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Box-Behnken response surface modeling assisted enantiomeric resolution of some racemic β-blockers using HPTLC and β-cyclodextrin as chiral mobile phase additive: Application to check the enantiomeric purity of betaxolol

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Abstract

Stereospecific separation method of (±) betaxolol, (±) carvedilol, and (±) sotalol using High Performance Thin Layer Chromatography (HPTLC) and β-cyclodextrin as chiral selector has been developed and validated. The Box-Behnken surface response design was selected for optimizing the operating variables based on 15 trials design. The optimized method involves separation on Fluka HPTLC silica gel plates 60 F_{254} (20 × 10 cm) using acetonitrile-methanol-acetic acid-water (3.4:3.6:0.18:1 v/v) as a mobile phase containing 0.57 mM β-cyclodextrin. Densitometric measurements were made at 220 nm for betaxolol and sotalol or at 245 nm for carvedilol. Maximum separation of the enantiomers of the three drugs was obtained by optimizing concentration of chiral selector, the mobile phase composition including acetonitrile amount in the organic part of the mobile phase and the volume of acetic acid added. The proposed method enables estimation of (−) and (+) enantiomers of betaxolol in drug substance and in various pharmaceuticals. The detection limit of betaxolol was 0.15 and 0.13 μg band^{-1} for (−) and (+) enantiomers, respectively. The detection limits were found to be 0.2 and 0.3 μg band^{-1} for carvedilol and sotalol, respectively, as racemate. In addition, the proposed method was applied in checking the enantiomeric purity of (−) BET in the presence of (+) BET at 1% level where the inactive (+) enantiomer was quantified with good accuracy and precision at 1% level in the active (−) enantiomer.

KEYWORDS

Box-Behnken design, enantiomeric purity, enantiomeric resolution, mobile phase additive, validated HPTLC, β-blockers, β-cyclodextrin

1 | INTRODUCTION

Most of chiral drugs including β-blockers are marketed as racemic mixtures. Although enantiomers have identical chemical structure, they usually show great alterations in their biological activities including pharmacokinetic and toxicology.[1]

Betaxolol (BET, Figure 1) is a cardio-selective β1-adrenergic receptor-blocking agent. Levobetaxolol is a single active (−) isomer of BET possessing cardiac beta

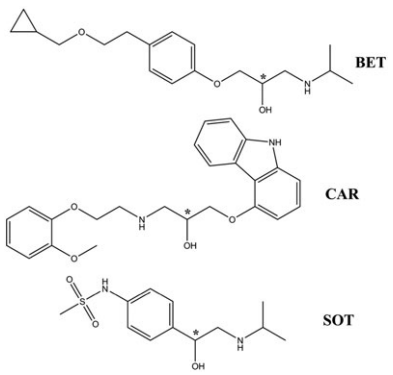

FIGURE 1 Chemical structures of betaxolol (BET), carvedilol (CAR), and sotalol (SOT)

blocking activity about 530 times more relative to its inactive (+) enantiomer.[2]

Carvedilol (CAR, Figure 1) is a racemic mixture in which nonselective β-adrenoreceptor blocking action resides in the (−) enantiomer whereas blocking α1-adrenergic receptors is due to both (+) and (−) enantiomers at the same potency.[3]

Sotalol (SOT, Figure 1) is a non-selective β -blocker which causes prolongation of cardiac repolarization, thus showing class III antiarrhythmic properties.[4] (−) SOT is the enantiomer that possesses the majority of the β-blocking activity. Meanwhile, both (−) and (+) SOT enantiomers have equal Class III antiarrhythmic activity.[2] It was proved that optically pure (+) SOT enantiomer caused high mortality rate as it only exerts antiarrhythmic class III activity without beta-blocking effects. Thus, β-blocking activity which is exerted only by the (−) enantiomer plays a vital role in both safety and efficacy of SOT.[5]

Separation of BET, CAR, and SOT enantiomers has been reported using HPLC with chiral stationary phase[6] and capillary electrophoresis.[7]Compared with these reported chromatographic techniques, High Performance Thin Layer Chromatography (HPTLC) shows the advantages of low expenses, simplicity of performance, and possibility of running several samples in the same run using small amount of solvents making it more ecofriendly.[8,9] It is advantageous in increasing sample throughput and the ease of comparison of the spots positions with those of reference standards analyzed simultaneously.[10] Application of TLC for the resolution of some β-blockers' enantiomers (other than BET, CAR, and SOT) has been performed using plates impregnated with different chiral selectors[11-14] and one TLC report[3] for the separation of CAR using impregnated silica plates but no reports on enantiomeric separation or quantification of BET and SOT by HPTLC. All the reported TLC methods uses silica plates impregnated with the chiral selector which involves tedious procedure of impregnating and drying the plates (which may also require pH adjustment in some cases) and checking the complete coverage of the plate with the impregnating layer (which may affect the method reproducibility). Thus, this work was directed to the development of a simple HPTLC method (compared with the reported HPLC and capillary electrophoresis) for the enantiomeric separation of some β-blockers using chiral mobile phase additive without the tedious plates' impregnation technique which may affect reproducibility. Although the use of cyclodextrins as chiral selectors in HPTLC based techniques offers the simplicity and the reproducibility required by many quality control laboratories for chiral separation and analysis, no previous reports have been found for their use in HPTLC separation and analysis of β-blockers. For optimizing the operating experimental parameters, Box-Behnken design (BBD) was selected instead of the time-consuming univariate optimization (one variable at a time). The theoretical background for multiple response optimization using experimental design is extensively discussed in literature.[15,16]

Box-Behnken design is one of the response surface design that includes three-level incomplete factorial design. The design points fall at combinations of the high and low factor levels and their midpoints, and it contains $N = (2f\ (f - 1)) + Cp$ experiments where f is the number of variables and Cp is the number of center points.[17] This paper describes a simple direct and economic HPTLC method for separation and identification of the enantiomers of (±) BET, (±) CAR, and (±) SOT using (β-CD) as chiral selector. In addition, accurate quantitation of (−) and (+) betaxolol enantiomers was performed in its marketed eye drops and tablets using the proposed method. Optimization of the operating experimental parameters was done using the BBD. The low LOD and LOQ values and the high-resolution factor between each pair of enantiomers suggest extending the applicability of the method for rapid routine determination of enantiomeric purity. So, enantiomeric purity of the active (−) BET enantiomer was checked by spiking it with the inactive enantiomer, (+) BET, where (+) BET was quantified with good accuracy and precision at the 1% level. Moreover, the method was validated as per the ICH guidelines[18] for the quantitation of the (−) and (+) enantiomers of BET.

2 | MATERIALS AND METHODS

2.1 | Materials and reagents

Pharmaceutical grade (−) BET and (+) BET certified to contain 99.85% and 99.00% and were purchased from selleckchem, US and Haoyuan Chemexpress Co., Ltd.,

Shanghai, China, respectively. (±) CAR and (±) SOT were obtained as generous gifts from EIPICO pharmaceutical industries company, Egypt, and certified to contain 99.85% and 99.80%, respectively. All reagents used were of analytical grade. Methanol, acetonitrile, and glacial acetic acid (El-Nasr Chemical Ind. Co., Egypt) and β-CD (Fluka, Germany) were used. Two commercial products namely Betopic ophthalmic suspension containing betaxolol HCl [(±) BET] 5.6 mg equivalent to 5.0 mg of betaxolol free base per mL, produced by Alcon Laboratories, USA, and Betaxolol tablets containing betaxolol HCl [(±) BET] 10 mg equivalent to 8.94 mg of betaxolol free base per tablet, produced by BORG Pharmaceutical Industries, BORG El Arab new city, Alexandria Egypt, were purchased from the commercial market

2.2 | Instrumentation and chromatographic conditions

HPTLC aluminum plates pre-coated with silica gel 60 F_{254} with dimensions of 20 × 10 cm and 250 μm thickness were purchased from Fluka (Darmstadt, Germany). Sample application was done using Camag 100 μL microsyringe and Camag Linomat IV applicator (Hamilton, Bonaduz, Switzerland), keeping the slit dimension at 6 × 0.2 mm and the scanning speed at 20 mm s^{-1}. The mobile phase was developed in the ascending mode in twin trough glass chamber (Camag, Switzerland) with dimensions of 20 cm × 10 cm. The chamber was saturated for 20 minutes at room temperature (25 ± 2°C) using 20 mL of the mobile phase. Densitometric scanning was performed at 220 nm for BET and SOT or at 245 nm for CAR on a Camag TLC scanner III operated in the reflectance-absorbance mode and controlled by CATS software (V 3.15, Camag). Deuterium lamp was utilized as the source of radiation emitting a continuous UV spectrum in the range of 190 and 400 nm. The HPTLC plates were developed with acetonitrile:methanol:acetic acid:water (3.4:3.6:0.18:1, v/v) as mobile phase containing 0.57 mM β-CD. For detection and quantitation, 10-μL volumes of either test or standard solutions to give final concentration within the linearity range were applied separately as compact bands with 6-mm width, 6 mm apart and 15 mm from the bottom of the plate. The plate was developed up to the top (over a distance of 8 cm) in the usual ascending way and the average development time is about 15 minutes. After development in the saturated chamber, the plate was air dried for 5 minutes and scanned at 220 nm for BET and SOT or at 245 nm for CAR

The magnified retardation factor (hR_F) values were calculated (R_F × 100) for the separated bands of the enantiomers of the three drugs. The chiral resolution factor [hR_F (+)/hR_F (−)] of the two separated bands obtained for each racemate was calculated as the ratio of the higher R_F value to the lower R_F value for the two enantiomers. Because pure enantiomers of CAR and SOT were not available for identification of the separated enantiomers by determination of their spot positions, the pure (−) enantiomer, levobetaxolol, was used as reference compound.

2.3 | Methods

2.3.1 | Preparation of stock solutions

Standard stock solutions containing 2 mg mL^{-1} of (−) BET, (+) BET, (±) CAR, and (±) SOT were prepared separately by dissolving the reference materials in methanol. For method development and optimization, standard mixture of (+) BET and (−) BET was prepared to contain 300 μg mL^{-1} of each enantiomer in methanol. Also, diluting the stocks of (±) CAR and (±) SOT with methanol was done to obtain standard solutions of 300 and 800 μg mL^{-1} of (±) CAR and (±) SOT, respectively. The stock solutions were kept in amber glass vessels at 4°C and were stable for a minimum of 7 days. A solution of 10 mM β-CD was prepared in distilled water and stored at 4°C

2.3.2 | Standard solutions for calibration

Standard solutions were diluted with methanol to prepare working solutions in concentration ranges 50 to 600 μg mL^{-1} and 40 to 600 μg mL^{-1} for (−) BET and (+) BET, respectively. Triplicate 10-μL portions from each working solution were spotted as bands to obtain final concentrations of 0.5 to 6 and 0.4 to 6 μg $band^{-1}$ for (−) BET and (+) BET, respectively.

2.3.3 | Sample preparation

Portions from Betopic ophthalmic suspension equivalent to 2 mg (±) BET were separately transferred into 10-mL volumetric flasks and diluted to volume with methanol to give final concentrations of 200 μg mL^{-1} of (±) BET. For Betaxolol tablets, 10 tablets were ground, and an amount equivalent to 25 mg (±) BET was transferred into 25-mL volumetric flask using about 15-mL methanol. Sonication for 15 minutes was done followed by completing the flasks to volume and filtration through Whatman, grade 1 filter paper. Dilution was made with methanol to give final concentration of 200 μg mL^{-1} of (±) BET. Spotting was done in order to achieve a final concentration of 2 μg mL^{-1} of (±) BET

2.4 | Box-Behnken statistical design

Since the number of factors to be investigated was three, BBD was selected. The process was performed on StatSoft STATISTICA 10 software. Based on the results of the preliminary experiments, three levels were given to the selected variables, and the optimization was performed using 15 trials (13 unique runs and two replications for the centre point, Table 1). The process behaviour can be described in the present study by quadratic model using Equation (1):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (1)$$

where Y is the chiral resolution factor, β_n are quadratic coefficients, and X_1, X_2, X_3 are the studied variables.

3 | RESULTS AND DISCUSSION

3.1 | Mechanism of chiral recognition

The interaction of a guest molecule with cyclodextrin encompasses the insertion of the more hydrophobic part of the guest molecule into the cyclodextrin cavity, while the more polar group (usually charged group) orients itself outside cyclodextrin cavity.[19] In general, the R (+)-configuration shows higher affinity to β-CD for all guest molecules that have a hydroxyl group attached to the asymmetric center in addition to a hydrogen atom, an aromatic/aliphatic cycle for inclusion, and a charged group for dissolution in water.[19] The literature reveals that the chiral recognition may occur because of formation of inclusion complexes, hydrogen-bonding, π-π interaction, hydrophobic interaction, or steric repulsion.[20]

3.2 | Preliminary trials for selection of variables

Preliminary studies show that a mobile phase containing seven parts organic solvent (mixture of acetonitrile and methanol) to one part of water containing β-CD as chiral selector in the presence of acetic acid could be adopted for the enantiomeric separation of the three drugs' racemates. The ratio of acetonitrile in the organic part of the mobile phase, the concentration of β-CD in the mobile phase, and the added volume of acetic acid were optimized using BBD. The presence of acetic acid was necessary for the chiral resolution of each enantiomeric pair, and when removed from the mobile phase or replaced with ammonia, no chiral separation was observed. This may confirm that for chiral recognition of the drugs, they should be charged (the amino group in each drug will be protonated in the presence of acetic acid).

TABLE 1 Three-factor BBD experimental runs with their corresponding design matrix codes[a]

Run	CD Concentration, mM	% ACN	Volume of HAC, mL
1	0.1 (−1)	30 (−1)	0.2 (0)
2	1.0 (+1)	30 (−1)	0.2 (0)
3	0.1(−1)	70 (+1)	0.2 (0)
4	1.0 (+1)	70 (+1)	0.2 (0)
5	0.1 (−1)	50 (0)	0.1 (−1)
6	1.0 (+1)	50 (0)	0.1 (−1)
7	0.1 (−1)	50 (0)	0.3 (+1)
8	1.0 (+1)	50 (0)	0.3 (+1)
9	0.55 (0)	30 (−1)	0.1 (−1)
10	0.55 (0)	70 (+1)	0.1 (−1)
11	0.55 (0)	30 (−1)	0.3 (+1)
12	0.55 (0)	70 (+1)	0.3 (+1)
13 (C)	0.55 (0)	50 (0)	0.2 (0)
14 (C)	0.55 (0)	50 (0)	0.2 (0)
15 (C)	0.55 (0)	50 (0)	0.2 (0)

[a]CD is the concentration (mM) of β-cyclodextrin in the mobile phase, ACN is the % of acetonitrile in the organic part of the mobile phase, and HAC is the volume (mL) of acetic acid added to the mobile phase; (C) is the center points.

It is well known that chiral recognition which arises from the formation of an inclusion complex between the guest molecule and the hydrophobic cavity of cyclodextrin is affected by temperature.[10] In the presented study, ambient temperature (25 ± 2°C) gave the best resolution for the enantiomers of the three drugs. When the temperature was reduced to 4°C, no enhancement in the separation of CAR and SOT enantiomers and incomplete separation of BET enantiomers was obtained. Meanwhile, upon increasing the temperature, a tendency toward partial or incomplete separation of the three drugs was noticed. Therefore, optimization of the experimental parameters was made at room temperature which also adds simplicity to the advantages of the proposed method.

3.3 | Box-Behnken design for optimization of the experimental variables

The studied factors were introduced into StatSoft STATISTICA 10 software with their corresponding levels that were prudently chosen to obtain an experimental domain in which the expected optimum variable levels are covered as much as possible, and a BBD was generated as presented in Table 1. The optimization trials were performed using the studied variables (The ratio of acetonitrile in the organic part of the mobile phase, the concentration of β-CD in the mobile phase, and the added volume of acetic acid) at the selected levels illustrated in Table 1. The three drugs' racemates were chromatographed using mobile phases prepared with different compositions as suggested by the BBD and chromatographed using the previously mentioned chromatographic conditions; then, the corresponding chiral resolution factors were calculated.

The obtained results for BET, CAR, and SOT were subsequently statistically analyzed using StatSoft STATISTICA 10 software. The results of this statistical analysis are depicted in the ANOVA table (Table 2) and pareto charts (Figure 2A shows pareto chart for BET as representative example). The obtained BBD results agree with the experimental data as indicated by the high values of both R^2 (0.914, 0.923, and 0.905) and adjusted R^2 (0.851, 0.866, and 0.834) for BET, CAR, and SOT, respectively. Figure 2B illustrates the desirability function graph for BET as representative example. From the ANOVA test results and the desirability function plots, it was found that the effect of all the studied variables on the chiral resolution factors of the racemic mixture of the three drugs was quadratic more than being linear (the quadratic factors only were significant and appeared bold in the ANOVA table showing *p*-values less than 0.05). In addition, no interactive effect was found between the studied variables as their obtained *p*-values

TABLE 2 ANOVA table for BBD results of BET, CAR, and SOT[a]

	BET				
Factor	**SS**	**df**	**MS**	**F**	**P**
(1) CD (L)	0.00180	1	0.00180	0.60317	0.47249
CD (Q)	**0.11458**	**1**	**0.11458**	**38.3978**	**0.00159**
(2) ACN (L)	0.00647	1	0.00647	2.17069	0.20065
ACN (Q)	**0.15140**	**1**	**0.15140**	**50.7358**	**0.00084**
(3) HAC (L)	0.01686	1	0.01686	5.65219	0.06336
HAC (Q)	**0.05540**	**1**	**0.05540**	**18.5668**	**0.00765**
1 L by 2 L	0.00005	1	0.00005	0.01873	0.89647
1 L by 3 L	0.00694	1	0.00694	2.32821	0.18756
2 L by 3 L	0.00640	1	0.00640	2.14460	0.20295
Error	0.01492	5	0.00298		
Total SS	0.33193	14			
Factor	**CAR**				
	SS	**df**	**MS**	**F**	**p**
(1) CD (L)	0.00061	1	0.00061	0.26702	0.62737
CD (Q)	**0.11585**	**1**	**0.11585**	**50.5067**	**0.00085**
(2) ACN (L)	0.00629	1	0.00629	2.74570	0.15841
ACN (Q)	**0.13564**	**1**	**0.13564**	**59.1335**	**0.00059**
(3) HAC (L)	0.01420	1	0.01420	6.19355	0.05525
HAC (Q)	**0.06160**	**1**	**0.06160**	**26.8560**	**0.00351**
1 L by 2 L	0.00047	1	0.00047	0.20865	0.66698
1 L by 3 L	0.00671	1	0.00671	2.92920	0.14767
2 L by 3 L	0.00562	1	0.00562	2.45225	0.17813
Error	0.01146	5	0.00229		
Total SS	0.31684	14			
Factor	**SOT**				
	SS	**Df**	**MS**	**F**	**p**
(1) CD (L)	0.00451	1	0.00451	0.92063	0.38137
CD (Q)	**0.08979**	**1**	**0.08979**	**18.3194**	**0.00786**
(2) ACN (L)	0.00588	1	0.00588	1.20092	0.32309
ACN (Q)	**0.18074**	**1**	**0.18074**	**36.8751**	**0.00174**
(3) HAC (L)	0.02176	1	0.02176	4.43988	0.08894
HAC (Q)	**0.11797**	**1**	**0.11797**	**24.0690**	**0.00445**
1 L by 2 L	0.01016	1	0.01016	2.07298	0.20947
1 L by 3 L	0.00148	1	0.00148	0.30229	0.60610
2 L by 3 L	0.00250	1	0.00250	0.51005	0.50706
Error	0.02450	5	0.00490		
Total SS	0.40777	14			

[a]CD is the concentration (mM) of β-cyclodextrin in the mobile phase, ACN is the % of acetonitrile in the organic part of the mobile phase, and HAC is the volume (mL) of acetic acid added to the mobile phase, L is linear effect, Q is quadratic effect, SS is sum of squares, df is degrees of freedom, and MS is mean of squares. Significant factors (*P*-value < 0.05) appear in bold.

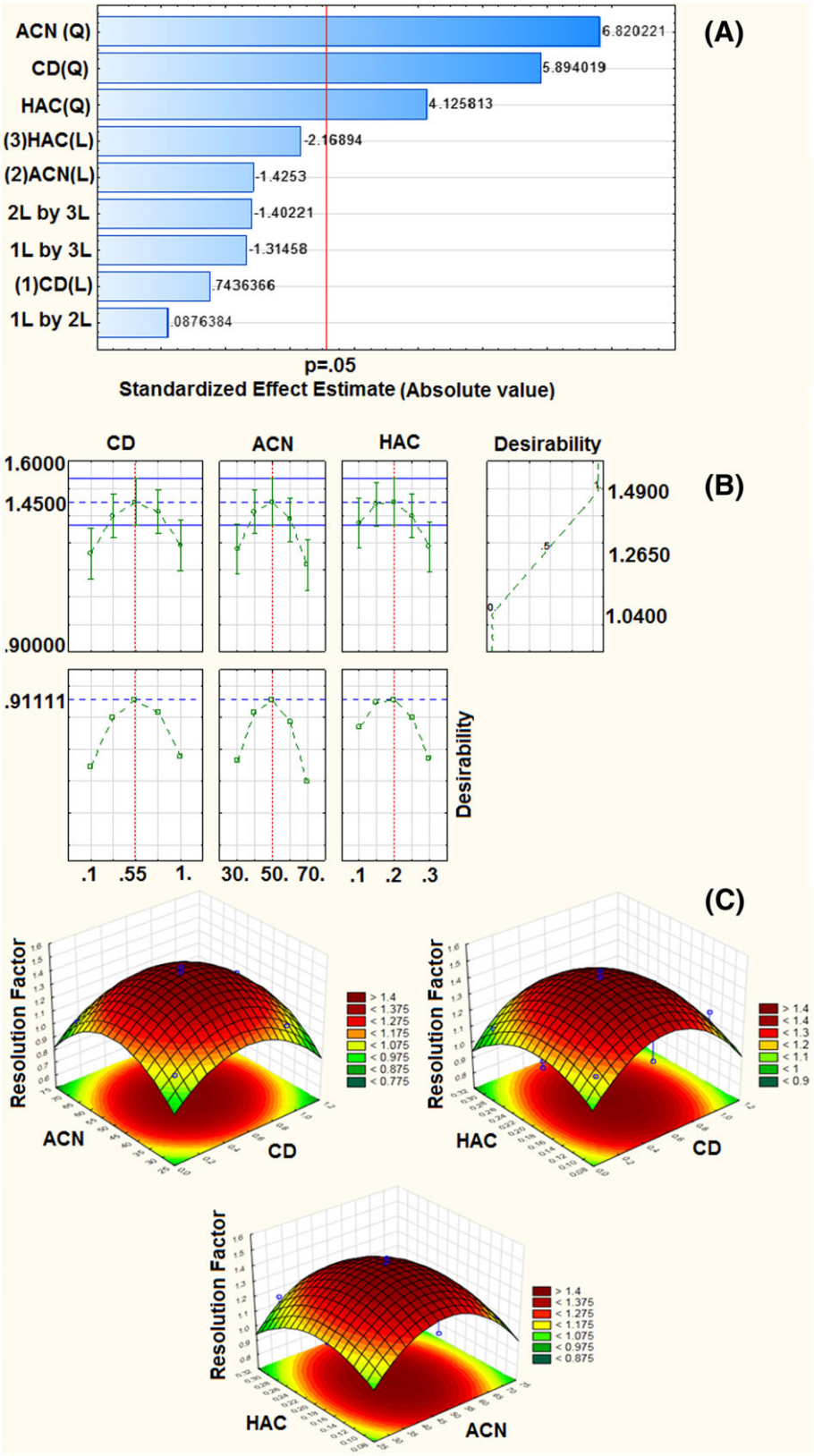

FIGURE 2 A, BBD Pareto chart; B, response desirability function profiling graph; and C, surface plots for BET

in the ANOVA table exceed 0.05. In addition, the response surface graphs demonstrate the relation between the significant variables that required to be optimized and chiral resolution factors of the three drugs' racemates (Figure 2C shows response surface graph for BET as representative example).

The optimum values of the studied variables for the three drugs could be obtained from the desirability function graphs. For each studied variable, its critical value calculated by BBD for the three drugs does not show great differences (RSD% less than 2) so the mean of the three optimum values of each variable (0.57 mM of β-CD, 48.85% of acetonitrile in the organic solvent part and 0.18 mL of HAC) was used for the concurrent enantiomeric separation of the three drugs. The percentage relative error (Er%) obtained for the predicted and observed optimum was found to be 0.17%, −0.53%, and − 0.70% for BET, CAR, and SOT, respectively.

The predictive equations for the chiral resolution factors of the three drugs are as follows:

$$Y = -0.7139 + 1.1689X_1 + 0.0529X_2 + 5.9472X_3 + 0.0004X_1X_2 - 0.9233X_1X_3 - 0.0199X_2X_3 - 0.8833X_1^2 - 0.0005X_2^2 - 12.250X_3^2 \text{ for BET}$$

$$Y = -0.6865 + 1.1174X_1 + 0.0495X_2 + 6.1808X_3 + 0.0012X_1X_2 - 0.9079X_1X_3 - 0.0187X_2X_3 - 0.8881X_1^2 - 0.0004X_2^2 - 12.9166X_3^2 \text{ for CAR}$$

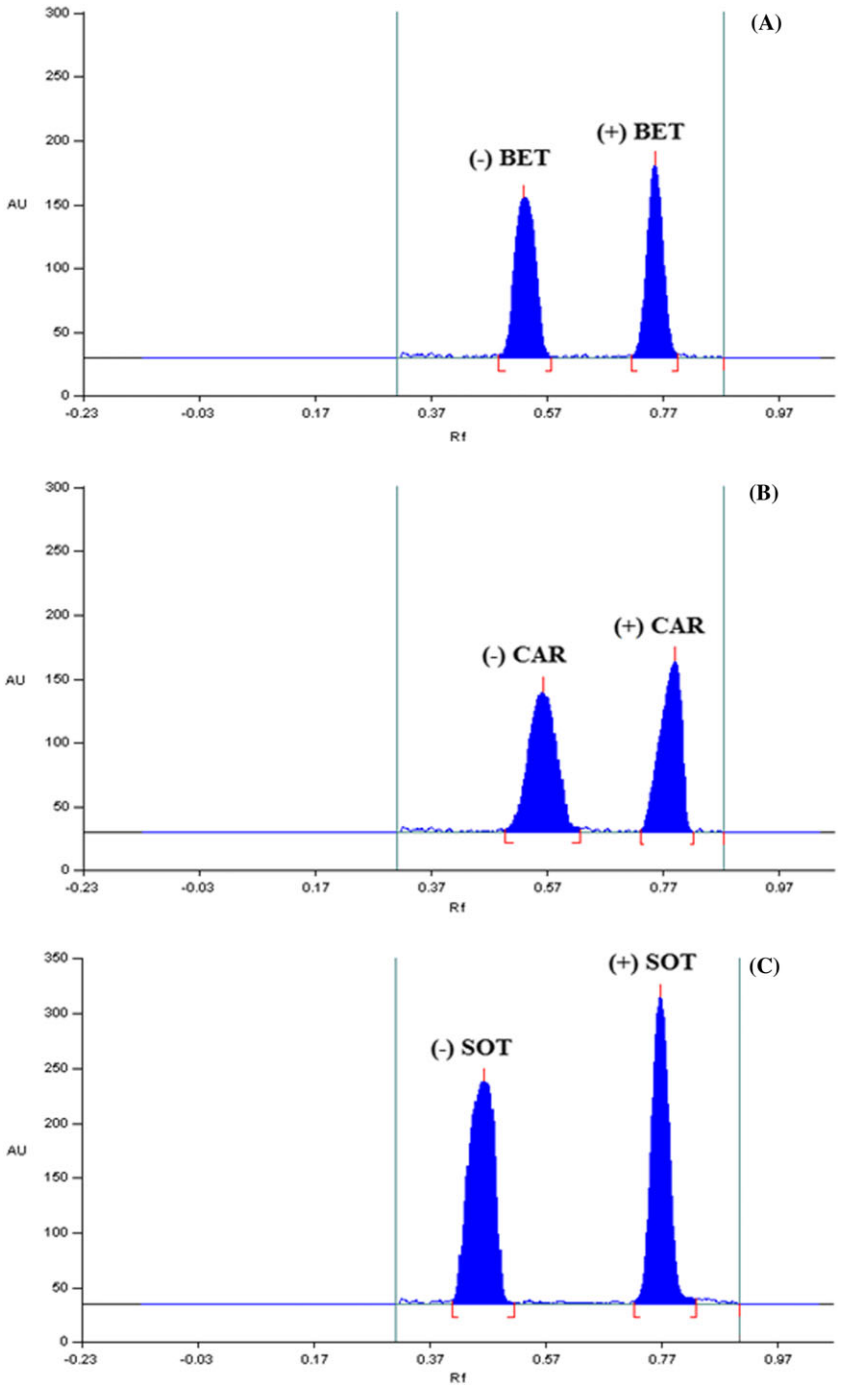

FIGURE 3 Densitograms showing the enantiomeric resolution of A, 6 μg band^{-1} (±) BET; B, 3 μg band^{-1} (±) CAR; and C, 8 μg band^{-1} (±) SOT obtained using the developed method under the optimized conditions

$$Y = -0.5645 + 0.7190X_1 + 0.0533X_2 + 7.4863X_3 + 0.0055X_1X_2 - 0.4263X_1X_3 - 0.0125X_2X_3 - 0.7819X_1^2 - 0.0006X_2^2 - 17.8750X_3^2 \text{ for SOT}$$

where Y is the chiral resolution factor, X_1 is the concentration (mM) of β-cyclodextrin in the mobile phase, X_2 is the % of acetonitrile in the organic part of the mobile phase, and X_3 is the volume (mL) of acetic acid added to the mobile phase.

Using the BBD optimized mobile phase, maximum separation of the (−) and (+) enantiomers of BET (R_F = 0.52 and 0.76), CAR (R_F = 0.57 and 0.80) and SOT (R_F = 0.47 and 0.77) was achieved using acetonitrile:methanol:acetic acid: water (3.4:3.6:0.18:1.00, v/v) containing 0.57 mM β-CD. The chromatograms illustrating good resolution of the enantiomers of the three drugs using the optimized experimental variables are shown in Figure 3.

3.4 | Method validation

Linearity was assessed by preparing six calibration standard solutions of the (−) or (+) enantiomers of BET in the concentration ranges 0.5 to 6 and 0.4 to 6 μg band^{-1} for (−) and (+) BET, respectively. Least-squares method was used to obtain the following regression equations:

Peak area = 171.31+ 1105.44X_1 and Peak area = −31.11 + 1260.97X_2 (where X_1 and X_2 are (−) BET and (+) BET concentration, respectively, in μg band^{-1}).

High correlation coefficients (>0.998) and F-values (1227.32 and 1489.39, for (−) and (+) enantiomers, respectively) suggest the good linearity obtained.[21] Detailed regression parameters are presented in **supporting table S1**.

Signal-to-noise approach was used to estimate limits of detection (LOD) and quantitation (LOQ) which represent the concentrations having ratios of three and 10, respectively. The found LOD and LOQ of (−) BET and (+) BET are 0.15, 0.13, and 0.5, 0.4 μg band^{-1}, respectively. In addition, LOD of (±) CAR and (±) SOT as racemates were found to be 0.2 and 0.3 μg band^{-1}, respectively.

Standard addition method was applied to assess accuracy. Pre-analyzed drug products (Betopic and Betaxolol) were spiked with known quantities of (−) BET and (+) BET at 80%, 100%, and 120% of label claim within their linearity ranges and analyzed, (Table 3). The percentage recoveries for the added concentrations were in the range of 98% to 102% with small percentage relative standard deviations (< 2%) indicating good accuracy of the proposed method.

Precision was checked by intra-day (repeatability) and inter-day (intermediate) precision studies for both BET enantiomers. Fresh solutions of (−) BET and (+) BET at concentrations (0.6: 6, 1: 4, 2: 2, 4: 1, and 6: 0.6 μg band^{-1}) were prepared (three replicates for each concentration level) and analyzed on the same plate in the same day (repeatability) and by different analysts on different days under the same experimental conditions (intermediate precision). The calculated RSD% values did not exceed 2% indicating good precision of the proposed method (**supporting table S2**).

The robustness of the proposed method was checked by making deliberate alterations in the studied experimental conditions, and the resolution between the (−)

TABLE 3 Recovery of (−) and (+) BET enantiomers added to the pharmaceutical dosage forms (n = 5) for evaluation of the accuracy of the method

Preparation		Amount Added[a] (μg Band^{-1})	Total Amount (μg Band^{-1})	Mean % Recovery	RSD (%)[b]	Er (%)[c]
Betopic	(−)	1.6	3.6	98.83	1.53	−1.17
		2	4.0	99.17	1.06	−0.83
		2.4	4.4	100.45	0.86	0.45
	(+)	1.6	3.6	101.13	1.32	1.13
		2	4.0	99.59	0.78	−0.41
		2.4	4.4	100.28	1.24	0.28
Betaxolol	(−)	1.6	3.6	100.61	0.95	0.61
		2	4.0	101.25	1.18	1.25
		2.4	4.4	99.73	1.34	−0.27
	(+)	1.6	3.6	98.75	1.72	−1.25
		2	4.0	100.94	1.28	0.94
		2.4	4.4	101.58	0.84	1.58

[a]Amount added to dosage form solution of nominal concentration 4 μg band^{-1} (±) BET.

[b]Percentage relative standard deviation.

[c]Percentage relative error.

and (+) enantiomers of the three drugs was evaluated. These changes include small variations in the concentration of β-CD in the mobile phase (0.57 ± 0.05 mM), ratio of acetonitrile to methanol in the organic part of the mobile phase (3.4 ± 0.1:3.6 ± 0.1**)**, volume of acetic acid added (0.18 ± 0.01 mL), and detection wavelength (220 ± 1 nm for BET and SOT and 245 ± 1 nm for CAR). The RSD% of chiral resolution factor [$hR_F(+)$/ $hR_F(-)$] of the two separated bands obtained for each racemate was less than 2% under all the conditions tested (**supporting table S3**).

The specificity of the proposed HPTLC method was ascertained by analyzing (−) BET and (+) BET in their standard and/or sample solutions with complete separation of their peaks and in the presence of other excipients normally present in dosage forms. Comparing the R_F values and spectra of the spots with those of the standards confirms also the specificity. The purity of the chromatographic peaks was checked by spectral comparison at three levels (peak apex (M) compared with both peak start (S) and peak end (E) positions of the spot). The results indicate peaks homogeneity.

For stability confirmation, no significant change in the content of (−) BET and (+) BET was observed (RSD% less than 2%) suggesting that these solutions were stable for a minimum of 6 hours at ambient temperature 25 ± 2°C and 7 days while refrigerated, which was sufficient for the whole analytical process.

3.5 | Enantiomeric purity

The proposed method was applied to quantitate the inactive (+) BET enantiomer in the presence of high concentration of the active (−) enantiomer at 1% level [0.5:50 μg $band^{-1}$, (+) BET:(−) BET]. Spiking at 1% level was performed in five replicates, and the (+) enantiomer was quantified with good accuracy where the mean %recovery was 101.15% and good precision (RSD% = 1.54). Thus, the proposed method could be successfully applied in checking the enantiomeric purity of the active (−) enantiomer in raw materials and in dosage forms in quality control laboratories where the inactive (+) enantiomer could be quantified at 1% level of the active enantiomer.

3.6 | Analysis of dosage forms

The proposed enantioselective HPTLC method was successfully applied for the analysis of Betopic ophthalmic suspension and Betaxolol tablets for their contents

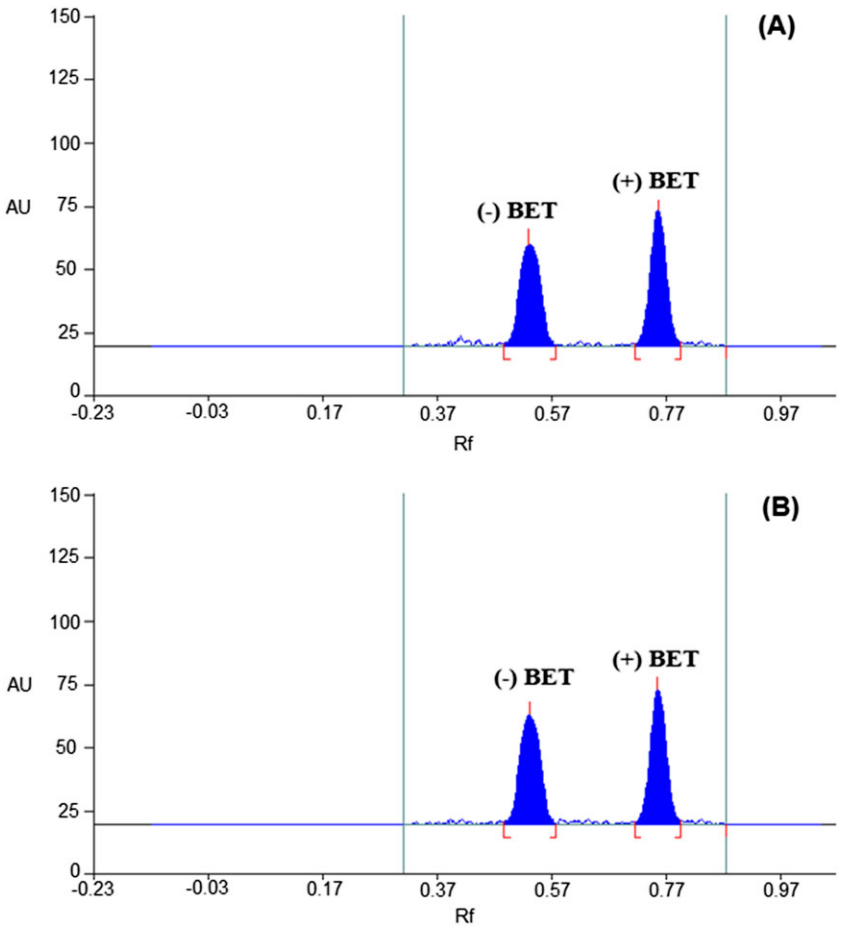

FIGURE 4 Densitograms of A, Betopic ophthalmic suspension (2 μg $band^{-1}$ of (±) BET) and B, Betaxolol tablets (2 μg $band^{-1}$ of (±) BET)

TABLE 4 Assay results for the determination of (-) and (+) BET enantiomers in their pharmaceutical formulations (n=5)

Preparation	(-) BET			(+) BET			Total Mean % recovery ± RSD%	
	Mean %recovery (SD)	RSD%	Er%	Mean %recovery (SD)	RSD%	Er%	Proposed method	Reported method[22]
Betopic® ophthalmic suspension[a]	50.79 (1.23)	1.21	0.79	49.78 (0.93)	0.93	-0.22	100.57 ± 1.32	101.55 ± 0.85
t[c]							1.39	
F[c]							2.41	
Betaxolol® tablets[b]	49.13 (0.53)	0.54	-0.87	49.30 (1.05)	1.06	-0.70	98.43 ± 0.80	100.17 ± 1.90
t[c]							1.88	
F[c]							5.84	

[a]Betopic® ophthalmic suspension labeled to contain betaxolol HCl [(±) BET] 5.6 mg equivalent to 5.0 mg of betaxolol free base per mL.

[b]Betaxolol® tablets labeled to contain betaxolol HCl [(±) BET] 10 mg equivalent to 8.94 mg of betaxolol free base per tablet.

[c]Theoretical values for t and F at P = 0.05 are 2.31 and 19, respectively.

of each drug enantiomer [(−) and (+) BET], Figure 4. The results obtained indicate that both Betopic ophthalmic suspension and Betaxolol tablets contain equal concentrations of both (−) and (+) BET enantiomers and they were in good agreement with the label claim as indicated by the good % recoveries obtained (Table 4). The method results of analyzing the total (±) BET in the two formulations were compared with a non-chiral HPTLC method for analyzing BET racemate.[22] The statistical comparison indicates no significant difference between the results of the proposed and the reported methods (Table 4).

4 | CONCLUSION

This work describes a multivariate design assisted simple HPTLC method (compared with the reported HPLC and capillary electrophoresis) for the enantiomeric resolution of the three racemic mixtures of β-blockers (BET, CAR, and SOT) using β-CD as chiral mobile phase additive without the tedious plates' impregnation step which may affect reproducibility which represents the first HPTLC attempt to separate β-blockers' enantiomers using the chiral selector as mobile phase additive. Important experimental parameters including ratio of acetonitrile in the organic part of the mobile phase, the concentration of β-CD in the mobile phase, and volume of acetic acid added to the mobile phase were carefully adjusted using three-factor BBD which enables fast optimization with the least number of experimental runs. The obtained 3D-surface response plots showed that the effect of the three studied variables on the chiral resolution of the three drugs was quadratic more than linear. The optimized method has been applied successfully for the enantiomeric resolution of the three racemic mixtures of BET, CAR, and SOT and the quantitation of (−) and (+) BET enantiomers in its drug substance and drug products and to check the enantiomeric purity of (−) enantiomer in the presence of its inactive enantiomer where the (+) enantiomer was accurately and precisely quantified at 1% level in the active enantiomer. The advantages offered by the proposed HPTLC chiral method suggest that it can be applied for chiral resolution of other chiral β-blocker drugs.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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