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 was used 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 (r²) 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.

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(CKd4/6) 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 1.6 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 ovariotomy in reducing uterine weight, by increasing serum LH and causing the retrogression of estrogen dependent tumors. As compared to ovariotomy 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
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, 5.00, 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 10 µl 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.
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.

**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 | 4099562 | 7.5 | 50 | 7.50 | 2524821 |

**Table 4: For letrozole**

| Amount of API Added (mg) | Actual API Added (mg) | Potency (As is basis) | Mean | Amount | % Recovery |
|-------------------------|-----------------------|-----------------------|------|--------|------------|
| Injection Area Counts   | Recovery (mg)         |                      |      |        |            |
| 50% accuracy            | 2.5                   | 2.50                  | 152463 | 2.47 | 98.8       |
|                         | 2.5                   | 2.50                  | 154284 | 2.5   | 100.0      |
|                         | 2.5                   | 2.50                  | 151312 | 2.46 | 98.4       |
| 100% accuracy           | 5                     | 5.00                  | 308443 | 5.01 | 100.2      |
|                         | 5                     | 5.00                  | 307618 | 4.99 | 99.8       |
|                         | 5                     | 5.00                  | 307264 | 4.99 | 99.8       |
| 150% accuracy           | 7.5                   | 7.50                  | 462031 | 7.5   | 100.0      |
|                         | 7.5                   | 7.50                  | 464570 | 7.54 | 100.5      |
|                         | 7.5                   | 7.50                  | 456456 | 7.41 | 98.8       |

**Table 5: For ribociclib**

| Ribociclib | Batch no | Area Counts | Potency (As is basis) | Mean | Amount | % Recovery |
|------------|----------|-------------|-----------------------|------|--------|------------|
|            |          | Injection Area Counts | Recovery (mg)         |      |        |            |
| 50% accuracy | 200   | 200.00      | 1360582               | 197.53 | 98.8 | Mean 99.1 |
|              | 200   | 200.00      | 1364716               | 198.13 | 100.0 | SD 0.83  |
|              | 200   | 200.00      | 1368218               | 198.64 | 98.4  | %RSD 0.840 |
| 100% accuracy| 400   | 400.00      | 2744501               | 398.44 | 99.9  | Mean 99.7 |
|              | 400   | 400.00      | 2746991               | 398.8  | 99.6  | SD 0.50  |
|              | 400   | 400.00      | 2721988               | 395.17 | 98.8  | %RSD 0.500 |
| 150% accuracy | 600   | 600.00      | 4071373               | 591.08 | 98.5  | Mean 98.8 |
|              | 600   | 600.00      | 4072024               | 591.17 | 98.5  | SD 0.22  |
|              | 600   | 600.00      | 4087242               | 593.38 | 98.9  | %RSD 0.220 |

*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            | 100.0 w/w | 5 ml to 50 ml & 1 ml to 1 ml | 5.0 |
| % Potency        | (as is basis) | 50.0 |

| Working standard | Ribociclib | Dilutions | ppm |
|------------------|------------|-----------|-----|
| Wt. taken (mg)   | 400.1      | Taken in to 100 ml | 400.1 |
| B. No            | 100.0 w/w  | 5 ml to 50 ml & 1 ml to 1 ml | 400.1 |
| % Potency        | (as is basis) | 50.0 |

Table 7: For system precision

| Ribociclib | Letrozole |
|------------|-----------|
| Standard area counts | Standard area counts |
| Injection | Area | Brack. Std. | Area |
| 1 | 2760981 | 309393 |
| 2 | 2757098 | 308271 |
| 3 | 2751049 | 307449 |
| 4 | 2752301 | 308183 |
| 5 | 2760385 | 307998 |
| 6 | 2753672 | 309959 |
| Mean | 2755914 | 30842 |
| SD | 4214.61 | 827.53 |
| % RSD | 0.153 | 0.269 |

| Ribociclib | Letrozole |
|------------|-----------|
| Standard area counts | Standard area counts |
| Injection | Area | Brack. Std. | Area |
| 1 | 2755914 | 30842 |
| 2 | 2754872 | 30842 |

Table 8: Method precision for ribociclib

| Ribociclib | Letrozole |
|------------|-----------|
| Standard area counts | Sample area counts |
| Injection | Area | Brack. Std. | Sample weight (mg) | Area counts injection | Mean area counts | % Label claim |
| 1 | 2760981 | 309393 | 500.2 | 2747629 | 2747629 | 99.7 |
| 2 | 2757098 | 308271 | 500.4 | 2711616 | 2711616 | 98.3 |
| 3 | 2751049 | 307449 | 500.2 | 2716991 | 2716991 | 98.6 |
| 4 | 2752301 | 308183 | 500.1 | 2733071 | 2733071 | 99.1 |
| 5 | 2760385 | 307998 | 500.4 | 2754107 | 2754107 | 99.9 |
| 6 | 2753672 | 309959 | 500.3 | 2773887 | 2773887 | 100.6 |
| Mean | 2755914 | 30842 | Mean | 2773887 | 2773887 | 99.4 |
| SD | 4214.61 | 827.53 | SD | 0.153 | 0.269 | 0.87 |

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 | 309393 | 500.4 | 305997 | 305997 | 99.2 |
| 3 | 307998 | 309393 | 500.2 | 309917 | 309917 | 100.5 |
| 4 | 308183 | 309393 | 500.1 | 307131 | 307131 | 99.6 |
| 5 | 308259 | 309393 | 500.4 | 303496 | 303496 | 98.4 |
| Mean | 308259 | 307694 | Mean | 305295 | 305295 | 99.5 |
| SD | 790.95 | 1523.35 | SD | 0.23 | 0.95 | 0.77 |
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).

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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