Thesis title: **Researches on chiral analysis of proton pump inhibitors with performant analytical methods**

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Summary

Chirality plays an essential role in the modern pharmaceutical research; the desired pharmacological effect being generally limited to one of the enantiomers of an active substance. In the last 25 years governmental regulations on drug safety and efficacy have become stricter about compounds that exhibit stereochemistry.

Proton pump inhibitors (PPIs) are among the most frequently prescribed medicine classes in modern therapy, being used in pathologies related to gastric hyperacidity. For example, Nexium (containing the enantiomerically pure esomeprazole as active ingredient) was in the first place on the list of the top selling drugs of the USA in year 2012.

PPIs are benzimidazole-derivative compounds; possessing an asymmetric sulphur in their structure, which generates their chiral character.

Based on the Index of medicinal products for human use of the National Agency for Medicines and Medical Devices of Romania (NAMMDR), there are pharmaceutical products available for five substances from this group: omeprazole, pantoprazole, lansoprazole, rabeprazole and esomeprazole (the *S* enantiomer of omeprazole). In other countries exist other enantiomerically pure forms as well, like dexlansoprazole (the *R* enantiomer of lansoprazole) and dexrabeprazole (the *R* enantiomer of rabeprazole), none of these being commercialized in Romania yet. The pharmacologically active forms of PPIs are achiral, consequently their enantiomeric form does not influence their pharmacodynamic activity. However enantiomeric form can have a importance during interactions with metabolizing enzymes, the eutomer forms having a more advantageous pharmacokinetics as their distomeric and racemic forms.

The aim of this thesis was the development of new modern chiral separation methods using capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC), which san serve as rapid, simple and efficient alternatives for the already published methods. Taking into account the stereoselective action and the high therapeutic prevalence of PPIs, we consider that this subject is of real actuality and can have major implications in the field of modern pharmaceutical research.

The first study describes the achiral analysis of four PPIs (lansoprazole, omeprazole, pantoprazole, rabeprazole), having similar structural and physico-chemical characteristics, using CE. At first, the electrophoretic behaviour of PPIs was evaluated using phosphate buffers as background electrolytes (BGEs) covering a large pH interval (2.5-9.0). The method was optimized using a face centred central composite design, with the purpose to obtain a method capable of separating simultaneously all of the four analytes. The analytical performances of the method were verified and applied for determinations from pharmaceutical formulations. We succeeded in developing a generic method for the analysis of the four studied PPIs, with good resolution values (above 2.5) and in a short analysis time (below 9 min.).

The second study consisted of the validation of an already published chiral separation method of omeprazole for the chiral impurity analysis of esomeprazole. As preliminary analysis the electrophoretic behaviour of omeprazole enantiomers and the possible mechanism of chiral resolution was studied using BGEs containing different cyclodextrin (CD) derivatives, as chiral selectors. The robustness of the chiral separation method was tested by applying a Plackett-Burman design, and afterwards the analytical performance of the method was verified. The validated method, capable of determination of 0,2% *R*-omeprazole in esomeprazole samples, was applied for analysis of esomeprazole-containing gastro-resistant tablets.

The third study aimed the development of two novel chiral separation methods of lansoprazole and rabeprazole enantiomers, respectively. We started with a complex screening of many CDs (neutral and ionized), as chiral selectors, in acidic (pH-4.0) and neutral (pH-7.0) BGEs. The latter one was chosen for method development based on the results of preliminary analysis. Complexation parameters as apparent complexation constants and complex mobilities of the enantiomer-CD complexes were calculated in the case of different selector systems with the aim to study the possible enantiomeric separation mechanisms. The mechanism of enantiomeric separation, when using sulfobutyl-ether-β-CD (SBE-β-CD) with different degrees of substitution (DS), and their dual CD systems containing an anionic CD and a neutral CD, was studied. Chiral resolution increased, while the analysis time decreased, when applying dual CD systems, especially when γ-CD was used as neutral component of the dual system, by comparing to the single SBE-β-CD systems. Method optimization was executed using SBE-β-CD DS 6.5/γ-CD chiral selector system, applying at first a fractional factorial experimental plan, followed by a central composite one. The two chiral separation methods were validated for the determination of the enantiomers of both compounds from racemic samples, and for the determination of distomers in *R*-lansoprazole and *R*-rabeprazole samples, respectively. Methods capable of determination of 0,15% *S* enantiomer as chiral impurity in eutomer samples within 10 minutes were obtained.

The last study describes the elaboration of a reverse phase HPLC method for the chiral separation of pantoprazole. During preliminary analysis, different chromatographic modes as polar organic, polar ionic and reverse phase were systematically compared using different macrocyclic antibiotic-based chiral columns (ristocetin A-based Chirobiotic R, teicoplanin-based Chirobiotic T, teicoplanin aglycone-based Chirobiotic TAG, and vancomycin-based Chirobiotic V). Best resolution values in acceptable analysis time were obtained when Chirobiotic TAG column and reverse phase conditions were applied. Method optimization was carried out using a face centred central composite design, finally succeeding in the baseline resolution of pantoprazole enantiomers within 10 minutes. The study of the effect of temperature on the chromatographic resolution revealed that the enantioseparation is enthalpically driven. The elution order of enantiomers was determined by combining different methods. In the first approach, HPLC-circular dichroism (HPLC-CD) coupled technique was used to apply an empirical method, when the inline CD signal of pantoprazole enantiomers was compared to the CD spectra of enantiomerically pure esomeprazole, serving as a reference compound due to its structural similarity to pantoprazole. The second approach consisted of comparing the online registered CD spectra of pantoprazole enantiomers with their quantum chemically calculated CD spectra. Finally, binding affinities of pantoprazole enantiomers to the chiral selector (teicoplanin aglycone) were determined by docking simulations. All methods showed the same elution order of enantiomers: *S*-pantoprazole followed by its *R*-isomer. The developed method was further validated and applied for determination of pantoprazole enantiomers from commercially available pharmaceutical product, and biological samples, the latter being executed using HPLC-MS/MS technique.

The results of these studies demonstrate the utility of experimental design in optimization and robustness testing of EC and HPLC methods and reveal the advantages of this modern approach in evaluation of the complex effects of different experimental parameters on the analytical responses followed.

At the same time, we succeeded to elaborate simple and fast separation methods, which can be applied for the achiral and chiral analyses of the studied PPIs.