

## **Abstract**

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**Title of Diploma thesis:** Application of alternative mobile phases in Hydrophilic interaction chromatography method.

This thesis was focused on the possibilities of using 1,3-dioxolan as an alternative organic component of the mobile phase in HILIC.

The changes in selectivity and efficiency of the separation were observed when using dioxolan and acetonitrile as the organic components of the mobile phase. 0.5 % acetic acid or 50 mM ammonium acetate pH 3.8, pH 6.8 and pH 9.8 were used as the aqueous components for the conventional HILIC. The mobile phase consisting of acetonitrile-dioxolan, acetonitrile-dioxolan with 0.5 % acetic acid and acetonitrile-dioxolan modified with 50 mM ammonium acetate aqueous solution pH 6.8 were tested for non-aqueous HILIC.

For the measurements on column ACQUITY UPLC BEH Amide (1.7  $\mu\text{m}$ , 2.1 mm x 100 mm, Waters) 5 different groups of compounds were used, including of phenolic acids, purine and pyrimidine bases, pyridine derivatives, water-soluble vitamins and beta-blockers. Retention, selectivity and the speed of analysis were compared. Measurements were performed using ACQUITY Ultra Performance LC with PDA detection at 280 nm, with the exception of beta-blockers which were measured at the wavelength of 220 nm and 270 nm. The measurements were carried out at isocratic elution at the constant flow of 0.4 ml/min, injection volume was 2  $\mu\text{l}$ .

Acetonitrile was evaluated as a more suitable organic component of the mobile phase for the tested compounds. Unlike dioxolan, acetonitrile allowed more efficient separation of the individual compounds of the groups. The different effect of the type of aqueous component on separation with dioxolan and acetonitrile was observed. The duration of separation was not varying significantly when using either of the solvents. In the beta-blockers group, labetalol was detected in separations with dioxolan. However, it was not detected in separations with acetonitrile. Neither the column nor the mobile phase composition tested appeared to be suitable for the non-aqueous HILIC.