

Fabrication and Use of 20 µm ID Nanobore Columns

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A widely used method for protein identification incorporates gel electrophoresis (2D-PAGE) followed by nanobore liquid chromatography/ nanoelectrospray ionization mass spectrometry (nLC-ESI-MS). Nanospray in combination with 75-100 µm inside diameter (ID) nanobore chromatography, provides typical limits of detection in the low femtomole to subfemtomole range with an ion trap MS. The improvement in detection limit, as compared to conventional LC, is due to low volume (nL) elution, increasing the relative analyte concentration by more than 3,000 fold.

Typical nLC columns are comprised of a packed particle (3-5 µm) bed within 75 to 100 µm ID capillary tubing. In this study, 20 µm ID columns were tested to further push detection limits. The most common fabrication method for nanobore columns is conventional slurry packing. Normally, this requires the use of high pressures, (10,000 psi or more) necessitating the use of cumbersome instrumentation. This becomes more the case as column ID and particle size decreases. Here we report the extension of two novel condensation column and frit fabrication methods suitable for the 20 µm format.^{1,2,3}

Frits were created inside fused silica nanospray emitters fabricated from 20 µm ID fused-silica tubing having a 10 µm ID tip (PicoTip, New Objective, Inc.) via a self assembly packing method.^{1,2} A slurry of 3 µm diameter non-porous silica was prepared in methanol. The PicoTip, loaded on a micromanipulator stage, was briefly dipped (< 1 sec) into the slurry. After approximately 60 sec, the methanol completely evaporates, leaving behind a packed tip. After drying, the tip was positioned in front of a focused CO₂ laser beam for fusion sintering. After a number of laser pulses, the sintered tip and frit were packed with reverse phase media using a multi-step "pre-pack" process.³

20 µm ID pre-pack columns were fabricated by conventional high pressure (2500-5000 psi) slurry packing of 3-5 µm diameter C18 media (ProteoPep, New Objective, Inc.). After packing was complete, a portion of the pre-pack column was excised and connected to the empty 20 µm fritted tip via a ZDV union. The distal end of the pre-pack column was connected to a high pressure pump, and the system was again brought to full pressure. The pre-pack material rapidly (< 1 seconds) transfers and packs into the second fritted-tip column. Using this process the second column packs much more tightly than the original pre-pack column, having a column length that is typically 15-20% shorter.³

The condensation method of packing nanobore columns proved to be very efficient and accurate in creating a high quality packed bed. Total bed length decreased by 20% or more with the same amount (mass) of packing material when compared to conventional packing, indicating a more tightly packed bed.

Two columns were analyzed, each with different dimensions: a 75 µm ID column with a tip size of 15 µm packed with a 5 cm bed and a 20 µm ID column with a tip size of 10 µm with a 5 cm bed. Nanobore columns were created from fritted fused-silica electrospray emitters (PicoFrit, New Objective) and packed with ProteoPep C18 (3-5 µm diameter, 300 Å pore). The performance characteristics of the capillary columns were assessed by the analysis of two standards: a test mix of five angiotensin variants and a tryptic digest of bovine serum albumin (BSA) (Michrom BioResources, Auburn, CA). Analyses were performed on an LC/MS system composed of an 1100 Capillary LC system (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). Samples (1 µl) were injected onto the columns using a high-pressure bomb (400 psi). The 20 µm ID column produces a high back pressure which translates to increased injection time when compared to a 75 µm ID column. Figures of merit include limit of detection (LOD) and signal-to-noise ratio (S/N).

To establish the sensitivity limit for product ion monitoring the LCQ was set to acquire a full scan and a MS/MS spectra of the [M+2H]²⁺ ion for angiotensin (m/z 459.5). All of the five angiotensin variants are

easily detected at a limit of detection of 10 fmol with a S/N of 28 with a 75 μm column. No consistent signal was detected with full scan MS at 1 fmol. The detection limits reached 100 amol in MS/MS mode. The limit of detection was further improved to 1 fmol in full scan with a column ID of 20 μm (S/N of 22). Lowering the column volume increases the relative concentration of the analyte within the column (Table 1).

Along with improved detection limits, small ID columns produce mass spectra with improved S/N. This should directly relate to increased sequence coverage for digested protein samples. BSA was used to illustrate this point. 10 fmol of BSA digest was injected on both the 75 and 20 μm column (Figure 1). The 20 μm column produced a much cleaner spectrum. An eluting peptide within the digest (sequence of CDDLEALKKK, molecular weight 1163.38) can be clearly identified at the 10 fmol level with the 20 μm ID column (Figure 1 Right)

The condensation method of packing nanobore columns proved to be very efficient and accurate in creating a high quality packed bed in 20 μm ID columns. Total bed length decreased by 20% or more when compared to conventional packing, indicating a more tightly packed bed.

Detection limits can be enhanced with the use of smaller ID columns. A 20 μm ID column improved the limit by more than one order of magnitude compared to a 75 μm ID column. This improvement in detection limit will directly relate to better sequence coverage for digested protein samples. Future challenges include the development of improved sample injection and loading schemes.

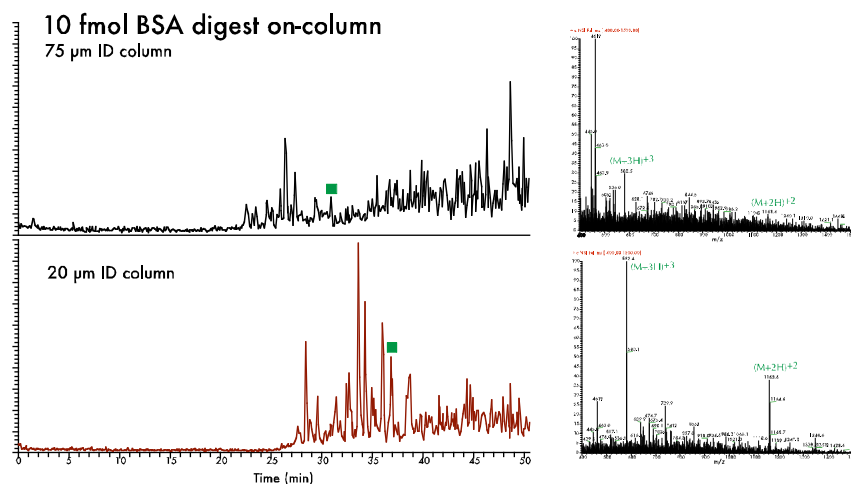


Figure 1 - Base peak reconstructed ion chromatogram for Full scan MS data of a 10 fmol BSA digest injection. Top: 75 μm ID column; Bottom: 20 μm ID column
Right: Shows the mass spectra of the peak highlighted by the green box which is the eluting peptide CDDLEALKKK

1. US Patent 5,997,746, Dec 7, 1999
2. US Patent 6,190,559, Feb 20, 2001
3. US Patent 6,395,183, May 28, 2002
4. Tomer & Moseley, Mass. Spec. Rev., 1994, 13, 431