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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF GEFITINIB BY RP-HPLC METHOD IN TABLET DOSAGE FORM

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ABSTRACT

A Simple Precise, Accurate and Rapid Reverse Phase HPLC method developed for the estimation of Gefitinib in tablet dosage form. An Hypersil, 250 x 4.6 nm, 5 µ, C_{18}, with mobile phase consisting of Methanol: Buffer(3.4 gm of Dihydrogen potassium phosphate was dissolved in 1000 ml of milli-Q water) in the ratio of 85:15 v/v was used. The flow rate was 1.5ml/min and the effluents were monitored at 247nm. The retention time was 4.087 min. The percentage assay of Gefitinib was 100%. The calibration curve of Gefitinib was found to be linear over the range of 0.14 to 0.52 mg/ml with correlation coefficient of 0.999. The precision percentage RSD was found to be 0.42%. Accuracy, robustness, ruggedness, system suitability, intermediate precision were validated for the developed method.

KEY WORDS

Estimation, Gefitinib, method development, RP-HPLC, validation.

INTRODUCTION

Gefitinib is a drug used in the treatment of certain types of cancer. Gefitinib is an EGFR inhibitor, like erlotinib, which interrupts signalling through the epidermal growth factor receptor in target cells. Gefitinib is the first selective inhibitor of epidermal growth factor receptor's (EGFR) tyrosine kinase domain [1-3]. Its chemical name was N-(3-chloro-4-fluoro-phenyl)-7- methoxy-6-(3-morpholin-4-ylpropoxy)quina zolin-4-amine with molecular weight was 446.902363 g/mol, and its molecular formula was $C_{22}H_{24}ClFN_4O_3$. The aim of the study was to develop a method and validate a suitable high precision and accurate analytical method for the estimation of Gefitinib in tablet dosage form by using Rp-Hplc method. Structure of Gefitinib was shown in **Fig-1**.

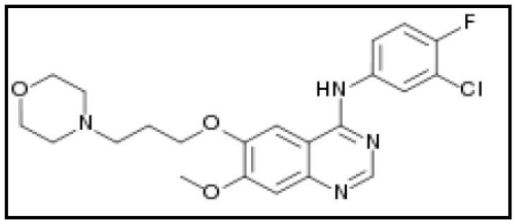

Fig 1: Structure of the drug molecule, Gefitinib

EXPERIMENTAL

Materials and methods

Working standards of Gefitinib was obtained from well reputed research laboratory, Dihydrogen potassium phosphate, methanol, were purchased from E.merck. Water (HPLC grade) was obtained from Milli-Q water purification system.

Instrument:

A Hypersil, 250x4.6nm, 5µ, C_{18} was used, the HPLC system was equipped with Empower software, and

PDA detector module equipped with automatic injection volume 20µl was used.

Chromatographic Condition:

The contents of the mobile phase were Methanol: Buffer (3.4 gm of Dihydrogen potassium phosphate was dissolved in 1000 ml of milli-Q water) in the ratio of 85:15 v/v was used. They were filtered before use through a 0.45 µm membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 1.5 ml/min. The run time was set at 10 min and the column temperature was 60°C.prior to the injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 247nm.and linear regression data was given in **Table-1**.

DRUG	GEFITINIB
Concentration range(mg/ml)	0.14 to 0.52
Slope (m)	39098632.06
Intercept (b)	231447
Correlation coefficient	0.999

Table 1: linear regression data for calibration curves

Preparation of solutions:

Standard solution:

Weigh and transfer accurately 250mg of Gefitinib working standard into 100 ml volumetric flask add about 70 ml of diluent, sonicate to dissolve and make up to the volume with diluent, and further dilute 1 ml of the resultant solution to 10 ml with diluent.

Sample solution:

Weigh and transfer accurately twenty tablets in to a clean and dry mortar and crush them to fine powder. Transfer accurately sample powder equivalent to 250mg of Gefitinib in to a 100ml volumetric flask, add 50ml of diluent sonicate for 15 minutes with occasional stirring and make up to the volume with diluent and filter through 0.45µ syringe filter.

Method validation:

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

Accuracy: The accuracy of the method was established from recovery experiments. The recovery was performed by adding Gefitinib to the placebo in the range of 80 % to 150% of test concentration i.e. 0.1mg/ml of Gefitinib. The solutions were injected into HPLC. The individual recovery and mean recovery values were also calculated.

Linearity: The linearity and range of the method was established by measuring the responses of the standard preparations of six different preparations of six different concentrations of Gefitinib i.e. 40%, 60%, 80%, 100%, 120% and 150%. Graph was shown in **Fig-2**.

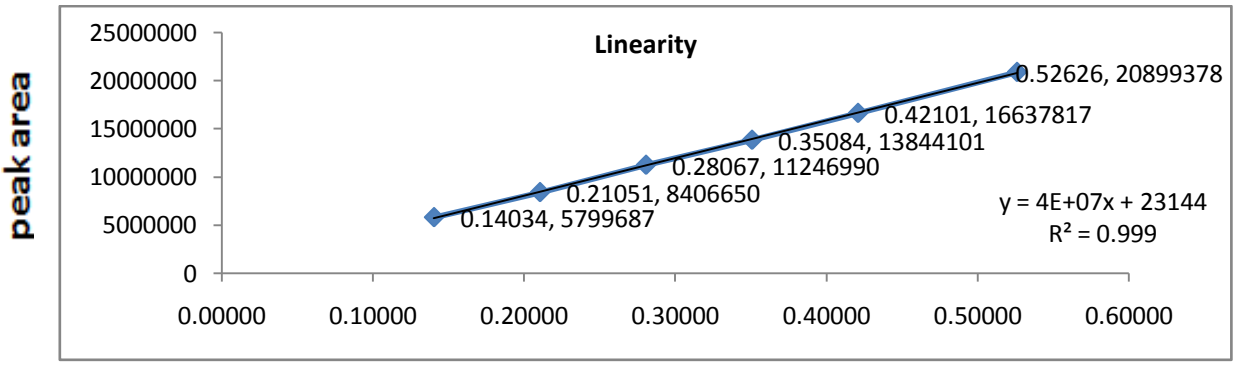

Fig 2: Graph for Linearity data of Gefitinib

Precision: Here the precision was established by using two methods:

a) Repeatability: The repeatability of the method was established by estimating the assay for six different sample preparations of the same batch.

b) Intermediate precision (Ruggedness): The intermediate precision of the method was established by estimating the assay of Gefitinib for six different sample preparations of the same batch by different analysts using a different HPLC with similar column on a different day.

Robustness: Robustness of the method was studied by changing wavelength, flow rate, temperature.

RESULTS AND DISCUSSION

From the typical chromatogram of Gefitinib as shown in the **Fig 3**, it was found that the retention time was 4.087 min, In the present developed HPLC method, the standard and sample preparations required less time and no tedious extraction were involved. A good linear relationship 0.999 was observed between a concentration range of 0.14 to 0.52 mg/ml, low values of the standard deviation are indicative of high precision method. The assay of Gefitinib tablets were found to be 100%, this indicates by high accuracy of the method. For Gefitinib, flow rate was 1.5 ml/min, retention time was found to be 4.087 min, accuracy % RSD was found to be 0.66%, and precision % RSD was found to be 0.42%, intermidiate precision and robustness results were summarised in **Table-2&3**.

Parameters	Gefitinib	
	Analyst1/ Day1/ column-1	Analyst2/ Day2/ column-2
No. of samples	6	6
Mean assay (%)	100.8	99.6
Std. Dev.	0.42	1.85
%RSD	0.42	1.9

Table 2: Observations for intermediate precision

S.No.	Parameters	Low		High	
		Assay%	%RSD	Assay %	%RSD
1	Flow rate	97.5	0.93	98.3	0.65
2	Wavelength	98.4	1.21	96.8	1.42
3	Temperature	96.9	0.23	97.8	0.49

Table 3: Percentage of RSD and Percentage of assay of robustness study of Gefitinib

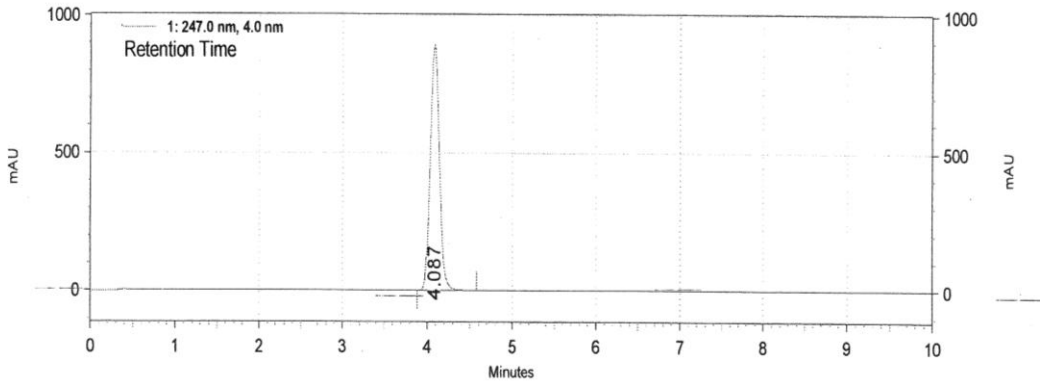

Fig 3: HPLC Chromatogram of Gefitib

CONCLUSION

The proposed method was found to be simple, sensitive, rapid and economical for the estimation of Gefitinib in tablet dosage form, The developed method was also checked for the performance characteristics and has also been validated. The assay by HPLC method adopted for Gefitinib was found to be precise, linear and accurate. It was also proved to be robust. Therefore, the proposed method can be used for routine analysis of estimation of Gefitinib in its tablet formulation.

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