Abstract

Hydrophilic interaction liquid chromatography is a frequently used separation method for analysis of polar compounds. It is an alternative method to reversed-phase chromatography, where these compounds show insufficient or very weak retention. A high number of stationary phases are currently available for HILIC and new ones are still being developed. The aim of this diploma thesis was to characterize and compare three relatively new HILIC columns containing an unmodified silica gel (HILIC-A), aminopropyl modified silica (HILIC-B) and polyhydroxyl chain modified silica (HILIC-N) as stationary phase. Based on the study of the effect of acetonitrile content in mobile phase on the retention of a model set of peptides, a multimodal retention mechanism was demonstrated. Analysis of 18 model analytes with different p*K*a values showed, that the composition of aqueous part of mobile phase (buffer), more specifically it‘s concentration and pH value, has a significant impact on retention of ionized analytes and peptides on the studied stationary phases. A significant contribution of ionic interactions to retention was observed on HILIC-B and HILIC-A columns. The retention of basic compounds on the HILIC-B column increased with increasing ionic strength, while it decreased on the HILIC-A column. Increasing the pH of the mobile phase buffer significantly increased the retention of dissociated acids on the HILIC-B column, while on the HILIC-A column significantly increased the retention of basic, positively charged compounds. The retentivity of studied columns was compared with 21 commercially available HILIC columns. Furthermore, the analysis of a mixture of peptides on the studied columns was optimized and compared using isocratic and gradient elution in order to shorten the analysis time.

Key words: HILIC, HILIC-A, HILIC-B, HILIC-N, buffer concentration, buffer pH, peptides