

New Analytical Method Development and Validation for Estimation of Lurasidone Hydrochloride by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

Research Article

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Abstract: A simple and reproducible method was developed for Lurasidone HCL by Reverse Phase High Performance Liquid Chromatography (RP-HPLC). Lurasidone HCL was separated on Agilent Extended C18 column (5 μ m, 4.6x150mm) and a mobile phase containing Acetonitrile: Formic acid buffer 0.1% (pH adjusted to 2.8 using triethylamine) in the ratio of 50:50 v/v was used for the separation and quantification. The flow rate was 1.0 mL/min. The retention time was found to be 2.33 $\pm$ 0.03 minutes. The developed method was validated according to ICH guidelines with different parameters such as Linearity, Precision, Accuracy, Robustness, Limit of detection (LOD), Limit of quantification (LOQ). The standard curve was linear over a working range of 10-60 μ g/ml and gave an average correlation factor 0.996 for Lurasidone HCL. The Limit of detection and Limit of quantification was found to be 2.1 μ g/ml and 8.5 μ g/ml respectively of Lurasidone HCL. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise.

Keywords – Lurasidone HCL, RP-HPLC, Validation, ICH Guidelines, Linearity, Accuracy

I. INTRODUCTION-

The analytical technique of High Performance Liquid Chromatography (HPLC) is used astronomically throughout the pharmaceutical industry. HPLC play an important and critical role in the field of pharmaceutical industries and analysis, since it is used to provide information on the composition of drug related samples and to detect the raw ingredient used to make them i.e., qualitative and quantitative.

Reversed-phase high-performance liquid chromatography (RP-HPLC) involves the separation of molecules on the basis of hydrophobicity. ^[1]

Lurasidone belongs to a class of medications known as atypical antipsychotics. Atypical antipsychotics like lurasidone are considered the standard of care for treating schizophrenia. Additionally, in clinical studies lurasidone was shown to be effective in improving mood in many people struggling with bipolar depression. ^[2]

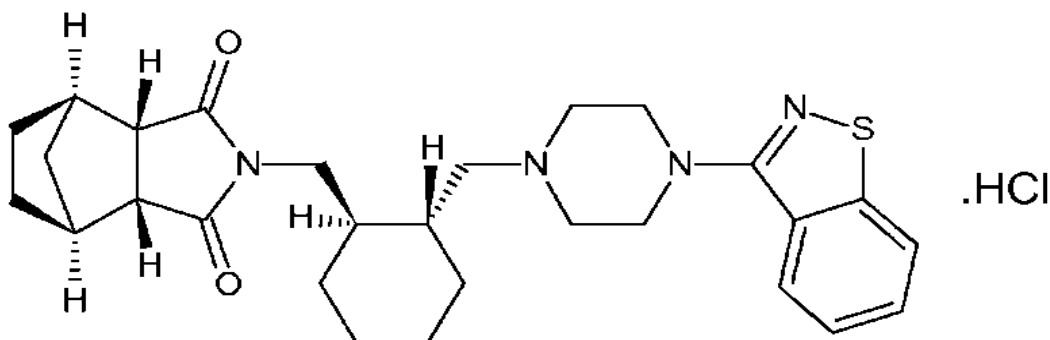


Figure 1: Structure of Lurasidone HCL

Lurasidone is approved by the U.S. Food and Drug Administration (FDA) for treatment of Schizophrenia since 2010. Lurasidone Hydrochloride (3aR,4S,7R,7aS)-2-[(1R,2R)-2-[4-(1,2-benzisothiazol-3-yl)piperazin-1-ylmethyl]cyclohexylmethyl]hexahydro-4,7-methano-2H isoindole-1,3-dione.HCl is a benzisothiazol derivative.^[6]

Lurasidone is soluble in water 45 mg/mL (20 °C), insoluble in Toluene, slightly soluble in ethanol, sparingly soluble in methanol and very sparingly soluble in Acetone.^[3]

The mechanism of action of Lurasidone Hydrochloride, as with other drugs having efficacy in schizophrenia, is unknown. It has been suggested that the efficacy of Lurasidone Hydrochloride in schizophrenia is mediated through a combination of central dopamine Type 2 (D2) and serotonin Type 2 (5HT2A) receptor antagonism. And it gives Antipsychotic Activity. Lurasidone may be useful for treating cognitive and memory deficits seen in schizophrenia for several reasons: **1)** unlike many other antipsychotics, lurasidone does not block the muscarinic acetylcholine receptors, an action well-known to impair learning and memory; **2)** lurasidone has prominent activity at 5-HT1A, 5-HT2A, 5-HT7, and Alpha 2C adrenergic receptors, all of which have been implicated in enhancement of cognitive function if modulated properly; **3)** due to its low liability for extra pyramidal symptoms, lurasidone is unlikely to require co-administration of anticholinergic, which impair cognition in their own right.^[4]

Lurasidone is metabolized in the liver via the enzyme CYP3A4. This means that its plasma concentrations may be increased when combined with CYP3A4 inhibitors like ketoconazole or grapefruit juice, possibly leading to more side effects. Co-administration of CYP3A4 inducers like rifampicin or St. John's wort can reduce plasma levels and consequently the effects of the drug.^[5]

Side effects are generally similar to other antipsychotics. The drug has a relatively well-tolerated side effect profile, with low propensity for extra pyramidal symptoms, QTc interval changes, and weight, lipid, and glucose-related adverse effects.^[5]

On literature survey it was found that very few analytical methods are available for the estimation of Lurasidone Hydrochloride. In view of the need for a suitable method for routine analysis of Lurasidone Hydrochloride, attempts are made to develop simple, precise and accurate analytical methods for its estimation. Analytical validation is the corner stone of the process validation. Without a proven measurement system it is impossible to confirm whether the manufacturing process has done what it purpose to do. Hence, there is a need to validate the new methods developed.

II. MATERIALS AND METHODS-

Chemicals and Reagents-

Formic Acid (HPLC grade), Acetonitrile (HPLC grade), Water (HPLC grade), Triethylamine (HPLC grade). Commercial tablets of Lurasidone HCl formulation was procured from local market. Lurakem-tablets 40 mg are manufactured by MSN Laboratories Pvt. Ltd. Telangana, India and marketed by Alkem laboratories Ltd. Mumbai, India.

INSTRUMENTATION-

Analysis was performed on the Agilent 1120 Compact LC HPLC system consisting of gradient pump (LC- 10AT VP pump) (40MPa or 400barr), rheodyne injector, UV variable wavelength detector, standard cell and Agilent syringe was used. The separations were achieved on an Agilent Extended C18 column (5µm, 4.6x150mm). Analytical weighing balance (Shimadzu AUX 220) was used for weighing, sonicator (EQUITRON230VAC, 50Hz), vacuum pump (SUPER FIT), filtration kit (TARSONS) and Nylon membrane filter (Merck Millipore) for solvents and sample filtration was used throughout the experiment. Double beam UV-Visible spectrophotometer (SHIMADZU-UV 1700) was used for wavelength detection. The EZ Chrome Elite software-single channel was used for acquisition, evaluation and storage of chromatographic data.

Buffer Preparation-

0.1% Formic acid buffer- It was prepared by dissolving 0.5ml of Formic acid in 500ml of HPLC grade water and the pH maintained to 2.8 with Triethylamine.

Chromatographic Condition-

The mobile phase consisting of Acetonitrile : Formic acid buffer 0.1% (pH adjusted to 2.8 using triethylamine) in the ratio of 50:50 v/v, was filtered through 0.45 μ membrane filter, sonicated and pumped from the solvent reservoir. The flow rate of the mobile phase was maintained at 1ml/min and the response was monitored at 316nm with the run time 8min. Injection volume was 20 μ L, with ambient temperature and retention time was 2.33 $\pm$ 0.03 min.

Preparation of Standard-

About 25mg of pure Lurasidone HCL was accurately weighed and transferred into a 25ml of volumetric flask and dissolve in 25ml of diluent to get 1mgmL⁻¹ stock solution. Working standard solution of Lurasidone HCL was prepared with the diluent. The standard solution was filtered through 0.45 μ membrane filter and degassed by sonication.

Preparation of Sample-

The content of the 10 tablets of Lurakem-40 were accurately weighed and transferred into a mortar and ground to fine powder. From this, tablet powder which is equivalent to 25mg of Lurasidone HCL was taken and transferred into a 25ml clean and dry volumetric flask and about 15ml of diluent was added and sonicated to dissolve it completely and the volume made up to the mark with the same diluent. The resulting solution was filtered using 0.45 μ membrane filter and degassed by sonication. This solution was further suitably diluted for chromatography.

Selection of detection wavelength-

For the selection of detection wavelength 10 μ g/ml Lurasidone HCL solution was prepared by appropriate dilution from the standard solution. This drug solution was then scanned in the range of 200-400nm. From the spectrum λ_{max} of Lurasidone HCL 316nm was selected for the analysis.

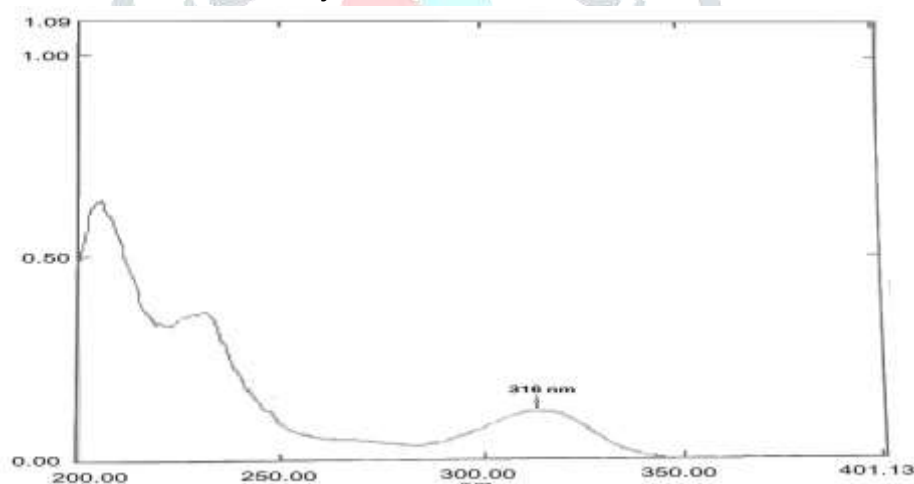


Figure 2: Absorption spectrum of Lurasidone hydrochloride

Calibration Curve for Lurasidone HCL-

Replicates of each Standard solutions (10, 20, 30, 40, 50 & 60 μ g.mL⁻¹) were injected using a 20 μ L fixed loop system and the solution were chromatographed for 8minutes using mobile phase at a flow rate of 1.0 ml/min. The graph was plotted for peak area vs. concentration for Lurasidone Hydrochloride.

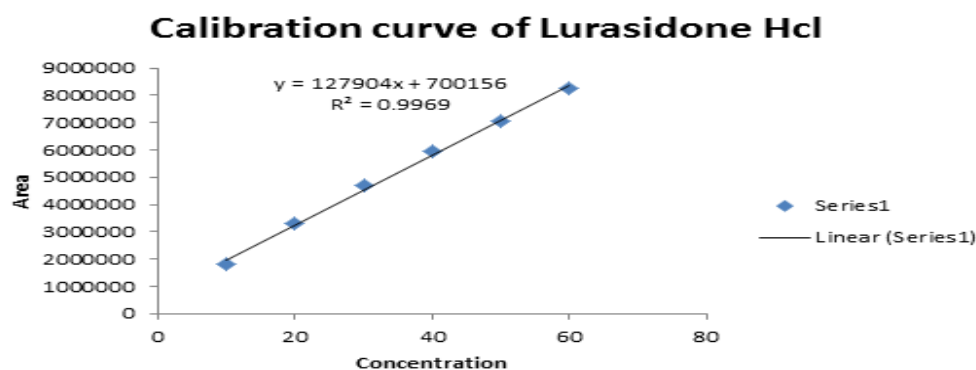


Figure 3: Calibration curve of Lurasidone Hcl

III. VALIDATION OF THE DEVELOPED HPLC METHOD-

The developed method was validated according to ICH guidelines with respect to accuracy, precision, linearity, specificity, robustness, limit of detection (LOD), limit of quantification (LOQ), ruggedness and system suitability

Specificity and selectivity

Specificity is a procedure to detect quantitatively the analyte in presence of the components that may be expected to be present in the sample matrix. The specificity of the method was determined by comparing test results obtained from analysis of sample solution containing excipients with that of test results those obtained from standard drug. While selectivity is a procedure to detect the analyte qualitatively in presence of components that may be expected to be presented in the sample matrix. The excipients in tablet formulation were spiked in pre weighted quantity of drugs and then absorbance was measured and calculations were done to determine the quantity of the drugs.

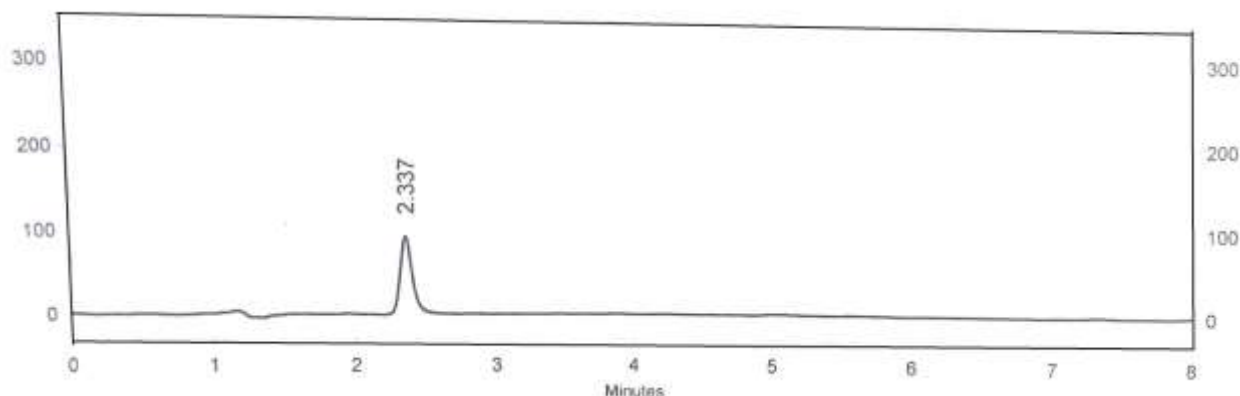


Figure 4: Standard chromatogram of Lurasidone Hcl

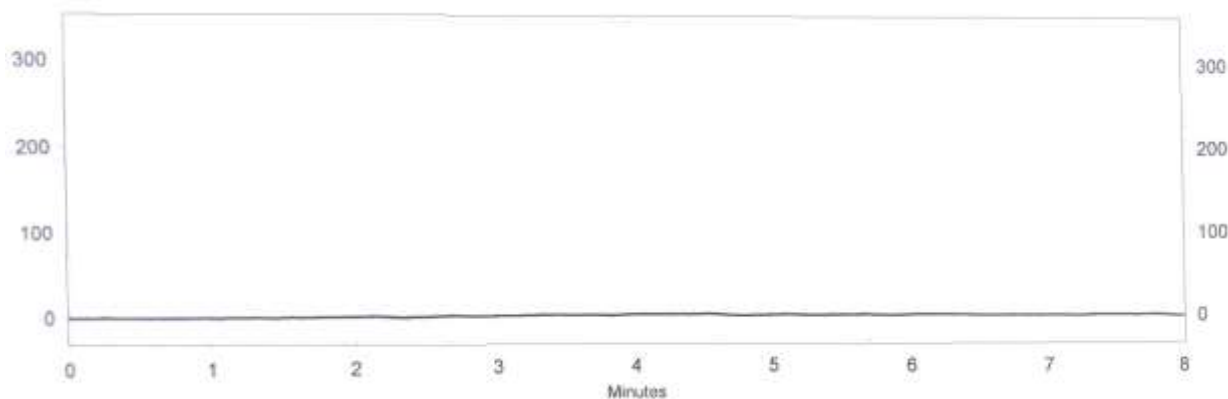


Figure 5: Blank chromatogram

Linearity

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. Linearity was tested for the range of concentrations 10-60 µg/ml. Six dilutions of each concentration were prepared separately. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient, regression data and calibration data values.

Table 1: Results of linearity of Lurasidone HCl

Sl No.	Concentration(µg/ml)	Area
1	10	1807423
2	20	3303759
3	30	4680095
4	40	5956094
5	50	7063986
6	60	8249340
Correlation coefficient (r ²)		0.9969
y-intercept		700156
Slope		127904

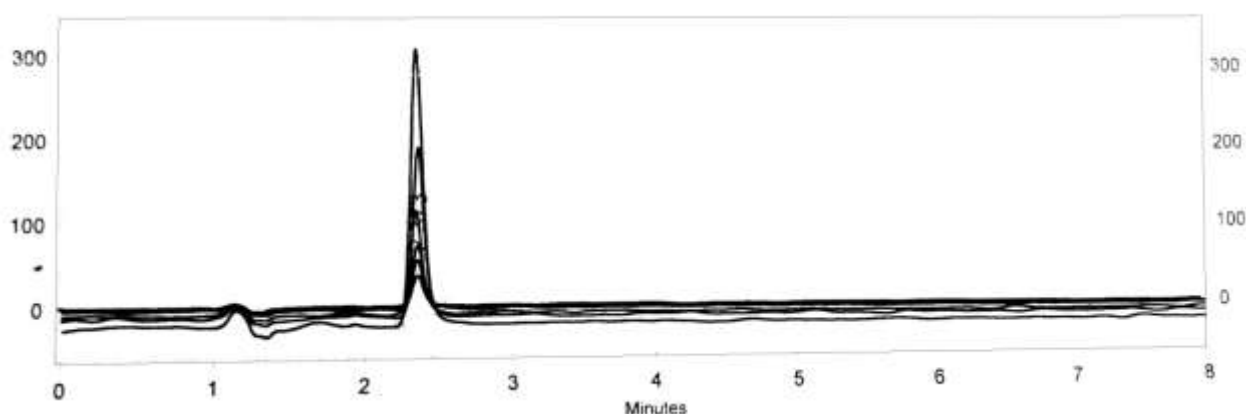


Figure 6: Chromatogram of linearity of Lurasidone HCl

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation (RSD). Variation of results within the same day (intra day), variation of results between days (inter day) were analyzed.

Intraday precision was determined by analyzing Lurasidone Hydrochloride for two times in the same day i.e. Morning and afternoon. Inter day precision was determined by analyzing the drug daily for two days i.e. Day 1 and Day 2.

Table 2: Interday and Intraday precision study of Lurasidone HCl

SL NO.	Intraday			Interday		
	Replicates	Area		Replicates	Area	
		Morning	Afternoon		Day 1	Day 2
1	Replicate 1	8209247	8206318	Replicate 1	8272647	8267411
2	Replicate 2	8293740	8220344	Replicate 2	8129246	8179285
3	Replicate 3	8246311	8112176	Replicate 3	8225200	8177312

4	Replicate 4	8330570	8293708	Replicate 4	8247491	8288873
5	Replicate 5	8209176	820023	Replicate 5	8113617	8270832
6	Replicate 6	8244214	8166908	Replicate 6	8298001	8277984
Average		8255543	8200063	Average	82143350	8243616
SD(Standard Deviation)		48155.7	60073.2	SD(Standard Deviation)	76157.4	51129.18
%RSD		0.58	0.73	%RSD	0.92	0.62

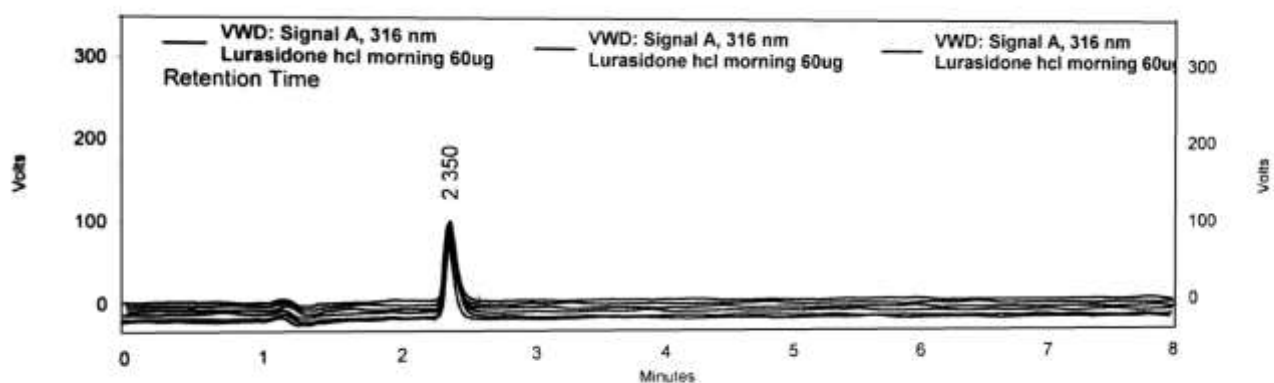


Figure 7: Chromatogram for Precision (Morning)

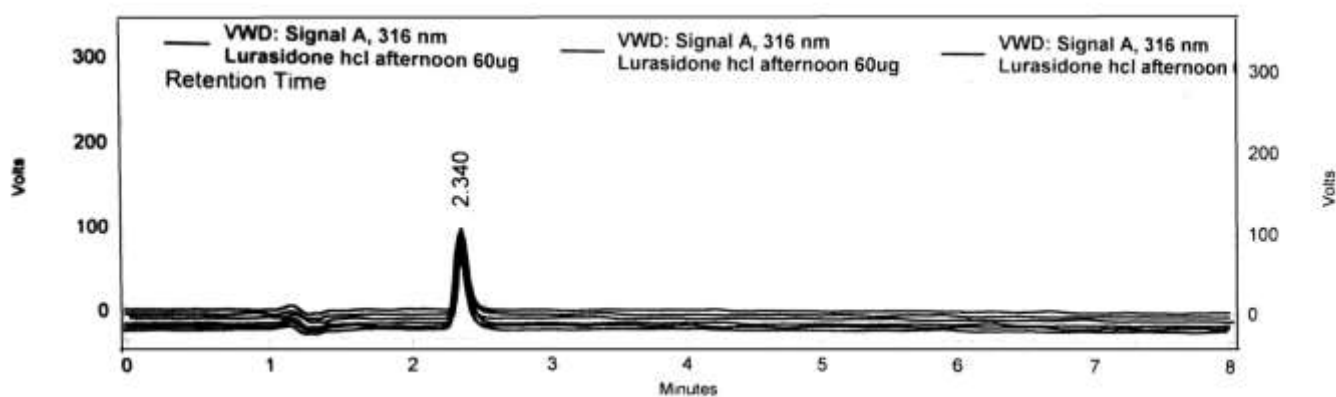


Figure 8: Chromatogram for Precision (Afternoon)

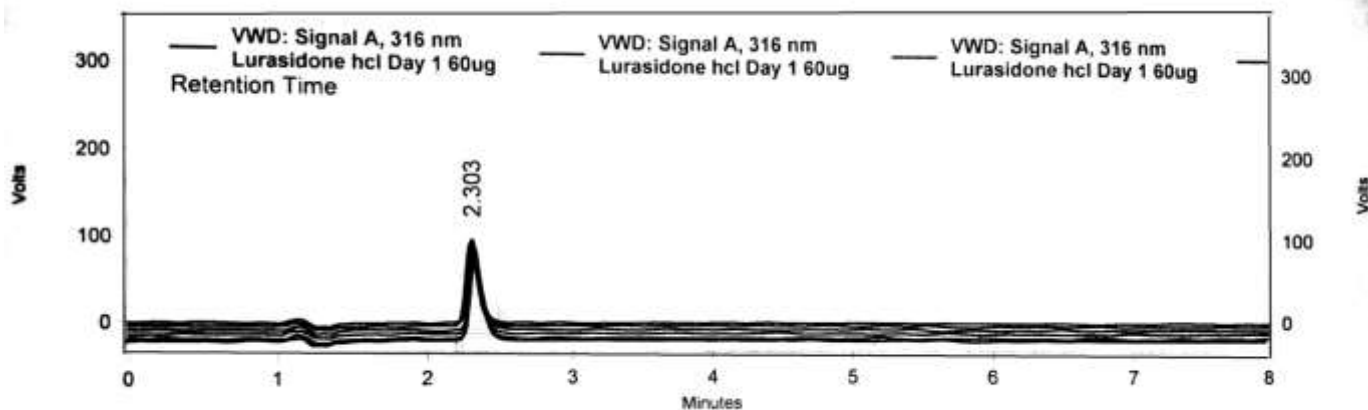


Figure 9: Chromatogram for Precision (Day 1)

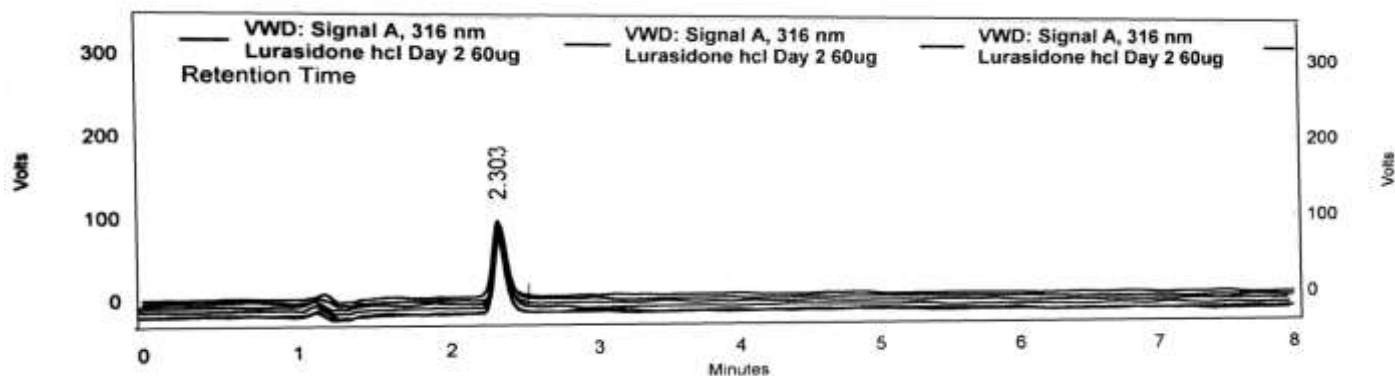


Figure 10: Chromatogram for Precision (Day 2)

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. The procedure for the preparation of the solutions for Accuracy determination at 80%, 100% and 120% level were prepared.

For 80% Accuracy for : Lurasidone Hydrochloride

32 µg of the pure drug was added to 40 µg of formulation

For 100% Accuracy for Lurasidone Hydrochloride

50 µg of the pure drug is added to 50 µg of formulation

For 120% Accuracy for Lurasidone Hydrochloride

72 µg of the pure drug is added to 60 µg of formulation

Table 3: Recovery study of Lurasidone HCl

Spiking %	Amount of sample (µg/ml)	Amt. of drug added (µg/ml)	Amt. recovered (µg/ml)	% Recovery	%RSD
80%	40	32	72.48	100.66%	0.23
100%	50	50	99.88	99.88	0.67
120%	60	72	130.90	99.16	0.50

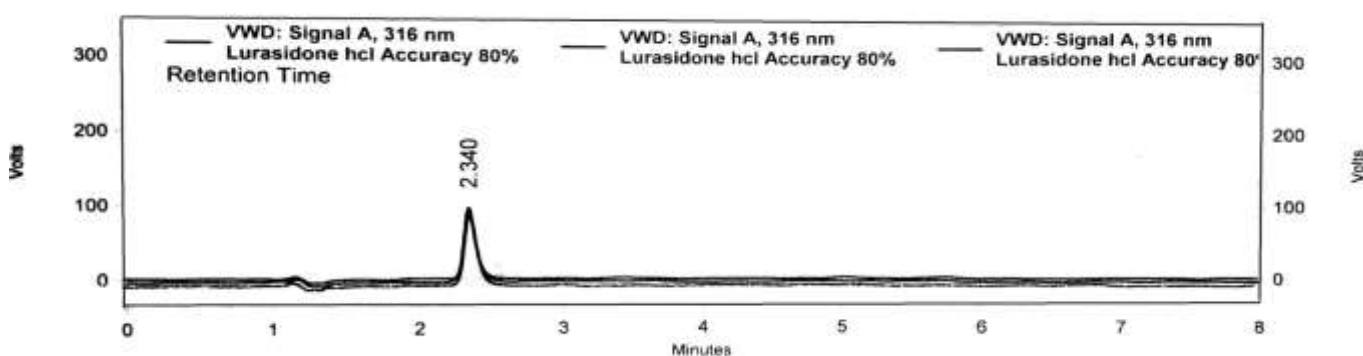


Figure 11: Chromatogram for Accuracy (80%)

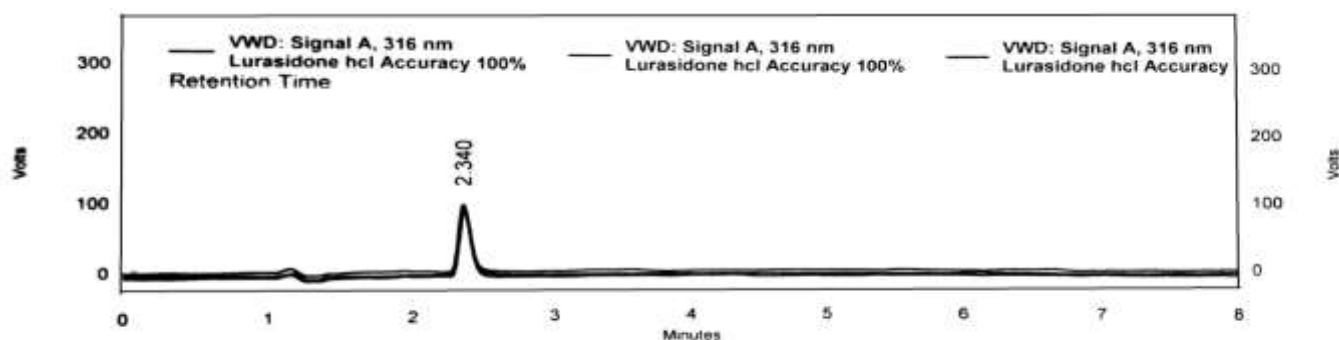


Figure 12: Chromatogram for Accuracy (100%)

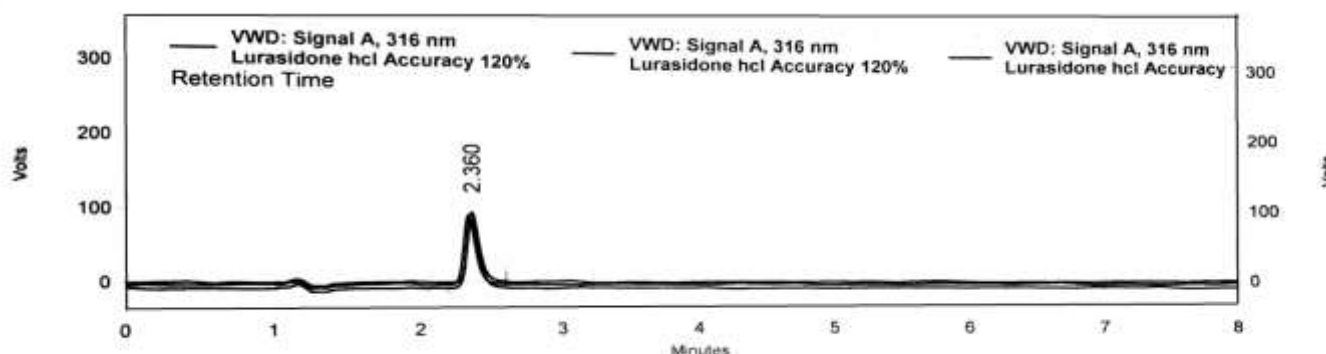


Figure 13: Chromatogram for Accuracy (120%)

Robustness

As defined by the ICH, the robustness of an analytical procedures describes to its capability to remain unaffected by small and deliberate variation in the chromatographic conditions such as making small changes in flow rate (± 0.1 mL/min), detection wavelength (± 2 nm).

Table 4: Robustness study of Lurasidone HCl

S.N.	Parameter	Optimized	Used	Retention time (minutes)
1.	Flow rate	1ml/min	0.9ml/min	2.56
			1.1ml/min	2.14
2.	Detection wavelength	316nm	314nm	2.33
			318nm	2.32

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of Detection is the lowest concentration in a sample that can be detected. The limit of quantitation is the lowest concentration of analyte in a sample that can be determined. LOD and LOQ were calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. A signal to noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively.

Table 5: LOD and LOQ for estimation of Lurasidone HCl

Name of drug	LOD $\mu\text{g/ml}$	LOQ $\mu\text{g/ml}$
Lurasidone HCL	2.15	8.5

IV. RESULTS AND DISCUSSION

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug LURASIDONE being non-polar is preferably analyzed by reverse phase columns and accordingly Agilent

Extended C18 column was selected. So the elution of the compound from the column was influenced by polar mobile phase. First of all, maximum absorbance was found to be at 316nm. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Acetonitrile: Formic acid buffer 0.1% (pH adjusted to 2.8 using triethylamine) in the ratio of 50:50 v/v. Injection volume was selected to be 20µl which gave a good peak area. Run time was selected to be 8 min because analyte elutes at around 2.33±0.03 min. The analytical method was found linear over the range of 10-60µg/ml. The percent recovery was found to be 99.16-100.66. Both Intraday and Interday precision was found to be well within range. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise.

V. CONCLUSION

A New validated RP-HPLC method has been developed for the quantitative determination of Lurasidone HCL in bulk and pharmaceutical tablet dosage forms. From the above results, method was found to be accurate, precise, linear, specific, system suitable, robust proved to be sensitive, convenient, cost effective, reproducible and have short run time which makes the method rapid for the estimation of Lurasidone HCL in oral solid dosage form. Hence it can be concluded that this method may be employed for the routine quality control analysis of Lurasidone HCL in pharmaceutical preparations and active pharmaceutical ingredient (API).

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