

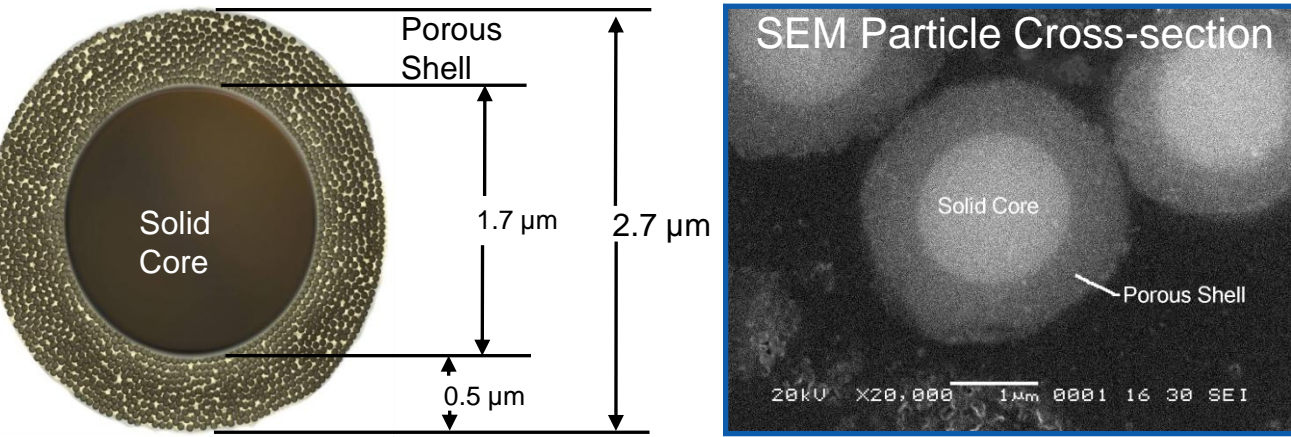
Novel Superficially Porous HILIC Column Packing Material for High Speed Separations of Biological Molecules

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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 core-shell or Fused-Core[®]) silica particles can be obtained with a variety of pore sizes, particle sizes, and shell thicknesses. Columns packed with 2.7 μm diameter Fused-Core particles (Halo[®]) have been shown to exhibit surprising efficiency. Columns packed with Fused-Core bonded phase particles can demonstrate performance superior to smaller-sized porous particles, or at performance equivalence, can be operated at lower column back pressures. High column efficiencies have been observed for the use of bare silica Fused-Core particle columns operated in hydrophilic interaction liquid chromatography separations (HILIC). Our objective has been to develop highly polar bonded-phase surface modifications of Fused-Core silica particles for HILIC separations that maintain the excellent efficiencies previously observed with bare Fused-Core silica, but that exhibit the many potential advantages of covalently-modified surface 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 were synthesized which are observed to be highly hydrophilic, exhibit typical HILIC retention properties, but with notably reduced ionic interactions. We demonstrate applications of such highly polar bonded phases for a variety of HILIC separations of small molecules, synthetic peptides, tryptic digests and small proteins. We compare the retention properties and column efficiencies obtained for these hydroxylated bonded Fused-Core HILIC packing materials using small and larger pore materials. These novel HILIC column packing materials are shown to exhibit advantages for very rapid and high resolution separations of small polar molecules, as well as larger molecules, including polypeptides, glycopeptides and enzymatically-released protein N-glycans.

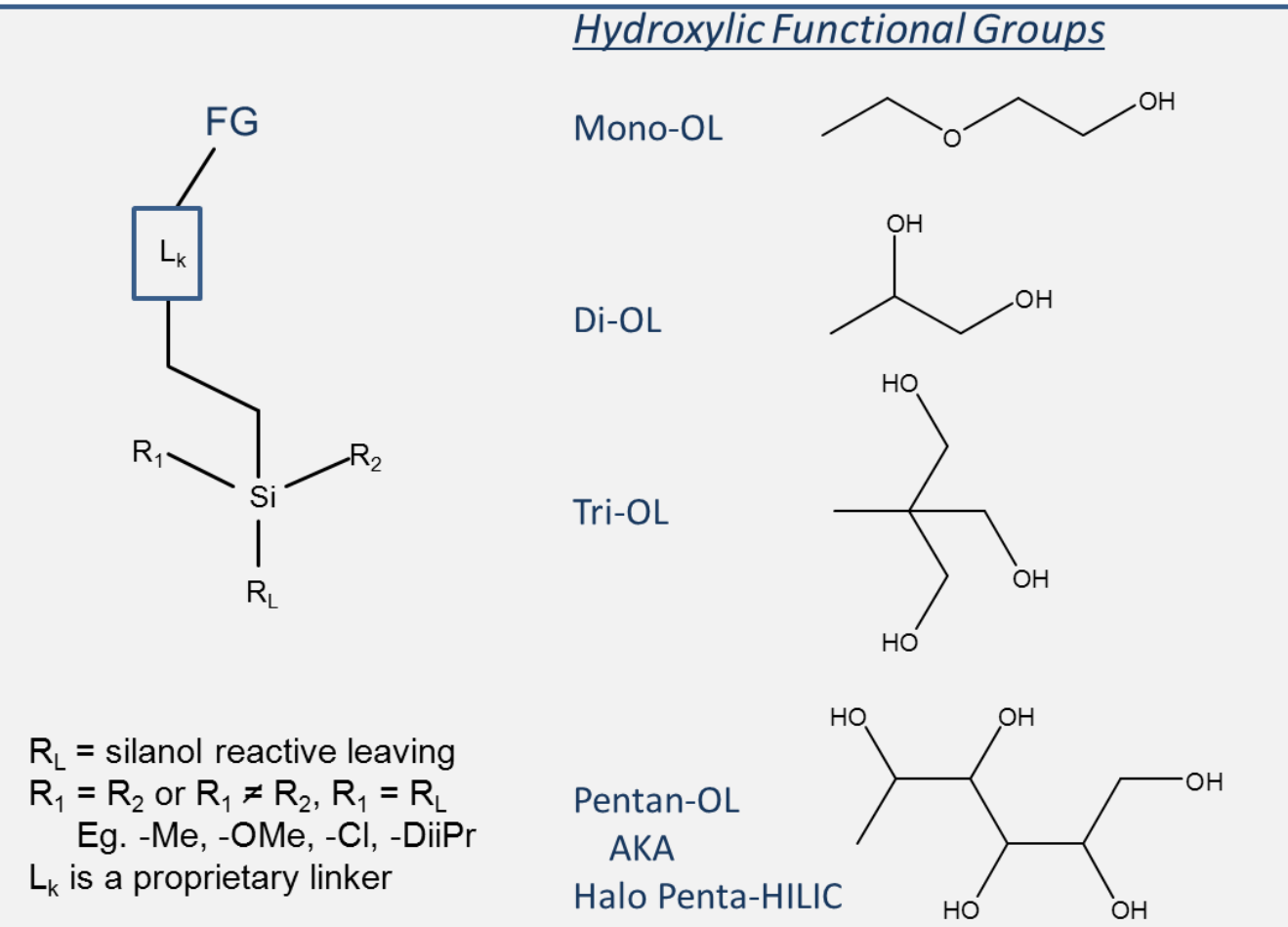
Superficially Porous Particles (Fused-Core[®])



Selection of HILIC Bonded Phases

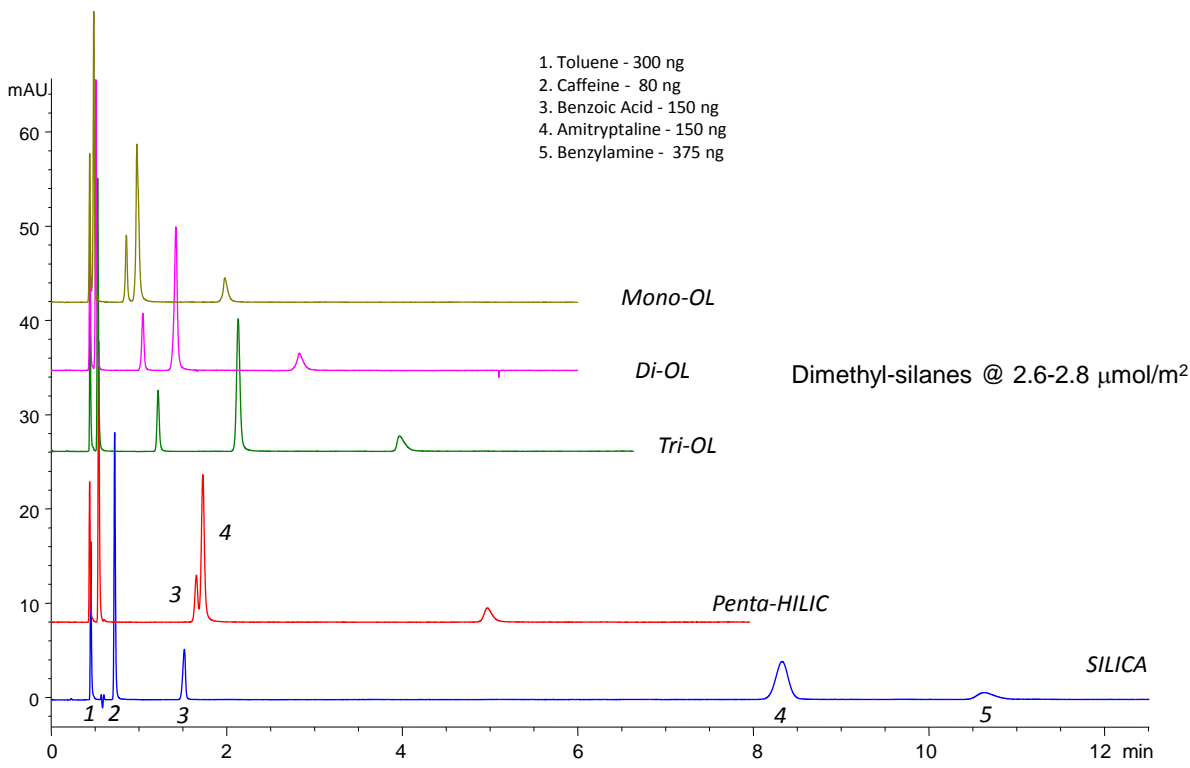
A range of functional group types were immobilized on Fused-Core silica particles. Based on performance properties, the Penta-HILIC structure has been selected for further development.

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



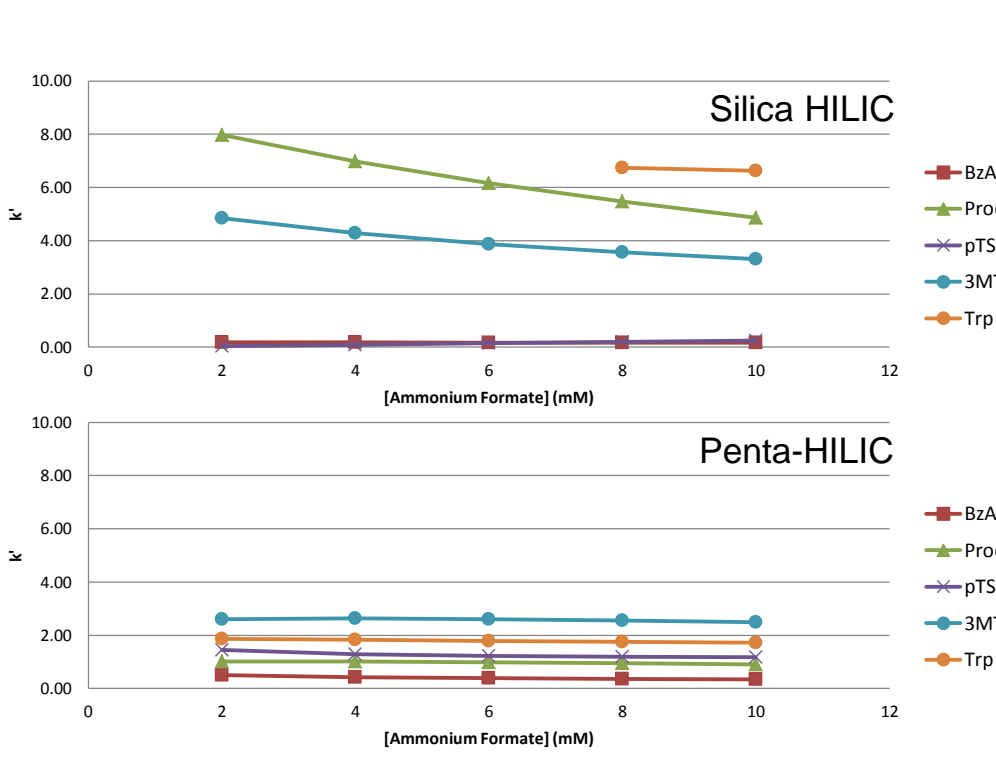
Features of Halo Penta-HILIC

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



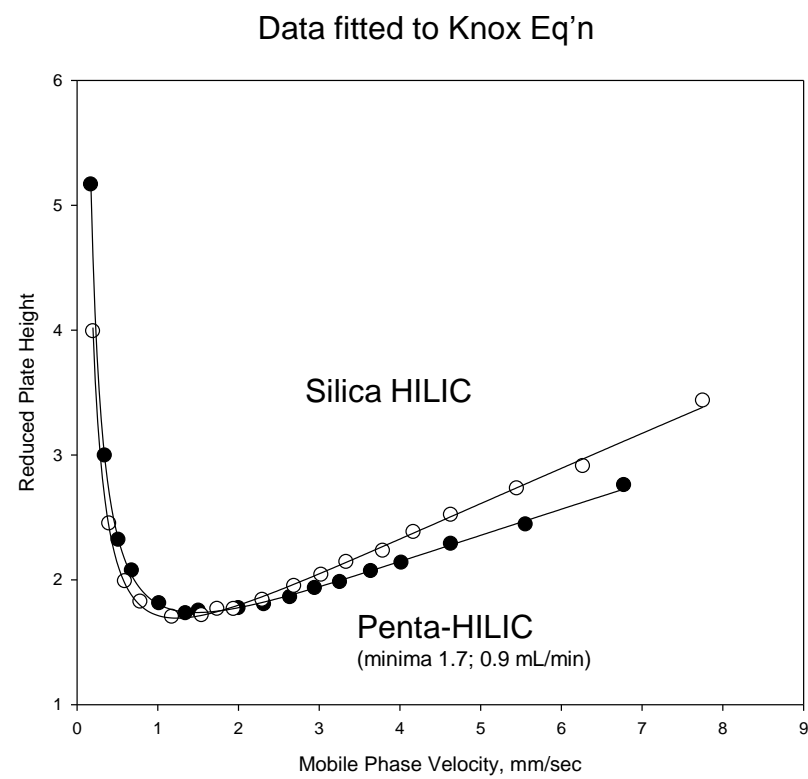
- The hydroxylic functional group family of bonded-phase surfaces demonstrates increasing retention with chain length (number of hydroxyls).

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



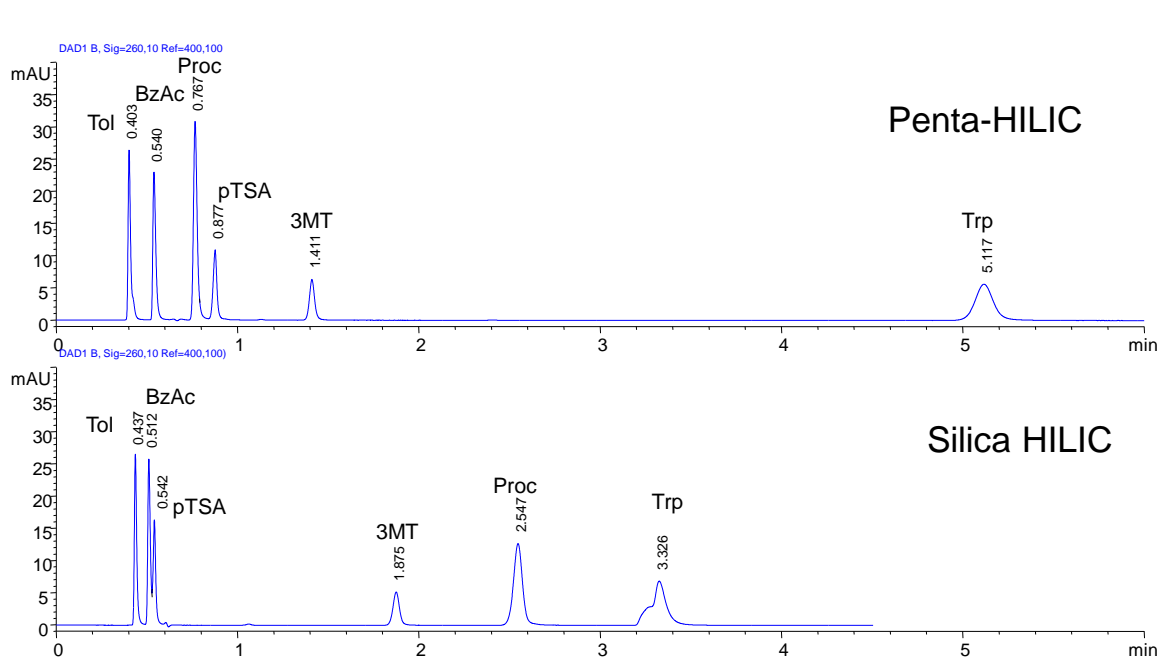
- Unlike bare Silica HILIC, the bonded-phase Halo Penta-HILIC surface exhibits little dependence of retention on ionic strength of the mobile phase.

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 °C; 1 μL , 50 ng Adenosine



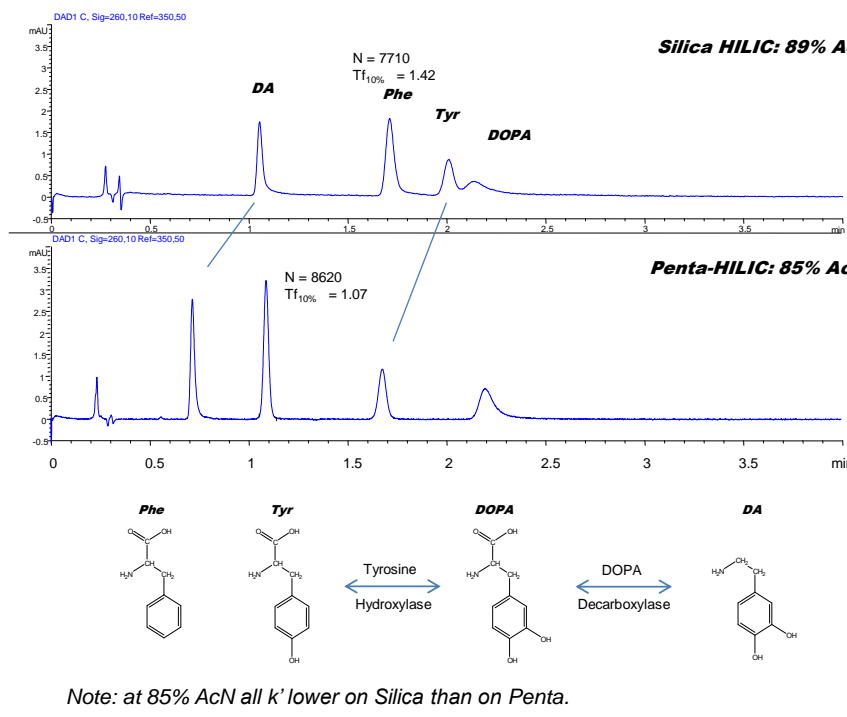
- The desirable mass transfer kinetics of the superficially porous particle are preserved by appropriate selection of HILIC bonded phase characteristics. Mass transfer properties for bare silica and Penta-HILIC are not significantly different with a small molecule probe.

HILIC Separation of A/B/Z on Silica and Penta-HILIC Silane Surfaces
90% Acetonitrile/10 mM NH_4Form , pH 3.0: 2.1 mm ID x 100 mm, 25 °C, 0.5 mL/min



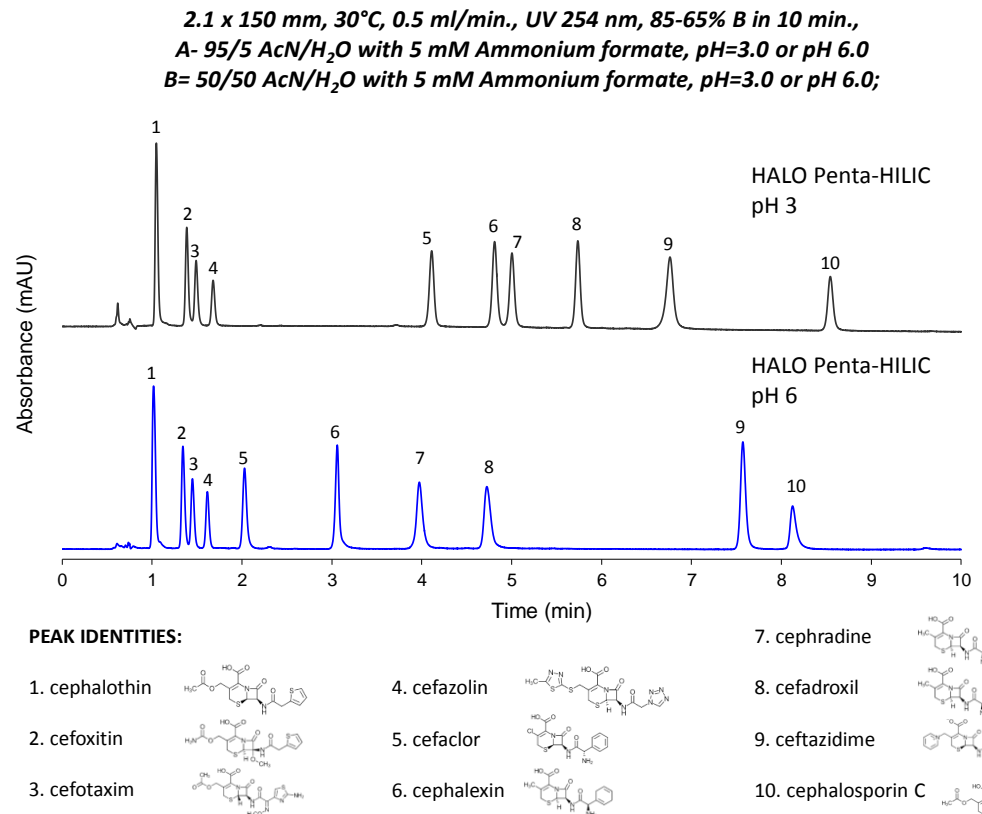
- The Halo Penta-HILIC particle exhibits good peak symmetry with various small molecules that varying in functional groups.

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



- 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.

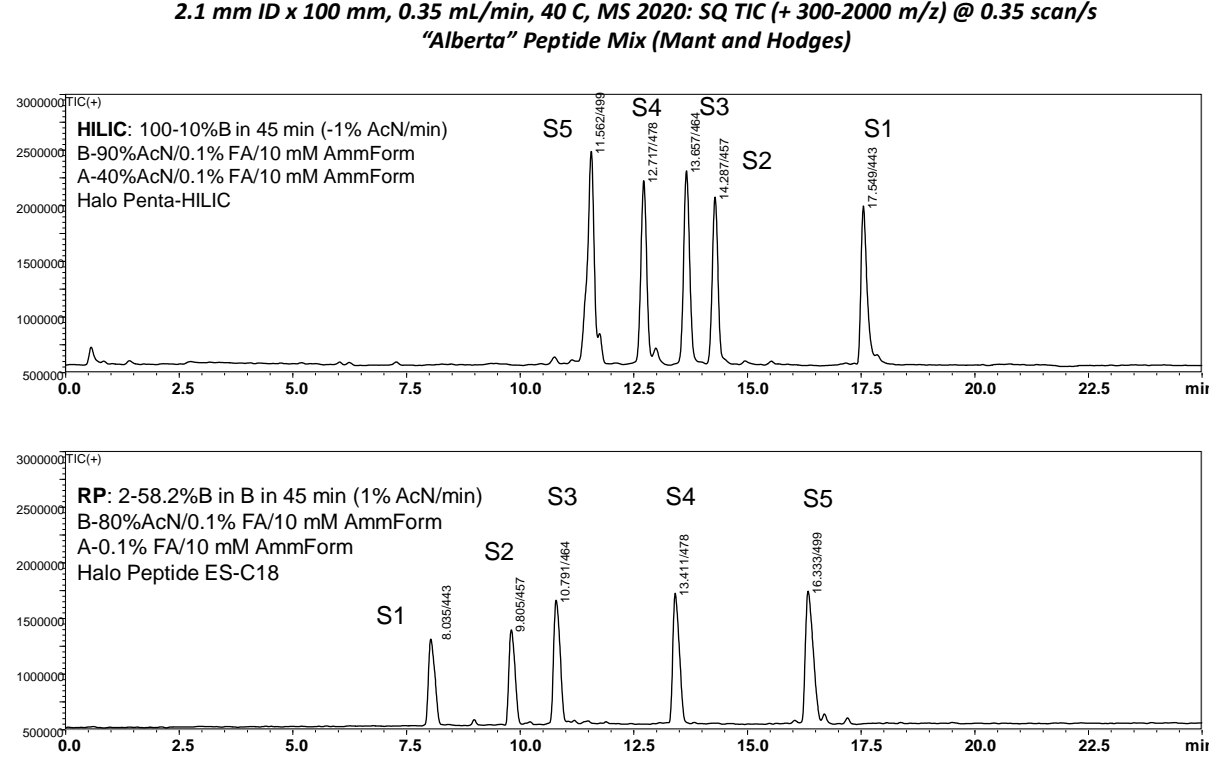
Cephalosporin Analysis on Penta-HILIC



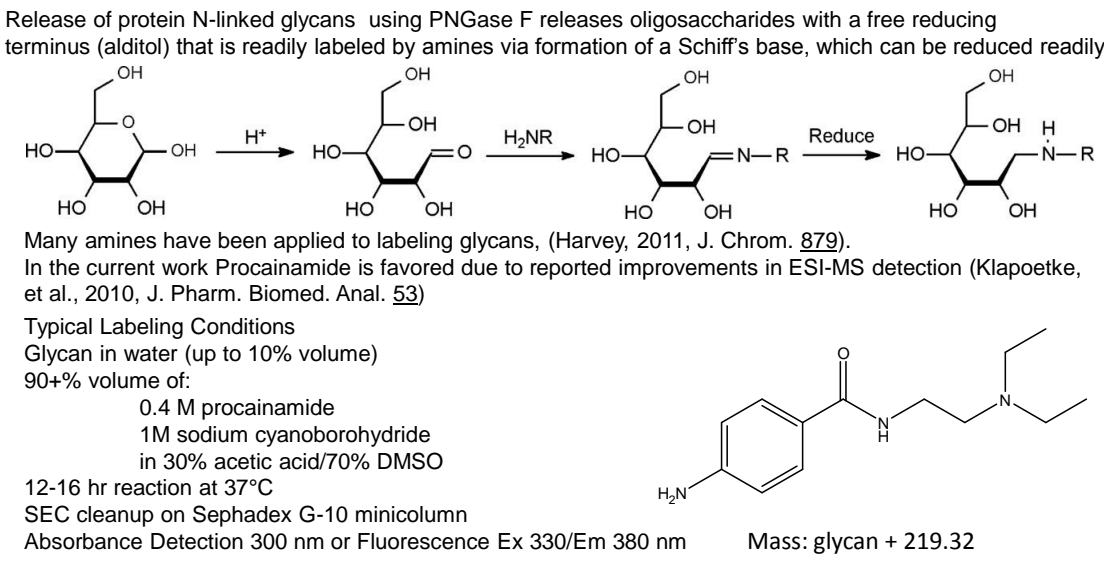
- Separations of this 10 component mixture can be conducted under conditions that manipulate selectivity, while demonstrating excellent peak shape and column efficiency.

Separations of Biomolecules

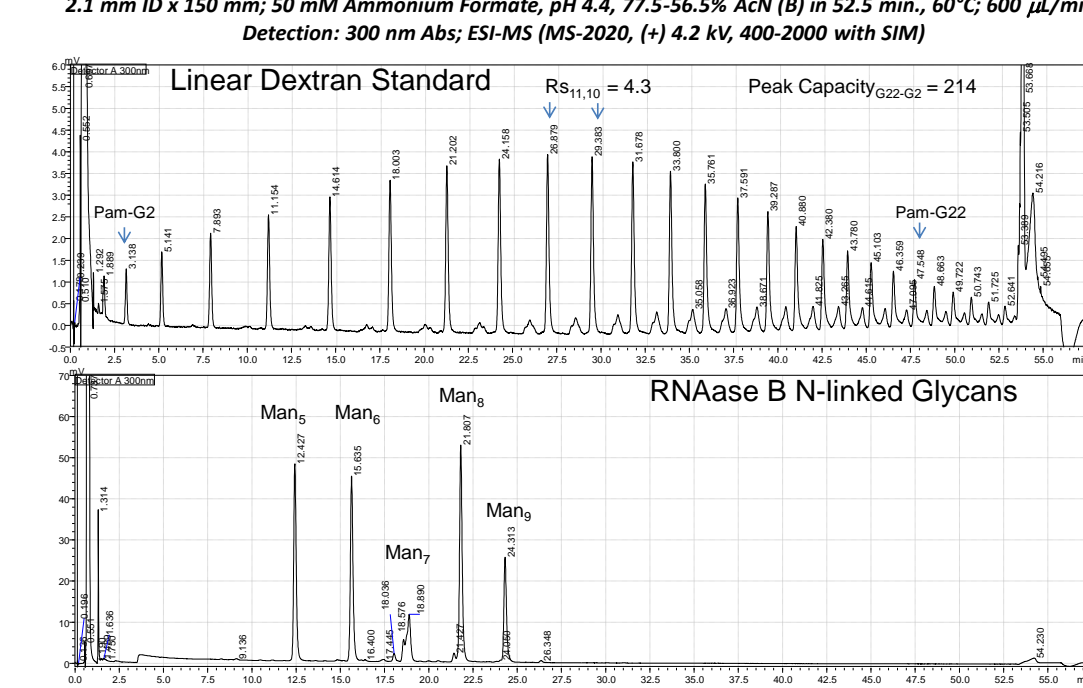
Comparing RP and HILIC Columns for Peptide Separations



Analysis of PNGase Released and Labeled N-Glycans



Penta-HILIC Separations of Labeled Oligosaccharides and Glycans



Penta-HILIC Separations of Abundant bov. a1-AGP N-glycans

