

## **Abstract**

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**Title of thesis:** Retention behaviour of catecholamines and their precursors under HILIC conditions

The aim of this diploma thesis was to study retention behaviour of catecholamines and their precursors under Hydrophilic Interaction Liquid Chromatography by using spectrophotometric and fluorometric detection. 5 analytes were examined – adrenaline, noradrenaline, dopamine, L-DOPA, and tyrosine. Retention behaviour studying began by using LUNA 3  $\mu\text{m}$  (PFP)2 column which possesses both RPLC and HILIC characteristics with 2 different mobile phases. The first one consisted of 25 mM acetic acid in mixture of acetonitrile and water and the second mobile phase was 25 mM acetate buffer (pH 4,50) in mixture of acetonitrile and water.

Subsequently, the effect of stationary phase was studied on neutral column XBridge BEH Amide and columns Avantor ACE HILIC-A and Sequant ZIC-HILIC with ionic interactions. Mobile phases consisted of high proportion of acetonitrile (70–90 %) and their composition was the following – 25 mM acetic acid in mixture of acetonitrile and water. Then, the effect of acetic acid concentration as a mobile phase additive on retention behaviour of analytes was studied on XBridge BEH Amide and Sequant ZIC-HILIC columns. Also, the effect of pH and mobile phase composition was studied by using Sequant ZIC-HILIC column and Avantor ACE HILIC-A (only effect of pH).

The best separation and baseline resolution of all peaks within 20 minutes was achieved with Sequant ZIC-HILIC column with the mobile phase 25 mM acetic acid in mixture of acetonitrile:water (80:20). However, instability in retention time was noticed and it could not be removed even by column regeneration, different mobile phase preparation strategies or prolongation of equilibration time.

**Keywords:** HPLC, HILIC, catecholamines, tyrosine