# Evaluation of Positively Charged Surface Phenyl-Hexyl Stationary Phase for Separation of Basic Small Molecule Drugs

William L. Miles, Barry E. Boyes, Ph.D.

Advanced Materials Technology, Inc.


HPLC 2024

July 25th, 2024

# Basic Analyte Challenges in RP-LC

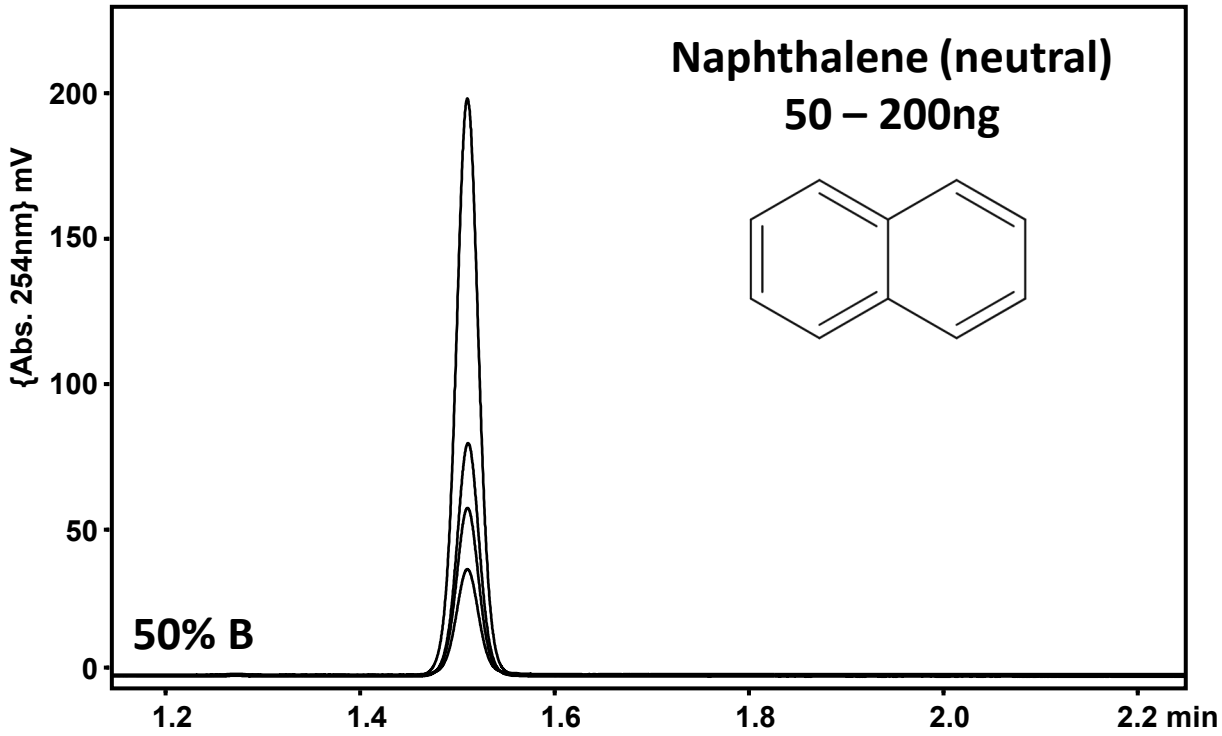
Achieving good peak shapes of ionized analytes by reversed-phase liquid chromatography (RP-LC) can be difficult

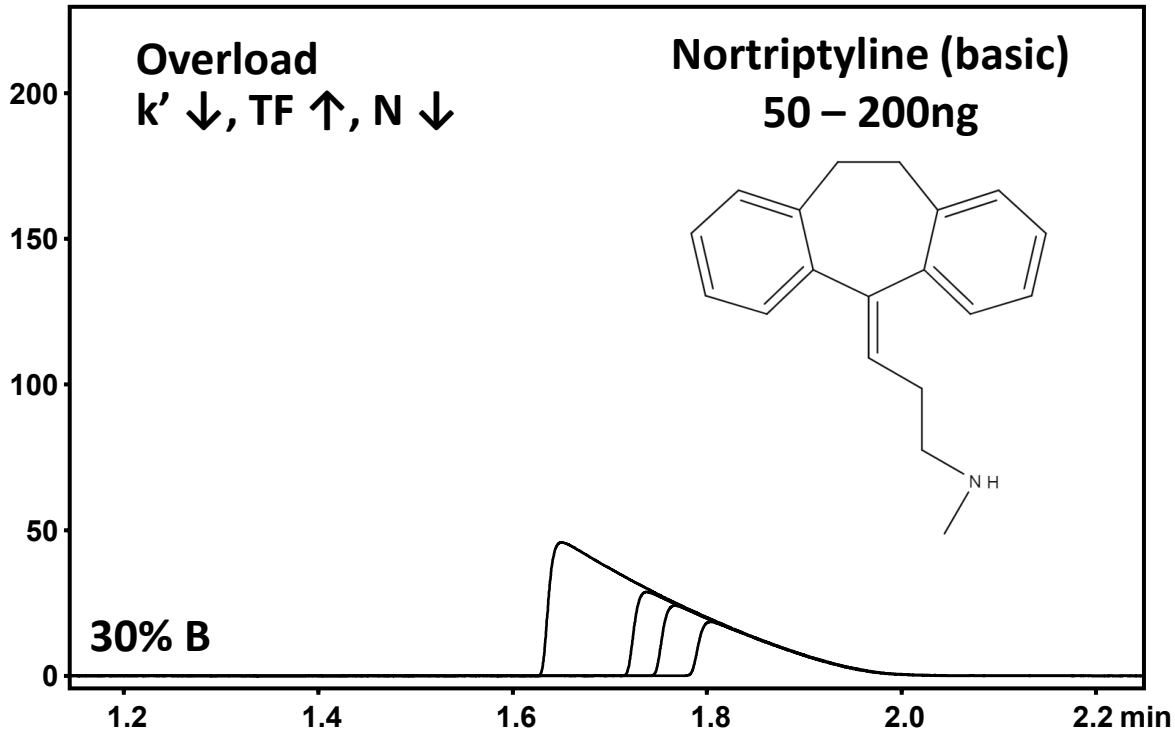
- Overload at low mass loads
- High asymmetry and low efficiencies
- Does match performance of neutral or non-ionized molecules

When using low ionic strength mobile phases, like those favored in liquid chromatography-mass spectrometry (LC-MS), these effects are worsened

# Neutral vs Basic Peak Shape

**90Å SPP C18 2.7µm, 2.1x100mm**
**MP A = H2O + 0.1% formic acid, MP B = ACN + 0.1% formic acid**
**x% B, 0.50mL/min, 35C, 1.0 µL inj, 254nm**



# Theories on Basic Analyte Overloading

Heterogeneous adsorption of basic solutes onto stationary-phase [1, Gritti et al. 2014]

Low Mass Loads: Adsorption onto a small number of high energy sites near the surface [1]

Higher Mass Loads: Adsorption onto a larger number of low energy sites, solute-solute repulsion [1]

Mutual repulsion of basic solutes adsorbed to bonded-phase [2, McCalley 2003]

[1] F. Gritti, G. Guiochon, Effects of the surface concentration of fixed charges in C18-bonded stationary phases on the adsorption process and on the preparative chromatography of small ionizable compounds, Journal of Chromatography A 1372 (2014) 42–54. https://doi.org/10.1016/j.chroma.2014.10.003.

[2] D.V. McCalley, Rationalization of Retention and Overloading Behavior of Basic Compounds in Reversed-Phase HPLC Using Low Ionic Strength Buffers Suitable for Mass Spectrometric Detection, Anal. Chem. 75 (2003) 3404–3410. https://doi.org/10.1021/ac020715f.

# Mobile Phases Considerations

## Common modifications to improve basic peak shapes in LC-UV

- Increase mobile phase pH
- Ion pairing reagents (trifluoroacetic acid)
- Increase ionic strength (ammonium formate, phosphate buffer)

## LC-MS is commonly used for analysis of pharmaceuticals

- Impurities, unknowns, and metabolites

## High sensitivity LC-MS (ESI) analysis require volatile, low ionic strength mobile phases for efficient ionization

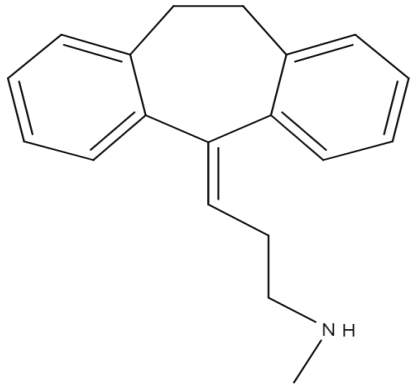
- 0.1% formic acid preferred

# Basic Small Molecule Pharmaceuticals

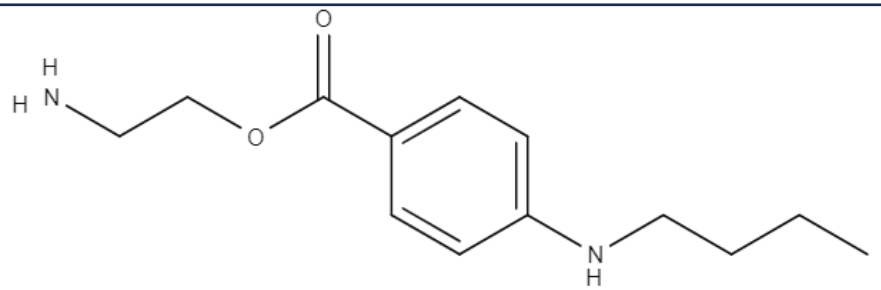
## Many small molecule pharmaceuticals contain basic functional groups

## Neutral and acidic pH separations (RP-LC)

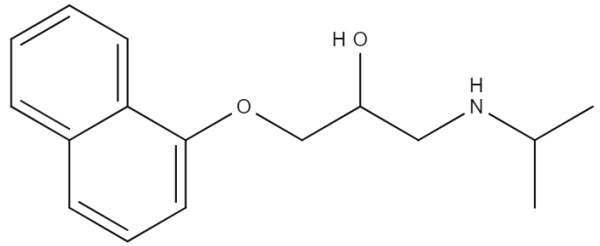
- Basic groups will be protonated, positive charge




**Tricyclic Antidepressants**
**Nortriptyline, 2°, pKa = 10.1**




**Local Anesthetics**
**Tetracaine, 1°, pKa = 8.5**




**Beta-Blockers**
**Propranolol, 2°, pKa = 9.5**

# Bonded-Phase Technology

## Addition of a positively charged ligand to the silica particle surface can improve basic peak shape

- Positive charge under acidic conditions
- Repulses cationic solutes, screens from negative interactions

## Many versions of this technology have been described

- Commonly found with C18 bonded-phase, on FPP and SPP's

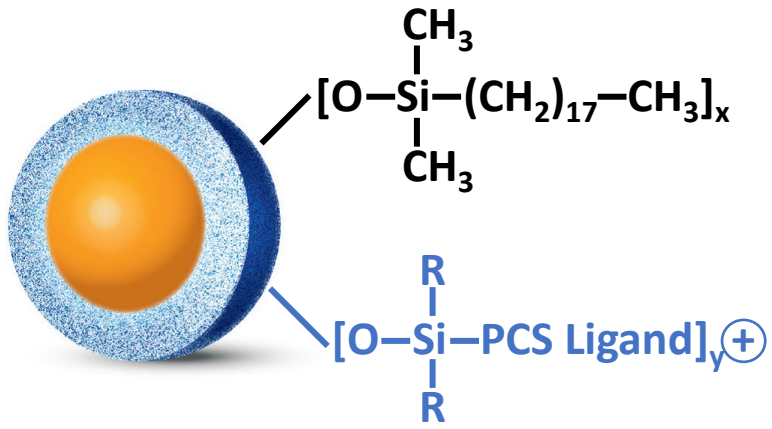
## These are reversed-phase chromatographic columns

- Not ion-pairing or mixed-mode

# Bonded-Phase Technology: Positively Charged Surface

## 90Å HALO PCS C18 2.7µm

- Superficially porous particle
- 125 $m^2/g$ surface area
- End capped




## Careful selection of the PCS ligand

- Correct ligand chemistry and coverage (µmol/$m^2$)
- [C18] > [PCS]

## Designed for separations of basic small molecules, using low ionic strength mobile phases

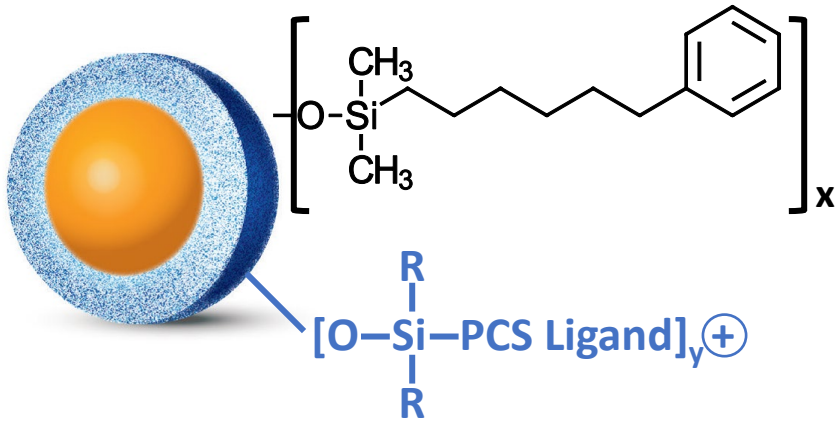
# Change in Primary Ligand: Phenyl-Hexyl

## HALO® PCS technology: Phenyl-Hexyl

- Common RP-LC bonded-phase
- Aromatic nature, with alternative selectivity

## 90Å HALO PCS Phenyl-Hexyl 2.7µm

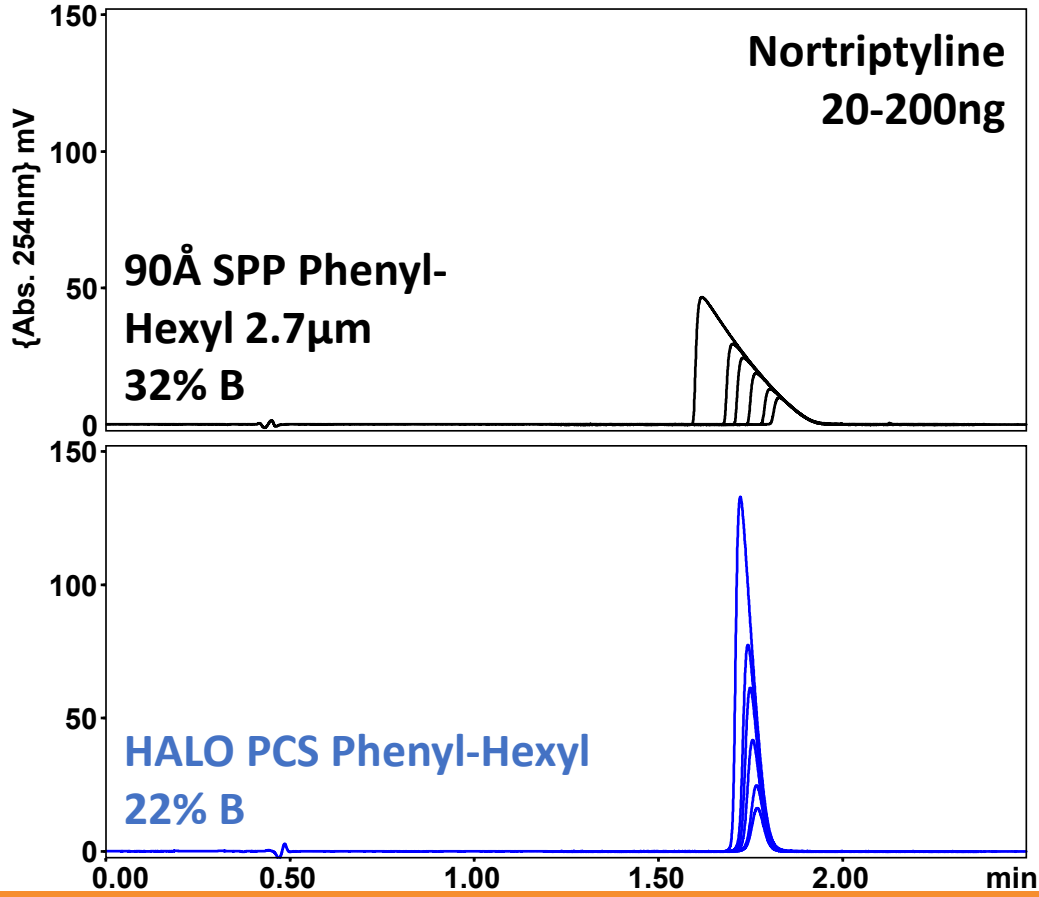
- Superficially porous particle
- 125 $m^2/g$ surface area
- End capped

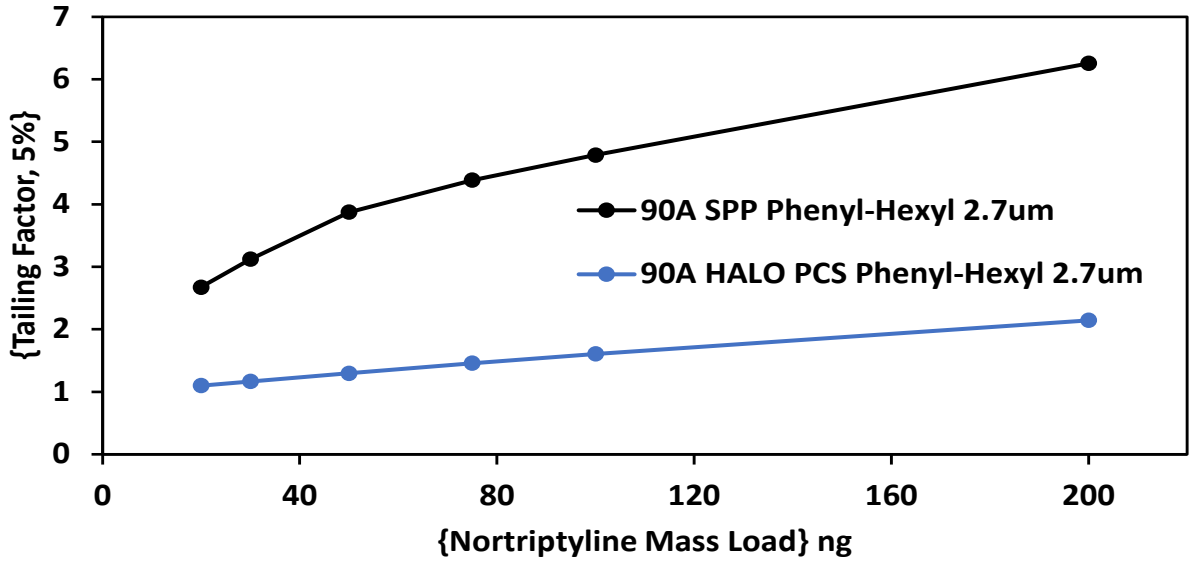



## Unique combination of SPP + positively charged ligand + Phenyl-Hexyl

# Improvements with HALO® PCS Phenyl-Hexyl

**2.1x100mm, x% B, 0.50mL/min, 35C, 1.0 µL inj, 254nm**
**MP A = H2O + 0.1% formic acid, MP B = ACN + 0.1% formic acid**




**Addition of positively charged ligand:**

- Improvements in peak tailing and plates
- Decrease in retention times
- PCS Phenyl-Hexyl and C18

# Evaluation of HALO® PCS Phenyl-Hexyl

## Separations of basic pharmaceuticals

- Differences in selectivity
- Load tolerance performance
- Imipramine applications (spiked and standard impurity)

## Comparisons “In-class”

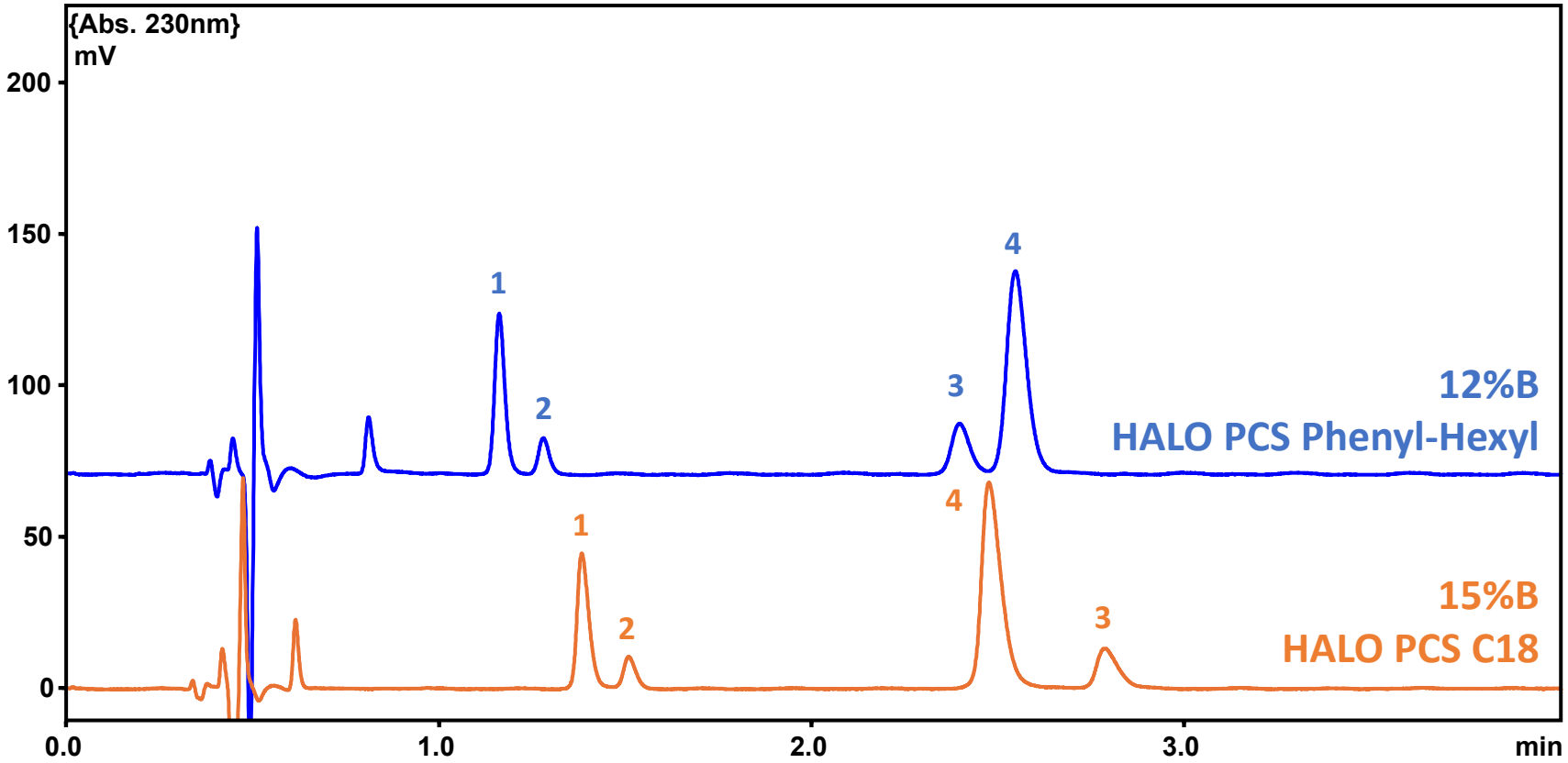
- Commercially available SPP’s with C18 bonded-phase and a charged surface modification
- 90-100Å pore size, 2.7µm particle size
- 2.1x100mm columns

## Mobile Phases: H2O and ACN with 0.1% formic acid

## Shimazu Nexera UHPLC PDA

# Changes in Basic Analyte Selectivity

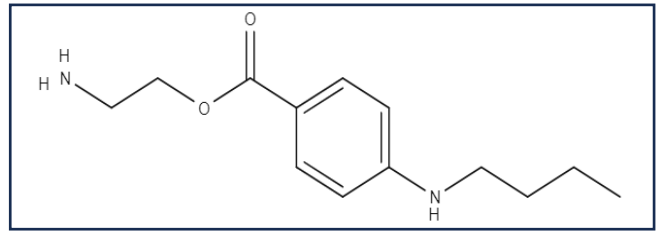
**2.1x100mm, x% B, 0.50mL/min, 30C, 1.0 µL inj, 230nm**
**MP A = H2O + 0.1% formic acid, MP B = ACN + 0.1% formic acid**



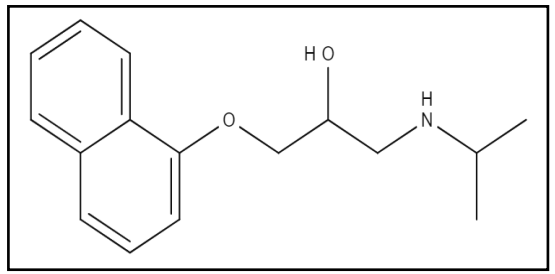

## Mixture of Beta-Blockers and Local Anesthetics (0.030 mg/mL)

## Peak Identities

1. Bisoprolol (beta-blocker)
2. Bupivacaine (local anesthetic)
3. Tetracaine (local anesthetic)

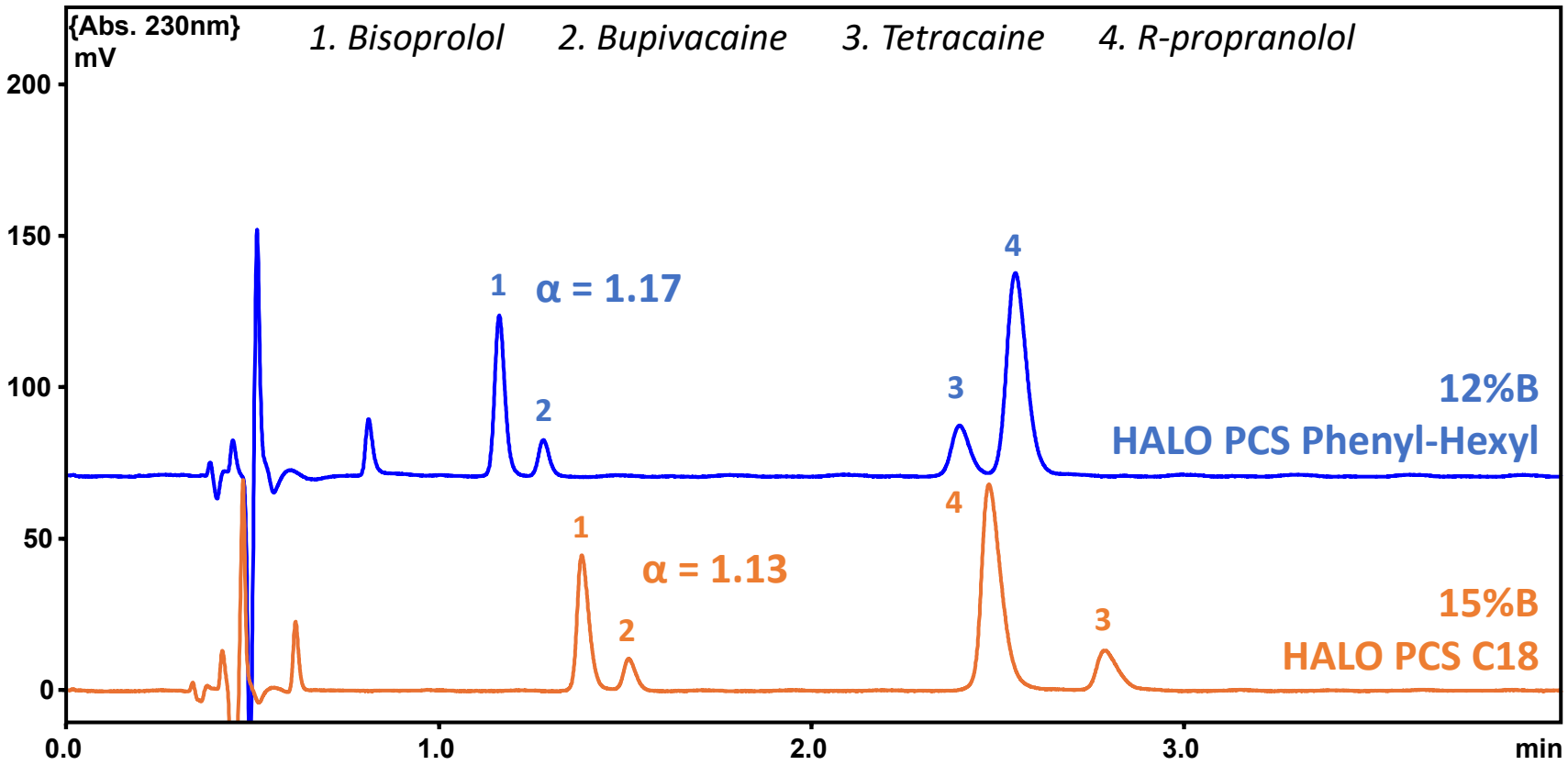


4. R-propranolol (beta-blocker)



# Changes in Basic Analyte Selectivity

**2.1x100mm, x% B, 0.50mL/min, 30C, 1.0 µL inj, 230nm**
**MP A = H2O + 0.1% formic acid, MP B = ACN + 0.1% formic acid**




**Mixture of Beta-Blockers and Local Anesthetics (0.030 mg/mL)**

**Retention:**

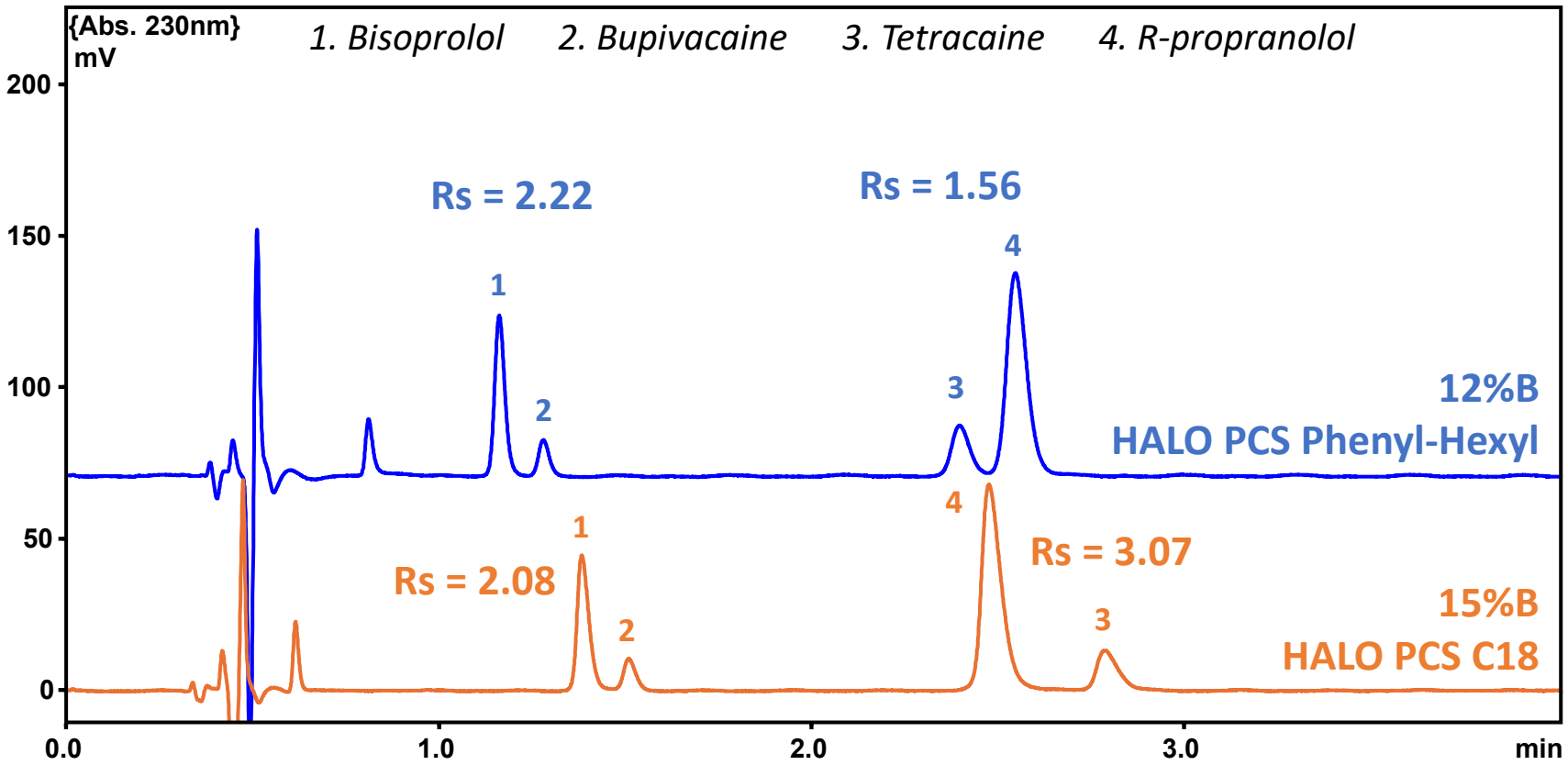
- Adjusted ACN %, closer Rt match

**Selectivity (α):**

- Retention order switch peaks 3 & 4
- Small change for peaks 1 & 2

# Changes in Basic Analyte Selectivity

**2.1x100mm, x% B, 0.50mL/min, 30C, 1.0 µL inj, 230nm**
**MP A = H2O + 0.1% formic acid, MP B = ACN + 0.1% formic acid**



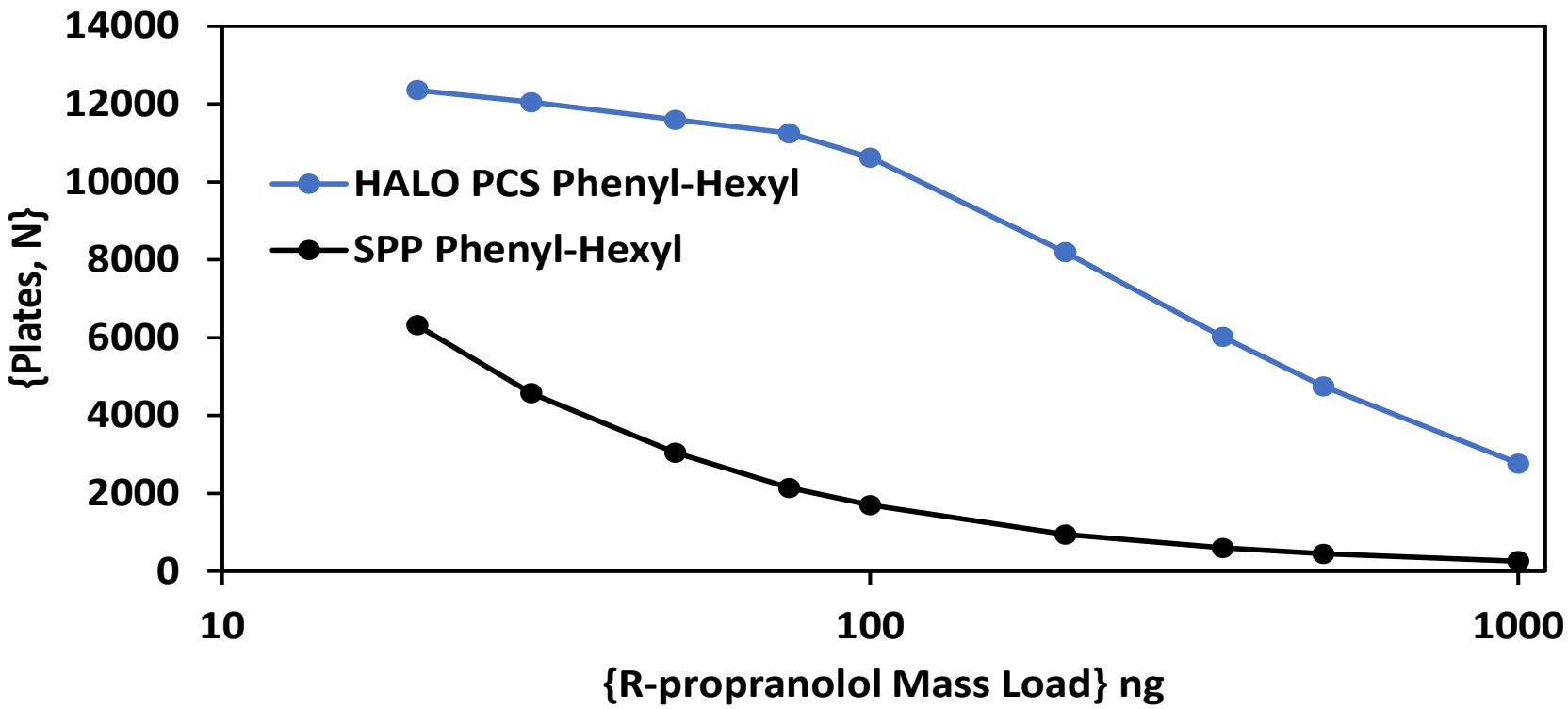

**Mixture of Beta-Blockers and Local Anesthetics (0.030 mg/mL)**

**Resolution (Rs):**

- Baseline resolution for all pairs
- Changes in selectivity lead to changes in resolution

# R-propranolol Load Tolerance

**2.1x100mm, x% B, 0.50mL/min, 35C, 1.0 µL inj, 280nm**
**MP A = H2O + 0.1% formic acid, MP B = ACN + 0.1% formic acid**




**20 to 1,000 ng injected**

**Retention Factor, k' 20ng**

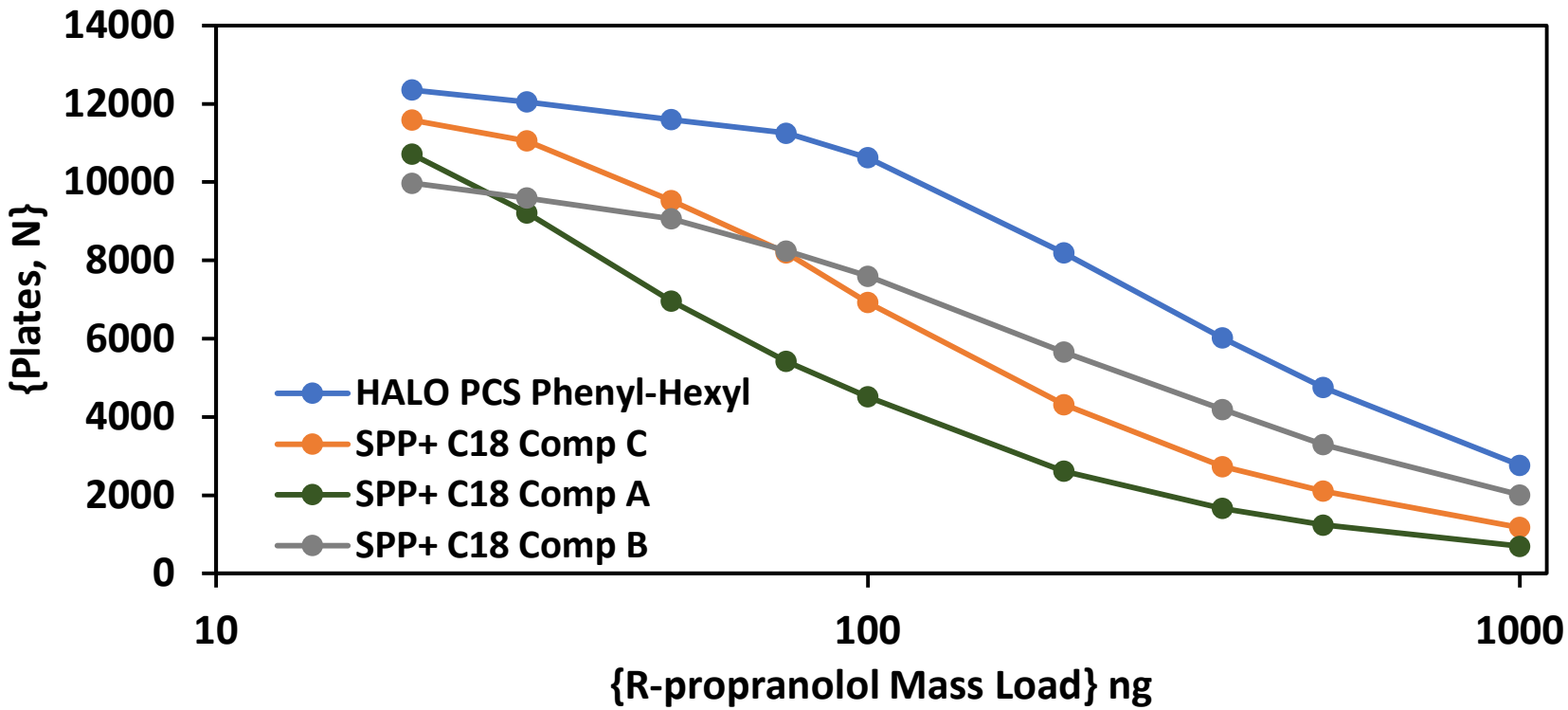
- HALO®PCS Phenyl-Hexyl = 3.18
- SPP Phenyl-Hexyl = 3.27

**7x increase in $N_{50\%}$ loss**

- HALO®PCS Phenyl-Hexyl= 339 ng
- SPP Phenyl-Hexyl = 49 ng

# R-propranolol Load Tolerance

**2.1x100mm, x% B, 0.50mL/min, 35C, 1.0 µL inj, 280nm**
**MP A = H2O + 0.1% formic acid, MP B = ACN + 0.1% formic acid**




**Retention factor, k' 20ng**

- HALO PCS Phenyl-Hexyl = 3.18
- SPP+ C18 Comp A = 2.99
- SPP+ C18 Comp B = 3.44
- SPP+ C18 Comp C = 3.29

**Overload seen for all columns as mass load increased (still ionized)**

**HALO PCS Phenyl-Hexyl demonstrates highest column efficiencies across entire range**

# Load Tolerance Comparison

**2.1x100mm, x% B, 0.50mL/min, 35C, 1.0 µL inj, 280nm**
**MP A = H2O + 0.1% formic acid, MP B = ACN + 0.1% formic acid**

| Column | R-propranolol | | Nortriptyline | |
|---|---|---|---|---|
| | N 20ng | {$N_{50\%}$} ng | N 20ng | {$N_{50\%}$} ng |
| HALO PCS Phenyl-Hexyl | 12357 | 339 | 12727 | 208 |
| SPP+ C18 Comp A | 10713 | 77 | 10364 | 63 |
| SPP+ C18 Comp B | 9972 | 269 | 9938 | 185 |
| SPP+ C18 Comp C | 11584 | 143 | 11868 | 112 |

**Retention factors matched**

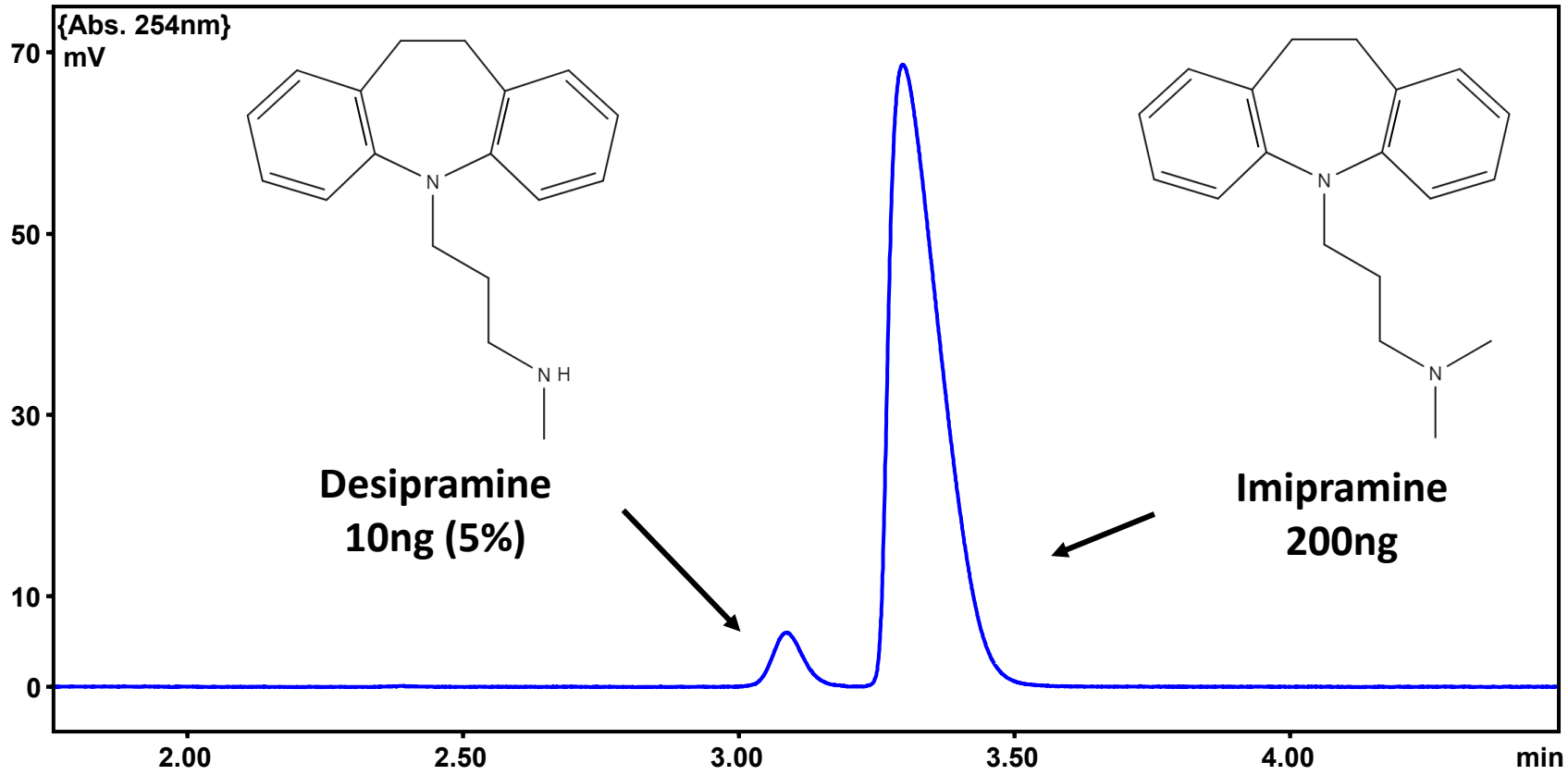
**Similar curves, but analyte dependence (propranolol > nortriptyline)**

**HALO® PCS Phenyl-Hexyl**

- Provides highest column efficiencies at all tested load
- Largest $N_{50\%}$ values for both analytes

# Imipramine Spiked Impurity Analysis

**2.1x100mm, 90Å HALO PCS Phenyl-Hexyl 2.7µm,**
**17% B, 0.50mL/min, 35C, 1.0 µL inj, 254nm**
**MP A = H2O + 0.1% formic acid, MP B = ACN + 0.1% formic acid**




**Isocratic separation of imipramine and closely related impurity**

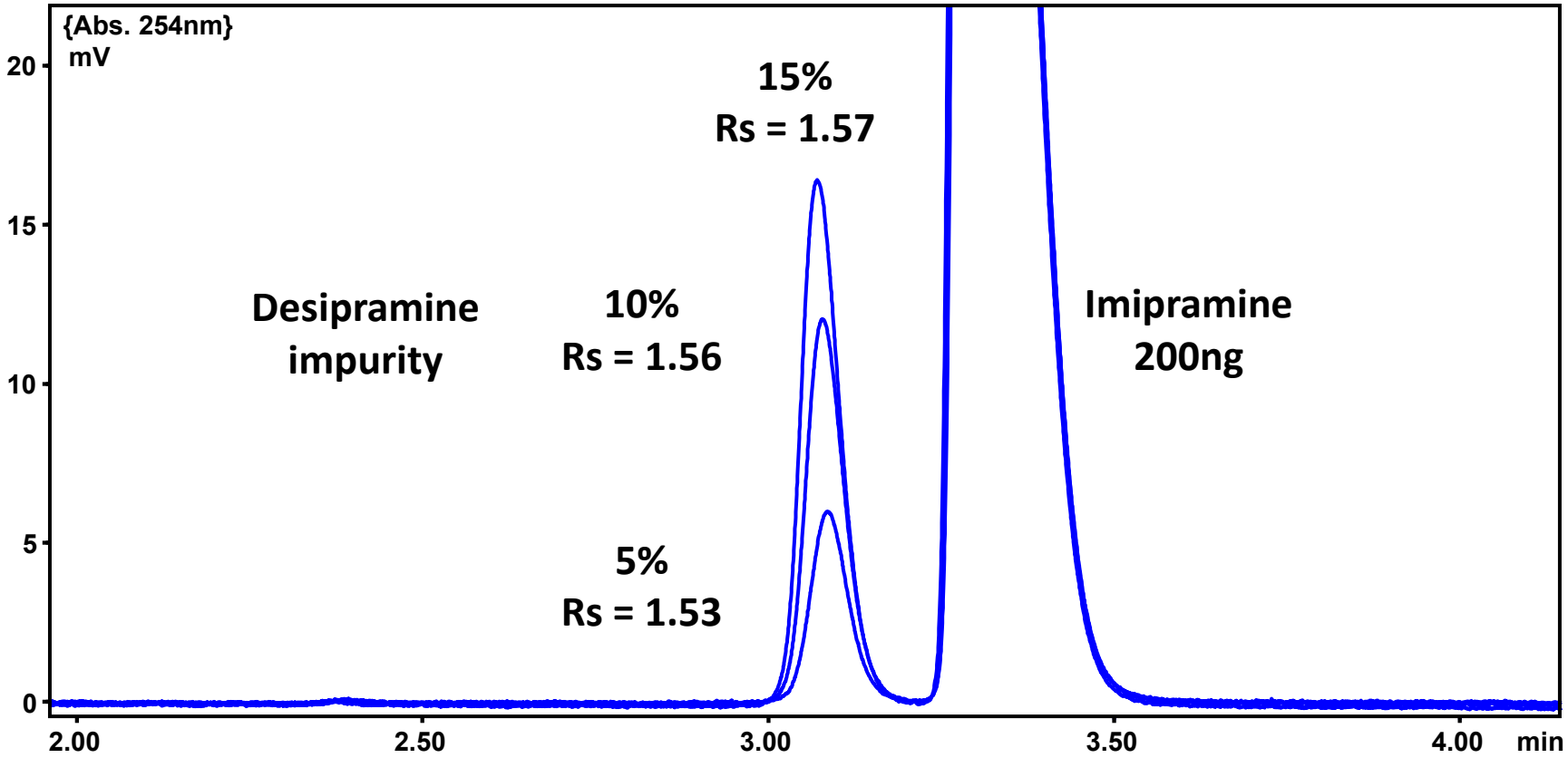
**Desipramine = N-demethylation impurity**

**Moderately high load**

- Tailing and loss of efficiency expected for imipramine

# Imipramine Spiked Impurity Analysis

**2.1x100mm, 90Å HALO PCS Phenyl-Hexyl 2.7µm,**
**17% B, 0.50mL/min, 35C, 1.0 µL inj, 254nm**
**MP A = H2O + 0.1% formic acid, MP B = ACN + 0.1% formic acid**



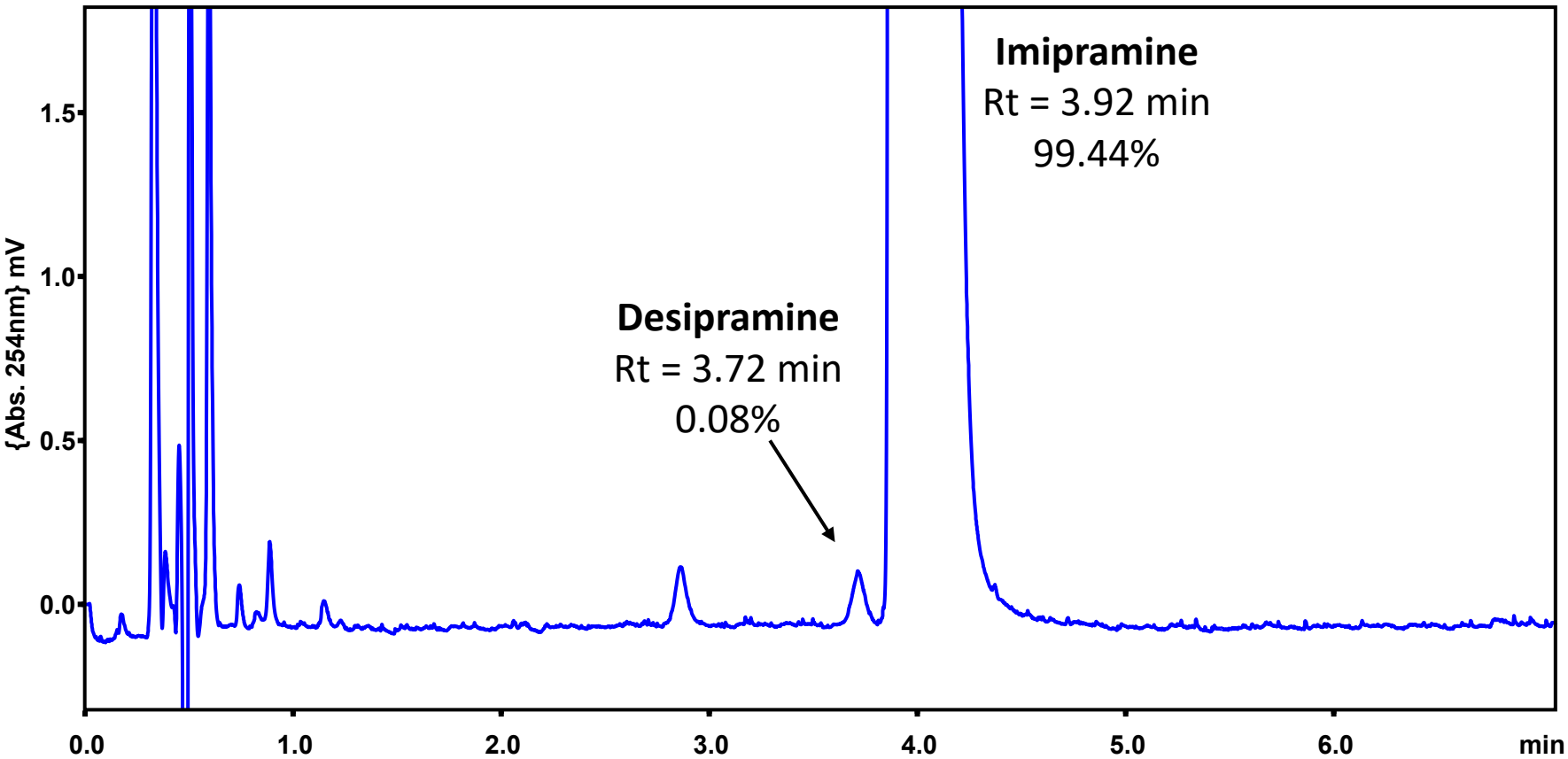

**Isocratic separation (17% ACN) of imipramine and closely related impurity**

**Baseline resolution is maintained as impurity concentration increased**

**Advantage of improved column efficiencies over a range of mass**

# Imipramine Impurity Analysis LC-PDA

**2.1x100mm, 90Å HALO PCS Phenyl-Hexyl 2.7µm,**
**16% B, 0.50mL/min, 35C, 2.0 µL inj, 254nm**
**MP A = H2O + 0.1% formic acid, MP B = ACN + 0.1% formic acid**




**LC-PDA Isocratic Separation**

**400ng inj. imipramine standard**

**Percent Area**

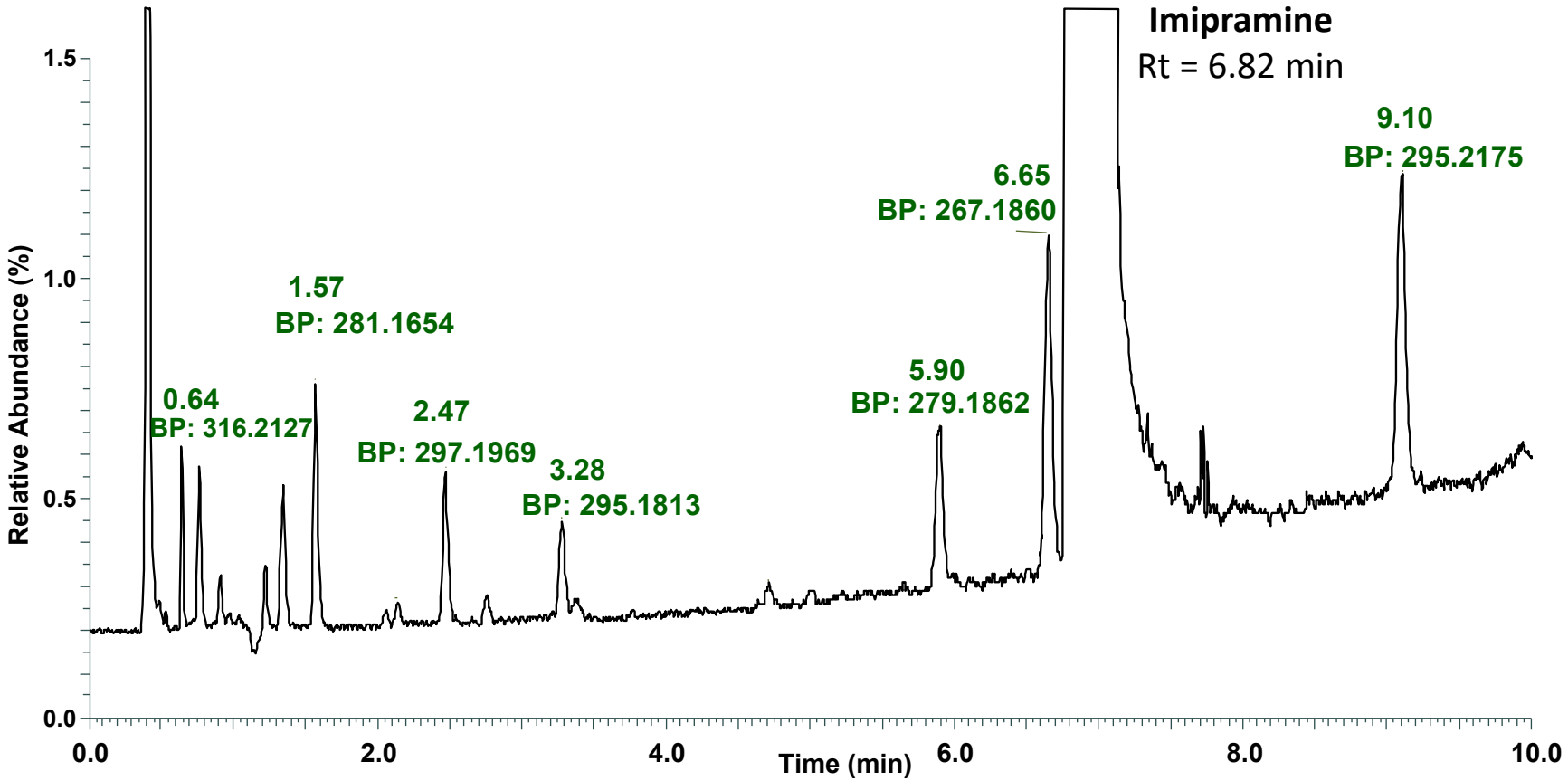
- 99.44% imipramine
- 0.56% impurities (0.01 – 0.33 %)
- Desipramine identified

**Improved load tolerance allows for detection of trace impurities**

# Imipramine Impurity Analysis LC-MS

**2.1x100mm, 90Å HALO PCS Phenyl-Hexyl 2.7µm,**
**10-25% B in 10min, 0.40mL/min, 35C, 2.0 µL inj,**
**MP A = H2O + 0.1% formic acid, MP B = ACN + 0.1% formic acid**

**Thermo Q Exactive Orbitrap HF, ESI: 3.3 kV, Tcap 320 C, Rs 45,000, 50 ms; ddMS$^2$ NCE 30%, Rs 30,000, 50 ms**




**LC-MS Gradient elution method (400ng)**

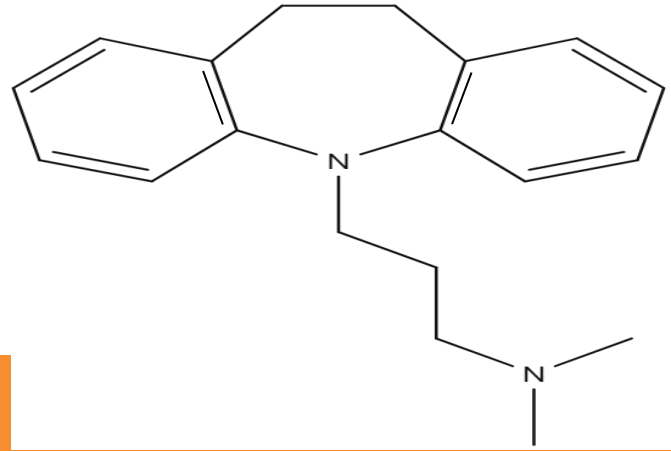
**Impurities ≤ 1% rel. abundance**

**High resolution accurate mass**
- Measuring exact mass
- Formula match impurities

**MS$^2$ Experiment**
- Structural details of impurities

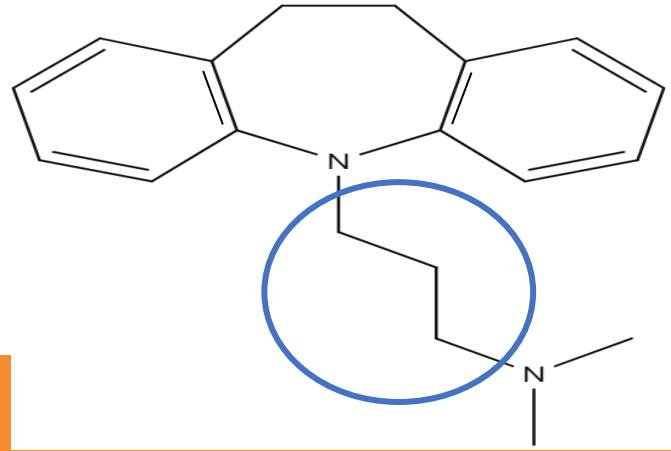
# Imipramine Impurity Analysis LC-MS

| Peak | {Rt} min | Measured m/z | Formula Match | Functional Group | $MS^2$ Confirmation |
|---|---|---|---|---|---|
| **Imipramine** | **6.86** | **281.2009** | **$C_{19}H_{25}N_2$** | **[Imip]** | **Y** |
| 1-4 | 0.76 – 1.57 | | | No TCA | N |
| 5 | 2.47 | 297.1969 | $C_{19}H_{25}N_2O$ | [Imip] +O, -OH alkyl | Y |
| 6 | 3.27 | 295.1815 | $C_{19}H_{23}N_2O_1$ | No TCA | N |
| 7 | 5.90 | 279.1863 | $C_{19}H_{23}N_2$ | [Imip] -2H, C=C alkyl | Y |
| Desipramine | 6.65 | 267.1860 | $C_{18}H_{23}N_2$ | [Imip] – $CH_3$, N-methyl | Y |
| 8 | 9.10 | 295.2175 | $C_{20}H_{27}N_2$ | [Imip] + $CH_2$ (butylamine) | Y |



# Imipramine Impurity Analysis LC-MS

| Peak | {Rt} min | Measured m/z | Formula Match | Functional Group | $MS^2$ Confirmation |
|---|---|---|---|---|---|
| **Imipramine** | **6.86** | **281.2009** | **$C_{19}H_{25}N_2$** | **[Imip]** | **Y** |
| 1-4 | 0.76 – 1.57 | | | No TCA | N |
| 5 | 2.47 | 297.1969 | $C_{19}H_{25}N_2O$ | [Imip] +O, -OH alkyl | Y |
| 6 | 3.27 | 295.1815 | $C_{19}H_{23}N_2O_1$ | No TCA | N |
| 7 | 5.90 | 279.1863 | $C_{19}H_{23}N_2$ | [Imip] -2H, C=C alkyl | Y |
| Desipramine | 6.65 | 267.1860 | $C_{18}H_{23}N_2$ | [Imip] – $CH_3$, N-methyl | Y |
| 8 | 9.10 | 295.2175 | $C_{20}H_{27}N_2$ | [Imip] + $CH_2$ (butylamine) | Y |



# Conclusions

## Changes in selectivity for HALO® PCS Phenyl-Hexyl compared to in-class C18's

- Another versatile option for separations

## Improvements in column efficiencies over a large mass range for basic small molecule pharmaceuticals

- Phenyl-Hexyl + positively charged surface appears to be favorable for separations of these aromatic, basic pharmaceuticals
- Impurities resolution and trace impurities

## Addition of positively charged ligand to the silica surface greatly improves basic peak shape

- LC-MS preferred mobile phases

# Acknowledgements

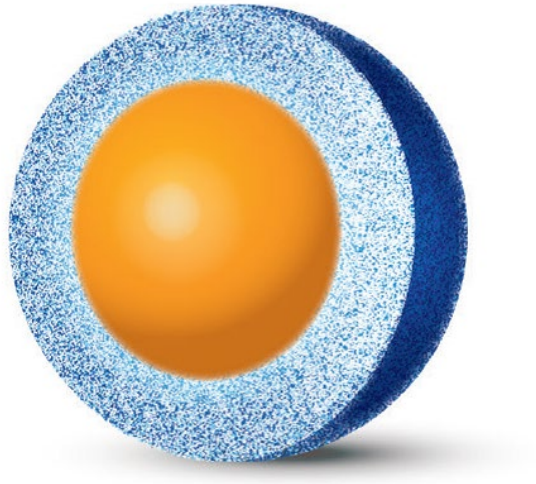

**Research and Development Team**
- Dr. Joesph DeStefano
- Timothy Langlois
- Mark Haynes

**Applications**
- Dr. Stephanie Schuster
- Conner McHale

**Engineering Team**
- Aggie Cesbron
- Kristen O'Donnell
- Matt Jackson
- Bryant McKnight

# Thank you for your time today!

## Questions



# advancedmaterialstechnology


halocolumns.com

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

(302) 992-8060


ISO 9001 QMS certified
All operations in Wilmington, DE
Global distribution