

## **Abstract**

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*Title of Diploma thesis: A study of HILIC mechanism on selected UHPLC stationary phases*

This diploma thesis deals with a study of HILIC mechanism of Ascentis HILIC Express (2.1 x 100 mm, 2.7  $\mu\text{m}$ , Sigma-Aldrich), ACQUITY UPLC BEH HILIC (2.1 x 100 mm, 1.7  $\mu\text{m}$ , Waters) and ACQUITY UPLC BEH AMIDE (2.1 x 100 mm, 1.7  $\mu\text{m}$ , Waters) stationary phases.

Totally 15 analytes had been selected for testing of the characteristics of the stationary phases. The analytes were divided into groups of acidic, basic and neutral substances. The measurement was performed on UHPLC instrument by Waters with detection by a PDA detector at the wave-length of 254 nm. Isocratic elution was used for the measurement. The flow was set to 0.4 ml/min, injection volume was 1-2  $\mu\text{l}$ . Mobile phase was composed of ultrapure water or buffer and acetonitrile.

For an easier evaluation, tables and charts were constructed from measured data. Lin-lin, log-lin and log-log plots were constructed. The stationary phases were evaluated in terms of selectivity and retention of the individual groups of analytes.

The influence of a percentage of water and acetonitrile in mobile phase on the retention of analytes was monitored. Further, the influence of pH was monitored.

For Ascentis HILIC Express and ACQUITY UPLC BEH HILIC stationary phases no substantial difference in selectivity was observed. For an ACQUITY UPLC BEH AMIDE stationary phase, formed by BEH particles modified by a trifunctional amide, the difference in selectivity was significant, compared to ACQUITY UPLC BEH HILIC stationary phase.

The effect of pH was observed on ACQUITY UPLC BEH AMIDE stationary phase.