Abstract

This diploma thesis deals with the characterization and comparison of two zwitterionic stationary phases of SeQuant ZIC-HILIC and Atlantis Premier BEH Z-HILIC columns. Analyses were performed in the hydrophilic interaction liquid chromatography (HILIC) separation mode. The ligand bound to both stationary phases of these columns is identical, sulfoalkylbetaine. The main difference between these stationary phases is in the particles to which the ligands are bound. The type of the particles significantly contributes to different interaction and separation properties of the stationary phases.

Firstly, for the basic characterization of these stationary phases, a Tanaka's test was performed. The results of this test showed that the SeQuant ZIC-HILIC column has greater cation-exchange properties than the Atlantis Premier BEH Z-HILIC column, which, has on the other hand greater anion-exchange properties. Both stationary phases showed a higher value of the parameter describing hydrophilicity than hydrophobicity, confirming their polar nature. The properties of the stationary phases were further examined and compared based on the analysis of model analytes. These analytes included a range of small polar analytes, acids, bases, ampholytes, neutral analytes, and their mixtures. Analyses were performed at various ratios of acetonitrile to 10mM acetate buffer of pH = 4,7. Initially, a 5mM acetate buffer of pH = 6,8 was tested, but it has turned out to be less suitable for our analyses. During the separation of most of the model analytes, the separation temperature was set to 25 °C. For the analysis of short oligonucleotides, the separation temperature was increased to 40 °C.

The results of the analyses of model analytes correlated with the results of the Tanaka's test. Increasing retention of polar analytes with increasing acetonitrile content in the mobile phase confirmed the presence of the HILIC retention mechanism. There were found suitable chromatographic conditions for the separation of most model analyte‘s mixtures within this work.