UNIVERSITY OF MEDICINE AND PHARMACY OF TÎRGU MUREȘ

DOCTORAL SCHOOL

PhD Thesis

Enantioselective method development for the optical purity determination of substances of pharmaceutical interest

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Abstract

More than half of the pharmaceuticals used in therapy today are chiral and about 90 % of these are marketed as an equimolar mixture of the enantiomers (racemates). It has long been established that the pharmacological activity of a given substance is strongly dependent upon its interaction with the biological matrix. Given the advanced homochiral structure of our organism, it is not surprising that the biological faith of the optical antipodes is seldom the same. Due to this stereoselective nature of our body, it is important to study the chirality of pharmaceutically active ingredients in an attempt to optimize therapeutical outcome and minimize the side effect profile. Bearing in mind the increased number of enantiopure substances recently introduced in therapy, the development of new, enantioselective methods is not only a necessity, but also a must.

The main objectives of this thesis were the development of enantioselective methods for pharmaceuticals where chirality plays an important role. In most of the reported cases, no suitable methods are presented in the literature for the chiral separation of the selected pharmaceuticals. Therefore, the primary objective of this work was the development, optimization and evaluation of electrophoretic and/or chromatographic chiral separation methods for the selected active pharmaceutical ingredients.

Another objective of the present work was the elucidation of the enantiorecognition and enantioseparation mechanisms of different cyclodextrin derivatives, used as chiral selectors. In order to achieve this goal, mathematical evaluation of electrophoretic and/or chromatographic data was undertaken, supplied with the analysis of diastereomeric associates by complementary techniques.

Development of enantioselective methods was achieved using capillary electrophoretic and/or high-performance liquid chromatographic methods. In both cases, different cyclodextrin derivatives were used as chiral selectors. In all cases, an initial chiral selector screening was followed by an univariate or multivariate optimization, analytical performance verification/method validation and applicability testing.

In the chiral selector phase, in several cases the elucidation of the chiral recognition mechanisms was also studied using the electrophoretic chiral separation theory introduced by Wren and Rowe, while in the case of chromatographic methods the classical van't Hoff analysis was performed. In order to elucidate the spatial structure of the diastereomeric associates and reveal its stoichiometry, complementary techniques such as mass spectrometry or nuclear magnetic resonance spectroscopy were also employed.

The first study presents the development and optimization of a capillary electrophoretic method for the simultaneous chiral separation of four, extensively used H1 antihistamines, namely: cetirizine, clorpheniramine, brompheniramine and promethazine, in less than 8 minutes, using a dual cyclodextrin system, composed of SBE-β-CD and β-CD. Applicability of the method was tested on tablets containing levocetirizine as active substance.

The second study presents the separation of the enantiomers of asenapine maleate, a newly introduced atypical antipsychotic for the treatment of schizophrenia, achieved using β-cyclodextrin as chiral selector. Using multivariate method optimization, the baseline separation of the enantiomers was achieved in less than 20 minutes. Spatial configuration of the diastereomeric associates, along with complex stoichiometry were also determined, using electron-spray ionization-mass spectrometry and nuclear magnetic resonance spectroscopy. Results revealed 1:1 binding stoichiometry, with either of the aromatic rings being accommodated in the cyclodextrin cavity.

Chiral separation of the immunomodulatory drug, pomalidomide, is presented in the next study using both electrophoretic and liquid chromatographic techniques. Capillary electrophoretic enantioseparation was achieved in less than 13 minutes, with resolution values as high as 4.87, using carboxymethyl-β-cyclodextrin as chiral selector. Exploration of the chiral separation mechanism revealed thermodynamic control in most cases, arising from different affinities of the solutes to the chiral selector.

Liquid chromatography with mass spectrometry enantioseparation was achieved in reverse-phase mode, using β-cyclodextrin based chiral stationary phase. Enantiomeric elution order was determined by an empirical approach, comparing circular dichroism signals of the separated pomalidomide enantiomers with that of enantiopure thalidomide. Enantioselective mechanisms were studied using variable temperature studies, indicating that chiral discrimination was dominated by enthalpic contribution. Given its high sensitivity, the method can be a good starting point for enantioselective bioanalytical determinations of the drug.

The fourth study presents the development and validation of a cyclodetrin-modified capillary zone electrophoretic method for the cost-effective enantiomeric quality control of the eutomer of the well-known antischistosomal drug, praziquantel. The study presents the development, optimization and validation of a fast and economical, sulfated-β-cyclodextrin based capillary electrophoretic method, capable of detection and quantification of trace amount of optical impurity. Applicability of the method was verified on *in-house* synthetised *R*-praziquantel batches and also on commercial, combination tablets containing racemic praziquantel. The method presented herein can be suitable for the cost-effective enantiomeric quality control of the future chiral switch of praziquantel.

Keywords: chiral separation; capillary electrophoresis; liquid chromatography; enantioseparation; resolution; enantiodiscrimination; nuclear magnetic resonance spectroscopy; mass spectrometry