# Novel Hydrophilic Bonded-Phase Fused-Core® Particles for Highly Efficient HILIC Separations

Barry E. Boyes[1,2], Timothy Langlois, Joseph J. DeStefano and Joseph J. Kirkland

1) Advanced Materials Technologies, Inc., Wilmington, DE USA;
2) Complex Carbohydrate Research Center, UGA, Athens, GA USA

# Fused Core Bonded Phase HILIC

## Opportunities

- HILIC has many potential advantages
- HILIC separation speed and robustness
- Bonded phases for broadened applications

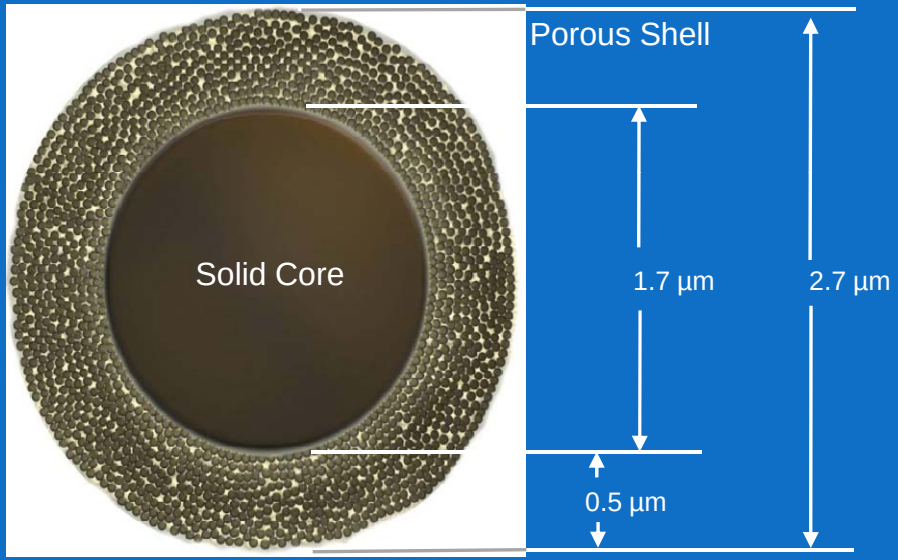
## Current State

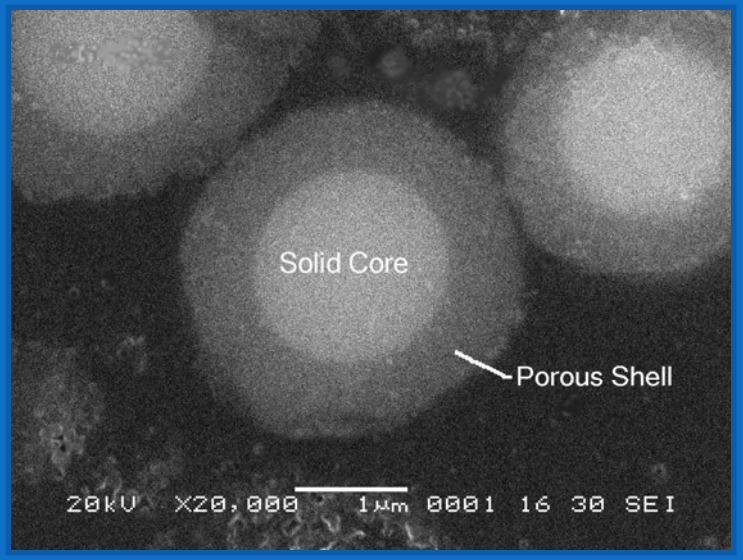
- Superficially porous particles can have strong performance advantages
- Silica HILIC has limitations
- HILIC bonded phases on superficially porous particles have a challenge of retention due to phase ratio available

## Development and characterization of Fused-Core BPs

- HILIC retention is complex
- Highly hydroxylic bonded phase is appealing
- Polymeric bonded phases likely to limit mass transfer kinetics – prefer use of a monolayer silane
- Maximizing Performance: Retention, Selectivity, Efficiency, Stability, Reproducibility

# Superficially Porous (Fused-Core) Particles

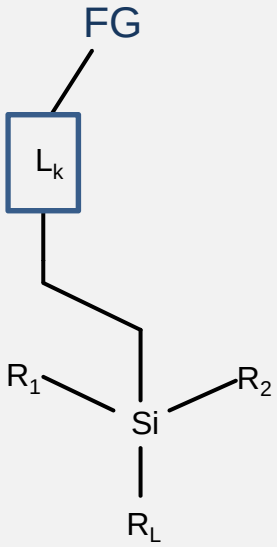



SEM Particle Cross-section

| x (µm) | y (µm) | $d_p$ (µm) | Pore Size (Å) | Surface Area ($m^2/g$) |
|---|---|---|---|---|
| 1.7 | 0.5 | 2.7 | 90 | 130 |
| 1.7 | 0.5 | 2.7 | 160 | 80 |

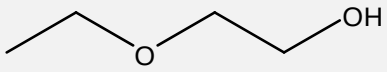
# *Silane Reagents for Hydrophilic Functional Groups*



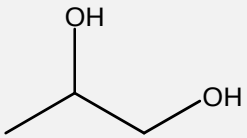

$R_L$ = silanol reactive leaving
$R_1 = R_2$ or $R_1 \neq R_2$, $R_1 = R_L$
Eg. -Me, -OMe, -Cl, -DiiPr

## *Hydroxylic Functional Groups*

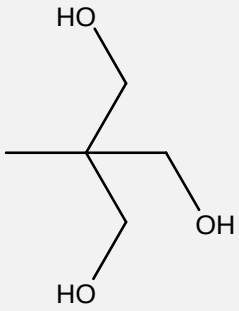
Mono-OL



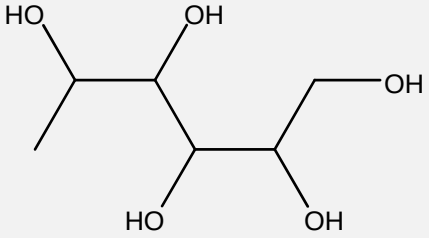

Di-OL




Tri-OL

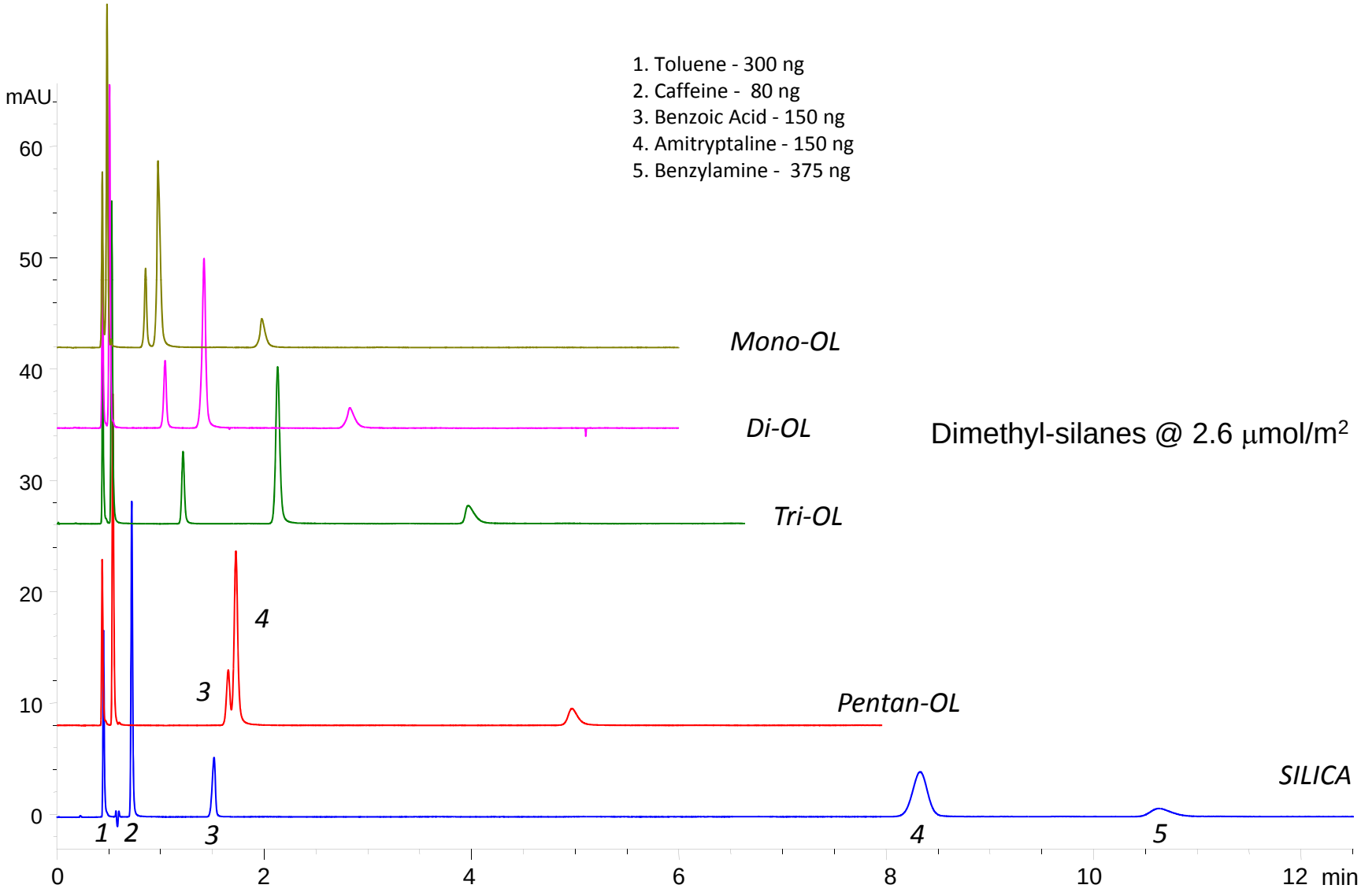



Pentan-OL



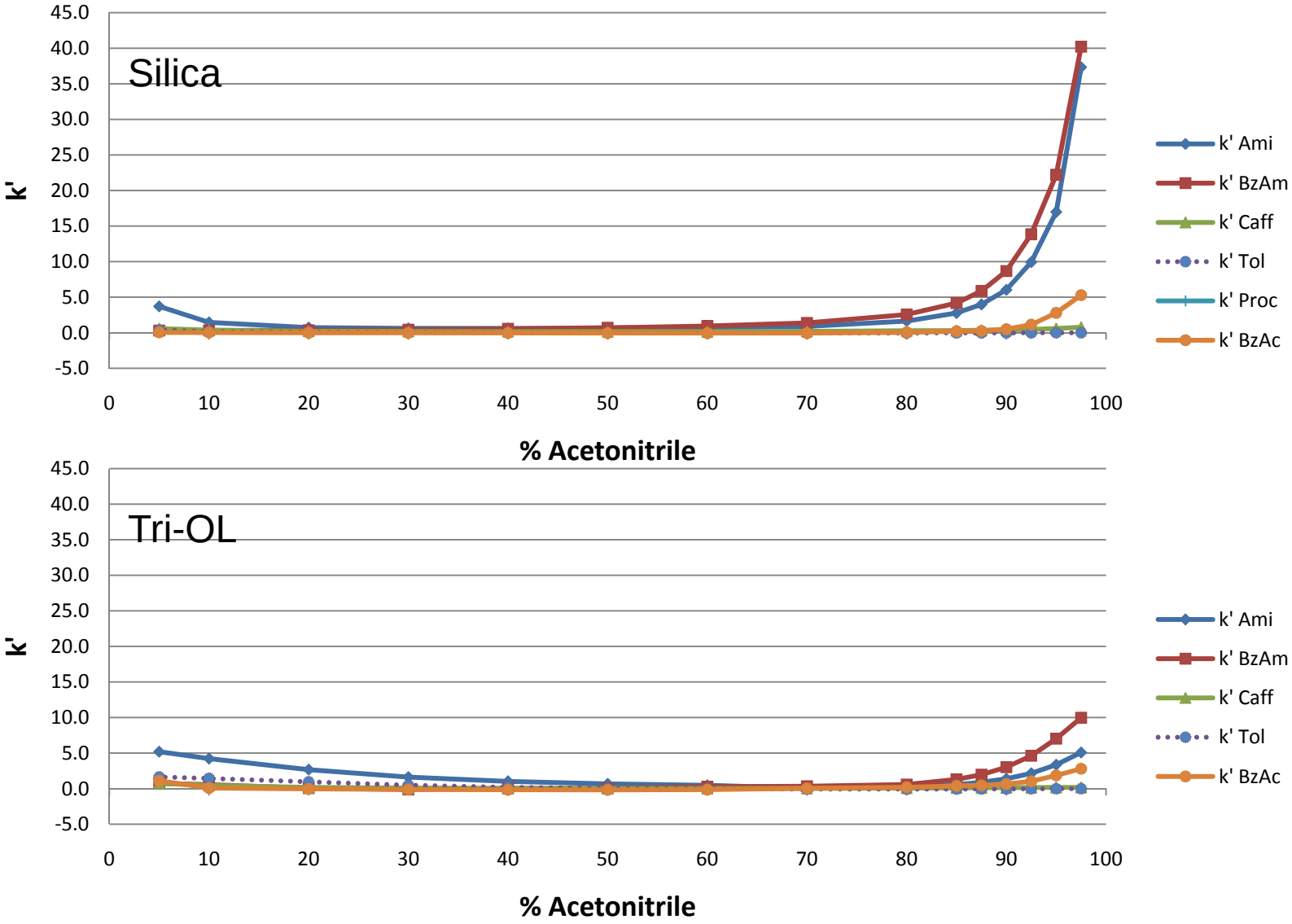
# *Effect of Surface Functional Groups on a HILIC Separation*

## *95% Acetonitrile/5 mM $NH_4OAc$, pH 4.0: 2.1 mm ID x 100 mm, 25 °C, 0.5 mL/min*



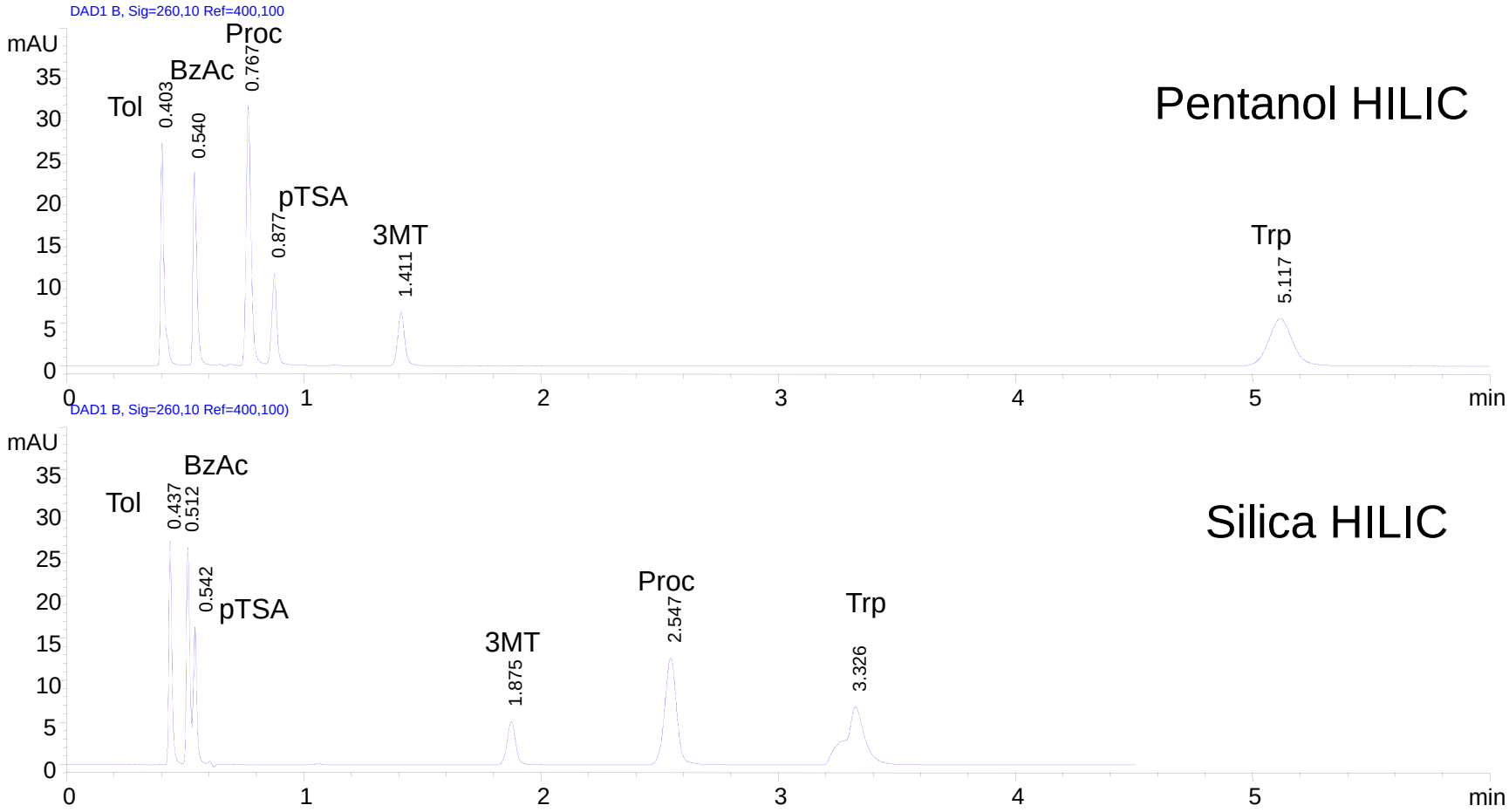
# *Effect of Mobile Phase Organic Composition on Retention*

## *5 mM $NH_4$Acetate, pH 4.0: 2.1 mm ID x 100 mm, 25 °C, 0.5 mL/min*



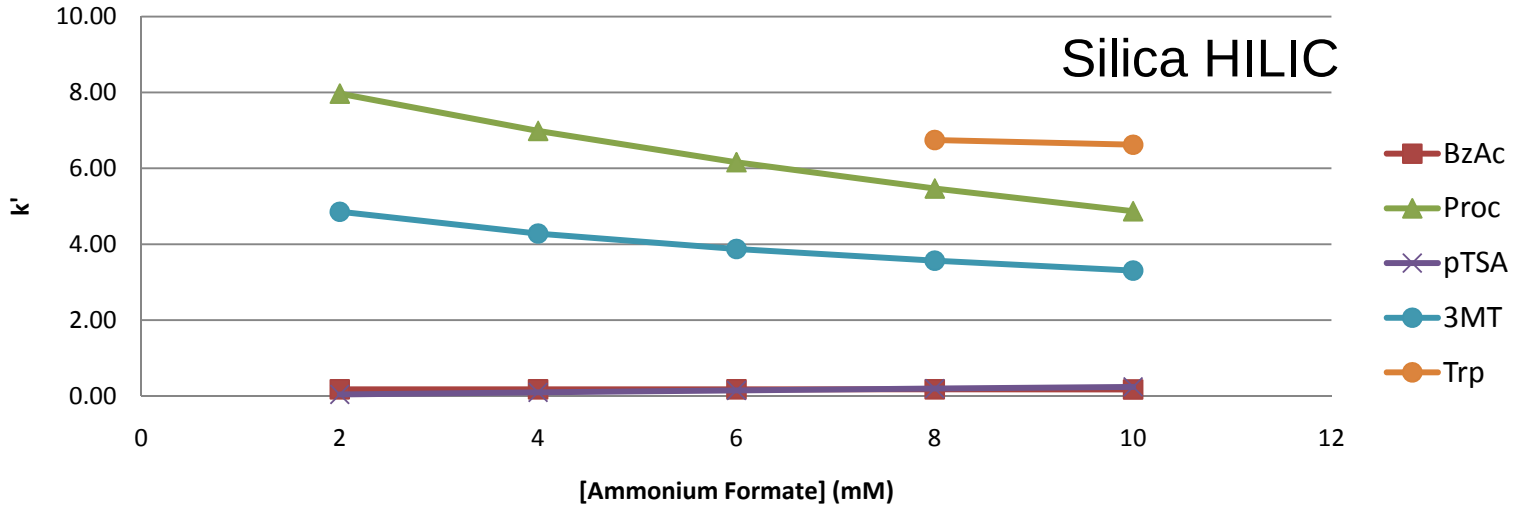
# *HILIC Separation of A/B/Z on Silica and Pentanol Silane Surface*

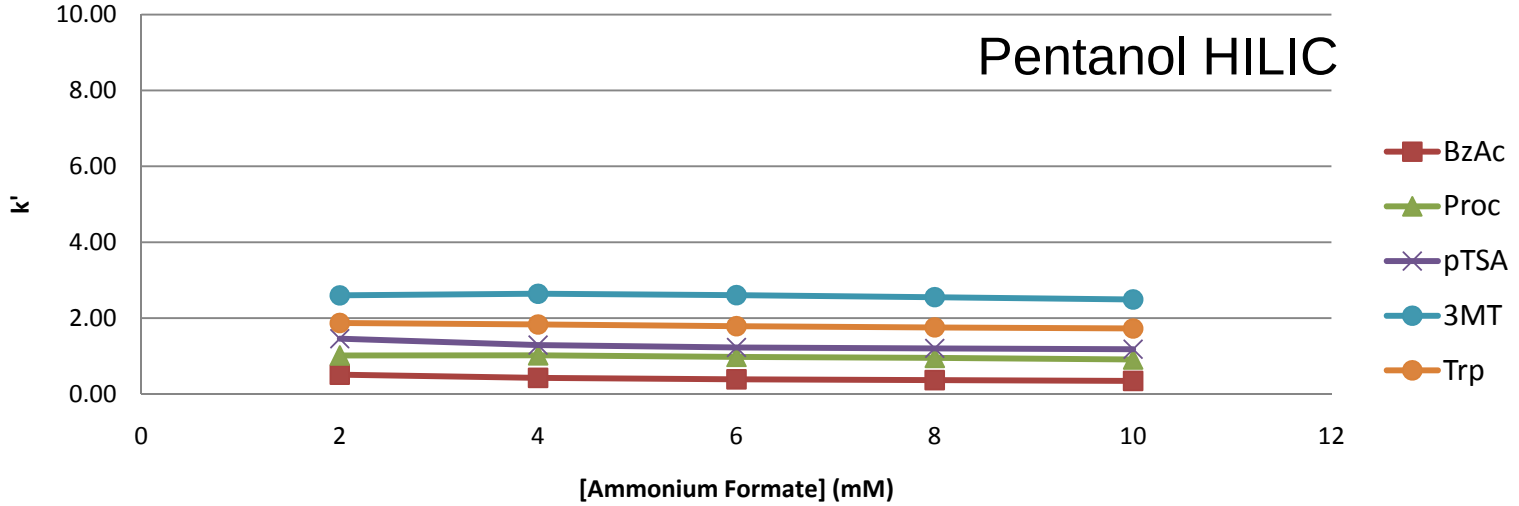
## *90% Acetonitrile/10 mM $NH_4$Form, pH 3.0: 2.1 mm ID x 100 mm, 25 °C, 0.5 mL/min*



# *Effect of Buffer Concentration on HILIC Separations*

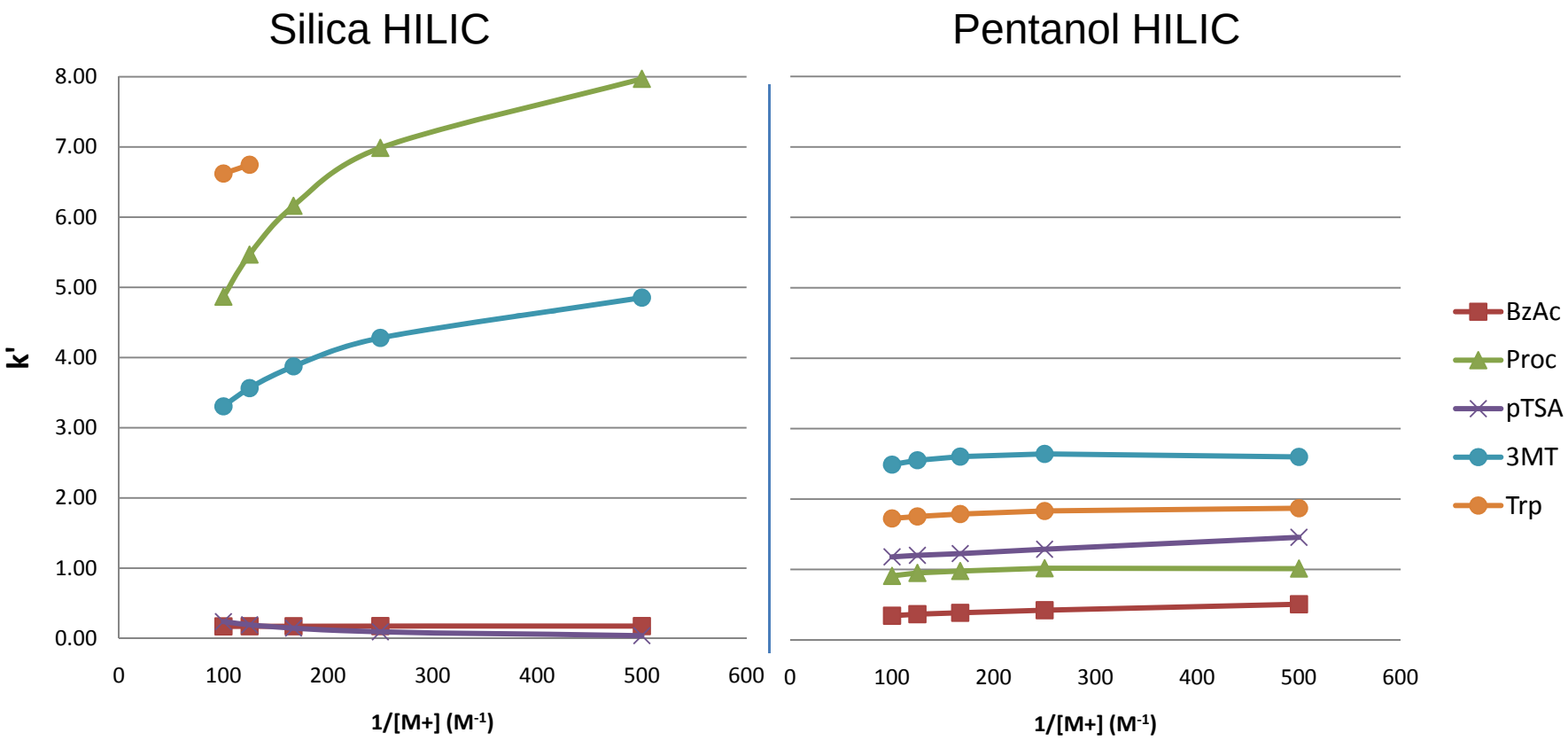
## *90% Acetonitrile/$NH_4$Form, pH 3.0: 2.1 mm ID x 100 mm, 25 °C, 0.5 mL/min*



# *Contribution of Ionic Strength on HILIC Separations*

## *90% Acetonitrile/$NH_4$Form, pH 3.0: 2.1 mm ID x 100 mm, 25 °C, 0.5 mL/min*



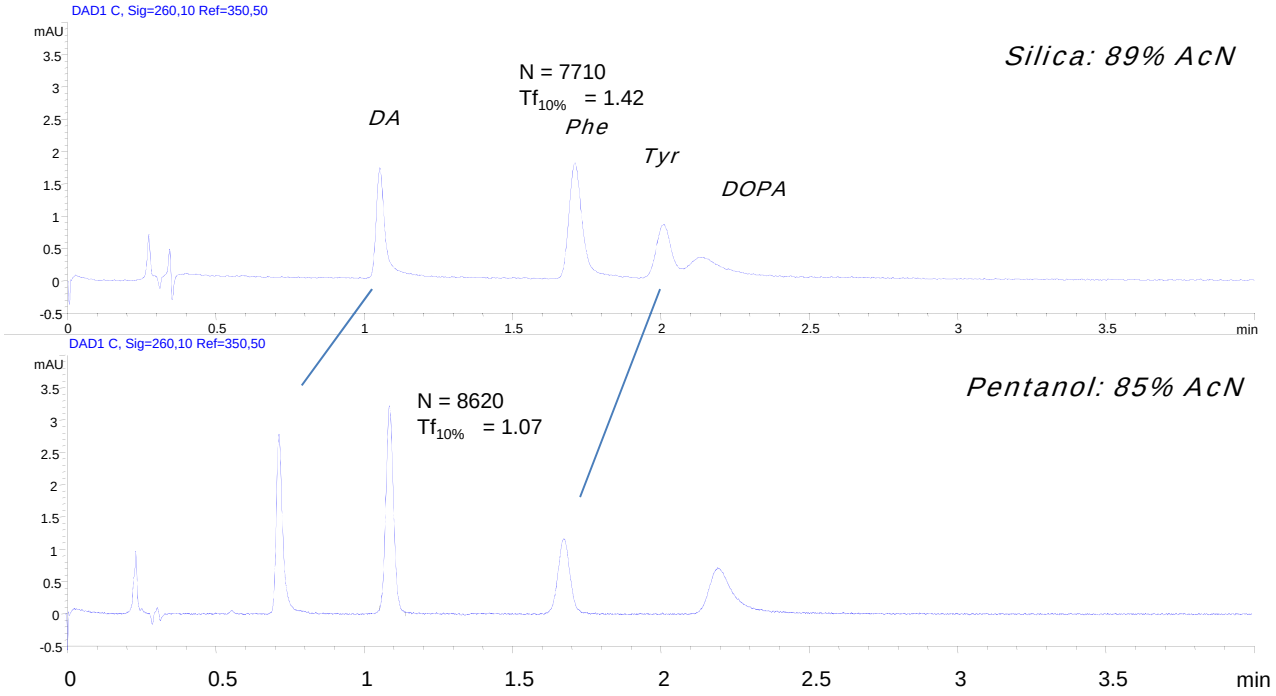

## Percent Contribution of Ionic Strength on Retention

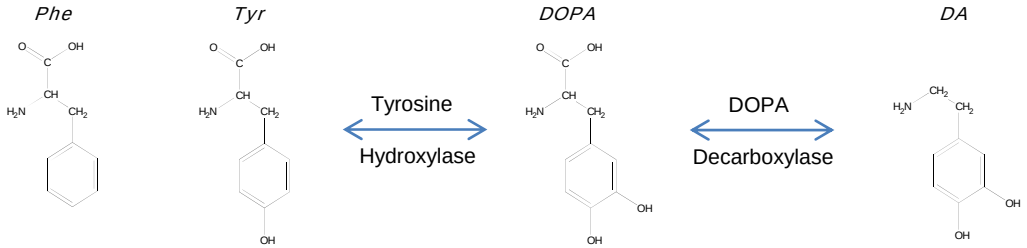
| | Proc | 3MT |
|---|---|---|
| 2 mM | 63 | 50 |
| 10 mM | 58 | 26 |

| | Proc | 3MT |
|---|---|---|
| 2 mM | 21 | 2 |
| 10 mM | 12 | 10 |

# *High Speed HILIC Separation of Catecholamines and Amino Acids*

***4.6 mm ID x 50 mm; 2 mL/min., 85% AcN/10 mM $NH_4$Form 3.0, 25 °C; 3 µL inj***



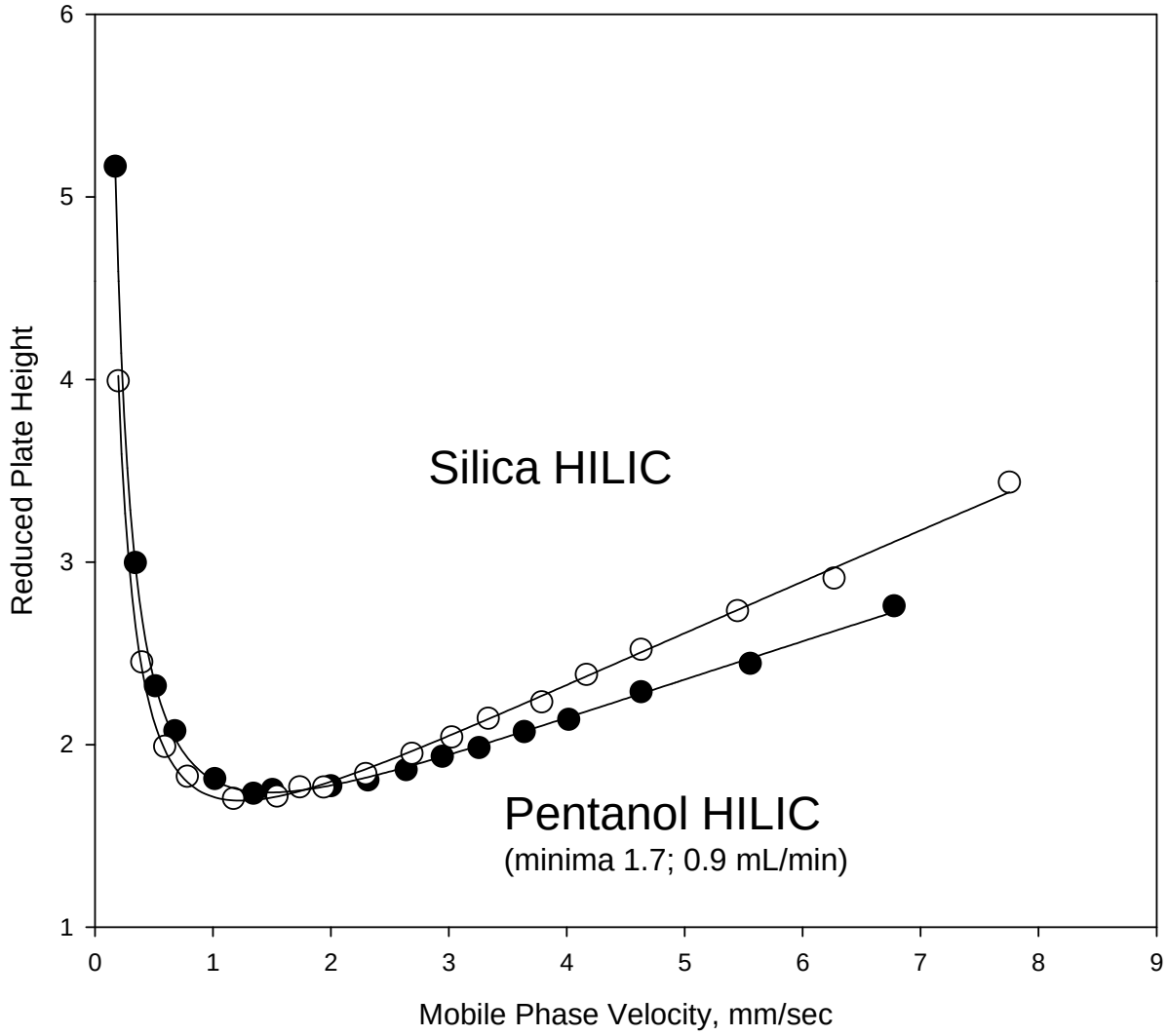




*Note: at 85% AcN all k' lower on Silica than on Pentanol.*

## *Effect of Linear Velocity on Pentanol Column Efficiency*

***4.6 mm ID x 50 mm; 90% AcN/10 mM $NH_4$Form 3.0, 25 °C; 1 μL, 50 ng Adenosine***

Data fitted to van Deemter curve

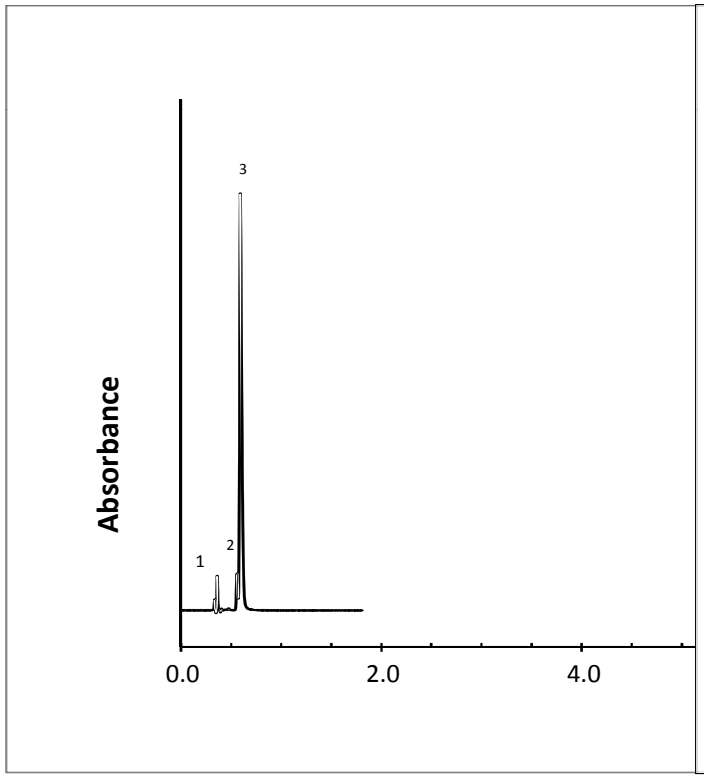


# *Pentanol HILIC Column Stability in Acidic Mobile Phase*

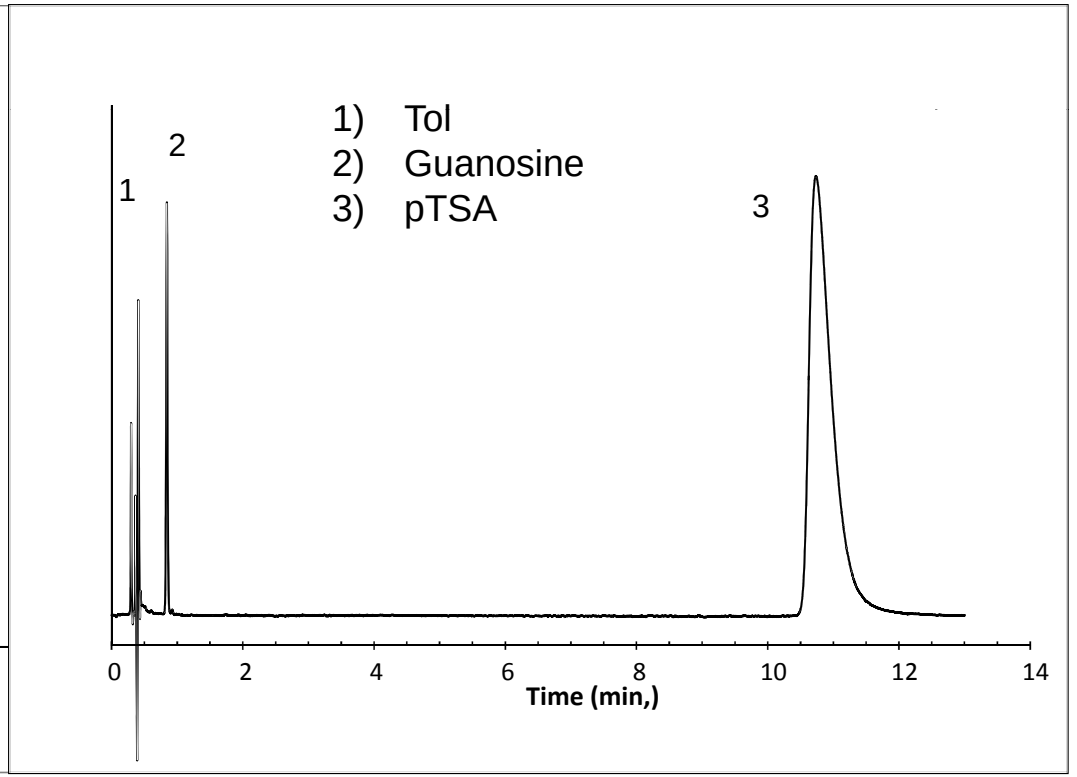
***4.6 mm ID x 50 mm***

***90% AcN/0.05% TFA, 60 °C, 1.5 mL/min.***

**Stability Test on SILICA HILIC Column**



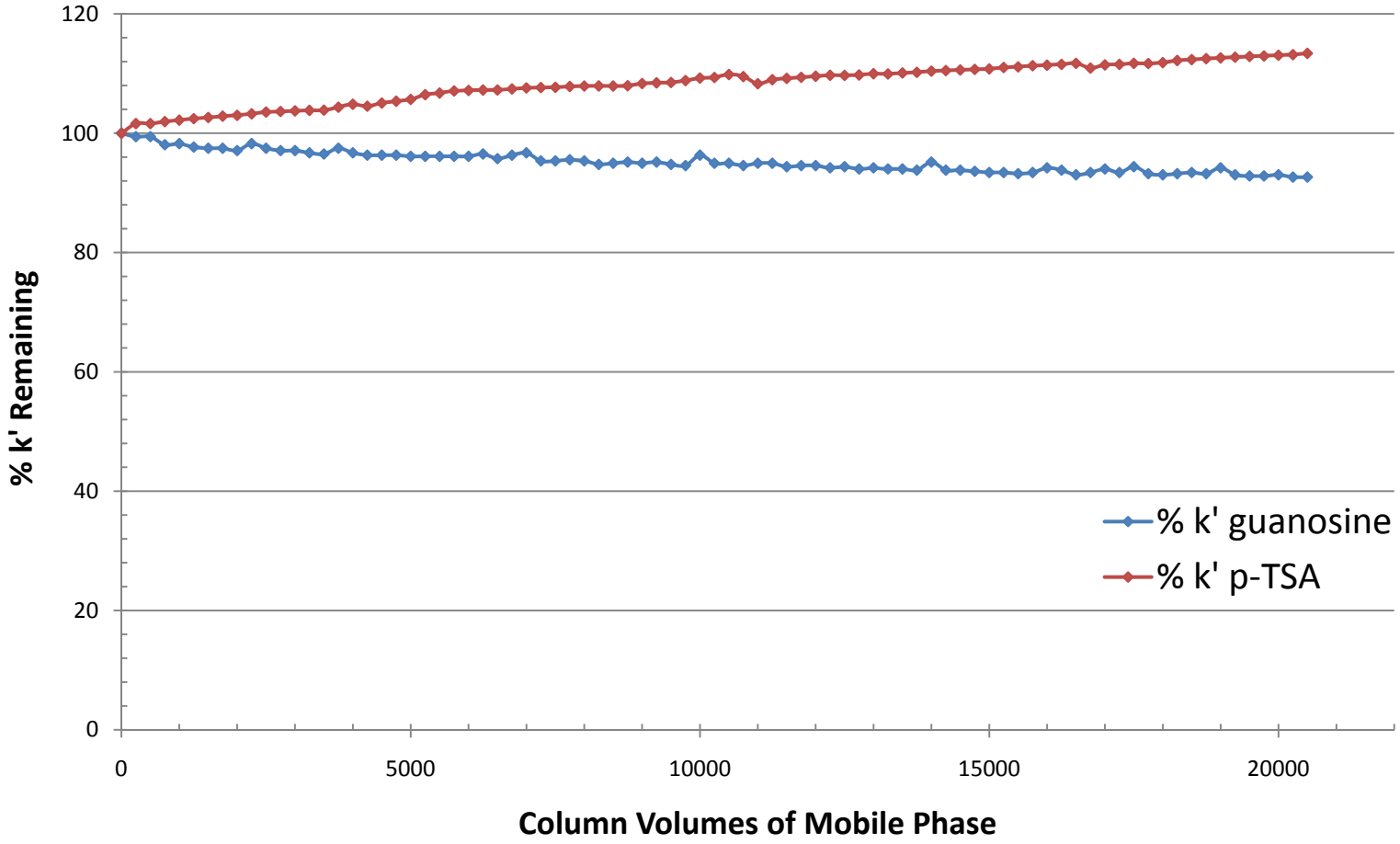

**Stability Test on Pentanol HILIC Column**



# *Pentanol HILIC Column Stability in Acidic Mobile Phase at 60 C*

***4.6 mm ID x 50 mm***

***90% AcN/0.05% TFA, 60 °C, 1 mL/min.***

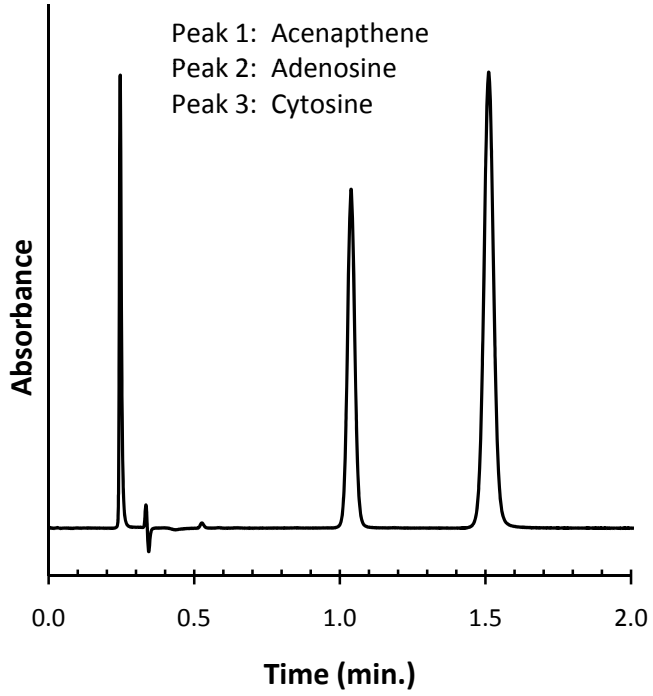


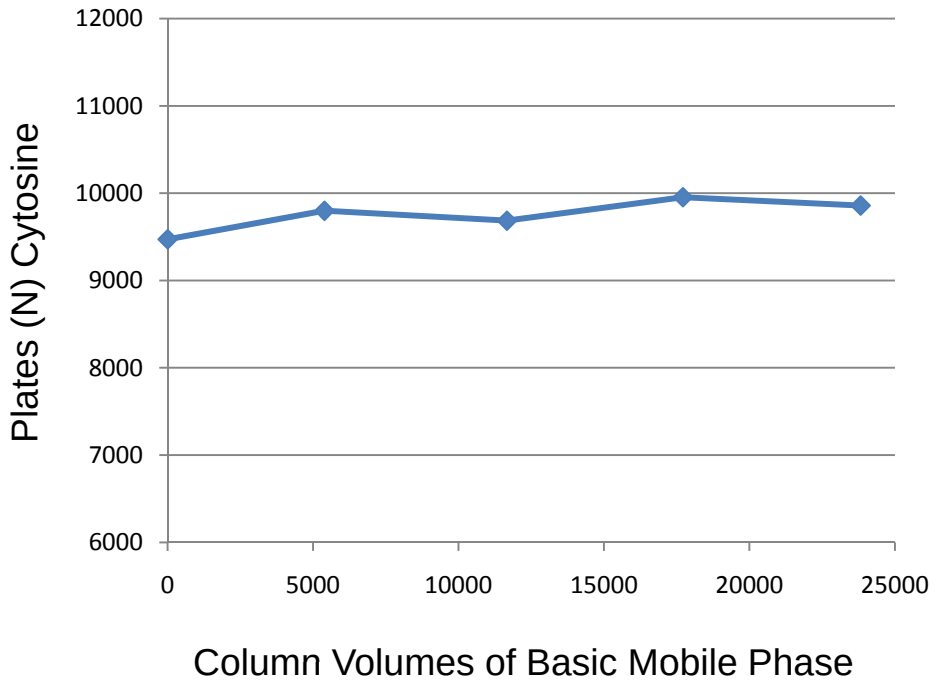
# *Pentanol HILIC Column Stability in Basic Mobile Phase*

***4.6 mm ID x 50 mm***

***Test: 90% AcN/0.1% $NH_4$Form pH 3.0, 1.8 ml/min, 78bar, 23 °C, 254 nm, 2.0 ul inj***

***Challenge: 50% AcN/50 mM $NH_4PO_4$, pH 9.0, 30 °C, 2 mL/min.***



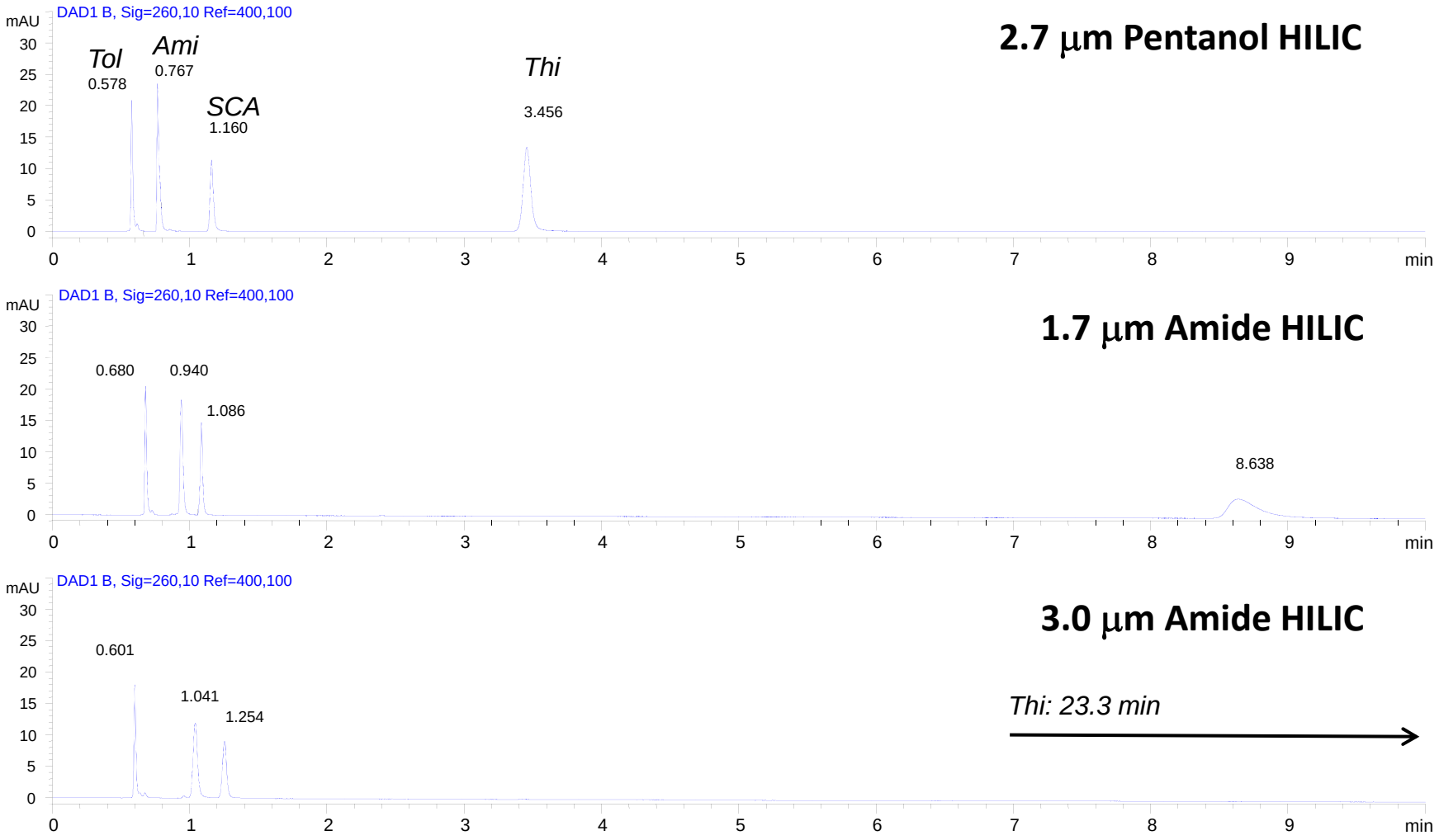



# *Comparative Retention of HILIC Columns*

***2.1 mm ID x 150 mm***

***Test: 90% AcN/10 mM $NH_4$Form pH 3.0, 0.5 mL/min, 23 °C***

***Toluene/Amitryptaline/Salicyclic Acid/Thiamine***



# Conclusions

## Hydroxylic Bonded Phases

- HILIC retention increased with the degree of hydroxylic substitution
- Larger monomeric hydroxylated silanes exhibited increased retention of all analytes, particularly acids and zwitterions
- The Pentan-OL ligand shows relatively high acid and zwitterion retention, with moderated base retention

## Fused-Core Pentanol Properties

- The densely bonded hydroxylated ligand exhibits HILIC retention insensitive to ionic strength over the tested range/conditions, not exhibiting typical silanol anionic character
- Column stability is high, at modest back pressures
- Column efficiency is as good, and sometimes better than, superficially porous materials
- Retention and performance is comparable to, and in some cases better than, amide-type bonded phases

# Acknowledgements

Technical and scientific assistance:
Dr. Stephanie Schuster
Dr. Bill Johnson
Jason Lawhorn

Financial Support:
NIH NIGMS GM099355

advancedmaterialstechnology