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Research Article

DEVELOPMENT AND VALIDATION OF LC METHOD FOR THE ESTIMATION OF GEFITINIB IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Gefitinib in tablet dosage form. An Inertsil ODS C-18, 5µm column having 250 x 4.6mm internal diameter in isocratic mode with mobile phase containing acetonitrile: methanol: tetrahydrofuran in the ratio of 20:70:10 (v/v/v) was used. The flow rate was 1.0ml/min and effluents were monitored at 251nm. The retention time for Gefitinib was 4.282min. The method was validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness. Limit of detection and limit of quantification were found to be 0.09ppm and 0.29ppm respectively and recovery of Gefitinib from tablet formulation was found to be 99.16%. The proposed method was successfully applied for the quantitative determination of Gefitinib in tablet formulation.

Keywords: Gefitinib, HPLC, Linearity, Validation, Robustness.

INTRODUCTION

Gefitinib is a drug used in the treatment of certain types of cancer. Gefitinib is an EGFR inhibitor, like erlotinib, which interrupts signaling through the epidermal growth factor receptor in target cells. It is marketed by AstraZeneca and Teva.

Gefitinib is the first selective inhibitor of epidermal growth factor receptor's (EGFR) tyrosine kinase domain. Thus gefitinib is an EGFR inhibitor. The target protein (EGFR) is also sometimes referred to as Her1 or ErbB-1 depending on the literature source. IUPAC name is *N*-(3-chloro-4-fluoro-phenyl)-7-methoxy-6-(3-morpholin-4-ylpropoxy)quinazolin-4-amine.

Molecular formula $C_{22}H_{24}ClFN_4O_3$
Molecular weight 446.9

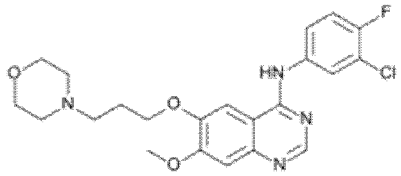


Fig. 1: Molecular Structure of Gefitinib

Literature survey revealed that numerous methods have been developed and reported for estimation of Gefitinib in pharmaceutical formulations.

Present study involves development of LC method using simple mobile phase which is sensitive and rapid for quantification of Gefitinib in tablet dosage forms as well as subsequent validation of developed method according to ICH guide lines.

EXPERIMENTAL

Instrument

The liquid chromatographic system consisted of Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Intersil ODS C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing purpose.

Reagents and materials

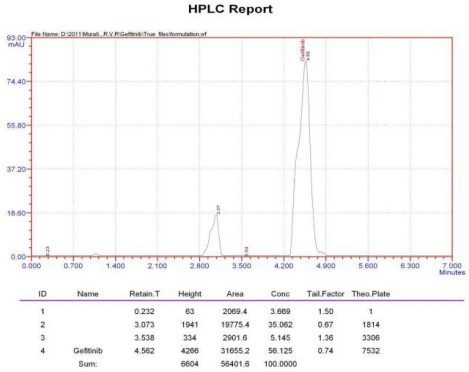
Methanol, acetonitrile and tetrahydrofuran of HPLC grade was purchased from E.Merck, Mumbai, India.

Preparation of Standard Stock Solution

A stock solution of Gefitinib was prepared by accurately weighing 10mg of drug into 100ml of volumetric flask and dissolved in the chosen solvent. Appropriate aliquot of this solution was further diluted with solvent to obtain final standard solution of 25ppm of Gefitinib. Resultant solution was filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper.

Preparation of sample Solution

The formulation tablets of Gefitinib were crushed to give finely powdered material. Powder equivalent to 10mg of drug was taken in 10 ml of volumetric flask containing 5ml of mobile phase and was shaken to dissolve the drug and then filtered through Ultipor N_{66} Nylon 6,6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent to obtain concentration of 20ppm.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		0.232	63	2069.4	3.669	1.50	1
2		3.073	1941	19775.4	35.062	0.67	1814
3		3.538	334	2901.6	5.145	1.36	3306
4	Gefitinib	4.562	4266	31655.2	56.125	0.74	7532
	Sum:		6604	56401.6	100.0000		

Fig. 2: HPLC chromatogram of Gefitinib formulation

Chromatographic conditions

The mobile phase consisting of acetonitrile : methanol : tetrahydrofuran were filtered through 0.45µm Ultipor N_{66} Nylon 6,6 membrane solvent filter, degassed and were pumped from the solvent reservoir in the ratio of 20:70:10(v/v/v),and was pumped into the column. The flow rate of mobile phase was maintained at 1.0ml/min and detection wavelength was set at 251nm with a run time of 7min. The volume of injection loop was 20µl. Prior to injection of the drug solution, the column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

Calibration curve

Appropriate aliquots of standard Gefitinib stock solution were taken in different volumetric flasks and resultant solution was diluted up to the mark with mobile phase to obtain final concentration of 5, 10, 15, 20, 25ppm of Gefitinib. These solutions were injected into chromatographic system. Chromatograms were obtained and peak area ratio was determined for each concentration of drug solution. Calibration curve of Gefitinib was constructed by plotting peak area ratio versus applied concentration of Gefitinib and regression equation was computed. Similarly the sample solution was chromatographed and concentration of Gefitinib in tablet sample was found out using regression equation.

Method validation

The method was validated for accuracy, precision, linearity, specificity, limit of detection, limit of quantification and robustness by following procedures.

Accuracy

The accuracy of the method was determined by calculating recovery of Gefitinib by the method of standard addition. Known amount of Gefitinib (10ppm, 5ppm and 15ppm) was added to a pre quantified sample solution and the amount of Gefitinib was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of Gefitinib

was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated.

Precision

The intra-day precision study of Gefitinib was carried out by estimating the correspondence responses six times on the same day with 25ppm concentration and inter-day precision study of Gefitinib was carried out by estimating the correspondence responses six times next day with 25ppm concentration.

Linearity and range

The linearity of the method was determined at seven concentration levels ranging from 5-25ppm for Gefitinib.

Specificity

Commonly used excipients (colloidal silicon dioxide, lactose, magnesium stearate, povidone, starch and talc) were spiked into a pre-weighed quantity of drug. The chromatogram was taken by appropriate dilutions and the quantity of drug was determined.

Limit of detection and limit of quantification

Limit of detection = 0.09ppm
Limit of quantification = 0.29ppm

Stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both the solutions were analyzed over a period of 8 hours at room temperature.

Robustness

Robustness of the method was studied by changing the composition of organic phase by ±5% and the P^H by ± 0.2, and also by observing the stability of the drugs for 24 hours at ambient temperature in the mobile phase.

RESULTS AND DISCUSSION

The UV spectra of Gefitinib showed that the drug absorbs appreciably at 251nm was selected as the detection wave length in liquid chromatography. Optimization of mobile phase was performed based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase acetonitrile: methanol: tetrahydrofuran in the ratio of 20:70:10 (v/v/v) was used. The retention time of Gefitinib was found to be 4.28min, which indicates a good base line.

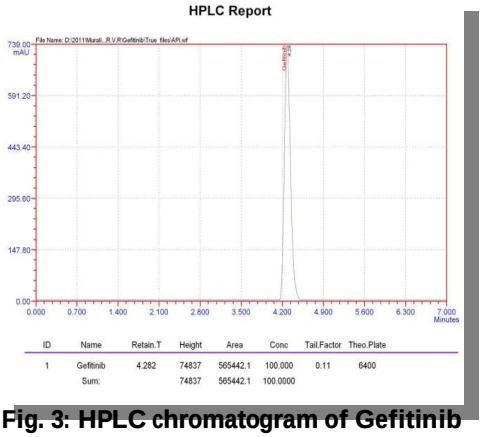


Fig. 3: HPLC chromatogram of Gefitinib

The number of theoretical plates was found to be 6400, which indicates efficient performance of the column. The asymmetric factor was found to be 0.11, which indicates asymmetric nature of the peak. The calibration curve for Gefitinib was obtained by plotting the peak area ratio versus the concentration of Gefitinib over the range of 5-25ppm, and it was found to be linear with regression coefficient of 0.999. The regression equation of Gefitinib concentration over its peak area ratio was found to be Y = 6275.09+ 22400.38 X, where X is the concentration of Gefitinib (ppm) and Y is the respective peak area. The data of regression analysis of the calibration curve was shown in Table 1. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The limit of detection and limit of quantization for Gefitinib was found to be 0.09ppm and 0.29ppm, indicating the sensitivity of the method. The system suitability and validation parameters were given in Table 2. The high percentage of recovery of Gefitinib was found to be 99.16% indicating that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of Gefitinib in tablet formulation. The result for Gefitinib was

comparable with a corresponding labelled amount (Table 3). The absence of additional peaks indicates no interference of the excipients used in the tablets.

Table 1: Regression analysis of the calibration curve

Parameters	Values
Calibration range (ppm)	5-25
Slope	22400.38
Intercept	6275.09
Correlation coefficient (r^2)	0.999

Table 2: System suitability and validation parameters

Parameters	Results
Theoretical plates (N)	6400
Retention time (min)	4.282
Asymmetric factor	0.11
LOD (ppm)	0.09
LOQ (ppm)	0.29
Accuracy (%)	99.98%
R.S.D. (%)	0.877%

Table 3: Assay results of formulation

Formulation	Labelled claim (mg)	% of Gefitinib in Tablet
Gefonib	10	25.86%

CONCLUSION

Proposed study describes new LC method for the estimation of Gefitinib in tablet formulation and serum. The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore the proposed method can be used for routine analysis of estimation of Gefitinib in its tablet formulation and serum.

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