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Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Lorcaserin Hydrochloride in Bulk and Tablet Dosage Form

Hemlata S. Bhawar*, Chetan C. Kedari, Sagar D. Magar

Department of Pharmaceutical Chemistry, Pravara Rural College of Pharmacy, Loni, Tal-Rahata, Dist-Ahmednagar, MS (413736)

ABSTRACT

In the current study a simple, precise, sensitive and accurate reversed phase liquid chromatography method was developed for the analysis and estimation of Lorcaserin HCL in bulk and tablet dosage form. The present study of Lorcaserin HCL was achieved by using Cosmosil C18 (250nm×4.6ID, Particle Size: 5 Micron) Column with mobile phase Methanol:10mM KH_2PO_4 Buffer (70:30) pH:3 at a flow rate 0.8ml/min with UV detection at 222nm. The retention time for Lorcaserin HCL was found to be 5.108 min. In Linearity the correlation coefficient (R^2) for Lorcaserin HCL was found to be 0.9995, slope is 42071 and intercept was found to be 21966 which are well within the acceptance criteria. The mean percent recovery for Lorcaserin HCL at three different levels for 50%, 100%, and 150% was found to be 100.65%, 98.84% and 100.34%. The %RSD (NMT 2%). In precision study interday (RSD is 0.26%) and intraday (RSD is 0.29%) are found. Forced degradation experiments was carried out by exposing standard form of Lorcaserin HCL for Acid-base hydrolytic, Oxidative, photolytic and thermal stress conditions. The method has been validated by System suitability parameters, Linearity, Accuracy and Percent recovery, Precision, Ruggedness, Robustness, LOD and LOQ.

Keywords: Lorcaserin hydrochloride, RP-HPLC, Validation.

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***Address for Correspondence:**

Hemlata S. Bhawar, Department of Pharmaceutical Chemistry, Pravara Rural College of Pharmacy, Loni, Tal-Rahata, Dist-Ahmednagar, MS (413736)

INTRODUCTION:

Lorcaserin Hydrochloride is highly selective $(5HT)2_C$ receptor agonist, is used for the treatment of obesity. It has been shown to reduce body weight and it is targeting the $(5HT)2_C$ receptor may alter body weight by regulating satiety. Lorcaserin is chemically derivative of benzazepine [1]. Lorcaserin is also evaluated for its ability to interact with number of other human GPCRs and neurotransmitter transporters [2]. The chemical name is (5R)-7-chloro-5-methyl-2, 3, 4, 5-tetrahydro-1H-3 benzazepine hydrochloride [3]. Lorcaserin is approved by many countries as a 10-mg tablet for BID dosing and it is indicated for long term weight management in adults with obesity [4]. In clinical trials 47.5% subjects who received 10-mg Lorcaserin once at daily they lost 5% or more of their body weight [5]. In introduction of stability indicating study, it is determine stability of drug substance which may affect purity potency and safety [6]. The values between 5% to 20% degradation of the drug substance considered as reasonable and acceptable generally for validation of chromatographic assays [7]. Different methods were described in the literature for the determination of Lorcaserin HCL in tablet and in API form. The techniques include validated UPLC MS/MS Assay for rapid determination of Lorcaserin in Plasma and brain tissue samples [8], Liquid chromatographic separation and thermodynamic investigation of Lorcaserin HCL enantiomers on Immobilized amylose based chiral stationary phase [9], development and validation of a HPLC-based bioanalytical method for Lorcaserin using solid phase extraction and application to a pharmacokinetic Study in rats [10] and content determination of Lorcaserin HCL by HPLC [11].

However, there is no stability indicating RP-HPLC method reported for estimation of Lorcaserin HCL. The aim of this work was to develop and validate RP-HPLC stability indicating method for estimation and determination of Lorcaserin HCL in bulk and pharmaceutical dosage form.

Chemical Formula and Structure of Lorcaserin HCl [12]:

$C_{11}H_{15}CL_2N$

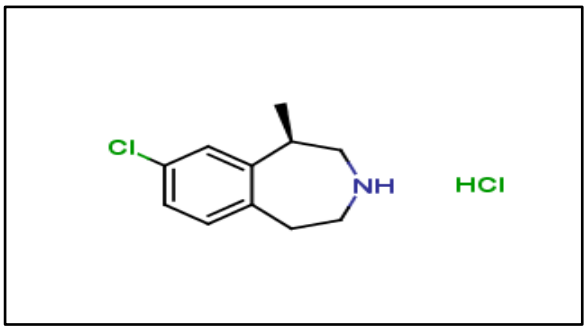


Fig-1: Chemical structure of Lorcaserin HCL

MATERIALS AND METHODS:

Chemicals and Solvents:

A HPLC grade Potassium dihydrogen phosphate, O-Phosphoric acid, Methanol, Hydrogen peroxide, Hydrochloric acid, Sodium hydroxide and purified water were procured from Loba Chemie Pvt. Ltd.

Chromatographic Conditions:

A High Performance Liquid Chromatographic System (Binary Gradient System) was used for the analysis. The Pump P-3000-M Reciprocating (40MPa), Column Cosmosil C18 (250mm×4.6ID, Partical size-5 micron), Detector UV-3000-M was used system manufactured by Analytical Technologies Ltd. Mobile phase containing Methanol:10mM KH_2PO_4 Buffer (70:30) pH:3 at a flow rate 0.8ml/min, sample volume used 20µl and pressure is 10-11MPa with UV detection at 222nm.

Mobile phase preparation:

Mixed a HPLC grade Methanol and 10mM KH_2PO_4 Buffer Solution (70:30) pH:3 in volumetric flask and filtered through 0.45µ filter under vacuum filtration.

Method of preparation of 10mM KH_2PO_4 Buffer Solution:

Weighed accurate 0.136g of KH_2PO_4 and was dissolved in 100ml of water and pH was adjusted to 3 using o-phosphoric acid (qs).

Diluent Preparation: Use Mobile phase as Diluent.

Preparation of standard stock solution:

Accurately weigh and transfer 10 mg of pure drug Lorcaserin hydrochloride in to clean and dry 10 ml volumetric flask. Add diluent and sonicated to dissolve it completely and made volume up to the mark with same solvent (Mobile Phase). This gives 1000 ppm solution.

Preparation of working standard solution:

From the above standard stock solution subsequent dilutions were made in mobile phase to give the concentrations 10, 20, 30, 40 and 50 ppm for Lorcaserin HCL.

Preparation of sample stock solution:

A sample solution of Lorcaserin HCL was prepared by using 20 tablets of Belviq 10mg (containing 10 mg of Lorcaserin HCL label claim). Make fine powder of tablets by crushing to it. Weigh and transfer sample powder quantity equivalent to 10 mg of Lorcaserin and dissolved in diluent. The resulting solution was sonicated for 15 Min the solution was filtered through 0.45µ membrane filter and made volume up to mark with mobile phase. Dilutions and final concentrations were made by using with mobile phase.

Preparation of working sample solution:

Aliquots from sample stock solution was pipetted and transferred in to a series of clean and dry 10 ml volumetric flask and diluents was added up to mark to get final concentration of Lorcaserin hydrochloride.

Selection of Wavelength:

UV spectrum of Lorcaserin diluent (Mobile phase composition) was recorded. From available spectrum wavelength selected as 222nm. At this wavelength, drug was showing good absorbance result of analysis is presented in fig.2.

Assay:

A 20 µL volume of these standard and sample solutions was used. After completion the setting of chromatographic conditions and stabilizing the instrument to obtain a steady baseline. The solution was injected and a chromatogram was recorded.

The percent assay for bulk drug was calculated by using regression equation and peak areas of drug used for calculation of percent assay for formulation the results of analysis are presented in Table 1.

METHOD VALIDATION:

System suitability test:

System suitability tests are integral part of method development and are used to ensure adequate performance of the chromatographic system shown in fig.3. The results given in table 2 were within acceptable limits.

Linearity:

The linearity of the method was determined by using a solution of five concentration levels ranging from 10 to 50ppm of Lorcaserin HCL. The calibration curve was constructed by area against concentration of drug and it is shown in fig.4. The Correlation coefficient for area verses concentration of analyte was calculated and is presented in Table 3.

Accuracy (Recovery study):

Accuracy of the method was determined by recovery experiments was conducted at three different levels 50%, 100% and 150% samples were prepared with Lorcaserin HCL. The recovery study calculated percentage recovery presented in Table 4 & 5. From the data obtained, added recoveries of standard drug found to be accurate.

Precision:

The precision of the method was determined by Interday and Intraday studies. In Interday studies a standard solution was injected for six times in two days from available data to calculated SD and %RSD. In Intraday studies a standard solution was injected for six times in same day from data to calculated SD and %RSD. The result shown in Table 6.

Ruggedness:

Ruggedness was determined by making small changes in flow rate and wavelength. By using a solution of five concentration level ranging from 10 to 50ppm of Lorcaserin HCL. The calibration curve was constructed by area against concentration of drug and it is shown in fig.5. The Correlation coefficient for area verses concentration of analyte was calculated and is presented in Table 7.

Robustness:

Robustness of this method was determined by deliberate small change in flow rate (±2units) and wavelength (±2nm) from data to calculated SD and %RSD result shown in Table 8 and 9.

Limit of Detection and Limit of Quantification:

The LOD and LOQ were separately determined based on standard deviation from accuracy and slope from Linearity. The results shown in Table 10.

Forced degradation studies:

Forced degradation studies are applied by different ways like Acidic, Base/Alkaline, Oxidative, Photolytic and Thermal degradation. For this Concentration of standard solution was made and used for degradation at different conditions injected in HPLC and chromatogram was recorded. The result of degradation was shown in Table 11.

RESULTS:

Table-1: Result of HPLC Assay for Lorcaserin HCL

Name of Drug	Composition (ppm)	% Assay	
		Bulk	Formulation
Lorcaserin HCL	30	100.33	99.89

Table-2: System suitability studies of Lorcaserin HCL

Lorcaserin HCL	
Property	**Observed values**
Retention time	5.108
Resolution (Rs)	0.00
Theoretical plates (N)	8095
Tailing factor/Asymmetry factor (T)	1.17

Table-3: Linearity results for Lorcaserin HCL

Drug	Lorcaserin HCL				
Conc. (ppm)	10	20	30	40	50
Area	426768	886084	1288349	1691790	2127452
Regression equation	y = 42071x + 21966				
Correlation (R^2)	0.9995				
Slope (m)	42071				
Intercept (c)	21966				

Table-4: Accuracy and Recovery data for Lorcaserin HCL

Lorcaserin HCL						
% Composition	Sample amount (ppm)	Amount added (ppm)	Final Volume (ppm)	Area	% Recovery	% Mean recovery
50%	20	10	30	1279472	100.78	
	20	10	30	1281955	101.37	
	20	10	30	1275310	99.79	100.65
100%	20	20	40	1683842	98.45	
	20	20	40	1691327	99.34	
	20	20	40	1686175	98.72	98.84
150%	20	30	50	2117367	99.98	
	20	30	50	2125830	100.65	
	20	30	50	2122413	100.38	100.34

Table-5: Statistical data of Accuracy and Recovery for Lorcaserin HCL

Lorcaserin HCL				
% Composition	Mean area std.	Mean area sample	SD	%RSD
50%	1288349	1278912	3357.667	0.26
100%	1691790	1687115	3829.952	0.23
150%	2127452	2121870	4257.550	0.20

Table-6: Precision results for Lorcaserin HCL

Lorcaserin HCL		
Sample No.	**Interday Precision (area)**	**Intraday Precision (area)**
1	1288349	1288349
2	1288804	1288804
3	1282054	1282054
4	1283639	1286095
5	1284512	1290500
6	1290410	1292806
Mean	1286295	1288101
SD	3336.67	3715.863
%RSD	0.26%	0.29%

Table-7: Ruggedness results for Lorcaserin HCL

Drug	**Lorcaserin HCL**				
Conc. (ppm)	**10**	**20**	**30**	**40**	**50**
Area	422048	882827	1282843	1697369	2123613
Regression equation	y = 42177x + 16438				
Correlation (R^2)	0.9995				
Slope (m)	42177				
Intercept (c)	16438				

Table-8: Robustness data for Lorcaserin at different flow rate and wavelength

Lorcaserin HCL		
Level	**Retention time**	**Tailing factor**
Change in Flow rate (ml/min)		
-1(0.7ml)	5.861	1.21
0(0.8ml)	5.164	1.18
+1(0.9ml)	4.605	1.19
Change in Wavelength (nm)	**Retention time**	**Tailing factor**
-2(220nm)	5.178	1.20
0(222nm)	5.164	1.18
+2(224nm)	5.153	1.19

Table-9: Statistical data of Robustness for Lorcaserin HCL

Lorcaserin HCL					
Change in Flow rate (ml/min)	**Conc. (ppm)**	**Area**	**Mean**	**SD**	**%RSD**
-1(0.7ml)	20	889961			
0(0.8ml)	20	886084			
+1(0.9ml)	20	883388	886478	3304.14	0.37
Change in Wavelength (nm)	**Conc. (ppm)**	**Area**	**Mean**	**SD**	**%RSD**
-2(220nm)	20	884240			
0(222nm)	20	886084			
+2(224nm)	20	885855	885393	1005.07	0.11

Table-10: LOD and LOQ data for Lorcaserin HCL

Sr.No.	**Drug**	**LOD (µg/ml)**	**LOQ (µg/ml)**
1	**Lorcaserin HCL**	0.263	0.798

Table-11: Stability data for Lorcaserin HCL

Sr.No.	**Stress condition**	**Retention time**	**Area of Peak**	**Degraded up to %**	**Actual % degradation**
1	**Standard Drug**	5.229	2127452	--	--
2	**Acidic (0.1N HCL)**	5.145	1993666	93.71	6.29
3	**Alkaline (0.1N NaOH)**	5.148	1985610	93.33	6.67
4	**Oxidation (3% H_2O_2)**	5.148	2051548	96.43	3.57
5	**Photolytic**	5.148	2063088	96.97	3.03
6	**Thermal**	5.143	2079926	97.77	2.23

Table-12: Characteristic parameters of Lorcaserin HCL for proposed RP-HPLC method

Parameters	RP-HPLC
Calibration range (µg/ml)	10-50 of Lorcaserin HCL
Detection Wavelength	222nm
Mobile Phase	Methanol:10mM KH_2PO_4 Buffer (70:30) pH:3
Flow rate	0.8ml/min
Retention time	5.108 min
Temperature	Ambient
Pressure	10-11MPa
Regression equation (Y)	Y= mx+c
Slope (m)	42071
Intercept (c)	21966
Correlation coefficient (r^2)	0.9995
Interday Precision (%RSD)	0.26%
Intraday Precision (%RSD)	0.29%
Limit of detection (mcg/ml)	0.263
Limit of quantification (mcg/ml)	0.798

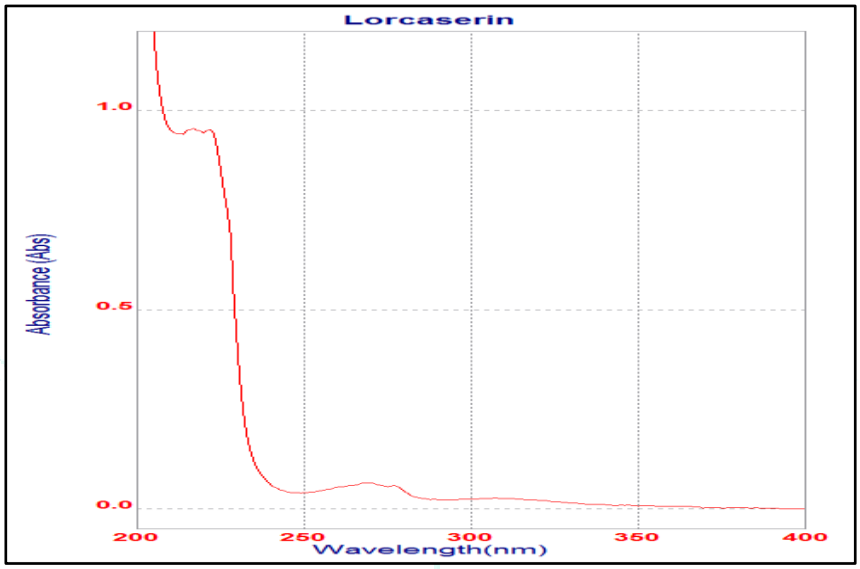
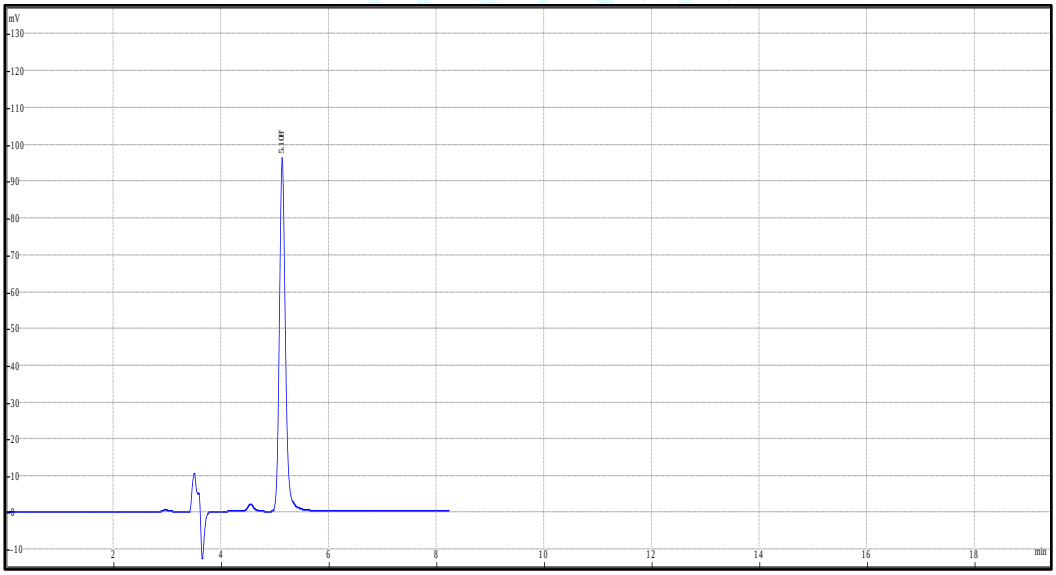


Fig-2: Wavelength detection of Lorcaserin hydrochloride.



Mobile phase: Methanol: Buffer (70:30) pH:3, **Run time:** 8.22min					
Flow Rate	**Time**	**Area**	**Resolution**	**T.PlateNum**	**Asymmetry**
0.8ml/min	5.108	1136912	0.00	8095	1.17

Fig-3: Optimized chromatogram for Lorcaserin HCL at (222nm)

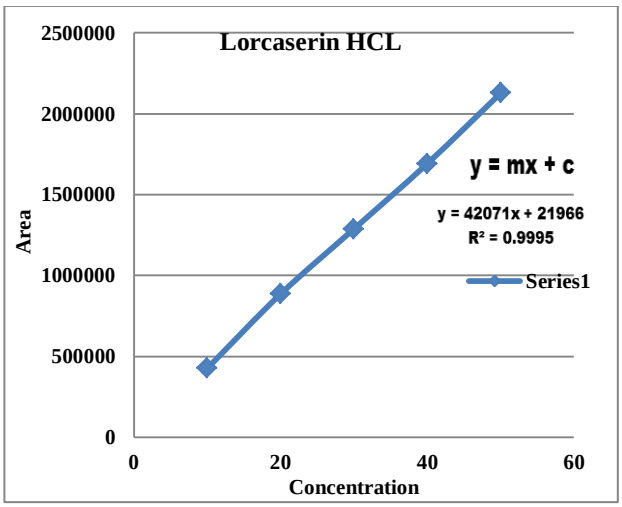


Fig-4: Linearity graph of Lorcaserin HCL

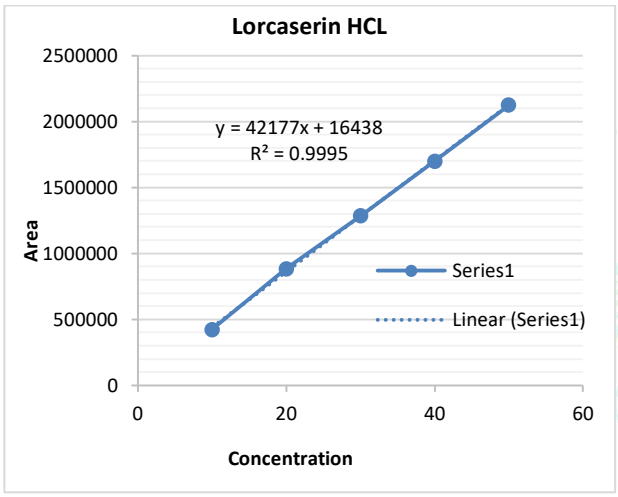


Fig-5: Ruggedness graph of Lorcaserin HCL

DISCUSSION:

In HPLC method, HPLC conditions were optimized and adequate elution of compound observed. The main objective of this study was to develop sensitive and rapid RP-HPLC method for determination and analysis of Lorcaserin HCL in bulk and Pharmaceutical dosage form by using Cosmosil C18 (250nm×4.6ID, Particle Size: 5 Micron) Column with mobile phase Methanol:10mM KH_2PO_4 Buffer (70:30) pH:3. A flow rate 0.8ml/min with UV detection at 222nm. The retention time for Lorcaserin HCL was found to be 5.108 min.

A good linear relationship (r^2 = 09995) was observed between the concentration of Lorcaserin HCL and respective peak areas in range of 10-50ppm. To analyse tablet formulations, RP-HPLC method has been developed. Lorcaserin HCL contained tablet was analyzed as per the procedure described above. The mean recoveries were found in the range of 98%- 102%. The low %RSD values (≤ 2) indicated that method was accurate and precise. In stability study Lorcaserin hydrochloride undergoes different parameters, comparatively more degradation was found with alkaline and acidic shows that the degradation product does not interfere with analytical determination of Lorcaserin HCL in Pharmaceutical dosage form. The degradation of drug was found to be within acceptance criteria.

CONCLUSION:

A simple, sensitive, rapid, linear, accurate, precise and stabilized method has been developed and validated for determination of Lorcaserin HCL in bulk and tablet formulation it is suitable for routine analysis work.

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