Asian Pac. J. Nursing and Health Sci., **2025; 8(2):1-11** **e-ISSN: 2581-7442**

Simultaneous Estimation of Ribociclib and Letrozole in Pure and Pharmaceutical Dosage Form by validated UV- VIS Spectroscopy Method

Sumit Singla[1*], Pankaj Arora[2]

[1]Research Scholar,Department of Pharmacy, Lords University, Alwar, Rajasthan, India.

[2]Professor, Department of Pharmacy, Lords University, Alwar, Rajasthan, India.

**Correspondence*

Sumit Singla,

Research Scholar,Department of Pharmacy, Lords University, Alwar, Rajasthan, India.

E-mail: singlasumit35@gmail.com

Abstract

A simultaneous UV-Visible spectrophotometric method was developed for Ribociclib and Letrozole. Both drugs showed linearity in the range of 2–10 µg/ml with correlation coefficients 0.9997 and 0.9998. Precision studies revealed %RSD < 2% for repeatability, intra-day, and inter-day precision. Accuracy results showed recoveries of 100.29% for Ribociclib and 101.16% for Letrozole, within acceptance limits (98–102%). The assay of marketed formulation confirmed values within the specification range (98–102%). LOD/LOQ values were low, confirming high sensitivity.

Keyworlds: Ribociclib, Letrozole, method development, ICH Guidelines

This is an Open Access article that uses a fund-ing model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Introduction

Ribociclib and letrozole in single-agent and combination formulations should be accurately quantified and characterized, thus playing the fundamental role of quality control of drugs, as well as pharmacokinetic investigations, bioequivalence analysis, and therapeutic monitoring [1]. Analytical methodologies should not only be sensitive, specific, reproducible, and validated as per tough regulatory guidelines like that of the International Council of Harmonization (ICH) [2]. Additionally, Ribociclib, a pyrrole [2,3-d][1,2,3,4]pyrimidine derivative, poses analytical difficulty since it has moderate polarity, difficult biodegradation processes and its photo-oxidative stability [3]. On the other hand, triazole-based molecule like letrozole has a reputation of being thermally and hydrolytically unstable [4]. Therefore, their quantification demands thoughtful optimization of the chromatographic conditions and mobile phase composition as well as detection parameters [5-6]. Analytical methods like the liquid chromatography (HC), ultra-performance liquid chromatography (UPLC) and the mass spectrometry (LC-MS/MS) are some of the techniques that have been instrumental in qualitative and quantitative analysis of such compounds in bulk and biological matrices [7-8]. Further, the knowledge of the degradation kinetics and the forced degradation behavior of Ribociclib and letrozole under stress conditions, such as acidic, basic, oxidative, thermal and photolytic degradations are important in the development of the stability indicating assay [9-10]. Although the forced degradation studies are known to determine only the degradation pathways, they also help in predicting the potential impurities and metabolites that might interfere in the efficacy of the therapies or cause toxicity [11]. These tests are used to guide the design of formulation that has a prolonged shelf- life, stable to different storage conditions and low incidences of adverse interactions when administered with a co-product [12]. At the same time, the demonstration of the analytical procedures in the process of validation based on ICH Q2 (R1) recommendations, including parameters of accuracy, preciseness, linearity, robustness, detection limits, is critical to the reproducibility and regulatory acceptability [13]. The current version of stability-indicating methods has to be highly sensitive and high-resolution of distinguishing active pharmaceutical substances (APIs) and degradation products [14]. Since the field of oncology drug development is rapidly changing accompanied by a rise in the complexity of combination medicine, both the comparative analysis and systematic review of Ribociclib and Letrozole

regarding the available analytical methods and stability profiles is necessary [15].

MATERIAL AND METHOD

Materials

Active Pharmaceutical Ingredients of Ribociclib & Letrozole

Chemicals Used

- Acetonitrile AR Grade (Fisher Scientific Ltd. India)
- Buffer Capsules
- DMSO

Equipments

- Analytical Balance (Model: Mettler AJ100)
- UV-Vis Spectrophotometer 1800 (Shizmazu Corporation ,Kyoto, Japan)
- Sonicator (Sonapros Ultrasonic Processor / Sonicator Oscar ultrasonic Pvt. Ltd Maharashtra, India)
- pH Meter (Instrument India, Mumbai)
- Indicator paper pH 1.0-14.0
- Water bath

Preparation of Standard Stock Solutions

Accurately weighed quantities of Ribociclib (100 mg) and Letrozole (100 mg) were transferred into two separate 100 mL volumetric flasks. Each drug was dissolved in 20 mL of dimethyl sulfoxide (DMSO) and the volume was made up to the mark with distilled water to obtain stock solutions containing 1000 µg/mL of Ribociclib and Letrozole, respectively.

Preparation of Working Standard Solutions

Aliquots from the above stock solutions were further diluted with distilled water to prepare working standard solutions of appropriate concentrations for method development and validation.

Selection of Detection Wavelength

Solutions of each drug in methanol were scanned in the range of 200–400 nm using a UV–Visible spectrophotometer. It was observed that Ribociclib and Letrozole showed significant absorbance maxima at 270 nm and 240 nm, respectively. These wavelengths were selected for simultaneous quantification.

Analysis of Marketed Formulation

Five tablets were weighed accurately, finely powdered, and a portion equivalent to 200 mg of Ribociclib and 2.5 mg Letrozole was transferred into a 100 mL volumetric flask. Methanol (20 mL) was added and the solution was sonicated for 30 minutes. The mixture was filtered through Whatman filter paper No. 41 and the filtrate was diluted to the mark with distilled water.

Preparation of Calibration Curve

Appropriate aliquots of the working standard solutions of Ribociclib and Letrozole were transferred to a series of 10 mL volumetric flasks and diluted to the mark with distilled water to obtain final concentrations in the range of 2–10 µg/mL for both drugs. The absorbance values were recorded at 270 nm and 240 nm.

Preparation of Synthetic Mixture

Synthetic mixtures of Ribociclib and Letrozole were prepared in a 2:1 ratio. Aliquots (1–5 mL) were transferred to separate 10 mL volumetric flasks and diluted with distilled water. The absorbances were measured at 270 nm and 240 nm.

METHOD DEVELOPMENT

The UV spectra of both drugs were scanned from 200–400 nm. Ribociclib showed λmax at 270 nm and Letrozole at 240 nm. These two wavelengths were selected for the development of the simultaneous equation method. Absorptivity values (ax1, ax2, ay1, ay2) were calculated at the selected wavelengths using Beer-Lambert's law.

METHOD VALIDATION

As per ICH Q2(R1) guidelines, the method was validated for Linearity, Accuracy, Precision, Specificity, LOD, LOQ, and Robustness.

Linearity

A good linear relationship between concentration and absorbance over a concentration range of Ribociclib and Letrozole was found 2-10µg/ml. The correlation coefficient was found to be 0.9997 for Ribociclib and 0.9998 for Letrozole these are near about 0.999, ensure that a good correlation existed between the absorbance and concentration. The regression data shows in (Table 1,2, 3& 4) (Fig. 2,3, 4 & 5).

Specificity

Specificity was evaluated by spiking the solution with common excipients (8% starch, 7% magnesium stearate, and 15% lactose) at 1000 µg/mL, followed by filtration. No significant interference in absorbance was observed.

Estimation of Ribociclib and Letrozole in synthetic mixture

The simultaneous equations were applied on the data obtained by the uv spectra. By using the above obtained absorptivity coefficient values given in table for the both drugs at wavelength at 270 nm and 240nm for Ribociclib and Letrozole respectively.The synthetic mixture of the combination of both the drugs was prepared in the ratio of 2:1 (Ribociclib and Letrozole). This ratio of synthetic mixture was selected on the basis of dosage strength of formulation in combination, which

is available in the market. Now the absorbance of the synthetic mixtures were measured at two wavelengths and the concentration of Ribociclib and Letrozole were calculated using following two equations.

Simultaneous Equation Method

For simultaneous estimation of Ribociclib and Letrozole using UV-visible spectroscopy, the following simultaneous equations based on Beer-Lambert's Law are applied:

$A_1 = a_{x1}C_x + a_{\gamma1}C_\gamma$

$A_2 = a_{x2}C_x + a_{\gamma2}C_\gamma$

Solving these equations gives:

$C_x=(A_1a_{\gamma2}–A_2a_{\gamma1})/(a_{x1}a_{\gamma2}–a_{x2}a_{\gamma1})$

$C_\gamma = (A_2a_{x1} – A_1a_{x2}) / (a_{x1}a_{\gamma2} – a_{x2}a_{\gamma1})$

Where:

A_1,A_2= Absorbance at λ_1 and λ_2

a_{x1},a_{x2}= Absorptivity of Ribociclib at λ_1 and λ_2

$a_{\gamma1}$, $a_{\gamma2}$ = Absorptivity of Letrozole at λ_1 and λ_2

Now equation become

$$\mathbf{C_x = (A_2\, a_{y1} - A_1\, a_{y2})/a_{x2}\, a_{y1} - a_{x1}\, a_{y2}} \text{.....Equ. 1}$$

$$\mathbf{C_y = (A_1\, a_{x2} - A_2\, a_{x1})/a_{x2}\, a_{y1} - a_{x1}\, a_{y2}} \text{.....Equ. 2}$$

where,

Cx = the Concentration of Ribociclib

Cy = the concentrations of Letrozole .

a_{x1} and a_{x2} = absorptivities of Ribociclib at λ_1 (λ_{max} of 270) and λ_2 (λ_{max} of 240).

a_{y1} and a_{y2} = absorptivities of Letrozole at λ_1 (λ_{max} of 270) and λ_2 (λ_{max} of 240).

A_1 and A_2 = the absorbance of the diluted samples at λ_1 and λ_2.

The concentration for synthetic mixture of Ribociclib and Letrozole was found to be 12.08 µg/ml and 6.97 µg/ml respectively (Table 12 & 13).

Precision

The %RSD obtained for repeatability study was found to be 0.829 and 0.031 for Ribociclib and Letrozole respectively. These values are below 2% indicates that the repeatability of method is satisfactory (Table 7).

Intra day

The % RSD values for intraday precision were found to be 0.010 for Ribociclib and 0.004 for Letrozole. The % RSD values of data obtained are well below 2% indicates that is Precise (Table 8).

Inter day

The % RSD values for interday were found 0.008 for Ribociclib and 0.005 for Letrozole. The % RSD values of data obtained are well below 2% indicates that is precise. (Table 9 &10)

Limit of detection and limit of quantitation

The LOD values for Ribociclib 0.424 µg/ml and 0.219 µg/ml at 240nm and 270 nm .The LOD values for Letrozole was found to be 0.204 µg/ml and 0.673 µg/ml at 240 nm and 270 nm (Table 6).

The LOQ values for Ribociclib was found to be 1.28µg/ml and 0.66µg/ml at 240nm and 270nm.The LOQ values for Letrozole was found to be 0.619µg/ml and2.039µg/ml at 240nm and 270nm. The obtained results of LOD and LOQ represents that sensitivity of method is very high.

Accuracy

The mean % recovery were found to be 100.29 % for Ribociclib and 101.16 %for Letrozole .These mean recovery values are well within the 98-102% indicates the method is accurate (Table 11).

Analysis of Marketed Formulation

Marketed formulation containing 400 mg Ribociclib and 2.5 mg Letrozole was analyzed. Tablets were powdered, dissolved in methanol, filtered, and analyzed. The % assay was found to be within 98-102% for both drugs. (Table14).

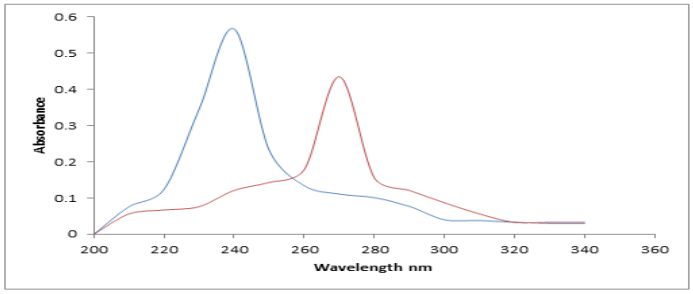

Fig. 1 Overlaps spectra of Letrozole and Ribociclib

Table 1:Linearity Data of Ribociclib at 270nm

Ribociclib at 270nm			
S.No.	**Actual Concentration PPM**	**Absorbance**	**Concentration Found PPM**
1	2	0.102	1.71
2	4	0.203	3.59
3	6	0.311	5.59
4	8	0.421	6.81
5	10	0.532	9.69
	STEYX	0.0036	
	SLOPE	0.0539	
	LOD	0.21904339	
	LOQ	0.66376786	
	c	0.0096	

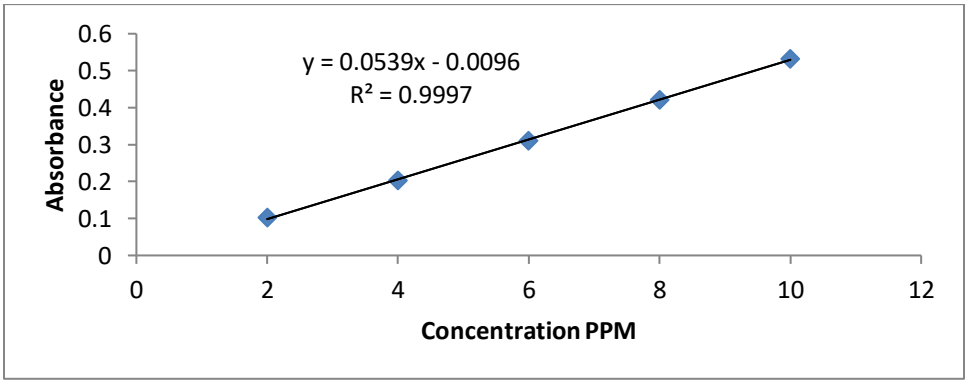

Fig. 2 Calibration curve of Ribociclib at 270nm

Table 2: Linearity Data of Ribociclib at 240nm

S.No.	**Actual Concentration PPM**	**Absorbance**	**Concentration Found PPM**
1	2	**0.054**	2.06
2	4	**0.098**	3.87
3	6	**0.15**	6.01
4	8	**0.202**	7.31
5	10	**0.245**	9.92
	STEYX	0.0031	
	SLOPE	0.0243	
	LOD	0.42368049	
	LOQ	1.28388027	
	c	0.004	

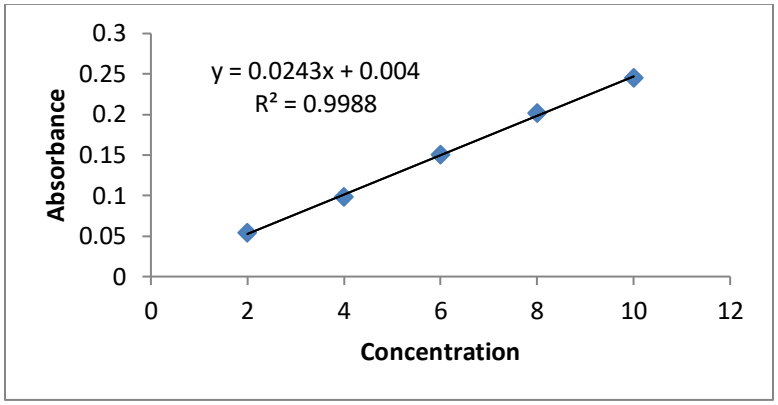

Fig. 3 Calibration curve of Ribociclib at 240 nm

Table 3:. Linearity Data of Letrozole at 270nm

S. No.	Actual Concentration	Absorbance	Concentration Found
1	2	**0.045**	1.21
2	4	**0.098**	3.16
3	6	**0.145**	4.90
4	8	**0.201**	6.40
5	10	**0.265**	9.31
	STEYX	0.0055	
	SLOPE	0.02715	
	LOD	0.673	
	LOQ	2.039	
	c	0.0121	

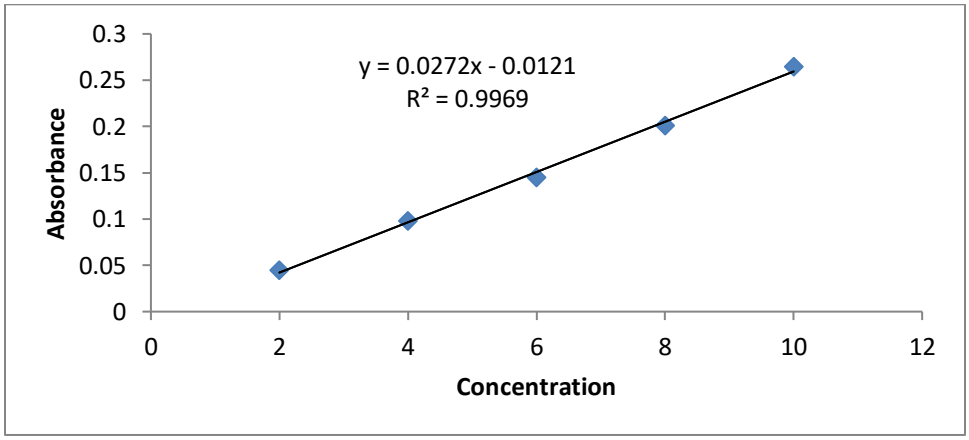

Fig. 4 Calibration curve of Letrozole at 270nm

Table 4: Linearity Data of Letrozole at 240nm

S.No.	Actual Concentration	Absorbance	Concentration Found
1	2	0.166	1.96
2	4	0.325	3.88
3	6	0.487	5.84
4	8	0.656	6.93
5	10	0.828	9.96
	STEYX	0.0051	
	SLOPE	0.08275	
	LOD	0.204	
	LOQ	0.619	
	c	0.0041	

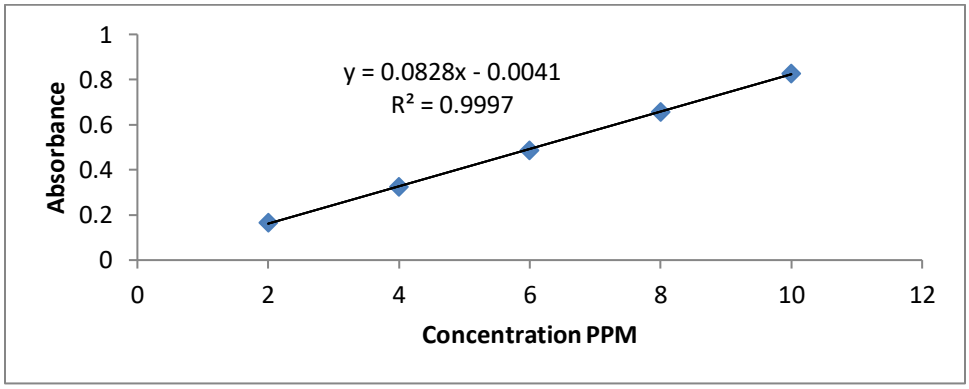

Fig. 5 Calibration curve of Letrozole at 240nm

Table 5: Regression analysis data for Ribociclib and Letrozole

S. No	Parameter	Ribociclib		Letrozole	
		240nm	**270nm**	**240nm**	**270nm**
1	Linearity (µg/ml)	10-50	10-50	10-50	10-50
2	Correlation coefficient(r^2)	0.9983	0.9988	0.9997	0.9969
3	Slope	0.0243	0.0539	0.0828	0.0272
4	Intercept	0.004	0.0096	0.0041	0.0121
5	LOD(µg/ml)	0.424	0.219	0.204	0.673
6	LOQ(µg/ml)	1.284	0.663	0.619	2.039

Table 6: Repeatability for Ribociclib and Letrozole

S.N.	Actual Concentration PPM	Ribociclib		Letrozole	
		Absorbance	Concentration found	Absorbance	Concentration found PPM
1	4	0.211	3.74	0.325	3.88
2	4	0.209	3.70	0.326	3.89
3	4	0.211	3.74	0.324	3.87
4	4	0.213	3.77	0.324	3.87
5	4	0.213	3.77	0.326	3.89
Mean			**3.744**	**Mean**	3.88
STDV			**0.031**	**STDV**	0.01
%RSD			**0.829**	**%RSD**	0.003116

Table 7: Intraday precision of Ribociclib and Letrozole

S.N.	Ribociclib			Letrozole		
	4 µg/ml	6µg/ml	8µg/ml	4 µg/ml	6µg/ml	8µg/ml
1	0.206	0.312	0.423	0.333	0.488	0.66
2	0.211	0.313	0.432	0.33	0.486	0.661
3	0.213	0.31	0.423	0.329	0.487	0.662
4	0.214	0.312	0.432	0.328	0.485	0.659
5	0.213	0.311	0.423	0.332	0.486	0.657
MEAN	0.21	0.3116	0.4266	0.33	0.4864	0.6598
STDV	0.0032	0.00	0.005	0.002	0.001	0.002
%RSD	0.015	0.004	0.012	0.006	0.002	0.003
Mean % RSD	0.010			0.004		

Table 8: Inter day precision Ribociclib

Day 1				Day 2			Day 3		
S.N.	**4 µg/ml**	**6 µg/ml**	**8 µg/ml**	**4 µg/ml**	**6 µg/ml**	**8 µg/ml**	**4 µg/ml**	**6 µg/ml**	**8 µg/ml**
1	0.206	0.312	0.423	0.213	0.317	0.425	0.211	0.316	0.423
2	0.211	0.313	0.432	0.216	0.316	0.432	0.213	0.312	0.425
3	0.213	0.31	0.423	0.212	0.316	0.427	0.213	0.313	0.427
4	0.214	0.312	0.432	0.211	0.312	0.432	0.216	0.314	0.429
5	0.213	0.311	0.423	0.213	0.314	0.426	0.215	0.314	0.427
MEAN	0.21	0.312	0.427	0.21	0.315	0.4284	0.21	0.3138	0.426
STDV	0.0032	0.00	0.005	0.002	0.002	0.003	0.0019	0.0015	0.0023
%RSD	0.015	0.004	0.012	0.009	0.006	0.008	0.009	0.005	0.005
Mean % RSD	0.010			0.008			0.006		
	MEAN % RSD			0.008					

Table 9: Inter day precision Letrozole

Day 1				**Day 2**			**Day 3**		
S.N.	**4 µg/ml**	**6 µg/ml**	**8 µg/ml**	**4 µg/ml**	**6 µg/ml**	**8 µg/ml**	**4 µg/ml**	**6 µg/ml**	**8 µg/ml**
1	0.325	0.487	0.656	0.333	0.488	0.66	0.328	0.487	0.654
2	0.325	0.479	0.66	0.33	0.486	0.661	0.327	0.489	0.658
3	0.326	0.486	0.662	0.329	0.487	0.662	0.326	0.478	0.657
4	0.332	0.481	0.655	0.328	0.485	0.659	0.326	0.485	0.658
5	0.326	0.485	0.657	0.332	0.486	0.657	0.328	0.486	0.659
MEAN	0.33	0.4836	0.656	0.33	0.4864	0.6598	0.33	0.485	0.6572
STDV	0.0029	0.003	0.003	0.002	0.001	0.002	0.0010	0.0042	0.0019
%RSD	0.009	0.007	0.004	0.006	0.002	0.003	0.003	0.009	0.003
Mean % RSD	0.007			0.004			0.005		
	Mean % RSD				0.005				

Table 10:Recovery study of Ribociclib and Letrozole

Parameters	**Ribociclib**			**Letrozole**		
Accuracy Level	**80%**	**100%**	**120%**	**80%**	**100%**	**120%**
Amount present	10	10	10	10	10	10
Amount Added	8	10	12	8	10	12
Amount recovered*	17.99	20.17	22.02	18.41	20.20	22.05
% Recovery	99.95	100.84	100.10	102.28	101.09	100.20
Mean	100.29			101.16		

*Mean of three determinations in each level

Table 11:Linearity and absorptivity Ribociclib and Letrozole mixture

Name of Drug	**Concentration PPM**	**Absorbance ʎ1 270**	**Absorbance ʎ2 240**	**Absorptivity ʎ1 270**	**Absorptivity ʎ2 240**
Ribociclib	2	0.102	0.054	0.027	0.051
	4	0.203	0.098	0.025	0.051
	6	0.311	0.15	0.025	0.052

	8	0.421	0.202	0.025	0.053
	10	0.532	0.245	0.025	0.053
				ax1	ax2
	Mean			0.025	0.0518
Letrozole	2	0.045	0.166	0.083	0.0225
	4	0.098	0.325	0.08125	0.0245
	6	0.145	0.487	0.081166667	0.024166667
	8	0.201	0.656	0.082	0.025125
	10	0.265	0.828	0.0828	0.0265
				ay1	ay2
	Mean			0.0820	0.02455
Mixture	**Absorbance**	0.877	0.798		

Table 12: Determination of concentration of unknown mixture

aX_1	aX_2	aY_1	aY_2
0.02525	0.051881667	0.082043333	0.024558333
A1	0.87	**A2**	0.798
CX mcg/ml	12.08	**Cy mcg/ml**	6.97

Table 13:Analysis of Ribociclib and Letrozole in combination dosage form

Formulation	Drug	Label claim(mg/tab)	% Drug found
Tablet 1	RBC	200	100.49
	LTZ	2.5	100.05

Table 14:Acceptance Criteria and Results of Ribociclib and Letrozole of UV-Visible Spectroscopy Method Development and Validation Parameters as per ICH Guidelines

Parameter	Purpose	ICH Guideline Requirement / Acceptance Criteria	Ribociclib	Letrozole
Linearity	Demonstrates proportional relationship between concentration and absorbance (Beer's law)	Linearity range: typically 80–120% of test concentration	2–10 µg/mL	2–10 µg/mL
Wavelength maximum			270	240
Intercept			0.009	0.027
Slope			0.053	0.012

Correlation coefficient		Correlation coefficient (r^2) ≥ 0.999	0.998	0.0996
Accuracy (Recovery)	Closeness of observed value to true value	% Recovery: 98–102% (API)	100.29	101.26
Precision (Repeatability)	Reproducibility of results under same conditions	%RSD ≤ 2% for absorbance values (n=6)	0.829	0.003
Intermediate Precision (Ruggedness)	Agreement of results under different conditions (analyst, instrument, day)	%RSD ≤ 2%	0,008	0.005
LOD (Limit of Detection)	Lowest concentration detected but not necessarily quantified	S/N ratio ~ 3:1 (or based on SD & slope: 3.3 × σ/S)	0.219	0.204
LOQ (Limit of Quantitation)	Lowest concentration quantified with acceptable precision & accuracy	S/N ratio ~ 10:1 (or based on SD & slope: 10 × σ/S)	0.663	0.619
Assay %	Ensure suitable for routine quality control analysis in dosage forms.	98% to 102%,	100.49	100.05

Conclusion

The validated UV-Vis method for simultaneous estimation of Ribociclib and Letrozole is accurate, precise, and reliable. It meets ICH Q2(R1) guidelines and is suitable for quality control of combination dosage forms.

The method developed was found to be simple, rapid, precise, accurate, and cost-effective. It can be successfully applied for routine analysis in quality control laboratories for bulk and dosage forms containing Ribociclib and Letrozole.

Acknowledgement

Authors are thankful to Novartis Pharma for providing API standard of Ribociclib and Letrozole. Authors are also thankful to the principal and Management, Lords university, College of Pharmacy, Alwar for providing required facilities for research work.

References

1. Bakshi M, Singh S. Development of validated stability-indicating assay methods—Critical review. J Pharm Biomed Anal. 2002;28(6):1011–40.
2. Peters FT, Drummer OH, Musshoff F. Validation of new methods. Forensic Sci Int. 2007;165(2–3):216–24.
3. Swartz ME, Krull IS. Handbook of analytical validation. CRC Press; 2012.
4. Nash RA, Wachter AH, editors. Pharmaceutical process validation. 3rd ed. CRC Press; 2003.
5. Bansal G, Soni A. Analytical challenges in forced degradation studies and stability indicating assays. Pharm Technol Eur. 2012;24(11):14–22.
6. Feret B, Shrivastava P, Shukla S, Kaushal A, Trivedi P. Simultaneous estimation of letrozole and ribociclib by validated HPLC method. Indian Drugs. 2014;51(6):40–5.
7. Shabir GA. Validation of high-performance liquid chromatography methods for pharmaceutical analysis. J Chromatogr A. 2003;987(1–2):57–66.
8. González O, Iriarte G, Rico E, Ferreiros N, Maguregui MI, Alonso RM. Bioanalytical chromatographic method validation according to current regulations. J Chromatogr A. 2013;1353:10–27.
9. Blessy M, Patel RD, Agrawal YK. Role of liquid chromatography in drug stability studies. J Liq Chromatogr Relat Technol. 2012;35(11):1575–601.
10. Sharma BK. Instrumental methods of chemical analysis. 26th ed. Goel Publishing House; 2013.
11. Fadeyi MO, Myers DP, Reddy YV, Araujo JA. Integrated analytical development for quality control of oncology combination products. J Pharm Sci. 2013;102(10):3388–98.
12. Pandey S, Sharma S. Analytical method validation for simultaneous estimation of ribociclib and letrozole: A regulatory approach. Asian J Pharm Anal. 2021;11(2):72–7.
13. United States Pharmacopeial Convention. <1225> Validation of compendial procedures. In: USP 43–NF 38. USP; 2020.

14. Rao RN, Nagaraju V. Stability-indicating HPLC methods for drug substances. J Pharm Biomed Anal. 2003;33(3):617–36.

15. Wu YS, Levons JK. Analytical tools for quality control of fixed-dose combinations. Am Pharm Rev. 2011;14(3):76–85.

Source of Support: Nil
Conflict of Interest: Nil