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RP-HPLC Method Development and Validation for the Determination of Lorlatinib in Bulk and Its Pharmaceutical Formulation

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ABSTARCT

A Simple, Precise, Accurate and rapid liquid chromatography (RP- HPLC) method has been developed for the determination of Lorlatinib in bulk and in tablet dosage form. A reverse phase Eclipse plus C18 column (250mmX4.6mm,3µm) with mobile phase consisting of potassium dihydrogen orthophosphate, acetonitrile and methanol (45:30:25%V/V) having pH 5.8 was adjusted by using orthophosphoric acid. The flow rate was 1.0mL and effluents were monitored at 310nm. The Retention time of Lorlatinib was 7.871/min. The method was linear over the concentration range of 50 to 150µg/ml. The percentage mean recovery for Lorlatinib was observed to be 99% to 102% and the RSD was observed under 1%. The method was carried out based on International Conference on Harmonization (ICH) guidelines. The proposed technique was a new method of analysis for assessment of Lorlatinib drug substance by RP-HPLC method. And the method was observed to be appropriate for the standard examination of Lorlatinib in pure drug substance.

KEYWORDS: Lorlatinib Acetonitrile, Methanol, Validation, RP-HPLC.

1.INTRODUCTION

Lorlatinib is chemically 7-amino-12-fluoro2, 10,16-trimethyl-15-oxo-10, 15,16,17- tetrahydro-2*H*-4,8-methenopyrazolomethenopyrazolo [4, 3 – *h*] [2, 5, 11] enzoxadiazacyclo – tetradecine -3-carbonitrile. Is a kinase inhibitor indicated for the treatment of patients with anaplastic lymphoma kinase (ALK)-positive metastatic non-small cell lung cancer (NSCLC). Lorlatinib is available in markets as conventional tablets with a trade name of LORBRENA. It has shown survival benefits in the treatment of lung cancer in phase III trials. Some high-performance liquid-chromatographic (HPLC) methods with ultraviolet (UV) have been developed. Some methods with tandem mass spectrometry (MS=MS) each with its own advantages and limitations has been reported for the assay of Lorlatinib or other ALK drugs in human plasma. The present article describes the quantitative determination and validation of Lorlatinib in bulk drug and in formulations by RP-HPLC method. The proposed method is simple and specific because it can determine Lorlatinib in the presence of its degradation products, excipients, and additives.

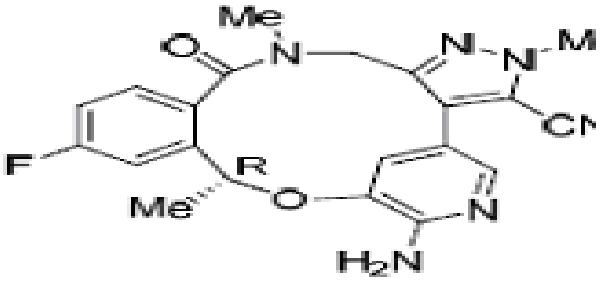

Fig 1,Chemical structure of Lorlatinib

2. MATERIALS AND METHODS

Chemicals and Solvents

The working standards of Lorlatinib was gifted from in Pfizer Health Care, Pvt Ltd, Chennai, 600119 India, Tablet dosage form LORBRENA containing 100 mg of Lorlatinib were procured from Jeevant Labs Ltd Bangaluru India. Methanol and acetonitrile of HPLC grade was purchased from Fisher Scientific Pvt. Ltd. and purified water was procured from Merck (India) Pvt. Ltd. All of other reagents were of analytical grade unless otherwise indicated.

Chromatographic conditions

The isocratic mode of elution was utilized and the mobile phase consisting of potassium dihydrogen orthophosphate, acetonitrile and methanol (50:30:20V/V) was used. For the filtration purpose 0.45 µm (HPLC grade) nylon filter paper was used. The column Eclipse plus C18 (250mm X 4.6mm, 3µm) was used for determination. The flow rate was $1mL^{-1}$ and ambient temperature was maintained throughout the process. The sample was injected as volume of 10µl. Prior to injection of solutions, column was fixed before 15min with mobile phase was running through the system. The UV detector was set at wavelength of 310nm.

Buffer preparation

Potassium dihydrogen orthophosphate (3.42 g) was dissolved in 1000 mL of HPLC grade water and adjusted to pH 5.8 with dilute orthophosphoric acid. The solution was filtered through 0.45 µm (HPLC grade) nylon filter paper and degassed. The resulting solution was sonicated.

Preparation of Mobile Phase

The above buffer solution 500ml (45%) was Mixed with Acetonitrile 300ml HPLC grade (30%) and Methanol 200ml HPLC grade (25%) sonicated for 5min. Filtered through 0.45µm (HPLC grade) nylon filter paper under vacuum filtration.

Preparation of Standard solution

About 50.0mg of Lorlatinib sample is transferred in to a 50ml volumetric flask. Dissolved in Acetonitrile and the volume is made up to the mark. Further 5.0 mL of this solution is transferred in to 50 mL volumetric flask and volume was made up to the mark. 50 mL.with Acetonitrile.

Preparation of Sample solution

Twenty tablets of LORBRENA containing 100 mg of Lorlatinib were accurately weighed, averaged and finely powdered and weight equivalent to one tablet was weighted and transferred to a 100 mL volumetric flask. Diluent Acetonitrile (100 mL) was added to the volumetric flask and was sonicated for 10 min for complete dissolution of the drug in the solution, filtered through a 0.45 µm (HPLC grade) nylon filter paper and made up to the volume with Acetonitrile. Filtrate (5.0 mL) was taken in a 50 mL volumetric flask and the volume as make up with Acetonitrile to the final concentration of 100 µg mL^{-1}.

Method Development

For estimation of Lorlatinib drugs in tablet formulations, primary test was performed. Different chromatographic conditions were estimated for the estimation of Lorlatinib in pure drug and dosage form. The pure drug substance of Lorlatinib was injected in to the HPLC system and run by using mobile phase. Potassium dihydrogen orthophosphate, acetonitrile and methanol, were tested to find the good Conditions for the Separation of Lorlatinib in focus to develop good symmetrical peak. In placed of Water, phosphate buffer was used. Acetonitrile and methanol gave satisfactory results. This mobile phase system was tried with different proportions. Finally, the optimal condition of the mobile phase was chosen as Phosphate butter (pH 5.8): acetonitrile and methanol in the proportion of 45:30:25% v/v was selected for the study. All measurements were carried out at ambient temperature of the column. The flow rate was optimizing by using various flow rate conditions were studied. The optimal flow rate was 1.0ml/min for the present work.

Validation of method

As per ICH guidelines the analysis was carried out. The parameters assessed were Specificity, Linearity, Precision, Accuracy, Limit of detection (LOD) and Limit of Quantification (LOQ).

Specificity

Specificity of a systematic technique is its capacity to measure precisely and particularly the concentration of analyte with no impedance from different types of diluents. Solvents of standard and sample solutions were injected in to liquid chromatography.

Linearity

For the validation of linearity, a minimum of 5 linearity levels of different concentration of Lorlatinib in the range of 50 to 150% of working level concentration of Lorlatinib were injected in to the HPLC system. Plotting the graph of the peak area responses against the concentration and determine the correlation coefficient (r^2), Y- intercept and % RSD of response factor. The results were shown in Table no 1.

Accuracy: The percentage of accuracy was 50%, 100%&150% each Level was injected three times. The data was represented in Table. No, 2.

Precision

Precision of the method was studied as system precision, Method precision and intermediate precision. The standard deviation and the %RSD were mentioned in Table no.3.

LOD and LOQ

The detection and quantification limits for the Lorlatinib was performed and calculated by using S/N (Signal to Noise) ratio method. The values were tabulated in Table no .4.

Robustness: The robustness study was carried out by change in the composition of mobile phase concentration and change in the pH was made to evaluate the impact on the method. The method was robust by change in mobile phase composition and pH change.

System suitability: System suitability test was conducted by using standard stock solution of Lorlatinib. And was injected five times in to HPLC system and the values were recorded.

3. RESULTS AND DISCUSSION

Lorlatinib can be effectively analyzed by the RP-HPLC method with Phosphate buffer (pH:4.2): acetonitrile and methanol (50:30:20%V/V) at wavelength of 310nm. The Rt for standard drug was found to be 7.871/min, and for sample was found to be 7.565/min. The total time of analysis was less than 15min. **Figure 2 and 3**.

Peak Table

Ch1 310nm 4nm

S. No	Peak Name	R_t	Area	Height	USP Plate Count	USP Tailing
1	Lorlatinib	7.871	1992213	249025	21409	1.0

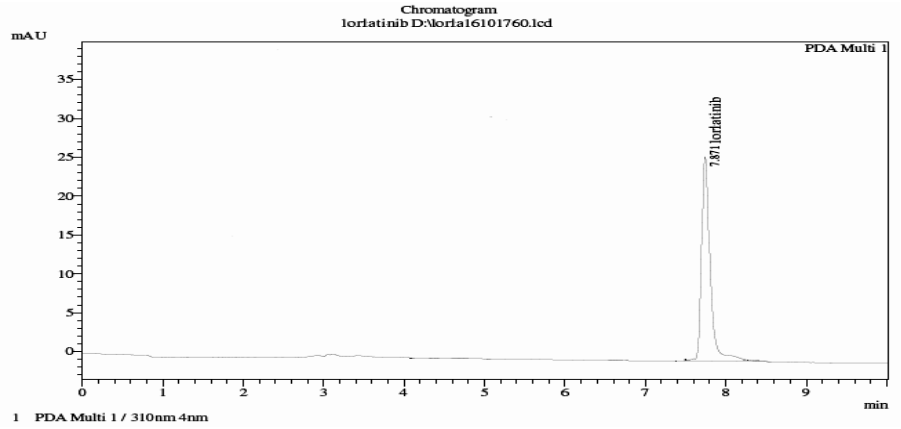

Figure. No. 2. Chromatogram of Standard drug Lorlatinib.

PeakTable

Ch1 310nm 4nm

S. No	Peak Name	R_t	Area	Height	USP Plate Count	USP Tailing
1	lorla	7.565	1444340	209125	25640	1.0

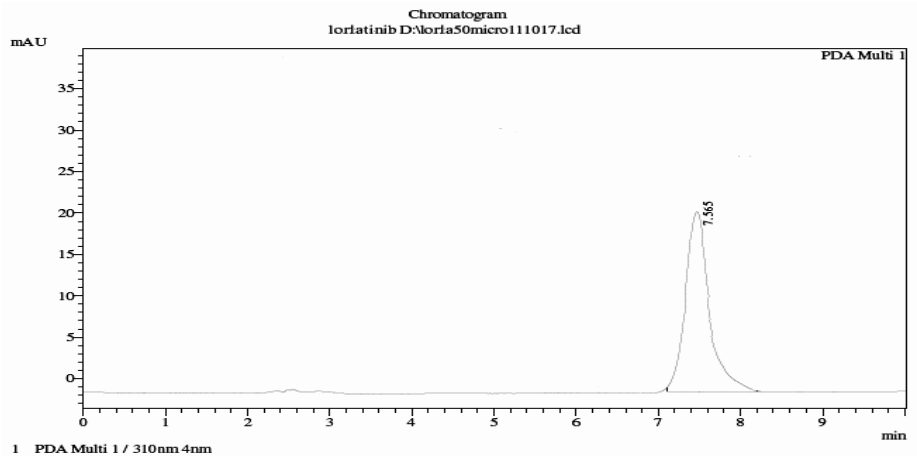

Figure. No. 3 Chromatogram of Sample drug Lorlatinib.

From the specificity of the method, it was observed that there is no impedance of different substances in the Rt of the analytical peak. The theoretical plates were 25640. The tailing factor was 1.0 within the limit. The linearity study was performed for the concentration range of 50-150µgm of Lorlatinib and the correlation coefficient was found to be 0.9994. (figure no 4, Table 1 and 2).

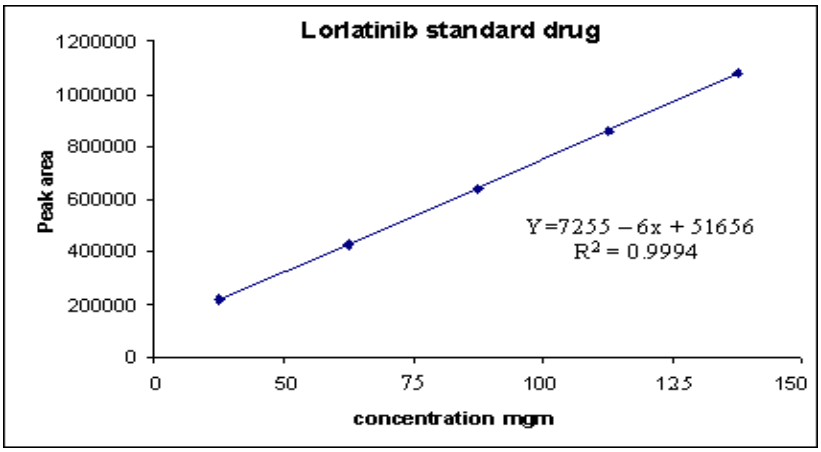

Figure. No. 4. Calibration curve of Lorlatinib.

Table. No. 1. Calibration data of Lorlatinib.

Sr No	Linearity Level	Concentration	Area
1	I	50µgm	219000
2	II	75 µgm	427000
3	III	100 µgm	640000
4	IV	125 µgm	859000
5	V	150 µgm	1080000
Correlation Coefficient			0.9994

Table No. 2. Linear regression parameters for the calibration of Lorlatinib

Parameters	Values
Accuracy	96.62%
Slope	7255
Intercept	51656
Linearity range	50-150 µgm
R	0.999
SE of intercept	7173
SD of intercept	16038
LOD	0.03
LOQ	0.05

Table no.3. Shows the accuracy study of Lorlatinib was performed for % recovery at 50%, 100% and 150%. The percentage recovery for Lorlatinib was found to be 100.5%.

Table No. 3. Results of recovery studies.

% Concentration (at specification level)	% Recovery	Mean recovery
50%	99.5%	99.8%
	98.7%	
	101.2%	
100%	101.5%	100.5%
	98.6%	
	101.4%	
150%	100.6%	100.3%
	99.1%	
	100.3%	

The precision study was performed for Lorlatinib by the present method and %RSD was calculated. The system precision was found to be 0.1%, 0.3% for intermediate system precision, and method precision was found to be 0.5%.RSD, which indicates that the system has good reproducibility.

Table No. 4. Precision data of the method.

Injection	System Precision	Intermediate precision	Method Precision
	Area	Area	Area
1	2173242	2174240	2172246
2	2172924	2171925	2170926
3	2166232	2165234	2163233
4	2172734	2176736	2172734
5	2170128	2174122	2174122
Average	2171052	2172451	2170652
SD	0.663	0.724	0.546
% RSD	0.1%	0.5%	0.3%

Robustness of the sample was prepared and run by changing the variations in mixture of mobile phase and changing in the pH concentration at ambient temperature for Lorlatinib. The validated method was robust in low concentration of mobile phase composition and pH concentration.

Table No. 5. Robustness of the method

Sr.No	Change in composition of mobile phase			Change in pH Concentration		
	Conc.	**Plate count**	**Tailing factor**	**Conc.**	**Plate count**	**Tailing factor**
1	10% less	20417	1.0	10% less	20408	1.0
2	Actual	21409	1.0	Actual	21410	1.0
3	10% more	22428	1.0	10% more	22310	1.0

The system suitability parameter like theoretical plates, Tailing factor (T) were calculated and was observed to be more than 20000. And the proposed RP-HPLC technique was accurate and precise as presented in the Table. No, 6.

Table No. 6: Specificity data of the method.

Sr. No	Parameters	Values
1	Retention time	7.871
2	Theoretical plates	21409
3	Tailing factor	1.0

SUMMARY AND CONCLUSION

The proposed technique was found to be specific, Precise, Accurate, rapid and economical for estimation of Lorlatinib in pure drug substance. This method was validated as per ICH guidelines. The sample recoveries in dosage forms were in good agreement with their respective label claims.

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