



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



Conner McHale
Technical Support Specialist
Advanced Materials Technology

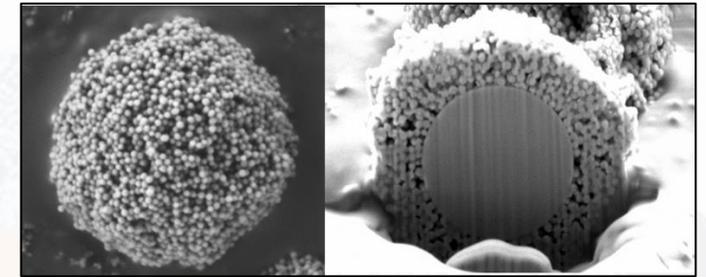
cmchale@advanced-materials-tech.com

Phone: 1-302-992-8060 *1124

Presentation Outline

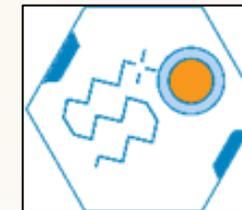
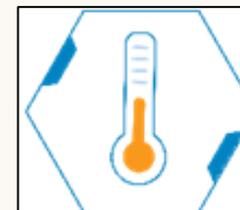
- **Advanced Materials Technology**

- 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

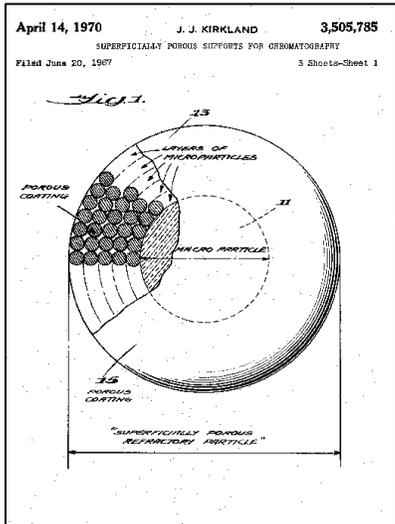
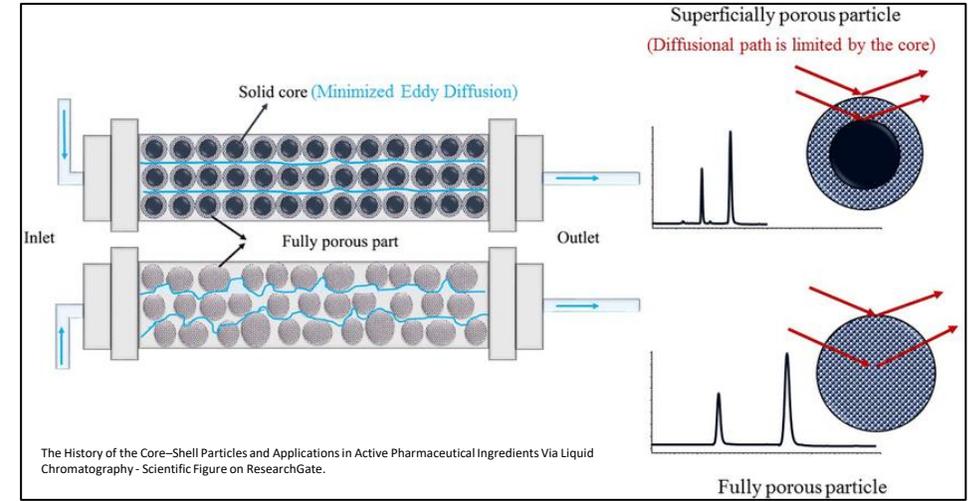
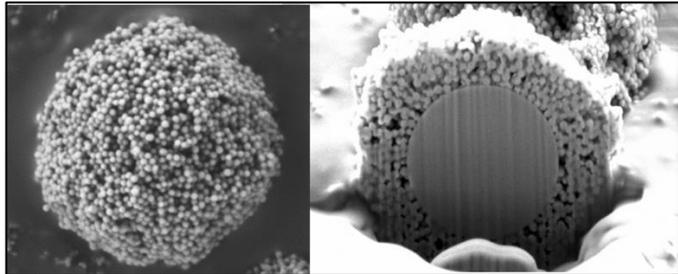
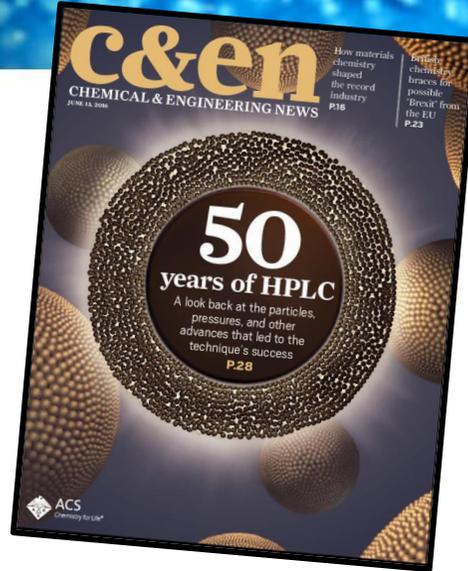


AMT is a company of innovators and continues to grow and deliver enabling materials to market. Our incredible team is our greatest resource.

Superficially Porous Particle Technology (SPP)



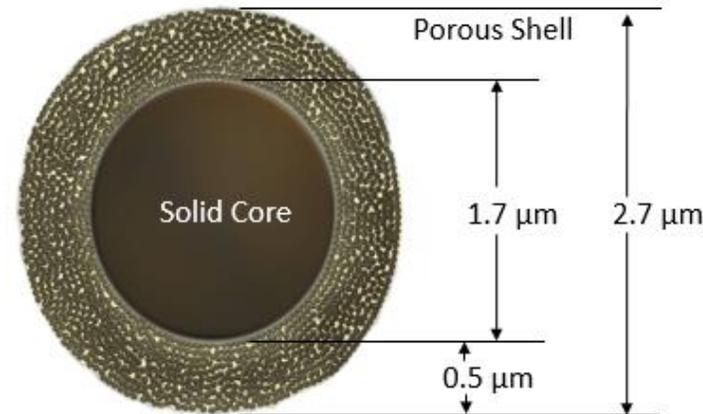
- High Purity Silica Particles (2, 2.7, 3.4, 5 μm)
- Bonded Phase Shell Fused to Solid Core
- Shell Consists of Different Pore Sizes (90, 160, 400, 1000 \AA)



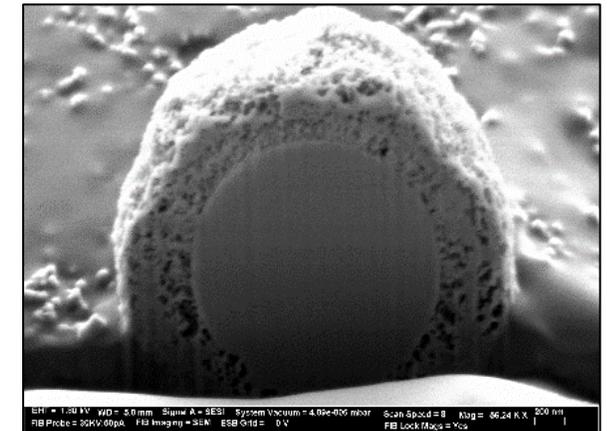
3,505,785
SUPERFICIALLY POROUS SUPPORTS FOR CHROMATOGRAPHY
Joseph J. Kirkland, Wilmington, Del., assignor to E. I. du Pont de Nemours and Company, Wilmington, Del., a corporation of Delaware
Filed June 20, 1967, Ser. No. 647,506
Int. Cl. B01d 15/08
U.S. Cl. 55-67 8 Claims

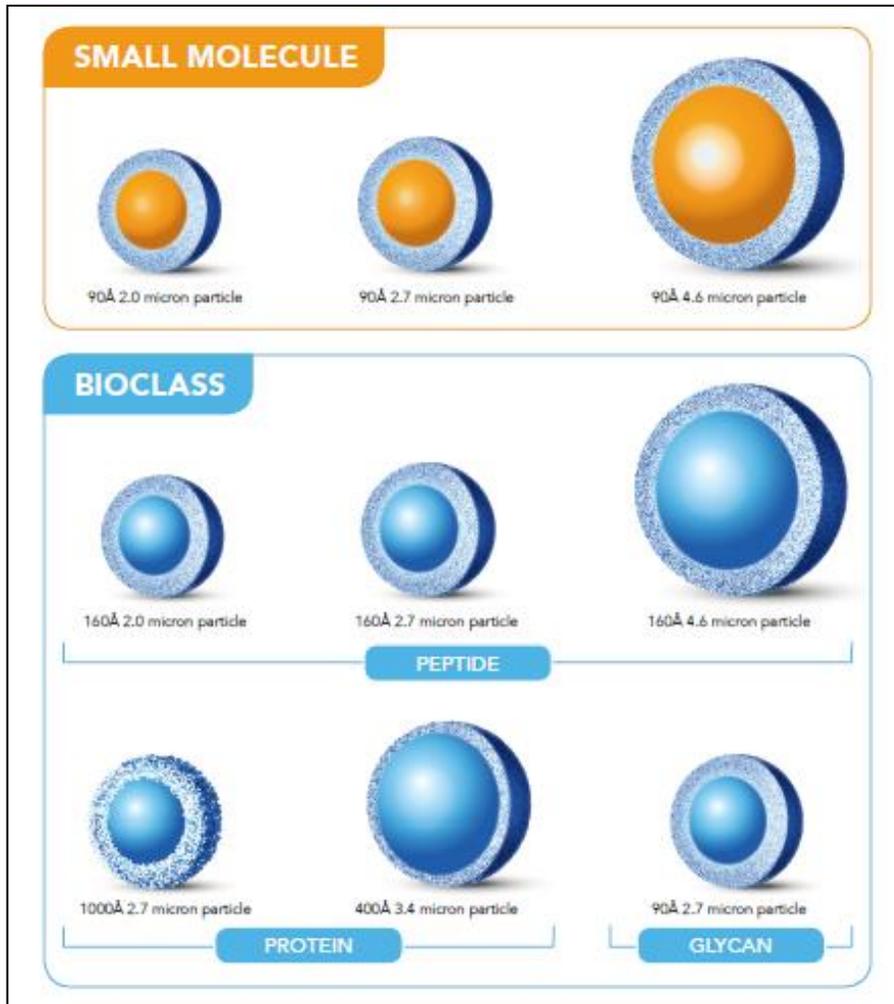
ABSTRACT OF THE DISCLOSURE
This invention relates to an improvement in chromatography and chromatographic columns. A novel packing of superficially porous refractory particles for use in chromatography has been prepared consisting of a plurality of discrete macroparticles with impervious cores and having 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



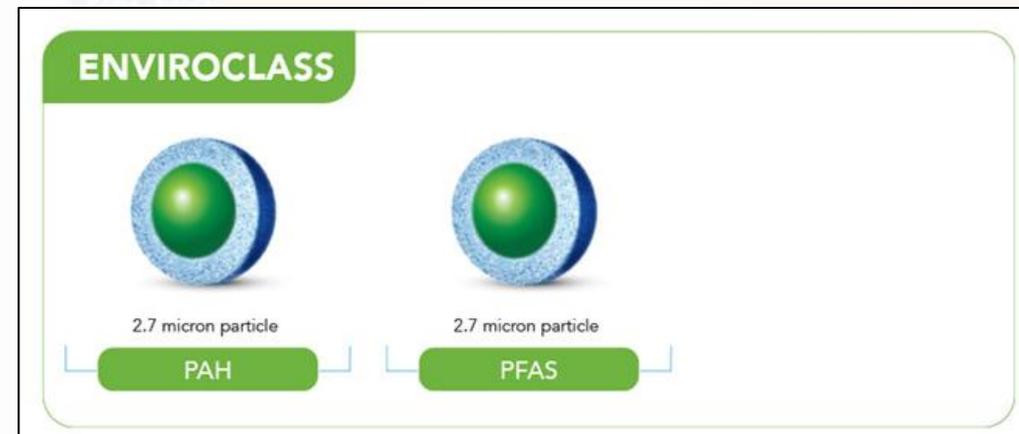
SEM Particle Cross-section





Portfolio of Products

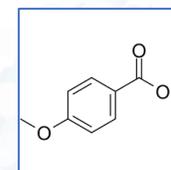
- Varying particle morphologies to meet separation needs (particle size, core size, shell thickness, pore size)
- Various chemistries for selectivity of analytes across small molecule to large molecule
- Many different column dimensions from capillary to semi-prep.



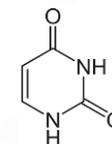
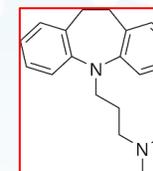
Method Development



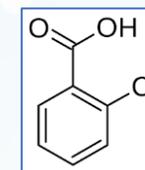
4 Methoxy Benzoic Acid



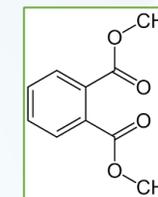
Imipramine



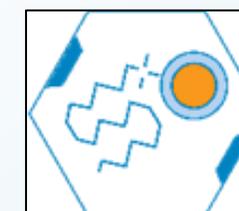
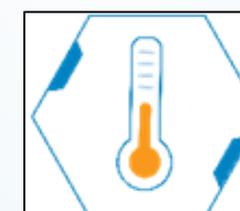
Uracil



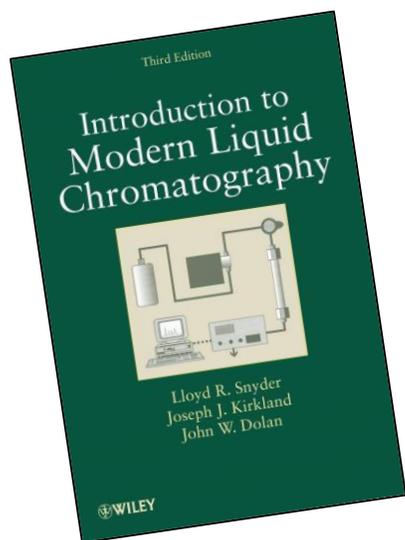
2 Chlorobenzoic Acid



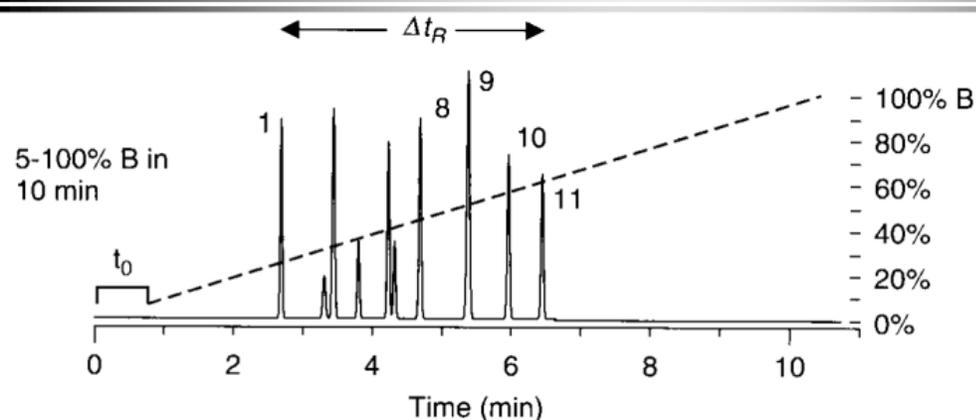
Dimethyl Phthalate



Isocratic or Gradient?



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



$$t_R = (6.5 - 2.7) = 3.8 \text{ min}$$

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

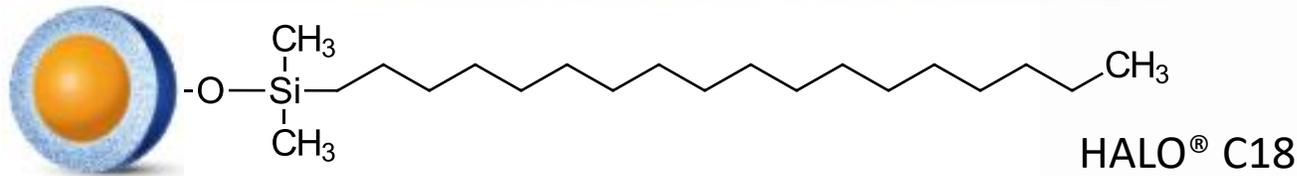
$$\Delta\phi = 0.01(100 - 5) = 0.95$$

- 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- μ m) C_{18} column, 2.0 mL/min, 30°C. Gradient indicated by (- - -).

● from IMLC3e, Fig. 9-15

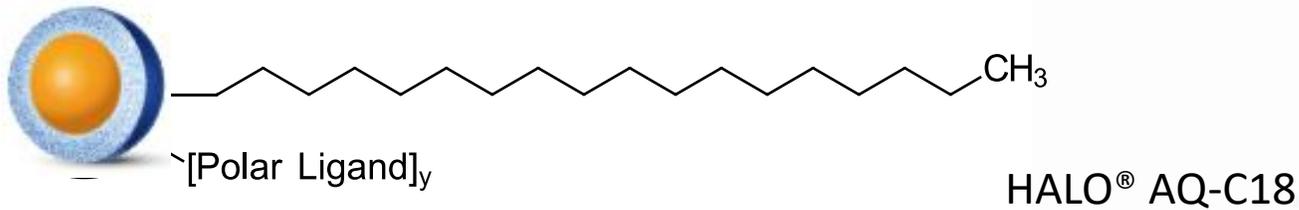
Gradient & computer-9

HALO Column Screening



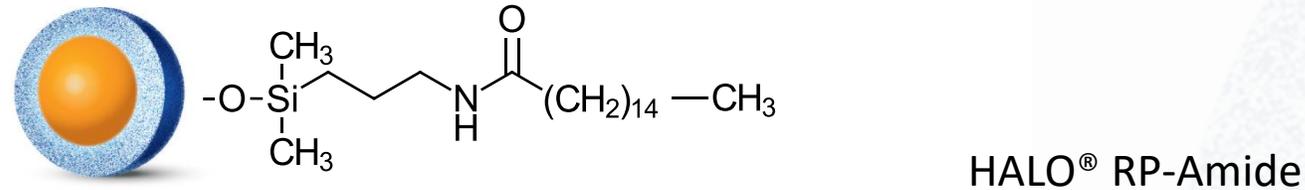
Features and Benefits

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



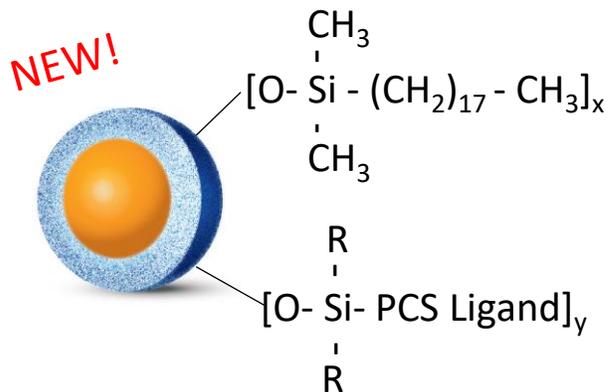
- Resistant to dewetting, making it 100% aqueous mobile phase compatible

- Enhanced retention and selectivity for polar molecules



- Complementary selectivity to alkyl phases

- Enhanced stability for minimum bleed and long life



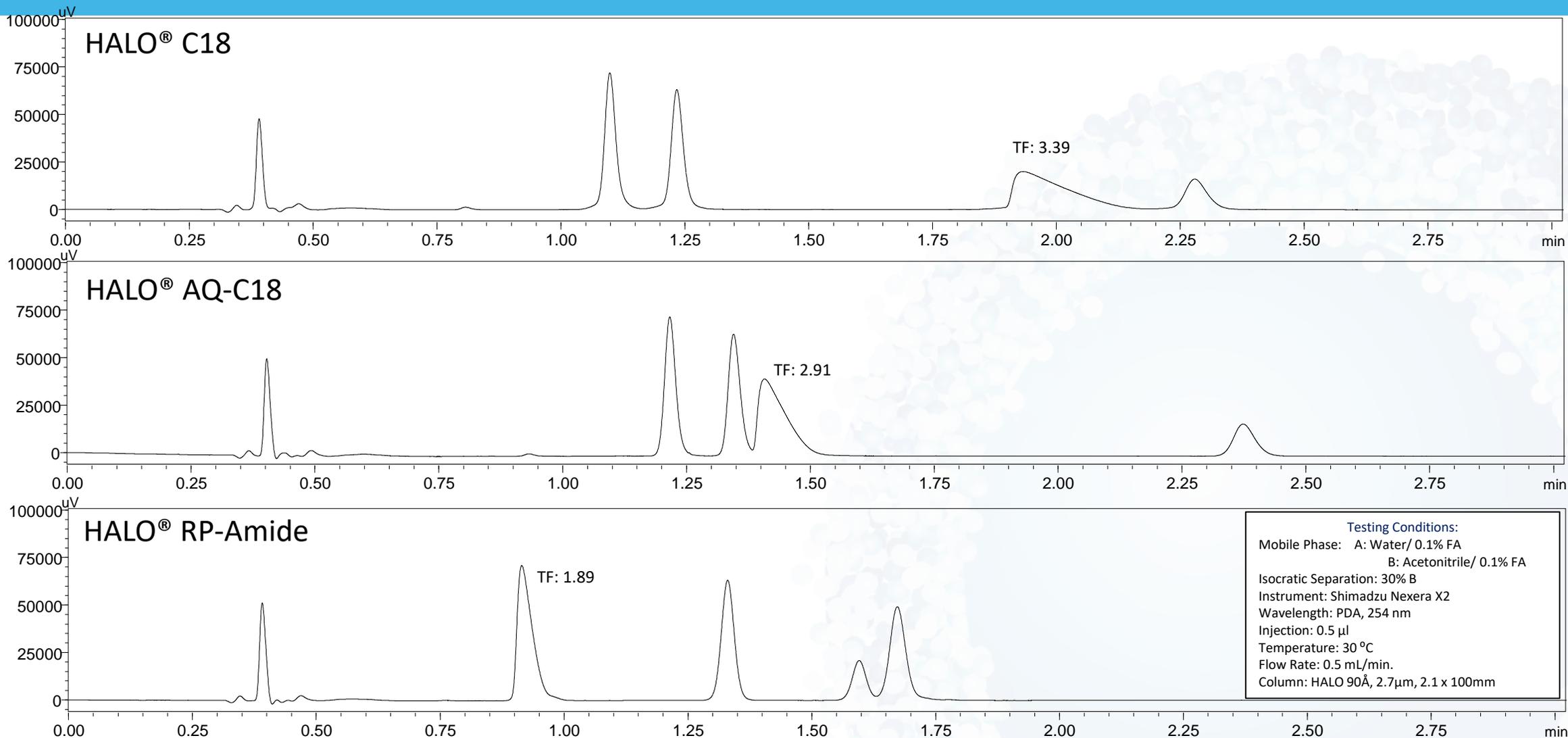
- Improved peak shape and increased loading capacity for basic compounds

- Ideal for low ionic strength mobile phases such as formic acid



Stationary Phase Screening

HALO®



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

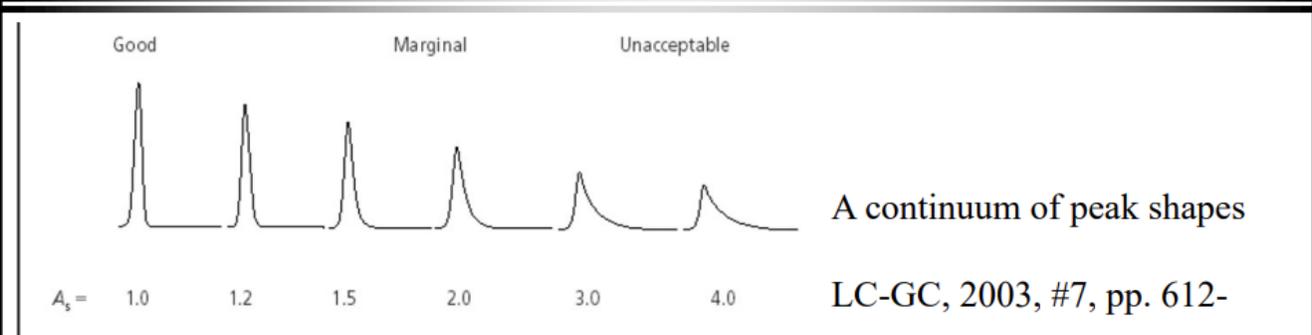


Figure 2: Examples of asymmetric peaks.

- 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

- **Positively Charged Surface = PCS**

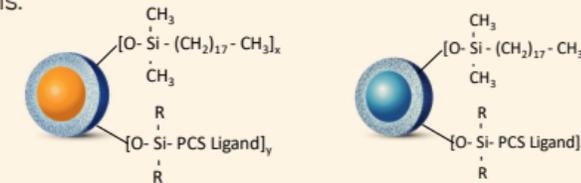
LC-MS Analysis with 0.1% Formic Acid

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 mobile 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

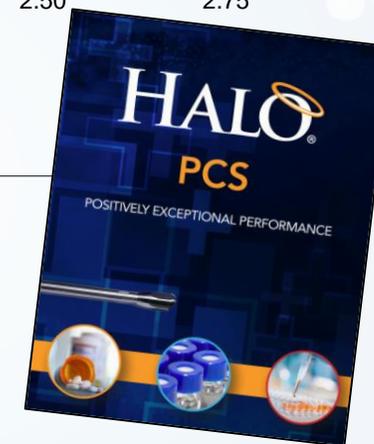
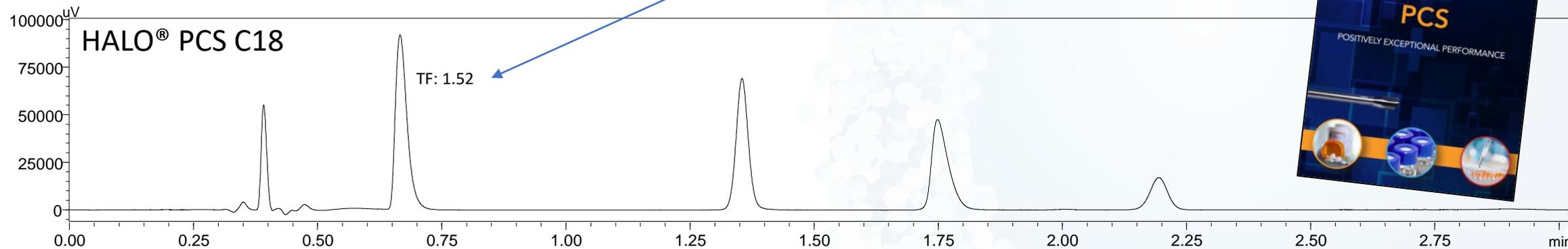
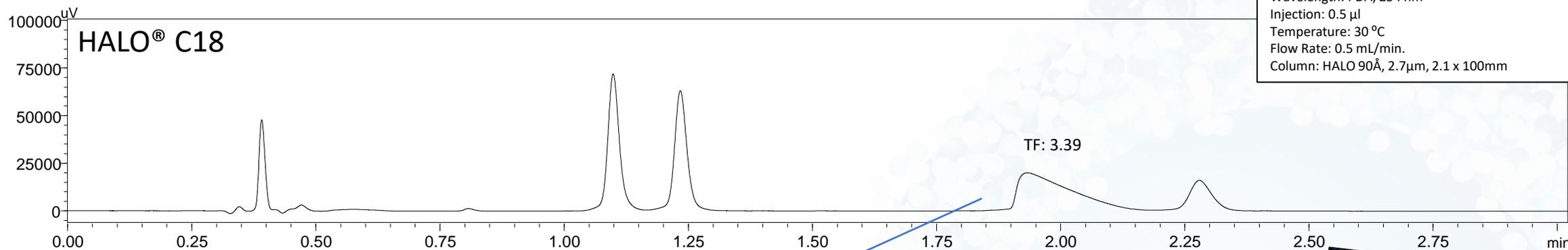
HALO 160 Å PCS C18



C18 vs. PCS C18

HALO®

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

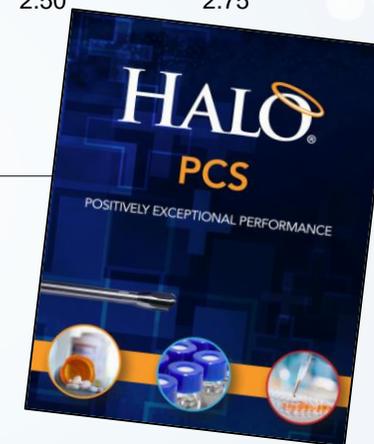
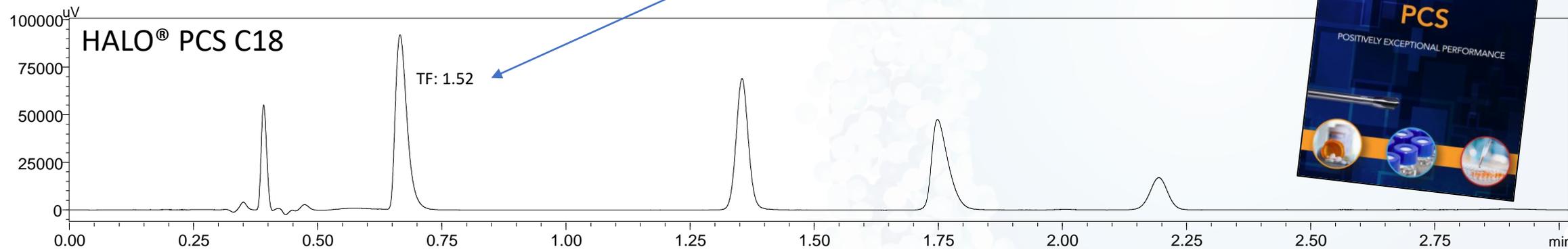
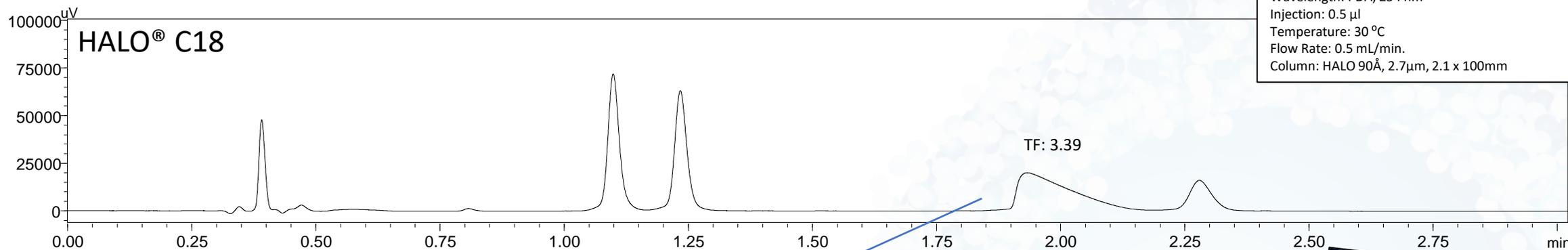




C18 vs. PCS C18

HALO®

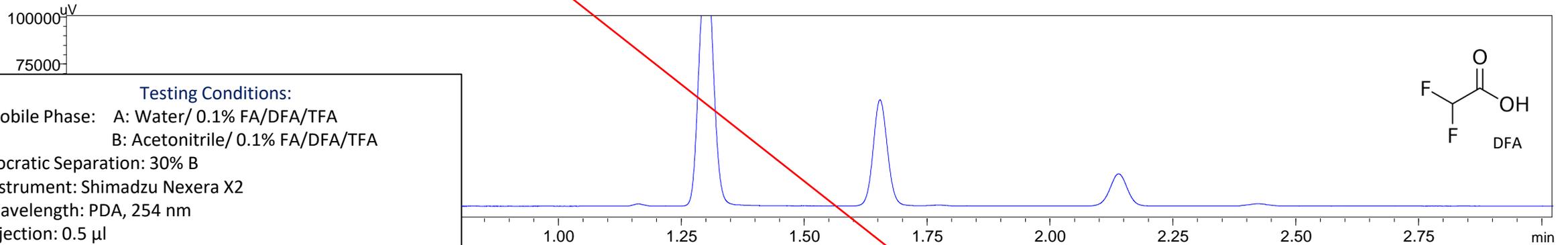
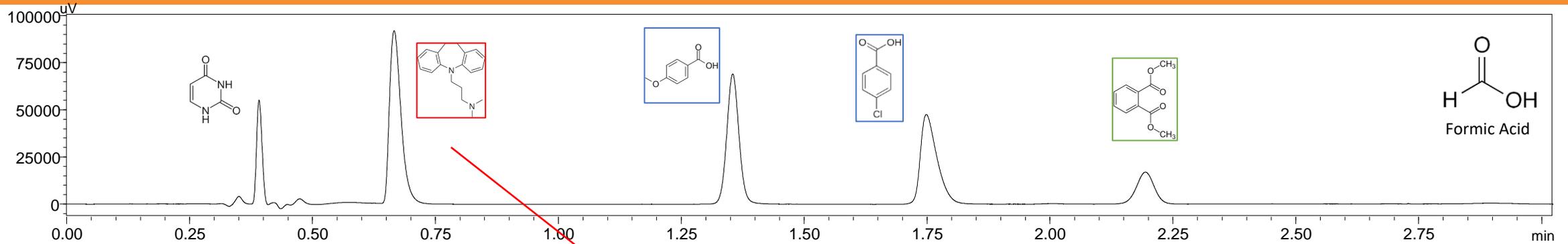
Testing Conditions:
Mobile Phase: A: Water/ 0.1% FA
 B: Acetonitrile/ 0.1% FA
Isocratic Separation: 30% B
Instrument: Shimadzu Nexera X2
Wavelength: PDA, 254 nm
Injection: 0.5 µl
Temperature: 30 °C
Flow Rate: 0.5 mL/min.
Column: HALO 90Å, 2.7µm, 2.1 x 100mm



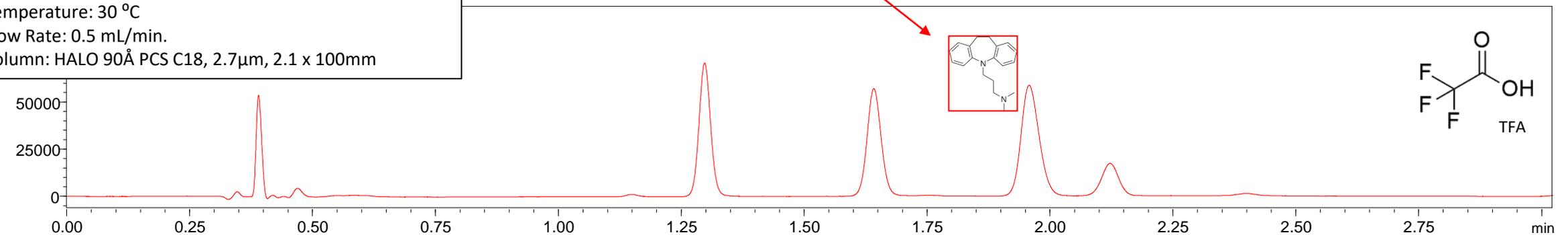


Mobile Phase/ pH Screening

HALO®



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)

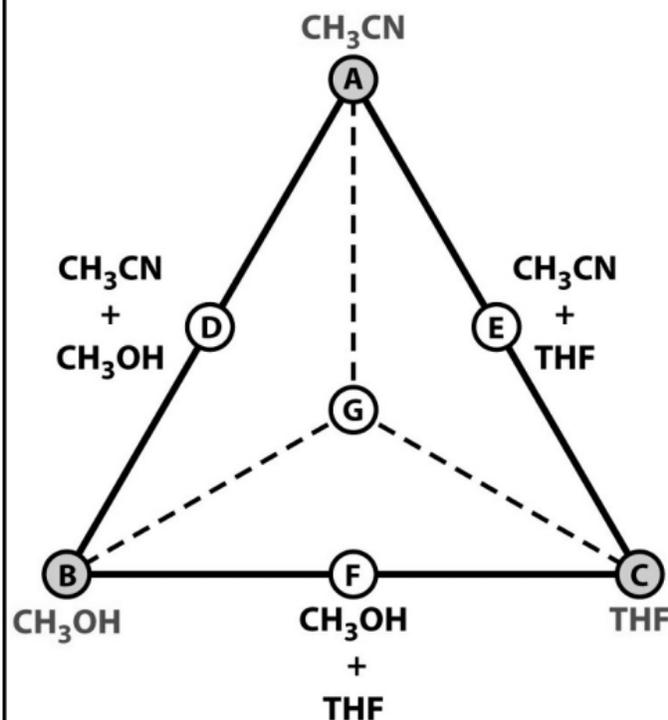


Figure 25-25
Quantitative Chemical Analysis, Seventh Edition
© 2007 W.H. Freeman and Company

- 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).

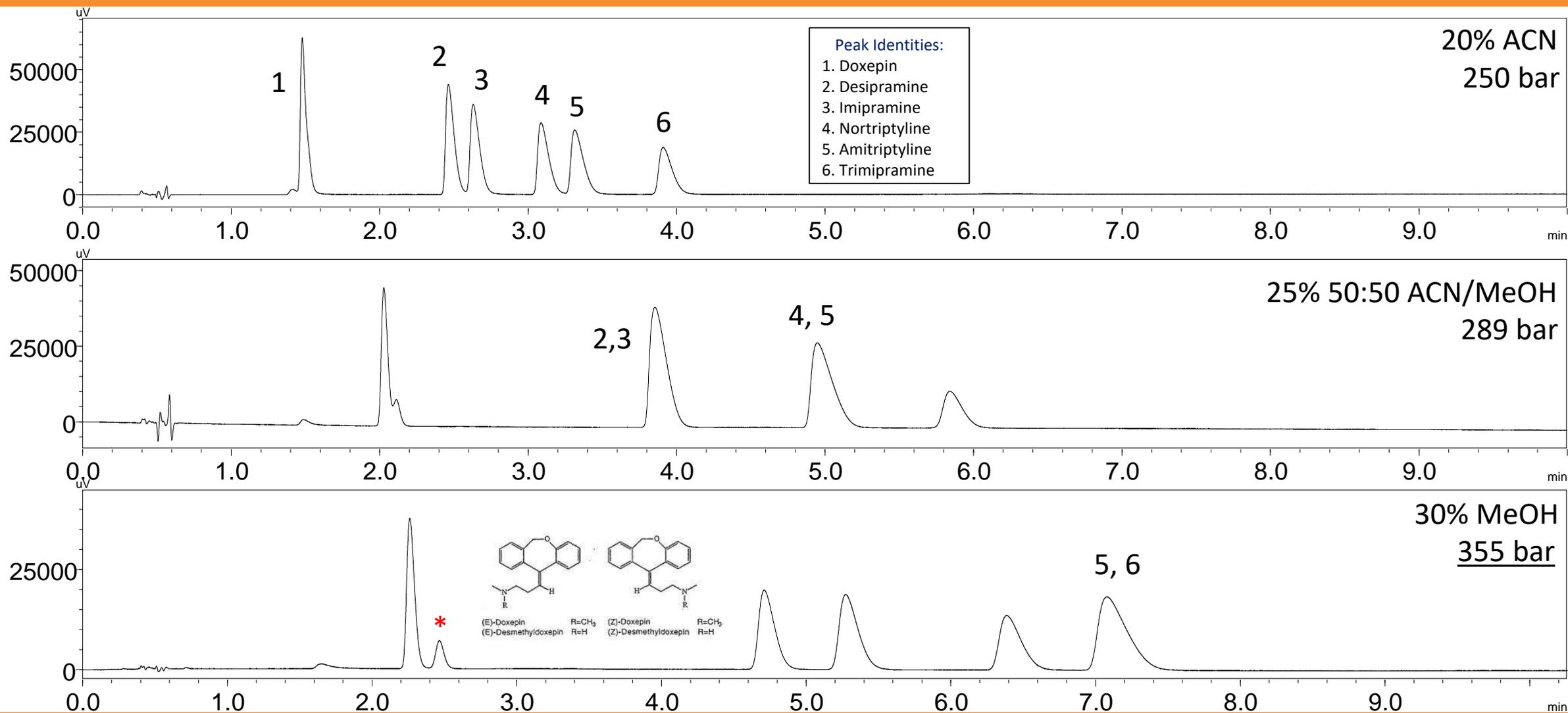
Optimization - 20

Tricyclic Antidepressants

ACN vs. MeOH

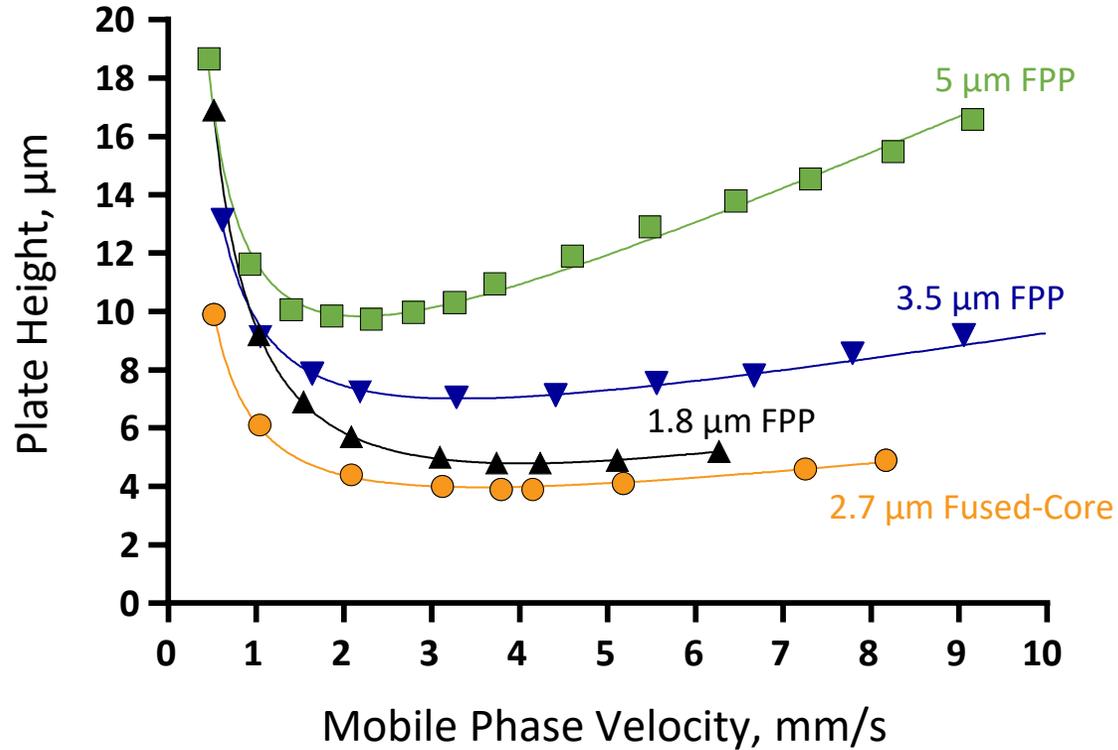


HALO®



How SPP Benefits Separations?

Speed and Efficiency



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

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

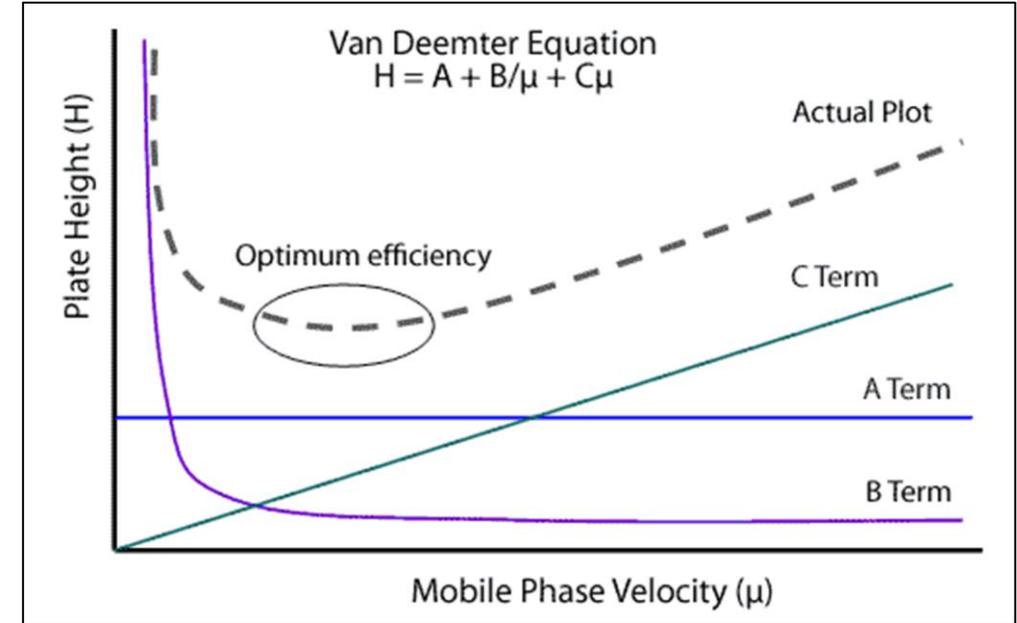
H = height equivalent to theoretical plate

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

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

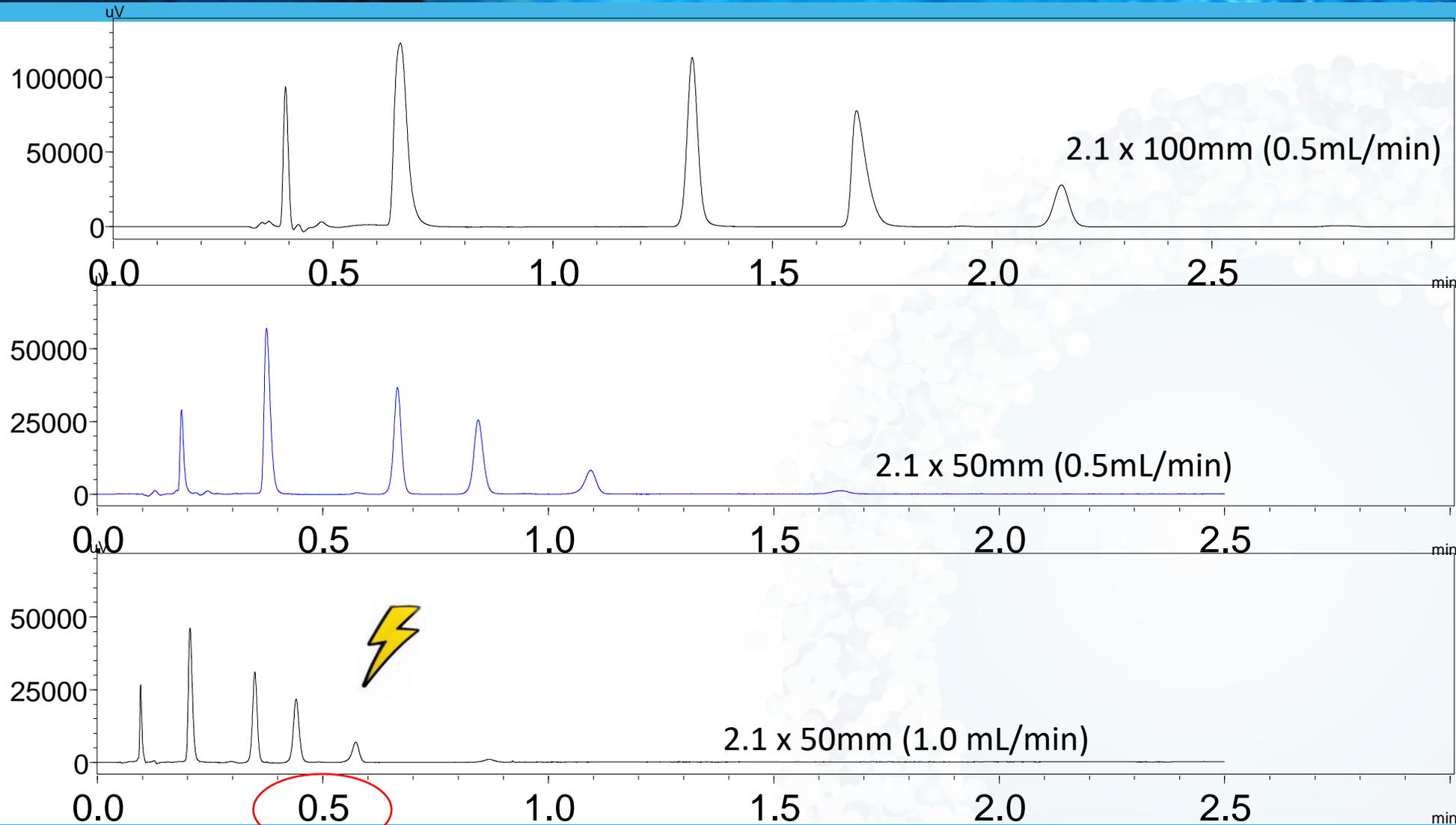
C = resistance to mass transfer term (kinetics of the analyte b/w mobile phase and stationary phase)

μ = mobile phase linear velocity (L/t₀)



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

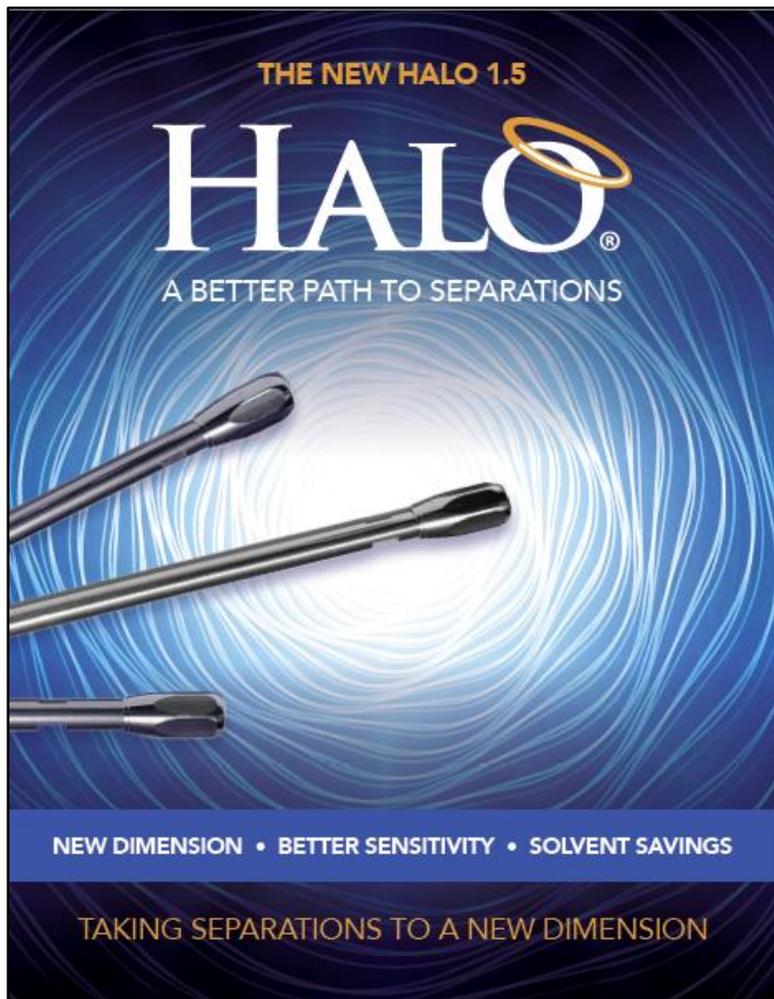
Speed vs. Resolution



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

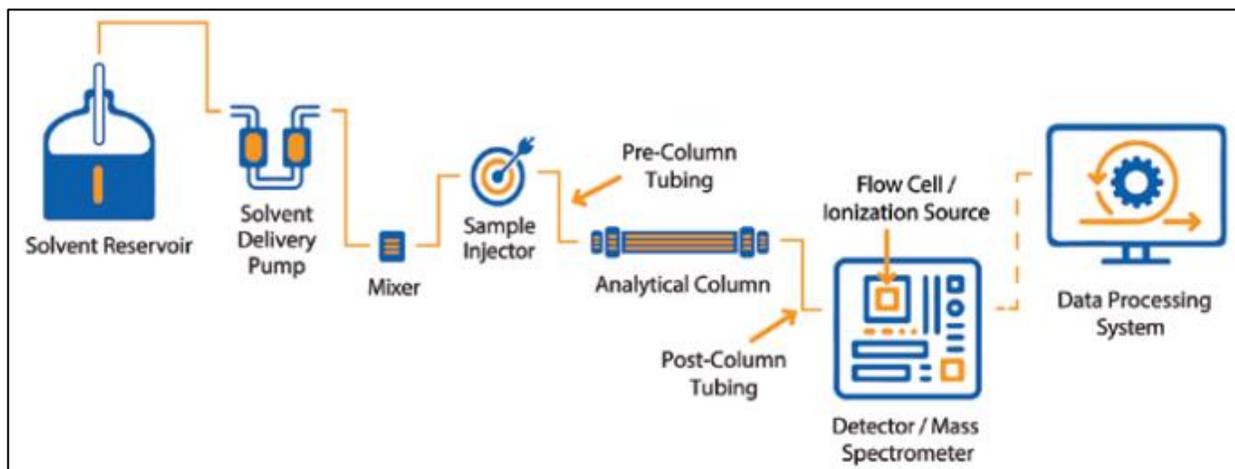
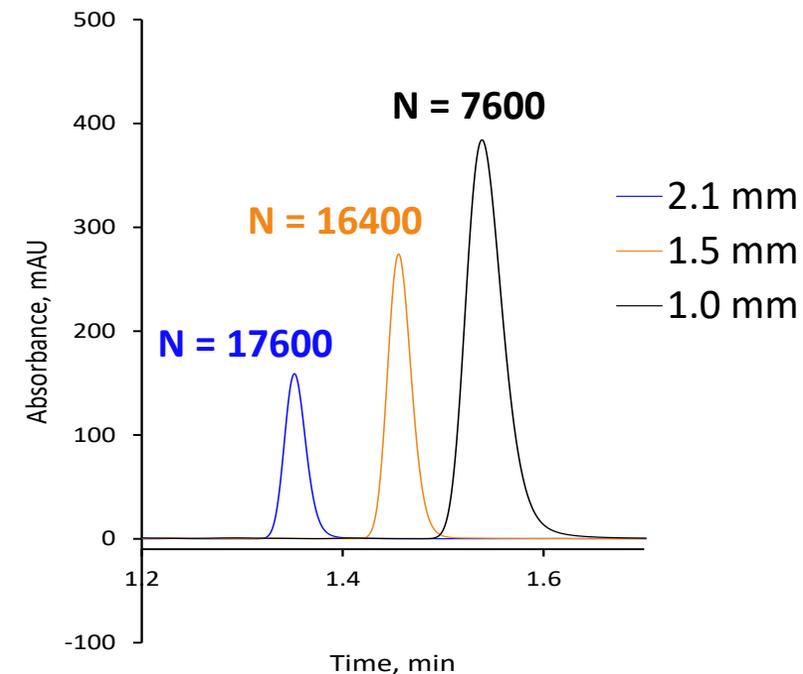
Advantages of the 1.5mm ID

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

The screenshot shows the HALO website homepage. At the top, there is a navigation bar with the HALO logo, a search bar, and links for Shop, Cart, My Account, and a phone number (302) 992-8060. Below the navigation bar, there are search boxes for Part Number and Global Site. The main content area features a large blue banner with the text "NEW DIMENSION IN CHROMATOGRAPHY - HALO® 1.5" and "A BETTER PATH TO SEPARATIONS WITH FUSED-CORE® HPLC COLUMNS". The banner includes a description of the technology and two buttons: "SHOP NOW" and "DOWNLOAD PDF".

The screenshot shows the HALO Facebook page. The page header includes the HALO logo, the company name "Advanced Materials Technology", and the tagline "Biotechnology Company". There is a "Shop on Website" button and the website URL "halocolumns.com". The page has tabs for Home, Posts, and Reviews. The About section includes the website URL "http://www.halocolumns.com/", the phone number "(302) 992-8060", and the company type "Biotechnology Company". A large Facebook logo is visible on the right side of the page.

The screenshot shows the HALO LinkedIn page. The header features the HALO logo and the LinkedIn logo. The company name "Advanced Materials Technology" is displayed, along with the tagline "INNOVATION YOU CAN TRUST - PERFORMANCE YOU CAN RELY ON" and the location "Research Services · Wilmington, DE · 597 followers". There is a button for "Following" and a "Learn more" link. The page navigation includes Home, My Company, About, Posts, Jobs, People, and Videos.

The screenshot shows the HALO YouTube channel. The header includes the HALO logo, the company name "Advanced Materials Technology", and the subscriber count "11 subscribers". There is a "SUBSCRIBE" button and the YouTube logo. The page has tabs for HOME, VIDEOS, PLAYLISTS, CHANNELS, and ABOUT. The "Uploads" section shows a list of videos with thumbnails, titles, and view counts. The videos include "LC Separations and Workflow Improvements for...", "Back to Basics for Bio Chromatography: Reversed...", "Reversed Phase Liquid Chromatography...", "Extra Column Volume in HPLC | HALO®", "Capillary Column Installation Instructions | HALO®", and "HALO® ENVIROCLASS: New Solutions for PFAS and PAH...".



Technical Resources

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



BIOPHARMACEUTICALS

Increased Sensitivity and Solvent Savings of Trazosinamide Tryptic Digest using a 1.5 mm ID Column

Black = 1.5 mm ID
Red = 2.1 mm ID

TEST CONDITIONS:
Column: HALO 160 Å ES-C18, 2.7 µm, 1.5 x 150 mm
Part Number: 52120-702
Column: HALO 160 Å ES-C18, 2.7 µm, 2.1 x 150 mm
Mobile Phase: A: Water/0.1% DFA
B: Acetonitrile/0.1% DFA
Gradient: Time [min] Z %B
40.0 50
Flow Rate: 0.2 mL/min for 1.5 mm ID
0.4 mL/min for 2.1 mm ID
Back Pressure: 310 bar (1.5 mm)
444 bar (2.1 mm)
Temperature: 60 °C
Injection Volume: 2 µL of 1.25 mg/mL trazosinamide tryptic digest
Sample Solvent: 1.5 M guanidine HCl/0.5% formic acid
LC System: Shimadzu Nexera S2
MS System: ThermoFisher Q Exactive

MS CONDITIONS:
Spray Voltage: 3kV; 3.8
Capillary temperature: 320 °C
Sheath gas: 35
Aux gas: 10
RF lens: 50

A separation of Trazosinamide tryptic digest is performed on a HALO 160 Å ES-C18 column using a ThermoFisher Q Exactive. By switching from a 2.1 mm ID to a 1.5 mm ID column there is an increase in overall sensitivity along with a significant reduction in solvent consumption highlighted with a long analysis time, such as with a peptide map. Extra column volume was reduced by optimizing the tubing from the column outlet to the MS source. The use of a 1.5 mm ID column delivers an increase in sensitivity and reduces solvent usage without having to invest into a specialized micro flow HPLC system.



Application Notes



Conference Papers



Product Literature



Technical Documents



Videos

Webinar: Reversed Phase Liquid Chromatography: Fundamentals and Strategies for Faster Method Development

Reversed Phase Liquid Chromatography: Fundamentals and Strategies for Faster Method Development

HALO

Reversed Phase Liquid Chromatography: Fundamentals and Strategies for Faster Method Development

Stephanie Schuster, Ph.D.
Senior Technical Support Scientist
Advanced Materials Technology, Inc.
Wilmington, Delaware, USA

Watch on YouTube

HALO
METHOD CONVERSION GUIDEBOOK

HALO
GUIDEBOOK ON REVERSED PHASE CHEMISTRIES & UTILIZING SELECTIVITY FOR HPLC SEPARATIONS!

HALO
LPH - C18
ENHANCED STABILITY FOR LOW PH APPLICATIONS

HALO

TECHNICAL REPORT: AMT-TR022001

TITLE: HIGH RESOLUTION LCMS SEPARATIONS OF EDIBLE OILS

MARKET SEGMENT: FOOD / BEVERAGE

AUTHORS:
Andrew Herron, PhD, Application Scientist

ABSTRACT
Edible oils, extracted from both plant and animal sources, have evolved into a multi-billion dollar industry and are being used in new applications every year. In 2018 over 382.05 million metric tons of edible oils were consumed and valued worldwide. Products such as biodiesel, pharmaceutical formulation applications, soaps, shampoos, and household cleaners are among a few. In recent years, the food industry has sought to incorporate more oils with higher nutritional value, but with often ambiguous results. The hydrophilic nature of oils often makes analysis problematic by C18 stationary phases due to limited selectivity. In this technical report we generate the TAG profile of four common edible oils, including sun, sesame, canola, and grape seed oil by LC/MS, to demonstrate how the HALO C18 column with its unique stationary phase offers superior selectivity and higher shape selectivity. This enables better separation of the hydrophilic long-chain molecules, such as TAGs.

INTRODUCTION
Often thought of as an essential part of a healthy diet, the nutritional value of edible oils has been a topic of debate, primarily due to their application. The major component of edible oils is triglycerides (TAGs), which consist of approximately 95% of the oil. The remaining 5-10% is a mixture of free acids, monoacylglycerols, diacylglycerols (DAGs), phospholipids, sterols and various hydrocarbons, including vitamins and antioxidants (Quaranta 2006).

The analysis of edible oils by LC/MS is difficult due to the high concentration of hydrophilic molecules, such as long chain fatty acids (LCFA) and steric, as well as DAGs and TAGs. In the food industry, the analysis of TAGs in the oil is a critical step to determine nutritional value, for example amount of unsaturation in the oil, as well as suitability for non-food-based applications. C18 columns, the most

(Rinaldi et al., 1998; Abul 2005; Sander and Vlas 1992). The structure and conformation of the C18 phase compared to C18, provides better phase thickness to enhance the interaction between the stationary phase, and long chain molecules, such as TAGs and DAGs (Sander and Vlas, 1992). In this application note we report the TAG profile of 4 common edible oils, and compare with previously published data, to demonstrate the utility of the HALO C18 for the analysis of long chain hydrophilic molecules, such as those found in edible oils.

KEY WORDS:
Edible oils, Triacylglycerides, Diacylglycerides, HALO C18, Hydrophilic, LC/MS, TAG, DAG.

Molecular Probes to Characterize HPLC Column Performance

Richard A. Henry¹, Stephanie Schuster¹, Connor Mohler¹ and William Johnson¹
¹Advanced Materials Technology, State College, PA 16802; Advanced Materials Technology Inc., Wilmington, DE

Presented at Pittcon 2019 Poster 1340-Z

Use of Chemical Probes in HPLC
Chemical probes are used to evaluate the performance of HPLC columns. They are small molecules that are used to test the selectivity and stability of the column. The probes are used to evaluate the column's ability to separate different types of molecules, such as hydrophobic, hydrophilic, and ionic. The probes are used to evaluate the column's ability to separate different types of molecules, such as hydrophobic, hydrophilic, and ionic.

Hydrophobic Subtraction Model
The hydrophobic subtraction model is used to evaluate the selectivity of HPLC columns. It is based on the idea that hydrophobic molecules will interact with the stationary phase, while hydrophilic molecules will not. The model is used to evaluate the column's ability to separate different types of molecules, such as hydrophobic, hydrophilic, and ionic.

Impact of Phase Polarity on OH Probes
The impact of phase polarity on OH probes is evaluated. The probes are used to evaluate the column's ability to separate different types of molecules, such as hydrophobic, hydrophilic, and ionic.

Impact of Hydrophobicity Plus Strong Hydrogen Bonding
The impact of hydrophobicity plus strong hydrogen bonding is evaluated. The probes are used to evaluate the column's ability to separate different types of molecules, such as hydrophobic, hydrophilic, and ionic.

RP-Amide Shows Different Selectivity to Match for OH Probe
The RP-Amide shows different selectivity to match for OH probe. The probes are used to evaluate the column's ability to separate different types of molecules, such as hydrophobic, hydrophilic, and ionic.

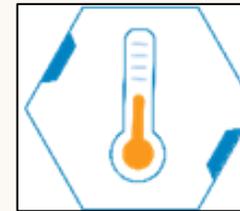
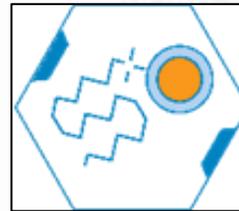
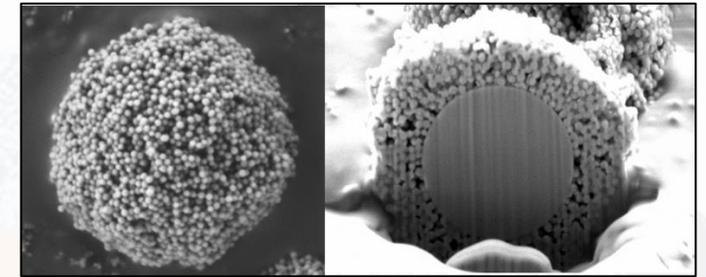
Enhanced Selectivity Values for OH Probe Mix in MOOH Mobile Phases
The enhanced selectivity values for OH probe mix in MOOH mobile phases are evaluated. The probes are used to evaluate the column's ability to separate different types of molecules, such as hydrophobic, hydrophilic, and ionic.

Amide Compounds (with PE electron)
The amide compounds (with PE electron) are evaluated. The probes are used to evaluate the column's ability to separate different types of molecules, such as hydrophobic, hydrophilic, and ionic.



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:

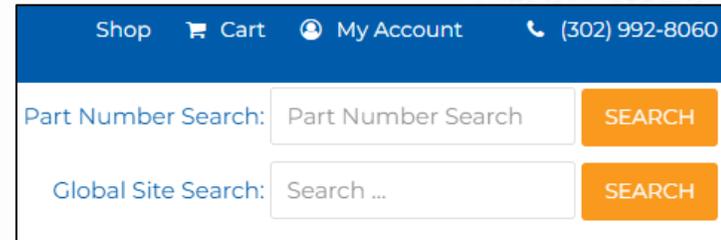
- www.halocolumns.com

Technical Support:

- support@advanced-materials-tech.com

Sales Questions/Sales Orders:

- sales@advanced-materials-tech.com



The screenshot shows the top navigation bar of the HALO website. It includes links for 'Shop', 'Cart', 'My Account', and a phone number '(302) 992-8060'. Below the navigation bar are two search boxes. The first is labeled 'Part Number Search:' and contains the text 'Part Number Search' and a 'SEARCH' button. The second is labeled 'Global Site Search:' and contains the text 'Search ...' and a 'SEARCH' button.



Conner McHale
Technical Support Specialist
Advanced Materials Technology

cmchale@advanced-materials-tech.com
Phone: 1-302-992-8060 *1124





advancedmaterialstechnology



halocolumns.com



3521 Silverside Road, Suite 1-K
Quillen Building
Wilmington, DE 19810



(302) 992-8060

