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Research Article

Development & Validation Of RP-HPLC Method For Analytical Evaluation Of Ribociclib In Bulk Drug And Pharmaceutical Dosage Form

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ABSTRACT

Purpose:
A new Reverse Phase high-performance liquid chromatography (RP-HPLC) method was developed for the quantitative determination of ribociclib bulk and its tablet dosage form. Method: The chromatographic separation was achieved on Symmetry ODS C18 (4.6×150mm, 5µm). The mobile phase selected was Methanol: Phosphate Buffer pH-4.2 in the ratio of 35:65% v/v at a flow rate of 1ml/min with column temperature maintained at 40°C and 10µl injection volume. The detection was carried out at 276 nm.
Results:
The retention time of Ribociclib was found to be 3.02 min. The developed HPLC method was validated as per ICH (Q2R1) guidelines. The HPLC method was linear over the range of 10-50µg/ml with a regression coefficient of 0.999.The %recovery for accuracy was found to be 100.1-100.4.The % purity for assay was found to be 99.57%.Conclusion: The developed HPLC method was a simple, sensitive, precise, accurate, robust and economical therefore, it can be applied for routine quality control analysis of Ribociclib in its tablet dosage form.

INTRODUCTION

HPLC is able to separate macromolecules and ionic species labile natural products, polymeric materials, and a wide variety of other high molecular weight poly functional group. HPLC is the fastest growing analytical technique for the analysis of the drugs. It's simplicity, high specificity, and wide range of sensitivity makes it ideal for the analysis of many drugs in both dosage forms and biological fluids. In this, the separation is about 100 times faster than the conventional liquid chromatography due to packing of particles in the range of 3- 10µm.(1-3) Ribociclib is an Antineoplastic Agent.Its IUPAC name is 7-cyclopentyl-N,N-dimethyl-2-{[5-(piperazin-1-

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yl)pyridin-2-yl]amino}-7H-pyrrolo[2,3-d]pyrimidine6-carboxamide and its structure is shown in Fig 1. Ribociclib is a unique cyclin-dependent kinase inhibitor that is used in combination with Ribociclib is in a class of medications called kinase inhibitors. Ribociclib is an orally available cyclin-dependent kinase (CDK) inhibitor targets at cyclin D1/CDK4 and cyclin D3/CDK6 cell cycle pathway, with potential antineoplastic activity. Ribociclib specifically inhibits CDK4 and 6, thereby inhibiting retinoblastoma (Rb) protein phosphorylation4 .Few HPLC5-8 methods were reported .The aim of the present work is to develop a simple, sensitive, precise,accurate,robust and economical therefore, it can be applied for routine quality control analysis of Ribociclib in its tablet dosage form and validate as per ICH guidelines9.

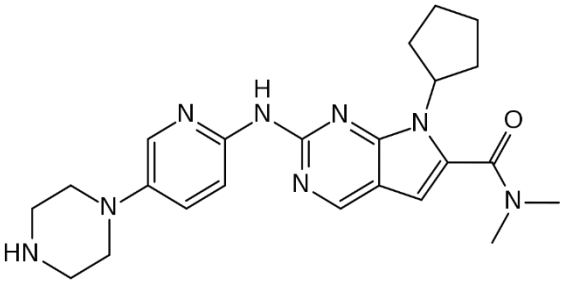

Fig. 1: Structure of Ribociclib.

METHODS AND MATERIALS

Table 1: Instruments used.

S. No.	Instruments And Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital Uultra sonicator	Lab man

Table-2: Chemicals used

S. No.	Chemical	Brand names
1	Ribociclib (Pure)	Dr. REDDY LABORATORIES LTD
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

HPLC METHOD DEVELOPMENT:

TRAILS

Preparation of standard solution: Accurately weigh and transfer 10 mg of Ribociclib working standard into a 10ml clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.3ml of the above Ribociclib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.(30□g/ml)

PREPARATION OF MOBILE PHASE:

Preparation of mobile phase:

Accurately measured 350 ml (35%) of Methanol and 650 ml of Phosphate buffer (65%) were mixed and degassed in digital ultrasonicator for 10 min and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatogram note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Acetonitrile: Water and Methanol: Phosphate Buffer pH-4.2 with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer pH 4.2 in proportion 35:65% v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, X- bridge column, Xterra, and C18 column. Symmetry C18 5µm (4.6×150mm) 5 µ was found to be ideal as it gave good peak shape and resolution at 0.8 ml/min flow.

SYSTEM SUITABILITY:

The standard solution of 30□g/mlwas injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was calculated.

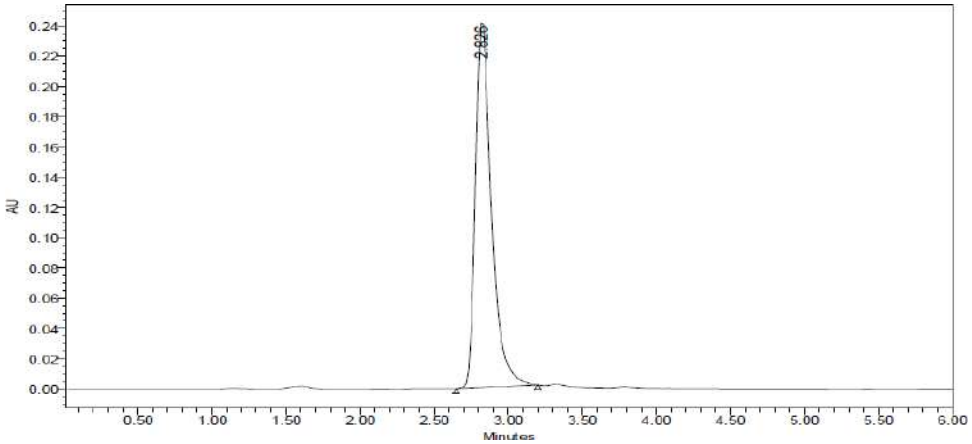

Fig. No. 2: Optimized chromatogram of ribociclib.

Specificity:

It was determined by check for interference from mobile phase(blank)

Assay:

Preparation of Sample Solution:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Ribociclib, sample into a 10mL clean dry volumetric flask add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.3ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. The sample solutions of 30 µg/ml of Ribociclib are injected for five times and the peak areas were recorded and calculate the % assay.

VALIDATION PARAMETERS:

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY:

Accurately weigh and transfer 10 mg of Ribociclib working standard into a 10 ml of clean dry volumetric flasks add about 10 ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent, (Stock solution, 1000ppm).

Preparation of Level – I (10ppm of Ribociclib):

0.1ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (20ppm of Ribociclib):

0.2ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (30ppm of Ribociclib):

0.3ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (40ppm of Ribociclib):

0.4 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (50ppm of Ribociclib):

0.5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Procedure:

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision:

REPEATABILITY:

The standard solution of 30□g/ml was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed o by different analyst maintaining same conditions.

Procedure

The standard solution of 30□g/ml was injected for five times by analyst 1 and analyst 2 and measured the area for all six injections in HPLC. The %RSD for the area of five replicate injections.

Accuracy:

Preparation of Standard stock solution: Accurately weigh and transfer 10 mg of Ribociclib working standard into a 10 ml of clean dry volumetric flasks add about 10 ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution-I 1000ppm)

For preparation of 50% solution:

Pipette 0.15 ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. The standard and sample solutions of containing concentrations were 50%.

For preparation of 100% solution:

Pipette 0.3 ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. The standard and sample solutions of containing concentrations were 100%.

For preparation of 150% solution:

Pipette 0.45 ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. The standard and sample solutions of containing concentrations were 150%.

Procedure:

Inject three replicate injections of standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Ribociclib and calculate the individual recovery and mean recovery values.

ROBUSTNESS:

The analysis was performed in different conditions to find the variability of test results. The standard solution of 100% was analyzed at 0.8 ml/min and 1.1 ml/min instead of 1.0 ml/min, remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded. The standard solution of 100% was analyzed by variation of mobile phase i.e. Methanol: Phosphate buffer pH-3.6 was taken in the ratio and 30:70, 25:75 instead 35:65, remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were record.

RESULTS AND DISCUSSION:

SYSTEM SUITABILITY:

System suitability of ribociclib was shown in Table 3

Table 3: Results of System suitability for Ribociclib

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ribociclib	2.824	1825658	132653	5426	1.6
2	Ribociclib	2.825	1836587	132658	5369	1.5
3	Ribociclib	2.827	1825654	135685	5359	1.6
4	Ribociclib	2.822	1835642	134857	5418	1.6
5	Ribociclib	2.830	1825787	136598	5356	1.5
Mean			1829866			
Std. Dev.			5714.466			
% RSD			0.312			

DISCUSSION:

%RSD of five different sample solutions should not more than 2,USP plate count and tailing factor was within limits.Hence the method was suitable.

SPECIFICITY:

It was determined by check for interference from mobile phase and shown in Fig.3.There was no other components were present at the elution time for ribociclib.

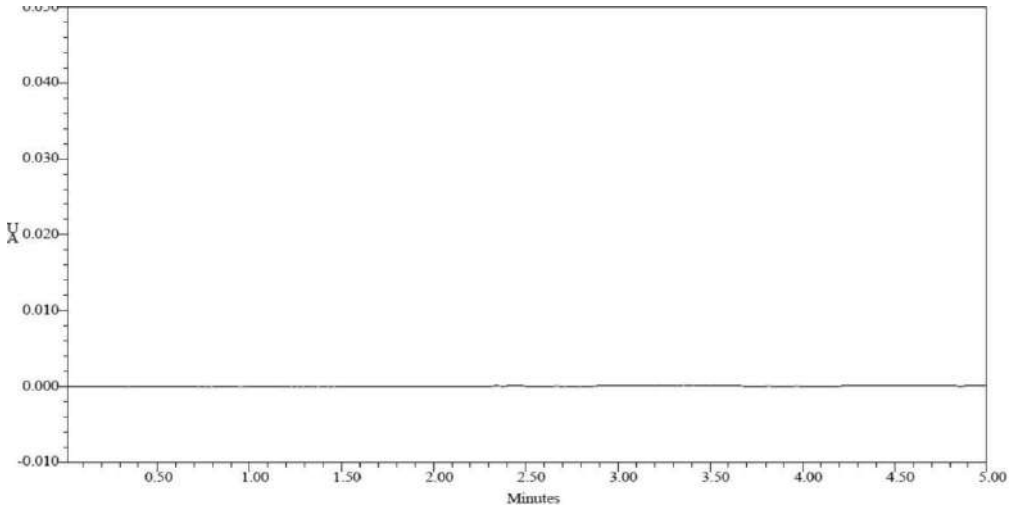

Fig. No. 3: Chromatogram showing blank (mobile phase preparation)

Assay

Table 4 Assay of Ribociclib(n=6)

Label claim	Amount found	Assay%±SD
0.5 mg	0.49mg	99.57±0.15

DISCUSSION:

Ribociclib % purity was found to be 99.57% .

LINEARITY :

Linearity data and calibration curve was shown in Table 5 and figure 4 respectively.

Table 5: Linearity data of Ribociclib

Concentration µg/ml	Average Peak Area
10	668748
20	1278875

30	1886598
40	2458644
50	3028547

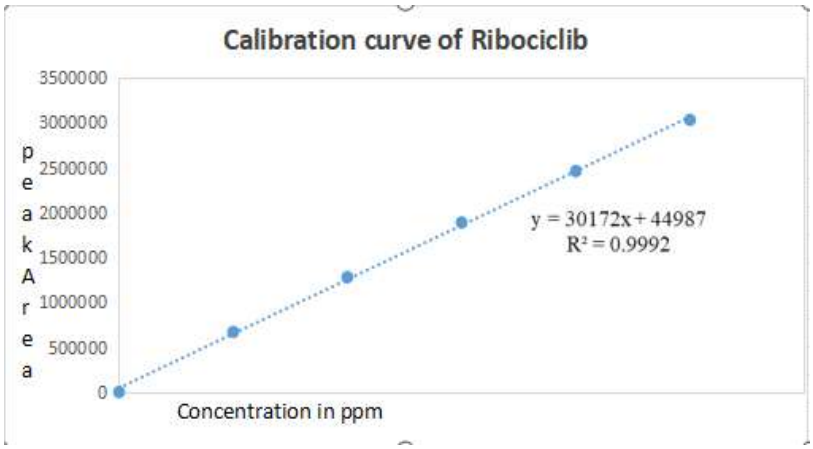

Fig No.4 : Calibration curve of ribociclib.

Precision: Repeatability and Intermediate Precision data was shown in Table 6 and Table 7 respectively.

Table 6: Results of Repeatability for Ribociclib

S. No	RT	Area (µV*sec)
1	2.824	1825463
2	2.827	1825685
3	2.833	1825426
4	2.833	1835687
5	2.836	1825642
Mean		1827581
Std. Dev.		4532.9
% RSD		0.248

Table 7: Results of Intermediate precision for Ribociclib

S. No	RT	Area (µV*sec)
1	2.823	1836524
2	2.827	1836875
3	2.828	1836958
4	2.828	1836597
5	2.825	1845689
6	2.822	1845784
Mean		1839737.8
Std. Dev.		4649.5
% RSD		0.252

DISCUSSION:

%RSD for Repeatability and Intermediate Precision was less than 2.Hence the method was precise.

ACCURACY:

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated and shown in Table 8.

Table 8 : The accuracy results for Ribocicli

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery
50%	952225.3	30	30.068	100.226%
100%	1863056	60	60.256	100.426%
150%	2764762	90	90.142	100.157%

DISCUSSION:

The %recovery for accuracy was found to be in the range of 100.1-100.4

Limit of detection and Limit of quantification (LOD & LOQ):

LOD & LOQ data for Ribociclib was found to be 2.6 µg/ml and 6.35 µg/ml and shown in Table 9.

Table 9: LOD & LOQ data for Ribociclib

Drug	LOD (µg/ml)	LOQ (µg/ml)
RIBOCICLIB	2.6	6.35

DISCUSSION:

Hence the method was sensitive

ROBUSTNESS

The robustness was performed for the flow rate variations from 0.8ml/min to 1.0ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Ribociclib by change in the Mobile phase ±5% and robustness data was shown in Table 10.

Table 10 : Results for Robustness.

Parameter	Retention Time	Tailing factor
Actual Flow rate of 1.0mL/min	2.826	1.6
Less Flow rate of 0.8mL/min	3.13	1.7
More Flow rate of 1.0mL/min	2.589	1.6
More Organic phase	2.514	1.6
Less Organic phase	3.344	1.7

DISCUSSION:

Hence the method was robust

CONCLUSION:

In the present investigation, a simple, sensitive, precise,accurate,robust and economical RP-HPLC method was developed for the quantitative estimation of Ribociclib in bulk drug and pharmaceutical dosage forms.The RP-HPLC method was more sensitive, accurate and precise compared to the Spectrophotometric methods.This method can be used for the routine determination of Ribociclib in bulk drug and in Pharmaceutical dosage forms.

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