Assessment of Stationary Phase Ligands on Positively Charged Particles by an Anionic Exchange Parameter

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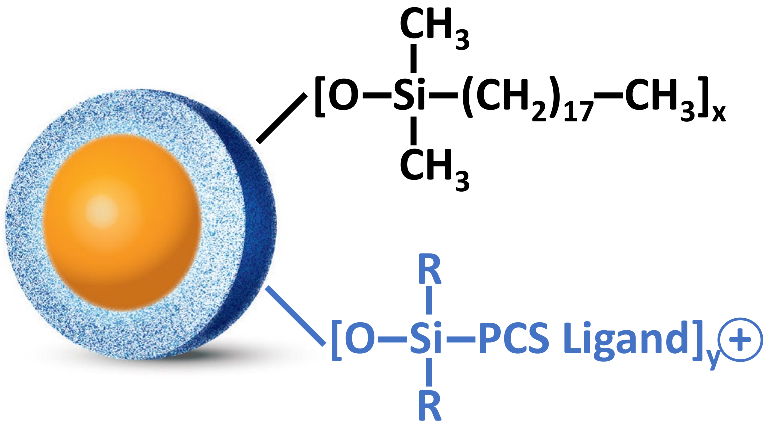
Cation Modified Stationary Phases

Further our understanding of the properties of cation-modified reversed-phase (RP) column packing materials to develop high performance LC and LC-MS applications.

- Combination of primary RP ligand with a low quantity of a positively charged surface modification
  - Many commercially available options
- Improve ionized basic solute peak shape and performance, while operating in 0.1 % (v/v) formic acid mobile phases
  - High sensitivity LC-MS applications

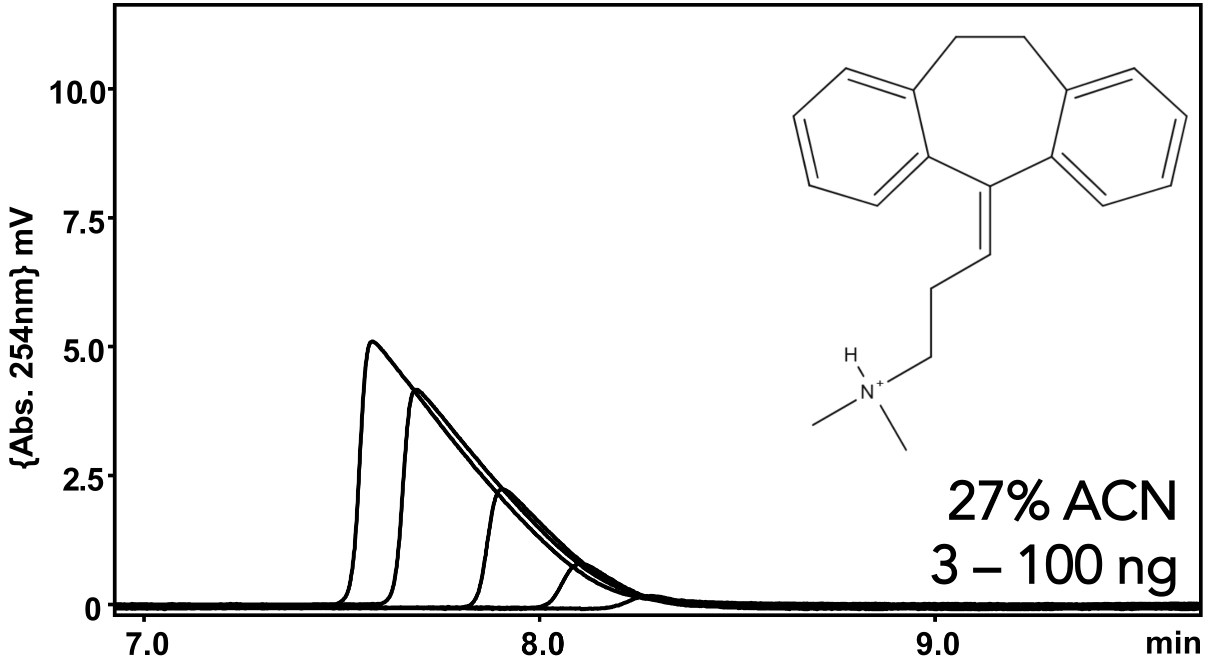


90Å HALO® PCS C18
2.7µm

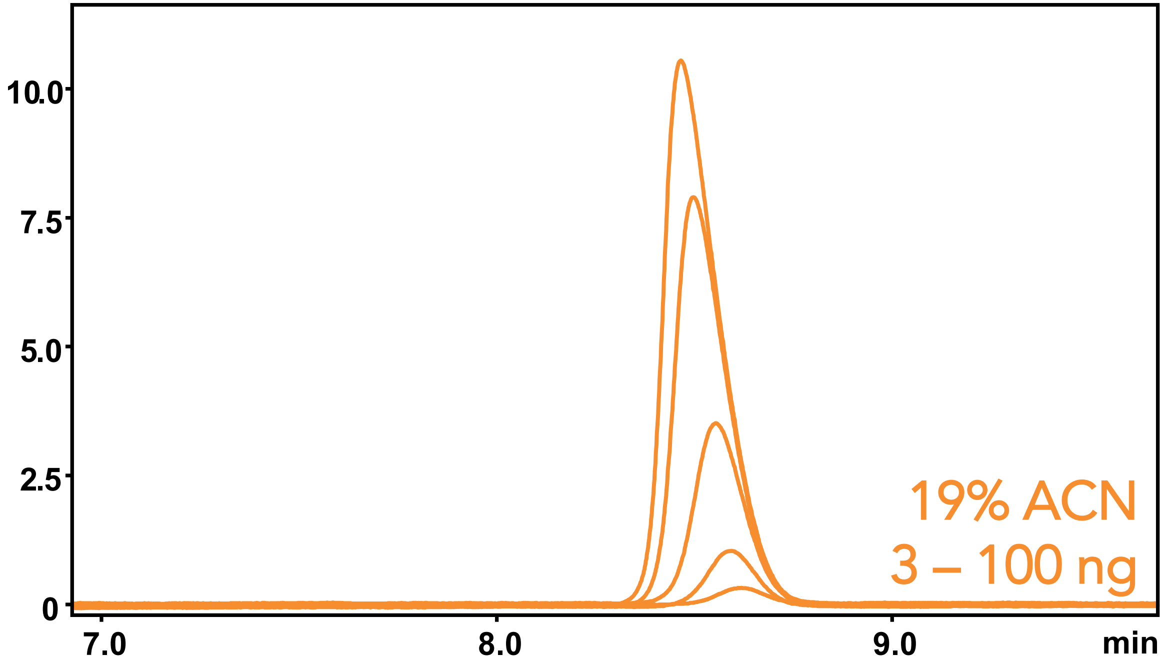
Ionized Basic Solute Overload

4.6x100 mm, 1.20 mL/min, 30° C, 4.0 µL Inj, 254 nm
MP - ACN/H2O + 0.1 % formic acid (v/v),
Amitriptyline 3 to 100 ng

Greater resistance to
ionized basic solute overload

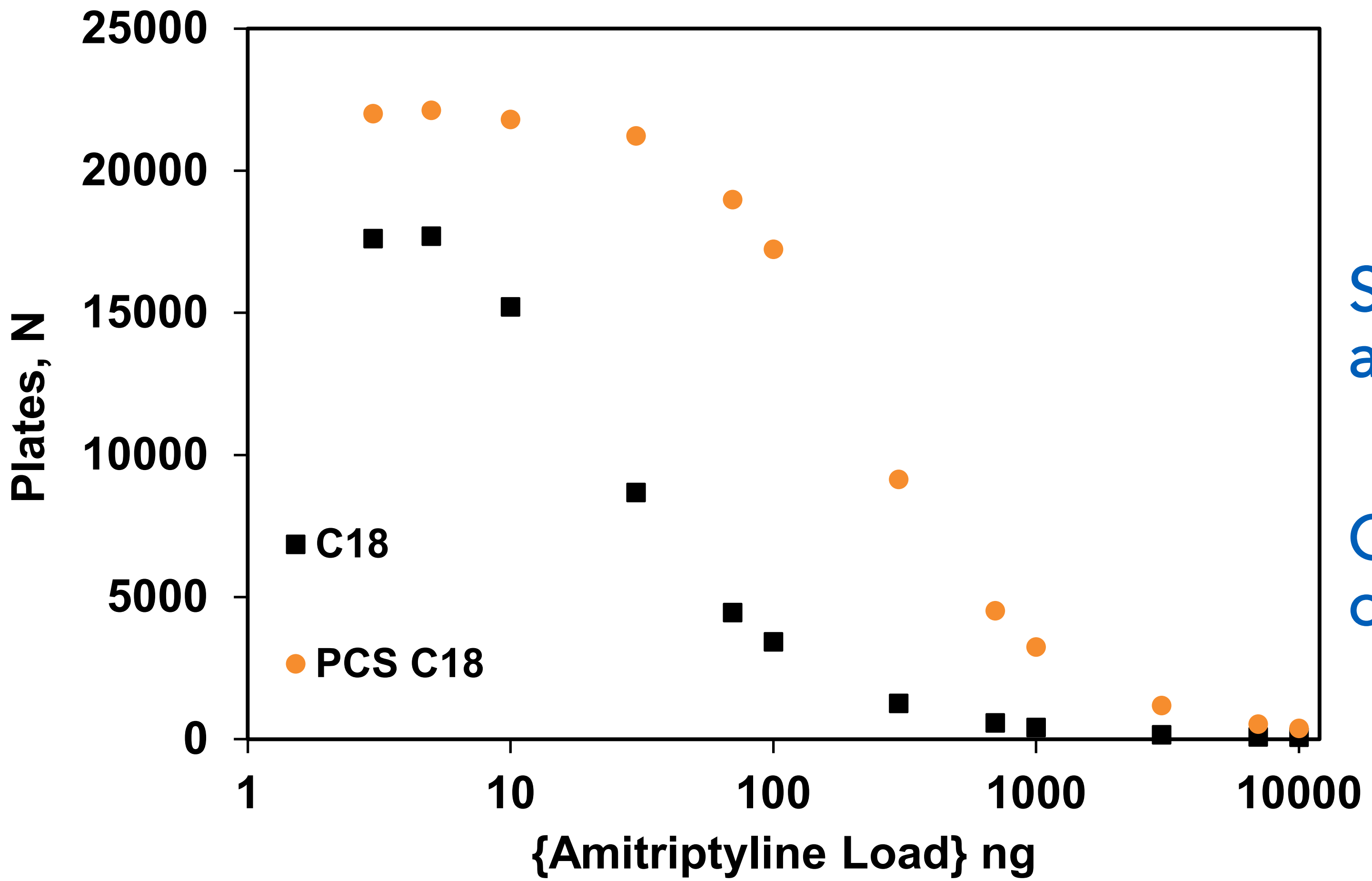


“Neutral” - 90Å C18 2.7µm SPP



Positively Charged - 90Å PCS C18 2.7µm

Ionized Basic Solute Overload

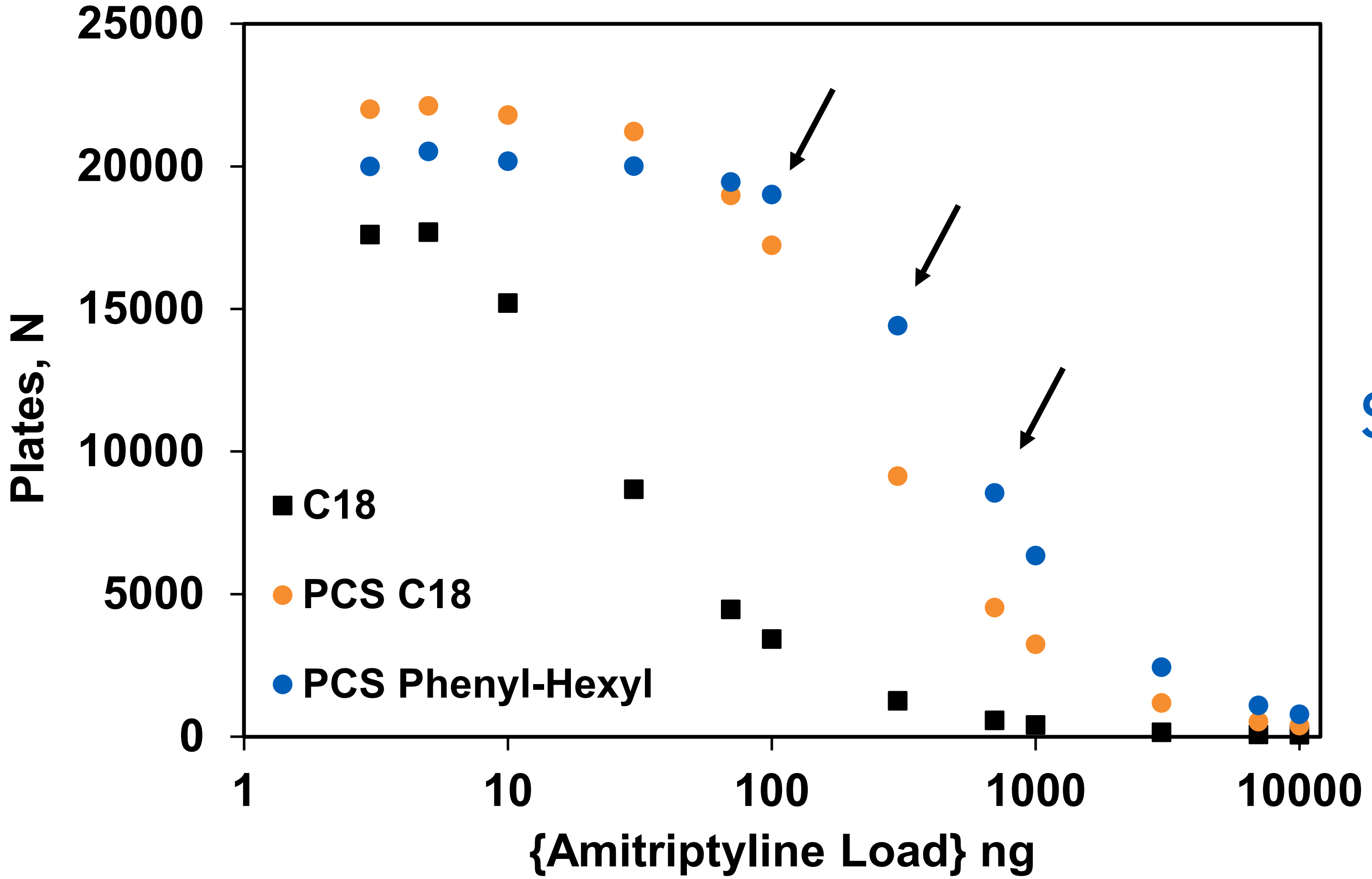


Similar trends for k' and TF

Overload still occurring

4.6x100 mm, 1.20 mL/min, 30° C, 4.0 μL Inj, 254 nm, 27 and 19 % ACN, MP - ACN/H2O + 0.1 % F.A. (v/v)

Effect of Primary Ligand



PCS Phenyl-Hexyl -

Similar improvements in k' and TF

4.6x100 mm, 1.20 mL/min, 30° C, 4.0 µL Inj, 254 nm, 27 / 19 / 16 % ACN, MP - ACN/H2O + 0.1 % F.A. (v/v)

Anionic Exchange Parameter

A 2024 publication by Jolliffe et. al. described an updated anionic exchange (AE) parameter, to better discriminate between the positive character of neutral and cation modified stationary phases [1].

- Tanaka Plots – New AE Parameter $\alpha_{4\text{-OBSA / Tol}}$ [1]
    - Replace α benzenesulfonic acid (BSA) with α 4-n-octylbenzenesulfonic acid (4-OBSA)
    - Measure selectivity of anion with toluene (Tol)

- Classified the positive character of phases into three groups: neutral, low to medium, and significant [1]

[1] *S. Jolliffe, J.K. Field, M.R. Euerby, P. Petersson, Characterization of reversed-phase liquid chromatographic columns containing positively charged functionality, Journal of Chromatography A 1730 (2024) 465059.*

Anionic Exchange Parameter

What can we learn from this new AE Parameter $\alpha_{4\text{-OBSA / Tol}}$ to help in developing stationary phases?

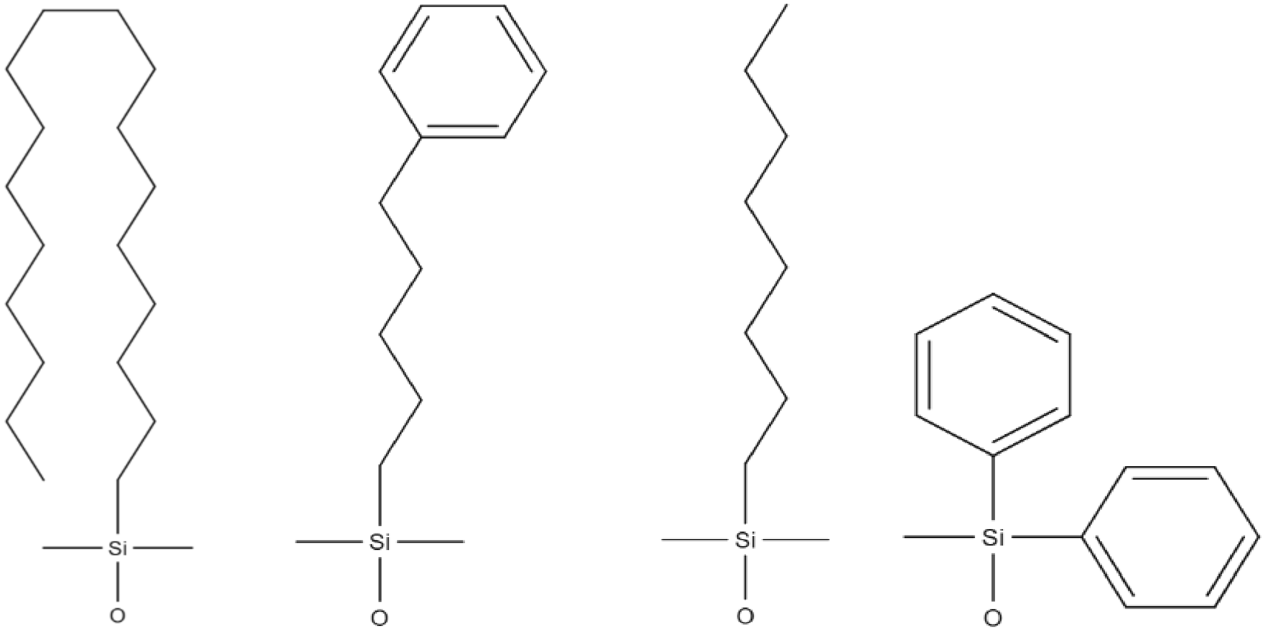
Measure $\alpha_{4\text{-OBSA / Tol}}$ for cation modified stationary phases bonded with different primary hydrophobic ligands

- Does the primary hydrophobic ligand play a role in determining the positive character of these phases?

Design of Experiments

90Å HALO® PCS 2.7µm (SPP)

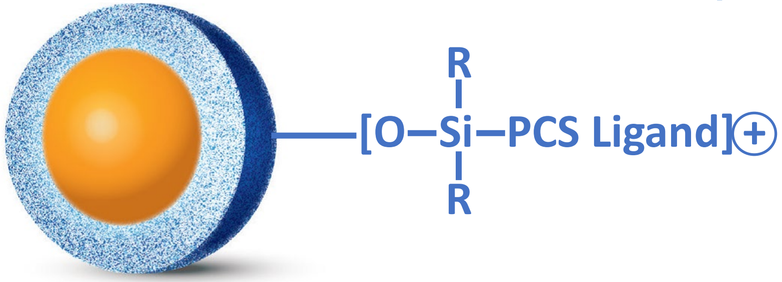
- Silica, 125 m^2/g surface area
- C18 and Phenyl-Hexyl commercially availa
- Controlled experiment
    - Surface area and coverage of PCS



C18 Phenyl-Hexyl *C8* *Diphenyl*

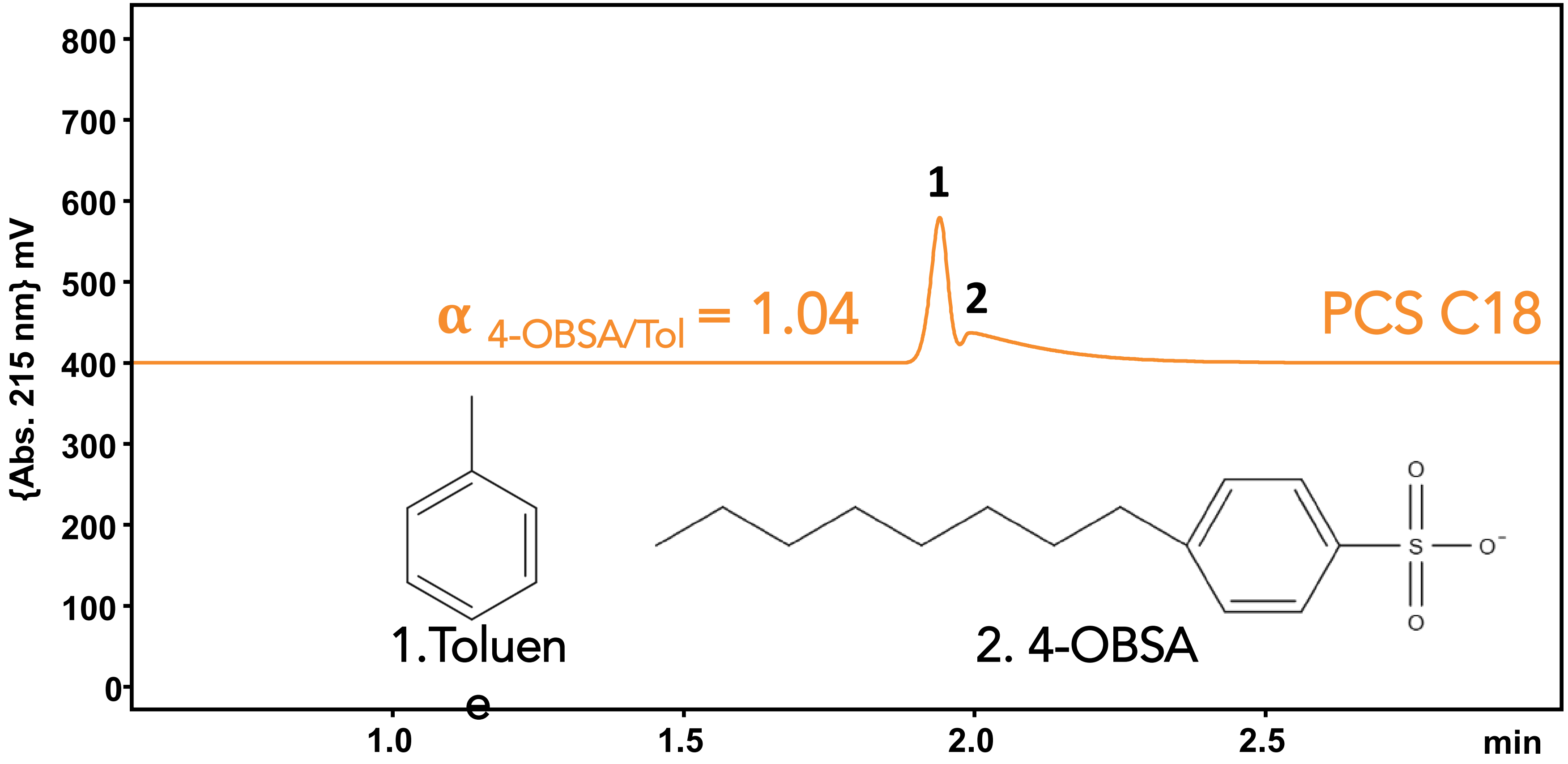
Experiments

- $\alpha_{4\text{-OBSA/Tol}}$ AE measurements
- Ionized solute overload studies
- LC-MS Beta Blocker Separation



Anionic Exchange Measurement

4.6x100 mm, 1.80 mL/min, 40° C, 1.0 µL Inj, 215 nm
MP – 50/50 ACN/H2O + 5mM KH_2PO_4 pH = 2.5 , Ref [1]
0.50 mg/mL



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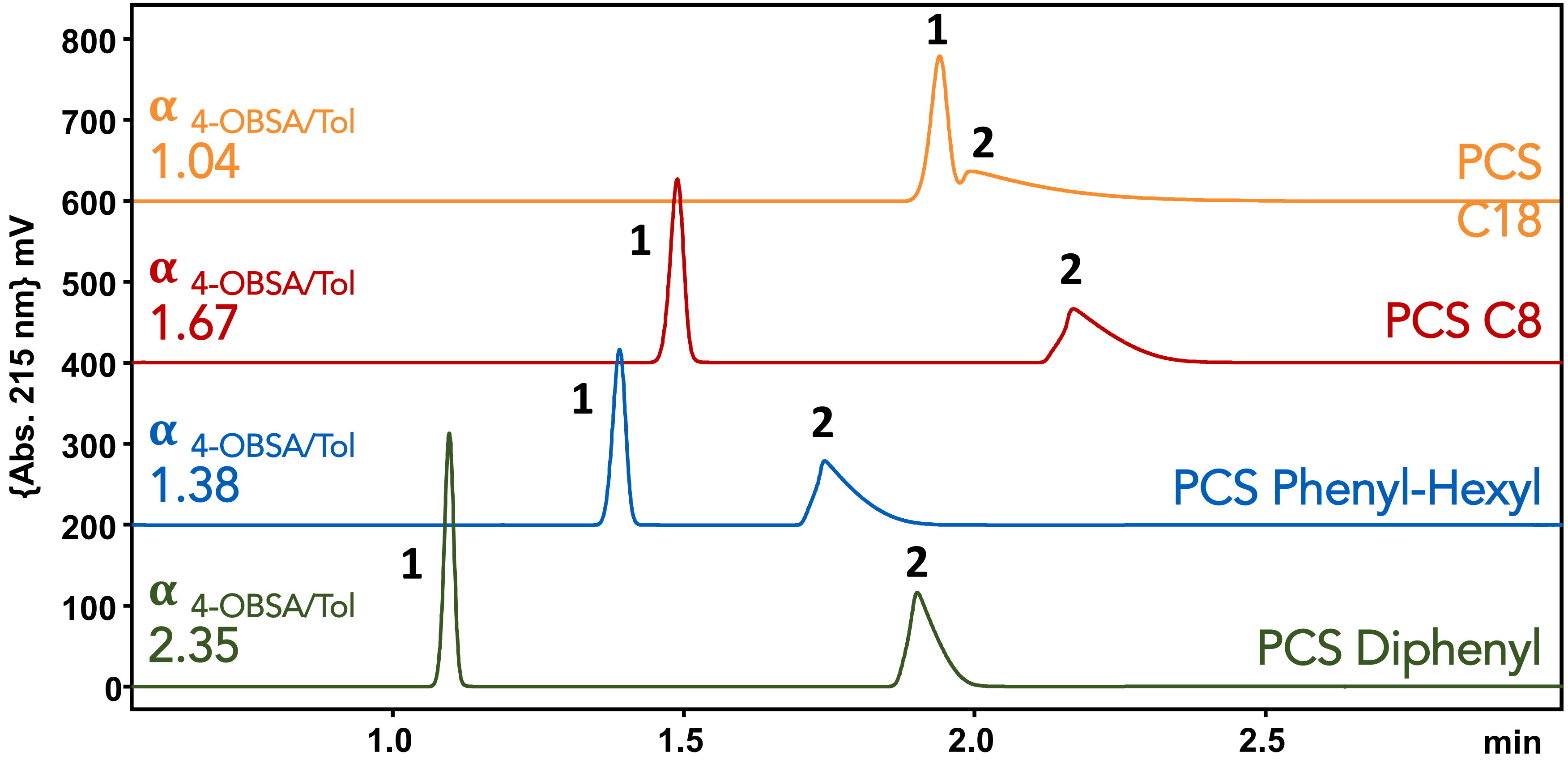
1. Toluene (0.50 mg/mL) 2. 4-OBSA (0.50 mg/mL)



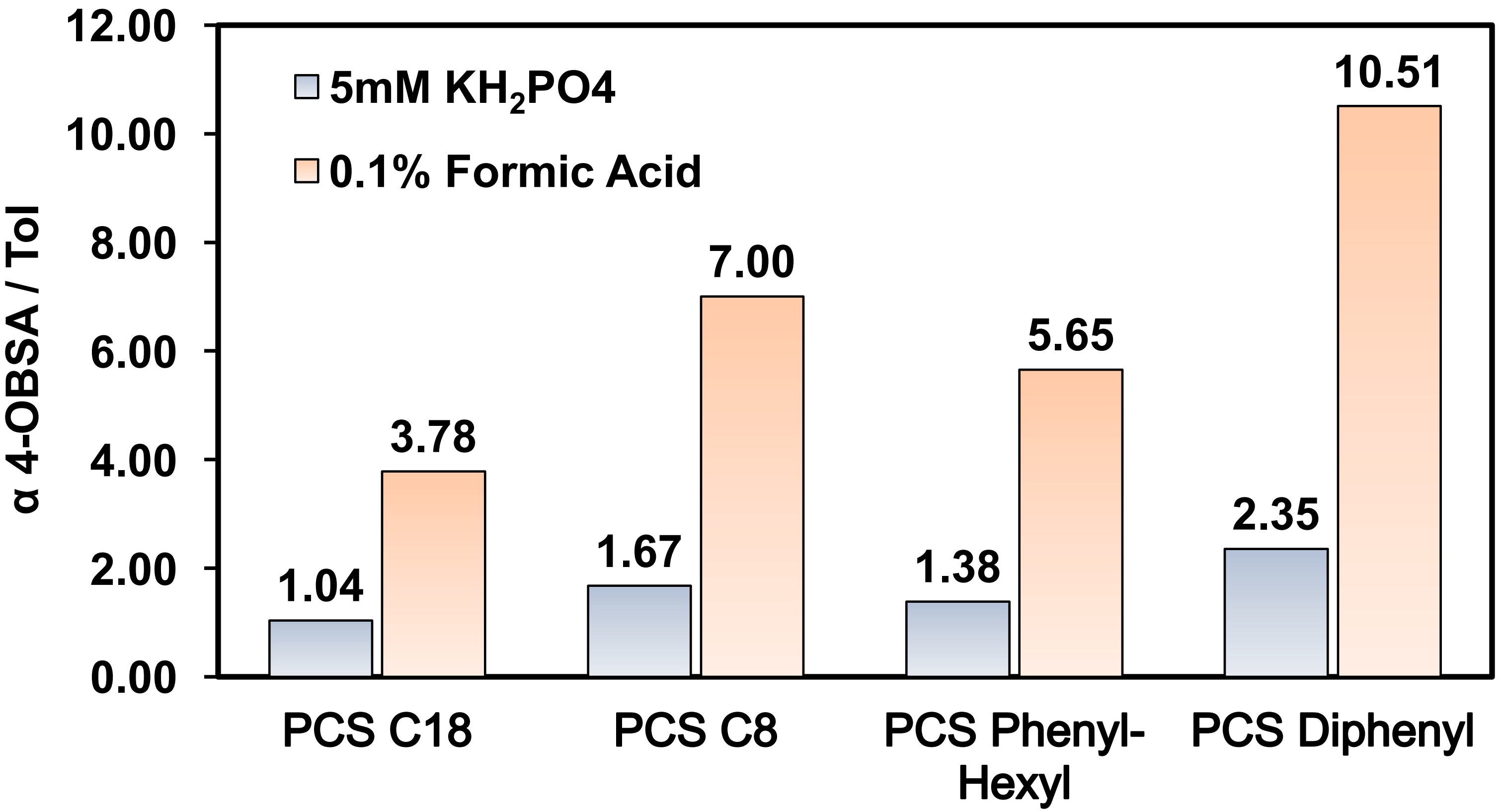
Anionic Exchange Formic Acid

Formic acid mobile phase

- Decrease in ionic strength
- 3-to-4-fold increase in $\alpha_{4\text{-OBSA/Tol}}$

Positive Character of PCS Phases

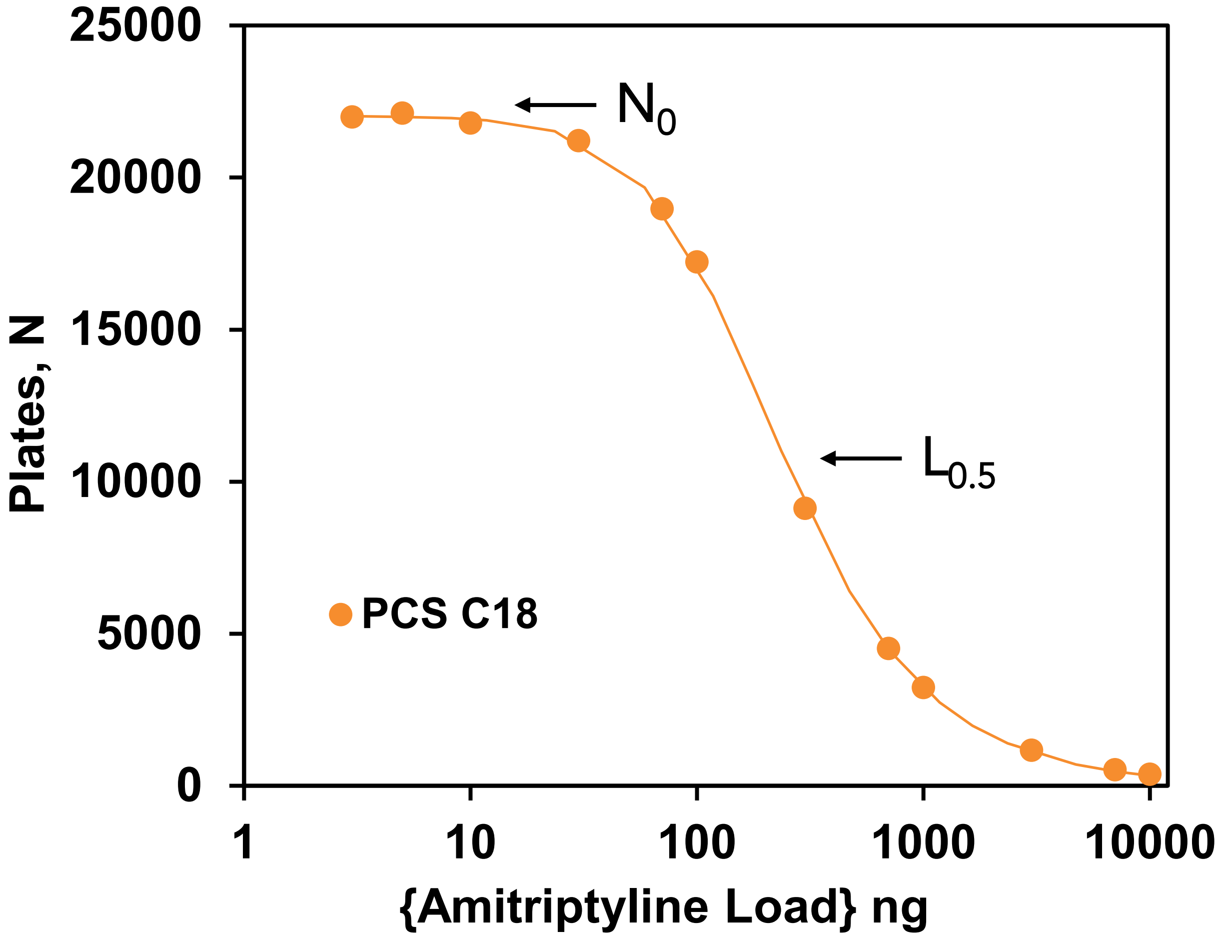
Diphenyl > C8 > Phenyl-Hexyl > C18



4.6x100 mm, 1.80 mL/min, 40° C, 1.0 µL Inj, 215 nm
MP – 50/50 ACN/H2O + 5mM KH_2PO_4 pH = 2.5 or 0.1% formic acid pH = 2.7

Ionized Solute Overload Studies

4.6x100 mm, 1.20 mL/min, 30° C, 4.0 µL Inj, 254 nm
MP - ACN/H2O + 0.1% formic acid, Amitriptyline 3 to 10,000 ng



Phase	ACN %	Calculated N_0	Calculated {$L_{0.5}$} ng
PCS C18	19	22025	236

Evaluate ionized solute overload through [2]

- Limiting plate count (N_0)
- Load capacity at 50% of N_0 ($L_{0.5}$)

Experimental results fit to universal overload equation to solve for N_0 and $L_{0.5}$ [2]

[2] J. Dai, P.W. Carr, D.V. McCalley, A new approach to the determination of column overload characteristics in reversed-phase liquid chromatography, Journal of Chromatography A 1216 (2009) 2474–2482.

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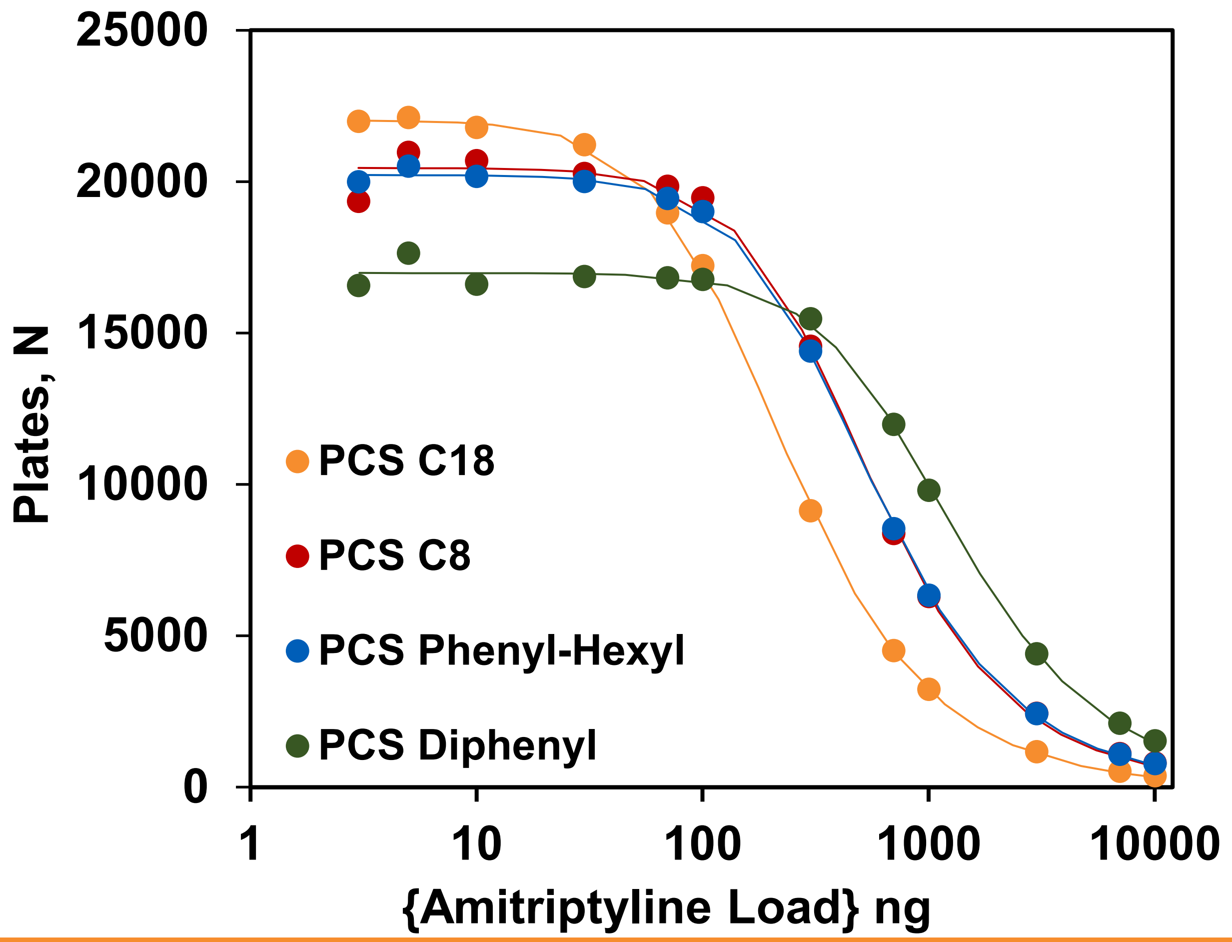


Phase	ACN %	Calculated N_0	Calculated $\{L_{0.5}\}$ ng
PCS C18	19	22025	236
PCS C8	16	20453	551
PCS Phenyl-Hexyl	16	20219	558
PCS Diphenyl	12	$\boxed{16984}$	$\boxed{1297}$

Similar trends for k′ and TF

Ionized Solute Overload Studies

	PCS			
	C18	**C8**	**Phenyl-Hexyl**	**Diphenyl**
α 4-OBSA / Tol	3.78	7.00	5.65	10.51
Amitriptyline (+)				
N_0	22025	20453	20219	16984
$L_{0.5}$	236	551	558	1297
Tetracaine (+)				
N_0	21732	20112	18709	13476
$L_{0.5}$	355	868	988	1691
Naphthalene sulfonic acid (-)				
N_0	14189	13504	14778	13961
$L_{0.5}$	240	483	419	769

4.6x100 mm, 1.20 mL/min, 30° C, 4.0 µL Inj, 254 nm
MP - ACN/H2O + 0.1% formic acid,

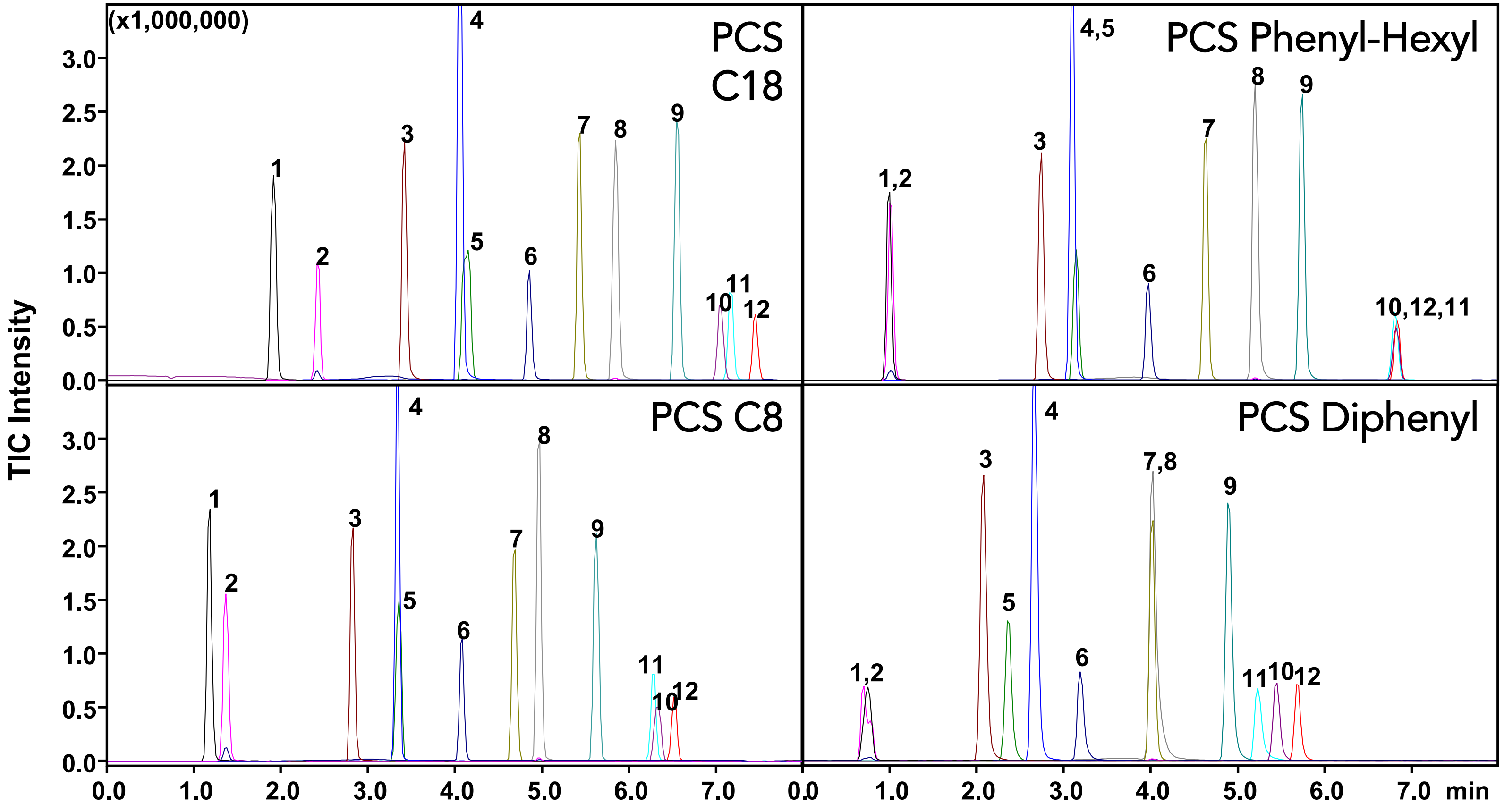
General trends of $\alpha_{4\text{-OBSA/Tol}}$

- Retention of ionized solutes
- Good correlation to $L_{0.5}$ (R^2 = 0.9496, 0.9168, and 0.9934)

Similar basic solute results for PCS C8 and Phenyl-Hexyl, despite difference in positive character

Beta Blocker Separation

2.1x100 mm, 0.40 mL/min, 30° C, 1.0 µL Inj, 0 – 50% B in 10 min, MP A – H_2O + 0.1% FA, MP B - MeOH + 0.1%FA, LC-MS ESI conditions [3]



1. Sotalol 2. Atenolol 3. Pindolol 4. Carteolol 5. Nadolol 6. Metoprolol 7. Acebutolol 8. Oxprenolol 9. Bisoprolol 10. Labetalol 11. Propranolol 12. Betaxolol 0.10 ng/mL

Average retention time

- C18 > Phenyl-Hexyl > C8 > Diphenyl
- Trends with positive character $\alpha_{4\text{-OBSA/Tol}}$

Hydrophilic vs hydrophobic solute retention

[3] P. Szpot, K. Tusiewicz, O. Wachełko, M. Zawadzki, Application of UHPLC-QqQ-MS/MS Method for Quantification of Beta-Adrenergic Blocking Agents (β-Blockers) in Human Postmortem Specimens, Molecules 29 (2024) 4585.

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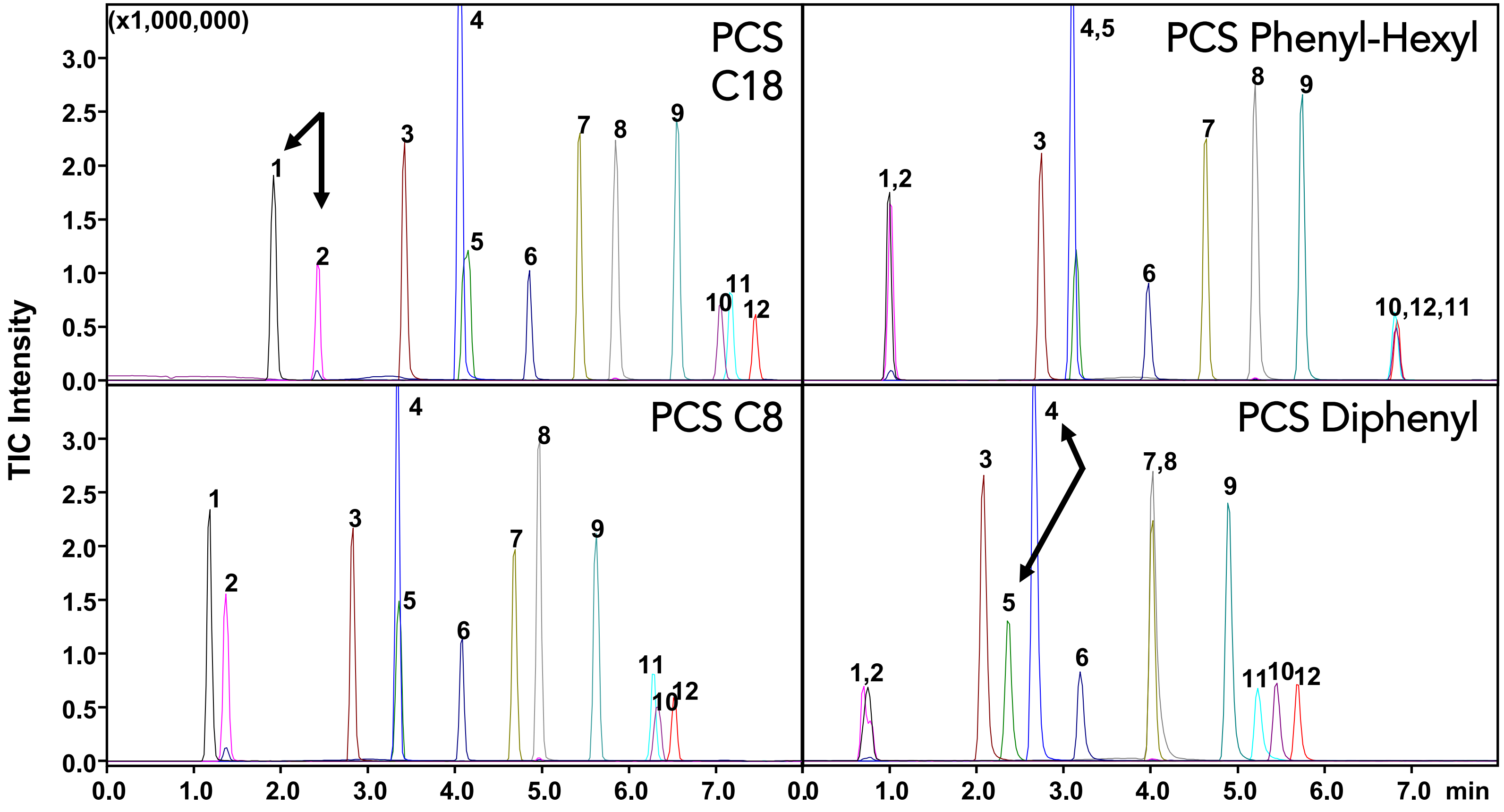

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Changes in selectivity observed

- Importance of primary hydrophobic ligand

Peaks 1 and 2 PCS C18

Peaks 4 and 5 PCS Diphenyl

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Conclusions

Primary hydrophobic ligand does affect the positive character of these cation modified stationary phases.

- Controlling for surface area and concentration of the positive charge

$\alpha_{4\text{-OBSA/Tol}}$ classifies HALO PCS columns as low to medium positive character, as designed [1].

As positive character of the phase increases, an increase in resistance to the effects of ionized solute overloading ($L_{0.5}$, tailing factor, and k') was observed.

Anionic exchange parameter was found to correlate to general retention and selectivity trends of ionized acids and bases.

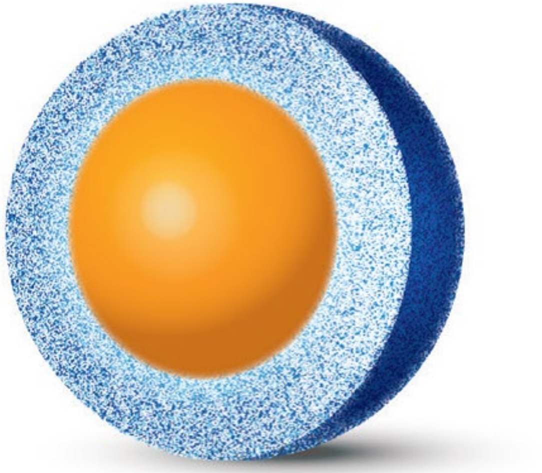
Acknowledgments

Research and Development Team
- Mark Haynes
- Bryant McKnight
- Timothy Langlois

Engineering Team
- Matt Jackson

Thank you for your time today!

Questions



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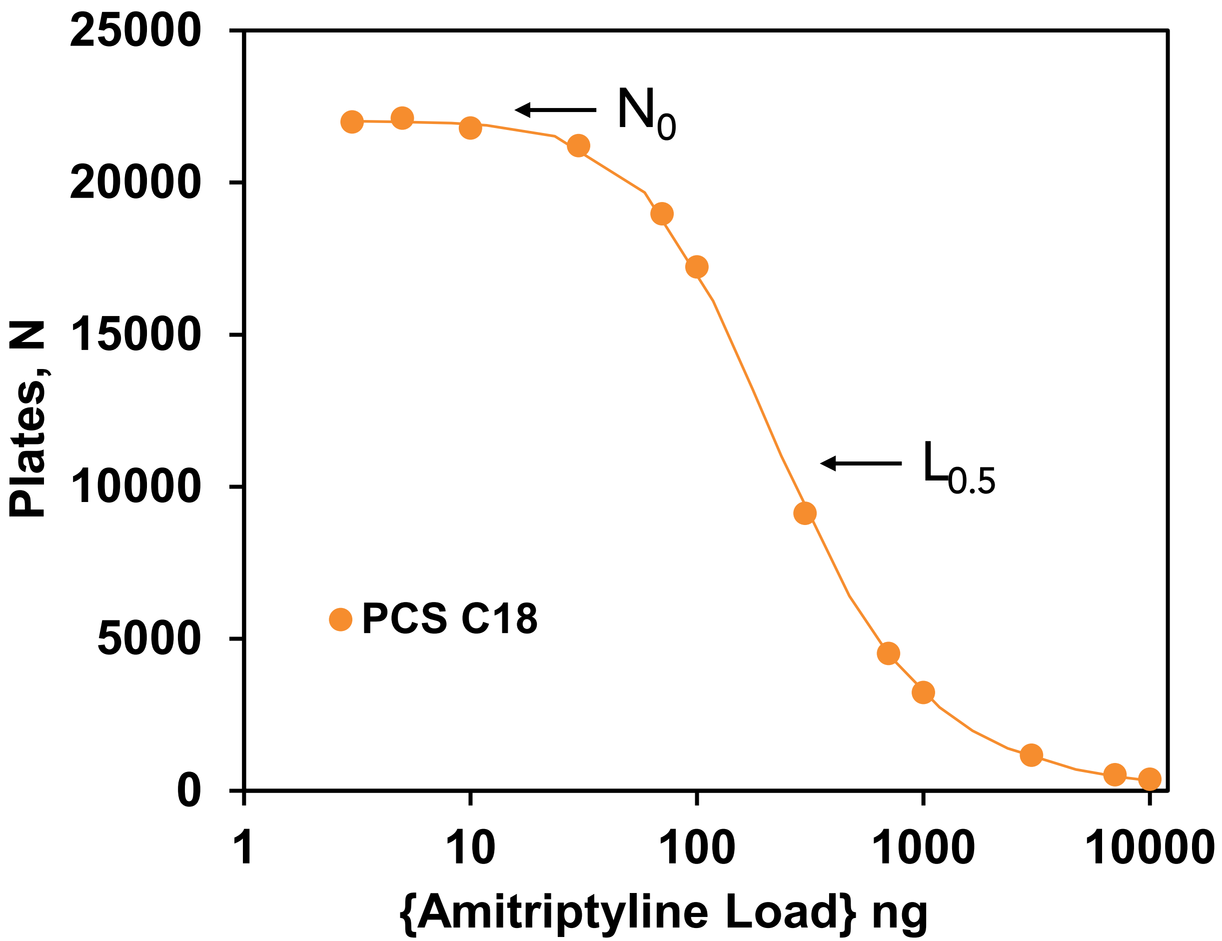

Phase	ACN %	Calculated N_0	Calculated $\{L_{0.5}\}$ ng
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Experimental results fit to universal overload equation to solve for N_0 and $L_{0.5}$ [2]

$$\frac{N}{N_0} = \frac{1 + bL'}{1 + bL' + (b+1)L'^2}$$

[2] J. Dai, P.W. Carr, D.V. McCalley, A new approach to the determination of column overload characteristics in reversed-phase liquid chromatography, Journal of Chromatography A 1216 (2009) 2474–2482.

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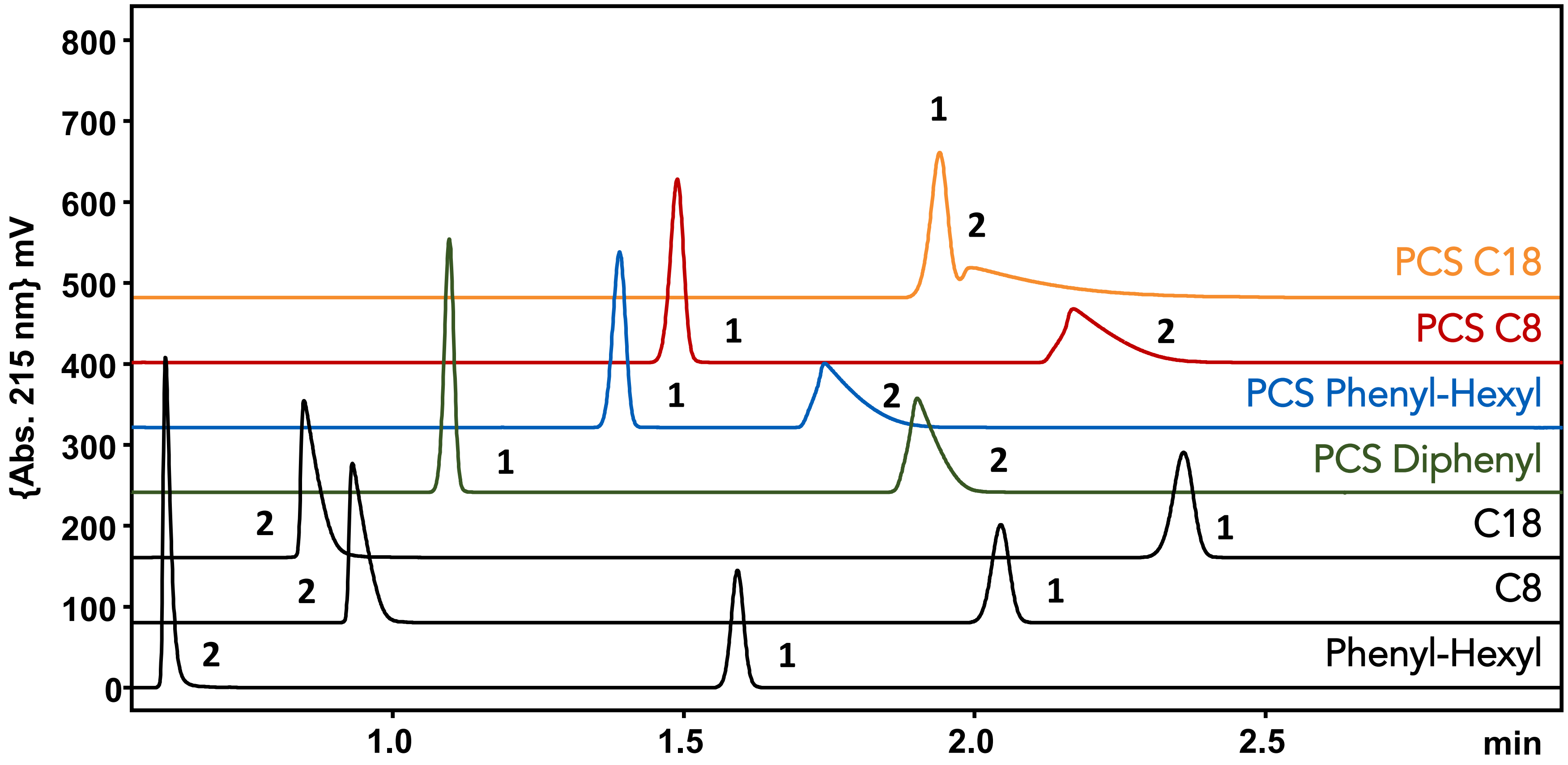

Alpha 4-OBSA/Tol
1.04
1.67
1.38
2.35
0.21
0.30
0.14

Anionic Exchange Measurement

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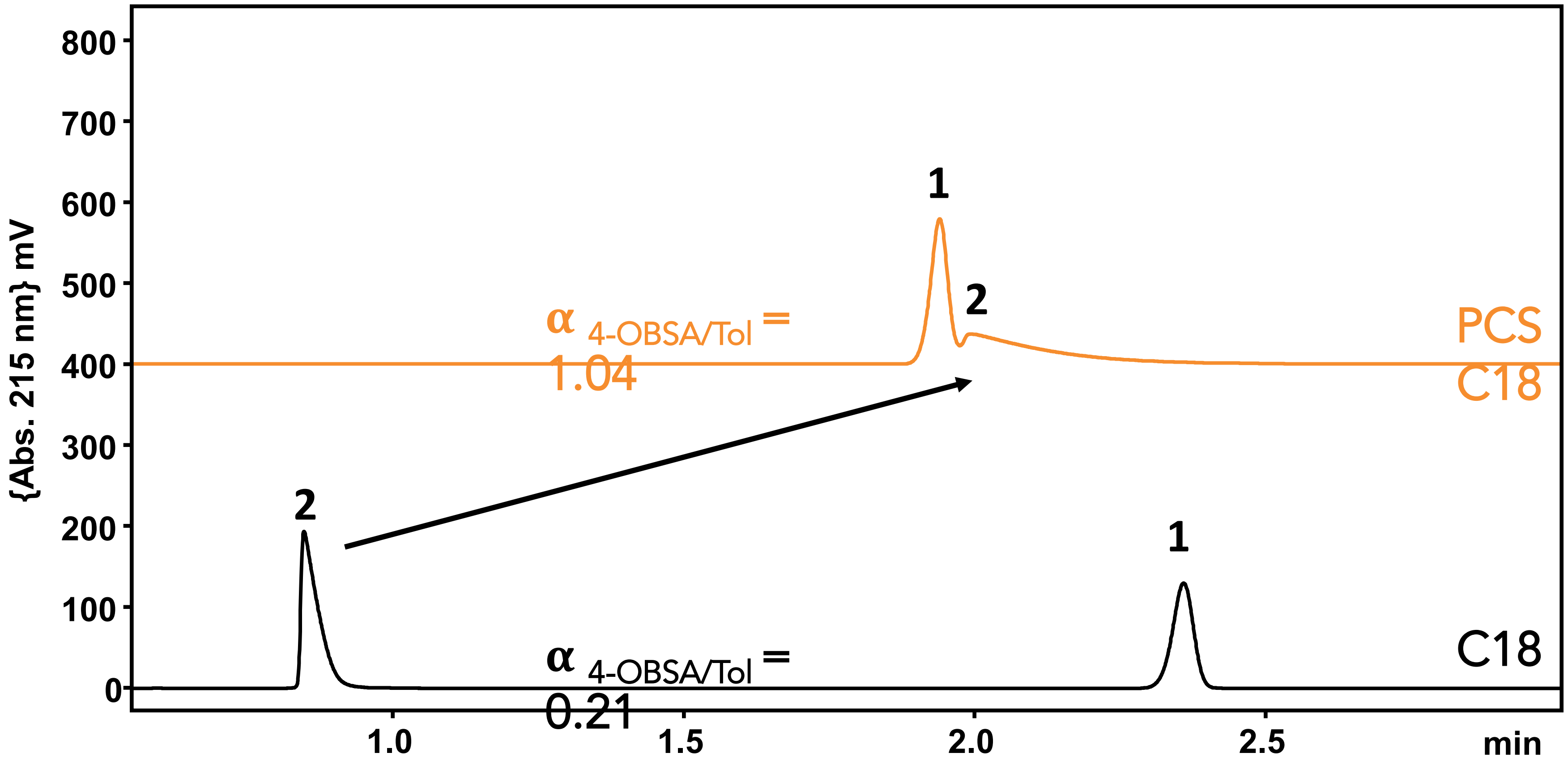

Ionized Basic Solute Overload

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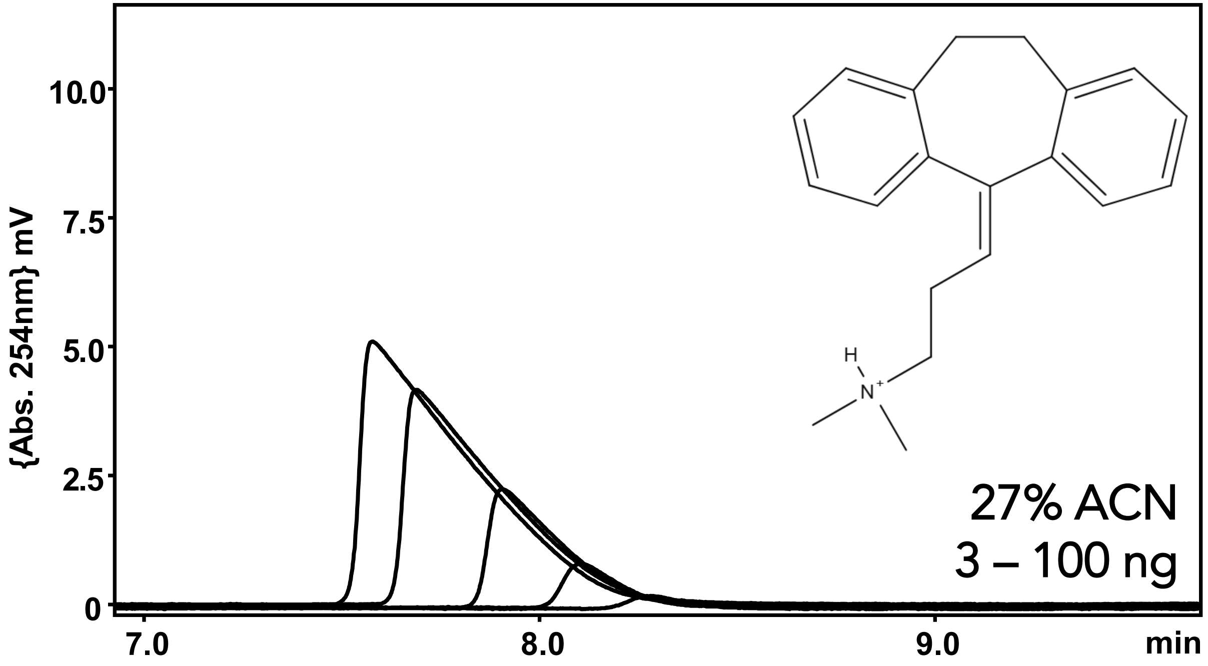

"Neutral" - 90Å C18 2.7μm SPP

Overload [Base] <<<< [Neutral]

Non-linear chromatography

Tailing Factor ↑ (1.50 → 5.43)
Plates, N ↓ (17,610 → 3,423)
Retention Factor, k' ↓ (11.1 → 10.1)