Enantiomeric Separation and Simulation Studies of Pheniramine, Oxybutynin, Cetirizine, and Brinzolamide Chiral Drugs on Amylose-Based Columns

IMRAN ALI,[1,2]* ZEID A. AL-OTHMAN,[2] ABDULRAHMAN AL-WARTHAN,[2] SYED DILSHAD ALAM,[1] AND JAVED A. FAROOQI[3]

[1]*Department of Chemistry, Jamia Millia Islamia, (Central University) New Delhi, India*
[2]*Department of Chemistry, College of Science, King Saud University, Riyadh, Kingdom of Saudi Arabia*
[3]*Department of Chemistry, School of Sciences, Indira Gandhi National Open University, New Delhi, India*

ABSTRACT Solid phase extraction (SPE)-chiral separation of the important drugs pheniramine, oxybutynin, cetirizine, and brinzolamide was achieved on the C_{18} cartridge and AmyCoat (150 x 46 mm) and Chiralpak AD (25 cm x 0.46 cm id) chiral columns in human plasma. Pheniramine, oxybutynin, cetirizine, and brinzolamide were resolved using n-hexane-2-PrOH-DEA (85:15:0.1, v/v), n-hexane-2-PrOH-DEA (80:20:0.1, v/v), n-hexane-2-PrOH-DEA (70:30:0.2, v/v), and n-hexane-2-propanol (90:10, v/v) as mobile phases. The separation was carried out at 25 ± 1 ºC temperature with detection at 225 nm for cetirizine and oxybutynin and 220 nm for pheniramine and brinzolamide. The flow rates of the mobile phases were 0.5 $mLmin^{-1}$. The retention factors of pheniramine, oxybutynin, cetirizine and brinzolamide were 3.25 and 4.34, 4.76 and 5.64, 6.10 and 6.60, and 1.64 and 2.01, respectively. The separation factors of these drugs were 1.33, 1.18, 1.09 and 1.20 while their resolutions factors were 1.09, 1.45, 1.63 and 1.25, and 1.15, respectively. The absolute configurations of the eluted enantiomers of the reported drugs were determined by simulation studies. It was observed that the order of enantiomers elution of the reported drugs was S-pheniramine > R-pheniramine; R-oxybutynin > S-oxybutynin; S-cetirizine > R-cetirizine; and S-brinzolamide > R-brinzolamide. The mechanism of separation was also determined at the supramolecular level by considering interactions and modeling results. The reported SPE-chiral high-performance liquid chromatography (HPLC) methods are suitable for the enantiomeric analyses of these drugs in any biological sample. In addition, simulation studies may be used to determine the absolute configuration of the first and second eluted enantiomers. *Chirality 26:136–143, 2014.*

KEY WORDS: SPE-chiral-HPLC; human plasma; modeling; mechanism of separation

INTRODUCTION

Due to health awareness and advancement of pharmaceutical science the need of optically active pure drugs (single enantiomer) is felt globally.[1] It is essential to know the stereochemistry of the chiral drugs before launching them into the market. In last few decades, enantioseparations and the need of chiral drugs have been active areas of research.[2] It is a great challenge to scientists and industrialists to separate and ensure optical purity of the enantiomers.[3] Various factors such as drug concentration, pH, ionic concentration, temperature, etc., are responsible for controlling the racemization process.[4] It is a well-known fact that one of the enantiomers is pharmaceutically active while the other may be inactive or toxic or ballast, leading to various side effects, toxicity, and problems in the human body.[5,6] US-FDA, European Committee for Proprietary Medicinal Products, Health Canada, and Pharmaceutical and Medical Devices Agencies of Japan have banned the marketing of all racemic drugs.[7–9] The enantiomeric differences at the pharmacokinetic level are mainly because of stereoselective drugs binding, absorption, clearance, excretion, etc. The interactions mainly occurred with different human plasma proteins.[10] Hence, pharmacokinetic studies and chiral purity testing need analytical methods with high resolution power and good efficiency. Chiral high-performance liquid chromatography (HPLC) fulfill all these needs easily.[11] Globally, the consumption of chiral drugs was U.S $33 billion in 1993, which reached U.S. $73 billion in 1997. Later on, the marketing cost of the chiral drugs rose to U.S. $172 billion in 2002. Bingyun and Haynie[12] described an enhancement in the consumption cost of enantiomeric drugs as U.S. $200 billion in 2008. Furthermore, it was expected to increase about U.S. $350 billion in 2013 followed by U.S. $1 trillion in 2020.[1,13]

Pheniramine and cetirizine and (Fig. 1) are H_1 receptor antagonist antihistamines used for curing hey fever, allergy, seasonal and perennial allergic rhinitis, and chronic idiopathic urticaria.[14–16] The enantiomers of cetirizine are R-levocetirizine and S-dextrocetirizine, with former active antipode (2-fold higher affinity for the H^1-receptor).[17–19] Furthermore, both enantiomers have different pharmacological effects. For example, S-dextrocetirizine is used for the treatment of urticaria, while R-levocetirizine is given for curing allergic disorders.[20,21] The dextro form of pheniramine is pharmaceutically active.[22] Another popular drug, oxybutynin, (Fig. 1) has been widely prescribed for the treatment of urinary tract disorders. The R-enantiomer is a more potent anticholinergic than the S-isomer.[23–25] Brinzolamide (Fig. 1) is a racemic drug widely used for curing open-angle glaucoma

*Correspondence to: Imran Ali, Department of Chemistry, Jamia Millia Islamia, (Central University) New Delhi, India and Department of Chemistry, College of Science, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia. E-mail: drimran_ali@yahoo.com; drimran.chiral@gmail.com
Received for publication 11 July 2013; Accepted 24 October 2013
DOI: 10.1002/chir.22276
Published online 26 January 2014 in Wiley Online Library (wileyonlinelibrary.com).

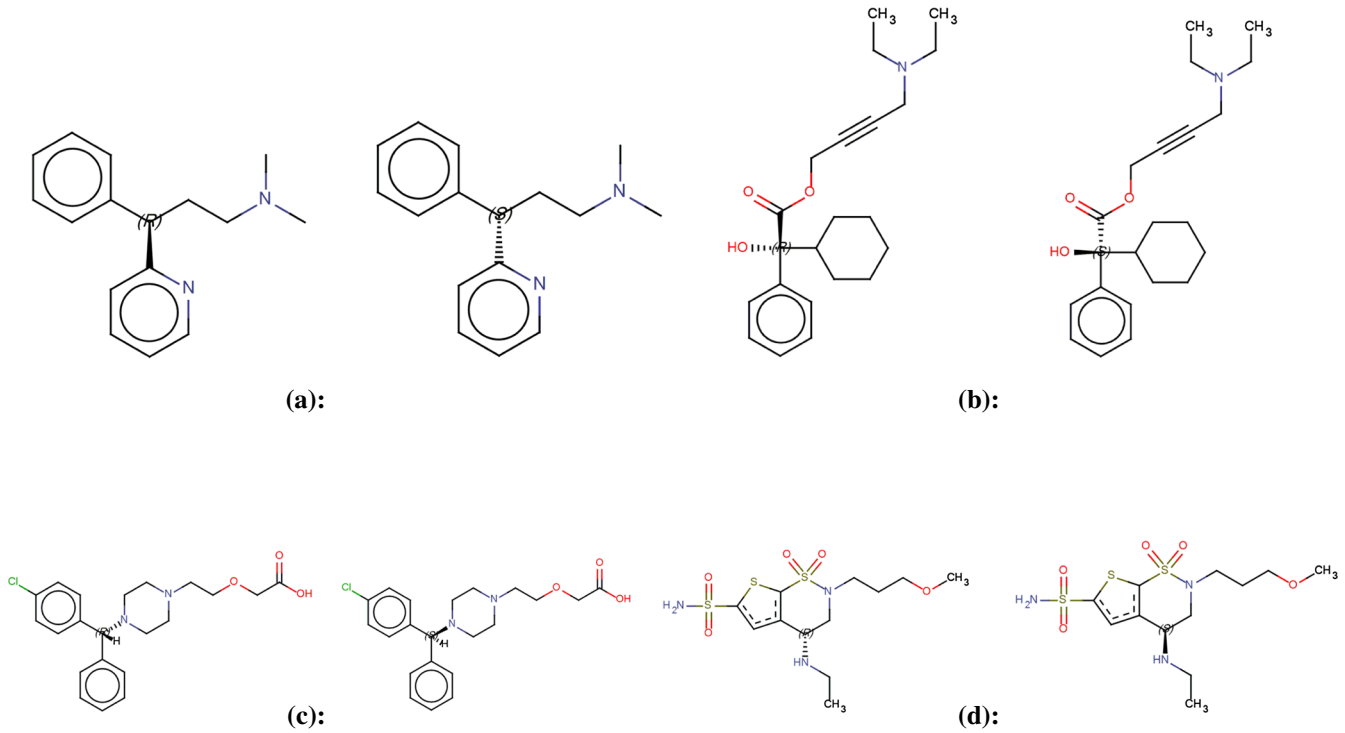


Fig. 1. Chemical structures of R- and (S)- enantiomers of (**a**): pheniramine, (**b**): oxybutynin (**c**): cetirizine, and (**d**): and brinzolamide.

or ocular hypertension. R-brinzolamide is pharmaceutically active. Generally, brinzolamide is a racemic mixture in pharmaceutical preparation, which needs chiral separation. These drugs are being used frequently for different health problems.

A thorough search of the literature was carried out and it was observed that a few papers are available on the chiral separation of cetirizine and pheniramine.[14,26–30] On the other hand, there is only one publication each on the chiral separation of oxybutynin and brinzolamide.[25] Furthermore, it was observed that these molecules were resolved on classical chiral columns. These methods have a high limit of detection with a long run time. Taking into consideration the importance of chiral analyses in human plasma, attempts have been made to develop solid phase extraction methods; 80% of chromatographers are using solid phase extraction **(**SPE) for sample preparation of the reported drugs in human plasma.[1,31–33] In addition, the chiral-HPLC method was also developed and used to monitor the enantiomeric ratio of these drugs in human plasma. Also, efforts have been made to ascertain the enantioseparation mechanism on chiral columns using amodeling approach. The results of these experiments are given herein.

EXPERIMENTAL

Chemicals and Reagents

Methanol, ethanol, n-hexane, 2-propanol, and diethyl amine of HPLC grade and sodium dihydrogenphosphate ($NaH_2PO_4.2H_2O$) of AR grade were supplied by Merck (Mumbai, India). Trifluoro acetic acid was obtained from Qualigens (Mumbai, India). Frozen Human Plasma (Mfg. License No. 504) was purchased from the Rotary Blood Bank (New Delhi, India). The standards of pheniramine, oxybutynin, cetirizine, and brinzolamide were purchased from Sigma-Aldrich (St. Louis, MO). The solution of these drugs of 1.0, 0.5, and 0.1 $mgmL^{-1}$ concentrations were prepared in methanol separately, respectively.

Instrumentation

The experiments were carried out on an HPLC system of ECOM (Prague, Czech Republic) consisting of a solvent delivery pump (model Alpha 10), manual injector, absorbance detector (Indtech. Instrument, Bombay, India) and Winchrome software. The columns used were AmyCoat [*tris*-(3,5-dimethylphenyl carbamate)] (150 x 46 mm id) and Chiralpak AD (25 cm x 0.46 cm id). These columns were purchased from Kromasil (Sweden) and Daicel Chemical Industries (Japan), respectively. An SPE unit was purchased from Varian (Palo Alto, CA). C_{18} Cartridges were obtained from Waters (Milford, MA). A pH meter from Control Dynamics (Model APX175 E/C) and centrifuge from Remi (model C-30BL) were used. Millipore water (Millipore-Q, Bedford, MA) was used to carry out these experiments.

Solid Phase Extraction (SPE)

To optimize sample preparation conditions, 1.0 mL each of pheniramine, oxybutynin, cetirizine, and brinzolamide of 1.0 $mg\,mL^{-1}$ concentration were mixed with 5.0 mL fresh-frozen human plasma, separately. The spiked human plasma samples were vortexed for 2.0 min and kept for 30.0 min. Then 15.0 mL acetone was mixed with each vortexed sample and kept for 30 min. These samples were centrifuged at 10,000 rpm (11,180 *g*) for 10.0 min to separate the supernatant. The supernatant was evaporated to dryness under vacuum and the residue was redissolved in 10.0 mL phosphate buffer (50 mM, pH 7.0). Sep-Pac C_{18} cartridges (1.0 mL Waters) were preconditioned with 2.0 mL methanol and 5.0 mL Millipore water. Then 10.0 mL phosphate buffer (50 mM, pH 7.0) having pheniramine, oxybutynin, cetirizine, and brinzolamide were passed through the cartridge with a 0.1 $mLmin^{-1}$ flow rate, followed by cartridges washing with 2.0 mL Millipore water at the same flow rate. The cartridges were dried with hot air and the reported drugs eluted by 10.0 mL methanol containing 0.1% trifluoro acetic acid at a 0.1 $mLmin^{-1}$ flow rate. The eluted methanol solutions for the reported drugs were concentrated under vacuum to 0.5 mL separately. These samples were further used for Chiral-HPLC analyses. To optimize SPE conditions, various experimental factors were optimized.

High-Performance Liquid Chromatography (HPLC)

The experiments were carried out on an HPLC system as described above. The standard solutions of 1.0 mgmL^{-1} of pheniramine, 0.5 mgmL^{-1} of oxybutynin, 1.0 mgmL^{-1} of cetirizine, and 0.1 mgmL^{-1} of brinzolamide were prepared in methanol. Then 5.0 µL of each solution was injected into the HPLC system. The mobile phases used in this study were n-hexane-2-PrOH-DEA (85:15:0.1, v/v), n-hexane-2-PrOH-DEA (80:20:0.1, v/v), n-hexane-2-PrOH-DEA (70:30:0.2, v/v), and n-hexane-2-propanol (90:10, v/v) for pheniramine, oxybutynin, cetirizine, and brinzolamide drugs, respectively. The separation was carried out at 25 ± 1 ºC with detection at 225 nm for cetirizine and oxybutynin and 220 nm for pheniramine and brinzolamide. The flow rates of the mobile phases were 0.5 mLmin-1. The mobile phase was filtered and degassed before use daily. The capacity (k), separation (α) and resolution (Rs) factors were calculated.

In Silico Studies

To determine the chiral recognition mechanism, the interactions of enantiomers of the reported drugs on AmyCoat [*tris*-(3,5-dimethylphenyl carbamate) amylose] chiral selector (Fig. 2) were determined by modeling studies.

Methodology

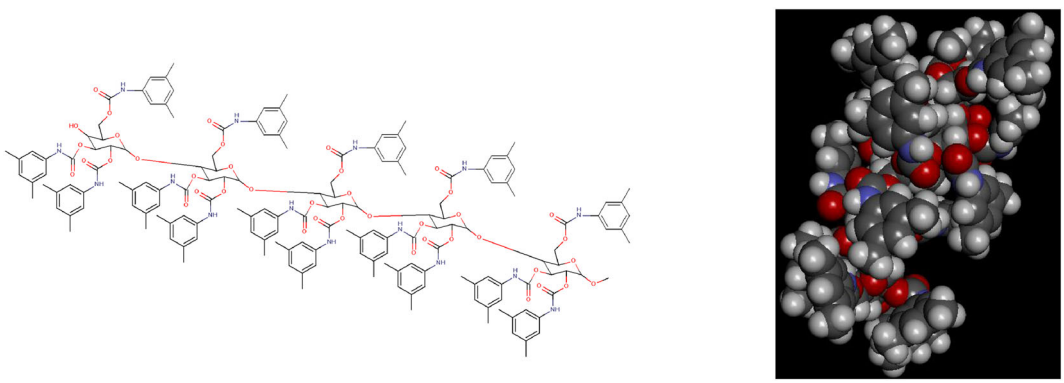
The modeling studies were carried out on a computer having these configurations: Intel Core i3 CPU (2.3 GHz) with XP-based operating system (Windows 2003). Marvin Sketch (v. 5.8.2) was used to draw the enantiomeric structures of the drugs. Marvin Sketch (v. 5.8.2) was used to draw the enantiomeric structures of the reported drugs. The structures were cleaned to 3D and saved in pdb format. The docking studies was performed with AutoDock 4.2 (Scripps Research Institute, La Jolla, CA) considering all the rotatable bonds of ligand as rotatable and *tris*-(3,5-dimethylphenyl carbamate) amylose as rigid.[34] Grid box size of 50 × 50 × 50 with 0.375 spacing was used. Docking was performed using an empirical-free energy function and Lamarckian Genetic Algorithm, with an initial population of 150 randomly placed individuals, a maximum number of 2,500,000 energy evaluations, a mutation rate of 0.02, and crossover rate of 0.80. Fifty independent docking runs were performed for each ligand and *tris*-(3,5-dimethylphenyl carbamate) amylose for lowest free energy of binding conformation from the largest cluster, which was written and saved in PDBQT format. These PDBQT files were converted to PDB file format.

RESULTS AND DISCUSSION

Solid Phase Extraction

To determine the efficiency of the reported SPE method, the percentage recoveries of the enantiomers of pheniramine, oxybutynin, cetirizine, and brinzolamide were calculated. The percentage recoveries of pheniramine, oxybutynin, cetirizine, and brinzolamide were determined by running blank experiments. The percentage recoveries of pheniramine, oxybutynin, cetirizine, and brinzolamide were 99.1, 99.0, 99.0, and 99.1%, respectively. The experimental errors were considered during the calculation of percentage binding of the reported drugs with human plasma proteins. The optimization of SPE was achieved by varying different experimental conditions such as concentration and pHs of phosphate buffer, flow rates of human plasma samples, phosphate buffer, and eluting solvents. In addition, various eluting solvents tried were methanol, ethanol, acetone, ethyl acetate, and dichloromethane. In order to achieve the maximum recoveries, 0.1 to 1% acetic acid or trifluroacetic acid was also added in the above-cited eluting solvents. As a result of exhaustive experimentation, the best experimental conditions are reported herein. The maximum percentage recoveries of pheniramine, oxybutynin, cetirizine, and brinzolamide from C_{18} cartridge were achieved using phosphate buffer (50.0 mM, pH 5.0, 7.0, and 9.0) at 0.1 mL min^{-1} as the flow rate. The best eluting solvent was methanol containing 0.1% trifluoro acetic acid for pheniramine, oxybutynin, cetirizine, and brinzolamide at a 0.1 mLmin^{-1} flow rate. The calculated percentage recoveries of the reported drugs on the C_{18} cartridge and percentage bindings with human plasma are given in Table 1.

The percentage recoveries of pheniramine enantiomers on the C_{18} cartridge were 25.18, 20.67, 32.59, 36.40, 39.00, and 42.10, respectively. These values clearly depicted maximum recovery at pH 9.0. Therefore, the human plasma bindings of the enantiomers were calculated by considering percentage recoveries at pH 9.0. Similarly, the percentage recoveries of oxybutynin enantiomers on the C_{18} cartridge were 11.24 and 13.17, 10.82 and 16.20, and 12.17 and 14.89 at pH 5.0, 7.0, and 9.0, respectively. These values clearly indicated maximum recovery at pH 9.0. Therefore, the human plasma bindings of the enantiomers were calculated by considering percentage recoveries at pH 9.0. The percentage recoveries of cetirizine enantiomers on the C_{18} cartridge were 17.59 and 20.68, 22.20 and 28.61, and 25.91 and 27.02 at pH 5.0, 7.0, and 9.0, respectively. These values clearly indicated maximum recovery at pH 9.0. Therefore, the human plasma bindings of the enantiomers were calculated by considering percentage recoveries at pH 9.0. The percentage recoveries of brinzolamide enantiomers on the C_{18} cartridge were 19.00 and 20.40, 6.28 and 17.59, and 4.70 and 15.90 at pH 5.0, 7.0, and 9.0, respectively. These values clearly showed maximum recovery at pH 9.0. Therefore, human plasma



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

Fig. 2. 2D (**a**) and 3D (**b**) structures of *tris*-(3,5-dimethylphenyl carbamate) amylose chiral selector.

TABLE 1. Percentage bindings of pheniramine, oxybutynin, cetirizine, and brinzolamide with human plasma protein

		Pheniramine		Oxybutynin		Cetirizine		Brinzolamide	
Sl. no.	pH of buffer	% Recovery 0.1%TFA in MeOH	% Binding with plasma protein	% Recovery 0.1%TFA in MeOH	% Binding with plasma protein	% Recovery 0.1%TFA in MeOH	% Binding with plasma protein	% Recovery 0.1%TFA in MeOH	% Binding with plasma protein
1	5.0	25.18 (20.67)	30.00 (36.51)	(13.17) 11.24	(36.33) 38.26	20.68 (17.59)	28.82 (31.91)	(20.40) 19.00	(29.10) 30.50
2	7.0	32.59 (36.40)	25.90 (31.40)	(16.20) 10.82	(33.30) 38.68	28.61 (22.20)	20.89 (27.30)	(17.59) 6.28	(31.91) 43.22
3	9.0	39.00 (42.10)	32.50 (37.67)	(14.89) 12.17	(34.61) 37.33	27.02 (25.91)	22.48 (23.59)	(15.90) 4.70	(33.60) 44.80

The values outside and inside of the parentheses belong to the R– and S–enantiomers, respectively.

bindings of the enantiomers were calculated by considering percentage recoveries at pH 9.0. Hence, human plasma bindings of the enantiomers of pheniramine, oxybutynin, cetirizine, and brinzolamide were 32.50 and 37.67, 34.61 and 37.33, 22.48 and 23.59, and 33.60 and 44.80, respectively.

Chromatography

The chromatographic parameters such as retention (k), separation (α), and resolution (Rs) factors were calculated for the enantiomers of pheniramine, oxybutynin, cetirizine, and brinzolamide using both AmyCoat and Chiralpak AD columns. The values of retention factors for the first and second enantiomers were calculated and are given in Table 2. It is clear from this table that the retention factors of pheniramine, oxybutynin, cetirizine, and brinzolamide were 3.25 and 4.34, 4.76 and 5.64, 6.10 and 6.60, and 1.64 and 2.01, respectively. The separation factors of these drugs were 1.33, 1.18, 1.09 and 1.22 while the values of resolution factors were 1.09, 1.45, 1.63 and 1.20, and 1.15, respectively. The values of separation and resolution factors were greater than one indicating complete resolution of the enantiomers of the reported drugs. Furthermore, it was observed that the chiral separations of these drugs were complementary to each other on AmyCoat and Chiralpak columns (Table 2). This is due to the fact that both these columns have the same chiral selector, i.e., *tris*-(3,5-dimethylphenyl carbamate) amylose. The chromatograms for these drugs in standard and human plasma samples using AmyCoat are shown in Figures 3 to 6, respectively.

HPLC Method Optimization

To optimize the chromatographic conditions, various combinations of solvent systems were tried. The alteration in flow rate, detection wavelength, and amount injected was also carried out. As a result of exhaustive experimentation, the best HPLC conditions were developed and reported herein. The experiment was carried out using mobile phases n-hexane-2-PrOH-DEA (85:15:0.1, v/v), n-hexane-2-PrOH-DEA (80:20:0.1, v/v), n-hexane-2-PrOH-DEA (70:30:0.2, v/v), and n-hexane-2-propanol (90:10, v/v) for pheniramine, oxybutynin, cetirizine, and brinzolamide drugs, respectively. The developed SPE-Chiral-HPLC methods were validated by carrying out five sets (n = 5) of the chromatographic procedure under identical conditions. The HPLC method was validated with respect to various parameters including linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy, selectivity, recovery, and robustness and ruggedness.

MECHANISM OF SEPARATION AT THE SUPRAMOLECULAR LEVEL

Among various chiral selectors, the best one is polysaccharide-based chiral selectors. AmyCoat and Chiralpak AD columns have amylose polysaccharide (Fig. 7), which is more helical than cellulose and others.[35] The separation of the reported drugs on AmyCoat and Chiralpak AD columns is due to the presence of chiral grooves. The enantiomers of these drugs get fitted stereospecifically to different extents. The fittings of the enantiomers are stabilized by various forces such as π-π interactions, hydrogen bondings, van der Waal's forces, steric effects, etc. A perusal of Table 2 indicates a poor resolution factor of brinzolamide. This may be due to the fact that this molecule does not have a phenyl ring avoiding the possibility of π-π interactions. Therefore, it may be concluded that π-π interactions are the major controlling forces in the chiral separation. This fact has already been published by our group.[36–38] Briefly, the enantiomers of these drugs fitted stereospecifically in the chiral groove. The mobile phase tends to carry these enantiomers along with it. As a result of the competition between stationary and mobile phases, the less retained enantiomer eluted first followed by the more retained enantiomer. Efforts

TABLE 2. Separation (α) and resolution (Rs) factors for pheniramine, oxybutynin, Cetirizine, and brinzolamide on AmyCoat and Chiralpak AD columns in standard and in human plasma samples

Sl. no.	Chiral drugs	Separation factors (α)	Resolution factors (Rs)	Mobile phase
1.	Pheniramine (standard)	1.33 (1.38)	1.09 (1.10)	n-hexane-2-Propanol-DEA (85:15:0.1,v/v)-do-0.5 mL/min at 220 nm
	Pheniramine (human plasma)	1.30 (1.32)	1.08 (1.09)	
2.	Oxybutynin (standard)	1.18 (1.17)	1.45 (1.45)	n-hexane-2-Propanol-DEA (80:20:0.1,v/v)-do-0.5 mL/min at 225 nm
	Oxybutynin (human plasma)	1.15 (1.15)	1.43 (1.44)	
3.	Cetirizine (standard)	1.09 (1.10)	1.63 (1.62)	n-hexane-2-propanol-DEA (70:30:0.2,v/v)-do-0.5 mL/min at 225 nm
	Cetirizine (human plasma)	1.06 (1.08)	1.60 (1.60)	
4.	Brinzolamide (standard)	1.22 (1.22)	1.25 (1.25)	n -hexane-2-propanol (90:10,v/v)-do-0.5 mL/min at 220 nm
	Brinzolamide (human plasma)	1.20 (1.19)	1.25 (1.25)	

The values outside and inside of the parentheses correspond to AmyCoat and Chiralpak AD column, respectively.

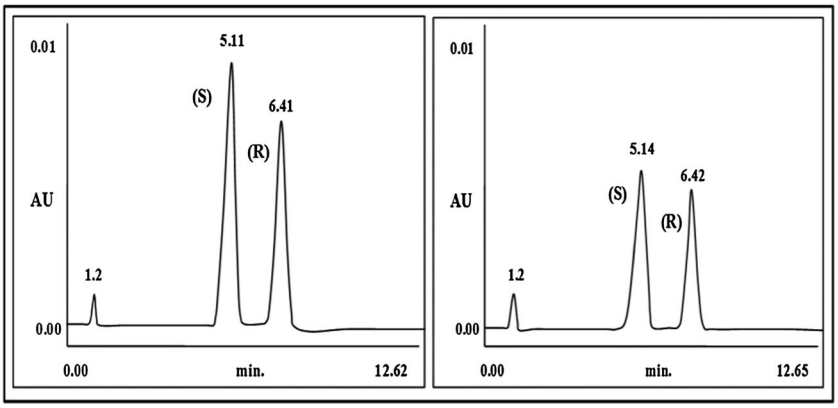


Fig. 3. HPLC chromatograms of pheniramine standard and in human plasma sample.

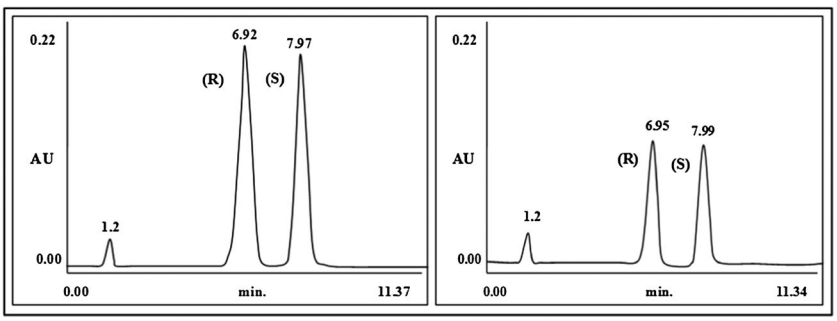


Fig. 4. HPLC chromatograms of oxybutynine standard and in human plasma sample.

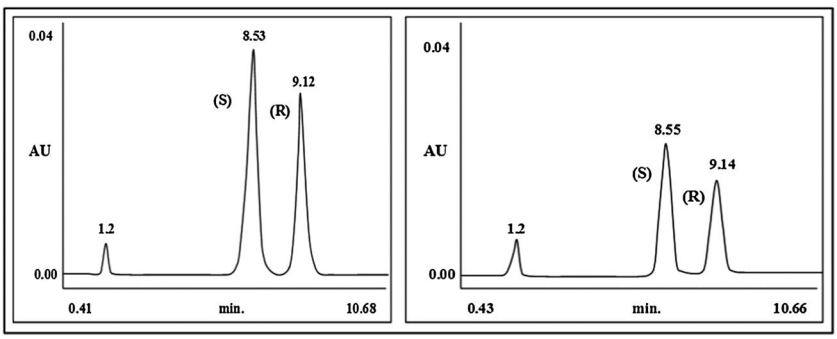


Fig. 5. HPLC chromatograms of cetirizine standard and in human plasma sample.

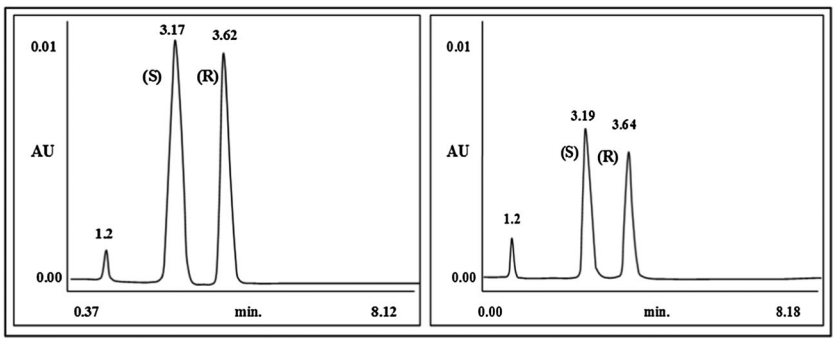


Fig. 6. HPLC chromatograms of brinzolamide standard and in human plasma sample.

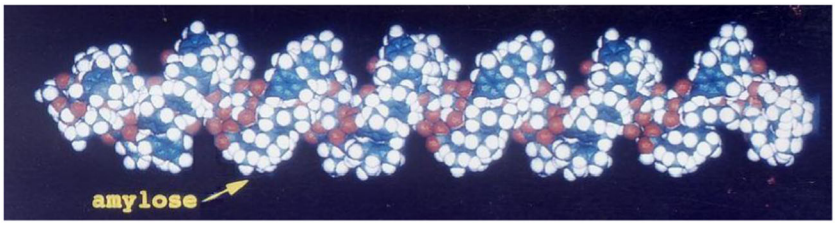


Fig. 7. 3D structure of amylase polymer.

have also been made to determine the interaction of the enantiomers with chiral selectors by a modeling approach. In silico studies of the chiral recognition mechanism is discussed in the following section.

Simulation Studies of the Drugs on AmyCoat Chiral Selector

In silico study is an attractive tool to assess the binding of molecule to receptor. In the present work, attempts were made to establish the enantiomeric interactions on a chiral stationary phase. Amylose-based columns have different types of functional groups responsible for various types of bondings. The docking energies of R- and S-enantiomers of pheniramine, oxybutynin, cetirizine, and brinzolamide were −6.55 and −5.25, –4.90 and −6.10, –7.95 and −7.43, and −8.55 and −8.13 kcal/mol, respectively. The differences of docking energies (ΔE) of R- and S-enantiomers of pheniramine, oxybutynin, cetirizine, and brinzolamide drugs were 1.30, 1.20, 0.52, and 0.42 kcal/mol, respectively (Table 3). It is clear from Table 3 that the energy values of R-enantiomers are greater than S-enantiomers of pheniramine, cetirizine, and brinzolamide. Contrarily, the S-enantiomer of oxybutynin has greater energy than the R-antipode. Therefore, it may be predicted that the first eluted enantiomers of pheniramine, cetirizine, and brinzolamide were of S- configuration. In the case of oxybutynin drug the first eluted enantiomers were of R- configuration.

The chromatographic experimental results (retention times) of the enantiomeric resolution can be correlated with the docking output. Δt_R of enantiomeric resolution was on the order of pheniramine (1.30 min) > oxybutynin (1.05 min) cetirizine (0.59 min) > brinzolamide (0.45 min). These results were verified by the order of docking energy differences (ΔE), i.e., pheniramine (1.30 kcal/mol) > oxybutynin (1.20 kcal/mol) > cetirizine (0.52 kcal/mol) > brinzolamide (0.42 kcal/mol). Therefore, close chiral resolution of brinzolamide is because of the low binding energy difference of the enantiomers (due to the absence of π-π interactions). On the other hand, the binding energy differences were quite good for enantiomers of pheniramine, oxybutynin, and cetirizine and, hence, well resolution.

R-pheniramine had one H-bond (2.99 Å), between nitrogen and oxygen atoms of pheniramine and amylose, respectively, while S-pheniramine had no H-bond with the chiral receptor. Similarly, R-Cetirizine showed one H-bond (2.073 Å) between the oxygen and nitrogen atoms of cetirizine and amylose, respectively, while H-bonds was absent in S-cetirizine. The S-enantiomer of brinzolamide was able to form one H-bond (1.947 Å) between the oxygen and nitrogen atoms of brinzolamide and amylose, respectively, while it lacked in R-antipode. In the case of both R- and S-enantiomers of oxybutynin, H-bonding was absent. On the other hand, the bondings such as van der Waal's forces, electrostatic, and hydrophobic interactions were also playing key roles. The docking models of the enantiomers of these drugs are shown in Figure 8.

TABLE 3. Modeling results of R- and S-enantiomers of pheniramine, oxybutynin, cetirizine, and brinzolamide drugs with *tris*-(3,5-dimethylphenyl carbamate) amylose chiral selector

Name of enantiomeric drugs	Binding energy	Total Internal	Docking energy	*$\Delta E = R_{DE} - S_{DE}$
Pheniramine				
(R)	−5.85	−0.7	−6.55	1.30
(S)	−4.57	−0.68	−5.25	
Oxybutynin				
(R)	−3.83	−1.07	−4.90	1.20
(S)	−4.69	−1.41	−6.10	
Cetirizine				
(R)	−5.57	−2.38	7.95	0.52
(S)	−5.15	−2.28	−7.43	
Brinzolamide				
(R)	−8.09	−0.46	−8.55	0.42
(S)	−7.62	−0.51	−8.13	

*$|R_{DE} - S_{DE}|$ = Difference in the docking energies of enantiomers.

VALIDATION OF CHIRAL HPLC AND SPE METHODS

The developed chiral HPLC and SPE methods were validated by carrying out five sets (n = 5) of the chromatographic and SPE extraction procedures under identical experimental conditions. It is interesting to note that the values of chromatographic parameters for the reported drugs (Table 2) were almost equal in standard solutions and human plasma extracts, which indicated the robustness of the chiral HPLC method. The validation of the developed method was ascertained by regression analysis using the Excel Microsoft program. The results for chiral chromatographic and SPE methods regression analysis are given in Table 4. A perusal of Table 4 indicates that the values of standard deviation (SD), correlation coefficient (CC), and confidence limit (CL) of chiral HPLC varied from ±0.10 to ±0.11, 0.9996 to 0.9999, and 99.0 to 99.2, respectively. On the other hand, these values for SPE method ranged from ±0.11 to ±0.13, 0.997 to 0.9998, and 99.0 to 99.1, respectively. The data of the peak area versus drug concentrations were treated by linear least square regression analysis. The linearity was calculated according to DIN 32645 with B.E.N. v. 2.0[39] and the values are given in Table 5. It is clear from Table 5 that the linearity ranges were 1.0–50 μg/L for cetirizine, pheniramine, and brinzolamide. On

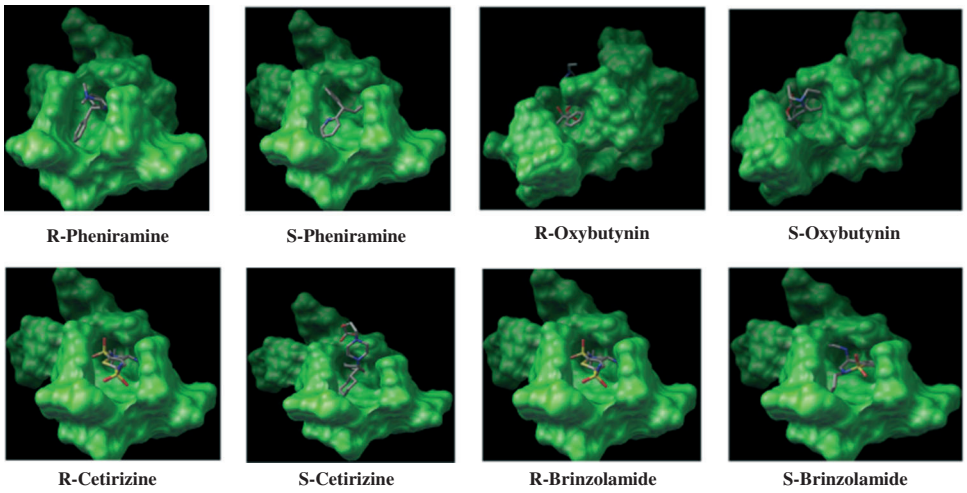


Fig. 8. Docking model of R- and S-enantiomers of pheniramine, oxybutynin, cetirizine, and brinzolamide with amylose chiral selector.

TABLE 4. Regression analysis data for the chiral chromatographic and SPE methods of pheniramine, oxybutynin, cetirizine, and brinzolamide drugs

	Chiral HPLC (peak area)			SPE (percentage recoveries)		
Compounds	SD	CR	CL	SD	CR	CL
Pheniramine	±0.11	0.9997	99.0	±0.12	0.9998	99.0
Oxybutynin	±0.11	0.9998	99.1	±0.12	0.9998	99.0
Cetirizine	±0.12	0.9996	99.0	±0.13	0.9997	99.0
Brinzolamide	±0.10	0.9999	99.2	±0.11	0.9998	99.1

CC: correlation coefficient, CL: confidence limit. and SD: standard deviation.

TABLE 5. Linearity of pheniramine, oxybutynin, cetirizine, and brinzolamide drugs

Compounds	Lin. range	CC	CL (%)
Pheniramine	1.0–50 μg/L	0.9998	99.0
Oxybutynin	1.0–40 μg/L	0.9998	99.1
Cetirizine	1.0–50 μg/L	0.9997	99.0
Brinzolamide	1.0–50 μg/L	0.9999	99.2

CC: correlation coefficient and CL: confidence limit.

the other hand, the values of linearity range of oxybutynin were 1.0–40 μg/L. The values of correlation coefficients varied from 0.9997 to 0.9999 while the values of confidence limit were 99.0–99.2% for all four drugs studied. The limits of detection of cetirizine, pheniramine, oxybutynin, and brinzolamide were 1.5, 1.6, 1.0, and 1.4 μg/L, respectively. On the other hand, limits of quantification of these drugs were 6.5, 6.8, 5.2, and 6.8 μg/L, respectively. These validation values indicated good reproducibility of the reported chiral HPLC and SPE methods.

APPLICATION OF THE DEVELOPED AND VALIDATED HPLC METHOD IN REAL-WORLD SAMPLES

The validity of the developed method was applied to analyze the enantiomeric ratio of pheniramine, oxybutynin, cetirizine, and brinzolamide drugs in human plasma by SPE-HPLC. The qualitative and quantitative analyses of the reported drugs were carried out using the above-mentioned HPLC conditions (Figs. 3–6). The reported method is new for the determination of the order of elution of the R- and S-enantiomer of pheniramine, oxybutynin, cetirizine, and brinzolamide in human plasma by HPLC. The reported method is also confirmed by in silico studies.

CONCLUSION

An eco-friendly, reproducible, accurate, inexpensive, and effective SPE-Chiral-HPLC method was described for the chiral separations of pheniramine, oxybutynin, cetirizine, and brinzolamide drugs in human plasma. For calculation of the percentage recoveries of pheniramine, oxybutynin, cetirizine, and brinzolamide drugs, five sets (n = 5) of SPE experiments were carried out under identical experimental conditions. The percentage recoveries were carried out at different pHs, i.e., 5.0, 7.0, and 9.0. The maximum percentage recoveries of pheniramine, oxybutynin, cetirizine, and brinzolamide were 32.50 and 37.67, 34.61 and 37.33, 22.48 and 23.59, and 33.60 and 44.80, respectively, at pH 9.0. The values for LOD for pheniramine, oxybutynin, cetirizine, and brinzolamide ranged from 1.0–2.5 ng/mL, while these values for LOQ were 5.0–10.0 ng/mL, respectively. The experimental results of the proposed method were compared with modeling output and both were in good agreement. Therefore, the reported method can be used for monitoring the chiral ratio of these drugs in any biological samples. In addition, the modeling approach is useful to ascertain the configuration of the separated enantiomers.

ACKNOWLEDGMENT

The authors thank King Saud University for funding this project through the Visiting Professor Program.

LITERATURE CITED

1. Ali I, Alam SD, Al-Othman ZA, Farooqi JA. Recent advances of chiral drugs by SPE-HPLC in biological samples. J Chromatogr Sci 2013;51: 645–654.
2. Aboul-Enein HY, Ali I. *Chiral separation by liquid chromatography and related technologies*. New York: Marcel & Dekker; 2003.

3. Deng X, Cock BD, Vervoort R, Pamperin D, Erwin A, Schepdael AV. Development of a validated capillary electrophoresis method for enantiomeric purity control and quality control of levocetirizine in a pharmaceutical formulation. Chirality 2012;24:276–282.
4. Ali I, Gupta VK, Aboul-Enein HY, Singh P, Sharma B. Role of racemization in optically active drugs development. Chirality 2007;19:453–463.
5. Ali I, Hussain A, Aboul-Enein HY, Bazylak G. Supramolecular systems-based HPLC for chiral separation of β-adrenergics and β-adrenolytics in drug discovery schemes. Curr Drug Discov Technol 2007;4:255–274.
6. Dai Z, Pittman CUJR, Li T. Enantiomeric separation of racemic 4-aryl-1,4-dihydropyridines and 4-aryl-1,2,3,4-tetrahydropyrimidines on a chiral tetraproline stationary phase. Chirality 2013;25:238–242.
7. Ali I, Gaitonde VD, Aboul-Enein HY, Hussain A. Chiral separation of β-adrenergic blockers on CelluCoat column by HPLC. Talanta 2009;78:458–63.
8. Simonyi M, Fitos I, Visy J. Chirality of bioactive agents in protein binding storage and transport process. Tips 1986;7:112–116.
9. FDA policy statement for the development of new stereoisomeric drugs. Chirality 1992;4:338–340.
10. Escuder-Gilabert L, Martínez-Gómez AM, Villanueva-Camañas RM, Sagrado S, Medina-Hernández MJ. Microseparation techniques for the study of the enantioselectivity of drug–plasma protein binding. Biomed Chromatogr 2009;23:225–238.
11. Mikus P, Valskov I, Havrnek E. Enantioselective analysis of cetirizine in pharmaceuticals by cyclodextrin-mediated capillary electrophoresis. J Sep Sci 2005;28:1278–1284.
12. Bingyun L, Haynie DT. Chiral drug separation. In: Lee S, editor. *Encyclopedia of chemical processing*. London: Taylor & Francis; 2006. p 449–458.
13. Ding-Qiang L, Xiu Li, Yan-Liang L, Jia-quan C, Shiba T. Resolution of chiral drugs research. Available at: http://eng.hi138.com/?i132501 (Accessed 2013-3-25 17:04:00).
14. Pflum DA, Wilkinson HS, Tanoury GJ, Kessler DW, Kraus HB, Senanayake CH, Wald SA. A large-scale synthesis of enantiomerically pure cetirizine dihydrochloride using preparative chiral HPLC. Org Process Res Dev 2001;5:110–115.
15. Golightly LK, Greos LS. Second-generation antihistamines, actions and efficacy in the management of allergic disorders. Drugs 2005;65:341–384.
16. Ganellin CR. The histamine receptor. In: Schwartz JC, Haas HL, editors. *Receptor biochemistry and methodology*, Vol. 16. New York: Wiley-Liss; 1992. p 1–56.
17. Gillard M, Van Der PC, Moguilevsky N, Massingham R, Chatelain P. Binding characteristics of cetirizine and levocetirizine to human H1 histamine receptors: Contribution of Lys191 and Thr194. Mol Pharmacol 2002;61:1–9.
18. Gupta A, Chatelain P, Massingham R, Jonsson EN, Hammarlund-Udenaes M. Brain distribution of cetirizine enantiomers: comparison of three different tissue-to-plasma partition coefficients. Drug Metab Dispos 2006;34:318–323.
19. Gupta A, Hammarlund-Udenaes M, Chatelain P, Massingham R, Jonsson EN. Stereoselective pharmacokinetics of cetirizine in the guinea pig: role of protein binding. Biopharm Drug Dispos 2006;27:291–297.
20. Tillement JP, Testa B, Brée F. Compared pharmacological characteristics in humans of racemic cetirizine and levocetirizine, two histamine H1-receptor antagonists. Biochem Pharmacol 2003;66:1123–1123.
21. Benedetti MS, Plisnier M, Kaise J, Maier L, Baltes E, Arendt C, McCracken N. Absorption, distribution, metabolism and extraction of [14C]levocetirizine, the R enantiomer of cetirizine, in healthy volunteers. Eur J Clin Pharmacol 2001;57:571–582.
22. Wu HL, Huang CH, Chen SH, Wu SM. Chiral quantitation of pheniramine, chlorpheniramine, and brompheniramine maleates by capillary zone electrophoresis. J Chromatogr Sci 1999;37:24–30.
23. Kachur JF, Peterson JS, Carter JP, Rzeszotarski WJ, Hanson RC, Noronha-Blob L. R and S-enantiomers of oxybutynin: pharmacological effects in guinea pig bladder and intestine. J Pharmacol Exp Ther 1988;247:867–872.
24. Noronha-Blob L, Kachur JF. Enantiomers of oxybutynin: *in vitro* pharmacological characterization at M1, M2 and M3 muscarinic receptors and in vivo effects on urinary bladder contraction, mydriasis and salivary secretion in guinea pigs. J Pharmacol Exp Ther 1991;256:562–567.
25. Walker TA. The chiral separation of oxybutynin enantiomers using an ovomucoid column. J Liq Chrom Rel Technol 2000;23:841–853.
26. Kowalski P, Plenis A. Comparison of HPLC and CE methods for the determination of cetirizine dihydrochloride in human plasma samples. Biomed Chromatogr 2007;21:903–911.
27. Liu Q, Zhang Z, Bo H, Sheldon RA. Direct separation of the enantiomers of cetirizine and related compounds by reversed-phase chiral HPLC. Chromatographia 2002;56:233–235.
28. Choi Sk, Lee SH, Kong HS, Kirn EJ, Hae-Young PC. Enantioselective determination of cetirizine in human urine by HPLC. Arch Pharm Res 2000;23:178–181.
29. Kang SW, Jang HJ, Moore VS, Park JY, Kim KA, Youm JR, Han SB. Enantioselective determination of cetirizine in human plasma by normal-phase liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry. J Chromatogr B 2010;878:3351–3357.
30. Schuster A, Bernhardt G, Eibler E, Buschauer A, Hesselink W. Chiral separation of 3-phenyl-3-(2-pyridyl)propylamines, andanalogous guanidines and guanidine-N-carboxylic acid esters with high-performance liquid chromatography and capillary zone electrophoresis. J Chromatogr A 1998;793:77–90.
31. Ali I, Al-kindy SMZ, Suliman FO, Alam SD. Fast analysis of flavonoids in apple juice on new generation halo column by SPE-HPLC. Anal Methods 2011;3:2836–2841.
32. Gergov M, Robson JN, Duchoslav E, Ojanpera I. Automated liquid chromatographic/tandem mass spectrometric method for screening beta-blocking drugs in urine. J Mass Spectrom 2000;35:912–918.
33. Ali I, Alam SD, Farooqi JA, Nagae N, Gaitonde VD, Aboul-Eneine HY. A comparison of β-blockers separation on C_{18} and new generation C_{28} columns in human plasma. Anal Methods 2013;5:3523–3529.
34. Morris GM, Goodsell GS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem 1998;19:1639–1662.
35. Ali I, Kümmerer K, Aboul-Enein HY. Mechanistic principles in chiral separations using LC and CE. Chromatographia 2006;63:295–307.
36. Ali I, Saleem K, Gaitonde VD, Aboul-Enein HY, Hussain I. Chiral separations of some β-adrenergic agonists and antagonists on AmyCoat column by HPLC. Chirality 2010;22:24–28.
37. Ali I, Al-Othman ZA, Hussain A, Saleem K, Aboul-Enein HY. Chiral separation of β-adrenergic blockers in human plasma by SPE-HPLC. Chromatographia 2011;73:251–256.
38. Al-Othman ZA, Ali I. Rapid and economic chiral-HPLC method of nebivolol enantiomers resolution in dosage formulation. Biomed Chromatogr 2012;26:775–778.
39. Goll M, Scbmitt G, Gangmann B, Aderjan RE. Excretion profiles of ethyl glucuronide in human urine after internal dilution. J Anal Toxicol 2002;26:262–266.