Improving Nanobore Column Duty Cycle via Trap-Column Injection: Evaluating the Effect of Trap-Column Injection Flow Rate on Analytical Separation

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Introduction
Sample trapped column injection is an injection strategy commonly employed in nanoflow LC/MS-based analysis of complex peptide mixtures. The approach of sample trapped column injection provides several inherent advantages. By effectively dosing and concentrating samples online, sample tags improve analytical column longevity and throughput. Additionally, the ability to load samples onto a trap column at a much higher flow rate than is feasible for a typical 75 µm ID nanoflow analytical column is a particularly attractive feature of this approach. Duty cycle can be significantly improved when the sample loading flow rate is decoupled from the gradient flow rate. Here we investigate the role sample trap column injection has on analyte recovery and overall chromatographic performance. Using a direct flow nano-LC-PG with the ability to deliver flow rates ranging from 50 nL/min to 20 µL/min coupled to an autosampler and commercially available peptide standards and protein digests, we evaluated the relationship between flow rate, analyte concentration and analytic composition to determine the effect on chromatographic performance.

Methods & Materials

Instrumentation
- Leap Technologies HTC Pal Autosampler
- VIO 400R micro valve
- 1.0 µL sample loop
- VICTOR-port valve with 2 position actuator
- Thermo Q-Exactive/Deca ion trap mass spectrometer
- MS/MS scans
- Full MS Scan
- 3 microscan spectra
- 390.00 - 1500.00 Da mass range
- Customized Digital PicoView nanospray source
- Eksigent nanoLC-3D pump
- Channel 1
  - Mobile Phase A: 98% water with 0.1% formic acid and 2% acetonitrile with 0.1% formic acid
  - Mobile Phase B: 98% water with 0.1% formic acid and 2% acetonitrile with 0.1% formic acid
- Channel 2
  - Mobile Phase A: 0.1% formic acid in water
  - Mobile Phase B: 0.1% formic acid in acetonitrile

Columns
Analytical Column
- Picoscale column (75 µm ID x 15 µm tip) packed to 10 cm with ProPac II 18.5 µm resin
- Trap Column
- Integral trap column (120 µm ID) packed to 2.5 cm with ProPac II 18.5 µm resin

Flow Rate Evaluation

**WHAT IS THE OPTIMAL FLOW RATE FOR SAMPLE TRAP LOADING?**

**HOW DOES FLOW RATE AFFECT PEAK SHAPE?**

**HOW DOES MAXIMIZING TRAP COLUMN CAPACITY AFFECT PEAK SHAPE?**

Samples
- Equimolar mix of four peptides, variable concentrations as indicated:
  - Angiotensin I, 1276 Da
  - Angiotensin II, 1296 Da
  - Val-Angiotensin I, 1282 Da
  - Neurotensin, 1672 Da
- BSA digests (Water Mass Prep): 300 pmol/L in 0.1% formic acid

Conclusions
- The maximum loading capacity of a 100 µm ID x 2.5 cm bead trap column was determined to be 110 ng using a mixture of 4 standard peptides. 110 ng Polyacrylamide represents column saturation for these column dimensions.
- Trap column saturation negatively impacts downstream analytical column performance.
- Cycle time improvements demonstrated using trap column loading flow rates up to 10 µL/min.
- Peptide recovery validated at flow rates ranging from 1–10 µL/min.
- Adjustment of injection time for flow rate is required for maximum peptide recovery.
- Increasing the amount of solvent flowing through the columns at higher flow rates can lead to loss of early eluting peptides.

Future Work
- Evaluate the loading capacity of trap columns with targeted MS/MS scans.
- Investigate trap column performance at flow rates higher than 10 µL/min.
- Incorporate studies for trap columns with other dimensions - for example 75 and 150 µm inner diameter.
- Evaluate the performance of trap columns for sample concentration.
- Study retention of early eluting hydrophilic peptides on different types of resin.