Novel Superficially Porous Bonded Phase for High Speed HILIC Separations

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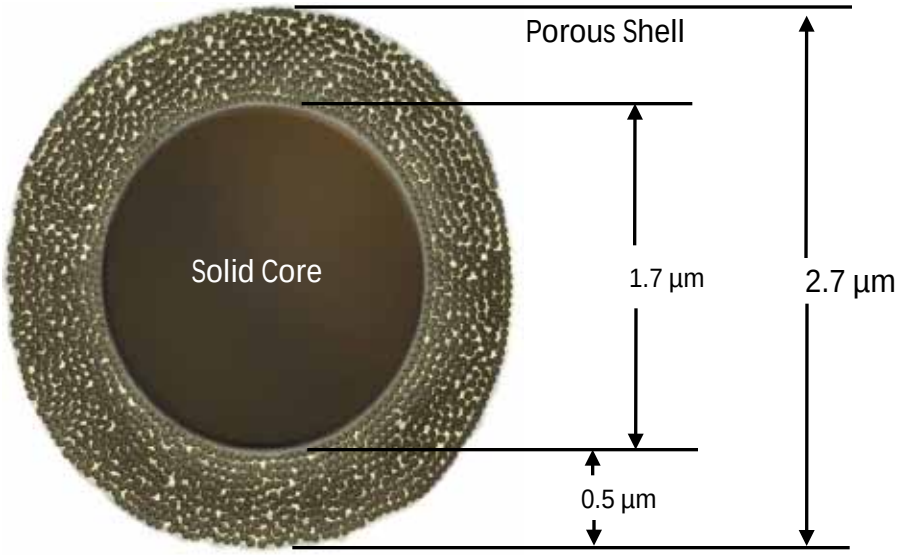
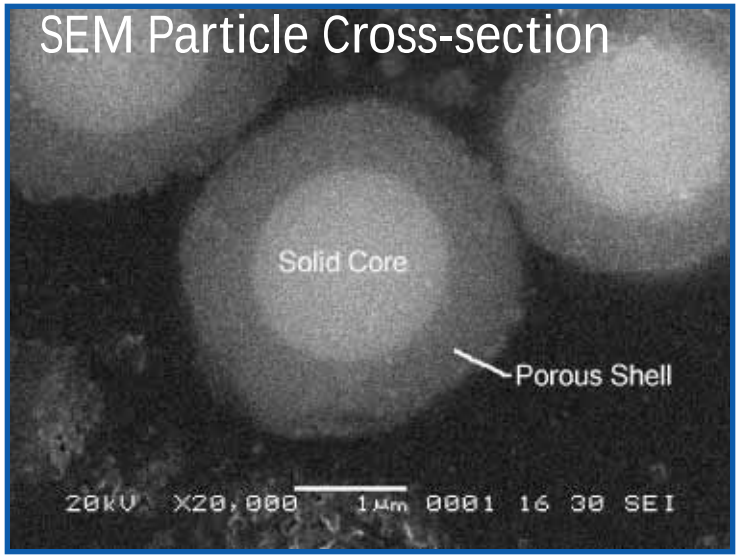
Introduction

During the past few years superficially porous silica particles have emerged as preferred materials for high efficiency and high speed separations in HPLC. Superficially porous (also known as Fused-Core®) silica particles can be obtained with a variety of bonded phases, pore sizes, particle sizes, and shell thicknesses. Columns packed with 2.7 µm diameter Fused-Core particles have been shown to exhibit surprising efficiency. These columns can demonstrate performance superior to equivalently sized porous particles. In addition, these columns show comparable performance to columns packed with sub-2-µm particles, but at less than half the back pressure. High column efficiencies have been observed for the use of bare silica Fused-Core particle columns operated in hydrophilic interaction liquid chromatography (HILIC) mode. The objective of this study has been to develop highly polar bonded-phase surface modifications of Fused-Core silica particles for HILIC separations that maintain the high efficiencies previously observed with bare Fused-Core silica, but that also exhibit the advantages of covalently-modified HILIC packing materials. To this end, a variety of highly polar covalent bonded phases were applied to Fused-Core silica particles. Novel hydroxylated bonded phases are observed to be highly hydrophilic, exhibiting typical HILIC retention properties. A selected material, commercialized as Halo Penta-HILIC , contains five hydroxyl groups on the bonded ligand and shows high efficiency and reduced ionic interactions with ionizable compounds, including bases, acids, and zwitterions. We demonstrate applications of such highly polar bonded phases for a variety of HILIC separations of small molecules, including antibiotics, antidepressants, drugs of abuse, and nucleosides.

Superficially Porous Particles (Fused-Core®)

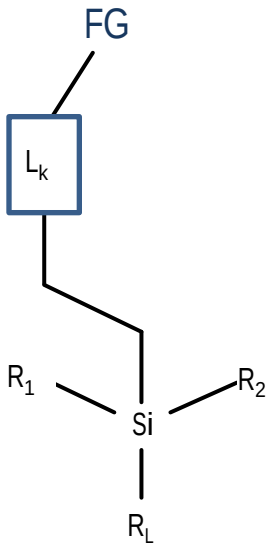




Selection of HILIC Bonded Phases

A range of functional group types were immobilized on Fused-Core silica particles. Based on performance properties, the Penta-HILIC structure has been selected for further development.

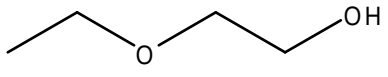
- Supports effective HILIC retention
- Exhibits desired kinetic advantages of SPP morphology
- Reduces unfavorable ionic/coulombic interactions



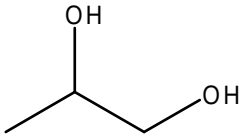
R_L = silanol reactive leaving
$R_1 = R_2$ or $R_1 \neq R_2$, $R_1 = R_L$
Eg. -Me, -OMe, -Cl, -DiiPr
L_k is a proprietary linker

Hydroxylic Functional Groups

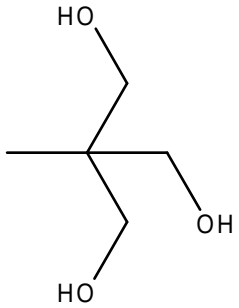
Mono-OL



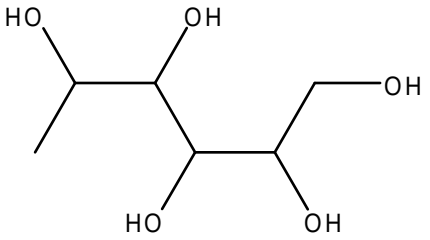
Di-OL



Tri-OL



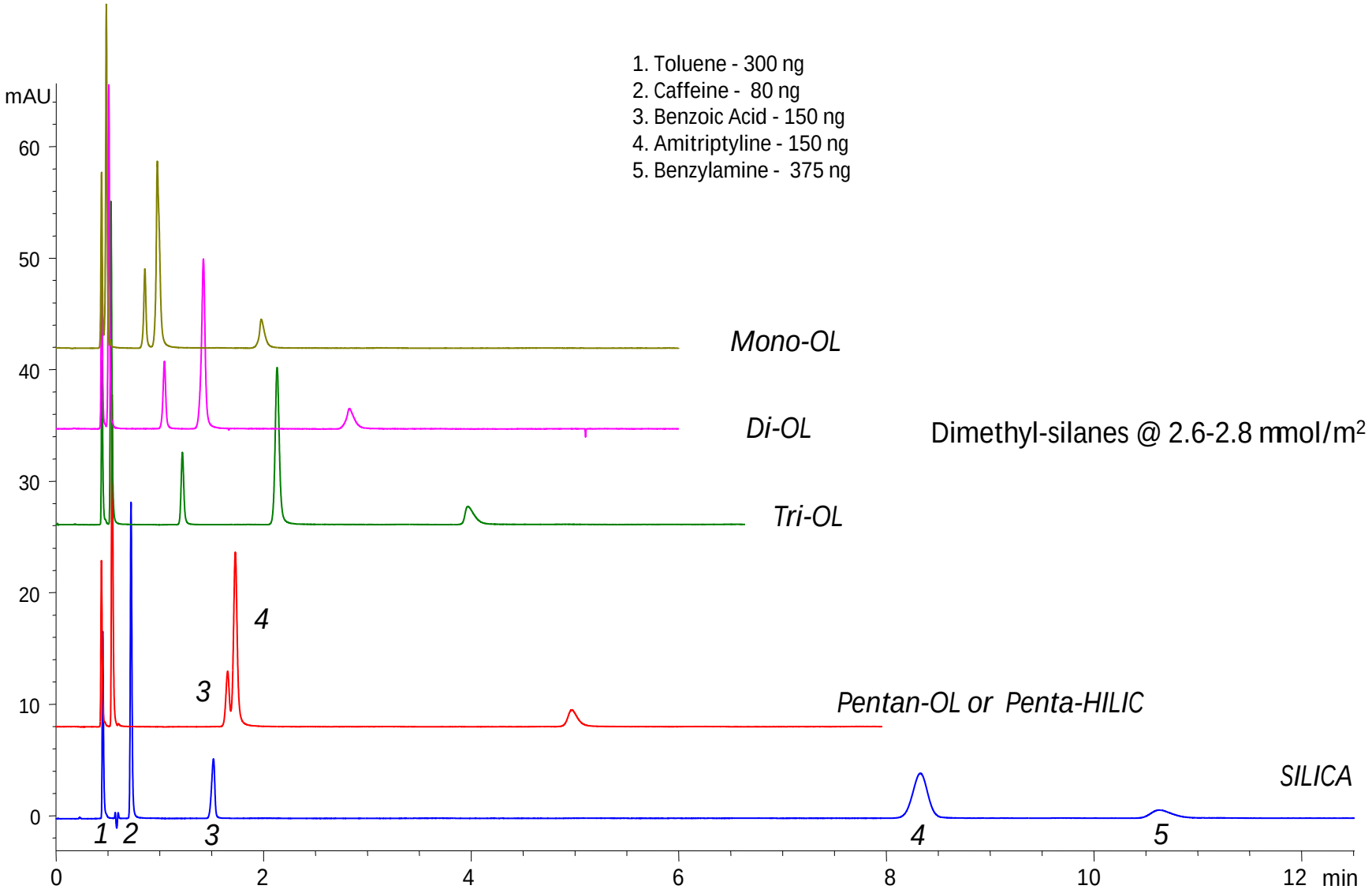
Pentan-OL
AKA
HALO Penta-HILIC



Features of HALO Penta-HILIC

Effect of Surface Functional Groups on a HILIC Separation

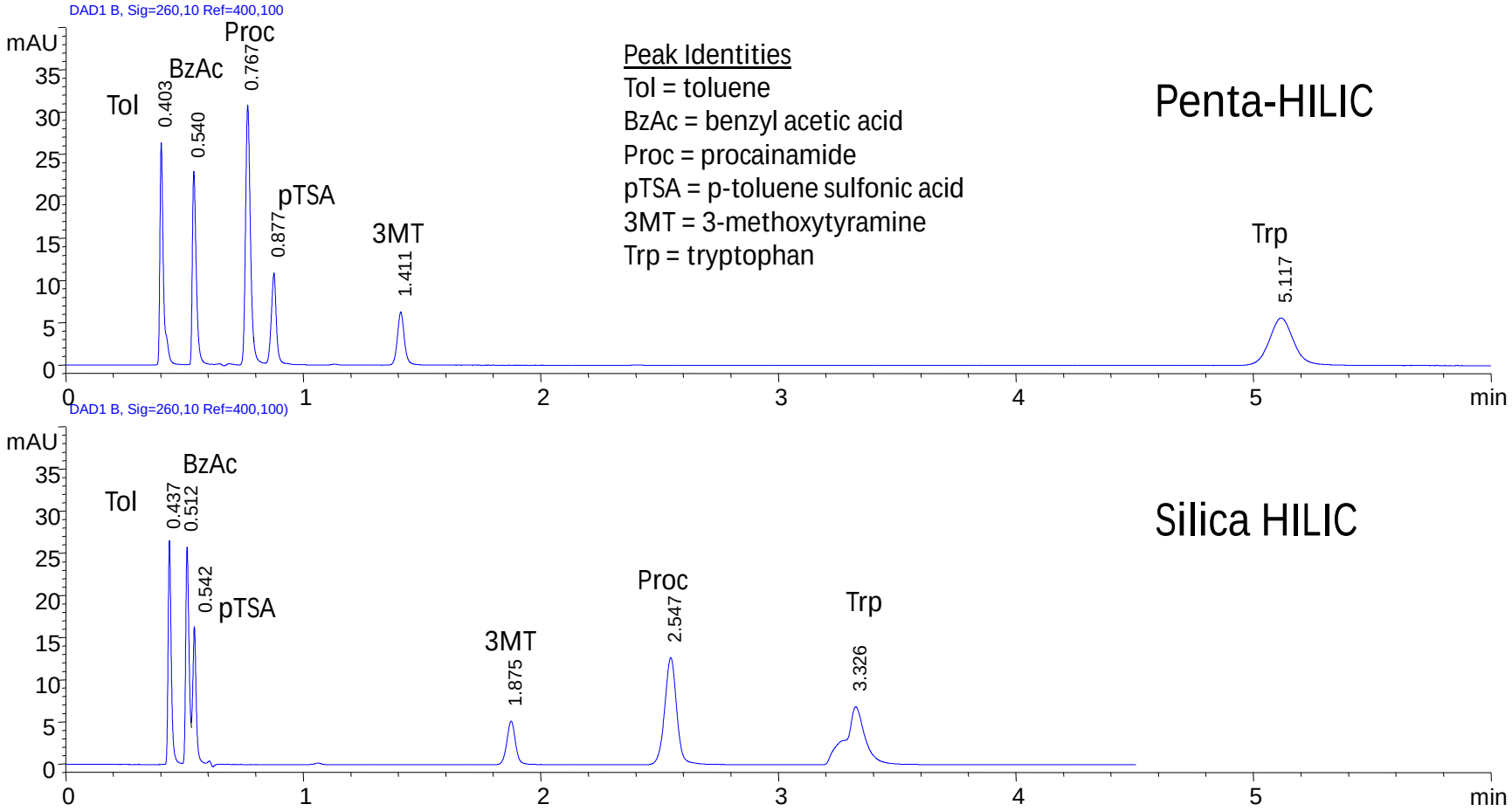
95% Acetonitrile/5 mM NH_4OAc, pH 4.0: 2.1 mm ID x 100 mm, 25 °C, 0.5 mL/min



- The hydroxylic functional group family of bonded-phase surfaces demonstrates increasing retention with chain length (number of hydroxyls).

HILIC Separation of A/B/Z on Penta-HILIC and Silica Surfaces

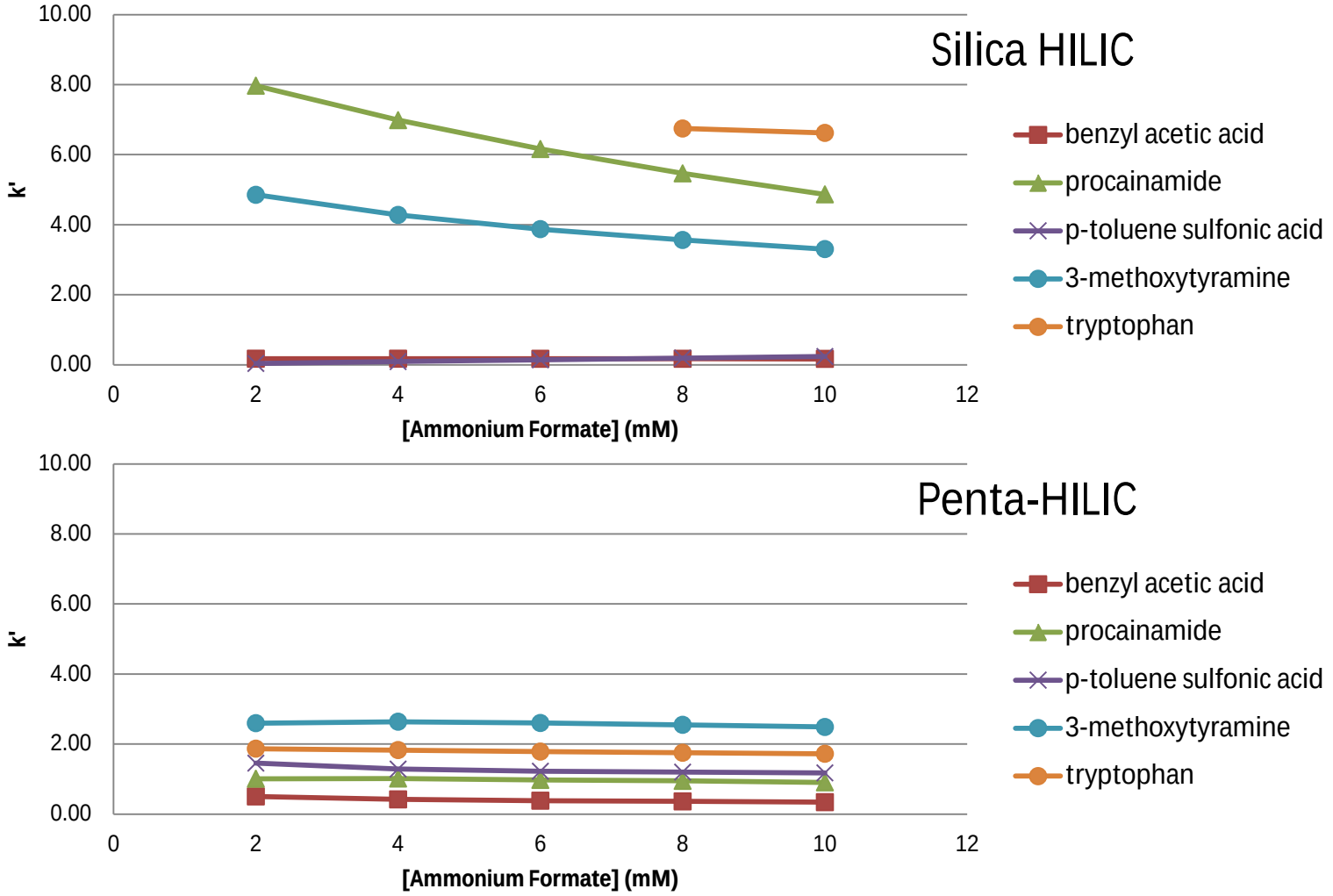
90% Acetonitrile/10 mM NH_4Form, pH 3.0: 2.1 mm ID x 100 mm, 25 °C, 0.5 mL/min



- The HALO Penta-HILIC particle exhibits high retention and good peak symmetry with various small molecules that vary in functional groups.

Effect of Buffer Concentration on HILIC Separations

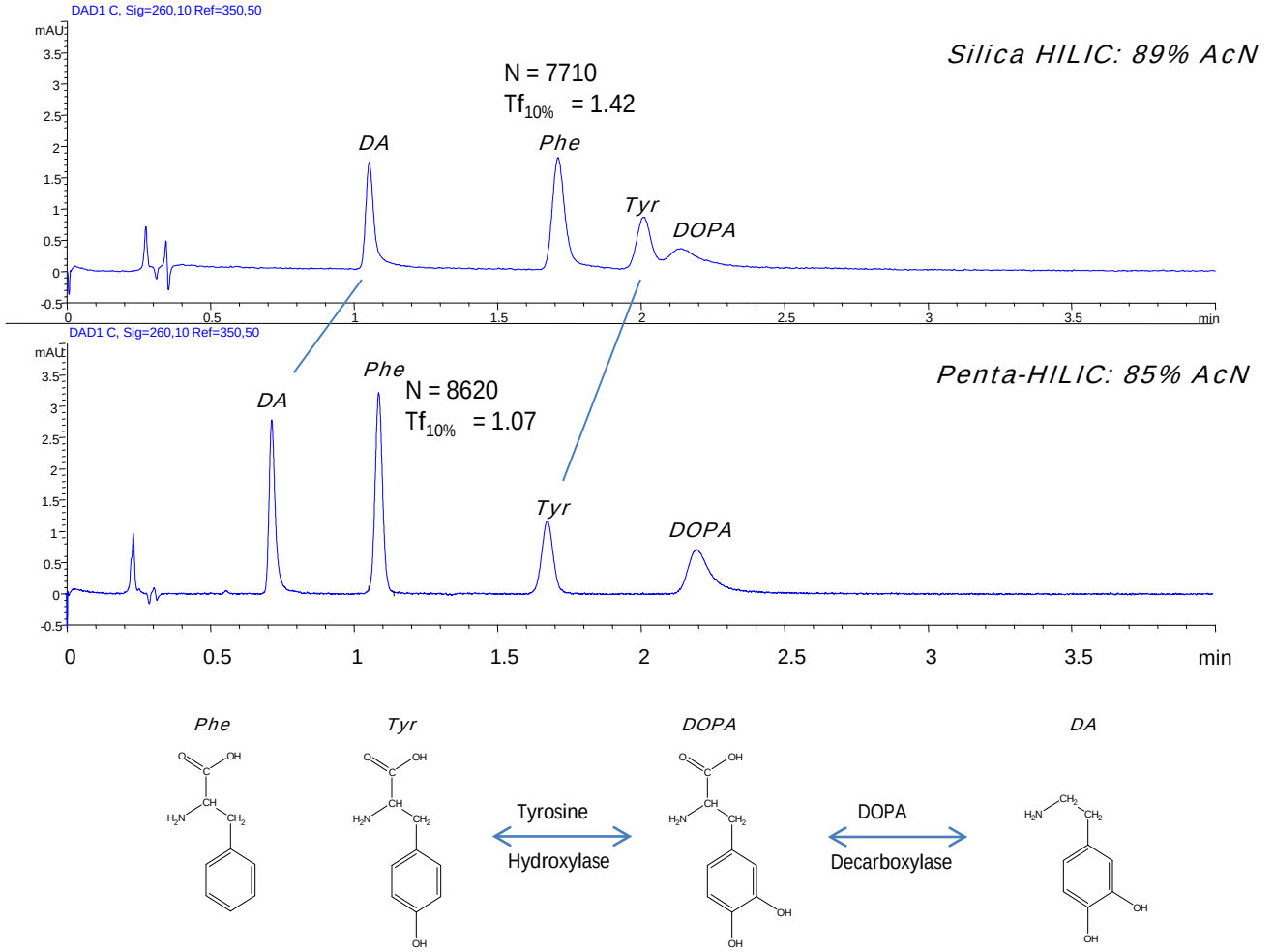
90% Acetonitrile/NH_4Form, pH 3.0: 2.1 mm ID x 100 mm, 25 °C, 0.5 mL/min



- Unlike bare Silica HALO HILIC , the bonded-phase HALO Penta-HILIC surface exhibits little dependence of retention on ionic strength of the mobile phase.

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 mL injection

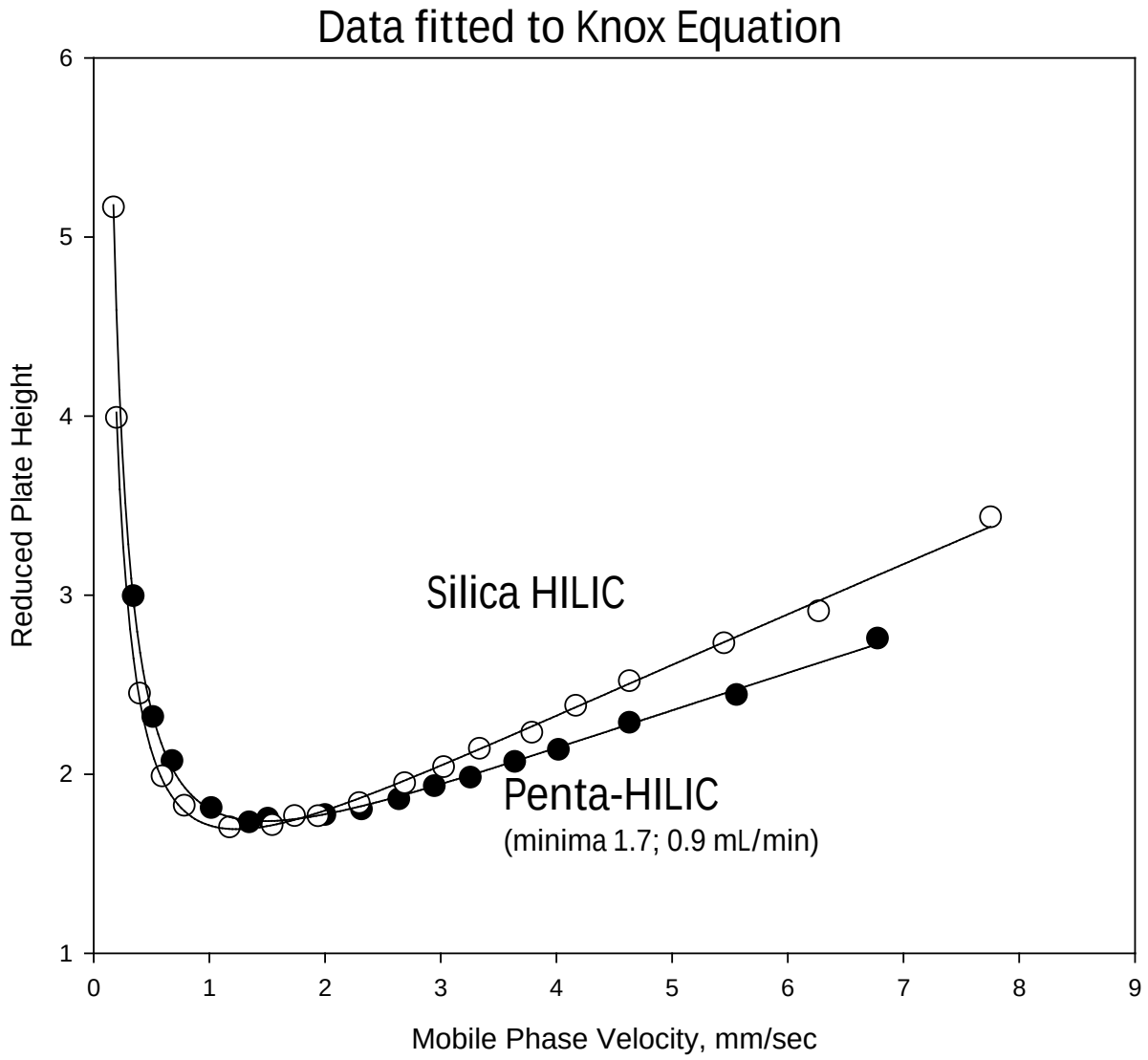


Note: at 85% AcN all k' lower on Silica than on Penta.

- Separations of catecholamines and related amino acids are accomplished at high speed, with good efficiency and peak shape, with modest ionic strength mobile phase modifier.

Effect of Linear Velocity on Penta-HILIC Column Efficiency

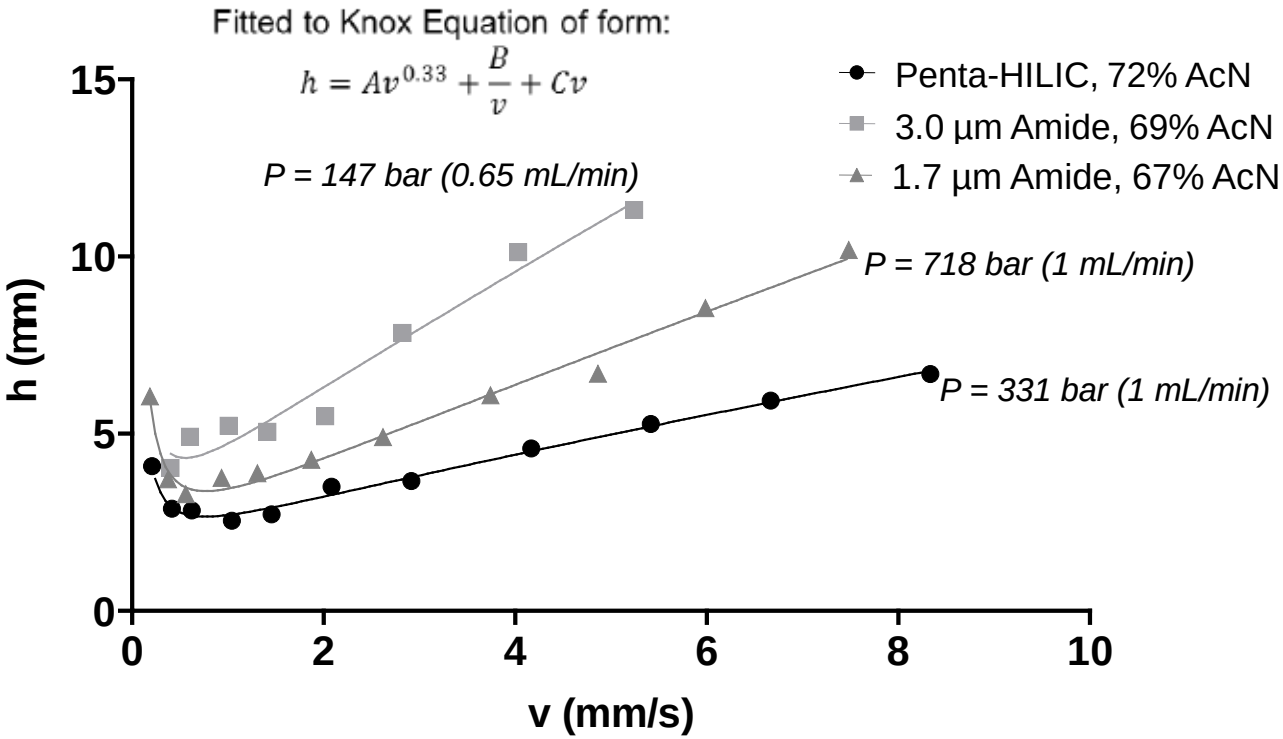
4.6 mm ID x 50 mm; 90% AcN/10 mM NH_4Form 3.0, 25 ℃; 1 mL, 50 ng Adenosine



- The desirable mass transfer kinetics of the superficially porous particle are preserved by appropriate selection of HILIC bonded phase characteristics. Mass transfer properties for bare silica and Penta-HILIC are not significantly different with a small molecule probe.

Column Efficiency Comparisons using Pam-G_5

2.1 (2.0 mm) ID x 150 mm, 60°C, k' ≈ 6,
50 mM Ammonium Formate Aqueous, pH 4.4
0.5 uL Injection (50 pmol), Abs. 300 nm



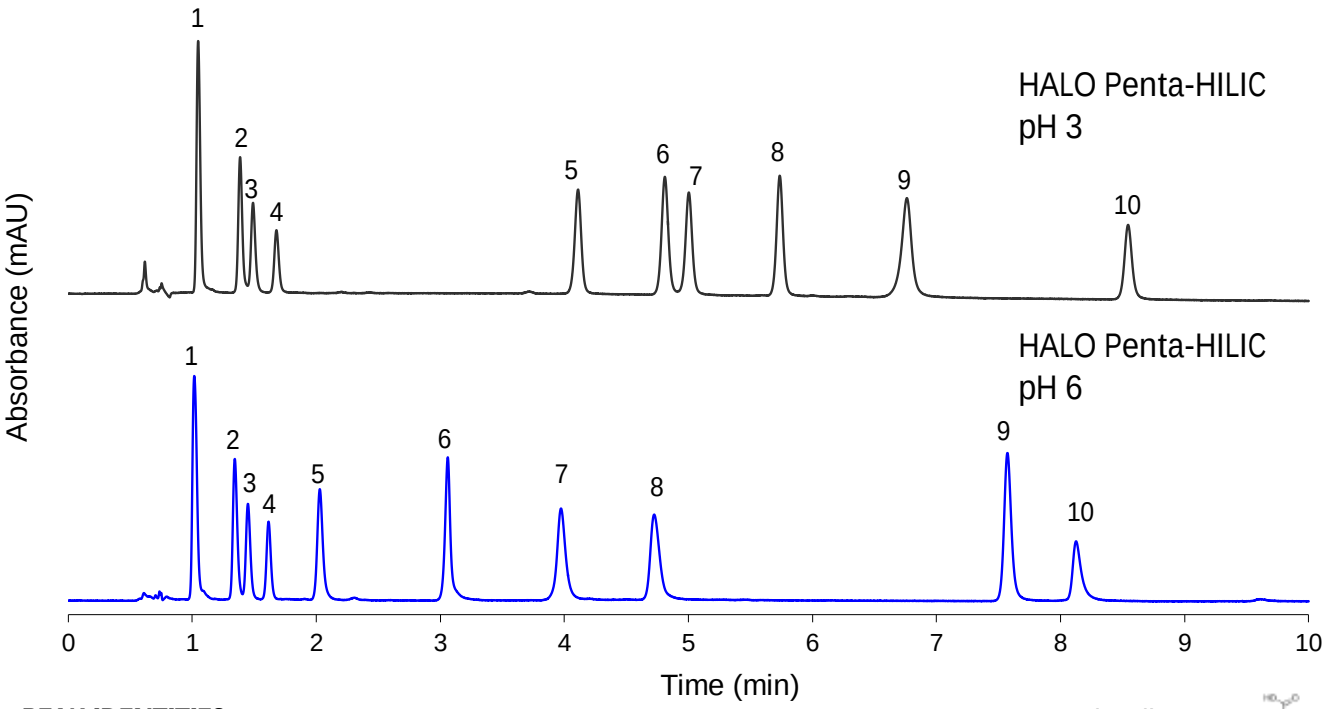
* Pam-G_5 denotes a maltopentaose (5 glucose unit) end-labeled saccharide.

- Column efficiency for separation of this modified glycan is high, and mass transfer efficiency is excellent, compared with current state of the art HILIC column packing materials.

Penta-HILIC Applications

Cephalosporin Analysis on Penta-HILIC

2.1 x 150 mm, 30°C, 0.5 ml/min., UV 254 nm, 85-65% B in 10 min.,
A- 95/5 AcN/H_2O with 5 mM Ammonium formate, pH=3.0 or pH 6.0
B= 50/50 AcN/H_2O with 5 mM Ammonium formate, pH=3.0 or pH 6.0;



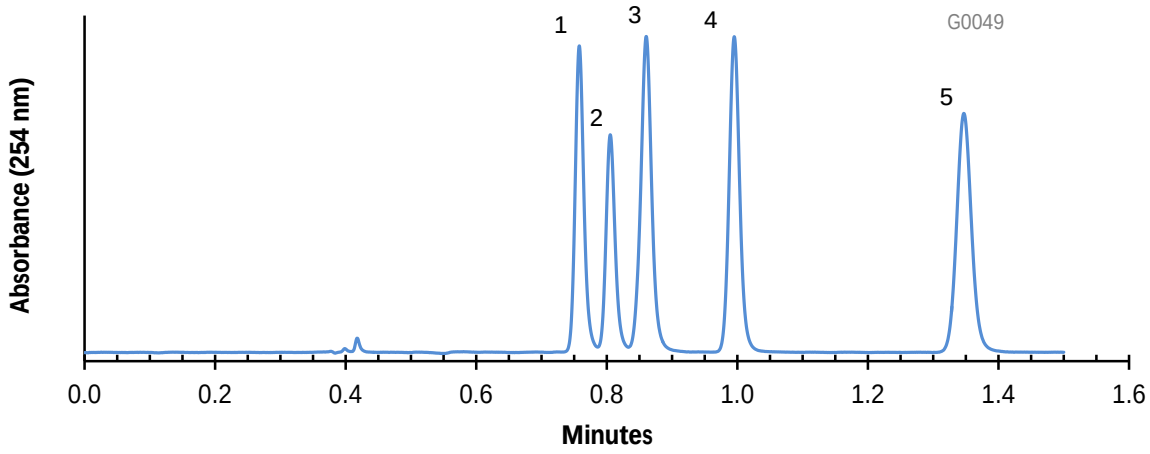
PEAK IDENTITIES:

1. cephalothin
2. cefoxitin
3. cefotaxim
4. cefazolin
5. cefaclor
6. cephalexin
7. cephradine
8. cefadroxil
9. ceftazidime
10. cephalosporin C

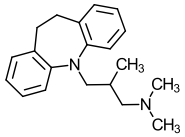
- Separations of this 10 component mixture can be conducted under conditions that manipulate selectivity, while demonstrating excellent peak shape and column efficiency.

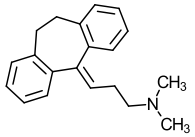
Separation of Antidepressants on HALO Penta-HILIC

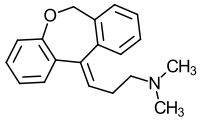
Column: 4.6 x 100 mm; Mobile phase: 7/93: A/B isocratic, A = 0.1 M Ammonium formate, pH=3.5 (adj.),
B = acetonitrile; Temperature: 30 °C; Detection: UV 254 nm, VWD;
Detector response: 0.02 sec.; Injection volume = 0.5 µL; LC System: Shimadzu Prominence UFLC XR

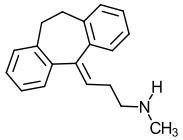


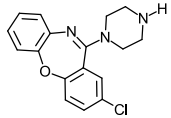
PEAK IDENTITIES:

1. Trimipramine 

2. Amitriptyline 

3. Doxepin 

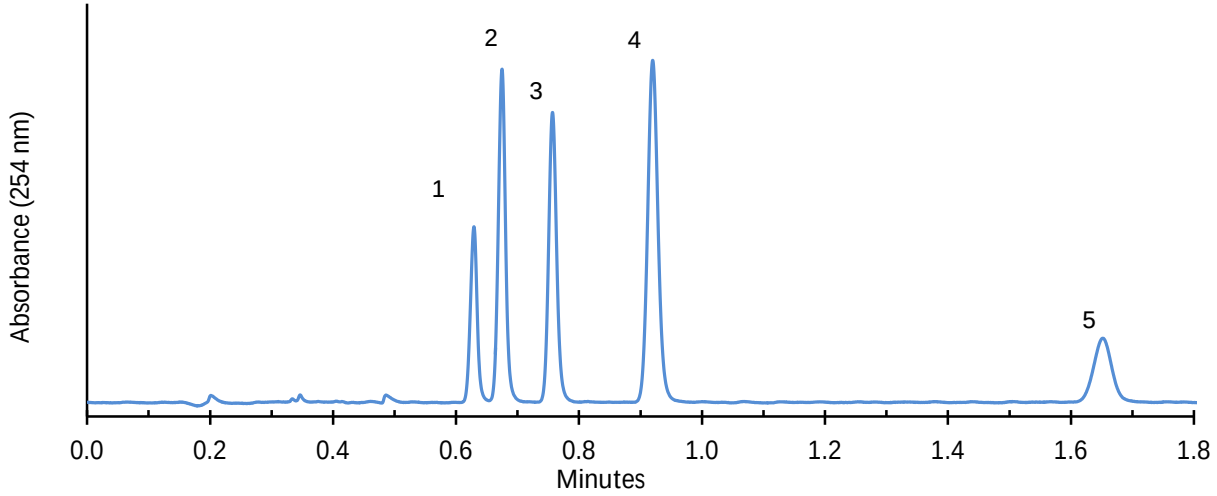
4. Nortriptyline 

5. Amoxapine 

- Highly similar structures can be resolved effectively at high speed, showing good peak shape and high column efficiency.

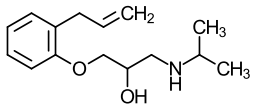
Separation of 5 Beta Blocker Drugs on HALO Penta-HILIC

Column: 4.6 x 100 mm; Mobile Phase: A= 0.04 M Ammonium formate buffer, pH=3.0
B= Acetonitrile; Isocratic: 10/90: A/B
Flow Rate: 3.0 mL/min.; Pressure: 215 bar; Temperature: 30° C; Detection: UV 254 nm, VWD; Injection Volume: 2.0 µL
Sample Solvent: mobile phase; Flow Cell: 2.5 µL semi-micro; LC System: Shimadzu Prominence UFLC XR

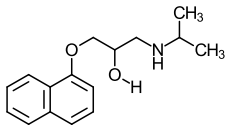


PEAK IDENTITIES:

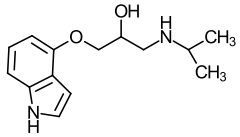
1. Alprenolol



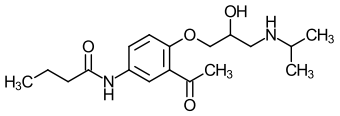
2. Propranolol



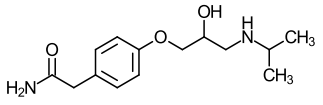
3. Pindolol



4. Acebutolol



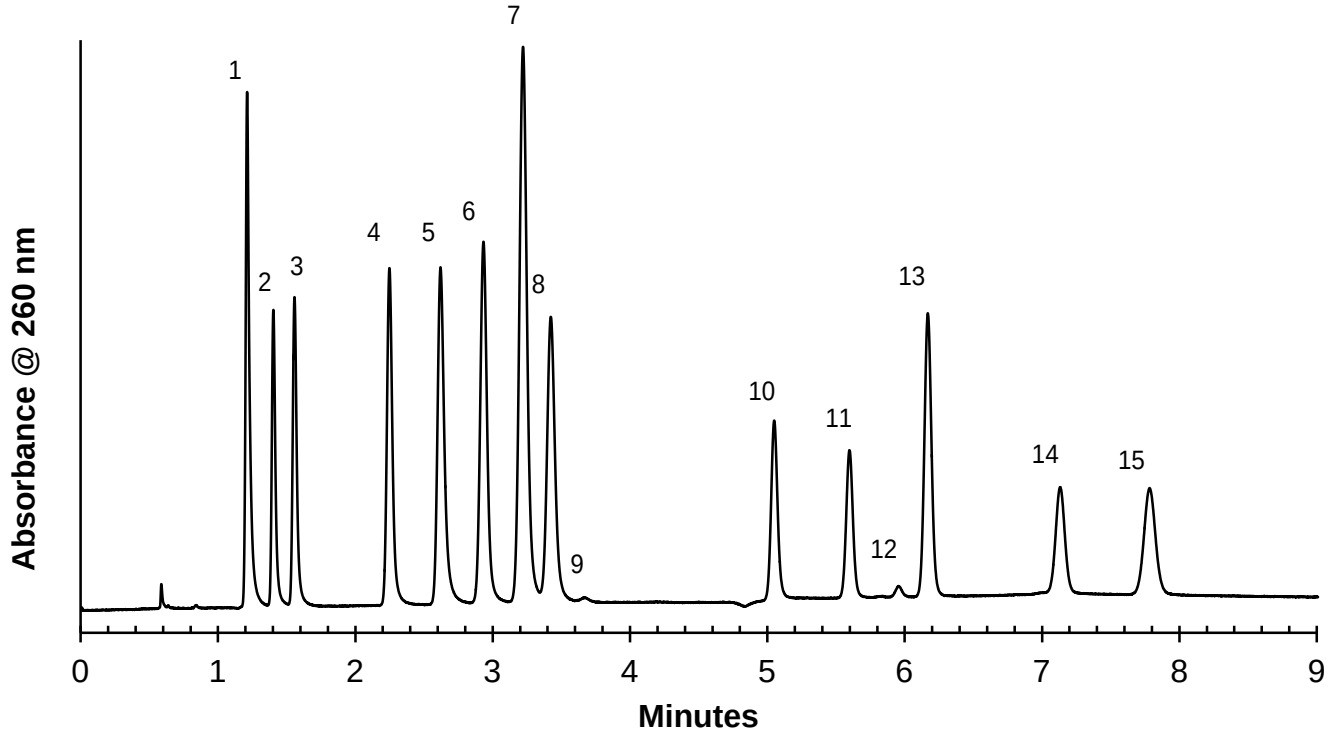
5. Atenolol



- These 5 beta-blockers show both symmetrical and efficient peak shapes.

Separation of Nucleosides and Bases with HALO Penta-HILIC

Column: 4.6 x 100 mm; Mobile phase: A/B gradient, A = 8/92 10 mM ammonium formate, pH 6/acetonitrile, B = 15/85 ammonium formate, pH 6/acetonitrile – gradient: 0 min., 0% B; 3.6 min., 0% B; 3.61 min., 30% B; 4.0 min., 50% B; 6.0 min., 70% B; Temperature: 35 °C; Detector response: 0.02 sec.;

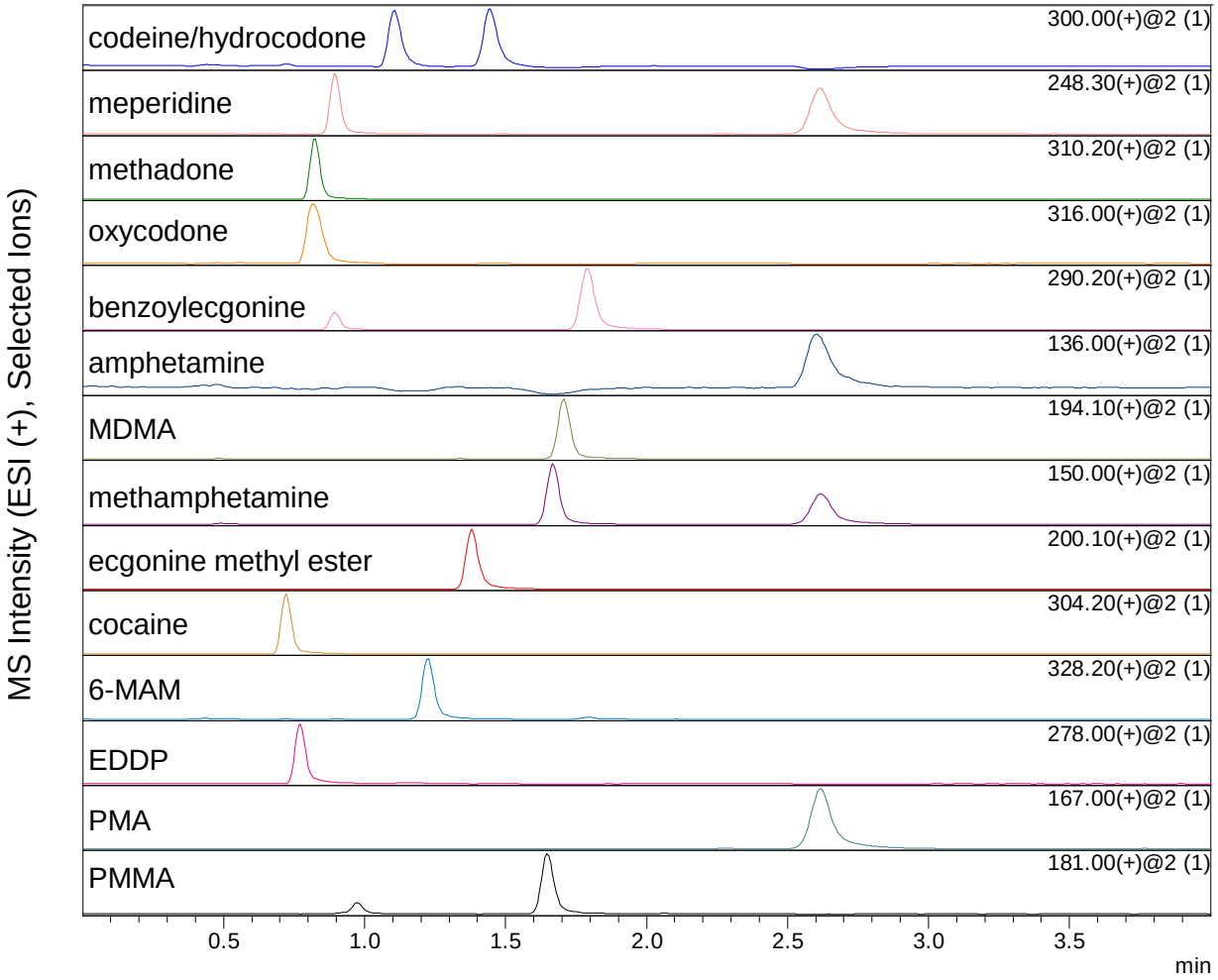


Peak Identities: 1) Thymine, 2) Uracil, 3) Thymidine, 4) 2'-Deoxyadenosine, 5) Adenine, 6) Uridine; 7) Adenosine, 8) Hypoxanthine, 9) Xanthine, 10) Cytosine, 11) 2'-Deoxycytidine, 12) Guanine, 13) 2-Deoxyguanosine, 14) Cytidine, 15) Guanosine

- Separations of this complex mixture can be conducted under selective conditions, showing excellent peak shape and high column efficiency.

Drugs of Abuse and Metabolites

2.1 x 100 mm HALO Penta-HILIC; 95/5 ACN/water with 5 mM Ammonium Formate pH 3; 0.5 mL/min; 1 µL [500 pg each except amphetamine and PMA 100 ng each] injected; SIM, unit resolution, Positive ion mode, 2 kV, 400 °C heat block, 225 °C capillary; Shimadzu Nexera and LCMS 2020



- Collection of common drugs of abuse show symmetrical peaks with good selectivity.

Conclusions

- A variety of highly polar bonded phase superficially porous silica particles were investigated for operation in HILIC separations.
- The Penta-HILIC material was selected for commercial development, based on high retention, good peak symmetry, limited apparent mixed-mode interactions, and desirable mass transfer properties.
- The Halo Penta-HILIC column packing material demonstrates superior separations at high flow rates for a variety of small molecule types, including acids, bases and zwitterions.
- High performance HILIC separations of antibiotics, antidepressants, beta-blockers, nucleosides, nucleobases, drugs, and drug metabolites illustrate the breadth of biological molecule and pharmaceutical compound separations that can be addressed.

Acknowledgements: This work was assisted by the efforts of Mr. Jason Lawhorn, Tim Langlois, Robert Moran and William Miles. Financial support from the NIH/NIGMS is gratefully acknowledged. Shimadzu has generously assisted with the use of the SPD-M30A detector.