HPLC-Fluorescence Method for the Enantioselective Analysis of Propranolol in Rat Serum Using Immobilized Polysaccharide-Based Chiral Stationary Phase

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ABSTRACT A stereoselective high-performance liquid chromatographic (HPLC) method was developed and validated to determine S-(−)- and R-(+)-propranolol in rat serum. Enantiomeric resolution was achieved on cellulose tris(3,5-dimethylphenylcarbamate) immobilized onto spherical porous silica chiral stationary phase (CSP) known as Chiralpak IB. A simple analytical method was validated using a mobile phase consisted of n-hexane-ethanol-triethylamine (95:5:0.4%, v/v/v) at a flow rate of 0.6 mL min^{-1} and fluorescence detection set at excitation/emission wavelengths 290/375 nm. The calibration curves were linear over the range of 10–400 ng mL^{-1} ($R = 0.999$) for each enantiomer with a detection limit of 3 ng mL^{-1}. The proposed method was validated in compliance with ICH guidelines in terms of linearity, accuracy, precision, limits of detection and quantitation, and other aspects of analytical validation. Actual quantification could be made for propranolol isomers in serum obtained from rats that had been intraperitoneally (i.p.) administered a single dose of the drug. The proposed method established in this study is simple and sensitive enough to be adopted in the fields of clinical and forensic toxicology. Molecular modeling studies including energy minimization and docking studies were first performed to illustrate the mechanism by which the active enantiomer binds to the β-adrenergic receptor and second to find a suitable interpretation of how both enantiomers are interacting with cellulose tris(3,5-dimethylphenylcarbamate) CSP during the process of resolution. The latter interaction was demonstrated by calculating the binding affinities and interaction distances between propranolol enantiomers and chiral selector. *Chirality 26:194–199, 2014.*

KEY WORDS: propranolol; enantioselective; Chiralpak IB; HPLC-FD; molecular modeling

INTRODUCTION

The use of a fluorescence detector coupled with high-performance liquid chromatography (HPLC) technique has allowed increased sensitivity and selectivity for the determination of propranolol enantiomers. To the best of our knowledge, the combinations of Chiralpak IB- HPLC-fluorescence (FL) detection with pharmacokinetic study have not been reported in single propranolol enantiomers analysis. Propranolol, 1[−isopropylamine-3-[1-naphthyloxy]-2-propanol] hydrochloride, is a nonselective βadrenergic antagonist (β-blocker), which is widely used in the treatment of several diseases such as cardiac arrhythmias, angina pectoris, sinus tachycardia, thyrotoxicosis, hypertrophic subaortic stenosis, and hypertension.[1] Propranolol has one chiral center and is administered as a racemic mixture. It is reported that the S-(−)- isomer is 100 times more potent as a β-blocking agent than the R-(+)-isomer.[1] Recently, it has been reported that a long term use of propranolol may cause the hypertensive patients to be a diabetic.[2]

Polysaccharide derivatives, being coated or immobilized on silica matrix, have become the first and broadest choice of selectors to be used as chiral stationary phases (CSPs) for both liquid and supercritical liquid chromatography.[3] Since 2004 three immobilized polysaccharide-derived CSPs have become commercially available: Chiralpak IA, Chiralpak IB, and Chiralpak IC. They are based on tris(3,5-dimethylphenylcarbamate) of amylose, tris(3,5-dimethylphenylcarbamate) of cellulose, and tris(3,5-dichlorophenylcarbamate) of cellulose, respectively. These CSPs are characterized by their facile use, high enantioselectivity, and high capacity.[4] Many factors can be responsible for the extent of interactions of stereoisomeric molecules with immobilized polysaccharide-based chiral stationary phase such as dipole–dipole interactions, electrostatic forces, hydrogen bonding, hydrophobic bonding, ion–dipole interactions, steric interferences (size, orientation, and spacing of groups), and Van der Waals forces. The nature and effects of some of these factors can influence the chromatography of enantiomers.[5,6]

Molecular modeling studies have succeeded in interpreting many ligand-receptor mechanisms depending on the basic interactions that occur between the ligand and its site of action. Moreover, in addition, some other factors such as the binding affinity of the ligand or receptor strain have been demonstrated. They help to interpret the mechanism by which the separation of enantiomers takes place and the reason for the potent biological activity of one enantiomer over the other.[7,8] The analytical methods reported for chiral separation of propranolol included capillary electrophoresis

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methods[9–11] and HPLC methods utilizing various chiral selector CSPs.[12–15] However, the reported capillary electrophoresis (CE) methods were concerned with the evaluation of different chiral selectors for resolution of propranolol enantiomers using UV detection. Moreover, the chromatography methods cited for the chiral separation and determination of propranolol in biological fluids have drawbacks of low sensitivity (UV detection) and deficiency of pharmacokinetic study. The main advantages of the developed HPLC-FL method with Chiralpak IB column are to provide a highly sensitive and selective method for the simultaneous analysis of propranolol enantiomers in rat serum. The developed method was of high precision, good accuracy, wide linear range of determination, and lower limit of detection of 3.0 ng mL^{-1}. The developed method was demonstrated to be applicable for conducting pharmacokinetic study. The effective separation of both S-(−)- and R-(+)-enantiomers of propranolol was studied by a molecular modeling technique, where the hydroxyl group of propranolol was found to be crucial in determining the stereochemistry of the structure.

EXPERIMENTAL

Apparatus and Reagents

Chromatography was performed on a Shimadzu (Japan) instrument consisting of one LC–20 AD pump, DGU–20 A3 / DGU–20 A5 on-line degasser, SIL-20A/20 AC autosampler, RF-10 AXL fluorescence detector, and CBM–20A system controller. The chiral stationary phase used in this study was the cellulose tris(3,5-dimethylphenylcarbamate) which is immobilized on 5-μm silica gel known as Chiralpak IB (250 x 4.6 mm i.d.) purchased from Chiral Technologies Europe (Cedex, France). The mobile phase consisted of n-hexane–ethanol–triethylamine (95:5:0.4%, v/v/v), which was filtered through a Millipore membrane filter (0.2 μm) from Nihon, Millipore (Japan), and degassed before use. The flow rate was 0.6 mL min^{-1} with fluorescence excitation wavelength 290 nm and emission wavelength 375 nm. (±) Propranolol, S-(−)- and R-(+)-propranolol were purchased from Sigma (St. Louis, MO). The internal standard NAN-190 (1-(2-Methoxyphenyl)-4-(4-phthalimidobutyl)piperazine) is the compound with a selective 5–HT_{1A} receptor antagonistic activity purchased from Sigma. HPLC-grade n-hexane, ethanol, and analytical grade triethylamine were purchased from BDH Chemicals (UK). Deionized water was purified using a cartridge system (Picotech Water Systems RTP, USA). Adult male Wistar rats were obtained from Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Preparation of Stock and Standard Solutions

Stock solutions containing 1 mg mL^{-1} of individual S-(−)- and R-(+)-propranolol were prepared in methanol. Working standard solutions (2 μg mL^{-1}) were prepared by dilution of an individual aliquot of stock solution with the same solvent. The internal standard NAN–190 was prepared in methanol to give a concentration of 0.4 mg mL^{-1} and was further diluted with methanol to give a working solution of 40 μg mL^{-1}. The solutions were stable for at least 7 days if kept in the refrigerator. Appropriate dilutions of the individual working solutions of propranolol and internal standard were made and used for constructing the calibration curves and spiking the rat serum.

Preparation of Standard Serum Sample

The quality control (QC) samples at three concentrations 30, 200, and 320 ng mL^{-1} were prepared by spiking the drug-free rat serum with appropriate volumes of individual S-(−)- and R-(+)-propranolol and stored frozen until analysis. Before spiking, the drug-free serum was tested to make sure that there were no endogenous interferences at the retention times of S-(−)- and R-(+)- propranolol as well as the retention time of the internal standard.

Propranolol Treatment and Serum Sampling

Adult Wistar albino male rats weighing 150–200 g (10–12 weeks old) were obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University. All animal procedures were performed in accordance with the NIH guidelines and approved by the Ethics Committee of the Experimental Animal Care Society, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. All animals were allowed to acclimatize in metal cages inside a well-ventilated room for 2 days prior \to the experiment. The animals were maintained under standard laboratory conditions (a temperature of 20 ± 3°C, a relative humidity of 55 ± 10%, and a 12-h light/dark cycle) and were fed a diet of standard commercial pellets and water ad libitum. The animals were randomly divided into six groups consisting of five rats each. Five groups were intraperitoneally (i.p.) injected with propranolol (40 mg kg^{-1}), and the rest group was i.p. injected with normal saline and considered as the control group to provide the blank rat serum. Blood samples from propranolol-treated groups were collected under light ether anesthesia from the orbital plexus 1, 2, 3, 4, and 5 h after injection. All blood samples were centrifuged (3000 rpm at 4°C) for 10 min to obtain the serum. The serum samples were placed on ice for immediate use or stored at −20°C until analysis.

Assay Method

Assay of rat serum was performed by placing a 300 μL serum into 1.5-mL Eppendorf tube and accurately measured aliquots of 15, 100, 160 μL of the individual working standard of S-(−)- and R-(+)-propranolol solutions were added. The internal working standard solution of 25 μL was then added to each tube and sonicated for 5 min then diluted to 1 mL with acetonitrile to give final concentration of 30, 200, and 320 ng mL^{-1} for each enantiomer. Each tube was vortexed for 5 min, then centrifuged at 10,000 rpm for 5 min. The supernatant solution was evaporated to dryness under gentle air then reconstituted by mobile phase to 1 mL, sonicated for 5 min, filtered if necessary through Millipore membrane filter (0.2 μm), then 20 μL of the final solution was injected into the HPLC system. Blank rat serum sample were processed by the same procedures using acetonitrile instead of propranolol enantiomers. The absolute recoveries of each enantiomer from serum was calculated by comparing drug peak area of the spiked analyte samples to unextracted analyte of stock solution, which had been injected directly into the HPLC system. Calibration curves were constructed by diluting stock solutions with pooled rat serum to yield six concentration points over the range of 10–400 ng mL^{-1} for each propranolol enantiomer.

Linearity, Precision, and Accuracy

Linear regression analysis of normalized drug/internal standard peak area ratio versus concentration gave slope and intercept data for each analyte, which were used to calculate the concentration of each analyte in the serum samples. Calibration standards at each concentration were analyzed in six replicates. The within-run and between-run precision (reported as RSD, %) and accuracy (reported as relative error, %) of the assay in serum were determined by assaying six quality control samples over a period of 3 d. The concentration represented the entire range of the calibration curve. The lowest level was at 3 times the expected limit of quantitation (LOQ) for each enantiomer. The second level was the midpoint of the calibration curve and the third level was 80% of the upper concentration. The regression equations were used to determine the concentrations in the quality control samples.

MOLECULAR MODELING STUDIES

Propranolol–β_2-Adrenergic Receptor Docking

Docking was done by Autodock Vina[16] since Autodock tools serve for visualization and measuring the distances of interactions.[17] Autodock Vina is new software aiming to improve the accuracy of the binding mode. It can predict the binding affinity of the ligand (Kcal mol^{-1}) that can be used for ranking of the resulting poses to predict which conformation could be the best for fitting and interactions. The crystal structure of the human β_2-adrenergic G-protein-coupled receptor complexed with carazolol, one of the best known

β_2-blockers, was downloaded from the protein data bank (pdb code = 2RH1) and saved as pdbqt format after building the Grid box.[18] This crystal structure gave us the chance to focus on the site in which carazolol was complexed, and helped in identification of residues that may be involved in the drug–receptor interactions. Both S-(−)- and R-(+)-enantiomers of propranolol were built, polar hydrogen's were added, minimized, and saved as pdbqt. A grid box was built to include all these residues. The similarity in structure between carazolol and propranolol, especially in their side-chains, was shown by alignment of the two structures, which confirms the similarity in steric and electrostatic features and probability of same mode of interaction. After docking of both S-(−)- and R-(+)-enantiomers of propranolol, visualizations with Autodock tools were performed to find a reasonable mechanism by which the S-(−)-enantiomer could be more potent than the R-(+)-enantiomer. Distances of interactions were calculated and used with the binding affinities for our interpretation.

Propranolol–Cellulose Tris(3,5-Dimethylphenylcarbamate) CSP Docking

Cellulose tris(3,5-dimethylphenylcarbamate) was constructed from two molecules and minimized to be used for docking. Energy minimization was done in order to relax the whole structure and to reduce the strain. Swiss pdb viewer software was used for the minimization in which the number of steepest descent = 20 steps, cutoff = 10,000 Å, and all atoms were selected to be minimized. GROMOS 43B1 is the force field that is used for minimization, then molecular docking for both the S-(−)- and R-(+)-enantiomers was done separately.

RESULTS AND DISCUSSION

Optimization of the Chromatographic Conditions

The resolution of propranolol enantiomers is affected by several factors. One of these factors is the type of mobile phase and its components plus the ratio of these components. The nature of the mobile phase could affect enantioselectivity, capacity factor, and resolution degree as well as the CSP stability and column life span, particularly with that based on polysaccharide derivates. In this study different solvents (standards and nonstandards)[4] and different ratios of these solvents with or without additive were tested. Finally, the suitable mobile phase chosen consisted of n-hexane-ethanol-triethylamine (95:5:0.4%, v/v/v) which gave good resolution (R_s = 3.37) for both isomers of propranolol (Table 1). The importance of ethanol in this mobile phase is to improve peak shape, to shorten retention time and enhance selectivity. However, further increase in the ethanol ratio in the mobile phase cause a dramatic decrease in the retention time of the drug which may be related to hydrogen bonding between both enantiomers of propranolol and CSP.[4] On the other hand, addition of 0.4% (TEA) to the mobile phase plays an essential role for the separation of propranolol isomers through suppression of the deleterious effect of residual free silanols on the silica surface which consequently improve the peak symmetry, resolution, and selectivity.[19] It is known that interactive forces such as π–π interactions, van der Waals forces, and hydrogen bonding may be responsible for the chiral resolution of propranolol isomers with Chiralpak IB column.[4]

TABLE 1. Chromatographic parameter data for propranolol enantiomers and NAN-190 as internal standard

Analyte	R_s [a]	α [b]	K [c]	T_R [d]
S-(−)-Propranolol	e	e	2.72 ± 0.04	19.47 ± 0.06
NAN–190 (IS)	3.37	1.24	3.36 ± 0.05	23.32 ± 0.07
R-(+)-Propranolol	2.74	1.16	3.88 ± 0.06	25.55 ± 0.09

[a] $Rs = (t_2\text{-}t_1)/0.5\ (W_2 + W_1)$, where t_2 and t_1 are the retention of the second and the first peaks while W_2 and W_1 are the base peak width of the second and first peaks.
[b] Separation factor, calculated as k_2/k_1, where $k = (t_R\text{-}t_0)/\ t_0$, where t_R is the retention of analyte and t_0 is the retention of solvent.
[c] Capacity factor, where $k = (t_R\text{-}t_0)/\ t_0$, where t_R is the retention of analyte and t_0 is the retention of solvent.
[d] T_R is the retention time, mean + SD, n = 10.
[e] Not calculated.

Method Validation

A specific method can accurately measure the analyte of interest even in the presence of potentially sample components (sample matrix). A major objective of determining specificity is to ensure "peak purity" of the main compound to be determined. Figure. 1A,B shows the chromatograms of a blank serum sample and the run of serum sample spiked with 1.0 μg mL^{-1} IS and 10 ng mL^{-1} of propranolol enantiomers, respectively, under the optimized conditions. The comparison of analyte standard solution chromatograms to those of a blank serum sample (spiked with the same standard solutions) showed that the matrix effect was minimal. Therefore, the deproteinization procedure by acetonitrile ensured that there was no matrix effect between serum samples. No impurity peaks were overlapped with the peaks of propranolol enantiomers and IS. These data collectively and evidently indicated the specificity of the developed method for the determination of targeted drug. A good linearity relationship was demonstrated

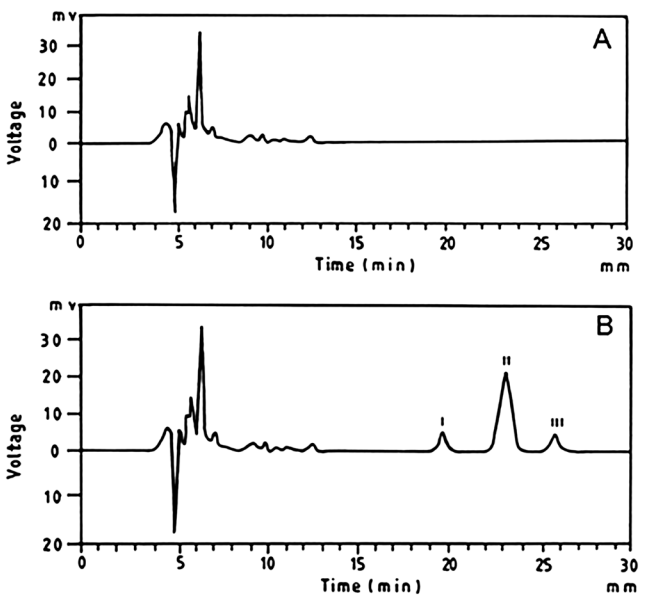

Fig. 1. Chromatograms of: (**A**) blank rat serum and (**B**) blank rat serum spiked with 10 ng mL^{-1} of S-(−)-propranolol (I), R-(+)-propranolol (III), and 1 μg mL^{-1} of NAN–190 (IS) (II). Chromatographic system column, Chiralpak IB (250 x 4.6 mm i.d.), Mobile phase consisted of n-hexane-ethanol-triethylamine (95:5:0.4 %, v/v/v) at a flow rate of 0.6 mL min^{-1} and fluorescence detection set at excitation/emission wavelengths 290/375 nm. Column temperature, ambient.

between peak area ratios of propranolol enantiomers and IS corresponding to serum concentrations over a range of 10–400 ng mL^{-1} for both enantiomers. The mean linear regression equation of the peak ratio *(y)* versus enantiomer concentration (ng mL^{-1}) in rat serum samples (×) showed correlation coefficients (R) = 0.9995, $y = 0.006\times + 0.023$ for S-(−)-propranolol and $y = 0.007\times + 0.021$ for R-(+)-propranolol, respectively. The good linearity of the calibration graphs and negligible scatter of experimental points are evident by the values of correlation coefficient and confidence intervals of the slope and intercept of the obtained data (Table 2).[20] The sensitivity was expressed as LOQ and limit of detection (LOD). LOQ is the injected amount that results in a peak with a height at least 10 times as high as the baseline noise level, and the LOD as peak height to base line ratio of 3:1.[21] Average values of LOD and LOQ of propranolol enantiomers in spiked rat serum samples were found to be 3.0 ng mL^{-1} and 10 ng mL^{-1}, respectively (Table 2). Blank serum was spiked with standard solution of propranolol enantiomers at 30, 200, 320 ng mL^{-1} and then 40 μg mL^{-1} of IS was added with the reconstitution step in the assay method. The sample used for the recovery runs was prepared as described above. The overall accuracy was assessed by the relative percentage error, which ranged from −5.2 to −2.5 for S-(−)-enantiomer and from −6.6 to −2.6% for R-(+)-enantiomer. The negative of the relative percentage error clearly exhibited that recoveries of both propranolol enantiomers were higher than 93.5% and less than 97.5%, indicating good overall accuracy. The intraday values of RSD were calculated based on six replicate runs of three different concentrations in 1 d; the interday values of RSD were calculated by using three sets from the same three standard solutions obtained in 5 different days. Results presented in Table 3 indicate that intra-assay RSD of 0.8–1.1% for S-(−)-enantiomer and 0.8–1.2% for R-(+)-enantiomer, while the inter-assay RSD for both enantiomers was 0.9–1.3 %.

Optimization of Rat Serum Sample Procedure

Prior to the determination of propranolol enantiomers in rat serum samples using the optimized HPLC conditions obtained, a serum sample pretreatment step had to be employed. Although many of the available SPE cartridges have been employed for the extraction of propranolol isomers including C_8, C_{18}, phenyl, CN, SCX, and WCX silica cartridges (55 μm, 70 Å, 100 mg, 1.0 mL), unknown peaks were observed and specificity of such methods is poor. A number of solvents (acetonitrile, ethanol, isopropanol, and trifluoroacetic acid) and a mixture of these solvents were evaluated as deproteinized solvents. Acetonitrile was selected since it provided the best data in terms of sample cleanup and high recoveries of both propranolol enantiomers. The mean recoveries using acetonitrile were 96.2 ± 1.2 for S-(−)- propranolol and 95.3 ± 1.5 for R-(+)- propranolol (n = 5).

TABLE 3. Accuracy and precision data for propranolol enantiomers in spiked rat serum

Analyte	Actual concentration (ng mL^{-1})	Found concentration (ng mL^{-1})	RSD (%)[c]	Error (%)[d]
Within-days[a]	30.0	28.9 ± 0.2	0.69	−3.57
S-(−)- propranolol	200.0	191 ± 2	1.10	−4.52
	320.0	312 ± 3	0.89	−2.46
R-(+)-propranolol	30.0	28.5 ± 0.2	0.70	−5.06
	200.0	190 ± 2	1.15	−4.82
	320.0	312 ± 3	0.93	−2.59
Between-days[b]	30.0	28.6 ± 0.3	1.05	−4.80
S-(−)- propranolol	200.0	190 ± 3	1.32	−5.23
	320.0	309 ± 3	0.91	−3.43
R-(+)-propranolol	30.0	28.2 ± 0.3	1.06	−6.06
	200.0	187 ± 2	1.28	−6.55
	320.0	311 ± 3	0.93	−2.00

[a]Mean ± SD based on n = 6.
[b]Mean ± SD based on n = 5.
[c]Expressed as %RSD: (SD/ mean) x 100.
[d]Calculated as (mean determined concentration / nominal concentration) x 100.

Pharmacokinetic Study

The described method was further applied to a pharmacokinetic study of both propranolol enantiomers in rats. Serum was collected 1, 2, 3, 4, and 5 h after propranolol treatment. The serum concentration-time curve (AUC) for propranolol enantiomers is presented in Figure 2, which shows the mean values of t_{max} and c_{max} were 1 h and 755.76 ± 18.97 ng mL^{-1}, respectively, for S-(−)-propranolol, while for R-(+)-propranolol the main values of t_{max} and c_{max} were 1 h and 362.22 ± 12.93 ng mL^{-1}. The variation in plasma level between the two

TABLE 2. Validation parameters for the determination of propranolol enantiomers using the proposed HPLC method

Parameters	S-(−)-propranolol	R-(+)-propranolol
Concentration (ng mL^{-1})	10.0 – 400.0	10.0 – 400.0
Intercept (a) ± CI[a]	0.023 ± 0.001	0.021 ± 0.001
Slope (b) ± CI[a]	0.006 ± 0.0005	0.007 ± 0.0006
Correlation coefficient (R)	0.9995	0.9995
$S_{y/x}$[b]	0.033	0.042
S_a[d]	0.00003	0.00003
S_b	0.0005	0.0005
LOQ (ng mL^{-1})[e]	10.0	10.0
LOD (ng mL^{-1})[f]	3.0	3.0

[a]Confidence intervals at P = 0.05.
[b]Standard deviation of the residual.
[c]Standard deviation of the intercept.
[d]Standard deviation of the slope.
[e]S/N = 10.
[f]S/N = 3.

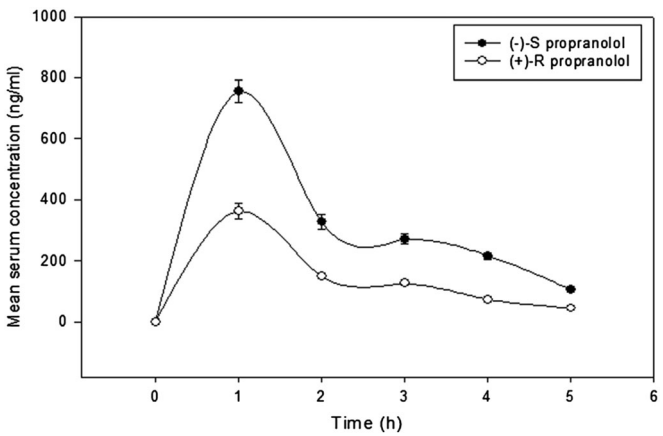

Fig. 2. Concentration-time profile of S-(−)-propranolol and R-(+)-propranolol in rat serum after intraperitoneal administration of 40 mg kg^{-1} of propranolol. Each point represents the mean ± SD, n = 5.

enantiomers may be related to the lower protein binding of S(−)-enantiomer and better stereospecific fitting towards its β-receptor, which consequently increases its plasma level.[22]

Molecular Modeling

The 3D crystal structure of β_2-adrenergic receptor in combination with carazolol can be used as guidance for docking residues and consequently defining key residues that might be involved in the interactions. By using the former structure, we were able to investigate all possible docking conformations for both S-(−)-, R-(+)- and visualized separately looking for the conformations that might have the best fit as well as the same orientation like carazolol. The OH group of S-(−)-enantiomer was believed to be positioned in a centered place between Asn 312 (L-asparagine amino acid 312) and Asp 113 (aspartic acid 113) allowing for possible hydrogen bond interaction between OH group of propranolol and –C = O groups of both Asn 312 and Asp 113. On the other hand, the R-(+)-enantiomer hydroxyl group positioned only towards Asn 312 as shown in Figure 3. This difference between the two enantiomers contributes to the retention time difference with cellulose in separating the two enantiomers. The effective separation of both S-(−)- and R-(+)-enantiomers of propranolol was studied by molecular modeling techniques to find a suitable relation between the retention time of both enantiomers and the mechanism by which they could be separated. The hydroxyl group here is very important for the interactions simply because of its role in determination of the stereochemistry of the structure. In the S-(−)-isomer, OH group is found in the same plane with the –NH and this will allow both of them to interact with cellulose tris (3,5-dimethyl phenylcarbamate) but with relatively long distances and large root of mean square deviation (RMSD). Energy minimization resulted in removing the steric clashes that could happen between the ligand and its site of action. Moreover, it gave the chance to relax the whole structure of cellulose tris(3,5-dimethylphenyl carbamate), and so makes it easy for Autodock Vina to calculate the affinities for the docked enantiomer. Computation of the force field of all bonds, angles, torsions, nonbonds, and electrostatics resulted in a total energy of 305.3 kcal mol^{-1}. Upon minimization, the energy was found to equal 143.9 kcal mol^{-1}. The docking of the S-(−)-isomer against the minimized cellulose tris(3,5-dimethylphenylcarbamate) was found to have affinity of −4.8 Kcal mol^{-1} for the best mode with an RMSD of 3.819 units. The observed interactions was mostly for the hydroxyl group of S-(−)-propranolol forming a hydrogen bond with the –C = O group of carbamate moiety with a distance of 2.26 Å (Fig. 4). On the other hand, docking of the R-(+)-enantiomer had affinity of −5.1 Kcal mol^{-1} for the best mode, with RMSD of 1.196. The –OH group is found in a separate plane away from –NH and the rest of the structure, and this enabled it to interact freely with most of the electron acceptor groups found in cellulose tris(3,5-dimethylphenylcarbamate). Moreover, the measured distances of the R-(+)-enantiomer were 2.15 Å, which are shorter than those formed with the S-(−)-enantiomer (Fig. 5A,B). These short distances will make the bonds stronger and consequently delay the retention time of the R-(+)-enantiomer, as in Figure 4B. From this we can observe that the R-(+)-enantiomers have better affinity toward cellulose tris(3,5-dimethylphenylcarbamate), low RMSD, and short distances, which means strong bonds, and all of these reasons could be the main cause for its higher retention time than that of S-(−)-enantiomer, allowing effective separation between the two enantiomers.

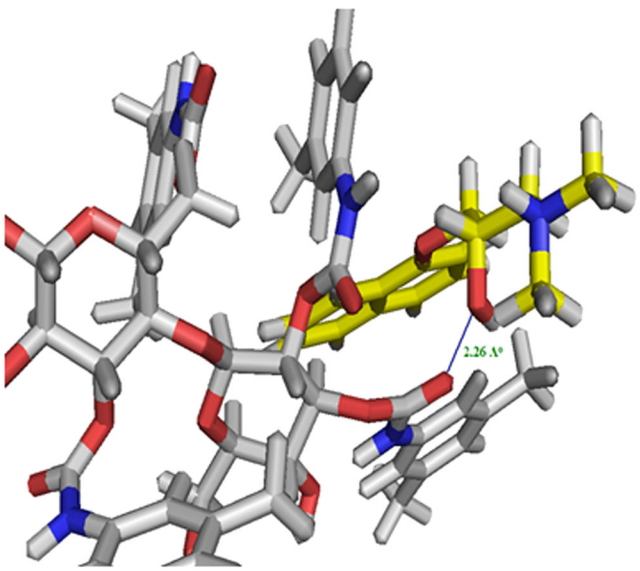

Fig. 4. Possible interactions of S-(−)-propranolol with measured distances of cellulose tris(3,5-dimethylphenylcarbamate).

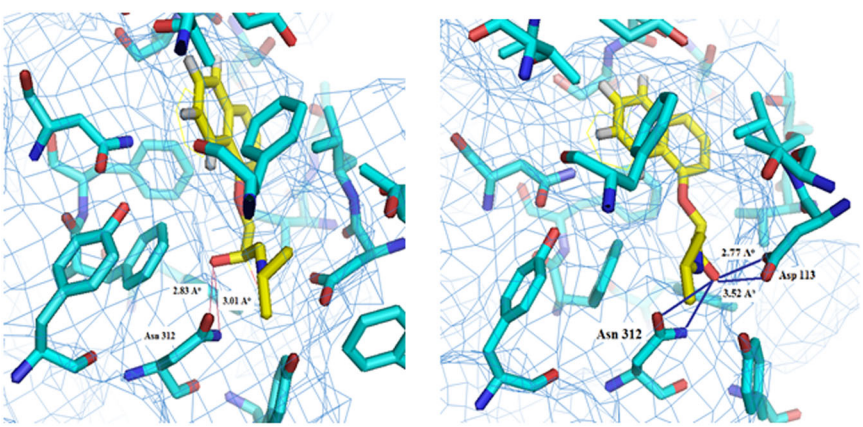

Fig. 3. (**A**) The possible interactions occur by the R-(+)-enantiomer of propranolol with its hydroxyl group toward Asn 312. (**B**) Orientation of the hydroxyl group of the S-(−)- enantiomer of propranolol between Asp 113 and Asn 312.

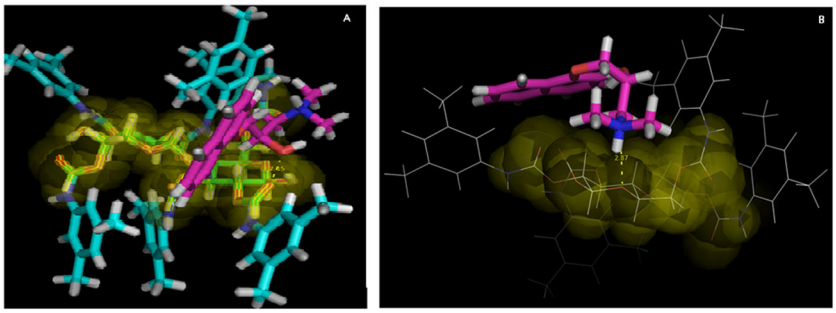

Fig. 5. Two different conformations (**A**,**B**) of R-(+)-enantiomer of propranolol showing the hydrogen bond formation with cellulose tris(3,5-dimethylphenylcarbamate) skeleton.

CONCLUSIONS

An enantioselective HPLC-fluorescence method using Chiralpak IB column was developed, for the first time, for the simultaneous analysis of propranolol enantiomers in rat serum. The use of a fluorescence detector coupled with an HPLC technique allowed increased sensitivity and selectivity for the determination of propranolol enantiomers. To the best of our knowledge, the combinations of Chiralpak IB-HPLC-FL detection with a pharmacokinetic study have not been reported in single propranolol enantiomers analysis. The developed method was used to remove endogenous interference by the simple technique of deproteinization as a sample cleanup procedure, thereby achieving a high degree of selectivity. Due to the minimal sample preparation, and its good precision and accuracy, this method appears to be very useful for the therapeutic and toxicological monitoring of propranolol isomers in clinical practice and for kinetic–metabolic studies of this drug. Under the optimized conditions, the developed method was of high precision, good accuracy, and wide linear range of determination, especially a low limit of detection. Finally, the developed method was demonstrated to be applicable for conducting pharmacokinetic studies. The effective separation of both S-(−)- and R-(+)-enantiomers of propranolol was studied by molecular modeling techniques to find a suitable relation between the retention time of both enantiomers and the mechanism by which they could be separated. The hydroxyl group of propranolol was found to be crucial in determining the stereochemistry of the structure.

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