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Synthesis of Quinoline-Based New Chiral Derivatizing Reagents and its use in the Derivatization and Enantioseparation of Few Structurally Similar β-Blockers using Liquid Chromatography and Structural Optimization using DFT

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In this work, quinoline based new chiral derivatizing reagent was synthesized by introducing chiral amino acid (*L*-proline) in the molecule. Synthesized chiral derivatizing reagent was characterized by spectroscopic techniques (^{1}H NMR, FT-IR, HRMS and CHNS analysis) and used in the synthesis of diastereomer of chosen β-blockers. The RP-HPLC system was used to separate synthesized diastereomers (indirect approach of enantioseparation). Acetonitrile and buffer solution was used as mobile phase for analysis. The effect of varying concentrations and pH of mobile phase was optimized for the separation of diastereomers. The density functional theory calculations were also carried out to develop the lowest energy-minimize optimized diastereomer structures and to design separation mechanisms and elution orders. The retention factor (k), selectivity factor (α), resolution factor (R_S), the limit of detection (0.192 ng mL^{-1}) and the limit of quantification (0.576 ng mL^{-1}) were calculated in the context of the method's validation in accordance with ICH guidelines.

Keywords: Quinoline, β-Blockers, Indirect-enantioseparation, Chiral derivatizing reagents, RP-HPLC.

INTRODUCTION

Quinolines are heterocyclic structures, which contain fused benzene with pyridine rings and also known as 1-azanapthalene and benzopyridine [1-3]. Quinoline is an essential molecule for industries and medicinal applications. Quinoline derivatives are applied in the treatment of bacterial infections, malaria, cancer, inflammation, fungal infections and leishmaniasis [2,3]. Several quinoline derivatives may also be applied as sensors, dyes, refinery anti-foaming agents, corrosion inhibitors and in other applications [4-10].

Due to the fused ring conjugated aromatic system, quinolines show very good UV-visible and fluorophoric activities [11-14]. Also, in the presence of the pyridine ring (nitrogen atom), quinoline acts as an excellent ligand and interacts with various analytes. Due to these properties, up to now, lots of quinoline derivatives-based chemosensors have been prepared. These chemosensors show great affinity to bind with target analytes (especially metal ions and small organic molecules) and offer remarkable detection under UV-visible or fluoresce spectroscopy [2,3,11-14]. The quinolines are naturally occurring, available at low cost and facile, easy to structure modification. Due to extraordinary properties, a new quinoline-based chiral derivatizing reagent is synthesized in this report. For this purpose, *L*-proline was introduced into 8-quinolinecarboxylix acid, under acylation and amide formation reaction. The chiral carboxylic group of *L*-proline was then converted to activate the ester of pentafluorophenol in order to prepare the final chiral derivatizing reagent (CDR).

β-Blockers (β-agonists and amino alcohol) are frequently used to treat respiratory and cardiovascular conditions [13]. Most of the amino alcohols are supplied for the market as racemic

mixtures. Usually, the (*S*)-enantiomer produces the desired pharmacological action, but mainly the (*R*)-enantiomer produces undesirable symptoms, including nausea, vomiting, fatigue, vertigo, depression, paresthesia, aching muscles, asthmatic wheeze and many more [15-18]. In present study, three structurally related β-blockers (sotalar, isoprenaline and terbutanline) were chosen.

The derivatization method for enantioseparation (indirect enantioseparation) has the advantage over the direct method (direct enantioseparation) [14]. It provides better stability in terms of permitting the use of various solvents in different harsh conditions (due to the use of chemically stable C_{18} stationary phase). Also, these methods provide better separation (resolution, separation factor, retention time, low LOD and LOQ values with highly-sensitive detection) with a standard analysis cost [13-16].

In this report, quinoline based, a new chiral derivatizing reagent, was synthesized and used to prepare diastereomers of racemic β-blockers under microwave irradiation (MWI). The CDR moiety turns racemic β-blockers into highly UV-visible sensitive (absorbing) molecules and the results provide a very sensitive detection using a PDA detector of HPLC. The synthesized diastereomers pair were injected into the C_{18} column of the HPLC and acetonitrile (ACN) and triethylamine phosphate (TEAP) buffer was used as the eluting phase. Besides this, the configuration and elution order was determined using the lowest energy structures of all the synthesized diastereomers. The accuracy, linearity, limit of detection (LOD) and limit of quantitation (LOQ) of the present method have all been confirmed.

EXPERIMENTAL

Racemic sotalar, isoprenaline, terbutaline and *L*-proline, pentafluorophenol (Pfp), 3-{[(ethylimino)methylidene]amino}-*N,N*-dimethylpropan-1-amine (EDC) were purchased from Sigma-Aldrich, India. Other analytical grade reagents and solvents used in the study were purchased from Merck (Mumbai, India).

Chromatographic system: HPLC system ((LC-20AD, Shimadzu) containing 20 µL manual injector, PDA detector, C_{18} column and LC solution analysis software was used for current studies. Other equipment such as microwave (Perkin-Elmer), pH meter, FT-IR spectrometer (Nicolet-6700, Thermo Scientific), NMR spectrometer 400 MHz (JEOL), UV spectrometer (Shimadzu, UV-2450 spectrophotometer) and elemental analyzer (Vario EL III) were used for characterization.

Preparation of stock solutions: The stock solutions of the racemic β-blockers (1 mmol/L) and CDR (1 mmol/L) were prepared, respectively in 1 M $NaHCO_3$ and ACN. The stock solution of triethylammonium phosphate buffer (TEAP; 10 mM; 3.5 pH) was prepared in distilled water and pH was maintained using phosphoric acid.

Synthesis of quinoline based CDRs: 8-Quinolinecarboxylic acid (8-QC, 346 mg, 2 mmol) was added in 10 mL dry DCM and then, under the stirring, oxalyl chloride (304 mg, 2.4 mmol) was added dropwise in the solution. After addition, a few drops of pyridine was added and the reaction was left to stir for 2 h at room temperature. After the reaction, DCM and the remaining oxalyl chloride were removed under reduced pressure [19,20]. The obtained solid (acyl chloride of quinoline; 8-QC-Cl) was then directly used for the following reaction.

The obtained solid was dissolved in 10 mL dry THF and the solution of 230 mg (2 mmol) *L*-proline and 244 mg DMAP in 20 mL dry THF was added to the reaction mixture dropwise. After addition, the reaction mixture was left to stir for 2 h at room temperature. The reaction progress was monitored using thin-layer chromatography. After the reaction, the THF was removed under reduced pressure. Ethyl acetate and 1N HCl solution was used to extract obtained solid compound (8-QC-N) [21,22]. The dried and solid compound was then purified using column chromatography.

Colour: Off white solid, yield: 98%; ^{1}H NMR (500 MHz, chloroform-d_6) δ ppm: 8.86 (dd, 1H), 8.24 (dt, 1H), 8.05 (dd, 1H), 7.86 (dd, 1H), 7.64 (t, 1H), 7.55 (dd, 1H), 4.47 (m, 1H), 3.71-3.61 (m, 2H), 2.27 (m, 1H), 2.06 (m, 1H), 1.96 (m, 1H), 1.86 (m, 1H).

Activation of carboxylic group (final CDR): The obtained purified compound (405 mg, 1.5 mmol) and 276 mg (1.5 mmol) pentafluorophenol (Pfp) were dissolved in 20 mL dry THF. In the reaction mixture, a solution of 310 mg (2 mmol) EDC and 200 mg (1.6 mmol) DMAP in 20 mL THF was added dropwise. The reaction mixture was then allowed to stir for 2 h at room temperature. After the reaction, the reaction mixture was washed with 1 N HCl solution and extracted with ethyl acetate [23,24]. The obtained compound (8-QC-Pfp or CDR) was then characterized using analytical techniques.

Colour: yellow white solid, yield 98%; UV (nm, in MeCN): 294 (λ_{max}); IR (KBr, cm^{-1}): 3519, 3515, 3043, 2636, 1818, 1720, 1650, 1581, 1556, 1505, 1464, 1431, 1387, 1368, 1321, 1249, 1200, 1038, 984, 832, 746, 716, 592. ^{1}H NMR (500 MHz, chloroform-d_6): δ ppm: 8.89 (dd, 1H), 8.25 (dt, 1H), 8.08 (dd, 1H), 7.88 (dd, 1H), 7.67 (t, 1H), 7.58 (dd, 1H), 4.53 (m, 1H), 3.75-3.63 (m, 2H), 2.34 (m, 1H), 2.10 (m, 1H), 1.98 (m, 1H), 1.86 (m, 1H). HRMS ($C_{21}H_{13}F_5N_2O_3$): 437.0894 ($M+H^+$); Anal. calcd. (found) % for $C_{21}H_{13}N_2O_3F_5$: C, 57.81 (57.98); H, 3.00 (3.56); N, 6.42 (6.26).

Synthesis of diastereomers: A reaction mixture was prepared by adding a solution of (*RS*)-sotalar (50 µL, 50 nmol), the chiral derivatizing reagent in ACN (56 µL, 56 nmol) and 5 µL of TEA in 2 mL vial. The 1:1.2 molar ratio of (*RS*)-sotalar and CDR were taken for the reaction. The microwave heating (MWI) was applied to prepare reaction mixture for 45 s at 80% (800 W) and then the reaction was cooled to room temperature [25]. Aliquots (10 µL) of the resulting solution of diastereomers were diluted 10 times with ACN and injected (20 µL) into the column. Similarly, the diastereomeric pairs of racemic isoprenaline and terbutaline were prepared. The chemical structures of the prepared diastereomers are shown in Fig. 1.

The effects of pH, excess of reagent, reaction duration and power of the microwave were considered while optimizing the experimental conditions for synthesizing diastereomeric pairs of racemic β-blockers.

RP-HPLC: The current work used a binary mobile phase combination of two modes.

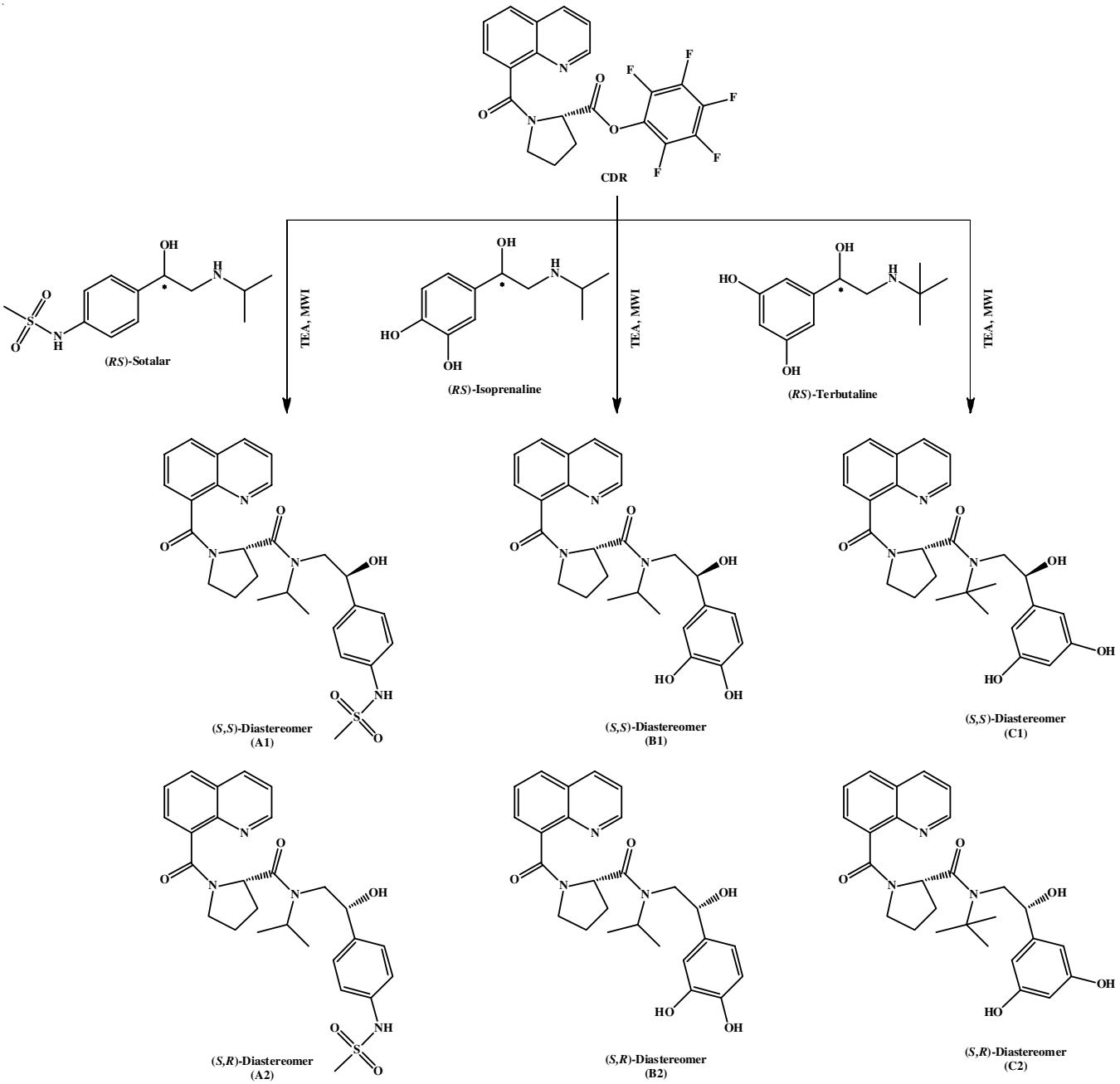

Fig. 1. Synthesis of diastereomers of racemic β-blockers using chiral derivatizing reagent (CDR)

(1) Isocratic mode: Acetonitrile (ACN) and triethylamine phosphate (TEAP) buffer (30 to 70%, 35 to 65%, 40 to 60%, 45 to 55%.

(2) Gradient mode: Acetonitrile (ACN) and triethylamine phosphate (TEAP) buffer (linear gradient from 80 to 20%, 70 to 30%, 60 to 40% and 55 to 45%).

Before use, the mobile phase was passed through a 0.45 mm filter and then nitrogen gas passed the mobile phase and degassed on the sonicator. The 294 nm wavelength was set for UV detection and 1.0 mL/min flow rate was applied to the RP-HPLC system.

Method development and validation: Studies were done to validate the (*RS*)-sotalar diastereomers prepared with quinoline based CDR regarding linearity, accuracy and precision. Followed by the least square method using the Microsoft Excel software, the calibration graphs calculated the slopes and correlation coefficients between the corresponding concentration range of 40-4000 ng mL^{-1} and the peak area (mAU) of diastereomer-A1 and A2.

RESULTS AND DISCUSSION

Synthesis of CDR and diastereomers: The synthesis of quinoline-based CDR proceeded in a three-step reaction (acylation, amidation and activation of the carboxylic group). For the acylation of 8-quinolinecarboxylic acid, oxalyl chloride was used as chlorinating reagent. According to the reported literature [19,20], oxalyl chloride is an excellent reagent for the acycliation of the carboxylic group. In the presence of

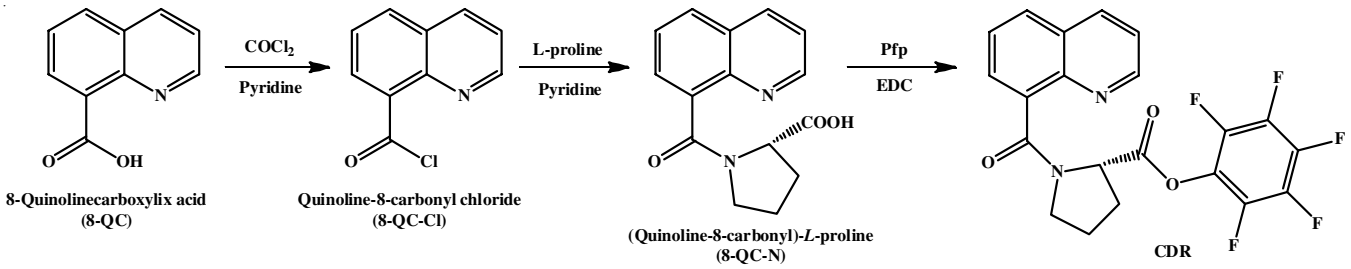

Fig. 2. Synthesis of quinoline based new chiral derivatizing reagent (CDR)

pyridine (catalyst), the oxalyl chloride gives a fast S_N^2 reaction and easily converts the carboxylic group into acyl chloride (100% yield; 8-QC-Cl; Fig. 2). Acyl chloride is very reactive toward the nucleophilic substitution reaction [26]; thus, under inert condition, acyl chloride of quinoline (8-QC-Cl) react very quickly with amino group compounds (in the current study *L*-proline) and yield more than 98% amide as product (8-QC-N; Fig. 2). *L*-proline is a conditional essential amino acid and is use as a precursor for protein synthesis and industrial applications. The five-membered aliphatic ring containing a nitrogen atom of *L*-proline makes it rigid and non-planer; thus, it generates a unique dihedral angle when two molecules are connected with this [27,28]. Also, the chiral carboxylic group of *L*-proline help to introduce chirality in the synthesized molecule. Literature shows the addition of amide bond on an aromatic system enhances the molecule's molar absorbance as well as absorbing UV-visible wavelength (bathochromic shift) [2,11,12]; thus, the UV-visible sensitivity increases when *L*-proline molecule is introduced in quinoline molecule.

In order to convert quinoline-*L*-proline amide (8-QC-N) into a reactive species toward nucleophilic attack, its carboxylic group was transformed into an ester of pentafluorophenol (Pfp) as shown in Fig. 2. The carboxylic group was activated using a potent coupling reagent (3-{[(ethylimino)methylidene]amino}-*N,N*-dimethylpropan-1-amine) (EDC), which tends to remove on –OH and -H groups from the carboxyl and hydroxyl groups, respectively, resulting in the formation of ester bond formation [14,29]. The addition of non-nucleophilic base 4-DMAP work as catalyst and enhance the progress of the reaction. None of the reaction doesn't take place on chiral carbon; thus, no racemization occurs in the synthesis of the CDR. The enantio-purity of the CDR was confirmed by the chiral column (cellulose).

The Pfp-modified CDR are extremely effective acylating agents [30] and efficiently produce the desired peptide (amide) bond [31], making it simple to synthesize six pairs of diastereomers of selected β-blockers, for instance, using (*RS*)-sotalar as an example (Fig. 1), in a shorter amount of time and under milder derivatization conditions. Racemization wasn't observed during the derivatization because the chiral centre was not the site of the reaction [32]. The chromatographic spectrum with two equal-sized peaks further demonstrate that there was no racemization during the derivatization. The synthesized CDR was examined for stability by varying different storage conditions. The CDR was unstable and quickly reacted and deactivated when exposed to basic conditions (pH 9-11) or in moist conditions, but shown to be very durable for more than six months in low temperature and neutral freezing conditions.

RP-HPLC analysis: The diastereomers of chosen β-blockers synthesized with quinoline-based CDR were cleanly separated by RP-HPLC as shown in Fig. 3. The obtained chromatographic data [retention time (t_R), retention factor (k), separation factor (α) and resolution (Rs)] of the separation of the diastereomeric pairs of β-blockers are shown in Table-1.

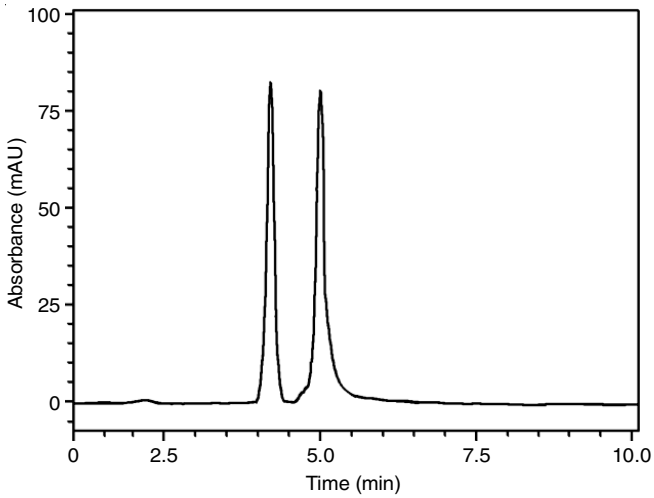

Fig. 3. Chromatographic separation of diastereomers of (*RS*)-sotalar prepared with quinoline based CDR

TABLE-1
CHROMATOGRAPHIC SEPARATION DATA OF DIASTEREOMERS OF β-BLOCKERS PREPARED WITH CDR

β-Blocker	Separation data for diastereomers prepared with CDR			
	k_1	k_2	α	*Rs*
(*RS*)-Sotalar	3.51	4.68	1.33	6.05
(*RS*)-Isoprenaline	5.78	7.08	1.22	4.68
(*RS*)-Terbutaline	4.45	5.14	1.15	4.25

The (*S,S*)-diastereomers (A1, B1, C1) was eluted before (*S,R*)-diastereomers (A2, B2, C2) of all the β-blockers. Fig. 3 shows sections from the specimen chromatogram that shows the resolution of the diastereomeric pairings of (*RS*)-sotalar. The elution time of first and second eluted diastereomers is given in Table-2. ACN and TEAP buffer (pH 3.5; 10 mM) in 30 min (70 to 30% in linear gradient mode) was the successful mobile phase. For optimization, experiments were run in the pH range of 2 to 6 and at buffer concentrations ranging from 5 to 25 mM. Beside the ACN, methanol was also tested as organic modifiers. Since acetonitrile has a lower viscosity (0.38 cP) than methanol (0.59 cP); thus, ACN containing mobile phase

TABLE-2
ELUTION TIME AND ORDER OF THE PREPARED DIASTEREOMERS

β-Blocker	Chromatographic separation data of diastereomers		
	First peak (time)	Second peak (time)	First eluted diastereomer
(*RS*)-Sotalar	4.06	5.12	A1
(*RS*)-Isoprenaline	6.11	7.28	B1
(*RS*)-Terbutaline	6.54	7.35	C1

elute diastereomers faster compared to methanol-containing mobile phase. As a result, ACN produced sharper peaks with lower retention periods [33,34]. The 1.0 mL/min flow rate of the mobile phase was found suitable for the separation of the synthesized diastereomers. The flow rate of the mobile phase was investigated by testing different mobile flow rates (0.5 to 2.0 mL/min) on the RP-HPLC system.

Elution order and DFT optimized 3D structures: The lowest energy-optimized 3D-structures of the prepared diastereomers were produced using DFT calculation on the Gaussian program. These structures were used to optimize the diastereomer's separation behaviour and elution order (A1-C2) during chromatographic separation.

Fig. 4 shows the optimized structures of the diastereomers of the chosen β-blockers. In this, the diastereomers of (*S*)-β-blocker have arranged in this way that the aromatic ring part of quinoline and β-blocker stabilize on maximum distance (due to restricted hindrance generated from chiral carbon of CDR moiety. While the reverse pattern was observed in diastereomers of (*R*)-β-blockers synthesized with CDR. In this, the aromatic ring has flexibility to reach near to each other and due to dipole-dipole interaction, these diastereomers are stabilized in a compact shape compared to the (*S*)-diastereomers of β-blockers. Thus, the (*S,S*)-diastereomers (A1, B1 and C1) have a bigger structure as compared to (*S,R*)-diastereomers (A2, B2, C2). Literature study shows bigger diastereomers have more surface to expose to the polar solvent and dissolve higher in the polar eluting solvent, thus eluting first from the RP-HPLC column [14,34,35]. While the compact diastereomers have less surface to expose (more hydrophobic); therefore, they interact more with the non-polar stationary phase of C_{18} column and stay longer in the column, thus eluting in the last [33]. The elution order with retention time of the prepared diastereomers of selected β-blockers are provided in Table-2.

Validation: In current study, the validation investigations were performed in accordance with ICH standards [29,36]. For RP-HPLC separation of diastereomers, A1 and A2, as a representative, the linearity, accuracy, precision, relative standard deviation (RSD), the limit of detection (LOD) and limit of quantification (LOQ) were established. The concentration range used for validation was 40-4000 ng mL^{-1}. The system-generated peak areas (obtained by RP-HPLC chromatogram) were used to quantify and investigate stabilities and recoveries. The calculated validation-data is provided in Table-3. For the first and second eluting diastereomers, the estimated recovery values are 99.24 and 99.89% for intra-day assay and 98.74 and 99.67% for inter-day assay. The LOD and LOQ were discovered to be 0.192 ng mL^{-1} and 0.576 ng mL^{-1}, respectively.

On comparing to the published reports [13-16,24,29,33,34], this work shows an excellent separation of diastereomers of the chosen β-blocker in terms of resolution (4.25-6.05), separation factor (1.15-1.33) and retention times (4.06-7.35 min)

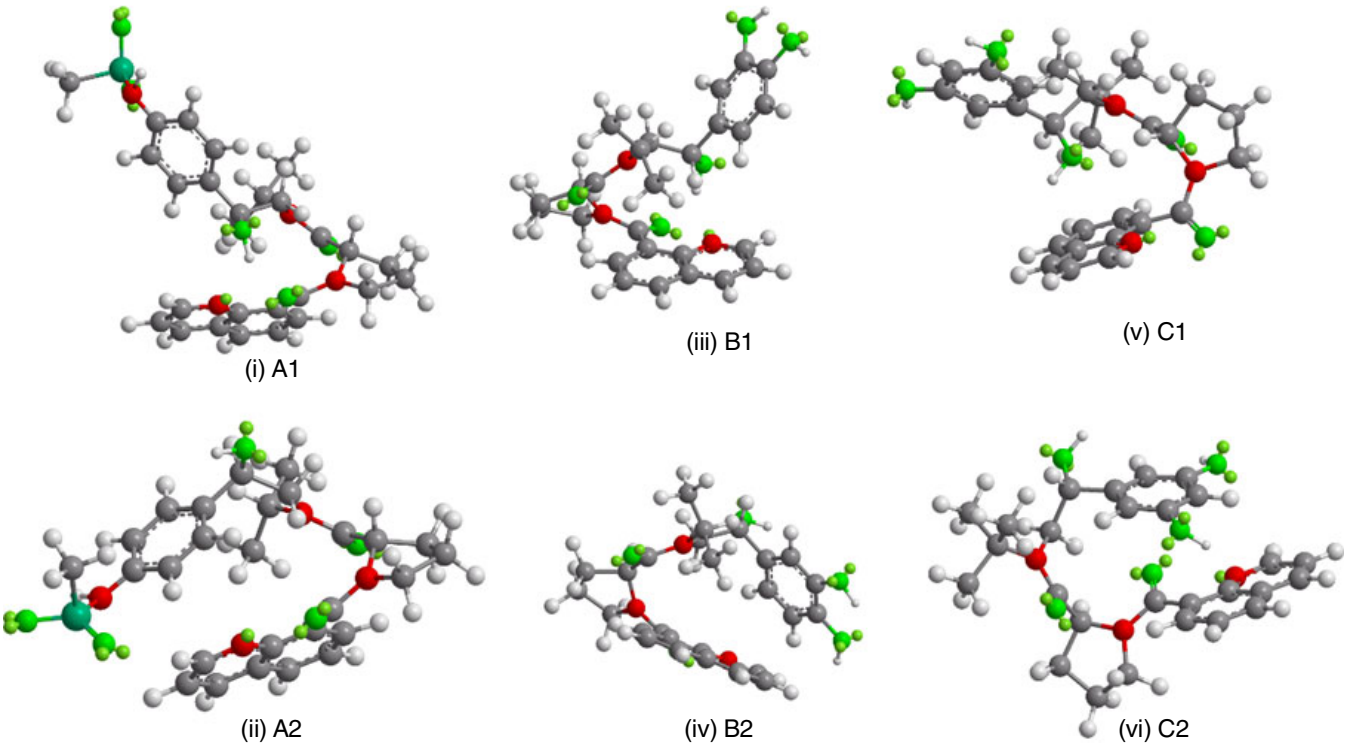

Fig. 4. DFT optimized 3D structures of diastereomers of (*RS*)-sotalar (A1 and A2), (*RS*)-Isoprenaline (B1 and B2) and (*RS*)-Terbutaline (C1 and C2) synthesized with CDR

TABLE-3
METHOD VALIDATION FOR RP-HPLC SEPARATION OF DIASTEREOMERS OF (*RS*)-SOTALAR PREPARED WITH CDR

Linearity	First eluting diastereomer (A1)			Second eluting diastereomer (A2)		
Range (ng mL^{-1})	20-2000			20-2000		
Slope	3.175			3.381		
Intercept	10.08			8.161		
Correlation coefficient (R^2)	0.998			0.997		
Accuracy and precision	First eluting diastereomer (A1)			Second eluting diastereomer (A2)		
Concentration of each diastereomer (ng mL^{-1})	Found conc. mean	Recovery (%)	RSD (%)	Found conc. mean	Recovery (%)	RSD (%)
Intra-day precision						
20	20.19	100.95	0.91	20.11	100.55	0.97
400	397.51	99.37	1.25	402.32	100.58	1.21
800	781.89	97.73	1.43	792.67	99.08	1.27
1600	1570.58	98.16	1.81	1589.64	99.35	1.44
2000	2000.07	100.01	1.88	1998.88	99.94	1.78
	Mean	99.24	1.45		99.89	1.33
Inter-day precision						
20	20.15	100.75	0.97	19.91	99.55	0.98
400	399.04	99.76	1.16	381.22	95.30	1.19
800	792.21	99.02	1.38	798.12	99.76	1.58
1600	1601.73	100.10	1.52	1586.92	99.18	1.82
2000	1982.14	99.10	1.79	1994.34	99.71	1.94
	Mean	99.67	1.36		98.74	1.49

Sensitivity: LOD (ng mL^{-1}) = 0.192; LOQ (ng mL^{-1}) = 0.576
[n (=5) is the number of replicates, SD = standard deviation, RSD = relative standard deviation]

and these values are found better then reported literature on chiral separation.

Conclusion

In summary, this is a successful report on the synthesis of quinoline based an efficient chiral derivatizing reagents. This report shows the easy synthesis of diastereomers of β-blockers with newly prepared CDR under MWI condition and their clean enantioseparation using RP-HPLC (indirect approach of enantioseparation). The mobile phase acetonitrile (ACN) with triethylamine phosphate (TEAP) buffer in gradient mode was better than the isocratic mode. In gradient mode, the 70:30 ratio (ACN:TEAP buffer) mobile phase provides fast and clean separation. The elution order and separation mechanism of the prepared diastereomers was established using DFT optimized structures and (*S,S*)-diastereomers of β-blocker elute first. The method was validated and showed sensitive detection of prepared diastereomers in terms of the limit of detection (LOD), limit of quantification (LOQ) and recovery. This method can determine enantiomeric purity and trace amounts of compounds from the pharmaceutical or organic synthesis sectors that include amino groups and are sold and given as racemic mixtures.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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