



Improving Chromatography for Basic Analytes Using Positive Charge Surface Material

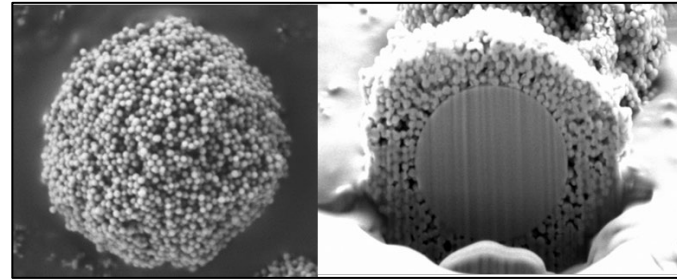


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Advanced Materials Technology

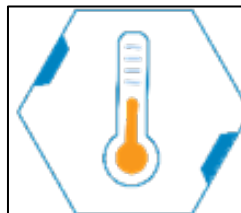
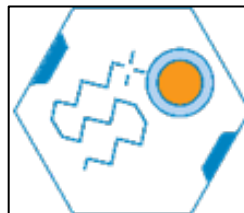
- Benefit of Fused-Core[®] Technology



Dealing with Basic Analytes/ Peak Tailing

- Ways to Improve Tailing
- HALO[®] PCS : Positively Charged Surface Chemistries

Applications



Founded in 2005 and dedicated to the invention and innovation of superficially porous particle technology for the mission of advancing chromatography

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

Expanded IP portfolio to include materials, products and technologies

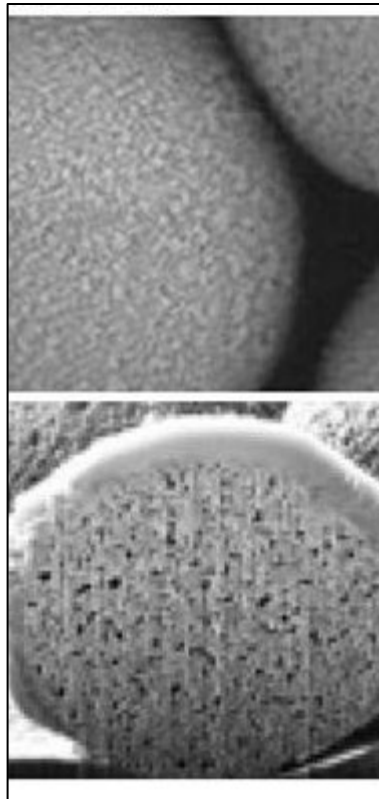
Facility

- ISO 9001 QMS certified company
- Fully equipped state of the art laboratories
- All operations handled in Wilmington, DE
 - R&D, Applications, QA/QC, Manufacturing, Sales and Marketing
- Global distribution

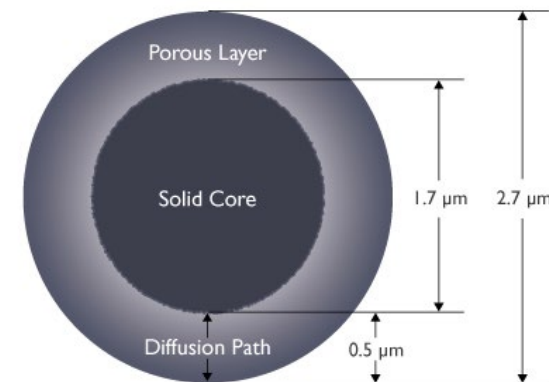
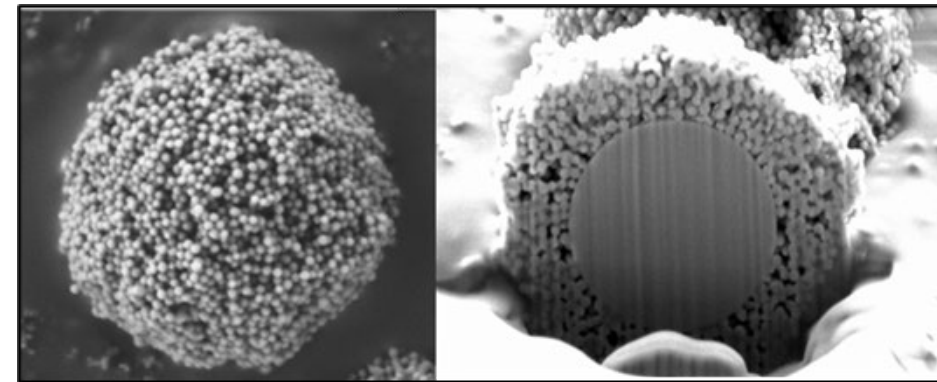


AMT is a company of innovators and continues to grow and deliver enabling materials to market. Our incredible team is our greatest resource.

HALO 1000 Å, 2.7 μm



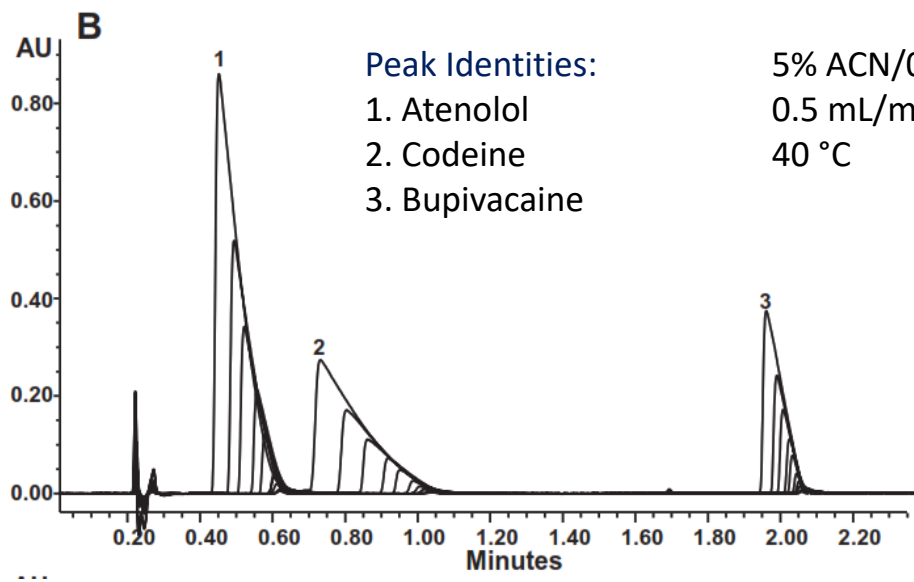
Fully Porous Particle (FPP)



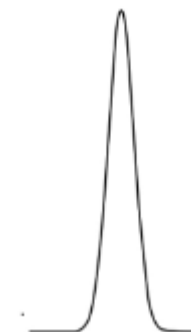
Superficially Porous Particle (SPP)

Tailing Peak Shape of Basic Compounds

- When basic compounds are run at low pH, they gain a proton and become positively charged.
- At high sample loads, the tailing will become significant, peak shape will suffer, and retention decreases - the “non-linear isotherm”.
- This is not a simple “silanol” concern.



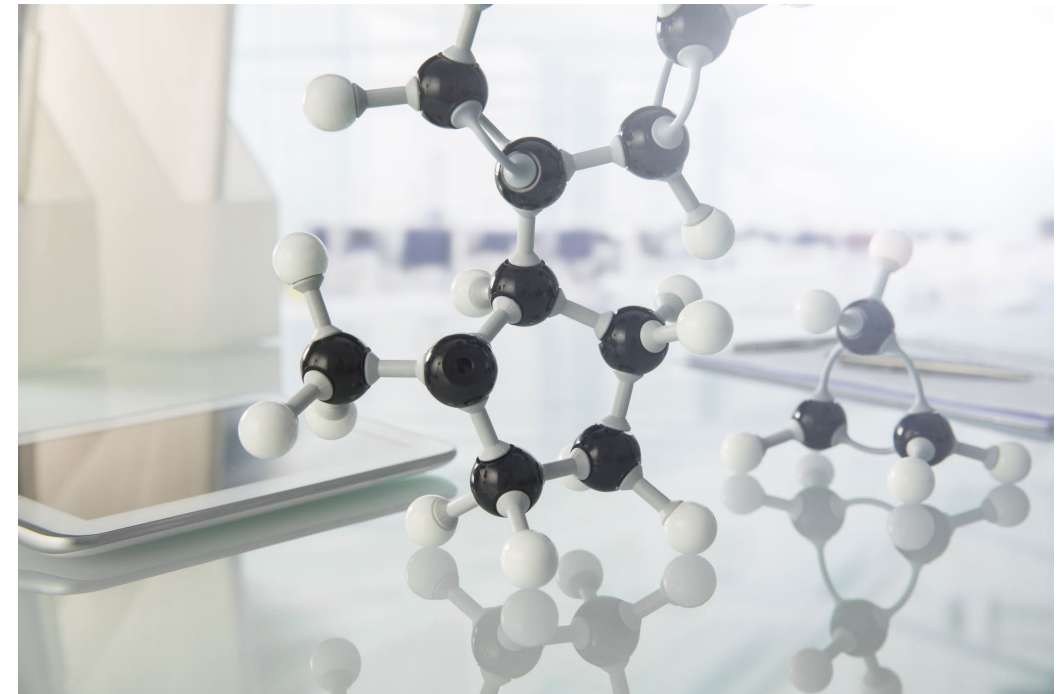
J. Chromatogr. A 1228 (2012) 221-231



Goal is to have symmetrical peak shapes across a wide range of sample concentrations

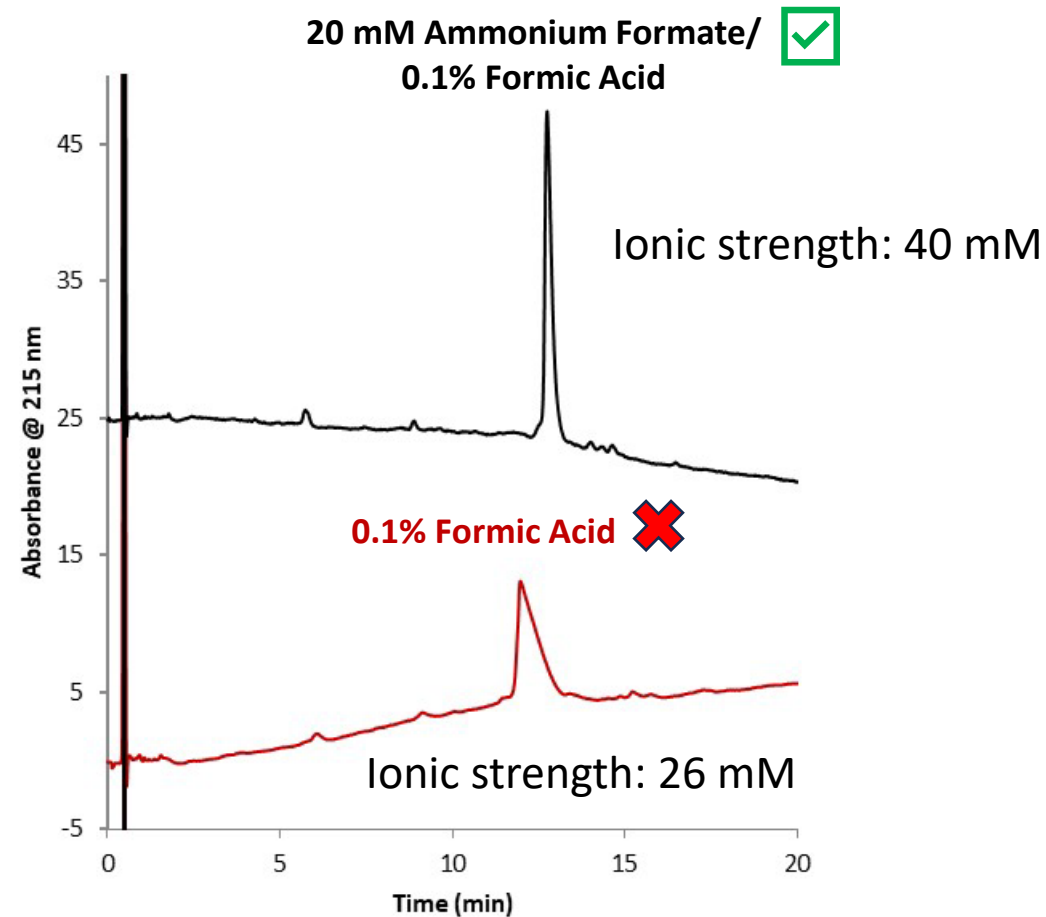
Solutions to Improve Tailed Peak Shape of Basic Compounds

1. Increase the ionic strength of the mobile phase by adding salt or buffer
2. Use an ion pair agent
3. Use a non-silica based column
4. Elevate the pH
5. Use a different stationary phase



1. Increase the ionic strength of the mobile phase by adding salt or buffer

1 µg of beta-amyloid 1-38 injected on column



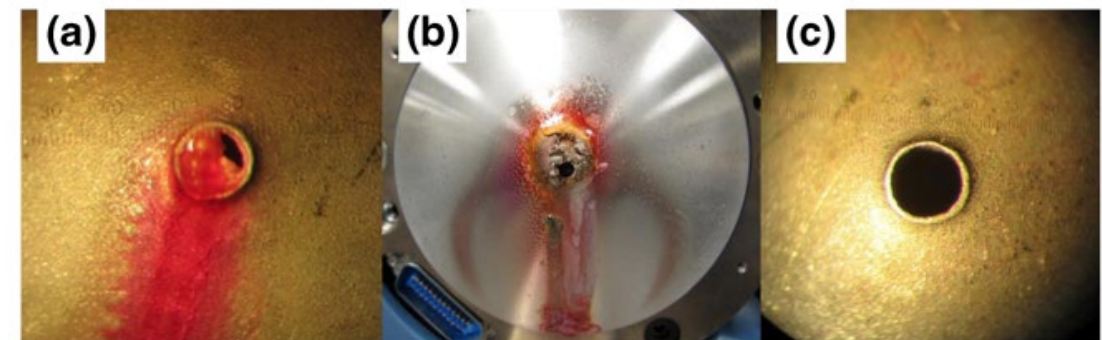
Using 20 mM Ammonium Formate/0.1% Formic Acid:

Pros:

1. Improved peak shape
2. Increased retention

Cons:

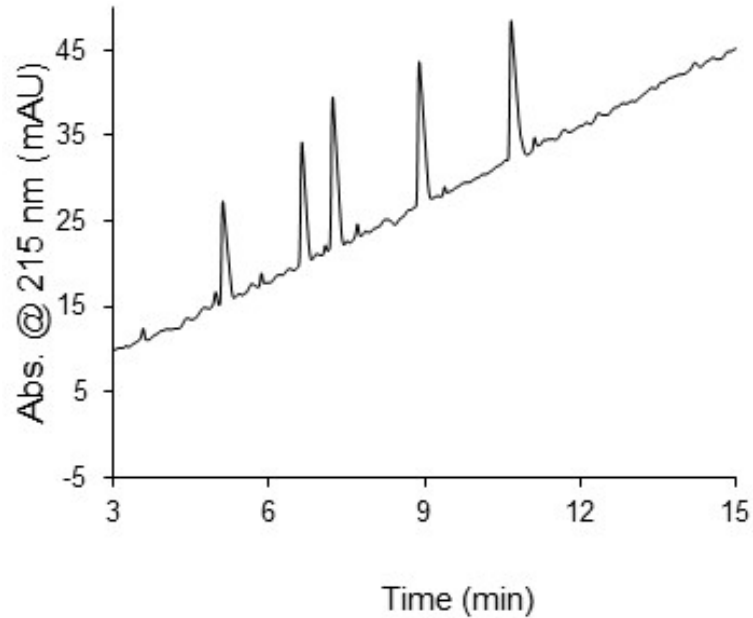
1. Additional step when prepping mobile phase
2. Reduced ionization efficiency with MS detection
3. “Belief” that MS source will need more frequent cleaning



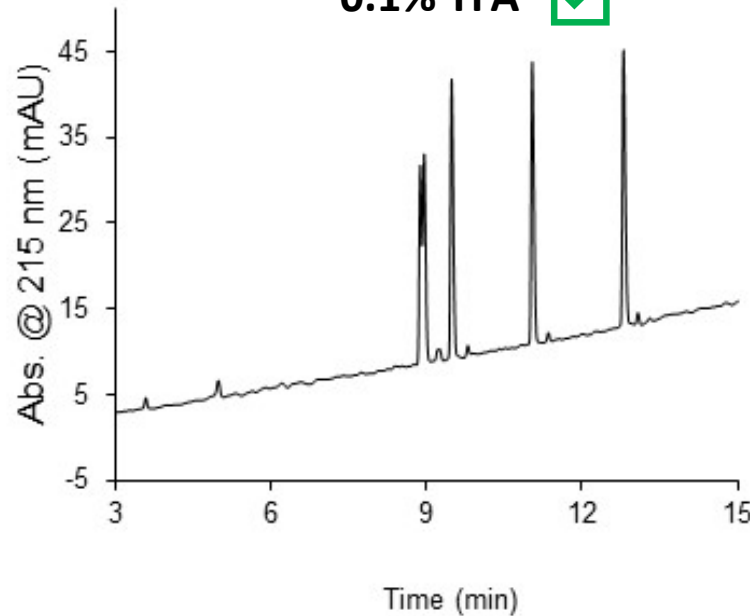
J. Am. Soc. Mass Spectrom. (2017) 28:2384Y2392

2. Use an ion pair agent

0.1% Formic Acid ❌



0.1% TFA ✅



Pros of using 0.1% TFA:

1. Improved peak shape
2. Increased retention

Column: HALO 160 Å ES-C18, 2.7 μ m, 4.6 x 100 mm; Flow rate: 2.0 mL/min; T= 30°C;

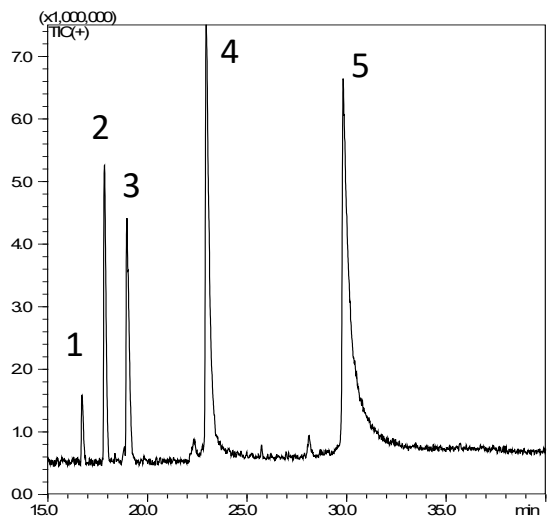
A: Water/acid modifier; B: ACN/0.1% TFA or Formic Acid

Gradient: 1.5% to 26% B in 15 min.; Detection: UV @ 215 nm; Injection: 8 μ L (800 ng) of synthetic peptides S1-S5

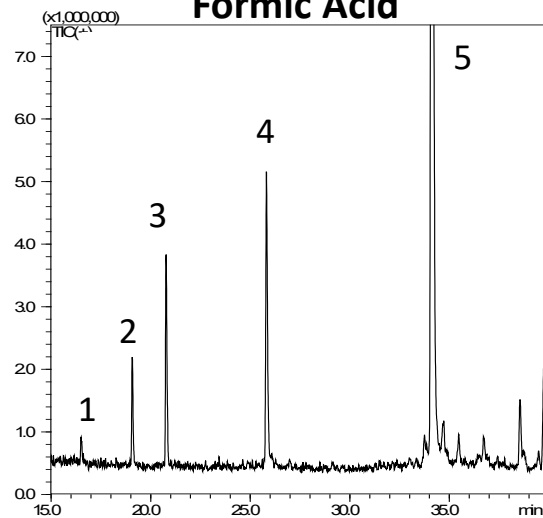
Con of using Ammonium Formate or TFA

Reduced ionization efficiency with MS detection compared to formic acid

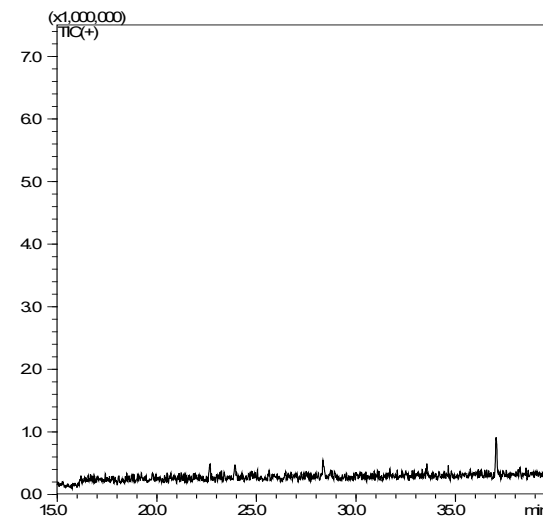
Formic Acid



Ammonium Formate/
Formic Acid



TFA



Peak Identities

1. Leucine Enkephalin
2. Angiotensin I
3. Substance P
4. β -endorphin
5. Mellitin

10FA					
	LeuEnk.	Angio. I	Subst. P	β -Endor.	Mellitin
Ret.Time	16.7	17.9	19.0	23.0	29.8
MIC(+)	9.3E+06	4.4E+07	3.0E+07	7.3E+07	1.0E+08
Relative	100%	100%	100%	100%	100%



10AFFA					
	LeuEnk.	Angio. I	Subst. P	β -Endor.	Mellitin
Ret.Time	16.5	19.1	20.8	25.8	34.1
MIC(+)	1.1E+06	6.1E+06	1.2E+07	2.0E+07	1.6E+08
Relative	12%	14%	41%	28%	150%



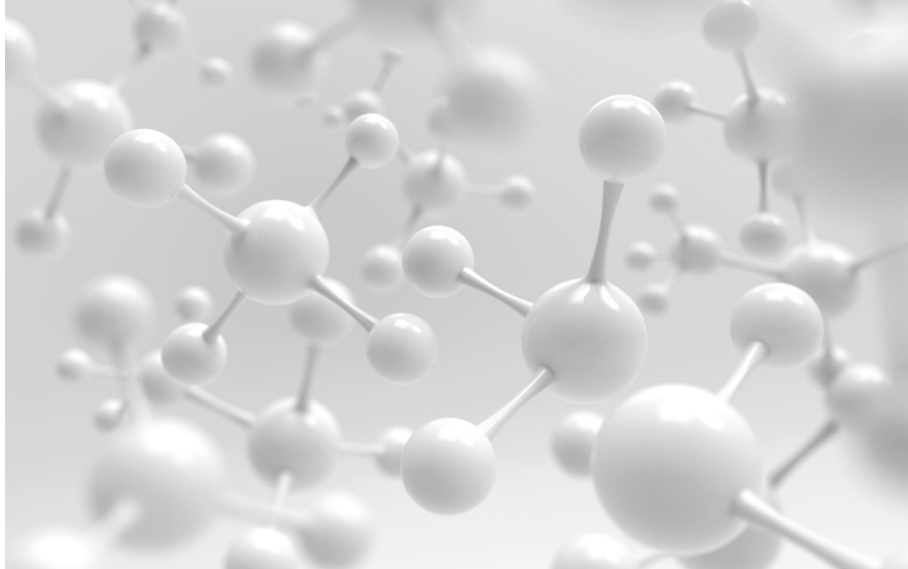
10TFA					
	LeuEnk.	Angio. I	Subst. P	β -Endor.	Mellitin
Ret.Time	19.0	22.5	23.7	28.2	36.9
MIC(+)	1.4E+05	8.8E+05	8.0E+05	1.0E+06	2.9E+06
Relative	1%	2%	3%	1%	3%



10 mM acid in mobile phases; 2.1 x 150 mm HALO 160 Å C18, 0.3 mL/min, 2-47% ACN in 40 min, 60°C, 300-1800 m/z, 4 kV, 0.33 s

3. Use a non-silica based column

- Use a column based on an organic polymer when operating at elevated pH
- Use a column based on a different metal oxide, such as alumina, titania, or zirconia



Pros:

1. Can improve peak shape at elevated pH
2. Non-silica packings will also exhibit the base non-linear isotherm

Cons:

1. Lower back pressure ratings with organic polymer columns
2. Some compounds perform poorly
3. Columns with metal oxides other than silica not widely used
4. Solvent compatibility concerns

4. Raise the pH

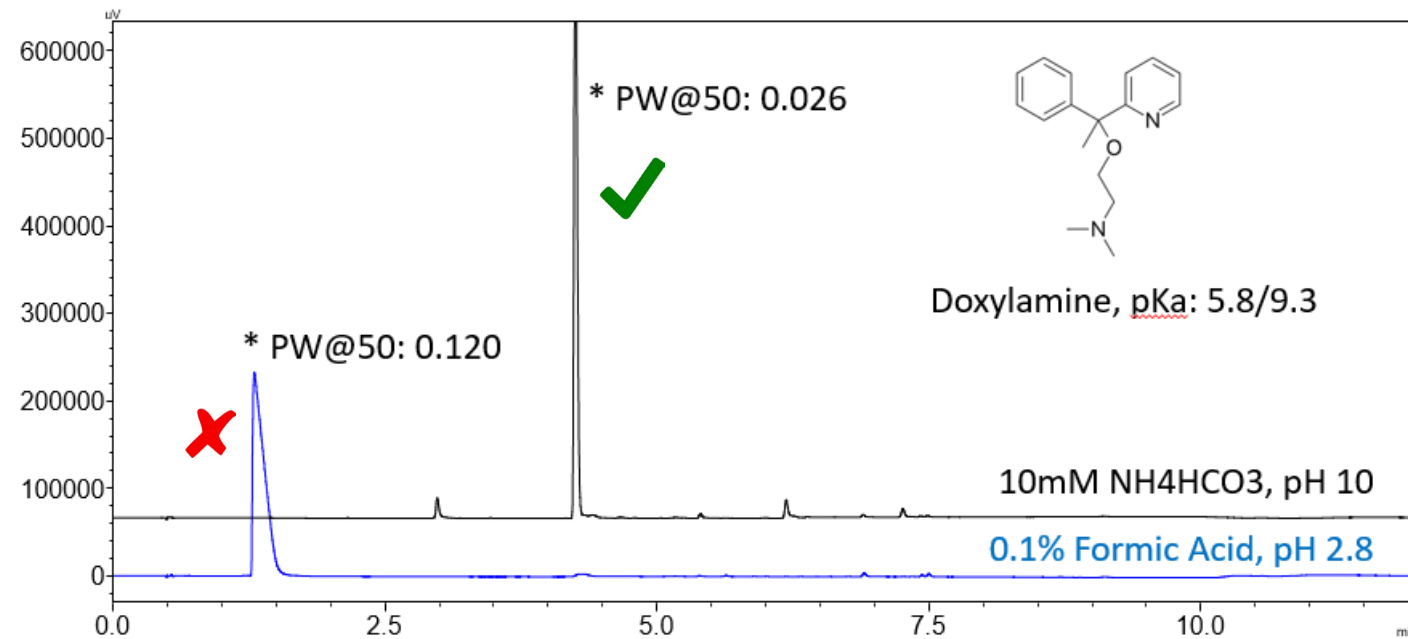
Pros:

1. Improved peak shape (deprotonate)
2. Increased retention bases (reduce charge)
3. "Hybrid silica" increasingly available

Cons:

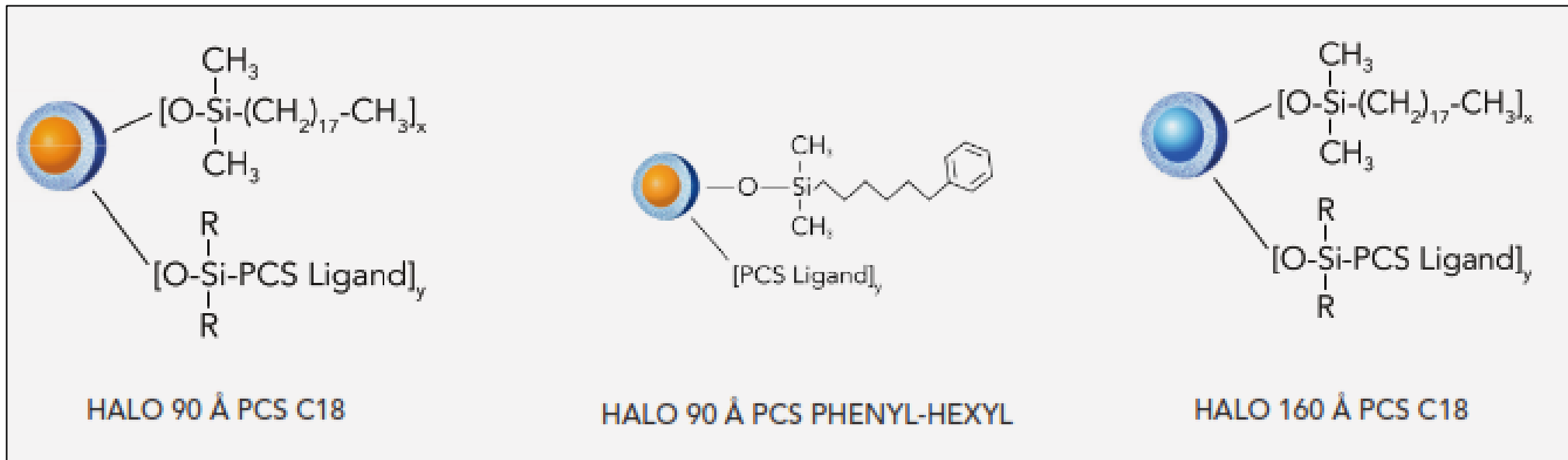
1. pH stability of *traditional silica* materials is poor
2. Sample stability (solubility, oxidation) with alkaline conditions

Testing Conditions:
Mobile Phase: A: Water/ additive
 B: Acetonitrile
Gradient: 10-90%B in 8 min
Instrument: Shimadzu Nexera X2 (103)
Temperature: 30 °C
Flow Rate: 0.4 mL/min.
Column: HALO 120Å ELV C18, 2.7µm, 2.1 x 100mm
Pre-Column Filter: EXP2/ Optimize Technologies



5. Use a different stationary phase

Introducing the HALO[®] PCS Phases: Positively Charged Surface



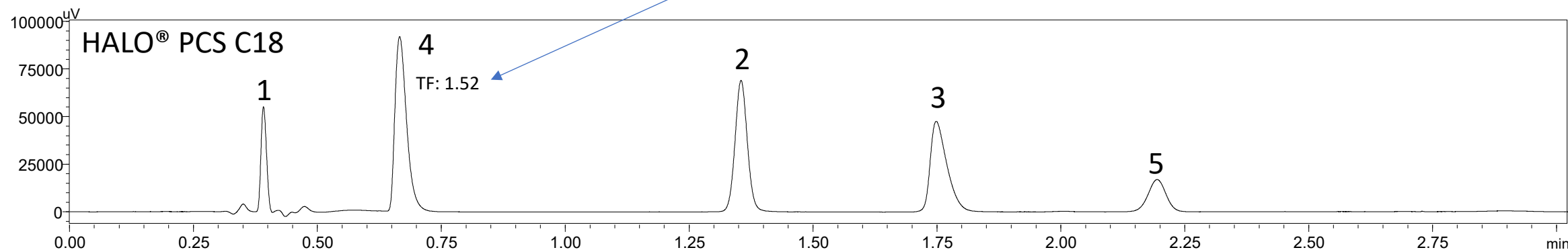
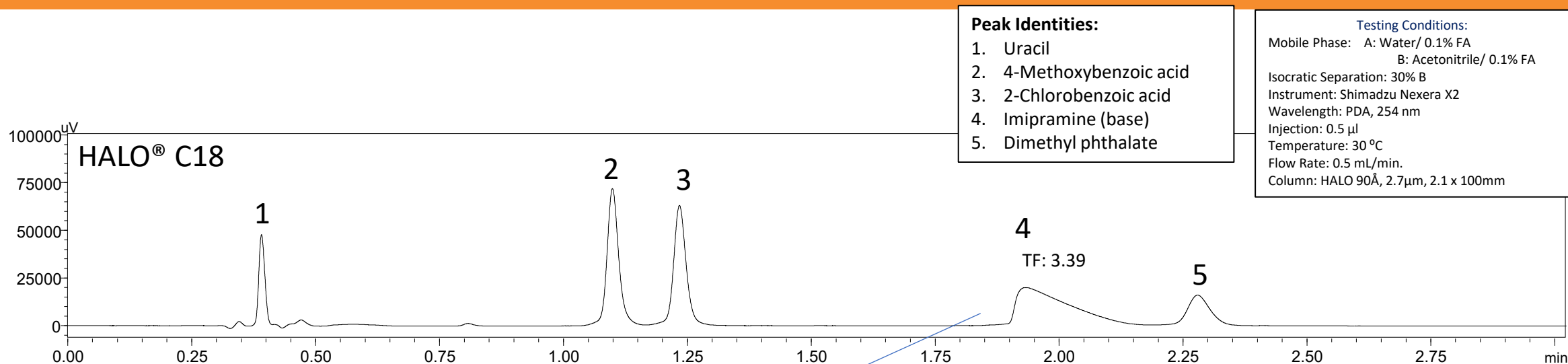
90 Å, 2.7 μm for Small Molecule Analyses

- Excellent peak shape and increased loading capacity for basic compounds
- Alternate L1 selectivity (PCS C18)
- Alternate L11 selectivity (PCS Phenyl-Hexyl)
- Built upon Fused-Core[®] technology for fast, efficient and reliable separations

160 Å, 2.7 μm for Peptide Separations

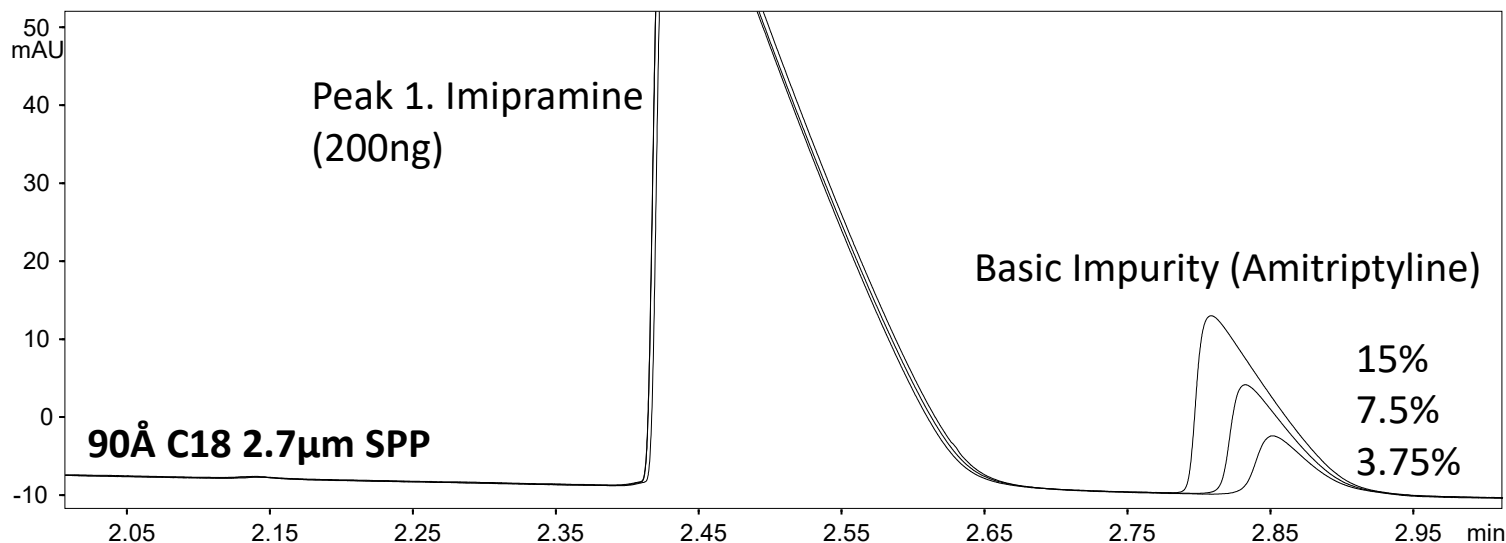
- Significantly improved peak widths and symmetry for basic peptides compared to traditional peptide C18 stationary phases
- Designed for performance with formic acid avoiding LCMS signal suppression from TFA
- Alternate L1 selectivity with optimized pore size for peptide separations

HALO[®] C18 vs. HALO[®] PCS C18

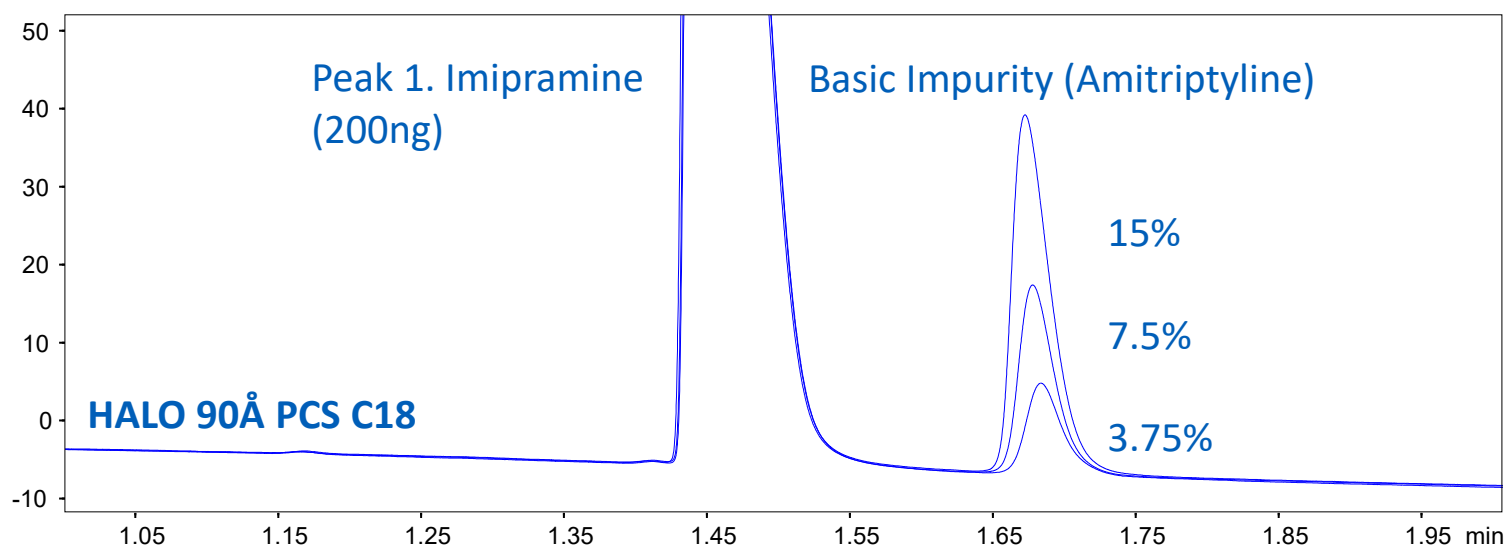


Why do we need a Positively Charged Stationary Phase? (2)

MP A = H₂O + 0.1% formic acid, MP B = ACN + 0.1% formic acid
 25 – 35 %B in 3.0 min, 0.40mL/min, 35C, 1.0μL inj, Abs. 254nm
 2.1x100mm



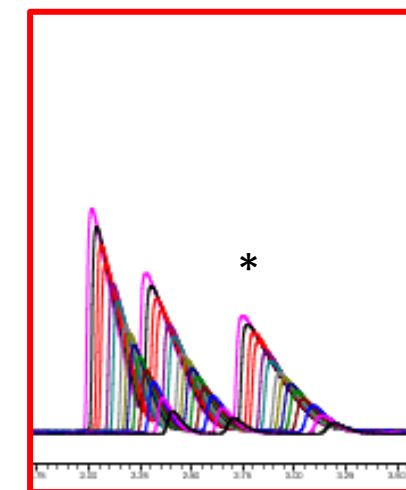
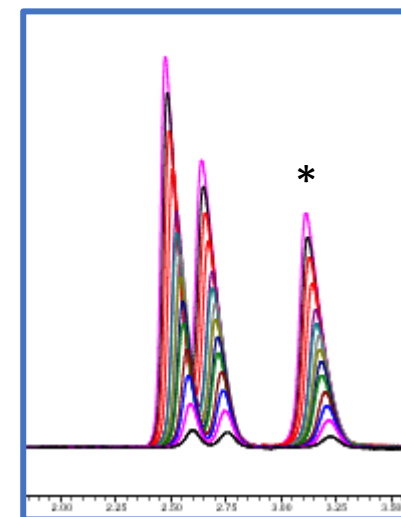
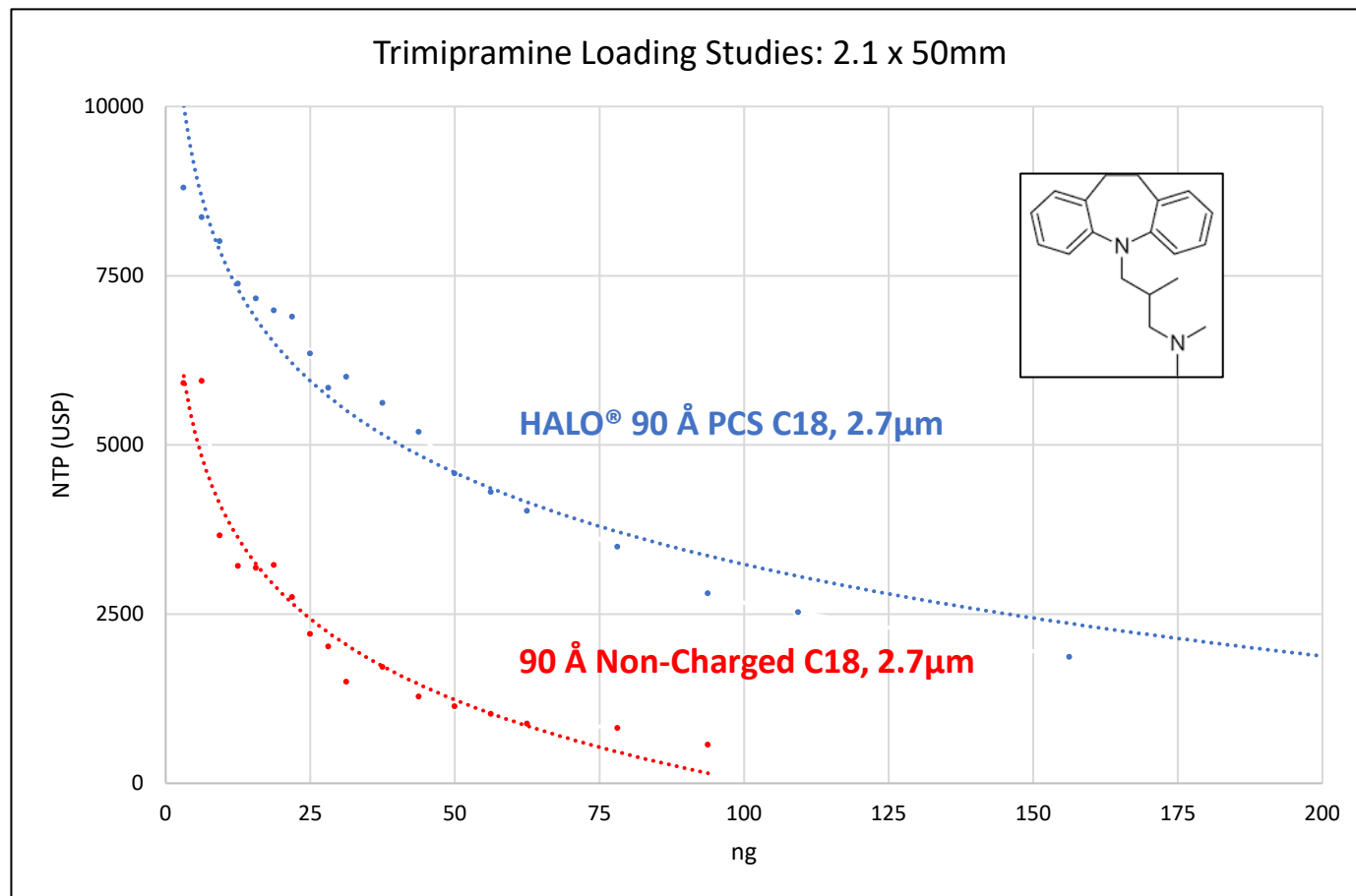
Column	Imipramine (200ng)		
	{Rt} min	TF 5%	{W 50%} min
C18	2.43	5.26	0.108
HALO PCS C18	1.44	2.44	0.044



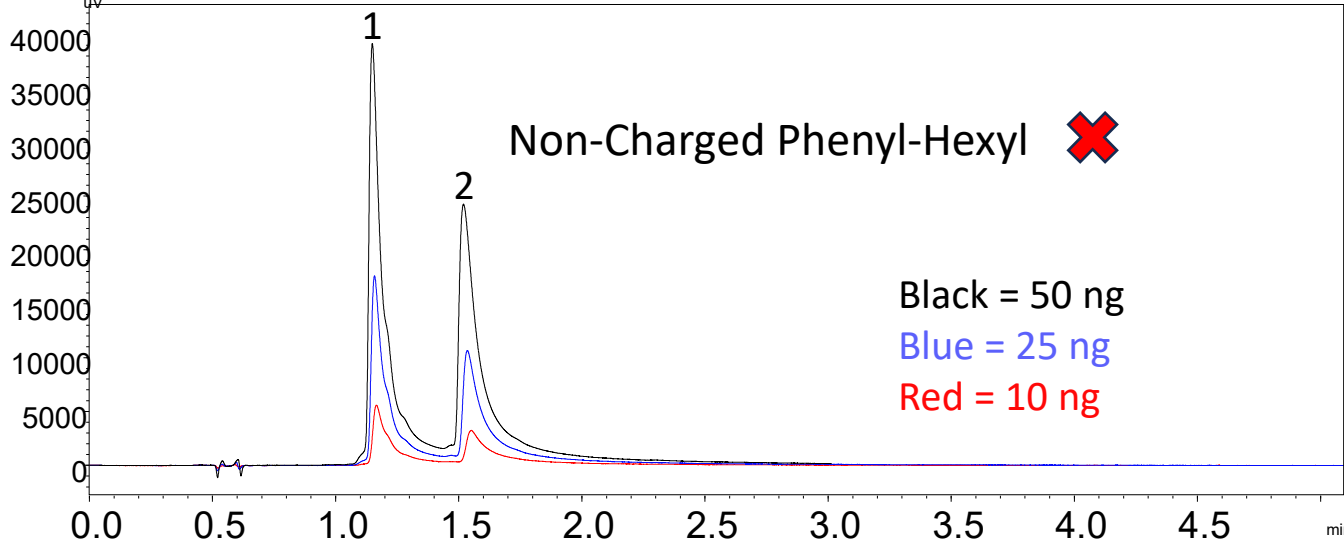
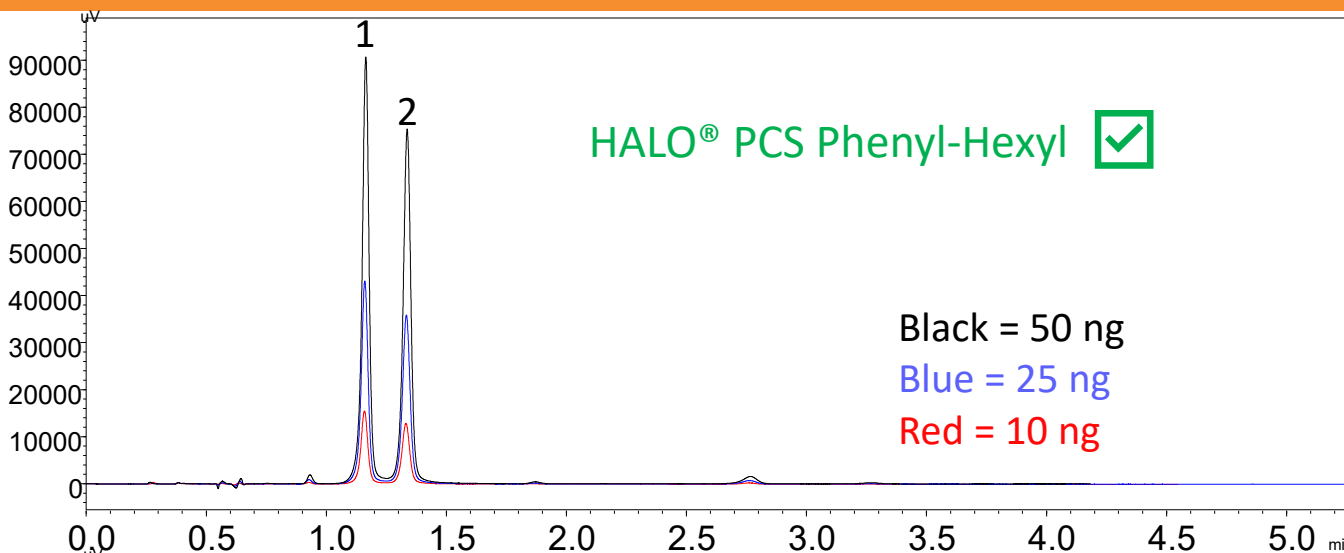
Column	Amitriptyline (30ng, 15%)			
	{Rt} min	TF 5%	{W 50%} min	Rs (USP)
C18	2.81	3.15	0.059	2.46
HALO PCS C18	1.67	1.60	0.029	3.57

HALO® PCS Loading Capacity

0.1% Formic Acid

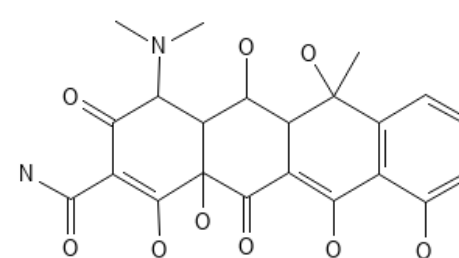


HALO[®] PCS Phenyl-Hexyl vs. standard (uncharged) HALO[®] Phenyl-Hexyl

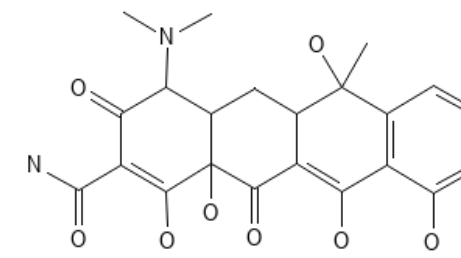


Testing Conditions:
Column: 2.7 μ m, 2.1 x 100 mm phase as labeled
Mobile Phase A: Water/ 0.1% Formic Acid
B: Acetonitrile/ 0.1% Formic Acid
Isocratic: 12% B HALO[®] PCS Phenyl-Hexyl
18% B HALO[®] Phenyl-Hexyl
Flow Rate: 0.4 mL/min
Instrument: Nexera
Injection: 0.2, 0.5, 1.0 μ L (10,25,50 ng)
Temperature: 35°C

- Sharp, symmetrical peaks are observed from 10-50 ng injected on the HALO[®] PCS Phenyl-Hexyl column
- Peak widths are 50% smaller with HALO[®] PCS Phenyl-Hexyl



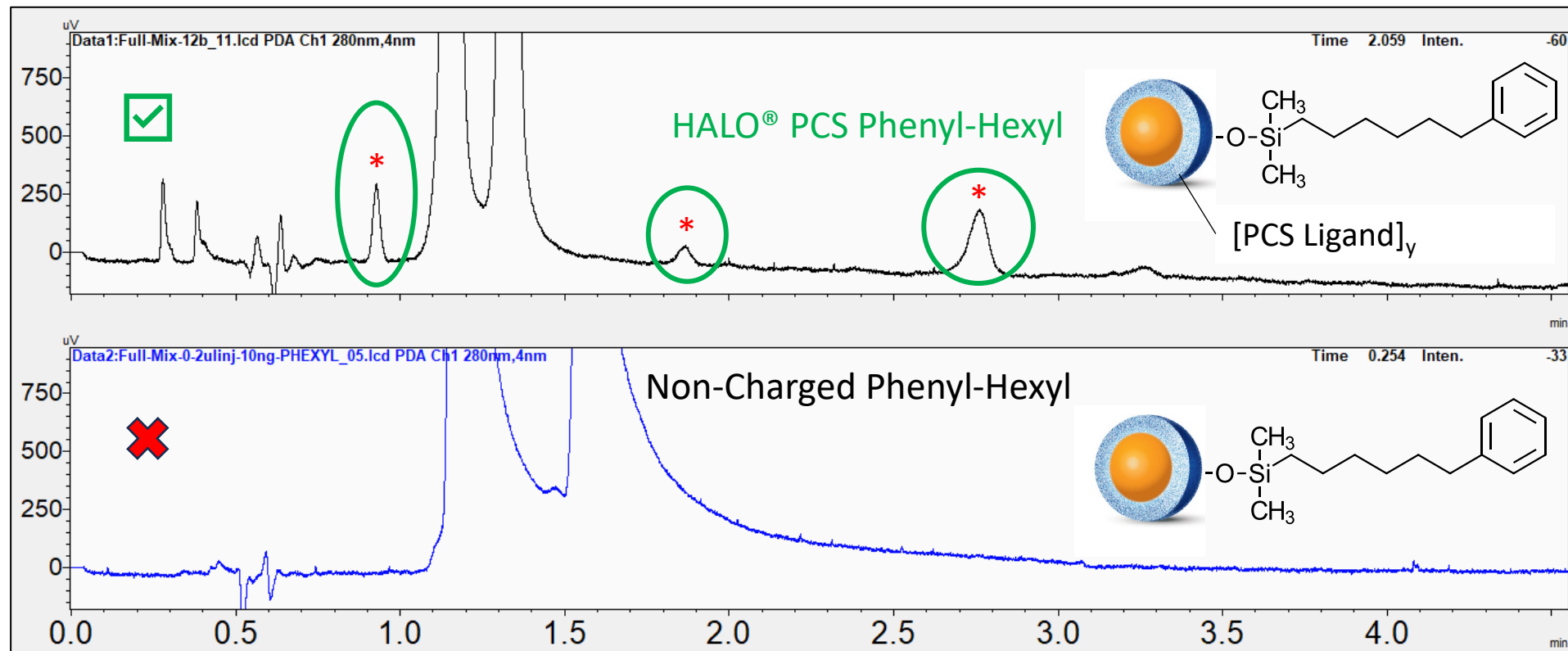
1. Oxytetracycline



2. Tetracycline

A Closer Look at the Baseline

At 10 ng of each tetracycline injected on column, the sharper, narrower peaks enable small impurities to be detected on the HALO[®] PCS Phenyl-Hexyl column.



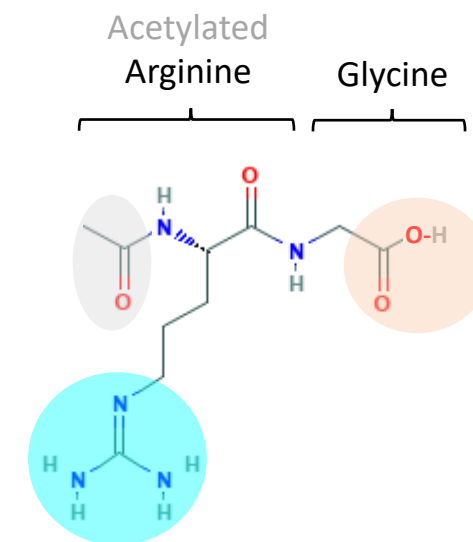


**HALO 160 Å PCS C18
Peptide**

Why HALO 160Å PCS C18?

HALO®

- Synthetic peptide analysis and protein digest analysis (e.g. via trypsin) share chromatographic challenges that are like the challenges of small-molecule basic analyte separations.
- The use of weakly acid mobile phases (e.g. LCMS-friendly formic acid) with 160Å PCS C18 can be employed for efficient separations of amphoteric (possessing basic & acidic characteristics) compounds such as peptides.
- Improvements in peptide peak shape were observed (versus 160Å C18).

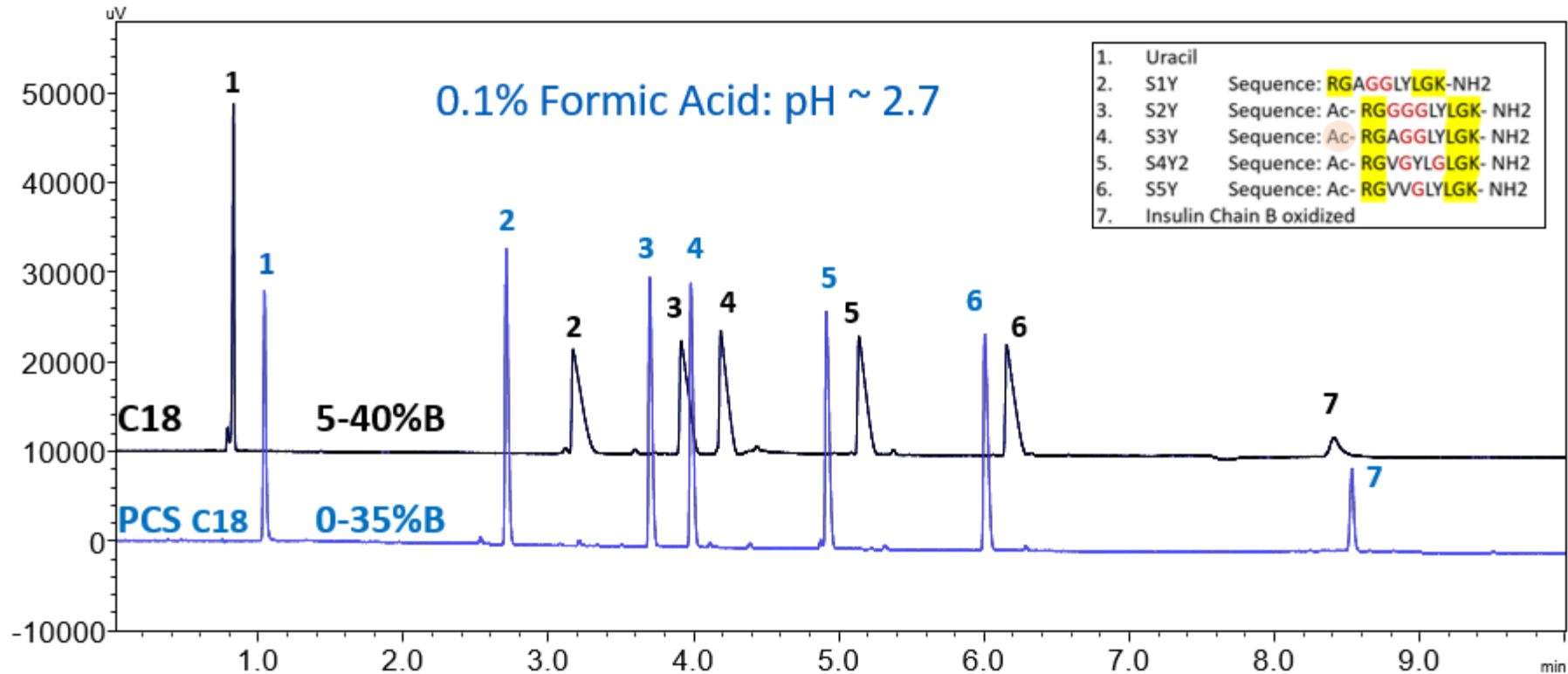


SPECIFICATIONS

Ligand: dimethyloctadecylsilane	Carbon Load 90 Å: 7.5%	Endcapped: Yes both 90 and 160 Å
Particle Size: 2.7 µm	Carbon Load 160 Å: 5.09%	Low pH Limit /Max T: 2/60 °C
Pore Size: 90 and 160 Å	Surface Area 90 Å: 135 m ² /g	High pH Limit/Max T: 7/40 °C
USP Designation: L1	Surface Area 160 Å: 90 m ² /g	

Peptide Analysis: 160Å PCS C18 vs 160Å C18

5µL Peptide Standard Analysis on 160Å 4.6x100mm 2.7µm

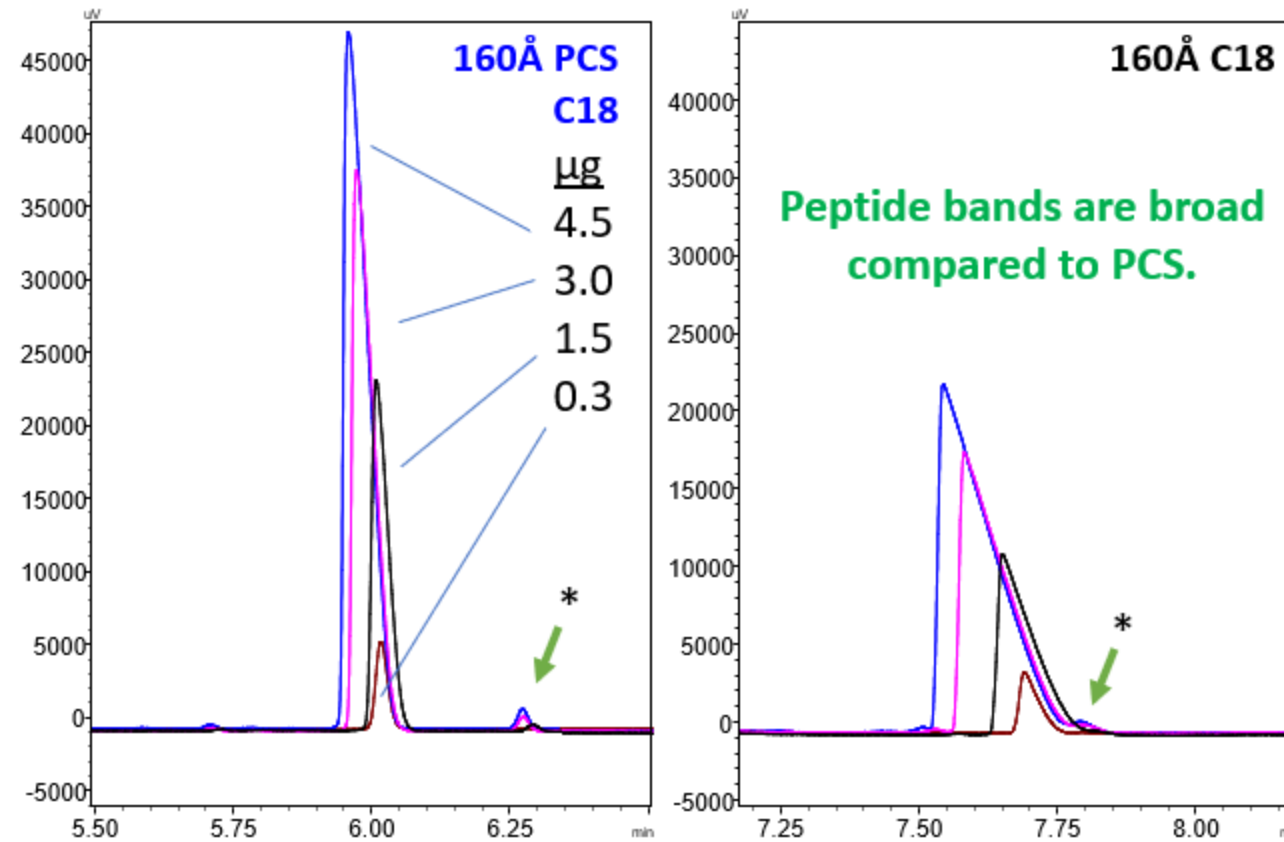


290 bar 1.5mL/min, 0-35%B in 10min, 30°C; 280nm A=0.1%Formic; B=0.1%Formic in ACN

Peptide Load Tolerance

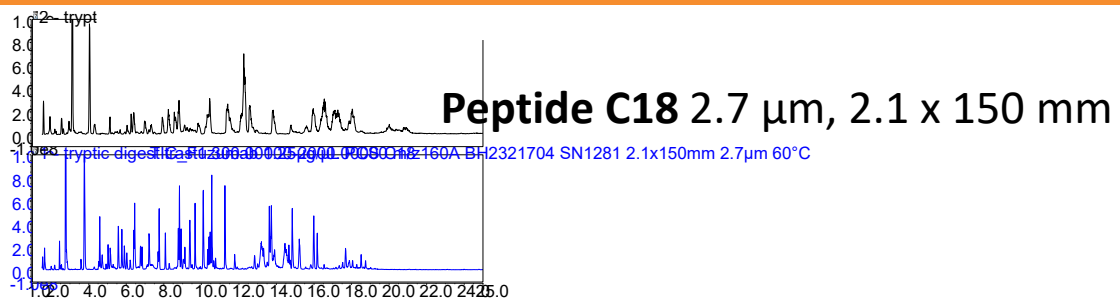
1, 5, 10, and 15 μ L injections of synthetic peptides (0.3 μ g/ μ L peptides) on 4.6x100mm

Ac-RGVVGLYLGK-NH2 (1102 Da)



1.5mL/min, 0-35%B in 10min, 30°C; 280nm A=0.1%Formic; B=0.1%Formic in ACN

Trastuzumab Tryptic Digest: Higher Peak Capacity with HALO[®] PCS C18 Peptide



$n_{PC} = 170$

0.4mL/min; 60°C; 3-50% in 30 min;
2 μg tryptic digest
A=0.1% Formic Acid in H₂O
B=0.1% Formic Acid in ACN
Shimadzu NexeraX2 -> diverter valve ->
QExactive HF (res=240,000)

HALO[®] PCS C18 Peptide 2.7 μm, 2.1 x 150 mm

$n_{PC} = 488$

MarvelXACT Post-Column Plumbing:

1. 50 μm x 350 mm from column to diverter valve
2. 50 μm x 350 mm from diverter valve to union
3. 50 μm x 150 mm from grounding union to HESI II

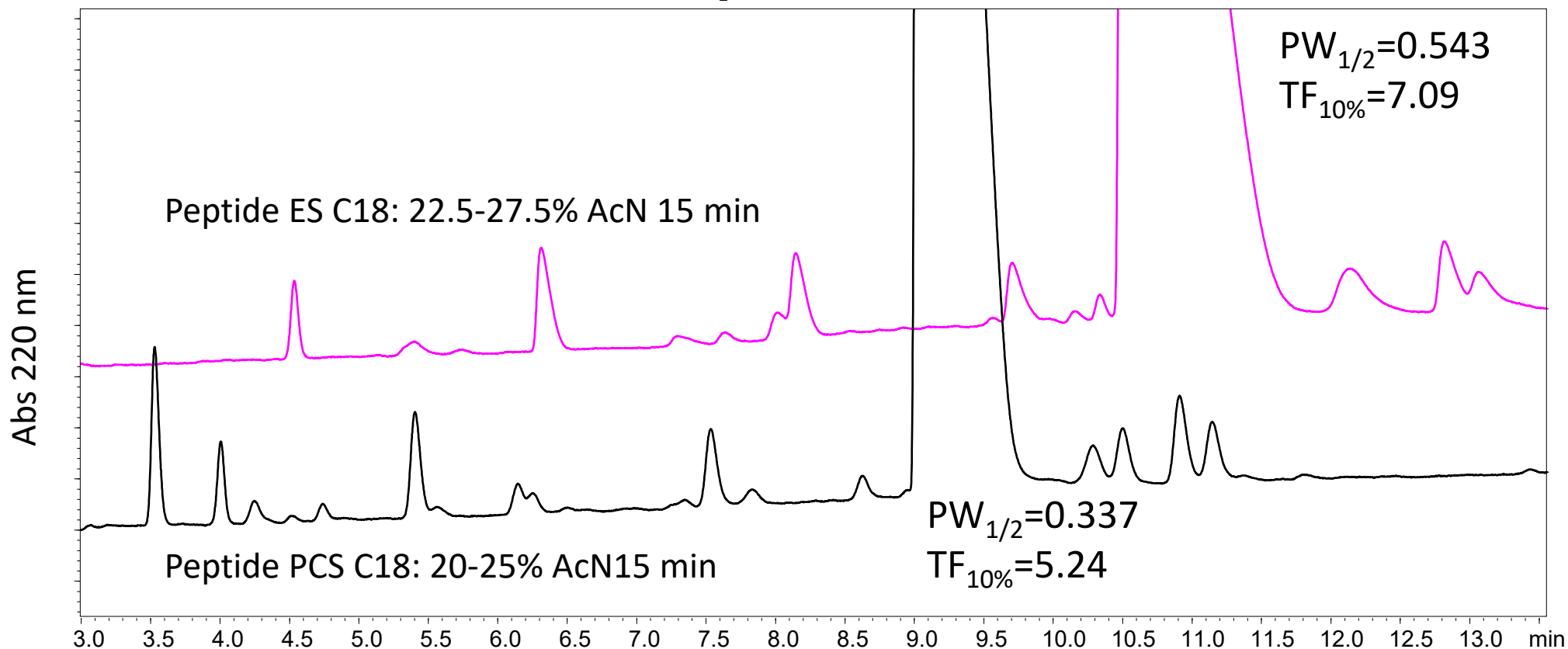
n_{PC} based on ID peptides

rGlucagon Deamidation with Formic Acid

Conditions: HALO[®] PCS C18 Peptide



0.4mL/min; 60°C; 2.1 x 150 mm; 3 ug injected in 10 mM HCl
A=0.1% Formic Acid in H₂O; B=0.1% Formic Acid in AcN



- Surface modified packing exhibits improved PW, tailing and resolution in this Formic Acid separation.

Technical Resources

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



BIOPHARMACEUTICALS

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

TEST CONDITIONS:
 Column: HALO 160 Å ES-C18, 2.7 µm, 1.5 x 150 mm
 Part Number: 92120-702
 Column: HALO 160 Å ES-C18, 2.7 µm, 2.1 x 150 mm
 Mobile Phase A: Water/0.1% DFA
 Mobile Phase B: Acetonitrile/0.1% DFA
 Gradient: Time (min) %B
 0.0 0.0
 40.0 50
 0.4 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: 40 °C
 Injection Volume: 2 µL of 1.25 mg/mL trastuzumab tryptic digest
 Sample Solution: 1.5 M guanidine HCl/0.5% formic acid
 LC System: Shimadzu Nexera X2
 MS System: ThermoFisher Q Exactive

MS CONDITIONS:
 Spray Voltage (kV): 3.8
 Capillary temperature: 320 °C
 Sheath gas: 35
 Aux gas: 10
 RP flow: 50

A separation of Trastuzumab 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.

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Webinar: 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

Molecular Probes to Characterize HPLC Column Performance

Richard A. Henry, Stephanie Schuster, Connor McKelvie and William Johnson
 Independent Consultant, State College, PA 16802
 Advanced Materials Technology Inc., Wilmington, DE

Presented at Pittcon 2019 Poster 1340-2

Use of Chemical Probes in HPLC

Hydrophobic Subtraction Model

Impact of Phase Polarity for OH Probes

Impact of Hydrophobic Plus Strong Hydrogen Bonding

RP-AMs Show Different Selectivity in MeOH for OH Probe

Probe Conclusions/Acknowledgements

Enhanced Selectivity Values for OH Probe Mix in MeOH Mobile Phases

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UNDER THE HALO SMALL MOLECULE CURRENT ISSUE

Extracolumn Dispersion Part 2: IMPACT TO ISOCRATIC AND GRADIENT UHPLC METHODS

In part 1 of this series, extracolumn dispersion (ECD) was introduced, its importance was discussed, and ways to measure and reduce it were described. In part 2, the impact of ECD on isocratic and gradient methods will be investigated with the use of dispersion plots so that one can clearly see the contributions of each term to the total system dispersion. The plots are generated from the **Van Deemter Dispersion Calculator**, referenced in the recent series of articles published in CMC North America 11. Equation 1 shows all the contributors to ECD:

$$\sigma_{total}^2 = \sigma_{column}^2 + \sigma_{injection}^2 + \sigma_{detector}^2 + \sigma_{tubing}^2 + \sigma_{mixing}^2 + \sigma_{flow}^2 + \sigma_{diffusion}^2$$

The Dispersion Calculator includes input fields for all of these terms, the method conditions, and UHPLC column details. Readers are encouraged to try the Dispersion Calculator for themselves so they can compare the "before" and "after" for whatever method conditions and instrument configurations are of interest. In all cases, the objective is to maximize the pie slice that corresponds to the column (yellow) so that the true efficiency of the column is observed or the maximum resolution is achieved.

ISOCRATIC UHPLC METHODS

Figure 1. Comparison of peak dispersion using an isocratic method for standard comparison in isocratic and gradient for a UHPLC.

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

HALO TECHNICAL REPORT: AMT-TR02001

TITLE: HIGH RESOLUTION LCMS SEPARATIONS OF EDIBLE OILS

MARKET SEGMENT: FOOD / BEVERAGE

AUTHORS:
 Andrew Herron, PhD, Application Scientist

ABSTRACT
 Edible oils, extracted from both plants and animal sources, have evolved into a multi-billion dollar industry and are being used in more applications every year. In 2018 over 382.05 million metric tons of edible oils were consumed and utilized worldwide. Products such as infant, pharmaceutical formulation applications, soaps, shampoos, and household cleaners are among a few. In recent years, the food industry has sought to integrate more oil 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 peak shape offers superior selectivity and higher shape selectivity. This, enabling 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 composition. 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 (Oliviero 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 (LCFAs) and water, as well as DAGs and TAGs. In the food industry, the analysis of TAGs in oils is a critical step to determine nutritional value, for example amount of unsaturations in the oil, as well as selectivity for non-ferrous-based applications. C18 columns, the most

(Ramsel et al., 1998; Abul 2000; Sander and Vias 1992). The structure and length 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 Vias, 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, non-ferrous-based applications, C18 columns, the most

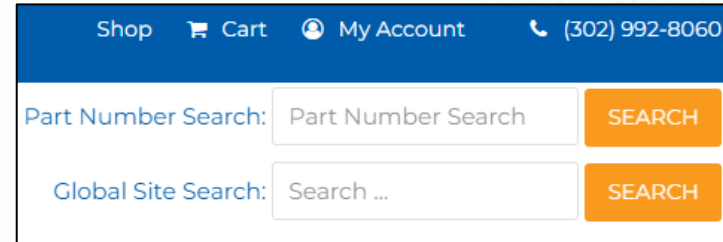
Questions?

Technical and Marketing Materials:

- www.halocolumns.com

Technical Support:

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