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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC METHOD FOR ESTIMATION OF GEFITINIB IN BULK AND ITS PHARMACEUTICAL FORMULATIONS

A. Sreedevi, A. Lakshmana Rao* and L. Kalyani

Department of Pharmaceutical Analysis, V.V. Institute of Pharmaceutical Sciences, Gudlavalleru- 521 356, Andhra Pradesh, India.

ABSTRACT

A simple, isocratic, accurate and precise stability indicating HPLC method has been developed and subsequently validated for the determination of Gefitinib in bulk and its pharmaceutical formulations. Separation was achieved on a Hypersil BDS C_{18} column (100 mm x 4.6 mm I.D., 5 µm particle size) as stationary phase using a mobile phase consisting of phosphate buffer (pH 3.6) and acetonitrile (55:45 v/v) at a flow rate of 1.0 mL/min and UV detection at 248 nm. The developed method is validated as per the ICH guidelines, which includes linearity, precision, accuracy, specificity, ruggedness and robustness. The current method demonstrates excellent linearity over the concentration range of 25-150 µg/mL for Gefitinib with correlation coefficient of 0.999. The mean recovery of the compound is 99.93%. The retention time was 4.179 min. The percentage assay of Gefitinib was 99.60%. The limit of detection and limit of quantification for Gefitinib was found to be 0.078 µg/mL and 0.238 µg/mL respectively. The drug Gefitinib was subjected to acidic, alkaline, oxidative, thermal, hydrolytic and photolytic stress conditions. The results showed that the proposed method is suitable for the determination of Gefitinib in bulk and pharmaceutical formulations.

Keywords: Gefitinib, Estimation, HPLC, Formulation.

INTRODUCTION

Gefitinib is an antineoplastic agent used for certain breast, lung and other cancers[1]. Chemically it is, *N*-(3-chloro-4-fluoro-phenyl)-7-methoxy-6-(3-morpholin-4-ylpropoxy) quinazolin-4-amine[2]. The molecular structure of Gefitinib was shown in Figure 1[3]. Gefitinib is first selective inhibitor of epidermal growth factor receptor's (EGFR)-tyrosine kinase inhibitor, which blocks signal transduction pathways implicated in the proliferation and survival of cancer cells. Many cells, including cancer cells, have receptors on their surfaces for epidermal growth factor (EGF), a protein that is normally produced by the body and that promotes the growth and multiplication of cells. When EGF attaches to EGFRs, it causes an enzyme called tyrosine kinase to become active within the cells[4]. Tyrosine kinase triggers chemical processes that cause the cells, including cancer cells, to grow, multiply and spread. Gefitinib attaches to EGFRs and thereby blocks the attachment of EGF and the activation of tyrosine kinase[5]. This mechanism for stopping cancer cells from growing and multiplying is very different from the mechanisms of chemotherapy and hormonal therapy.

Literature survey revealed that few analytical methods such as UV[6], Visible[7], HPLC[8-10] and LC-MS[11] methods were reported for the estimation of Gefitinib. Hence an attempt has been made to develop and validate a simple, rapid and accurate RP-HPLC method for the determination of Gefitinib in bulk drug and its pharmaceutical formulations.

EXPERIMENTAL

Instrumentation

The analysis of the drug was carried out on Waters HPLC system equipped with 2695 pump and 2996 photodiode array detector was used and a Hypersil BDS C_{18} column (100 mm x 4.6 mm I.D., 5 µm particle size) was used. The

output of the signal was monitored and integrated using Empower 2 software. The instrument is equipped with an auto sampler. A 10 µL rheodyne injector port was used for injecting the samples.

Chemicals and solvents

The working standard of Gefitinib was provided as gift sample from Spectrum Labs, Hyderabad, India. The market formulation GEFTIB Tablets (Gefitinib 250 mg) was procured from local market. HPLC grade acetonitrile and water were purchased from E.Merck (India) Ltd, Mumbai, India. Potassium dihydrogen phosphate, orthophosphoric acid and triethylamine of AR grade were obtained from S.D. Fine Chemicals Ltd, Mumbai, India.

Buffer preparation

Accurately weighed and transferred about 2.72 grams of potassium dihydrogen phosphate into a 1000 mL beaker and mixed well. 1 mL of triethylamine was added and diluted up to 1000 mL with HPLC water. Adjusted pH to 3.6 (±0.5) with orthophosphoric acid solution. Filtered the solution through 0.45 µm membrane filter.

Mobile phase preparation

A mixture of phosphate buffer and acetonitrile in the ratio of 55:45 v/v was used as mobile phase. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum.

Diluent preparation

The dilutions are made with a mixture of acetonitrile and water in the ratio of 70:30 v/v.

Standard solution preparation

10 mg of Gefitinib was accurately weighed, transferred to 10 mL volumetric flask and is dissolved in 7 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume is made up to 10 mL with diluent to get a concentration of 1 mg/mL stock solution. Further pipetted 1 mL of the above stock solution into a 10 mL volumetric flask and diluted up to the mark with diluent to obtain required concentration.

Sample solution preparation

Twenty commercial tablets were weighed and powdered. A quantity of the powder equivalent to 10 mg of Gefitinib was accurately weighed, transferred to 10 mL volumetric flask and is dissolved in 7 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume is made up to 10 mL with diluent to get a concentration of 1 mg/mL stock solution. Further pipetted 1 mL of the above stock solution into a 10 mL volumetric flask and diluted up to the mark with diluent to obtain required concentration of Gefitinib in pharmaceutical dosage form. Inject 10 µL of the above solutions into the HPLC system. All experiments were conducted in triplicate.

Chromatographic conditions

A Hypersil BDS C_{18} column (100 mm x 4.6 mm I.D., 5 µm particle size) was used for analysis of Gefitinib at a column temperature of 30^{0}C. The mobile phase was pumped through the column at a flow rate of 1.0 mL/min. The sample injection volume was 10 µL. The photodiode array detector was set to a wavelength of 248 nm for the detection and chromatographic run time was 8 minutes.

RESULTS AND DISCUSSION

Method Development[12]

To develop a suitable and reliable HPLC method for the determination of Gefitinib, different mobile phases were employed to achieve the best separation and resolution. The method development was carried out with Hypersil BDS C_{18} column (100 mm x 4.6 mm I.D., 5 µm particle size) as stationary phase and a mixture of phosphate buffer and acetonitrile in the ratio of 55:45 v/v was used as mobile phase respectively. The eluent flow rate was 1.0 mL/min and UV detection was performed at 248 nm. The retention time of Gefitinib is 4.179 minutes and the peak shape was good. The results of optimized HPLC conditions were shown in Table 1. The typical chromatogram of Gefitinib standard using the proposed method is shown in Figure 2. Gefitinib shows significant UV absorbance at wavelength 248 nm. Hence this wave length has been chosen for detection in analysis of Gefitinib.

Method Validation

The developed HPLC method is validated in accordance with ICH guidelines[13-14] for the analysis of Gefitinib using the following parameters.

Linearity

Several aliquots of standard solution of Gefitinib was taken in different 10 mL volumetric flasks and diluted up to the mark with diluent such that the standard curve was obtained for Gefitinib in the concentration range of 25-150 µg/mL. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient of standard

curve were calculated and given in Figure 3 to demonstrate the linearity of the method. Evaluation of the drug was performed with UV detector at 248 nm, peak area was recorded for all the peaks. The response for the drug was linear and the regression equation was found to be y=826.8x-802 and the correlation coefficient value of Gefitinib was found to be 0.999. The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated. The linearity results were shown in Table 2.

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solution using the developed HPLC method. The LOD and LOQ for Gefitinib were found to be 0.078 μg/mL and 0.238 μg/mL respectively, which indicate the sensitivity of the method.

Specificity

The specificity of the proposed method involved by injecting blank and placebo using the chromatographic conditions defined for the proposed method. It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention times of standard and sample.

Precision

The precision was determined for Gefitinib in terms of system precision and method precision. For system precision evaluation, a standard solution of fixed concentration was injected at various time intervals and %RSD for Gefitinib was 0.9% (limit %RSD < 2.0%). In addition, the method precision was studied by injecting the same concentration of standard solution on consecutive days and the %RSD for Gefitinib was 0.54% (limit %RSD < 2.0%). The results are showing that the proposed method was precise. Results are summarized in Table 3 and Table 4.

Accuracy

The accuracy of the method was determined by recovery studies of Gefitinib at three concentration levels. A fixed amount of preanalyzed sample was taken and standard drug was added at 50%, 100% and 150% levels. The standard concentration was fixed as 100 μg/mL and three concentration levels of 50 μg/mL, 100 μg/mL and 150 μg/mL were added to the standard concentration. Each level was repeated three times. The content of Gefitinib per tablet was calculated. The percentage recovery ranges from 99.61-100.14% and the mean recovery of Gefitinib was 99.93%. The overall %RSD of 0.75 and the low RSD values of Gefitinib indicate the method is accurate. The results of recovery studies were shown in Table 5.

System suitability

A system suitability test was performed to evaluate the chromatographic parameters like retention time, theoretical plates and tailing factor were calculated and compared with standard values. The retention time of Gefitinib was 4.179 minutes, cuts down on overall time of sample analysis and the method was more cost effective as it utilizes very less quantity of mobile phase. The number of theoretical plates was 3498 and tailing factor was 1.21 for Gefitinib, which indicates efficient performance of the column. The summary of system suitability parameters were shown in Table 6.

Analysis of marketed formulation

The proposed method was applied for the determination of Gefitinib in pharmaceutical formulaton of Gefitinib tablets. 10 μL of each standard and sample solution were injected and from the peak area of Gefitinib, amount of drug present in samples were computed. The % assay of Gefitinib was found to be 99.60. The assay obtained is more than 99% and no interference of impurity peak observed in Gefitinib peak. The assay results were shown in Table 7.

Ruggedness and robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments by different operators using different columns of similar types. Robustness of the method was determined by making slight changes in the chromatographic conditions like changes in flow rate and mobile phase composition. It was observed that there were no marked changes in the chromatograms, which demonstrated that the HPLC method so developed is rugged and robust.

Solution stability

The stability of solution under study was established by keeping the solution at room temperature for 24 hrs. The result showed no significant change in concentration and thus confirms the stability of the drug in the mobile phase used for the analysis.

Degradation studies

Control sample

20 tablets were accurately weighed and finely powdered. A quantity of the powder equivalent

to 10 mg of Gefitinib was accurately weighed, transferred to 10 mL volumetric flask and is dissolved in 7 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume is made up to 10 mL with diluent to get a concentration of 1 mg/mL stock solution. Further pipetted 1 mL of the above stock solution into a 10 mL volumetric flask and diluted up to the mark with diluent to obtain 100 µg/mL concentration of Gefitinib.

Acidic degradation studies

20 tablets were accurately weighed and finely powdered. A quantity of the powder equivalent to 10 mg of Gefitinib was accurately weighed, transferred to 10 mL volumetric flask and is dissolved in 7 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume is made up to 10 mL with diluent to get a concentration of 1 mg/mL stock solution. To 1 mL of stock solution of Gefitinib, 1 mL of 2N hydrochloric acid was added and refluxed for 30 minutes at 60^{0}C. The resultant solution was diluted to obtain 100 µg/mL solution and 10 µL solution were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Alkaline degradation studies

20 tablets were accurately weighed and finely powdered. A quantity of the powder equivalent to 10 mg of Gefitinib was accurately weighed, transferred to 10 mL volumetric flask and is dissolved in 7 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume is made up to 10 mL with diluent to get a concentration of 1 mg/mL stock solution. To 1 mL of stock solution of Gefitinib, 1 mL of 2N sodium hydroxide was added and refluxed for 30 minutes at 60^{0}C. The resultant solution was diluted to obtain 100 µg/mL solution and 10 µL solution were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Oxidative degradation studies

20 tablets were accurately weighed and finely powdered. A quantity of the powder equivalent to 10 mg of Gefitinib was accurately weighed, transferred to 10 mL volumetric flask and is dissolved in 7 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume is made up to 10 mL with diluent to get a concentration of 1 mg/mL stock solution. To 1 mL of stock solution of Gefitinib, 1 mL of 20% hydrogen peroxide (H_2O_2) was added and refluxed for 30 minutes at 60^{0}C. The resultant solution was diluted to obtain 100 µg/mL solution and 10 µL solution were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Thermal degradation studies

20 tablets were accurately weighed and finely powdered. A quantity of the powder equivalent to 10 mg of Gefitinib was accurately weighed, transferred to 10 mL volumetric flask and is dissolved in 7 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume is made up to 10 mL with diluent to get a concentration of 1 mg/mL stock solution. The standard Gefitinib solution was placed in oven at 105^{0}C for 6 hours. The resultant solution was diluted to obtain 100 µg/mL solution and 10 µL solution were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Hydrolytic degradation studies

20 tablets were accurately weighed and finely powdered. A quantity of the powder equivalent to 10 mg of Gefitinib was accurately weighed, transferred to 10 mL volumetric flask and is dissolved in 7 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume is made up to 10 mL with diluent to get a concentration of 1 mg/mL stock solution. The standard Gefitinib solution was refluxed in water bath for 6 hours at 60^{0}C. The resultant solution was diluted to obtain 100 µg/mL solution and 10 µL solution were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photolytic degradation studies

20 tablets were accurately weighed and finely powdered. A quantity of the powder equivalent to 10 mg of Gefitinib was accurately weighed, transferred to 10 mL volumetric flask and is dissolved in 7 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume is made up to 10 mL with diluent to get a concentration of 1 mg/mL stock solution. The standard solution of Gefitinib is exposed to UV light by keeping the beaker in UV chamber for 7 days or 200 Watt hours/m^2 in photo stability chamber. The resultant solution was diluted to obtain 100 µg/mL solution and

10 µL solution were injected into the system and the chromatograms were recorded to assess the stability of the sample.

HPLC studies of Gefitinib under different stress conditions indicated the following degradation behavior. In acidic degradation, the degradation product of Gefitinib was appeared at retention time of 3.850 minutes and the % degradation is 3.27%. In alkaline degradation, the degradation product of Gefitinib was appeared at retention time of 3.857 minutes and the % degradation is 1.83%. In oxidative degradation, the degradation product of Gefitinib was appeared at retention time of 3.838 minutes and the % degradation is 5.24%. In thermal degradation, the degradation product of Gefitinib was appeared at retention time of 3.855 minutes and the % degradation is 2.27%. In hydrolytic degradation, the degradation product of Gefitinib was appeared at retention time of 3.960 minutes and the % degradation is 0.11%. In photolytic degradation, the degradation product of Gefitinib was appeared at retention time of 3.901 minutes and the % degradation is 1.15%. The results of degradation studies are given in Table 8. The typical chromatograms of degradation behavior of Gefitinib in different stress conditions are shown in Figure 4 to Figure 9.

CONCLUSION

A simple, fast, sensitive, accurate, precise, reliable and reproducible stability indicating HPLC method was developed and validated for the estimation of Gefitinib in pharmaceutical formulations. As there is no interference of blank and placebo at the retention time of Gefitinib shows specificity of the method. Validation of this method was accomplished, getting results meeting all requirements. Hence this method was found to be suitable for the routine quality control analysis of the Gefitinib in bulk and its pharmaceutical dosage forms.

Table 1: Optimized chromatographic conditions of Gefitinib

Parameter	Condition
Mobile phase	Phosphate buffer:acetonitrile (55:45 v/v)
pH	3.6
Diluent	Acetonitrile:water (70:30 v/v)
Column	Hypersil BDS C_{18} column (100 mm x 4.6 mm, 5 µ)
Column temperature	30^{0}C
Wave length	248 nm
Injection volume	10 µL
Flow rate	1.0 mL/min
Run time	8 min

Table 2: Linearity results of Gefitinib

Concentration (µg/mL)	Area
25	19619
50	40414
75	60279
100	81544
125	103000
150	123641

Table 3: System precision data of Gefitinib

S. No.	Area of Gefitinib
1	869039
2	848468
3	865656
4	868431
5	862517
6	869664
Average	863963
SD	8041
%RSD	0.90

Table 4: Method precision data of Gefitinib

S. No.	Area of Gefitinib
1	868090
2	876882
3	866932
4	863993
5	866760
6	864329
Average	867831
SD	4710
%RSD	0.54

Table 5: Recovery studies of Gefitinib

Level	Concentration added (µg/mL)	Concentration found (µg/mL)	% Recovery	Mean recovery
50%	50	50.03	100.06%	99.93%
100%	100	100.14	100.14%	
150%	150	149.42	99.61%	

Table 6: System suitability parameters of Gefitinib

Parameter	Results
Linearity range (µg/mL)	25-150
Correlation coefficient	0.999
Theoretical plates (N)	3498
Tailing factor	1.21
LOD (µg/mL)	0.078
LOQ (µg/mL)	0.238
Retention time (min)	4.179

Table 7: Assay results of Gefitinib

Formulation	Label claim	Amount found	%Assay
GEFTIB	250 mg	249 mg	99.60%

Table 8: Degradation studies of Gefitinib

Stress conditions	Degradation time	Area of peak	% Degradation	% of active drug present after degradation
Standard Drug	-	869664	-	-
Acidic	30 mins	841215	3.27%	96.72%
Alkaline	30 mins	853736	1.83%	98.16%
Oxidative	30 mins	824041	5.24%	94.75%
Thermal	6 hours	849845	2.27%	97.72%
Hydrolytic	6 hours	868707	0.11%	99.88%
Photolytic	7 days	859626	1.15%	98.84%

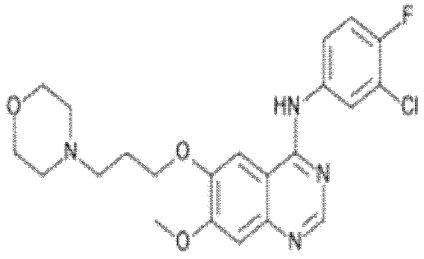

Fig. 1: Molecular structure of Gefitinib

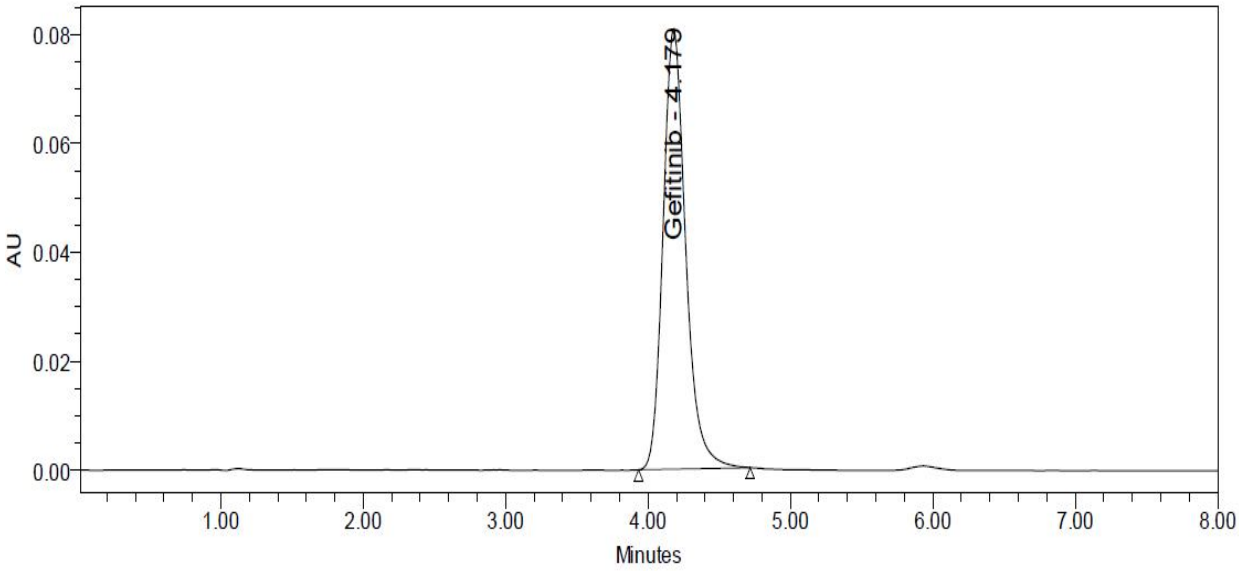

Fig. 2: Typical chromatogram of Gefitinib

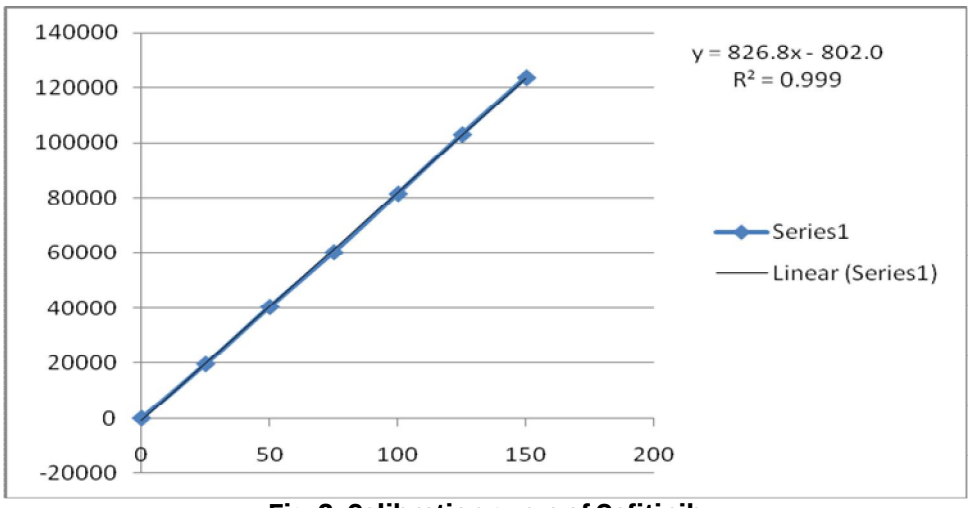

Fig. 3: Calibration curve of Gefitinib

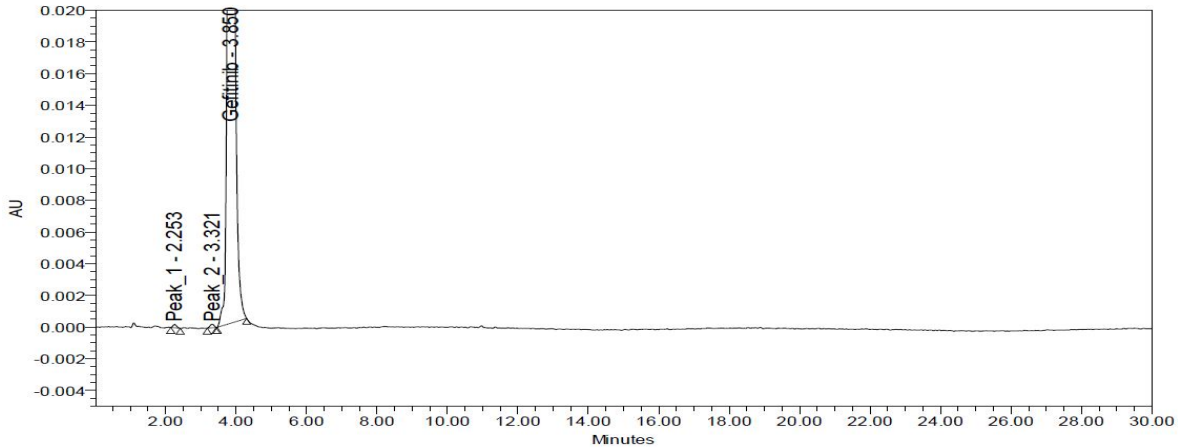

Fig. 4: Acidic degradation chromatogram of Gefitinib

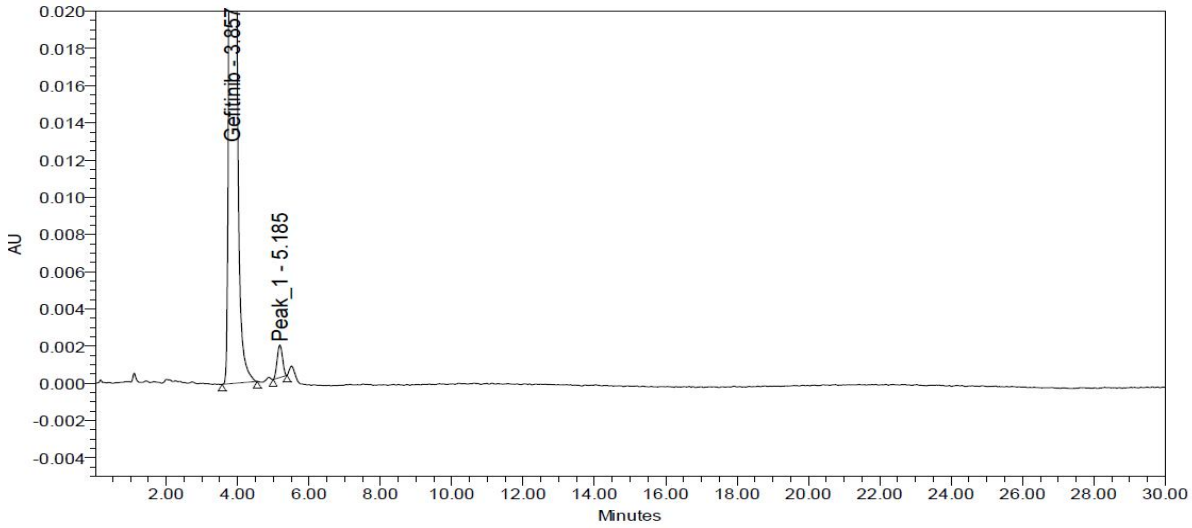

Fig. 5: Alkaline degradation chromatogram of Gefitinib

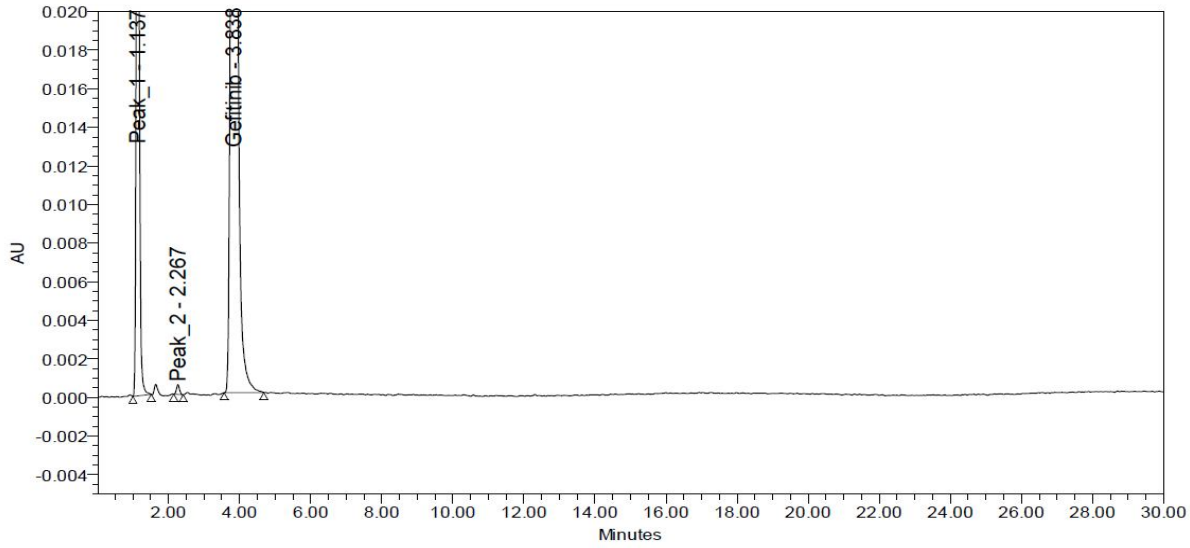

Fig. 6: Oxidative degradation chromatogram of Gefitinib

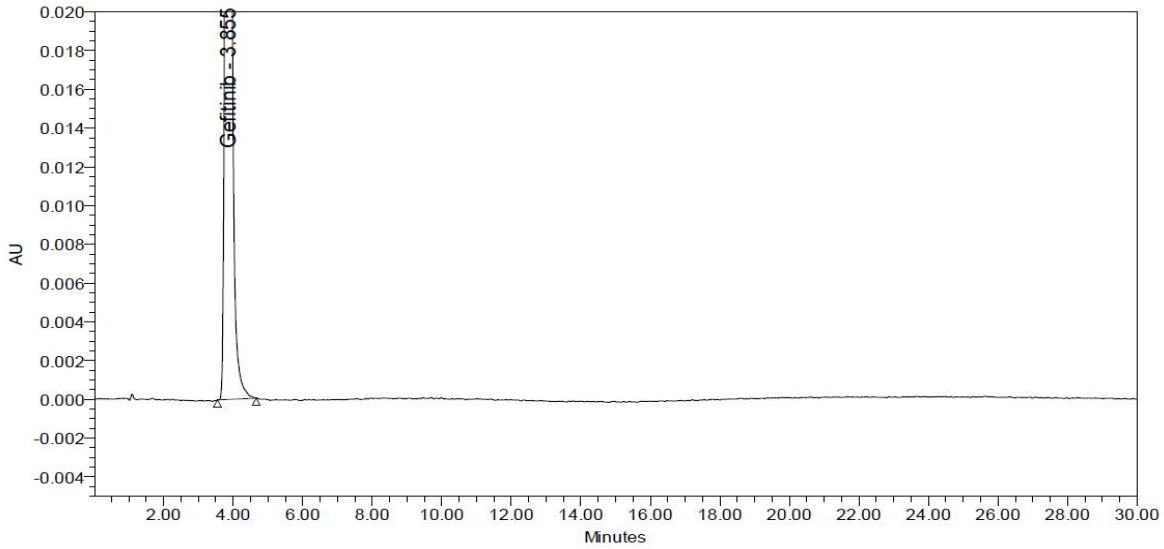

Fig. 7: Thermal degradation chromatogram of Gefitinib

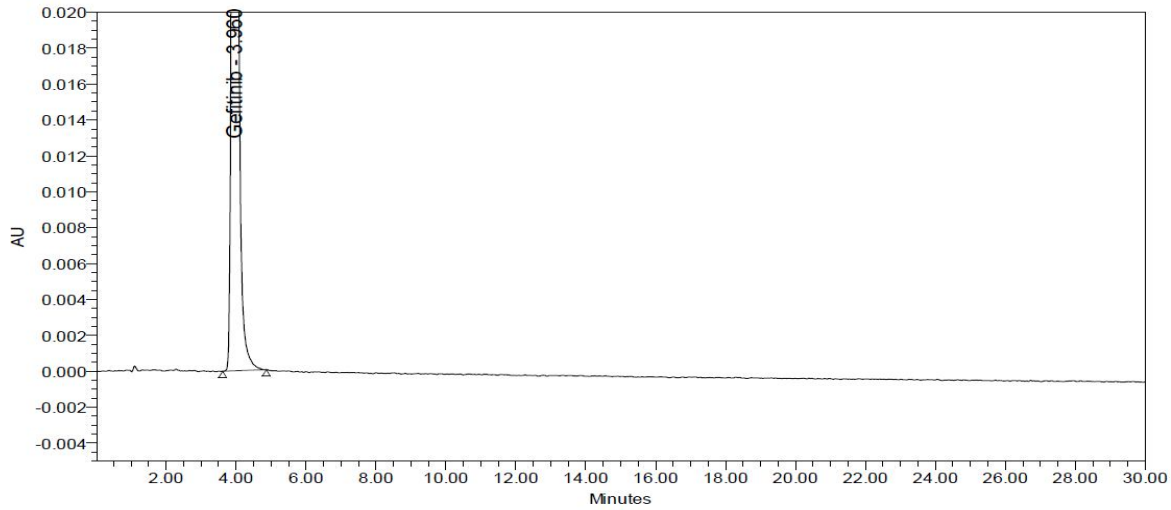

Fig. 8: Hydrolytic degradation chromatogram of Gefitinib

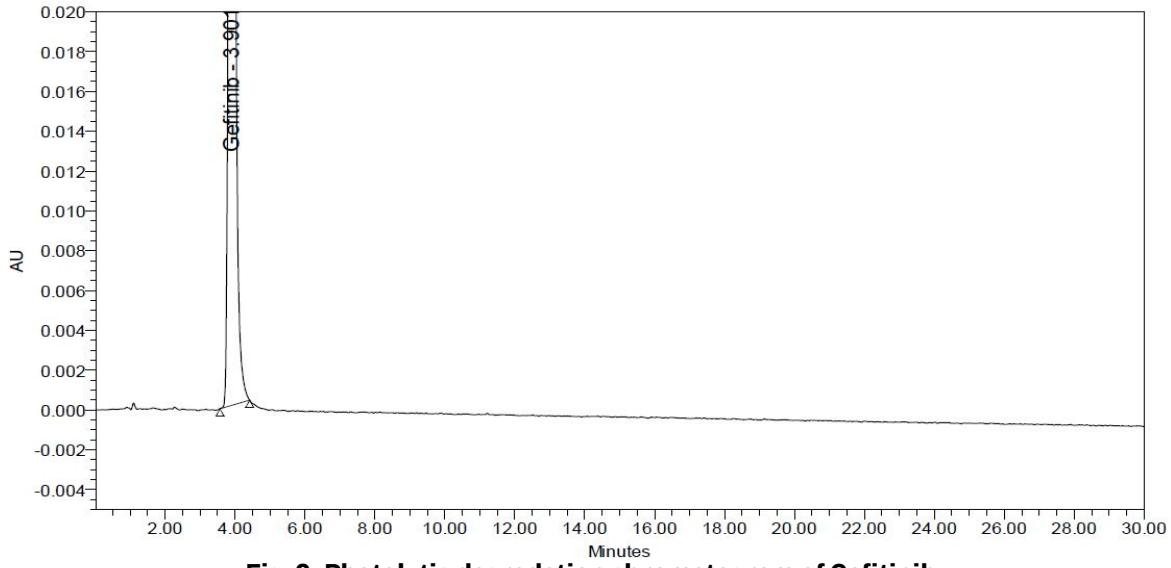

Fig. 9: Photolytic degradation chromatogram of Gefitinib

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