Review of PhD. Thesis

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Title: Contributions to method development in capillary electrophoresis and supercritical fluid chromatography for the analysis of pharmaceutical compounds

Reviewer: RNDr. Václav Kašička, CSc. (PhD.), Institute of Organic Chemistry and Biochemistry, The Czech Academy of Sciences, Prague, Czechia

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The submitted Ph.D. thesis of Petra Riasová deals with the development of new advanced high-performance separation methods, capillary electrokinetic chromatography (CEKC) and supercritical fluid chromatography (SFC). The developed methods were validated and applied for separation and quantification of structurally related pharmaceutical compounds in complex matrices. The topic of the thesis is very up-to-date and extremely important, because new techniques for the fast and highly sensitive analysis of structurally related biomolecules and drugs in complex matrices of real world samples are very much needed.

The author has set four challenging objectives:

1. The development and validation of a micellar EKC method (MEKC) for the determination of non-steroidal anti-inflammatory drug indomethacin and its structurally related impurities.
2. The development and validation of the first CE method for the base-line separation of all main structurally similar flavonolignans and the flavonoid taxifolin in silymarin complex.
3. The evaluation and improvement of retention prediction of structurally similar analytes in serially coupled chiral and achiral stationary phases in SFC, based on a limited number of initial analyses.
4. Attempt to apply the retention prediction in serially coupled stationary phases in SFC for the development of a novel method for the separation of the most important silymarin components.

I am pleased to state that all these ambitious objectives were successfully met. Two new CEKC methods and two SFC methods have been developed, validated and applied for a high efficient separation and high sensitive analysis of different types of structurally related pharmaceutical compounds. The following relevant particular results were achieved:

i) Using a design of experiment (DoE) approach, a new MEKC method with sodium dodecyl sulfate-based micellar pseudophase was developed and for the first time applied for determination of indomethacin and its three structurally related impurities, 4-chlorobenzoic acid, 5-methoxy-2-methyl-3-indoleacetic acid, and 3,4- dichloroindomethacin, in complex pharmaceutical matrices.

ii) Employing a univariate optimization, a new CEKC method with monomolecular pseudophase composed of heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin was developed for determination of the main components of silymarin. Baseline separation was achieved for all analytes, including the diastereomers of silybin and isosilybin. After validation, the method was applied for analysis of dietary supplements containing Milk Thistle extract.

iii) Based on SFC analysis of structurally similar achiral and chiral compounds and using five chiral and four achiral columns, an improvement of retention prediction of analytes on coupled column systems in SFC was achieved by adjusting the flow rate and backpressure.

iv) Using the best performing strategy, a new SFC method has been developed for the separation of major flavonolignan components of silymarin. In spite of extensive optimization of several parameters (organic modifier, flow rate, additives concentration, backpressure, and temperature), a baseline separation was not achieved.

The reviewer appreciates the detailed description of the developed methods and a clear presentation of the achieved results. High quality of these results is confirmed by the fact that they were published in three research articles in prestigious international peer-reviewed journals, Electrophoresis and Journal of Chromatography A and the fourth paper is under preparation. Petra Riasová is the first author of all of them. This documents her substantial contribution to the achieved results.

From the formal point of view, the thesis is presented in a good and clear graphical form with several illustrative figures and tables. The introductory part of the thesis shows a good knowledge of the author within the studied research areas.

Publication of the results in the peer-reviewed journals simplifies my role as a reviewer of the thesis. In fact, the results have been already reviewed and confirmed as correct ones. Nevertheless, fulfilling the role of a reviewer I have the following comments and questions for the general discussion during the public defense of the thesis.

1. In the revised version of the thesis, some references were updated, but some relevant papers are still missing. For example, in the Introduction, second paragraph, for the statement that "CE has been widely used for the separation of related substances in pharmaceutical analysis", several nice comprehensive reviews should be cited, see e.g.:

   Q. F. Zhu and G. K. E. Scriba. Analysis of small molecule drugs, excipients and counter ions in pharmaceuticals by capillary electromigration methods - recent developments. *J. Pharm. Biomed. Anal.* 147:425-438, 2018.
   S. Krait, M. L. Konjaria, and G. K. E. Scriba. Advances of capillary electrophoresis enantioseparations in pharmaceutical analysis (2017-2020). *Electrophoresis* 42 (17-18):1709-1725, 2021.
   C. Fanali, S. la Posta, A. Gentili, B. Chankvetadze, and S. Fanali. Recent developments in electromigration techniques related to pharmaceutical and biomedical analysis - A review. *J. Pharm. Biomed. Anal.* 235:115647, 2023.

   Unfortunately, none of them is presented here or later in the text in the context of application of CE methods for drug analysis.
2. Has the fourth manuscript been finished and submitted to any journal? If yes, to which journal?
3. Where the author see the further potential for increasing the speed and sensitivity of the developed CE methods?
4. What type of compounds are preferably analyzed by CE methods and for what compounds the SFC methods are more suitable?
5. What are the plans of the author in her future scientific carrier?

Conclusion

To summarize, my general evaluation of the thesis of Petra Riasová is positive. The thesis brings new significant results and represents a valuable contribution to the development of new CE and SFC methods and their application for high-efficient separation and high-sensitive analysis of structurally related pharmaceutical compounds in complex matrices. The author proved a good knowledge of the studied topics. She developed new methods and showed her ability of their creative application. For that reason, I recommend to accept the thesis of Petra Riasová for the defense and to consider the thesis as a basis for awarding her with the scientific degree Ph.D.

Prague, 16.8.2024 RNDr. Václav Kašička, CSc. (PhD.)