ABSTRACT

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Department of Pharmaceutical Chemistry and Drug Control
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Title of Doctoral Thesis **Analysis of biologicaly active substances using liquid chromatography**

Presented dissertation thesis is dedicated to the development of methods using liquid chromatography and involves thin-layer chromatography, high performance liquid chromatography and up-to date modification – ultra-high performance liquid chromatography. The paper deals with isolation techniques of tested substances from biological matrix or pharmaceutical preparation. All developed methods are confirmed by means of validation procedures which include primarily precision, accuracy, selectivity linearity and sensitivity. Thesis is divided into two thematic parts. In the first one derivatives of amphetamine are evaluated in biological material. The second part deals with evaluation of pharmaceutical preparations.
Firstly the Theoretical part is devoted to theoretical principles of chromatography, it is focused to liquid chromatography, especially HPLC, TLC, UHPLC. There are described principles of methods and their characteristic and also used instrumentation with the brief description. Also mainly used stationary phases and new trends in their development are mentioned. Comprehensive chapter deals with treatment of samples before analysis follows. One part is dedicated to the isolation of substances from biological matrix, the most common types of isolation techniques are described and new trends of these techniques are added. The second part deals with isolation of active ingredients from pharmaceutical preparations. The final section describes treatment of samples using derivatization. In this part there are described the ways of derivatization techniques and the most common reagents mainly used for fluorimetric detection. An integral part of the development of analytical method is validation therefore one of the last chapters is devoted to the validation parameters that are most commonly used during the validation process. The theoretical part is concluded with a chapter on individual biologically active substances which were the subject of the dissertation. Their chemical and pharmacological properties or metabolic processes are described.
The experimental part is divided into two sections. In the first one composes commented and also published works, they deal with TLC for separatin of methamphetamine, amphetamine and their major metabolites with visual detection (reagent Fast black K).The obtained results were compared with commercially available test strips for the detection of methamphetamine in urine. The second publication deals with the development and validation of HPLC method with fluorescence detection for the evaluation of methamphetamine and its metabolites in urine. SPE isolation of substances from biological matrix was supplemented by dansyl chloride precolumn derivatization.
The second section in detail described the development of new methods for the analysis of pharmaceutical preparations by HPLC or UHPLC. Active substances, related substances and preservatives were evaluated in pharmaceutical preparations. The first paper deals with the evaluation of acyclovir and its three related substances (guanine, diguanin and diacetylaciclovir) together with preservatives (methyl parahydroxybenzoate, propyl parahydroxybenzoate) in cream. UHPLC with UV detection with gradient elution at a higher temperature was used for separation of the individual components of the cream. The developed method was validated. The active ingredient risperidone with four pharmacopoeial impurities were analyzed in another study using UHPLC with UV detection. The method was validated and used for evaluation of risperidone in tablets. Both developed methods brought significant reduction in analysis time compared with conventional methods, which also led to desirable savings of organic solvents. The final new HPLC method serves to analysis of sodium picosulfate in drops. The degradation products and preservatives were evaluated besides the main active ingredient. Two isocratic HPLC methods with different stationary phases were developed depending on the preservative substance in the pharmaceutical preparation. Both methods were validated with a satisfying results.

At the end of the thesis is given the list of publications which is added by the overview of posters of communication presented during the postgraduate study.