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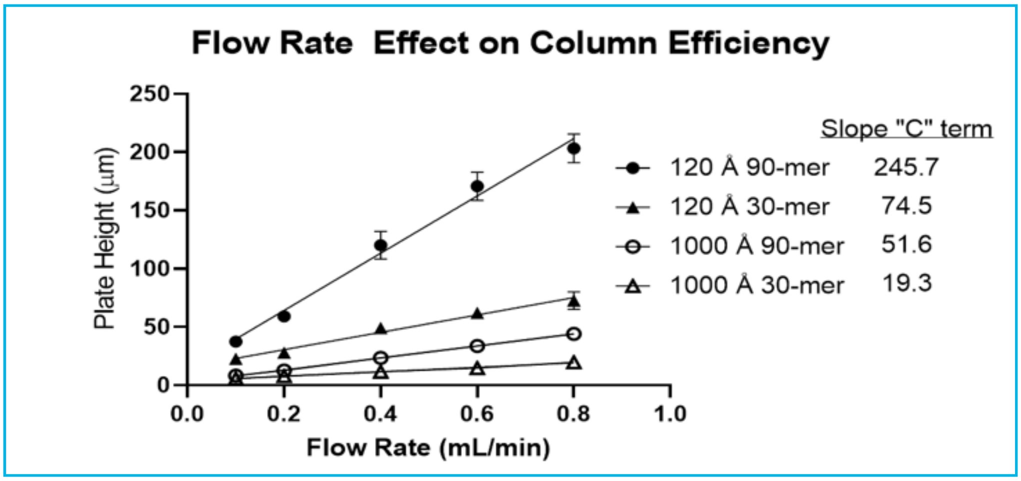
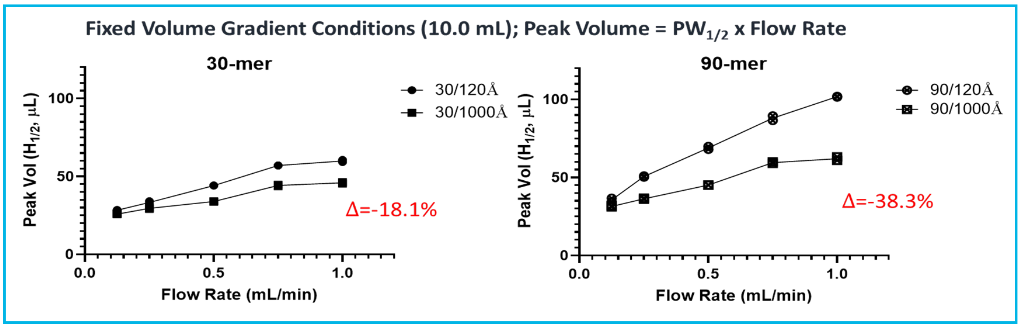
High Efficiency Separation Across Flow Rates





TEST CONDITIONS:

Column: HALO 120 Å OLIGO C18, 2.7 µm, 2.1 x 100 mm
Part Number: P2A62-602
Column: HALO 1000 Å OLIGO C18, 2.7 µm, 2.1 x 100 mm
Part Number: P2762-602
Mobile Phase A: (95)/5 (10mM DiBA/100mM HFIP pH - 8.4)/MeOH
Mobile Phase B: Acetonitrile
Flow Rate: Variable for Van Deemter Plot
Back Pressure: Variable for Van Deemter Plot

Temperature: 60 ºC
Injection: 1µL of ssDNA (10µg/mL)
Sample Solvent: Methanol
Wavelength: PDA, 260 nm
Flow Cell: 1 µL
Data Rate: 40 Hz
Response Time: 0.05 sec.
LC System: Shimadzu Nexera X2

Oligonucleotide ion-pair reversed-phase (IP-RP) retention and band width are highly sensitive to variables such as temperature, ion pair concentration and identity, solvent strength, and linear velocity, which is influenced by system pressure. Interpreting gradient elution band widths requires caution, as pressure effects on retention complicate the analysis of flow rate relationships to column efficiency, particularly in the context of the Van Deemter equation. At a constant gradient volume of 10.0 mL, with varied flow rate, band dispersion was compared under TEAA conditions for different pore sizes and oligonucleotide chain lengths in gradient elution with acetonitrile (AcN); notably, band dispersion was lower on the larger pore SPP relative to the smaller pore material, with a more pronounced difference observed for the large oligonucleotide. To address retention pressure dependence, a method similar to that recently described by Stoll et al. (J. Chromatogr. A, 1744 (2025), 465687) can be employed, where column efficiency is evaluated at constant retention by adjusting solvent strength—estimated via the linear solvent strength (LSS) relationship. Columns packed with two pore size variants of 2.7 µm particles exhibit very similar permeabilities; however, the larger pore material demonstrates significantly higher efficiency for the 90-mer at high flow rates and improved band width for the 30-mer at lower flow rates compared to the smaller pore packing. This behavior is attributed to resistance to mass transfer caused by restricted diffusion in smaller pores, a limitation that persists despite the favorable diffusion path provided by superficially porous particle (SPP) morphology.