ABSTRACT

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Title of Doctoral Thesis: The development of new methods of ultra-high performance supercritical fluid chromatography for pharmaceutical applications

A compilation of seven articles and three book chapters dealing with the applicability of supercritical fluid chromatography (SFC) methods in the field of pharmaceutical analysis is presented in this dissertation thesis.

The first part focuses on pharmaceutical quality control and especially impurity control. The presented articles are dealing with the searching for generic screening approach for the development of SFC methods and with the subsequent optimization and validation of these methods. The screening conditions were compared based on developed mathematical model. The generic combination of diol stationary phase and 0.1 % ammonium hydroxide in methanol as modifier of CO_2-based mobile phase was proposed as a starting point for SFC method development based on the obtained results.

Several challenges were described during optimization of UHPSFC methods and their solution was proposed. The benefits of acetonitrile added to the modifier, advantages of column coupling and the usage of LC-dedicated C18 column instead of SFC-dedicated C18 stationary phase were emphasized. In the next step, all developed methods were validated according to international guidelines ICH. Validated parameters included linearity, limit of detection and quantification, range, selectivity, accuracy, and precision at specific concentration levels. Moreover, validation using total error approach was applied on several selected methods.

The interlaboratory precision has to be proven before the use of SFC method in pharmaceutical laboratories. Therefore, a comparative study using SFC method for the determination of active pharmaceutical ingredient salbutamol and its impurities was carried out in 19 laboratories in 9 countries. Statistic evaluation confirmed the method reproducibility with results comparable or even better than for LC method.

Retention time shifts were observed for analytes during long-term SFC project dealing with impurity control. These unrepeatable retention times can cause inapplicability of such methods in strictly controlled pharmaceutical quality control. This issue was addressed in another of presented studies in which the retention times of 70 analytes were observed during analysis over 1 year on set of 8 columns. These retention time shifts were compared on each column and their practical implications were described.

SFC as well as LC method was developed for the determination of impurities in agomelatine tablets. Both these methods were fully validated and subsequently compared in selected parameters. Similar approach was used for the development of chromatographic method for the determination of vitamin E in dietary supplements. Vitamin E exists in nature in 8 forms and dietary supplements can contain natural extracts and/or synthetic tocopherol acetate. Therefore, the developed method had to be able to separate these 9 structurally close compounds. The developed LC method was the fastest LC method for the determination of tocopherols and tocotrienols published so far. Nevertheless, the analysis time of the SFC method was still more than two times shorter. Therefore, the SFC method was validated and subsequently applied on 8 dietary supplements.

The second part of this dissertation thesis is a compilation of a review article and three book chapters summarizing theoretical aspects of SFC technique and practical findings about its use in several application fields such as pharmaceutical analysis, bioanalysis, and others.

Obtained results proved a high potential of fast SFC methods for their application even in strictly control field of pharmaceutical analysis.