

ABSTRACT

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Title of Doctoral Thesis: Modern Trends in Sample Preparation of Biological Material for Analysis of Selected Biologically Active Compounds

This doctoral thesis deals with the determination of selected drugs, their metabolites and other biologically active substances in biological materials. This area of instrumental analysis turns out to be very attractive recently. The reason for this is the need to monitor and optimise treatment and the goal is to achieve the most effective therapy with minimum side effects of drugs for every individual; there are also toxicological reasons.

The thesis concentrates on the entire analytical phase of laboratory screening, i.e. not just on development and validation of chromatographical methods of analysis, but also (and especially) on preparation of biological samples prior to the actual analysis. This phase is crucial for analytes assessment, since it determines not only correct results of the laboratory examination, but also influences the lifespan of laboratory equipment, especially chromatographic columns. These days mostly traditional Liquid-Liquid Extraction (LLE) and Solid-Phase Extraction (SPE) methods are used during samples preparation. These are very work-intensive, time-consuming and difficult to automate. They also have high consumption of organical solvents as well as the actual sample material. To eliminate the above mentioned drawbacks many new sample preparation methods are being developed recently.

The introduction of the theoretical part of this thesis deals with various pre-analytical influences, which can significantly impact the result of the analysis. There is often lack of attention paid to pre-analytical phase of laboratory examination, despite the fact that this is the point, where enormous amount of errors can occur. These errors can have harsh influence on the outcome of the analysis.

Further on the theoretical part focuses on sample preparation techniques – their execution can again influence the correct result significantly. Traditional methods are

described, however most attention is paid to modern extraction techniques. These are developing rapidly in the past few years and lead to full automation, miniaturization and minimalization of pre-analysis lead time.

Important part of this doctoral thesis is a published article related to the use of modern extraction sorbents, so-called Restricted-Access Materials (RAM), which allow on-line extraction directly in the chromatographic equipment and also column-switching technique in high-performance liquid chromatography (HPLC) analysis of biologically active substances in complex matrices.

Experimental part of this doctoral thesis is divided into two parts. First deals with matters of vitamin A and vitamin E detection in biological material. HPLC method with UV detection and on-line extraction has been developed and validated. The extraction takes place directly in the chromatographical system on special RAM extraction sorbents, which are included in the system using column-switching technique (CSW). This allows vitamin A and vitamin E to be determined simultaneously in both plasma and serum.

Second topic of the experimental part deals with the matters of chromatographic determination of statins and their metabolites in serum and in particular lipoprotein fractions. Two articles have been published on this topic. First one describes the beginning of development of chromatographic methods for the determination of statins in our laboratory. Second one deals with the development and validation of Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry Method (UPLC-MS/MS) for simultaneous determination of simvastatin, atorvastatin, their metabolites and lacton forms in biological material after prior SPE.

All developed methods are described thoroughly in previously published articles. Their full wording is included under appendices and short commentary can be found in the chapter „Results and Discussion“.