Chiral separation of terbutaline by supercritical fluid chromatography with peaks purity determination by UPLC-MS and modeling for chiral recognition mechanism

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Abstract:

Background: Terbutaline is the drug of choice for asthma patients but it exist in racemic mixture. (*R*)-(-)-terbutaline is 200 times more active than (*S*)-(+)-terbutaline and it is not advisable to prescribe racmix xiture due to certain side effects of (*S*)-(+)-terbutaline. Therefore, fast, effective and reproducible separation method is the need of today.

Results:

Chiral separation was achieved on Chiralpak IE and Chiralpak IG columns (250 mm x 4.6 mm, 5 µm) using CO_2-MeOH (60:40) with 0.2% triethylamine mobile phase. The flow was 1.0 mL/min with detection at 223 nm using a PDA detector. The values of retention, separation and resolution factors were in the range of 1.88 to 2.38, 1.14 to 1.26 and 0.91 to 1.17; with best separation with Chiralpak IE. The tailing factors and number of theoretical plates were in the range of 1.0 to 1.23 and 487 to 3699. The purity of the separated peaks was determined by UPLC-MS; indicating 100% purity of the peaks. The chiral recognition was determined by modeling with binding affinities -5.0 and -6.0 of *S*- and *R*-enantiomers; indicating *S*-enantiomers elution first followed by *R*-enantiomers. The major forces responsible for the chiral resolution were hydrogen bonding and π-π interactions.

Conclusion:

Due to the great demand for optically active pure drugs and high economic pressure on analytical techniques, the chiral separation of terbutaline was achieved on inexpensive supercritical fluid chromatography. The reported method may be used to prepare optically active pure terbutaline drugs (*R*-enantiomers) at a pilot scale.

Keywords: Terbutaline, Chiral-SFC, UPLC-MS purity, Mechanism of separation, Modelling.
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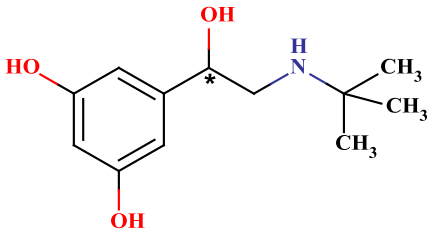
1. Introduction

Nowadays, asthma is the most common chronic disease found in all age groups and about 300 million people are suffering from this disease globally. Terbutaline is the most effective drug to treat this disease [1-3]. Terbutaline is a selective β_2-adrenoceptor agonist. Terbutaline sulfate, chemically β-[(tert-butylamino) methyl]-3,5-dihydroxy-benzyl alcohol ($C_{12}H_{19}NO_3$) is a synthetic β_2-adrenoceptor (β_2AR) agonist and generally used as a bronchodilator (Figure 1). It is also prescribed and used for the treatment of serious and long-term bronchial asthma, bronchitis, emphysema, and various types of chronic disruptive pulmonary diseases [4-8]. Terbutaline is a fast-releasing bronchodilator and can be administered orally or by nebulization. It is absorbed completely if administered orally [9]. It is proved that like other β_2-agonist, (*R*)-(-)-terbutaline is 200 times more active than (*S*)-(+)-terbutaline [10,11]. Terbutaline has an asymmetric center at the α-carbon and is generally prescribed and used as a racemate (Figure 1) [12,13]. It is well known, although, that of the two enantiomers, one is pharmacologically active while the other may be poisonous or inert, resulting in adverse effects or toxicity and possibly even damaging to human health in certain situations. Owing to these facts, the trading and marketing of all racemic medications has been prohibited by the US Food and Drug Administration, the European Committee for Proprietary Medicinal Products, the Pharmaceutical and Medical Devices Agencies of Japan, and Health Canada. [14-17].

Along with pharmaceutical activities, terbutaline has several side effects such as tachycardia, nervousness, anxiety, headache, tremors, hyperglycemia, hypotension hypokalemia, and, rarely, pulmonary edema, etc. [18]. Probably, these side effects may be due to (*S*)-(+)-terbutaline enantiomer which is less pharmaceutically [10-11]. Under such situations, the prescription of racemic terbutaline is not safe and the medicine should be resolved first into

enantiomers then optically pure drug should be given to the patients. For these reasons, there is a great demand to develop fast and economic separation methods due to the great pressure of the economy [19-21]. Some methods have been found to report enantiomeric resolution of terbutaline [22-25]. A critical evaluation of these methods confirmed that they are time-consuming and costly. Besides, some methods are not eco-friendly as these release a large amount of hazardous solvents. In view of these facts, efforts were made to explore the best method from the economic and environmental point of view. Among many chiral stationary phases (CSPs) polysaccharide-based CSPs are the best ones due to their remarkable recognition powers [26-29]. During the search for analytical techniques for this purpose, supercritical fluid chromatography (SFC) was considered the best due to its ease of operation and inexpensive nature. Furthermore, the purity of the peaks was ascertained by Chiral-LC-MS.



Terbutaline

Figure 1: Chemical structure of terbutaline (β_2-adrenoreceptor).

2. Experimental:

2.1 Chemicals and reagents

The standard drug terbutaline (batch no. BCBV3715), and trimethylamine were purchased from Sigma-Aldrich. Methanol was procured from Honeywell. Ethanol was supplied by Advent Chembio Pvt., Ltd. n-Hexane was of Prayog Fine Chem. Dichloromethane, ammonium acetate and formic acid were obtained from Merck Life Sciences Pvt., Ltd., India.

2.2 Instrumentation

Supercritical Fluid chromatography (UPC2) with a PDA detector (Waters), LC-2030C 3D with LabSolutions software version 6.89 was utilized to carry out the current work. Chiralpak IA [Amylose tris(3,5-dimethyl-phenylcarbamate)], Chiralpak IB [Cellulose tris(3,5-dimethylphenylcarbamate)], Chiralpak IC [Cellulose tris(3,5-dichlorophenylcarbamate)], Chiralpak IE [Amylose tris(3,5-dichlorophenylcarbamate)] and Chiralpak IG [Amylose tris(3-chloro-5-methylphenylcarbamate)]. All these columns were of the 250 mm x 4.6 mm, 5 µm dimensions and obtained from Daicel Chiral Technologies were used. The autosampler used was LH 40 with Fsupplied by Shimadzu, Japan. The samples of the separated peaks from SFC were collected by auto sample collector. These samples were heated in a water bath for 10 minutes at 60 ℃to remove CO_2. These samples were used to test the purity of the separated peak. The purity of SFC peaks was determined by Ultra-Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) of Waters with Mass Lynx software version 4.48. Acquity HSS-T3 (100 x 2.1 mm, 1.8 µm) column from Waters India Ltd. was used for this purpose. The mobile phase comprised of 5 mM ammonium hydroxide and acetonitrile with gradient mode. The detection of terbutaline was achieved at 223 nm using a PDA detector.

2.3 Molecular docking

The 3D structures of terbutaline enantiomers were prepared in Marvin Sketch and saved in pdb format. The geometry of both receptors and ligands was optimized in Avogadro software. Subsequent preparation of receptor in AutoDock (ADT) 4.2 by adding polar hydrogens, charges and ligands by detecting and choosing roots, led to PDBQT files for the amylose receptor and ligands. The pdbqt formatted files of the enantiomers were docked with the pdbqt file of the chiral selector amylose tris-(3,5 dichlorophenylcarbamate) (Chiralpak IE) using AutoDock (ADT) 4.2.

All the coordinates were set (x = 30.054, y = 22.75, and z = 4.171) with spacing (Angstrom) = 0.375 for docking purposes. The numerous docking runs were applied for both enantiomers with a chiral selector for the small free energy of binding confirmation from the large bunch. The post-docking analysis involved a meticulous examination of ligand conformations and interactions, visualized using PyMOL. The Post-docking analysis of the ligand conformations, interactions and number of hydrogen bonds was visualised by PyMOL.

3. Results and discussion:

3.1 Chiral separation and chromatographic data

Chiral separation of terbutaline was carried out using SFC. The samples were made in a mixture of dichloromethane and methanol (50:50, v/v) with a concentration of 1.0 mg/mL. An 8 µL sample of terbutaline drug was injected into to chiral SFC system. The mobile phase consisted of CO_2/0.2% TEA in MeOH (80:20) with 3.0 mL/min of flow rate. The temperature was 35 °C and the pressure was 1500 psi. The detection was achieved with PDA at 276 nm. Among all the chiral columns mentioned the best separation of the enantiomers was with Chiralpak IE (4.6 x 250 mm, 5 µm). However, the partial separation was also observed with the Chiralpak IG (4.6 x 250 mm, 5 µm) column. The retention times of the first peak with the Chiralpak IE column ranged from 1.02 to 6.82 while these values for the second peak ranged from 1.15 to 7.66. The values of the retention factor for the first peak were in the range of 1.10 to 7.31 while the values for the second peak were in the range of 1.20 to 7.34. The values of the separation factors were in the range of 1.09 to 1.16. The values of the resolution factors were in the range of 0.90 to 1.17; with the best separation in CO_2-MeOH (60:40) with 0.2% trimethylamine mobile phase. The retention times of the first peak with the Chiralpak IG column ranged from 1.01 to 1.15 while these values for the second peak ranged from 1.21 to 1.35. The values of the retention factor for the first peak

were in the range of 1.53 to 1.88 while the values for the second peak were in the range of 2.03 to 2.38. The values of the separation factors were in the range of 1.26 to 1.47. The values of the resolution factors were in the range of 0.80 to 0.91; indicating incomplete resolution in all the mobile phases. All these values are given in Table 1. The base-lined separation of terbutaline with Chiralpak-IE column using CO_2-MeOH (60:40) with 0.2% trimethylamine mobile phase is shown in Figure 2.

Table. 1: SFC chiral separation of terbutaline Chiralpak IE and IG columns.

Columns and mobile phases	tr_1 (min)	tr_2 (min)	k_1	k_2	α	Rs
Chiralpak-IE column						
CO_2-MeOH (60:40)	1.02	1.15	4.10	4.75	1.16	0.92
CO_2-MeOH (60:40) with 0.2% formic acid	1.00	1.10	1.10	1.20	1.09	0.90
CO_2-MeOH (60:40) with 0.2% triethylamine	6.82	7.66	7.31	8.34	1.14	1.17
Chiralpak-IG column						
CO_2-MeOH (60:40)	1.02	1.25	1.55	2.13	1.37	0.81
CO_2-MeOH (60:40) with 0.2% formic acid	1.01	1.21	1.53	2.03	1.47	0.80
CO_2-MeOH (60:40) with 0.2% triethylamine	1.15	1.35	1.88	2.38	1.26	0.91

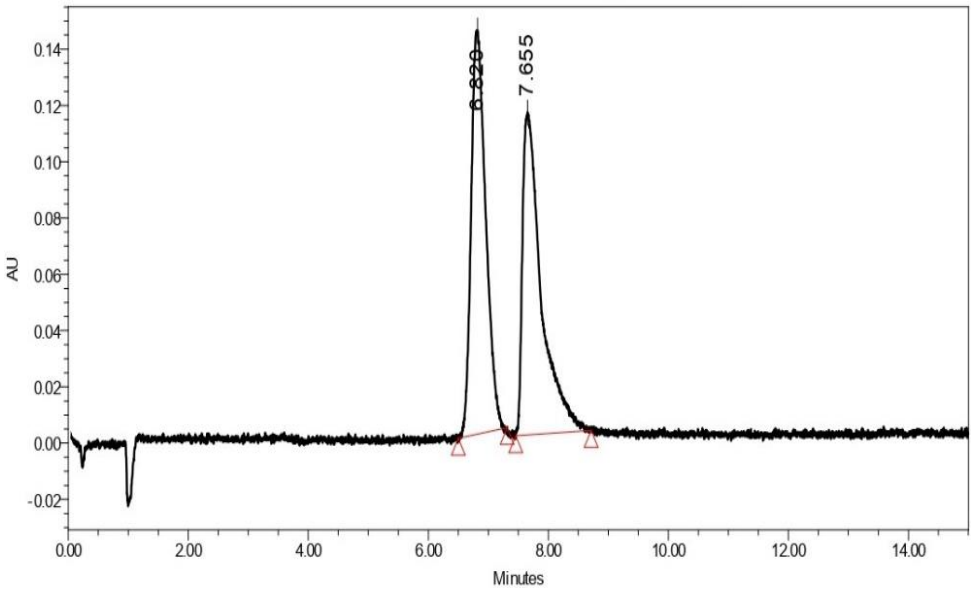


Figure 2: SFC chromatogram of terbutaline using mobile phase CO_2 and 0.2%TEA in MeOH (60:40) on Chiralpak-IE column.

The magnitudes of the numbers of theoretical plates and tailing factors of terbutaline chiral separation with the Chiralpak-IE column and Chiralpak-IG column using different mobile phases are recorded in Table 2. A perusal of this Table indicates that these values varied from 600 to 3548 for the first enantiomer and 1453 and 3699 for the second enantiomer with the Chiralpak IE column. On the other hand, these values varied from 487 to 1576 for the first enantiomer and 1508 and 1543 for the second enantiomer with the Chiralpak IG column. A comparison of these values indicates that Chiralpak IE is better than Chiralpak IG column. The values of the tailoring factors varied from 1.0 to 1.10 for the first enantiomer and 1.02 and 1.15 for the second enantiomer with the Chiralpak IE column. On the other hand, these values varied from 1.37 to 1.78 for the first enantiomer and 2.24 and 2.54 for the second enantiomer with the Chiralpak IG column. A comparison of these values again indicates that Chiralpak IE is better than Chiralpak IG column. Therefore, based on these calculations it is confirmed that Chiralpak IE gave better separation than the Chiralpak IG column.

Table 2: Numbers of theoretical plates and tailing factors of the separated enantiomers.

Mobile phases	**Theoretical plate number**		**Tailing Factor**	
	1st enantiomer	**2nd enantiomer**	**1st enantiomer**	**2nd enantiomer**
Chiralpak-IE column				
CO_2-MeOH (60:40)	605	1532	1.10	1.15
CO_2-MeOH (60:40) with 0.2% formic acid	600	1510	1.05	1.10
CO_2-MeOH (60:40) with 0.2% triethylamine	3548	3699	1.0	1.10
Chiralpak-IG column				
CO_2-MeOH (60:40)	590	1536	1.53	2.37
CO_2-MeOH (60:40) with 0.2% formic acid	487	1508	1.37	2.24
CO_2-MeOH (60:40) with 0.2% triethylamine	1576	1543	1.78	2.54

3.2 Optimization of SFC

The different combinations of solvents were tried to achieve the best chromatographic conditions. The different changes in flow rate, amounts injected and detection wavelength, were also carried out. We also tested and optimized the use of additives to the mobile phase like formic acid and triethylamine in different ratios in the mobile phases. The partial separation was attained on the Chiralpak-IG column using all the three mobile phase conditions such as i) a mixture of CO_2 and 0.2% formic acid in methanol (60:40); ii) a mixture of CO_2 and methanol (60:40); and 3) mixture of CO_2 and 0.2% triethylamine in methanol (60:40). Similarly, partial separation was also attained on Chiralpak-IE column using mobile with acidic condition. A split was observed on the Chiralpak-IB column in basic condition. There was no separation detected on the Chiralpak IA column. Finally, a good separation with baselined was achieved on the Chiralpak-IE column using a mixture of CO_2 and 0.2% formic acid in methanol (60:40). As a result of thorough experimentation, the finest SFC conditions were developed and reported herein. The mobile phase used in this study was CO_2 and 0.2% TEA in MeOH (60:40). The combined flow rate of the mobile phase was 3.0 mL/min. The separation was carried out at 35°C temperature with PDA detection (PDA 210-400 nm) terbutaline.

Attempts were made to explain the better separation of Chiralpak IE in comparison to Chiralpak IG. The structures of the CSPs (Chiralpak IE and Chiralpak IG) are shown in Figure 3. A perusal of this Figure indicates the difference between two CSPs. The only difference is of methyl group in Chiralpak IG CSP on meta position in place of the chlorine group in Chiralpak IE. The two chlorine atoms are making Chiralpak IE the better CSP than Chiralpak IG. This may be due to the fact one methyl group on the Chiralpak IG column creates a steric effect due to the bigger size of the methyl group in comparison to a chlorine atom. This steric effect decreasing π-

π interactions in Chiralpak IG compared to Chiralpak IE. These are the reasons for better chiral separation of terbutaline enantiomers on Chiralpak IE.

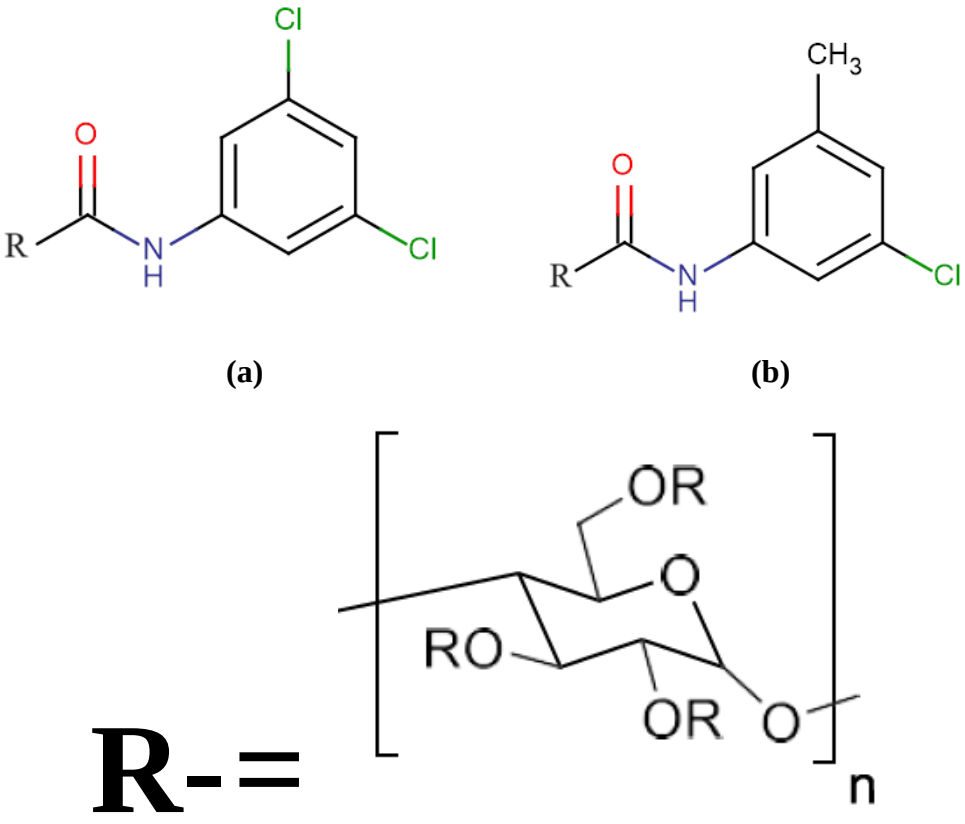


Figure 3: Structures of (a): Chiralpak IE (amylose tris(3,5 dichlorophenylcarbamate) and (b): Chiralpak IG (amylose tris(3-chloro-5-methylphenylcarbamate) columns.

3.3 Validation of chiral SFC method

The SFC chromatographic validation was done as mentioned in the experimental part. Under the System suitability test, resolution, tailing factors, retention times, and the peak area (%RSD) were calculated and the results are summarized in Table 3. These findings indicated low RSD values of 1)-peak area < 2.0% and 2)-retention time < 1.0 %. The tailing factors of terbutaline enantiomers were 1.0 and 1.15 on the Chiralpak IE column, while 1.37 and 2.54 on the Chiralpak IG column, respectively.

There was no peak present in the blank solution; showing good specificity. The complete interpretation outcomes for the slopes and the linearity are briefed in Table 3. Excellent linearity was observed in terbutaline drugs in an amount range of 10.0 to 1000 µg/mL on Chiralpak IE and Chiralpak IG. The outcome of the correlation coefficient and regression coefficient (r^2) was> 0.999 on Chiralpak IE, while 0.994–9979 on Chiralpak IG. The terbutaline enantiomers limits of detection on Chiralpak IE and Chiralpak IG were 24.79 and 32.65 µg/mL, and 76.91 and 68.80 µg/mL. The terbutaline enantiomer's limit of quantitation on Chiralpak IE and Chiralpak IG were 75.12 and 98.94 µg/mL, and 233.07 and 208.49 µg/mL. The values are given in Table 3.

Table 3: Details output of system suitability and linearity parameter.

Parameters	**Terbutaline**			
	Chiralpak-IE column		**Chiralpak-IG column**	
	First enantiomer	**Second enantiomer**	**First enantiomer**	**Second enantiomer**
Tailing Factor	1.00	1.15	1.37	2.54
Resolution	-	1.76	-	0.92
%RSD peak area	0.16	0.15	0.11	0.12
%RSD (RT)	0.25	0.19	0.23	0.22
Linearity range (µg/mL)	625.76	637.21	235.94	249.85
Y-intercept	-33337	-26873	-50977	-33238
Correlation Coefficient (r)	0.9997	0.9999	0.9959	0.9940
Regression Coefficient. (r2)	0.9993	0.9997	0.9979	0.9970
LOD (µg/mL)	32.65	24.79	76.91	68.80
LOQ (µg/mL)	98.94	75.12	233.07	208.49

The intra-day precision percentage assay was computed for the enantiomers of terbutaline on Chiralpak IE and found in the range of 99.27–100.16 and 99.45–100.06, while, on Chiralpak IG 96.29–97.49 and 96.85–97.15 separately, and respectively. Similarly, the % RSD for all the assays of terbutaline in intra-day precision on Chiralpak IE was 0.16–0.42 and 0.15–0.36 while, on Chiralpak IG 0.73–0.86 and 0.77–0.81. These values are given in Table 4.

Table 4: Intra-day precision (3 replicates).

Level	50%		100%		150%	
Parameters	% Assay Mean	% RSD	% Assay Mean	% RSD	% Assay Mean	% RSD
Terbutaline (Chiralpak IE)						
Enantiomer-1	100.16	0.42	99.27	0.16	99.91	0.38
Enantiomer-2	99.92	0.36	100.06	0.15	99.45	0.26
Terbutaline (Chiralpak IG)						
Enantiomer-1	96.58	0.86	96.29	0.73	97.49	0.83
Enantiomer-2	96.91	0.79	97.15	0.81	96.85	0.77

The inter-day precision percentage assay was calculated for terbutaline enantiomers on Chiralpak IE and found in the range of 99.86–100.81 and 99.29–100.62. These values on Chiralpak IG were found in the range of 95.89–97.12 and 96.17–97.03. The % RSD for all the assay of terbutaline enantiomers in inter-day precision was 0.32 and 0.49; and 0.51 and 0.36 on Chiralpak IE and Chiralpak IG columns. These values are given in Table 5.

Table 5: Inter-day precision.

Samples	**Chiralpak IE**		**Chiralpak IG**	
	Enantiomer-1	Enantiomer-2	Enantiomer-1	Enantiomer-2
Sample-1	100.39	100.42	96.56	96.31
Sample-2	100.81	100.52	96.81	96.95
Sample-3	100.28	99.29	96.19	97.03
Sample-4	100.32	100.13	97.06	96.17
Sample-5	99.86	100.44	95.89	96.62
Sample-6	100.58	100.62	97.12	96.79
%RSD	**0.32**	**0.49**	**0.51**	**0.36**

The percentage recoveries obtained were 98 and 102% for the enantiomers on Chiralpak IE. The percentage recoveries were calculated for terbutaline enantiomers and observed in the range of 98.92–100.12 and 98.82–100.28, separately and respectively. Similarly, the percentage recoveries were gotten from 96 and 99% for all enantiomers on Chiralpak IG. The percentage recoveries were calculated for terbutaline enantiomers and observed in the range of 96.19–98.18 and 96.29–98.25, separately and respectively. These values are given in Table 6. The robustness

outputs are mentioned in Table 7. The differences in retention time (Rt) and peak area were <2.0; showing the robustness of the method.

Table 6: Recovery study of terbutaline drug.

Level	Terbutaline (% Recovery)			
	Chiralpak-IE column		Chiralpak-IG column	
	Enantiomer-1	Enantiomer-2	Enantiomer-1	Enantiomer-2
50	99.06	99.32	96.52	97.15
50	99.45	99.64	96.43	96.71
50	99.12	99.36	96.19	96.42
100	100.06	99.24	96.72	97.31
100	100.12	99.98	96.59	96.25
100	99.26	100.28	97.02	96.47
150	98.92	99.66	97.18	96.88
150	99.78	98.82	96.78	96.91
150	99.36	99.42	96.67	96.29

Table 7. Robustness study for chiral SFC method.

Parameters	Terbutaline (Variation in %RSD)							
	Chiralpak-IE column				Chiralpak-IG column			
	Enantiomer-1		Enantiomer-2		Enantiomer-1		Enantiomer-2	
	RT (min)	Area	RT (min)	Area	RT (min)	Area	RT (min)	Area
Flow Rate (mL/min) 3.2	0.59	0.32	0.43	0.29	0.51	0.38	0.49	0.32
Flow Rate (mL/min) 2.8	0.46	0.28	0.26	0.48	0.39	0.31	0.33	0.45
Column Temp. (40 °C)	0.56	0.61	0.35	0.48	0.49	0.65	0.29	0.47
Column Temp. (30 °C)	0.48	0.54	0.27	0.42	0.46	0.52	0.25	0.41

4. SFC-separated peaks purity determination by UPLC-MS

The purity of any drug is an utmost parameter for safe medication. Consequently, the purity of the SFC-separated peaks of terbutaline was ascertained by UPLC-MS using Acquity HSST3 (100 x 2.1 mm, 1.8 µm) column with a mixture of 5 mM ammonium acetate and acetonitrile as mobile phase, gradient mode and 223 nm using PDA detection. Both the separated peaks of terbutaline eluted at 4.39 minutes. The purity chromatograms of terbutaline using TIC

and PDA are given in Figure 4. It is clear from this Figure that the peaks are with 1.0 and 1.10 tailing factors. There was no other peak in this chromatogram; showing the high quality of the separated peaks. Such type of terbutaline is called HPLC grade terbutaline. The mass spectrum of the separated peaks is shown in Figure 5. There is clear one peak at 226.33 m/z value, which is the mass of the studied molecule terbutaline. There was no extra peak in the spectrum; showing again the high purity of the separated enantiomers of terbutaline. Such quality of drugs should be called LC-MS grade.

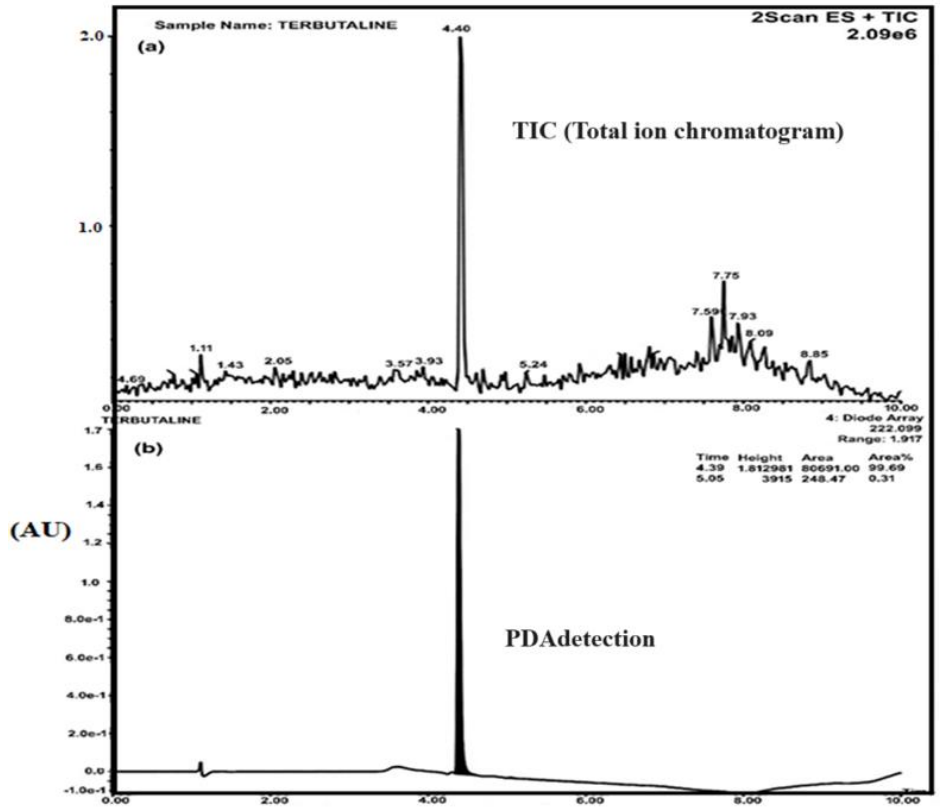


Figure 4: UPLC-MS chromatograms of the terbutaline-separated peaks with detection (a): TIC and (b): PDA.

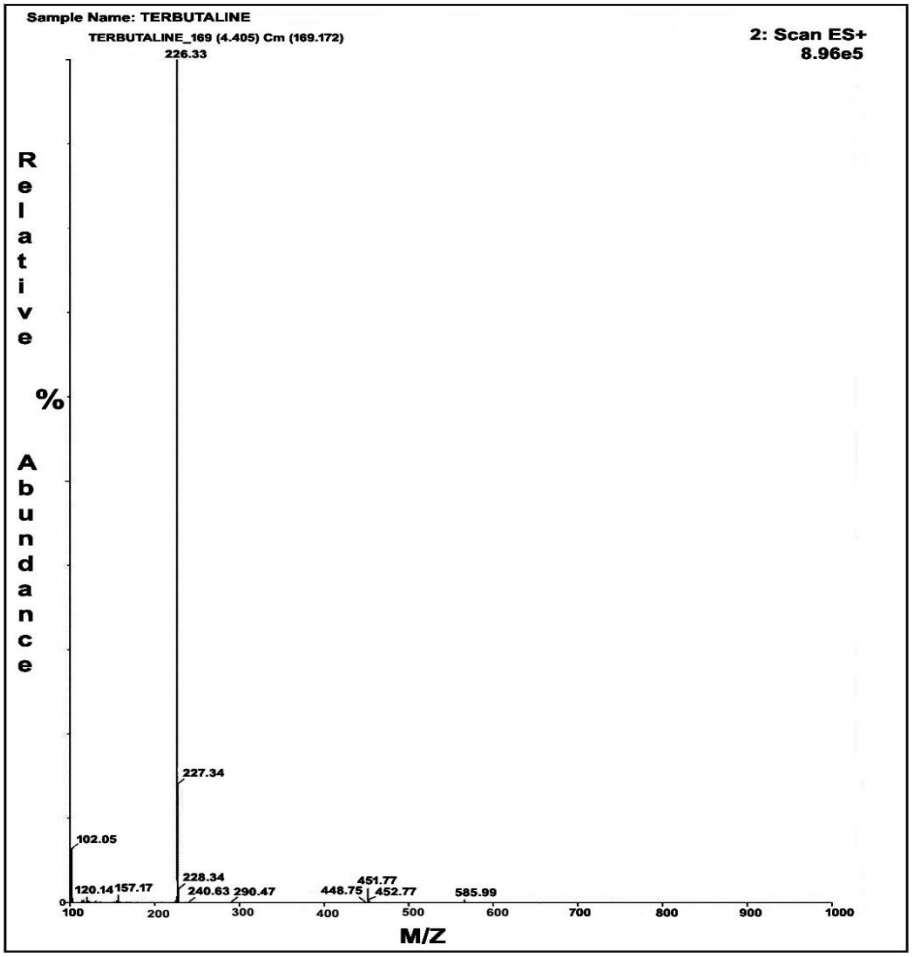


Figure 5: MS spectrum of the terbutaline-separated peaks.

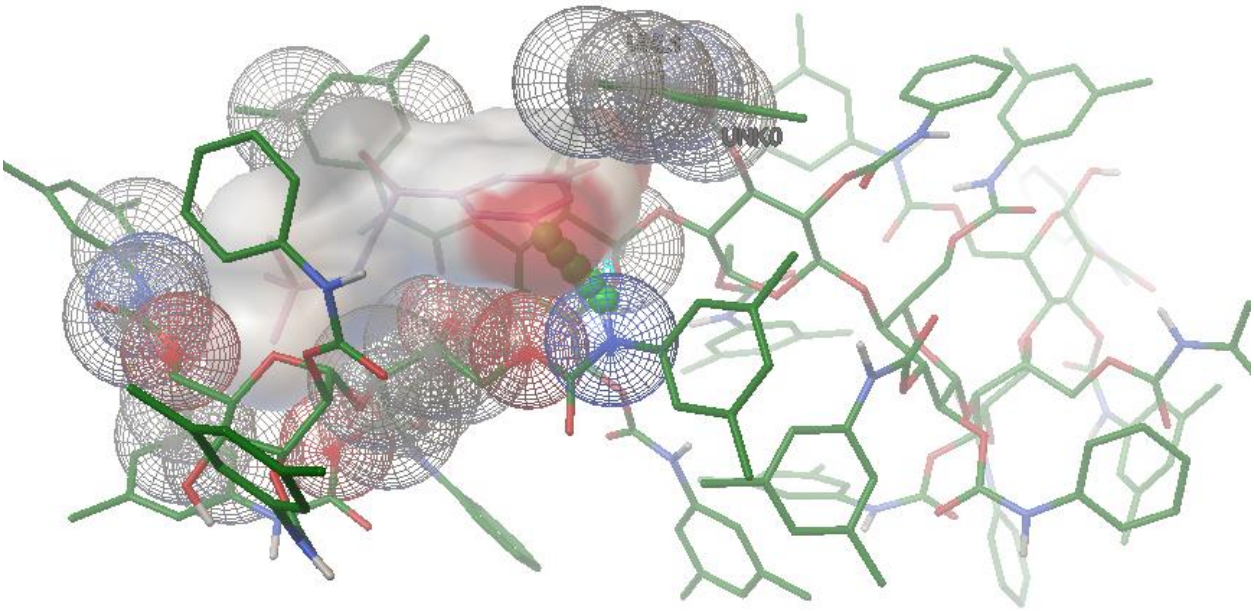
5. Chiral recognition mechanism at the supramolecular level

Chiral recognition is very significant in enantiomeric separation by the fact that it explores new methods and approaches to resolve more similar types of racemates. Moreover, this may be used to predict the elution of the chiral peaks. The chiral recognition mechanism was determined by molecular modeling using the procedure described in the experimental section. The modeling was carried out with the chiral selector Chiralpak IE (amylose tris(3,5 dichlorophenylcarbamate); the best polysaccharides-based CSP. The results are given in Table 8 and the molecular models are shown in Figure 6. It is clear from Table 8 that the *R*-enantiomer shows only one hydrogen

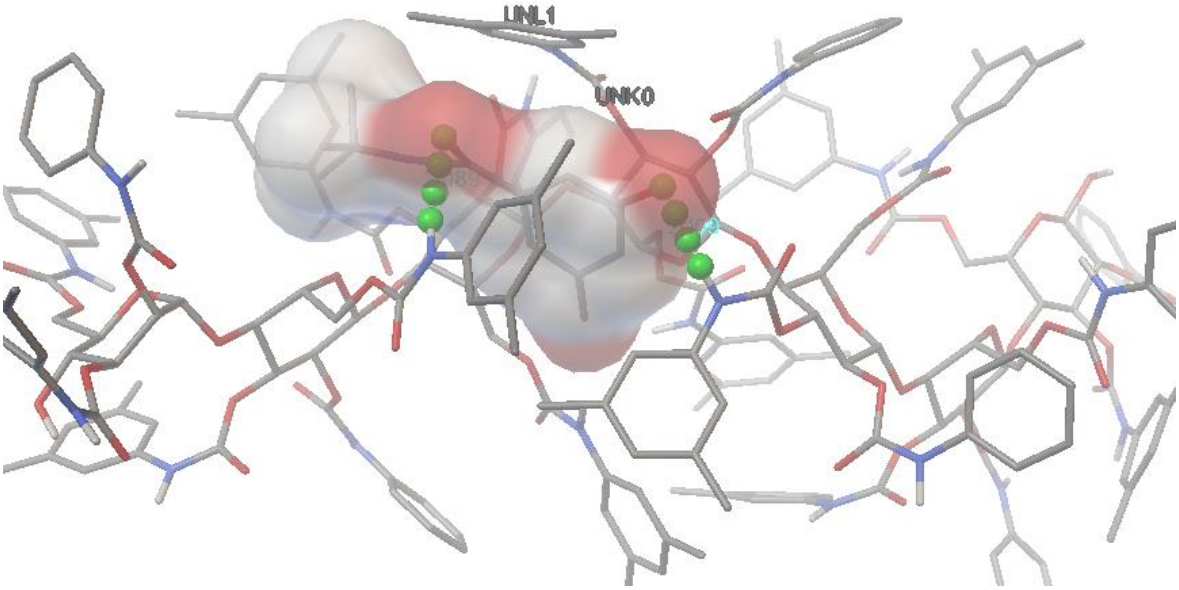
bond with 1.98 bond length A°. The residues involved in CSP were UNL1:HN and O of the OH group. The binding affinity was -6.99 Kcal/mole. On the other hand, the *S*-enantiomer shows two hydrogen bonds with 2.09 and 2.11 A° as the bond lengths. The residues involved were UNL1:HN and O of the OH group in these hydrogen bonds. The binding affinity was -7.41 Kcal/mole. A comparison of the number of hydrogen bonds and binding affinities confirmed that *R*-enantiomers retained less than *S*-enantiomers; leading to the first peak of *R*-enantiomers and a second peak of *S*-enantiomers.

Table 8: Modelling result of terbutaline with Chiralpak IE column.

Enantiomers	**Binding affinities (Kcal/mole)**	**No. of hydrogen bond**	**H bond length (A°)**	**Residues involved in H bonds**
R-	-6.99	1	1.98	Amylose: :UNL1:HN & O of OH group
S-	-7.41	2	2.09 2.11	Amylose: :UNL1:HN & O of OH group Amylose: :UNL1:HN & O of OH group



(a)



(b)

Figure 6: Models of (a): *R*- and (b): *S*-enantiomers with Chiralpak IE [(amylose tris(3,5 dichlorophenylcarbamate)] CSP.

In addition to the hydrogen bondings, efforts are made to explore other types of bonding involving aromatic moieties. Based on our experience and previous studies [27-34]. It was found that π-π interactions also played a significant role in the chiral separation of terbutaline enantiomers. A pictorial diagram of π-π interactions is shown in Figure 7. It is clear from this Figure that π-π interactions are because of the phenyl ring of the enantiomers of terbutaline and the CSP. A greater number of π-π interactions were observed in *S*-enantiomers; again indicating the strong bonding of these enantiomers in comparison to the *R*-enantiomer. These facts are supported by the modeling results. In addition to this, other forces such as van der Wall forces, steric effect and dipole-dipole interactions are responsible for the chiral separation of terbutaline.

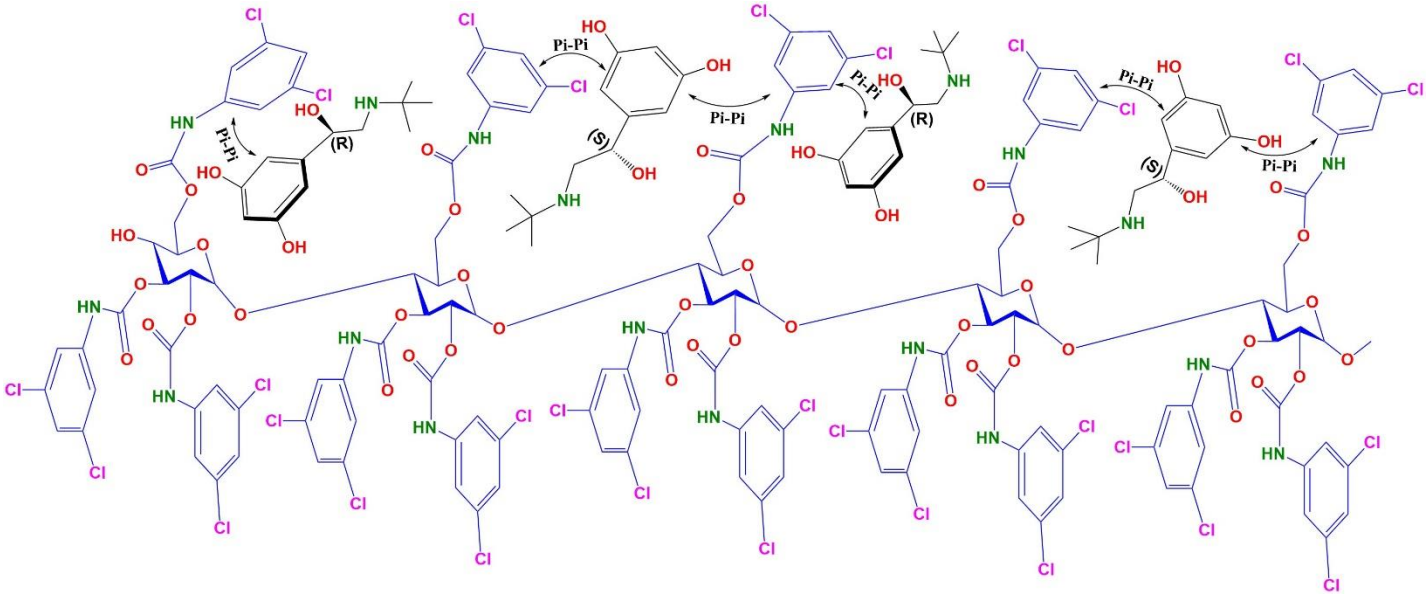


Figure 7: A supramolecular model showing the π-π interactions among terbutaline enantiomers and amylose tris(3,5 dichlorophenylcarbamate) chiral selector.

6. Conclusion:

Inexpensive, eco-friendly and fast separation of terbutaline enantiomers was attained on amylose-based CSPs. The separation was achieved within 9 minutes. Among using various CSPs

i.e. Chiralpak IA, IB, IC, IE, and IG (250 mm x 4.6 mm, 5 µm) columns, the best column was Chiralpak IE giving baselined separation. The purity of the separated peaks determined by UPLC-MS confirmed that the separated enantiomers were 100% chromatographically pure (*R*)-enantiomer can be marked as the optically active drug; being 200 times more active than *S*-enantiomer. The developed chiral mechanism is very useful for transferring this lab-based method at the pilot scale. The developed method is fast and safer for the environment, and analysts along with time-saving. The established method can be useful for Scientists, R&D persons, academicians, researchers and pharmaceutical industries, worldwide.

7. Acknowledgment:

This work was funded by the Researchers Supporting Project Number (RSP2024R429) at King Saud University, Riyadh, Saudi Arabia.

Conflict of interest:

The authors declare no conflict of interest.

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