Research Article

Development and Validation of Ribociclib by UPLC in Bulk and Tablet Dosage Form

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ABSTRACT

The objective of the present study was to develop a new stability indicating RP-UPLC method for the estimation of Ribociclib in bulk and tablet dosage form. The chromatographic separation was achieved with Phenomenex C8 (100mmx 2.1mm,1.7µm) column. The column was maintained at 300C, the mobile phase used was Sodium phosphate buffer: Acetonitrile in the ratio of (70:30 v/v). A flow rate of 1mL/min maintained with injection volume of 20µl. The λmax of the drug was found to be 284nm. The retention time was found to be 1.358min of Ribociclib. Linearity was observed in the range of 50-150 µg/mL concentration and R2 value was found to be 0.9997. The %RSD of intra-day precision and inter-day precision was found to be 0.3% and 0.1% respectively. The LOD and LOQ were found to be 1.79µg/mL and 5.43µg/mL, respectively. Stability indicating studies were carried out under various stress conditions as per ICH guidelines.

Keywords: Ribociclib, Sodium Phosphate, Acetonitrile, Linearity, UPLC.

INTRODUCTION

Ribociclib is a specific cyclin-dependent kinase inhibitor that works by inhibiting 2 proteins recognized as cyclin-dependent kinase 4 and 6 (CDK4/6) to slow down cancer growth. Cancer cells may be able to grow and divide too quickly if these proteins are overactive. The prevention of cancer cells from continuing to proliferate uncontrollably may be achieved by more precisely targeting CDK4/6.[1]

Structure:

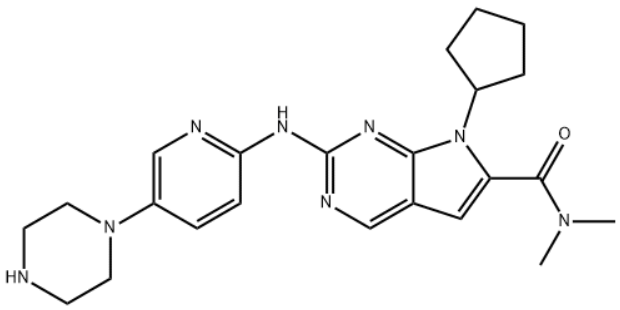

Figure 1: Ribociclib structure

Based on our literature review we observed that there is no UPLC work carried out for Ribociclib however, Sertić M, Nigović B, Crnković S, Lovrić M, Silovski T, Ekpenyong O, Bočkor L, Turković L, carried out LC-MS with other drug combination for Ribociclib.

There are many Bio analytical works that have been carried out for Ribociclib J. Li, N. Sanai, J. Wu, X. Bao being one of them.

Here we have developed a UPLC method which is Rapid, Precise, Accurate, and Linear for the estimation of Ribociclib.[2-21]

MATERIALS AND METHODS

Instrument:

The experiment was conducted using Agilent 1290 InfinityII LC system equipped with Nicolet evolution 100 UV- visible detector, binary system with auto sampler. The software used was open lab EZ chrome. The pH was measured by Thermo scientific pH meter.

Chemicals and Reagents:

Ribociclib was found as a gift sample by Fourrts India Pvt Laboratories. Sodium Phosphate and acetonitrile were arranged by CMR College of Pharmacy.

Buffer Preparation:

Weigh 11.9 grams of sodium phosphate monobasic precisely and moved into a 1000-milliliter volumetric flask. The mixture was sonicated to dissolve, and the pH was subsequently adjusted to 3.0 with "diluted orthophosphoric" acid. 0.45µm membrane filter was used for filtering.

Preparation of Mobile Phase:

Degassed by sonication after combining 700 mL of buffer and 300 mL of acetonitrile.

Standard solution Preparation

A 100 ml volumetric flask with an accurate weight and 100 mg of Ribociclib was filled with 70 ml of mobile phase. The material was then dissolved, and the stock solution was used for mixing up the rest of the volume with the mobile phase. To prepare 100 µg per ml of Ribociclib, 5 ml to 50 ml of mobile phase were diluted, accordingly.

Preparation of sample solution:

Twenty tablets containing 200 mg of Ribociclib each were weighed, placed in a mortar, ground into a fine powder,

and well combined. 500 ml of volumetric flask containing weighed crushed powder equal to 500 mg of Ribociclib; dissolve in 350 ml of mobile phase using sonication for 30 minutes; make up the volume along with mobile phase. The sample was centrifuged at 5000 rpm for 10 mins. 5mL of the sample stock solution was diluted and then mixed with 50mL of the diluted solution and a mobile phase to prepare a 100µg per mL sample solution.

Method Development:

To obtain an optimized chromatogram different, phases like Methanol, Acetonitrile, Water, Acetone were tried in different ratios with different volumes as well. The wavelength finalized was 284nm.[22]

Column: Phenomenex C8 (100×2.1mm ID) 1.7µm

Mobile Phase: Acetonitrile: Sodium Phosphate Buffer

Buffer Ratio: 70:30

Detection Wavelength: 284nm

Column Oven Temperature: 30^{0}C

Injection Volume: 20µL

Run Time: 10min

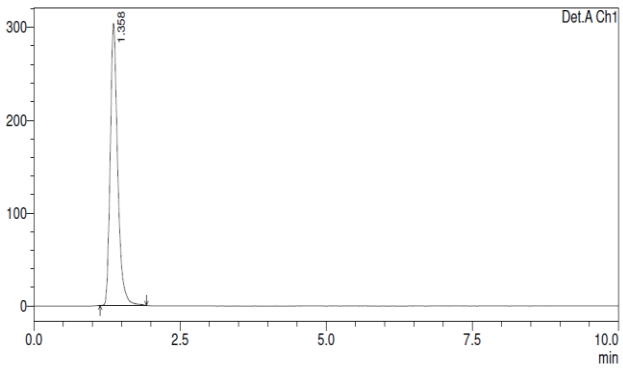

Figure 2: Chromatogram of Ribociclib

Method Validation:

System suitability, linearity, accuracy, precision, and other ICH requirements were followed in the validation of the devised method.[23]

System Suitability:

Tests for system compatibility are an essential component of liquid as well as gas chromatographic approaches. They are employed to check that the resolution and reproducibility of the chromatographic equipment are suitable for carrying out the analysis.

Specificity:

The capacity to precisely evaluate the analyte in the incidence of elements that would be predicted to be expressed in the sample matrix is known as specificity. These typically include matrix, degradants, and contaminants. Ensuring that a peak response is caused by a single component only, it measures the level of interference from other things including other active components, contaminants, excipients, as well as degradation products.

Linearity and Range:

In the current work, an acceptable linearity range for Ribociclib over the 50-150 ppm range was demonstrated. The calibration curve was planned for five different levels of drug vs corresponding peak area, regression equation was calculated (figure 2), y = 0.33117x ±661701. The calibration curve's linearity was developed by the high value of correlation coefficient, r^2: 0.9997 justifies the outstanding correlation connecting the peak area and levels of Ribociclib and is presented in Table 1.

Table 1: Linearity table for Ribociclib

S. No	Concentration	Peak Area
1	50	1009147
2	80	1953325
3	100	2667662
4	120	3312980
5	150	4306850

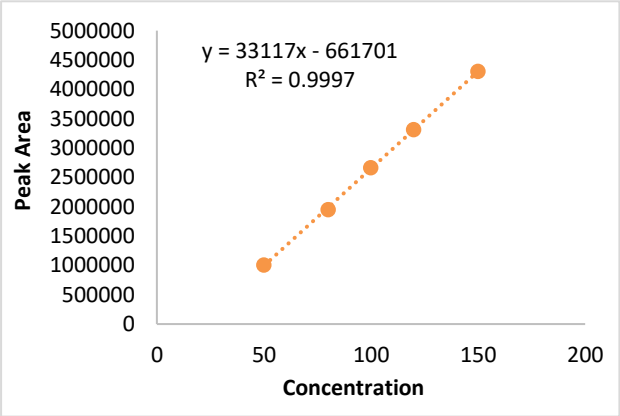

Figure 3: Linearity Graph of Ribociclib

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

The LOD obtained was 1.47µg/mL and the quantification limit were found to be 4.48µg/mL.

Accuracy:

Three levels of accuracy were achieved: 50%, 100%, and 150%.

Method precision and Intermediate precision:

Six independent determinations were made to test the method's precision. Ribociclib was determined six times, and the average assay was 99.2, with a 0.3% RSD. RSD < 2.0 denotes the accuracy of the procedure. By having independent analysts undertake chromatographic analysis of the samples on two different days, the ruggedness was confirmed.

Table 2: Accuracy result of Ribociclib

Solution Name		%Recovery
Recovery-50%	01	101.2
	02	100.3
	03	100.6
Recovery-100%	01	100.1
	02	99.9
	03	101.2
Recovery-150%	01	99.9
	02	101.8
	03	102.0
Average		101.0
Std dev		0.81
%RSD		0.8

Assay of marketed formulation:

After calculating the average weight of twenty commercial tablets, they were ground into a fine powder. Next, 10 mg of Ribociclib powder was added to a 50 mL volumetric flask and dissolved using mobile phase. Using Whatman #41 filter paper, 100 ml of volumetric flask was filled with the supernatant liquid. Following that, mobile phase was used to dilute 10 mL of the aforementioned solution to 100 mL. After injecting the sample solution, the peak area was noted. Asay results are shown in table 3.

Table 3: Precision results for Ribociclib

S. No.	Sample Number	%Assay
1	1	99.5
2	2	100.0
3	3	100.1
4	4	100.3
5	5	100.7
6	6	100.7
Average		99.2
Std Dev		0.34
%RSD		0.3

Robustness:

Small modifications to the buffer's pH, flow rate, detection at a different wavelength, and the chromatographic conditions of the exploratory environment were made to test the adaptability of the developed approach. As seen in Table 4, the approach is resilient because there were no appreciable variations to the chromatographic pattern when the alterations were applied under experimental settings.

Table 4: Robustness data for Ribociclib

Parameter Name	%RSD	Theoretical Plates	Tailing factor
Lower flow rate (0.8ml/min) (±2ml/min)	0.3	3964	1.43
Higher flow rate (1.2ml/min) (±2ml/min)	0.4	3956	1.45
Low Column Oven Temperature(25°C) (±5^{O}C)	0.9	3945	1.43
High Column Oven Temperature(35°C) (±5^{O}C)	0.7	3976	1.42

Stability Studies:

Table 5: Stability studies of Ribociclib

Control	% Degradation
Acid	99.88
Base	99.81
Peroxide	90.27
Photolytic	99.73
Thermal	99.80

Results:

The results showed that the retention time of Ribociclib was 1.358 minutes, and the concentration range of 50-150µg/mL was found to be linear. The created approach has been proven to be precise, sensitive, accurate, reliable, and efficient through validation in accordance with ICH requirements. Stress conditions such as acidity, base, lightness, oxidation, and heat were applied to Ribociclib in order to generate well-resolved breakdown products from the pure medication with varying retention times.

DISCUSSION

Ribociclib showed response between 50-150µg/mL indicating the method's ability to be linear. The co-relation coefficient was found to be 0.999 which indicates there is greater co-relation between peak area and concentration of the drug. The slope and Y intercept was found to be 33117 and 661701 respectively. Precision tells about the closeness of agreement between a series of measurements of the same sample. The %RSD values were found to be 0.3 and 0.1% respectively. The obtained values were within the limits (NMT 2%) confirming good precision of the method. The mean % recovery was found to be 101% which was within the acceptance range of 98-102%. Analyte concentrations with an estimable response (SNR 3) are referred to as LODs, while levels with a precision lower than a specified value (RSD ≥ 3.0% or SNR 10) are referred to as LOQ. The LOD and LOQ were found to be 1.79 and

5.43µg/mL respectively. Drug was made to go through various stress conditions like acid, base, light, heat, oxidation out of which oxidation was the one where the drug degraded more and the % purity was found to be 90.27%.

CONCLUSION

Based on the aforementioned experimental findings and parameters, it was determined that the recently developed method for estimating Ribociclib was straightforward, accurate, and inexpensive. Its shorter retention time also makes it more acceptable and practical, and it can be used in the near future for routine analysis in research institutions, industry quality control departments, approved testing laboratories, biopharmaceutical and bioequivalence studies, and clinical pharmacokinetic studies.

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Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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