Enhanced Nanobore LC-MS Using Methanol Gradient Elution with Peptide Mixtures

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Introduction

Although conventional online nanoelectrospray employs mobile phase gradients of water and acetonitrile (ACN), enhanced LC-MS performance has been reported for peptide analyses using a methanol (MeOH) organic modifier (Giorgianni et al. *Anal. Chem.* 2004, 76, 7028). Using two nanobore fused-silica columns of different sorbents with fritted integral emitters, an LC-MS peptide analysis compared efficacies of methanol and acetonitrile as organic modifiers. For a 5-component angiotensin sample, methanol yielded superior performance with enhanced MS S/N ratios and peak intensities. In a 100 fmol bovine serum albumin (BSA) digest, greater sequence coverage was obtained for methanol using a sample trap.

Methods & Materials

Instrumentation and Components

- Ion trap mass spectrometer (LCQ Deca™, Thermo Electron)
- Nanospray Source (PicoView® 150, New Objective)
- HPLC Pump (1100 Series, Agilent) with 20:1 flow splitter (Resulting flow rate ≈ 250 nL/min)
- 2 PicoFrit® columns (New Objective); 360 µm OD, 75 µm ID, 15 µm ID pulled tip, each containing a 10 cm bed of distinct sorbents:
  - ProteoPep II™ – 5 µm 300 Å (New Objective)
  - Jupiter™ Proteo™ – 4 µm, 90 Å (Phenomenex)
- 2 IntegraFrit™ Sample Traps (New Objective) containing above sorbents
- TurboSequest, BioWorks™ Browser (Thermo Electron)

Sample Preparation

- A 10 ng/µL solution of 5 angiotensins was prepared by diluting a commercially available mixture with 500 µL 2% acetonitrile (a 40-fold dilution generated a final concentration/peptide ≈ 283 fmol/µL)
- A 100 fmol/µL BSA tryptic digest was prepared using 100 µL commercially available 1,000 fmol/µL standard in 900 µL 2% ACN
- Two separate gradients employed MeOH and ACN organic modifiers

Figure 1A *PicoView® 150 on a Thermo Finnigan™ LCQ Deca™*

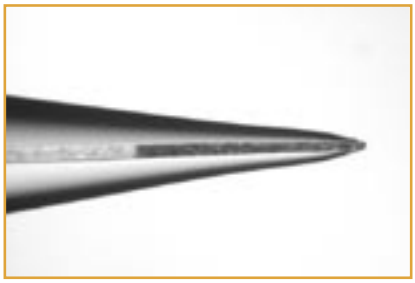

Figure 1B *Integral frit and emitter of a PicoFrit® C18 column*

Figure 1C *IntegraFrit™ Sample Trap and cartridge*

Originally published on June 7 , 2005, at the 54th Annual American Society for Mass Spectrometry Conference in San Antonio, TX, U.S.A.

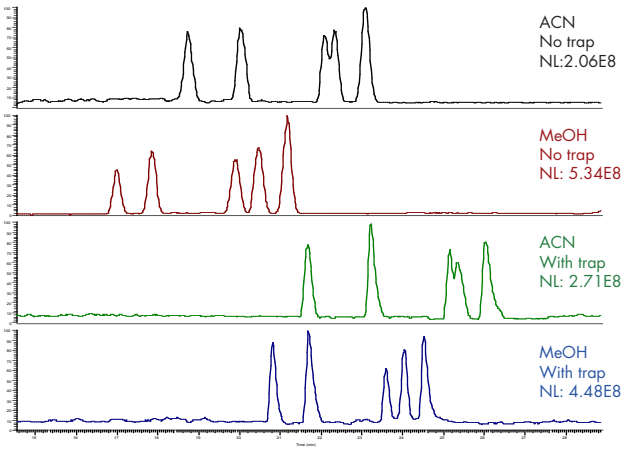

Figure 2 *Base peak chromatogram of 5-angiotensin test mixture using a PicoFrit® column: (75 µm ID x 15 µm tip) containing 10 cm of ProteoPep II™*

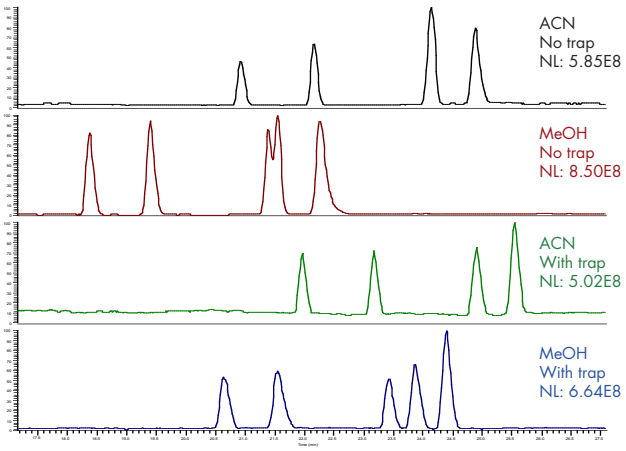

Figure 3 *Base peak chromatogram of 5-angiotensin test mixture using a PicoFrit® column: (75 µm ID x 15 µm tip) containing 10 cm of Jupiter™ Proteo™*

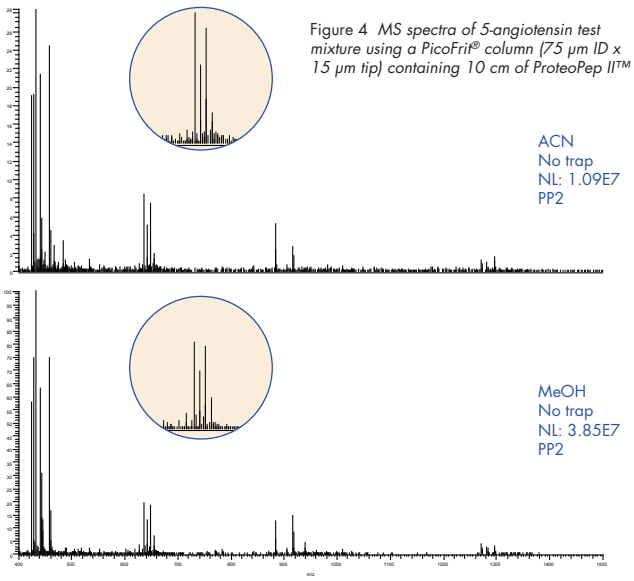

Figure 4 *MS spectra of 5-angiotensin test mixture using a PicoFrit® column (75 µm ID x 15 µm tip) containing 10 cm of ProteoPep II™*

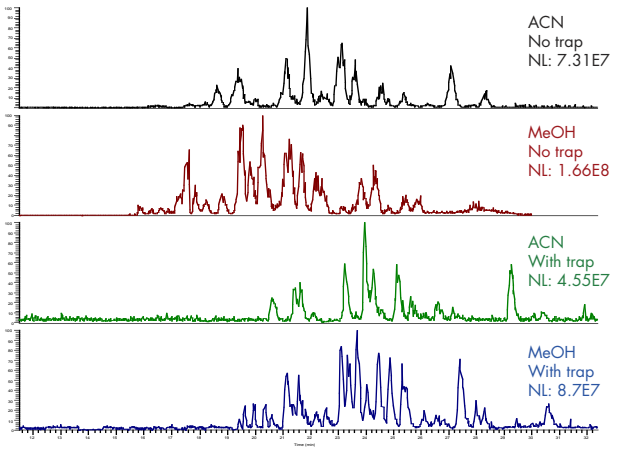

Figure 5 *Base peak chromatogram of 100 fmol BSA digest using a PicoFrit® column: (75 µm ID x 15 µm tip) containing 10 cm of ProteoPep II™*

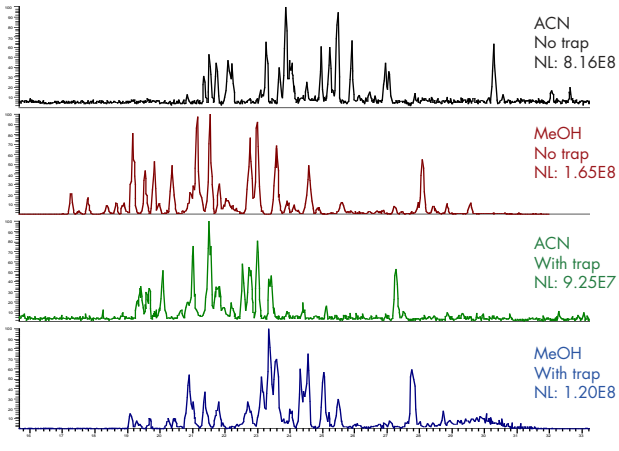

Figure 6 *Base peak chromatogram of 100 fmol BSA digest using a PicoFrit® column: (75 µm ID x 15 µm tip) containing 10 cm of Jupiter™ Proteo™*

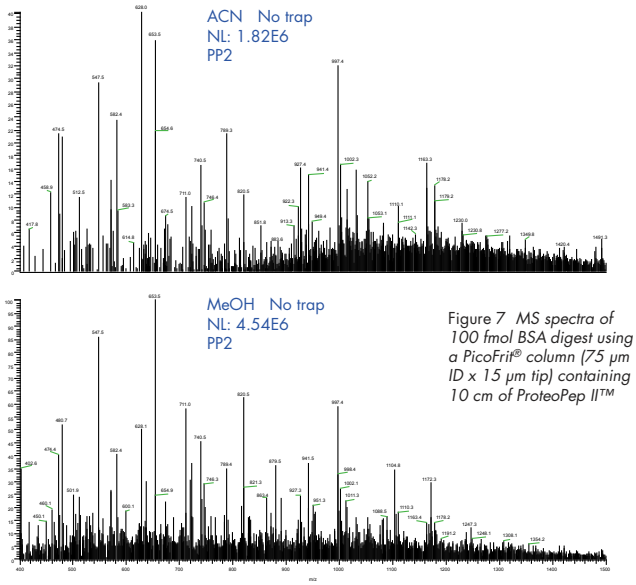

Figure 7 *MS spectra of 100 fmol BSA digest using a PicoFrit® column (75 µm ID x 15 µm tip) containing 10 cm of ProteoPep II™*

Results

Figures 2 and 3 display chromatographic separations of 5 angiotensins. Figures 5 and 6 display a tryptic BSA digest for each column configuration with ACN and MeOH. In shorter time, methanol produced superior separation for each column both with and without a sample trap. On average, peak height of the 5 peptides increased at least 2X for all column configurations after switching from ACN to MeOH. For the PicoFrit® column containing ProteoPep II™, using methanol with and without a sample trap generated a 1.5X S/N increase. Example spectra are displayed in Figures 4 and 7. A S/N decrease was observed for PicoFrit columns containing Jupiter™ Proteo™.

A methanol pre-rinse improved the charge state distribution for ProteoPep II sorbent (Figure 8), but subtle charge state distribution changes were observed when transitioning between ACN and MeOH. Negligible FWHM differences were detected for each column configuration when switching organic modifiers. BSA digest sequence coverage was also unaffected by changing ACN to MeOH in the absence of a sample trap. When a sample trap was used, sequence coverage increased 1.8X for both columns when changing from ACN to MeOH.

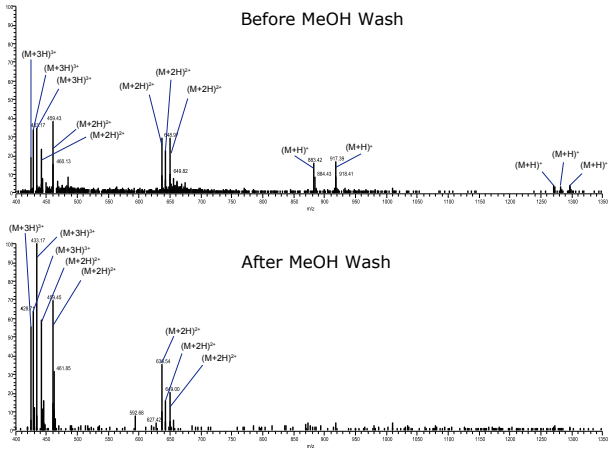

Figure 8 *MS spectra before and after methanol wash of the 5-angiotensin mixture using an ACN organic modifier*

Conclusions

- For both column configurations, methanol generated optimal chromatographic peak separation
- The ProteoPep II™ column generated a 1.5X increase in MS peak intensity with significant S/N improvement
- A column pre-wash with MeOH improves charge state distribution with more angiotensin in a single charge state
- Utilizing the sample trap with MeOH generated the best sequence coverage of all configurations under evaluation