HPLC Approaches to Improve Peak Shape for Basic Analytes

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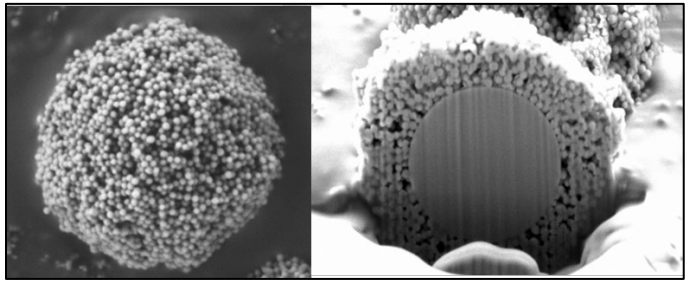
Outline

Advanced Materials Technology

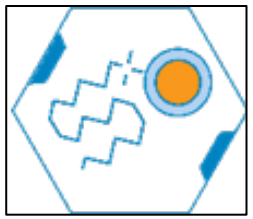
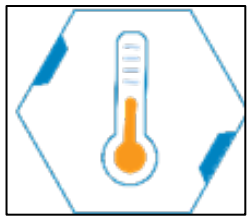
- Benefit of Fused-Core® Technology



Dealing with Basic Analytes

- Approaches to Improve Peak Tailing/ Retention
- HALO® Elevate
- HALO® PCS : Positively Charged Surface Chemistries

Applications

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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®*

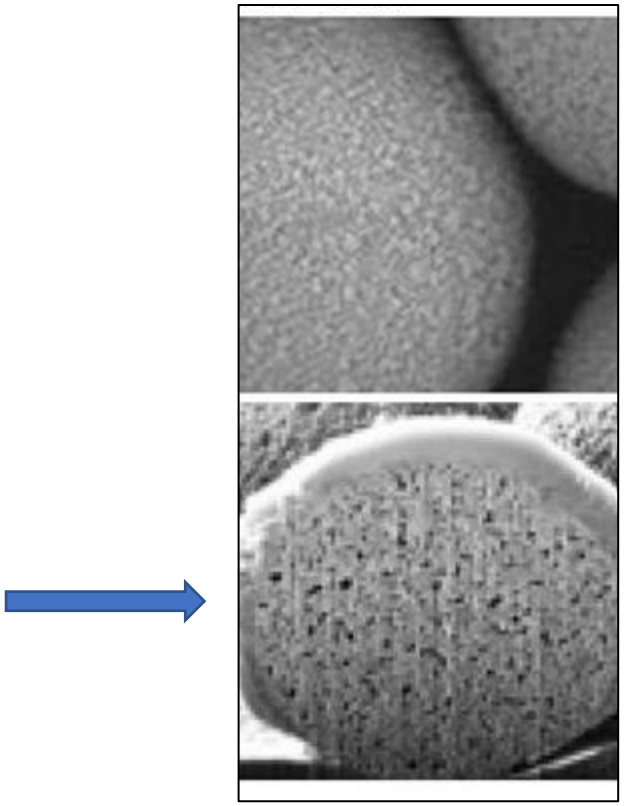
Expanded IP portfolio to include materials, products and technologies

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

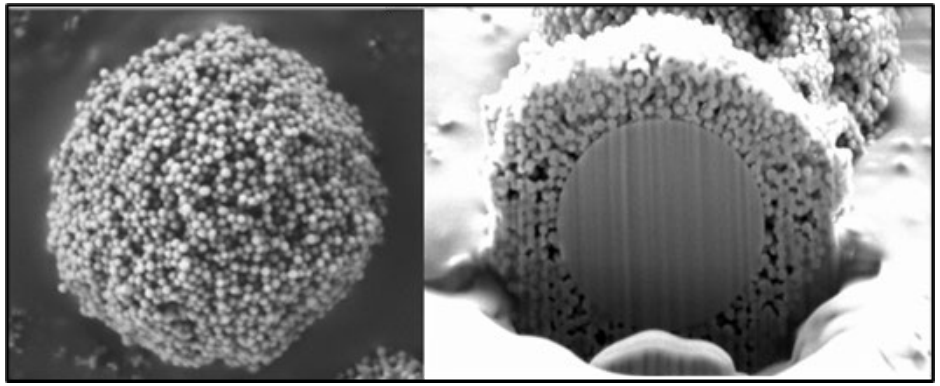
AMT is a company of innovators and continues to grow and deliver enabling materials to market. Our incredible team is our greatest resource.

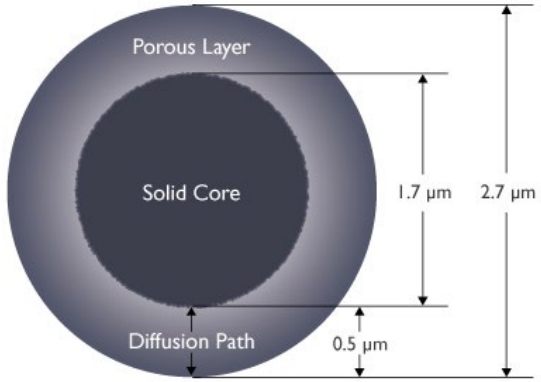
SPP Technology



Fully Porous Particle (FPP)

HALO 1000 Å, 2.7 µm





Superficially Porous Particle (SPP)

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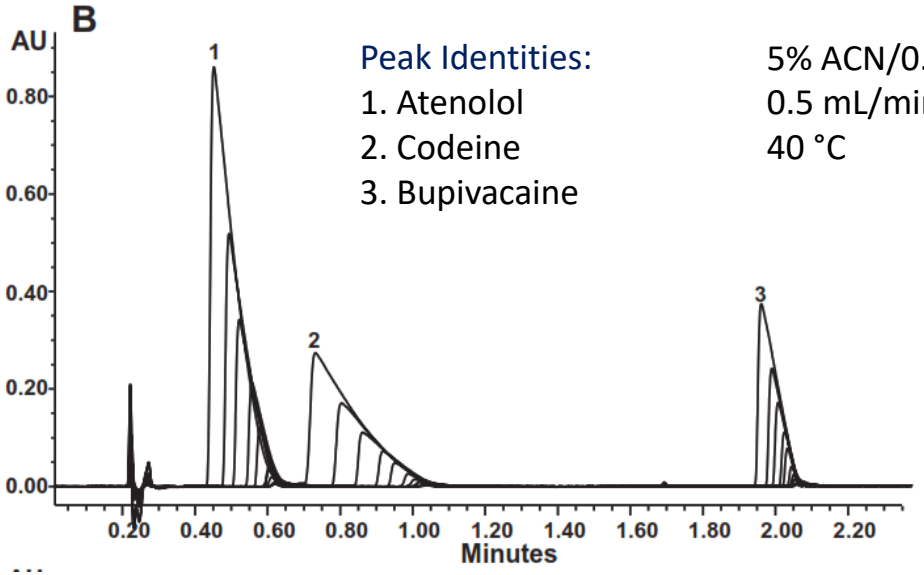
Tailing Peak Shape of Basic Compounds

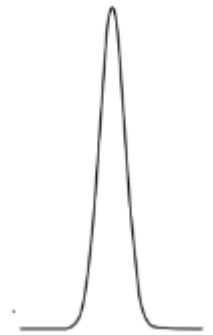
- When basic compounds are run at low pH, they gain a proton and become positively charged.
- At high sample loads, the tailing will become significant, peak shape will suffer, and retention decreases - the “non-linear isotherm”.
- This is not a simple “silanol” concern.



Peak Identities:
1. Atenolol
2. Codeine
3. Bupivacaine

5% ACN/0.1% formic acid for 1 min, then 5-95% ACN/0.1% formic acid in 3 min
0.5 mL/min; 230 nm
40 °C

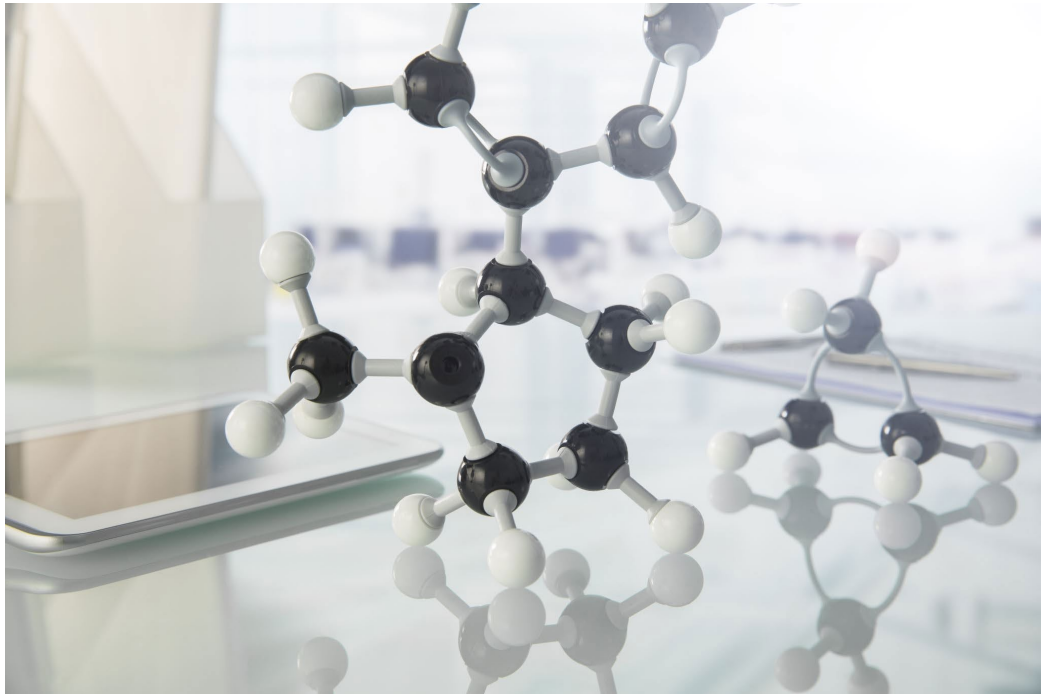
J. Chromatogr. A 1228 (2012) 221-231



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

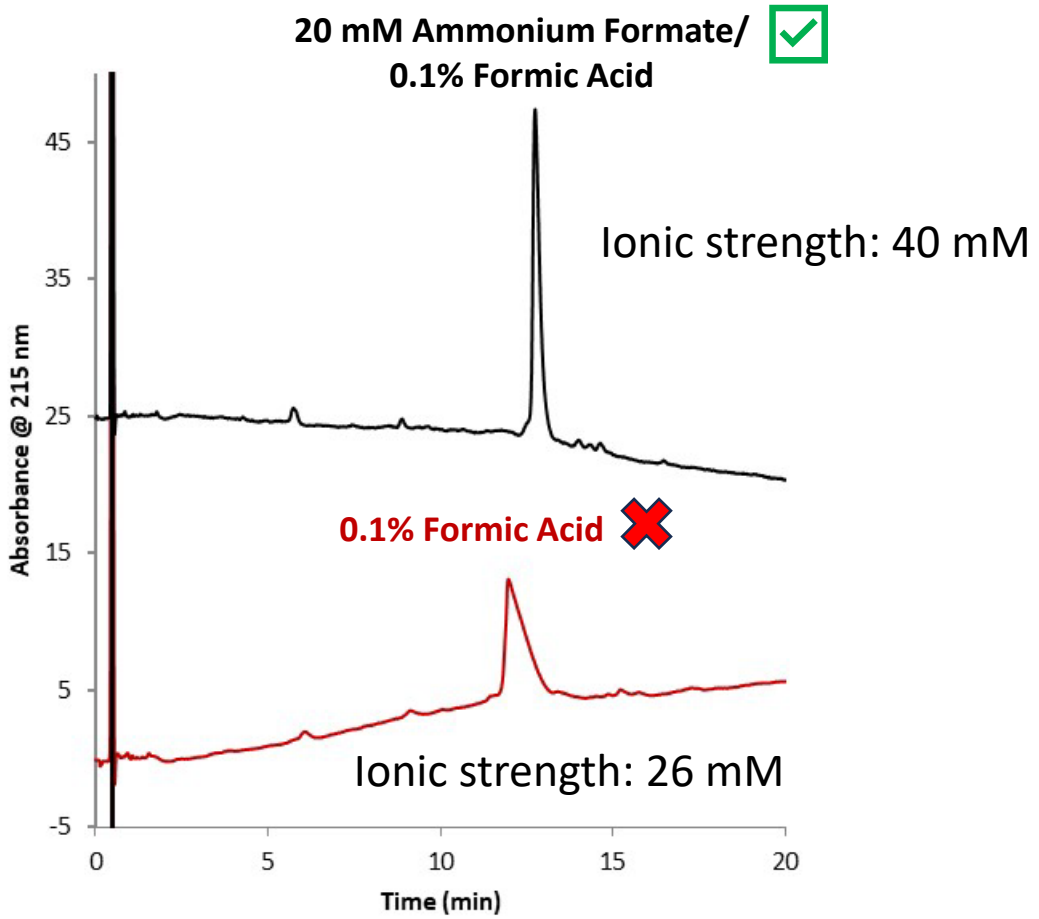
Solutions to Improve Tailed Peak Shape of Basic Compounds

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. Increase 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



HALO 160Å ES-C18, 2.1 x 100 mm; Flow rate: 0.5 mL/min; T= 60°C; A: Water/acid modifier; B: ACN/0.1% Formic Acid; Gradient: 20 mM Ammonium Formate/0.1% Formic Acid : 24% to 27% B in 20 min.; 0.1% Formic Acid : 20% to 24% B in 20 min

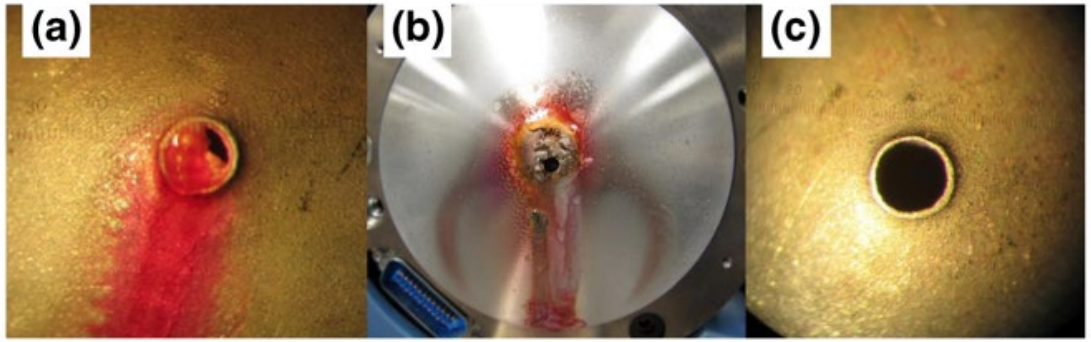
Using 20 mM Ammonium Formate/0.1% Formic Acid:

Pros:

1. Improved peak shape
2. Increased retention

Cons:

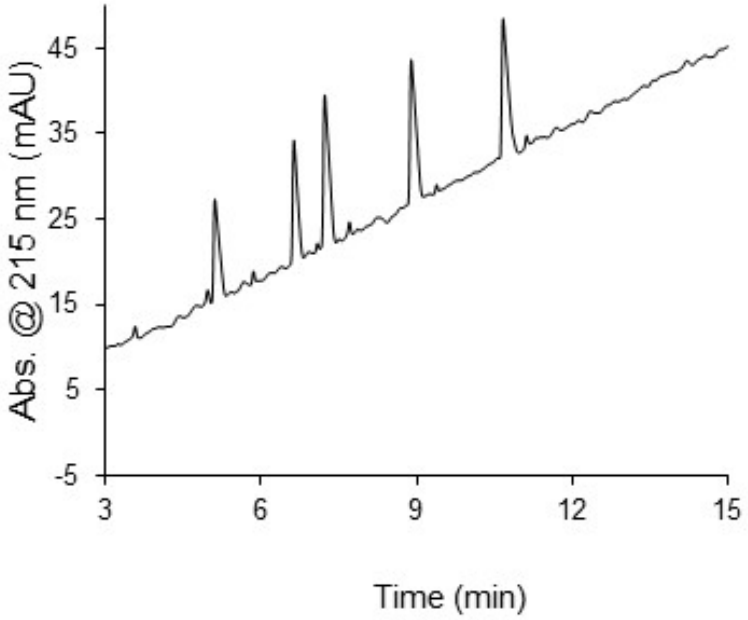
1. Additional step when prepping mobile phase
2. Reduced ionization efficiency with MS detection
3. "Belief" that MS source will need more frequent cleaning



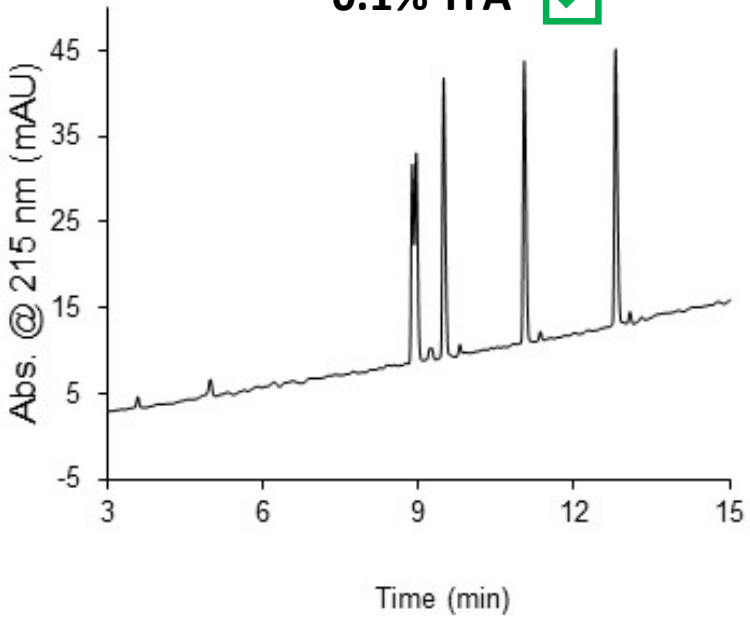
J. Am. Soc. Mass Spectrom. (2017) 28:2384Y2392

2. Use an ion pair agent

0.1% Formic Acid ✖



0.1% TFA ☑



Pros of using 0.1% TFA:

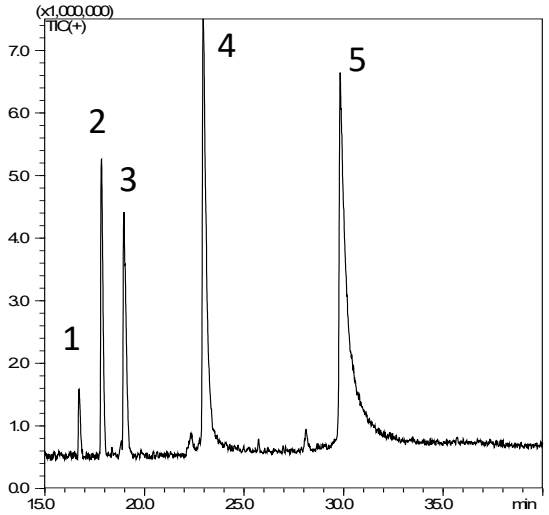
1. Improved peak shape
2. Increased retention

Column: HALO 160 Å ES-C18, 2.7 µm, 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 µL (800 ng) of synthetic peptides S1-S5

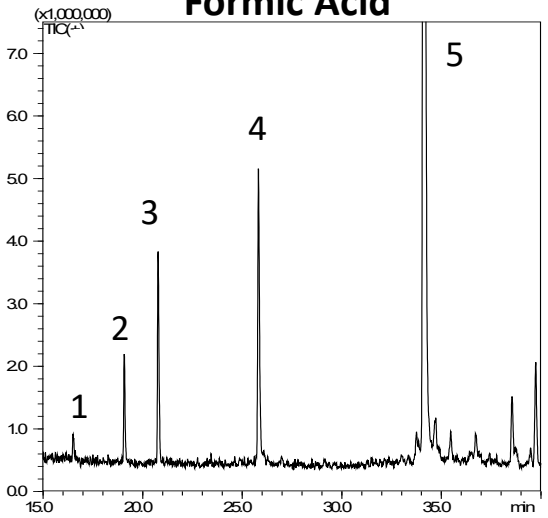
Con of using Ammonium Formate or TFA

Reduced ionization efficiency with MS detection compared to formic acid

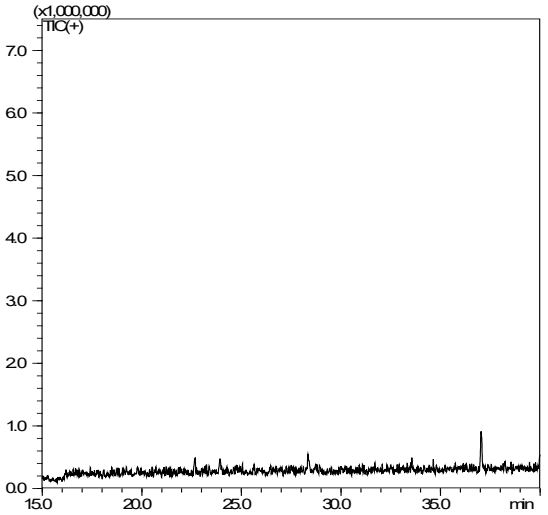
Formic Acid



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%

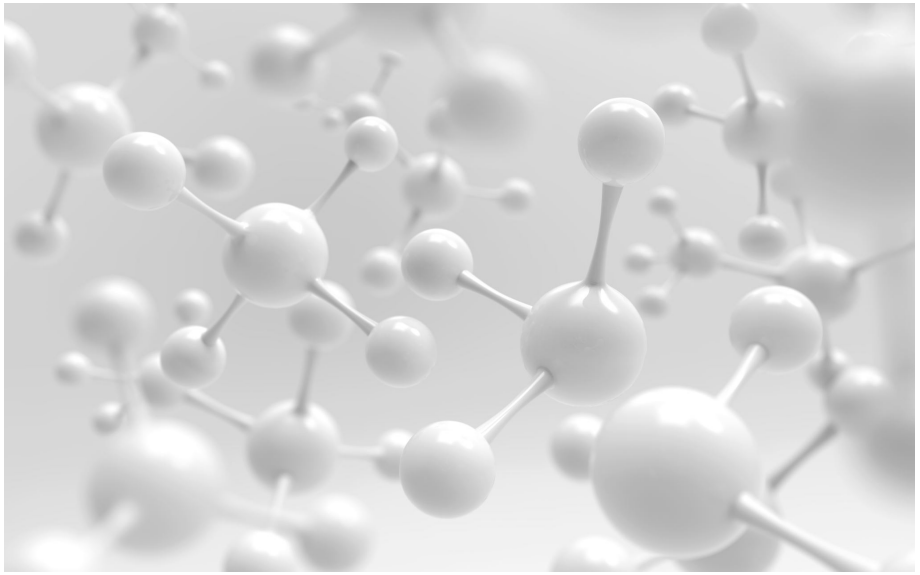
10AFFA	LeuEnk.	Angio. I	Subst. P	β-Endor.	Mellitin
	16.5	19.1	20.8	25.8	34.1
	1.1E+06	6.1E+06	1.2E+07	2.0E+07	1.6E+08
	12%	14%	41%	28%	150%

10TFA	LeuEnk.	Angio. I	Subst. P	β-Endor.	Mellitin
	19.0	22.5	23.7	28.2	36.9
	1.4E+05	8.8E+05	8.0E+05	1.0E+06	2.9E+06
	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

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4. Raise the pH

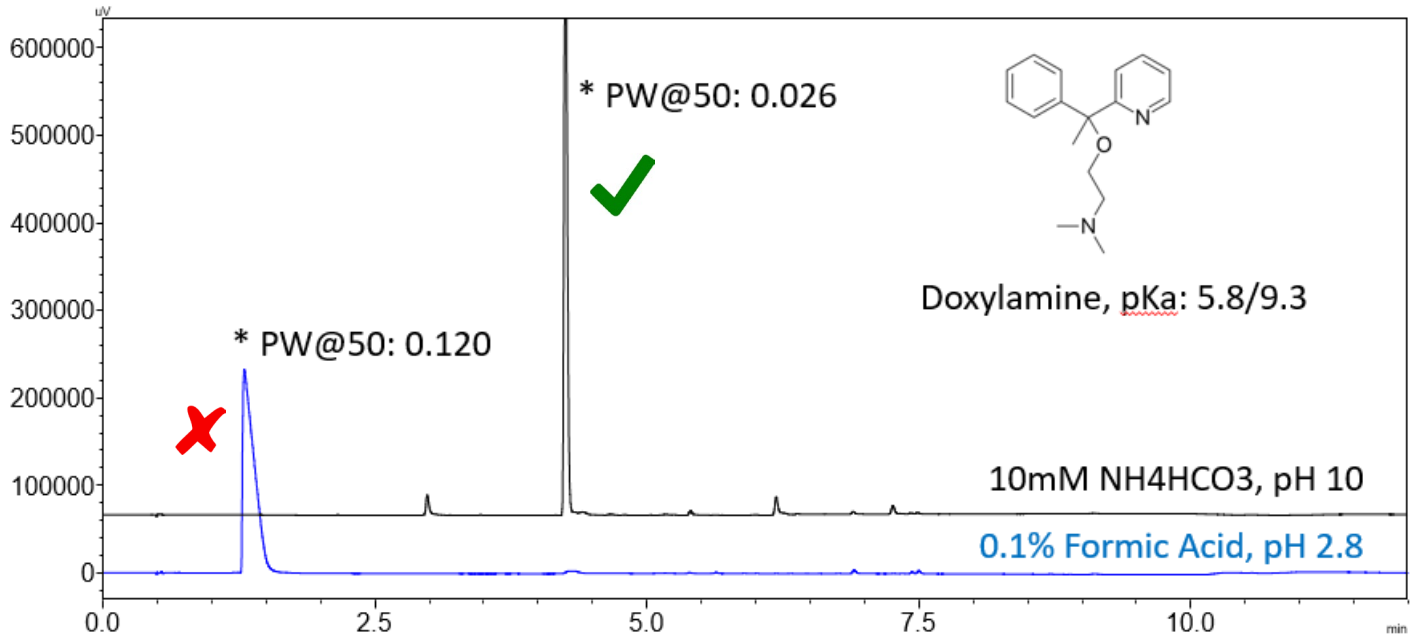
Pros:

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

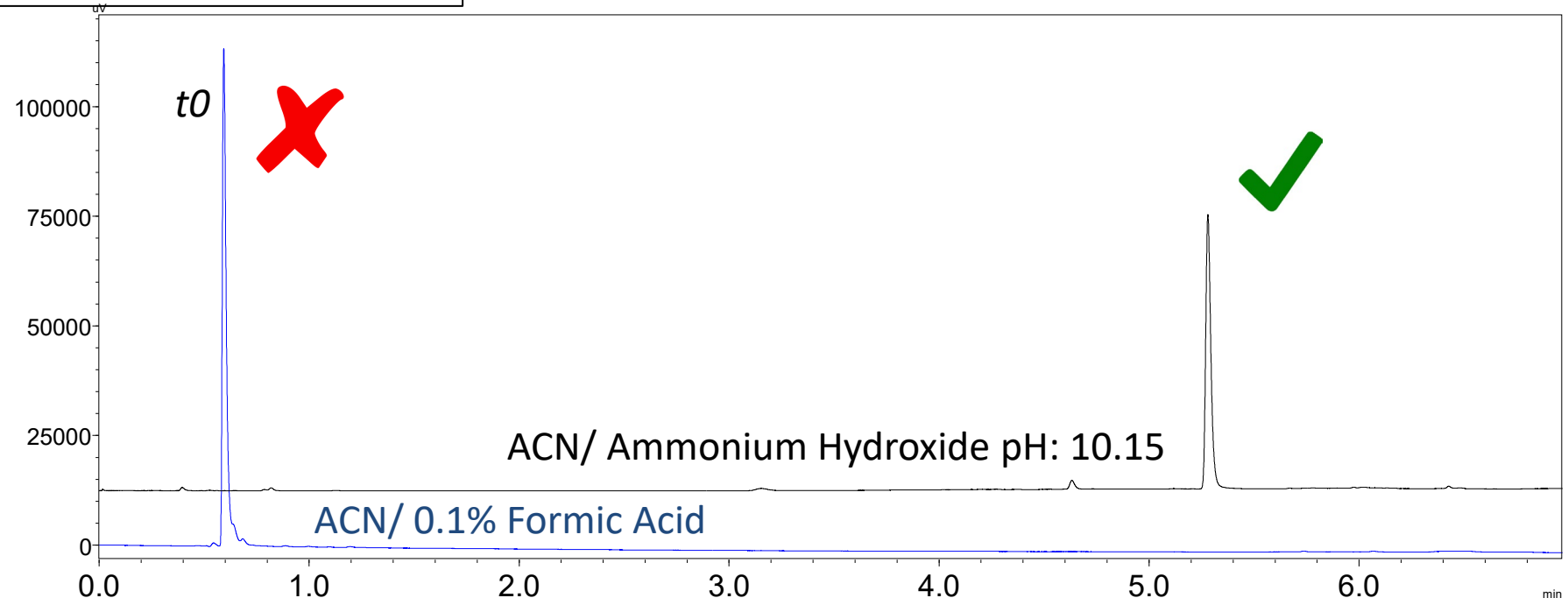
Testing Conditions:
Mobile Phase: A: Water/ additive
B: Acetonitrile
Gradient: 10-90%B in 8 min
Instrument: Shimadzu Nexera X2 (103)
Temperature: 30 °C
Flow Rate: 0.4 mL/min.
Column: HALO 120Å ELV C18, 2.7µm, 2.1 x 100mm
Pre-Column Filter: EXP2/ Optimize Technologies

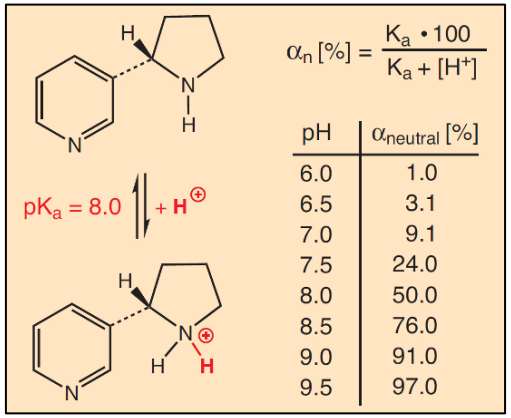


HALO 120 Å Elevate: Nicotine

Testing Conditions:
Column: HALO 120 Å ELV C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92272-602
Mobile Phase A: Water/ as listed
B: Acetonitrile
Gradient: 5-60%B in 10min (2 min. hold)
Instrument: Nexera
Injection: 0.1 µl Nicotine (50 ng)
Temperature: 30°C
Flow Rate: 0.4 mL/min.

- No retention using low pH/0.1% formic acid
- Great retention using pH 10.15 since pKa is 8.0

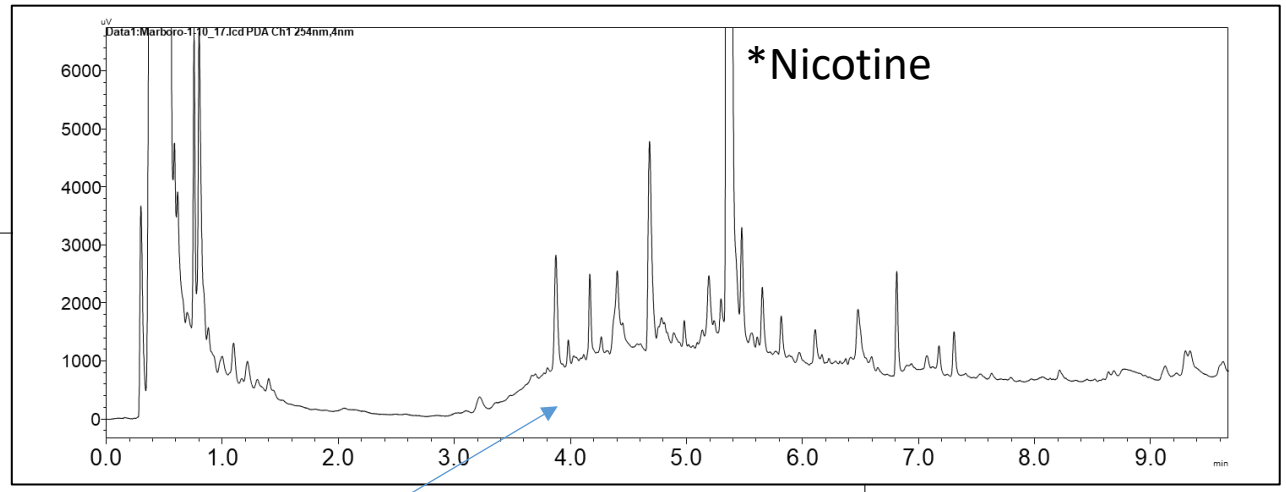


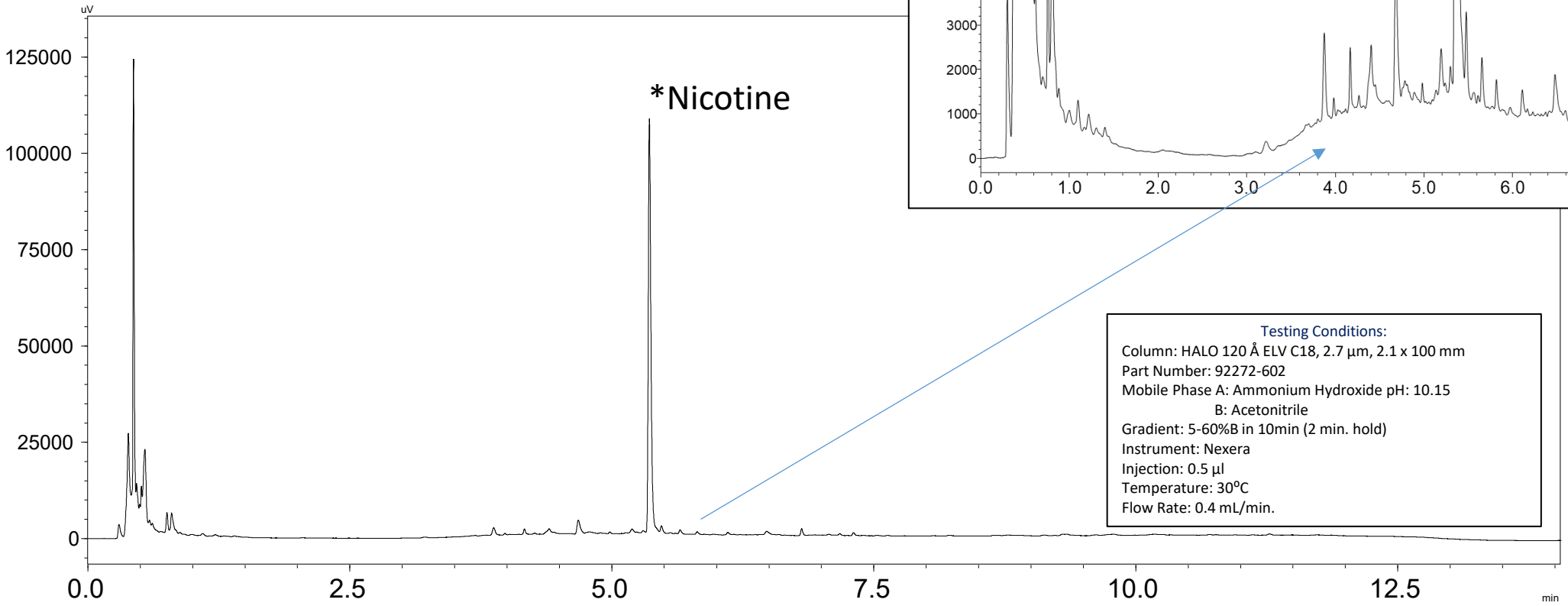


pH	$\alpha_{neutral}$ [%]
6.0	1.0
6.5	3.1
7.0	9.1
7.5	24.0
8.0	50.0
8.5	76.0
9.0	91.0
9.5	97.0

Nicotine Analysis of Cigarette Tobacco

- Many low concentration peaks observed when zoomed in near the baseline
- Work in progress to ID using MS

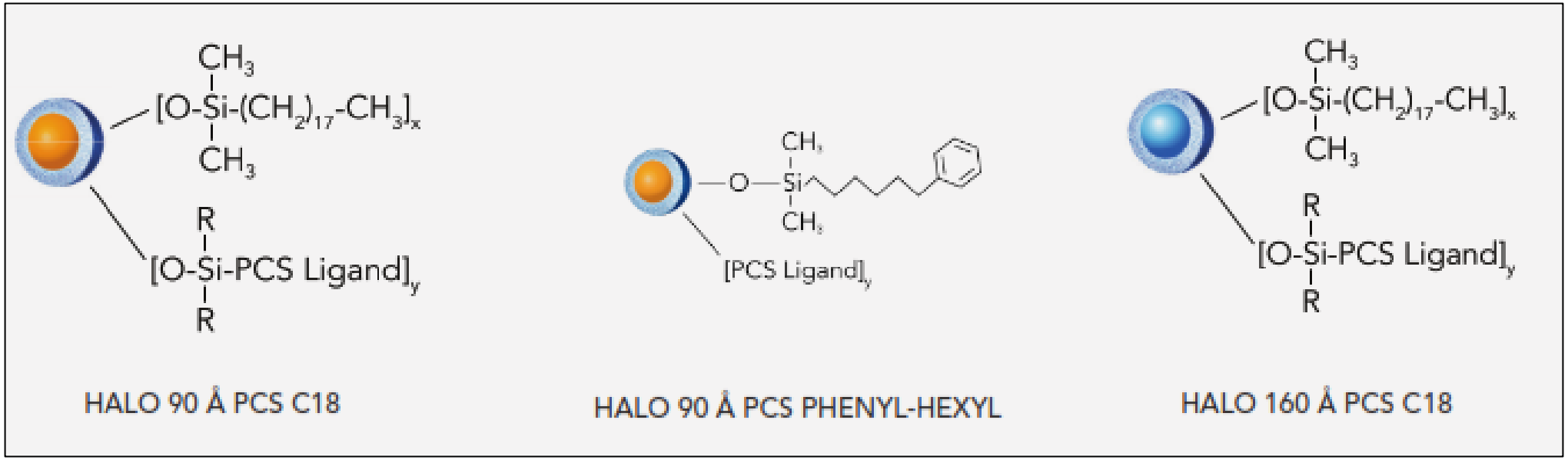




Testing Conditions:
Column: HALO 120 Å ELV C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92272-602
Mobile Phase A: Ammonium Hydroxide pH: 10.15
B: Acetonitrile
Gradient: 5-60%B in 10min (2 min. hold)
Instrument: Nexera
Injection: 0.5 µl
Temperature: 30°C
Flow Rate: 0.4 mL/min.

5. Use a different stationary phase

Introducing the HALO® PCS Phases: **P**ositively **C**harged **S**urface



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

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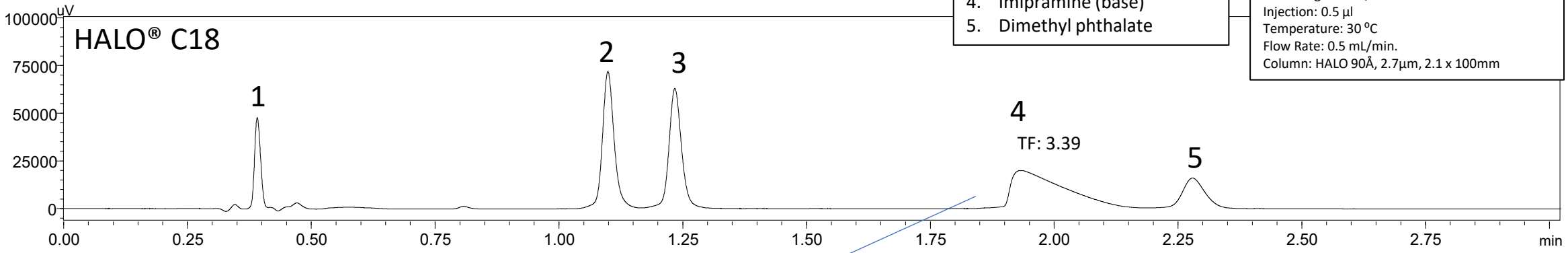
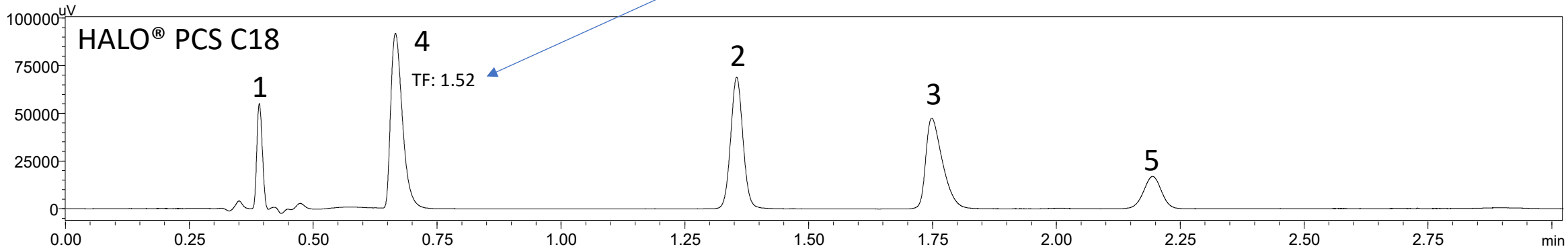
HALO® C18 vs. HALO® PCS C18

Peak Identities:
1. Uracil
2. 4-Methoxybenzoic acid
3. 2-Chlorobenzoic acid
4. Imipramine (base)
5. Dimethyl phthalate

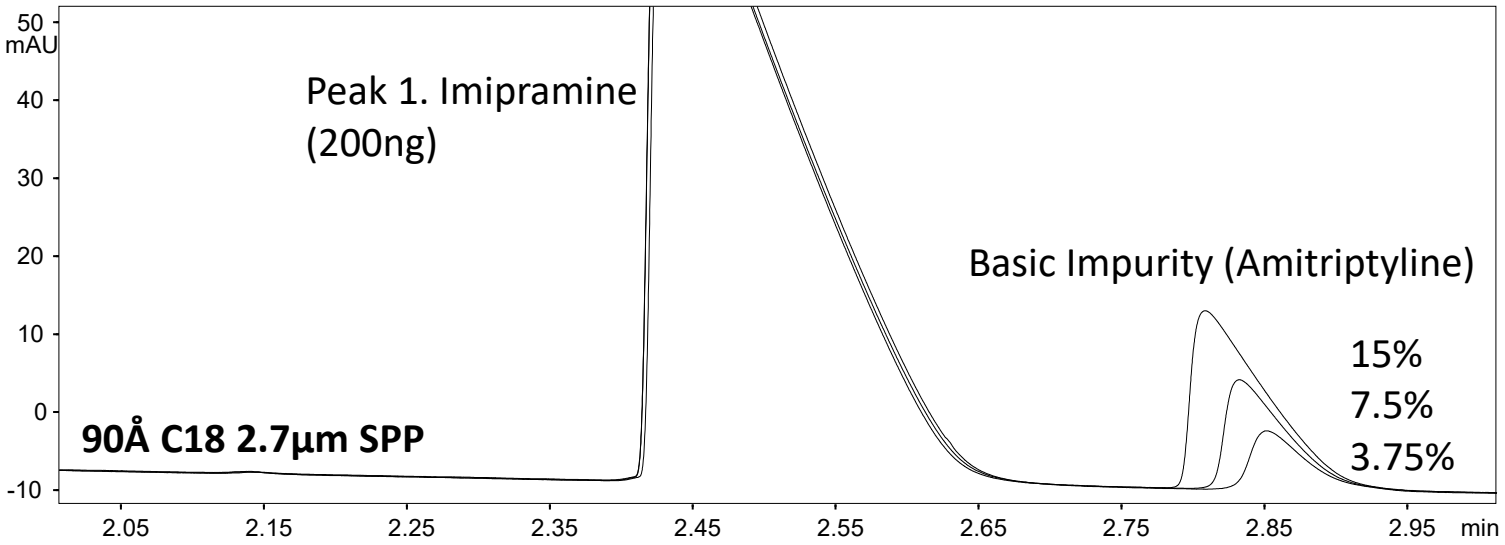
Testing Conditions:
Mobile Phase: A: Water/ 0.1% FA
B: Acetonitrile/ 0.1% FA
Isocratic Separation: 30% B
Instrument: Shimadzu Nexera X2
Wavelength: PDA, 254 nm
Injection: 0.5 µl
Temperature: 30 °C
Flow Rate: 0.5 mL/min.
Column: HALO 90Å, 2.7µm, 2.1 x 100mm

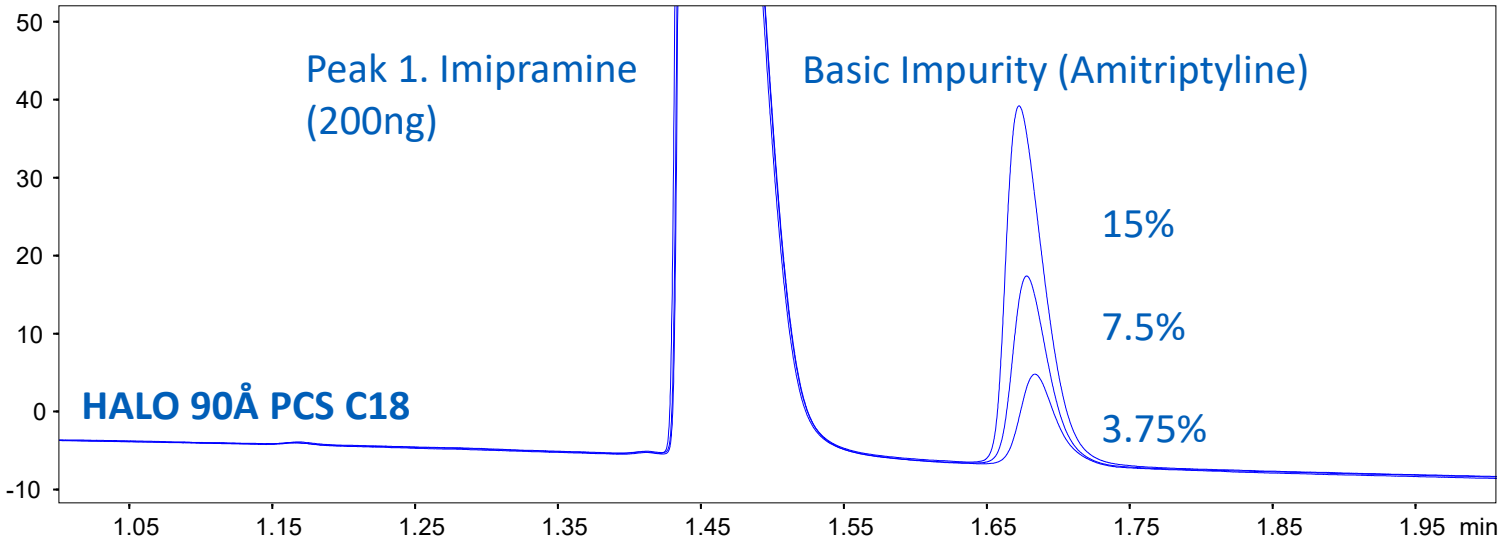




Why do we need a Positively Charged Stationary Phase? (2)

MP A = H_2O + 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



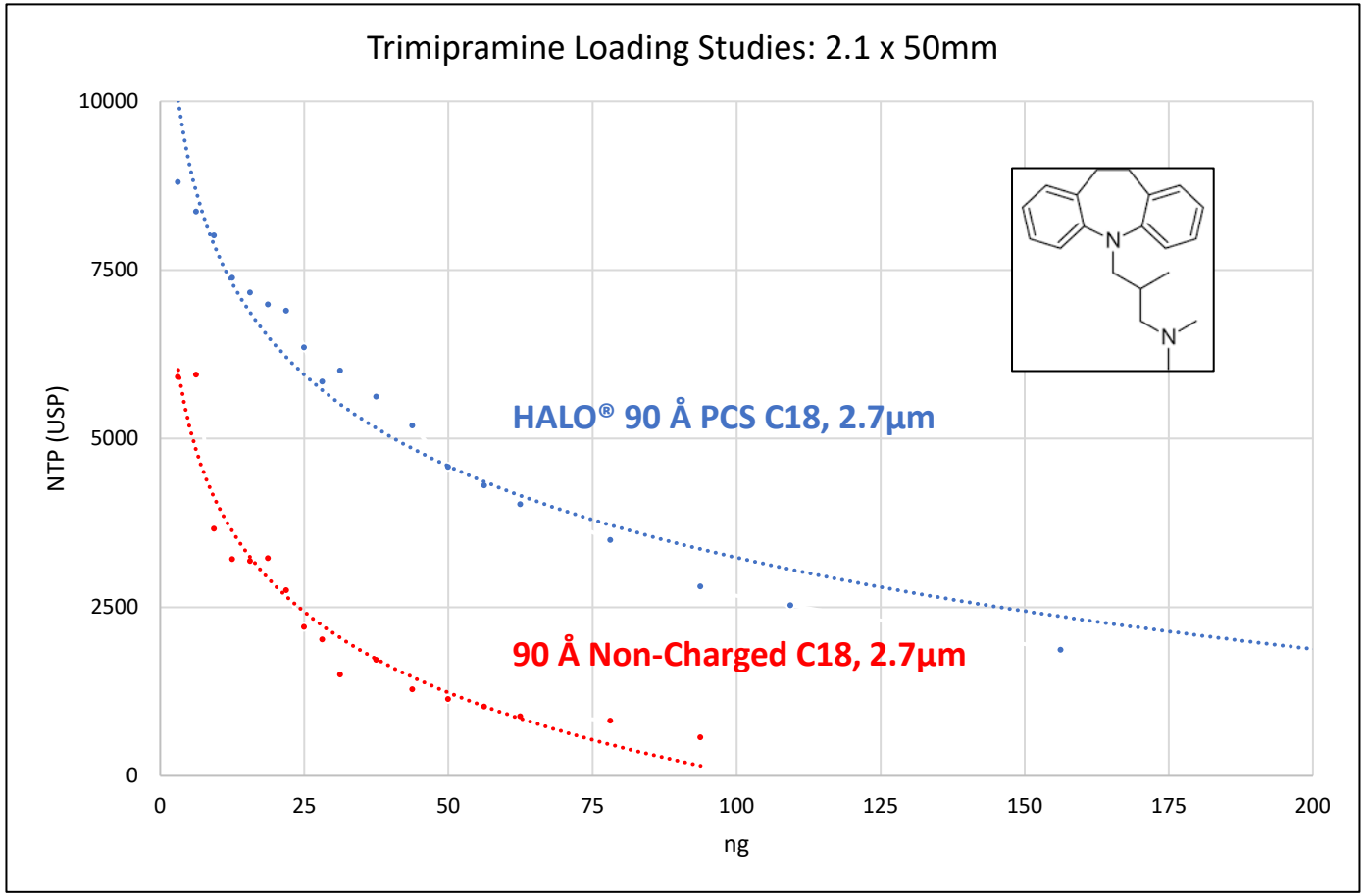


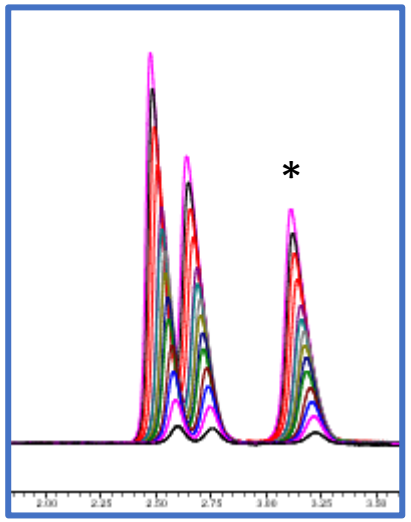
Column	Imipramine (200ng) {Rt] min	TF 5%	{W 50%} min
C18	2.43	5.26	0.108
HALO PCS C18	1.44	2.44	0.044

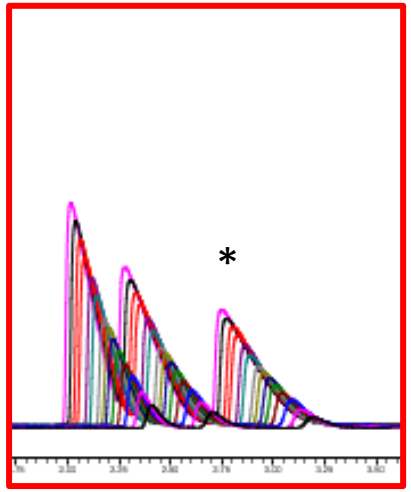
Column	Amitriptyline (30ng, 15%) {Rt] min	TF 5%	{W 50%} min	Rs (USP)
C18	2.81	3.15	0.059	2.46
HALO PCS C18	1.67	1.60	0.029	3.57

HALO® PCS Loading Capacity

0.1% Formic Acid







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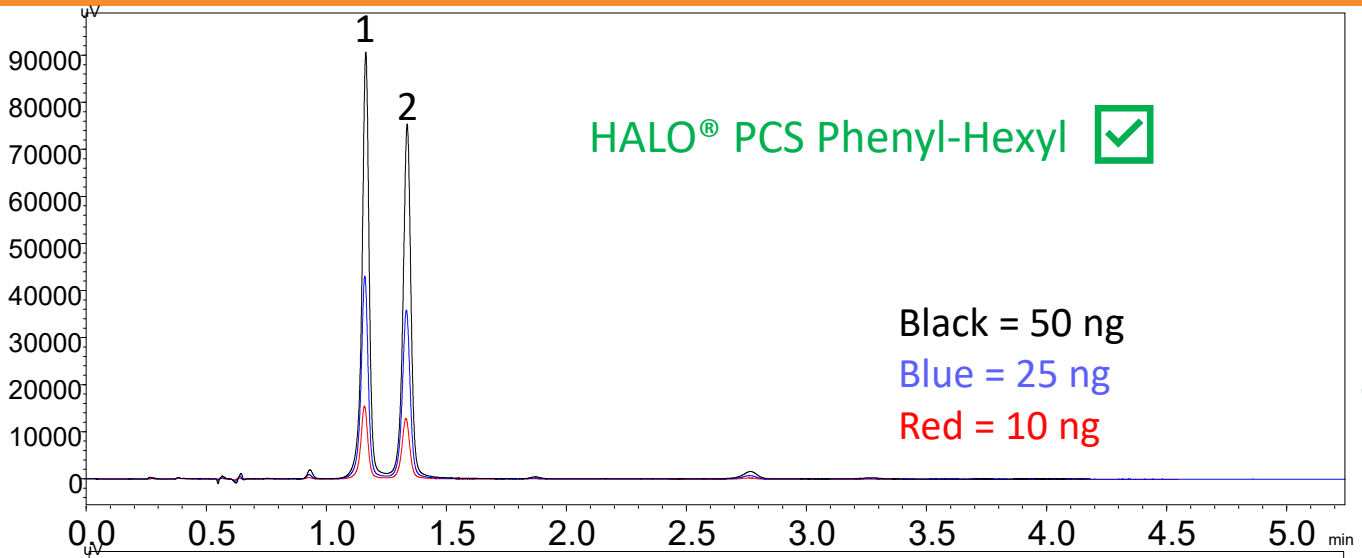
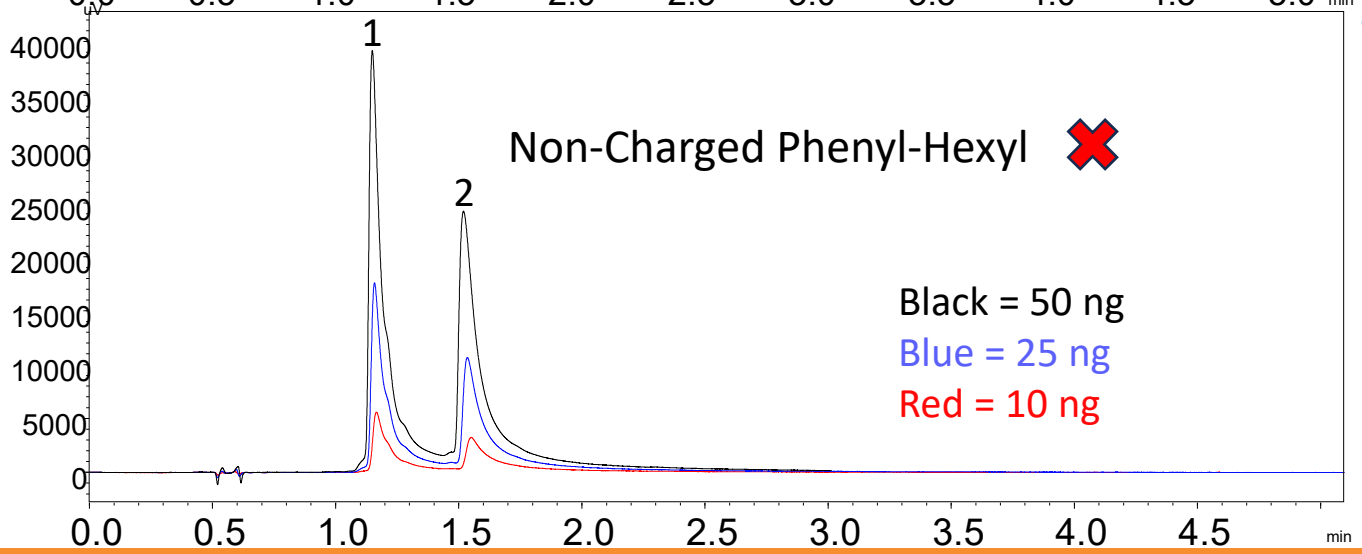
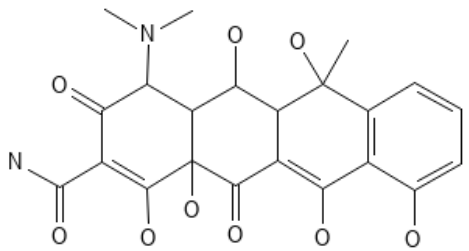
HALO® PCS Phenyl-Hexyl vs. standard (uncharged) HALO® Phenyl-Hexyl



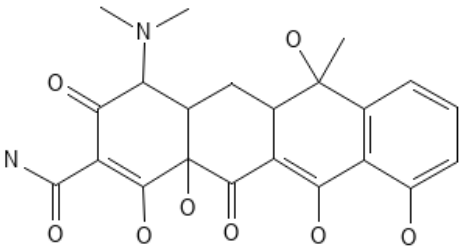


Testing Conditions:
Column: 2.7 µm, 2.1 x 100 mm phase as labeled
Mobile Phase A: Water/ 0.1% Formic Acid
B: Acetonitrile/ 0.1% Formic Acid
Isocratic: 12% B HALO® PCS Phenyl-Hexyl
18% B HALO® Phenyl-Hexyl
Flow Rate: 0.4 mL/min
Instrument: Nexera
Injection: 0.2, 0.5, 1.0 µL (10,25,50 ng)
Temperature: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



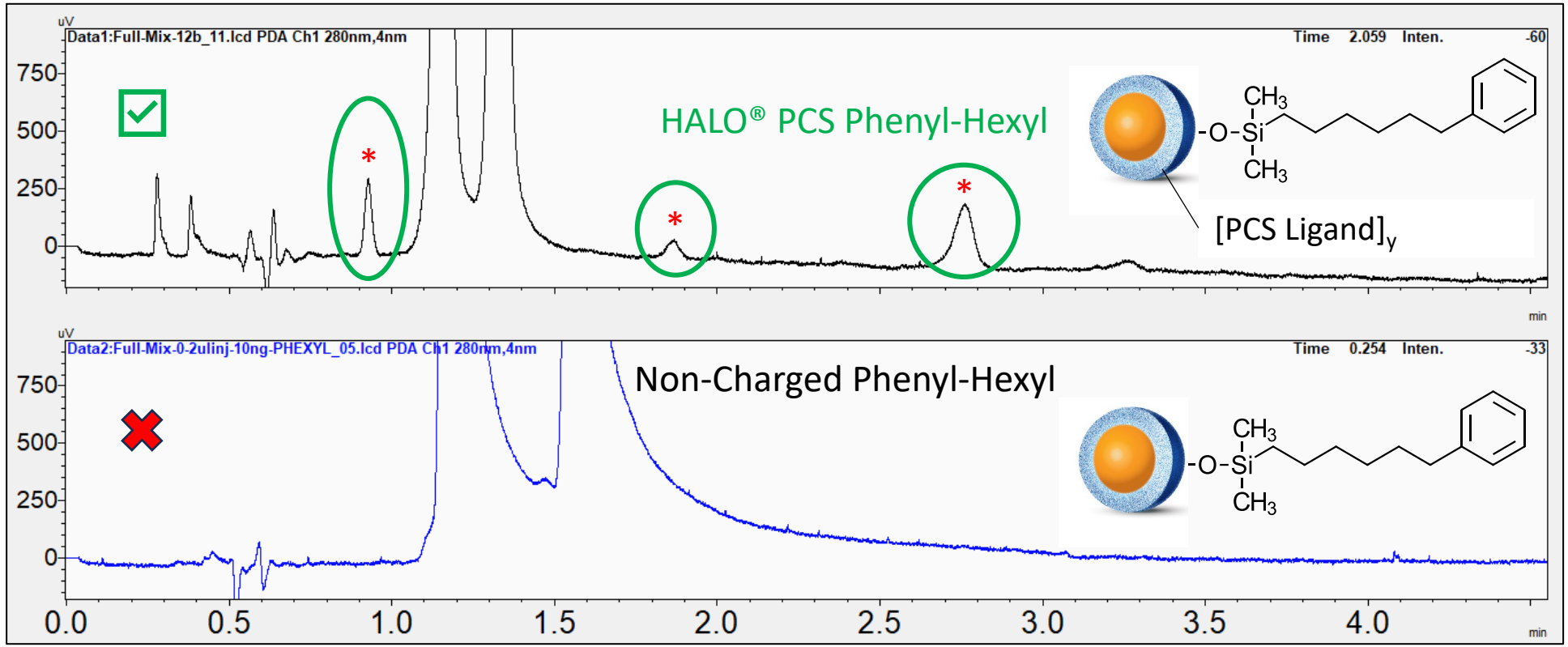
1. Oxytetracycline



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.

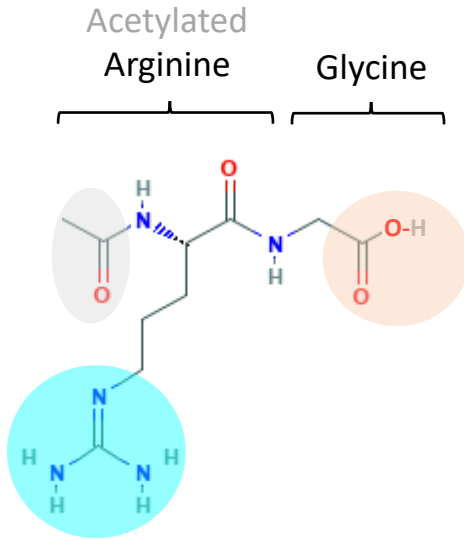


HALO 160 Å PCS C18 Peptide

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

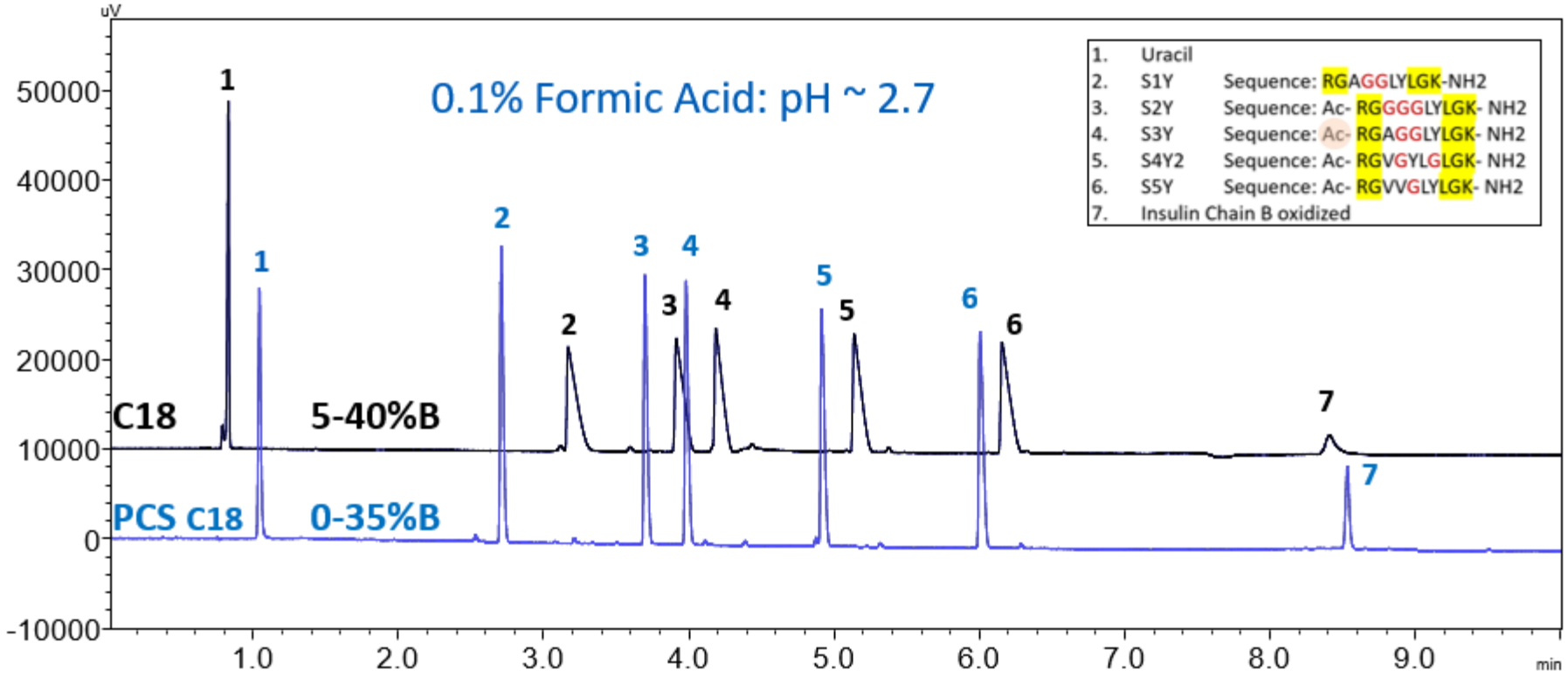
Ligand: dimethyloctadecylsilane
Particle Size: 2.7 µm
Pore Size: 90 and 160 Å
USP Designation: L1

Carbon Load 90 Å: 7.5%
Carbon Load 160 Å: 5.09%
Surface Area 90 Å: 135 m^2/g
Surface Area 160 Å 90 m^2/g

Endcapped: Yes both 90 and 160 Å
Low pH Limit /Max T: 2/60 °C
High pH Limit/Max T: 7/40 °C

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

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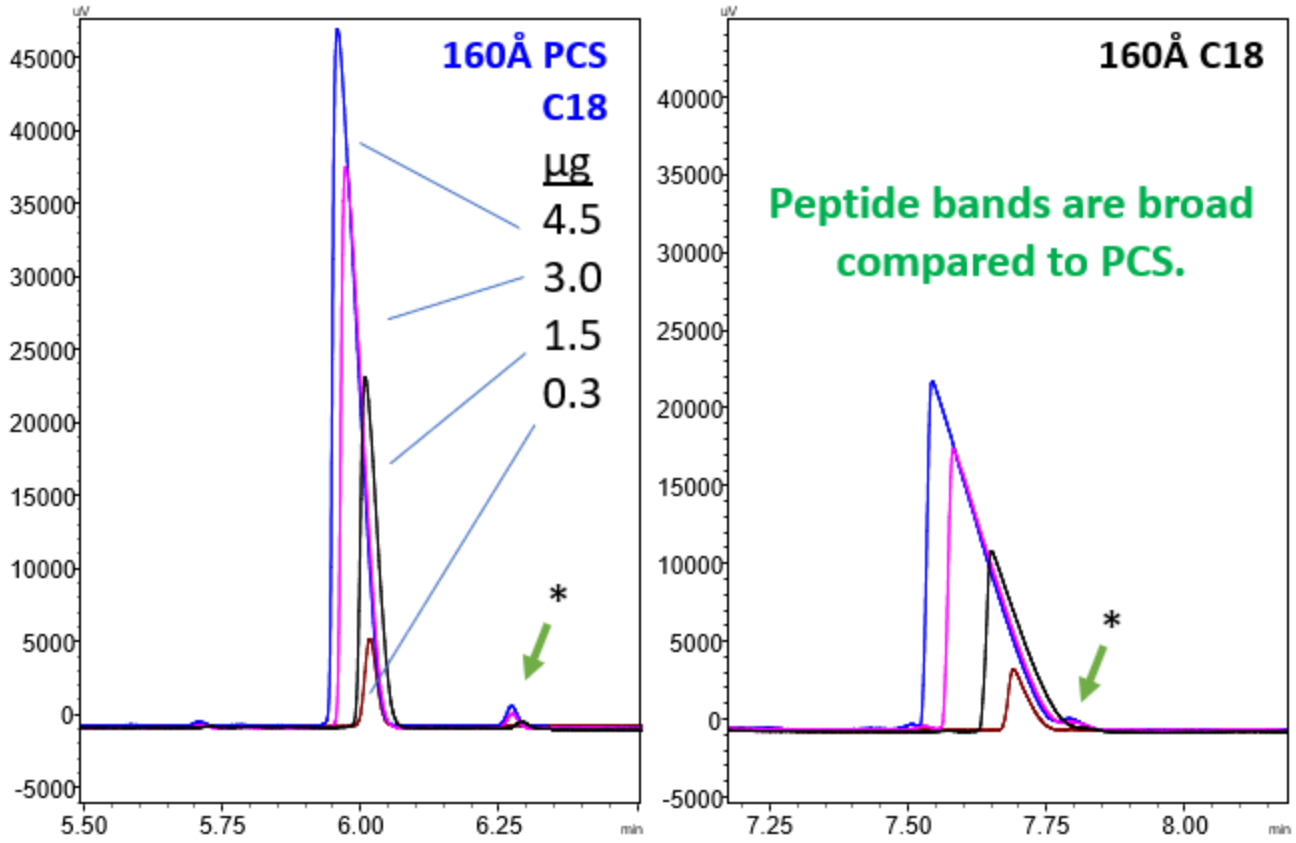
Peptide Load Tolerance

1, 5, 10, and 15µL injections of synthetic peptides (0.3µg/µL 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

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Conclusion

- Several things to consider when improving chromatographic peak shape/ separations
  - Mobile phase, pH, stationary phase, sample load, particle technology

- HALO® PCS is ideal for basic peak shape improvement under low ionic strength mobile phases such as formic acid
    - 90Å/ 160Å pore size
    - C18/ Phenyl-Hexyl

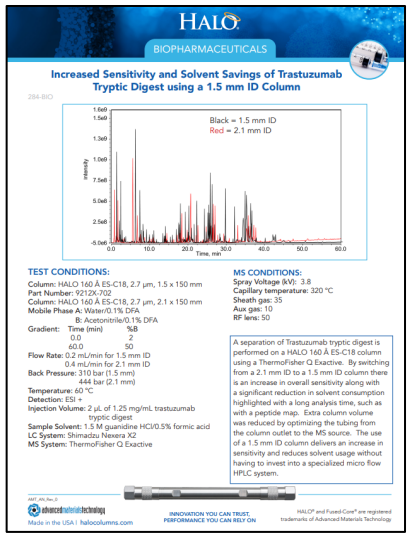
- HALO® Elevate is ideal for high pH separations, ideal for basic analytes
  - Wide pH range (2-12)

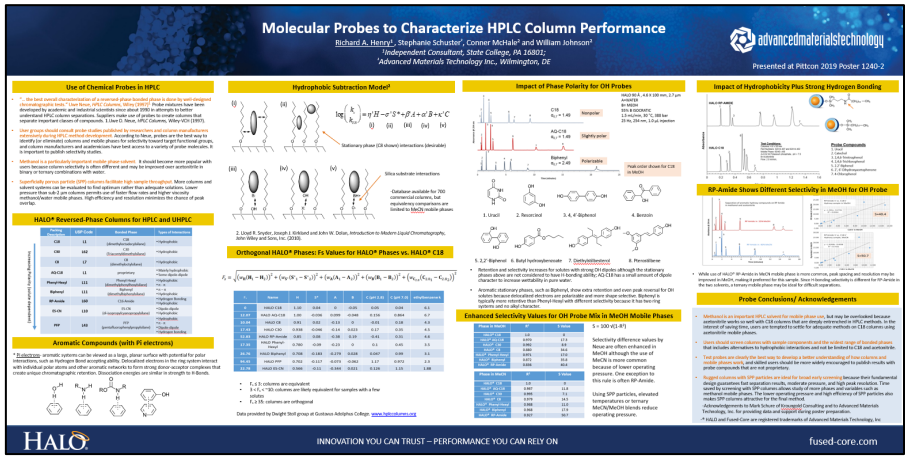
Technical Resources

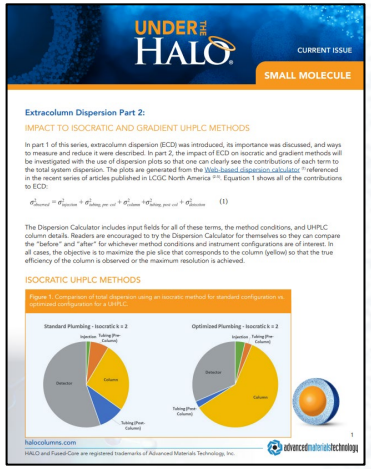
HALO® HPLC Columns for Chromatography Separation | LC Columns (halocolumns.com)

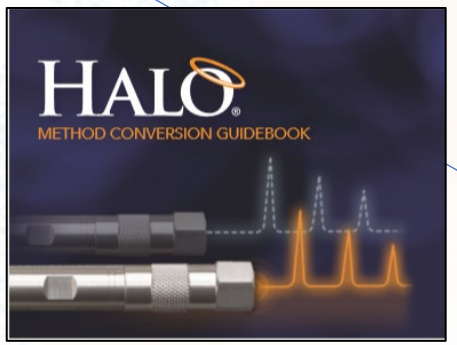
HALO®

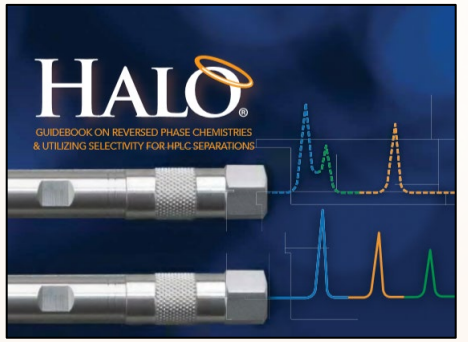
Application Notes | Conference Papers | Product Literature | Technical Documents | Videos











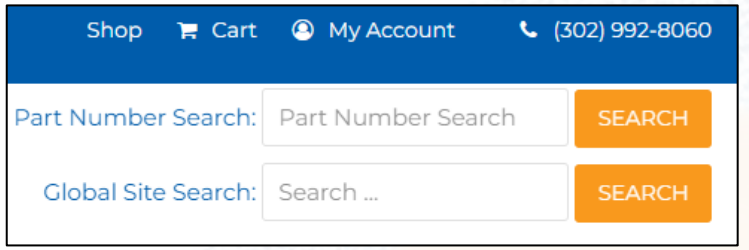
Questions?

Technical and Marketing Materials:

- www.halocolumns.com

Technical Support:

- support@advanced-materials-tech.com



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