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Original Research Article

DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR DETERMINATION OF ERDAFITINIB IN MARKETED FORMULATIONS

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Abstract

A simple and precise RP-HPLC method for the estimation of Erdafitinib in tablet dosage form was developed and validated. The chromatographic separation of the drug was done with a Hypersil™ ODS C18 Column (150 mm × 4.6 mm i.d., particle size 5 µ) using 20mM sodium acetate buffer (pH 4. ±0.02), methanol and acetonitrile (60:10:30 v/v/v) as a mobile phase. The instrument was set at flow rate of 1.0 mLmin^{-1} at ambient temperature and the wavelength of UV-visible detector at 310nm. The method showed excellent linearity over a range of 5-35 µgmL^{-1} for the drug. The correlation coefficient for Erdafitinib was noted to be 0.9999. The mean recovery values were found to be 99.77% and 100.88%. The results suggest that the proposed method could be suitable for quantitative determination of Erdafitinib in pharmaceutical preparations and also for quality control in bulk manufacturing. The F-test and t-test at 95% confidence level were applied on data for statistical analysis.

Introduction

Erdafitinib (ET) is a pan-fibroblast growth factor receptor (FGFR) inhibitor and has been approved for patients with urothelial carcinoma that has susceptible FGFR3 or FGFR2 genetic alterations (1-2). FGFRs are transmembrane tyrosine kinase receptors that are expressed in normal tissues and have a role in the processes as cell migration, proliferation and differentiation (3). There are four FGFRs (FGFR 1 to 4), and their activation pathway is mediated by fibroblast growth factor (FGF) ligands (4). Deregulation of FGFR pathway due to genetic alteration or a mutation have been reported to be involved in many human malignancies (5), and their inhibition is an important strategy for cancer treatment (6,7). Unfortunately, most of FGFRs inhibitors are not selective and have activity against other tyrosine kinases like vascular endothelial growth factor (VEGFR) and platelet derived growth factor (PDGFR) leading to potential additional toxicities (8). ET is the first treatment targeting susceptible FGFR genetic alterations for patients with metastatic bladder cancer (2). It inhibits FGFR phosphorylation and suppresses FGFR-related signal transduction pathways, leading to the prevention of tumor cell proliferation and cell death (9,10). The structure of ET is shown in Figure 1.

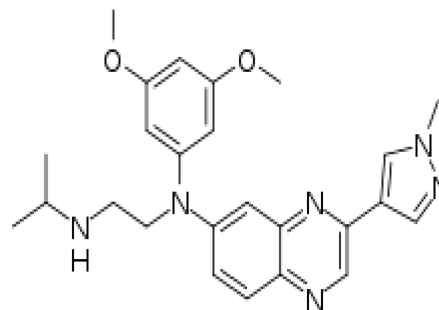

Figure 1: The structure of ET

The objective of this work was to develop a simple liquid chromatography method, which could serve as an assay for determination of ET in marketed dosage forms. A survey of literature revealed only one analytical method for the quantitative determination of ET in biological fluids that is mainly based on liquid chromatographic estimation using mass spectrometry detectors (11). No HPLC method is presently available for determination of the ET in pharmaceutical dosage forms. Hence, an attempt was made to develop a simple, precise and accurate method for the estimation of ET in pharmaceutical dosage form. This research article describes the development and validation of an isocratic reversed phase HPLC method for determination of ET in pharmaceutical dosage forms as per ICH guidelines.

Experimental

Chemicals and Reagents

ET standard were obtained from Alembic Pharmaceuticals Ltd. (Baddi). Methanol and acetonitrile were procured from Rankem, RFCL Limited, New Delhi, India. Ammonium acetate was procured from Central Drug House (P) Limited, New Delhi, India. The 0.45-mm pump nylon filter was purchased from Advanced Micro devices (Ambala, India). HPLC grade water was used. 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. Hypersil™ ODS C18 Column (150 mm × 4.6 mm i.d., particle size 5 μ), eluted with mobile phase at the flow rate of 1.0 $mLmin^{-1}$ was used.

Chromatographic Conditions

The mobile phase consisted of 20mM sodium acetate buffer (pH 4.0±0.02), methanol and acetonitrile (60:10:30 v/v/v). The buffer was filtered through 0.45-mm nylon filter and degassed in ultrasonic bath prior to use. Measurements were made with injection volume 10 µL and UV detection at 310 nm. All analyses were performed at ambient temperature.

Standard and Sample Solutions Preparation

Preparation of Standard Stock Solution

Accurately weighed 50mg of ET (99.11%) was transferred into a 50mL volumetric flask and dissolved in the mobile phase. Volume was made up to the mark with mobile phase. A standard solution was prepared from the stock solution by transferring 5mL of stock solution to a 50mL volumetric flask and diluting with mobile phase to get a solution of 100 μgmL^{-1} of ET.

Preparation of Sample Solutions

The method was used for estimation of ET in the marketed tablet formulation (BALVERSA™, Janssen Pharmaceuticals). For sample preparation, mobile phase was used as diluent. Twenty tablets were weighed and powdered finely. The Tablet powder equivalent to 5mg of ET was transferred in to 50mL volumetric flask and dissolved in 20mL of mobile phase. Volume was made up to the mark with diluent. The solution was ultrasonicated for 25 min and filtered through a 0.45-micron membrane filter. The solution was further diluted with mobile phase to obtain desired concentration and was subjected to HPLC analysis as described earlier. From the peak area of ET, the amount of drugs in samples was calculated.

Method Validation

The optimized chromatographic conditions were validated by evaluating specificity, range, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness and system suitability parameters in accordance with the ICH guidelines Q2 (R1). To assess the linearity and range of the developed method, seven different mix standard concentrations (5, 10, 15, 20, 25, 30 and 35 μgmL^{-1}) of ET were prepared. The analyses were performed in triplicate. The peak area values were plotted against corresponding concentrations. The accuracy and precision were measured by performing the assay of samples (spiked placebos) prepared at three concentration levels of 50%, 100% and 150% of the standard concentration, with 3 replicates for each concentration. The % recovery and % relative standard deviation (RSD) were calculated for each of the replicate samples. LOD and LOQ of the method were calculated based on the standard deviation of the response (σ) and slope approach as defined in ICH guidelines. The LOD was calculated using the formula 3.3*σ/slope, and the LOQ was calculated using the formula 10*σ/slope. Robustness of the method was investigated under a variety of conditions including flow rate, pH and percentage of solvent in the mobile phase (12-18).

Results and Discussion

In this work, a liquid chromatography method for the determination of ET in bulk drug and pharmaceutical formulations with UV detection was developed and validated as per ICH guidelines for analytical method validation, Q2 (R1).

Method Development

The main objective of this work was to develop a HPLC method for determination of ET within a short run time between 5-6 min and symmetry between 0.80 and 1.20. The stationary and mobile phases play an important role on theoretical plates, peak shape, symmetry and resolution. To obtain symmetrical peaks with better resolution and peak purity, various chromatographic conditions were investigated and optimized for the determination of ET; such as mobile phases with different composition, pH and stationary phases with different packing material etc. Finally, the mobile phase containing buffer (20mM sodium acetate, pH 4.0±0.02), methanol and acetonitrile in the ratio of 60:10:30v/v/v was selected and found to be optimal with more theoretical plates (≥18254) and short retention time (3.38, below 5 min). Based on the optimal mobile phase,

a highly symmetrical and sharp characteristic peak of ET was further obtained on Hypersil™ ODS C18 Column (150 mm × 4.6 mm i.d., particle size 5 µ) with 1.0 $mLmin^{-1}$ flow rate. A typical HPLC chromatogram of standard solution of ET is given in Figure 2.

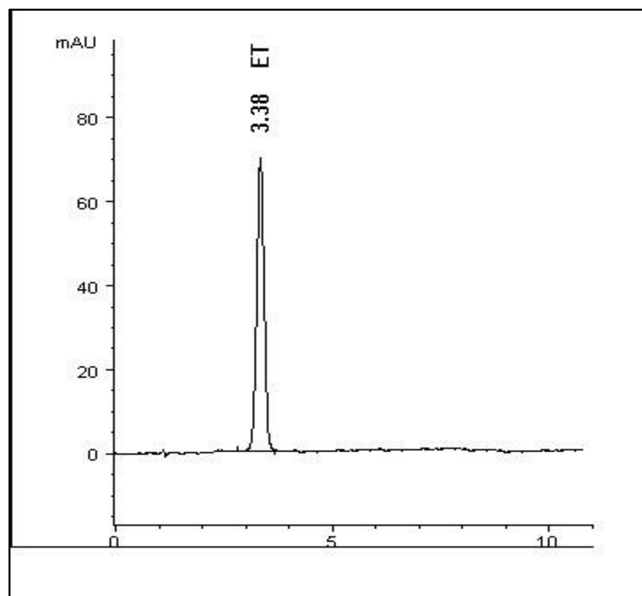

Figure 2: HPLC chromatogram of standard solution of ET

Method Validation

An optimized method must be validated before actual use. System suitability testing was performed as per ICH guidelines for analytical method validation, Q2 (R1). The validation studies were performed as prescribed in the following sections. Linear regression data demonstrated an excellent relationship over a concentration range of 5-35 μgmL^{-1} for ET. The linear regression equations for ET was found to be y = 2.04x – 1.2857. The regression coefficient value (r^2) was found to be 0.9999 indicating a high degree of linearity. The linearity curve of ET is given in Figure 3 and linearity parameters for the ET are given Table 1. The specificity studies depicted the complete absence of any other excipients as no peak was reported during the retention time of ET. Standard deviation and slope based method was adopted for determining the LOD and LOQ which was respectively found to be 0.2 and 5μgmL^{-1} for ET. The values indicate that the method is sensitive. The lower values of % RSD was found to be 0.37 and 0.92 for intra-day and inter-day precisions respectively indicate (Table 2) 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. Recovery study was carried out using standard addition method at three different levels of 50%, 100% and 150%. The average % recoveries for ET in marketed formulation were found to be between 99.77and 100.88 (Table 3). The results revealed that there was no interference.

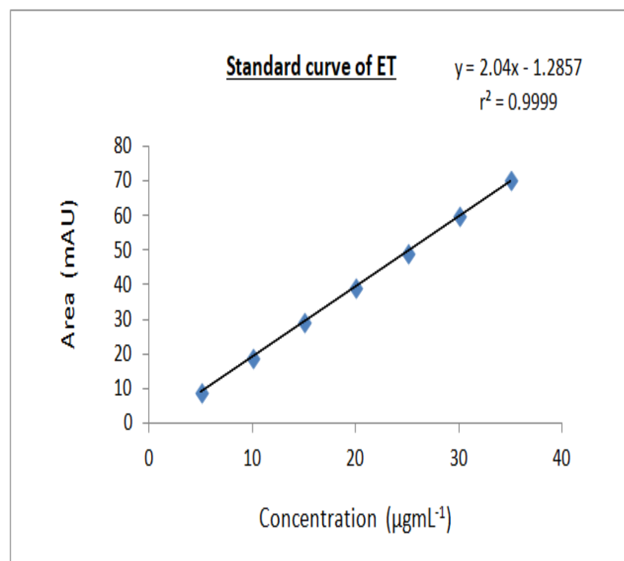

Figure 3: Standard curve of ET

Table 1: Linearity parameters for ET

Linearity Parameter	ET
Range	5-35 μgmL^{-1}
Slope	2.04 ± 0.12
Intercept	-1.2857 ± 0.05
Regression coefficient (r^2)	0.9999 ± 0.002
f-test	1.02 (4.23)[a]
t-test	0.31 (2.57)[a]

Table 2: Statistical treatment of the precision data

Parameter	ET
Intra day or Repeatability (%RSD)	0.37
Inter day (%RSD)	0.92
f-test	2.82 (5.10)[a]
t-test	1.02 (2.07)[a]

Table 3: Percent recovery data ET

% simulated dosage nominal	% Mean (n=3)	RSD (%)
50	100.88	0.89
100	99.77	1.23
150	99.92	0.97

The developed method was successfully applied to analyze ET in marketed tablet formulation. The amounts recovered were expressed as percentage of the label claim. Analysis of marketed tablets (BALVERSA™, Janssen Pharmaceuticals) was carried out using an optimized mobile phase and HPLC conditions. The average percentage of drug contents of tablets obtained by the proposed method for ET was noted to be 100.59% which comply with the official specifications.

System Suitability Parameters

For system suitability parameters, six replicates of standard solution were injected. All critical parameters

met the acceptance criteria on all days. Parameters such as resolution, tailing factor, theoretical plates, capacity factor, and retention volume of the peaks were calculated. The results are shown in Table 4.

Table 4: System suitability data for ET

Parameters	ET
Retention time (min)	3.38±0.02
Injection precision RSD (%)	0.87
Resolution	-
Tailing factor	1.02
Theoretical plates	18254
Capacity factor	0.65
Retention volume	3.38

Conclusion

A simple isocratic reversed phase HPLC method for estimation of ET was developed and validated as per ICH guidelines. Validation experiments proved that the HPLC method is linear in the proposed working range as well as accurate, precise and specific. The good recovery percentage of tablet forms suggests that the excipients have no interference in the determination. The RSD (%) was also less than 2 showing a high degree of precision of the method. The proposed method was also found to be robust with respect to flow rate and composition of mobile phase. In addition, simple isocratic elution and easy extraction procedure offered rapid and cost-effective analysis of the drugs. F-test and t-test were applied to the data at 95% confidence level, and no statistically significant difference was observed. The proposed method can be used for routine analysis of ET in marketed dosage forms and in the quality control in bulk manufacturing as well.

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