Trap/Column Combination to Optimize Sensitivity and Sequence Coverage

James P. Murphy III and Gary A. Valaskovic
New Objective Inc, Woburn, MA 01801-1023

Introduction

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. Although on-column injections prove to be the most sensitive, trapping cartridges permit higher loading rates and compatibility with auto sampler methods. Trap cartridges can yield guard-column functionality, with in-line enrichment and desalting of samples. Traditional trap columns typically have inner diameters (ID) of 300 µm to 1 mm, with a short (1-2 mm) bed length. The development of a novel nanobore trap with an ID of 100 µm and a bed length of 25 mm has shown a comparative increase in sensitivity. The longer bed length, contributing a reasonable degree of separating power, may also be used to modify the selectivity of an LC method by incorporating an alternative packing material from the analytical column.

Methods

Analysis were performed on an LC/MS system composed of an 1100 Capillary LC (Agilent, Palo Alto, CA) interfaced with a LCQ™ Deca ion trap mass spectrometer (Thermo Electron, San Jose, CA) with a PicoView™ nanospray ion source (New Objective, Woburn, MA) (Figure 1). Chromatography was performed with a 75 µm ID. PicoFrit™ column (New Objective) packed with ProteoPep™ C18 (5 µm diameter, 300 A pore, 5 cm bed) (Figure 2). An IntegraFrit™ sample trap (100 µm ID., 25 mm bed length) (Figure 2) was switched in line with the column through the use of a 10-port valve (Valco Instruments, Houston, TX). A variety of reverse phase packing materials were studied within the trap, including AQUASIL C18, BetaBasic C18, BioBasic® C18, BioBasic® C8 (Thermo Hypersil-Keystone) Jupiter™ Proteo C12 (Phenomenex, Torrance, CA) and ProteoPep™ C18.

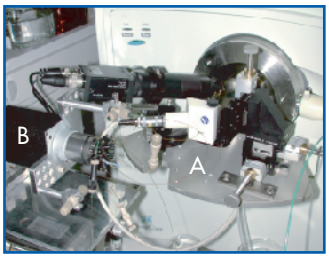

Figure 1 - (A) The PicoView™ nanospray source with (B) a 10 port Valco valve.

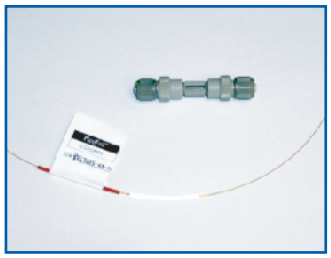

Figure 2 - (A) PicoFrit™ column and (B) capillary sample trap.

Experimental Parameters

LC:
(A) Water, 0.1% Formic acid
(B) Acetonitrile, 0.1% Formic acid

Source Conditions:
Nanospray voltage: 1.7kV
Emitter Position: 3mm from inlet

Mass Spectrometer:
Full MS Scan: 400-1500 amu
Microscans: 3
Heated capillary: 135ºC

Triple Play Scan Function:
Performed on 3 most intense ions
Dynamic Exclusion: On
Repeat Count: 2
Repeat Duration: 0.50 min
Exclusion Duration: 10 min

MS^n:
Collision Energy: 30%
q: 0.25
Activation Time: 30 ms

Samples were injected into a 5 µl sample loop (position A in Figure 3) and subsequently flushed onto the trap column with 10 µl of solvent at a flow rate of 1.6 µL/min (position B in Figure 3). The flow rate onto the sample trap can be increased to a maximum of 5 µL/min with no loss in sensitivity.

After sample loading, the PEEK™ tee incorporated within the flow path of the LC supplied a split flow LC gradient (25:1) resulting in a 200 nl/min flow through the sample trap and the 75 µm ID. analytical column during sample elution (position A in Figure 3)

Sample Injection

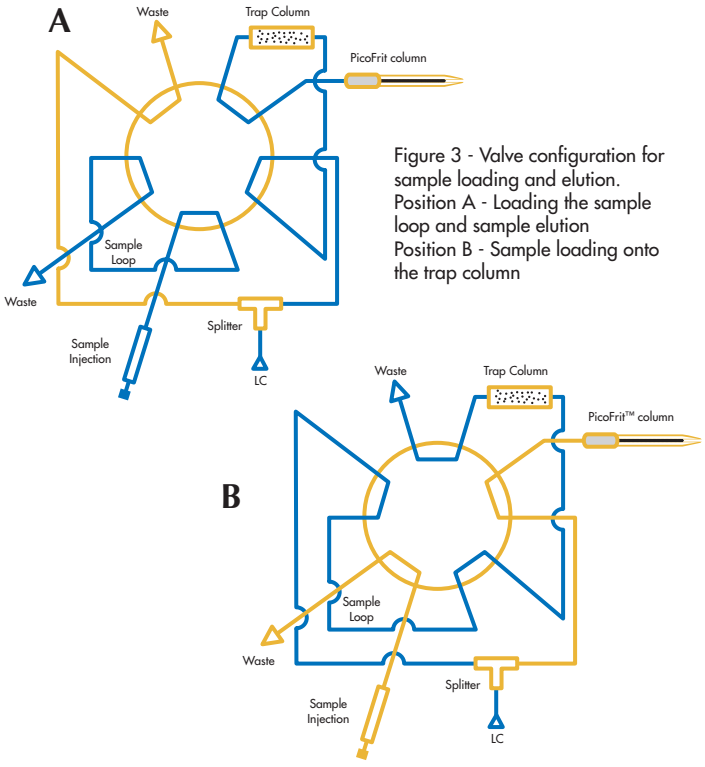

Figure 3 - Valve configuration for sample loading and elution.
Position A - Loading the sample loop and sample elution
Position B - Sample loading onto the trap column

Results

The performance characteristics of the sample trap were assessed by several standards including a test mix of five angiotensin variants and five different tryptic digests of bovine serum albumin (cow), phosphorylase B (rabbit), beta-lactoglobulin (cow), myoglobin (horse) and carbonic anhydrase (cow). Standards were purchased from Michrom BioResources (Auburn, CA). Figures of merit included retention characteristics and sequence coverage.

Resolution

Traditionally the same chromatographic media is used in a trap/analytical column combination. Varying the trap media may alter retention characteristics of an analysis. Figure 4 shows the analysis of 5 angiotensin variants performed with a trap/analytical combination of solely ProteoPep™ (top) and the same analysis performed with a sample trap filled with BioBasic™ C18 (middle). The combination of the BioBasic/ProteoPep resulted in wider peak widths (a 2 fold increase at FWHM) and lower resolution as the variants are no longer baseline resolved. A sample trap with Jupiter Proteo C12 however only showed a moderate change (bottom).

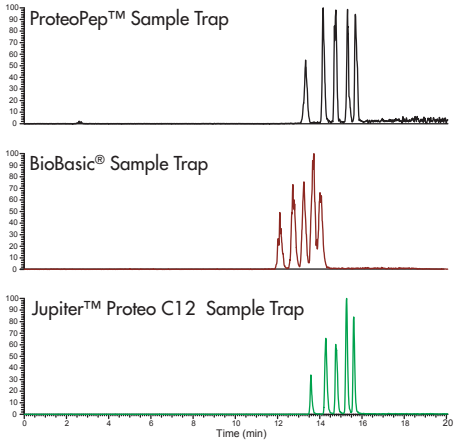

Figure 4 - Base peak reconstructed ion chromatogram for full scan MS data of 5 angiotensin variants on a ProteoPep™ PicoFrit™ column with a ProteoPep sample trap (top), a BioBasic™ sample trap (middle) and and Jupiter™ Proteo sample trap (bottom).

Sequence Coverage

A similar experiment was performed with a bovine serum albumin (BSA) tryptic digest. The combination of BioBasic/ProteoPep exhibited different retention characteristics for the BSA digest compared to the ProteoPep/ProteoPep combination (Figure 5). The use of alternate chemistry allowed several co-eluting peptides from the ProteoPep/ProteoPep run to be separated and identified a peptide not found with identical chromatographic media. This alternate selectivity may allow for increased MS/MS identification of peptides and sequence coverages.

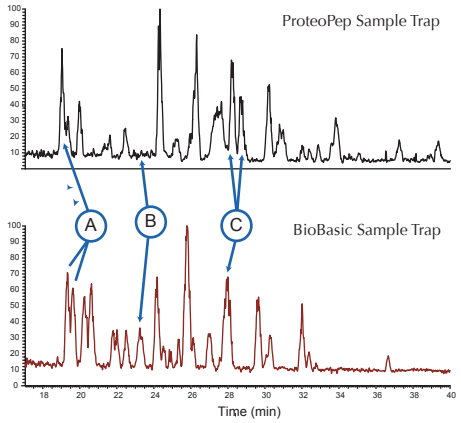

Figure 5 - Base peak reconstructed ion chromatogram for full scan MS data of 100 fmol of BSA tryptic digest on a ProteoPep™ PicoFrit™ column with a ProteoPep™ sample trap (Top) and a BioBasic™ sample trap (Bottom).

(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)

To test the effect of alternate trapping chemistry on sequence coverage five different chromatographic materials were used to analyze a complex solution of five different tryptic digests ranging in concentration of 20 fmol/µl to 200 fmol/µl (Figure 6). The MS/MS data was matched to database information using SEQUEST™ software package (Thermo Electron). The percent sequence coverage is presented in Table 1.

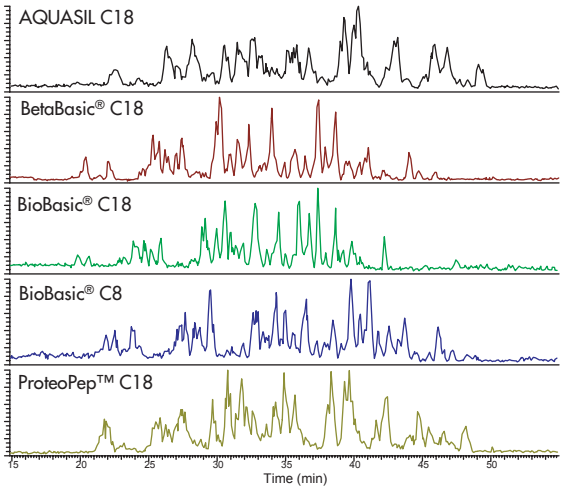

Figure 6 - Base peak reconstructed ion chromatogram for full scan MS data of a trypsin digest mixture on a ProteoPep™ PicoFrit™ column incorporated with five different sample trap materials.

	AQUASIL C18	BetaBasic C18	BioBasic C18	BioBasic C8	ProteoPep C18
Bovine Serum Albumen	16.2	16.5	15.3	15.0	18.5
Phosphorylase B	20.0	24.4	18.4	20.0	22.3
Beta-Lactoglobulin	18.0	14.0	12.4	11.6	11.2
Myoglobin	31.4	61.1	37.7	39.2	36.4
Carbonic Anhydrase	7.0	17.4	7.0	12.4	7.0

Table 1 - Sequence coverage for the trypsin digest mixture using five different sample trap materials. The percent sequence coverage is the average of three chromatographic runs for each trap.

Alternate reversed phase trapping media appears to have a minimal effect on sequence coverage as the dynamic exclusion feature of the LCQ™ Deca allows multiple co-eluting peptides to be properly identified. The minor differences in sequence coverage can be contributed to a small diversity of peptides identified with a specific trap/analytical column combination. The peptide with the sequence of VGDANPALQK (molecular weight of 1012.1, pI of 5.81) in carbonic anhydrase, for instance, is only identified with the sample trap containing BetaBasic C18 or BioBasic C8 media (Figure 7).

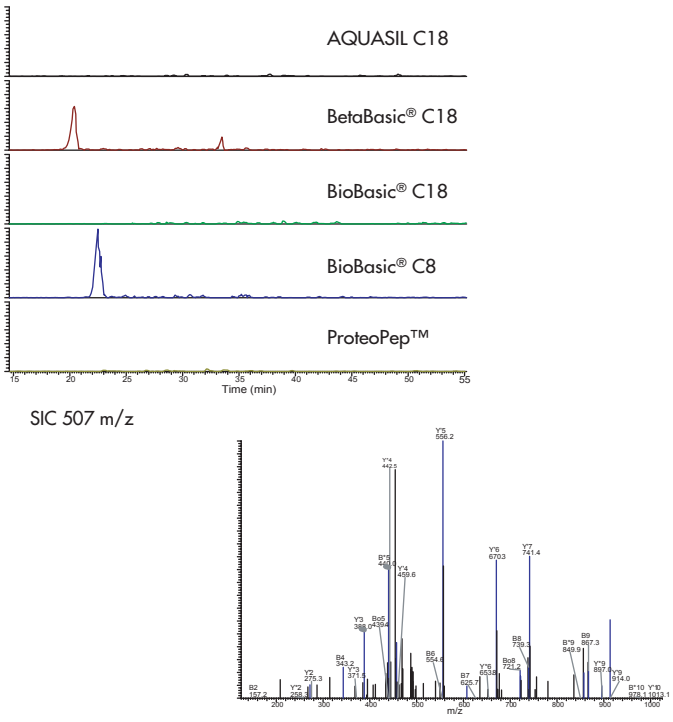

Figure 7 - Base peak reconstructed ion chromatogram for m/z 507 $(M+2H)^{+2}$ of a trypsin digest mixture on a ProteoPep™ PicoFrit™ column incorporated with five different sample trap materials (top).

MS/MS spectrum of m/z 507. (bottom)

Conclusions

Sample trapping permits the use of different chromatographic media within an analytical method without the labor of changing analytical column chemistry.

A varied trap/analytical column combination may result in a loss of chromatographic resolution. This resolution loss appears to be the result of mixed zone elution, in which a given peptide eluting late from the trap can catch-up to earlier eluting peptides that are strongly retained by the analytical column. In a simple mixture, the loss in resolution is a disadvantage, but for complex mixtures zone elution may provide alternate selectivity for LC-MS/MS identification.

Alternate reverse phase trapping media showed to have a minimal effect on sequence coverage of a complex tryptic digest. The small differences in sequence coverage can be contributed to a modest diversity of peptides identified with a specific trap/analytical column combination.

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