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# DEVELOPMENT OF STABILITY INDICATING NEW ANALYTICAL RP-HPLC METHOD AND VALIDATION FOR THE DETERMINATION OF RIBOCICLIB IN BULK FORM AND MARKETED PHARMACEUTICAL DOSAGE FORM

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## ABSTRACT

**Objective:** The current investigation was pointed at developing and progressively validating novel, simple, responsive and stable RP-HPLC method for the measurement of Ribociclib in active pharmaceutical ingredient and Marketed Pharmaceutical Dosage form of Ribociclib. **Methods:** A simple, selective, validated and well-defined stability that shows isocratic RP-HPLC methodology for the quantitative determination of Ribociclib. The chromatographic strategy utilized Symmetry ODS ($C_{18}$) RP Column, 250 mm x 4.6 mm, 5µm, using isocratic elution with a mobile phase of Phosphate Buffer (0.02M) and Acetonitrile were consists of 48:52% v/v (pH-2.80). A flow rate of 1.0 ml/min and a detector wavelength of 248 nm utilizing the UV detector were given in the instrumental settings. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines. **Results:** LOD and LOQ for the two active ingredients were established with respect to test concentration. The calibration charts plotted were linear with a regression coefficient of R2>0.999, means the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness were determined as a part of method validation and the results were found to be within the acceptable range. **Conclusion:** The proposed method to be fast, simple, feasible and affordable in assay condition. During stability tests, it can be used for routine analysis of the selected drug.

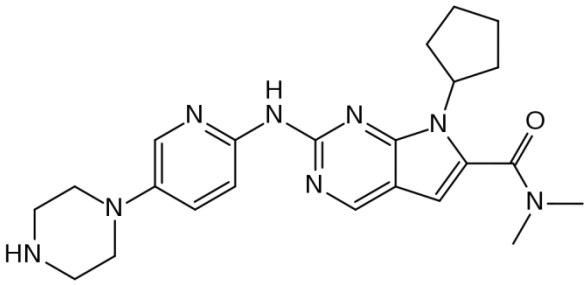
**KEYWORDS:** Ribociclib, RP-HPLC, Method Development, Validation, Accuracy, Robustness.

## INTRODUCTION

Ribociclib is a selective cyclin-dependent kinase inhibitor, a class of drugs that help slow the progression of cancer by inhibiting two proteins called cyclin-dependent kinase 4 and 6 (CDK4/6). These proteins, when over-activated, can enable cancer cells to grow and divide too quickly. Targeting CDK4/6 with enhanced precision may play a role in ensuring that cancer cells do not continue to replicate uncontrollably. Ribociclib was approved by the U.S. FDA in March, 2017 as Kisqali.

**Synonyms**: Ribociclib, 1211441-98-3, LEE011, LEE-011, Ribociclib (LEE011), LEE 011, Kisqali, LEE011A, UNII-TK8ERE8P56, TK8ERE8P56.

**Chemical Structure**




**IUPAC Name**: 7-cyclo pentyl-N, N-dimethyl-2-[(5-piperazin-1-yl pyridin-2-yl) amino] pyrrolo [2, 3-d] pyrimidine-6-carboxamide.

**Molecular Formula:** $C_{23}H_{30}N_8O$

**Medical Uses:** RIBOCICLIB (rye boe SYE klib) is a medicine that targets proteins in cancer cells and stops the cancer cells from growing. It is used to treat breast cancer. This medicine may be used for other purposes; ask your health care provider or pharmacist if you have questions.

**MARKETED FORMULATION**

| S. No. | Drug Name | Label Claim | Brand Name | Company |
|---|---|---|---|---|
| 1 | Ribociclib | 200mg | Kryxana 200mg Tablet | Novartis India Ltd |

**AIM & OBJECTIVE**

Review of literature for Ribociclib gave information regarding its physical and chemical properties, various analytical methods that were conducted alone and in combination with other drugs.

Literature survey reveals that certain chromatographic methods were reported for simultaneous estimation of Ribociclib and single method is available for such estimation by RP-HPLC.

Validation is a necessary and important step in both framing and documenting the capabilities of the developed method. The utility of the developed method to determine the content of drug in commercial formulation was also demonstrated. Validation of the method was done in accordance with USP and ICH guideline for the assay of active ingredient.

The method was validated for parameters like system suitability, linearity, precision, accuracy, specificity, ruggedness and robustness, limit of detection and limit of quantification. This method provides means to quantify the component. This proposed method was suitable for the analysis of Pharmaceutical dosage forms.

**The Primary Objective of Proposed Work is:**

To develop new simple, sensitive, accurate and economical analytical method for the estimation of Ribociclib in bulk and marketed pharmaceutical dosage form.

To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Ribociclib in bulk and marketed pharmaceutical dosage form.

**PLAN OF WORK**

- To develop a new analytical method for the estimation of Ribociclib by RP-HPLC in bulk and marketed pharmaceutical dosage form.
- The dissertation work has been carried out in the following steps.

**MATERIALS AND METHODS**

**INSTRUMENTS USED**

**Table-1: List of Instrument used.**

| S. No. | Instruments/ Equipments/Apparatus |
|---|---|
| 1. | **Waters HPLC** with Empower2 Software with Isocratic with UV-Visible Detector. |
| 2. | **ELICO SL-159** UV – Vis spectrophotometer |
| 3. | Electronic Balance **(SHIMADZU ATY224)** |
| 4. | Ultra Sonicator **(Wensar wuc-2L)** |
| 5. | Thermal Oven |
| 6. | Symmetry RP $C_{18}$, 5µm, 250mm x 4.6mm i.d. |
| 7. | $P^H$ Analyzer **(ELICO)** |
| 8. | Vacuum filtration kit **(BOROSIL)** |

**CHEMICALS / REAGENTS USED**

**Table-2: List of Chemicals used.**

| S. N. | Name | Specifications | | Manufacturer/Supplier |
|---|---|---|---|---|
| | | Purity | Grade | |
| 1. | Doubled distilled water | 99.9% | HPLC | Sd fine-Chem ltd; Mumbai |
| 2. | Methanol | 99.9% | HPLC | Loba Chem; Mumbai. |
| 3. | Dipotassium hydrogen orthophosphate | 96% | L.R. | Sd fine-Chem ltd; Mumbai |
| 4. | Acetonitrile | 99.9% | HPLC | Loba Chem; Mumbai. |
| 5. | Potassium dihydrogen orthophosphate | 99.9% | L.R. | Sd fine-Chem ltd; Mumbai |
| 6. | Sodium hydroxide | 99.9% | L.R. | Sd fine-Chem ltd; Mumbai |
| 7. | Hydrochloric acid | 96% | A.R. | Sd fine-Chem ltd; Mumbai |
| 8. | 3% Hydrogen Peroxide | 96% | A.R. | Sd fine-Chem ltd; Mumbai |

**SOLUBILITY OF RIBOCICLIB**

**Table-3: Results of Solubility.**

| SOLVENTS | SOLUBILITY |
|---|---|
| Ethanol | Soluble |
| DMSO | Soluble |
| Dimethyl Form amide | Soluble |
| Water | Highly Soluble |
| Acetonitrile | Soluble |
| Methanol | Slightly Soluble |

**METHOD DEVELOPMENT AND ITS VALIDATION FOR RIBOCICLIB BY RP-HPLC**

**Selection of Wavelength**

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Ribociclib, so that the same wave number can be utilized in HPLC UV detector for estimating the Ribociclib. While scanning the Ribociclib solution we observed the maxima at 248 nm. The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450. The scanned UV spectrum is attached in the following page,

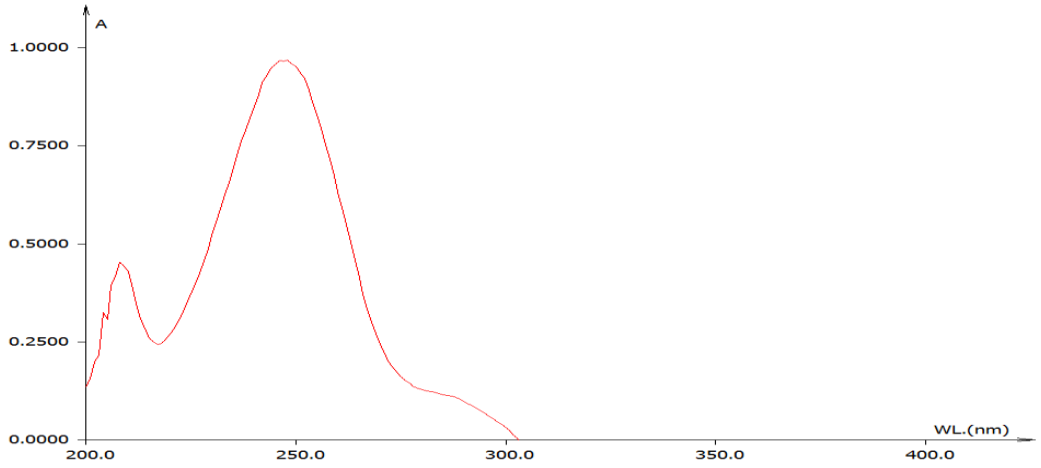



**Figure-1: UV Spectrum for Ribociclib.**

**Preparation of Standard Solution**

Accurately weigh and transfer 10 mg of Ribociclib working standard into a 10ml of 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 0.5ml of the above Ribociclib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

**Procedure**

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

**Preparation of Sample Solution**

Twenty tablets were taken and the average weight was calculated as per the method prescribed in I.P. The weighed tablets were finally powdered and triturated well. A quantity of powder of Ribociclib equivalent to 10mg were transferred to clean and dry 10 ml volumetric flask and 7 ml of HPLC grade methanol was added and the resulting solution was sonicated for 15 minutes. Make up the volume up to 10 ml with same solvent. Then 1 ml of the above solution was diluted to 10 ml with HPLC grade methanol. One ml (0.5 ml) of the prepared stock solution diluted to 10 ml and was filtered through membrane filter (0.45µm) and finally sonicated to degas.

**Optimization of Chromatographic Conditions**

The chromatographic conditions were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

**Summary of Optimized Chromatographic Conditions**

The Optimum conditions obtained from experiments can be summarized as below:

**Table-4: Summary of optimised Chromatographic conditions.**

| | |
|---|---|
| Mobile phase | Phosphate Buffer (0.02M): Acetonitrile = 48:52 (pH-2.80) |
| Column | Symmetry ODS ($C_{18}$) RP Column, 250 mm x 4.6 mm, 5µm |
| Column Temperature | Ambient |
| Detection Wavelength | 248 nm |
| Flow rate | 1.0 ml/ min. |
| Run time | 08 min. |
| Temperature of Auto sampler | Ambient |
| Diluent | Mobile Phase |
| Injection Volume | 20µl |
| Mode of Elution | Isocratic |
| Retention time | 3.649 minutes |

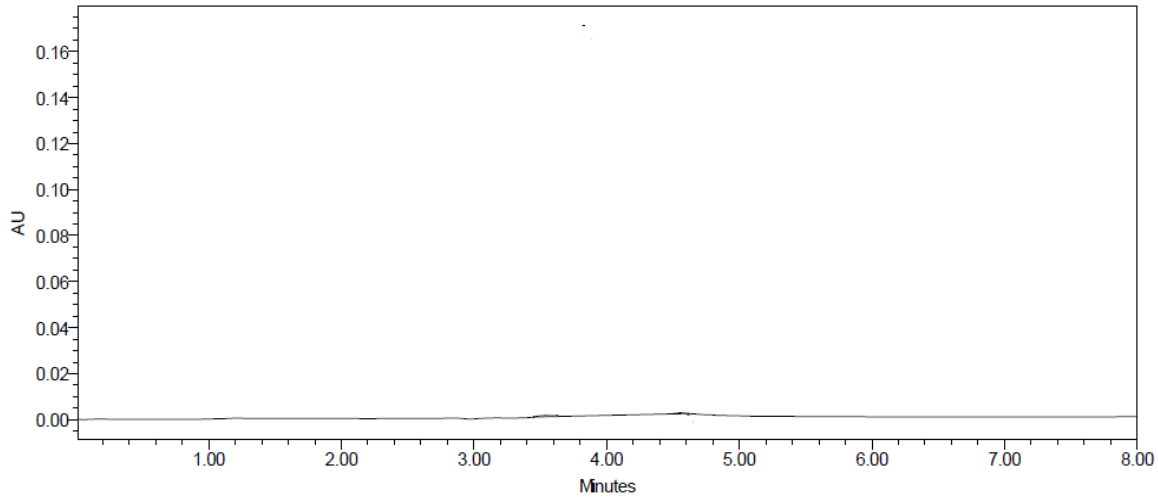



**Fig-2: HPLC Spectrum of Ribociclib (Blank Solution).**

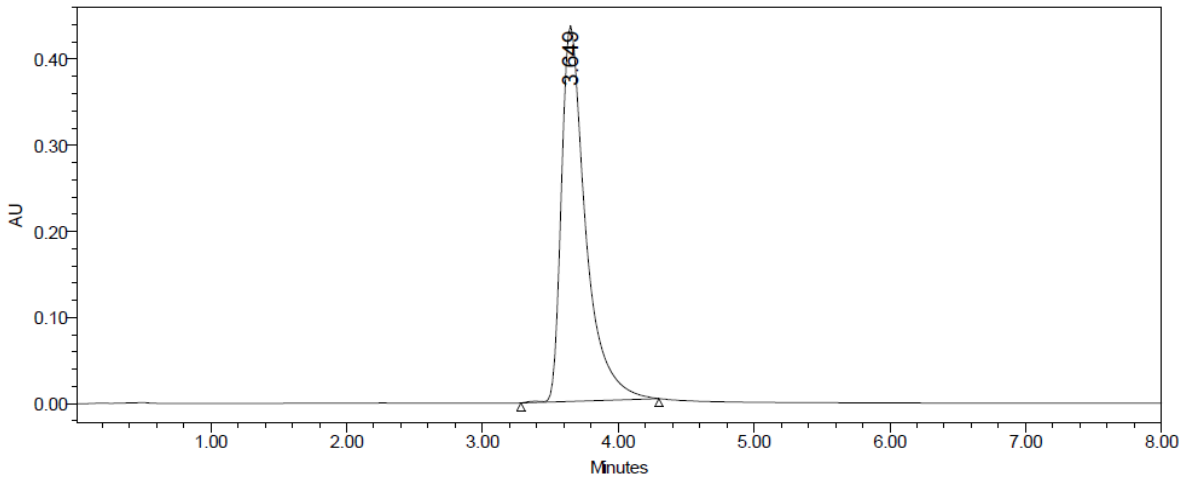



**Fig-3: Chromatogram of Ribociclib in Optimized Chromatographic Condition.**

**Table-5: Peak Results of Optimised Chromatogram.**

| S.No. | Drug Name | Rt | Peak Area | Tailing Factor | Plate Count |
|---|---|---|---|---|---|
| 1 | Ribociclib | 3.649 | 584624 | 1.42 | 4765 |

**Acceptance Criteria**

- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

**Preparation of 0.02M Potassium Dihydrogen Orthophosphate Solution**

About 2.72172grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC Grade water. The pH was adjusted to 2.80 with diluted orthophosphoric acid Solution.

**Preparation of Mobile Phase**

480mL (48%) of above Phosphate buffer solution and 520mL of HPLC Grade Acetonitrile (52%) were mixed well and degassed in ultrasonic water bath for 15 minutes. The resulted solution was filtered through 0.45 µm filter under vacuum filtration.

**METHOD VALIDATION**

**Accuracy**

**Preparation of Standard Solution**

Accurately weigh and transfer 10 mg of Ribociclib working standard into a 10ml of clean dry volumetric flasks 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 0.5ml of the above Ribociclib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

**Recovery Study**

To determine the accuracy of the planned technique, recovery studies were distributed by adds completely different amounts (80%, 100%, and 120%) of pure drug of Ribociclib were taken and extra to the pre-analyzed formulation of concentration 50µg/ml. From that proportion recovery values were calculated. The results were shown in table-15.

**Table-6: Accuracy Readings.**

| Sample ID | Concentration (µg/ml) | | Peak Area | % Recovery of Pure drug | Statistical Analysis |
|---|---|---|---|---|---|
| | Amount Added | Amount Found | | | |
| $S_1$ : 80 % | 40 | 40.141 | 502647 | 100.352 | Mean= 100.3947% S.D. = 0.071319 % R.S.D.= 0.071038 |
| $S_2$ : 80 % | 40 | 40.191 | 503214 | 100.477 | |
| $S_3$ : 80 % | 40 | 40.142 | 502656 | 100.355 | |
| $S_4$ : 100 % | 50 | 50.044 | 614215 | 100.088 | Mean= 99.98533% S.D. = 0.183045 % R.S.D.= 0.183071 |
| $S_5$ : 100 % | 50 | 49.887 | 612451 | 99.774 | |
| $S_6$ : 100 % | 50 | 50.047 | 614254 | 100.094 | |
| $S_7$ : 120 % | 60 | 60.192 | 728547 | 100.32 | Mean= 100.311% S.D. = 0.408574 % R.S.D.= 0.407308 |
| $S_8$ : 120 % | 60 | 59.939 | 725698 | 99.898 | |
| $S_9$ : 120 % | 60 | 60.429 | 731211 | 100.715 | |

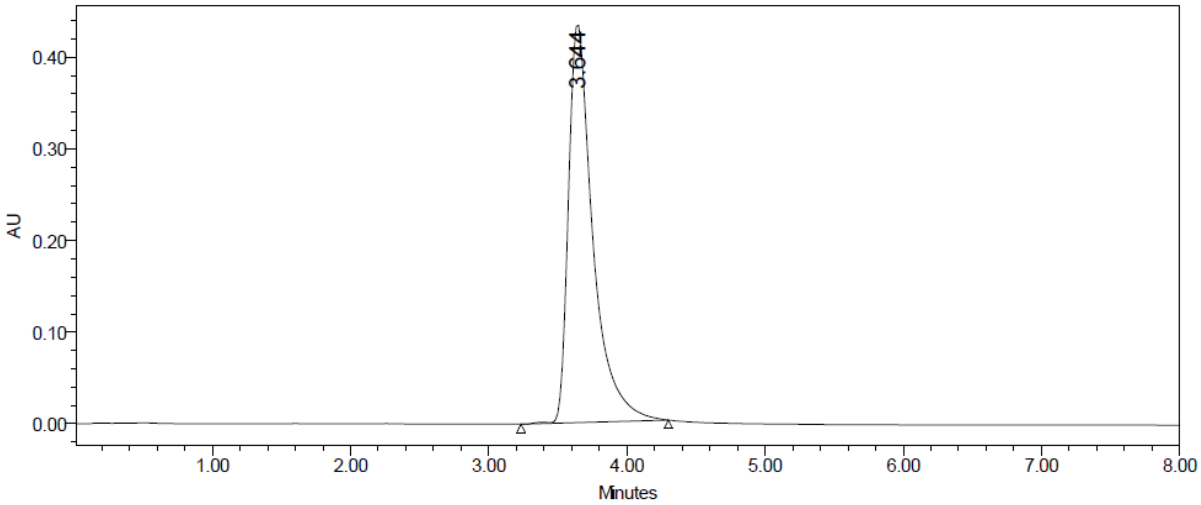



**Fig-4: Chromatogram of 80% Accuracy-1.**

**Table-6: Results of 80% Accuracy-1.**

| Drug Name | Rt | Peak Area | Tailing Factor | Plate Count |
|---|---|---|---|---|
| Ribociclib | 3.644 | 502647 | 3865 | 1.25 |

**Precision**

**Repeatability**

**Preparation of Ribociclib Product Solution for Precision**

Accurately weigh and transfer 10 mg of Ribociclib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

**Procedure**

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

The exactitude of every technique was determined one by one from the height areas & retention times obtained by actual determination of six replicates of a set quantity of drug. Ribociclib (API). The % relative variance was calculated for Ribociclib square measure bestowed within the table-25.

**Table-7: Repeatability Readings.**

| HPLC Injection Replicates of Ribociclib | Retention Time (Minutes) | Peak Area |
|---|---|---|
| Replicate – 1 | 3.649 | 5674158 |
| Replicate – 2 | 3.684 | 5654715 |
| Replicate – 3 | 3.687 | 5665841 |
| Replicate – 4 | 3.688 | 5654578 |
| Replicate – 5 | 3.688 | 5652284 |
| Replicate – 6 | 3.687 | 5641487 |
| **Average** | | **5657177** |
| **Standard Deviation** | | **11369.72** |
| **% RSD** | | **0.200979** |

**Acceptance Criteria**

- % RSD for sample should be NMT 2.
- The % RSD for the standard solution is below 2, which is within the limits hence method is precise.

**Intermediate Precision/Ruggedness**

**Intra-Day & Inter-Day**

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Ribociclib revealed that the proposed method is precise.

**Procedure**

**Analyst 1:** The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

**Analyst 2:** The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

**Table-8: Results of Ruggedness for Ribociclib Analyst 1.**

| S. No. | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Ribociclib | 3.687 | 584968 | 65982 | 4985 | 1.42 |
| 2 | Ribociclib | 3.688 | 582479 | 66354 | 4876 | 1.46 |
| 3 | Ribociclib | 3.688 | 586236 | 67425 | 4896 | 1.48 |
| 4 | Ribociclib | 3.687 | 586985 | 65982 | 4986 | 1.47 |
| 5 | Ribociclib | 3.684 | 582679 | 65932 | 5016 | 1.45 |
| 6 | Ribociclib | 3.649 | 583989 | 65874 | 4987 | 1.43 |
| **Mean** | | | **584556** | | | |
| **Std. Dev.** | | | **1846.658** | | | |
| **% RSD** | | | **0.315908** | | | |

**Acceptance Criteria**

- % RSD of Six different sample solutions should not more than 2.

**Table-9: Results of Intermediate Precision Analyst 2 for Ribociclib.**

| S. No. | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Ribociclib | 3.649 | 598698 | 66985 | 5265 | 1.49 |
| 2 | Ribociclib | 3.684 | 596847 | 67458 | 5168 | 1.47 |
| 3 | Ribociclib | 3.687 | 596354 | 66985 | 5436 | 1.46 |
| 4 | Ribociclib | 3.688 | 598676 | 67854 | 5369 | 1.45 |
| 5 | Ribociclib | 3.688 | 596874 | 68521 | 5247 | 1.48 |
| 6 | Ribociclib | 3.687 | 598989 | 67898 | 5375 | 1.42 |
| **Mean** | | | **597739.7** | | | |
| **Std. Dev.** | | | **1168.098** | | | |
| **% RSD** | | | **0.195419** | | | |

**Acceptance Criteria**

% RSD of Six different sample solutions should not more than 2.

**Linearity & Range**

**Preparation of Drug Solutions for Linearity**

- Accurately weigh and transfer 10 mg of Ribociclib working standard into a 10ml of clean dry volumetric flasks 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 0.5ml of the above Ribociclib stock solutions into a 10ml volumetric flask and dilute up to the mark with Mobile Phase.

**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.
- The calibration curve showed good linearity in the range of 0-70µg/ml, for Ribociclib (API) with correlation coefficient ($r^2$) of 0.999 (Fig-30). A typical calibration curve has the regression equation of y = 11266.x + 50416 for Ribociclib.

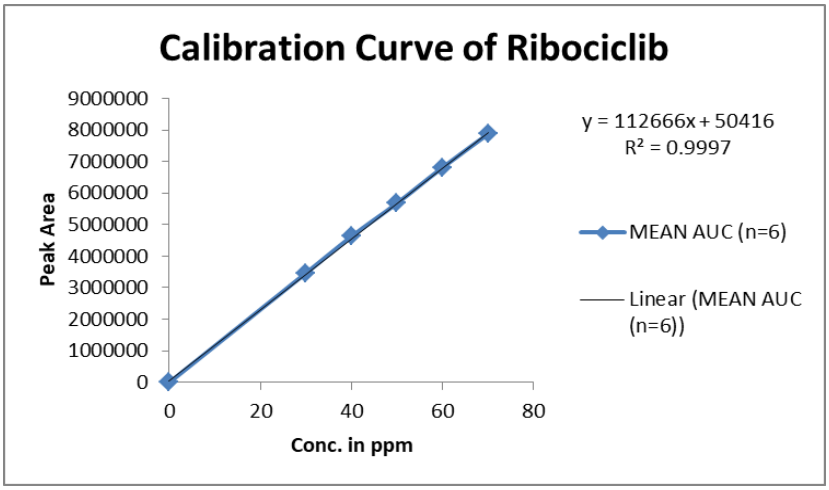



**Fig-5: Calibration Curve of Ribociclib (API).**

**Table-10: Linearity Results.**

| CONC.(µg/ml) | MEAN AUC (n=6) |
|---|---|
| 0 | 0 |
| 30 | 3465974 |
| 40 | 4626478 |
| 50 | 5682284 |
| 60 | 6815478 |
| 70 | 7878721 |

**Linearity Plot**

- The plot of Concentration (x) versus the Average Peak Area (y) data of Ribociclib is a straight line.
- Y = mx + c
- Slope (m) = 112666
- Intercept (c) = 50416
- Correlation Coefficient (r) = 0.99

**Validation Criteria:** The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

**Conclusion:** Correlation Coefficient (r) is 0.99, and the intercept is 50416. These values meet the validation criteria.

**Table-11: Results for Robustness.**

| Parameter Used for Sample Analysis | Peak Area | Retention Time | Theoretical Plates | Tailing Factor |
|---|---|---|---|---|
| Actual Flow rate of 1.0 mL/min | 584624 | 3.649 | 1.42 | 4765 |
| Less Flow rate of 0.9 mL/min | 598676 | 3.687 | 1.49 | 4856 |
| More Flow rate of 1.1 mL/min | 612543 | 3.649 | 1.46 | 4965 |
| Less organic phase | 578642 | 3.688 | 1.49 | 4758 |
| More organic phase | 569896 | 3.684 | 1.47 | 4962 |

**Acceptance Criteria**

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

**5. LOD & LOQ**

- **LOD:** The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.
- **LOD = 3.3 × σ / s**
- **LOQ:** The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.
- **LOQ = 10 × σ/S**

| | |
|---|---|
| SE of Intercept | 48846.22527 |
| SD of Intercept | 109223.4801 |
| LOD | 3.199168 |
| LOQ | 9.694449 |

**Observation**

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 3.19 & 9.69 µg/ml respectively.

**6. System Suitability Parameter**

System quality testing is associate degree integral a part of several analytical procedures. The tests square measure supported the idea that the instrumentation, physics, associate degree analytical operations and samples to be analyzed represent an integral system that may be evaluated intrinsically. Following system quality check parameters were established. The information square measured shown in Table-40.

**Preparation of Standard Solution**

- Accurately weigh and transfer 10 mg of Ribociclib working standard into a 10ml of 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 0.5ml of the above Ribociclib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

**Procedure**

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

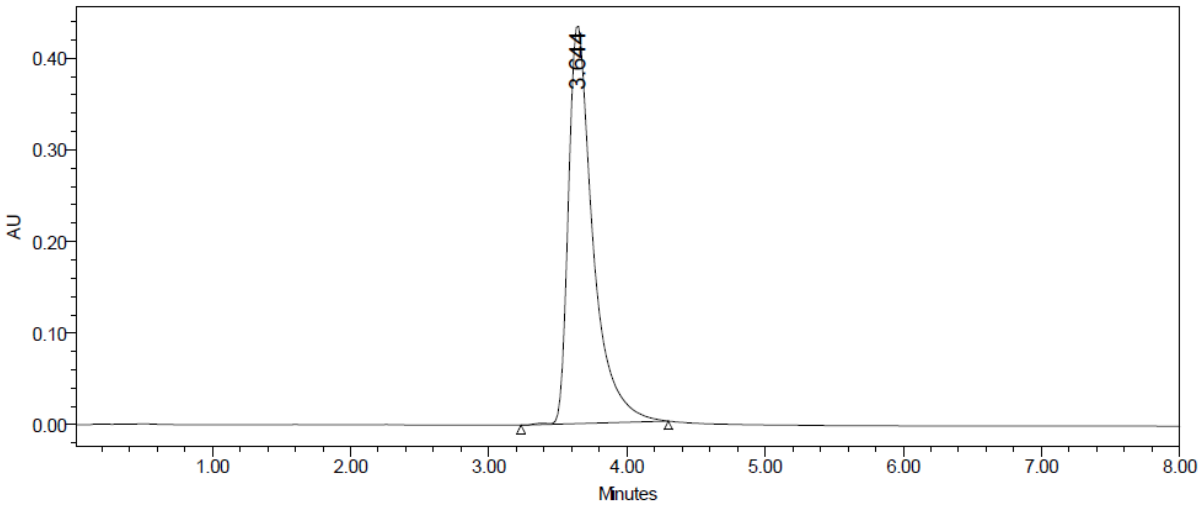



**Fig-6: Chromatogram Showing SST Injection – 1.**

**Table-12: Results of System Suitability for Ribociclib.**

| S. No. | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Ribociclib | 3.644 | 584635 | 65847 | 4857 | 1.48 |
| 2 | Ribociclib | 3.645 | 582695 | 65421 | 4955 | 1.42 |
| 3 | Ribociclib | 3.644 | 587432 | 65369 | 4875 | 1.47 |
| 4 | Ribociclib | 3.662 | 589687 | 65748 | 4796 | 1.46 |
| 5 | Ribociclib | 3.660 | 582547 | 65398 | 4952 | 1.49 |
| 6 | Ribociclib | 3.660 | 589656 | 652418 | 4896 | 1.47 |
| **Mean** | | | **586108.7** | | | |
| **Std. Dev.** | | | **3275.654** | | | |
| **% RSD** | | | **0.558882** | | | |

**Acceptance Criteria**

- % RSD of five different sample solutions should not more than 2.
- The % RSD obtained is within the limit, hence the method is suitable.

**Specificity**

Specificity can be determined by comparing the chromatograms obtained from the drugs with the chromatogram obtained from the blank solution. Blank solution was prepared by mixing the excipients in the mobile phase without drug. Drug solutions were prepared individually and the sample containing one drug was also prepared. Now these mixtures were filtered by passing through 0.45 µ membrane filter before the analysis. In this observation no excipient peaks were obtained near the drug in the study run time. This indicates that the proposed method was specific.

The chromatograms representing the peaks of blank, Ribociclib and the sample containing the one drug was shown in following figures respectively.

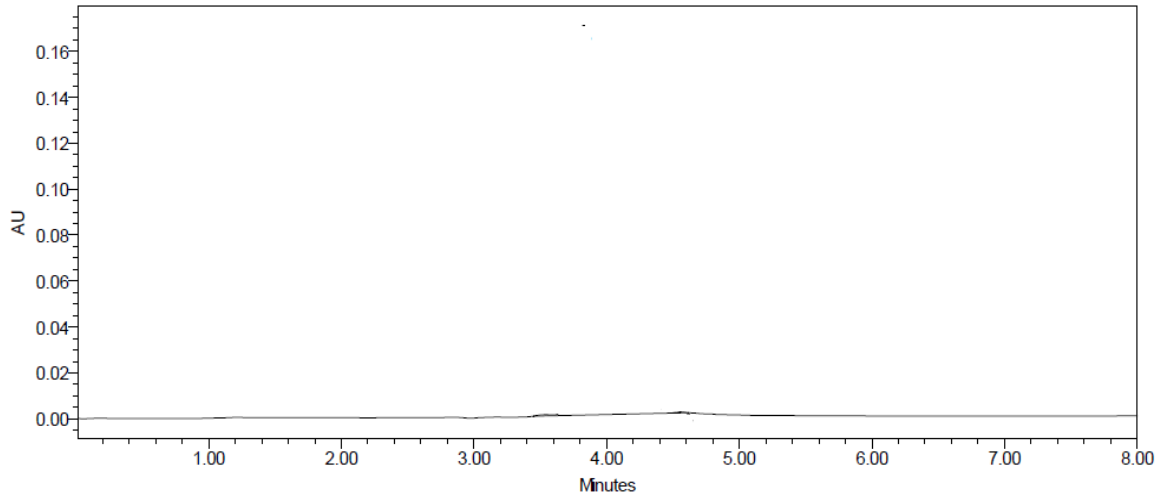
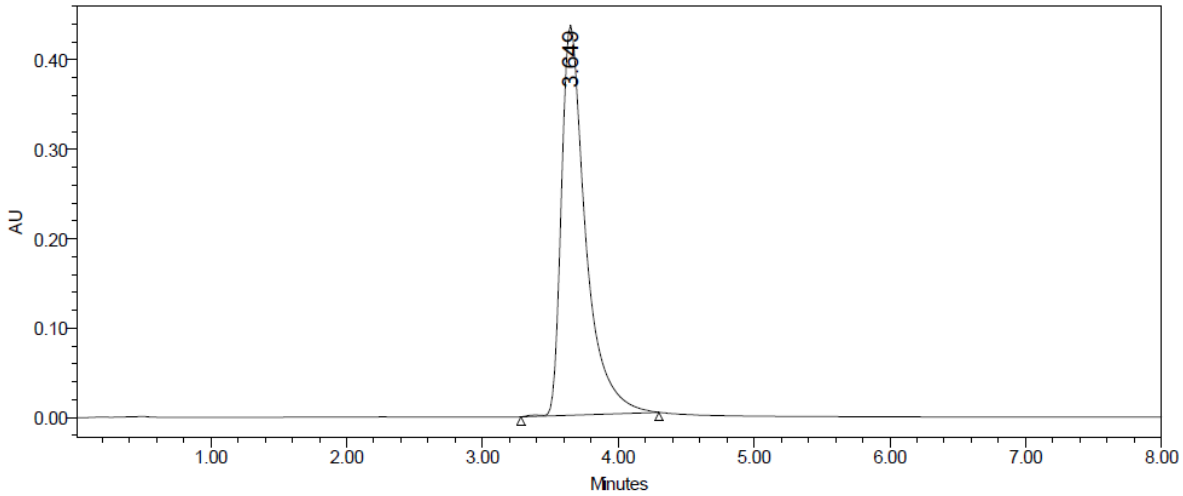
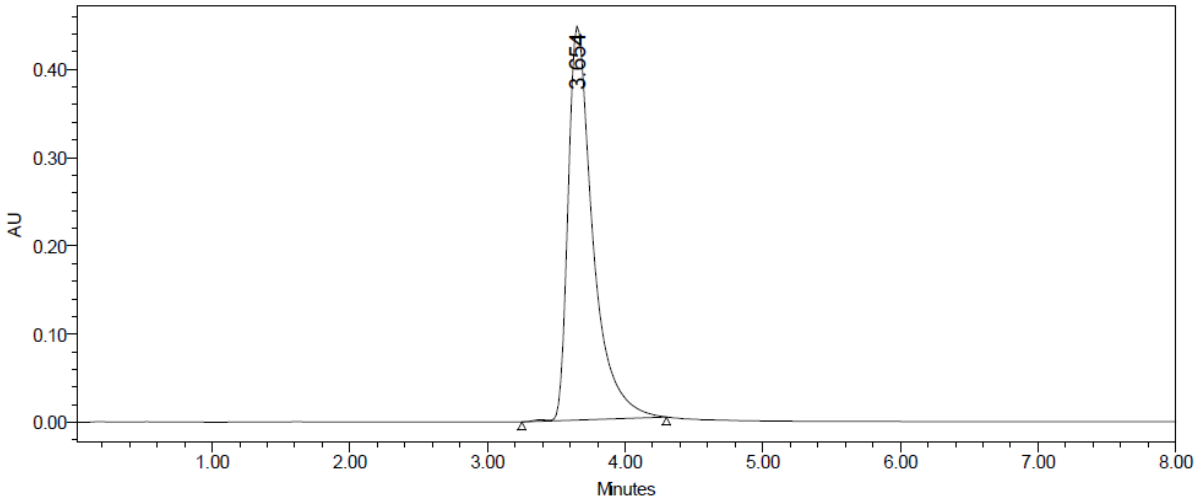



**Fig-7: Chromatogram for Blank Solution.**




**Fig–8: Chromatogram of Ribociclib Standard Solution.**




**Fig–9: Chromatogram of Ribociclib Sample Solution.**

**Observation:** In this test method blank, standard solutions were analyzed individually to examine the interference. The above chromatograms show that the active ingredient was well separated from blank and their excipients and there was no interference of blank with the principal peak. Hence the method is specific.

**Estimation of Ribociclib in Pharmaceutical Dosage Form**

Twenty Tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 25 mg of drugs were transferred to 25 ml volumetric flask, make and solution was sonicated for 15 minutes, there after volume was made up to 25 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with mobile phase. The solution was filtered through a membrane filter (0.45 μm) and sonicated to degas. The solution prepared was injected in five replicates into the HPLC system and the observations were recorded. The data are shown in Table-32.

**ASSAY**

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg Wt.} = \text{mg}$$

Where:

AT = Peak Area of drug obtained with test preparation

AS = Peak Area of drug obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

**Table-13: Recovery Data for Estimation Ribociclib**

| Brand Name of Ribociclib | Labelled amount of Drug (mg) | Mean (± SD) amount (mg) found by the proposed method (n=6) | Assay % (± SD) |
|---|---|---|---|
| Kryxana 200mg Tablet (200mg) (Novartis India Ltd) | 200mg | 199.286 (± 0.369) | 99.465 (± 0.348) |

**Result & Discussion**: The amount of drugs in Ribociclib Tablet was found to be 199.286 (± 0.369) mg/tab for Ribociclib & % assay was 99.465 (± 0.348).

**FORCED DEGRADATION STUDIES**

Following convention was entirely clung to for constrained corruption of Ribociclib Active Pharmaceutical Ingredient (API).

The API (Ribociclib) was subjected to pressure conditions in different approaches to watch the rate and degree of corruption that is probably going to happen over the span of capacity as well as after organization to body.

This is one kind of quickened dependability contemplates that encourages us deciding the destiny of the medication that is probably going to occur after prolonged stretch of time stockpiling, inside a brief timeframe as contrast with the

constant or long haul steadiness testing.

The different debasement pathways contemplated are Acid/corrosive hydrolysis, Alkali/fundamental hydrolysis, Thermal/warm Degradation, photolytic corruption/ Degradation and oxidative Degradation/corruption.

**Results of Degradation Studies**

The results of the stress studies indicated the Specificity of the method that has been developed. Ribociclib was stable in photolytic and peroxide stress conditions. The result of forced degradation studies are given in the following table-49.

**Table-14: Results of Forced Degradation Studies of Ribociclib API.**

| Stress Condition | Time | Assay of active substance | Assay of degraded products | Mass Balance (%) |
|---|---|---|---|---|
| Acid Hydrolysis (0.1 M HCl) | 24Hrs. | 98.76 | 1.24 | 100.0 |
| Basic Hydrolysis (0.I M NaOH) | 24Hrs. | 98.63 | 1.37 | 100.0 |
| Thermal Degradation (50 $^0$C) | 24Hrs. | 93.98 | 6.02 | 100.0 |
| UV (248nm) | 24Hrs. | 98.84 | 1.16 | 100.0 |
| 3 % Hydrogen Peroxide | 24Hrs. | 94.61 | 5.39 | 100.0 |

**SUMMARY**

The analytical method was developed by studying different parameters.

First of all, maximum absorbance was found to be at 248nm and the peak purity was excellent.

Injection volume was selected to be 20µl which gave a good peak area.

The column used for study was Symmetry ODS (C18) RP Column, 250 mm x 4.6 mm, 5µm particle size because it was giving good peak.

Ambient temperatures were found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time.

Mobile phase is Phosphate Buffer (0.02M) and Acetonitrile were taken in the ratio of 48:52 % v/v (pH-2.80) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study.

Methanol was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery.

Run time was selected to be 8.0 min because analyze gave peak around 3.649min and also to reduce the total run time.

The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range.

The analytical method was found linearity over the range of 30-70ppm of the Ribociclib target concentration.

The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

**CONCLUSION**

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Ribociclib in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps.

Ribociclib was found to be soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide, highly

soluble in water and ethanol.

Phosphate Buffer (0.02M) and Acetonitrile were taken in the ratio of 48:52 % v/v (pH-2.80) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is 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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