

UNIVERSITY OF MEDICINE AND PHARMACY OF TÎRGU MUREŞ

Summary of the thesis

The dependency of parameters of separation on experimental conditions and the chemical structure in the case of various classes of biologically active substances with cumulate isomerisms

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The topic of the present study, the chemical structure (chirality, inclusive) of pharmaceutical drugs, is of current and acute importance in the discovery and research of medicines. The development of new active therapeutic compounds, especially their discovery, represents an extremely laborious and costly process, which is why anticipating the physicochemical and biological properties of chemical compounds is of major importance.

The stereochemistry of medicine molecules influences both the interaction of chiral molecules with the receptors and their metabolic biotransformation, due to the fact that chiral molecules present complementarities that are different from the receptor proteins and the enzymes that participate to their metabolism, the enantiomers presenting different pharmaceutical properties, as well as different pathways of metabolism.

Given the fact that, currently, the industry that invests most in research is the pharmaceutical one and that, among the medicines used in treatment, half contain at least one chiral centre, and among the latter only a third are commercialised as active optical isomer, the possibility of efficient methods of separation represents a real necessity, and the knowledge of all the parameters that influence the separation of the isomer forms is essential.

Once Romania adhered to the Convention regarding the elaboration of the European Pharmacopoeia, abiding by the standards of quality imposed by the European Pharmacopoeia has become mandatory, consequently, the discovery and improvement of some methods of enantioseparation and of control of optical purity for chiral pharmaceutical drugs is of primary ongoing relevance.

Within the framework of this research topic, we have studied the connection between the separation parameters and the working temperature, the chemical structure of the studied compounds, as well as the interrelations that arise among these, in the case of compounds with radically different chiral chemical structures, respectively, in order to discover the common features of these interactions.

The theoretical part of the present study consists of three chapters, which investigate aspects related to the methods of chiral analysis currently used, the main divisions of chiral selectors, the parameters which characterise chiral separation, the

current state of knowledge as regards the dependency of one separation parameter on all the others, as well as the chemical structure of the studied compounds.

The experimental part, structured, in its turn, in three chapters, comprises original practical connections, chromatographic and electrophoretic, as a result of which the influence of the working conditions and of the chemical structures on the parameters that characterise the entire process of chiral separation has been studied.

The chiral separations of the studied compounds have been made using up-to-date methods of analysis, chromatographic (GC, HPLC, SFC), as well as electrophoretic (CZE). The stereospecific analyses have been conducted using both chiral stationary phases (GC-SGE Cydex B ; SFC- Chirasil Dex; HPLC - Ultron ES OVM), and nonchiral simple, mobile phases, in the composition of which chiral selectors (α -CD, β -CD, RAMEB- β -CD, 2HP- β -CD, γ -CD, SBE- β -CD, MA- β -CD, PMMA- β -CD) have been added.

I. Research on the influence of temperature on the separation parameters

Temperature is one of the most important parameters that determine the processes of separation of the components in a mixture, but the influence it has on the other parameters of separation, such as efficiency and the resolution of the chromatographic column respectively, has long been neglected, and the findings of existing research are often contradictory.

In this chapter, we have investigated the effect the temperature has on the rest of the parameters (α , R_s , N) which intervene in the chromatographic and electrophoretic separation of some chiral compounds with radically different chemical structures: glutethimide, trans-stilbene oxide, methyl ester of the acid 2,4-dichloro-phenyl propionic, amlodipine and indapamide.

II. Research on the influence of chemical structure on the separation parameters

In this chapter, we have studied the influence of derivatization on enantioseparation, starting from the fact that, due to the chemical structure and the physicochemical properties dependent on it (polarity, volatility), there are various chiral compounds which cannot be separated by their mere interaction with a chiral environment, making their derivatization for separation necessary.

In order to understand the mechanism of chiral separation, the thermodynamic parameters ΔH° , ΔG° and ΔS° have been calculated, as they characterise the process of separation and allow the comparative study of structural effects, in the case of a high number of chemical substances with radically different structures (glutethimide, trans-stilbene oxide, methyl ester of the acid 2,4-dichloro-phenyl propionic, ibuprofen, amlodipine and indapamide), as well as within the same classes of compounds (ibuprofen, ketoprofen and carprofen).

III. Research on the simultaneous, mixed influence of temperature and chemical structure on the separation parameters

Following the separate analysis of the dependency of the separation parameters on temperature and chemical structure, both of the analysed compounds and of the chiral selector used, we have studied their common features, in the attempt to find that ideal

value of a separation parameter by which all the other separation parameters are not influenced negatively, value at which the relation benefit-risk is optimal.

A new method of chromatographic separation (HPLC) of drops from all the pairs of enantiomers of three nonsteroidal anti-inflammatory drugs most often currently used in the pain treatment has been developed: ibuprofen, ketoprofen and carprofen with resolutions higher than $R_s > 1,5$, in a single analysis in less than 30 minutes.

The chromatographic and electrophoretic techniques applied offer excellent opportunity for investigating the dependency of the separation parameters on the working temperature, as well as on the chemical structure, respectively, thus making possible the recognition of quantitative relations that form among the parameters of separation methods, the partition factors and the respective steric energies.

Understanding the dependency of all the separation parameters on the working temperature, and on the chemical structure of the compounds to be separated, respectively, helps towards choosing the optimal conditions for analysis, paying attention to the technical particularities of the applied methods as well, thus reducing the time necessary for the production of chiral separation.

All the methods of chiral separation developed in this thesis are simple, quick, precise, reproducible and, last, but not least, cost-effective.

As a result of the research conducted for the present thesis, the objectives have been met, the present study bringing new and substantial contributions to the knowledge and understanding of the connection between the separation parameters, on the one hand, and the temperature and chemical structure of chiral compounds, on the other.

Keywords: chiral drugs, enantiomers, chemical structure, temperature, thermodynamic parameters, GC, SFC, HPLC, CZE, chiral stationary phase, cyclodextrins.