A Comprehensive Study on Nanobore Injection Methods for Proteomics

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Introduction

A widely used method for protein identification incorporates two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) followed by nanoelectrospray ionization mass spectrometry (nESI-MS). Nanospray, in combination with nanobore chromatography (< 100 µm i.d. columns), provides typical limits of detection in the subfemtomole to attomole range when combined with ion trap mass spectrometry.

Typically, an in-gel digested protein sample is injected onto a nanobore column by one of two methods: direct on-column injection using a pressure bomb, or on-line sample pre-concentration in a sample trap cartridge. The time intensive bomb injection method is highly sensitive, minimizing sample handling and maximizing sample utilization. Low back pressure trapping cartridges permit higher loading rates and compatibility with autosampler methods. Trap cartridges can also yield guard-column functionality, as in-line enrichment and desalting of samples increases nanobore column lifetime. Incorporating a trap cartridge with an alternative packing material may also be used modify the selectivity of a method.

Methods

Analysis were performed on an LC/MS system composed of an 1100 Cap LC (Agilent, Palo Alto, CA) interfaced with a LCQ™ Deca ion trap mass spectrometer (Thermo Finnigan, San Jose, CA). The LCQ Deca was outfitted with a PicoView™ nanospray ion source (New Objective, Woburn, MA). (A in Figure 2) Chromatography was performed with a PicoFrit™ column (New Objective) packed with ProteoPep™ C18 (5 µm particle with 300 A pore). (A in Figure 1) A PicoFrit column combines a nanobore LC column with a fritted electrospray emitter to eliminate post column analyte loss and band broadening. Two columns were analyzed each with different dimensions: a 75 µm i.d. column with a tip size of 15 µm packed with a 10 cm bed and a 20 µm i.d. column with a tip size of 10 µm packed with a 5 cm bed.

Experimental parameters:

- Nanospray voltage: 1.7kV (75 i.d. µm column) and 1.0kV (20 µm i.d. column)
- Tip Position: 2mm away from inlet
- Heated capillary: 140°C
- Microscans: 3
- HPLC Solvents: (A) Water, 0.1% Formic acid (B) Acetonitrile, 0.1% Formic acid

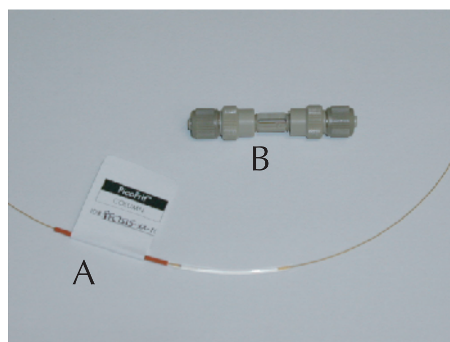

Figure 1 *- PicoFrit™ column (A) and capillary sample trap (B).*

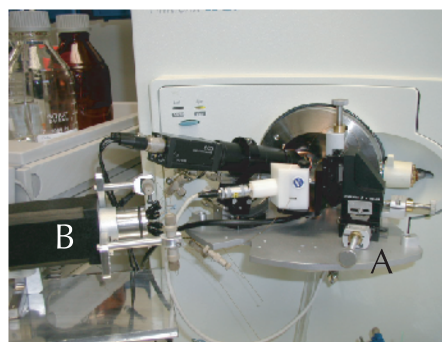

Figure 2 *- The PicoView™ nanospray source (A) with a 10 port Valco valve (B).*

Bovine Serum Albumin Tryptic Digest

BSA was used to test differences in sequence coverage for the two injection methods. The acquisition method for the LCQ™ Deca involved one MS precursor scan from 350 to 1500 amu followed by three data-dependent MS/MS scans (isolation width 3 amu, 30% collision energy) on the three most abundant ions in the MS scan. The resultant reconstructed base peak ion chromatograms for a trap injection and an on-column injection are shown in Figure 7(a) and (b) respectively. More early elution, hydrophilic peptides were observed for the on-column injection.

The tandem MS data were used to search sequence databases using the Sequest™ algorithm. The database search resulted in an amino acid coverage of 64.20% and 74.35% for a trap injection and an on-column injection respectively, a difference of 10.15%.

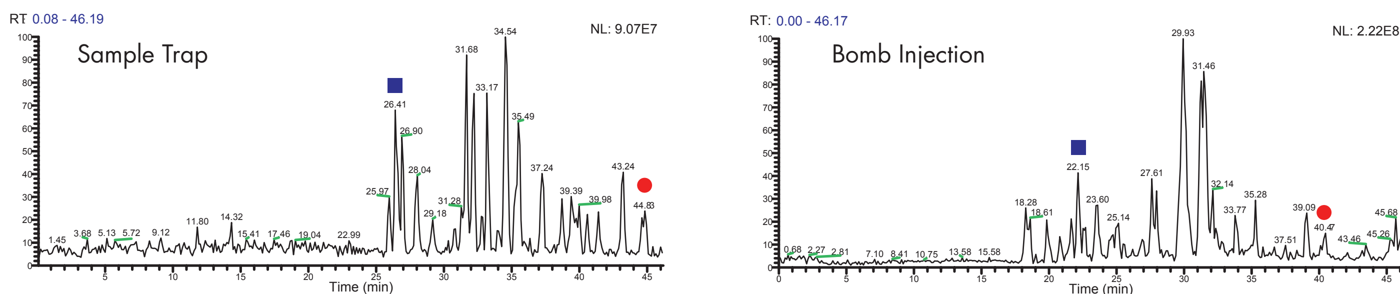

Figure 7 *- Base peak reconstructed ion chromatogram of 100 fmol BSA tryptic digest for (a) an injection with a sample trap and (b) an on column injection. The two colored shapes are associated with identical peptides on the two different chromatograms.*

DTHKSEIAHR	FKDLGEEHFK	GLVLIAFSQY	LQQCPFDEHV	KLVNELTEFA
KTCVADESHA	GCEKSLHTLF	GDELCKVASL	RETYGDMADC	CEKEQPERNE
CFLSHKDDSP	DLPKLKPDPN	TLCDEFKADE	KKFWGKYLYE	IARRHPYFYA
PELLYANKYN	GVFQECCQAA	DKGACLLPKI	ETMREKVLTS	SARQRLRCAS
IQKFGERALK	AWSVARLSQK	FPKAEFVEVT	KLVTDLTKVH	KECCHGDLLE
CADDRADLAK	YICBBZBTIS	SKLKECKDPC	LLEKSHCIAE	VEKDAIPEDL
PPLTADFAED	KDVCKNYQEA	KDAFLGSFLY	EYSRRHPEYA	VSVLLRLAKE
YEATLEECCA	KDDPHACYTS	VFDKLKHLVD	EPQNLIKZBC	BZFEKLGEYX
XXALIVRYTR	KVPQVSTPTL	VEVSRSLGKV	GTRCCTKPES	ERMPCTEDYL
SLILNRLCVL	HEKTPVESKV	TKCCTESLVN	RRPCFSALTP	DETYVPKAFD
EKLFTFHADI	CTLPDTEKQI	KKQTALVELL	KHKPKATEEQ	LKTVMENFVA
FVDKCCAADD	KEACFAVEGP	KLVVSTQTAL	A	

Figure 8a *- Sequence coverage for the trap injection of the BSA trypic digest.*

DTHKSEIAHR	FKDLGEEHFK	GLVLIAFSQY	LQQCPFDEHV	KLVNELTEFA
KTCVADESHA	GCEKSLHTLF	GDELCKVASL	RETYGDMADC	CEKEQPERNE
CFLSHKDDSP	DLPKLKPDPN	TLCDEFKADE	KKFWGKYLYE	IARRHPYFYA
PELLYANKYN	GVFQECCQAA	DKGACLLPKI	ETMREKVLTS	SARQRLRCAS
IQKFGERALK	AWSVARLSQK	FPKAEFVEVT	KLVTDLTKVH	KECCHGDLLE
CADDRADLAK	YICBBZBTIS	SKLKECKDPC	LLEKSHCIAE	VEKDAIPEDL
PPLTADFAED	KDVCKNYQEA	KDAFLGSFLY	EYSRRHPEYA	VSVLLRLAKE
YEATLEECCA	KDDPHACYTS	VFDKLKHLVD	EPQNLIKZBC	BZFEKLGEYX
XXALIVRYTR	KVPQVSTPTL	VEVSRSLGKV	GTRCCTKPES	ERMPCTEDYL
SLILNRLCVL	HEKTPVESKV	TKCCTESLVN	RRPCFSALTP	DETYVPKAFD
EKLFTFHADI	CTLPDTEKQI	KKQTALVELL	KHKPKATEEQ	LKTVMENFVA
FVDKCCAADD	KEACFAVEGP	KLVVSTQTAL	A	

Figure 8b *- Sequence coverage for the on-column injection of the BSA trypic digest.*

Trap/Analytical Coulmn Combinations

Sample trapping permits the use different chemistries within an analytical method. To examine the change in retention characteristics a sample trap filled with BioBasic™ C18 was used in conjuction with a ProtepPep™ analytical column with 5 cm of packing and compared with a ProtepPep sample trap. The combination of the BioBasic/ProteoPep resulted in wider peak widths and lower resolution in the analysis of the five angiotensin variants (Figure 9) and a mix of different retention characteristics in the analysis of the BSA digest (figure 10)

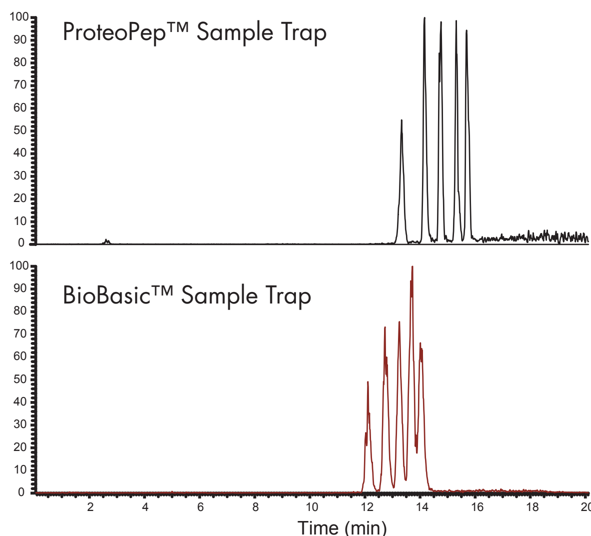

Figure 9 *- Base peak reconstructed ion chromatogram for full scan MS data of 5 angiotensin variants on a ProteoPep™ PicoFrit™ column with a ProteoPep sample trap (Top) and a BioBasic™ sample trap (Bottom)*

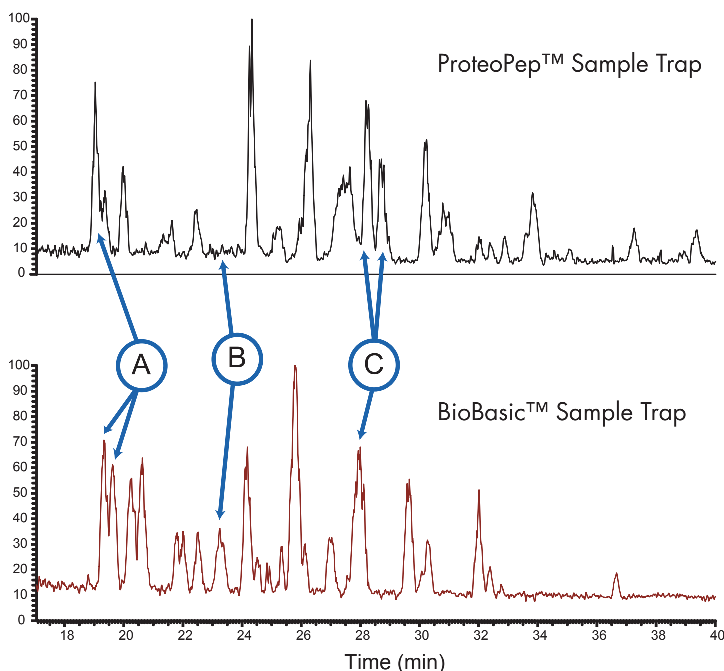

Figure 10 *- Base peak reconstructed ion chromatogram for full scan MS data of 100 fmol of BSA tryptic digest on a ProteoPep™ PicoFrit™ column with a ProteoPep sample trap (Top) and a BioBasic™ sample trap (Bottom).*

(A) Shows two co-eluting peptides (top) are separated with the BioBasic/ProteoPep combination (bottom)
(B) Shows a peptide not identified with the ProteoPep/ProteoPep combination (top) was identified using a BioBasic sample trap (bottom)
(C) Shows two peptides which are separated (top) co-elute with a BioBasic sample trap (bottom)

Conclusions

On-column injection yields optimal sensitivity, providing a lower detection limit with greater sequence coverage for tryptic peptides. Sample loading, however is fully manual proves to be very time consuming. Detection limits can be further improved with the use of smaller i.d. columns, a 20 µm i.d. column improved over an order of magnitude compared to a 75 µm i.d. column.

Sample trap injection provides reasonably high sensitivity, with the added advantage of autosampler compatibility. Even though these initial results suggest a limit of detection that is 2-3 fold compromised, at the 100 fmol level sequence coverage is nearly comparable.

Trap chemistries with different retention characteristics from the analytical column can be used to provide alternate selectivity for a method.

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