

High-Efficiency High-Sensitivity Peptide Analysis with Reversed-Phase Nano-LC Monolithic Columns Coupled to ESI-MS

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The high-sensitive identification and characterization of large numbers of components in protein digests is a major need in proteomics. Nanoflow chromatographic techniques offer the advantages of good selectivity, sensitivity and low sample and mobile phase consumption. However, analysis of limited amounts of biological or medical samples (laser capture microdissected cells, immunoprecipitated proteins, etc.) is still very challenging with use of commercially available particulate nano- and capillary columns. The inner diameter of commercially available capillary LC columns ranges from 75 μm to 300 μm . Columns with even smaller I.D. can decrease chromatographic band dilution and increase electrospray sensitivity in peptide nano-LC analysis for a given amount of sample but such columns are difficult to pack with particles media and difficult to operate. This work explores the use of 20 μm I.D. polymeric monolithic capillary columns for the analysis of peptide mixtures and complex biological samples with reversed-phase nano-LC-ESI-MS.

The separation of model peptide mixtures and protein digests by isocratic and gradient elution in nano-LC-ESI-MS is demonstrated. Monolithic capillary columns were prepared in silanized fused-silica capillaries of 20 μm I.D. by thermally-induced *in situ* polymerization. Monoliths were polymerized from styrene monomer and divinylbenzene as a crosslinker in the presence of n-octanol and tetrahydrofuran as porogens and azobisisobutyronitrile as initiator. The role of polymerization conditions and mobile phase composition on chromatographic performance was investigated.

To demonstrate the resolving power of the 20- μm i.d. monolith, Fig. 1 shows gradient nano-LC-ESI-MS analysis of a tryptic digest of a model 10-protein mixture containing hundreds of peptides at the level of 10-40 femtomoles. Separation with subsequent on-line ESI-MS was performed with a 30-minute gradient. A planar ion density map demonstrates the complexity of the sample and the high resolving power of the method with typical narrow elution windows of 15 – 40 seconds. MS/MS data-dependent scanning followed by TurboSEQUENT searching against a database created for the selected model proteins allowed identification of all proteins in the mixture with a high score.

To illustrate the effectiveness of the nano-LC monolithic columns for the analysis of low abundant analytes, a tryptic digest of bovine catalase was injected at the 10-attomole level on the monolithic nanocapillary column. Figure 2 presents MS/MS spectra with TurboSEQUENT database searching for this level injected on the column. Among fourteen peaks corresponding to catalase tryptic peptides

observed in the MS mode at 10 amol of the digest injected on the column, three provided good MS/MS fragmentation and high SEQUEST score.

To demonstrate the potential of the monoliths in the high-sensitivity nano-LC-ESI-MS/MS system, a tryptic digest of protein, from an extract of a breast ductal carcinoma tissue section was analyzed. 10^3 cell equivalent of the digested protein extract was injected onto the 20 μm i.d. monolithic column, followed by a one-hour gradient. Even though a low amount of sample material was injected on the column, MS/MS data-dependent acquisition, followed by database searching with TurboSEQUEST (Bioworks 3.0, ThermoFinnigan) allowed identification of 36 proteins. Fifty fully tryptic peptides were matched with SEQUEST delta correlation (delta Cn) greater than 0.08 and correlation (Xcorr) greater than 2.0, 1.5, and 3.0 for charged states of +1, +2, +3, respectively, with a search against the whole protein human NCBI database. The list of proteins identified by this approach included vimetin, alpha-tubulin 6, glyceraldehyde-3-phosphate dehydrogenase and member M of H2A histone family.

High efficiencies (up to 250,000 N/m) for peptide separations on the monolithic columns are shown. High sensitivity (5-10 attmol of tryptic peptides) in the MS and MS/MS modes is demonstrated in nano-LC-ESI-MS using 20 μm I.D. monolithic reversed-phase columns. Wide linear dynamic range (~4-5 orders of magnitude) is shown. Gradient elution nano-LC-ESI-MS with data dependent MS/MS fragmentation of protein mixture tryptic digests yielding high sequence coverage and analysis of breast cancer tissue samples demonstrate the potential of the method for proteomics.

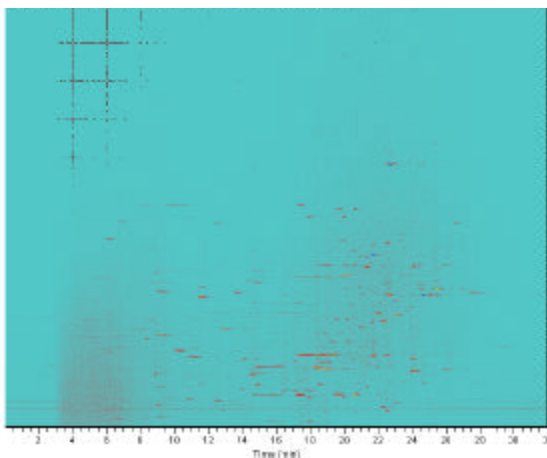


Figure 1: Planar ion density map of gradient nano-LC-ESI-MS of a tryptic digest of a 10-protein mixture on the monolithic column (10– 40 fmol injected on the column).

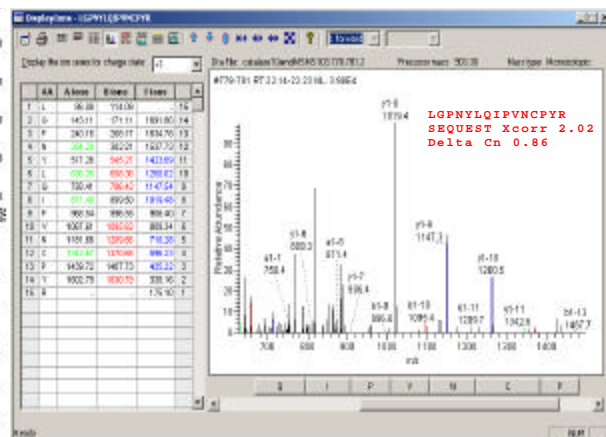


Figure 2: SEQUEST match for the ESI-MS/MS spectrum of the selected bovine catalase tryptic peptide LGPNYLQIPVNCPIR (10 amol of digest injected on the column).