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. 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 high-porosity 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.

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.

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
**nLC/ESI-Mass Spectrometry**

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.

**MS/MS Results**

Data-dependent MS/MS of 914.4 m/z

Data-dependent MS/MS of 993. m/z

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**References**


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