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Impact of mass spectrometer-friendly mobile phases on reverse-phase columns selectivity

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Despite all C18 reverse-phase (RP) columns being based on the same general principle and even with the same non-polar ligand as stationary phase, many RP columns exhibit different selectivity. Due to strong variability in chromatographic behavior, RP columns are extensively tested and compared for LC/UV applications in conjunction with mobile phases containing 0.1% TFA (trifluoroacetic acid) or phosphate, and therefore are not optimal for MS analysis. In the current investigation, we tested 28 different brands of RP columns at different temperatures, and compared their performance and selectivity using MS-compatible mobile phases versus a "standard" mobile phase containing 0.1% TFA. Analytes tested were human insulin (~5,808 m.w.), glucagon (~3483 m.w.), C-peptide (~3020 m.w.) and angiotensin 1 (~1,296 mw). To conduct the current study, we built a simple and inexpensive system for automated column testing based on a column switching system from Valco Instruments. While all the tested columns demonstrated low bleed, stable background and also excellent analyte peak shape in the presence of 0.1% TFA, we found that only a fraction of the columns tested could provide good peak shape without TFA for all tested peptide analytes. For some columns, low concentrations of TFA in the mobile phase (which is compatible with LC/MS analysis) may improve peak shape. In many cases however, this did not completely eliminate peak broadening and tailing. We also noticed that silica pore size had only a minor influence on peak shape and utilization of wide pore silica (300A) could not be considered an efficient strategy for improvement of the peak shape for peptide analytes tested. The C18 columns tested demonstrated almost identical selectivity when using 0.1% TFA and 0.015% TFA in the mobile phase, while utilization of MS–compatible mobile phases for peptide separations with formic acid instead of TFA demonstrated strong, unpredictable variability in column selectivity with changes in the retention order of the peptides used in our study. These results emphasize the necessity of column testing if maximization of LC/MS assay performance (by enhancing of peak shape and maximizing column separation efficiency) is the goal.

Biography

Eduard Rogatsky completed his M.Sc in physical chemistry from Belarus State University, PhD in bioanalytical chemistry from Bar-Ilan University (Israel) in 1999, and postdoctoral studies at Albert Einstein College of Medicine, NY. He joined the faculty there in 2001, and is currently a Senior Associate Scientist and Director of Mass Spectrometry in the Biomarker Analytical Resource Core Laboratory, Einstein-Montefiore Institute for Clinical and Translational Research, Bronx, NY, USA.

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