Separation of Ketoprofen Racemate by TLC Plate with Different Chiral Selectors

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Abstract— Separation of enantiomers has become a well-established technique in many fields of science; it is a matter of great importance in pharmaceutical, chemical and biotechnology. In fact, the biological and pharmacological activity of chiral compounds depend on their configuration; one of the enantiomers is pharmacologically active and even can be toxic. It is thus desirable to have reagents and separation techniques to separate the enantiomers and analyze the enantiomeric purity. A variety of chromatographic methods have been developed for optical resolution recently. Direct and simple separation of the enantiomers of ketoprofen is carried out on TLC plate used different chiral selectors in the mobile phase such as: quinidine, quinine carbamate, vancomycin and cyclodixtrin. The success of enantioseparation of racemic ketoprofen is due to the difference in characteristics and selectivity of each selector..

Keywords — Chiral Selector, Enantioseparation, ketoprofen, TLC.

I. INTRODUCTION

Ketoprofen is a non-steroidal anti-inflammatory drug (NSAID) used for the treatment of a wide range of painful and inflammatory illnesses. Ketoprofen is a white or almost white crystalline powder having empirical formula $C_{16}H_{14}O_3$, "Fig.1" with molecular weight of 254,3g/mol and melting point 94° to 97°C. It has pKa of 5,94. It is practically insoluble in water, freely soluble in alcohol, acetone, and dichloromethane. The chemical name of ketoprofen is [2-(3 benzoylphenyl)-propionic acid]..

It contains a single chiral centre, so it exists in two isomeric forms, the S-(+) and R-(-) enantiomers. [1], [2]

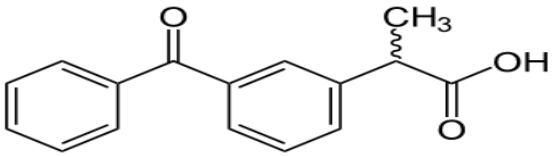

Figure.1- Chemical Structure Of Ketoprofen

Almost half of the drugs in use are chiral. It is well known that the pharmacological effect is restricted in most of the cases to one of the enantiomers.

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The pharmacologically inactive enantiomer (distomer) can show unwanted side effects; in some cases antagonistic and even toxic effects are observed, this is why the separation of enantiomers is required. [3]

Thin-layer chromatography is a very versatile technique, which has brought much advancement in various fields of science. Compared to other chromatographic techniques, TLC has been used less frequently for chiral separations. It might not be able to compete with HPLC or GC regarding separation efficiency; however, it shows several advantages. TLC is a very simple, inexpensive, rapid and flexible technique; many samples can be processed parallel on one plate and very selective detection can be carried out by using spray reagents. For chiral separation, in principal chiral stationary phases or chiral mobile phases can be used. [3], [4]

The interactions used in TLC to achieve enantiomeric separation are dependent on the additive in the mobile phase or the stationary phase used in the separation, can be found hydrogen bonds, hydrophobic effects, steric congestion, inclusion phenomena in cavities, charge transfer, π-π interactions, and dipole-dipole interactions. It is possible to classify the chiral selectors into families according to the nature of the chiral selector and interactions implemented during the recognition process stereoselective such as Pirkle type selectors and similar which allow the separation of enantiomers by forming diastereomeric complexes based on attractive interactions, Selectors by ligand exchange processes involving metal ions, cavity selectors: cyclodextrins involve the formation of inclusion complexes hydrophobic or hydrophilic by crown ether, the natural and synthetic polymer selectors involve a combination of attractive interactions and the formation of inclusion complex to induce separation, the protein selectors having a combination of hydrophobic effects and polar interactions and it found marcocyclic antibiotic selectors are the most used and give the best results as chiral selectors, it appears that the mechanisms of inclusion in the hydrophobic cavity, dipole-dipole interactions, hydrogen bonds, π--π, electrostatic interactions play a role in the chiral recognition. [3], [4], [5]

This article will present the enantiomer separation to one of non steroidal anti-inflammatory drug (NSAID) by TLC using different chiral additives in the mobile phase, quinidine and quinine carbamate the ligand exchange selectors involve the formation of stable complex with metallic ligand, cyclodextrins the cavity selectors which are based on inclusion of the bulky hydrophobic groups of the analyte into the hydrophobic cavities, hydrogen bonds, dipole–dipole interactions and vancomycin the marcocyclic antibiotic selectors. Additionally, know the most appropriate selector to separate the enantiomers of ketoprofen.

II. MATERIALS AND METHODS

A. Materials

The materials used in this study were; ketoprofen racemate was purchased from Saidal Algiers, TLC plate 10 x 20 cm (glass) used in experiments were coated with a thin layer of silica gel, spectrophotometer set at 220 nm, chirals selectors: quinidine, quinine carbamate, β cyclodextrin and vancomycin. All solvents and chemicals used were of analytical reagent for HPLC grade.

B. Analysis

The mobile phase comprised 80 mM Ammonium acetate buffer and methanol. The solution of racemate ketoprofen was prepared by dissolving in methanol [2, 6], and injected (2µl) on TLC plate, then it was placed inside a pre-equilibrated glass chamber containing mobile phase at room temperature. In order to separate the enantiomers of ketoprofen we added a small quantity of the chiral selector in the mobile phase. When the ascending solvent front neared the top margin, the plate was removed from the chamber and dried with a hair-drier. The spots were invisible so we used the spectrophotometer to detect.

III. RESULTS AND DISCUSSION

The R_f values of ketoprofen enantiomers for each experience are given in the table 1.

Table1- separation of ketoprofen racemate by TlC plate with different chiral selectors

N°	Mobile phase	R_f values
1	Methanol/ Ammonium acetate	0,30
2	Methanol/Ammonium acetate + quinidine	0,38
3	Methanol/Ammonium acetate + quinine carbamate	R_{f1} = 0,41 R_{f2}= 0,51
4	Methanol/Ammonium acetate + vancomycin	R_{f1}= 0,48 R_{f2}= 0,59
5	Methanol/Ammonium acetate + β-cyclodextrin	R_{f1}= 0,63 R_{f2}= 0,64

Through experimental work we have acquired one value of R_f when using the mobile phase with quinine selector, evidence to the emergence of a single spot of the racemic mixture of ketoprofen, thus the separation is impossible. But we can see two spots that means two values for R_f, this is an obvious of a separation chiral process when we used quinine carbamate, despite that the molecule has an average size and this is due to the effects occurring between solute and chiral selector. Vancomycin is a big molecule containing 18 chiral centers and 3 cavities, in addition to this the existence to polar groups near the cycle leads to the presence of the forces of attraction with the solvent and increases the selective property. Consequently, the formation of complexes with enantiomers of ketoprofen allows the success of separation process. The β cyclodextrin selector has 5 chiral centers, high capacity to form stable complexes and the presence of stereostructures obstruction resulting from the cavity which facilitates the separation of ketoprofen racemate.

IV. CONCLUSION

From the results obtained, we can say that the separation of ketoprofen racemate using TLC plate is controlled by several factors during the separation:

- Stationary phase properties (silica gel) and its ability to form interfacial bonds with each enanatiomers.
- Mobile phase properties, the elution strength and forming hydrogen bonds with compounds.
- Molecular weight and stereochemistry structure of solutes and chirals selectors used.

In addition to this, the vancomycin macrocyclic antibiotic and quinine carbamate are the best chiral selectors for the separation of the ketoprofen enantioners.

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