IJBPAS, February, 2025, 14(2): 595-607

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF RIBOCICLIB AND LETROZOLE IN BULK AND PHARMACEUTICAL DOSAGE FORM

P. POOJITHA[1*], KIRAN JYOTI[2], MAHESH[3], CH. GOPINATH[4]

[*1, 2, 3, 4] Department of Pharmaceutical analysis, JNTU OTPRI, Anantapur, Andhra Pradesh

***Corresponding Author: Dr. P. Poojitha: E Mail: pallachurupoojitha@gmail.com**

Received 4th Nov. 2023; Revised 5th Dec. 2023; Accepted 12th April 2024; Available online 1st Feb. 2025

https://doi.org/10.31032/IJBPAS/2025/14.2.8639

ABSTRACT

High Performance Liquid Chromatography (HPLC) is one mode of chromatography, one of the most used analytical techniques. Letrozole is indicated to treat postmenopausal women with hormone receptor (HR) positive early breast cancer, postmenopausal women with early breast cancer who have previously been treated with tamoxifen, and postmenopausal women with HR+ or unknown advanced breast cancer. Letrozole, given with Ribociclib, is indicated to treat pre and postmenopausal women with HR+ and human epidermal growth factor 2 (HER2) negative advanced or metastatic breast cancer. The present aim of the study is to development of Letrozole and Ribociclib HPLC method and validation of the test substance. The estimation of Letrozole and Ribociclib was done by RP-HPLC. The assay of Letrozole and Ribociclib was performed with tablets and the % assay was found to be 98.29 and 99.55 which shows that the method is useful for routine analysis. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

Keywords: Assay, Chromatography, Letrozole, Limit, Ribociclib

1. INTRODUCTION

High Performance Liquid Chromatography (HPLC) is one mode of chromatography, one of the most used analytical techniques. Chromatographic process can be defined as separation technique involving mass-transfer between stationary and mobile phase **[1, 2]**. Letrozole, is an oral non-steroidal type II aromatase inhibitor. It is a third generation aromatase inhibitor like exemestane and anas trozole, meaning it does not significantly affect cortisol, aldosterone, and thyroxin **[3]**. Letrozole is indicated to treat postmenopausal women with hormone receptor (HR) positive early breast cancer, postmenopausal women with early breast cancer who have previously been treated with tamoxifen, and postmenopausal women with HR+ or unknown advanced breast cancer. Letrozole, given with Ribociclib, is indicated to treat pre and postmenopausal women with HR+ and human epidermal growth factor 2 (HER2) negative advanced or metastatic breast cancer **[4]**. 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).

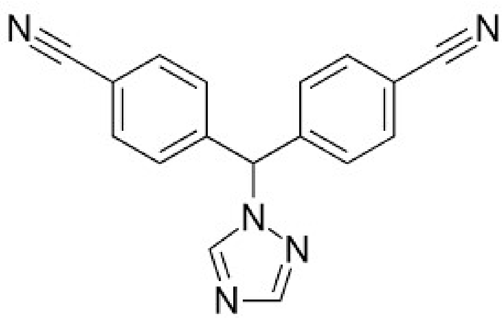

Figure 1a: Structure of Letrozole

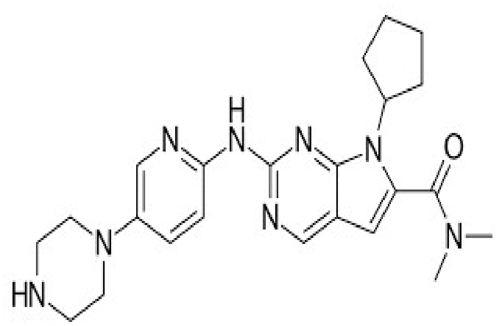

Figure 1b: Structure of Ribociclib

2. Materials:

Letrozole (LET) and Ribociclib (RIC), was obtained from Novartis Health care, India. Acetonitrile, Methanol, HPLC Grade water, and Ortho phosphoric acid from Sigma Aldrich USA.

3. HPLC Method Development [5]:

3.1 Wave length selection:

UV spectrum of 10 μg / ml Ribociclib and Letrozole in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 227. At this wavelength both the drugs show good absorbance.

3.2 Optimization of Column:

Waters C18 (4.6 x 250mm, 5.0μm) was found to be ideal as it gave good peak shape and resolution at 1.2 ml/min flow.

3.4 Optimized Chromatographic Conditions:

Instrument used: High performance liquid chromatography equipped with Auto Sampler and PDA or UV detector.

Temperature: Ambient

Column: Waters C_{18} (4.6 x 250mm, 5.0μm)

Buffer: 0.1% ortho phosphoric acid buffer

Mobile phase: 25% buffer: 75% Methanol

Flow rate: 1.0 ml per min

Wavelength: 227 nm

Injection volume: 20 μl

Run time: 10min.

3.5 Preparation of Buffer and Mobile Phase:

3.5.1 Preparation of 0.1% Ortho phosphoric acid buffer:

Pipetted 1 ml of ortho phosphoric acid in 100 ml HPLC water.

3.5.2 Preparation of mobile phase:

Mix a mixture of above buffer 250 ml (25%) and 750 ml Methanol HPLC (75%) and degas in ultrasonic water bath for 5 minutes. Filter through 045 μ filter under vacuum filtration.

Diluent Preparation:

Use the Mobile phase as Diluents.

3.6 Validation Parameters [6, 7]:

3.6.1 Assay [8, 9]:

Standard Solution Preparation:

Accurately weigh and transfer 200 mg of Ribociclib and 2.5 mg of Letrozole working standard into a 100ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution.) Further pipette 1.5 ml of Ribociclib and Letrozole of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 200 mg of Ribociclib and 2.5 mg Letrozole equivalent weight of the sample into a 100ml clean dry volumetric flask add about 70ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of Ribociclib & Letrozole of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure:

Inject 20 μL of the standard, sample into the chromatographic system and measure the areas for the Ribociclib and Letrozole peaks and calculate the %Assay by using the formulae.

3.6.2 Linearity [10, 11]:

Preparation of Level – I (100 ppm & 1.25ppm of Ribociclib & Letrozole):

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

Preparation of Level – II (200 ppm &2.5ppm of Ribociclib & Letrozole):

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

Preparation of Level – III (300 ppm & 3.75ppm of Ribociclib & Letrozole):

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

Preparation of Level – IV (400 ppm & 5 ppm of Ribociclib & Letrozole):

2ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – V (500 ppm & 6.25ppm of Ribociclib & Letrozole):

2.5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

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.

3.6.3 Precision [12, 13]:

Preparation of stock Solution:

Accurately weigh and transfer 200 mg of Ribociclib and 2.5 mg of Letrozole working standard into a 100ml clean dry volumetric flask add about 70ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of Ribociclib and Letrozole the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure:

The standard solution was injected for six times and measured the area for all five injections in HPLC. The mean and percentage relative standard deviation were calculated from the peak areas.

3.6.4 Intermediate Precision [14]:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day within the laboratory. Pipette 1.5 ml of Ribociclib & Letrozole the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure:

The standard solution was injected for five times and measured the area for all six injections in HPLC. The %RSD for the area of five replicate injections were calculated.

3.6.5 Accuracy [15]:

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same. Pipette 1.5 ml of Ribociclib and Letrozole the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 100 mg of Ribociclib and 1.25 mg of Letrozole working standard into a 100ml clean dry volumetric flask add about 70 ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of Ribociclib and Letrozole the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 200 mg of Ribociclib and 2.5 mg of Letrozole working standard into a into a 100ml clean dry volumetric flask add about 70ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of Ribociclib & Letrozole the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer 300 mg of Ribociclib and 3.75 mg of Letrozole equivalent weight of tablet powder into a 100ml 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 1.5 ml of Ribociclib and Letrozole the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents

Procedure:

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

3.6.6 Limit of Detection [16]:

Preparation of Ribociclib solution:

Accurately weigh and transfer 200 mg of Ribociclib working standard into a 100ml clean dry volumetric flask add about 70ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of Ribociclib the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of 0.013µg/ml solution:

Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Further pipette 0.1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Letrozole solution:

Accurately weigh and transfer 2.5 mg of Letrozole working standard into a 100ml clean dry volumetric flask add about 70ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of Letrozole the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation 0.002µg/ml solution:

Further pipette 0.1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Further pipette 0.7ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

3.6.7 Limit of Quantification:

Preparation of Ribociclib solution:

Preparation of 0.044µg/ml solution:

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Further pipette 0.2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Letrozole solution:

Preparation of 0.008µg/ml solution:

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Further pipette 0.28of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

3.6.8 Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a) The flow rate was varied at 0.9 ml/min to 1.1ml/min.

Standard solution 300 and 3.75 µg/ml of Ribociclib & Letrozole prepared and analysed using the varied flow rates along with method flow rate.

b) The Organic composition in the Mobile phase was varied from 67.5% to 82.5%

Standard solution 300 and 3.75 µg/ml of Ribociclib and Letrozole was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

4. RESULTS AND DISCUSSION

4.1 System Suitability:

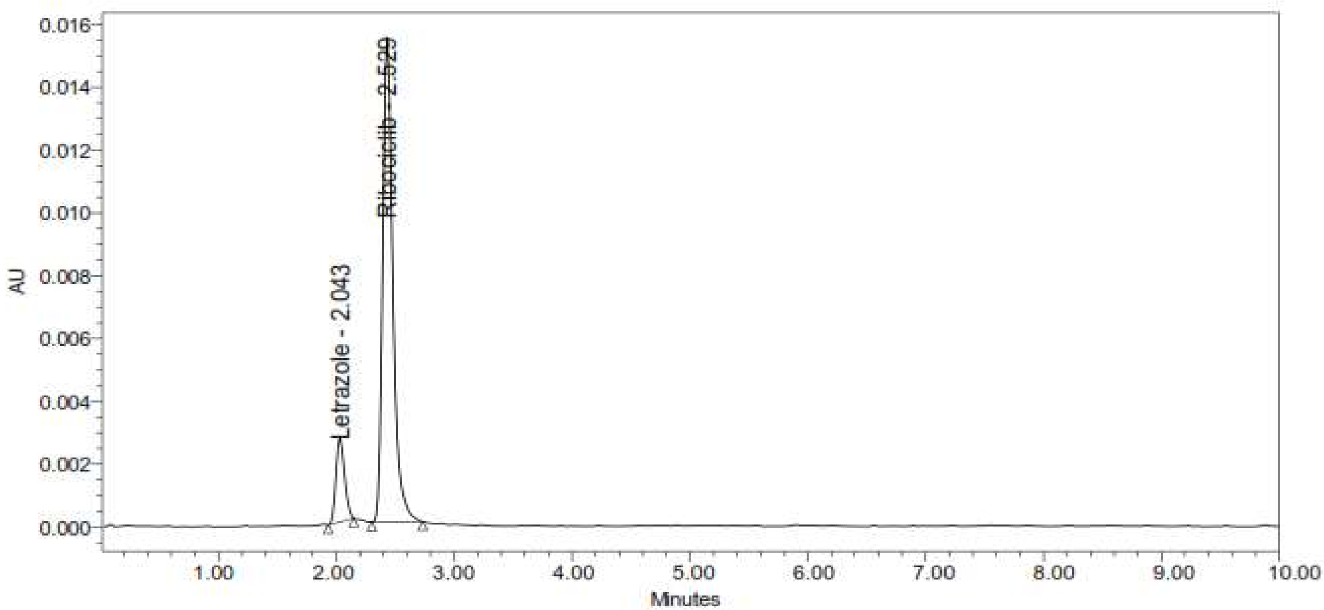

Figure 2: Chromatogram for system suitability

Table 1: Results of system suitability parameters

S. No.	Name	RT (min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Letrozole	2.043	181180	38253	-	1.02	6932.07
2	Ribociclib	2.529	646973	102033	8.19	0.98	8618.83

Acceptance criteria:

Resolution between two drugs must be not less than 2.

Theoretical plates must be not less than 2000.

Tailing factor must be not more than 2.

It was found from above data that all the system suitability parameters for developed method were within the limit.

5. Validation Parameters:

5.1 Assay:

Standard and sample solution injected as described under methodology. The corresponding chromatograms and results are shown below.

Table 2: Results of Assay for Letrozole and Ribociclib

Drug	Label Claim (mg)	% Assay
Letrozole	2.5	99.58
Ribociclib	200	99.61

5.2 Linearity:

The linearity range was found to lie from 100 µg/ml to 500 µg/ml of Ribociclib, 1.25 µg/ml to 6.25µg/ml 0f Letrozole and chromatograms are shown below.

Table 3: Area of different concentration of Letrozole and Ribociclib

S. No	Letrozole		Ribociclib	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	1.25	55850	100	200485
2	2.5	112430	200	434774
3	3.75	171371	300	653981
4	5	226421	400	854921
5	6.25	285508	500	1086071

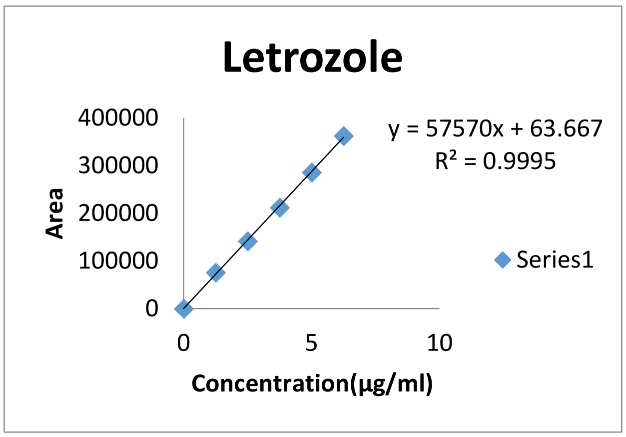

Figure 3a: Calibration graph for Letrozole

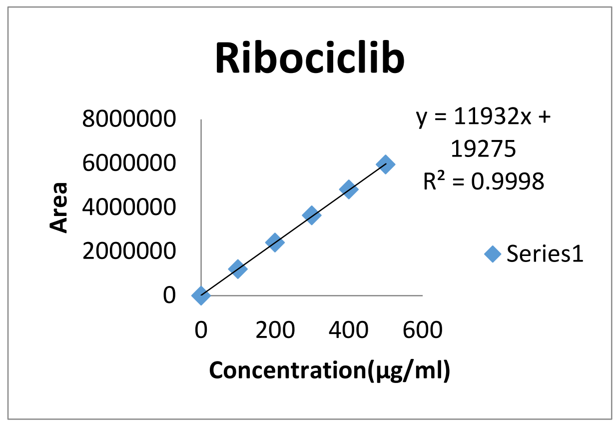

Figure 3b: Calibration graph for Ribociclib

Acceptance criteria:

Correlation coefficient (R^2) should not be less than 0.999

Inference: The correlation coefficient obtained was 0.999 which is in the acceptance limit.

5.3 Precision:

Precision of the method was carried out for both sample solutions as described under experimental work. The corresponding chromatograms and results are shown below.

Table 4: Results of Precision

Injection	Area of Letrozole	Area of Ribociclib
Injection-1	188313	651495
Injection-2	186597	646216
Injection-3	186341	656415
Injection-4	186517	655243
Injection-5	186785	656618
Injection-6	186528	652235
Average	186846.83	653037
Standard Deviation	732.42	3966.05
%RSD	0.39	0.61

Acceptance criteria: %RSD for sample should be NMT 2.

Inference: The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

5.4 Intermediate Precision

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

Table 5: Results of Intermediate precision for Letrozole

Injection	Area of Letrozole	Area of Ribociclib
Injection-1	186307	658768
Injection-2	187952	654831
Injection-3	187181	649669
Injection-4	186797	644577
Injection-5	189558	654694
Injection-6	187804	658902
Average	187599.8	653573.5
Standard Deviation	1139.4	5556.9
%RSD	0.61	0.85

Acceptance criteria: %RSD of five different sample solutions should not more than 2

Inference: The %RSD obtained is within the limit, hence the method is rugged.

5.5 Accuracy:

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Table 6: Accuracy (recovery) data for Letrozole

% Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	91538	1.25	1.248	99.92	99.09
100%	182946	2.5	2.49	99.26	
150%	271357	3.75	3.69	98.09	

***Average of three determinations**

Table 7: Accuracy (recovery) data for Ribociclib

% Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	325703	100	100.09	101.87	100.73
100%	669000	200	199.95	99.51	
150%	978498	300	300.12	100.82	

***Average of three determinations**

Acceptance Criteria: The percentage recovery was found to be within the limit (97-103%).

Inference: The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

5.6 Limit of Detection for Letrozole and Ribociclib

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

Table 8: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Letrozole	58	173	2.98
Ribociclib	58	171	2.95

Acceptance criteria: Signal to noise ratio shall be 3 for LOD solution

Inference: The result obtained is within the limit.

5.7 Limit of Quantification for Letrozole and Ribociclib

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

Table 9: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Letrozole	58	577	9.95
Ribociclib	58	579	9.98

Acceptance criteria: Signal to noise ratio shall be 10 for LOQ solution

Inference: The result obtained is within the limit.

5.8 Robustness:

The standard and samples of Letrozole and Ribociclib were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 10: Results for variation in flow for Letrozole

S. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	7608.51	1.00
2	1.0	6932.07	1.02
3	1.1	7384.51	1.02

Table 11: Results for variation in flow for Ribociclib

S. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	9343.22	0.96
2	1.0	8618.83	0.98
3	1.1	8679.82	0.98

*** Results for actual flow (1.0ml/min) have been considered from Assay standard.**

Table 12: Results for variation in mobile phase composition for Letrozole

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2071.65	1.78
2	*Actual	2947.68	1.71
3	10% more	2994.24	1.77

Table 13: Results for variation in mobile phase composition for Ribociclib

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3224.22	1.68
2	*Actual	3826.77	1.61
3	10% more	3824.86	1.61

*** Results for actual Mobile phase composition have been considered from Accuracy standard.**

Inference: The Retention time, USP plate count, USP tailing factor obtained for change of flow rate, variation in mobile phase was found to be within the acceptance criteria. Hence the method is robust.

6. CONCLUSION: The estimation of Letrozole and Ribociclib was done by RP-HPLC. The assay of Letrozole and Ribociclib was performed with tablets and the % assay was found to be 98.29 and 99.55 which shows that the method is useful for routine analysis. The linearity of Letrozole and Ribociclib was found to be linear with a correlation coefficient of 0.998 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.43 and 0.68 for Letrozole and Ribociclib which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.61 and 0.85 for Letrozole and Ribociclib which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.80% and 99.17% for Letrozole and Ribociclib. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ is 3 and 10.The LOD and LOQ for Letrozole was found to be 2.98 and 9.95 and LOD and LOQ for Ribociclib was found to be 2.95 and 9.98. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

7. Conflict of Interest: None

8. REFERENCES:

[1] Skoog *et al*., Principles of Instrumental Analysis. Barkhanath publishers, 8th edition, Pg. no. 973-995.

[2] Hobart.H.Willard *et al*., Instrumental methods of analysis, CBS Publications and Distributors, New Delhi, 1st edition ,1986, Pg. no 529-563.

[3] Bhatnagar AS. The discovery and mechanism of action of letrozole. Breast Cancer Res Treat. 2007;105 (Suppl 1):7-17.

[4] De Laurentiis M, Caputo R, et., al. Safety and Efficacy of Ribociclib in Combination with Letrozole in Patients with HR+, HER2- Advanced Breast Cancer: Results from the Italian Subpopulation of Phase 3b CompLEEment-1 Study. Target Oncol. 2022 Nov;17(6):615-625.

[5] Namratha, S., and V. A. "Method Development and Validation of Lopinavir In Tablet Dosage Form Using Reversed-Phase High-Performance Liquid Chromatography". Asian Journal of Pharmaceutical and Clinical Research, vol. 11, no. 16, Dec. 2018, pp. 125-8.

[6] Interna ICH of technical requirements for the registration of pharmaceuticals for human use, validation of analytical parameters; methodology adopted in 1996, Geneva.

[7] ICH Guidelines Q2B, Validation of Analytical Procedure: Definitions, published in March 1996, Geneva, Switzerland.

[8] Sunkara Namratha, A. Vijayalakshmi. A Validated Stability Indicating UPLC Method for Simultaneous Determination and Degradation studies of Sofosbuvir and Velpatasvir in Pharmaceutical Dosage Forms. Research J. Pharm. and Tech 2021; 14(3):1658-1662.

[9] Teresa I, Lucas, Bishara RH, Robert HS. A Stability Program for the Distribution of Drug Products. Pharmaceutical Technology Resource Guide 2004; 86-9.

[10] Singh S, Bakshi M. Development of validated stability-indicating assay methods critical review. J Pharm Biomed Anal 2002; 28: 1011-40.

[11] ICH Harmonised Tripartite Guideline Stability Testing of New Drug Substances and Products Q1A (R2) 2003; 2: 1-24.

[12] Posocco B, Buzzo M, Poetto AS, Orleni M, Gagno S, Zanchetta M, Iacuzzi V, Guardascione M, Puglisi F, Basile D, Pelizzari G, Marangon E, Toffoli G. Simultaneous quantification of palbociclib, ribociclib and letrozole in human plasma by a new LC-MS/MS method for clinical application. PLoS One. 2020 Feb 7;15(2):e0228822. doi: 10.1371/journal.pone.0228822.

[13] Turković L, Bočkor L, Ekpenyong O, Silovski T, Lovrić M, Crnković S, Nigović B, Sertić M. Development and Validation of a Novel LC-MS/MS Method for the Simultaneous Determination of Abemaciclib, Palbociclib, Ribociclib, Anastrozole, Letrozole, and Fulvestrant in Plasma Samples: A Prerequisite for Personalized Breast Cancer Treatment. Pharmaceuticals (Basel). 2022 May 16;15(5):614. doi: 10.3390/ph15050614.

[14] Hegde, A.R., Padya, B.S., Soman, S. *et al*. A simple, precise, and sensitive HPLC method for quantification of letrozole in rat plasma: development, validation, and preclinical pharmacokinetics. J Anal Sci Technol 12, 25 (2021). https://doi.org/10.1186/s40543-021-00276-4.

[15] Prasanthi, S. & Himabindu, G. & Kumar, Yagati. (2022). Bioanalytical Method For Simultaneous Estimation Of Ribociclib And Letrozole And Its Application To Pharmacokinetic Studies Using Ultra-Performance Liquid Chromatography. International Journal of Applied Pharmaceutics. 14. 95-102.

[16] Posocco B, Buzzo M, Poetto AS, Orleni M, Gagno S, Zanchetta M, *et al*. (2020) Simultaneous quantification of palbociclib, ribociclib and letrozole in human plasma by a new LC-MS/MS method for clinical application.