

#### 4. Conclusion

The main goal of this thesis was to consider using capillary liquid chromatography as an important analytical method for separation (and quantification) of biologically active and/or pharmaceutically important compounds, both achiral and chiral. Miniaturized separative system enabled an easy variation of experimental set-up and in this way helped to understand the separation mechanism.

New CLC method was developed for separation and quantification of recently synthesized pharmaceutically important acridine thioderivatives in a reaction mixture. Through the optimization procedure the impact of various experimental factors on retention, separation efficiency, resolution and quantification were tested. Under the optimized separation conditions the purity of the thioacridine derivatives was determined.

Chiral CLC systems were created with teicoplanin and vancomycin, two glycopeptides (macrocyclic antibiotics) that belong to the group of modern multimodal chiral selectors (CSs). These CSs were used either bonded on a silica gel support, as chiral stationary phases (CSPs), or free, added to the mobile phase.

Teicoplanin bonded CSP was proved to be suitable for separation of  $\beta$ -blockers, especially in polar organic mobile phase. The best results were obtained in MeOH/HAc/TEA 100/0.01/0.01 (v/v/v). The polar organic separative mode offered higher

separation efficiency, when compared to reversed phase mode, while selectivity of both systems was similar.

The teicoplanin bonded CSP was considerably less suitable for enantioseparation of more polar profen NSAIDs and chlorophenoxypropionic acid derivatives (CPPAs).

Addition of teicoplanin to mobile phases was restricted by its rather low solubility. Nevertheless, combination of achiral stationary phase C18 and mobile phase consisted of MeOH/0.1% TEAA, pH 5.0, 30/70 (v/v) with 0.1 mM teicoplanin resulted in baseline enantioselective separation of two (of the total number of three) CPPAs. Higher concentration of teicoplanin in the mobile phase, which would be advantageous, could not be reached because of its low solubility.

Comparison of the results obtained in the systems with teicoplanin bonded CSP and teicoplanin free in the solution showed that better chiral resolution and shorter analysis time are obtained with the teicoplanin bonded CSP.

The study with vancomycin CS led to opposite results. Better enantioseparation of profen NSAIDs was achieved when this chiral selector was added to mobile phase. The results can be explained by better accessibility of the interaction sites of vancomycin if it is free in the solution. Two achiral reversed stationary phases with different lengths of alkyl chains, namely C8 and C18, were compared in a system with MeOH/0.1% TEAA, pH 5.0, 50/50 (v/v) to which vancomycin was added in the concentration range 0 – 4 mM. In general, higher retentions of all

studied profen NSAIDs were obtained if the column with shorter alkyl chain was used. The increase in the vancomycin concentration resulted in increased retention, which was accompanied by improved enantioresolution. The results can be explained by stronger interaction of the vancomycin-analyte associate with the stationary phase at higher CS concentration.

Lower resolution values of the enantiomers of profen NSAIDs derivatives were achieved if vancomycin bonded CSP was used. The only exception of this general trend was flobufen that was better enantioresolved on the CSP than in the system with vancomycin added to the mobile phase. This result can be related to its different structure - with an aromatic part further from the chiral centre and an additional keto group in its molecule.

The results obtained in the thesis demonstrate high ability of capillary liquid chromatography to solve a wide spectrum of various analytical problems that involve both the achiral and chiral separations. The possibility of an easy variation of the separation arrangements can serve as a tool for better understanding of the mechanism of separation and chiral discrimination.