

Abstract

Charles University in Prague, Faculty of Pharmacy in Hradec Králové

Department of Analytical Chemistry

Candidate: Nikola Klvanová

Supervisor: RNDr. Hana Vlčková, Ph.D.

Title of Diploma thesis: Comparison of selectivity and retention of the analytes on two HILIC stationary phases

This diploma thesis deals with an influence of ACN concentration, pH and buffer concentration in mobile phase on the retention of tested analytes and selectivity of two analytical columns Luna HILIC (diol) column (3 μm , 3 mm x 100 mm) and Ascentis ES Cyano column (3 μm , 2.1 mm x 100 mm). A set of 28 analytes including neutral, basic and acidic compounds was chosen for this study. Detection was performed by PDA detector at the wave-length of 245 nm. Measurement was performed isocratically at the mobile phase flow rate of 0.7 ml/min and 0.3 ml/min. Mobile phase was consisted of a mixture of acetonitrile and water component (buffer or acetic acid). The column temperature was set up to 30 °C, autosampler temperature to 4 °C. Injection volume was 5 μl or 3 μl .

For easier evaluation of results, measured data are presented in the charts and tables. The influence of the ACN content in mobile phase was evaluated based on the plots of the retention factor that showed increasing retention with increasing ACN content (typical HILIC behavior) in mobile phase (mainly > 85%) for all analytes on Luna HILIC (diol) column. The same behavior was observed for both basic and acidic compounds on Ascentis ES Cyano column. Neutral compounds observed decreasing retention in the range from 5 to 30% of ACN (typical RP behavior) and weak HILIC behavior in the range from 80 to 95% of ACN in mobile phase. The influence of buffer concentration on the retention of compounds was detected. The retention of shikimic acid was increased with the increasing buffer concentration on both columns. Other acidic compounds were influenced minimally. On the other hand, the retention of basic compounds, such as atenolol and propranolol, was decreased. The influence of buffer concentration on the retention of neutral compounds was minimal on both columns. The differences selectivity of tested columns at pH 3.8 and 6.8 were insignificant. The most significant differences of selectivity were observed for the basic compounds. Retention mechanism was evaluated based on the lin-log and log-log plots. However, the results have not

confirmed a contribution of adsorption or partitioning mechanism, therefore a complex retention mechanism was showed.