ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové	
Training Workplace	Department of Analytical Chemistry
Doctoral Degree Program	Bioanalytical Methods
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Title of Doctoral Thesis	Analytical Chemistry Methods for the Analysis of Biologically Active Substances, Pharmaceuticals and the Environment

This work focuses on the use of analytical techniques in pharmacy, laboratory diagnostics, and ecology. The bioanalysis section deals with the determination of endogenous substances in various biomatrices, their impact on analysis, and addresses the issue of bioanalytical method validation. Specific experimental studies then focus on the determination of vitamin K, a less-studied fat-soluble vitamin. Determining vitamin K, which plays a crucial role in the human body, is challenging in many aspects, particularly due to its very low concentrations, difficult deproteinization, tendency to adhere to surfaces, and strong photosensitivity. The solution lies in the use of appropriate sample preparation, which is also utilized in conjunction with modern, selective, and sensitive techniques such as mass spectrometry. Our goal was to develop a simple, quick, and sensitive methodology that could be used in routine laboratories as well as for research purposes. The new UHPLC-MS/MS method for determining various forms of vitamin K (K1, MK4, MK7, MK9) in human and mouse serum and lipoprotein fractions meets these requirements and has already been used in several clinical studies. Furthermore, the experimental part addresses the analysis of vitamins A and E in breast milk, particularly the impact on their levels during milk processing using various techniques. Another area we addressed in the review work was the issue of determining kynurenine – a inflammation marker in various biological matrices. The publication should serve researchers beginning the analysis of this substance for easier orientation in the issue of its analysis, presenting its levels in individual matrices and the main aspects of its determination in connection with clinical significance.

Conversely, exogenous substances – drugs are the focus of the pharmaceutical part of this dissertation. In a preformulation study for determining the adjustable release of terbinafine, we addressed the most suitable variant of the polymer carrier. This work introduced a new method using a biphenyl column for the separation of terbinafine and its degradation products and confirmed the practical potential of polymer carriers based on poly(lactic-co-glycolic acid) for the topical administration of terbinafine. Furthermore, a method for determining cyclosporine was developed, which was used in selecting the appropriate technique for incorporating cyclosporine into inorganic porous excipients.

The third part, concerning environmental analysis, specifically contaminants in water bodies, focuses on substances commonly referred to as “substances/contaminants of concern.” These include pesticides, pharmaceuticals, photoprotection agents, and perfluoroalkyl and polyfluoroalkyl substances known mainly by the abbreviation PFAS. Monitoring the levels of these substances in the environment is essential to determine the extent of their spread and whether measures need to be implemented to limit their future use.

At the end of the dissertation, an overview of publications and other outputs is provided.