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Research Article

RP-HPLC Method Development and Validation for Determination of Abemaciclib in Bulk Drug Substance and Pharmaceutical Dosage Forms

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Abstract:
A simple, economic, rapid and precise method for the quantification of Abemaciclib in bulk drug substance and pharmaceutical dosage forms was developed and validated using RP-HPLC. The chromatographic separation was achieved using Ascentis® C-18 HPLC column (250mm x 4.6mm i.d., particle size 5µ) with mobile phase containing water: methanol: acetonitrile (35:45:20 v/v/v) with flow rate of 1.2mL/ min was used and effluents were monitored at 295nm. The retention time of Abemaciclib were found to be 3.72 min. The linearity was in the range of 6-42µg/mL. The limit of detection and limit of quantification were found to be 0.6 and 1.8 µg/mL, respectively. The % recoveries were found to be in the range of 99.65±0.77. In addition, the f-test and t-test at 95% confidence level were subjected on data for statistical analysis. The proposed method was validated and successfully applied to the estimation of Abemaciclib in marketed formulations.
Keywords: Abemaciclib, Estimation, Bulk drug substance, Pharmaceutical dosage forms

Introduction

The chemical name of Abemaciclib (AM) is N-[5-[(4-ethylpiperazin-1-yl) methyl]pyridine-2-yl]-5-fluoro-4-(7-fluoro-2-methyl-3-propan-2-benzimidazole-5-yl)pyrimidine-2-amine and its molecular formula is $C_{27}H_{32}F_2N_8$. AM is used alone to treat a certain type of hormone receptor-positive, advanced breast cancer or breast cancer that has spread to other parts of the body in people who have already been treated with an antiestrogen medication and chemotherapy. AM is in a class of medications called kinase inhibitors. It works by blocking the action of an abnormal protein that signals cancer cells to multiply. This helps slow or stop the spread of cancer cells (1-4). A number of analytical methods are already in existence for evaluation of these drugs in various marketed dosage forms as well as in biological fluids (5-13). Amongst the reported methods, electrophoresis methods are less sensitive and accurate as compared to HPLC methods. Literature survey reveals that there are few HPLC methods has been published for the determination of AM in marketed dosage form. The reported methods are complicated due

to use of mobile phase systems along with buffer with pH. Keeping in view the present scenario, there was a need to develop simple, more economic method with shorter analysis time with use of simple mobile phase. Therefore, the main aim of present work was to develop ICH guidelines compliant simple, economic and less time-consuming RP-HPLC method for estimation of AM in marketed pharmaceutical dosage forms.

Materials and Methods

Chemicals and Reagents

Methanol and acetonitrile were procured from Thermo Fisher Scientific India Pvt. Ltd. New Delhi, India. Tri fluoro acetic acid (TFA) was procured from Central Drug House (P) Limited, New Delhi, India. Mili Q water was used throughout the study. Other chemicals used in this study were of analytical or HPLC grade.

Instrumentation

The analysis was carried out on Waters Alliance e-2695 separating module (Waters Co., MA, USA) using photo diode array detector (waters 2998) with auto sampler and column oven. The instrument was controlled by Empower software (version 6.00.00.00) installed with equipment for data collection and acquisition.

Chromatographic conditions

The isocratic mobile phase consisting of water: methanol: acetonitrile in a ratio of 35:45:20 (v/v/v) was used at a flow rate of 1.2mL/min. Analysis was performed with UV-visible detector at 295 nm on ambient temperature (14-15).

Preparation of Standard Stock Solution

Accurately weighed AM (100mg each) and transferred in to 100mL volumetric flasks and dissolved in the methanol. The volume was diluted with methanol to give working solution of 100µg/mL of AM.

Preparation of Sample Solution

Twenty tablets (Ramiven tablet) were weighed and finely powdered. Tablet powder equivalent to 100 mg of AM was transferred to a 100 mL volumetric flask and dissolved in 20 mL of methanol. The volume was made up to the mark with methanol and mixed well. The solution was sonicated for 15 min and filtered through 0.45µ membrane filter. The solutions were further diluted as per requirement with methanol. The amount of drugs in samples was calculated from the peak area of AM (14-15).

Method Validation

The optimized chromatographic conditions were validated by evaluating specificity, range, linearity, accuracy, precision, robustness, limit of detection (LOD), limit of quantitation (LOQ) and system suitability parameters in accordance with the ICH guidelines (16-18).

Results and Discussion

Optimization of Chromatographic Conditions

Selection of mobile phase was done by hit and trial method by using number of mobile phase combinations. Finally the optimum mobile phase containing water: methanol: acetonitrile (35:45:20 v/v/v) was selected because of ability to resolve the peaks of AM at 3.72**.** Quantification was achieved with UV detection at 295nm. The atypical HPLC chromatogram of AM is given in **Fig 1** (16-18).

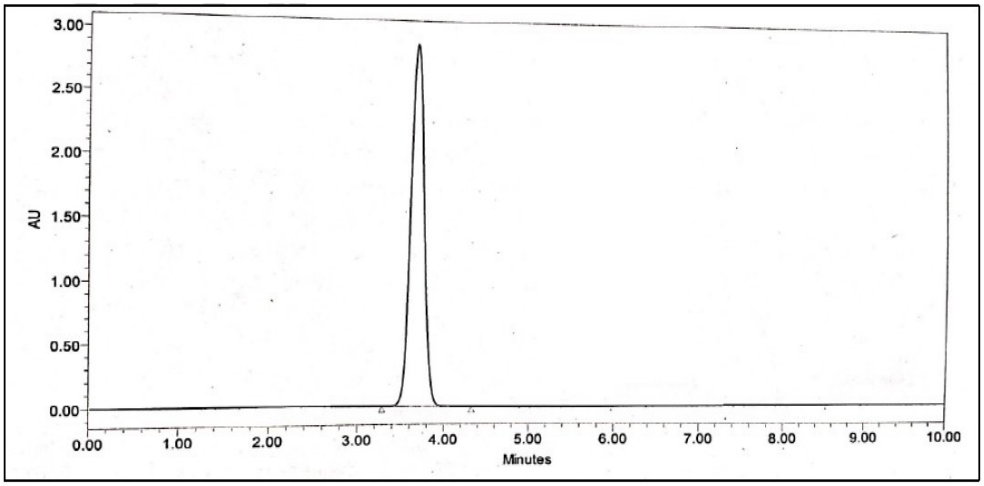

Figure 1: HPLC chromatogram of AM

Method Validation

Linearity and Calibration standards

Linear regression data showed a good relationship over a concentration range of 6-42 µg/mL for AM. The linear regression equation for AM was found to be y = 68648x – 15706 and the regression coefficient value (r^2) was found to be 0.9995 for AM indicating a high degree of linearity.

Specificity

The specificity studies revealed the absence of any interference by excipients since none of the peaks appeared at the same retention time of AM. The interaction study in standard solution was also carried out by comparing peak of each drug individually and in drug mixture. Results indicated that the analytes did not interact with each other.

LOD and LOQ

The LOD and LOQ of AM were found to be 0.6 and 1.8 µg/mL, respectively. The values indicate that the method is sensitive and selective.

Stability studies

The stability of the analyte solution was determined by comparing the analyte solution at 3rd day and 7th day with that of the freshly prepared solution at 1st day. The differences determined on 3rd day and 7th day were ±0.98, and ±1.59, respectively (16-18).

Accuracy and precision

For evaluation of accuracy of the developed method, recovery studies were carried out using standard addition method at three different levels. The average % recoveries for AM in marketed formulation were found to be 99.65±0.77**.** The results revealed that there was no interference of excipients. The results of accuracy are given in **Table 1**. The intra-day and inter-day precisions were assessed by analyzing standard solutions of AM. The lower values of % RSD was found to be 0.76 and 1.02 for intra-day and inter-day precisions respectively indicate that the method is precise. The results showed that the calculated value is less than the critical value, hence there is no significant difference between the results of linearity and precision on three consecutive days (15-16).

Table 1: Percent recovery data of AM

% simulated dosage nominal	% Mean	RSD (%)
50	99.52	0.56
100	100.12	0.82
150	99.32	0.92

Analysis of Tablets

Analysis of marketed formulation was carried out using an optimized mobile phase and HPLC conditions. The average percentage of drug contents obtained by the proposed method for AM was noted to be 99.50% which comply with the official specifications.

Conclusion

A simple, economic and less time-consuming RP-HPLC method for estimation of AM was developed and validated. The method is simple because there is no need to prepare buffer and maintain its pH throughout the experiment. The method is economic because it excludes the use of buffers and saves instrument working hours thus giving a long life to costly HPLC equipment and columns. The method gives good retention of the AM with a short analysis time (<4.0 min). The results obtained indicate that the proposed method is accurate, selective, linear and reproducible as per ICH guidelines. In addition, simple isocratic elution and easy extraction procedure offered rapid and cost-effective analysis of the drugs. The *f*-test and *t*-test were applied to the data at 95% confidence level, and no statistically significant difference was observed. The method has been successfully tested for the analysis of marketed tablets and can be adopted for the routine analysis of formulations.

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