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DEVELOPMENT AND VALIDATION OF A SENSITIVE REVERSED-PHASE HPLC METHOD FOR THE DETERMINATION OF GEFITINIB IN BULK AND IN ITS PHARMACEUTICAL FORMULATION

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ABSTRACT

A rapid and sensitive RP-HPLC method with UV detection at 205 nm for routine analysis of Gefitinib in bulk and pharmaceutical formulations was developed. Chromatography was performed with mobile phase containing a mixture of acetonitrile and 0.5% M ammonium dihydrogen phosphate buffer in the ratio of 30 : 70, v/v with flow rate 1.0 mL/min. The calibration curve of Gefitinib was found to be linear over the range of 0.05 to 0.15 mg/mL with correlation coefficient of 0.99. Sensitivity, accuracy, range, precision, robustness, ruggedness, stability, specificity, LOD, LOQ and system suitability parameters were validated for the developed method.

Key words: Gefitinib, RP-HPLC, Method development, Validation.

INTRODUCTION

Gefitinib is a drug used in the treatment 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 Astra Zeneca and Teva. Gefitinib is the first selective inhibitor of epidermal growth factor receptor's (EGFR) tyrosinekinase domain[1-3]. 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 $C_{22}H_{24}ClFN_4O_3$ and molecular weight is 446.9. Literature survey revealed that numerous methods have been developed and reported for estimation of Gefitinib in pharmaceutical formulations. Present study involves development

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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[4].

EXPERIMENTAL

Material and methods

Chemical and reagents

Gefitinib (active pharmaceutical ingredient, API) and reference material were procured from a reputed pharmaceutical company Astra, India. All chemicals and solvents of HPLC grade were purchased from RANKEM, New Delhi, India. Water (HPLC grade) was obtained from Milli-Q water purification system.

Instrumentation

Shimadzu Separations module with PDA/UV detector connected to LC solution software and data acquisition was performed by Class LC software[6,7]. Analysis was carried out at 205 nm with a reverse phase YMC-ODS-AQ (150 X 4.6 mm, 5 μm) at ambient temperature. The mobile phase was a mixture of acetonitrile and 0.5% ammonium dihydrogen phosphate in the ratio of 30 : 70 (v/v) and the flow rate was 1.0 mL/min with runtime 20 min. The mobile phase was degassed and filtered through 0.45 μm membrane filter before pumping into the HPLC system.

Preparation of solutions

Preparation of drug/test stock solution

10.27 mg of Gefitinib standard was accurately weighed and transferred to a 10 mL volumetric flask (1.027 mg/mL) dissolved and finally made up to the mark with diluent water. Further dilutions were prepared with the mobile phase to the required concentrations from the stock solutions. The sample was dissolved and finally made up to the mark. Further dilutions required were made from the stock solution.

Experimental procedure for method validation[5,8,9]

System suitability

System suitability was assessed by replicate analysis of six injections of the Gefitinib standard solution at a concentration of 0.1 mg/mL and the chromatogram was obtained. The system suitability parameters such as tailing factor, theoretical plate count and

reproducibility (% RSD of analyte retention time and area of the six replicates) were calculated from the chromatogram.

Specificity

The analyte was subjected to forced degradation studies using water, acid, alkali treatments, oxidative and reductive degradation for demonstration of specificity of the method. Gefitinib was analyzed under these conditions for purity, indicating that the developed HPLC method effectively separated the degradation products from the Gefitinib standard peak.

Acid degradation

From the stock solution 1.0 mL of Gefitinib standard was accurately transferred to a 10 mL volumetric flask. 1 mL of 1 N HCl was added to the volumetric flask. The flask was placed on a water bath maintained at 60°C for 60 min. It was cooled to room temperature, dissolved in the diluent and the volume was made up to mark.

Base degradation

From the stock solution 1.0 mL of Gefitinib standard was accurately transferred to a 10 mL volumetric flask. 1 mL of 1 N NaoH was added to the volumetric flask. The flask was placed on a water bath maintained at 60°C for 60 min. It was cooled to room temperature, dissolved in the diluent and the volume was made up to mark.

Oxidative degradation

From the stock solution 1.0 mL of Gefitinib standard was accurately transferred to a 10 mL. 1 mL of 6% H_2O_2 was added to the volumetric flask. The flask was placed on a water bath maintained at 60°C for 60 min. It was cooled to room temperature, dissolved in the diluent and the volume was made up to mark.

Linearity

To demonstrate the linearity 5 standard solutions of Gefitinib were prepared with concentration ranging from 50 to 150% to the target assay concentration. The peak area ratio of the drug was considered for plotting the graph. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision

Reproducibility: The reproducibility of the injection was assessed by using 6

injections of the standard solution and the area of relative standard deviation of the replicate injection was calculated.

Precision of the method

To demonstrate the precision of the method, 5 samples from the same batch of formulation were analyzed individually and the assay content (Gefitinib) of each sample was estimated. The average assay for 5 determinations was calculated along with RSD for replicate determinations.

Accuracy

To validate the accuracy of the test method recovery experiments were conducted at a concentration range of 80, 100 and 120%. Test solutions were prepared from target test assay concentration (0.1 mg/mL). Each test solution was prepared in triplicate and analysis was also performed in triplicate. The assay content value at the beginning of validation was considered as the true value (100) for recovery calculations. The percentage assay, percentage recovery, mean percentage recovery, were calculated from the data obtained.

Stability test

Test and standard solutions of 1.0 mg/mL were prepared from the stock solutions and were stored at ambient. The analysis of each solution was repeated at periodic intervals covering a time period of 48 hrs. The areas from each of the experiment were taken and the percentages were calculated.

Stability of mobile phase

Mobile phase was prepared as per the above-mentioned method and kept at room temperature for 5 days. Test and standard solutions were prepared with the same mobile phase and analyzed by HPLC at an interval of 24 hours for 2 days. Percentage assay and % RSD were calculated.

Robustness

System suitability tests were performed after making deliberate changes in the mobile phase flow rate (± 0.2 mL/min), mobile phase composition (± 5 mL), and buffer pH (± 0.2) buffer conc. 1.0 to 2 % organic solvent 1.0 to 2.0 % column temperature (± 3ºC) from developed HPLC conditions.

LOD and LOQ

This approach can only be applied to analytical procedures which exhibit baseline

noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 or 2 : 1 is generally considered acceptable for estimating the detection limit. Quantification limit can approach only be applied to analytical procedures that exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical signal-to-noise ratio is 10 : 1.

Ruggedness

Ruggedness of the method was demonstrated through the study of the following variations

1. Analyst to analyst variation
2. Column to column variation
3. Day to day variation

RESULTS AND DISCUSSION

Method development and optimization

Optimization of the method was carried out by fixing one parameter and altering the other parameters. Gefitinib was analyzed by using polar solvents like KH_2PO_4 buffer: acetronitrile in one case and (NH_4) H_2PO_4 : acetronitrile: methanol in another case as it is a polar molecule. In both cases Gefitinib was analyzed using reverse phase column (YMC-ODS-AQ 150 x 4.6 mm, 5 µ). Various buffer strengths viz., 0.5%, 0.1%, 0.2%, different flow rates like 0.5 mL/min, 0.8 mL/min, 1.0 mL/min were used with different experimental conditions. Different mobile phase compositions 50 : 50, 60 : 40, 70 : 30 v/v with isocratic elutions were used. Gefitinib was also analyzed using acetonitrile: buffer in a ratio of 70 : 30 v/v with isocratic elution. Experiments were conducted to optimize the HPLC method for Gefitinib in order to get reproducibility, better peak shape and rapid analysis. All the experiments were monitored using UV detector at a wavelength of 254 nm. Optimum mobile phase ratio for the analysis was found to be 70 : 30 of buffer and acetonitrile with a flow rate of 1.0 mL/min. A typical chromatogram is shown (Fig. 1). Experiments were conducted by changing the pH of the buffer. At buffer strengths (0.2%) with peak tailing

and low plate count were observed. Best separation and good peak shape was observed with buffer strength of 0.5%.

Method validation

System suitability

The % RSD of the peak area and retention time of Gefitinib were within 2% indicating system suitability. The efficiency of the column as expressed by the number of theoretical plates for 6 replicate injections was found to be 8989 ± 25 and USP tailing factor was 1.2.

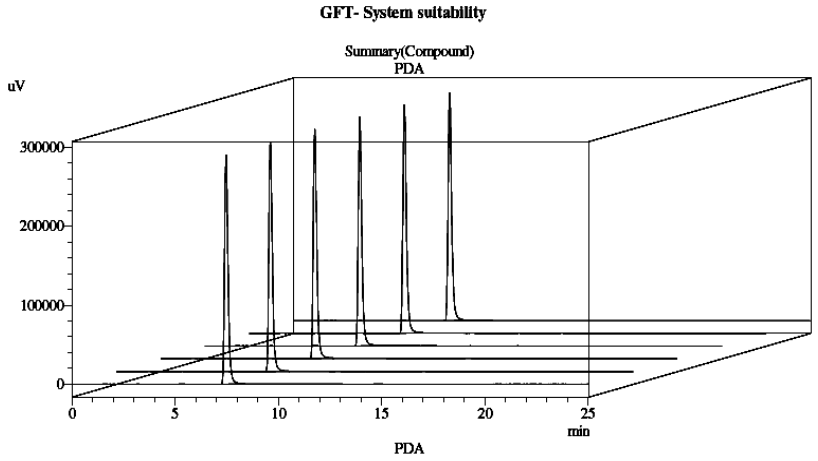

Fig. 1: Gefitinib system suitability

Linearity

The calibration curve constructed was evaluated by using correlation coefficient. The peak area of the Gefitinib was linear in the range of 0.05 to 0.150 mg/mL. The average area for each of the concentration obtained was plotted against the concentration of the analyte. The correlation coefficient for the data was calculated as 0.999 for Gefitinib indicating a strong correlation between the concentration and the area under the curve. A linear regression graph was drawn between the concentr ation of the analyte and the areas. The regression line was observed to be in the form of y = 3E + 07x for Gefitinib. The slope of the regression line was found to be 33851. Data indicated that the difference between the estimated and actual areas was significant. These experiments indicated that there was a linear relation between the amount of analyte and the areas within the range studied (50% to 150%).

Table 1: Concentration vs area

Injection	% Level	Conc. (mg/mL)	Average area
1	50	0.05135	1740147
2	80	0.08216	2818246
3	100	0.1027	3436064
4	120	0.12324	4157333
5	150	0.15405	5290357

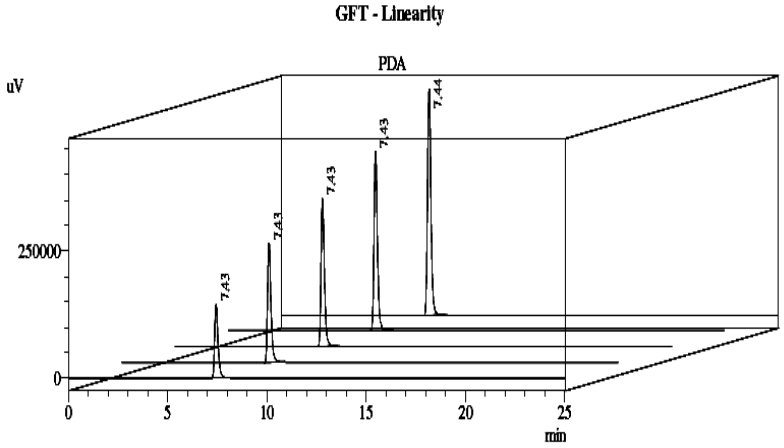

Fig. 2: Gefitinib linearity

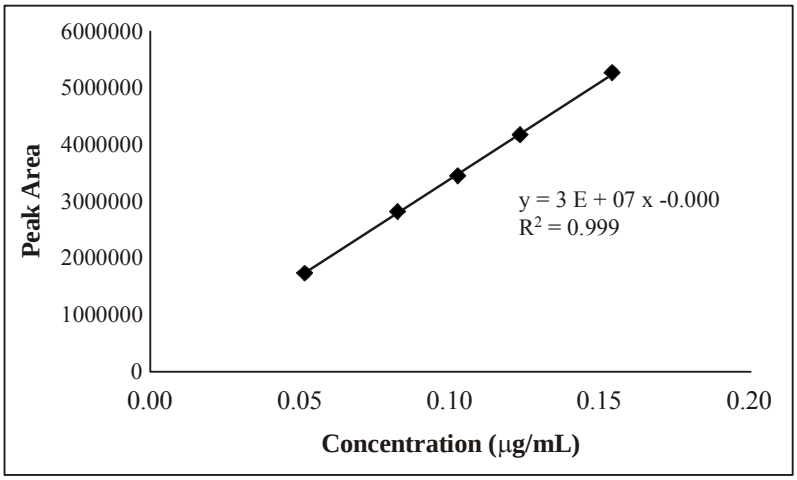

Fig. 3: Gefitinib Linearity graph

Accuracy

Accuracy of the method was expressed in terms of recovery of added compound. Percentage recovery was calculated by multiplying the ratio of the measured concentration with 100. Mean % recovery and % RSD were also calculated and were found to be 100 and 0.15 respectively. It can be observed that the developed HPLC method is accurate.

Table 2: Accuracy-results of recovery experiments

Conc. (mg/mL)	Area	Recovery (mg/mL)	% Recovery
0.08216	2784511	0.08190	99.7
0.08216	2816516	0.08287	100.9
0.08216	2819975	0.08298	101.0
0.1027	3442025	0.10188	99.2
0.1027	3430103	0.10152	98.9
0.1027	3451161	0.10216	99.5
0.12324	4161327	0.12374	100.4
0.12324	4158771	0.12367	100.3
0.12324	4155895	0.12358	100.3
		Mean	**100**
Intercept	**89761**	**Sdv**	**0.74**
Slope	**32903205**	**% RDS**	**0.74**

Precision

The precision of the method was calculated from the reproducibility of the area of standard solutions and percentage assay of six Gefitinib test samples. The results showed that the precision of the method is good.

Specificity (Forced degradation) and stability

Accelerated degradation studies under different conditions viz., acid treatment; base treatment, photolytic, oxidation and reduction were conducted to demonstrate the specificity. Photo stability tests showed that the active substance is not light sensitive and no degradation products were formed during the oxidation treatment whereas degradation products were observed during other treatments. The chromatogram is given in Fig. 4 and the results are tabulated.

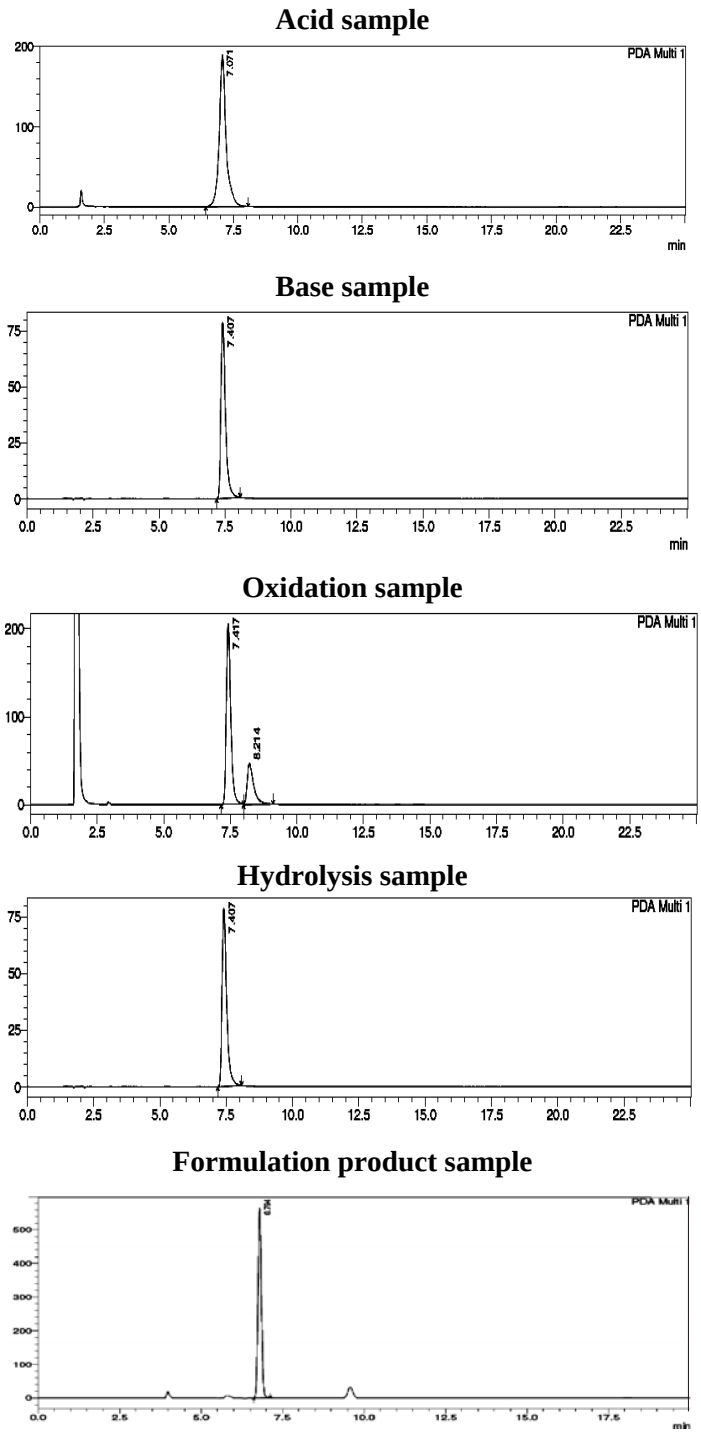

Fig. 4: Chromatograms of Gefitinib subjected for degradations

Stability of standard solution

Standard solutions were stable for 2 days and the results are given.

Table 3: Stability of solutions

Time point	Area	% Initial
Initial	3463662	100
R.T 48 hrs	3452191	99.7

Table 4: Results of analysis (Formulation recovery assay)

Drug	Amount mg/tab		% Recovery
	Labelled	Found	
Gefitinib	250	248.7	99.48

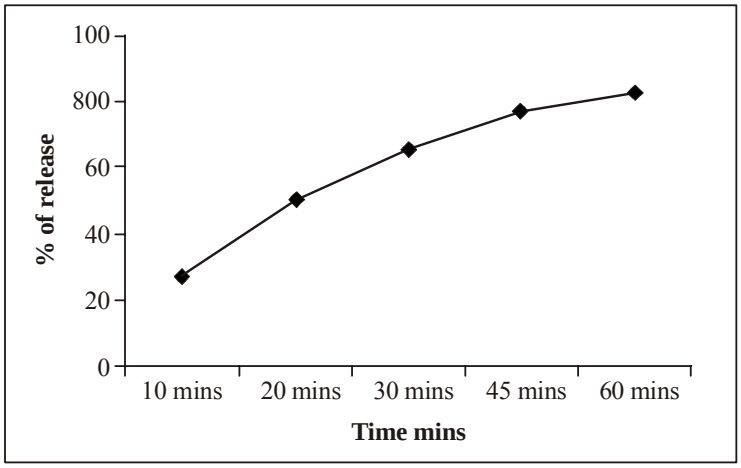

Fig. 5: Drug release

Ruggedness

The results were well within acceptable limits of 98-102 % (98.1-100.7 %) with percentage RSD less than 2.0 % for all the studied parameters viz., column, day and analyst. These results indicated that the developed HPLC method is rugged.

LOD and LOQ

The limits of detection and quantification of Gefitinib were found to be 0.07% and 0.2% respectively.

Application of the method to dosage forms

The method was validated for different parameters and was applied for the estimation of Gefitinib in pharmaceutical dosage forms. Tablets procured from formulation department were evaluated for the amount of Gefitinib present in the formulation. Samples were analyzed in triplicate and the amount of Gefitinib in was found to be 248.7 mg/mL (Labeled claim is 250 mg). Mean assay was found to 99.48. The developed HPLC method is sensitive and specific for the quantitative determination of Gefitinib in dosage forms.

CONCLUSION

A rapid and accurate RP-HPLC method was developed for the determination of Gefitinib in bulk and pharmaceutical dosage forms. The method was evaluated for specificity, linearity, accuracy, range, precision, LOD, LOQ, ruggedness and robustness as per ICH guidelines and proved to be economical and effective for the quality control of the Gefitinib in the given application. The measured signal was observed to be accurate, precise and linear with a correlation coefficient of 0.9999. The proposed method is observed to be rapid and selective, when compared to the methods reported in the literature, the retention time of Gefitinib was found to be very less. The method is also cost effective with respect to solvent consumption.

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