

# **ABSTRACT**

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**Title of diploma thesis: Evaluation of the selected active substance in the preparation using HTLC I**

The purpose of the project was to create a suitable method applicable to a given column and also to obtain sufficient sensitivity for detection and quantification of the test substances, further reduce the consumption of an organic solvents and hence the environmental burden. In this thesis I developed and validated UHTLC method for the determination of sodium picosulphate, its decomposition products and sodium benzoate. There was used the ZirChrom®-PBD column of 100 x 4,6 mm with UV detection by 263 nm for the separation of substances at increased temperature. The mobile phase (MF) consisted of acetonitrile (ACN) with a buffer in the ratio 1:19. The buffer contained 25mM phosphoric acid at a pH of 5,0 adjusted with ammonia. The column space was heated at 80 °C, the flow rate was set at 0,2 ml/min and 4 µl injection volume was selected. The method has been evaluated as sufficiently appropriate: sensitive, accurate, precise, linear and selective. For the evaluation of the robustness Plackett-Burman design was used. It was found that the temperature, pH of the mobile phase and the flow rate of MF had the greatest influence on the retention time of analyzed substances. The retention time of the analytes was decreased with increasing values of these parameters, but at the same time the resolution between the peaks was deteriorated.

The developed method can be used for the study of drug stability.