Rapid High-Resolution LC-MS Analyses of Peptides and Tryptic Digests using New Fused-Core® Particle Columns

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Reversed-phase HPLC (RP-HPLC) with online electrospray interface (ESI) mass spectrometry has general utility for peptide structure analysis and identification of sequences and modifications. There continues to be a need for faster separations that maintain high resolution, while using mobile phases that support efficient peptide ion generation and low chemical noise in MS detection. Highly efficient and fast RP-HPLC separations can be obtained using superficially-porous (fused-core) silica particles. Novel fused-core column packing materials of 2.7 micron particle diameter, with a 0.5 micron thick porous shells and an effective pore size of 16 nm, have recently been developed. Such materials are optimally designed for separations of peptides and proteins up to 15-20 kD molecular weight. We have investigated the utility of such materials for rapid LC-MS analyses of synthetic peptide mixtures and tryptic digests of simple and complex protein samples using various MS friendly mobile phases, including formic acid and formic acid/ammonium formate modified mobile phases. The addition of varying amounts of ammonium formate to formic acid (pH 3) progressively improves retention, peak shape and peak widths for basic peptides, up to 20 mM in the mobile phase, without negative impact on MS detection. The addition of ammonium formate also improves column mass load tolerance, which is of great benefit for trace impurity analysis.