Long- & Mixed-Column Nanobore Chromatography for Complex Proteomic Analysis

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Introduction

Coupling columns of same or different resin materials is often employed in complex proteomic digest analysis. Despite enhanced separation, these multidimensional columns are costly, time-consuming to produce, and initiate post-column loss by dead-volume introduction. Confounding factors of column-coupling can be eliminated via transparent, true zero-dead-volume (ZDV) unions that achieve flush connections and rapid swap-out facility during system maintenance. In the current investigation, two conventional 10 cm-bedded columns were coupled and connected to the bed terminus of a third 10 cm nanobore column with integrally fritted tip. Analytical merit of this extended column was compared with a single 30 cm-bedded column with integrally fritted tip and the same resin material. These novel unions supported chromatographic data collection with zero dead-volume, negligible resolution loss, and comparable caliber as the single 30 cm-bedded column.

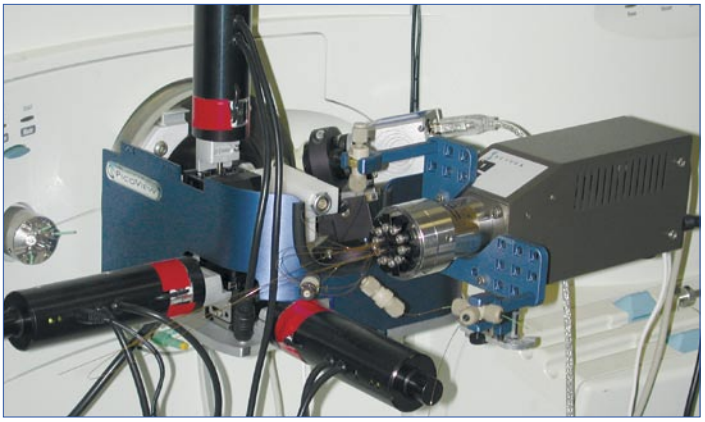

Figure 1 *Digital PicoView 150 nanospray source mounted on the Thermo Finnigan LCQ Deca mass spectrometer with Scivex 6-port valve*

Methods & Materials

Instrumentation & Components

- Ion-trap mass spectrometer (LCQ Deca™, Thermo Fisher Scientific)
- Customized nanospray source (Digital PicoView® 150, New Objective, Inc.)
- NanoLC Pump (Eksigent™)
- Six-port automatic nano-valve (Scivex) with 0.5uL sample loop
- PicoFrit® columns (360 µm OD, 75 µm ID, 15 µm tip ID, New Objective), each containing ProteoPep™ II (New Objective) 5.0 µm-diameter particles packed to 10 cm- and 30 cm- bed lengths
- IntegraFrit™ Columns (360 µm OD, 75 µm ID, New Objective), containing ProteoPep™ II (New Objective) 5.0 µm-diameter particles packed to 10 cm- and 20 cm- bed lengths

Sample Preparation

- A commercially available bovine serum albumin (BSA) standard was diluted to 200 fmol/µL in an aqueous solvent of 2% ACN, 0.1% formic acid
- A commercially available mixture of 5 angiotensins was diluted to 0.1 ng/peptide concentration with 2% ACN, 0.1% formic acid aqueous solvent
- Samples were analyzed via online nanobore ESI-MS in positive-ion-mode

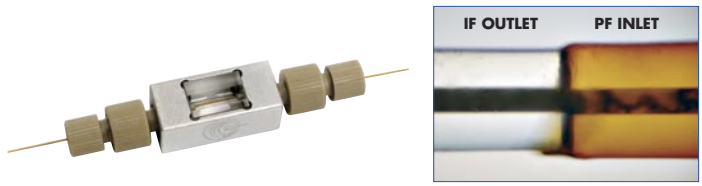

Figure 2 *A) PicoClear™ Union, and B) Expanded view of the zero-dead-volume connection achieved inside the clear union body. Note the excellent column-to-column alignment.*

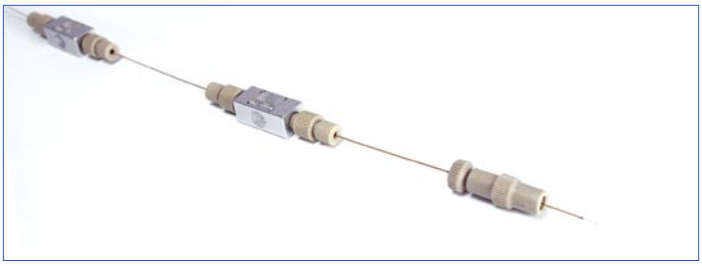

Figure 3 *Two 10 cm IntegraFrit columns configured with a 10 cm PicoFrit column via two PicoClear Unions to form a single 30 cm column*

Results

All column combinations were employed in analyzing the angiotensin standard. Data collected using the 30 cm ProteoPep II (PP2)-packed PicoFrit column resulted in FWHMs between 8.4 – 10.2 seconds. The 20 cm IntegraFrit column + 10 cm PicoFrit column combination displayed FWHMs between 13.2 – 14.4 seconds. The two 10 cm IntegraFrit column + 10 cm PicoFrit displayed FWHMs between

Angiotensin	MW	Sequence
[Ile^7]-Angiotensin III	897.1	RVYIHPI
[Val^4]-Angiotensin III	917.1	RVYVHPF
[Asn^1,Val^5]-Angiotensin II	1,031.0	NRVYVHPF
[Val^5]-Angiotensin I	1,282.5	DRVYVHPFHLA
Angiotensin I	1,296.0	DRVYIHPFHL

Table 1 *5-Angiotensin composition*

12.6 – 14.4 seconds. Figure 5 illustrates three chromatograms from each column combination for analyzing the angiotensin standard; 0.25 ng total peptide were subjected to a 300nL/min flow rate over a 70 minute gradient from 2% - 50% organic modifier concentration.

Figure 6 illustrates the three chromatograms produced in the BSA digest analysis through each column combination; 100fmol BSA were subjected to a gradient identical to that used for angiotensin. Data collected using the 30 cm ProteoPep II (PP2)-packed PicoFrit column allowed 71.8% sequence coverage. The 20 cm IntegraFrit column + 10 cm PicoFrit column supported 58.6% sequence coverage. The two 10 cm IntegraFrit column + 10 cm PicoFrit column yielded 65.1%. sequence coverage.

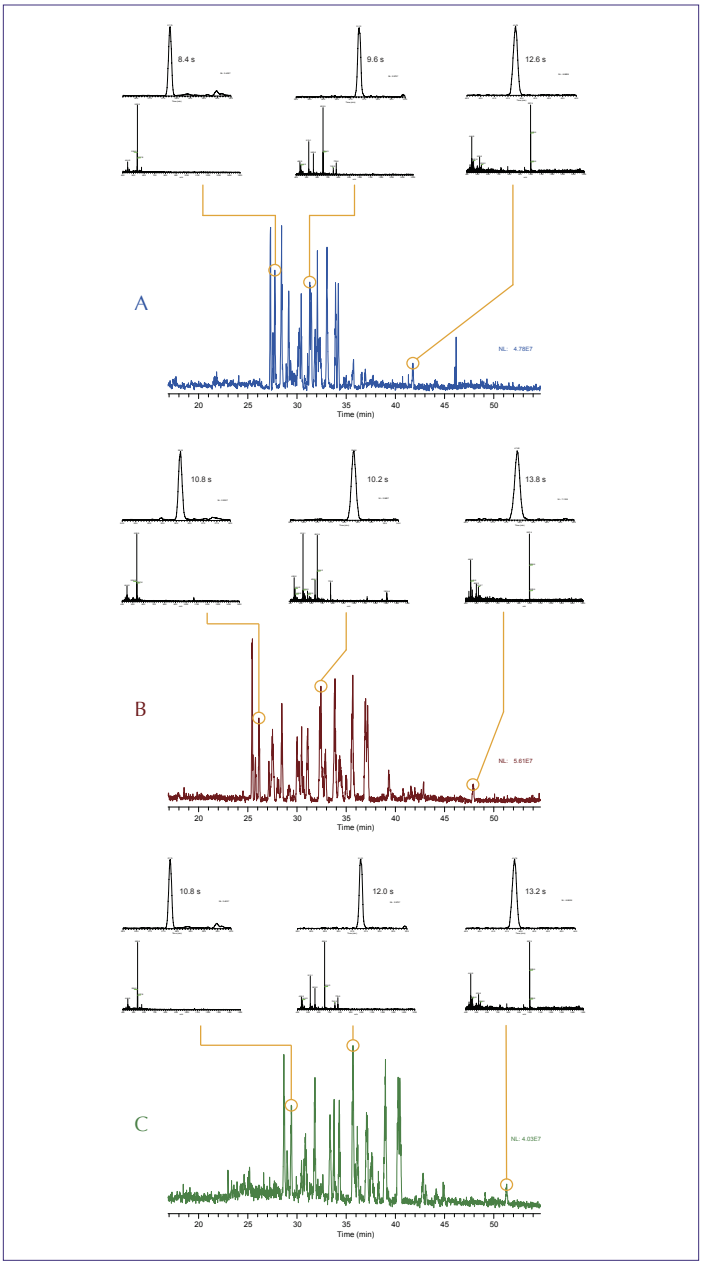

Figure 6 *Expanded regions of BSA tryptic digest chromatographic peaks. A) Chromatogrphic region, as collected with 30 cm PicoFrit column, B) Chromatographic region, as collected with 20 cm IntegraFrit + 10 cm PicoFrit, and C) Chromatographic region, as collected using two 10 cm IntegraFrit columns coupled to a 10 cm PicoFrit column.*
Injection: 100 fmol BSA, Flow rate: 300 nL/min., Gradient: 2% - 50% B over 70 min.

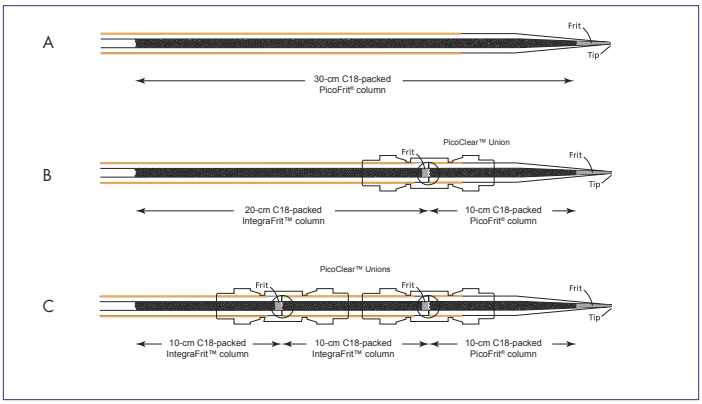

Figure 4 *Schematic diagrams of PicoClear union-column combinations. A) PicoFrit column with 30 cm bed, B) 20 cm IntergaFrit coupled to a 10 cm PicoFrit column with a PicoClear Union, and C) Two 10 cm IntegraFrit columns coupled to a 10 cm PicoFrit column via two PicoClear Unions*

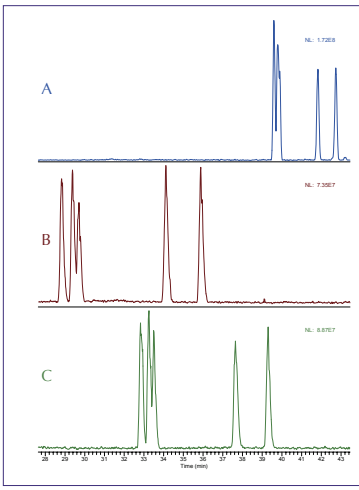

Figure 5 *Angiotensin chromatographic data collected with each column-union configuration A) Chromatogram collected with a 30 cm PicoFrit column, B) chromatogram collected with 20 cm IntegrFrit column + 10 cm PicoFirt column, and C) chromatogram collected using two 10 cm IntegraFrit columns + 10 cm PicoFrit column*

Injection: 0.25 ng total peptide
Flow rate: 300 nL/min.
Gradient: 2% - 50% B over 70 min.

Conclusions

- Minimal resolution loss and post-column loss were observed for columns combined using transparent, true ZDV unions
- Negligible sequence coverage differences were recorded between each column, although the integral 30 cm column provided the best overall score
- Transparent, true zero-dead-volume (ZDV) unions ensure clean connections between columns without dead volume
- Connecting columns containing different resins will be explored in future work
- Nanobore columns having "semi-disposable" integral guard columns are a viable next step