A Novel Fused-Core Silica Particle Based Nanobore Column for Ultra-high Performance Nanoelectrospray Mass Spectrometry

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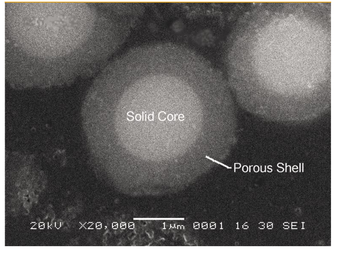
Introduction

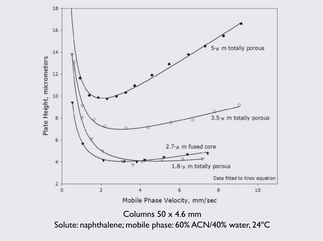
The analytical performance of nanobore columns, each with an integral nanospray emitter, packed with novel Fused-CoreTM silica particles is investigated. The particles consist of a 0.5 µm porous silica layer fused to a 1.7 um solid silica core with a C18 bonded phase. Decreased axial diffusion (porous layer limited) enables fast separations. With a particle diameter of 2.7 µm, the operating pressure for nanobore columns packed with these particles is within conventional pressure limits. The porous layer alleviates the loading capacity deficiency typical of pellicular media. Loading capacity, peak shape and peak capacity for 75 µm ID "packed tip" columns are presented. The application of this novel media in high-speed peptide separations at conventional pressures is also investigated.

Fused-Core Particle



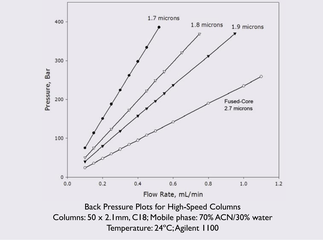
Fused-Core particles are produced by fusing a porous silica layer onto a solid silica particle. The short (0.5 µm) diffusion path created by this porous "halo" reduces axial dispersion and minimizes peak broadening, especially when separating larger molecules. These particles are manufactured for commercial use by Advanced Materials Technology and marketed under the brand name of HALO®.

Van Deemter Plots



Columns packed with smaller size particles provide greater separation efficiency (lower Plate Height). In addition, columns packed with smaller particles can be run at higher mobile phase velocity for faster separations, while sacrificing less in efficiency. Unique Fused-Core particles (HALO) give similar performance to 1.8 µm totally porous particles.

Pressure versus Flow Rate for Columns Packed with Different Size Particles



Column back pressure is inversely proportional to the particle size of the column packing. Therefore, columns packed with 2.7 µm Fused-Core particles will generate significantly less back pressure than columns packed with sub-2 µm particles and are generally well within the acceptable operating range of most HPLC systems, even when operating at high mobile phase velocity. Columns packed with sub-2 µm particles usually require special high pressure equipment.

PicoFrit Column

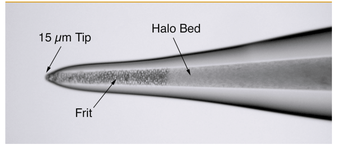


Photo of a PicoFrit® column packed with 2.7 µm "HALO" Fused-Core particles. The column ID is 75 µm and the tip ID is 15 µm. Note the tight integration of the 2.7 µm media with the non-porous silica contained in the fritted region. A 10 cm column typically shows a back pressure of less than 2,500 p.s.i.(300 nL/min, 2% B).

Experimental

Liquid Chromatography:

- Eksigent NanoLC, typical flow rate = 300 nL/min
- Mobile Phase A = 0.1% Formic Acid
- Mobile Phase B = 0.1% Formic Acid in Acetonitrile
- Manual Scivex Injection Valve with 1 µL loop
- Gradient as stated in figures, typically 2% to 50% B in 30 minutes

Column:

- 75 µm ID PicoFritTM (New Objective, Inc.) packed with 2.7 µm HALO C18 (Advanced Materials Technology, Inc.)
- Column bed length of 5 or 10 cm as stated in figures
- Back pressure (10 cm column, 300 nL/min, 2%B) typically 2400 p.s.i.

Mass Spectrometer:

- LCQ Deca (ThermoFinnigan)
- 3 microscans/spectra
- Mass range 300-1500 typical for digests, 400-1500 for peptide standards
- ESI voltage: 2.2. to 2.5 kV typical
- Digital PicoView Nanospray Source (New Objective, Inc.)
- Sequest running within BioWorks (1.4) Browser

Samples:

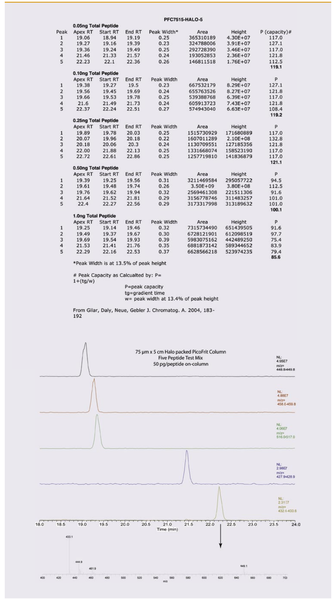
Waters MassPrep Standards

Nine Peptide Mix (MassPrep1 86002337) and BSA Digest prepared by serial dilution (0.1% formic acid) to concentrations of 20, 100, 200 femtomol (fmol) per microliter.

Five Peptide Standard (load capacity study)

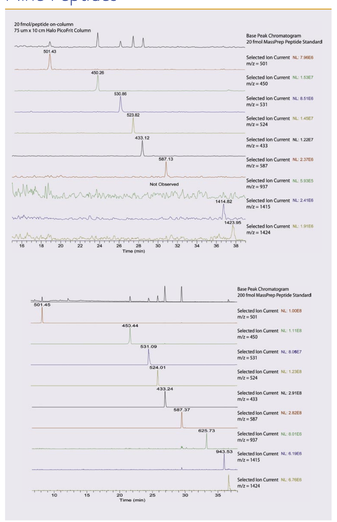
Five angiotensin variants (Sigma-Aldrich) were prepared by serial dilution (0.1% formic acid) to 0.05, 0.1, 0.25, 0.5 and 1.0 ng per microliter.

Sample Mass Loading Study



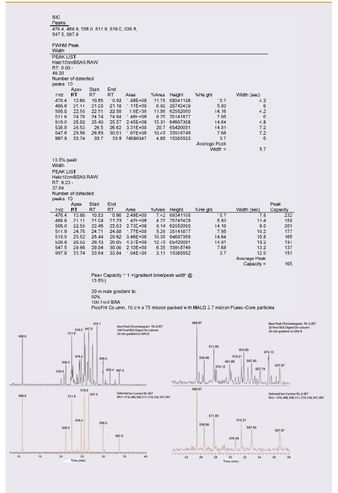
Loading capacity of a 75 µm x 5 cm column. (A) A loss in peak capacity was observed when 0.50 ng of sample was injected, indicating the beginning of column overload. Loading capacity on the Fused-Core particles is similar to what has been observed on totally porous particles with surface areas of between 250 and 300 m2/gram. (B) Example selected ion chromatogram from the 0.05 ng (50 pg) load. The sample is an mixture of five angiotensin peptide variants ranging in molecular weight from 897 to 1296 Da..

LC/MS of a Standard Sample Containing Nine Peptides



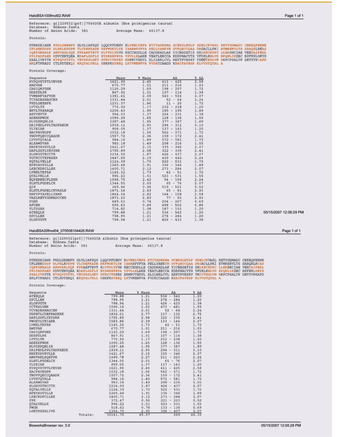
Analysis of a nine peptide mixture (2% to 50 % B in 30 min) using a 75 µm x 10 cm long column. Molecular weights for the peptides range from 898 to 2845 Da. Excellent peak width and shape was observed with both the 20 fmol (A) and 200 fmol (B) samples. The LC/MS data on the 20 fmol (A) sample indicates excellent sensitivity for most peptides in the sample, although for larger peptides (MW > 1757 Da) ion current was significantly reduced.

Peak Capacity of BSA Tryptic Digest



Analysis of a BSA tryptic digest at the 100 fmol (A) and 20 fmol (B) levels. The column was 75 µm x 10 cm; the gradient was from 2% to 50% B in 30 minutes. The bottom (red) chromatogram in each figures shows a summed selected ion current for 8 peptides used in the peak capacity measurement summarized in the results table. For the 100 fmol load, the average peak width (FWHM) was 5.7 sec, the average peak capacity was 165.

LC/MS/MS Sequence Coverage for BSA Tryptic Digest



The MS/MS data from the gradient elutions runs at 100 and 20 fmol of BSA was converted to a DTA file and analyzed using the Sequest Algorithm in BioWorks. Sequence coverage of 62% (A) and 46% (B) was obtained for on-column injections of 100 fmol and 20 fmol respectively.

Conclusions

Nanobore columns packed with novel 2.7 µm Fused-Core particles appear to offer some advantages over conventional, totally porous particles for LC-MS applications. They generate significantly higher efficiency (lower Plate Height) and maintain their efficiency at faster mobile phase velocity than conventional 5 µm and 3 µm particles. In addition, the 2.7 µm Fused-Core particles deliver peak capacity, sensitivity and analysis time similar to sub-2 µm totally porous particles, but generate less than half the back pressure. This eliminates the need for ultra high pressure LC pumps and permits columns packed with Fused-Core particles to be used with most any HPLC system. Nanobore packed-tip columns loaded with Fused-Core particles demonstrate high separation efficiency in the analysis of tryptic peptide samples.