A New stability indicating Method Development and Validation of liquid chromatography for the estimation of Ribociclib in pharmaceutical formulation

M. Prasada Rao[*1], CHEEKATI VENKATA VINEETH [2]

1) Principal, M.A.M College of Pharmacy, Kesanupalli, Narasaraopet, Guntur, Andhra Pradesh, India
2) 2nd year M.Pharmacy student, M.A.M College of Pharmacy, Kesanupalli, Narasaraopet, Guntur, Andhra Pradesh, India
* Corresponding author: prins2mam@gmail.com

ABSTRACT:

In the pharmaceutical industry, all manufactured products need to be of the highest quality to ensure the least risk to patients. To guarantee that goods pass certain standards, researchers, manufacturers and developers use various technical equipment and analytical techniques, including liquid chromatography, during the development process. Liquid chromatography is an analytical technique that is used to separate a certain sample into its individual components. HPLC is simple, specific, rapid, precise and accurate; it can be successfully and efficiently adopted for routine quality control analysis of drugs in bulk and pharmaceutical dosage form. In the present study a reverse phase high performance liquid chromatography method was developed and validated for the estimation Ribociclib in pharmaceutical formulations. To assess the effect of method parameters on chromatographic separation of the Ribociclib, statistically designed experiments were performed by varying different method parameters such as buffer concentration, pH of mobile phase, flow rate, and column temperature. The separation was performed on Spherisorb ODS C18 Column (250 x 4.6 mm and 5µm) at room temperature using Acetonitrile: 0.2 M sodium perchlorate in the ratio of 55:45 (v/v) in isocratic condition at a flow rate of 1.0 mL/min. The detection was performed by an ultraviolet detector (UVD) at 223 nm with total run time of 6 min. Calibration curves were linear in the concentration range of 10-100 µg/mL for with correlation coefficients of 0.999. LOD and LOQ were found to be 0.02 µg/mL and 0.066 µg/mL proves the sensitivity of the developed method. The method can effectively separate the degradation compounds during the stress study and the standard drug Ribociclib was found to be stable in all the stress degradation conditions. The developed method was able to determine the contents of the Ribociclib commercial dosage forms and hence the method was used for the routine analysis of Ribociclibin bulk drug as well as in pharmaceutical formulations.

Keywords: Ribociclib, Ultraviolet, Analytical method development &High-Performance Liquid Chromatography.

INTRODUCTION:

A drug may be defined as a substance meant for diagnosis, cure, mitigation andprevention, treatment of diseases in human beings or animals, for altering in structure or functionof the body of human beings or animals[1]. Pharmaceutical chemistry[2-6] is a science thatmakes use of general laws of chemistry to study drugs. Every country has legislation[7] on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" such as I.P.[8], U.S.P. [9], B.P.[10] and Martindale: The Extra Pharmacopoeia[11].Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and the quality of medicament depends[12]. Quality[13]is important in every product or service but it is vital in medicine as it involves life.Physico-chemical methods[14, 15] are used to study the physical phenomena that occur as a result of chemical reactions. Among the physico-chemical methods, the most important are optical and chromatographic methods[16, 17]

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 phosphorylation[18]. Inhibition of Rb phosphorylation prevents CDK-mediated G1-S phase transition, thereby arresting the cell cycle in the G1 phase, suppressing DNA synthesis and inhibiting cancer cell growth. Overexpression of CDK4/6, as seen in certain types of cancer, causes cell cycle deregulation[19].

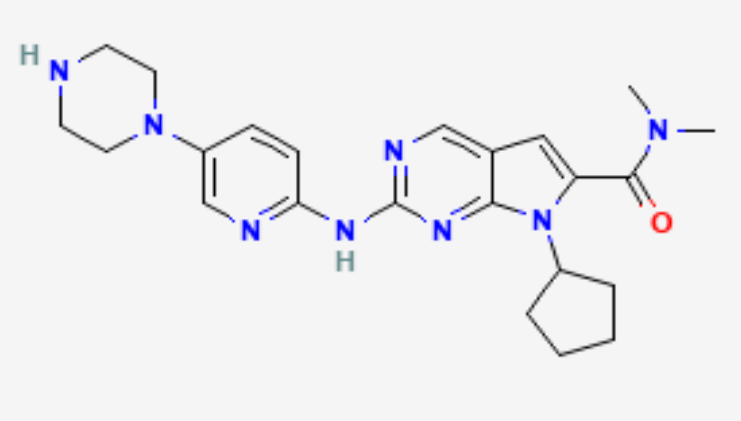

Figure 1: Molecular structure of Ribociclib

Ribociclib may cause fetal harm when given to a pregnant woman. The substance is mainly metabolized by CYP3A4 and subsequently by various phase II enzymes, resulting in a large number of metabolites[20]. Those with highest blood plasma concentrations in humans are

called CCI284 (an unspecified N-hydroxylation product), LEQ803 (the N-demethylation product) and M1 (a glucuronide). All metabolites have negligible clinical activity.Ribociclib has a slight tendency to accumulate in the body. It is eliminated with an average biological half-life of 32 hours, mostly (69%) via the feces, but also (23%) via the urine.

The literature survey for the available analytical methods for the analysis of Ribociclib confirms that there are very few analytical methods available for the estimation of Ribociclib in biological samples in single or in combination with its active metabolites in biological samples using LCMS or UPLC MS. There is no analytical method reported for the estimation of Ribociclibin pharmaceutical formulations[21-25].

MATERIALS AND METHODS

Instrumentation:

The author has attempted to develop a liquid chromatographic method for the simultaneous estimation of Ribociclib using isocratic Shimadzu HPLC equipment comprising of binary LC 10AT vp pumps, SIL 10AD vp Auto sampler, CTO 10A vp column oven, and Prontosil ODS C18 Column (250 x 4.6 mm and 5µm), and an SPD 10Avp UV-Visible detector. All the components of the chromatographic system were controlled using SCL-10A vp System controller. Data acquisition was done using LC Solutions version 1.23SP 1 software.

Chemicals and solvents:

The working standard drug Ribociclib (99.01% purity) along with the formulation dosage form (Kryxana® - 200 mg) were obtained from Novartis Healthcare Pvt Ltd in Madhapur, Hyderabad. HPLC grade Methanol, Water and Acetonitrile were purchased from Merk chemicals private limited, Mumbai. The buffer solutions used for the study were AR Grade and purchased from Merck Specialties Private Limited, Mumbai, India.

Preparation of standard drug solution:

Preparation of standard stock solution was the primary step prior to experimental work. A standard stock solution of 1000µg/mL was prepared by weighing accurately 10mg of the standard drug Ribociclib and was taken in a 10mL volumetric flask having little amount of Methanol. Dissolve the drug in the solvent and make up to the mark. Then it was filtered through 45µ filter paper to remove un-dissolved particles or any solid substances. The solution was preserved safely and used when required. For the comparison with test formulation, standard solution was prepared and used at 10 different concentrations from 10 to 100µg/mL in chronologically.

Preparation of formulation solution:

Tablets of Kryxana® brand containing 200 mg of Ribociclib was powdered using a sterile mortar and pestle. Then an amount of tablet powder equivalent to 50 mg of Ribociclib was accurately weighed and dissolved in 50 mL solvent using sonicator and filtered through 0.45 µ membrane filter. Then it was diluted while doing the formulation analysis.

HPLC Method Development:

Selection of wavelength:

To select an appropriate monitoring wavelength, the standard solutions ofw10µg /mLwas prepared and scanned by the UV-Vis spectrophotometer. The obtained wavelength maxima were selected as suitable wavelength for the detection.

Selection of stationary phase:

Since the Ribociclib is a Polar drug, a non-polar C18 column was selected for the separation of the drug. Different columns of different companies, manufactures and configurations were tested.

Analytical Method Validation:

The method was validated with respect to linearity, accuracy, precision, repeatability, selectivity, and specificity, according to the ICH guidelines. Validation studies were carried out by replicate injections of the sample and standard solutions into the chromatograph.

Specificity:

Specificity of the method was checked by injecting the solution into the chromatograph. Specificity of the method was assessed by comparing the chromatogram of Ribociclib(standard), blank and sample solutions to those obtained for tablet solutions. Retention time of the Ribociclib in standard solution, and in the sample, solution was compared to determine the specificity of the method.

System suitability:

The system suitability was determined by making six replicate injections of the standard solution and analyzing Ribociclibfor its peak area, peak USP tailing factor, and number of theoretical plates. The proposed accepted criteria are not more than 2% for RSD%, not less than 2 for resolution, not more than 2 for USP tailing factor, and not less than 2000 for the number of theoretical plates.

Sensitivity of the method:

The limit of detection (LOD) and limit of quantitation (LOQ) were defined as the lowest concentration of analyte in a sample that can be detected and quantified. The standard solutions of Ribociclib for LOD and LOQ were prepared by diluting them with suitable solvent. The LOD and LOQ were determined by the signal-to-noise (S/N) ratio for each compound through analyzing a series of diluted solutions until the S/N ratio yield 3 for LOD and 10 for LOQ, respectively.

Linearity and Range:

The calibration curve in the developed method was constructed from LOQ concentration. Ribociclib standard stock solution of 1 mg/mL was used for preparation of subsequent aliquots. Sample solution was loaded and 20µL was injected into column. All measurements were repeated for each concentration.

Trail .NO	Parameter	Condition	Trail.NO	Parameter	Condition
I	MP	Methanol: Acetonitrile 80:20 (v/v)	**IV**	MP	Acetonitrile: Water 25:75 (v/v)
	Wavelength	223nm		Wavelength	223 nm
	Stationary Phase	Kromosil ODS C18 Column (250 x 4.6 mm and 5µm)		Stationary Phase	Spherisorb ODS C18 Column (250 x 4.6 mm and 5µm)
	Flow Rate	1.0 mL/min		pH of MP	5.1
				Flow Rate	1.0 mL/min
II	MP	Methanol: Acetonitrile 25:75 (v/v)	**V**	MP	Methanol: Acetonitrile: Water 20:70:10 (v/v)
	Wavelength	223 nm		Wavelength	223 nm
	Stationary Phase	Sperbo Waters C18 Column (250 x 4.6 mm and 5µm)		Stationary Phase	Spherisorb ODS C18 Column (250 x 4.6 mm and 5µm)
	Flow Rate	1.0 ml/min		pH of MP	5.2
				Flow Rate	1.0ml/min
III	MP	Water:Acetonitrile 25:75 (v/v)	**VI**	MP	Acetonitrile: 0.2 M sodium perchlorate 55:45 (v/v)
	Wavelength	223 nm		Wavelength	223 nm
	Stationary Phase	Prontosil ODS C18 Column (250 x 4.6 mm and 5µm)		Stationary Phase	Spherisorb ODS C18 Column (250 x 4.6 mm and 5µm)
	pH of MP	5.1		pH of MP	4.9 with 9M perchloric acid
	Flow Rate	1.0mL/min			

Table 01: Method Development trails HPLC method development

***Precision*:**

The precision studies were carried out by estimating response of Ribociclib six times at a standard concentration of 60 µg/mL and results are reported in terms of %RSD. The intra-day and inter-day precision studies were carried out by estimating the corresponding responses six times on same day for intraday and interday for three different days and it was expressed as the percentage relative standard deviation (%RSD) which was calculated as per the following expression

%RSD = (standard deviation / mean) x 100.

***Accuracy/ Recovery*:**

Accuracy of method was observed by recovery result from two placebos preparations accurately spiked with different concentration of Ribociclib. Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 50%, 100% and 150% to the pre analyzed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated by using the formula.

***Ruggedness*:**

Two laboratory analysts carried out the precision of Ribociclib at a standard concentration of 60 µg/ml was prepared by different analysts in the laboratory conditions, the prepared solution was analyzed in the optimized conditions. Peak area that obtained was used for the determination of ruggedness of the method. Ruggedness was expressed in terms of %RSD which must be less than 2.

***Robustness*:**

Robustness of the proposed method included six deliberate variations to some chromatographic parameters. The modifications include different mobile phase ratios and different detector wavelengths and different percentage in the mobile phase (in the range of ± 5 of the nominal value and the normal (%). The % change in each of the changed condition was calculated.

***Formulation analysis*:**

This proposed method was applied to the determination of Ribociclibin commercially available tablets. The sample solution at a concentration of 60 µg/ml of Ribociclib was analyzed in the optimized conditions. The % assay was calculated for Ribociclib using the standard calibration values.

***Forced degradation study*:**

S. No.	Degradation type	Experimental conditions	Time
1	Acid Hydrolysis	50mg of drugs were mixed with 50ml of 0.1N HCl solution. The solution was neutralized and diluted up to standard concentration (100 %) and was analyzed in the developed method condition	24 Hours
2	Base Hydrolysis	50mg of drugs were mixed with 50ml of 0.1N NaOH solution. The solution was neutralized and diluted up to standard concentration i.e 100% and was analyzed in the developed method condition	24 Hours
3	Oxidative Degradation	50mg of drugs were with 50ml of 3% Peroxide solution. The solution was neutralized and diluted up to standard concentration (100 %) and was analysed in the developed method condition	24 Hours
4	Photolytic Degradation	50mg of drug sample was kept in UV light [254nm]. After the selected time of light expose, the drug solution was prepared and was analyzed	24 Hours
5	Thermal Degradation	50mg of drug sample was kept in oven at 60^0C. After the selected time of light expose, the drug solution was prepared and was analyzed	24 Hours

Table 02: Methodology for forced degradation study

RESULTS AND DISCUSSION

HPLC Method Development:

The present work aimed to develop a simple and accurate HPLC method for the quantification of Ribociclib in pharmaceutical formulations. The ultraviolet absorption spectra of the Ribociclibdemonstrated that the maximum absorption at a wavelength near 223 nm,

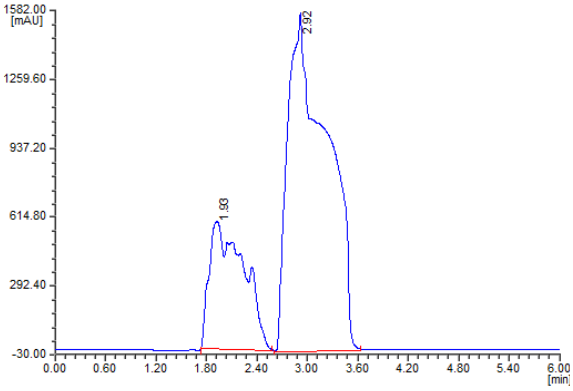

Figure 2: Chromatogram observed for Ribociclib trail 1

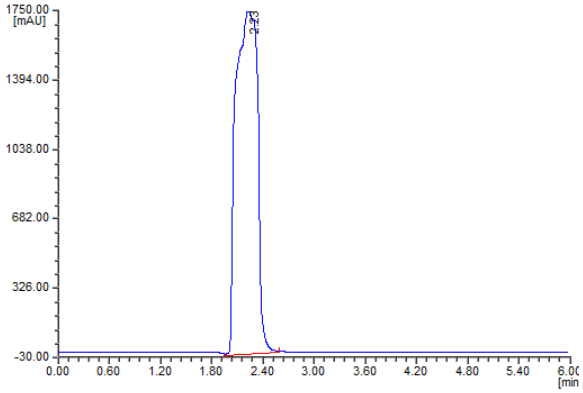

Figure 3: Chromatogram observed for Ribociclibin trail 2

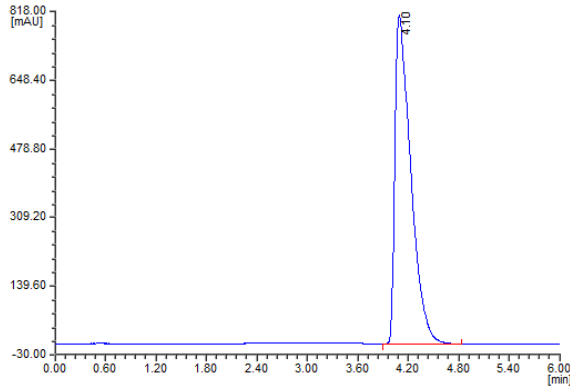

Figure 4: Chromatogram observed for Ribociclib in trail 3

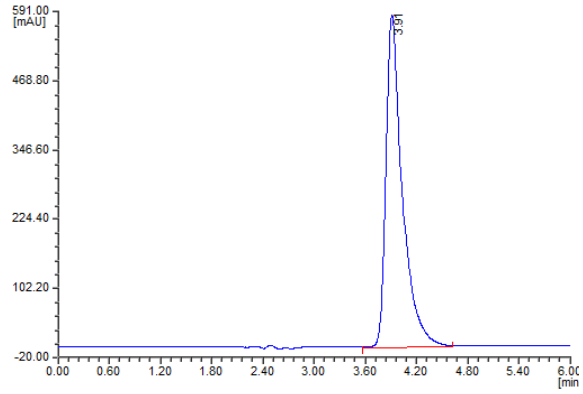

Figure 5: Chromatogram observed for Ribociclibin trail 4

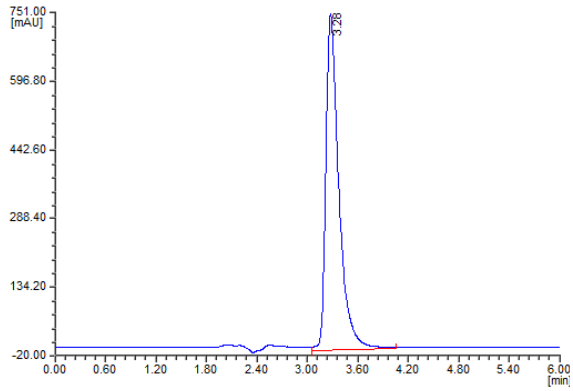

Figure 6: Chromatogram observed for Ribociclib in trail 5

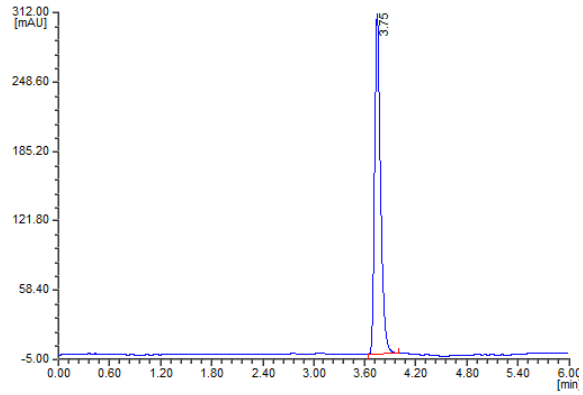

Figure 7: Optimized Chromatogram observed for Ribociclib in trail 6

In these conditions, single sharp symmetric peak with acceptable system suitability was observed (Figure 7). Hence these conditions were found to be suitable and further valuation was carryout using these conditions. The optimized conditions, well resolved, retained and accepted system suitability was observed. The optimized conditions were given in table 3.

S.No	Parameter	Results
1	Mobile phase	Acetonitrile: 0.2 M sodium perchlorate 55:45 (v/v)
2	Wavelength	223 nm
3	Stationary Phase	SpherisorbODS C18 Column (250 x 4.6 mm and 5µm)
4	pH of MP	4.9 with 9 M perchloric acid
5	Flow Rate	1.0mL/min
6	Pump Mode	Isocratic
7	Pump Pressure	12.7±6 MPa
8	Run time	6 min

Table 03: Optimized chromatographic conditions

Method Validation:

The method was validated with respect to linearity, accuracy, precision, repeatability, selectivity and specificity, according to the ICH guidelines.

Specificity:

Sharp peak was obtained for Ribociclibat retention times of 3.75 min. This peak was not detected in the blank solution.

System suitability:

In the optimized conditions, peak tailing factors of was found to be 0.92 for Ribociclib whereas the number of theoretical plates was found to be 9654. This method met the accepted requirements. Table 4 shows the results of the system suitability results of the proposed method.

S. NO	Parameter	Results
1	Api Concentration	60 µg/mL
2	RT	3.75 min
3	Resolution	Not applicable
4	Area	669584.3
5	Theoretical Plates	9654
6	Tailing Factor	0.92

Table 04: system suitability results of Ribociclib

Sensitivity of the method:

LOD and LOQ were found to be 0.02 µg/mL and 0.066 µg/mL for Ribociclib. Results confirmed that the method was sensitive and can be useful for the detection and analysis of drugs at very lowest concentrations. Figure 10 shows LOD chromatogram of Ribociclib in the developed method.

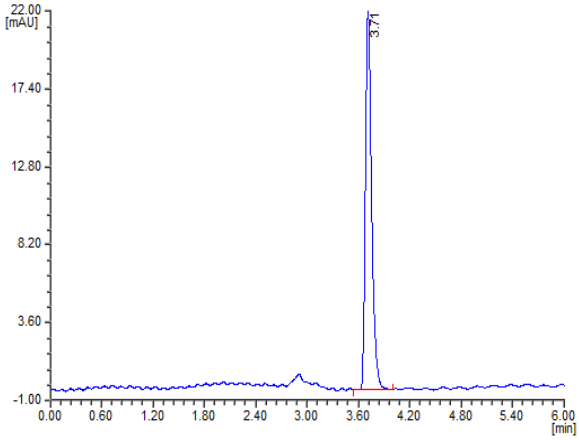

Figure 10: LOD chromatogram for the proposed method

Linearity and Range:

Linearity was observed in the concentration range of 10-100µg/ml for Ribociclib with Linear regression equation of y = 11293x - 780.7 (R^2 = 0.999). Linearity graph was given in figure11.

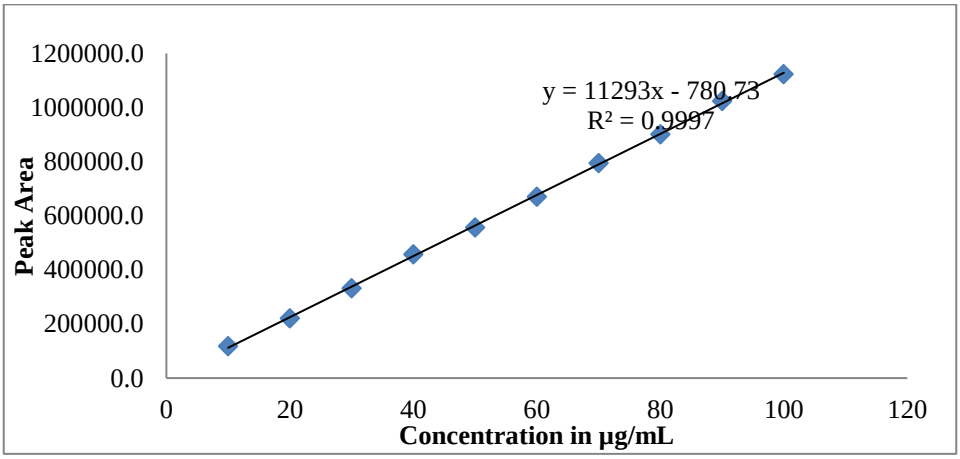

Figure 11: Linearity graph

Precision:

The %RSD was found to be 0.10 and 0.38 for Ribociclibin intraday precision and interday precisionrespectively. %RSD was found to be within the acceptance limit of less than 2. Hence the developed method was found to be precise. Results were given in table 5 for intraday and interday precision respectively.

S. No	Intraday Precision	Interday Precision
1	669584.3	666906.0

2	667598.9	666263.7
3	670957.2	666931.5
4	670415.8	668404.6
5	670112.4	664081.4
6	670542.8	664507.9
% RSD	**0.18**	**0.24**

Table 05: Precision results

Accuracy/ Recovery:

Table 6.5 shows the recovery results of Ribociclib in the developed method. The %recovery was fond to be within the range of 98.29 to 99.74 %. The %RSD in each spiked level was found to be 0.29, 0.50 and 0.55 % for Ribociclib in 50 %, 100 % and 150 % spiked level respectively. The results found to be with in the acceptance limit of 98-102 and % RSD of <2 which sense to conformation that the proposed method was accurate. Table6gives the accuracy results.

% Recovery	Concentration in µg/ml			Amount Found	% Recovery	% RSD
	Target	Spiked	Total			
50%	40	20	60	59.550	99.25	0.29
	40	20	60	59.802	99.67	
	40	20	60	59.472	99.12	
100%	40	40	80	78.968	98.71	0.50
	40	40	80	78.632	98.29	
	40	40	80	79.416	99.27	
150%	40	60	100	98.660	98.66	0.55
	40	60	100	99.740	99.74	
	40	60	100	99.120	99.12	

Table 6: Accuracy results

Ruggedness:

Ruggedness was expressed in terms of %RSD which must be less than 2. The %RSD was found to be 0.52 in the developed method. Results found to be within the acceptance limit confirms that the method is rugged.

Robustness:

%Change was found to be in the range of 0.33 to 1.46 % for Ribociclib and the results were found to be within the acceptance limit of less than 2. This confirms that the small change in the analytical conditions doesn't influence the results and hence the proposed method was found to be suitable for the analysis of Ribociclibwhen small change in the analytical conditions.

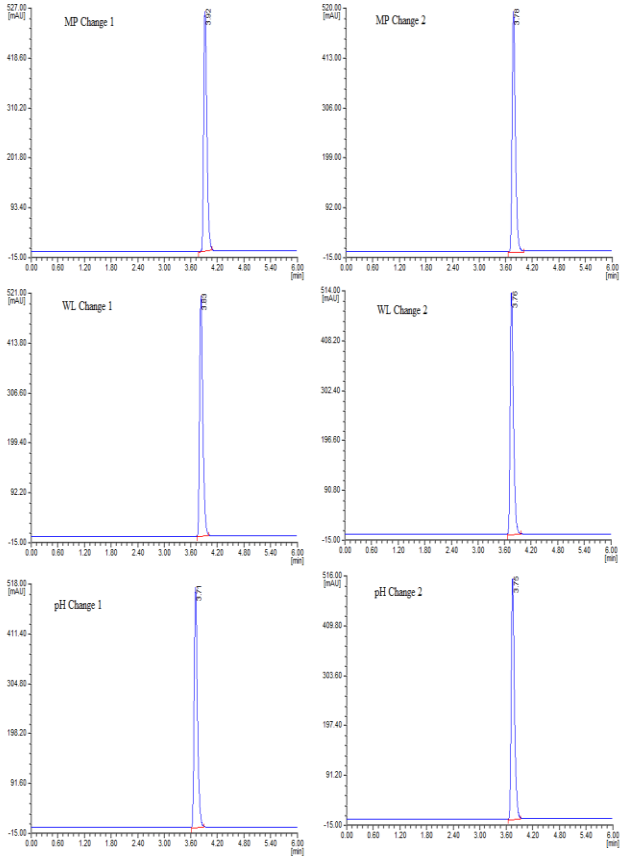

Figure 12: Robustness chromatograms

Forced Degradation studies:

In all the stress degradation conditions i.e acidic, base, peroxide, thermal and UV light conditions, the standard drug Ribociclib was effectively separated, identified and quantified. The % assay of Ribociclib was found to be very high and the % degradation was found to be very less in the developed method. The degradation products were found to be 3, 1, 1, 2 and 2 in acidic, base, peroxide, thermal and UV light conditions respectively. All the degradation products were effectively separated and there is no overlap of degradation compounds with the standard drug. Hence the developed method was found to be stability indicating. The forced degradation study results were given in table 7.

S No	Condition studied	No of degradation compounds separated	% Assay	% Degradation
1	Acid	3	92.35	9.25
2	Base	1	93.08	3.23
3	Peroxide	1	96.52	2.85
4	Thermal	2	97.92	4.25
5	UV light	2	94.32	6.29

Table 7: Forced degradation study results

Formulation analysis:

The % assay in formulation analysis was found to be 98.72 forRibociclib in the developed method. More than 98% assay was observed in the developed method. Hence the method was found to be suitable for the routine analysis ofRibociclib in bulk drug as well as formulations. Results of the formulation analysis were given in table 8 and formulation chromatograms were given in figure 13.

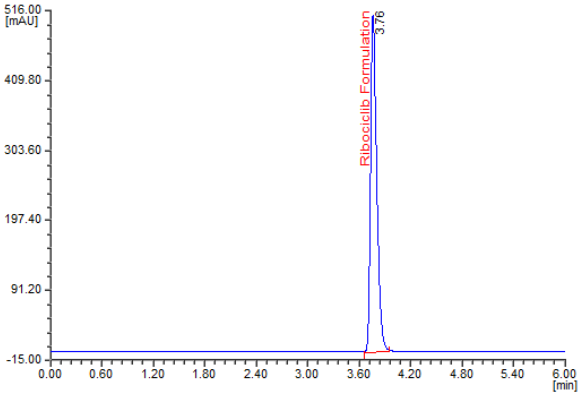

Figure 13:Formulation chromatogram

S.No	Drug	Brand	Label claim	Concentration prepared	Concentration found	% Assay
1	Ribociclib	Kryxana®	200 mg	60 µg/mL	59.23 µg/mL	98.72

Table 8: Formulation results

CONCLUSION

A stability indicating reversed phase-high performance liquid chromatography (RP-HPLC) method was developed and validated for analysis of Ribociclib. Retention time of the Ribociclib was found to be 3.75 min with run time of 10 min. The mobile phase consisting of acetonitrile, 0.2 M sodium perchlorate in the ratio of 55:45 (v/v) at flow rate of l.0 mL/min was employed in this study and separation was achieved on Spherisorb ODS C18 Column (250 x 4.6 mm and 5µm) and UV detection at 223 nm. The calibration curves were linear in the concentration range of 10 to 100 µg/mL with regression coefficient (r^2) of 0.999. The limits of detection (LOD) and the limits of quantification (LOQ) were found to be 0.02 µg/mL and 0.066 µg/mL respectively. The method was statistically validated in accordance with international conference on harmonization (ICH) guidelines. The method can effectively separate the stress degradation compounds formed during the stress study and the % drug content was observed to be very high in all the stress studies. Hence based on the statistical analysis of the data it has been unequivocally construed that the method is reproducible and selective for the routine analysis of Ribociclib in bulk drug and tablet dosage form.

REFERENCES

1. R. S. Satoskar, S. D. Bhandarkar and S. S. Ainapure. "Pharmacology and Pharmacotherapeutics", 17th edition, Popular Prakashan, Mumbai, India, 2001.
2. "Burger's Medicinal Chemistry and drug discovery", 6th edition, Wiley Interscience, New Jersey, 2007.
3. "Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry", 11th edition, Lippincott Williams & Wilkins, New york, 2004.
4. A. Korolkovas. "Essentials of Medicinal Chemistry", 2nd edition, Wiley Interscience, New Jersey, 1988.
5. "Goodman and Gilman's The Pharmacological Basis of Therapeutics", 9th edition, McGraw-Hill health professions division, New york, 1996.
6. Foye's "Principles of Medicinal Chemistry", 6th edition, Lippincott Williams & Wilkins, New york, 2008.
7. Drugs & Cosmetics Act, 1940 & Rules, 1945, 2nd edition, Susmit publishers, Mumbai, India, 2000.
8. Indian Pharmacopoeia, Ministry of Health & Family Welfare, Government of India, New Delhi, 1996.
9. The United States Pharmacopoeia- the National Formulary, United States Pharmacopoeial convention, Rockville, 2007.
10. British Pharmacopoeia, The Stationary Office, London, 2005.
11. "Martindale - The Extra Pharmacopoeia", 33rd edition, The Pharmaceutical Press, London, 2002.
12. A. H. Beckett and J. B. Stenlake. "Practical Pharmaceutical Chemistry", Volume I and II, CBS Publishers & Distributors, New Delhi, India, 2000.
13. P. D. Sethi. "Quantitative Analysis of Drugs in Pharmaceutical Formulations". 3rd edition, CBS Publishers & Distributors, New Delhi, India, 1997.
14. H. H. Willard, L. L. Merrit, J. A. Dean and F. A. Settle. "Instrumental Method of Analysis", 7th edition, CBS Publishers & Distributors, New Delhi, India, 1986.
15. R. A. Day and A. L. Underwood. "Quantitative Analysis", 6th edition, PHI learning private limited, New Delhi, India, 2009.

16. G. Ramana Rao, S. S. N. Murthy and P. Khadgapathi. Gas chromatography to pharmaceutical analysis (Review). *Eastern Pharmacist.* 30(353): 35 (1987).

17. G. Ramana Rao, S. S. N. Murthy and P. Khadgapathi. High performance liquid chromatography and its role in pharmaceutical analysis (Review). *Eastern Pharmacist.* 29 (346): 53 (1986).

18. Ll-Yord R. Snyder, Joseph J. Kirkland and Joseph L. Glajch. Practical HPLCMethod development. John Wiley & Sons, INC, U.S.A. 2nd Edition, New York, 1997.
19. Satinder Ahuja and Michael W. Dong. Handbook of Pharmaceutical Analysis by HPLC, Elsevier academic press, 1st Edition, Vol. 6, 2005.
20. M. Thompson, S. L. R. Ellison and R. Wood. Harmonized guidelines for single laboratory validation of methods of analysis. Pure and Applied Chemistry, 74(5):835-855 (2002).
21. VamseekrishnaGorijavolu, Ajay Kumar Gupta, YA Chowdary and B Raviteja, Estimation of Ribociclib in Human Plasma Samples by LC-ESI-MS/MS, Journal of Chemical and Pharmaceutical Research, 2017, 9(11):61-66.
22. Sreelakshmi Ma, R. L. C. Sasidhara and RavitejaB, Simultaneous Estimation of RibociclibandPalbociclib in Bulk Samples by Reverse PhaseHigh Performance Liquid Chromatography, International Journal of Pharmacy and Biological Sciences-IJPBSTM (2019) 9 (2): 413-421.
23. Alejandra Martínez-Chávez, Hilde Rosing, Michel Hillebrand, Matthijs Tibben, Alfred H. Schinkel, and Jos H. Beijnen, Development and validation of a bioanalytical method for the quantification of the CDK4/6 inhibitors abemaciclib, palbociclib, and ribociclib in human and mouse matrices using liquid chromatography-tandem mass spectrometry, Anal Bioanal Chem. 2019; 411(20): 5331–5345.
24. Bao X, Wu J, Sanai N, Li J, Determination of total and unbound ribociclib in human plasma and brain tumor tissues using liquid chromatography coupled with tandem mass spectrometry, Journal of Pharmaceutical and Biomedical Analysis, 2019, 166:197-204.
25. 25. Bianca Posocco, Mauro Buzzo, Ariana Soledad Poett, Marco Orleni, Sara Gagno, Martina Zanchetta, Valentina Iacuzzi, Michela Guardascione, Fabio Puglisi, Debora Basile, Giacomo Pelizzari, Elena Marangon, and Giuseppe Toffoli, Simultaneous quantification of palbociclib, ribociclib and letrozole in human plasma by a new LC-MS/MS method for clinical application, PLoS One. 2020; 15(2).