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

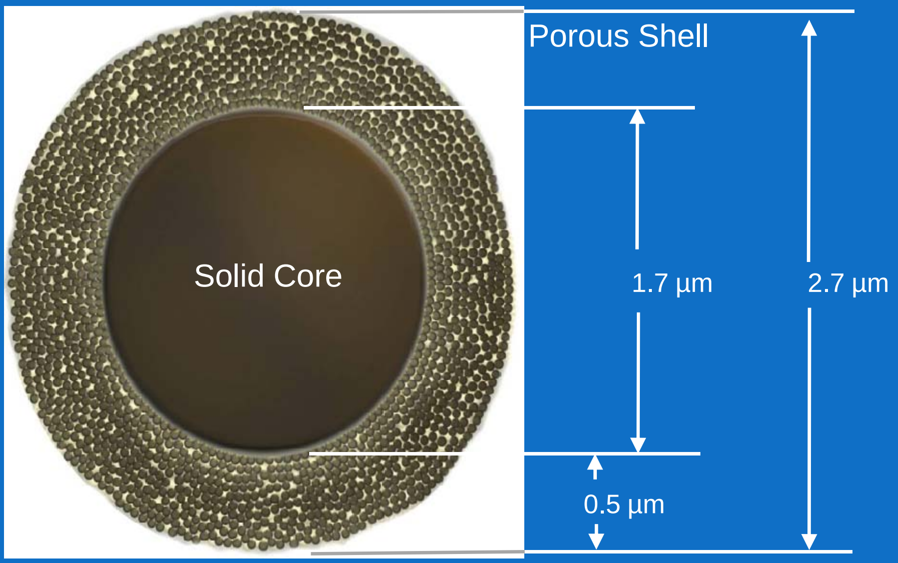
Current State

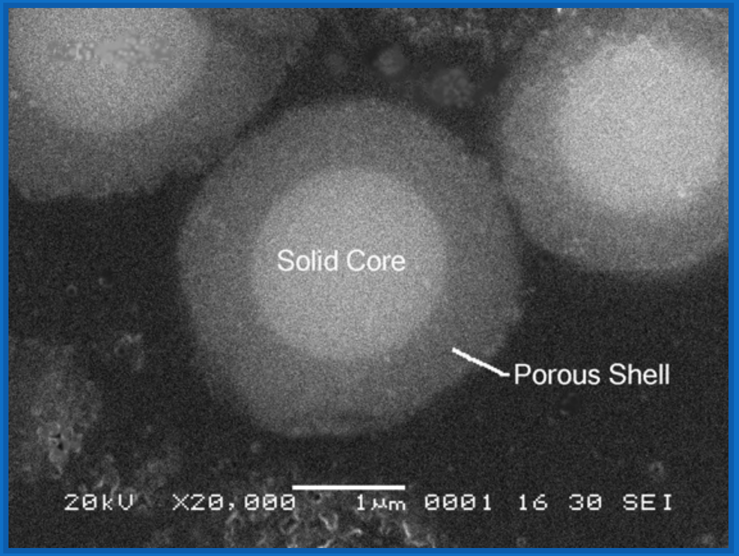
- Superficially porous particles can have strong performance advantages
- Silica HILIC has limitations
- HILIC bonded phases on superficially porous particles have a challenge of retention due to phase ratio available

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

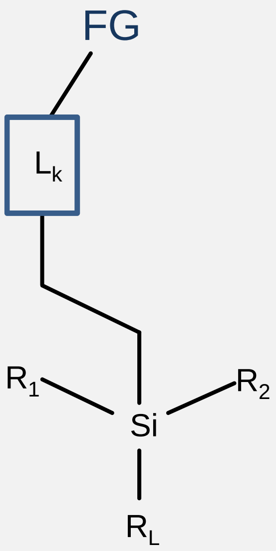




SEM Particle Cross-section

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

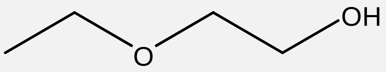
Silane Reagents for Hydrophilic Functional Groups



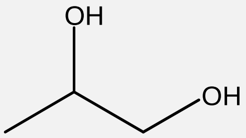
R_L = silanol reactive leaving
$R_1 = R_2$ or $R_1 \neq R_2$, $R_1 = R_L$
Eg. -Me, -OMe, -Cl, -DiiPr

Hydroxylic Functional Groups

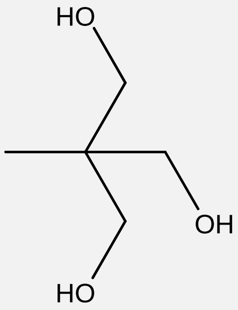
Mono-OL



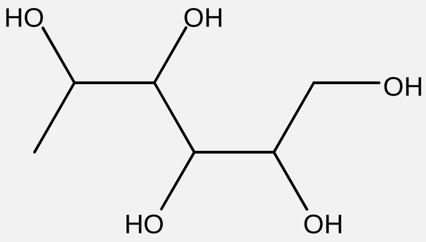
Di-OL



Tri-OL

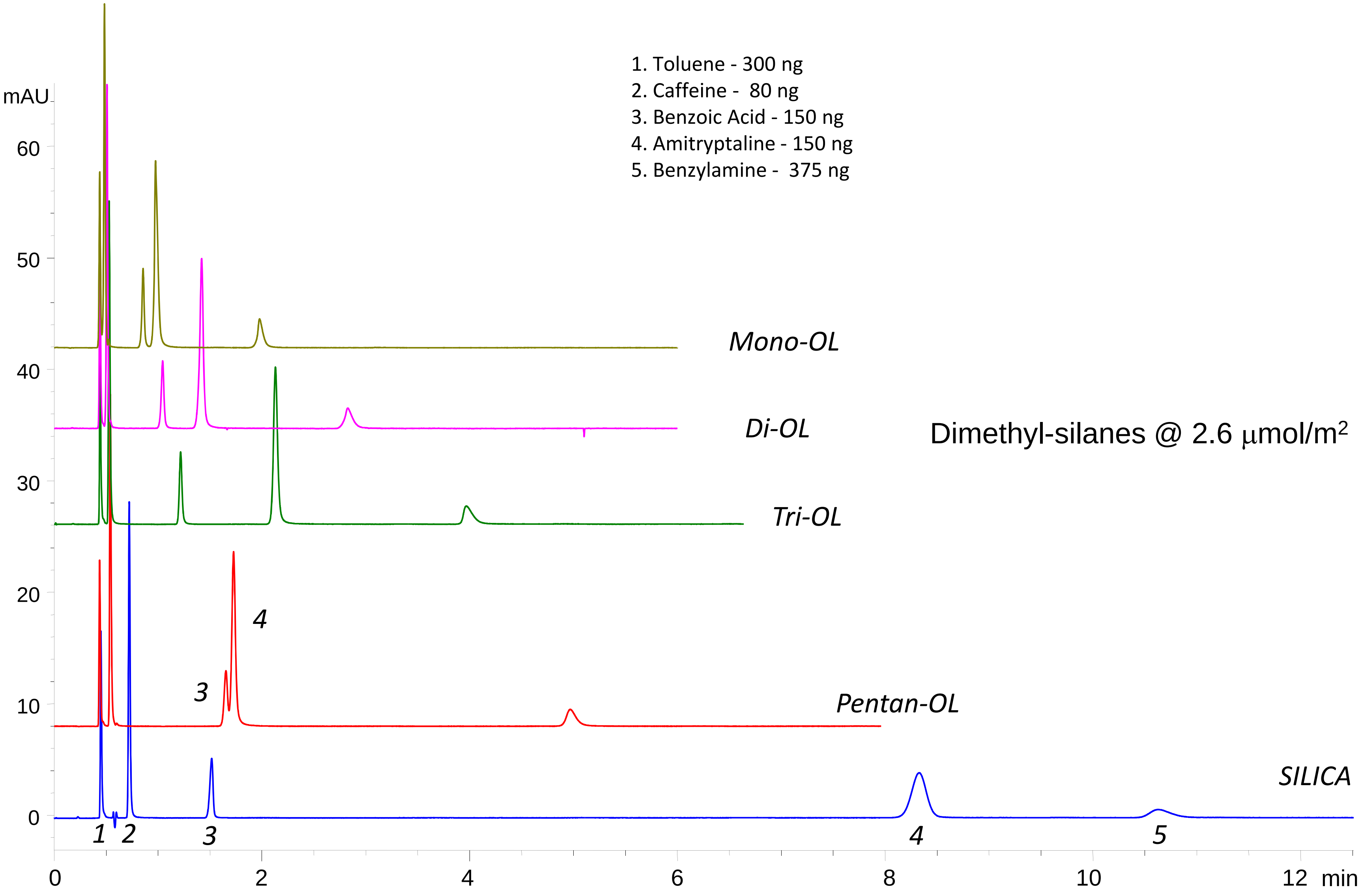


Pentan-OL



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

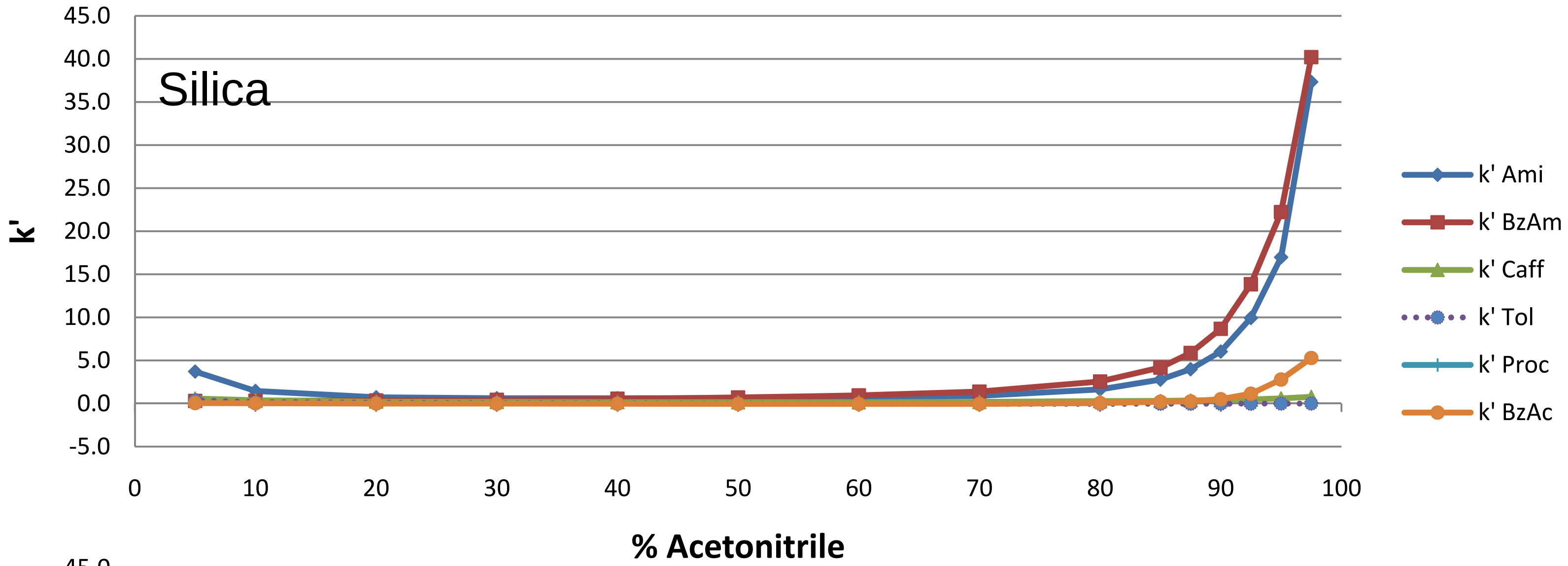


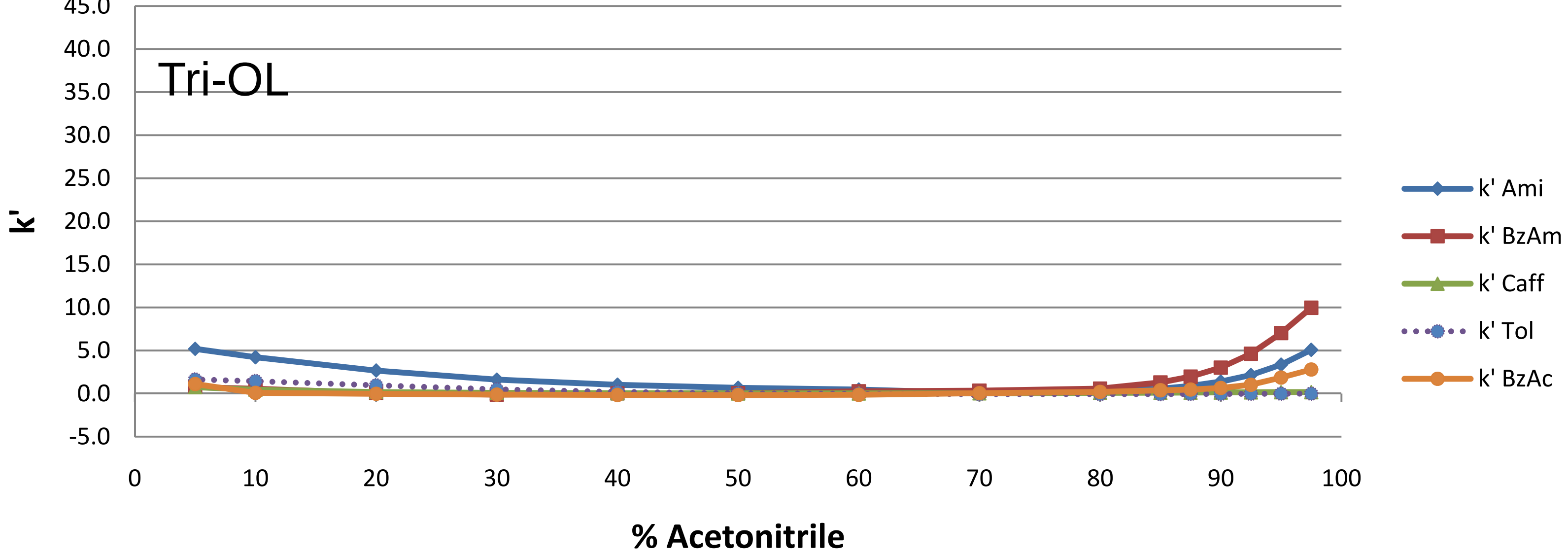
1. Toluene - 300 ng
2. Caffeine - 80 ng
3. Benzoic Acid - 150 ng
4. Amitryptaline - 150 ng
5. Benzylamine - 375 ng

Dimethyl-silanes @ 2.6 µmol/m²

Effect of Mobile Phase Organic Composition on Retention

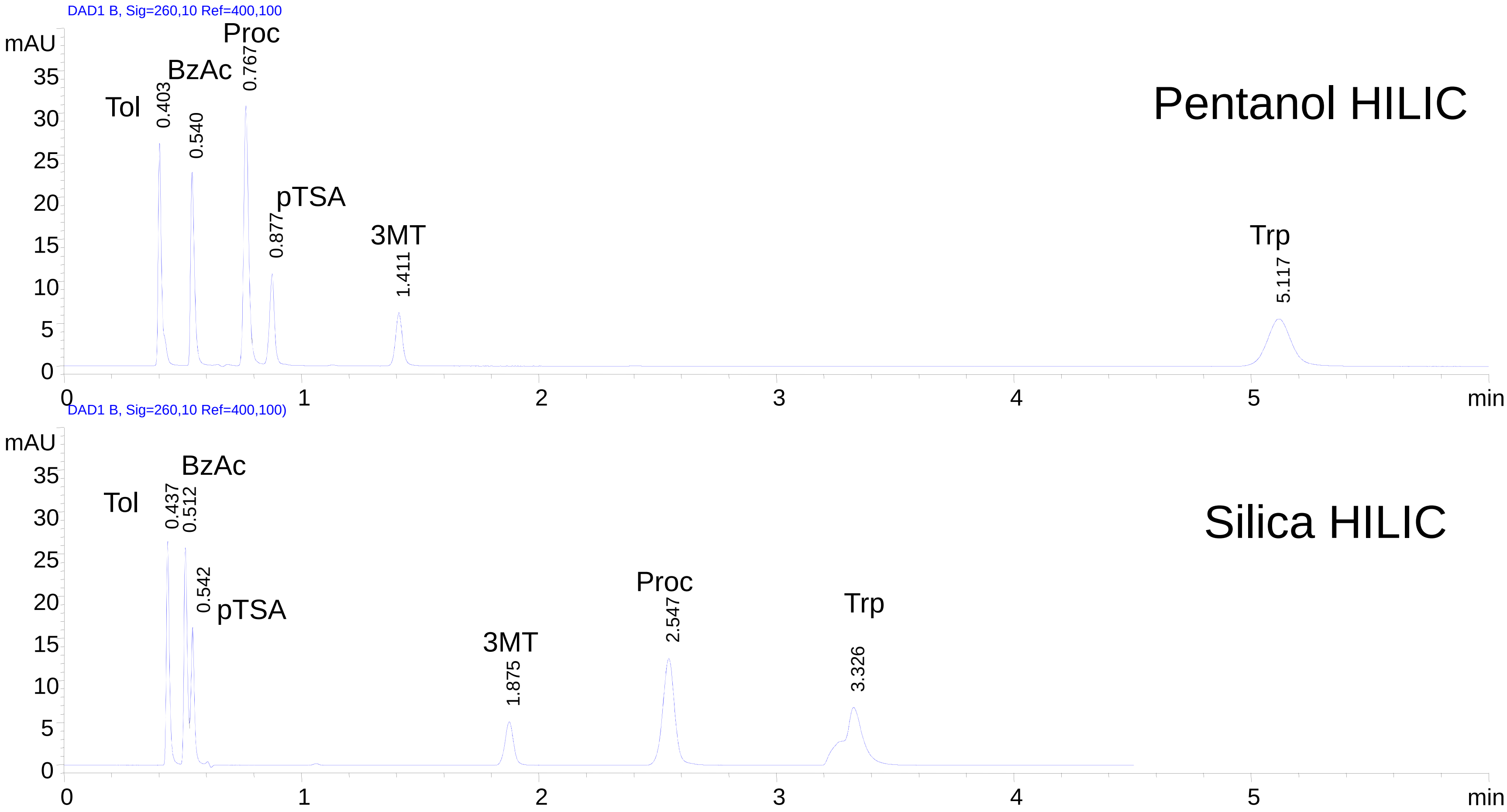
5 mM NH_4Acetate, pH 4.0: 2.1 mm ID x 100 mm, 25 °C, 0.5 mL/min





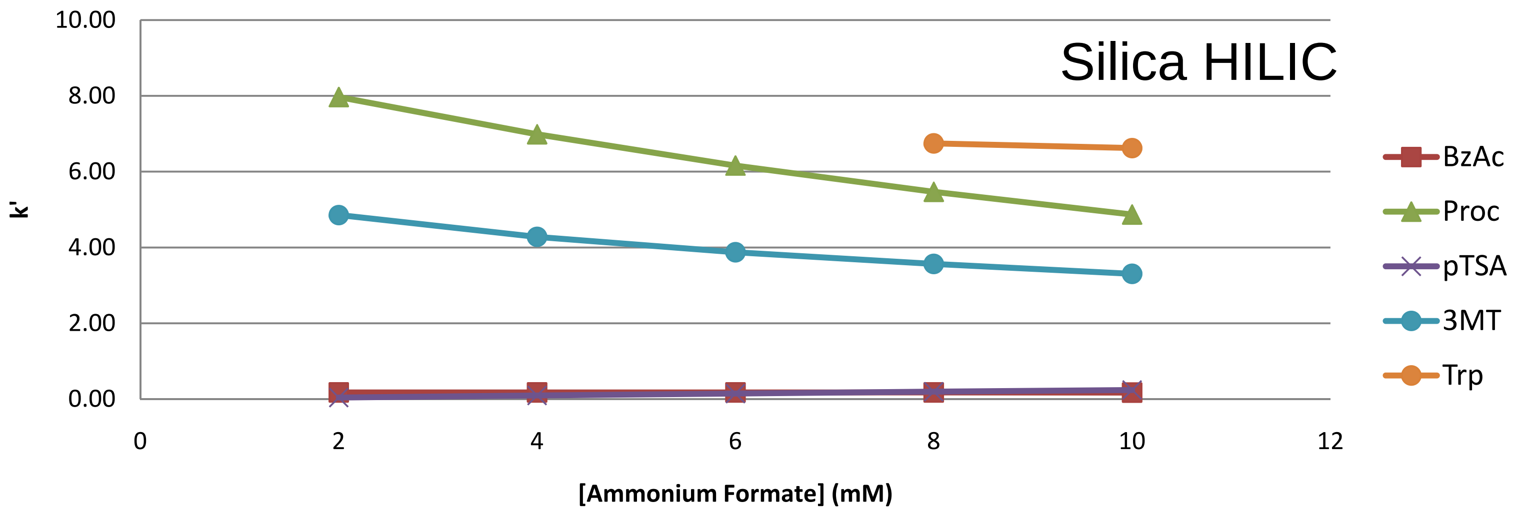
HILIC Separation of A/B/Z on Silica and Pentanol Silane Surface

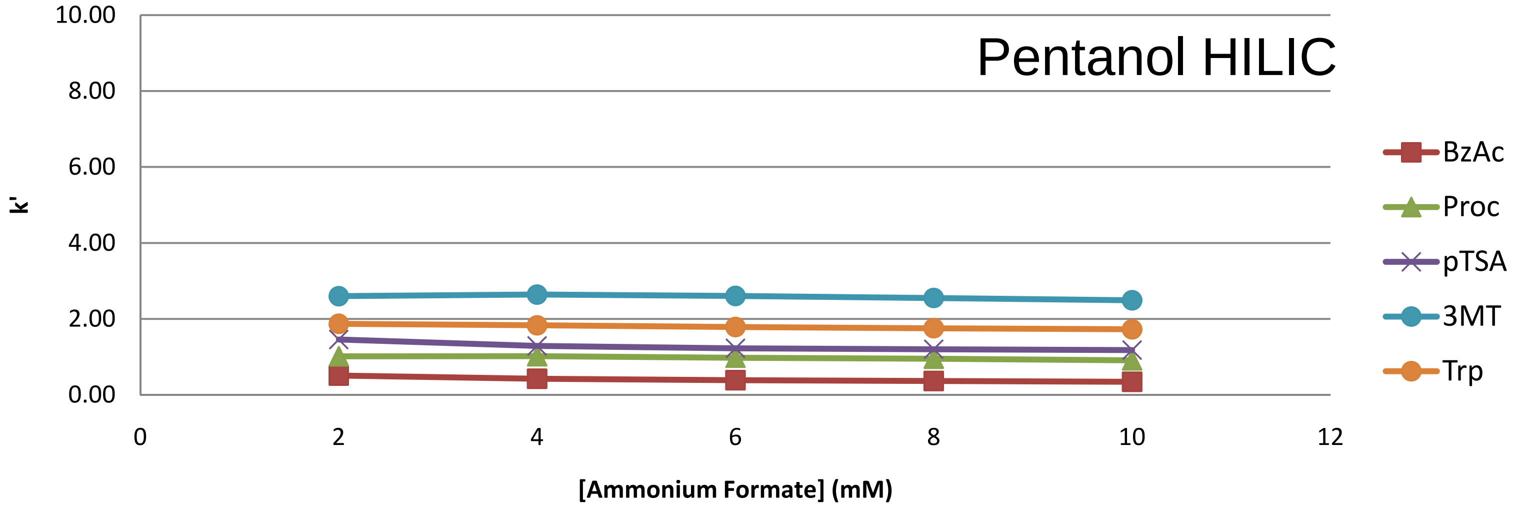
90% Acetonitrile/10 mM NH_4Form, pH 3.0: 2.1 mm ID x 100 mm, 25 °C, 0.5 mL/min



Effect of Buffer Concentration on HILIC Separations

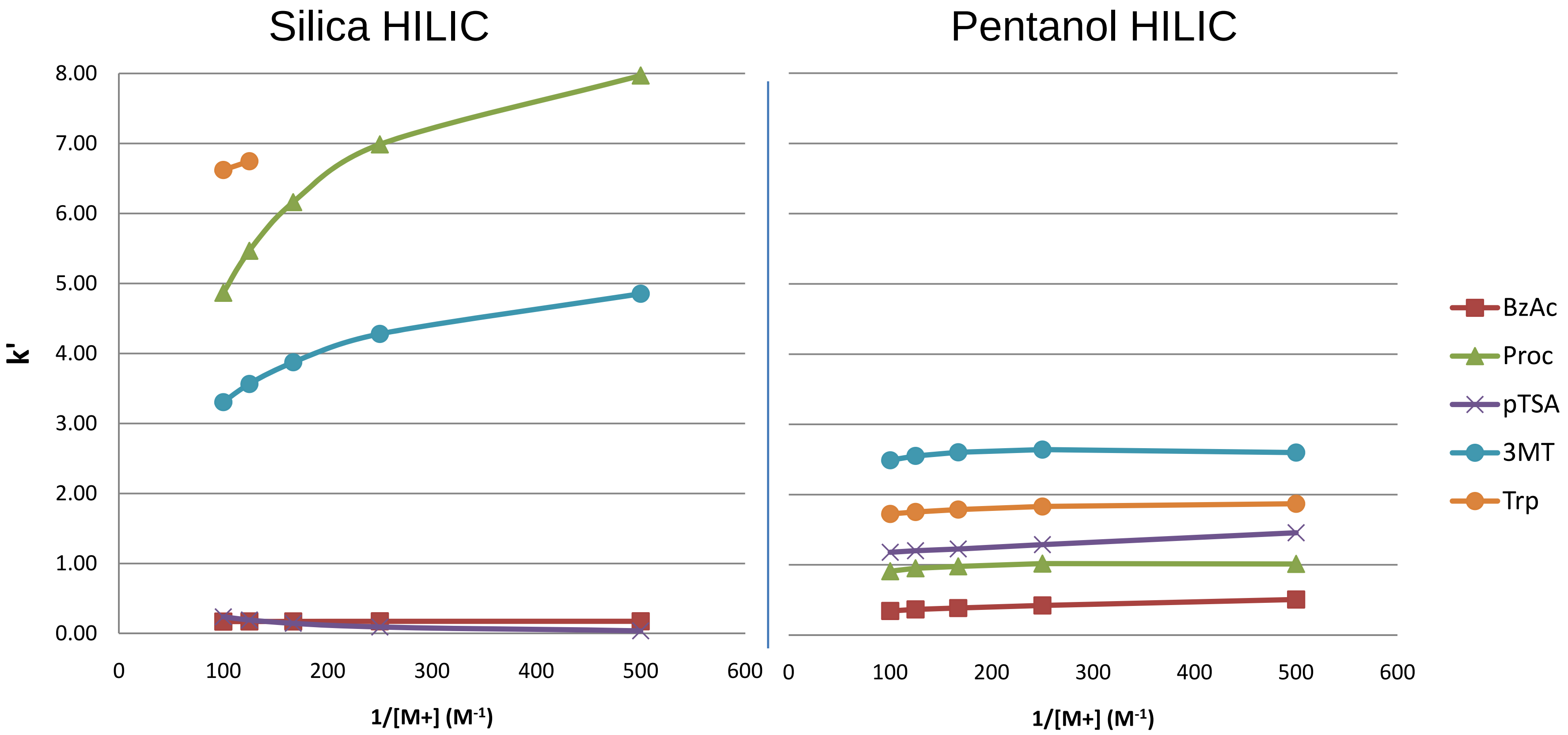
90% Acetonitrile/NH_4Form, pH 3.0: 2.1 mm ID x 100 mm, 25 °C, 0.5 mL/min





Contribution of Ionic Strength on HILIC Separations

90% Acetonitrile/NH_4Form, pH 3.0: 2.1 mm ID x 100 mm, 25 °C, 0.5 mL/min



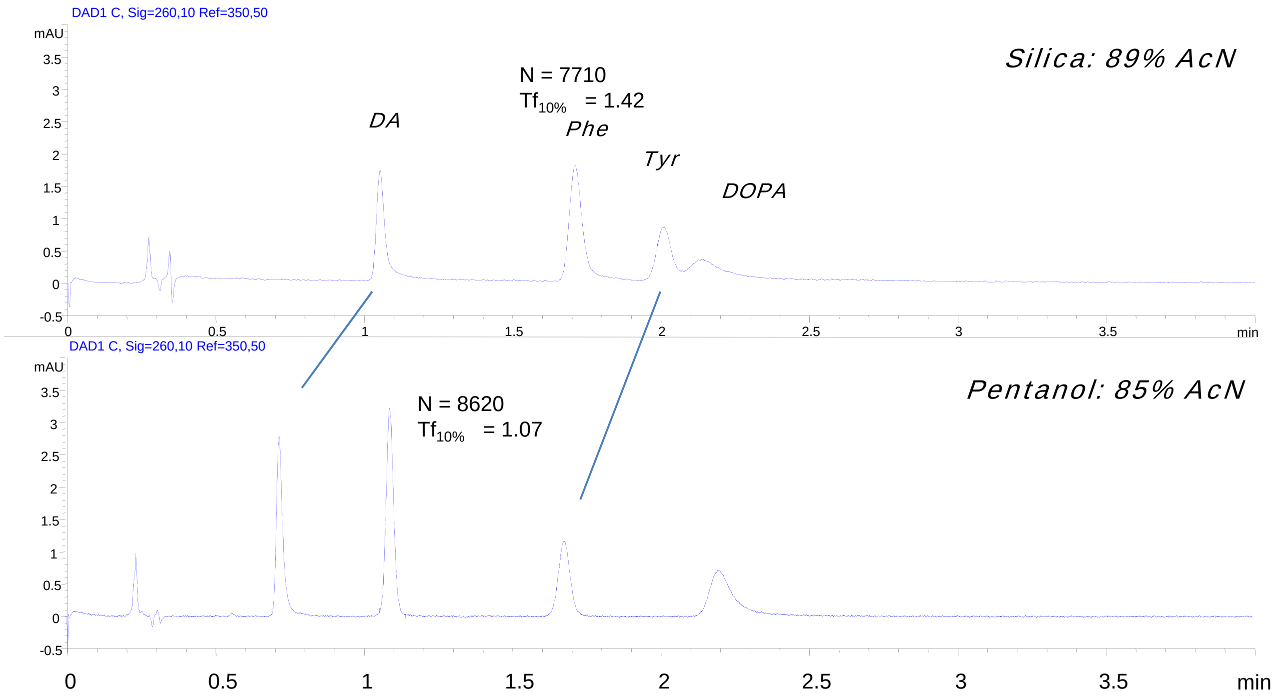
Percent Contribution of Ionic Strength on Retention

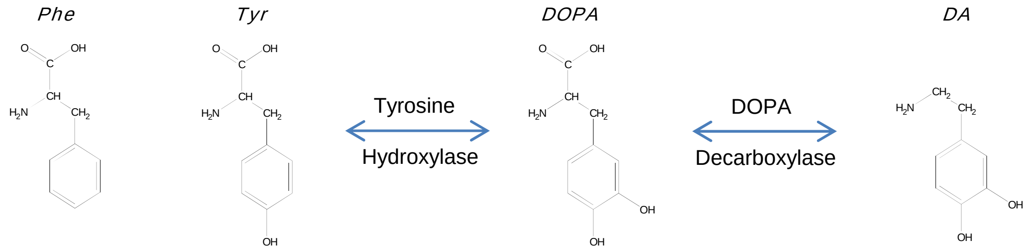
	Proc	3MT
2 mM	63	50
10 mM	58	26

	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_4Form 3.0, 25 °C; 3 μL inj



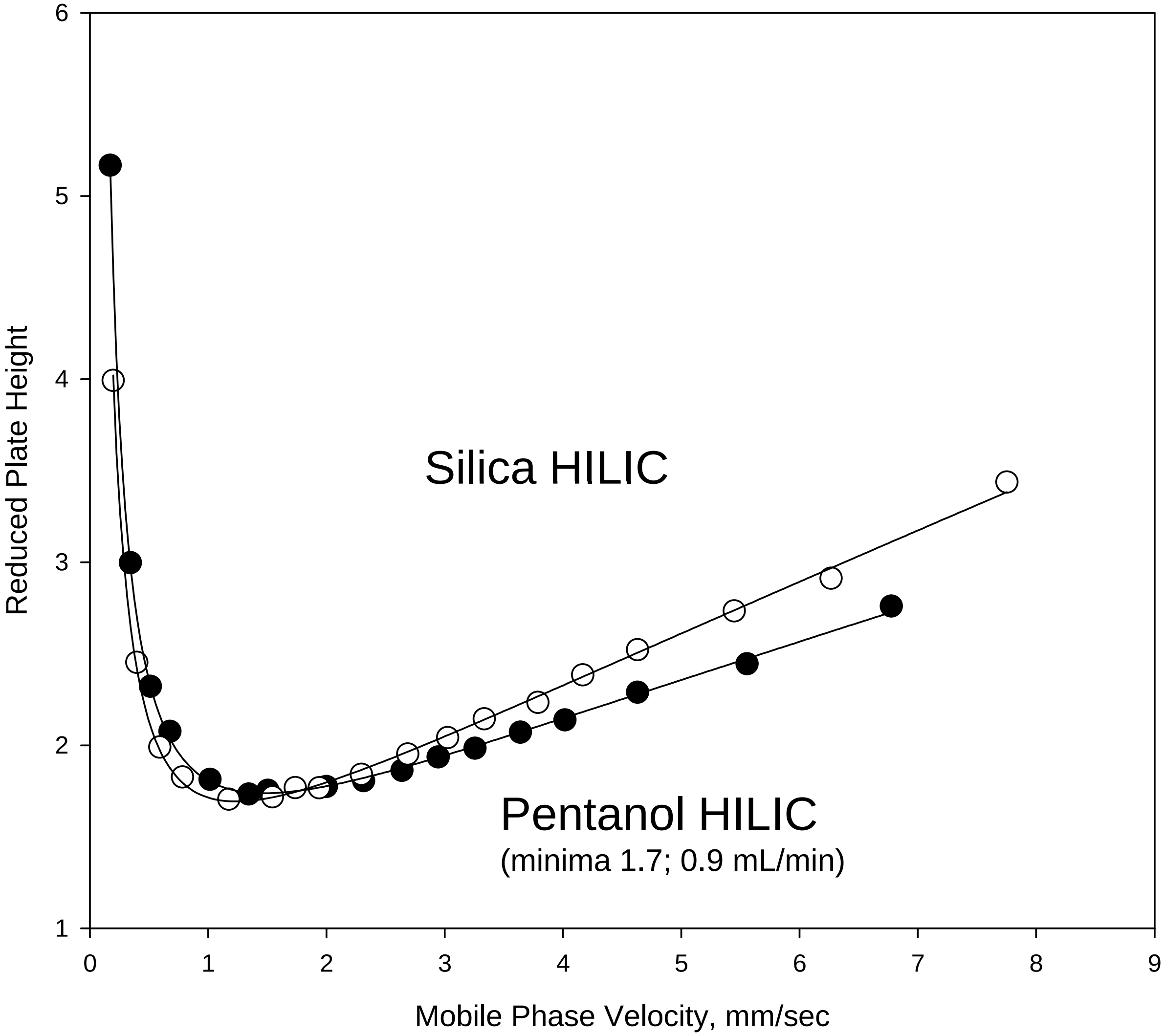


Note: at 85% AcN all k’ lower on Silica than on Pentanol.

Effect of Linear Velocity on Pentanol Column Efficiency

4.6 mm ID x 50 mm; 90% AcN/10 mM NH_4Form 3.0, 25 °C; 1 µL, 50 ng Adenosine

Data fitted to van Deemter curve

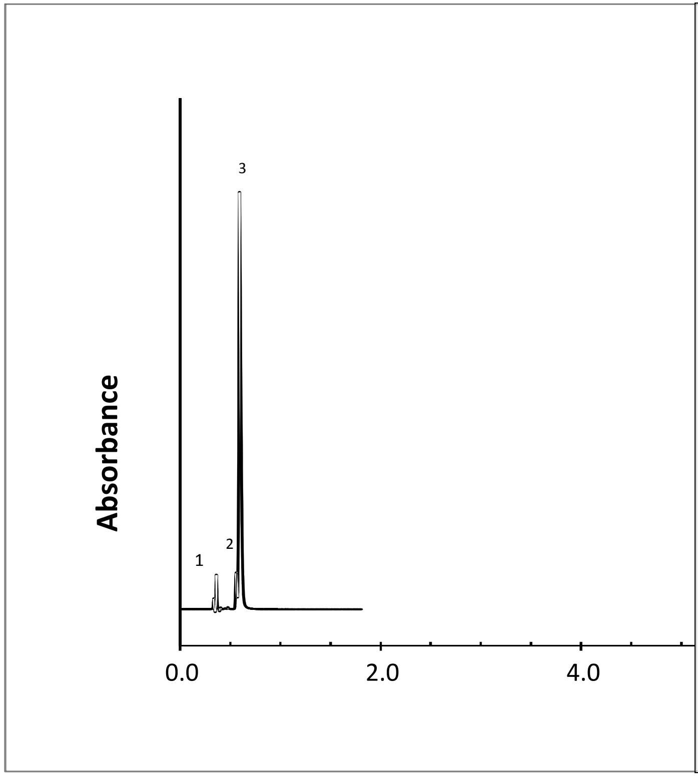


Pentanol HILIC Column Stability in Acidic Mobile Phase

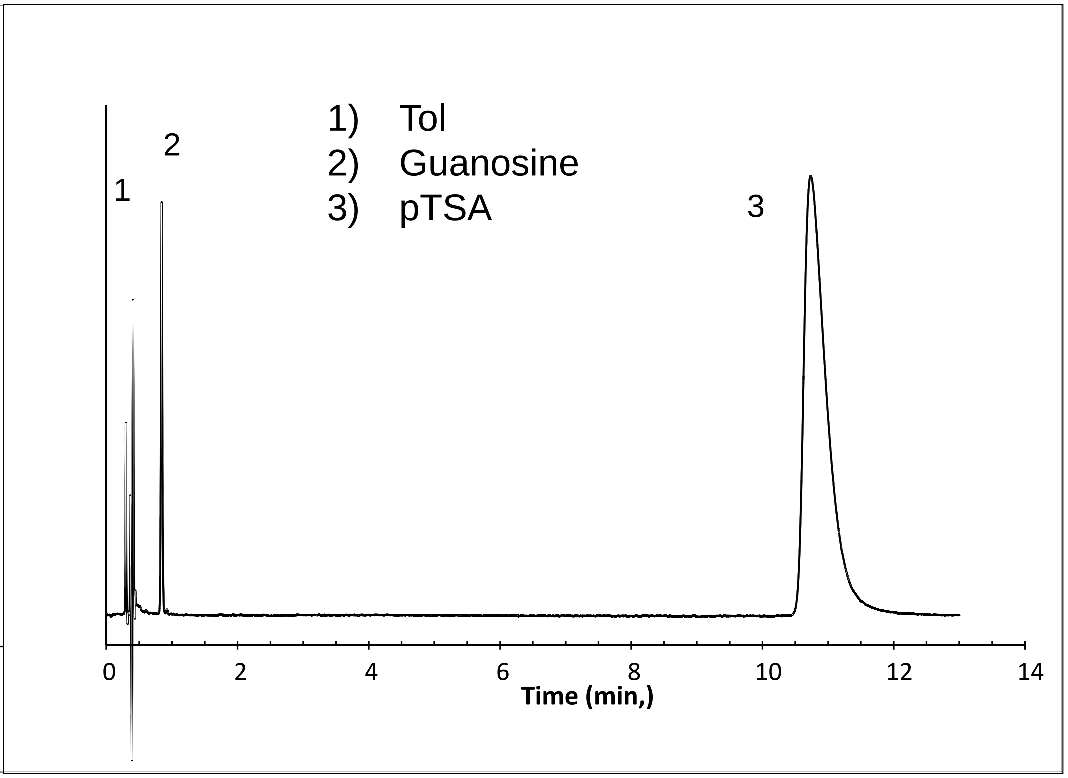
4.6 mm ID x 50 mm

90% AcN/0.05% TFA, 60 °C, 1.5 mL/min.

Stability Test on SILICA HILIC Column



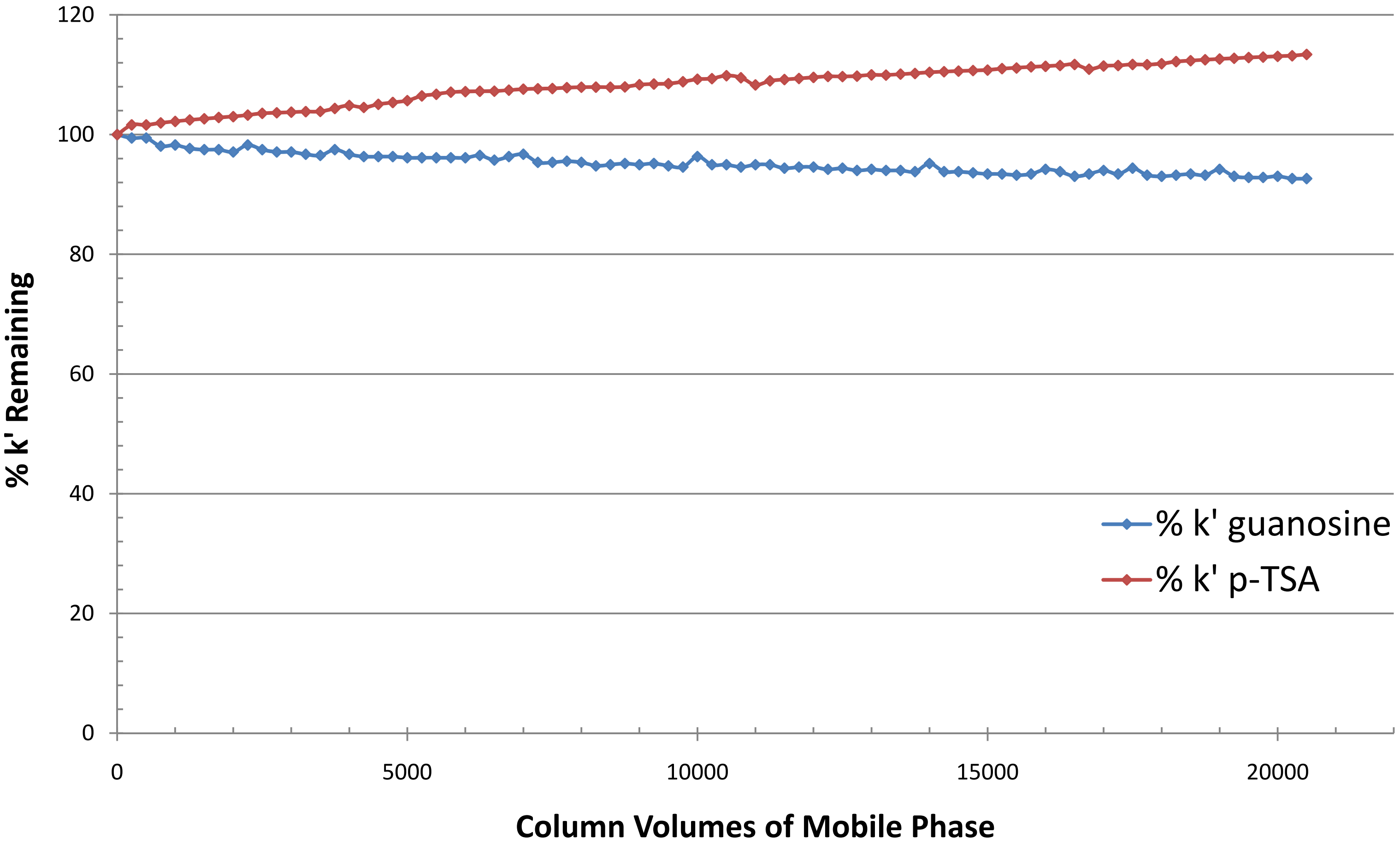
Stability Test on Pentanol HILIC Column



Pentanol HILIC Column Stability in Acidic Mobile Phase at 60 C

4.6 mm ID x 50 mm

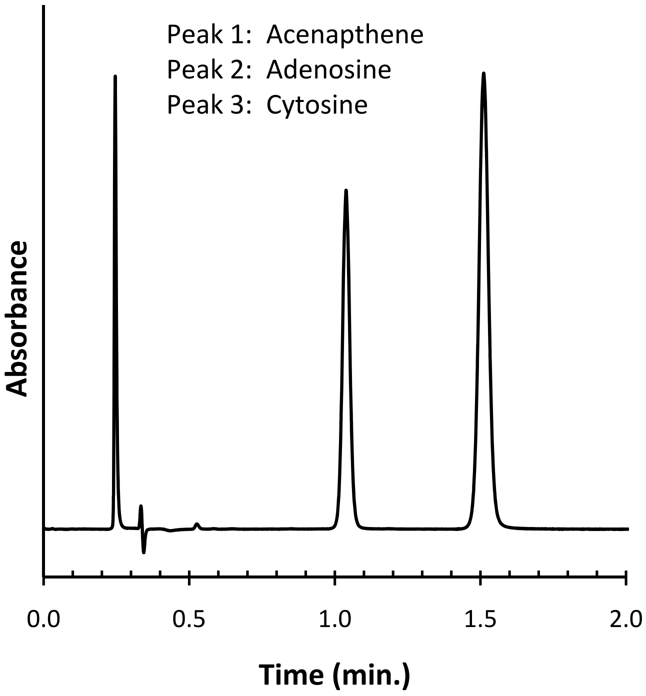
90% AcN/0.05% TFA, 60 °C, 1 mL/min.

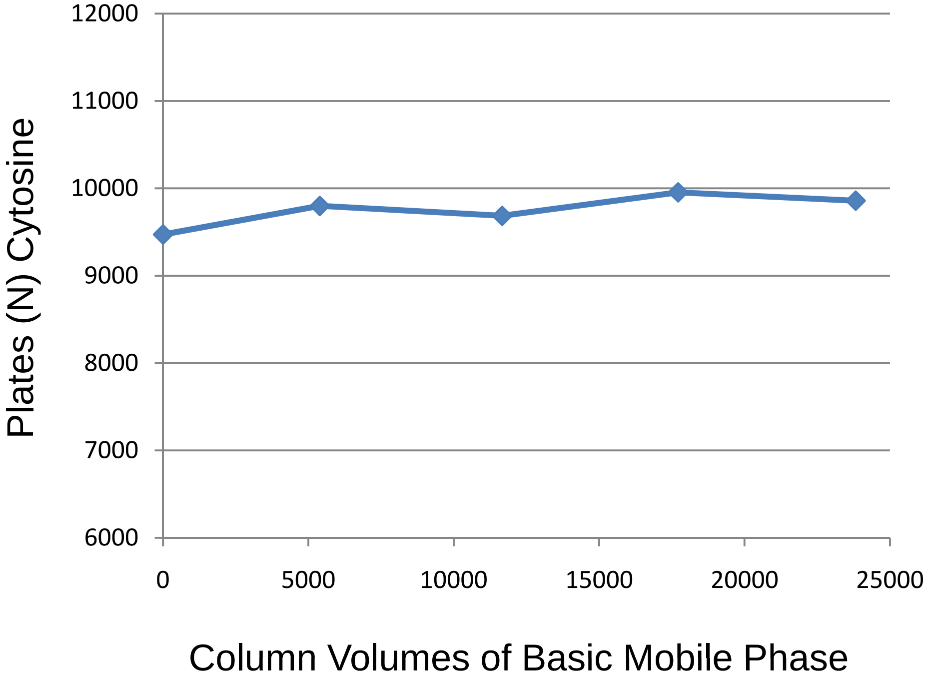


Pentanol HILIC Column Stability in Basic Mobile Phase

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$_4$PO$_4$, pH 9.0, 30 °C, 2 mL/min.



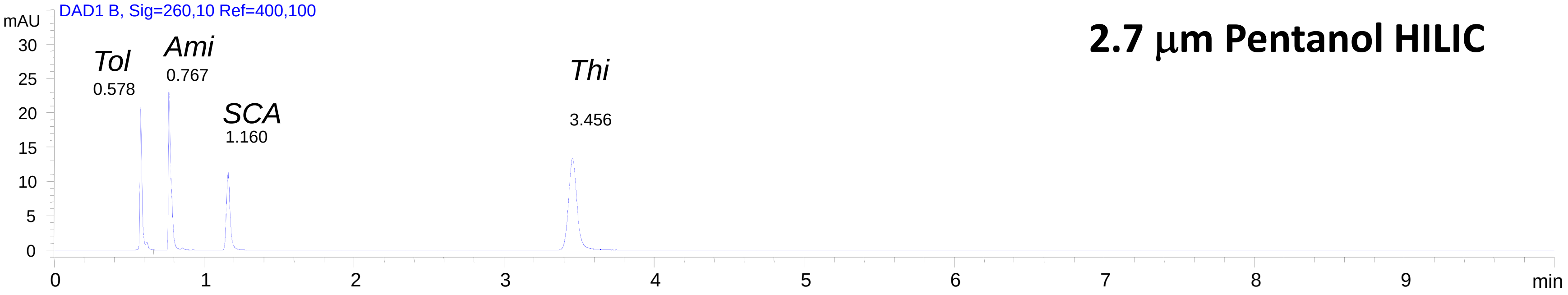


Comparative Retention of HILIC Columns

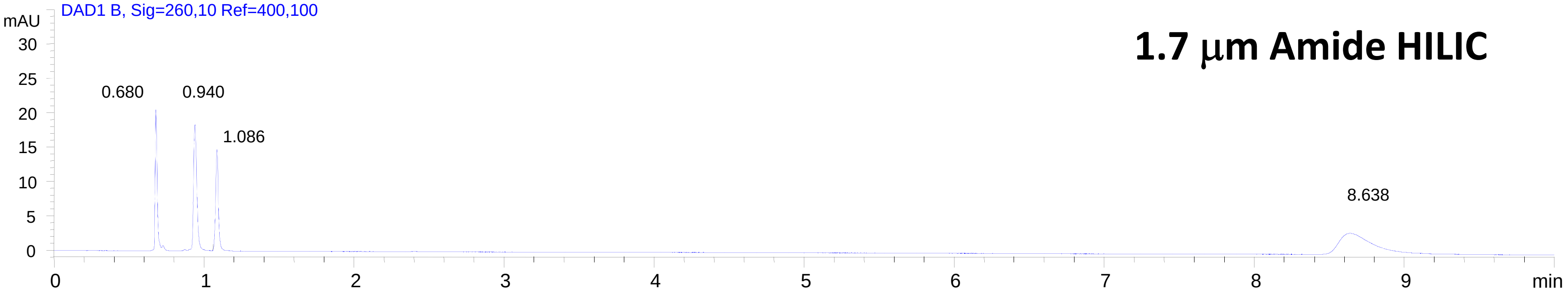
2.1 mm ID x 150 mm

Test: 90% AcN/10 mM NH_4Form pH 3.0, 0.5 mL/min, 23 °C

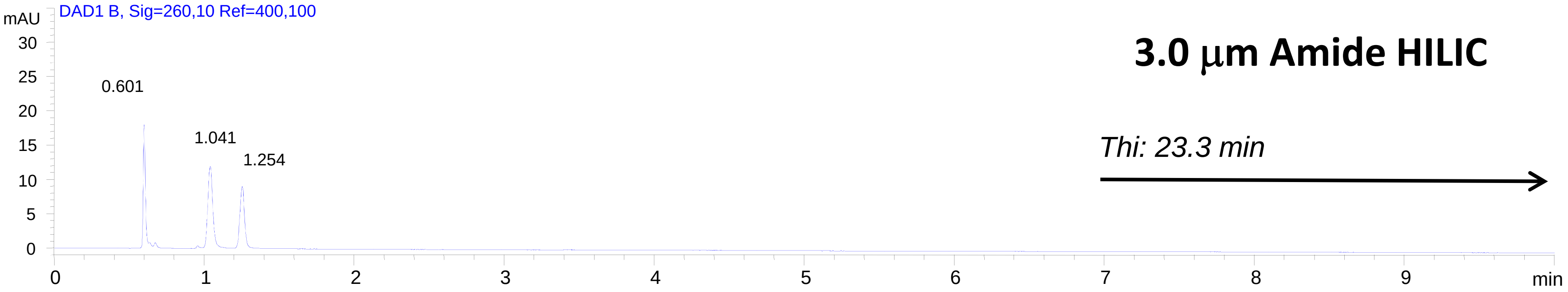
Toluene/Amitryptaline/Salicyclic Acid/Thiamine



2.7 µm Pentanol HILIC



1.7 µm Amide HILIC



3.0 µm Amide HILIC

Thi: 23.3 min

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

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