Nanobore Gradient LC/MS and MS/MS Using POROS®-Packed PicoFrit® Columns for Femtomole Sensitivity Peptide Analysis

Introduction

With their low back-pressure, high-porosity frits, PicoFrit® emitters for electrospray ionization (ESI) allow packing of reverse-phase HPLC particulate media. Figure 1 shows unpacked and packed PicoFrit emitters. Post-column band spreading or losses are eliminated in this integral LC column/ESI emitter approach. The only post-column plumbing is the mass spectrometer itself. The highest sensitivities reported for LC-MS utilize this sheathless interface approach.[1,2] PicoFrits are ideal for femtomole (10–15 mol) sensitivity peptide/proteomics analysis.

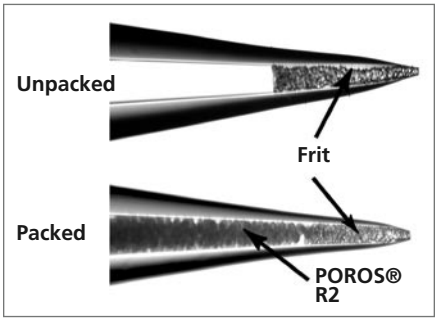


FIGURE 1 PicoFrit® columns, unpacked and packed with media

Column fabrication

Washed and decanted to remove fine particles, 10 µm POROS® R2 media was prepared in filtered MeOH as a dilute slurry. Fused-silica tubing (360 µm OD x 75 µm ID x 50 cm, with an integral 15 µm tapered and fritted tip (New Objective part number PF360-75-15-N)) was pressure packed at 400 psi using a capillary packing bomb.[3] The system was pressurized for approximately five minutes, resulting in an approximately 10 cm packed column. Packing uniformity was monitored by light microscopy.

Column installation

The packed PicoFrit® column was connected to a gradient HPLC pumping system modified for sub-microliter per minute flow rates with the use of an electrically grounded, pre-column T flow-splitter.[3] Samples were injected on-column by disconnecting the column from the pump line and inserting it into a head-space pressurization bomb similar to that used for packing.[3] The tip end of the PicoFrit column was mounted in close proximity (less than 0.5 mm) to the inlet orifice of an ion-trap mass spectrometer (Thermo Finnigan LCQ™ Classic) using a custom-built stage. The required ESI high-voltage contact (1–3 kV) was established at the head of the column by a platinum wire through the arm of a second PEEK™ tee. This second tee also served as the column coupling point to the filtered outlet of the flow-splitter.

Chromatography was performed at a nominal column flow rate of 215 nL/min. A gradient of water to acetonitrile (both 0.05 M HOAc) was run at 2% B/min (B = acetonitrile/0.05 M HOAc). Peptides of interest eluted in the range of 20–40% B. Columns were washed with a high percentage of B (more than 50%) between injections.

20 fmol/peptide
200 nL inj. @ 100 fmol/µL/peptide
215 nL/min, 2% B/min gradient
in10 cm x 75 µm column
3.18E7 Base peak
Full ms:[395-1300]

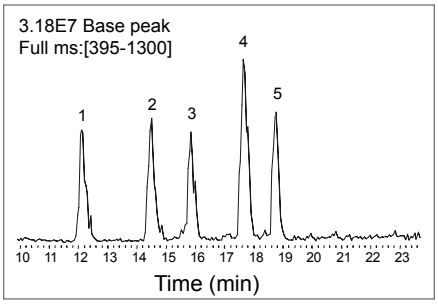
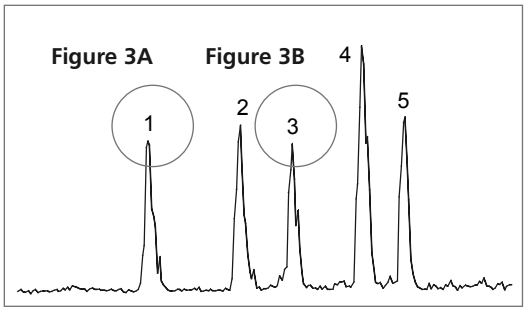


FIGURE 2 Base-peak ion chromatogram (A = water, B = acetonitrile; 50 mM HOAc) of five angiotensin peptides at 20 femtomole per peptide

Results

Figure 2 and Figure 3 show a base-peak ion chromatogram and representative MS and MS/MS spectra of a test mixture of five different angiotensin type I, II, and III peptides (Sigma-Aldrich Corporation). Two hundred nL of the 100 fmol/µL/peptide mixture yielded a total of 20 fmol/ peptide available for analysis. Note the excellent peak symmetry and reasonable peak width (12–16 s FWHM), even though this low flow rate is below the "perfusion" regime desired for high-performance separations from POROS® media.[4]



Data courtesy of William S. Lane and Daniel P. Kirby, Harvard Microchemistry Facility, Cambridge, Mass.

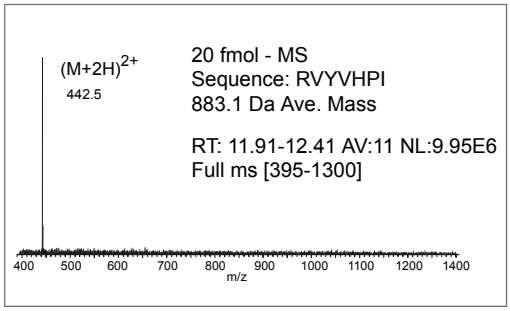


Figure 3A High-quality, full-scan MS spectrum of peak #1, with no other peptides or contaminants observed

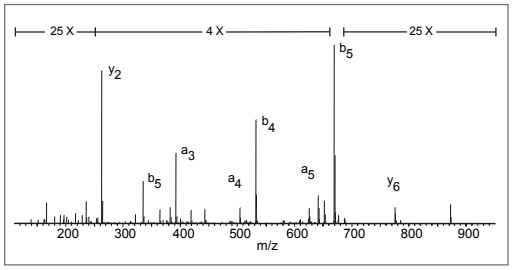


Figure 3B Good chromatographic resolution permits full-scan MS/MS of peak #3 with straightforward interpretation. Such data provides sufficient sequence information (y = C terminus ions; a, b = N terminus ions) for peptide and, in many cases, subsequent protein identification.5 Scale expansion factors are denoted at the top of the figure.

References

1. M. R. Emmett and R. M. Caprioli, *Journal of the American Society for Mass Spectrometry* 5 (1994): 605.
2. P. E. Andren, M. R. Emmett, and R. M. Caprioli, *Journal of the American Society for Mass Spectrometry* 5 (1994): 867.
3. K. B. Tomer, M. A. Moseley, L. J. Deterding, and C. E. Parker, *Mass Spectrometry Reviews* 13 (1994): 431.
4. N. Afeyan et al. *Journal of Chromatography* A 519 (1990): 1.
5. S. D. Patterson and R. Aebersold, *Electrophoresis* 16 (1995): 1791.