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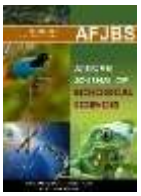

A Stability Indicating Liquid Chromatographic Assay Method for the Simultaneous Determination of anti-cancer drugs in bulk and Pharmaceutical Dosage Forms

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Abstract:

Combination Dosage Forms generally refers to pharmaceutical formulations which contains two or more active ingredients in a single dosage unit or formulation. These combination dosage forms serve several important purposes in health care and play a crucial role by improving treatment outcomes, enhancing patient adherence and optimizing therapeutic regimens for various medical conditions. In this present strategy a stability indicating assay method was developed and validated as per ICH Guidelines for the simultaneous quantitation of Letrozole and Ribociclib in commercially available pharmaceutical dosage forms. The proposed method is based on Isocratic elution using Octa Decyl Silane (ODS) C_{18} (250*4.6 mm, 5 µm) column with a flow rate of 0.9 ml/min. The linear ranges for Letrozole and Ribociclib were found to be in the range of 1.25 -6.25µg/ml and 100-500 µg/ml. During the process of validation the intra and Interday Precision % RSD value was found to be less than 2 and recovery was in the range of 98-102%. The method was successfully applied for routine quality control analysis of dosage forms

Keywords: Ribociclib, Letrozole, Validation, Isocratic

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1. Introduction:

Combining two or more active ingredients 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[1]. This type of dosage forms also can improves patient adherence to treatment, as it is generally easier for patients to remember to take one medication rather than multiple separate ones and this type of dosage 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[2]. Multi component dosage forms allow for targeting multiple aspects of the disease process simultaneously leading to more comprehensive treatment and this approach is commonly used in antiretroviral therapy[3]**.**

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[4].

Letrozole chemically 4,4'-((1H-1,2,4-triazol-1-yl)methylene)dibenzonitrile having molecular formula $C_{17}H_{11}N_5$ and molecular weight 285.3 g/mol is an aromatase inhibitor used to treat breast cancer in postmenopausal women. Letrozole is an aromatase inhibitor used in the treatment of breast cancer[5]. Aromatase inhibitors work by inhibiting the action of the enzyme aromatase, which converts androgens into estrogens by a process called aromatization. As breast tissue is stimulated by estrogens, decreasing their production is a way of suppressing recurrence of the breast tumor tissue. Letrozole is a third generation type II aromatase inhibitor used to treat estrogen dependant breast cancers[6].

Ribociclib and letrozole combination is used to treat hormone receptor (HR)-positive, HER-2 negative advanced or metastatic (cancer that has spread) breast cancer in pre/perimenopausal women, postmenopausal women[7-12]. The Chemical Structures of Ribociclib and Letrozole were shown in Figure 1 and Figure 2.

2. Methodology

2.1 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.

2.2 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 Decyl Silane (ODS) C_{18} (250*4.6 mm, 5 µm) was used during analysis. The Injection volume was 10µl for both standard and samples and before analysis standard and sample solutions were filtered through 0.22µm filters. Run time of 10 min was found to be sufficient for the separation of the drugs followed by washing with buffer for a period of 4 min between the runs

2.3 Procedures

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

2.3.2 Preparation of 0.1% Ortho phosphoric acid buffer: Pipette out 0.1 ml of ortho phosphoric acid and dissolve in 100 ml HPLC water.

2.3.3 Preparation of mobile phase: Mix a mixture of above buffer 350 ml (35%) and 650 ml Methanol HPLC (65%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration and mobile phase was used as diluent.

2.3.4 Preparation of Sample Solution: 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. 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.

2.4 Validation Parameters

2.4.1 Linearity: Accurately weigh and transfer 200 mg of Ribociclib & 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. From the above solution 0.5 ml, 1 ml, 1.5 ml, 2 ml and 2.5 ml has taken in 10 ml of volumetric flask and dilute up to the mark with diluents which gives the concentration range of 100 -500 ppm for Ribociclib and 1.25 -6.25 ppm of Letrozole. 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.

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

2.4.3 Intermediate precision/Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method Precision was performed on different day within the laboratory. Accurately weigh and transfer 200 mg of Ribociclib& 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. 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. 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.

2.4.4 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. 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.

2.4.5 Limit of Detection:

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. Further pipette 1.5 ml of Ribociclib the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent. 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. Further pipette 1.5 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 Diluents. Further pipette 0.7ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents

2.4.6 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.

3. Results and Discussion:

3.1 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.

3.2 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.

3.3 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

3.4 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 results are shown in table 4

3.5 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.

3.6 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 percentage recovery was found to be within the limits and method is accurate

3.7 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

3.8 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.

4. 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.

Conflict of Interest:

The authors declare that there is no conflict of Interest

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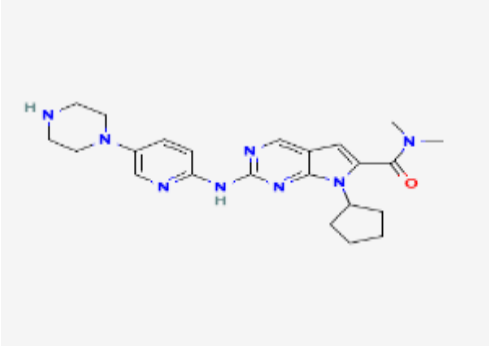

Figure 1: Chemical Structure of Ribociclib

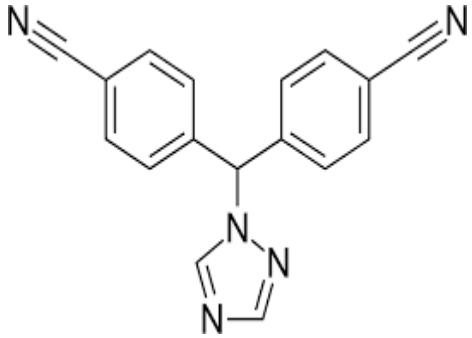

Figure 2: Chemical Structure of Letrozole

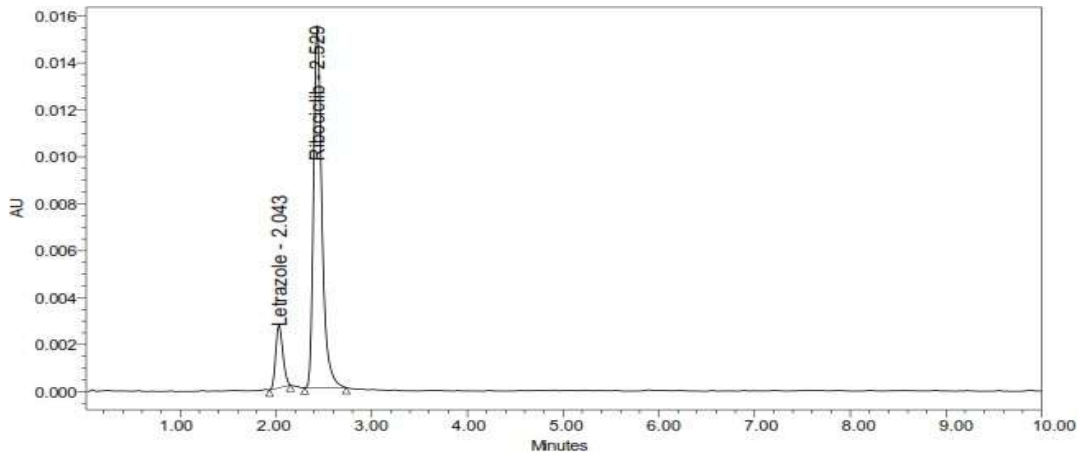

Figure 3: Chromatogram for system suitability

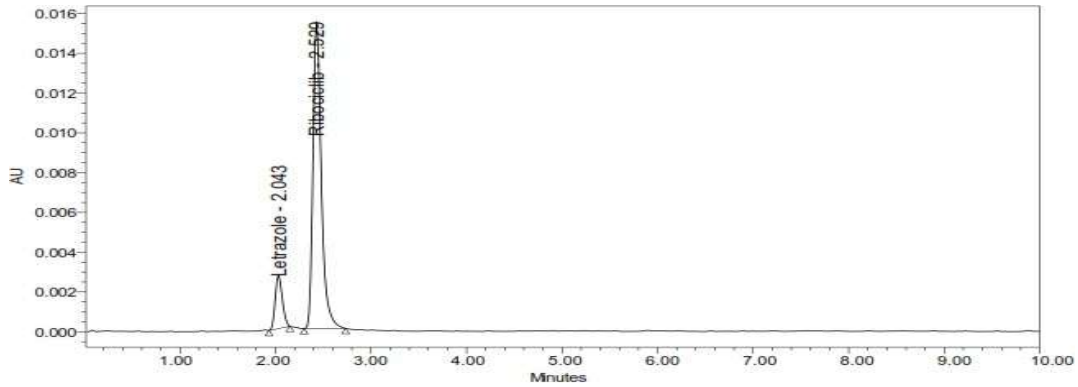

Figure 4: Chromatogram for Standard

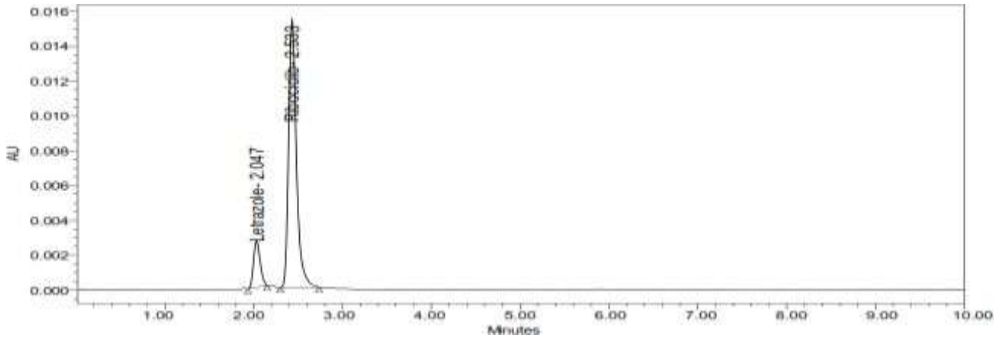

Figure 5: Chromatogram for Sample

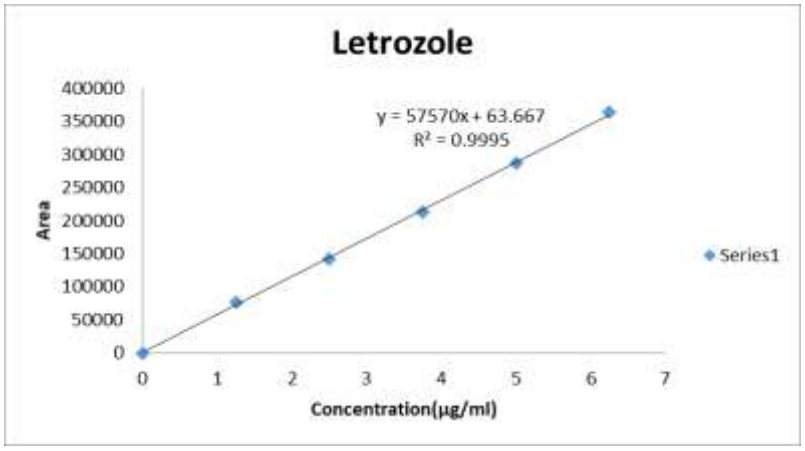

Figure 6: Calibration graph for Letrozole

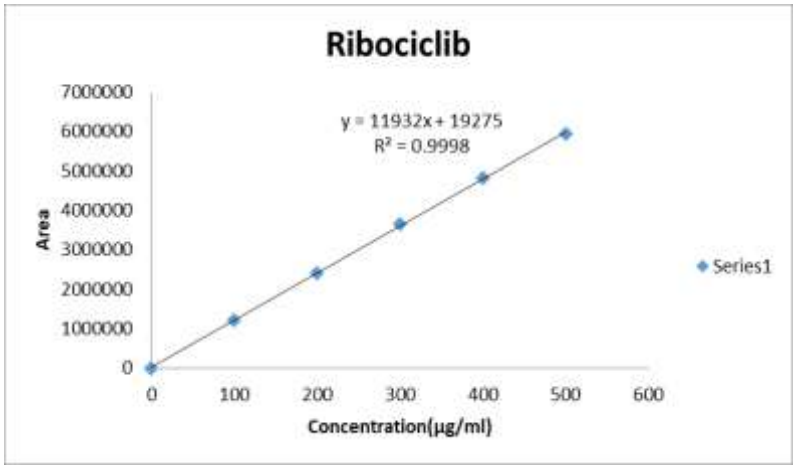

Figure 7: Calibration graph for Ribociclib

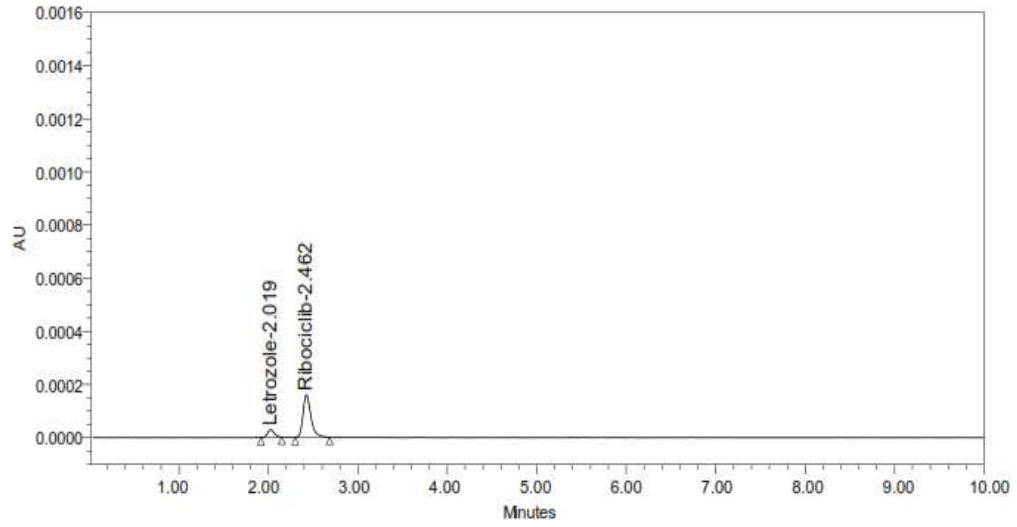

Figure 8: Chromatogram of Letrozole, Ribociclib showing LOD

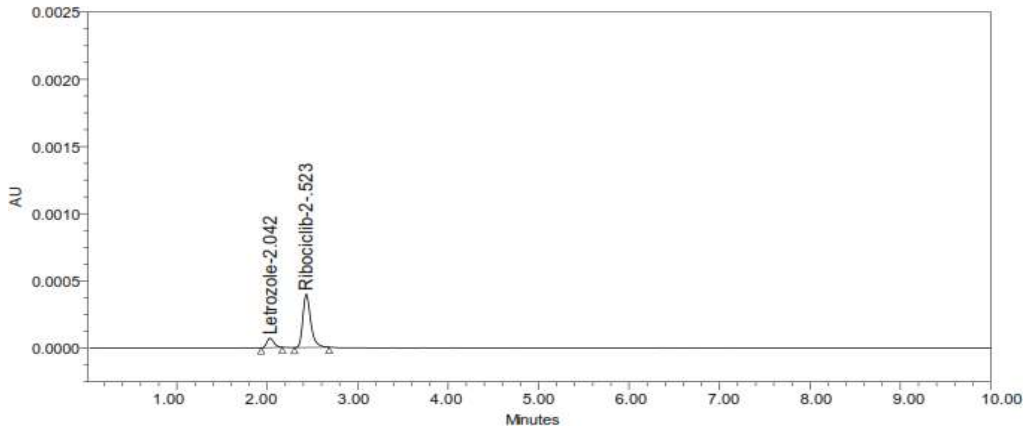

Figure 9: Chromatogram of Letrozole, Ribociclib showing LOQ

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

Table 1: Results of system suitability parameters

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

Table 2: Results of Assay for 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 3: Area of different concentration of Letrozole and Ribociclib

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 4: Results of Precision

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**

Table 5: Results of Intermediate precision for Letrozole

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

Table 6: Accuracy (recovery) data for Letrozole

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

Table 7: Accuracy (recovery) data for Ribociclib

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

Table 8: Results of LOD

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

Table 9: Results of LOQ