PhD thesis: Applications of experimental design in the development of capillary electrophoresis methods for the determination of chiral and achiral impurities in pharmaceutical substances
PhD student: Adriana Modroiu
PhD coordinator: Prof. Gabriel Hancu

The determination of chiral purity and the profile of related chemical impurities represents an important aspect of drug analysis, ensuring quality and therapeutic safety. Currently, the trend of using optically pure enantiomers in therapy has significantly increased as the effect of stereochemistry on the pharmacological profile of chiral drugs has been better understood and recognized. Notably, over the past 10 years, approximately 95% of chiral drugs approved by the FDA have been in the form of optically pure enantiomers.

The aim of this thesis was to develop and optimize analytical methods based on capillary electrophoresis (CE) for the determination of chiral purity and chemically related impurity profiles in chiral drugs, using Analytical Quality by Design (AQbD) methodology and Design of Experiments (DoE) approaches to ensure the efficiency, reliability, and applicability of these methods in pharmaceutical analysis.

The model molecules were silodosin (SLD) and tamsulosin (TAM), belonging to the α-adrenergic blockers class, and omeprazole (OME), from the proton pump inhibitor class. These substances are used in therapy as optically pure enantiomers (*R*-SLD, *R*-TAM, *S*-OME), and in the case of OME, the racemic mixture (*R,S*-OME) is also used.

CE was chosen as the analytical method due to its advantages, such as high separation efficiency, rapid method development, and low consumption of analytes, reagents, and chiral selectors. Moreover, CE is considered a "green" method compared to HPLC, due to its low consumption of organic solvents.

Traditionally, univariate strategies (OFAT – One Factor at a Time) are used for analytical method development, but this approach presents some limitations, requiring a relatively large number of experiments and not characterizing the interactions between parameters. The use of DoE methodology involves simultaneously studying several experimental parameters, analyzing multidimensional effects, interactions between parameters, and their influence on selected analytical responses, with a relatively small number of experiments. The application of DoE in analytical method development is essential in the context of AQbD methodology, allowing their development through the application of scientific methods and risk assessment.

The first study aimed to determine the chiral purity of SLD using CE. Preliminary experiments, consisting of a complex cyclodextrin (CD) screening, led to the selection of CM-β-CD as the optimal chiral selector and the establishment of initial conditions for the screening phase. A full factorial design (FFD) at two levels was applied to identify critical process parameters; final

method optimization was achieved using a central composite design (CCD), identifying the following optimal analytical conditions: 100 mM phosphate buffer, pH 2.9, 40 mg/mL CM-β-CD, temperature 17°C, voltage 28 kV. The optimized method allowed the detection of chiral impurities at concentrations as low as 0.02% *S*-SLD. Method robustness was evaluated using a Plackett-Burman design (PBD), with pH proving to be a critical parameter that requires particular attention during electrolyte preparation. The method was validated and successfully applied to the analysis of a pharmaceutical formulation.

The second study describes the development of a CE method for the determination of chiral purity and chemically related impurities of TAM. A dual CD system, composed of two anionic derivatized CDs, S-β-CD and CM-α-CD, was selected based on the results of preliminary experiments. A factorial screening design with resolution V+ was used to identify critical method parameters, while final optimization and definition of the design space were performed using a CCD and Monte Carlo simulations. Optimized experimental conditions were: 30 mM phosphate buffer, pH 3.0, 40 mg/mL S-β-CD and 7 mg/mL CM-α-CD, temperature 18°C, voltage -23 kV. The method allowed the determination of chiral impurities and three other chemical impurities at levels as low as 0.1%.

The third study deals with the determination of the impurity profile of OME using CE. Preliminary experiments led to the selection of micellar electrokinetic chromatography (MEKC) mode, with the addition of an organic solvent, using a "pseudostationary" phase formed from sodium dodecyl sulfate (SDS) micelles and n-butanol as an organic modifier in a borate buffer. A three-level symmetric screening design ($3^7//16$) was used to evaluate the effect of critical parameters on the method's attributes. Method optimization was achieved through Response Surface Methodology using an orthogonal central composite design (OCCD). Failure risk maps allowed the definition of the method's operational design region (MODR), from which the optimized conditions were selected: 72 mM borate buffer, pH 10.0, 96 mM SDS, 1.45% v/v n-butanol, temperature 21°C, voltage 25 kV. The developed method allows the simultaneous determination of OME and seven chemically related impurities and was applied to the analysis of pharmaceutical formulations.

The application of AQbD methodology and DoE techniques led to the development of three new CE methods published for the first time in the scientific literature: for the control of SLD chiral purity, for the control of TAM chiral purity and chemically related impurities, and for the analysis of chemically related impurities in OME. These studies highlight the advantages of AQbD in analytical method development, allowing a deeper understanding of the investigated systems and improving the efficiency and reliability of procedures.

The developed methods were successfully applied to the analysis of pharmaceutical formulations and can be used in the industry to ensure the quality and safety of medicinal products.

The studies emphasize the importance of systematic optimization and knowledge management in designing analytical procedures, aligning with the latest pharmaceutical standards.