Introduction
Sample trapped column injection is an injection strategy commonly employed in nanofluid LC/MS-based analysis of complex peptide mixtures. The approach of sample trapped column injection provides several inherent advantages. By effectively desalting and concentrating sample onto a trap column at a much higher flow rate than is feasible for a typical 75 pm ID nanofluid analytical column, a particularly attractive feature of this approach is that it allows the use of high performance capillary LC columns. Using a direct flow nano-LC pump with a trap column is a particularly attractive feature of this approach. Daily cycle can be significantly improved when the sample loading flow rate is decoupled from the gradient flow rate. Here we report the use of a trap column injection has on analyte recovery and overall chromatographic performance. Using a direct flow nano-LC pump with the ability to deliver flow rates ranging from 50 nl/min to 250 µl/min coupled to an autosampler and commercially available peptide standards and protein digestes, we evaluated the relationship between flow rate, analyte concentration and analyte composition to determine the effect on chromatographic performance.

Methods & Materials

Instrumentation
- Leap Technologies HTC PAL Autosampler
- Thermo LTQ Deca ion trap mass spectrometer
- MRM scan - Full MS Scan
- 3 Micromass spectra
- 390.00 - 1500.00 Da mass range
- Customized Digital PicoView nanospray source
- Elite nanofL-15D 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
- PicoPack column (75 µm ID x 15 µm tip) packed to 10 cm with Proteopac HILIC 5 µm resin
- Trap Column
- Innosil trap column (100 µm ID) packed to 2.5 cm with Proteopac II HILIC 5 µm resin

Sample Preparation
- Equimolar mix of four peptides, variable concentrations as indicated
- Angiotensin I, 1276 Da
- Angiotensin II, 1045 Da
- Val-Angiotensin I, 1282 Da
- Neurotensin, 1672 Da
- Brain digest (Waters Madigest)
- 300 fmol/µl in 0.1% formic acid

Future Work
- Evaluate the loading capacity of trap columns with targeted MRM 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 hydrophobic peptides on different types of 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?

Trap Column Capacity Evaluation

HOW MUCH SAMPLE CAN BE LOADED ONTO THE TRAP COLUMN?