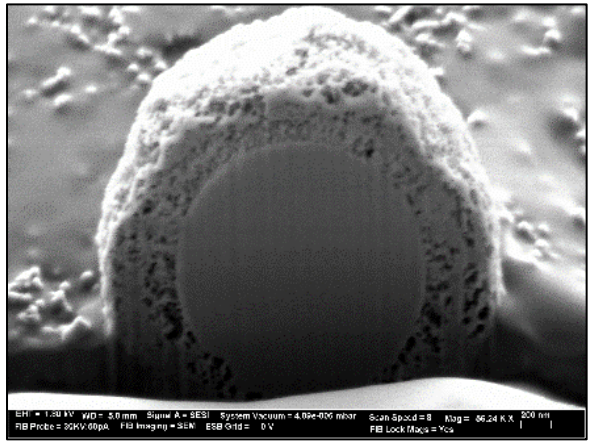
ABSTRACT OF THE DISCLOSURE

This invention relates to an improvement in chromatography and chromatographic columns. A novel packing of superficially porous refractory particles for use in chromatography has been prepared consisting of a plurality of discrete macroparticles with impervious cores and having irreversibly joined thereto a coating of a series of sequentially adsorbed like monolayers of like colloidal inorganic microparticles. The coating is characterized by being uniform and of predetermined thickness. In preferred embodiments, the cores would be ceramics, preferably glass spheres, and the coating would consist of monolayers of colloidal refractory particles, preferably silica, in a structure of predetermined thickness and porosity.

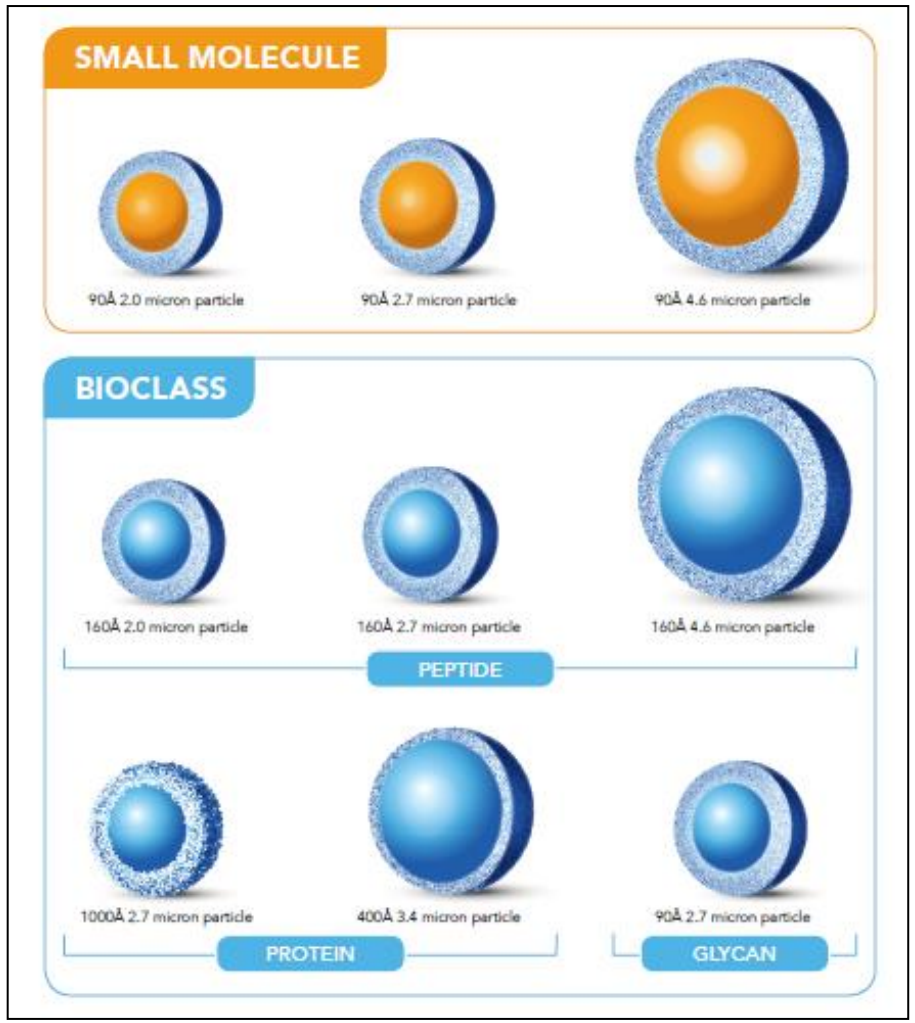
Shell with pores



SEM Particle Cross- section

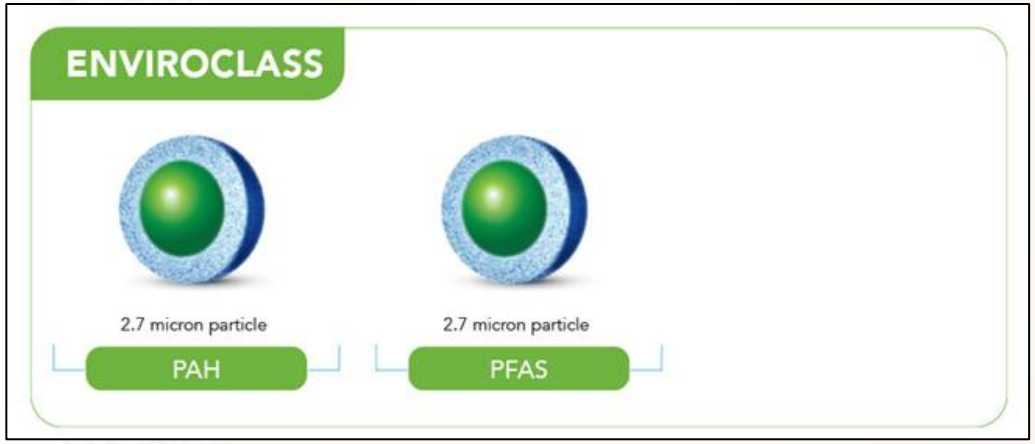


AMT Product Portfolio



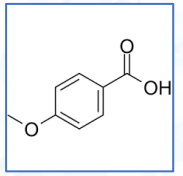
Portfolio of Products

- Varying particle morphologies to meet separation needs (particle size, core size, shell thickness, pore size)
- Various chemistries for selectivity of analytes across small molecule to large molecule
- Many different column dimensions from capillary to semi-prep.

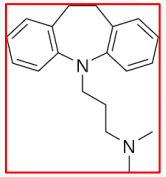


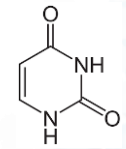
Method Development

4 Methoxy Benzoic Acid

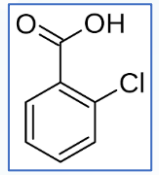


Imipramine

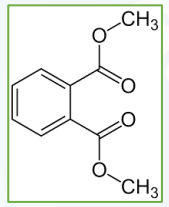




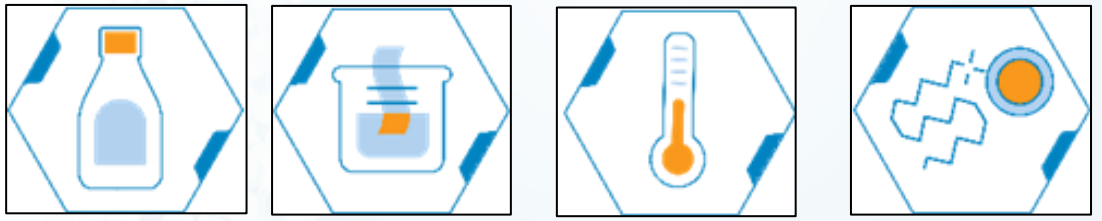
Uracil



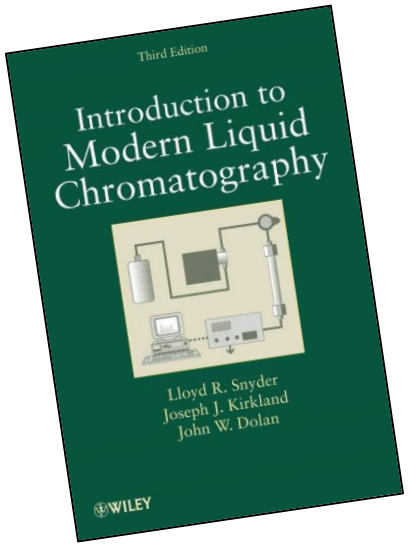
2 Chlorobenzoic Acid



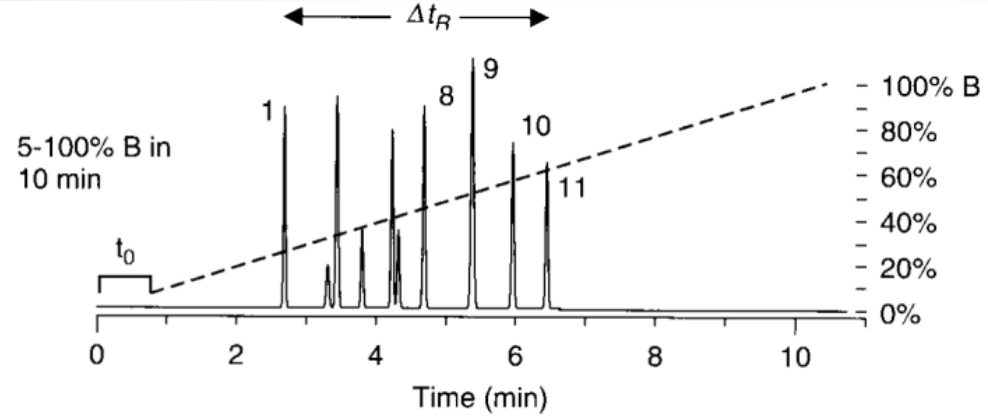
Dimethyl Phthalate



Isocratic or Gradient?



Use a standard gradient run to determine whether isocratic or gradient elution is best for a given sample



$t_R = (6.5 - 2.7) = 3.8$ min

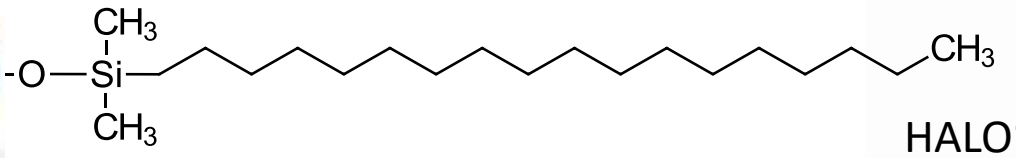
$(t_R)_{avg} = (6.5 + 2.7)/2 = 4.6$

$\Delta\phi = 0.01(100 - 5) = 0.95$

- Value of $\Delta t_R/t_G$: ≤ 0.25, isocratic; 0.25-0.40, either isocratic or gradient; ≥ 0.40, gradient
- In this example the "irregular" sample of Figure 9.4 was separated with the recommended initial conditions of Table 9.3: 5-100% acetonitrile in 10 min, 100 x 4.6-mm *(3-μm)* C_{18} column, 2.0 mL/min, 30°C. Gradient indicated by (- - -).
- from IMLC3e, Fig. 9-15

Gradient & computer-9

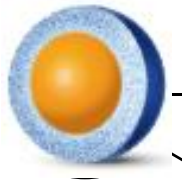
HALO Column Screening

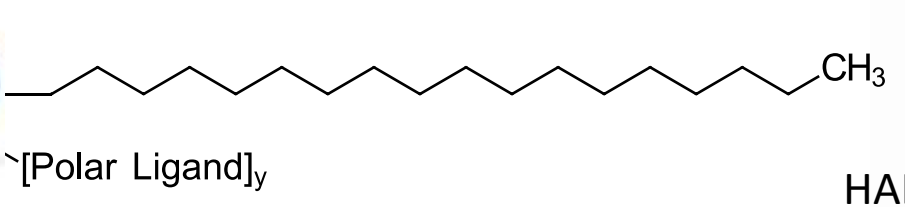


HALO® C18

Features and Benefits

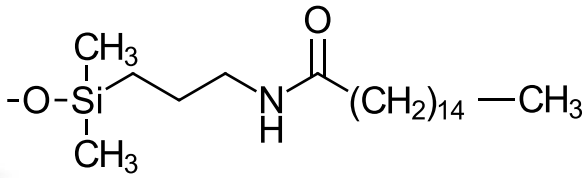
- The standard for retaining and separating a broad range of analytes polarities





HALO® AQ-C18

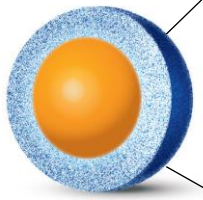
- Resistant to dewetting, making it 100% aqueous mobile phase compatible
- Enhanced retention and selectivity for polar molecules

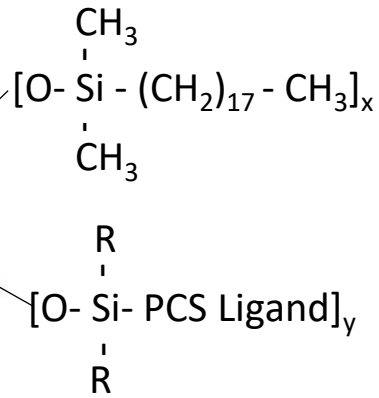


HALO® RP-Amide

- Complementary selectivity to alkyl phases
- Enhanced stability for minimum bleed and long life

NEW!

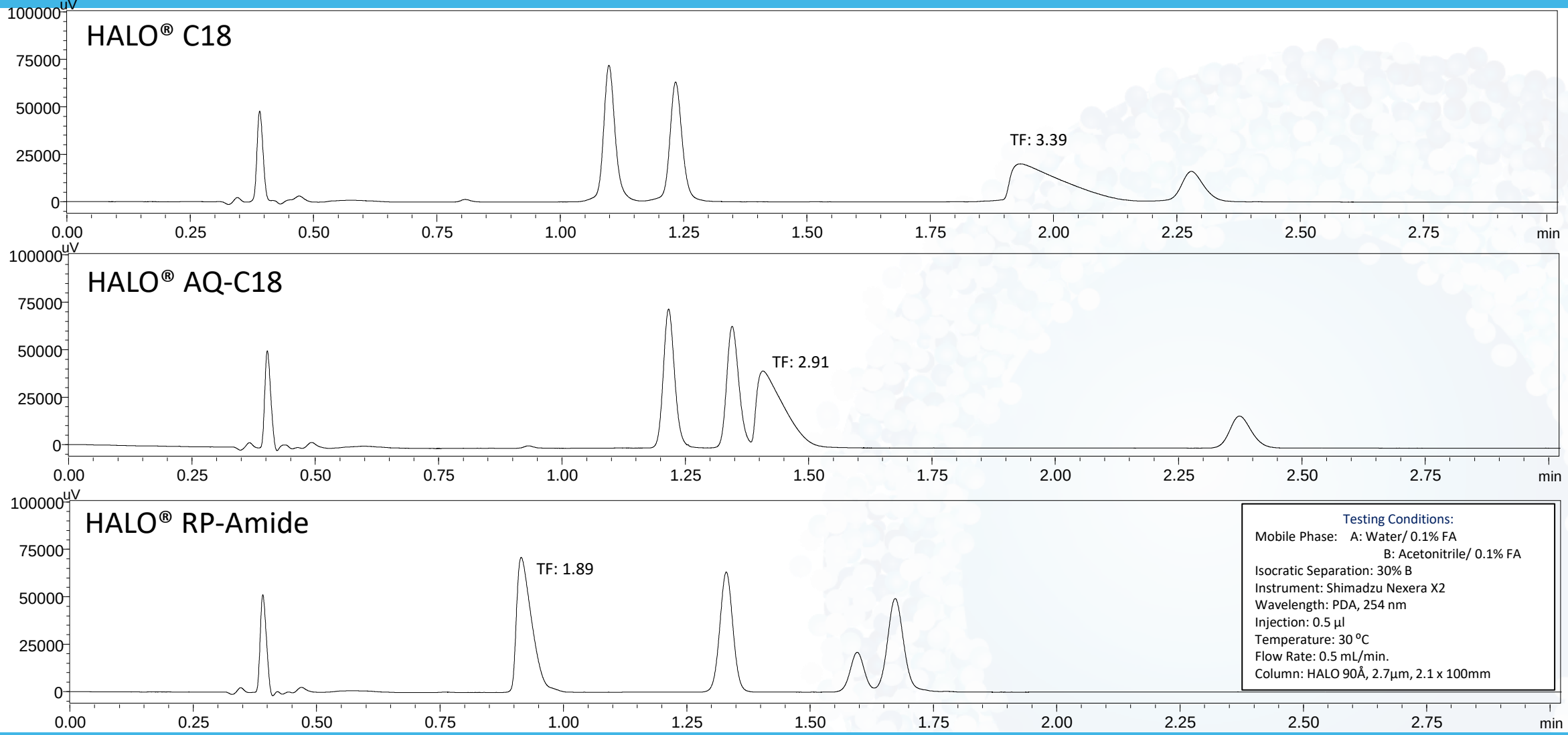




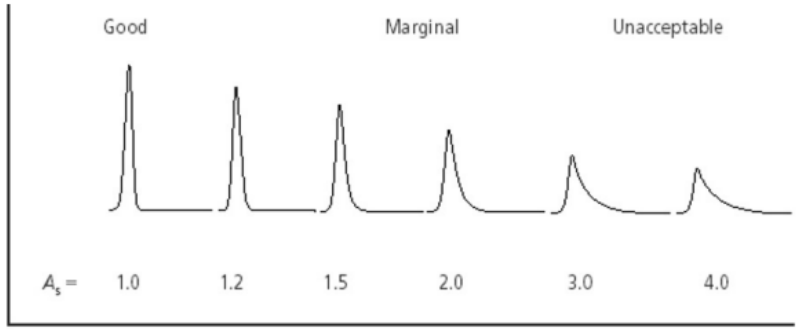
HALO® PCS C18

- Improved peak shape and increased loading capacity for basic compounds
- Ideal for low ionic strength mobile phases such as formic acid

Stationary Phase Screening



If tailing peaks are observed, a mobile phase additive may be needed.



A continuum of peak shapes

LC-GC, 2003, #7, pp. 612-

Figure 2: Examples of asymmetric peaks.

- If basic compounds tail (due to interactions with silanols), try adding a competing base such as
  - » 10 mM triethylamine or triethylammonium chloride (salt form)
- If acidic compounds tail, try adding an acid to suppress their ionization.
  - » acetic acid (1% v/v) or phosphoric acid (0.3%)
- Alternatively, try switching to a column whose stationary phase is "base-deactivated" in one way or another.
  - » e.g., highly pure ("Type-B") silica with few metallic impurities

Optimization - 16

Introduction to HALO PCS

- **P**ositively **C**harged **S**urface = **PCS**

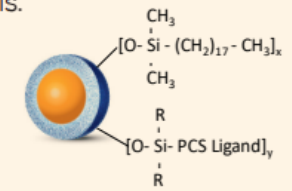
LC-MS Analysis with 0.1% Formic Acid

Bonded Phase	Analyte Type		
	Neutral	Acid	Base
HALO C18	✓	✓	✘
HALO PCS C18	✓	✓	✓

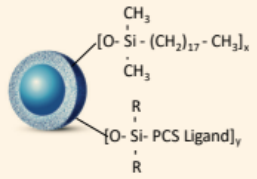
- HALO PCS C18 fills the gap for separations of basic analytes in LC-MS analysis using formic acid mobiles phases.
- Many pharmaceuticals are basic in nature (anti-depressants, beta-blockers, etc…).

POSITIVE RESULTS FOR BASIC COMPOUNDS

Built upon proven Fused-Core® technology for speed and efficiency, the HALO® PCS C18 is a positively charged surface chemistry designed to deliver improved peak shapes for basic compounds. Ideal for use with low ionic strength mobile phases, HALO® PCS maintains peak symmetry at higher loading capacities and provides an alternate selectivity from other C18 bonded phases. Available in both a 90 Å and 160 Å pore size for small molecule and peptide analysis.



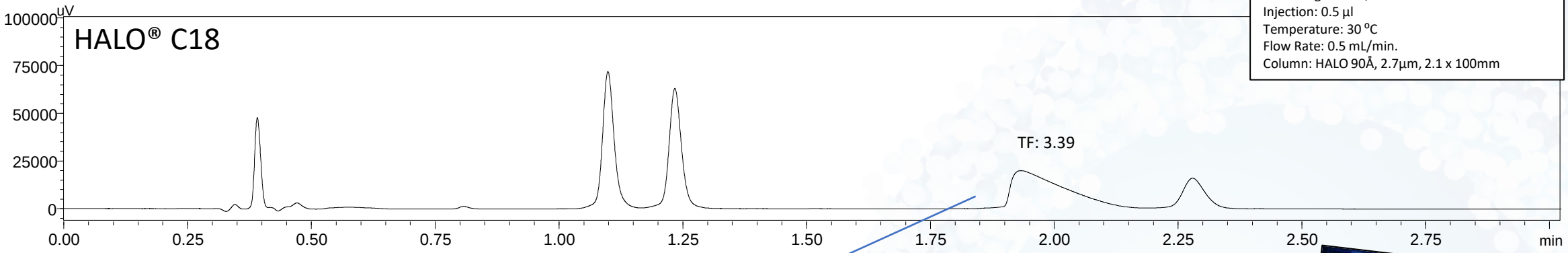
HALO 90 Å PCS C18

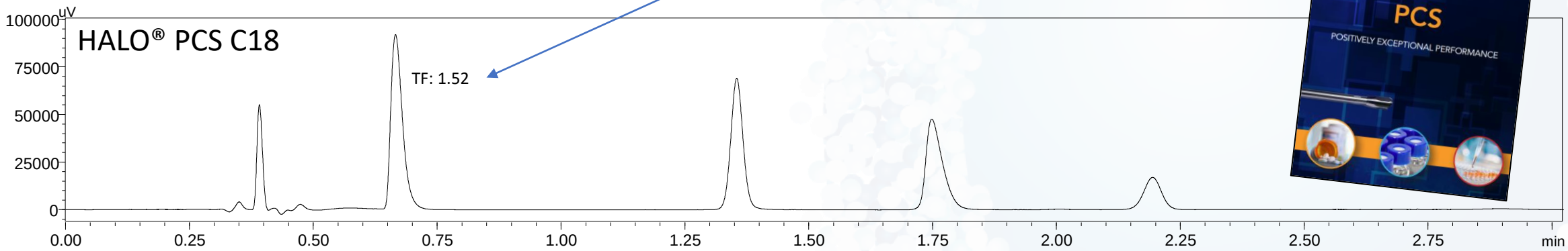


HALO 160 Å PCS C18

C18 vs. PCS C18

Testing Conditions:
Mobile Phase: A: Water/ 0.1% FA
B: Acetonitrile/ 0.1% FA
Isocratic Separation: 30% B
Instrument: Shimadzu Nexera X2
Wavelength: PDA, 254 nm
Injection: 0.5 µl
Temperature: 30 °C
Flow Rate: 0.5 mL/min.
Column: HALO 90Å, 2.7µm, 2.1 x 100mm





C18 vs. PCS C18

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Injection: 0.5 µl
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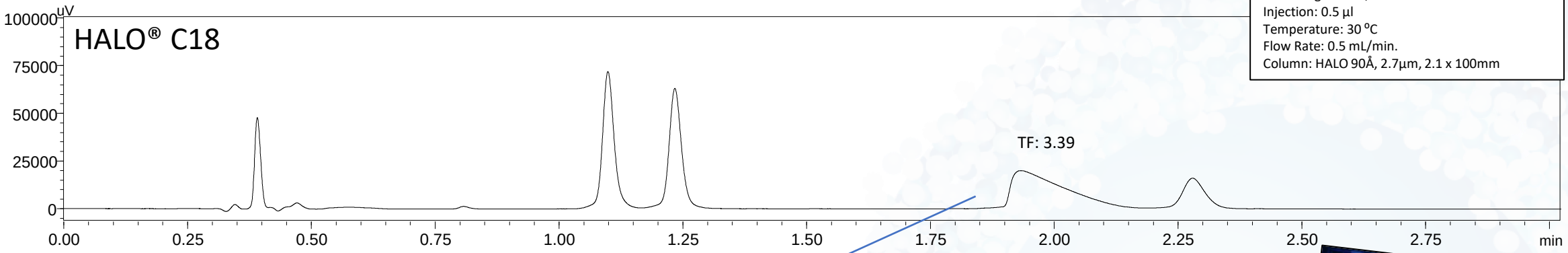
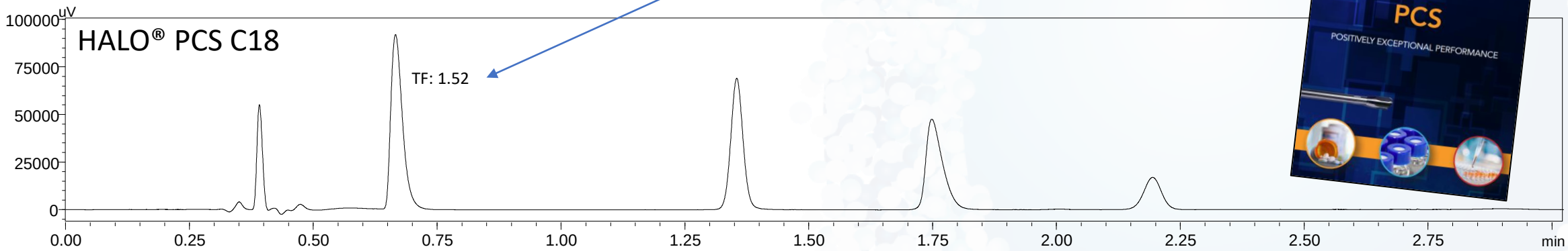
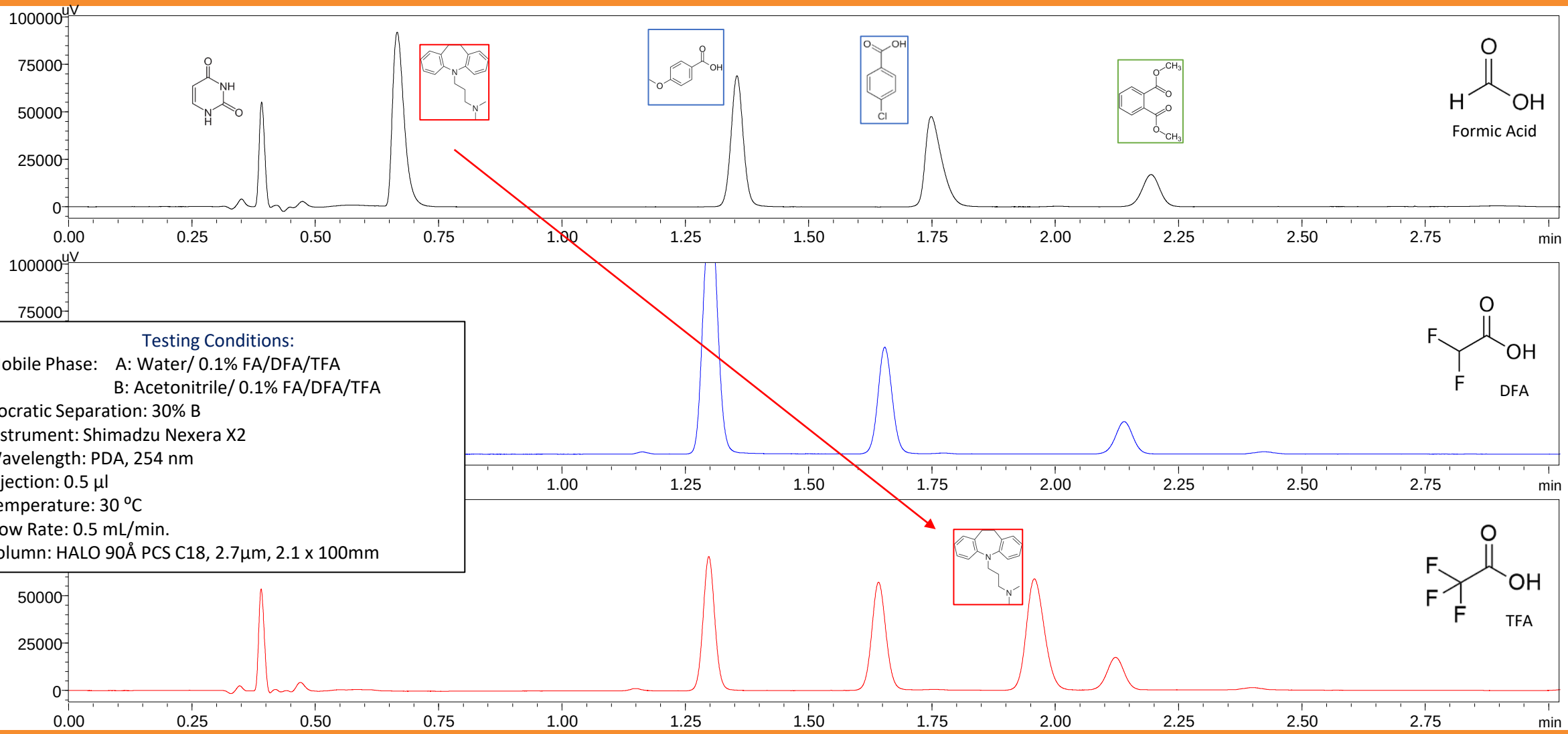




Mobile Phase/ pH Screening



Testing Conditions:

Mobile Phase: A: Water/ 0.1% FA/DFA/TFA
B: Acetonitrile/ 0.1% FA/DFA/TFA
Isocratic Separation: 30% B
Instrument: Shimadzu Nexera X2
Wavelength: PDA, 254 nm
Injection: 0.5 µl
Temperature: 30 °C
Flow Rate: 0.5 mL/min.
Column: HALO 90Å PCS C18, 2.7µm, 2.1 x 100mm

Systematic approach to selectivity adjustment via solvent type (RPLC)

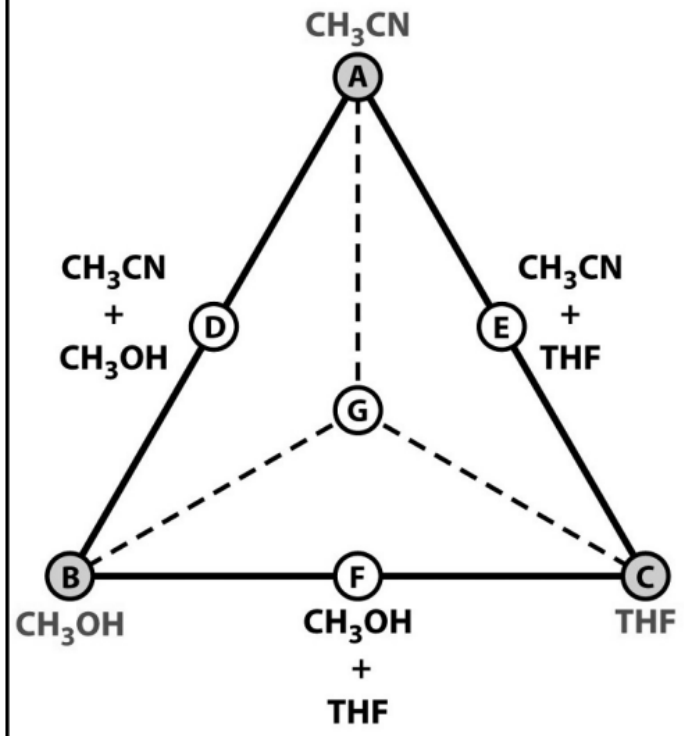
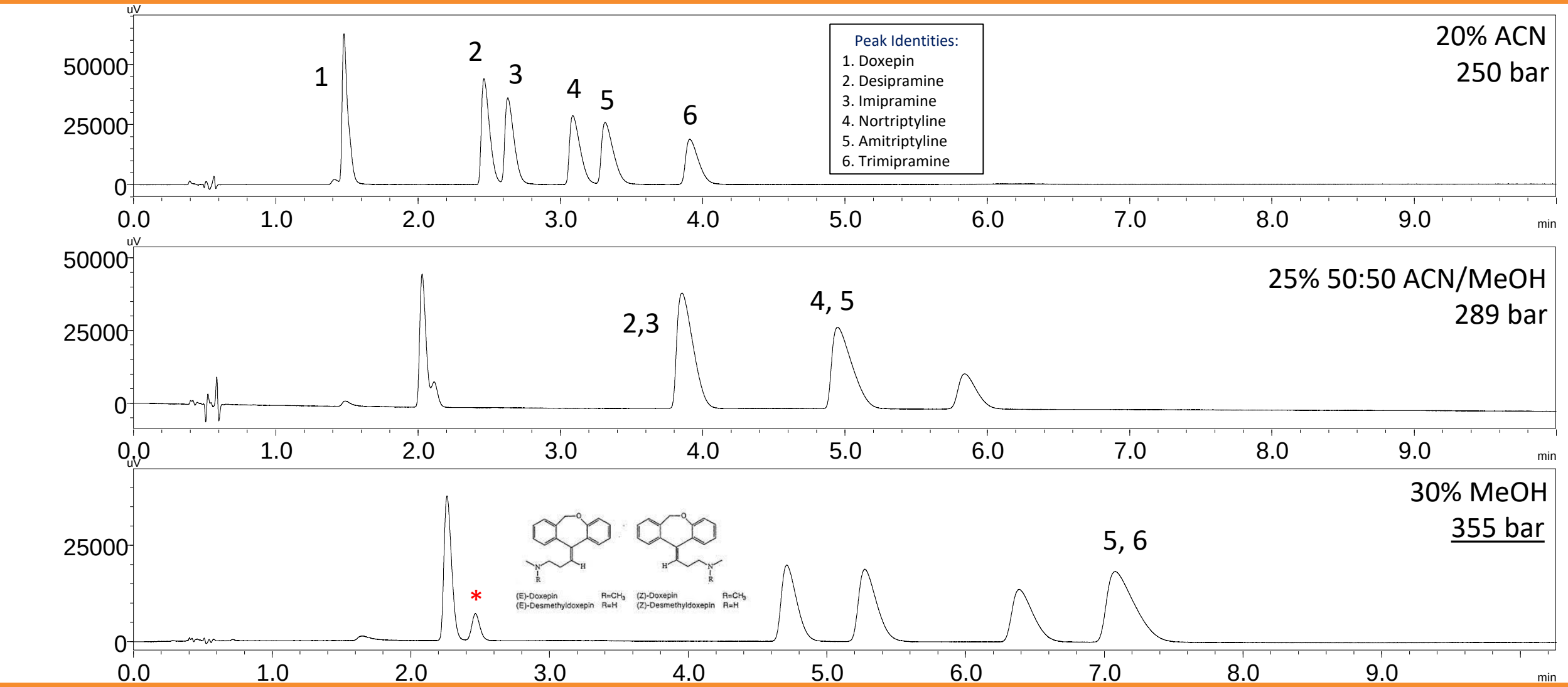


Figure 25-25
Quantitative Chemical Analysis, Seventh Edition

- 1. If ACN/water mixtures do not provide adequate selectivity after retention has been optimized (vertex A), *switch to an isoeluotropic mixture* of MeOH/water.
- 2. Adjust %MeOH to fine-tune selectivity and retention (vertex B). If separation is adequate, STOP!
- 3. Switch to an isoeluotropic mixture of THF/water; adjust %THF to fine-tune selectivity and retention. If separation is adequate, STOP!
- 4. If necessary, continue experiments with isoeluotropic ternary (D,E,F) and quaternary mobile phases (G).

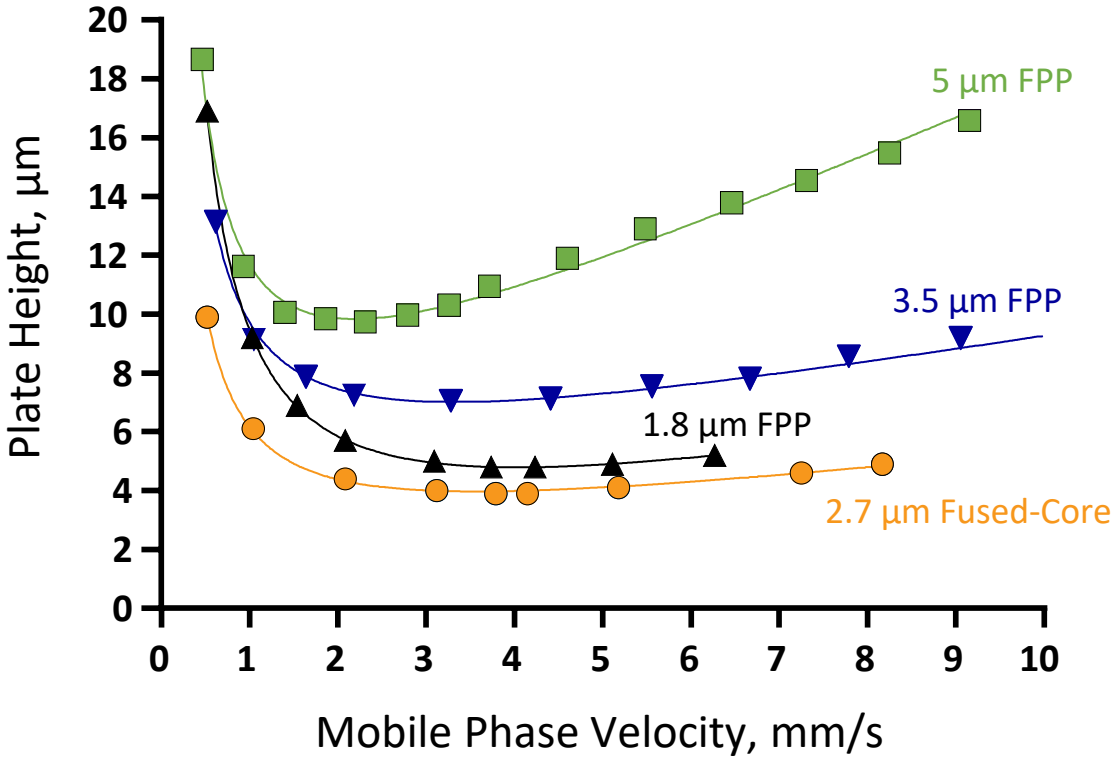
Tricyclic Antidepressants

ACN vs. MeOH

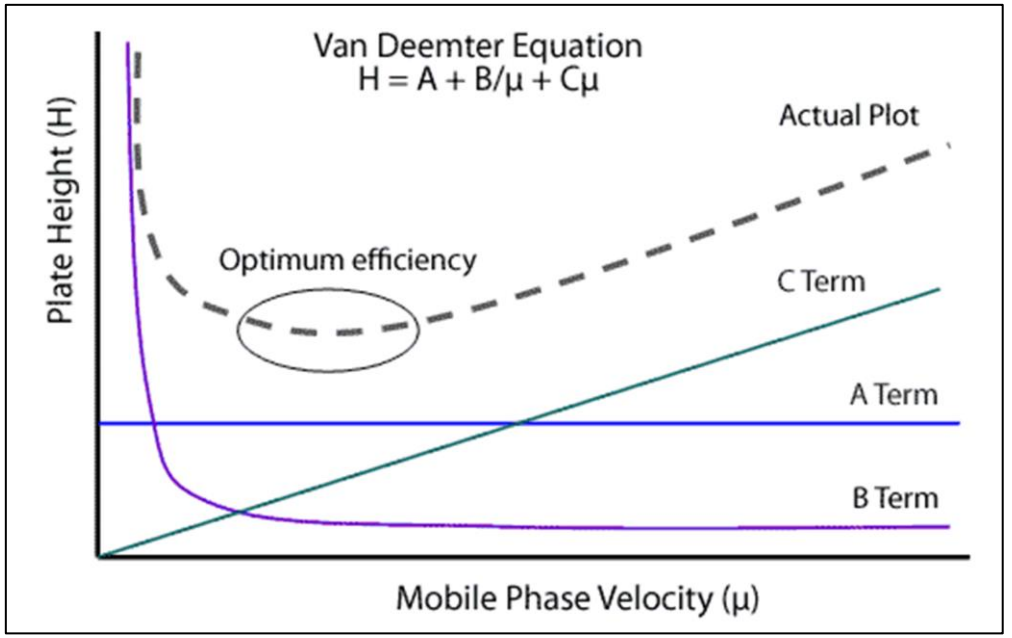


How SPP Benefits Separations?

Speed and Efficiency



J.J. DeStefano, T.J. Langlois, & J.J. Kirkland, ***J. Chromatogr. Sci.***, 2008, 46(3), 254-260



$$H = A + \frac{B}{\mu} + C\mu$$

Effect of Particle Size and Type

Columns: 4.6 x 50 mm
5 µm FPP C18
3.5 µm FPP C18
1.8 µm FPP C18
2.7 µm HALO C18

Solute: naphthalene
Mobile phase: 60% ACN/40% water
Temperature:24 °C

van Deemter Equation

H = height equivalent to theoretical plate

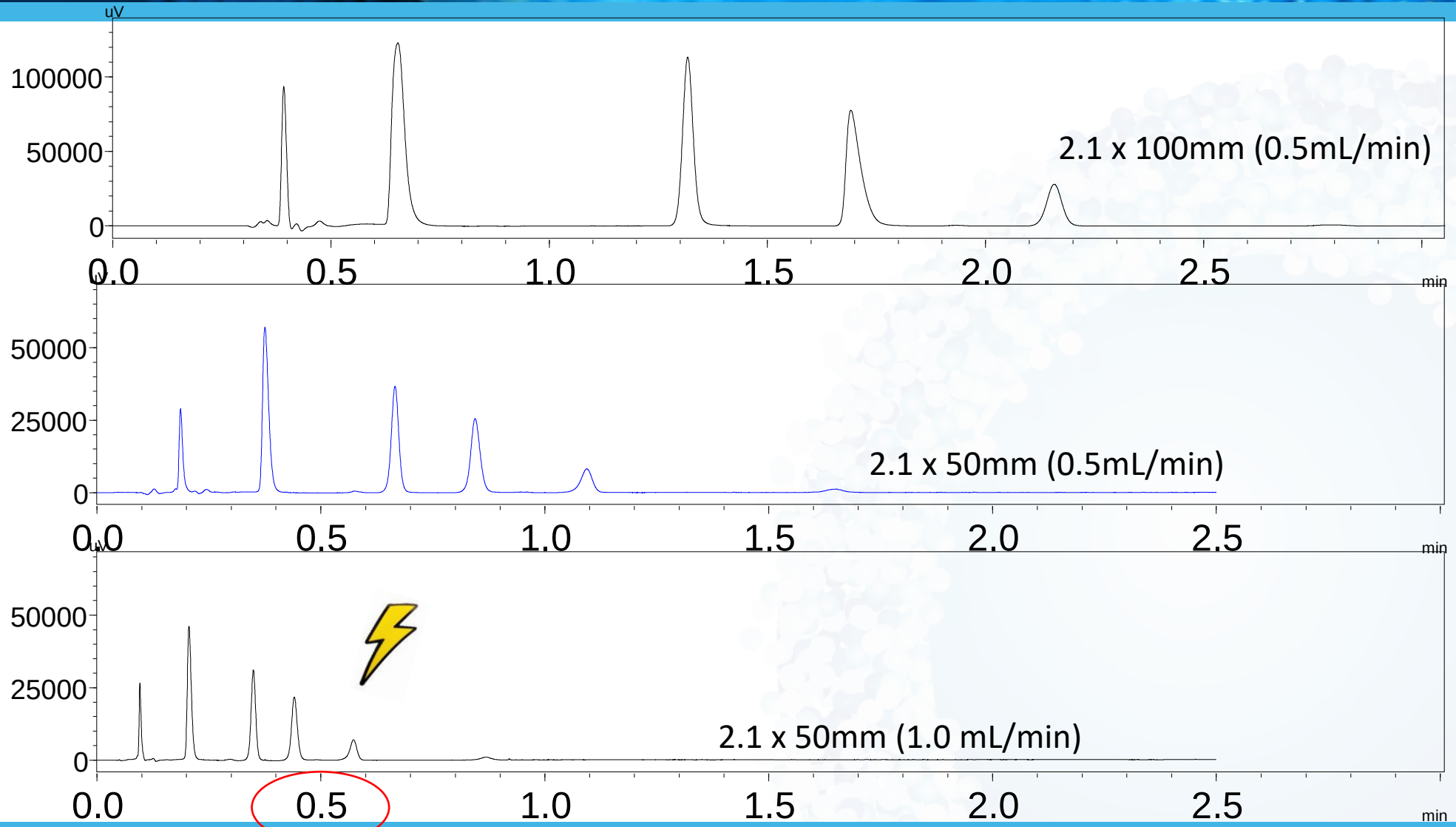
A = eddy diffusion term (particle size and how well bed was packed) **30 - 40% smaller**

B = longitudinal diffusion term **25 - 30% smaller**

C = resistance to mass transfer term (kinetics of the analyte b/w mobile phase and stationary phase)

µ = mobile phase linear velocity (L/t_0)

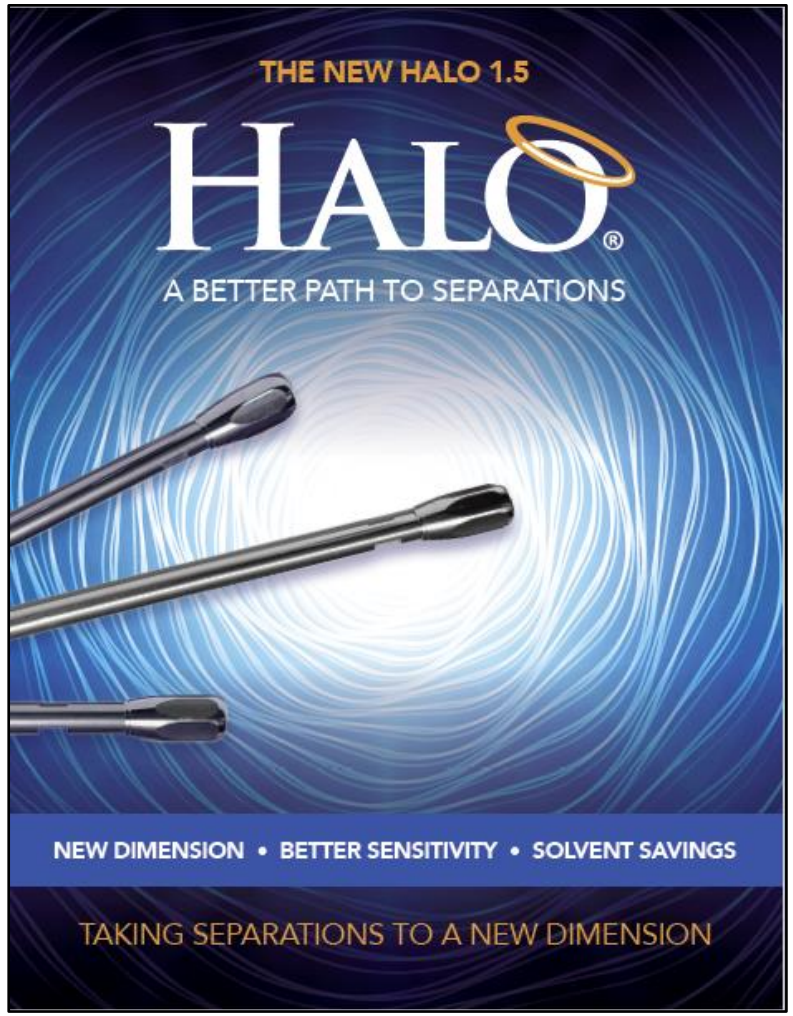
Speed vs. Resolution



A NEW DIMENSION IN SEPARATIONS

HALO®

MORE PERFORMANCE FROM UHPLC AND LCMS SYSTEMS



- More sensitivity from conventional UHPLC systems
- Higher ionization efficiencies from LCMS systems
- Reduced solvent consumption compared to 2.1 mm id columns (and greater)
- Easy to implement microflow solution

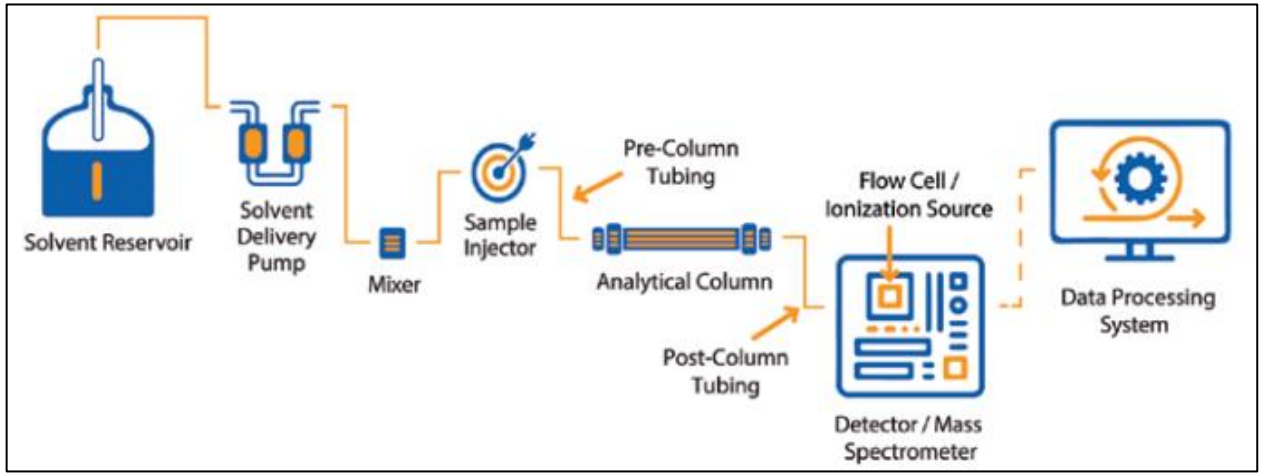
Advantages of the 1.5mm ID

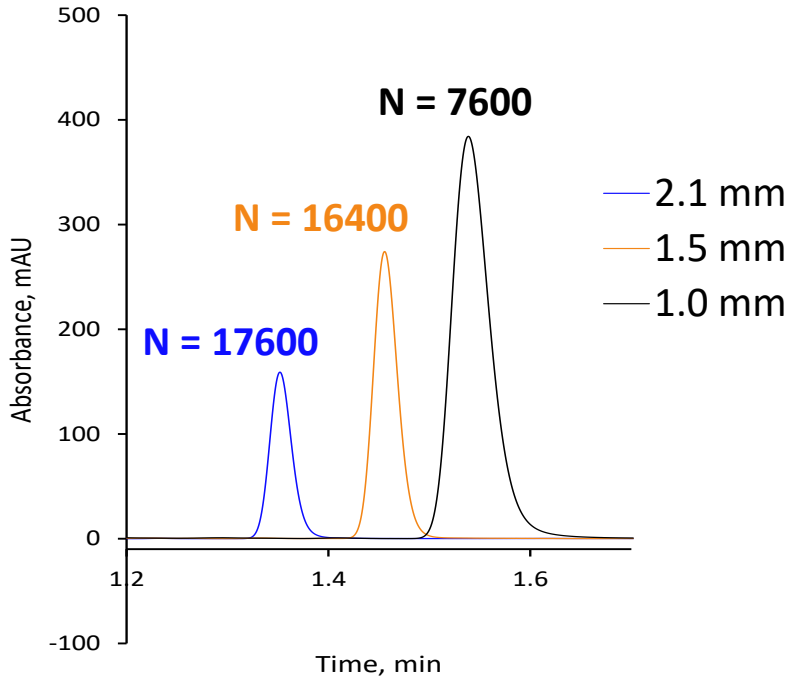
Why stop at the 1.5mm ID instead of going lower

- Efficiency is lost from ECV
- Peak widths are increased

The 1.5 maintains efficiency

- The 2.1 is more efficient but at the cost of signal
- The 1.0 has more signal but is less efficient
- The 1.5 bridges the gap between analytical and microflow systems

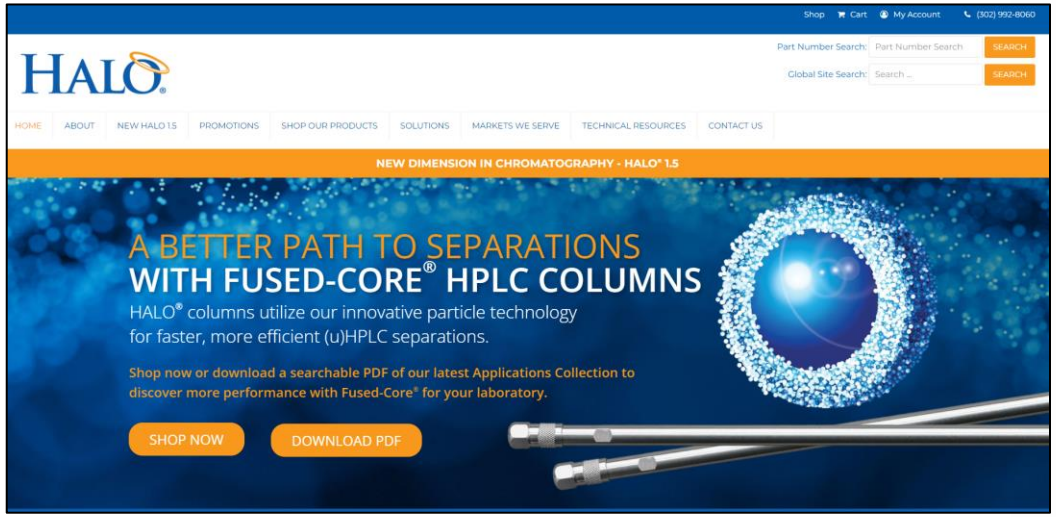


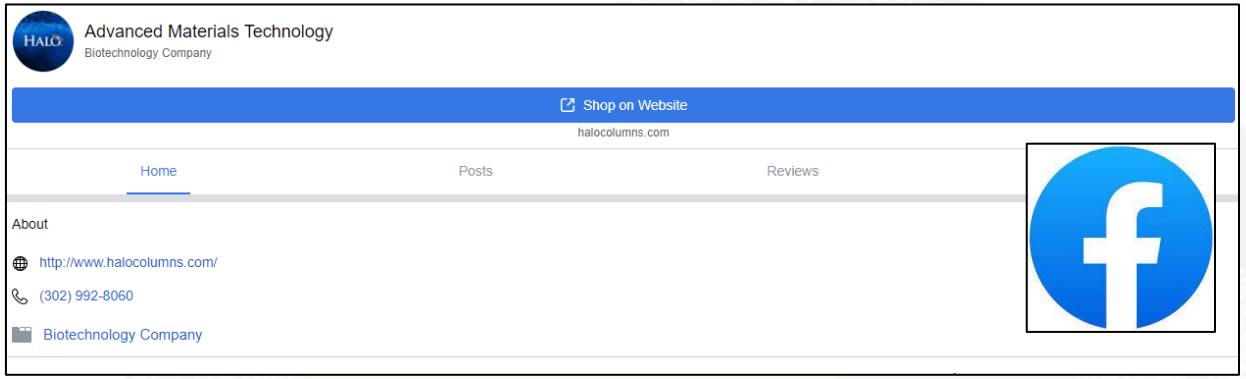


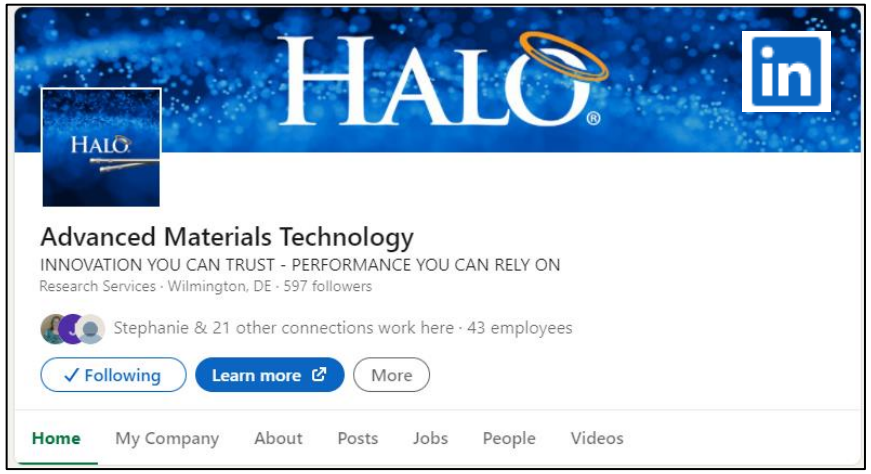
Website, LinkedIn, YouTube, Facebook

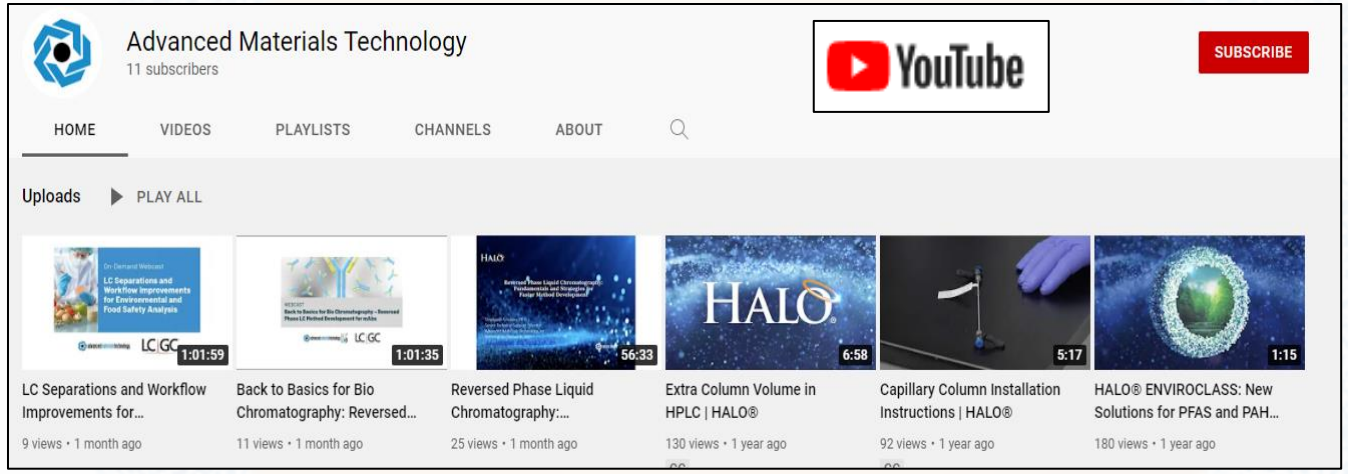
HALO®

[HALO® HPLC Columns for Chromatography Separation | LC Columns (halocolumns.com)]









Technical Resources

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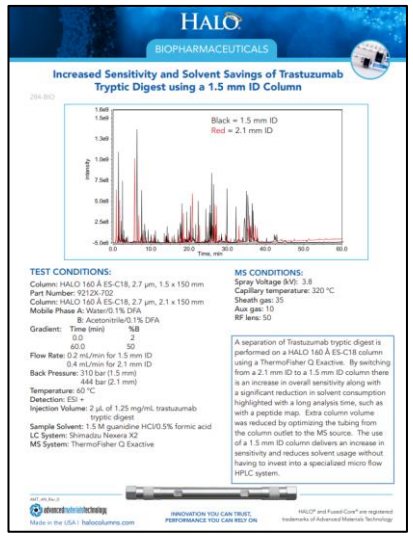


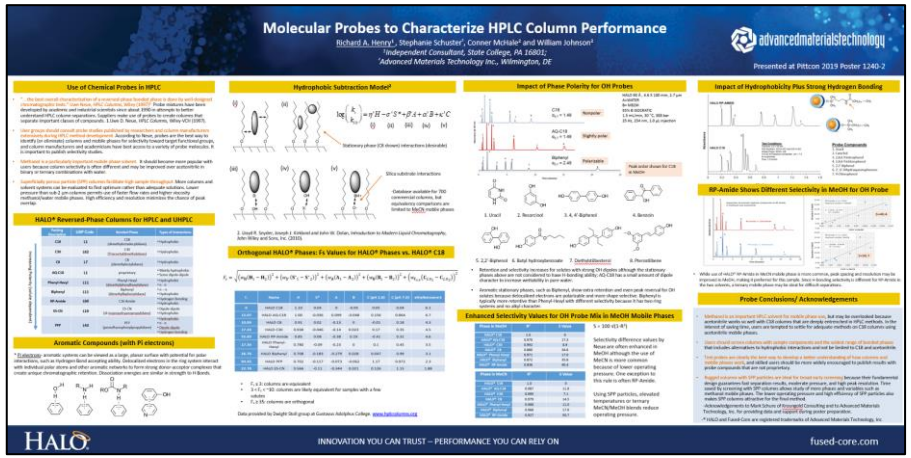
Application Notes

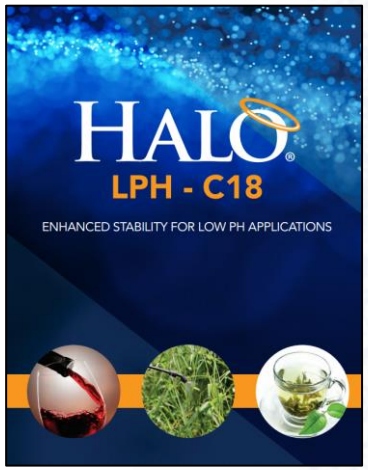
Conference Papers

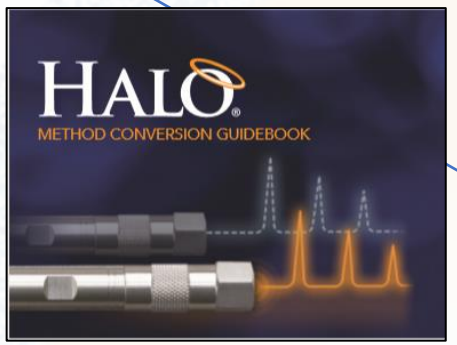
Product Literature

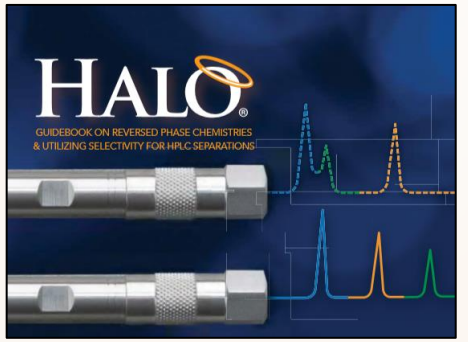
Technical Documents

Videos

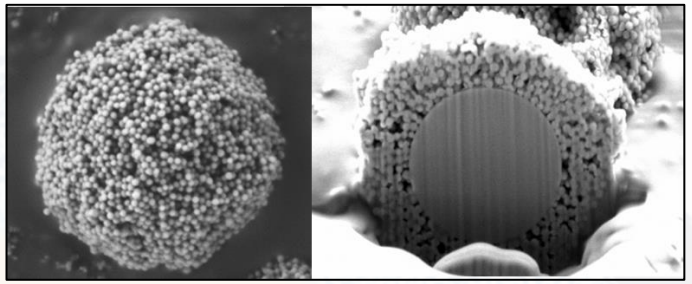




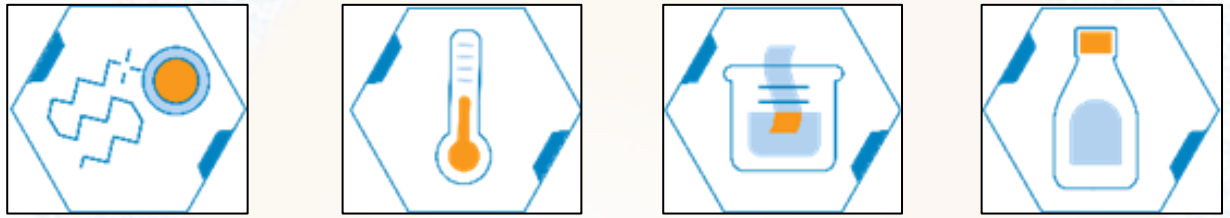




Conclusion



- Advantages of SPP vs. FPP
  - Benefits of the Fused Core particle technology
- Method Development
  - C18 and beyond!
  - Increase speed on SPP
  - Mobile Phase Optimization (MeOH vs. ACN)
  - Column Dimension



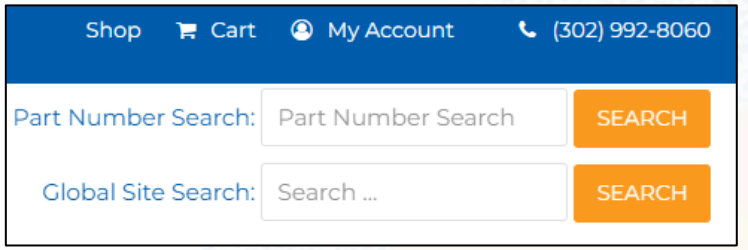
- HALO 90 Å PCS C18, 2.7 µm

- Technical Resources/ Support

Questions?

Sales, Technical and Marketing Materials:

- www.halocolumns.com



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