# ABSTRACT

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Title of diploma thesis: **Evaluation of chosen active substance in the preparation by UHPLC V**

In this thesis there was developed and validated UHTLC method for the determination of sodium picosulphate, its related substances and sodium benzoate. The purpose of the project was to reduce the consumption of an organic solvents burdensome environment and simultaneously achieve sufficient sensitivity for the detection and quantification of the test substances. The LiChroCART® 125-4 LiChrospher® 60 RP-select B (5 µm) column with UV detection by 263 nm was used for the separation of substances at high temperature. The mobile phase was consisted of acetonitrile and buffer in the ratio 1:9. The buffer contained a triethylamine (TEA) at a final pH of 5,0 adjusted with acetic acid. The column oven was heated at 100 ° C, the flow rate was set at 0.4 ml / min and 2 µl injection volume was chosen.

The method has been evaluated as sufficiently sensitive, precise, accurate, linear and selective. The evaluation of the robustness was tested by using Plackett-Burman design. It was found, that according to the tested parameters concentration of acetonitrile in the mobile phase, temperature and flow rate had the greatest influence on retention. Retention time (RT) was reduced with increasing values of these parameters. Remaining parameters had only a little influence at the separation. Conversely, the triethylamine volume, pH and the concentration of acetonitrile in the mobile phase had the greatest influence at the resolution between the individual peaks.

The developed method can be used for the study of drug stability as well as for the routine analysis.