

Optimization of Nanobore Capillary Trapping Column Geometries for Analysis of Peptide Mixtures

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Introduction

Nanobore reversed-phase HPLC has become a mainstay for protein identification by modern tandem mass spectrometry. Enhanced robustness and versatility is obtained with trapping columns for on-line preconcentration and desalting of samples. Trapping columns of varying sizes and packing materials are available, yet few configurations accommodate 75 μm internal diameter (ID) packed-tip columns frequently employed in high-sensitivity work. Using a novel fluoropolymer and PEEKTM-based zero-dead-volume fitting with packed-tip, silica-fritted capillary columns, variable trap column geometries, and column chemistry combinations were investigated toward optimizing chromatographic performance in a typical nanobore RP-HPLC-MS system.

Methods & Materials

LC-MS analysis of a peptide mixture was performed using a Q-ToF PremierTM (Waters Corporation) equipped with a nanoAQUITY UPLCTM (Waters Corporation) and 10 cm x 75 μm analytical columns possessing 15 μm ID tips. Trap columns were positioned in-line of the analytical column and upstream of a micro-tee union used both as a vent and liquid junction. Several trapping column geometries were custom-fabricated in-house; columns ranged from 5 mm – 25 mm lengths and 75 μm to 250 μm IDs with in-column silica frits. A 1 mm x 380 μm ID trap was fabricated within optically transparent unions with novel fluoropolymer cores (PicoClearTM, New Objective). Each trap column contained a 100 nL total bed volume which was controlled while trap geometries were varied. Optically-transparent unions facilitated complete inspection of trap columns to confirm bed volume and integrity of capillary junctions. To minimize chromatographic variation between various trap-column formats, pre-cut and polished glass capillary tubing was used throughout the system. For studies of trap column-column geometry, both the analytical column and various trap columns were packed with 5 μm -diameter ProteoPepTM II C18 sorbent (New Objective).

MassLynxTM (Waters Corporation) was used to control an automated 5 μL trap injection of an enolase digestion. For each ~40 min. run, 125 fmol were injected across trap columns at 4 $\mu\text{L}/\text{min.}$ for 5 min. 100% A-Buffer (0.1% formic acid). Once the loop was switched out of line, B-Buffer (0.1% formic acid in ACN) concentration was increased from 1-51% over 10 min. at 300 nL/min.; after holding the B-Buffer concentration at 51% for 5 min. and washing at 95% B for 5 min., the gradient was concluded by returning to initial aqueous and organic modifier concentrations. Full-scan MS data for the 395 Da-1990 Da range were acquired in 0.2 second scans with a 0.02 second interscan delay. This rapid scan speed obtained superior data for fast-eluting peaks.



Figure 1 PicoClearTM clear unions as sample-trap cartridges. A) 10 mm trap column; B) 25 mm trap column. Transparent fittings allow visual monitoring of the trap for debris and integrity.

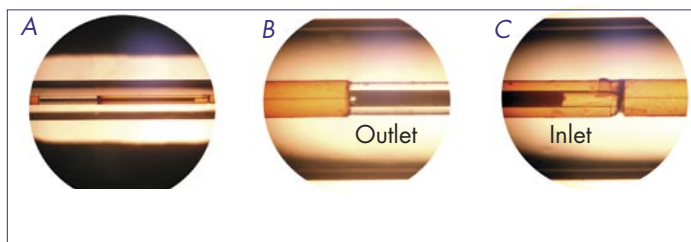


Figure 2 (A) Low (2.5x objective) and (B, C) high magnification (10x objective) photomicrographs of the clear trap assembly taken directly through the trap body. Shown is a 150 μm ID trap packed inside a length of fused silica tubing (380 μm OD) fritted at the outlet end. The length of inlet and outlet tubing are 20 μm ID. Note the precise axial alignment of the connection tubing with respect to the trap bore, ensuring a high-performance connection.

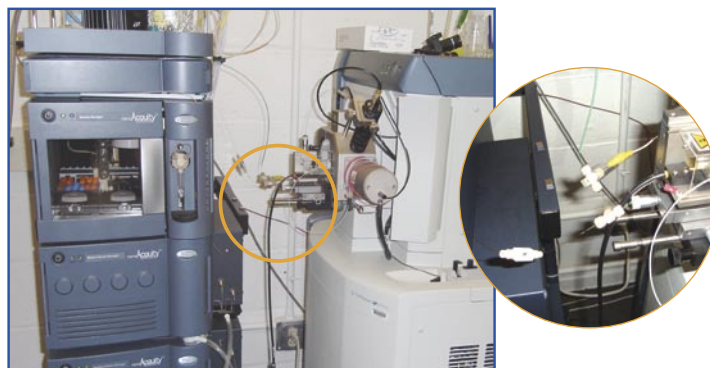


Figure 3 Waters nanoACQUITY UPLCTM System and Q-ToF PremierTM with PicoClearTM union, trap split (Upchurch[®] MicroTee), and liquid junction visible (inset). Pre-cut fused-silica capillaries ensured clean mating of fittings, with easy confirmation of the trap column and union integrity.

Results

The first experiments assessed the impact of altered media types between the trap and analytical columns. Testing different reversed-phase chemistries in trap and analytical columns revealed poor combinations significantly increase chromatographic peak width compared to peaks obtained with identical chemistries. For instance, peak shape and overall elution profile deteriorates using Vydac® C4 (G.W. Grace & Co. - Conn) or POROS® 10R2 (Applied Biosystems, Inc.) in the 5 mm x 250 µm trap column, as compared with ProteoPep™ II. Analyte refocusing on the analytical column mandated a trap chemistry possessing a significantly different retention value; merely selecting a different reversed-phase media was insufficient to achieve refocusing.

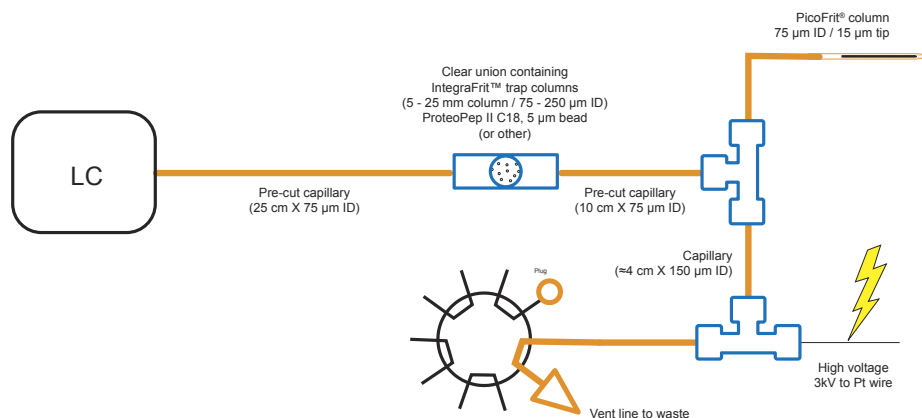


Figure 4 Fluid path diagram for experiments. Fused-silica capillary was connected from the injection valve on the nanoAcquity to the PicoClear union housing the trap columns. This, in turn, was connected to a MicroTee fitting, allowing for flow diversion to waste for trapping, or to the analytical column. The liquid junction was positioned on the vent line, ensuring good electrical contact with minimal sample loss. This arrangement has proven to be both robust and versatile.

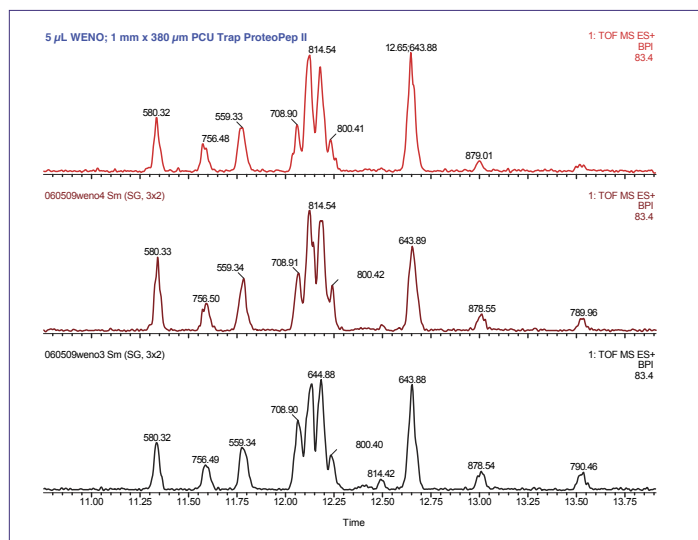


Figure 5 Trap column was constructed with a PicoClear™ union with 1 mm gap between two IntegraFrit™ columns packed with ProteoPep™ II C18 reversed phase media. At a back-pressure of ~900 psi at 4 µL/min and a run pressure of 480 psi with 100% A-Buffer, good peak resolution results (note the 12 minute peak triplet)

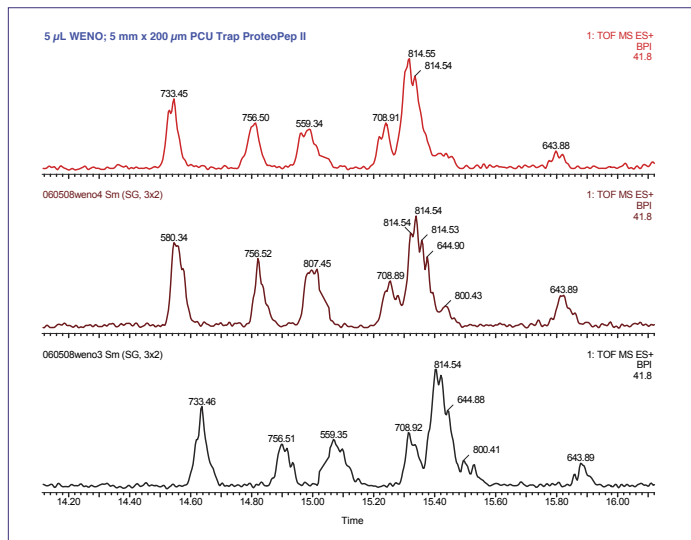


Figure 6 Trap column was constructed in a 200 µm ID IntegraFrit™ column with 5 mm of ProteoPep™ II C18. Pre-cut and polished tubing ends produced visually-verifiable fittings through the optically-clear PicoClear™ union. At a back-pressure of ~670 psi at 4 µL/min and a 460 psi run pressure, this geometry displayed worse resolution in the 12-minute triplet but slightly improved resolution at 14.5 minutes.

Maintaining consistent trap column bed volume offered various challenges with increasing the glass capillary trap IDs, but utilization of novel transparent unions facilitated visual verification of integrity and bed length in each trap-column geometry. Critical to success was high-quality, custom-fabricated glass capillary trap columns, each with machine-cut, polished ends for flush mating with the HPLC system via commercially-available, pre-cut capillary tubing; flush mating established near-perfect and visually-verifiable unions which minimized chromatographic degradation at normal operating pressures. Current results with the 5 mm x 100 µm ID trap demonstrate sensitivities in the sub-femtomole range. The data also reveal trap columns packed directly in the 1 mm x 380 µm fluoropolymer trap column generate excellent chromatographic signal and peak resolution. Absence of the channeling effect along nanobore capillary column walls may explain the enhanced performance attributed to intimate contact between the flexible fluoropolymer and sorbent.

Optimum peak shape and resolution were observed with the narrowest 25 mm x 75 µm ID trap column coupled to the 75 µm ID pulled-tip capillary analytical column. This configuration effectively increased the analytical column by 25%. With decreasing column length and increasing inner diameter, peak resolution gradually deteriorated. Consistent with expectations, operating pressures also decreased as trap column ID increased. Because greater surface area exists at the head of wider trap columns, particulate matter is more easily captured, promoting increased column longevity in proportion to increasing ID.

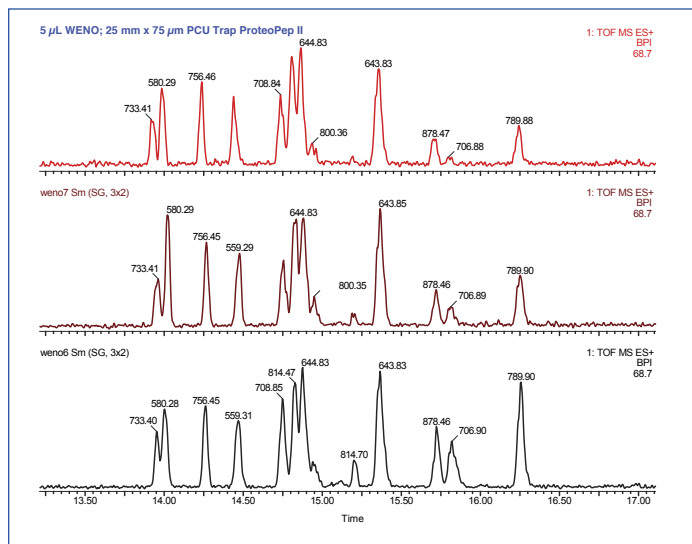


Figure 8 Trap was constructed in a 75 µm ID IntegraFrit™ column packed with 25 mm of ProteoPep™ II C18. At a trapping pressure of ~1600 psi and run pressure of ~550 psi, improved resolution was observed in the baseline-resolved triplet and earlier peaks. Peak height and shape are optimized in this configuration by effectively adding a 25% length increase in the 75 µm ID analytical column. Higher operating pressure combined with a smaller cross-section in the trapping column can predispose this configuration to clogging.

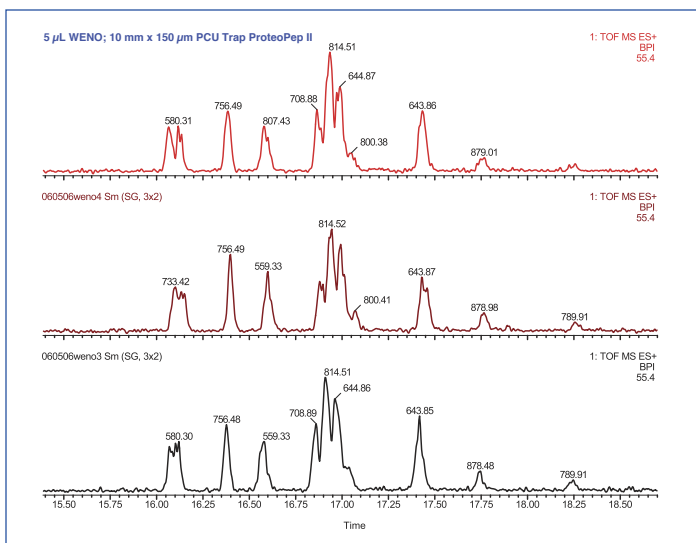


Figure 7 Trap was constructed in a 150 µm ID IntegraFrit™ column packed with 10 mm of ProteoPep™ II C18. At a trapping pressure of ~800 psi and a run pressure of 450 psi, improved resolution is observed in the triplet area as well as the earlier peak, now appearing as a doublet.

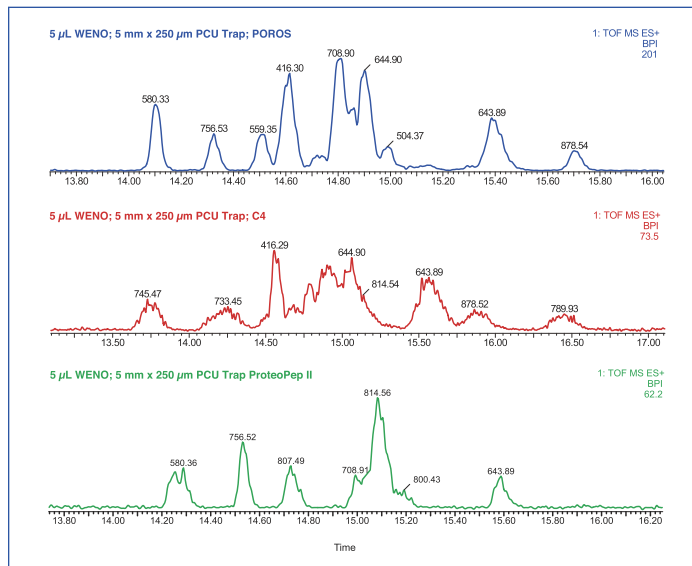


Figure 9 Trap was constructed in a 250 µm ID IntegraFrit™ column packed to 5mm with different RP-media. At a back-pressure of ~680 psi at 4 µL/min and ~460 psi run pressure, Trace A displays a 5 µL injection under aforementioned conditions with POROS® 10R2 trap media. Trace B displays the same with Vydac® C4 trap media. Trace C displays results using ProteoPep™ II trap media.

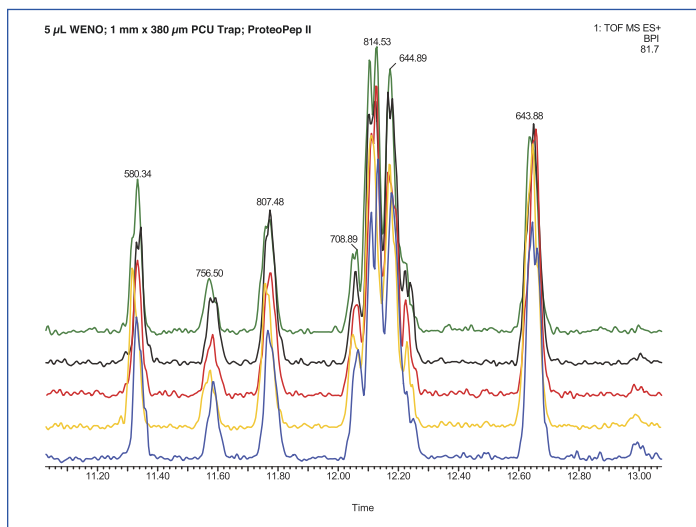


Figure 10

Five superimposed chromatograms from the 1 mm x 380 µm ID trap. Even with the rapid 5% B/min. gradient, excellent reproducibility is observed.

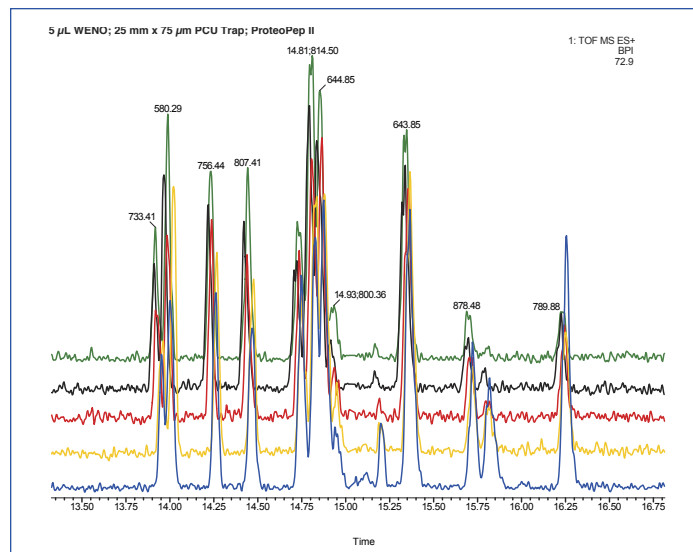


Figure 11 Five superimposed chromatograms from the 25 mm x 75 µm ID trap collected at 5% B/min. displayed poorer reproducibility compared to the 380 µm ID trap column; overall performance is acceptable given the rapid gradient.