Femtomole Peptide Analysis by PicoFrit® Nanobore LC/MS at Low Pressures

Introduction

The inherent chemical specificity and sensitivity of electrospray ionization mass spectrometry (ESI-MS) has led to the development of integrated nanoscale liquid chromatography (nLC) ESI systems.[1,2] In this approach, an appropriate ESI emitter is fabricated directly on the nLC column outlet. Method development has been severely limited by the difficult fabrication of suitable integrated nLC-ESI columns. Furthermore, instrumentation necessary for the generation of suitable sample injection and subsequent chromatography has been specialized and complex. The combination of a tapered, fritted fused-silica needle packed with a highporosity reverse-phase media eliminates these difficulties. This device, the PicoFrit®, provides purification, concentration, and separation at low column pressures. Low-pressure operation eliminates the need for specialized HPLC hardware, provides for short nLC run times, and allows direct integration with common syringe pumps and/or auto-samplers as shown in Figure 1.

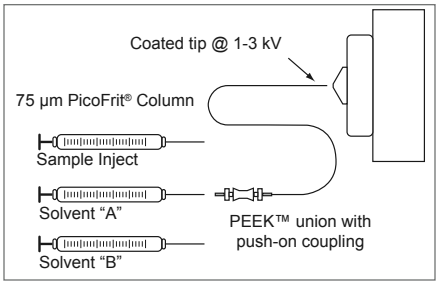

FIGURE 1 Linking a hand inject, rinse or syringe pump and a PicoFrit® on a Thermo Finnigan LCQ™

Column Fabrication

PicoFrit® columns were fabricated from fused-silica tubing with a 30 µm ID tip, an integral high-porosity frit, and multi-layer conductive coating (PF360-75-30-CE) as shown in Figure 2. Columns were syringe-packed with 10 µm POROS®, R2 phase media as follows: Approximately 200 ml of freshly ultrasonicated POROS slurry in MeOH (5 mg/ml) was drawn into a 500-ml gas-tight Luer-lock syringe (Hamilton Company). The distal end of a 50-cm ESI column was inserted into the barrel of the syringe using Luer/fused-silica adapter components from Upchurch Scientific®. Columns were packed by hand pressure alone; packing progress was monitored by light microscopy. When the desired length (about 5 cm) was reached, the column was rinsed with 50 ml of MeOH. Columns were dried for long-term storage with dry nitrogen at 500 psi for 15 minutes. Prior to use, columns were re-hydrated with MeOH and equilibrated with 1% acetic acid.

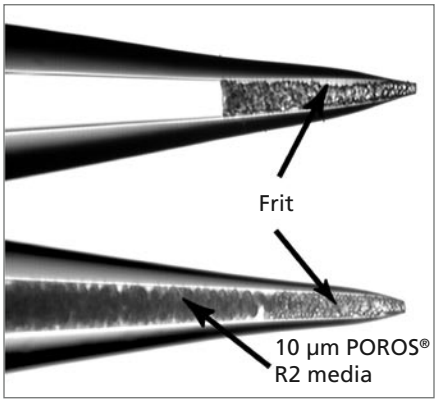

FIGURE 2 Unpacked (top) and pre-packed (bottom) PicoFrit® columns

Advantages of PicoFrit® Combined Column/Emitter

- Provides routine high-sensitivity, low-fmol limit of detection, for peptides on an ion trap in MS/MS mode
- Dirty and/or dilute peptide mixtures now easily run in ESI mode
- Zero post-column effects (e.g. sample loss, resolution loss)
- Capable of fast sample turn-around (< 5 min/run) for high throughput
- Packing the column in the tip eliminates problems with clogged tips
- Operable at low (syringe pump) pressures, eliminating the need for specialized hardware

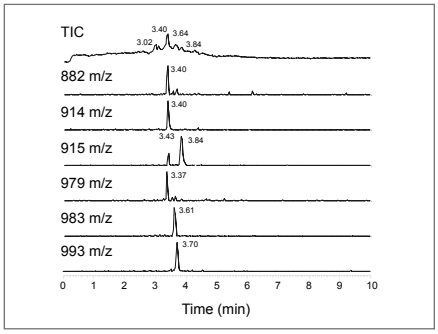

FIGURE 3 75 m x 47 mm (200 nL) POROS® R2, 30 m PicoFrit® @ 1 L/min 30:70:1 (ACN:H2O:HOAc) (10 L inj. 79 - 165 fmol/peptide)

nLC/ESI-Mass Spectrometrey

LC-ESI columns were mounted on a LCQ™ ion-trap mass spectrometer (Thermo Finnigan Inc.) using an inline tip adapter, model ADPT-TLC (New Objective, Inc.) shown in Figure 4. High-voltage (1-3 kV) contact was established with the coated end of the needle using a spring contact mechanism. The distal end of the column was attached to a modified PEEK™ union (Upchurch Scientific). The other end of the union was fitted with a tubing sleeve that provided for rapid push-on coupling to a variety of stainless-steel needles on gas-tight syringes (SGE Inc.). On-column sample injection, washing, and elution were performed by manually swapping syringes. Injection was performed by hand, while solvent flow for washing and elution (0.2 to 1 ml/min) utilized the LCQ syringe pump. MS/MS data was acquired in a data-dependent manner. A test sample consisting of a mixture of six synthetic class I kb murine peptides (7 to 8 bases long, covering a mass range of 881 to 992 Da) was prepared in 1% acetic acid, final concentrations ranging from 7.9 to 16.5 fmol/ml per peptide.

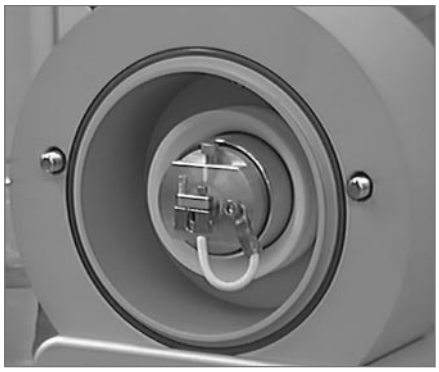

FIGURE 4 ADPT-LTC on the Thermo Finnigan LCQ™

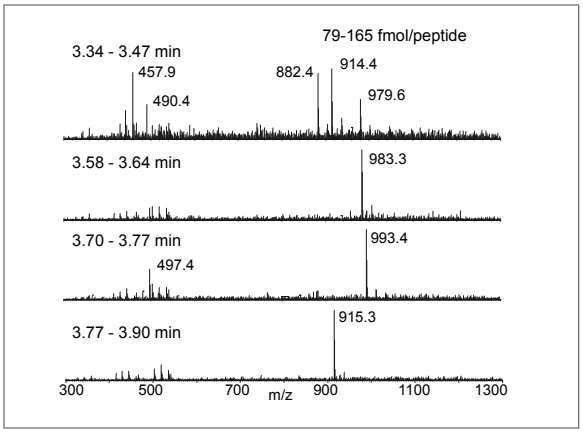

FIGURE 5 MS Results

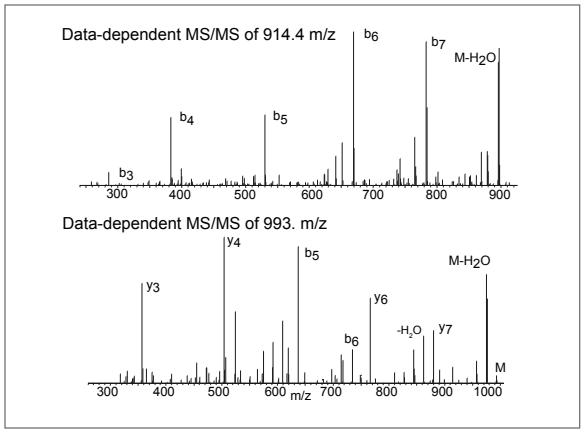

FIGURE 6 MS/MS Results

MS/MS Results Acknowledgments

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References

1) M. R. Emmett and R. M. Caprioli. "Micro-Electrospray Mass Spectrometry: Ultra-High-Sensitivity Analysis of Peptides and Proteins," *J. Am. Soc. Mass Spectrom.* 1994, 5, 605-613

2) M. T. Davis, D. C. Stahl, S. A. Hefta, T. D. Lee. "A Microscale Electrospray Interface for On-Line Capillary Liquid Chromatography/Tandem Mass Spectrometry of Complex Peptide Mixtures." *Anal. Chem.* 1995, 67, 4549-4556