PhD Thesis: Research on the chiral separation of medicinal substances used in cardiovascular therapy
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Chirality is a property of the living world that govern our existence. This property is also found in the case of many medicinal substances, with implications on their pharmacological profile, as the therapeutic effect is usually limited to one of the enantiomers called the ***eutomer***, while the other enantiomer called the ***distomer***, may be "inactive", with a lower potency than the eutomer, or sometimes it may show different pharmacological effects or even be responsible for the adverse effects of the racemic mixture.

Cardiovascular drugs are one of the most prescribed classes of pharmaceuticals, with cardiovascular disease being one of the leading causes of morbidity and mortality globally. Many medicinal substances used in the treatment of cardiovascular diseases have at least one chiral center in the molecule, and in some cases their enantiomers differ in terms of pharmacological profile.

Based on these aspects, the development of new enantioselective methods for the chiral separation of enantiomers of substances of pharmaceutical interest represents a challenge and a pressing necessity of modern pharmaceutical research.

In the present study we chose three chiral model substances used in the treatment of cardiovascular diseases: an asymmetric dihydropyridine (***amlodipine - AML***), an arylalkylamine derivative (***verapamil - VER***), and a dimethylphenoxy propane amine derivative (***mexiletine - MXL***). The substances were selected based on their stereochemical properties; all three substances are used in therapy as racemic mixtures, but there are proven differences between the pharmacokinetic and pharmacodynamic profiles of the enantiomers.

As chiral separation method we chose ***capillary electrophoresis (CE)***, based on its advantages related to the high efficiency of separations, rapid method development, low consumption of analytes, reagents and chiral selectors and great flexibility in choosing and changing the chiral selectors. Due to the relatively low consumption of organic solvents, this method lends itself successfully to the concept of "green chemistry". As chiral selectors we used ***cyclodextrin derivatives (CD)*** (natural and derivatized, neutral and ionized), considering that they are the most effective chiral selectors in CE, with advantages related to their commercial availability, high affinity to a large number of drug substances, acceptable solubility in aqueous solutions and low UV absorption

The methods were optimized using different approaches depending on the particularities of the method: univariate approach ("One Factor A Time" - OFAT) respectively ***experimental design (DoE)*** (screening DoE, optimization DoE).

To study the complex interactions and the geometry of the complexes formed between CD and the enantiomers of the analyzed substances, we applied molecular modeling techniques.

The main objective of the work aimed at the development of new fast and efficient CE chiral analysis methods for the separation of enantiomers of model substances, optimization of analytical conditions, verification of the analytical performance of the optimized methods and their applicability to typical pharmaceutical forms.

Study I aimed the development of a simple and efficient method for the separation of AML by CE using CDs as chiral selectors. Following an initial CD screening at four pH levels, CM-β-CD, an anionic derivatized CD, was selected as the optimal chiral selector at a buffer pH of 9.0. The use of a basic buffer and an anionic CD as a chiral selector for the separation of a substance with a basic character resulted in a short analysis time and high chiral resolution. The values of buffer concentration and pH, CD concentration, temperature, applied voltage and injection pressure were optimized using an orthogonal DoE following their influence on the chiral resolution. Using the optimized electrophoretic conditions, a separation of AML enantiomers was achieved with a chiral resolution of 2.65 in an analysis time of less than 5 minutes. The analytical performances of the method were verified in terms of precision, reproducibility, linearity, accuracy, sensitivity, and robustness. The developed method has been successfully used to determine the enantiomeric ratio of AML in several commercial pharmaceutical preparations.

Study II aimed to develop a simple and efficient method for the separation of VER by CE using CDs as chiral selectors. Following an initial CD screening at four pH levels, TM-β-CD, a neutral derivatized CD was chosen as the optimal chiral selector at a buffer pH of 5.0. To optimize the analytical parameters, we initially applied a univariate technique (OFAT), one parameter being varied individually, while the others were kept unchanged. After identifying the significant parameters affecting the enantioseparation (CD concentration, temperature, voltage), a Box-Behnken DoE was applied to establish the optimal analytical conditions. Applying the optimized method, chiral separation of VER enantiomers was achieved with a chiral resolution of 1.58 and a migration time of approximately 4 min, the migration order being *R*-VER followed by S-VER. The analytical performances of the method were evaluated in terms of precision, reproducibility, linearity, accuracy, sensitivity, and robustness by applying a Plackett-Burman DoE. Molecular modeling studies have been used to characterize complex enantioselective chiral selector-enantiomer interactions. The applicability of the method was verified by determining the enantiomeric ratio of VER in pharmaceutical products.

Study III aimed to develop a simple and efficient method for the separation of MXL by CE using CDs as chiral selectors. Based on a complex screening of CDs at three pH levels, TM-β-CD, a neutral derivatized CD, was chosen as the optimal chiral selector at a buffer of pH 5.0. To identify significant parameters interfering with enantioseparation, a fractional factorial screening DOE was applied; the classification of significant parameters on chiral

separation was performed using Pareto charts as well as ANOVA statistical analysis. Buffer concentration, CD concentration and voltage were found to be significant factors and were introduced into a Face Centered Composite Design DoE used to optimize analytical conditions. Chiral separation of MXL enantiomers under optimized conditions was achieved with a chiral resolution of 1.52 and a migration time of less than 5 minutes. The analytical performances of the method were evaluated in terms of precision, reproducibility, linearity, accuracy, sensitivity, and robustness by applying a Plackett-Burman DoE. The applicability of the method was verified by determining the enantiomeric ratio of MXL in pharmaceutical products. The migration order of the enantiomers (*S*-MXL migrates first, followed by *R*-MXL) and the chiral discrimination mechanism were elucidated by applying molecular modeling calculations.

The thesis describes the development of three new methods for chiral separation by CE of some optically active compounds used in therapy, which can be successfully used in their preliminary enantiomeric analysis.

Throughout this thesis, DoE approaches were used in the development of chiral separation methods by CE; the studies are carried out falling within the current trends regarding the application of multivariate approaches in the field of medicinal substance analysis. The thesis presents three different approaches in the optimization of chiral CE methods: the use of a single screening design (orthogonal factorial design) - AML, the use of OFAT technique followed by an optimization design (Box-Behnken) - VER and the use a screening design (fractional factorial design) followed by an optimization design (face centered composite design) – MXL.

Also, the CD-chiral analyte molecular modeling studies used to establish the migration order of the enantiomers and characterize the stereoselective interactions bring added value to the elaborated methods.