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Research Article

A NEW ROBUST ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF RIBOCICLIB AND LETROZOLE IN SOLID DOSAGE FORM (TABLET)

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ABSTRACT

Objective: The objective of the study was to develop a new robust, sensitive, precise, accurate RP-HPLC analytical method and validate for simultaneous estimation of ribociclib and letrozole in solid dosage form (tablet).

Methods: The chromatographic separation was carried out on Waters, symmetry C18 (150 mm×4.6 mm with 3.5 µm), mobile phase used was a mixture of buffer and acetonitrile in the ratio of 80:20, with flow rate of 1ml/min and injection volume of 10 µL for the assay. The detection was done using PDA at 260 nm, with run time of 5 min. The retention time for the drugs ribociclib and letrozole was detected to be 2.648 min and 3.151 min, respectively. The method was validated according to ICH guidelines.

Results: The linearity of letrozole and ribociclib was observed to be in the range of 0.50–7.50 and 40.01–600.15, Correlation coefficient (r^2) 0.999 and 0.9983, respectively. Accuracy for ribociclib and letrozole is carried out by repeatable concentrations of 50%, 100%, and 150. Validation factors of robustness and ruggedness were detected to be in limits.

Conclusion: The developed method was simple, rapid, and consistent; it can be used for the simultaneous estimation of ribociclib and letrozole tablet dosage form in routine analysis.

Keywords: RP-HPLC, Ribociclib, Letrozole, Method validation and simultaneous estimation.

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INTRODUCTION

Chemically ribociclib is 7-cyclopentyl-N, N-dimethyl-2-{[5-(1-piperazinyl)-2-pyridinyl] amino}-7H- pyrrolo[2,3-d] pyrimidine- 6-carboxamide (Fig.1). Slight yellow to brown. It is freely soluble in dichloromethane; slightly soluble in ethanol; practically insoluble in water. Ribociclib holds cyclin-dependent kinase4 and 6(CDk4/6) inhibitor2 helps to slow the progression of cancer [1-3]. The drug regulates cell cycle progression through phosphorylation of the retinoblastoma protein (pRb). The combination of ribociclib with anti-estrogen results in increased inhibition of tumor growth. 200 mg tablets of the drug are available for oral administration.

Chemically letrozole is 4,4-((1H-1,2,4-triazole-1-yl) methylene) dibenzo nitrile [4], orally active, nonsteroidal selective aromatase inhibitor, used for the treatment of postmenopausal women with breast cancer and being an antiestrogen [5]. Letrozole is soluble in organic solvents such as dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF), which should be purged with an inert gas. The solubility of letrozole in these solvents is approximately 16 mg/ml, sparingly soluble in aqueous buffers [4]. It acts by irreversible binding to the heme of its cytochrome P450 unit. The action is distinct and does not reduce secretion of corticosteroids. Letrozole is considered as equally effective as ovariectomy in reducing uterine weight, by increasing serum LH and causing the retrogression of estrogen dependent tumors. As compared to ovariectomy treatment with letrozole will not cause in the level of serum FSH.

Survey of literature revealed that various RP-HPLC analytical methods were available for the determination of letrozole in combination with vilazodone/palbociclib and also for the determination of ribociclib individually or in combination with palbociclib [2]. Hence, an attempt was made to develop a simple, rapid, and validated method for the simultaneous estimation of ribociclib and letrozole in combination tablet dosage form.

METHODS

Chemicals and reagents

Acetonitrile (HPLC grade), Ortho Phosphoric Acid (HPLC grade), HPLC grade, Water (Milli Q or equivalent)

Instrumentation and chromatographic condition

Separation was carried out using the column Waters, Symmetry C18, 150 mm × 4.6 mm, 3.5 µm, with mobile phase Acetonitrile: Ortho Phosphoric Acid Buffer in ratio 20:80, was degassed and filtered by using 0.45 µm membrane filter in a vacuum filter system. Separation was carried out at room temperature by injecting 10 µl with flow rate 1.0 ml/min. The analytes were quantified with PDA detector at 260 nm.

Preparation of solutions

Buffer 1 ml of O-phosphoric acid buffer dissolved in 1 L of HPLC water.

Diluent mobile phase used as diluent.

Standard solution

400 mg of ribociclib and 5 mg of letrozole working standards were taken into a 100 ml volumetric flask, 70 ml of diluents was added and sonicated for 15 min to dissolve the contents, the final volume was made with diluent. 5 ml of above solution was pipetted out and diluted to 50 ml with diluent.

Preparation of sample solution

Average weight of five tablet taken, then three tablets were powered into power form, 500 mg of powder was taken in a 100 ml volumetric flask, 70 ml of diluent was added and sonicated for 30 min to dissolve

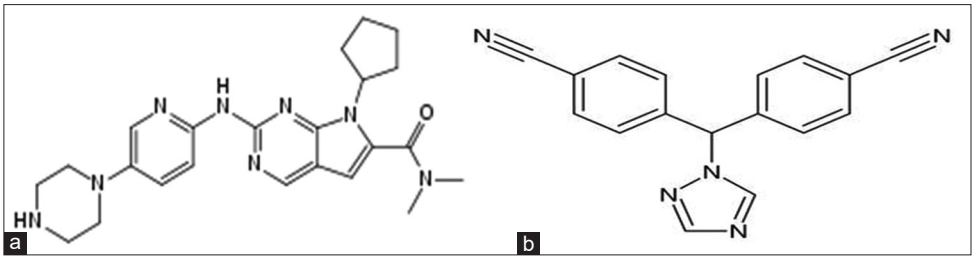

Fig. 1: (a) Ribociclib, (b) Letrozole

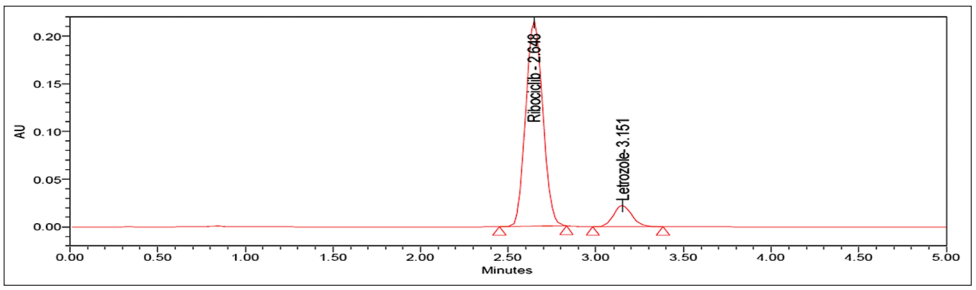

Fig. 2: Optimized chromatogram. Retention time of ribociclib is about 2.648 min. Retention time of letrozole is about 3.151 min

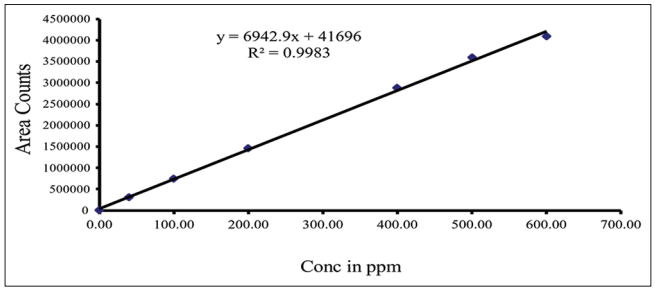

Fig. 3: Linear plot for ribociclib

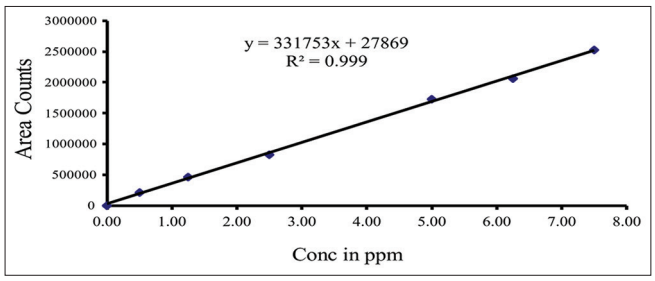

Fig. 4: Linear plot for letrozole

Table 1: Working standard for letrozole

Std. Name	Letrozole	% Potency	100.0
STD wt. (mg)	5.00		
Dilutions	Wt taken in mg into 100 mL	Concentration	50
	5 mL 50 mL	(ppm)	5
	1 mL 1 mL		5

Table 2: Working standard for ribociclib

Std. Name	Ribociclib	% Potency	100.0
STD wt. (mg)	400.10		
Dilutions	Wt taken in mg into 100 mL	Concentration	4001
	5 mL 50 mL	(ppm)	400.1
	1 mL 1 mL		400.1

the contents completely. 5 ml of above solution was pipette out and diluted to 50 ml with diluent.

Procedure for assay

Six replicates of standard and sample were analyzed. 10 µl of blank, standard solution and sample solution was injected into the HPLC system. From the peak area of the chromatogram, the percentage purity of the sample was calculated.

Method validation

The optimized chromatographic condition is applied for quantitative determination and the method was validated by considering some parameters linearity, precision, accuracy (% recovery), robustness, system precision, method precision, and ruggedness [5,6].

Specificity

The specificity of an analytical method is its ability to quantify with precision and especially the analyte of interest in the presence of other components that are likely to be present in the sample. The blank, standard, sample, and placebo solutions were injected to check the interference.

Linearity

It is the ability of the method to produce the test results which are directly proportional to the concentration of analyte in the sample solution. Six concentrations for the drugs letrozole and ribociclib at 0.50, 1.25, 2.50, 5, 6.25, and 7.50 µg/mL and 40.01, 100.03, 200.05, 400.10, 500.13, and 600.15 µg/mL, respectively, were prepared from the standard solution and 10µl of each was injected.

Accuracy

The accuracy of an analytical method is the proximity of the test results derived by that method to the true value. This is sometimes termed trueness. It is proposed that accuracy should be determined using not less than nine determinations over a minimum of the three concentration levels, covering the specified range (three concentrations/three replicates each of total analytical procedures). Accuracy was studied using the standard addition method. For letrozole recovery studies, 2.5 mg of standard was added for 50%, 5 mg was added for 100%, and 7.5 mg was added for 150%, for ribociclib, 200 mg was added for 50%, 400 mg was added for 100%, and 600 mg for 150% was added to the previously prepared test solutions.

Table 3: Linearity for ribociclib and letrozole

Ribociclib				Letrozole			
mL of lin stock	**Vol. made up to**	**µg/mL**	**Area count**	**mL of Lin stock**	**Vol made upto**	**µg/mL**	**Area count**
0	0	0.00	0	0	0	0.00	0
0.5	50	40.01	303014	0.5	50	0.50	214633
1.25	50	100.03	739150	1.25	50	1.25	464156
2.5	50	200.05	1461298	2.5	50	2.50	832584
5	50	400.10	2880851	5	50	5.00	1726321
6.25	50	500.13	3596077	6.25	50	6.25	2062896
7.5	50	600.15	4089562	7.5	50	7.50	2524821
	Correl Coeff.		0.99915	Correl Coeff.	0.99953	0.99953	0.99953
	Slope		6942.87	Slope	331753.49	331753.49	331753.49
	Intercept		41695.91	Intercept	27868.66	27868.66	27868.66

Linear regression analysis curves (n=6)

Table 4: For letrozole

	Letrozole							
			Area Counts	**Potency (As is basis)**		**100.0**		
	Amount of API Added (mg)	**Actual API Added (mg)**		**Mean**	**Amount**	**% Recovery**		
			Injection	**Area Counts**	**Recovered (mg)**			
50% accuracy	2.5	2.50	152463	152463	2.47	98.8	Mean	99.1
	2.5	2.50	154284	154284	2.5	100.0	SD	0.83
	2.5	2.50	151312	151312	2.46	98.4	%RSD	0.840
100% accuracy	5	5.00	308443	308443	5.01	100.2	Mean	99.9
	5	5.00	307618	307618	4.99	99.8	SD	0.23
	5	5.00	307284	307284	4.99	99.8	%RSD	0.230
150% accuracy	7.5	7.50	462031	462031	7.5	100.0	Mean	99.8
	7.5	7.50	464570	464570	7.54	100.5	SD	0.89
	7.5	7.50	456456	456456	7.41	98.8	%RSD	0.890
					Mean	99.6		
					SD	0.436		
					% RSD	0.44		

*SD: Standard deviation, *% RSD: % relative standard deviation

Table 5: For ribociclib

Ribociclib								
	Batch no		**Area Counts**	**Potency (As is basis)**		**100.0**		
	Amount of API Added (mg)	**Actual API Added (mg)**		**Mean**	**Amount**	**% Recovery**		
			Injection	**Area Counts**	**Recovered (mg)**			
50% accuracy	200	200.00	1360582	1360582	197.53	98.8	Mean	99.1
	200	200.00	1364716	1364716	198.13	99.1	SD	0.28
	200	200.00	1368218	1368218	198.64	99.3	%RSD	0.280
100% accuracy	400	400.00	2744501	2744501	398.44	99.6	Mean	99.4
	400	400.00	2746991	2746991	398.8	99.7	SD	0.50
	400	400.00	2721988	2721988	395.17	98.8	%RSD	0.500
150% accuracy	600	600.00	4071373	4071373	591.08	98.5	Mean	98.6
	600	600.00	4072024	4072024	591.17	98.5	SD	0.22
	600	600.00	4087242	4087242	593.38	98.9	%RSD	0.220
					Mean	99.0		
					SD	0.404		
					% RSD	0.41		

*SD: Standard deviation, *% RSD: % relative standard deviation

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is recurrent to multiple samplings of samples that are not homogenous in nature.

System precision

Six replicates of standard solution of both the drug were analyzed by the same analyst, on the same equipment on the same day. Area of the peak and % RSD were calculated.

Method precision

Six different standard solutions for both the drug were prepared from homogenous sample solution and were analyzed by the same analyst, on the same equipment on the same day. The assay results and % RSD were calculated.

Robustness

The robustness determines the influence of small but steady variation in the chromatographic conditions. The robustness of the method was

Table 6: For working standards

Working standard	Letrozole		Dilutions	ppm
Wt. taken (mg)	5		Taken in to 100 ml	50.0
B. No			5 ml to 50 ml	5.0
% Potency	100.0	w/w (as is basis)	1 ml to 1 ml	5.0
Working standard	**Ribociclib**		**Dilutions**	**ppm**
Wt. taken (mg)	400.1		Taken in to 100 ml	4001.0
B. No			5 ml to 50 ml	400.1
% Potency	100.0	w/w (as is basis)	1 ml to 1 ml	400.10

Table 7: For system precision

Ribociclib		Letrozole
Standard area counts		Standard area counts
Injection	Area	Area
1	2760981	309393
2	2757098	308271
3	2751049	307449
4	2752301	308183
5	2760385	307998
6	2753672	306959
Mean	2755914	308042
SD	4214.61	827.53
% RSD	0.153	0.269

*SD: Standard deviation, *% RSD: % relative standard deviation

determined by changing the flow rate (0.9 and 1.1mLmin_1) of the mobile phase, pH of ortho phosphate buffer, ratio of composition of the mobile phase and wavelength.

Limit of detection and limit of quantitation

Limit of detection is the lowest amount of sample detected by an analytical procedure but not quantified exactly. Limit of quantitation is the lowest amount of sample that can be quantified by an analytical procedure.

As per ICH guidelines, in the simultaneous analytical method development study, limit of detection and limit of quantitation were based on the standard deviation of the response and slopes using signal-to-noise ratio.

RESULTS AND DISCUSSION

Several trials have been performed for the development of the method by changing the column, flow rate, mobile phase ratio, and wavelength.

The chromatogram of optimized method is shown in Fig. 2.

Optimized trials

Optimized chromatographic condition

Parameters	Optimized conditions
Mobile phase ratio	Acetonitrile and ortho phosphoric acid (20:80)
Column	Waters, symmetry C18, 150 mm×4.6 mm, 3.5 µm
Flow rate	1.0 ml/min
Wavelength	260 nm
Injection Volume	10 µL
Elution mode	Isocratic
Run time	5 min
Column temperature	25°C

Table 8: Method precision for ribociclib

Ribociclib						
Standard area counts			Sample area counts			
Injection	Area	Brack. Std.	Sample weight (mg)	Area counts injection	Mean area counts	% Label claim
1	2760981	2748621	500.2	2747629	2747629	99.7
2	2757098		500.4	2711616	2711616	98.3
3	2751049		500.2	2716991	2716991	98.6
4	2752301		500.1	2730071	2730071	99.1
5	2760385		500.4	2754107	2754107	99.9
6	2753672		500.3	2773887	2773887	100.6
Mean	2755914	2754872			Mean	99.4
SD	4214.61	4733.01			SD	0.862
% RSD	0.153	0.172			% RSD	0.87

*SD: Standard deviation, *% RSD: % relative standard deviation

Table 9: Method precision for letrozole

Letrozole						
Standard area counts			Sample area counts			
Injection	Area	Brack. Std.	Sample weight (mg)	Area counts injection	Mean area counts	% label claim
1	309393	304867	500.2	305295	305295	99.0
2	308271		500.4	305997	305997	99.2
3	307449		500.2	309917	309917	100.5
4	308183		500.1	307131	307131	99.6
5	307998		500.4	303496	303496	98.4
Mean	308259	307694	500.3	308831	308831	100.1
SD	709.95	1523.35			Mean	99.5
% RSD	0.23	0.495			SD	0.763
					% RSD	0.77

*SD: Standard deviation, *% RSD: % relative standard deviation

Table 10: Limit of detection (LOD)

Sample name	Sample concentration	Retention Time	Area (µV*sec)	% Area	USP tailing	s/n	USP plate count	USP resolution
Ribociclib	0.4001	2.639	17081	71.29	1.09	7	3179	
Letrozole	0.005	3.140	2630	28.71	1.15	5	3839	2.52

Table 11: Limit of quantitation

Sample name	Sample concentration	Retention Time	Area (µV*sec)	% area	USP tailing	s/n	USP plate count	USP resolution
Ribociclib	4.005	2.629	302070	90.69	1.09	27	3095	
Letrozole	0.05	3.131	31002	9.31	1.10	22	3980	2.58

Table 12: For letrozole

Variation	Standard mean area	SD of Standard	RSD of Standard	Mean %label claim of sample	SD %label claim of sample	RSD %label claim of sample
Flow plus	288126	5001.4	1.736	100.7	0.058	0.06
Flow minus	371291	4037.32	1.08	99.9	0.3	0.3
Org plus	313139	3702.98	1.183	99.5	0.265	0.27
Org minus	344854	2773.86	0.804	99.6	0.361	0.36
pH plus	284983	2683.53	0.926	99.4	0.681	0.69
pH minus	346080	3405.23	0.984	99.8	0.954	0.96
Wave plus	296935	1617.65	0.545	100.2	0.577	0.58
Wave minus	325860	673.78	0.207	99.6	0.889	0.89

Table 13: For ribociclib

Variation	Standard mean area	SD of Standard	RSD of standard	Mean %label claim of sample	SD %label claim of sample	RSD %label claim of sample
Flow plus	2528147	16839.14	0.666	99.9	0.586	0.59
Flow minus	3331018	18882.69	0.567	100.2	0.503	0.5
Org plus	2769764	13196.04	0.476	99.7	0.929	0.93
Org minus	2909548	9672.9	0.332	100.3	0.306	0.31
pH plus	2532149	14183.74	0.56	100.2	0.208	0.21
pH minus	3348280	24029.87	0.718	100	0.2	0.2
Wave plus	2649954	4215.42	0.159	99.9	0.404	0.4
Wave minus	3328400	3245	0.097	99.9	0.058	0.06

Validation of proposed method

Linearity

Linear correlation was prevailed between peak area versus concentration of letrozole and ribociclib**.** Calibration curves were linear in the concentration range from 0.50 µg/mL and 7.50 µg/mL for letrozole and 40.01 µg/mL and 600.15 µg/mL for ribociclib. Linearity of the calibration curves was validated from the value of correlation coefficients of the regression analysis. The value of r^2 was 0.99953 for letrozole and 0.9983 for ribociclib. The results of the linearity experiment are listed in Tables 1-3 and the calibration plot is given in Figs. 3 and 4 for ribociclib and letrozole, respectively.

Accuracy

The experiments were performed in accordance to standard addition method. The percentage recovery and % RSD were calculated for both ribociclib and letrozole. The % RSD was found to be within limit (Tables 4 and 5).

Precision

System precision: Six injections of standard solutions of both the drugs were injected. The results are listed in the Tables 6 and 7.

Method precision

Method precision determined with developed method (n=6).

The precision of the method was given in the Tables 8-11.

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection for letrozole and ribociclib was found to be 0.005 and 0.4001 µg/mL, while the limit of quantitation for letrozole and ribociclib was found to be 0.05 and 4.005 µg/mL, respectively.

Robustness

The intend changes in the method do not have more impact in the peak tailing, theoretical plates, and the percent assay of letrozole and ribociclib. It has been performed by changing the composition of the mobile phase, flow rate, wave, and pH of buffer (Tables 12 and 13).

CONCLUSION

The suggested RP-HPLC method was rapid, more precise, robust, and sensitive. This RP-HPLC method accommodates the utilization of the economically and simply available mobile phase and PDA detector. The validated method can be used for the routine analysis of both the drugs from bulk and different formulations and will help in therapeutic drug monitoring (TDM) and bioavailability studies.

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CONFLICT OF INTERESTS

Declared none.

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