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Chiral separation and modeling of baclofen, bupropion, and etodolac profens on amylose reversed phase chiral column

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Abstract

Chiral resolution of baclofen, bupropion, and etodolac profens was obtained with amylose derivatized chiral reversed stationary phase (carbamate groups). The eluent used for bupropion and etodolac was MeOH–water (20:80, *v*/v) and for baclofen was water–methanol (95:5, *v*/v). The eluent run rates, finding wavelength and temperature, were 1.0 mL/min, 220 nm and 27 ± 1 °C for all the eluents. The magnitude of the retardation factors for S- and R-enantiomers of baclofen, bupropion, and etodolac were 1.37, 2.62, 2.25, 3.25, 1.8, and 3.0. The magnitudes of separation and resolution factors were 1.90, 1.44, and 1.67 and 2.77, 2.35, and 2.04. Limits of detection and quantitation were 1.0–2.0 and 5.1–10.0 μg/mL. Chiral recognition mechanisms were recognized by simulation and high-performance liquid chromatography (HPLC) experiments. It was seen that hydrogen interactions, hydrophobic interactions, and π–π exchanges were the chief interactions for chiral recognition mechanisms. The described methods may be exploited for the chiral separation of baclofen, bupropion, and etodolac profens in any unknown sample.

KEYWORDS

amylose chiral column, baclofen, bupropion, chiral HPLC-separation, docking studies, etodolac

1 | INTRODUCTION

The chiral separation of racemic drugs became important since the guidelines of the US Food and Drug Administration (FDA) for marketing of optically active drugs were published.[1] The FDA formulated certain guidelines due to the dissimilar pharmaceutical properties of the enantiomers. In several research papers, it was mentioned that one enantiomer is pharmaceutically active while the other is dormant or lethal or ballast. A mostly inactive enantiomer creates a range of side effects and harm in the human body.[2] This is due to certain features, such as a dissimilar stereoselective metabolism, allocation rate, excretion, and clearance in the human body. Consequently, medical practitioners, researchers, academicians, industry, and government are looking for a practical approach to the chiral separation of racemates. Now there are guidelines for selling racemic drugs all over the world. The most crucial agencies issuing these guidelines are the FDA, the European Medicines Agency, and Canadian and Japanese health agencies.[3-6]

Aryl propionic acids, so-called profens, are nonsteroidal antiinflammatory (NSAID) medicines. These are sold for curing puffiness and arthritis.[7,8] The most regularly used profens are baclofen, bupropion, and etodolac (Figure 1).[9] The WHO in 2001 reported profens as the most usually prescribed medicines in about 100 countries and, hence, incorporated in the list of indispensable medicines.[10] Approximately 30 million people use NSAIDs every day for many diseases.[11]. The yearly world sale market of these drugs is $12 billion US, and is assumed to increase to $30 billion US by the end 2017.[12] Profens show serious problems despite their wide applications.[13-17] The serious undesirable

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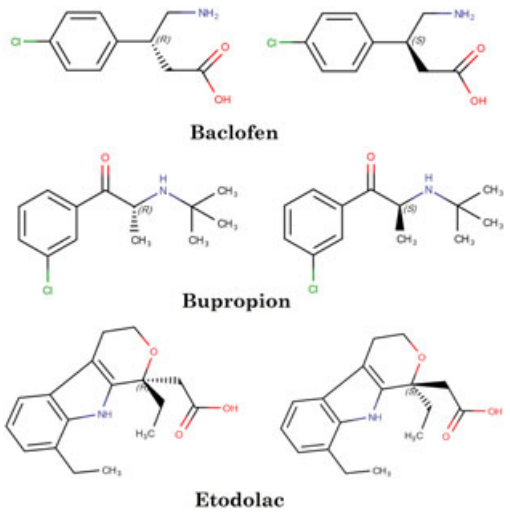

FIGURE 1 Enantiomeric structures of baclofen, bupropion and etodolac

effects are ulceration, gastric bleeding, vomiting, [18-20] dyspepsia,[21] bowel swelling,[22] and mucus injury in the small intestine.[13] Profens also create risk to the kidneys concerning fluid and salt preservation. Profens are also accountable for heart-related issues, producing ten and two times an additional probability of a heart assault.[14,23] As per a research described in the *American Journal of Medicine*, ~107,000 patients are admitted to hospitals yearly due to gastric problems after consumption of profens.[24] Out of these, ~16,500 persons died yearly.[25] Profens also show risk effects on the central nervous system after interaction with fluoroquinolones in combination therapy.[26] Every so often, people suffered coma, resulting in death.[16] The 50% side effects and toxicities may be reduced by describing optically active profens. Besides, it is not known which enantiomer is creating these problems. There are chances that all these side effects and problems may be controlled by prescribing the optically pure profens.

A thorough look for scientific articles in the literature[27-33] confirm that only some research articles described chiral resolution of baclofen, bupropion, and etodolac. Most of the articles described the enantiomeric resolution of a single drug, while some used costly and toxic solvents along with a high resolution time. Therefore, there is an immense need to produce fast, economic, and efficient enantiomeric resolution of the separation of these molecules. Additionally, there is also a requirement to describe the chiral recognition mechanism. As a result, chiral separation methods were produced for baclofen, bupropion, and etodolac on an amylose derivatized chiral reversed stationary phase. In addition, a chiral recognition mechanism was evaluated by simulation of the column with simulation studies to describe the enantiomeric mechanism. These results of these studies are presented in this article (Figure 1).

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

The (±)-mixtures of baclofen, bupropion, and etodolac were extracted from the commercial drugs available in the market. Methanol of high-performance liquid chromatography (HPLC) grade was provided by Merck (Bombay, India). Ionized water was obtained through a Millipore Milli-Q (Bedford, MA) water production machine. Standard solutions of 1.0 mg/L concentration of bupropion and etodolac were made in MeOH, while of baclofen was made in methanol–water (5:5, *v*/v), methanol, respectively.

2.2 | Instrumentation

HPLC runs were made on a machine manufactured by Shimadzu (Japan). The HPLC machine comprised software Class VP, pump (LC-10 AT VP), and ultraviolet range detector (SPD-10A). The stationary phase used was tris-(3,5-dimethylphenyl carbamate (carbamate groups), packed in a steel column (15.0 x 0.46 cm), with trade name AmyCoat-RP (Kromasil, Bohus, Sweden).

2.3 | HPLC conditions

All the experiments were made on an HPLC machine as depicted with 20.0 μL of profens solution loaded onto the HPLC machine. The eluent used for bupropion and etodolac was methanol–water (20:80, *v*/v) and for baclofen was methanol–water water–methanol (59:5, *v*/v). The eluent run rate, finding wavelength, and temperature were 1.0 mL/min, 220 nm, and 27 ± 1 °C for all the eluents. The eluents were cleaned and degassed every day before use. The eluents were cleaned through a nylon membrane (25 mm diameter with 0.45 μm pore size). The retention (k), separation (α), and resolution (Rs) factors were ascertained.

2.4 | Simulation studies

The exchanges of enantiomers of the reported profens with amylose derivatized chiral selector (Figure 2).were ascertained by docking studies. The results achieved were utilized to fix the chiral recognition mechanisms.

2.5 | Procedure

The docking study of the compounds baclofen, bupropion, and etodolac were done with an Intel dual CPU (1.86 GHz) with Windows XP operating software. Marwin Sketch software was exploited to sketch the structures of different antipodes. The structures were

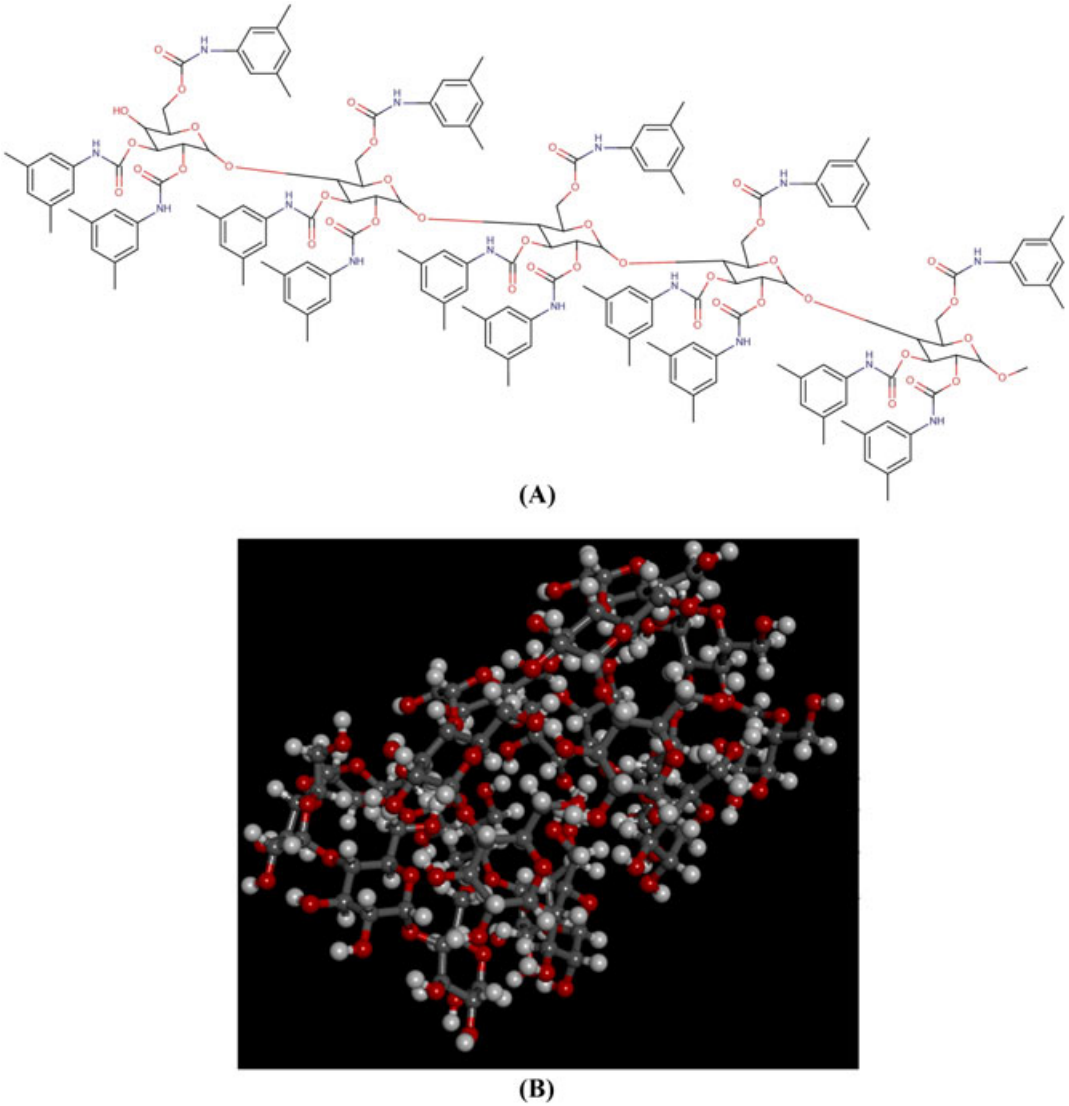

FIGURE 2 2D (**a**) and 3D (**b**) structures of tris-(3,5- dimethylphenyl carbamate) amylose chiral selector

sparked to 3D and saved in a PDB file.[34] After that, the structure of amylose derivatized chiral stationary phase was sketched using Marwin Sketch software. The structure of amylose derivatized chiral stationary phase was docked using AutoDock Tools (ADT) 4.2 by handing over Gastegier charges, integrating nonpolar hydrogen atoms, and saving in PDBQT file format. Docking was done with AutoDock 4.2 allowing for all the rotatable bonds of the ligands as a rotatable and rigid receptor.[35] Using the same tool, an enantiomer (ligand) was edited to be saved in PDBQT format. The lattice box size of 60 Å × 80 Å × 110 Å with spacing (0.375 Å) was used. After saving both files in PDBQT format, Vina software was used to obtain the binding energy/affinity between the receptor tris-(3,5-dimethylphenyl carbamate) amylose and ligand (enantiomer). After using Vina software, the output file was opened in PyMOL to do virtual screening, molecular docking, and a binding site study and to obtain an image of interaction and the bond length of the hydrogen bond between crown ether and enantiomer. The plugin represents a crossing point among PyMOL and two docking programs of AutoDock Vina and AutoDock (4.2). The joint effect of the two softwares completed wide use of a Python script compilation (AutoDock Tools) for fixing docking experiments. Additionally, Ligplot software was applied for the assessment of hydrophobic interactions. Fifty self-determining docking experiments were done for all the enantiomers separately and the chiral selector for low free energy of fastening conformation from a large cluster, which was written and saved in PDBQT layout. The PDBQT files had been transformed to the PDB file format.

3 | RESULTS AND DISCUSSION

3.1 | Chromatography

The HPLC measurements of retention (k), separation (α), and resolution (Rs) factors were ascertained for the enantiomers of baclofen, bupropion, and etodolac on amylose derivatized chiral stationary phase. The magnitude of the enantioselectivity for S- and R-enantiomers of baclofen, bupropion, and etodolac were 1.37, 2.62, 2.25, 3.25, 1.8, and 3.0. The magnitudes of separation and resolution factors were 1.90, 1.44, and 1.67 and 2.77, 2.35, and 2.04. These magnitudes are given in Table 1. The magnitude of separation and resolution factors were >1, showing acceptable chiral separation of all three racemic drugs. The separated peaks of HPLC are shown in Figure 3. Further, a serious examination of HPLC measurements and the peaks established good chiral separation of all the enantiomers of the three racemic drugs.

3.2 | Chiral HPLC method optimization

The different compositions of the eluents were tried in order to optimize the HPLC conditions. The various organic solvents tried with water were methanol, ethanol, acetonitrile, and propanol in different amounts. The various pHs of these combinations were also adjusted and tested. The ultraviolet wavelength varied was 210–300 nm. The concentrations of injection used were 5–25 μL. The optimization was fixed by using temperatures from 15–40 °C. As a consequence of comprehensive testing, the best chiral chromatographic conditions were produced and described. Chiral chromatographic methods were validated via accuracy, selectivity, precision, robustness, limits of detection and quantitation, and linearity.

3.3 | Simulation study of dipeptides on AmyCoat chiral selector

The chiral mechanism was determined by a docking study of the enantiomers with amylose chiral selector. The chiral selector showed various functionalities, which interacted with the enantiomers of the profens. The docking affinities (energies) of R- and S-enantiomers of baclofen, bupropion, and etodolac were −3.4 and −3.3, −3.4 and −3.2, −3.8 and −3.9, K cal/mol (Table 2). It is apparent from Table 2 that the various interactions were hydrogen bondings and hydrophobic interactions. The elution order may be predicted by considering the binding affinities (kcal mol^{-1}) as reported. The binding energies of R-enantiomers are higher that S-enantiomers for all three drugs. Therefore, it may be assumed that S-enantiomers eluted first following R-enantiomers. The different interactions among various residues of the chiral selector and enantiomers are outlined below.

TABLE 1 The capacity (k), separation (α), and resolution (Rs) factors of the enantiomers of of baclofen, bupropion and etodolac on tris-(3,5-dimethylphenyl carbamate) amylose chiral selector

Profens	k_1 S-enantiomer	k_2 R-enantiomer	α	Rs
Baclofen	1.37	2.62	1.90	2.77
Bupropion	2.25	3.25	1.44	2.35
Etodolac	1.8	3.00	1.67	2.04

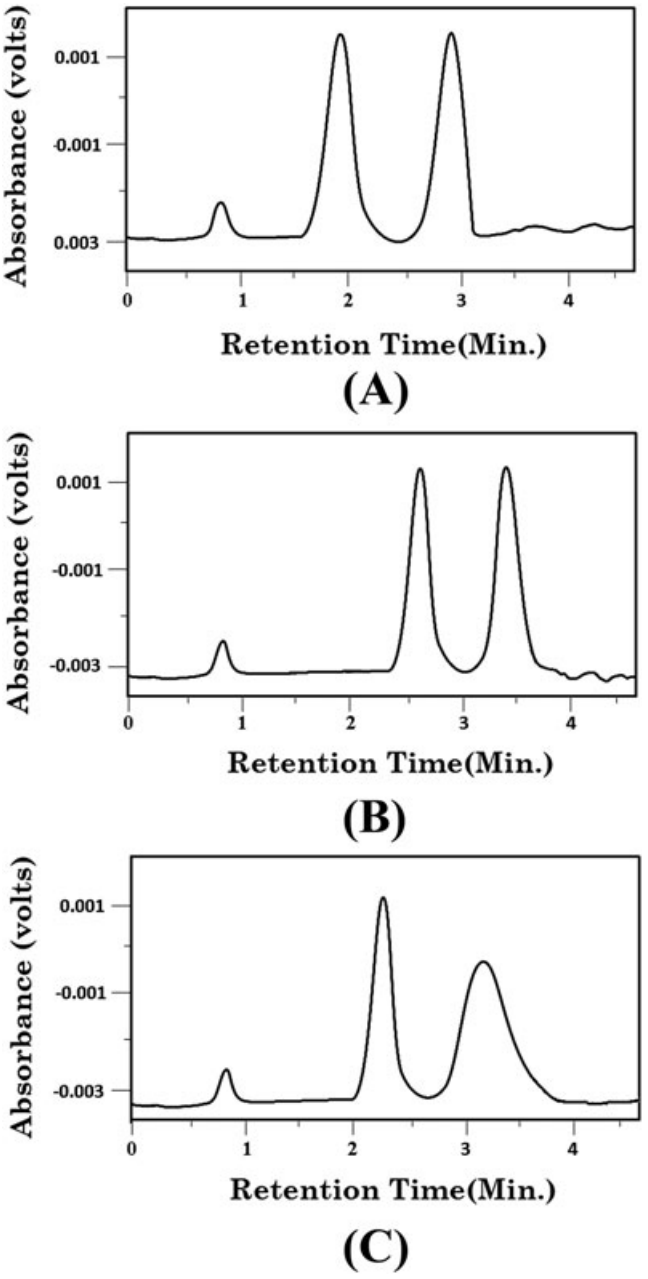

FIGURE 3 Chiral resolution of (**a**): baclofen, (**b**): bupropion and (**c**) etodolac on AmyCoat RP column (150 x 4.6 6 mm, 5.0 μm)

TABLE 2 Modeling results of the enantiomers of baclofen, bupropion, and etodolac with tris-(3,5-dimethylphenyl carbamate) amylose chiral selector

S. no.	Enantio-mers	Binding affinity (kcal mol^{-1})	No. of hydrogen bonds	Residues involved in H-bonding (*Bond length in Å*)	Residues involved in hydrophobic interactions
Baclofen					
1.	R	-3.4	2	UNK.390::H of NH_2 group(2.8) UNK.390::H of NH_2 group(1.9)	Unk 159,161,163::C2, Unk 159::C3, Unk 163::C4, Unk 157,159::C5, Unk 157,287:: C7, Unk 287::C8, Unk 287,288::C9, Unk 157,287::C10, Unk 155,158::N, Unk 284,285::O1, Unk 155::O2
2.	S	-3.3	2	UNK.348::H of NH_2 group (2.0) UNK.388::H of NH_2 group (2.7)	Unk 158,159::C1, Unk 191::C2, Unk 162::C3, Unk 191::C4, Unk 158::C5, Unk 156,157::C7, Unk Unk 129,314,316::C9, Unk 157,316, 287::C10, Unk 157,158::N, Unk 128, 192::O1, Unk 313,315::O2
Bupropion					
3.	R	-3.4	2	UNK`.388::H of NH group (2.5) UNK`.289::H of -OH group (*2.3*)	Unk 188::C1, Unk 158::C2, Unk 191, 158::C3, Unk 157,316::C4, Unk 155,287, 288::C5, Unk 157,159, 161:: C7, Unk 157,158::C8, Unk 158,192: :C10, Unk 287,316::C12, Unk 287::C13
4.	S	-3.2	2	UNK`.390::H of NH group (2.0) UNK`.289::H of -OH group (*2.4*)	Unk 185,189::C1, Unk 185,131::C2, Unk 189::C3, Unk 188::C4, Unk 307, 308::C5, Unk 182,184,185,186,308, 310::C6, Unk 131::C8, Unk 188, 189::C10, Unk 310::C11, Unk 310::C12, Unk 310::C13, Unk 131::O
Etodolac					
5.	R	-3.8	2	UNK`.353::H of -OH group (*2.8*) UNK`.647::H of -OH group (*2.4*)	Unk 163::C1, Unk 158,162::C2, Unk 158, 159::C5, Unk 153::C6, Unk 161,157:: C7, Unk 158,159::C8, Unk 155,287::C9, Unk 157,158, 316::C10, Unk 157,158,159::C11, Unk 157,158::C12, Unk 316::C17, Unk 158::N, Unk 284::O3
6.	S	-3.7	2	UNK`.353::H of -OH group (*2.8*) UNK`.647::H of -OH group (*2.1*)	Unk 159,162::C1, Unk 162::C2, Unk 159::C4, Unk 157,287::C6, Unk 158, 159::C7, Unk 162::C8, Unk 157::C9, Unk 158,159::C11, Unk 88,190,191, 192::C14, Unk 128,129,191,192, 313,315::O3

3.4 | Baclofen

3.4.1 | R-enantiomer

Oxygen of the –OH group in tris-(3,5-dimethylphenyl carbamate) amylose::H of $–NH_2$ group in baclofen (*2.8 Å*). Oxygen of –OH group in tris-(3,5-dimethylphenyl carbamate) amylose::H of $–NH_2$ group in baclofen (*1.9 Å*).

3.4.2 | S-enantiomer

Oxygen of the –OH group in tris-(3,5-dimethylphenyl carbamate) amylose::H of $–NH_2$ group in baclofen (*2.7 Å*). Oxygen of (−O-) group in tris-(3,5-dimethylphenyl carbamate) amylose::H of $–NH_2$ group in baclofen (*2.0 Å*).

3.5 | Bupropion

3.5.1 | R-enantiomer

Oxygen of –OH group in tris-(3,5-dimethylphenyl carbamate) amylose::H of –NH group in bupropion (*2.5 Å*). Oxygen of (−CO-) group in bupropion::H of –OH group in tris-(3,5-dimethylphenyl carbamate) amylose (*2.3 Å*).

3.5.2 | S-enantiomer

Oxygen of –OH group in tris-(3,5-dimethylphenyl carbamate) amylose::H of –NH group in bupropion (*2.0 Å*). Oxygen of (−CO-) group in bupropion::H of –OH group in tris-(3,5-dimethylphenyl carbamate) amylose (*2.4 Å*).

3.6 | Etodolac

3.6.1 | R-enantiomer

Oxygen of –O- group in etodolac::H of –OH group in tris-(3,5-dimethylphenyl carbamate) amylose (*2.8 Å*). Oxygen of (−COOH) group in etodolac::H of –OH group in tris-(3,5-dimethylphenyl carbamate) amylose (*2.4 Å*).

3.6.2 | S-enantiomer

Oxygen of –O- group in etodolac::H of –OH group in tris-(3,5-dimethylphenyl carbamate) amylose (*2.8 Å*). Oxygen of (−COOH) group in etodolac::H of –OH group in tris-(3,5-dimethylphenyl carbamate) amylose (*2.1 Å*).

3.7 | Mechanisms of resolution at the supramolecular level

The chiral recognition mechanism is explained by chromatographic and modeling results. Amylose derivatized chiral selector is helical in shape (Figure 2).[36-40] It was seen that the amylose based chiral selector has wide range chiral separation capacity.[41-43] The chiral separation of profen enantiomers on amylose derivatized chiral selector is due to the chiral grooves in the structure of the stationary phase. The antipodes of these profens enantiomers set enantioelectively. The setting of the antipodes is controlled hydrogen, hydrophobic, π–π, van der Waal's, and steric effects interactions. It is attractive to report that the reported profens have a benzene ring facilitating π–π interactions among the antipodes and chiral amylose derivatives. It is already established that π–π interactions are crucial in the chiral separation of many aromatic racemates.[44-51] The setting of all the antipodes of profens in the derivatized amylose grooves is depicted in Figures 4–9. These were developed using modeling analyses. Two antipodes of each profen are involved in chiral grooves by electrostatic forces of attraction between amino groups, and carboxylic acid groups of profens and amino groups, amide groups, hydroxyl groups, and oxide groups of the derivatized amylose. Briefly, the enantiomers of the reported profens get set enantioelectively in derivatized amylose chiral grooves. Contrarily, the eluent attempted to take forward these antipodes. Consequently, the competitive forces of stationary and mobile phase resulted in the different retention of the antipodes. Low retained enantiomer eluted first and more retained enantiomer eluted last.

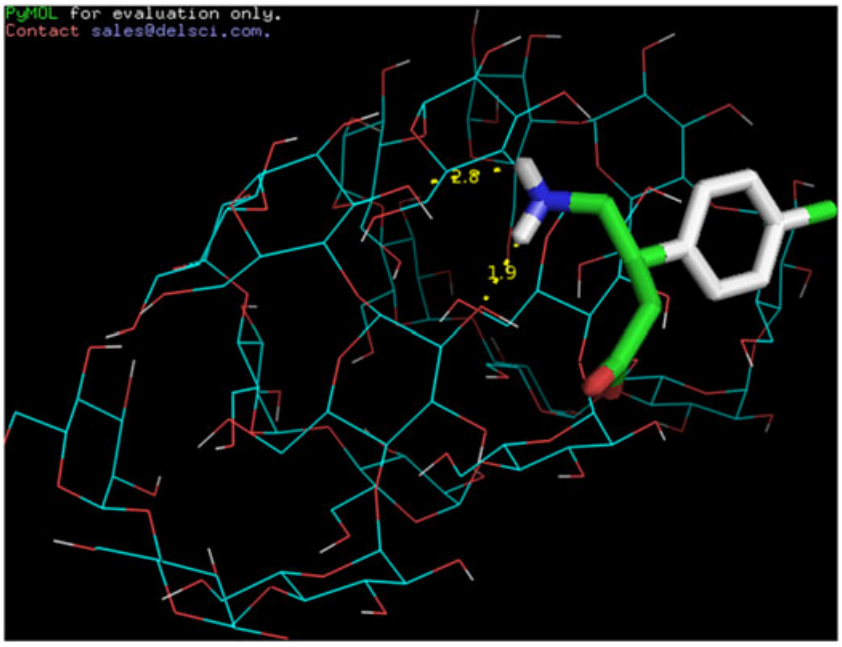

FIGURE 4 Docking model of R-baclofen with tris-(3,5-dimethylphenyl carbamate) amylose chiral selector

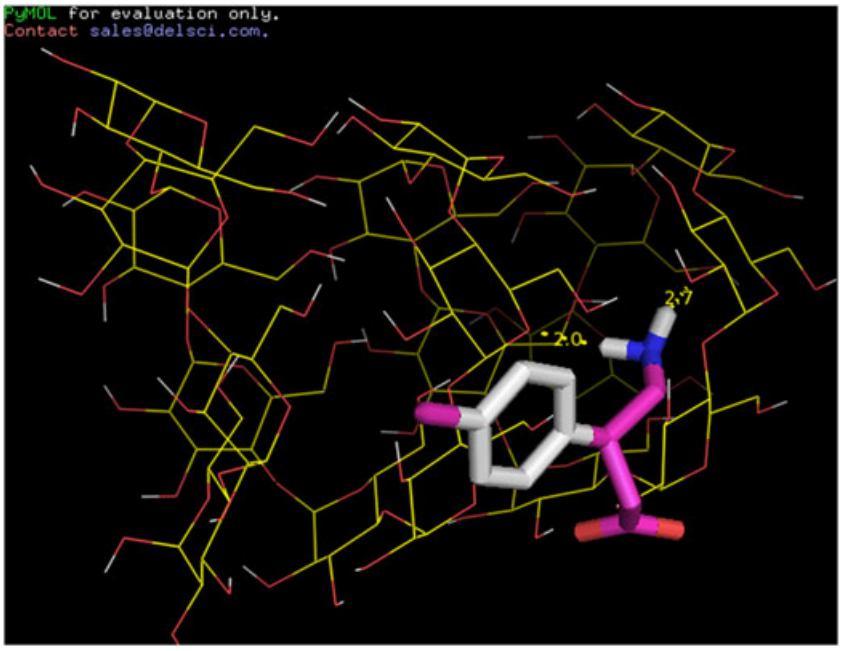

FIGURE 5 Docking model of S-baclofen with tris-(3,5-dimethylphenyl carbamate) amylose chiral selector

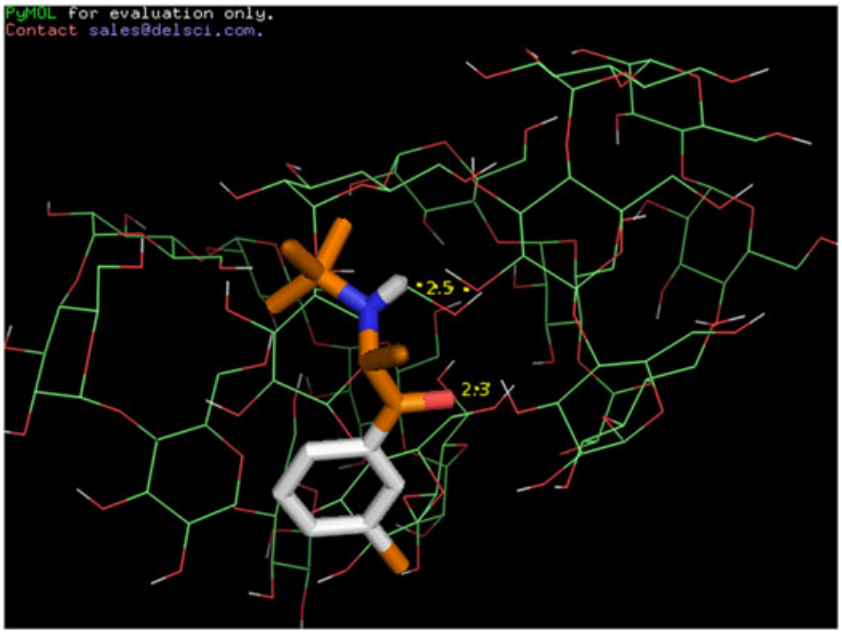

FIGURE 6 Docking model of R-bupropion with tris-(3,5-dimethylphenyl carbamate) amylose chiral selector

3.8 | Validation of the chiral HPLC method

The developed chiral chromatographic method was validated by five sets of experiments ($n = 5$) in similar conditions. The validation was carried out by accuracy, precision, linearity (2.0–200.0 μg/mL), limit of detection and quantification, specificity, and robustness. The validation was carried out as per standard methods. The produced chiral HPLC methods were fairly good specific, as shown in Figure 3. No effect of the supplemented impurities (in standard solution) was seen on the retention time chromatograms of these profens. Therefore, the conclusion pointed to good specificity of the chiral separation. The accuracy was determined by interpolation of replicates ($n = 5$) peak areas. The percent error was estimated and observed from 0.55–1.0% for each enantiomer. This range indicated a good accuracy of the developed method. The validation calculations were carried out with Excel software. The outcomes of the enantiomeric chromatographic method were determined in terms of percent relative standard deviation (%RSD), correlation coefficient (CC), and confidence limit (CL). The validation magnitudes showed good reproducible results.

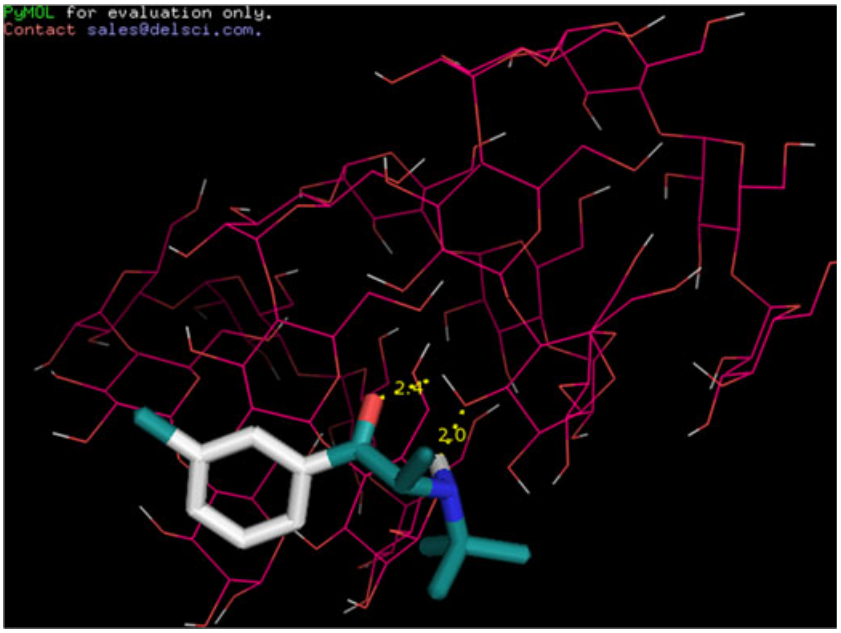

FIGURE 7 Docking model of S-bupropion with tris-(3,5-dimethylphenyl carbamate) amylose chiral selector

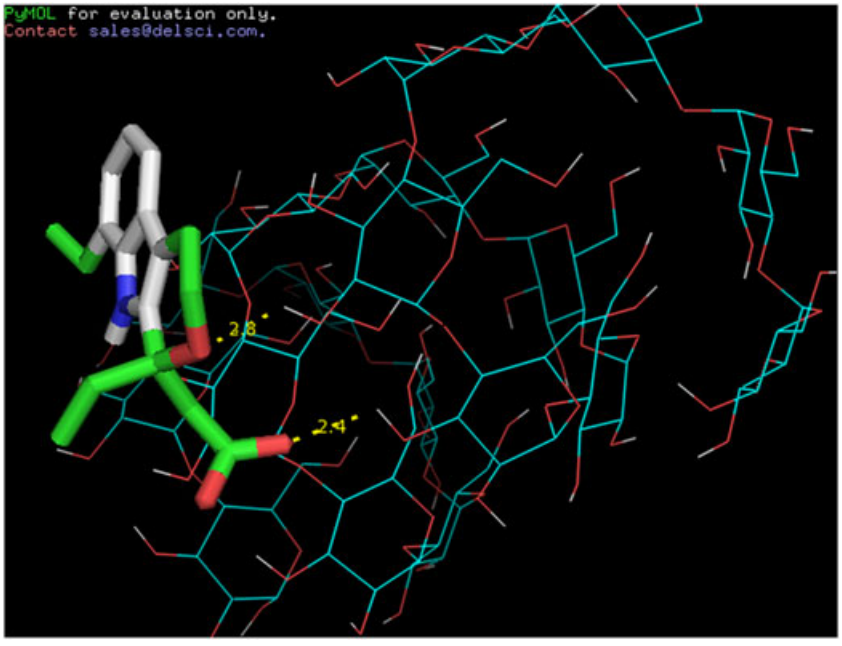

FIGURE 8 Docking model of R-etodolac with tris-(3,5-dimethylphenyl carbamate) amylose chiral selector

3.9 | System suitability test

Test of suitability was done with five loadings ($n = 5$) of the standard solution onto the HPLC injector. The calculated values were for tailing of the peaks, separation, percent RSD of the area of the peaks, and chromatographic times (Table 3). The values in Table 3 are indicative of low RSD, <1.5% (peak area) and <1.5% (chromatographic time). The peaks tailing of S- and R-enantiomers of baclofen and bupropion were 0.90 and, 1.0. Contrarily, the S- and R-enantiomers of etodolac showed 1.2 and 1.45 magnitudes.

3.10 | Specificity

The specificity of the methods was ascertained for the impurities and degradation of profen. The eluent was loaded onto a machine as blank. It was observed that no peak was seen in the blank situation. It confirmed these methods to be specific.

3.11 | Linearity

The linearity was ascertained by plotting the areas of the peaks versus various amounts of the profens. The slope, y intercepts, regression coefficients (r^2), and correlation

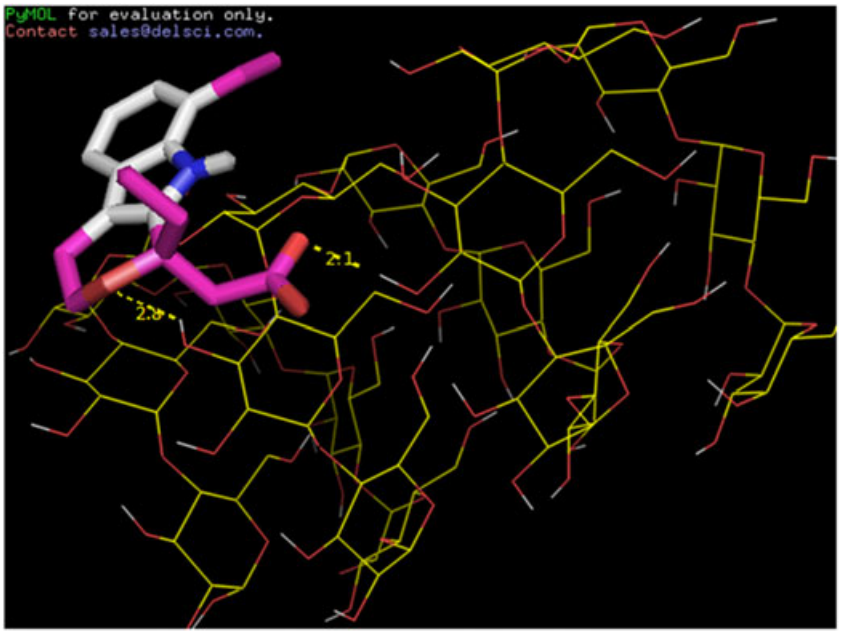

FIGURE 9 Docking model of S-etodolac with tris-(3,5-dimethylphenyl carbamate) amylose chiral selector

TABLE 3 Summary of the system suitability and linearity parameter

HPLC method	Baclofan		Bupropion		Etodolac	
Parameters	S	R	S	R	S	R
System suitability						
Tailing factor	0.90	1.0	0.90	1.0	1.2	1.45
Resolution	—	2.88	6.43	7.90	—	2.67
% RSD peak area	0.80	0.70	0.71	1.00	1.0	1.4
% RSD Retention time	0.50	0.30	0.60	0.90	0.35	0.95
Linearity						
Slope	4237.2	435.3	355.2	395.0	464.0	422.1
Y-intercept	−5730.0	−750	−730.1	−955	3136	−750.2
Corr. coeff. (r)	0.9985	0.9986	0.9967	0.9988	0.9980	0.9978
Regression coeff (r^2)	0.9866	0.9883	0.9854	0.9996	0.9856	0.9867
LOD (μg/mL)	1.0	1.0	1.0	1.0	1.1	2.0
LOQ (μg/mL)	5.1	5.1	5.1	5.1	5.5	10.0

coefficients (r) were estimated (Table 4). Superior linearity was seen for all the enantiomers of the reported profens in 2.0–200.0 μg/mL concentrations. The regression constant (r^2) was greater than 0.98.

3.12 | Limit of detection (LOD) and quantification (LOQ)

The limits of detection and quantification were ascertained as a signal-to-noise ratio of 3 for limit of detection and 10 for limit of quantitation. Limit of detection of R- and S-enantiomers of baclofen and bupropion was 1.0 μg/mL. Contrarily, these were 1.1 and 2.0 μg/mL for the S- and R- enantiomers of etodolac (Table 3).

3.13 | Precision

The intraday and interday precision was ascertained by standard procedures. The obtained data are summarized in Tables 4 and 5. The intraday precision was ascertained in a single day, while interday precision was done on the next day. The percent assay for intraday precision was calculated for the S- and R- enantiomers of baclofen, bupropion, and etodolac. These percent mean assays and

TABLE 4 Intraday precision

Baclofen	1.0 mg		1.5 mg		2.0 mg	
	% Mean assay	%RSD	% Mean assay	%RSD	% Mean assay	%RSD
S	100.0	0.55	99.9	0.55	99.0	0.56
R	99.9	0.55	99.7	0.55	99.0	0.56
Bupropion						
S	100.0	0.55	99.9	0.55	98.0	0.56
R	99.9	0.55	99.7	0.55	99.0	0.56
Etodolac						
S	99.00	0.58	98.6	0.58	98.0	0.60
R	98.0	0.65	97.5	0.65	97.0	0.69

TABLE 5 Interday precision

	Baclofen (mean % assay)		Bupropion (mean % assay)		Etodolac (mean % assay)	
Samples	S	R	S	R	S	R
Sample - 1	100.0	100.0	100.0	100.0	99.99	98.9
Sample - 2	100.0	100.0	100.0	100.0	99.98	98.9
Sample - 3	100.1	100.1	100.1	100.1	99.99	98.8
Sample - 4	100.0	100.0	100.0	100.0	99.98	98.9
Sample - 5	100.1	100.1	100.1	100.1	99.99	99.9
% RSD	0.55	0.55	0.55	0.55	0.70	0.74

%RSD are given in Table 4. Similarly, the values of interday percent mean assays and %RSD for these enantiomers are given in Table 5. These values of intraday and interday assays were fine.

3.14 | Recovery

The recovery was ascertained using three different spiked amounts of baclofen, bupropion, and etodolac profens. The spiked concentration of each drug was 50–100.0 μg/mL. The recoveries of the antipodes are given in Table 6. The percent recoveries were 99–101% for all six enantiomers.

TABLE 6 Recovery study

Concentration (μg/mL)	Baclofen (% recovery)		Bupropion (% recovery)		Etodolac (% recovery)	
	S	R	S	R	S	R
50	100.0	101.0	100.0	101.0	100.0	99.0
60	100.0	101.0	100.0	101.0	100.0	99.0
70	100.0	101.0	100.0	101.0	100.0	99.0
80	100.0	101.0	100.0	101.0	100.0	99.0
90	100.0	101.0	100.0	101.0	100.0	99.0
100	100.0	101.0	100.0	101.0	100.0	99.0
% RSD	0.55	0.56	0.55	0.56	0.71	0.75

TABLE 7 Robustness study for the new chiral HPLC method

Baclofen (variation in % RSD)	S		R	
	RT	Area	RT	Area
Flow rate (1.0 mL/min)	0.53	0.75	0.53	0.75
Flow rate (0.8 mL/min)	0.55	0.78	0.55	0.78
Column temperature (27 °C)	0.53	0.75	0.53	0.75
Column temperature (20 °C)	0.50	0.71	0.50	1.0
Bupropion (variation in % RSD)				
Flow rate (1.0 mL/min)	0.54	0.77	0.54	0.77
Flow rate (0.8 mL/min)	0.57	0.80	0.57	0.80
Column temperature (27 °C)	0.54	0.76	0.54	0.76
Column temperature (20 °C)	0.51	0.73	0.52	1.10
Etodolac (variation in % RSD)				
Flow rate (1.0 mL/min)	0.56	0.78	0.60	0.84
Flow rate (0.8 mL/min)	0.57	0.79	0.62	0.86
Column temperature (27 °C)	0.56	0.78	0.60	0.84
Column temperature (20 °C)	0.60	0.80	0.60	1.20

3.15 | Robustness

The robustness was ascertained by minor variation of one experimental variable at a time, while maintaining the other variables fixed. The changes were observed constant in the peaks, which might be affected by the presentation of the method. These values are presented in Table 7. The changes in run times (R_t) and the areas of the peaks were greater than 2, confirming a robust method.

4 | CONCLUSION

The chiral separation of baclofen, bupropion, and etodolac profens was achieved successfully on derivatized amylose reversed stationary phase. Limits of detections and quantitation were 1.0–2.0 and 5.1–10.0 μg/mL. Chiral recognition mechanisms were recognized by simulation and HPLC

experiments. It was seen that hydrogen interactions, hydrophobic interactions, and π–π exchanges were the chief interactions for the chiral recognition mechanism. The described methods may be exploited for the chiral separation of baclofen, bupropion, and etodolac profens in any unknown sample.

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