# Abstract

The aim of this thesis has been the research on reactivity of protic metabolites with chloroformates and its application for GC-MS analysis of biofluids. The research was conducted in three separate studies and the results are three new, original methods for GC-MS determination of low-molecular protic metabolites in biological material especially in biofluids.

The first study explores the discovery of fast derivatization of alicyclic hydroxyl groups by fluoroalkyl chloroformates (FCFs) under anhydrous conditions [1]. FCF fully converts the hydroxyl group into a corresponding carbonate and the step can easily be coupled with liquid-liquid microextraction (LLME) of the arising derivatives into organic phase. The reaction of the alicyclic OH group with FCFs was tested on 12 clinically relevant steroids and 4 tocopherols. The analytical properties of determined analytes were described and the method was validated for the GC-MS determination of 6 diagnostic sterols and 4 tocopherols in human serum and amniotic fluid. The new method was further successfully used for determination of sterols and tocopherols in tissues of the bug *Pyrrhocoris apterus* [2].

The second study was focused on the reactions of protic, particularly urinary metabolites with FCFs, mainly heptafluorobutyl chloroformate (HFBCF). Reaction products of 153 urinary metabolites with HFBCF and two internal standards were investigated in detail by GC-MS and LC-HRMS. The new procedure was validated for 132 metabolites in human urine and was successfully evaluated by GC-MS analysis of a certified urine sample containing known concentrations of diagnostic organic acids and by analysis of urine samples obtained from 100 healthy volunteers. A urine sample volume of 25 µl allows direct determination of 112 metabolites in the sample set [3]. The analytical protocol was further prepared for the chapter in the book [4].

The third study describes a rapid GC-MS method for determination of acidic urinary biomarkers arising after human exposure to industrial pollutants. The exposition-biomarkers of benzene, toluene, styrene, xylenes, alkoxyalcohol, carbon disulfide, fural, and *N,N*-dimethylformamide were examined after the reaction with different alkyl chloroformates (RCFs). Whereas most of the analytes provided a single, expected product, some provided another, unusual product. Their structure was clearly confirmed by LC-HRMS and by derivatization with isotopically labelled agents. The method was validated for determination of 14 biomarkers of exposure in human urine and successfully evaluated by the analysis of a reference urine material.

New knowledge, acquired by the research of the reactivity of alicyclic hydroxyl group and other protic function groups with alkyl chloroformates, enables perspective use particularly in the field of GC-MS based metabolomics.