Iranian Journal of Pharmaceutical Sciences
2023: 19 (1): 11- 23
www.ijps.ir

Original Article

Analytical Characterization of USFDA Drug Lorcaserin Recently Banned – Scope for Its Existence in the Pharma Industry

Vinodhini Chandrasekar[a*], Ramya Jonnalagadda[a], Hindu Kalluru[a]

[a]*Department of Pharmaceutical Chemistry, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, Pour, Chennai, Tamil Nadu, India.*

Abstract

Lorcaserin is an anti-obesity agent used to treat chronic obesity. It is a selective 5-HT receptor agonist. Lorcaserin tablet formulation is not marketed in India but was available in the US and the patent expiration happens to be in 2023, there is greater scope for the launch of generic drugs in India as well as other countries. The literature review reveals that there are several clinical data for the estimation of Lorcaserin was reported so far but there are few Analytical reports available. Therefore, the attempt at method development and validation of Lorcaserin raw material was undertaken by various analytical methodologies such as Titrimetry, UV, and HPLC methods. But Lorcaserin tablet (Belviq) was withdrawn from US Market on Feb 13, 2020, due to cancer risk. In this view, the synthetic mixtures were prepared and evaluated by the proposed HPLC Method. The developed titrimetry, UV, and HPLC methods are easy to perform and specific to Lorcaserin and pay a wider way to the characterization of newer drug substances and formulations.

Keywords: Lorcaserin; Characterization; Titrimetry; UV; HPLC; Synthetic mixtures.

1. Introduction

Lorcaserin is an antiobesity drug used to treat chronic weight management. It suppresses appetite and food intake. It is a selective 5-Hydroxy Tryptamine receptor agonist. Induction of 5-HT receptors stimulates the release of 2-melanocortin stimulating hormone that acts on melanocortin 4-receptors to control appetite [1].

Lorcaserin's chemical name is R-8-chloro-1-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride hemihydrate. The empirical formula is C11H15Cl2N.0.5H2O and the molecular weight of the hemihydrate form is 241.16 g/mol (**Figure 1**) [2].

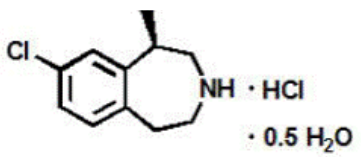

Figure 1. Structure of Lorcaserin.

Corresponding Author: Chandrasekar V., Department of Pharmaceutical Chemistry, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai, Tamil Nadu, India,
E-mail: vinpharma79@gmail.com, vinodhini.c@sriramachandra.edu.in
Cite this article as: Chandrasekar V, Jonnalagadda R, Kalluru H., Analytical Characterization of USFDA Drug Lorcaserin Recently Banned – Scope for Its Existence in the Pharma Industry, Iran. J. Pharm. Sci., 2023, 19 (1): 11- 23.
DOI: 10.22037/ijps.v19.43112

Lorcaserin is given orally at a dose of 10 mg twice a day with or without food [3]. Weight loss of 3.23 kg and body mass index of 1.16 kg/m^2 was observed in comparison to placebo in randomized clinical trials [4]. The most common side effects are headache, upper respiratory tract infection, and nasopharyngitis [5]. Concomitant use of Lorcaserin with serotonergic drugs may cause serotonin syndrome and neuroleptic malignant syndrome (NMS)-like reactions. Contraindicated with potent CYP3A4 inhibitor (e.g. Ketoconazole) and CYP2D6 inhibitor (e.g. Quinidine) [2].

The extensive literature review revealed that there are several clinical data for the estimation of Lorcaserin was reported so far [6-13]. But there is only one analytical method ultra performance liquid chromatography (UPLC)-mass spectroscopy (MS)-MS was reported in rat plasma and brain tissues [14-17]. Amal A. Bajrai et al [14], developed the UPLC-MS-MS assay and validated it for the quantitative analysis of Lorcaserin in rat plasma and brain tissue. The assay was successfully applied in a pharmacokinetic study of lorcaserin after oral administration in rats. Sadhana J. Rajput et al [15] developed a rapid, precise, accurate, specific, and simple high-performance liquid chromatography method for the estimation of lorcaserin. That study used metoprolol as an internal standard and validated as per the regulatory requirements hydrochloric in human plasma. Another study by Chetan Kedari et al [16], reviewed an antiobesity drug Lorcaserin hydrochloride, and focus on an estimate of Lorcaserin hydrochloride in bulk and tablet dosage form by using RP-HPLC and ultraviolet (UV) spectrophotometric methods. In a patent, a method for detecting the content of Lorcaserin hydrochloride through high-performance liquid chromatography (HPLC) was described [17].

The focus was only on the analytical characterization of raw material and its synthetic mixtures of Lorcaserin including solubility, pH, and loss on drying to identify qualitatively Lorcaserin raw material. Also characterized the structure of Lorcaserin raw material procured using various spectral studies such as infrared (IR) spectroscopy, nuclear magnetic resonance (NMR), and MS. Quantitative estimation by Titrimetry, UV, and HPLC methods have a vital role in Lorcaserin content determination was also explored.

2. Materials and Methods

2.1. Qualitative analysis

2.1.1. Solubility

Solubility of Lorcaserin in various solvents at a concentration of 1% solutions such as water, 0.1 M HCl, 0.1 M NaOH, Petroleum ether, and Hexane were determined.

2.1.2. Determination of pH

The determination was carried out at a temperature of 25±2 ºC, using Apparatus: Digital pH meter. pH value of the Lorcaserin bulk drug was determined using various solvents like water, 0.1 M NaOH, and 0.1 M HCl.

2.1.3. Loss on drying

The test was carried out on Hot Air Oven, Make Technico, Serial No: 1982. About 1 g of the Lorcaserin raw material was uniformly distributed in a petri-dish and kept in a hot air oven for 1 hour at 110 ˚C.

2.1.4. Spectral studies

2.1.4.1. Infra-Red Spectroscopy

IR spectroscopy was performed by the KBr pellet method The pellet was scanned in the range of 4000-400 cm^{-1} and the spectrum was recorded using Instrument name: FT-IR, model no: 4100, Make: JASCO.

2.1.4.2. Mass Spectroscopy

Mass spectra were recorded using AGILENT MSD VL Mass spectrophotometer using electron impact ionization technique.

2.1.4.3. NMR Spectroscopy

The proton and carbon NMR was recorded using DMSO as solvent at a radio frequency of 400 MHz using Instrument name: Bruker Model no: Ultrashield 400, Make: TM.

2.2. Quantitative analysis

2.2.1. Precipitation titration

2.2.1.1. Chemicals Required

NaCl, CaCO3, NaHCO3, K2Cr2O4 and AgNO3. Apparatus Required: Burette, Conical flask 250 mL, Desiccator, Volumetric flask 500 ml, Amber bottle, graduated cylinder 100 ml, Wash bottle. Preparation of 5% K2CrO4 (indicator): 1.0 g of K2Cr2O4 was dissolved in 20 ml of distilled water.

2.2.1.2. Preparation of 0.1 M AgNO3 solution

Dissolved 17.0 gr in sufficient water to produce 1000 ml. Standardized the solution in the following manner. Weighed accurately about 0.1 gr of sodium chloride, previously dried at 110 ºC for 2 hours, and dissolved in 5 ml of water. About 5 ml of acetic acid, 50 ml of methanol, and 0.15 ml of eosin solution were added, stirred with a magnetic stirrer, and titrated with the silver nitrate solution. Each ml of 0.1 M silver nitrate is equivalent to 0.005844 gr of NaCl.

2.2.1.3. Determination of Chloride in a solid sample

The Lorcaserin raw material was dried for 1 hour at 110 °C and later cooled in a desiccator. About 0.25 gr was weighed, dissolved in about 100 ml distilled water, and transferred into a 250 ml conical flask. A little amount of NaHCO3 was added until the caseation of effervescence. About 2 ml of K2CrO4 was added as an indicator and titrated until the first appearance of red Ag2Cr2O4. A small amount of chloride-free CaCO3 in 100 ml distilled water containing 2 ml of K2CrO7 was used to determine the indicator blank.

2.2.2. UV Spectroscopy

2.2.2.1. Equipment used

Analytical balance – Shimadzu AX200, UV - Visible Spectrophotometer - Shimadzu UV1800. Distilled water as diluent.

2.2.2.2. Preparation of standard solution

Weighed accurately about 10 mg of Lorcaserin bulk drug into a 10 ml volumetric flask. Dissolved and diluted to volume with distilled water to get a concentration of 1000 µg/ml. Diluted 0.1 ml of this solution with distilled water into a 10 ml volumetric flask to get a concentration of 10 µg/ml.

2.2.2.3. Determination of ʎ max

From the stock solution, about 0.1 ml was pipetted out and transferred into three 10 ml standard flasks and made up the volume with distilled water, 0.1 M HCl, and 0.1 M NaOH respectively. The above solutions were scanned in the range of 200-400 nm and the ʎmax was determined.

2.2.2.4. Determination of linearity range

From the stock solution about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6ml was pipetted out into a series of six standard flasks and the volume was made with distilled water and the absorbance of the solutions was measured at its ʎmax. The procedure was repeated in a similar manner using 0.1 M HCl and 0.1 M NaOH respectively. The linearity graph of Lorcaserin raw material was constructed using a concentration on the x-axis and absorbance on the y-axis. From the graph, the linearity range and correlation coefficient were predicted.

2.2.3. High-Performance Liquid Chromatography

2.2.3.1. Solvents required

All the solvents used were HPLC-grade Methanol, Acetonitrile, and Water. Equipment used: Analytical balance: Shimadzu (AX220), HPLC Model: LC-2010 HT, Make: Shimadzu, Data handling system – Class-VP, Sonicator: Model: PCI.

2.2.3.2. Preparation of mobile phase

Preparation of buffer (10 mM): About 0.78 g of ammonium acetate was dissolved in water to 1000 ml, and filtered using 0.45µ. A mixed 850ml of Acetonitrile with 150 ml buffer and 1 ml of formic acid was added. It was well-sonicated for 10 mins.

2.2.3.3. Diluent

Acetonitrile: Water [50:50]

2.2.3.4. Chromatographic Parameters

Column: C18 Nova-Pak column (150×4.6 mm), particle size: 4µ Mobile phase: Acetonitrile: Buffer: formic acid (85:15:0.1).

Flow Rate: 1 ml/min Injection volume: 20 µL Detection: UV at 222 nm.

2.2.3.5. Preparation of standard solution

Weighed accurately 10 mg of Lorcaserin bulk drug into a 10 ml standard flask. Dissolved and made up the volume to 10 ml with methanol. About 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, and 0.6 ml were pipetted from the stock solution into separate 10 ml standard flasks, and the volumes were made up with the diluent.

2.2.3.6. Preparation of Synthetic Mixtures

Synthetic Mixture 1 (SM-1): SM-1 of Lorcaserin and Chitosan was prepared in a ratio of 1:1. Lorcaserin (100 mg) and Chitosan (100 mg) were accurately weighed and physically mixed in a container.

Synthetic Mixture 2 (SM-2): SM-2 of Lorcaserin and Chitosan was prepared in a ratio of 1:2. Lorcaserin (100 mg) and Chitosan (200 mg) were accurately weighed and physically mixed in a container.

2.2.3.7. Evaluation of system suitability

Injected 20 µl of the standard solutions in the chromatograph recorded the chromatograms

and measured the peak responses. The system is suitable for analysis, if and only if, the relative standard deviation of the area counts of Lorcaserin peak, determined for five replicate injections of standard solution is NMT 2.0%, Tailing factor NMT 2, and Theoretical plates NLT 2000.

2.2.3.8. Validation Protocol – HPLC Method [18]

System suitability data: The standard solution at the concentration of 10 mcg/ml was prepared and analyzed as per the proposed method. Relative Standard Deviation of area counts of Lorcaserin peak obtained from the five replicates. The parameters like the United States Pharmacopeia (USP) tailing factor and theoretical plates were calculated.

Precision:

System precision (Intraday): The system precision was evaluated by measuring the peak response of Lorcaserin for replicate injections of standard solution, prepared as per the proposed method. About 10 mg of Lorcaserin working standard was weighed in a 10 ml volumetric flask, dissolved to volume with mobile methanol. Further, 0.1 ml of the solution was diluted to 10 ml with diluent. Blank (diluent) was injected and standard solution (10 µg/ml) was injected five times for system suitability.

Intermediate Precision: The method precision was determined by preparing the standard solution of a single batch of Lorcaserin drug substance 5 times and analyzing it as per the proposed method by different analysts on a different day.

Linearity of response: About 10 mg of Lorcaserin bulk drug was weighed in a 10 ml volumetric flask, dissolved to volume with methanol. From the stock solution 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, and 0.6 ml were pipetted out into 10 ml volumetric flasks and made up the volume with the diluent. All the above 6 linearity preparations were injected and chromatograms were recorded.

Stability: Lorcaserin standard, SM-1, and SM-2 were evaluated at room temperature and 4 ºC for 48 hr.

Robustness: The robustness of the method was evaluated by deliberately varying the chromatographic condition viz. Flow rate ± 10% mobile phase ± 2%, and wavelength ±2 nm. About 10 mg of Lorcaserin working standard was weighed in a 10 ml volumetric flask, dissolved to volume with the mobile phase. Further 0.1 ml, 0.3 ml, and 0.6 ml of this solution were diluted to 10 ml with the diluent. Blank (diluent) was injected and the standard solution was injected 3 times of different concentrations (10, 30, 60 µg/ml) and chromatograms were recorded.

Change in Flow Rate (-10%): The flow rate changed to 0.8 ml/min and the rest of the parameters followed as per the method.

Change in Flow Rate (+10%): The flow rate changed to 1.2 ml/min and the rest of the parameters followed as per method.

Change in Wavelength (-2 nm): The wavelength changed to 220 nm and the rest of the parameters followed as per the method.

Change in Wavelength (+2 nm): The wavelength changed to 224 nm and the rest of the parameters followed as per the method.

Change in Mobile Phase Ratio [Acetonitrile: Water (80:20)]: The mobile phase ratio changed to Acetonitrile: Water (80:20) and the rest of the parameters followed as per the method.

Change in Mobile Phase Ratio [Methanol: Water (90:10)]: The mobile phase ratio changed to Methanol: Water (90:10) and the rest of the parameters followed as per the method.

3. Results and Discussion

3.1. Qualitative Analysis

The solubility of Lorcaserin raw material (1% concentration) in various solvents revealed that Lorcaserin is freely soluble in water, 0.1 M HCl, 0.1 M NaOH, and insoluble in non-polar solvents such as petroleum ether and hexane. The pH of 1% concentration of Lorcaserin raw material was determined using calibrated pH meter and was found to be 5.7,1.72 and 11.40 in water, 0.1 M HCl, and 0.1 M NaOH respectively. The LOD of Lorcaserin raw material was determined as per the proposed methodology and the % weight loss was found to be 0.11 % w/w.

The IR spectrum recorded for Lorcaserin raw material shows the presence of the following absorption which confirms qualitatively that the procured raw material as Lorcaserin N-H (str) - 3365.25 cm-1, C-H (str) aromatic – 3124 cm-1, C-H (str) [CH3 grp] - 2503.15 cm-1 C=C (str) aromatic – 1592.91 cm-1, C-H (def) - 829.241 cm-1, N-H (def) – 613.252 cm-1 The NMR spectrum recorded in the range of 0-12 ppm for Lorcaserin raw material revealed the presence of following chemical shift and thus the procured raw material was confirmed quantitatively. Ar-H – δ7.3 ppm, CH3 – δ 1.15 ppm, CH2 – δ 2.508 ppm, CH – δ 3.1 ppm, NH – δ 3.5 ppm. The 13C NMR spectrum recorded in the δ range of 0-200ppm revealed the presence of 11 carbon atoms in the structure and characterized as follows Alkyl C – C – δ 2- 55 ppm, Aromatic C = C – δ 110-175 ppm. The mass spectrum using the electron impact ionization technique showed (M+1) peak at m/e – 19.61 revealing the molecular weight of Lorcaserin raw material as 195. The peak at 237.1 was formed to be due to (M+1) + acetonitrile used as a solvent. The above spectral data confirmed the procured raw material as Lorcaserin qualitatively (**Figure 2a-d**).

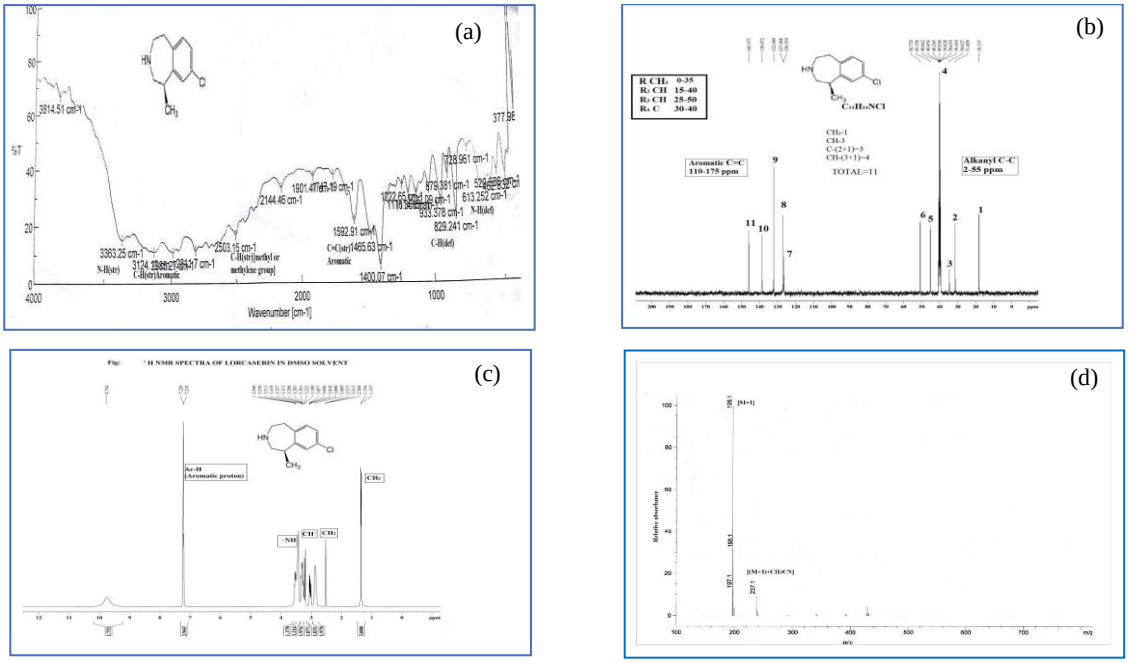

Figure 2. a) IR spectrum of Lorcaserin raw material using KBr pellet method, b) ^{1}H NMR spectrum of Lorcaserin raw material in DMSO solvent, c) .^{13}C NMR spectrum of Lorcaserin raw material in DMSO solvent, d) Mass spectrum of Lorcaserin raw material.

3.2. Quantitative analysis

The precipitation titration as per Mohr's method was performed on Lorcaserin raw material and the percentage of chloride present was found to be 9.1106% w/w.

3.2.1. Ultra Violet Spectroscopy

The absorption maximum was determined for Lorcaserin raw material in 3 different solvent systems such as distilled water, 0.1 M HCl, and 0.1 M NaOH, and was found to be 222 nm, 222 nm, (222 and 227 nm) respectively (**Table 1**). The linearity graph was constructed for Lorcaserin raw material in 3 different solvents at its predicted ʎmax and was found to be linear in the range of 10-100 µg/ml (distilled water and HCl) and 10-60 µg/ml (NaOH). The correlation coefficient was found to be 0.995, 0.993, (0.994 and 0.992) at 222 nm in water, 222 nm in 0.1 M HCl, 222 nm, and 227 nm in 0.1 M NaOH respectively (**Figure 3**).

3.2.2. High-Performance Liquid chromatography

Several mobile phases were tried in trial and error form to resolve Lorcaserin, better resolution with short retention time was obtained with the system containing acetonitrile: 20 M ammonium acetate buffer: formic acid (85:15:0.1) as mobile phase at a flow rate of 1.0 ml/min. The retention time was 3.00 min. The detection wavelength was determined from UV spectroscopy analysis and found to be 222 nm. At the above HPLC conditions mentioned complete resolution of the peaks with a clear baseline was obtained with 2.88 mins retention time. The blank chromatogram represents that there was no peak found after 2 minutes of run time by the proposed method. The system suitability parameter such as theoretical plates, and asymmetry (tailing factor) was calculated from the chromatogram (**Figure 4, Table 2**) and was found to be 3724.69 and 1.333 respectively [acceptance limit, number of theoretical plates NLT 2000 and tailing factor NMT 2.0 as per USP]. The intraday precision was studied by injecting five replicates of 10 mcg/ml concentration at a proposed chromatographic condition, retention time, and peak area were recorded and tabulated in **Table 2**.

Table 1: Graph linearity of Lorcaserin raw material in water (222nm), 0.1MHCl (222nm), 0.1MNaOH (222 nm) and 0.1MNaOH (227nm).

Conc. (µg/ml) in water	Abs at 222nm	Conc. (µg/ml)in 0.1M HCl	Abs at 222nm	Conc. (µg/ml)in 0.1M NaOH	Abs at 222nm	Conc. (µg/ml)in 0.1M NaOH	Abs at 227 nm
0	0	0	0	0	0	0	0
10	0.309	10	0.396	10	0.352	10	0.326
20	0.62	20	0.625	20	0.506	20	0.465
30	0.893	30	0.935	30	0.82	30	0.75
40	1.196	40	1.326	40	1.125	40	1.026
50	1.484	50	1.516	50	1.296	50	1.181
60	1.78	60	1.806	60	1.492	60	1.358
70	2.071	70	2.086	70	1.919	70	1.757
80	2.318	80	2.341	80	1.979	80	1.824
90	2.507	90	2.549	90	2.256	90	2.093
100	2.694	100	2.718	100	2.408	100	2.258
y=0.0275x+0.0658		**y =0.0274x+0.112**		**y=0.0242x+0.0757**		**y =0.0225x+0.0589**	
R^2 =0.9955		**R^2 =0.9934**		**R^2 =0.9924**		**R^2 =0.994**	

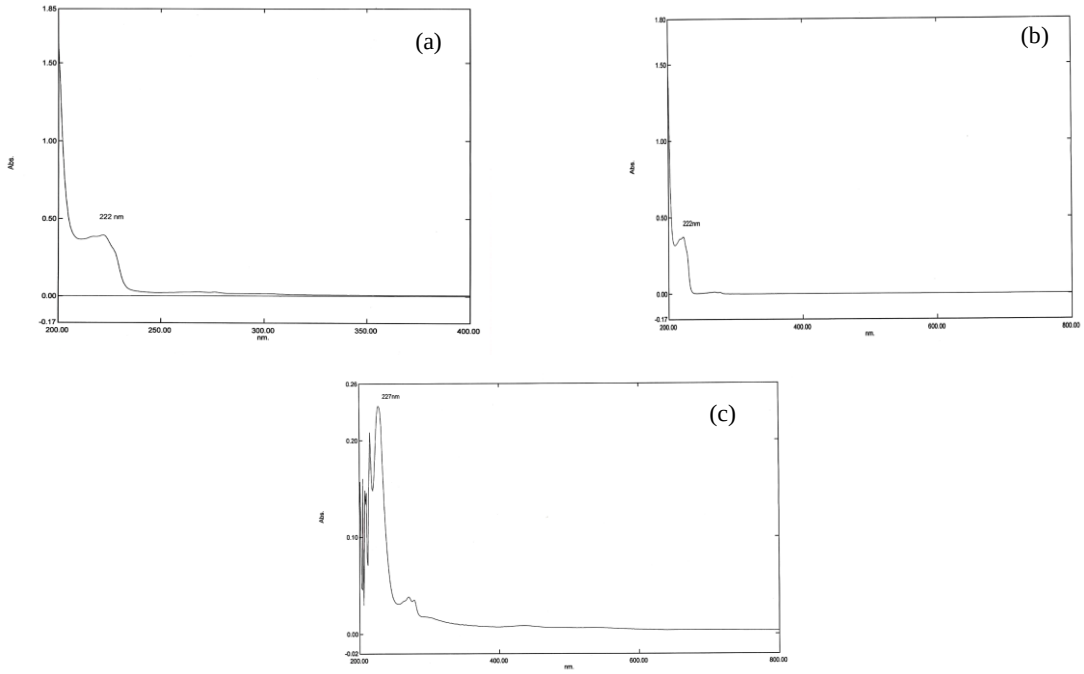

Figure 3. a) Absorbance maximum in water at 222nm, b) Absorbance maximum in 0.1M HCl (222nm), c) Absorbance maximum in 0.1M NaOH (222nm and 227nm).

Table 2: Precision.

S.No	Conc. (µg/ml)	Lorcaserin		SM-1		SM-2	
Intraday Precision		**Rt**	**Peak Area**	**Rt**	**Peak Area**	**Rt**	**Peak Area**
1	10	2.883	471484	2.875	424241	2.892	439941
2	10	2.883	472059	2.867	425312	2.867	433435
3	10	2.883	472179	2.867	424087	2.867	433343
4	10	2.883	473141	2.867	423295	2.867	433260
5	10	2.883	472867	2.892	423525	2.867	431266
S.D		**662.7156**		**785.3859**		**1993.283**	
RSD		**0.140303**		**0.185192**		**0.458807**	
Interday Precision		**Rt**	**Peak Area**	**Rt**	**Peak Area**	**Rt**	**Peak Area**
1	10	2.858	471281	2.767	446079	2.767	433606
2	10	2.858	470108	2.767	445835	2.788	434588
3	10	2.858	472614	2.767	445854	2.792	434265
4	10	2.858	472355	2.798	446328	2.767	435820
5	10	2.858	471603	2.798	445791	2.767	434910
S.D		**658.6636**		**225.5511**		**818.1933**	
RSD		**0.138435**		**0.050575**		**0.188247**	

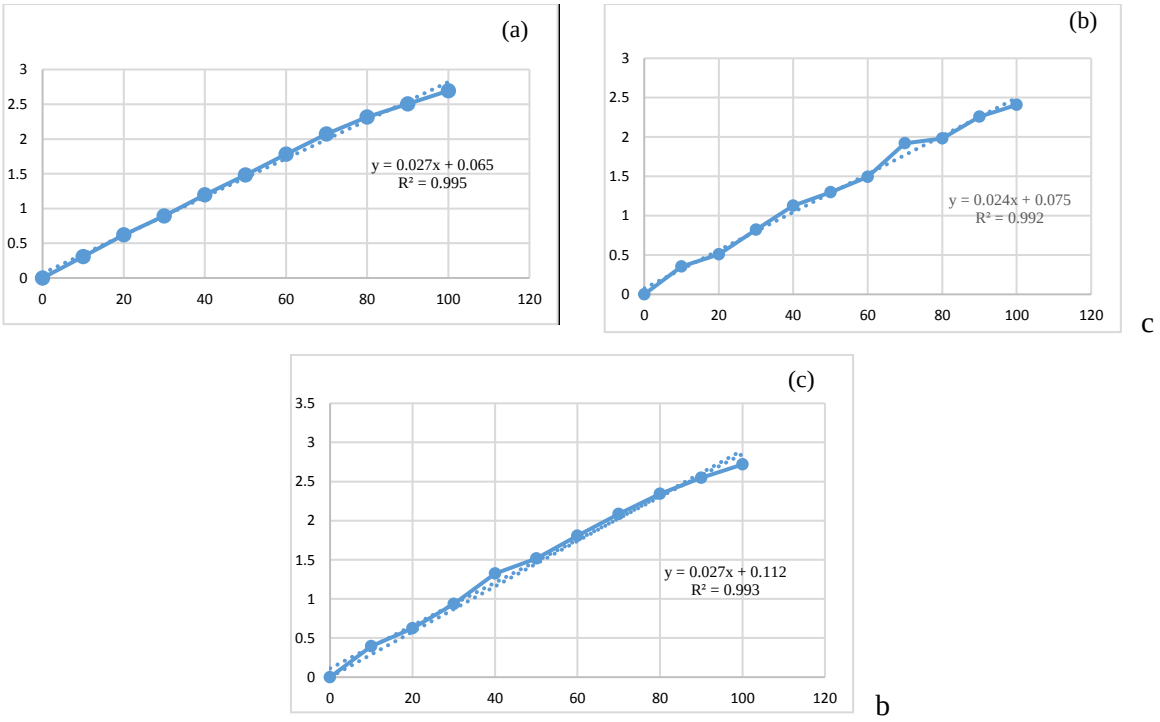

Figure 4. a) Linearity graph of Lorcaserin raw material in water (222nm). X-axis: Absorbance, Y-axis: Concentration µg/ml, **b)** Linearity graph of Lorcaserin raw material in 0.1M HCl (222nm). X-axis: Absorbance, Y-axis: Concentration µg/ml, **c)** Linearity graph of Lorcaserin raw material in 0.1M NaOH (222 nm). X-axis: Absorbance, Y-axis: Concentration µg/ml.

The standard deviation and RSD for Retention time and area count were calculated and were found to be zero. This showed that the proposed method is system precise. The interday precision was determined using a standard solution at 10mcg/ml concentration prepared on the next day of intraday precision analysis using the proposed chromatographic condition. Five replicate injections were made and recorded chromatograms and tabulated in **Table 2.** The SD and RSD were calculated and were found to be zero which indicated that the proposed method is precise. The linearity range was assessed by injecting 6 different concentrations of Lorcaserin raw material (10-60 mcg/ml) at the proposed chromatographic condition. Retention time and peak area were recorded. The graph was constructed using a concentration on the x-axis and peak area on the y-axis and the correlation coefficient was predicted as 0.991 and obeyed Beer's law in the range of 10-60mcg/ml. These data show that the method is sensitive for the determination of lorcaserin. The LOD and LOQ were measured by using an equation and were presented in **Table 3**. The blank chromatogram with no interference peaks of mobile phase and diluent after 2 mins of run time and the peak purity of analyte indicates that the method is specific for the analysis of lorcaserin. The stability of lorcaserin standard, SM-1, and SM-2 was evaluated at room temperature and 4ºC for 48 hr. The relative standard deviation was found below 2.0%. It showed that both standard and synthetic mixtures (SM-1 and SM-2) were stable for up to

48 hr at both room temperature and 4ºC in **Table 4**. The robustness of the method was evaluated by deliberately varying the flow rate ± 0.2ml, detector wavelength ± 2nm, and mobile phase ratio. At these varied conditions, Lorcaserin raw material robustness was performed in three different concentrations (10, 30, 60 mcg/ml) as per the proposed method and the chromatograms were recorded. The % RSD values are within the limit and tabulated in **Table 5** shows the method is having precision under a given set of conditions.

Table 3: Linearity, LOD & LOQ.

S.No	Concentration (µg/ml)	Lorcaserin Std		SM-1		SM-2	
		Rt	Peak Area	Rt	Peak Area	Rt	Peak Area
1	10	2.8	470565	2.767	424944	2.767	435523
2	20	2.883	773964	2.750	867503	2.750	930365
3	30	2.858	1318984	2.742	1315494	2.742	1375988
4	40	2.85	1696920	2.773	1755314	2.733	1802383
5	50	2.833	2125614	2.750	2197746	2.725	2231592
6	60	2.817	2822601	2.773	2760376	2.725	2711893
R^2 Value		**0.987**		**0.997**		**0.999**	
LOD		**62.1**		**63.6**		**61.4**	
LOQ		**188.2**		**192.7**		**186.17**	

Table 4: Stability.

Time (hr)	Lorcaserin Std			SM-1			SM-2		
	Rt	Peak Area (R.T)	Peak Area (4ºC)	Rt	Peak Area (R.T)	Peak Area (4ºC)	Rt	Peak Area (R.T)	Peak Area (4ºC)
0	2.883	0	0	2.867	0	0	2.867	0	0
1	2.883	471355	471988	2.892	423525	423295	2.867	432966	433435
2	2.883	471603	471033	2.867	424077	426079	2.867	433343	433260
3	2.883	470962	470220	2.875	424241	425835	2.892	434914	439941
4	2.883	471221	472119	2.883	423544	421854	2.892	432541	431266
5	2.883	470986	471634	2.883	423118	426328	2.892	433699	433343
6	2.883	472630	471769	2.867	422312	425791	2.867	433435	433143
12	2.883	471023	472201	2.867	422991	423606	2.883	432992	434921
24	2.883	472281	472692	2.867	423568	424188	2.883	433114	433260
36	2.883	472108	472620	2.867	424087	423265	2.867	433343	434966
48	2.883	470614	471980	2.867	424419	425820	2.867	434921	432941
%RSD (R.T)		**0.14**			**0.15**			**0.18**	

Table 5: Robustness.

Parameters	Change in flow rate (0.8ml/min)		Change in flow rate (1.2ml/min)		Change in Wavelength (220nm)		Change in wavelength (224nm)		Change in mobile phase ratio [Acetonitrile: water (90:10)]		Change in mobile phase ratio [Acetonitrile: water (80:20)]	
Concentration (µg/ml)	**Rt (mins)**	**Peak area**	**Rt (mins)**	**Peak area**	**Rt (mins)**	**Peak area**	**Rt (mins)**	**Peak area**	**Rt (mins)**	**Peak area**	**Rt (mins)**	**Peak area**
10	3.667	542327	2.45	401377	2.95	479082	2.95	459944	3.492	436997	2.842	474483
30	3.633	1648787	2.425	1088813	2.917	1315356	2.908	1249248	3.45	1310858	2.808	1276932
60	3.583	3138020	2.4	2066124	2.883	2589392	2.875	2366704	3.408	2397966	2.775	2450520
	SD:0.042253		**SD:0.025**		**SD:0.03350**		**SD:0.0375**		**SD:0.03350**		**SD:0.042**	
	RSD:1.164		**RSD:1.030**		**RSD:1.148**		**RSD: 1.283**		**RSD:1.192**		**RSD:1.217**	

The analytical characterization of Lorcaserin raw material was carried out to assess the qualitative and quantitative analytical results by various analytical techniques such as determination of solubility, pH, Loss on drying, Identification by spectral studies like IR, ^{1}H NMR, ^{13}C NMR, and Mass spectroscopy and also using quantitative analysis like Titrimetry (Precipitation titration), UV spectroscopy and High-performance liquid chromatography. To date, there is only little number of quantification methods available for Lorcaserin. The HPLC method was validated concerning Precision, Stability, linearity, and robustness as per ICH guidelines. The limit of detection and limit of quantification of the currently developed method is sensitive and fast enough to carry out regular quality control lorcaserin. The value of regression coefficient r^2=0.9998 confirmed the linear relationship between the concentration of the drug and the area of the peak. This method is precise enough to perform continuous and regular quantification of Lorcaserin raw material and synthetic mixtures. The minimal variation in % RSD for interday and intraday precision indicates the complete harmony among repeated injection, repeated analysis, intraday, and interday study.

The strong correlation between the standard solutions and synthetic mixtures concerning retention time and peak area establishes the accuracy of the method. The proposed method of analysis was highly selective and specific as peaks of the analyte in synthetic mixtures spiked was well resolved. The minor deliberate changes made in various experimental parameters did not significantly affect the peak area, indicating the robustness of the developed method. The stability of Lorcaserin in various conditions has been studied in this method for 5 h both at room temp (bench-top) and under refrigeration at 4° C to establish the stability of the analyte during the process of analysis. The %RSD values show that there were no considerable changes in their concentration after 5 h indicating that samples are stable during analysis. The results are consolidated in **Table 6**.

Table 6: Summary of results of Lorcaserin raw material.

S. No	Parameter	Observation/Acceptance criteria				
A.	Solubility	Freely Soluble in water, 0.1M HCl, 0.1M NaOH				
B	pH	Water				5.7
		0.1M NaOH				11.40
		0.1M HCl				1.72
C.	Loss on Drying	110ºC for1 hour				0.11%w/w
D.	Spectral studies	IR, ^{1}H NMR, ^{13}C NMR, Mass				Complies
E.	Precipitation Titration (Chloride content by Mohr's method)	9.1106%w/w				
F.	UV Spectroscopy	Solvent	Water	HCl	NaOH	NaOH
		Wavelength (nm)	222	222	222	227
		Linearity Range (µg/ml)	10-100	10-100	10-60	10-60
		Slope	0.0275	0.0266	0.0242	0.0225
		Intercept	0.0658	0.112	0.0757	0.0589
		Linear Equation	Y=0.0275x+ 0.0658	Y=0.0266x+ 0.112	Y = 0.0242x+ 0.0757	Y= 0.0225x+ 0.0589
		Correlation Co-efficient	0.9955	0.9938	0.9924	0.994
G. HPLC ANALYSIS						
i.	Blank	No peak eluted before 2mins.			-	
ii.	System suitability					
	Tailing factor	1.333			Not more than 2.0	
	Theoretical plate	3724.69			Not less than 2000	
iii.	System precision (Intra-day precision)	RSD=0			Not more than 2.0 %	
iv.	Inter-day precision	RSD=0			Not more than 2.0 %	
v	Linearity range	10-60mcg/ml r^2= 0.991			Closer to 1.0	
vi. Robustness						
a)	Change in flow 0.8ml/min	RSD=1.164			Not morethan 2%	
b)	Change in flow1.2ml/min	RSD=1.030			Not morethan 2%	
c)	Change in Wavelength (220nm) (-2nm)	RSD=1.148			Not morethan 2%	
d)	Change in Wavelength (224nm) (+2nm)	RSD=1.213			Not morethan 2%	
e)	Change in Mobile phase (90:10)	RSD = 1.192			Not morethan 2%	
f)	Change in Mobile phase (80:20)	RSD = 1.217			Not morethan 2%	

4. Conclusion

The comprehensive method development and validation study inferred that the HPLC method was simple isocratic, fast, precise, linear, and robust and met the respective acceptance criteria over the already reported method in 2019. The proposed methods are available for various applications in the development of formulation analysis specific to the Lorcaserin drug with a high degree of accuracy and precision also as the patent expires in 2023, this article extends the scope for researchers and pharmaceutical industries to explore more shortly.

Acknowledgments

None.

Conflict of interest

The authors declare to have no conflict of interest.

Funding

None.

References

[1]. Venkatesh P, Venkat BR, Venu GK, Vinodhini S, Vinodhini T, Vinodhini C, Chitra K. A Review on Lorcaserin – A Selective 5-HT Serotonin Receptor Agonist in Obesity Management. *Int J Cur Res Rev* (2017) 9 (17): 37-40.

[2]. Gustafson A, King C, Rey JA. Lorcaserin (Belviq): A Selective Serotonin 5-HT2C Agonist in the Treatment of Obesity. *P T* (2013) 38 (9): 525-534.

[3]. Jason CG, Halford, Joanne AH. Lorcaserin and the role of 5-HT 2c agonist in the treatment of obesity. *Clin. Med. Ther* (2011) (3): 347-354.

[4]. Chan EW, He Y, Chui CS, Wong AY, Lau WC, Wong IC. Efficacy and safety of lorcaserin in obese adults: a meta-analysis of 1-year randomized controlled trials (RCTs) and narrative review on short-term RCTs. *Obes Rev* (2013) 14 (5): 383-392.

[5]. Aronne L, Shanahan W, Fain R, Glicklich A, Soliman W, Li Y, Smith S. Safety and efficacy of lorcaserin: a combined analysis of the BLOOM and BLOSSOM trials. *Postgrad Med* (2014) 126 (6): 7-18.

[6]. Smith SR, Prosser WA, Donahue DJ, Morgan ME, Anderson CM, Shanahan WR. APD356-004 Study Group. Lorcaserin (APD356), a selective 5-HT (2C) agonist, reduces body weight in obese men and women. *Obesity (Silver Spring)* (2009) 17 (3): 494-503.

[7]. Steven. R. Smith, Neil J.Weissman, Christen M.Anderson, Matilde S. Emil Chuang, Scott Stubbe, Harold Bays, William R. Shanahan. Multicenter, Placebo-controlled Trail of Lorcaserin For Weight Management. *N Engl J Med* (2010) 15 (363): 245-256.

[8]. Hurren KM, Berlie HD. Lorcaserin: an investigational serotonin 2C agonist for weight loss. *Am J Health Syst Pharm* (2011) 68 (21): 2029-2037.

[9]. Kang JG, Park CY. Anti-Obesity Drugs: A Review about Their Effects and Safety. *Diabetes Metab J* (2012) 36 (1): 13-25.

[10]. O'Neil PM, Smith SR, Weissman NJ, Fidler MC, Sanchez M, Zhang J, Raether B, Anderson CM, Shanahan WR. Randomized placebo-controlled clinical trial of lorcaserin for weight loss in type 2 diabetes mellitus: the BLOOM-DM study. *Obesity (Silver Spring)* (2012) 20 (7): 1426-1436.

[11]. Chan EW, He Y, Chui CS, Wong AY, Lau WC, Wong IC. Efficacy and safety of lorcaserin in obese adults: a meta-analysis of 1-year randomized controlled trials (RCTs) and narrative review on short-term RCTs. *Obes Rev* (2013) 14 (5): 383-392.

[12]. Fleming JW, McClendon KS, Riche DM. New obesity agents: lorcaserin and phentermine/topiramate. *Ann Pharmacother* (2013) 47 (7-8): 1007-1016.

[13]. Neil J. Weissman, Matilde Sanchez, Gary G. Koch, Steven R. Smith,William R. Shanahan, Christen M. Anderson. Echocardiographic Assessment of Cardiac Valvular Regurgitation With Lorcaserin From Analysis of 3 Phase 3 Clinical Trials. *Circ Cardiovasc Imaging* (2013) 9 (6): 560–567.

[14]. Bajrai AA, Ezzeldin E, Al-Rashood KA, Raish M, Iqbal M. A Validated UPLC-MS-MS Assay for the Rapid Determination of Lorcaserin in Plasma and Brain Tissue Samples. *J Anal Toxicol* (2016) 40 (2): 133-139.

[15]. Sadhana J. Rajput, Mansi A. Sathe, Swati D. Patel. Development and Validation of a HPLC-based Bioanalytical Method for Lorcaserin using Solid Phase Extraction and Application to a Pharmacokinetic Study in Rats. *Indian J Pharm Sci* (2018) 80 (2): 235-241.

[16]. Chetan Kedari, Hemlata Bhavar, Sagar Magar. A review on estimation of lorcaserin hydrochloride in bulk and tablet dosage form. *World J. Pharm. Res* (2019) 8 (1): 677-682.

[17]. Method for detecting content of lorcaserin hydrochloride through high performance liquid chromatography (HPLC) method. Available from: https://patents.google.com/patent/CN103901151A/en.

[18]. Guideline ICH. Validation of analytical procedures: text and methodology Q2(R1) (2005). Available from: http://www. ich. org/cache/compo/363-272-1. html. 2017.