ABSTRACT

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Doctoral Degree Program	Pharmaceutical Analysis
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Title of Doctoral Thesis	Contributions to method development in capillary electrophoresis and supercritical fuid chromatography for the analysis of pharmaceutical compounds

The goal of this thesis is to contribute to the field of separation science by investigating various approaches of method development for the separation of structurally similar pharmaceutical compounds, using capillary electrophoresis (CE) and supercritical fluid chromatography (SFC) as separation techniques. The theoretical part outlines the main principles and aspects of method development in CE and SFC and introduces the relevant analytes studied throughout the thesis. The experimental part consists of four publications and brief introductions to them.

The first publication describes the development of a micellar electrokinetic chromatography method for the separation of indomethacin and its three impurities and includes method validation and application. The method was developed using a multivariate approach, where a quarter-fraction factorial design was used for screening and a face-centred central composite design for optimization. Baseline separation of indomethacin and its three impurities was achieved within 10 min. The method was validated in terms of linearity, precision, and accuracy, and applied to pharmaceutical samples.

The second work focused on the optimization, validation, and subsequent application of a CE method for the determination of the main components of silymarin. In contrast to previous work, univariate optimization was used. The first approach focused on the optimization of a cyclodextrin-modified micellar electrokinetic chromatography method. However, because of the poor repeatability of migration times, the optimization continued with an electrokinetic chromatography method, with heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin as additive in the background electrolyte. Baseline separation was achieved for all analytes, including the diastereomers of silybin and isosilybin. The electrokinetic chromatography method was validated for linearity, precision, and accuracy, and applied to dietary supplements containing Milk Thistle extract.

The third publication describes the evaluation and improvement of retention prediction of analytes on coupled column systems in SFC. A test set consisting of structurally similar compounds, including chiral compounds, diastereomers, and structural isomers, was analysed on a selection of five chiral and four achiral columns. Analyses were performed both on individual and coupled columns. First, an equation providing the best correlation between predicted and experimental retention factors was selected. Secondly, the prediction precision was improved by adjusting the flow rate and backpressure.

The best performing strategy was applied in the fourth paper to select columns for the separation of silymarin flavonolignans by means of SFC. The method was initially optimized in terms of the selection of an organic modifier. In a following step, the content of organic modifier, flow rate, additives concentration, backpressure, and column temperature were varied using a quarter-fraction factorial design. Further optimized parameters were sample solvent and chemistry of mobile phase

additives. However, despite the extensive optimization, a baseline separation of all analytes could not be achieved.

This work demonstrates various methods and approaches for the separation of structurally similar compounds. Both multivariate and univariate optimization were successfully used to achieve baseline separation for the developed CE methods. However, even after optimizing the coupled column selection and applying multivariate optimization, the SFC separation of silymarin components was not achieved.