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NOVEL ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ANALYSIS OF LURASIDONE HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC

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ABSTRACT

A convenient, simple, specific, accurate, precise, rapid, inexpensive isocratic Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the quantitative determination of Lurasidone HCl in pharmaceutical tablet dosage forms. RP-HPLC method was developed by using Welchrom C_{18}Column (4.6 X 250mm, 5µm), Shimadzu LC-20AT Prominence Liquid Chromatograph. The mobile phase composed of 10mM Phosphate buffer (pH-3.0, adjusted with triethylamine): acetonitrile (50:50v/v). The flow rate was set to 1.0 $mL.min^{-1}$ with the responses measured at 235nm using Shimadzu SPD-20A Prominence UV-Vis detector. The retention time of Lurasidone HCl was found to be 4.333 minutes. Linearity was established for Lurasidone HCl in the range of 10-50µg.$mL^{-1}$with correlation coefficient 0.9999.The LOD and the LOQ were found to be0.0653µg.$mL^{-1}$and 0.1980µg.mL^{-1}respectively. The amount of Lurasidone HCl present in the formulation was found to be 99.90 %. The validation of the developed method was carried out for specificity, linearity, precision, accuracy, robustness, limit of detection, limit of quantitation. The developed method can be used for routine quality control analysis of Lurasidone HCl in pharmaceutical tablet dosage form.

KEY WORDS: Lurasidone HCl, Isocratic RP-HPLC, Method Validation, ICH guidelines.

INTRODUCTION

Lurasidone HCl is a potent atypical antipsychotic drug. It is white to off-white powder. Lurasidone HCl belonging to the chemical class of benzisothiazol derivatives. Its chemical name is (3aR,4S,7R,7aS)-2-{(1R,2R)-2-[4-(1,2-benzisothiazol-3-yl)piperazin-1ylmethyl]cyclohexylmethyl}hexahydro-4,7-methano-2H-isoindole-1,3- dionehydrochloride. Tablets of Lurasidone HClare intended for oral administration only. Each tablet contains 20 mg, 40 mg, 60 mg, 80 mg, or 120 mg of Lurasidone hydrochloride. Lurasidonehydrochloride is FDA approved for the treatment of schizophrenia and depressive episodes associated withbipolar I disorder (bipolar depression) 10.4 million American adults have been suffering from bipolar I disorde. Lurasidone may be useful for treating the cognitive and memory deficits seen in schizophrenia. Lurasidone has activity at several serotonin receptors that are involved in learning and memory, and unlike most other antipsychotics, lacks any anti-cholinergic effects which are known to impair cognitive processes and memory.

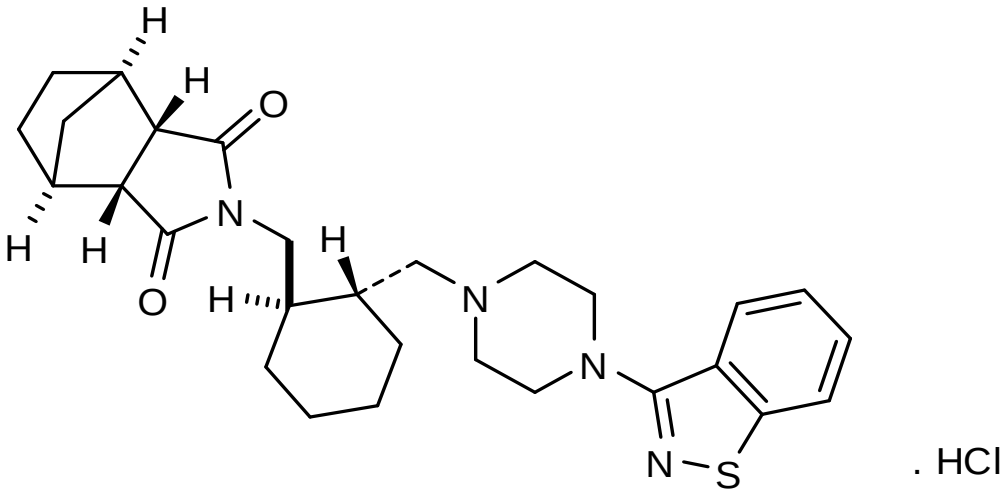

Fig. 1: Structure of Lurasidone HCl.

Literature survey reveals that determination of Lurasidone hydrochloride in biological fluids and rat plasma, methods such as LC/MS/MS [1-2],UV spectrophotometric [3-5], Pharmaco-Kinetic and Pharmaco-Dynamic [6-7] and HPLC [8]. Infact no isocratic Reversed phase high performance liquid chromatography method has been found to estimate the Lurasidone HCl in tablet dosage form hitherto. Hence the present method was developed to quantify Lurasidone HClin tablet dosage form by isocratic RP-HPLC.

EXPERIMENTAL

Chemicals and Reagents

The reference sample of Lurasidone HCl standard was kindly supplied as gift sample by Hetero Drugs Ltd., Hyderabad, Andhra Pradesh, India. All the chemicals were analytical grade. Potassium dihydrogen orthophosphate and phosphoric acid from Rankem Ltd., Mumbai, India, while acetonitrile (HPLC grade) and triethylamine (HPLC grade) from Merck Pharmaceuticals Private Ltd., Mumbai, India. Ortho phosphoric acid used was of HPLC grade and purchased from Merck Specialties Private Ltd., Mumbai, India. Commercial tablets of Lurasidone HCl formulation was procured from local market. LATUDA-tablets 40 mg are manufactured by Hetero drugs Pvt. Ltd. Hyderabad, A.P.

Instruments

Quantitative HPLC was performed on a isocratic high performance liquid chromatography (Shimadzu LC-20AT Prominence Liquid Chromatograph) with a LC-20AT VP pump, manual injector with loop volume of 20 µL (Rheodyne), programmable variable wavelength Shimadzu SPD-20A Prominence UV-Vis detector and Welchrom C_{18} Column (4.6 X 250mm, 5µm particle size). The HPLC system was equipped with "Spinchrome" software. In addition an electronic balance (Shimadzu TX223L), digital pH meter (Systronics model 802), a sonicator (spectra lab, model UCB 40), UV-Visible Spectrophotometer (Systronics model-2203) were used in this study.

Chromatographic conditions

Lurasidone HCl was analyzed by various reversed phase columns like C_8 and C_{18} columns. Among C_8 and C_{18} columns, C_{18}(250mmX4.6mm, 5µm) column was selected. Various combinations of acetonitrile, phosphate buffer and methanol with triethylamine as column modifier were tested. The mixture of 10mM Phosphate buffer (pH adjusted to 3.0 using triethylamine) and Acetonitrile in ratio of 50:50 v/v was selected as mobile phase and UV detection wavelength was 235 nm with a flow rate of 1mL.min^{-1}. Injection volume was 20µL, with ambient temperature, run time was 8 min. and retention time was 4.333 min. The resulting HPLC chromatogram was shown in Fig. 3.

Preparation of mobile phase

A 10mM Phosphate buffer was prepared by dissolving 6.056 g of potassium dihydrogen orthophosphate in 445mL of HPLC grade water. To this 55mL of 0.1M phosphoric acid was added and pH was adjusted to 3.0 with triethylamine. The above prepared buffer and

acetonitrile were mixed in the proportion of 50:50 v/v and was filtered through 0.45 µm nylon membrane filter and degassed by sonication.

Preparation of Standard solution

About 10 mg of pure Lurasidone HCl was accurately weighed and dissolved in 10mL of mobile phase at to get 1 $mg.mL^{-1}$ stock solution. Working standard solution of Lurasidone HCl was prepared with mobile phase. The final volume was made with the mobile phase. The standard solution was filtered through 0.45 µm nylon membrane filter and degassed by sonication.

Preparation of Sample solution

The content of 10 tablets of LATUDA-40 were accurately weighed and transferred into a mortar and ground to a fine powder. From this, tablet powder which is equivalent to 100 mg of Lurasidone HCl was taken and the drug was extracted in 100mL of mobile phase. The resulting solution was filtered using whatman grade No.1 filter paper and degassed by sonication. This solution was further suitably diluted for chromatography.

Selection of detection wavelength

For the selection of analytical wavelength 10µg/mL Lurasidone HCl solution was prepared by appropriate dilution from standard solution and scanned in the range of 200 to 400 nm. From the spectrum λ_{max} of Lurasidone HCl 235 nm was selected for the analysis.

Calibration curve for Lurasidone Hydrochloride:

Replicates of each calibration standard solutions (10,20,30,40,50µg.mL^{-1}) were injected using a 20µL fixed loop system and the chromatograms were recorded. Calibration curves were constructed by plotting concentration of Lurasidone HCl on X-axis and peak areas of standard Lurasidone HCl on Y-axis and regression equations were computed for Lurasidone HCl. The calibration data is presented in Table 2.

VALIDATION OF THE PROPOSED METHOD

The developed method of analysis was validated as per the ICH Q2(R1)[9] for the parameters like system suitability, specificity, linearity, precision, accuracy, robustness and system suitability, limit of detection (LOD) and limit of quantitation (LOQ).

System suitability

System suitability tests are an integral part of chromatographic method which was used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution at the concentration level 10µg mL^{-1} for Lurasidone HCl to check the reproducibility of the system. At first the HPLC system was stabilized for 40 min. One blank followed by sixreplicates of a single calibration standard solution of Lurasidone HCl was injected to check the system suitability. To ascertain the systems suitability for the proposed method, the parameters such as theoretical plates, peak asymmetry, retention time and parameters were taken and results are presented in Table 1.

Specificity

The effect of wide range of excipients and other additives usually present in the formulations of Lurasidone HCl in the determinations under optimum conditions was investigated. The specificity of the RP-HPLC method was established by injecting the mobile phase and placebo solution in triplicate and recording the chromatograms. The common excipients such as lactose anhydrous, microcrystalline cellulose and magnesium stearate have been added to the placebo solution and injected and tested. The representative chromatogram of placebo is shown in Fig. 2. The specificity results are presented in Table 4.

Linearity

The linearity graphs for the proposed assay methods were obtained over the concentration range of 10-50 µg.$mL^{-1}$of Lurasidone HCl. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient, regression data and calibration data values and the results are presented in Table 2 and Table 3. The representative chromatograms indicating the Lurasidone HCl are shown in Fig. 5 to 9. A calibration curve was plotted between concentration and area response and statistical analysis of the calibration curve is shown in Fig. 10.

Precision

Intra-day and inter-day precision study of Lurasidone HCl was carried out by estimating corresponding responses 3 times on the same day and on 3 different days for the concentration of 10µg/mL. The percent relative standard deviation (% RSD) was calculated which is withinthe acceptable criteria of not more than 2.0. The results for intra-day and inter-day precision are presented in Table 5 and Table 6 respectively.

Accuracy (Recovery studies)

The accuracy of the method was determined by calculating recovery of Lurasidone HCl by the method of addition. Known amount of Lurasidone HCl at 80%, 100% and 120% was added to a pre quantified sample solution. The recovery studies were carried out in the tablet in triplicate each in the presence of placebo. The mean percentage recovery of Lurasidone HCL at each level was not less than 99% and not more than 101%. The results are presented in Table 7.

Robustness

The Robustness was evaluated by the analysis of Lurasidone HCl under different experimental conditions such as making small changes in flow rate (± 0.2 mL/min), detection wavelength (±5nm), and Mobile phase composition (±5%). The results are presented in Table 8.

LOD and LOQ

Limit of Detection is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. The limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. Limit of Detection and Limit of Quantitation were calculated using following formula LOD= 3.3(SD)/S and LOQ= 10 (SD)/S, where SD=the standard deviation of response (peak area) and S= the slope of the calibration curve. The LOD and LOQ values are presented in Table 9.

RESULTS AND DISCUSSION

The mobile phase consisting of 10mM phosphate buffer (pH-3.0): acetonitrile (50:50 % v/v at1mL.min^{-1} flow rate was optimized which gave sharp peak, minimum tailing factor with short run time for Lurasidone HCl. The retention time for Lurasidone HCl was 4.333 min. UV spectra of Lurasidone HCl showed that the drug absorbed maximum at 235 nm, so this wavelength was selected as the detection wavelength. System suitability parameters &optimized chromatographic conditions are shown in Table 1. The calibration curve for Lurasidone HCl was found to be linear over the range of 10-50 µg.mL^{-1}. The data of regression analysis andthe calibrationdata are shown in Table 2 and Table 3. The developed method was applied to the assay of Lurasidone HCl tablets. The experimental results are given in Table 4. The results were very close to labelled value of commercial tablets. The representative standard and sample chromatograms of Lurasidone HCl are shown in Fig. 3

and 4 respectively. The regression equation was found to be Y= 63.07 x+0.878with correlation coefficient is r^2=0.9999 which indicates this method has good linearity. The representative chromatograms indicatingthe Lurasidone HCl are shown in Fig. 5 to 9. The linearity of the graph is shown in Fig. 10. The specificity was studied for the examination of the presence of interfering components,while the comparison of chromatograms there was no interference from placebo (Fig. 2) with sample peak. They do not disturb the elution or quantification of Lurasidone HCl, furthermore the well-shaped peaks also indicate the specificity of the method. Therefore, it was concluded that the method is specific. The specificity results are summarized in Table 4. Precision was studied to find out intra and inter day variations in the test methods of Lurasidone HCl for the three times on the same day and different day. The intra-day and inter-day precision obtained was % RSD (< 2.0) indicates that the proposed method is quite precise and reproducible and results are shown in Tables 5 and 6. Recovery studies of the drug were carried out for the accuracy parameter at three different concentrations levels i.e., multiple level recovery studies. A known amount of Lurasidone HCl standard was added into pre-analyzed sample and subjected them to the proposed HPLC method. The % recovery was found to be within the limits as listed in Table 7. Generally the mean percentage recovery of Lurasidone HCl at each level was not less than 99% and not more than 101%. In this case percentage recovery of Lurasidone HCl was found to be in the range of 99.60 % to 99.80%.The method precision was done and the low %RSD values indicates that the proposed method which was in good agreement with precision. Robustness was done by small changes in the chromatographic conditions like mobile phase flow rate, temperature, mobile phase composition etc., It was observed that there were no marked changes in the chromatograms. Infact the parameters are within the limit which indicates that the method has robustness and suitable for routine use. The Robustness results are presented in Table 8. The limit of detection (LOD) and limit of quantitation (LOQ) was calculated based on the standard deviation (SD) of the response and the slope (S) of the calibration curve at levels approximating the LOD and LOQ. The limit of detection (LOD) was 0.0653µg/mL and the limit of quantitation (LOQ) was 0.1980µg/mL which shows that this method is very sensitive. The results are presented in Table 9.

Table 1: Optimized chromatographic conditions and system suitability parameters for proposed HPLC method for Lurasidone HCl.

Parameter	Chromatographic conditions
Instrument	SHIMADZU LC-20AT prominence liquid chromatograph
Column	WELCHROM C_{18} Column(4.6 X 250mm, 5µm)
Detector	SHIMADZU SPD-20A prominence UV-Vis detector
Diluents	10mM Phosphate Buffer(pH-3): Acetonitrile (50:50 v/v)
Mobile phase	10mM Phosphate Buffer(pH-3): Acetonitrile (50 : 50 v/v)
Flow rate	1mL.min^{-1}.
Detection wave length	By UV at 235nm.
Run time	8 minutes
Column back pressure	98kgf
Temperature	Ambient temperature(25^{o}C)
Volume of injection loop	20µL
Retention time (R_t)	4.333 min
Theoretical plates[th.pl] (Efficiency)	13,871
Theoretical plates per meter[t.p/m]	277426
Tailing factor (asymmetry factor)	1.238

Table 2: Linear regression data of the proposed HPLC method of Lurasidone HCl.

Parameter	Method
Detection wavelength(λ max)	By UV at 235nm
Linearity range (µg/ml)	10-50µg.mL^{-1}
Regression equation (Y=a+bx)	Y=63.07x+0.878
Slope(b)	63.07
Intercept(a)	0.878
Standard deviation of slope (S_b)	0.0416
Standard deviation of intercept (S_a)	1.2615
Standard error of estimation (Se)	1.7430
Correlation coefficient (r^2)	0.9999
% Relative standard deviation* i.e., Coefficient of variation(CV)	0.1305
Percentage range of errors* (Confidence limits) 0.05significance level 0.01 significance level	 0.2604 0.0052

*Average of 6 determinations; Acceptance criteria < 2.0.

Table 3: Calibrationdata of the proposed HPLC method of Lurasidone HCl.

S.No	Concentration, µg.mL^{-1}.	Retention time,(R_t) min.	Peak area, mV.s.
1	0	-	0
2	10	4.333	637.785
3	20	4.333	1275.367
4	30	4.333	1913.157
5	40	4.333	2551.333
6	50	4.333	3183.910

Table 4: Specificity study.

Name of the solution	Retention time (R_t)min.
Mobile phase	No peaks
Placebo	No peaks
Lurasidone HCl 10 µg.mL^{-1}	4.333 min.

Table 5: Results of Precision study (Intra-day).

Sample	Concentration (µg.mL^{-1})	Injection no.	Peak area	%RSD(acceptance criteria< 2.0)
Lurasidone HCl	10	1	637.785	0.1040
		2	636.554	
		3	637.456	
		4	636.278	
		5	637.678	
		6	637.759	

Table 6: Results of Precision study (Inter-day).

Sample	Concentration (µg.mL^{-1})	Injection no.	Peak area	%RSD (acceptance criteria < 2.0)
Lurasidone HCl	10	1	637.787	0.1276
		2	635.812	
		3	637.425	
		4	636.522	
		5	635.826	
		6	636.819	

Table 7: Recovery data of the proposed Lurasidone HCl by RP-HPLC method.

Recovery level	Amount taken (mg)	Amount added (mg)	Total Amount (mg)	% recovery (mg)	Mean % Recovery	%RSD#
80%	8	5	13	12.95	99.61	0.26
100%	10	5	15	14.98	99.86	0.17
120%	12	5	17	16.96	99.76	0.060

[#]acceptance criteria< 2.0.

Table 8: Robustness results of Lurasidone HCl.

S. No	Parameter	Optimized	Used	Retention time (R_t), min	Plate count	Peak asymmetry[#]	Remark
1.	Flow rate (±0.2mL.min^{-1})	1.0 mL.min^{-1}	0.8 mL.min^{-1}	4.333	13850	1.286	*Robust
			1.0 mL.min^{-1}	4.256	13853	1.294	*Robust
			1.2 mL.min^{-1}	4.168	13855	1.295	*Robust
2.	Detection wavelength (±5nm)	235 nm	230nm	4.258	13853	1.294	*Robust
			235nm	4.254	13853	1.286	*Robust
			240nm	4.256	13853	1.295	*Robust
3.	Mobilephase composition (±5%)	50:50v/v	55:45v/v	4.199	13855	1.286	*Robust
			50:50v/v	4.254	13856	1.294	*Robust
			45:55v/v	4.257	13853	1.295	*Robust

Table 9: Limit of Detection (LOD) and Limit of Quantitation (LOQ).

Limit of Detection(LOD)	0.0653 µg.mL^{-1}
Limit of Quantitation(LOQ)	0.1980µg.mL^{-1}

Table 10:Assay results of Lurasidone HCl formulation.

S. No	Formulations	Labelled amount	Amount found	% Assay ±RSD*
1	LATUDA	40mg	39.962 mg	99.905±0.10

***** Average of 6 determinations.

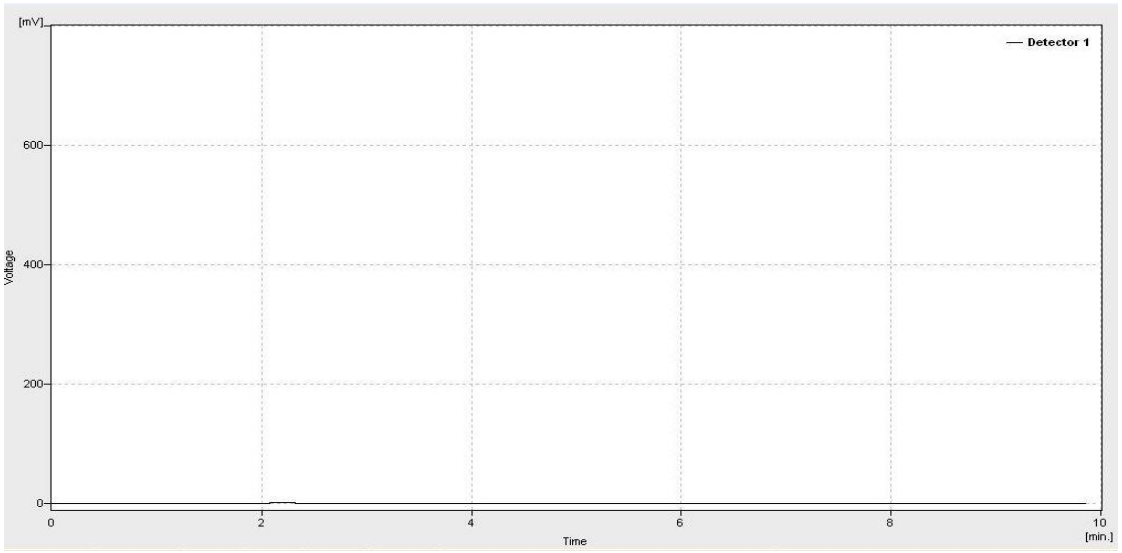

Fig 2: Chromatogram of placebo.

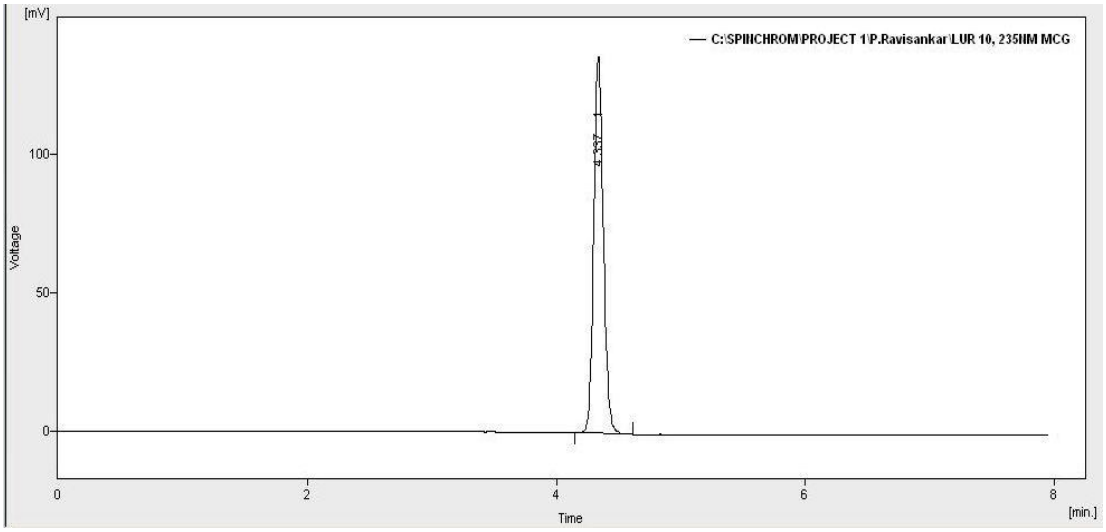

Fig. 3: A typical chromatogram of Lurasidone HCl standard.

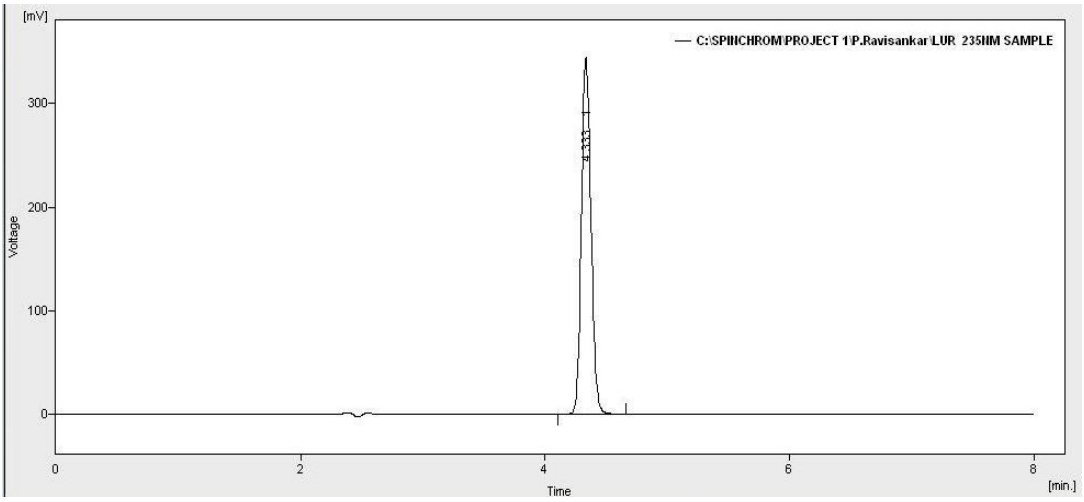

Fig. 4: Chromatogram of market formulation (LATUDA-40mg Tablets) of Lurasidone HCl.

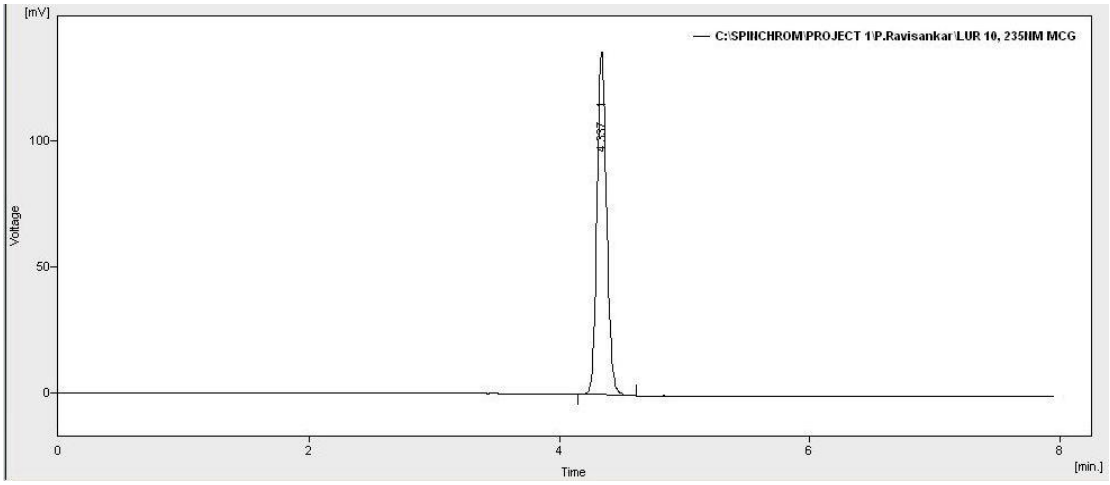

Fig. 5: Standard chromatogram of Lurasidone HCl (10 µg.mL^{-1}).

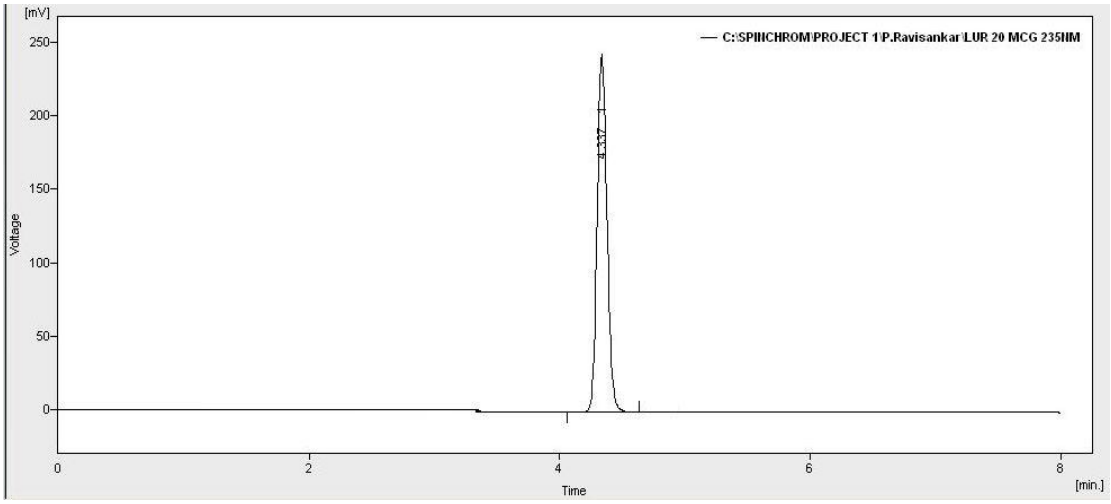

Fig. 6: Standard chromatogram of Lurasidone HCl (20µg.mL^{-1}).

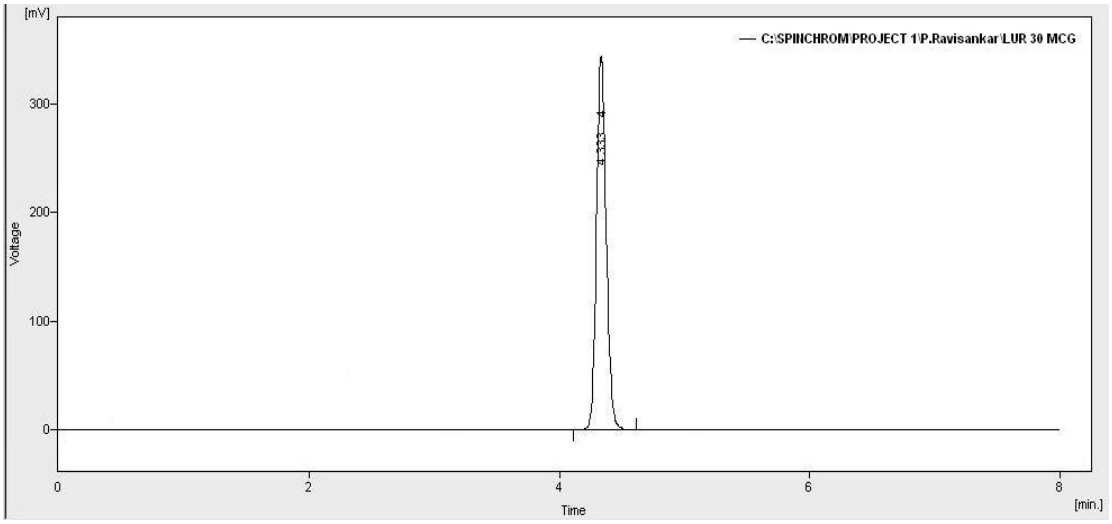

Fig. 7: Standard chromatogram of Lurasidone HCl (30 µg.mL^{-1}).

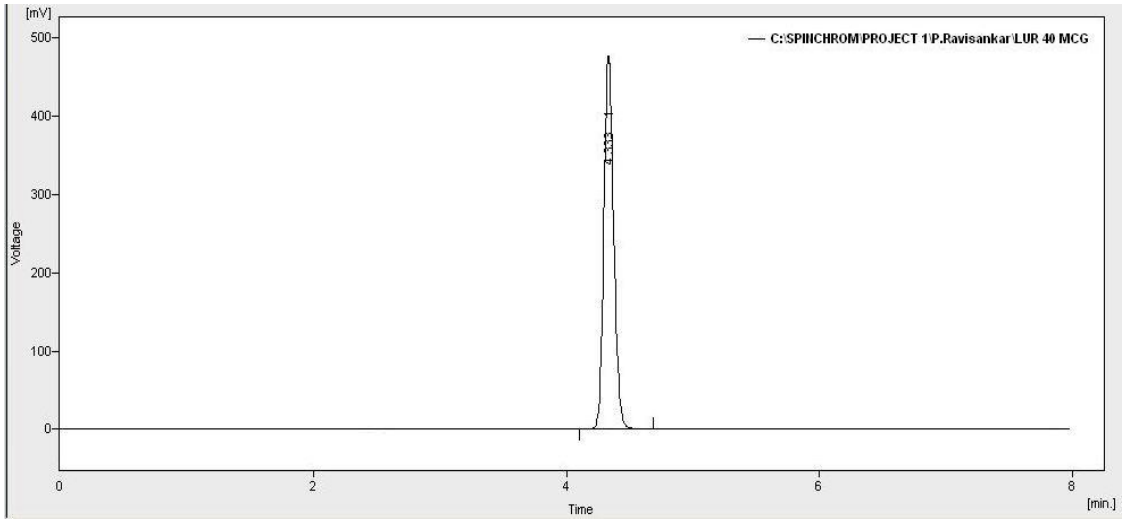

Fig. 8: Standard chromatogram of Lurasidone HCl (40 µg.mL^{-1}).

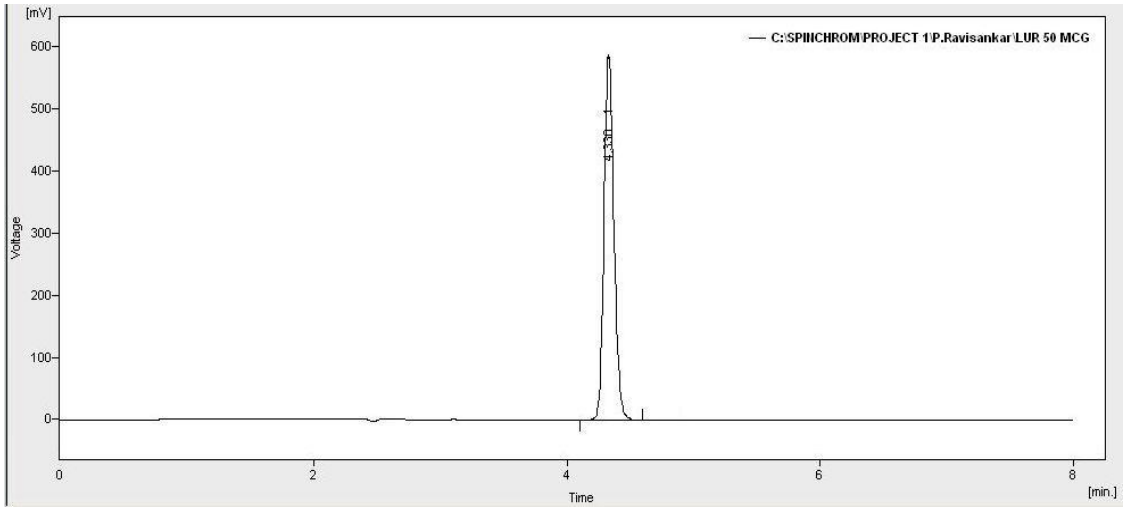

Fig. 9: Standard chromatogram of Lurasidone HCl (50µg.mL^{-1}).

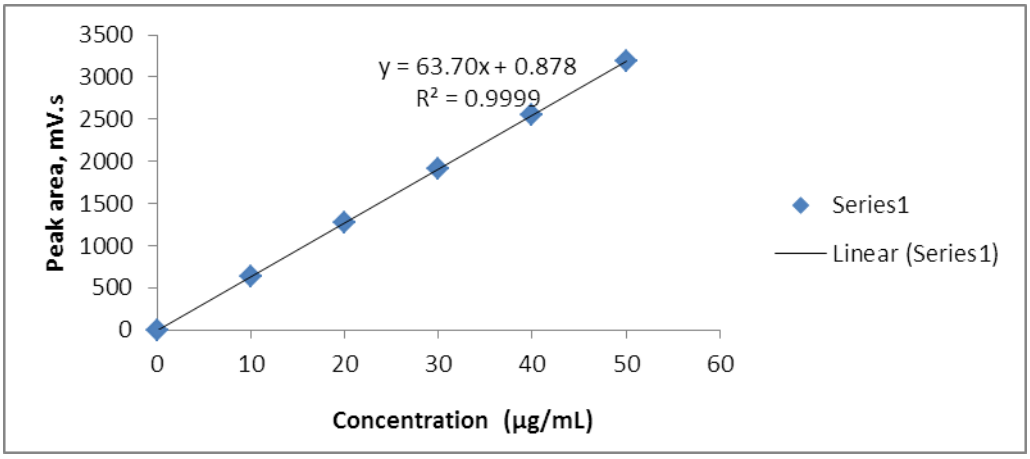

Fig. 10: Calibration plot of Lurasidone HCl

CONCLUSION

A New validated RP-HPLC method has been developed for the quantitative determination of Lurasidone HCl in bulk and pharmaceutical tablet dosage forms. Statistical analysis of the results shows that the proposed procedure has good precision and accuracy. The method was completely validated shows satisfactory results for all the method validation parameters tested and method was free from interference of the other active ingredients and additives used in the formulation.In fact results of the study indicate that the developed method was found to be simple, reliable, accurate, linear, sensitive, economical, and reproducible and have short run time which makes the method rapid. Hence it can be concluded that this method may be employed for the routine quality control analysis of Lurasidone HCl in active pharmaceutical ingredient (API) and pharmaceutical preparations.

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