Rapid Nanobore LC-ESI-MS Peptide Analysis Using Sub-3 µm-Diameter Resins

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Introduction

Optimizing resolution through small-diameter resin particles in analytical high-performance liquid chromatography (HPLC) has been measurably saddled by soaring operating pressures. The current investigation assesses the performance of integrally fritted-tip columns packed to 1 , 2 , and 5 cm bed lengths with 1.7-2.5 µm-diameter reverse-phase resins. Analytical figures-of-merit, including full-width-at-half-maximum (FWHM), peak resolution, and operating pressures of short-bed columns possessing sub-3 µm particle-size resins will be compared with a 10 cm-bedded column containing conventional 5 µm-diameter reverse-phase resin. When packed to reduced bed lengths, small-particle resins reveal identical separation behavior, stable, acceptable back-pressures, and reduced run times relative to longer-bedded columns containing 5 µm-diameter resin particles.

Methods & Materials

Instrumentation & Components:

- Ion-trap mass spectrometer (LCQ Deca™, Thermo Electron)
- Customized nanospray source Digital PicoView® 150, New Objective, Inc.) (Figure 1)
- NanoLC Pump (Eksigent™)
- Automatic 6-Port Valve (Scivex) containing a 0.5 µL sample loop (Figure 1)
- PicoFrit® columns (360 µm OD, 75 µm ID, 15 µm tip ID, New Objective), each containing one of three reverse-phase resins:
  - o ACQUITY™ (Waters) 1.7 µm-diameter bead, C18, packed to 1 cm, 2 cm, and 5 cm bed lengths
  - o ProteoPep™ II (New Objective) 5.0 µm-diameter bead, C18, packed to a 10 cm bed length
  - o HALO™ (Mac-Mod Analytical) 2.7 µm-diameter bead, C18, packed to a 5 cm bed length

Sample Preparation:

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

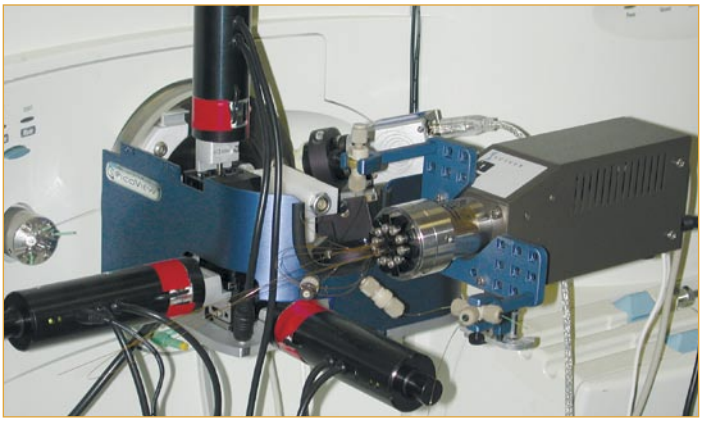

Figure 1 *Digital PicoView 150 nanospray source mounted on the Thermo Finnigan LCQ Deca mass spectrometer with Scivex 6-port valve*

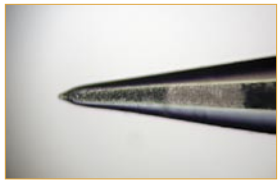

Figure 2 *PicoFrit® column packed with ProteoPep™ II C18; 5 µm bead, 10-cm bed*

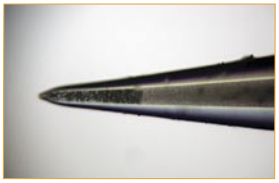

Figure 3 *PicoFrit® column packed with HALO™ C18; 2.7 µm bead, 10-cm bed*

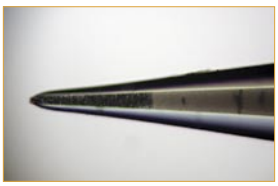

Figure 4 *PicoFrit® column packed with ACQUITY™ C18; 1.7 µm bead, 10-cm bed*

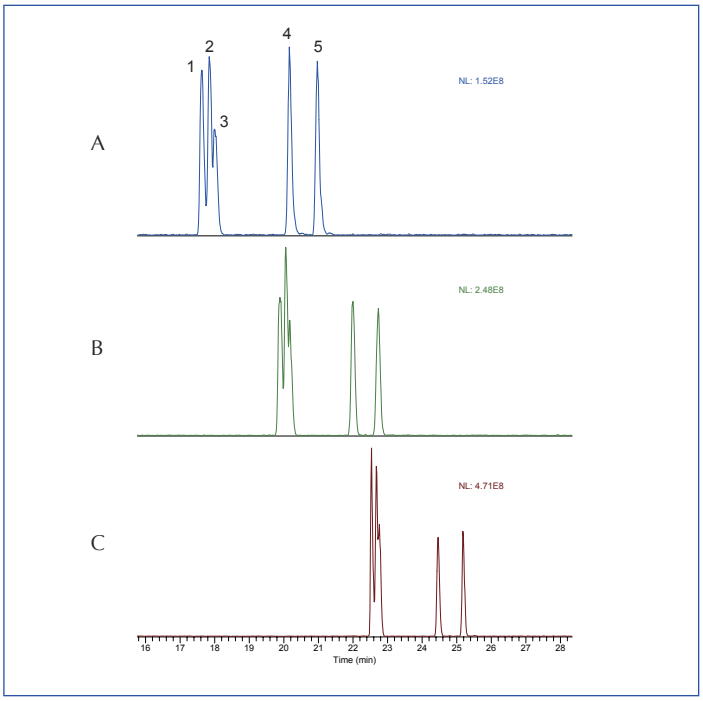

Figure 5 *Chromatograms from 0.05 ng peptide sample injected on A) 10 cm ProteoPep II PicoFrit column, B) 5 cm HALO PicoFrit column, and C) 5 cm ACQUITY PicoFrit column*

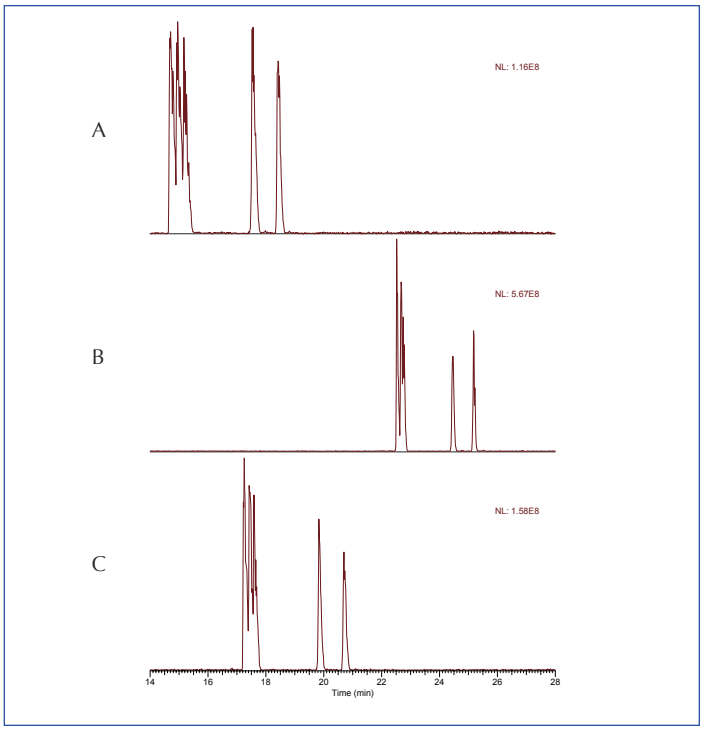

Figure 6 *Chromatograms from 0.05 ng peptide sample injected on A) 1 cm ACQUITY PicoFrit column. B) 2 cm ACQUITY PicoFrit column, and C) 5 cm ACQUITY PicoFrit column*

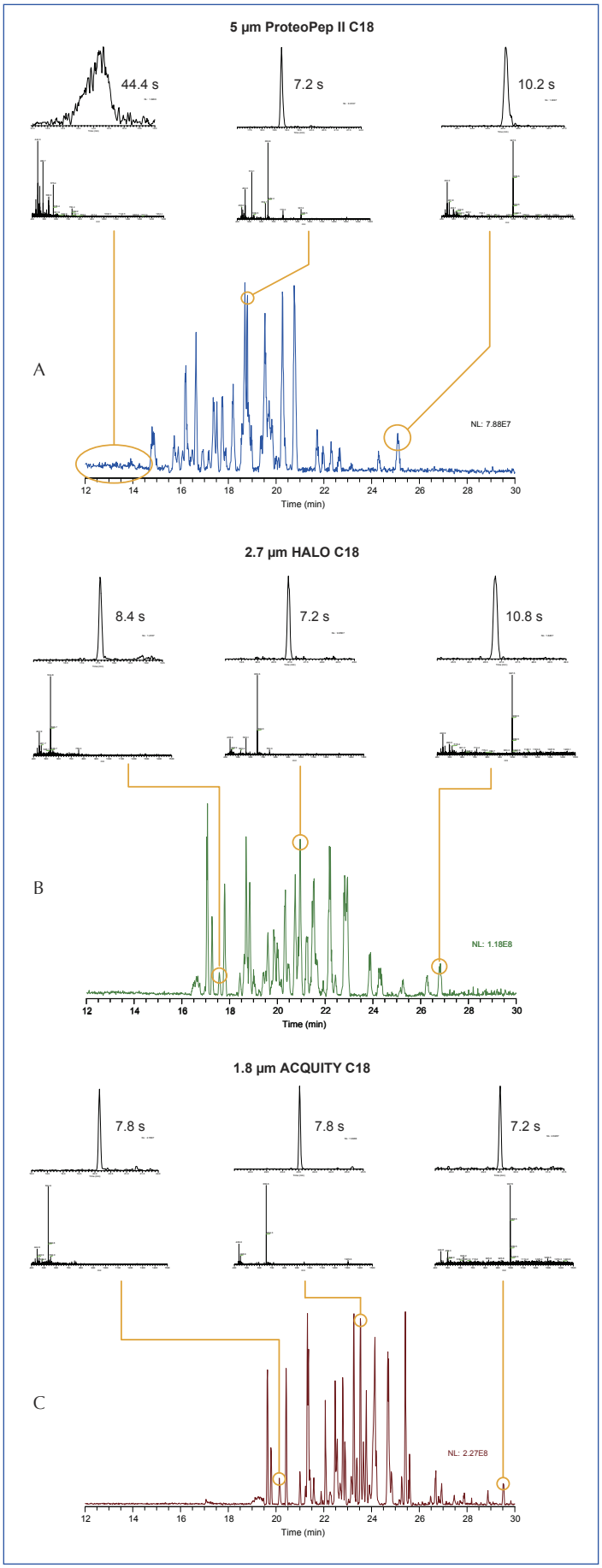

Figure 7 *RIGHT Expanded chromatographic regions for A) ProteoPep II-packed PicoFrit column. B) HALO-packed PicoFrit column, and C) ACQUITY-packed PicoFrit column. Flow rate: 300 nL/min., Gradient: 2% B to 50% B over 29 min.*

MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLIA
FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLK PDPNTLCDEF
KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC
LLPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE
FVEVTKLVTD LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE
CCDKPLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL
GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS
RSLGKVGTRC CTKPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC
TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT
ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
STQTALA

Figure 8 *BSA Sequence coverage for 5 cm ACQUITY column*

Peak	Angiotensin	MW	Sequence
1	[IIe^{7}]-Angiotensin III	897.1	RVYIHPI
2	[Val^{4}]-Angiotensin III	917.1	RVYVHPF
3	[Asn^{1},Val^{5}]-Angiotensin II	1,310.0	NRVYVHPF
4	[Val^{5}]-Angiotensin I	1,282.5	DRVYVHPFHLA
5	Angiotensin I	1,296.0	DRVYIHPFHL

Table 1 *5-Angiotensin composition*

Column	Back Pressure
10-cm ProteoPep II	730
5-cm ACQUITY	1,500
5-cm HALO	1.100
2-cm ACQUITY	760
1-cm ACQUITY	460

Table 2 *Column back-pressures recorded at 300 nL/min of 98% water*

Results

All columns were employed in analyzing the angiotensin standard. Data collected using the 10cm ProteoPep II (PP2)-packed column resulted in FWHMs between 9.0-10.2 seconds; the ACQUITY-packed 5 cm column displayed FWHMs between 8.4-10.2 seconds. Figure 1 illustrates the three chromatograms of the angiotensin standard on each column. Two additional columns containing 1 cm and 2 cm ACQUITY-packed beds were then compared to the 5 cm column; the 1 cm column produced FWHMs between 10.2-13.2 seconds; the 2 cm column generated FWHMs between 8.4-10.2 seconds. Figure 2 shows chromatograms for these columns. To explore the impact of reduced particle size on column back-pressure, data for each column were recorded and tabulated in Table 2.

Additionally, all columns were employed in analyzing the BSA digest standard; Figure 3 illustrates the chromatograms generated by each column. MS/MS data were collected and processed for sequence coverage via TurboSEQUEST™ in the BioWorks™ Browser. Sequence-coverage analysis revealed the 10 cm-PP2 column supported 57.6% coverage, the 5 cm ACQUITY column offered 60.9% coverage and the 5 cm HALO column produced 54.7% sequence coverage.

Conclusions

- Smaller particle sizes of ACQUITY and HALO resins increase both peak resolution and sensitivity
- ACQUITY improves sequence coverage and enhances separation for the BSA digest
- 5 cm HALO was comparable to a 10 cm ProteoPep II column for sequence coverge, though improved separation was observed with the HALO column
- Back-pressures recorded for reduced-bed-length, reduced-particle-size columns were within acceptable operating parameters for nanobore chromatography columns (≤ 2000 psi)

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