Received: 8 June 2017 | Revised: 12 October 2017 | Accepted: 17 October 2017
DOI: 10.1002/chir.22793

REGULAR ARTICLE

WILEY

Liquid chromatographic separation and thermodynamic investigation of lorcaserin hydrochloride enantiomers on immobilized amylose–based chiral stationary phase

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Abstract
A novel liquid chromatographic method was developed for enantiomeric separation of lorcaserin hydrochloride on Chiralpak IA column containing chiral stationary phase immobilized with amylose tris (3.5-dimethylphenylcarbamate) as chiral selector. Baseline separation with resolution greater than 4 was achieved using mobile phase containing mixture of n-hexane/ethanol/methanol/diethylamine (95:2.5:2.5:0.1, v/v/v/v) at a flow rate of 1.2 mL/min. The limit of detection and limit of quantification of the *S*-enantiomer were found to be 0.45 and 1.5 μg/mL, respectively; the developed method was validated as per ICH guideline. The influence of column oven temperatures studied in the range of 20°C to 50°C on separation was studied; from this, retention, separation, and resolution were investigated. The thermodynamic parameters ΔH°, ΔS°, and ΔG° were evaluated from van't Hoff plots,(Ink′ $_{versus}$ 1/T) and used to explain the strength of interaction between enantiomers and immobilized amylose–based chiral stationary phase

KEYWORDS
enantiomer and chiral separation, immobilized amylose, lorcaserin hydrochloride, van't Hoff plots

1 | INTRODUCTION

Obesity is a life-threatening disorder in which there is an increased risk of morbidity and mortality arising from concomitant diseases such as type 2 diabetes, hypertension, stroke, and cancer. Lorcaserin hydrochloride (APD356) is chemically (*R*)8-chloro-1-methyl 2,3,4,5-tetrahydro-1H-3-benzazepine as shown in Figure 1, which is novel, selective, and potent anti-obesity drug that targets the activation of the serotonin 5HT2C receptor and is intended to promote weight loss in obese population by acting as agonist at the intended target.[1,2] 5HT2C has been reasonably demonstrated to underlie the anorexigenic effect of lorcaserin,[3,4] it as some abuse potential also, and is listed as a Schedule IV drug in the Controlled Substances Act.[5]

The recommended dosage for initial monotheraphy is 10 mg/day, administered orally in the form of tablet; lorcaserin hydrochloride is enantiomerically active *R*-enantiomer that shows higher affinity than *S*-enantiomer, so *S*-enantiomer could be present as chiral impurity.[6,7] A literature survey reveals that an LC-ESI-MS/MS assay method is reported for determination of synthetic drug in natural and herbal slimming product,[8] another one is UPLC–MS-MS assay in Plasma and Brain tissue samples.[9] However, an extensive literature survey revealed that no LC method has been reported for quantitative determination and enantiomeric separation of lorcaserin in bulk drug and formulation. Therefore, it was felt necessary to develop an accurate, precise, and robust enantioselective normal-phase HPLC method for separation of lorcaserin enantiomers.

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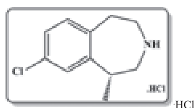

FIGURE 1 Chemical structure of lorcaserin hydrochloride

Here, we present a work describing the development and validation of normal phase LC method to determine the enantiomeric purity of lorcaserin hydrochloride on chiral stationary phase immobilized with amylose tris-(3,5-dimethylphenylcarbamate) on silica gel as shown in Figure 2, as chiral selector, the chromatographic retention, separation, and resolution were investigated at different column oven temperatures. The data were used to derive apparent thermodynamic parameters, which in turn explain the some of the mechanistic aspects of interaction of enantiomer with chiral stationary phase (CSP).[10]

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

Lorcaserin hydrochloride and its *S*-isomer obtained as gratis sample from Mylan Inc.laboratories Hyderabad, HPLC grade *n*-hexane,2-propanol and methanol were procure from Rankem Fine Chemicals (India), diethylamine was procure from Merck, (India) and ethanol from Changshu Hongsheng Fine Chemical Co Ltd (China).

2.2 | Equipment

The present work was performed by using HPLC system (model 1100 series, Agilent Technology) equipped with quaternary pump, degasser, auto sampler, column oven, and PDA detector. The chromatographic and the integrated data were recorded using Dell computer system as workstation controlled using Chromeleon 7.2 as data-acquiring software (Thermo-Fisher). Ruggedness was performed on Shimadzu: Prominence HPLC system equipped with quaternary pump, degasser, auto sampler, column oven and PDA detector and controlled using Chromeleon 7.2 as data-acquiring software.

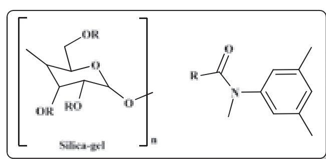

FIGURE 2 Chemical structure of tris-(3, 5-dimethylphenylcarbamate) of amylose immobilized on silica gel

2.3 | Preparation of system suitability solution

A system suitability solution of lorcaserin hydrochloride and its *S*-enantiomer was prepared by dissolving 5.0 mg of each substance in 5 mL of methanol than further dilute 1 mL of this solution to 10 mL with methanol to obtain final concentration of 0.1 mg/mL.

2.4 | Preparation of standard solution

A standard solution was prepared by dissolving 25.0 mg of lorcaserin hydrochloride and its enantiomer in 25 mL of methanol than further dilute 1 mL of this solution to 100 mL with methanol to obtain final concentration of 10 μg/mL.

2.5 | Preparation of sample solution

A sample solution was prepared by dissolving 50.0 mg of lorcaserin hydrochloride in 5 mL of methanol sonicate to dissolved, than diluted up to 25 mL with methanol the test concentration is 2 mg/mL.

2.6 | Chromatographic conditions

The chromatographic separation was achieved by using Chiralpak IA (25 cm × 4.6 mm I.D., 5 μm particle size) (Daicel Chemical Industries, Ltd, Tokyo, Japan) as chiral column. The mobile phase used was a mixture of n-hexane/ethanol/methanol/diethylamine in the ratio of (95:2.5:2.5:0.1, v/v/v/v). By using 1.2 mL/min as flow rate, the column oven temperature was maintained at 25°C, with injection volume of 10 μl, and detection wavelength was 220 nm.

2.7 | Method development and thermodynamic study

The objective of this chromatographic method development was to achieve the baseline separation for both the isomers of lorcaserin for accurate quantification of *S*-enantiomer as chrial impurity, for method development racemic mixture was prepared (0.1 mg/mL) in methanol. To develop a robust and rugged method, different stationary phases (CSPs) such as Chiralpak AD-RH, Chiralcel OD-H, Chiralcel OJ-H,Chiralpak AD-H, and Chiralpak IA were used to achieve the chromatographic separation of the 2 isomers with different mobile phases. Numerous experiments were conducted to select the best stationary and mobile phase that could give the optimum resolution and selectivity for both the isomers.

Thermodynamic study was performed under normal-phase condition at different column oven temperatures between 20°C and 50°C; from this, retention, separation, and resolution were investigated. Retention factors (K') were calculated from the formula $(t_R - t_0)/t_0$, where t_R is the retention time of a particular enantiomer and t_0 is the retention time of an unretained peak. Separation factor (α) for pair of enantiomer of lorcaserin was calculated from k_2'/k_1', where k_1' and k_2' are the retention factor for the first and second eluted enantiomer, respectively; from this, as change in enthalpy ΔH°, change in entropy ΔS°, and change in Gibbs free energy ΔG° were evaluated. [11]

2.8 | Method validation

The proposed method was validated as per ICH guideline; following parameters were covered accuracy, precision, limit of detection (LOD) and quantification, and linearity and robustness. [12-14]

2.9 | System suitability

System suitability was evaluated by injecting system suitability solution once and 6 replicate injections of standard solution.

2.9.1 | Precision

The precision of the method can be verified by performing method precision (repeatability) and intermediate precision studies; repeatability, study was performed by injecting 6 individual preparation of lorcaserin hydrochloride spiked with 0.5 % of *S*-enantiomer; % RSD of area obtain for *S*-enantiomer content was calculated; intermediate precision was performed by using different analyst, different instrument by performing the analysis on different days.

2.10 | Linearity

Linearity was evaluated for both the enantiomers by preparing different concentration in the range of LOQ to 200% of specification limit; a calibration curve was obtain by plotting peak response versus analyte concentration. The correlation coefficient (r), slope, and intercept were calculated

2.11 | LOD and limit of quantification

Limit of detection and limit of quantification (LOQ) were determined from signal-to-noise ratio of 3:1 and 10:1, respectively. By injecting a series of diluted solution of known concentration, precision was determine at LOQ level by injecting 6 preparation.

2.11.1 | Accuracy

The accuracy of the method was evaluated for *S*-enantiomer in triplicate at 4 concentration levels, that is, LOQ, 50%, 100%, and 150% of specification limit (0.5%); the percentage recovery was calculated at each level.

2.12 | Robustness

The robustness of the method was studied by deliberately altering the different variable and evaluates the system suitability parameter; the variable studied was flow rate ± 0.2 mL/min, composition of organic solvents (methanol and ethanol) in the mobile phase ± 10% absolute, and concentration of diethylamine ± 20%.

2.13 | Solution stability and mobile phase stability

Stability of both the enantiomer in solutions was studied by keeping the solutions in tightly capped volumetric flask at room temperature on laboratory bench for 48 hours. Content of *S*-enantiomer was checked at every 6 hours intervals up to 48 hours. Mobile phase stability was determined by analyzing freshly prepared lorcaserin solution by using mobile phase prepared before 48 hours.

3 | RESULTS AND DISCUSSION

3.1 | Method development

The main purpose of this study was to get the baseline separation of both the enantiomers. The observations were that separation of lorcaserin isomers could not be achieved on Chiralpak AD-RH, Chiralcel OJ-H, and Chiralcel OD-H column; little separation was observed on Chiralpak AD-H column; relatively better peak shape and resolution were achieved on Chiralpak IA column.

With later column initial efforts were made using mobile phase containing mixture of *n*-hexane and 2-propanol in the proportion of 90:10, v/v. Peak obtain as late eluting broad peak but by adding 0.1% diethylamine as modifier 2 isomers were eluted at about 6.2 to 6.4 minutes with resolution of 0.58. To get better resolution, amount of 2-propanol in mobile phase was reduced to *n*-hexane:2-propanol:diethylamine in proportion of 95:05:0.1, v/v/v. By using this mobile phase, the resolution between 2 isomers (retention time about 8.0 and 8.5 min) was improved to 0.84. By using *n*-hexane/

2-propanol/diethylamine in proportion of 98:02:0.1, v/v/v, the resolution was improved to 1.66. To get better resolution, methanol was incorporated in mobile phase *n*-hexane/2-propanol/methanol/diethylamine in proportion of 95:2.5:2.5:0.1, v/v/v/v. By using this mobile phase, resolution between 2 isomers (retention time 7.34 and 8.20 min) was found to be 2.09 but peak shape showing somewhat fronting, so to improve the peak shape and column efficiency, 2-popanol was replaced with ethanol so the final optimized condition was described as follows The separation was achieved using Immobilized Chiralpak IA column (25 cm × 4.6 mm I.D., 5 μm particle size) with mobile phase containing mixture of *n*-hexane/ethanol/methanol/diethylamine (95:2.5:2.5:0.1, v/v/v/v). The flow rate of the mobile phase was 1.2 mL/min, the column oven temperature was maintained at 25°C, injection volume was 10 μl, and detection wavelength was 220 nm. The method development summary is shown in Table 1.

The fundamental basis for separation of enantiomer on chromatographic system is transformation of enantiomers to diastereomers or creation of diastereomeric relationship between analyte and CSP by forming reversible short-lived, transient diastereomeric complexes on surface of CSP.[15] The energetic differences between 2 diastereomeric complexes were the fundamental basis for stereoselective retention in chromatographic system. The complexes are formed as a result of hydrogen bonding, dipole-dipole interactions, π- π bonding, electrostatic interactions, and inclusion complex formation. Chiralpak IA has given the best resolution, it is 3,5-dimethylphenylcarbamate derivative of amylose immobilized on silica gel. The 2 methyl group at 3,5 position of phenyl ring act as electron donating substituents, so inductively, it would increase the electron density of carbonyl oxygen of carbamate group, so it would increase the retention of enantiomers. From these observations, we could interpret that the separation of lorcaserin enantiomer may be due to the interaction between the polar groups of lorcaserin enantiomer (—NH) and the polar carbamate group on the CSP. The carbamate group on the CSP interacts with (—NH) group of lorcaserin enantiomer through hydrogen bonding; in addition, the dipole- dipole interaction occurs between the C═O group in carbamate on the CSP by accepting electron from —Cl group on lorcaserin enantiomer that act as electron donor.[16] Okamoto et al reported that solute having aromatic functionality could provide additional stabilizing effect on the solute-CSP complex by insertion of aromatic portion in to the chiral cavity.[17] Amylose forms a helical structure and possesses more defined grooves, making it different than cellulose derivative. These polysaccharides contain large number of chiral

TABLE 1 Method development summary

Column (CSP) and Mobile Phase	Remark
Chiralpak AD-RH 10 mM ammonium acetate buffer:MeOH (80:20)	Broad peak for both enantiomer at same RT (α = 0.0)
Chiralpak AD-RH 10 mM ammonium acetate/EtOH (80:20)	Broad peak for both enantiomer at same RT (α = 0.0)
Chiralcel OD-H H/IPA/DEA (90:10:0.1; v/v/v)	Late eluting broad peak (dump shape) (α = 0.21)
Chiralcel OJ-H H/IPA/DEA (90:10:0.1; v/v/v)	Late eluting broad peak (dump shape) (α = 0.38)
Chiralpak AD-H H/EtOH/MeOH/DEA (95:2.5:2.5:0.1; v/v/v)	No base line separation Rs = 0.98 (α = 1.07)
Chiralpak IA H/IPA (90:10; v/v)	Late eluting broad peak (α = 0.98)
Chiralpak IA H/IPA/DEA (90:10:0.1; v/v/v)	No base line separation Rs = 0.58 (α=1.03)
Chiralpak IA H/IPA/DEA (95:05:0.1; v/v/v)	No base line separation Rs = 0.84 (α = 1.06)
Chiralpak IA H:IPA:DEA (98:02:0.1; v/v/v)	No base line separation Rs = 1.66 (α = 1.09)
Chiralpak IA H/IPA/MeOH/DEA (95:2.5:2.5:0.1; v/v/v)	No base line separation Rs = 2.09 (α = 1.15)
Chiralpak IA H/EtOH/MeOH/DEA (95:2.5:2.5:0.1; v/v/v)	Base line separation Rs ≥ 4.0 (α = 1.30)

active sites and thus relative high probability of interaction with the solute leading to separation of 2 enantiomers. Peak tailing may result due to extra high active sites. Addition of small amount of diethylamine basic modifier in the mobile phase was helpful when analytes contains amino basic functions, reduces peak tailing by masking the residual silanol group of the CSP, and improves peak shape.

3.2 | Method validation

System suitability is the confirmation of suitability and reproducibility of chromatographic system for analysis; the system was deemed as suitable as shown in Table 2.

The method was found to be reproducible with relative standard deviation (RSD) of less than 5% for peak area, and resolution was found more than 4.0 as shown in Figure 3A. Precision was studied from the %RSD for the isomer content, for repeatability study was within 1%, and for intermediate precision within 1.5%, which gives better precision of the method as shown in Table 3.

The LOD and LOQ for *S*-enantiomer were found to be 0.45 and 1.5 μg/mL and for *R*-enantiomer 0.56 and 1.98 μg/mL; the %RSD value of precision at LOQ level was 3.8 and 4.7, respectively, as shown in Table 5. The accuracy was determine form calculating recovery from spike amount, it was found to be in the range of 94.86% to 98.03% as shown in Table 4; the overall RSD at all level was found to be 6.4 %, the spike chromatogram at specification limit as shown in Figure 3B.

The detector response was found to be linear in the range of LOQ to 200% of specification limit for both the isomers. The calibration curve was plotted between concentration and the peak area for *R*-enantiomer; correlation coefficient obtain was 0.997 with equation of calibration curve $Y = 7132.6 \times -10577.2$ and for *S*-enantiomer correlation of 0.998 with equation of calibration curve $Y = 6862.4 \times -17904.3$; the above result

TABLE 2 System suitability report

Analyte	R_t	α	Rs	N	T
R-Lorcaserin	7.68	1.23	...	7069	1.16
S-Lorcaserin	9.42	...	4.60	9281	1.14

Abbreviations: α, selectivity; N, number of theoretical plates; Rs, USP resolution; R_t, retention time; T, tailing factor.

TABLE 3 Precision data of lorcaserin

Spike Test Prep	Method Precision *S*-enantiomer, %	Intermediate Precision *S*-enantiomer, %
Prep-1	0.50	0.50
Prep-2	0.50	0.49
Prep-3	0.50	0.50
Prep-4	0.49	0.50
Prep-5	0.50	0.49
Prep-6	0.50	0.50
Average	0.50	0.50
STDV	0.004	0.005
%RSD	0.82	1.04

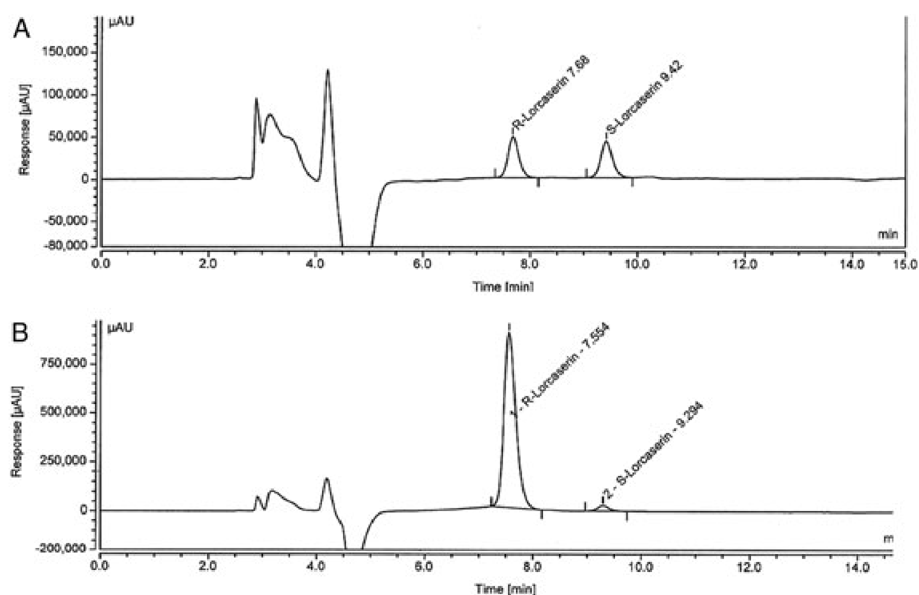

FIGURE 3 Representative chromatograms of (A) resolution of racemic mixture of lorcaserin enantiomers and (B) lorcaserin spike with *S*-enantiomer at specification level

TABLE 4 Recovery results of *S*-enantiomer of lorcaserin

Spike Levels	Added Amount, μg/mL	Recovered amount, μg/mL	Recovery, %	%RSD
LOQ	1.02	1.00	98.03	5.2
50%	5.25	4.98	94.86	1.2
100%	10.21	10.05	98.43	0.9
150%	15.42	14.99	97.21	1.4

shows excellent goodness of fit between peak area and concentration over working range as shown in Table 5. The robustness was evaluated from the chromatographic resolution between both the enantiomer under varied conditions. The deliberate changes in method conditions did not significantly affect the resolution between the isomers, and elution order remains the same, which gives better significant of method. Robustness data at each condition were shown in Table 6, demonstrating optimum robustness. Solution stability and mobile phase stability was evaluated from the enantiomer content and was within ±10% during solution stability experiments, so concluded that it is stable in diluent as no significant change was observed up to 48 hours.

3.3 | Investigation of thermodynamic parameters

The separation of enantiomer on CSP is based on formation of complex between analyte and CSP; separation can be based on free and complexed states energy balanced, and it would be studied from thermodynamic considerations. The equilibrium binding constant (k_i) that measures the binding strength may be based on standard Gibbs free energy change (ΔG):

$$\Delta G^{\circ} = \text{-RT} \ \text{In} k_i, \quad (1)$$

TABLE 6 Robustness result of developed method

Experimental Condition	Resolution >4.0	USP Tailing <2.0	%RSD <5.0%
Flow rate (mL/min)			
1.0	4.40	1.1	1.8
1.2	4.60	1.1	1.1
1.4	4.51	1.2	1.9
Column temperature (°C)			
20	5.50	1.3	1.3
25	4.56	1.1	1.5
30	4.19	1.2	1.8
Ethanol (%) in mobile phase			
2.3	4.79	1.2	1.8
2.5	4.59	1.1	1.4
2.8	4.25	1.2	1.6
Methanol (%) in mobile phase			
2.3	4.81	1.2	1.9
2.5	4.60	1.1	1.2
2.8	4.35	1.2	2.1
Diethyl amine (%) in mobile phase			
0.08	4.81	1.1	1.9
0.10	4.58	1.1	1.5
0.12	4.35	1.2	1.8

TABLE 5 Validation result

Validation Parameter	*R*-isomer	*S*-isomer
System precision (n = 6, %RSD)		
Retention time	0.09	0.1
Peak area	1.2	1.1
LOD and LOQ		
Limit of quantification (μg/mL)	1.98	1.50
Limit of detection (μg/mL)	0.56	0.45
Precision at LOQ level (% RSD)	4.7	3.8
Linearity		
Calibration range	LOQ-200%	LOQ-200%
Correlation coefficient	0.997	0.998

where R is the universal gas constant (8.3144 J/[mol·K]), T is the absolute temperature in Kelvin, k_i is the binding constant, and i denotes the corresponding enantiomers. The large energy difference is set free upon solute and CSP association; because of favorable energetic state of bound versus free solute, this will give large association constant.[18-20]

The Gibbs free energy is composed of enthalpic and entropic contributions (ΔH° and ΔS°), the enantiomeric strong binding driven by intermolecular interaction as measured by the enthalpy change ΔH. The process of complexation is usually paid off by an entropic cost ΔS, shown as following Gibbs-Helmholtz equation:

$$\Delta G^{\circ} = \Delta H^{\circ} - T\,\Delta S^{\circ}. \quad (2)$$

This gives Van't Hoff equation, which gives the clear way for straightforward determination of thermodynamic parameter of enantioseparation, such as the standard enthalpy (ΔH) and entropy (ΔS) of transfer of the solute from the mobile phase to the CSP.[21-23]

$$\text{In } k' = -\Delta H^{\circ}/RT + \Delta S^{\circ}/R + \text{In } \Phi, \quad (3)$$
$$\text{In } k' = -\Delta H^{\circ}/RT + \Delta S^{\circ},$$

$$\text{In } \alpha = -\Delta\Delta H^{\circ}/RT + \Delta\Delta S^{\circ}/R, \quad (4)$$

where k represents the retention factor; R is the universal gas constant (8.3144 J/[mol·K]); T is the absolute temperature; ΔH and ΔS are the molar enthalpy and molar entropy of the adsorption; ΔΔH and ΔΔS are the differences ΔH2– ΔH1 and ΔS2–S1; and Φ is the column phase ratio. The slope and intercept are -ΔH/R and ΔS/R + lnΦ (ΔS*). For the linear plot of lnα versus 1/T, the slope and intercept are –ΔΔH/R and ΔΔS/R, respectively. The logarithm of retention factor (*Ink*) was plotted versus inverted temperature in Kelvin.[24-28]

TABLE 7 Influence of temperature on chiral separation

T (°C)	t_1	t_2	k'_1	k'_2	α	R_s
20	8.205	10.458	2.28	3.18	1.39	5.50
25	7.668	9.401	2.07	2.76	1.34	4.66
30	7.058	8.388	1.82	2.36	1.29	4.17
35	6.596	7.619	1.64	2.05	1.25	3.80
40	6.177	6.97	1.47	1.79	1.22	3.28
45	5.810	6.430	1.32	1.57	1.19	2.73
50	5.474	5.951	1.19	1.38	1.16	2.24

Abbreviation: α, selectivity; k'_1 & k'_2, retention factor for both enantiomers; Rs, USP resolution; t_1 and t_2, retention time.

The effect of column oven temperature on the retention, separation, and resolution of enantiomers of lorcaserin was studied in the range of 20°C to 50°C by using optimized composition of mobile phase; 7 experiments at an interval of 5°C were conducted. The lower limit was set as −5°C to the set temperature of the method, as expected, the retention times for all the compound were decreased, as the temperature of column oven increased from 20°C to 50°C, it is observed that retention factor (k'), separation factor (α), and resolution (Rs) for lorcaserin enantiomer decreases linearly, the separation factor (α) decreased from 1.39 to 1.16, and resolution (Rs) decreased from 5.50 to 2.24, as the temperature of column oven increased from 20°C to 50°C as shown in Table 7 and Figure 4.

In enantiomeric separation, as shown in Table 7, the relation between chromatographic retention and column temperature can be described by the Van't Hoff plots. In this work, linear plots of lnk versus 1/T were obtained having the regression coefficient r^2 = 0.999 for both the enantiomer as shown in Table 8 and Figure 5. The plots of lnα versus 1/T and resolution versus column temperature were also linear having the regression coefficient r^2 = 0.992 and 0.991 as shown in Figures 6 and 7. From Van't Hoff plots, values of ΔΔH and ΔΔS were thus calculated. Table 8 shows the Van't Hoff plots and thermodynamic parameters for the chiral separations.

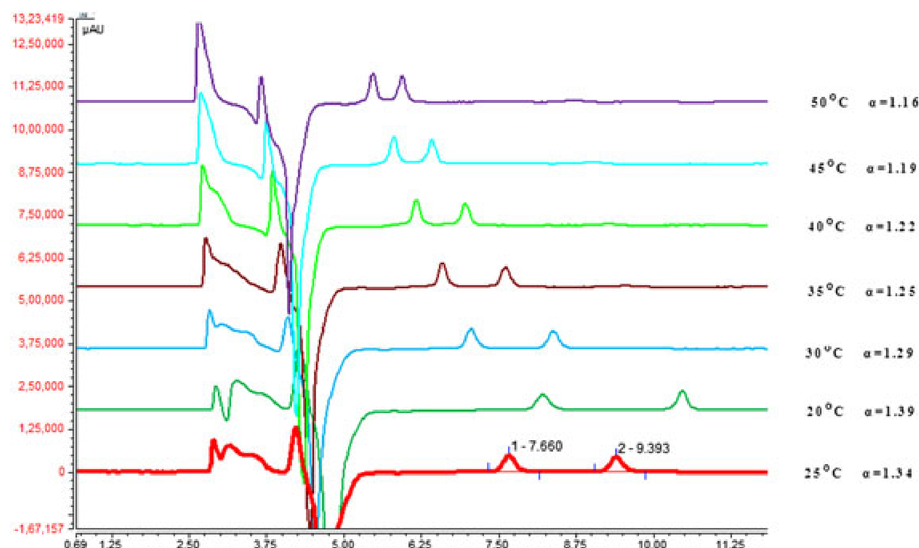

FIGURE 4 Effect of column temperature on retention (tR), separation (α), and resolution (Rs) of lorcaserin enantiomers on Chiralpak IA using *n*-hexane/ethanol/methanol/diethylamine (95:2.5:2.5:0.1 v/v/v/v) as optimum mobile-phase composition

TABLE 8 Thermodynamic parameter

Parameter	*R*-lorcaserin		*S*-lorcaserin
Slope	899.3		1149
Intercept	2.705		3.418
r^2	0.999		0.999
ΔH	7.48		9.55
ΔΔH (KJ/mol))		−2.08	
ΔS	0.0225		0.0284
ΔΔS		0.006	
ΔΔG (KJ/mol))		−2.07	

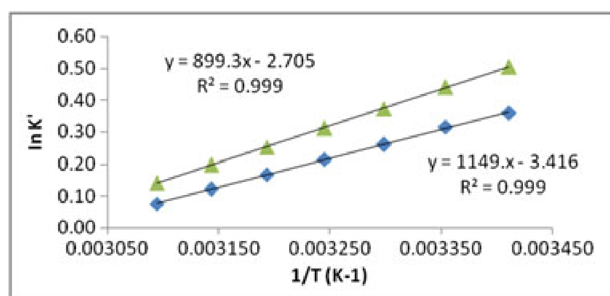

FIGURE 5 Van't of plot for lorcaserin enantiomers

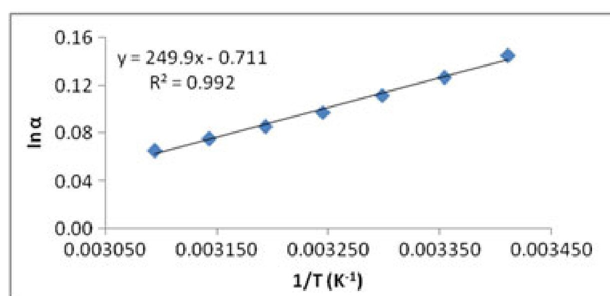

FIGURE 6 Plot of Inα vs 1/T (K^{-1})

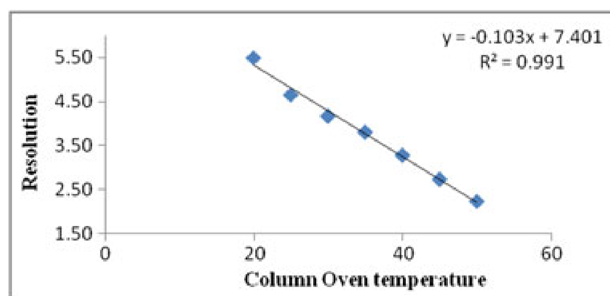

FIGURE 7 Plot of oven temperature vs resolution

The linear plots indicate that these thermodynamic parameters are constant within the experimental temperature. No significant changes in the composition of the stationary phase, that is, the enantioselective mechanism, remained unchanged.

From the values of ΔΔH and ΔΔS, it seems that the adsorption process between solute and stationary phase was somewhat enthalpically controlled. It should be noted the biggest absolute values of ΔΔH, indicating the difference in association energy between the 2 enantiomers with the CSP, which shows the best enantiomeric separation on the column used for study. The interactive force between the used mobile phase and stationary phase was estimated from the absolute value of ΔΔH. When the value is greater than 1.0 kJ/mol, the more retained enantiomers would suffer π—π interactions or hydrogen bonding, which shows somewhat strong chiral recognition. For lorcaserin, the value obtain was (ΔΔH = −2.08 KJ/mol), which shows strong π—π interactions or hydrogen bonding with CSPs giving chiral separation. Also shows strong preference to exothermic adsorption processes can be inferred from a negative sign. This gives the strength of interaction between enantiomers and immobilized amylose based CSP, from this the chiral recognition was enthalpically controlled.

4 | CONCLUSION

A novel, simple, specific, precise, and robust normal phase chiral HPLC method was developed by studying the effect of column oven temperature on retention and separation on Chiralpak IA column with the evaluation of apparent thermodynamic parameters derived from Van't Hoff plots (Ink' $_{\text{versus}}$ 1/T; Inα $_{\text{versus}}$ 1/T) were used to explain some aspect of chiral recognition mechanism of Chiralpak IA column for both the enantiomer of lorcaserin. The highly linear and high value of enthalpy suggested that the stationary phase confirmation does not change in studied temperature, and high value shows interaction of solute with stationary phase Chiralpak also shows that strong preference to exothermic adsorption processes can be inferred from a negative sign, which shows the enthalpically controlled chiral recognition. The baseline separation with Rs > 4.0 was achieved between the 2 enantiomers. All validation parameter shows acceptable result hence developed method efficiently used for quantitative determination of enantiomeric purity of lorcaserin hydrochloride in bulk drugs and formulation product.

ACKNOWLEDGMENTS

The authors are thankful to the management of Wockhardt for giving opportunity to pursue further

study and to the Chairman, Maulana Azad Education Trust's Y.B. Chavan College of Pharmacy, Aurangabad, for providing the research facility.

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How to cite this article: Wani DV, Rane VP, Mokale SN. Liquid chromatographic separation and thermodynamic investigation of lorcaserin hydrochloride enantiomers on immobilized amylose–based chiral stationary phase. *Chirality*. 2017;1–9. https://doi.org/10.1002/chir.22793