Investigation of Elastomer Core Trap Column and Maximizing Sample Loading Capacity

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Introduction

Numerous injection strategies exist (direct, trapped, multi-dimensional) for nanobore LC/MS based analysis of complex peptide mixtures. Compared to direct on-column injection, sample trapped-column injection provides multiple advantages. By effectively desalting and concentrating samples on-line, sample traps improve analytical column longevity and throughput. Unfortunately trap-column injection suffers from comparatively degraded sensitivity and a loss of chromatographic performance. A novel elastomer-core trapping column format is evaluated in terms of chromatographic and mass spectrometric figures of merit. Using a BSA tryptic digest and a three peptide mixture, the performance of this sample trap configuration was assessed by evaluating loading capacity, operating back pressure, peak shape and sample recovery relative to a conventional format, fused-silica. In the process of evaluating trapping column format, the importance of matching the sorbent chemistry of the trapping column to the analytical column was observed and is demonstrated.

Methods

Chromatography

- Leap Technologies HTC Pal Autosampler
  - VICI 6-port micro valve
  - 1.0 µL Sample loop
- Eksigent nanoLC-2D pump
  - Channel 1 - Isocratic loading
    - Mobile Phase A = 0.1% formic acid in 2% acetonitrile/98% water
    - Mobile Phase B = 0.1% formic acid in 2% acetonitrile/98% water
    - Sample trap loading - 5 µL/min. for 5 minutes
    - On-Column loading - 2 µL/min. for 10 minutes
  - Channel 2 – Gradient elution
    - Mobile Phase A = 0.1% Formic Acid in Water
    - Mobile Phase B = 0.1% Formic Acid in Acetonitrile
    - 300 nL/min. Gradient elution
    - 25 Minute gradient to 50% B
- VICI® 10-port micro valve for column switching

Mass Spectrometry

- Thermo LCQ Deca™ Ion Trap
  - 3 microscans/spectra
  - 375.00 – 1600.00 Da mass range for MS spectra
  - Top-3 data dependent mode with dynamic exclusion for MSMS spectra
- New Objective Digital PicoView® Nanospray Source (DPV-150)
- XCalibur™ v1.3 and Bioworks™ for data analysis

Columns

- Analytical Column (AC)
  - 75 µm ID PicoFrit® Column (New Objective) with a 15 µm integral frit slurry packed to 10 cm with 5µm ZORBAX® 300SB-C18 (Agilent)
- Trapping Columns
  - IntegraFrit™ Sample Trap Column (IFST)
    - 150 µm ID packed to 20 mm (New Objective) with 5 µm ZORBAX® 300SB-C18 (Agilent)
    - Column housing - PEEK™ sample trap column assembly containing a NanoFilter capsule with a 1µm titanium frit at the inlet and a PEEK capsule union at the outlet
  - PicoClear™ Sample Trap Column (PCST)
    - 300 µm ID packed to 4.0mm with 5µm ZORBAX® 300SB-C18 (Agilent)
    - Column housing – PicoClear™ Union body (New Objective) with a 50 µm ID IntegraFrit™ (New Objective) plumbed in each side

Samples

- BSA digest (Waters MassPREP™)
  - 300 fmol/µL in 0.1% formic Acid
- Equimolar mix of three peptides
  - Asn1, Val5-angiotensin II, angiotensin II and bradykinin (Sigma Aldrich)
  - 0.05 ng/µL, 0.10 ng/µL, 0.25 ng/µL, 0.50 ng/µL, 1.0 ng/µL and 10 ng/µL in 100% water
Investigation of Elastomer Core Trap Column and Maximizing Sample Loading Capacity

**Figure 1** Upschurch Sample Trap Cartridge, assembled, with 150 mm ID x 20 mm IntegraFrit Sample Trap Column inserted.

**Figure 2** PicoClear Union with 300 µm ID x 4.0 mm packed bed inside PicoClear elastomer core.

**Figure 3** HTC Pal autosampler equipped with automatic VICI 6-port micro valve plumbed with 1.0 mL loop, Eksigent nanoLC-2D pump, VICI 10-port micro valve—for switching between trap loading and gradient elution—and LCQ Deca ion trap mass spectrometer.

**Figure 4** (A) Channel 1 on nanoLC-2D pump loading sample trap in forward direction, and (B) Channel 2 on nanoLC-2D pump gradient eluting off of the sample trap in the forward direction, onto the PicoFrit Analytical Column.

**Figure 5** The ratio of \( \frac{w_b}{w_a} \) at 10% peak height indicates the asymmetry of a chromatographic peak. A perfectly symmetrical peak will have a value of 1, a tailing peak will have a value greater than 1 and a value less than 1 indicates fronting.
Figure 6  Column loading capacity at six different amounts of Asn1, Val5-Angiotensin II peptide: 0.05 ng, 0.10 ng, 0.25 ng, 0.50 ng, 1.0 ng and 10 ng (A) PicoFrit Analytical Column, on-column injection; (B) IntegraFrit Sample Trap Column, trap injected; (C) PicoClear Sample Trap Column, trap injected.

Figure 7  Plot of FWHM peak width in seconds for Asn1, Val5-Angiotensin II, angiotensin II and bradykinin each at six different amounts on three different column formats (A Analytical column, on-column injection; B IntegraFrit Sample Trap injection, and; C PicoClear sample trap injection). The slope of the plots for the three different formats indicates the traps have a higher loading capacity than the analytical column. The capacity of the PicoClear sample trap slightly exceeds the capacity of the IntegraFrit sample trap as demonstrated by the slope of the plots.

Figure 8  SIC of 7 BSA peptides on three different column formats at m/z 739.9, 751.8, 554.2, 922.4, 582.5, 507.9, 997.4 (A) PicoFrit Analytical Column (B) IntegraFrit Sample Trap (C) PicoClear Sample Trap.
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Figure 9 Separation of 300 fmol BSA digest on three different column formats in triplicate. Mean (n=3) peak width (FWHM) and mean peak asymmetry at 10% peak height is shown. Both trap column formats show an increase in peak width and peak tailing, as indicated by peak asymmetry values greater than 1, compared to on-column injection. The PicoClear sample trap column performs as well as the IntegraFrit sample trap in terms of peak shape, width and signal to noise.

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Figure 10 Peptide sequence coverage of BSA digest on three column formats. Peptides not detected are indicated in black italics. Sequence coverage for each format is indicated as a percentage of amino acids identified from the total. The PicoClear sample trap shows improved sequence coverage by almost 78%, as compared to 72% for the IntegraFrit sample trap. This improvement in peptide recovery can be attributed to the different geometry (300 mm ID vs. 150 mm ID).
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Results

• Matching the sorbent chemistry of the sample trap to the sorbent chemistry of the analytical column results in improved peak resolution.
• Employing sample traps in-line with an analytical column improves the overall loading capacity.
• Sample traps improve peptide recovery as compared with on-column injection.
• The PicoClear™ Sample Trap has a higher loading capacity than the IntegraFrit sample trap.
• The PicoClear Sample Trap demonstrated improved peptide recovery compared to the IntegraFrit™ Sample Trap.
• The PicoClear Sample Trap Column is a new column format offering improved overall performance as compared to a traditional fused-silica sample trap (IntegraFrit).

Future Work

• Investigate benefits of matching sample trap chemistry to analytical column chemistry further using other commercially available chemistries.
• Evaluate PicoClear sample trap with different bed volumes to determine its effect on performance and recovery.
• Optimize sample trap elution to minimize band broadening.

Figure 11 Separation of 300 fmol BSA digest (A) IntegraFrit Sample Trap, chemistry matched with PicoFrit Analytical Column (B) IntegraFrit Sample Trap, chemistry matched with PicoFrit Analytical Column (C) IntegraFrit Sample Trap, chemistry not matched to PicoFrit Analytical Column. Matching the C18 chemistry of the sample trap to the C18 chemistry of the analytical column results in better overall chromatographic performance as demonstrated here, qualitatively, by improved resolution.