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ORIGINAL ARTICLE

A Stability Indicating Liquid Chromatographic Assay Method for the Simultaneous Determination of anti-cancer drugs in bulk and Pharmaceutical Dosage Forms

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Abstract

Combination dosage forms are pharmaceutical preparations that include two or more active therapeutic agents within a single unit. These formulations are widely used in clinical practice because they help simplify treatment regimens, improve patient adherence, and enhance overall therapeutic effectiveness. In the present study, a stability-indicating assay method was developed and validated according to ICH guidelines for the simultaneous estimation of Letrozole and Ribociclib in combined pharmaceutical formulations. Chromatographic separation was performed using an ODS C18 column (250 × 4.6 mm, 5 µm) under isocratic conditions with a flow rate of 0.9 mL/min. The method demonstrated linearity for Letrozole across 1.25–6.25 µg/mL and for Ribociclib across 100–500 µg/mL. Validation results confirmed that both intraday and interday precision showed %RSD values below 2%, and accuracy studies yielded recoveries within 98–102%, meeting acceptable analytical standards. Thus, the developed method is precise, accurate, and reliable, making it suitable for routine quality control of these combination dosage forms.

Keyword: Ribocicilib, Letrozole, Method Validation, Isocratic

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Introduction

Combining More than one active component with complementary mechanisms of action can lead to synergistic effects; enhancing therapeutic efficacy and can simplify medication regimens by reducing the number of pills or doses a patient needs to take (Minghao et al., 2016). Dosage forms of these category can improves patient adherence to treatment, and is easier for patients to remember to take one medication rather than multiple separate ones and this category of dosage forms are particularly beneficial for patients with chronic conditions who may need to take multiple medications daily. By combining multiple drugs into a single dosage form, it may be possible to achieve therapeutic effects at lower doses of each drug, potentially reducing the risk of side effects associated with higher doses (Jarde et al., 2011). Multi component dosage forms allow for targeting multiple aspects of the disease process

simultaneously leading to more comprehensive treatment and the particular strategy is used in antiretroviral therapy (Tan et al., 2014).

Multi Component dosage forms can be customized to meet the specific needs of individual patients by adjusting the doses and ratios of the active ingredients. This flexibility allows healthcare providers to tailor therapy based on factors such as disease severity, patient preferences, and drug interactions.

Ribociclib chemically 7-Cyclopentyl-N,N-dimethyl-2-{[5-(1-piperazinyl)-2-pyridinyl]amino}-7H-pyrrolo [2,3-d]pyrimidine-6-carboxamide is a kinase inhibitor having molecular formula $C_{23}H_{30}N_8O$ and molecular weight 434.5 g/mol used to treat HR+, HER2- advanced or metastatic breast cancer. 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 (Riggins et al ., 2005).

Letrozole chemically 4,4'-((1H-1,2,4-triazol-1-yl) methylene)dibenzonitrile having molecular formula C17H11N5 and molecular weight 285.3 g/mol is an aromatase inhibitor used to treat breast cancer in postmenopausal women. Letrozole is a third-generation, type II aromatase inhibitor widely used in the management of estrogen- dependent breast cancer (Raymond et al., 2014). Aromatase inhibitors function by blocking the activity of the aromatase enzyme, which is responsible for converting androgens into estrogens through the process of aromatization. Since the growth of breast tissue is stimulated by estrogen, reducing its synthesis helps suppress the recurrence and progression of hormone- dependent breast tumors. Letrozole, a third- generation type II aromatase inhibitor, is widely used in the treatment of estrogen-dependent breast cancers (Lee et al., 2011). The combination of Ribociclib and Letrozole is clinically employed for the treatment of hormone receptor (HR)- positive, HER2-negative advanced or metastatic breast cancer in premenopausal, perimenopausal, and postmenopausal women (Kala et al., 2017; Sreelakshmi et al., 2019; Bao et al., 2019; Kaplan et al., 2018; Mondal et al., 2009; Dange et al., 2018). The Chemical Structures of Ribociclib and Letrozole were shown in **Figure 1** and **Figure 2**.

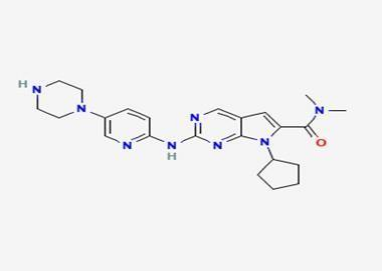

Figure 1: Chemical Structure of Ribociclib

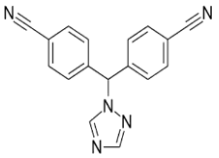

Figure 2: Chemical Structure of Letrozole

Methodology

Materials and Methods

All chemicals used throughout this study were of analytical grade. The solvents used for analysis were of HPLC Grade. Pure Drugs of Ribociclib and Letrozole were kindly gifted from Nutech Biosciences Pvt Ltd, Hyderabad and marketed formulations are purchased from local market.

Instrument and Software

Waters 2695 HPLC System connected to PDA Detector using Empower 2 software for data acquisition and processing. The HPLC Column was Octa Decayl SilaIne (ODS) C18 (250*4.6 mm, 5 µm) was used during analysis. The Injection volume was 10µl for both standard and samples and before analysis reference and working samples were filtered through 0.22µm filters. Run time of 10 min seems to be sufficient for separating the dosage forms followed by washing with buffer for a period of 4 min between the runs.

Procedures

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 nm. At this wavelength both the drugs show good absorbance.

Preparation of 0.1% Ortho phosphoric acid buffer:
Accurately measure 0.1 mL of ortho-phosphoric acid and dilute it to 100 mL with HPLC-grade water.

Preparation of mobile phase:
Combine 350 mL of the prepared buffer (35%) with 650 mL of HPLC-grade methanol (65%). Degas the mixture in an ultrasonic bath for 5 minutes, then filter it through a 0.45 µm membrane using vacuum filtration. The resulting mobile phase was also used as the diluent.

Preparation of Sample Solution:

Accurately weigh and transfer a quantity of sample equivalent to 200 mg of Ribociclib and 2.5 mg of Letrozole into a clean, dry 100 mL volumetric flask. Add approximately 70 mL of diluent and sonicate until the sample is completely dissolved, then make up the volume to 100 mL with the same diluent. From this stock solution, pipette 1.5 mL into a 10 mL volumetric flask and dilute to volume with the diluent.

Validation Parameters

Linearity:
Accurately weigh 200 mg of Ribociclib and 2.5 mg of Letrozole and transfer them into a clean 100 mL volumetric flask. Add about 70 mL of diluent, sonicate until dissolved, and then make up the volume to 100 mL with diluent.From this stock, pipette 0.5, 1.0, 1.5, 2.0, and 2.5 mL into separate 10 mL volumetric flasks and dilute to volume. These dilutions yield concentrations of 100–500 ppm for Ribociclib and 1.25–6.25 ppm for Letrozole. Inject each level, record the peak areas, and prepare a calibration curve by plotting concentration versus peak area to determine the correlation coefficient..

Precision:
Accurately weigh 200 mg of Ribociclib and 2.5 mg of Letrozole working standards and transfer them into a clean, dry 100 mL volumetric flask. Add approximately 70 mL of diluent, sonicate until fully dissolved, and then make up the volume to 100 mL with the same diluent. From this stock solution, pipette 1.5 mL into a 10 mL volumetric flask and dilute to volume.

The resulting standard solution was injected six times into the HPLC system, and the peak areas were recorded. The %RSD of the five replicate injections was within acceptable limits.

Intermediate precision/Ruggedness:
Intermediate precision (ruggedness) was assessed by performing the precision study on a different day within the same laboratory. Accurately weigh 200 mg of Ribociclib and 2.5 mg of Letrozole working standards and transfer them into a clean, dry 100 mL volumetric flask. Add approximately 70 mL of diluent, sonicate to achieve complete dissolution, and then make up the volume to 100 mL with the same diluent. From this stock solution, pipette 1.5 mL into a 10 mL volumetric flask and dilute to volume. The prepared solution was injected five times into the HPLC system, and the peak areas were recorded. The %RSD of the replicate injections was found to be within acceptable limits.

Accuracy
For the accuracy study, three concentration levels—50%, 100%, and 150%—were prepared independently for the analytes, and their chromatograms were recorded. The standard solution along with the 50%, 100%, and 150% accuracy samples were injected into the system. The amount added and amount found for Ribociclib and Letrozole were calculated, and the individual as well as mean recovery values were determined.

Limit of Detection:
Accurately weigh 200 mg of Ribociclib working standard and transfer it into a clean, dry 100 mL volumetric flask. Add approximately 70 mL of diluent, sonicate until fully dissolved, and then make up the volume to 100 mL with the same diluent.

From this primary stock solution, pipette 1.5 mL into a 10 mL volumetric flask and dilute to volume. Then, transfer 0.5 mL of this intermediate solution into another 10 mL volumetric flask and dilute to volume. Finally, pipette 0.1 mL of the resulting solution into a 10 mL volumetric flask and dilute to the mark with diluent.

Preparation of Letrozole solution: Accurately weigh 2.5 mg of Letrozole working standard and transfer it into a clean, dry 100 mL volumetric flask. Add approximately 70 mL of diluent, sonicate until fully dissolved, and then make up the volume to 100 mL with the same diluent.

From this stock solution, pipette 1.5 mL into a 10 mL volumetric flask and dilute to volume. Then pipette 0.1 mL of this solution into another 10 mL volumetric flask and dilute to the mark. Finally, transfer 0.7 mL into a separate 10 mL volumetric flask and dilute to volume with diluent

Limit of Quantitation:

Preparation of Ribociclib solution: Pipette out 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: Pipette out 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent. Further pipette 0.2 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Results and Discussion

System Suitability:

Resolution between two drugs was more than 2 and Theoretical plates more than 2000 and tailing factor was found to be less than 2. From the data it was found that all the system suitability parameters for developed method were within the limit and results are tabulated in **Table 1** and chromatogram was shown in **Figure 3**.

Linearity:

The linearity was found to be in the range of 100 µg/ml to 500 µg/ml for Ribociclib, 1.25 µg/ml to 6.25µg/ml for Letrozole and linearity results are tabulated in **table 3** and calibrations graphs are represented in **Figure 6 and Figure 7** and correlation coefficient value was found to be 0.999 which was found to be within acceptable limits and results are tabulated in **table 3**.

Precision:

Precision of the method was carried out for both sample solutions as described under experimental work and results are tabulated in **Table 4**. The % RSD for the standard solution is below 1, which is within the limits hence method is precise and re- sults are shown in **table 4**.

Intermediate Precision (Ruggedness):

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 and results are shown in **table 5**. %RSD of five different sample solutions should not more than 2 and %RSD obtained is within the limit, hence the method is rugged.

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	2094603	196622	4.06	1.71	2947.68
2	Ribociclib	2.529	3694090	286174	4.38	1.61	3826.77

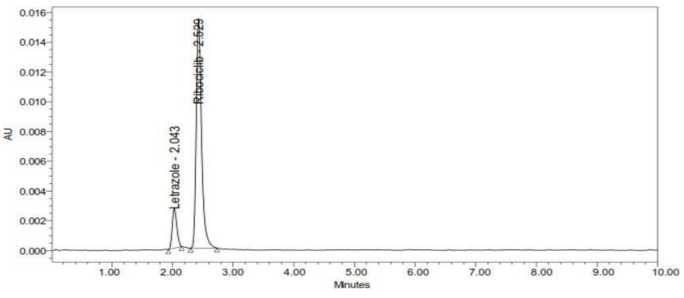

Figure 3: Chromatogram for system suitability

Assay:

Standard and sample solution injected as described under experimental work. The corresponding chromatograms and results are shown below in **Figure 4** and **Figure 5** and results are tabulated in **Table 2**.

Table 2: Results of Assay for Letrozole and Ribociclib

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

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	75814	100	1206413
2	2.5	141758	200	2408658
3	3.75	212645	300	3640611
4	5	286395	400	4819318
5	6.25	363215	500	5937929

Table 4: Results of Precision

Injection	Area of Letrozole	Area of Ribociclib
Injection-1	210170	3705273
Injection-2	210522	3710274
Injection-3	210720	3715132
Injection-4	211598	3725737
Injection-5	211775	3728935
Injection-6	210258	3715688
Average	210840.5	3716839.83
Standard Deviation	685.85	9017.74
%RSD	0.33	0.24

Table 5: Results of Intermediate precision for Letrozole

Injection	Area of Letrozole	Area of Ribociclib
Injection-1	211763	3732160
Injection-2	212690	3745179
Injection-3	213061	3747032
Injection-4	213193	3751496
Injection-5	214043	3757903
Injection-6	212258	3715688
Average	212834.667	3741576.33
Standard Deviation	792.67	15274.66
%RSD	0.37	0.41

Accuracy:

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated and results are tabulated in **Table 6** and **Table 7** for Letrozole and Ribociclib. The percent- age recovery was found to be within the limits and method is accurate

Limit of Detection:

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio and results are shown below along with chromatograms in **figure 8** and tabulated in **Table 8** and result is obtained within the limit

Limit of Quantification:

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio and chromatogram was shown in **figure 9** and results are tabulated in **Table 9**.

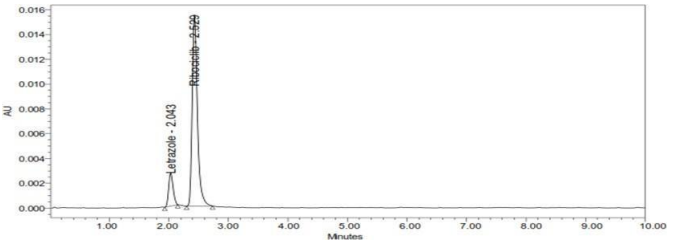

Figure 4: Chromatogram for Standard

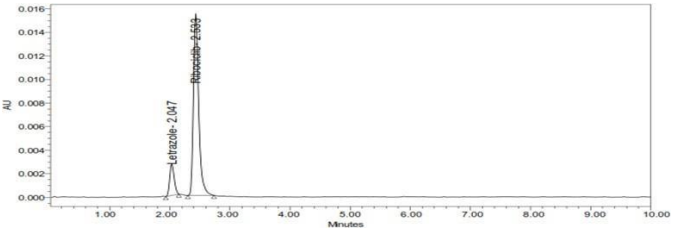

Figure 5: Chromatogram for Sample

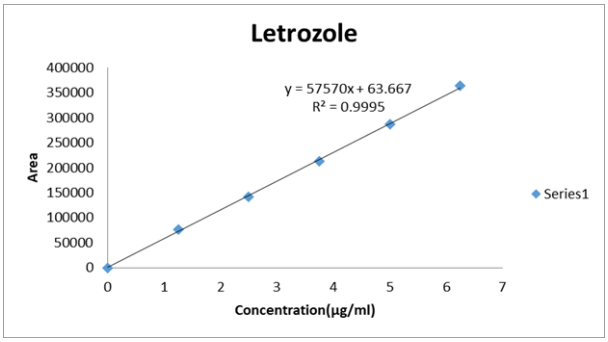

Figure 6: Calibration graph for Letrozole

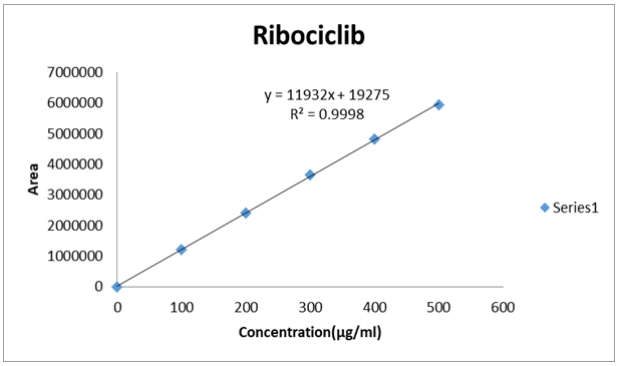

Figure 7: Calibration graph for Ribociclib

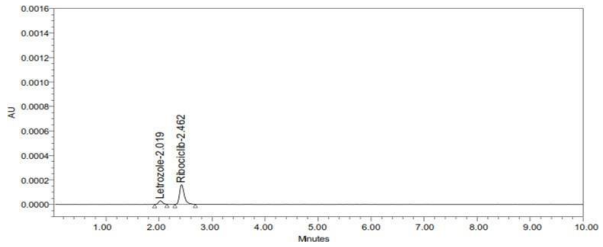

Figure 8: Chromatogram of Letrozole, Ribociclib showing LOD

Table 6: Accuracy (recovery) data for Letrozole

% Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	105464	1.25	1.248	99.92	
100%	207981	2.5	2.49	99.26	99.09
150%	308308	3.75	3.69	98.09	

Table 7: Accuracy (recovery) data for Ribociclib

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1882082	100	100.09	101.87	
100%	3676645	200	199.95	99.51	100.73
150%	5588103	300	300.12	100.82	

Table 8: Results of LOD

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Letrozole	55	164	2.98
Ribociclib	55	162	2.95

Table 9: Results of LOQ

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Letrozole	55	548	9.96
Ribociclib	55	549	9.98

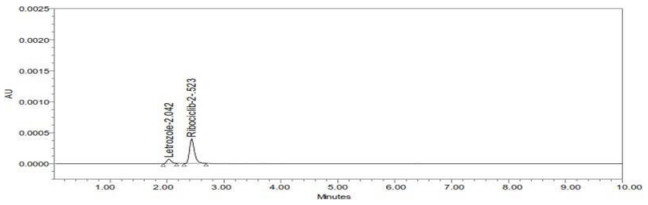

Figure 9: Chromatogram of Letrozole, Ribociclib showing LOQ

Conclusion

The Proposed method successfully separated and simultaneously quantified all the components. The procedure used for analysis was simple and fast and can be used for routine quality control analysis. Validation parameters were found to meet the specified ICH Standards allowing the method to pharmaceutical fields. The method was proved to be excellent for quality control and serves as a promising foundation for further development particularly in terms of compatibility and its application.

Disclosure

Ethics approval and consent to participate

Not Applicable

Competing interests

NIL

Funding

Nil

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