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Development and Validation of Novel HPLC Method for Analytical Evaluation of Ribociclib

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ABSTRACT

A simple and new isocratic high-performance liquid chromatography (HPLC) method was developed for the quantitative determination of ribociclib in its tablet dosage form. The chromatographic separation was achieved on HemochromIntsil (C18, 25cm × 4.6mm id, 5µ) column. The mobile phase selected was buffer (potassium dihydrogen phosphate and triethylamine (0.1% v/v) adjusted to pH 3.0 with orthophosphoric acid) and acetonitrile in the ratio of (60:40) v/v at a flow rate of 0.8ml/min with column temperature maintained at 40°C and 20µl injection volume. The detection was carried out at 276 nm. The retention time of Ribociclib was found to be 3.02 minutes. The developed HPLC method was validated as per ICH (Q2R1) guideline. The HPLC method was linear over the range of 2.5-15µg/ml with a regression coefficient of 0.9999. The results of validation parameters indicate that the developed HPLC method was specific, accurate, precise, rapid, reliable, and reproducible, therefore, it can be applied for routine quality control analysis of Ribociclib in its tablet dosage form.

INTRODUCTION

Ribociclib is an anticancer drug used in the treatment of metastatic breast cancer in association with hormonal therapy for the management of patients. Ribociclib is an orally administered highly selective Cyclin-Dependent Kinases CDK4 and CDK6 inhibitor, it reversibly combines with cyclin D, and the bipartite complex of these elements phosphorylates pivotal tumor suppressors and transcription factors, thus contributing to cell cycle progression. Ribociclib in combination with letrozole prolongs progression-free survival (PFS) for patients with ER-positive/HER2-negative advanced breast cancer[1-2]. Ribociclib was approved by the U.S. Food and Drug Administration (FDA) in 2017[3,4]. The chemical name of ribociclib is 7-cyclopentyl-*N*,*N*-dimethyl-2-[(5-piperazin-1-ylpyridin-2-yl)amino]pyrrolo[2,3-d]pyrimidine-6-carboxamide (Fig 1). The molecular formula is $C_{23}H_{30}N_8O$. The molecular weight is 434.5 g/mol.[5]

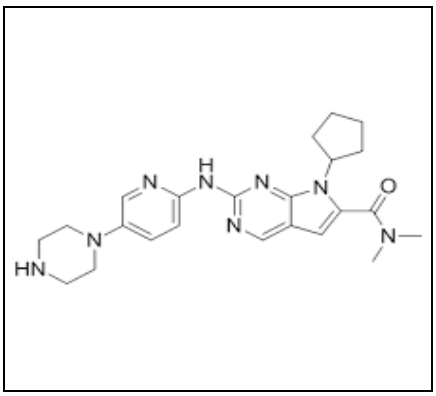


Figure No. 1: Structure of Ribociclib

Literature survey revealed LC-MS/MS and LC-ESI-MS/MS method to quantify ribociclib, only one RP-HPLC method was reported for simultaneous estimation of Ribociclib and Palbociclib in Bulk samples[6,7]. Hence attempts were made to develop a simple, rapid, precise, and accurate reverse phase chromatographic method to estimate ribociclib in the tablet dosage form. The proposed method was optimized and validated as per ICH guidelines [8]. The main objective is to give an overview of the mechanism of Reversed-Phase High-Performance Liquid Chromatography and to explain the basis of the retention mechanism and achieve high-speed separation without loss of reproducibility.

MATERIALS AND METHODS

Chemicals and Reagents

Ribociclib with defined potency was procured from Central Drugs Testing Laboratory, Mumbai. KRYXANA™ (200mg) tablets were also procured from Central Drugs Testing Laboratory, Mumbai. Acetonitrile (HPLC grade) from Merck Life Science, methanol (HPLC grade) from Molychem, triethylamine from Rankem, orthophosphoric acid was from Avra. Ultra purified HPLC grade water was obtained from the Milli - Q® system (Millipore, Milford, MA, USA) water purification unit. The mobile phase was filtered using 0.45µ nylon filters by Millipore (USA) and was sonicated and degassed using a sonicator.

Instrumentation

Perkin Elmer UV/VIS Spectrometer Lambda 25 connected to a computer loaded with software Perkin Elmer UV Win Lab was used for all the spectrophotometric measurements. The chromatography was performed on Thermo Scientific Ultimate 3000 system using chameleon 7.4.2 software with LC instrument control. Precision parameter to be performed on the different instruments was done on Perkin Elmer Flexar HPLC using software Tc Nav/Ver 6.3.2. HemochromIntsil (C18, 25cm × 4.6mm id, 5µ) column was used as a stationary phase. LAQUA, Horiba scientific pH meter was used to maintain the ionic concentration.

Determination of wavelength

The standard solution 10 µg/ml of Ribociclib was scanned in the range of 400-200 nm against diluent as a blank. Ribociclib showed maximum absorbance at 276 nm and 342 nm as shown in **Figure 2.** But the greater intensity was observed at 276 nm. So, the suitable wavelength selected for the HPLC analysis of ribociclib was 276nm.

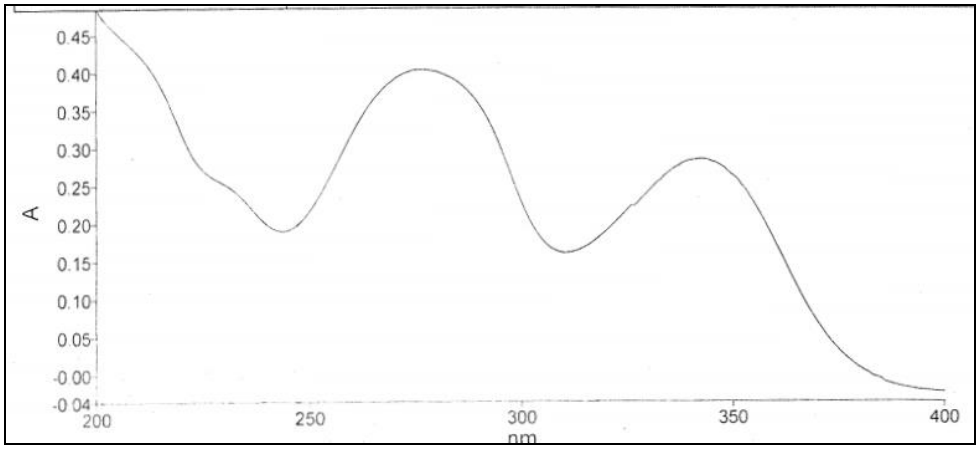


Figure No. 2: UV Spectra of Ribociclib.

Mobile Phase preparation

Phosphate buffer (25 mM) and acetonitrile in a ratio of 60:40 v/v were used as a mobile phase for the present study. Two different ports were used for running of mobile phase in isocratic form. Phosphate buffer was prepared by dissolving 3.40 gm of KH_2PO_4 and 1 ml of triethylamine into 1000 ml HPLC grade water followed by adjustment of PH to 3 with orthophosphoric acid. The mobile phase was vacuum filtered through 0.45µm high flow nylon membrane filters and was sonicated and degassed using an ultra sonicator.

Diluent Preparation

A mixture of acetonitrile and distilled water in the ratio of 1:1 v/v was used for the preparation of standard and sample solutions.

Preparation of Standard Solution

A standard stock solution of 100 µg/ml was prepared by accurately weighing 10.0 mg of ribociclib and transferring it into a 100.0 ml volumetric flask. To this add 50.0 ml of diluent and dissolve the drug properly by sonicating for about 5 min, make up the solution to 100 ml with the diluent. Working standard solution of concentration 10 µg/ml of ribociclib was obtained by pipetting out 1.0 ml of standard stock solution and transferring it into 10.0 ml volumetric flask and making up to the mark with the diluent.

Preparation of Sample Solution

An amount of sample equivalent to 200 mg of Ribociclib in the tablet formulation was accurately weighed and transferred in 200.0 ml volumetric flasks followed by the addition of

100 ml of diluent. The mixture was then subjected to sonication for 15 min, frequently subjected to vortex for the complete dissolution of the drug. The solution was cooled to room temperature and further volume was made up to the mark with diluent. Sample solution of concentration 10 µg/ml of ribociclib was obtained by pipetting out 1.0 ml of the above solution and transferring it into 100.0 ml volumetric flask and making up to the mark with the diluent.

Chromatographic conditions

HemochromIntsil (C18, 25cm × 4.6mm id, 5µ) column was used for analysis at 40°C column temperature. The mobile phase consisted of pH 3 buffer and acetonitrile in the ratio of 60:40 v/v. The mobile phase was pumped through the column at a flow rate of 0.8 ml/min. The sample injection volume was 20 µl. The UV detector was set to 276 nm for detection and the chromatographic runtime was 10 minutes.

Method optimization

Molecular structure and solubility data show that ribociclib is a highly basic non-polar compound. HemochromIntsil column was selected for the better retention of ribociclib. Here different compositions of mobile phase solvents are used in the trial and error method. Initially, the trials were made with a mobile phase consisting of water and acetonitrile in the ratio of 50:50 v/v % with a flow rate of 0.8 ml/min. But low retention time was observed. Further changes were made in the mobile phase consisting of buffer triethylamine (0.1% v/v) adjusted to pH 3.0 with orthophosphoric acid) and acetonitrile in the ratio of (55:45). The results show a slight increase in the retention time. Later buffer consisting of potassium dihydrogen phosphate and triethylamine (0.1% v/v) adjusted to pH 3.0 with orthophosphoric acid) and acetonitrile in the ratio of mobile phase to (60:40) was introduced. Better symmetrical peak shape with acceptable system suitability testing parameters was found. The selected mobile phase and column were then preceded to method validation as per ICH guidelines.

Method Validation Studies

The method was validated for system suitability, specificity, linearity, precision, accuracy and recovery, LOD, LOQ, and robustness parameters were estimated as per ICH guidelines

System Suitability Testing

System suitability parameters were evaluated and analyzed to check the system performance by injecting a working standard solution (six replicate) of concentration of 10 µg/ml and blank preparation (single injection) into the HPLC. The chromatograms were recorded to evaluate SST parameters like %RSD of Retention time, Tailing factor, Theoretical plates. Data of system suitability studies are summarized in **Table 1**.

Table No.1: System suitability and system precision study of Ribociclib.

Sr No	Peak Area	Retention Time	Theoretical plate	Tailing Factor
1	1142429	3.02	13603	0.830
2	1146067	3.02	13479	0.830
3	1148939	3.02	13473	0.830
4	1148115	3.03	13554	0.830
5	1148089	3.03	13580	0.830
Average	1146727.8	3.0238	13537.8	0.830
Std deviation	2625.9728	0.0010954	59.06	0.00
% RSD	0.23	0.04	0.44	0.00
Limit	NMT 2.0%	NMT 1.0%	NMT 2.0%	NMT 2.0%

Specificity

For specificity solutions of blank, a standard of 10 µg/ml and a sample of 10 µg/ml were injected and their chromatograms were recorded as represented in **Figure 3,4,5** respectively.

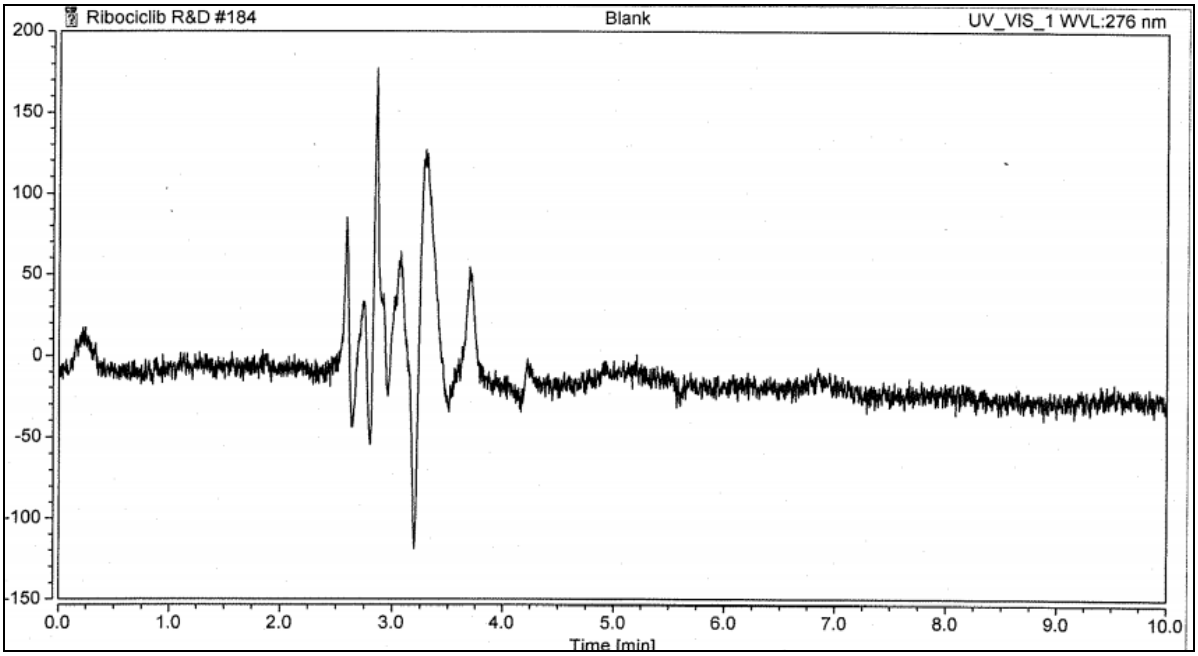


Figure No. 3: Chromatogram of Blank solution

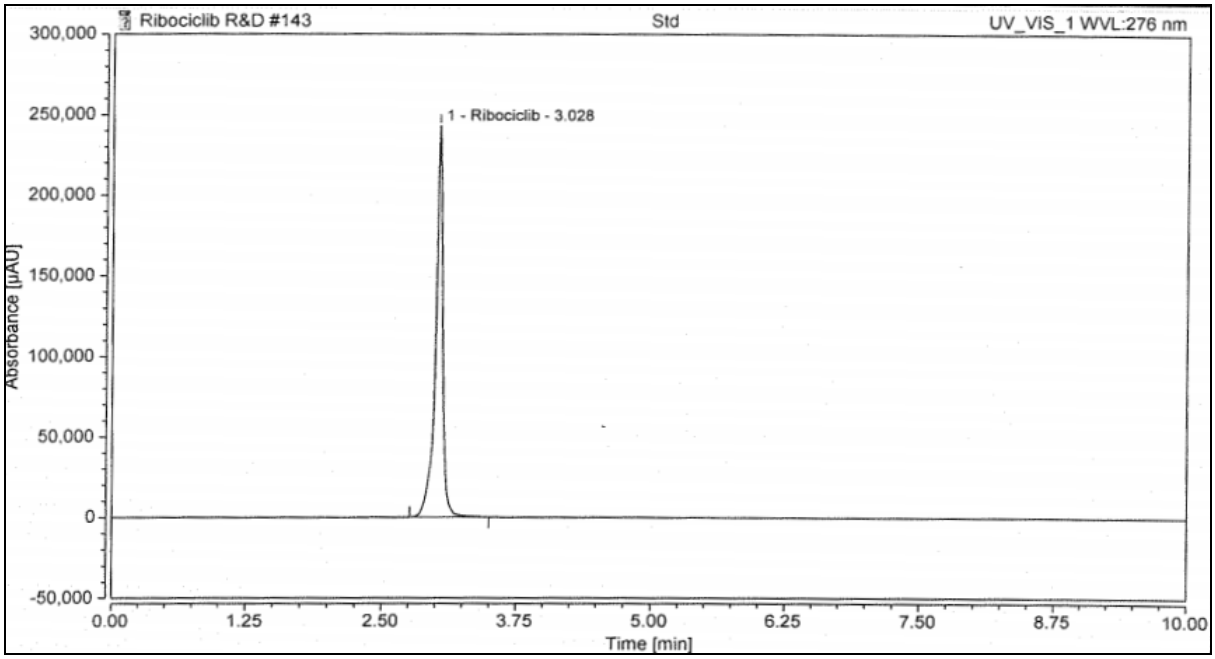


Figure No. 4: Chromatogram of Standard solution

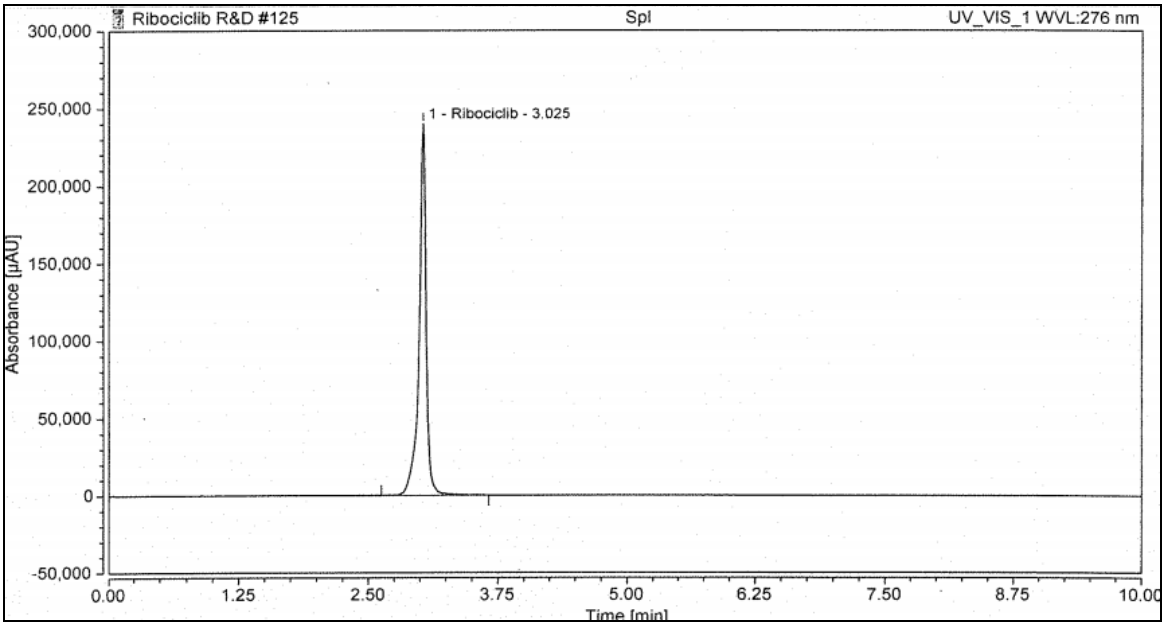


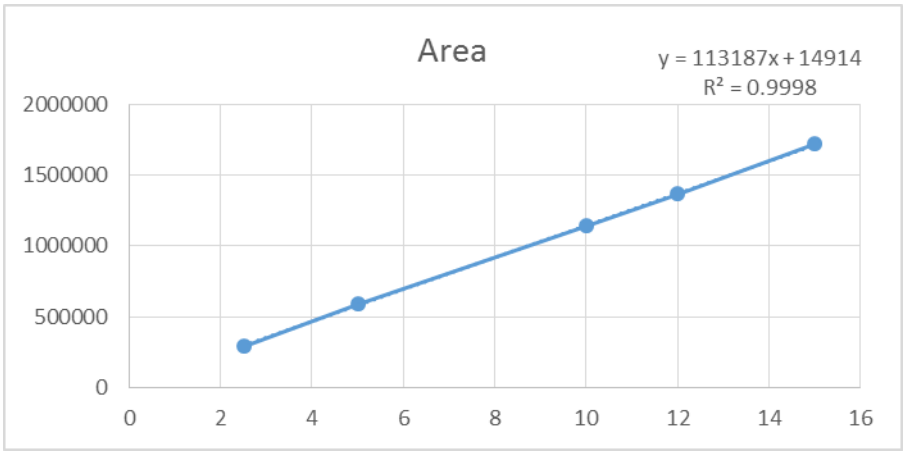
Figure No. 5: Chromatogram of Sample solution.

Linearity

The linearity of the method was obtained within the concentration range of 2.5-15 µg/ml for ribociclib. The linearity graph was plotted by taking the concentration of the drug on the X-axis and the corresponding peak area on the Y-axis as shown in **Figure 6**. The linearity data is summarized in **Table 2**.

Table No. 2: Linearity data of Ribociclib.

Concentration	Area
2.5	294036
5	590431
10	1140882
12	1367063
15	1718968



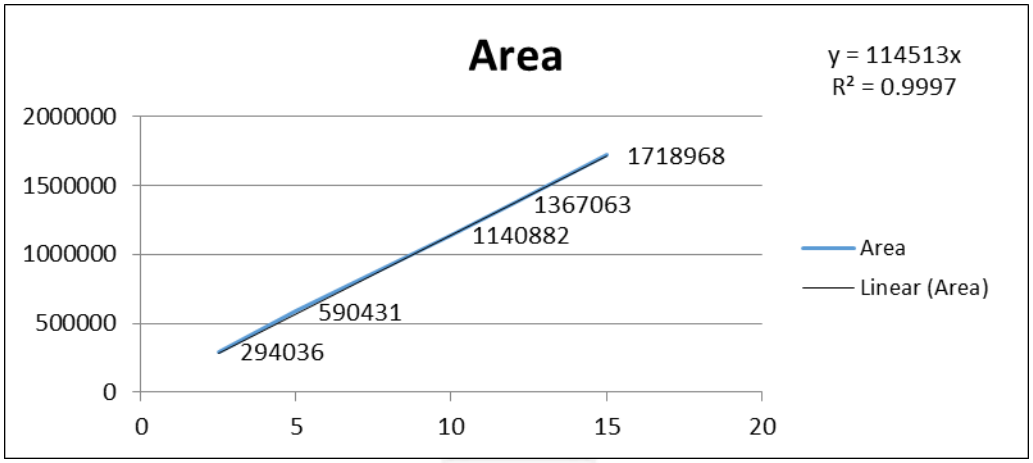


Figure No. 6: Linearity graph of Ribociclib

Precision

"The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample".

System Precision: This was performed by injecting five replicate injections of a standard solution (10 µg/ml). The average, SD, %RSD of an area in six replicate injections was calculated and reported. The results are summarized in **Table 1**.

Method Precision (Assay Repeatability): This was performed by injecting five replicate injections of standard solution (10 µg/ml) and six sample preparation of ribociclib (10 µg/ml) in triplicates into the HPLC system. Its % Assay, average, SD, %RSD were calculated and reported. The mean assay percentage results are summarized in **Table 3.**

Intermediate Precision: This was performed on two different days and different HPLC instruments. Five replicates of standard solution (10 µg/ml) and three sample preparation (10

µg/ml) in triplicates were injected into the HPLC system. Its % Assay, average, SD, %RSD were calculated and reported. Results are summarized in **Table 4.**

Table No. 3: Method Precision (Assay Repeatability) data ofRibociclib.	
Sample No.	% Assay
1	99.94
2	100.82
3	100.58
4	99.75
5	99.94
6	100.34
AVERAGE	100.21
SD	0.383
%RSD	0.38
Limit	NMT 2%

Table No. 4: Intermediate precision data of Ribociclib.

Sample No.	% Assay Day-1	% Assay Day-2	%Assay HPLC-1	% Assay HPLC-2
1	99.87	99.38	99.87	101.46
2	100.76	99.94	100.76	101.16
2	100.51	100.86	100.51	101.77
AVERAGE	100.38	100.06	100.38	101.45
SD	0.373	0.608	0.373	0.30
%RSD	0.37	0.61	0.37	0.299
LIMIT	NMT 2%	NMT 2%	NMT 2%	NMT 2%

Accuracy and Recovery:

Accuracy is a measure of how close is the experimental value to the true value. Accuracy was determined by the method of standard addition method, by calculating of % mean recovery of the sample at Four different levels 100, 110, 120, 130%. At each level, three determinations

were performed, the amount recovered, % recovery, and % RSD were taken into consideration. Accuracy results at various levels of concentration are summarized in **Table 5.**

LOD and LOQ:

Limit of detection (LOD) and limit of quantification (LOQ) of ribociclib were determined from the calibration curve method using the following formulas:

$$LOD = 3.3 \times \propto/s, LOQ = 10 \times \propto/s$$

Where $\propto$ is the standard deviation of the response of the regression line and s is the slope obtained from the calibration curve After calculating, solutions of desired concentration for LOD and LOQ were prepared and injected. The chromatograms obtained were recorded as represented in **Figures 7, 8.**

Table No. 5: Accuracy data of Ribociclib.

Level	Found mg	Found %	Recovery	Average	Std dev	% RSD	Mean Reco (%)
100%	199.85	99.9	99.9	99.2	0.369	0.372	99.70
	198.46	99.2	99.2				
	198.73	99.4	99.4				
110%	219.55	109.8	99.8	100.1	0.269	0.269	
	220.18	110.1	100.1				
	218.99	109.5	99.5				
120%	239.31	119.7	99.7	99.7	0.044	0.044	
	239.20	119.6	99.7				
	239.41	119.7	99.8				
130%	259.15	129.6	99.7	99.9	0.104	0.104	
	259.63	129.8	99.9				
	259.61	129.8	99.8				

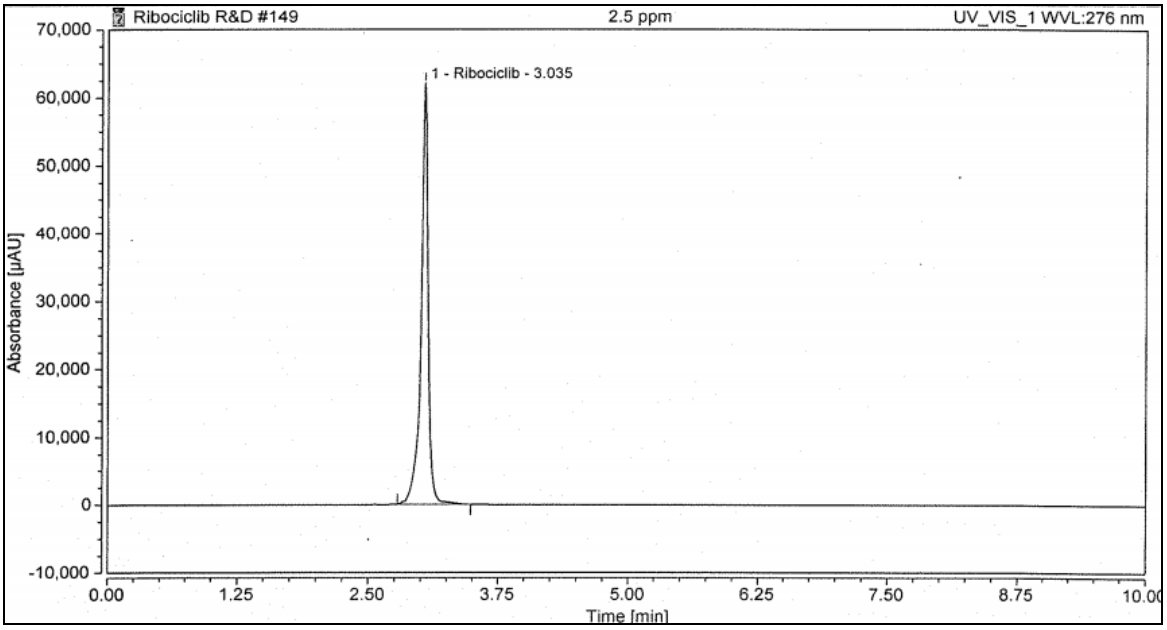


Figure No. 7: Chromatogram of LODof ribociclib

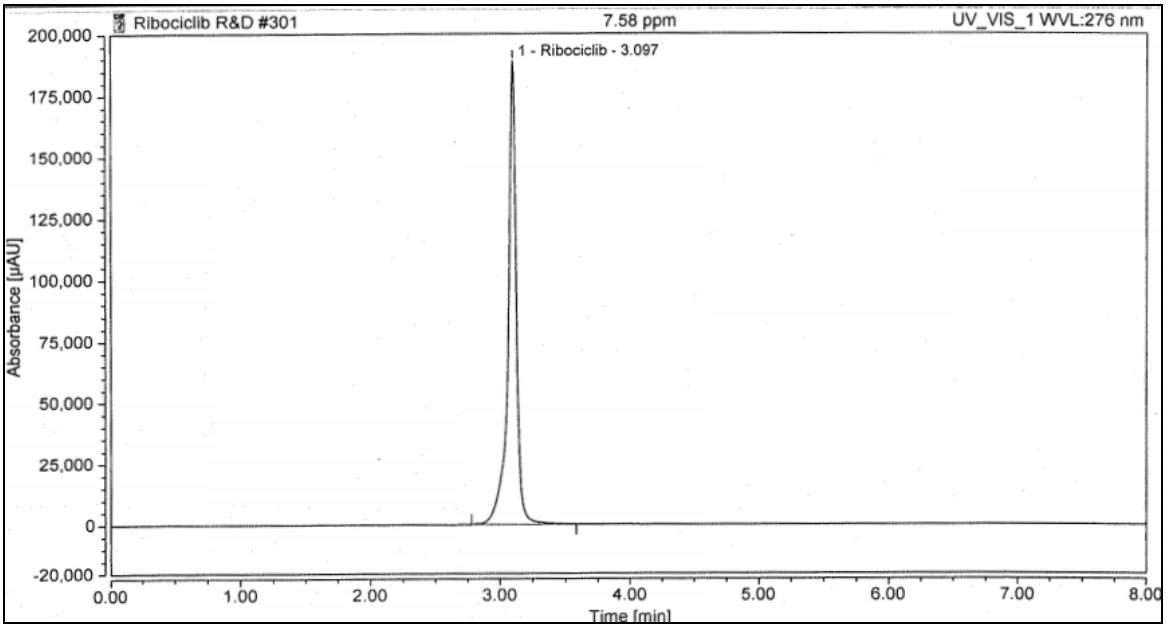


Figure No. 8: Chromatogram of LOQ of ribociclib

Robustness:

It is defined as a small or deliberate change in the parameter that should not affect any method. This was performed by a change in flow rate (± 0.2 ml/min), change in column temperature (±3 °C), change in wavelength (±2 nm), Change in Mobile phase (± 2). Ones

ample of 10 µg/ml was prepared and injected in triplicate along with five replicate injections of a standard solution of 10 µg/ml under different chromatographic conditions. Its % assay, average, SD, %RSD were calculated and reported, results are summarized in **Table 6**.

Table No. 6: Robustness data of ribociclib.

Parameter	Change in parameter (±)	% Assay Estimation	AVERAGE	SD	% RSD	LIMIT
Flow rate (±0.2 ml/min)	0.6	99.93	99.81	0.15132746	0.15161553	NMT 2%
	0.8	99.86				
	1	99.64				
Column temperature (±3°C)	37	100.23	100.4366667	0.4291076	0.42724198	
	40	100.93				
	43	100.15				
Wavelength (±2 nm)	274	99.02	99.11333333	0.08144528	0.08217389	
	276	99.17				
	278	99.15				
Mobile Phase (±2)	58:42:00	99.7	99.46333333	0.47184037	0.47438624	
	60:40:00	99.77				
	62:38:00	98.92				

Assay:

The optimized method was applied on tablets having a label claim of ribociclib 200 mg. The assay was performed on the above solution wherein five replicate injections of standard preparation 10 µg/ml and six sample preparation 10 µg/ml in triplicate were injected into the HPLC system. Its % assay, average, SD, %RSD were calculated and reported, results are summarized in **Table 7.**

Table No. 7: Assay results of ribociclib.

Sample No.	Weight of standard (mg)	Sample weight (equivalent to 200 mg ofRibociclib)	Mean Area of the standard at 276 nm	Area of a sample at 276 nm	% Assay
1	10.08	466.6	1142333.6	1141791.3	99.94
2		463		1143011.7	100.82
3		464		1142688	100.58
4		468		1143118.7	99.75
5		467.4		1143785.7	99.94
6		465		1142475	100.34
Mean					100.21
± SD					0.4645
% RSD					0.4636

RESULT AND DISCUSSION

An RP-HPLC method for quantitative estimation of ribociclib was developed and validated as per ICH guidelines. The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible. As there is no interference of blank at the retention time of ribociclib hence method is specific. Linearity is observed over a concentration range of 2.5-15 µg/ml for ribociclib. The correlation coefficient was found to be 0.99992.

The relative standard deviation for system suitability testing and system precision studies was found to be less than 2%. Theoretical plates were found to be greater than 2000, also the tailing factor was reported to be less than 2. In Method precision (assay repeatability) studies of ribociclib average assay percentage were found to be 100.21% which was within the limit i.e., between 98% to 102%. The relative standard deviation for all intermediate precision parameters was found to be within the limit.

The accuracy studies were shown as % recovery at 100% to 130% level for ribociclib. The mean percent recovery was found to be 99.70 % which was within the limit. Hence the method was found to be accurate. The present method can detect and quantify the analyte at a lower concentration. Limit of detection and limit of quantification values were estimated as

following LOD = 2.5 µg/ml, LOQ = 7.58 µg/ml where standard deviation (∝=8517.339) and slope (s = 113186.75) values were obtained by calibration curve method. By analyzing robustness, resultant values were found to be within a limit that is less than 2%, thus the developed method was confirmed to be robust. The results obtained from the assay show that the percentage recoveries were high and SD values are very low, which confirms that the method is suitable for routine analysis of ribociclib in its pharmaceutical preparation.

CONCLUSION

The RP-HPLC method development was found to be simple, precise, rapid, accurate for the quantification of ribociclib in a tablet dosage form. The method was reliable in terms of system suitability, linearity, precision, accuracy and recovery, robustness, and assay. All the verification parameters were within the range according to ICH Q2A (R1) guidelines. Hence, we can conclude that the proposed RP-HPLC method can be used for routine analysis in the Pharmaceutical industry.

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