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A New RP-HPLC Method for Simultaneous Estimation of Ribociclib and Letrozole in Its Pure and Tablet Dosage Form as Per ICH Guidelines

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A B S T R A C T

A simple, precise, and reliable RP-HPLC method was developed and validated for the simultaneous estimation of Letrozole and Ribociclib in bulk and pharmaceutical dosage forms in accordance with ICH Q2(R1) guidelines. Chromatographic separation was achieved using a Waters C18 column (4.6×250mm, 5 µm) with a mobile phase of 0.1% ortho phosphoric acid buffer and methanol (25:75 v/v) at a flow rate of 1.0 mL/min, and detection wavelength of 227 nm. Retention times were 2.04 min for Letrozole and 2.52 min for Ribociclib, with system suitability parameters meeting all acceptance criteria. Validation results demonstrated excellent linearity (R^2 = 0.999), high accuracy (recoveries 99–101%), precision (%RSD < 1%), and sensitivity with low LOD and LOQ values. Robustness and ruggedness studies confirmed the method's reliability under deliberate variations in flow rate and mobile phase composition, while intermediate precision showed consistent performance across days and instruments. Overall, the developed method is simple, cost-effective, accurate, and sensitive, making it highly suitable for routine quality control, stability studies, batch release testing, and regulatory applications for Letrozole and Ribociclib formulations.

Keywords: C18 column, Robustness, ruggedness, reliable RP-HPLC, LOD, LOQ

A R T I C L E I N F O

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1. Introduction

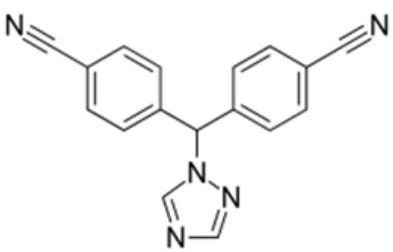

Fig.1: Letrozole

Table.1: Drug profile of Letrozole

IUPAC Name	4-[(4-cyanophenyl)(1H-1,2,4-triazol-1-yl)methyl]benzonitrile
Categories	Letrozole is an aromatase inhibitor used to treat breast cancer in postmenopausal women.
Chemical Formula	$C_{17}H_{11}N_5$
Molecular weight	285.30
Melting point	175-178 °C
Solubility	4.95e-04 g/l

pKa	4.33
Mechanism of action	Letrozole is a non-steroidal type II aromatase inhibitor. It blocks the active site, and therefore the electron transfer chain of CYP19A1. This competitive inhibition prevents the conversion of androgens to estrogen. This action leads to a reduction in uterine weight and elevated leuteinizing hormone. In postmenopausal women, the action of aromatase is responsible for the majority of estrogen production. With reduced availability of estrogen, estrogen-dependent tumors regress. Third generation aromatase inhibitors do not significantly affect cortisol, aldosterone, and thyroxine levels.

Letrozole, or CGS 20267, is an oral non-steroidal type II aromatase inhibitor first described in the literature in 1990. It is a third generation aromatase inhibitor like exemestane and anastrozole, meaning it does not significantly affect cortisol, aldosterone, and thyroxin.

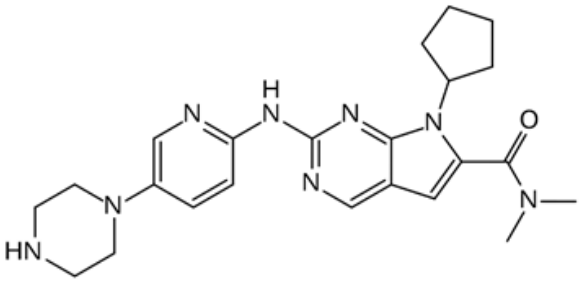

Fig.2: Ribociclib

Table.2: Drug profile of Ribociclib

IUPAC Name	7-cyclopentyl-N,N-dimethyl-2-{[5-(piperazin-1-yl)pyridin-2-yl]amino}-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide.
Formula	C27H36N8O5
Molecular weight	552.63
Freely soluble at pH 1	Methanol
pKa(strongest basic)	7.6
Refractivity	108.89
Categories	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).

Ribociclib is an anti-cancer medication. It works by blocking proteins (kinases) which are important for the growth and division of cells. This slows the growth of cancer cells and eventually kills them.

2. Materials and Methods

Table 3: List of Equipment's used

S.N	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module.2487 UV detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000+
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 4: List of Materials Used

S.N	Chemical	Brand
1	Letrozole	Novartis Health care
2	Ribociclib	Novartis Health care
3	Ortho phosphoric acid	FINAR chemical Ltd
4	Water and Methanol for HPLC	Standard solutions Ltd
5	Acetonitrile for HPLC	Standard solutions Ltd
6	HCl, H_2O_2, NaOH	Merck

HPLC Method Development:

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.

Optimization of Column:

Waters C_{18} (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.

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.

Optimized chromatogram is shown in the **fig 2.** System suitability parameters are shown in **fig 2** and the results are shown in **Table 6.**

Preparation of buffer and mobile phase:

Preparation of 0.1% Ortho phosphoric acid buffer:

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

Preparation of mobile phase: Mix a mixture of above buffer 250 ml (25%) and 750 ml Methonol 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.

Validation parameters:

1. Assay:

Standard Solution Preparation:

Accurately weigh and transfer 250 mg of Ribociclib & 3.13 mg of Letrozole working standard into a 250ml 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 6 ml of Ribociclib & 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 250 mg of Ribociclib & 3.13 mg of Letrozole working standard into a 250ml 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 6 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 & Letrozole peaks and calculate the %Assay by using the formulae. The chromatograms were recorded as show in **Fig 3, 4** and results are shown in **Table- 7.**

2. Linearity:

Preparation of stock solution: Accurately weigh and transfer 250 mg of Ribociclib & 3.13 mg of Letrozole working standard into a 250ml 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)

Preparation of Level–I (200ppm & 2.5ppm 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 (400 ppm &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 (600 ppm & 7.5ppm 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 (800 ppm & 10 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 (1000 ppm & 12.5ppm 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.

Precision:

Preparation of stock Solution: Accurately weigh and transfer 250 mg of Ribociclib & 3.13 mg of Letrozole working standard into a 250ml 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 6 ml of Ribociclib & Letrozole of 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 %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate precision/ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day within the laboratory.

Preparation of stock solution:

Accurately weigh and transfer 250 mg of Ribociclib & 3.13 mg of Letrozole working standard into a 250ml 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 6 ml of Ribociclib & Letrozole of 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 was found to be within the specified limits.

5. Accuracy: 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.

Preparation of Standard stock solution:

Accurately weigh and transfer 250 mg of Ribociclib & 3.13 mg of Letrozole working standard into a 250ml 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 6 ml of Ribociclib & Letrozole of 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 125 mg of Ribociclib & 1.56 mg of Letrozole working standard into a 250ml 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 6 ml of Ribociclib & Letrozole of 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 250 mg of Ribociclib & 3.13 mg of Letrozole working standard into a 250ml 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 6 ml of Ribociclib & Letrozole of 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 375 mg of Ribociclib & 4.69 mg of Letrozole working standard into a 250ml 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 6 ml of Ribociclib & Letrozole of 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 & Letrozole and calculate the individual recovery and mean recovery values.

Limit of detection:

Preparation of Letrozole solution:

Preparation of 0.01µg/ml solution: Accurately weigh and transfer 3.13 mg of Letrozole working standard into a 250ml clean dry volumetric flask add about 100ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 6 ml of Letrozole the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent. Further pipette 0.1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent. Further pipette 0.7ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Ribociclib solution:

Preparation 0.48µg/ml solution: Accurately weigh and transfer 250 mg of Ribociclib working standard into a 250ml 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 6 ml of Ribociclib the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent. Further pipette 0.1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 0.8ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents

Limit of quantification:

Preparation of 0.14µg/ml solution:

Accurately weigh and transfer 3.13 mg of Letrozole working standard into a 250ml clean dry volumetric flask add about 100ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 6 ml of Letrozole the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent. Further pipette 2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent. Further pipette 0.9of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of 0.90µg/ml solution:

Accurately weigh and transfer 250 mg of Ribociclib working standard into a 250ml clean dry volumetric flask add about 100ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 6 ml of Ribociclib the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent. Further pipette 0.1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent. Further pipette 1.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

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.

The flow rate was varied at 0.8 ml/min to 1.2ml/min: Standard solution 600 & 7.5 µg/ml of Ribociclib & Letrozole prepared and analysed using the varied flow rates along with method flow rate.

The Organic composition in the Mobile phase was varied from 67.5% to 82.5%: Standard solution 600 & 7.5 µg/ml of Ribociclib & Letrozole was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

3. Results and Discussion

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, 5.0 m)

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 ml

Run time : 10min

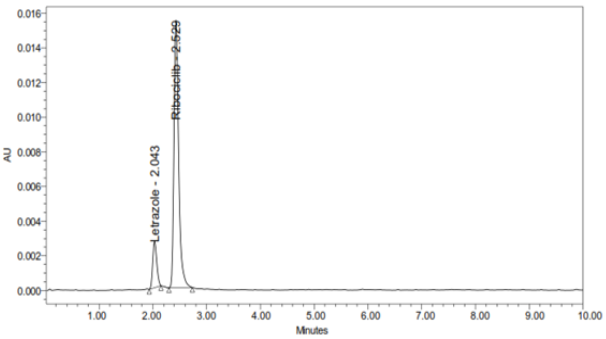

Fig.3: Chromatogram for system suitability

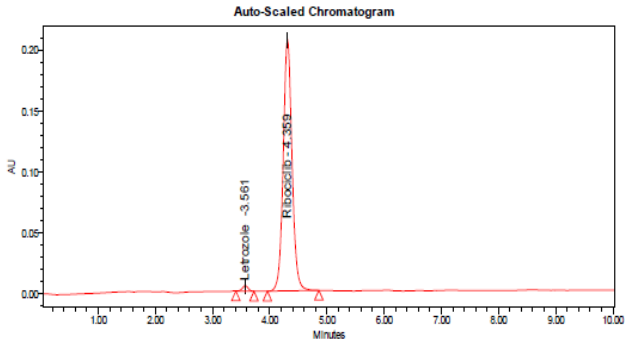

Fig.4: Chromatogram for Sample

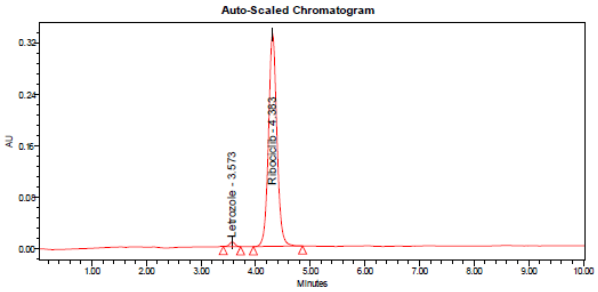

Fig.5: Chromatogram for Standard

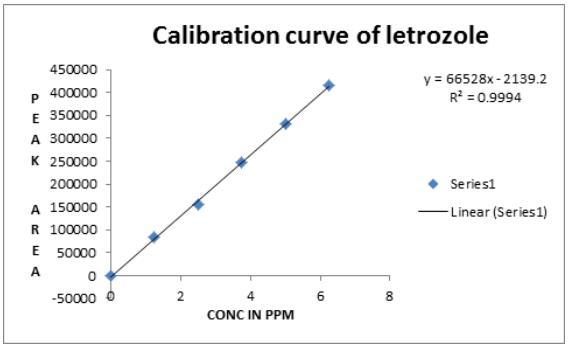

Fig.6: Calibration graph for Letrozole

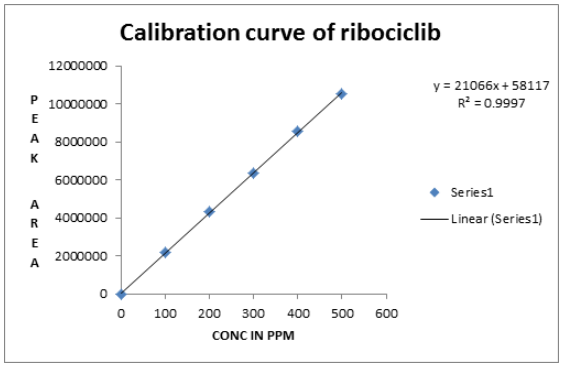

Fig.7: Calibration graph for Ribociclib

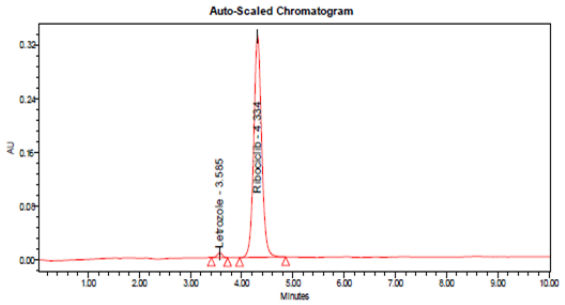

Fig.8: Chromatogram for Precision -6

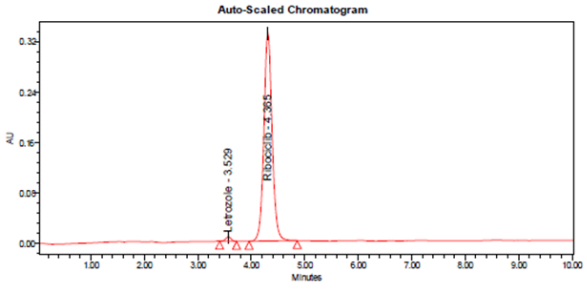

Fig.9: Chromatogram for ID Precision -6

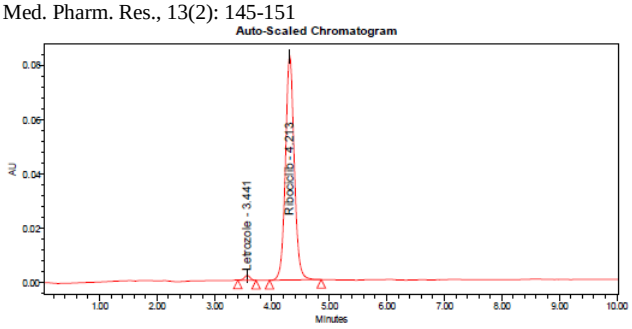

Fig.10: Chromatogram for Accuracy 50%-3

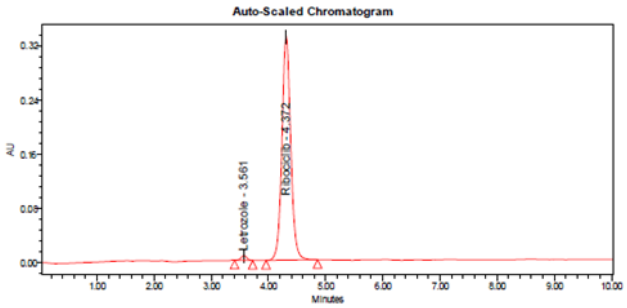

Fig.11: Chromatogram for Accuracy 100%-3

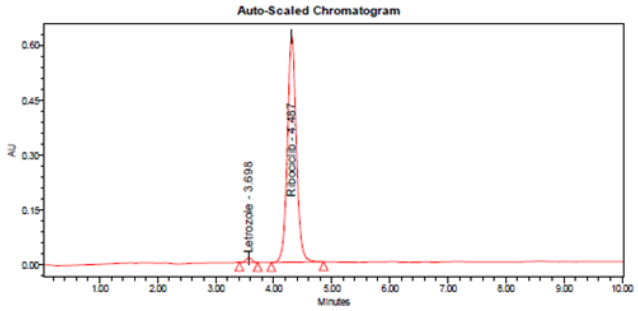

Fig.12: Chromatogram for Accuracy 150%-3

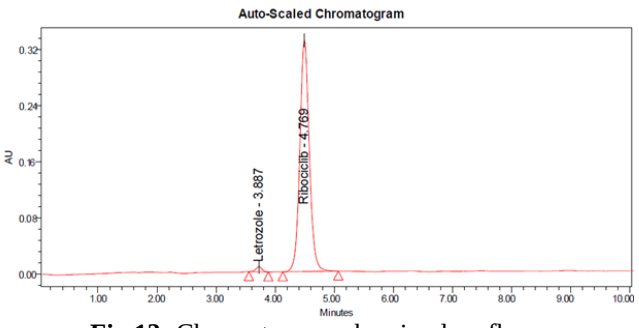

Fig.13: Chromatogram showing less flow

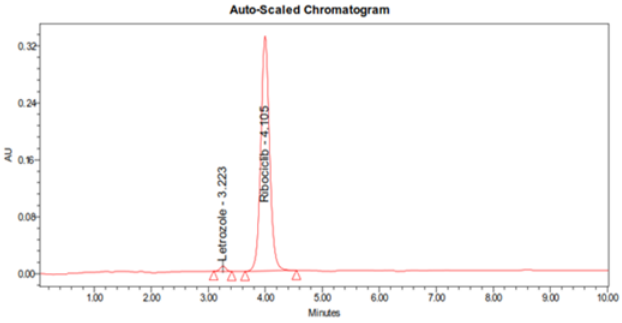

Fig.14: Chromatogram showing more flow

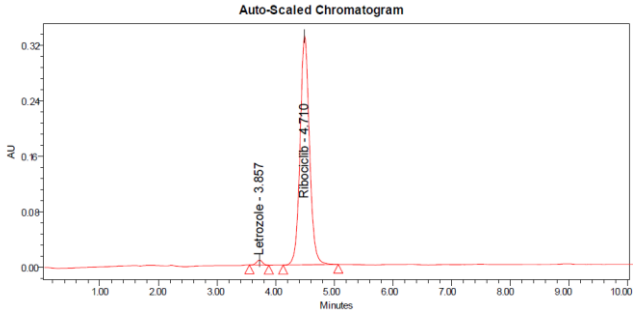

Fig.15: Chromatogram showing less organic composition

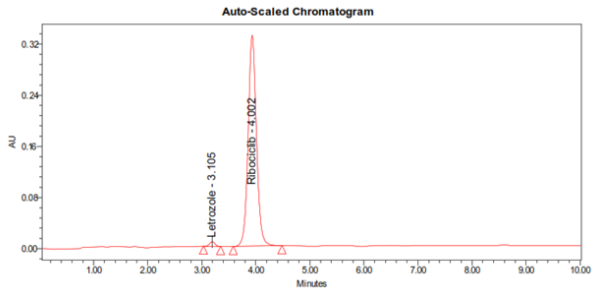

Fig.16: Chromatogram showing more organic composition

Table 10: Results of Precision

Injection	Area of Letrozole	Area of Ribociclib
Injection-1	248254	6329542
Injection-2	249854	6323654
Injection-3	249548	6328542
Injection-4	249521	6326541
Injection-5	248542	6334785
Injection-6	249547	6329652
Average	249211	6328786
Standard Deviation	647.9852	3708.0043
%RSD	0.3	0.1

Table 12: Results of Intermediate precision for Letrozole

Injection	Area of Letrozole	Area of Ribociclib
Injection-1	250658	6327423
Injection-2	250695	6316331
Injection-3	250745	6328942
Injection-4	250265	6313654
Injection-5	250854	6314123
Injection-6	250746	6319478
Average	250660.5	6319991.8
Standard Deviation	204.6995	6104.7083
%RSD	0.1	0.10

Table 14: Accuracy (recovery) data for Letrozole

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	124685	1.56	1.56	100.1	100.1
100%	249428	3.13	3.12	99.8	
150%	374528	4.69	4.71	100.4	

Table 15: Accuracy (recovery) data for Ribociclib

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	3162462	125	124.5	99.6	99.8
100%	6332572	250	249.4	99.8	
150%	9533542	375	375	100.1	

Table 16: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Letrozole	77	210	2.73
Ribociclib	77	228	2.96

Table 17: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Letrozole	77	750	9.94
Ribociclib	77	766	9.95

4. Conclusion

A simple, precise, and robust RP-HPLC method was successfully developed and validated for the simultaneous estimation of Letrozole and Ribociclib in bulk and pharmaceutical dosage forms as per ICH Q2(R1) guidelines. Chromatographic separation was optimized using a Waters C18 column (4.6 × 250 mm, 5 µm) with a mobile phase of 0.1% ortho phosphoric acid buffer and methanol (25:75 v/v), at a flow rate of 1.0 mL/min and detection wavelength of 227 nm. The method yielded sharp, well-resolved peaks with retention times of 2.04 min for Letrozole and 2.52 min for Ribociclib, meeting all system suitability requirements (resolution >2, theoretical plates >2000, tailing factor <2). Validation results confirmed high accuracy with recoveries of 99.4% and 99.5%, excellent linearity (R^2 = 0.999), precision with %RSD values below 0.5%, and sensitivity with acceptable LOD and LOQ values. Accuracy at 50%, 100%, and 150% concentration levels demonstrated recoveries within the acceptance range of 97–103%. Robustness and ruggedness studies confirmed that small variations in flow rate and mobile phase composition did not significantly affect chromatographic performance, while intermediate precision showed reproducibility across different days and systems. Overall, the developed RP-HPLC method is simple, reliable, cost-effective, and stability-indicating, making it suitable for routine quality control, assay validation, stability testing, and regulatory applications of Letrozole and Ribociclib formulations.Its reproducibility, sensitivity, and compliance with ICH guidelines establish the method as a dependable analytical tool for ensuring consistent quality and therapeutic efficacy of these combination formulations.

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