

ABSTRACT

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Title of diploma thesis: **Study of UHPLC separation depending on temperature**

This thesis analyzes the UHPLC separation and its dependence on temperature. The aim of this project was to map the temperature dependence of the individual chromatographic parameters: retention time, symmetry factor, separation factor and resolution.

Seven different commonly used methods were applied to analyze different chemical substances. These include acids, bases and neutral substances.

Temperature range at Kinetex C18 column was 25 °C to 130 °C, 150 x 2.10 mm. The manufacturer guarantees stability of this column at up to 60 °C. As a part of the analysis, the column was exposed to a temperature of 130 °C for a short period of time. No degradation of the column was observed.

The research demonstrated that in all cases, the retention time along with the separation factor decrease as the temperature increases. Similarly, the resolution worsened as the temperature increased and it was only calculated if the individual substances were sufficiently separated throughout the whole temperature range tested. The time of analyzes was shorter as the temperature increased.

The order of separated substances was the same even at higher temperatures. Only when analyzing sodium picosulfate, its degradation products and sodium benzoate, the separation order of impurities A and B changed at 115 °C. The same alteration was observed when analyzing codeine-phosphate sesquihydrate, morphine-hydrochloride and their derivated substances. Here, the order of separation of methylnorphine-hydrochlorid and pholcodine-hydrochloride also changed at 115 °C.