FRIEDRICH-SCHILLER-
UNIVERSITÄT
JENA
Fakultät für Biowissenschaften
Institut für Pharmazie

Universität Jena · Institut für Pharmazie · Philosophenweg 14 · 07743 Jena

Prof. Dr. Gerhard K. E. Scriba

Philosophenweg 14
D-07743 Jena, Germany

Telefon: 0 36 41 9-498 30
Telefax: 0 36 41 9-498 02
E-Mail: gerhard.scriba@uni-jena.de

Sekretariat: Theresa Hildebrandt
Telefon: 0 36 41 9-498 00
E-Mail: Theresa.Hildebrandt@uni-jena.de

Jena, 25. Juli 2024

Evaluation of the Ph.D. thesis of Ms. Petra Riasová
"Contributions to method development in capillary electrophoresis and supercritical fluid chromatography for the analysis of pharmaceutical compounds"

Ms. Petra Riasová submitted a publication-based thesis, which contains four manuscripts, three of which have been published between 2018 and 2022 in peer-reviewed international analytical journals with good impact factors in the field (between 2.9 to 4.759). The 4th manuscript is in preparation at the time of the submission of the thesis. Manuscript 1 deals with indomethacin and manuscripts 2 - 4 describe the research on silymarin. All manuscripts describe the application of the liquid separation techniques to the analysis of compounds with (closely) related structures so that it is coherent with regard to the individual topics.

The introduction gives the general background for the need of analytical methods for the determination of the purity of drugs is described followed by the aims and scope of the thesis. In the theoretical part, the basics of CE and SFC as analytical techniques as well as the instrumental set-up are described as well as the application of design of experiments (DoE) in analytical chemistry. Subsequently, the analytes are introduced, i.e. silymarin flavonolignans and indomethacin and impurities, including a brief review of published analytical methods for the compounds.

Manuscript 1 describes the application of MEKC to the analysis of indomethacin and three related substances also mentioned as potential impurities in the European Pharmacopoeia. SDS was used as micellar pseudostationary phase in a phosphate buffer-based electrolyte. Optimization of the experimental parameters including buffer pH and concentration, SDS concentration, MeOH modifier content, and separation voltage was performed using a DoE approach composed of an initial fractional factorial design for identification of the significant parameters and a central composite face-centered design for final method optimization. This was followed by method validation according to the ICH guideline Q3A and applied to drug substance and a topical gel.

The second manuscript deals with the development of a CE method for the analysis of silymarin flavonolignans with special emphasis on the separation of the diastereomeric compounds silybin A and B as well as isosilybin A and B. Eventually, a system based on trimethyl-β-cyclodextrin as chiral selector in a borate buffer was further optimized, so that base-line separations of the diastereomers could be achieved. The method was validated and applied to the analysis of dietary supplements containing milk thistle extracts.

Manuscripts 3 and 4 focus on the coupling of columns in SFC. Thus, manuscript 3 deals with the prediction of the retention behavior of analytes in systems with two coupled columns. While not often applied in HPLC, coupling of columns is easily possible in SFC because of the low viscosity of super/subcritical fluids and the resulting low backpressure. First, a set of test compounds (racemic drugs, diastereomers and positional isomers) was analyzed on the individual columns and subsequently the predictability of the elution of the compounds was evaluated for various combinations of two columns out of five polysaccharide-based chiral columns and four achiral columns. The best predictability (deviation < 10 %) was found for an equation that had been derived earlier for serially connected columns in HPLC. Other approaches resulted in relative deviations of up to 82 %. Column coupling was subsequently applied to the separation of the silymarin flavonolignans, but separations were relatively poor.

As a follow-up, manuscript 4 describes further efforts for the separation of the silymarin flavonolignans applying column coupling in SFC. Two combinations of serially coupled chiral columns were selected for further method optimization using a DoE approach. Unfortunately, no baseline separation could be achieved so far for all analytes. A large problem was the peak broadening due to the coupled columns.

The thesis is concluded by a short summary of the most important findings of the four projects and some perspectives that might be considered in continuation of the research areas.

All manuscripts are well written and describe novel and important data as can be concluded from the fact that three papers have already been published in international peer-review journal. Thus, other international experts have also concluded novelty and importance of the findings. Ms. Riasová is the first author of these publications as well as co-author of another three publications, which are not part of the thesis, demonstrating that she has contributed to further research. Moreover, she has presented data of her research at national and international scientific meetings.

Summarizing, the quality and quantity of the thesis as well as the additional scientific activities clearly fulfill all requirements so that I strongly recommend to award the Ph.D. degree to the Ms. Riasová.

Question 1: How would you rate and compare the developed methods by CE and SFC for the analysis of silymarin flavonolignans? What are their advantages and disadvantages?

Question 2: What could be reasons for band broadening in CE? What are differences with regard to band broadening in SFC versus CE?

Question 3: What do you consider advantages of analyte quantitation by the “2 standard calibration technique” versus the quantitation using one standard solution, which is usually applied by pharmacopoeias (comparison of peak area in the test solution and peak area in standard solution)?

Prof. Dr. Gerhard Scriba