LOVELY PROFESSIONAL UNIVERSITY

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DISSERTATION-II FINAL REPORT

For the Partial Fulfilment of the Degree of Masters of Sciences (Chemistry)

On the project entitled as

Enantioseparation of amino containing pharmaceuticals using indirect approach on TLC and HPLC

Submitted by

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Under the guidance of

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TOPIC APPROVAL PERFORMA

School of Chemical Engineering and Physical Sciences

Program : P266-H::M.Sc. (Hons.) Chemistry

COURSE CODE : CHE688 **REGULAR/BACKLOG :** Regular **GROUP NUMBER :** SCRGD0037

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PROPOSED TOPIC : Enantioseparation of amino containing pharmaceuticals using indirect approach on TLC

Qualitative Assessment of Proposed Topic by PAC		
Sr.No.	**Parameter**	**Rating (out of 10)**
1	Project Novelty: Potential of the project to create new knowledge	7.00
2	Project Feasibility: Project can be timely carried out in-house with low-cost and available resources in the University by the students.	7.25
3	Project Academic Inputs: Project topic is relevant and makes extensive use of academic inputs in UG program and serves as a culminating effort for core study area of the degree program.	6.50
4	Project Supervision: Project supervisor's is technically competent to guide students, resolve any issues, and impart necessary skills.	8.00
5	Social Applicability: Project work intends to solve a practical problem.	6.50
6	Future Scope: Project has potential to become basis of future research work, publication or patent.	7.00

PAC Committee Members		
PAC Member 1 Name: Dr. Gurpinder Singh	UID: 13608	Recommended (Y/N): Yes
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PAC Member 3 Name: Dr. Rekha	UID: 14537	Recommended (Y/N): NA
DRD Nominee Name: Dr. Runjhun Tandon	UID: 19532	Recommended (Y/N): Yes
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Final Topic Approved by PAC: **Enantioseparation of amino containing pharmaceuticals using indirect approach on TLC**

Overall Remarks: Approved

PAC CHAIRPERSON Name: 11800::Dr. Ramesh Chand Thakur **Approval Date:** 13 Oct 2016

CERTIFICATE

This is to certify that this research was carried out by Jaspreet Kaur under the supervision of Dr. Manisha Singh at the department of Chemistry, Lovely Professional University, for the award of M.Sc. (Hon's) Chemistry.

Supervisor
Dr. Manisha Singh
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DECLARATION

I hereby declare that this capstone named **"Enantioseparation of amino containing pharmaceutical using indirect method on TLC"** submitted for M.Sc (Hon's) chemistry degree to the department of Chemistry, Lovely Professional University is entirely done by me.

Date:

Jaspreet Kaur

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INTRODUCTION

Levofloxacin was used as chiral derivatising reagent (CDR) for enantioseparation of racamic drug the analytical method is simple, cost-effective and less time consuming. Racamic drug separation was observed under different conditions like pH of reaction mixture, concentration of mobile phase, polarities of solution or reaction mixture and temperature. TLC experiment has been done for the enantioseparation of racemic drug mixture in which pure drug was extracted by method of recrystalisation.

I.1. Basic Terminology: (Optical isomers, Chirality, Enantiomers, Enantioseparation.....)

An object which is non-superimposable on its mirror image is called as chiral. This property of the molecule is termed as **chirality**. If an object having superimposable relationship with its mirror image is termed as achiral. The term chirality derived from Greek word kheir, meaning is "hand"i.e. those object which having "handedness"i.e. the object and mirror image relationship of left and right hand. Thechiral object and its mirror whichare non-superimposable on each other are called **enantiomers**. In other words, a pair of molecules that is not identical to its mirror images which are related to each other in same way as our left and right hand. A pair of enantiomers has identical physical and chemical properties in achiral environment but when theseare placed in chiral environment or kept under plane polarized light they behave differently.The mixture of two enantiomers in equal proportions (50/50) is called a racemic mixture or a racemate. The method by which both enantiomers can separatedfrom racemic mixture is termed as **enantioseparation**.

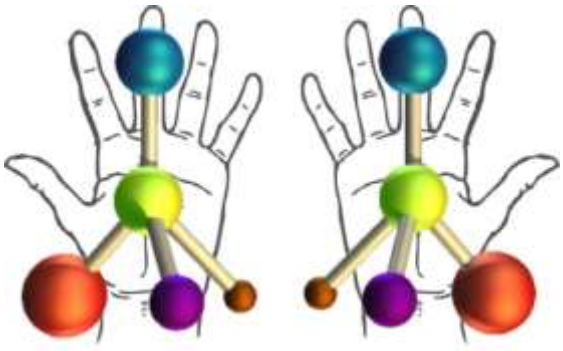

Figure 1.Chiral molecules containing a tetrahedral stereocenter

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1.2. Thin Layer Chromatography (TLC)

The term "Thin layer chromatography" was coined by Egon Stahl [3] in Germany in the late 1950s; his first TLC laboratory manual popularized TLC in 1962. The development stages of TLC have been reviewed in one of the review article [4]. Thin-layer chromatography (TLC) is a simple, rapid, versatile, sensitive and inexpensive analytical technique for the separation of substances. The mobile phase in TLC is a liquid containing a single solvent or a mixture of solvents and the stationary phase is an active solid, known as the sorbent. TLC can be used as both an analytical and a preparative technique. TLC is a micro analytical procedure and provides the separations and at least tentative identification of substance nanogram range.

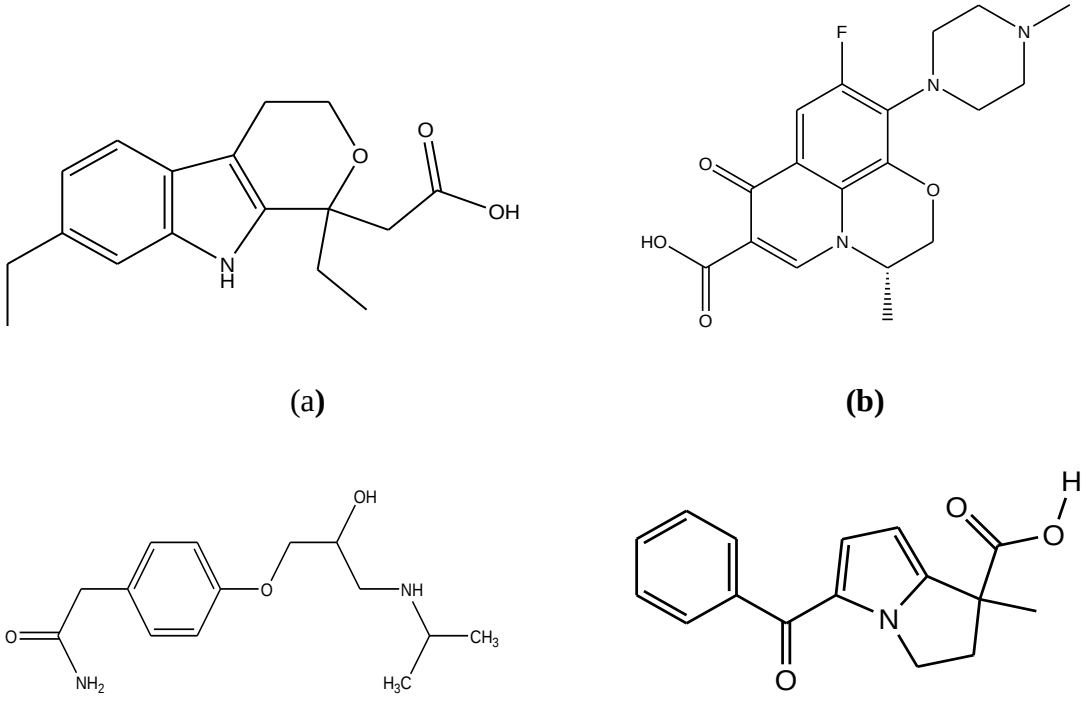
Among various chromatographic separation techniques, thin layer chromatography (TLC) is one of the economical viable and advantageous techniques. Studies on resolution of enantiomers by TLC revealed the significant role of this technique for routine analysis and determination of optical purity in synthetic and analytical laboratories.

1.3. β –blocker

β -blockers are 2-hydroxy propyl amines whose enantiomers show significant difference in their pharmacological effects and activities [1, 2]. Atenolol is a very well-known cardio selective β-blocking agent, which available in market as racemic mixture, and is used to treat hypertension, coronary heart disease, arrhythmias, sinus tachycardia and myocardial infarction, where it acts preferentially upon the beta-adrenergic receptors in the heart [1]. It causes fewer side effects like cold extremities, dizziness and tiredness, decrease in heart rate, depression, and nightmares. Literature reveals that pharmacological action of atenolol is largely confined to (*S*)-(-)-isomer [3] whereas (*R*)-(-)-isomer is inactive in nature.

Etodolac and ketorolac are well known nonsteroidal anti-inflammatory drugs (NSAID),which is available in market as racemic mixture,Etodolac can increase your risk of fatal heart attack and also cause stomach or intestinal bleeding, constipation, vomiting etc.These conditions can occur without warning while you are using etodolac, especially in older adults. ketrolac is used as short term pain relief drug but it cause some side effects like itching, tearing, abnormal thinking and sweating etc,.Similar like atenolol, etodolac and ketorolac is largely confined to (*S*)-(+)-isomer

[3] whereas (*R*)-(-)-isomer is inactive in nature. Several reports have been observed in which etodolac has been separated by two different analytical method direct and indirect method of separation. Several reports have been observed in which etodolac has been separated by two different analytical method direct and indirect method of separation. Direct chiral separation of two enantiomers has been done by using chiral stationary phases but in indirect method of separation diasteromers were prepared with optically pure chiral selector followed by separation of enantiomer using HPLC [4].Indirect separation of enantiomers has been achieved by using several chiral derivatizing reagents (CDR) because it can provide an excellent detector response for the analysis of biological samples viz., plasma, urine, blood etc. Even indirect method of separation can provided the better result and resolution than direct method. The derivatization time for etodolac (or some other β-blocker) is reported to be 30 min at room temperature using different CDRs, e.g. using levofloxacin which has been one of the most successful reagents.



(a**)** **(b)**

(c) (d)

Fig 2:- Structure of (a) Etodolac (analyte) and (b) Levofloxacin (Chiral Selector)

(c) Atenolol (analyte) (d) ketorolac

1.4. Strategies of Enantioseparation of Racamic Drug:-

Enantioseparationcan be achieved by two different method direct and indirect enantioseparation of biological active compound.

Indirect Enantioseparation: -

This method basically involve to the formation of pairs of diastereomers followed by separation in achiral environment.

Use of Chiral derivatizing reagents (CDRs)(Levofloxacin):-

In current study, I focused only on indirect method of enantioseparation because direct separation already done in our previous paper.

LITERATURE REVIEW

Chiral derivatizing reagent (Sanger's reagent) has been used for enantioresolution of six beta-blocker using dinitrophenyl-L-Pro-N hydroxysuccinimide ester, N-succinimidyl- (S)-2-(6-methoxynaphth-2-yl) propionate by Reversed-phase HPLC [5]. Marfey's reagent has been used for derivatization in rat plasma for determination of enantiomers of atenolol by reversed –phase liquid chromatography [6].Thin layer chromatographic method has been used for enantioseparation of atenolol, propranolol and salbutamol by different approaches of impregnation using Cu (II)-L-amino acid complexes in ligand exchange chromatography [7].Chiral derivatization and high-performance liquid chromatography (HPLC) has been used for the preparative resolution of theβ-blocker drug atenolol [8].

Liquid chromatography and other techniques related have been used for separation of some unusual amino acid by using chiral derivatizating reagent [9]. A method has been developed for the simultaneous chiral separation followed by purity determination of chiral switches of atenolol and amlodipine usingHPLC [10].

Highly enantioselective CALB-catalyzed kinetic resolution of building blocks for β-blocker atenolol [11]. C_8 column has been used for the enantioseparation of atenolol using ligand exchange chromatography [12]. A validated RP-HPLC-UV method was developed for simultaneous determination of Buparvaquone, atenolol, propranolol, quinidine and verapamil in situ intestinal permeability studies in rat [13]. Direct TLC Resolution of the enantiomers of three β-blocker by Ligand Exchange with Cu (II)–L-Amino Acid complex, using four different approaches [14].

RP-LC Resolution of (*RS*)-Atenolol via Diasteriomerization with Marfey's Reagent and its Structural Variants under Conventional and Microwave Heating [15].

(*RS*)-Etodolac has been used for a "novel approach for enantioseparation from pharmaceutical formulations", the confirmation of diasteremers were separation by the LC MS and density functional theory [16].

Etodolac has been resolving by the exploration of an efficient method using chiral isopinocamphenol or L-cinchonidinium salt diasteromeric esters is described herein[17].High performance liquidchromatography (HPLC)–tandem mass spectrometry has been used for the determination of etodolac in human body plasma [18].

SCOPE OF THE STUDY

The scope of the current study has been concluded as:

1. In recent study, TLC has good scope for the separation of direct and indirect method of separation of enantiomers by using different approach and chiral derivatizations reagent (CDR).

2. The indirect method for separation of diasteriomers is very simple and cost-effective.

3. The direct method for chiral separation was single step enantioseparation of different chiral drugs.

4. The indirect method involve the formation of diastereomersusing optically pure chiral derivatizing reagent (CDR) followed by separation of diastereomers using TLC and HPLC.

5. Chiral stationary phases are commercially available in market which can be used for HPLC enantioresolution of chiral pharmaceutically important compounds.

6. Chiral derivatizingreagentshas also been used for the separation of diasteromers followed by HPLC. Therefore, this technique may also apply for the enantioseparation of various amino containing chiral pharmaceuticals.

OBJECTIVES OF THE STUDY

1. To separate certain amino group containing chiral compounds via diastereomers formation i.e. indirect approach of enantioseparation.

2. To employ certain new chiral derivatizing reagents (CDRs) for the derivatization of two enantiomers of chiral compounds in which only one enantiomer is useful for us while other one may cause some side effects, toxic and may be inactive to body.

3. Chiral derivatizing reagents on TLC better technique than on column chromatography because it has been found to take lesser time than column and provide excellent separation.

4. Chiral derivatizing reagents (CDRs) have been used for the separation of racemate mixture of chiral drugs as well as trace analysis of enantiomers in real samples like urine, blood etc.

5. The separation of diastereomers will be carried out using liquid chromatography (TLC).

6. The limit of detection will also be determined with serial dilution method.

EXPERIMENTAL WORK

5.1 **Material and Methods**

Various drugs like Atenolol (50 mg, uncoated tablet),Etodolac (400 mg, coated tablet) was obtained from Ipca laboratories Ltd. (Mumbai, India). Levofloxacin (500mg, coated tablet)was obtained from CIPLA Ltd. (Baddi, India) and solvent which used toprepare stock solution likeMeOH, DCM pure, Acetone, C_4H_9OH, CH_3CN, was from Analytical CS for synthesis and silica gel was from (Molychem). All other reagents and apparatus were provided by laboratories (LPU). Most of solution and solvent were prepared in MeOH.Double distilled water was used for the preparation of all stock solutions.

5.2. General Procedure:-

Extraction and purification of active pharmaceuticals from Pharmaceutical formulation

The extraction and purification of active pharmaceuticalswasdone from their tablets as per procedure described earlier [19].

(i) Extraction of Levofloxacin (CDR)

To ten tablets of levofloxacin pure (500mg each), were firstly remove the coating then grind to a fine powder and extracted with MeOH (50ml). After that filter the solution and took filtrate for evaporation on Rota vapor and left to cool until crystals appeared. Then recrystallized the compound with MeOH and chloroform (7:3) ml recorded the yield.

(ii) Extraction of Atenolol

To fourteen tablets of atenolol pure (50mg each), were grind to a fine powder and extracted with MeOH (50ml). After that filter the solution and took filtrate for evaporation on Rota vapor and left to cool until crystals appeared. Then recrystallized the compound with MeOH and chloroform (7:3) ml recorded the yield.

(iii) Extraction of Etodolac

To ten tablets of etodolac pure (400mg each), were grind to a fine powder and extracted with MeOH (50ml). After that filter the solution and took filtrate for

evaporation on Rota vapor and left to cool until crystals appeared. Then recrystallized the compound with MeOH and chloroform (7:3) ml recorded the yield.

(iv) **Extraction of Ketorolac**

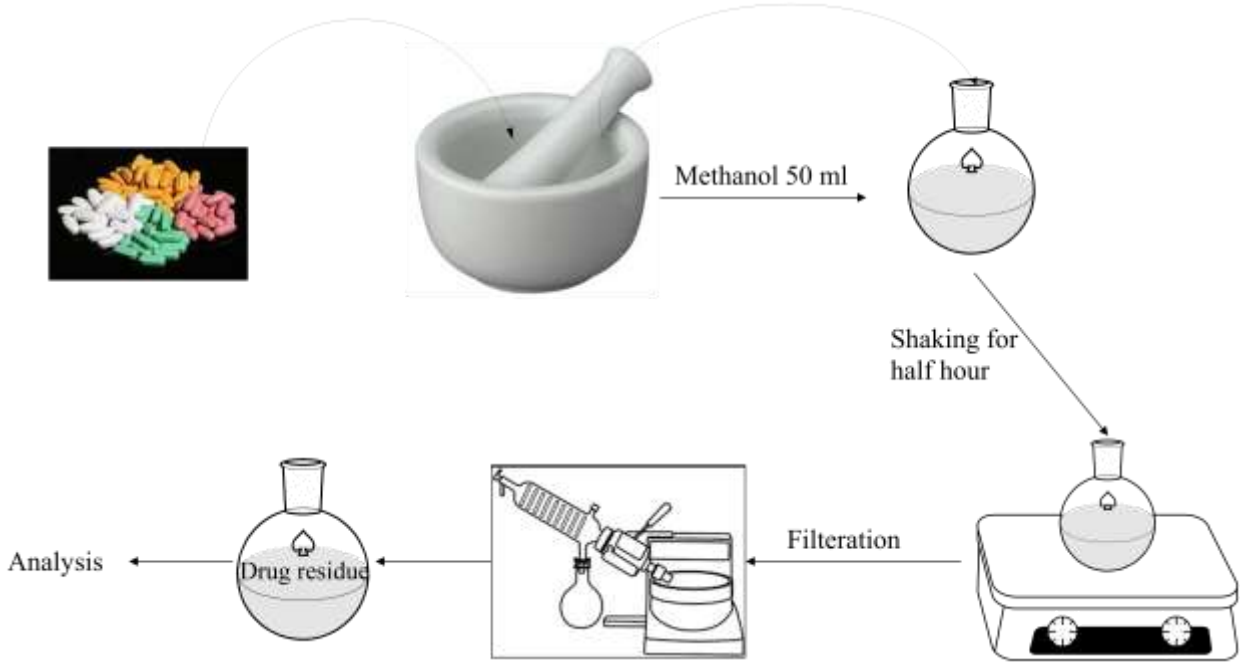


Fig 3:- Layout of extraction.

5.3. Preparation of stock solutions: -

All stock solutions of (racemic) β-blockers, (*RS*)-etodolac and (*S*)-Levofloxacin (50 mM) were prepared in 0.1 M $NaHCO_3$ for derivatization reactions. Solution of $NaHCO_3$ (0.1 M) wasprepared in purified water.

5.4. Preparation of TLC plates:-

An unmodified TLC plates were prepared by spreading slurry of silica gel (10g) dissolve in distilled water (20ml), after that TLC plates put into oven for activation.

5.5. Preparation of solution of Analyte :-

Solutions of analyte (10^{-2} M) were prepared by dissolving appropriate amount in dichloromethane (DCM). For proper dissolution, sometimes ammonia buffer solution was added to it.

5.6. Mobile phases for TLC development:-

Following mobile phases with different ratio were tested for the development of TLC plates:

Sr. No.	Solvent system	Ratio
1.	CH_3CN-MeOH-H_2O	6:1:1
2.	CH_3CN-MeOH-H_2O–$CHCl_3$	7:1:1:1
3.	C_4H_9OH- CH_3COOH – H_2O	4:1:1
4.	MeOH – CH_2Cl_2	4:6
5.	CH_3CN – CH_3OH – $CHCl_3$	3:3:4
6.	C_2H_5OH – $CHCl_3$	2:1
7.	CH_3CN – CH_3OH – $CHCl_3$	3:1:1

Table1. Different solvent ratio used for separation.

5.7. SYNTHESIS OF DIASTEREOMERS OF ATENOLOL

Atenolol (*RS*) has been derivatizated by using different chiral reagent and formation of diatereomeric mixture and that mixture was separated by using HPLC.

Figure 4:- Reaction setup of Synthesis of diateromers of atenolol

The solution of atenolol (*RS*)(2M, 0.532g, 532mg) prepared in $NaHCO_3$ (0.1M) was added to solution of levofloxacin (1M, 0.361g, 361mg) in DCM (dichloromethane) and then added (DCC) dicyclohexylcarbodiimide (2mM of 0.412g, 412mg) as a catalyst. The reaction mixture was kept on magnetic stirrer at temperature 40-50^{0}C for 2.5hrs with constant stirring. Before workup check the reaction progress by TLC. After completion of reaction cool the mixture at room temperature then filter the mixture and evaporate the filtrates by using Rota vapors.

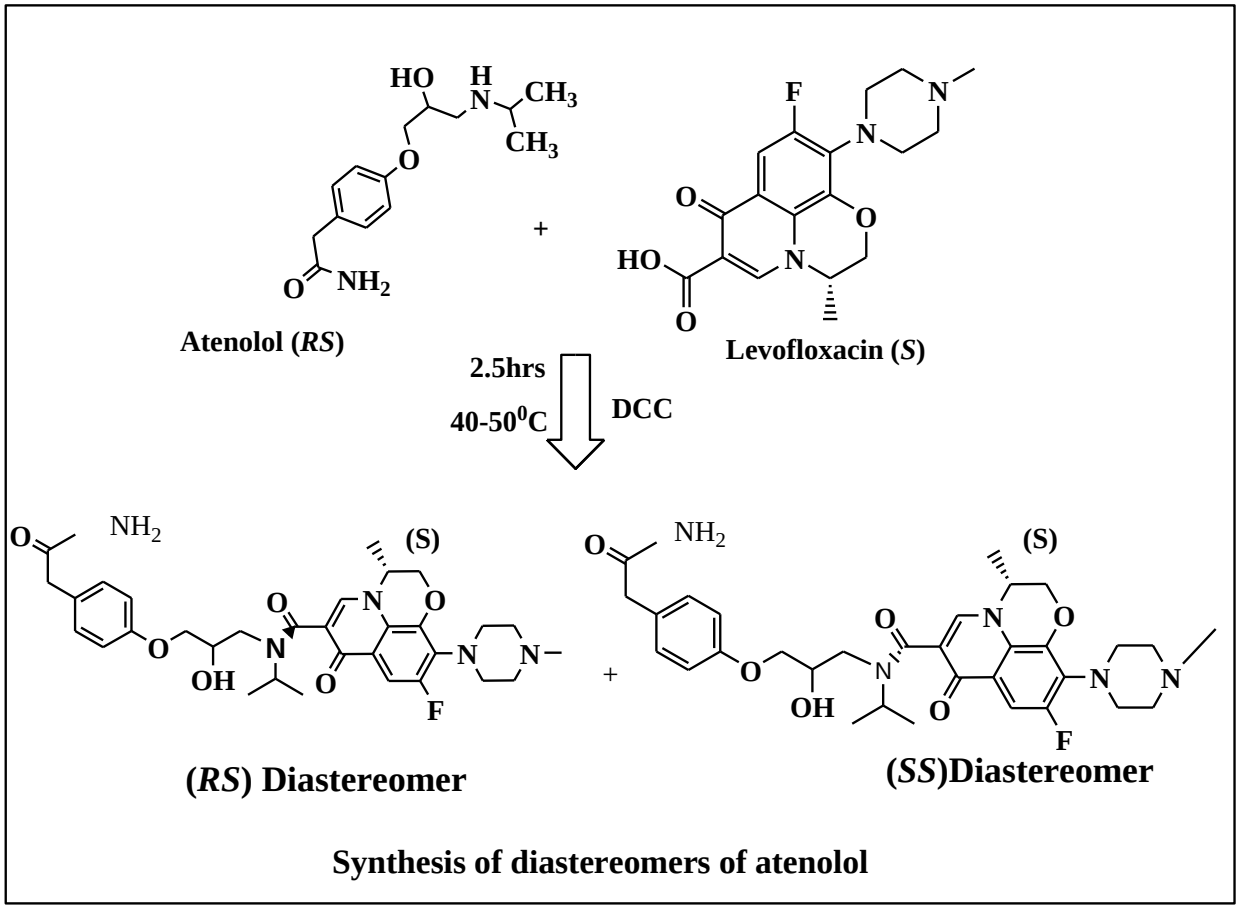


Figure 5:- The structures of diastereomer of atenolol formed from the chiral ligand (LEVO)

SYNTHESIS OF DIASTEREOMERS OF ETODOLAC

Etodolac(*RS*) has been derivatizated by using different chiral reagent and formation of diatereomeric mixture and that mixture was separated by using HPLC.

Figure 6:- Reaction setup of Synthesis of diateromers of etodolac

The solution of etodolac(*RS*)(1M, 0.287g, 287mg) prepared in $NaHCO_3$ (0.1M) was added to solution of levofloxacin (1M, 0.361g, 361mg) in DCM (dichloromethane) and then added (DCC) dicyclohexylcarbodiimide (1mM of 0.206g, 206mg) as a catalyst. The reaction mixture was kept on magnetic stirrer at temperature 40-50^{0}C for 2.5hrs with constant stirring. Before workup check the reaction progress by TLC. After completion of reaction cool the mixture at room temperature then filter the mixture and evaporate the filtrates by using Rota vapors.

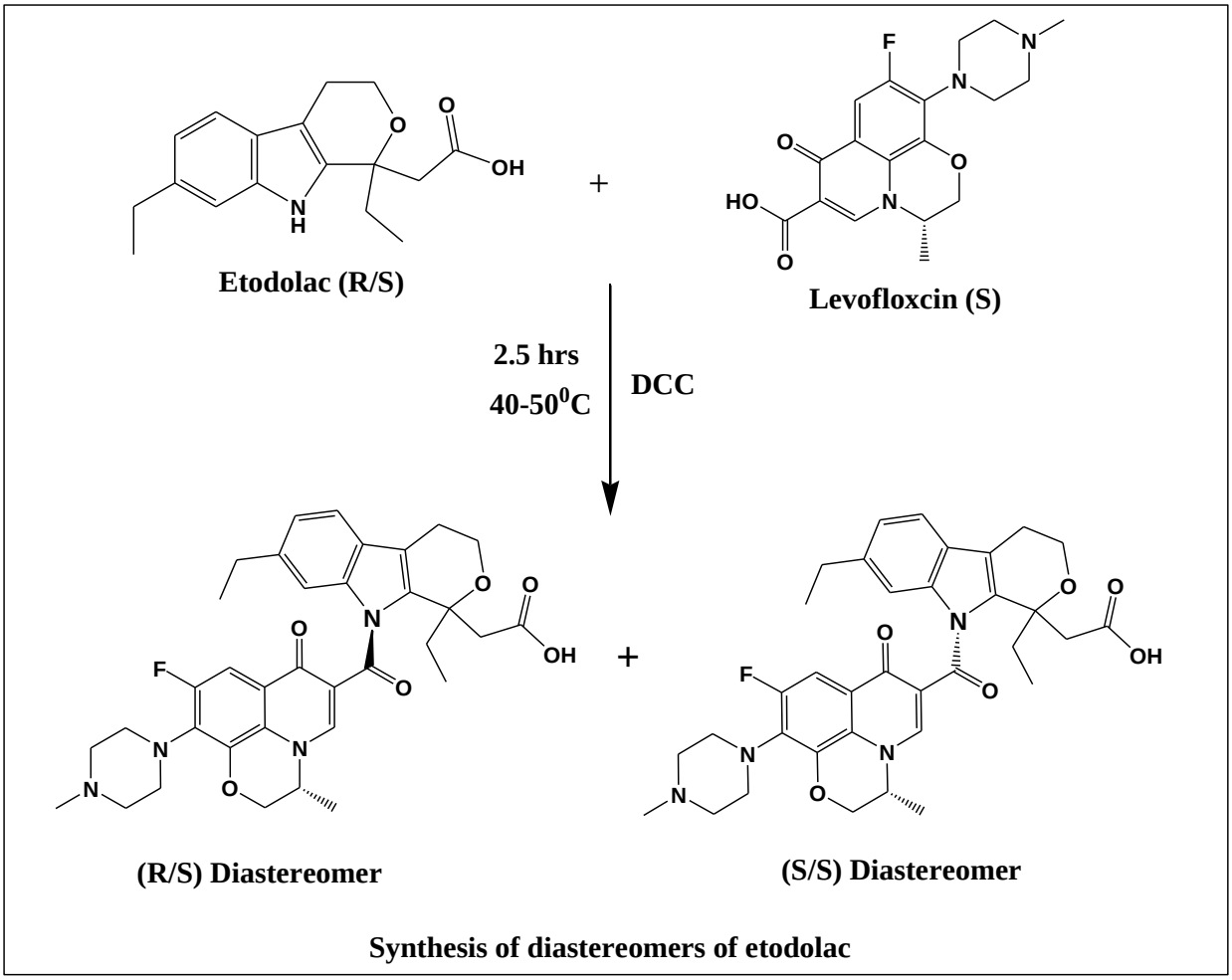


Figure 7:- The structures of diastereomer of Etodolac formed from the chiral ligand (LEVO)

SYNTHESIS OF DIASTEREOMERS OF KETOROLAC

Ketorolac (*RS*) has been derivatizated by using different chiral derivatizing reagent and formation of diatereomeric mixture and that mixture was separated by using HPLC.

The solution of Ketorolac (*RS*) (1M, 0.255g, 255mg) prepared in $NaHCO_3$ (0.1M) was added to solution of levofloxacin (1M, 0.361g, 361mg) in DCM (dichloromethane) and then added (DCC) dicyclohexylcarbodiimide (1mM of 0.206g, 206mg) as a catalyst. The reaction mixture was kept on magnetic stirrer at temperature 40-50^{0}C for 2.5hrs with constant stirring. Before workup check the reaction progress by TLC. After completion of reaction cool the mixture at room temperature then filter the mixture and evaporate the filtrates by using Rota vapors.

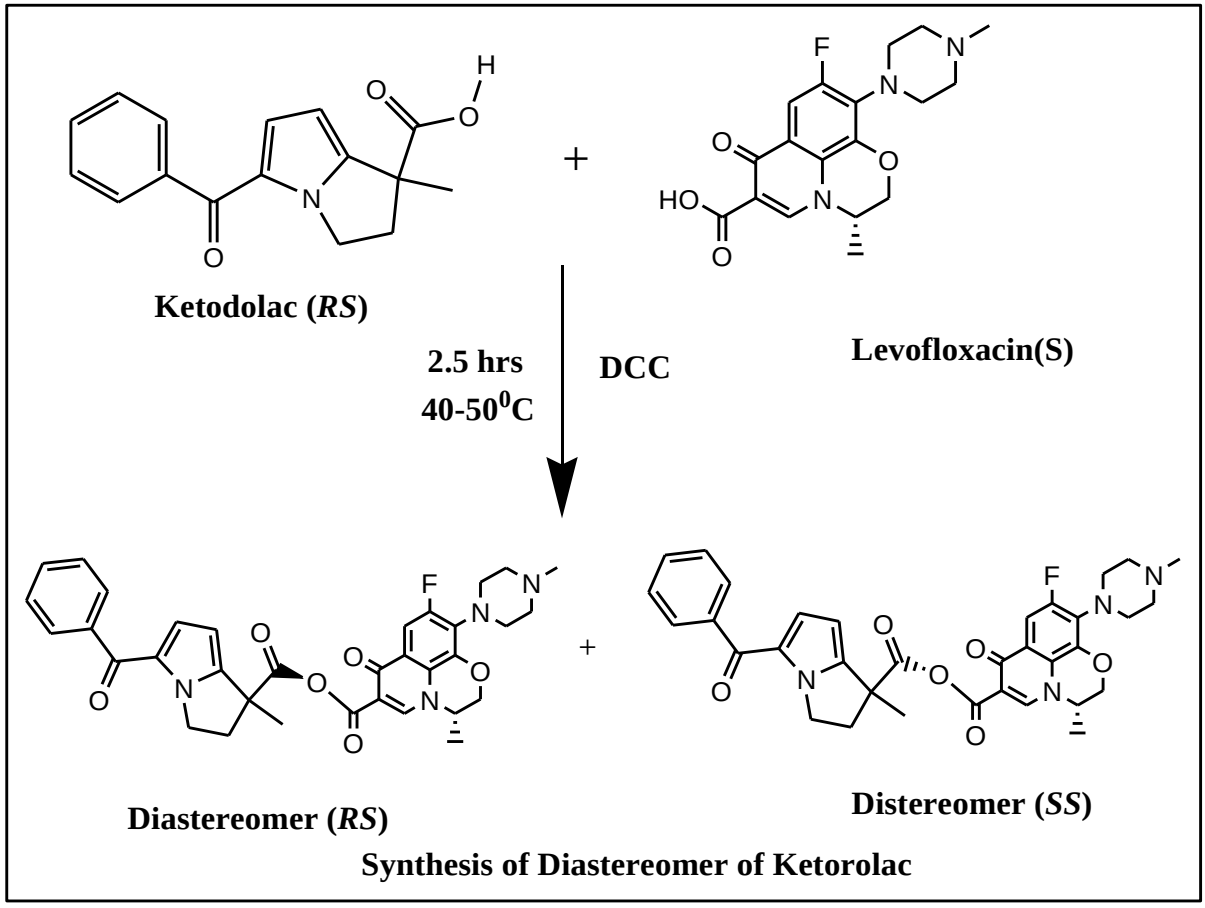


Figure 8:- The structures of diastereomer of Ketorolac formed from the chiral ligand (LEVO)

RESULT AND DISCUSSION

ENANTIOSEPARATION OF ATENOLOL

Thin Layer Chromatography (TLC)

Enantioseparationalong with actual chromatograms and their respective results are shown below

Preparation of stock solution for analytes (Atenolol): - Prepared the solution of (10^{-2} M) reaction mixture(R/S, S/S) in MeOH and $NaHCO_3$, then mark a spot on plain TLC plates and run intoMeCN-MeOH-H_2O in the ratio(6:1:1). Afterward, the TLC plate was dried at room temperature and placed it in an iodine chamber for spot location.

Result: - Two different spot are located at different R_f value.

R_f for 1st spot = 3.1/5.5= 0.56, **R_f for 2nd spot =** 4.7/5.5 =0.854

Preparation of stoke solution with different solvent: -

Prepared the solution of (10^{-2} M) atenolol (RS), and reaction mixture (R/S, S/S) in MeOH and $NaHCO_3$, then mark a spot on plain TLC plates and run into C4H9OH- CH3COOH–H_2Oin the ratio(4:1:1). Afterward, the TLC plate was dried at room temperature and placed it in an iodine chamber for spot location.

For reaction mixture R_f value 1st spot = 2.7/5= 0.54, **R_f value for 2nd spot** = 4.2/5=0.84

For Atenolol R_f value = 2.9/5=0.58 but spots are not clearly separated due to solvent effect.

Result: - In right side result of reaction mixture and obtained two different spot on TLC at different $R_f$valueand in left side one spot obtained which is not clearly obsevered.

High performance liquid chromatography (HPLC)

The retention times (t_R) retention factor (k), separation factor (α) and resolution (Rs) of the resolved diastereomers using successful mobile phase I are summarized in Table 1. A full chromatogram of the run showing the enantioseparation of (RS)-Atl (two similar peaks) and one extra peak indicate the peak of excess CDR is shown in Chromatogram C1. Enantioseparation of (RS)-Atl was confirmed by comparing the peak area ratios of two similar peaks.

Chromatographic parameters concluded in Table 2 like separation factor is greater than 1 and resolution greater than 1.5 indicates this separation is a baseline separation of enantiomer. However, retention factor values are large which is undesirable and somehow time

consuming. These values should be as small as possible to save the analysis time.

Table 2: Chromatographic parameters for the separation of diastereomers of (RS)-Atenolol prepared with levofloxacin

Peaks	**Retention time (t_R)**	**Peak area**	**Retention factor (K_1& K_2)**	**Separation Factor ($\alpha=K_2/K_1$)**	**Resolution (Rs)**
t1	42.0	95.85	19.30	1.11	8.2
t2	46.1	96.06	21.37		

ENANTIOSEPARATION OF ETODOLAC

Thin Layer Chromatography (TLC)

Enantioseparation along with actual chromatograms and their respective results are shown below

Preparation of stock solution for analytes (Etodolac): - Prepared the solution of (10^{-2} M) reaction mixture(R/S, S/S) in DCM, then mark a spot on plain TLC plates and run intoMeCN-MeOH-H_2Oin the ratio (6:1:1) and MeCN-MeOH-$CHCl_3$in the ratio(3:1:1). Afterward, the TLC plate was dried at room temperature and placed it in an iodine chamber for spot location.

Result: - Two different spot are located at different Rf value.

(a) R_f for 1st spot = 5.4/5.5= 0.981, **R_f for 2nd spot =** 1.2/5.5 =0.218

(b) R_f for 1st spot = 5.3/5.7= 0.929, **R_f for 2nd spot =** 1.3/5.7 =0.228

(a)

(b)

High performance liquid chromatography (HPLC)

The retention times (t_R) retention factor (k), separation factor (α) and resolution (Rs) of the resolved diastereomers using successful mobile phase I are summarized in Table 2. A full chromatogram of the run showing the enantioseparation of (RS)-Etl (two similar peaks) and one extra peak indicate the peak of excess CDR is shown in Chromatogram C2. Enantioseparation of (RS)-Etl was confirmed by comparing the peak area ratios of two similar peaks.

Chromatographic parameters concluded in Table 3like separation factor is greater than 1 and resolution greater than 1.5 indicates this separation is a baseline separation of enantiomer. However, retention factor values are large which is undesirable and somehow time consuming. These values should be as small as possible to save the analysis time.

Table 3: Chromatographic parameters for the separation of diastereomers of (RS)-Etodolac prepared with levofloxacin

Peaks	**Retention time (t_R)**	**Peak area**	**Retention factor (K1 & K2)**	**Separation Factor (α=K2/K1)**	**Resolution (Rs)**
t1	7.22	13.02	2.70	1.10	5.8
t2	7.99	11.89	2.99		

ENANTIOSEPARATION OF KETOROLAC

Thin Layer Chromatography (TLC)

Enantioseparation along with actual chromatograms and their respective results are shown below

Preparation of stock solution for analytes (Ketorolac): - Prepared the solution of (10^{-2} M) reaction mixture(R/S, S/S) in DCM, then mark a spot on plain TLC plates and run into MeCN-MeOH-H_2Oin the ratio (6:1:1) and MeCN-MeOH-$CHCl_3$in the ratio (3:1:1). Afterward, the TLC plate was dried at room temperature and placed it in an iodine chamber for spot location.

Result: - Two different spot are located at different Rf value.

(a) R_f for 1st spot = 4.4/4.5= 0.977, **R_f for 2nd spot =** 1.0/4.5 =0.22

(b) R_f for 1st spot = 4.9/5.0= 0.98, **R_f for 2nd spot =** 2.5/5.0 =0.5

(a) **(b)**

Effect of Temperature and pH

Earlier studies on enantioseparation of different chiral compounds by indirect method of separation using chiral derivatizating reagent on TLC plate showed to affect the enantioseparation on variation in temperature and pH. The chromatograms on TLC were developed at 15 ± 2, 20 ± 2, and 25 ± 2°C to study the effect of temperature on separation. Separation was found to be temperature dependent as the temperature lowers at15±2℃ then the sparation was good and spots were clearly visible. But as the temperature increases above 25 ± 2℃ then spots were elongated like eight shaped spots. It may be due to fast movement of analyte on chiral stationary phase (chiral TLC) resulting in lesser interaction time with chiral selector and other factor may be solvent system having two or more than two components, if one of the component is volatile in nature then chances of evaporation will be more at higher temperature which in turn provide different mobile phase composition rather than original. Thus, volatile nature of the component affects the chiral interaction of analyte and chiral selector. In literature, pH of mobile phase and reaction conditions were also found to influence the enantioseparation. Solutions of the chiral selectors were adjusted to pH range 6-9 for to find out the optimum condition for good resolution.

Characterizations

IR Data of Chiral Drug

Sr. No.	Compound	-COOH	C=C	C=O	NH_2	-F atom
1.	Levofloxacin	3441 cm^{-1}	2916 cm^{-1}	1708 cm^{-1}	1288 cm^{-1}	1087 cm^{-1}
2.	Etodolac	3327 cm^{-1}	2929 cm^{-1}	1720 cm^{-1}	1294 cm^{-1}	1085 cm^{-1}
3.	Ketorolac	3325 cm^{-1}	2929cm-1	1626cm^{-1}	1260cm^{-1}	1046cm^{-1}

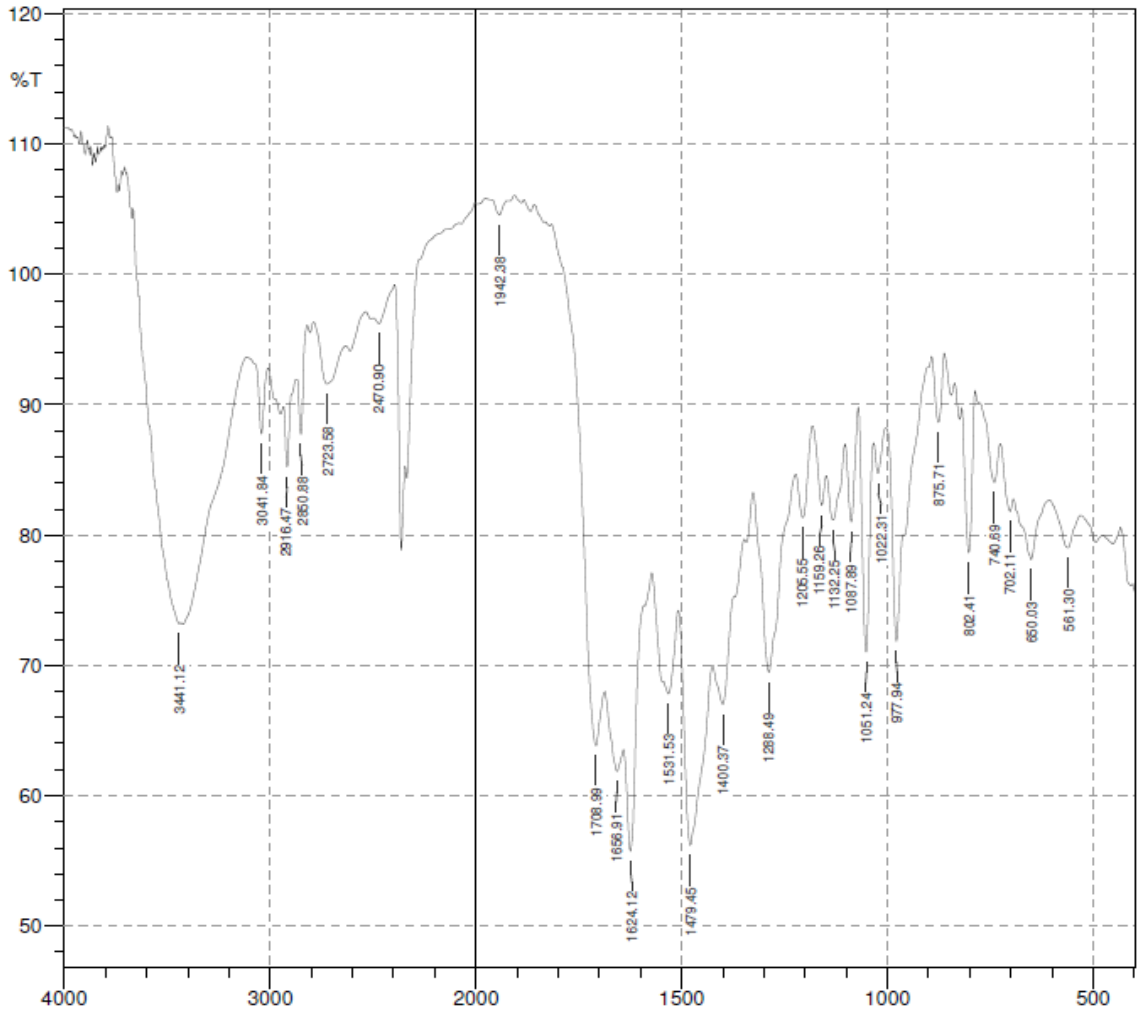
CONCLUSION

The recent work describes improved analytical method for the synthesis of diastereomers by the reaction of analytes (Atenolol, Etodolac, Ketodolac) withlevofloxacine using N,N'-dicyclohexylcarbodiimide (DCC) which is used as dehydrating agent for the preparation of amide. The amidic diastereomers so formed were separated on TLC. The simplicity, flexibility, versatility and sensitivity make the TLC technique more advantageous for the enantioseparation of diasteriomer via indirect method. There is a clear evidence of "TLC being a complimentary technique to HPLC" as it is inexpensive, simple, less time consuming and easily approachable, but for diastereomeric separation or indirect separation HPLC provide excellent separation by gradient mode of separation and detection can be achieved using diverse class of detector.

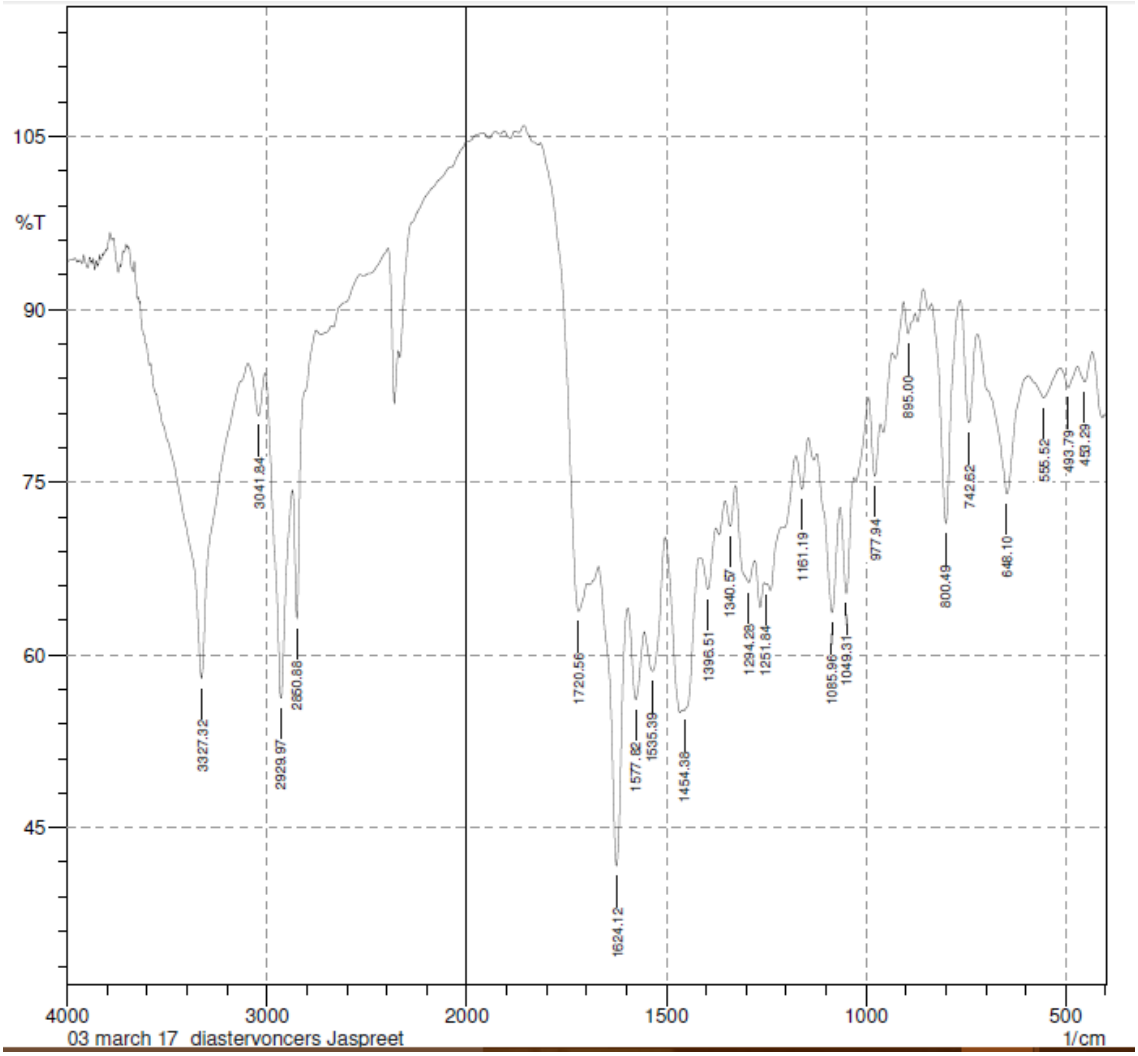
TLC using formation of diastereomer methods provided a simple, sensitive, rapid and economical approach to enantiomeric resolution of certain analytes including those from pharmaceutical preparations along with a method to recover the pure enantiomer for further use or application in small amounts and can be practiced both in analytical laboratory and industrial purpose for routine analysis and R&D activities in comparison to high-performance liquid chromatography and capillary electrophoresis that have high equipment and running costs. Thus, the methods have potential applications in quality control in pharmaceutical formulations of these pharmaceutically and biologically important compounds.

APPENDIX

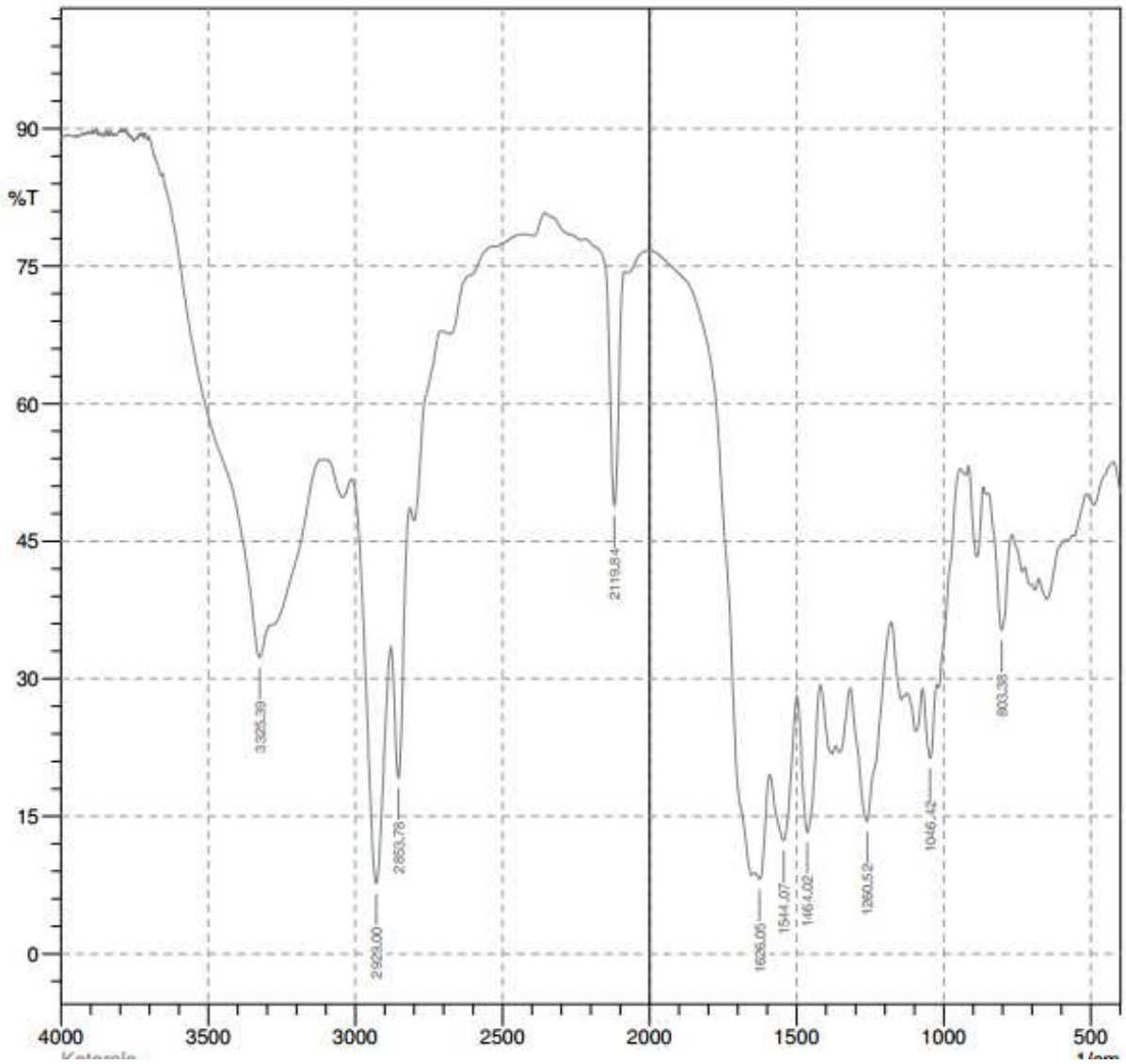
IR Spectra of levofloxacin

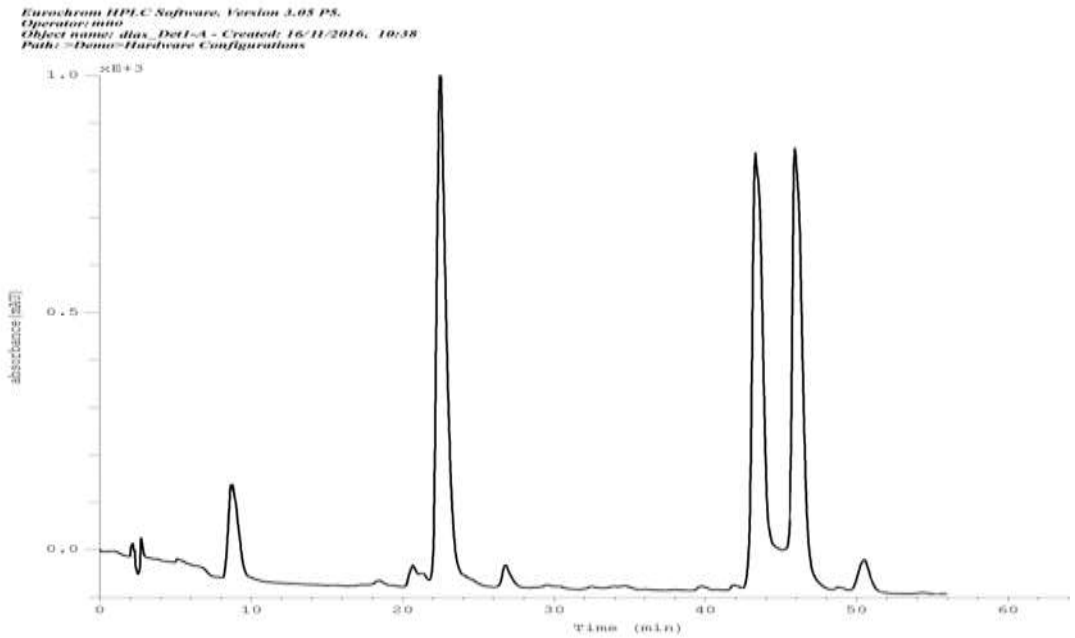


IR Spectra of Etodolac diasteromers

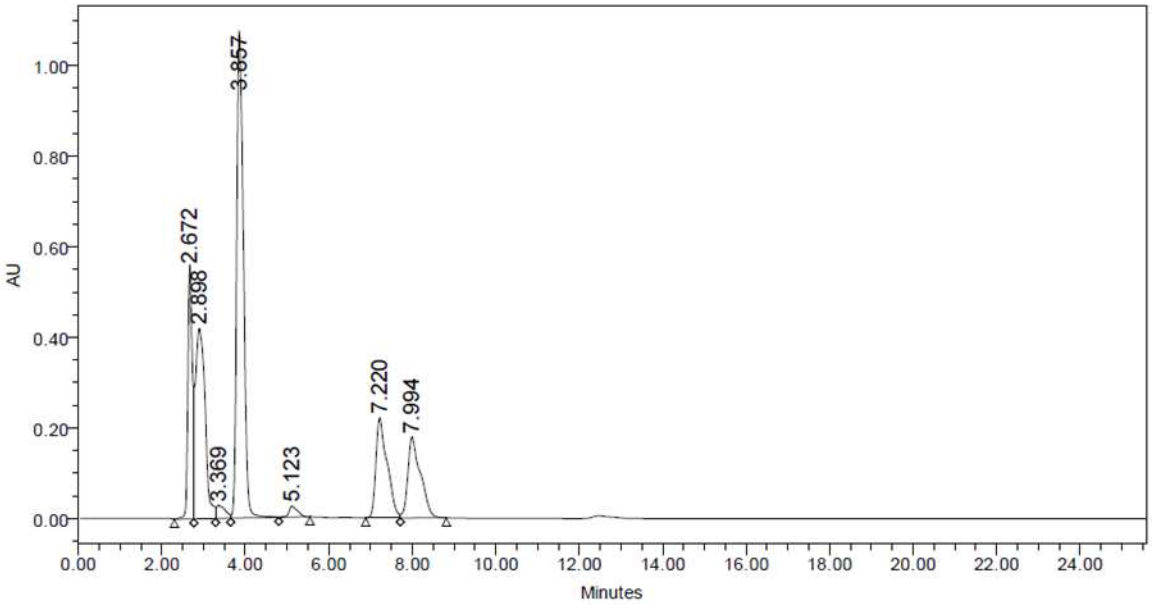


IR Spectra of Etorolac diasteromers





C1 :- Chromatogram showing resolution of diastereomers of (RS)-Atenolol prepared with levofloxacin



C2 :- Chromatogram showing resolution of diastereomers of (RS)-Etodolac prepared with levofloxacin

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