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Quality by design approach in high performance liquid cromatography and software-aided chromatograhic method development

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Introduction: The Analytical Quality by Design (AQbD) concept is a modern, science-based approach used for chromatographic method development. This approach aims at a predefined objective and focuses on the understanding and control of analytical processes. Depending on the purpose of the development, systematic experiments are carried out to construct virtual models using computer programs based on statistical principles or fundamental chromatographic theories. The virtual model is the representation of quality attributes as a function of method parameters, ensuring the identification of optimal operating conditions, and providing additional information for the identification of critical parameters necessary for evaluating method robustness. The recommendations of the authorities on the application of AQbD principles are summarized in the preliminary version of the recently published ICH Q14 guideline.

Aim: The main objective of the present Ph.D. thesis was to demonstrate the applicability of AQbD approach in the case of chromatographic method development, according to the preliminary version of the ICH Q14 guideline. The secondary aim was to present the opportunities and advantages offered by the chromatographic fundamentals-based modeling software (DryLab, Molnár Institute) in the development of analytical methods for the determination of chemically related substances.

Material and methods: Reversed-phase high-performance and ultra-high-performance liquid chromatography (RP-HPLC and RP-UHPLC) were used for the determination of related substances of albendazole and ezetimibe, on traditional reversed-phase columns and chiral stationary phases, respectively. To model and optimize the chromatographic separation processes, DryLab modeling software was used, which operates on mechanistic principles, using systematic experiments to build virtual separation models. The method development process was carried out according to the enhanced approach proposed in the preliminary version of the ICH Q14 guideline.

Results: Study 1: UHPLC method development for the determination of albendazole and its chemically related substances, in accordance with AQbD principles.

In this study the development of a RP-UHPLC method was carried out by optimizing the chromatographic method proposed in Ph. Eur. for the determination of the related substances of albendazole. Based on systematic experiments and by applying the modeling program, a suitable stationary phase was selected, and the separation process was optimized in regard to the mobile phase pH, ternary composition, column temperature, and gradient structure. Optimal operating conditions and robustness ranges were identified to ensure method suitability. The method was validated and tested by real sample analysis to demonstrate its applicability. The result of the development process is a selective, efficient, and robust RP-UHPLC method for the determination of albendazole and its related substances. Based on the advantages listed above, the developed method is suitable for replacing the Ph. Eur. method, which presents low performance and poor selectivity.

Study 2: Chromatographic method development for determination of related substances of ezetimibe and comparison of stationary phases, based on AQbD principles.

In the second study a RP-HPLC method development was carried out for the determination of related substances of ezetimibe. Using the modeling software we investigated the effect of temperature, ternary composition, gradient structure, and stationary phase structure on the separation process. Emphasis was placed on column comparison, to identify common operating conditions, which are independent of the chemical structure of the stationary phases. By comparing separation models, chromatographic conditions were identified that can provide the same selectivity and efficiency on six different columns, demonstrating column equivalence and providing high flexibility. The applicability of the method was demonstrated by validation and analysis of real samples. The result of the development process is a selective, efficient, and robust RP-HPLC method for the determination of related substances of ezetimibe. Moreover, a simple AQbD-based methodology was presented to demonstrate the equivalence of stationary phases. The results showed that the structure of the stationary phase is not always a critical parameter, but a deep understanding of the separation processes is essential to ensure the efficiency and flexibility of chromatographic methods.

Study 3: The applicability of chromatographic retention modeling on chiral stationary phases in reversed-phase mode: a case study for ezetimibe and its achiral related substances.

The third study aims to demonstrate the applicability of mechanistic modeling in the case of chiral stationary phases used in reversed-phase mode, for the separation of ezetimibe and its achiral related substances. The separation process was investigated using a cellulose-based chiral column and a beta-cyclodextrin-based stationary phase. The accuracy of the virtual models was higher for the beta-cyclodextrin-based column, which is explained by the similar mechanism of inclusion complex formation to that described by the solvophobic theory. The deviation of the retention times from the values calculated by the modeling program may be due to secondary polar effects between the analyte and the stationary phases, which are excluded from the solvophobic theory. Based on the results obtained chiral stationary phases may provide orthogonal selectivity in the separation of achiral related substances compared to traditional reversed-phase columns.

Conclusion: These three studies demonstrated that the application of the AQbD principles through the use of modelling software is a science-based approach that provides thorough understanding of the chromatographic separation process, involving efficiency, robustness and flexibility. The results of the presented studies together with other publications contribute to the paradigm shift whereby empirical, trial-and-error-based development will gradually be replaced by the science-based AQbD approach.