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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF GEFITINIB (ANTI – CANCER DRUG) IN PHARMACEUTICAL TABLET DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

An RP-HPLC method was developed and validated for the estimation of Gefitinib in pharmaceutical dosage form tablets. The chromatographic system was equipped Agilent column 4.6x250mm internal diameter with 5 micron particle size column and UV detector set at 246nm, in conjunction with a mobile phase of Triflouroacetic acid (0.1%) and Methanol in the ratio of 35:65. at a flow rate of 1ml/min. The retention time of Gefitinib was found to be 3.8 minute. The separation was performed at ambient temperature. The injection volume was 10µl. The separation was performed at ambient temperature. Linearity in concentration range of 5-30µg mL-1 with regression 0.9999. The Percentage recoveries were found in the range of 100.1– 101.87 %. The proposed method was validated in accordance with ICH parameters the method is precise, accurate, selective and rapid and applied for analysis of the same in laboratory prepared mixtures.

KEY WORDS

RP-HPLC, Gefitinib, Validation, Concentration and ICH.

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INTRODUCTION

Gefitinib (Figure No.1) is an anti neo plastic 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 No.1. Molecular Formula : C 22H 24 CIFN 4 O[3], Molecular Weight : 446.902 g/mol, white to yellow coloured powder, sparingly soluble in aqueous media but readily soluble in organic solvents, 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.

EXPERIMENTAL SECTION

Instrumentation and Chromatographic conditions

The analysis was performed by using Agilent column 4.6x250mm internal diameter with 5 micron particle size column and UV detector set at 246nm, in conjunction with a mobile phase of triflouroacetic acid (0.1%) and Methanol in the ratio of 35:65. at a flow rate of 1ml/min. The retention time of Gefitinib was found to be 3.8 minute. The separation was performed at ambient temperature. The injection volume was 10µl.

Reagents and Solutions

Methanol, Triflouroacetic acid of HPLC grade and Milli Q water were used in analysis.

Mobile Phase Preparation

Preparation of buffer (0.1% Triflouroacetic acid)

1750µL of Ortho-phosphoric acid was diluted to 1000mL using water and pH was adjusted to 3.0 by using C6H15N (Triethylamine). This solution was filtered through a 0.45µm nylon filter paper (PALL) and degassed by ultrasonicator.

Preparation of Mobile Phase

Transferred 1000ml of above solution and 1000ml of methanol to the mobile phase bottles separately. HPLC experiments were carried out using binary pump A containing Triflouroacetic acid (0.1%) and pump B containing Methanol in the ratio of 35:65.

Diluent preparation

Mixture of triflouroacetic acid and Methanol was used as diluent. Transferred 35mL of Triflouroacetic acid buffer to a 100mL beaker and add 65mL of Methanol.

Preparation of Standard solution

Standard Stock solution

50 mg of gefitinib standard was accurately weighed and transferred to a 50ml volumetric flask, dissolved in 10 ml of methanol and sonicate for 10mins and made up with methanol to give a solution containing 1000µg/ml.

Standard solution

From this stock solution, pipette out 5ml, placed in to 50ml volumetric flask and volume was made up to mark with diluent to give a solution containing 100µg/ml. Filter the solution through 0.45µ filter and inject.

Procedure for analysis of tablet formulation

Twenty tablets were weighed and finely powdered. An accurately weighed quantity of the powder equivalent to 100 mg of Gefitinib was transferred to 100 ml volumetric flask containing 40 ml of mobile phase and the contents of the flask were sonicate for 15 min, to ensure the complete solubility of the drug, then the mixture was made up to 100ml with mobile phase.

The resulting solution was thoroughly mixed and filtered through a 0.45µm membrane filter.

Method Validation

The method was validated for Specificity, linearity, accuracy, intra-day and inter-day precision, robustness and ruggedness in accordance with ICH guidelines.

Specificity

The specificity of the method was evaluated with regard to interference due to presence of any other excipients. The specificity of the method was tested under normal conditions and the results of the tests proved that the components other than the drug did not produce a detectable signal at the retention place of Gefitinib. Thus, the HPLC method presented in this study is selective.

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 3.8minutes, 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 3799 and tailing factor was 1.2099 for Gefitinib, which indicates efficient performance of the column.

Linearity

Linearity of the proposed HPLC method for determination of Geftinib was evaluated by analyzing a series of different concentrations of standard drug. In this study five concentrations were chosen ranging between 5-30µg mL-1. By using the stock solution 'B', aliquots of 5, 10, 15, 20, 25 and 30µg/ml were prepared with diluent six dilutions of each of the above mentioned concentrations were prepared separately and from these six dilutions, 20 µl each concentration was injected three times and obtained information on variation in the peak area response of pure analytes was plotted against corresponding concentrations and result was shown in Table No.1.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes was compared with the signals of blank samples. A signal-to-noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively. The values of LOD and LOQ were given in Table No.2.

Accuracy

Accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its range. Accuracy is performed in three different levels, each level in triplicate for Gefitinib using standards at 50%, 100% and 150%. Each sample is analyzed in triplicate for each level. From the results, % recovery is calculated (Table No.3).

Precision

Precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision expressed as % RSD.

Method reproducibility was demonstrated by repeatability and intermediate precision measurements of peak area, retention time and peak symmetry parameters of HPLC method for each title ingredients.

Repeatability (of results of measurements) - the closeness of the agreement between the results of successive measurements of the same substance carried out under the same conditions (same measurement procedure, the same observer, the same measuring instrument, used under the same conditions, the same location, and repetition over a short period of time) of measurement.

The repeatability and intermediate precision were carried out at 100% concentration for Gefitinib. The obtained results within and between the days of trials were in acceptable range indicating good precision of the proposed methods were given in Table No.4 and 5.

RESULTS AND DISCUSSION

The objective of the proposed work was to develop a method for the determination of Gefitinib to validate the methods according to ICH guidelines and applying the same for its estimation in laboratory prepared mixtures. HPLC methods developed were found to be rapid, simple, precise, accurate and economic for routine estimation of Gefitinib in laboratory prepared mixtures. In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compound. Initially, various mobile phase compositions were tried, to separate title ingredient. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time. The system with tri flouro acetic acid: methanol (PH 3.0) (35:65 v/v) with 1ml.min-1 flow rate is quite robust. The optimum wavelength for detection was 246 nm at which better detector

response for the title drug was obtained. The retention time for Gefitinib was found to be 3.8 min respectively. The calibration was linear in concentration range of 5-30µg mL-1 with regression 0.9999, intercept 100006 and slope 759565 for Gefitinib. The mean recoveries were found in the range of 100.1 - 101.87 %. The low values of % R.S.D indicate the method is precise and accurate.

Sample to sample precision and accuracy were evaluated using three samples of five different concentrations, which were prepared and analyzed on same day. Day to day variability was assessed using three concentrations analyzed on three different days over a period of three days. These results show the accuracy and reproducibility of the assay. The % R.S.D. reported was found to be less than 2 %. The proposed method was validated in accordance with ICH parameters and the applied for analysis of the same in laboratory prepared mixtures. The proposed methods are accurate, simple, rapid and selective for the estimation of Gefitinib in laboratory prepared mixtures. Hence, these methods can be conveniently adopted for the routine analysis of Gefitinib in quality control laboratories.

Table No.1: Linearity data for Gefitinib

S. No	Gefitinib	
	Concentration in µg/ml	Peak area
1	5	5203009
2	10	8231162
3	15	12515723
4	20	16107083
5	25	20417132
6	30	23757913

Table No.2: LOD and LOQ of Gefitinib

S. No	Parameters	µg/ml
1	LOD	1.481
2	LOQ	4.489

Table No.3: Data for Recovery Study

S.No	Recovery levels %	Standard area	Area of Gefitinib	% Recovery
1	50	8231162	8214874	99.80
2	100	8231162	8291399	100.73
3	150	8231162	8240493	100.1

Table No.4: Intraday (Repeatability) study results by HPLC

S.No	Morning		After Noon	
	Injection	**Area**	**Injection**	**Area**
1	Injection-1	19761211	Injection-1	20685542
2	Injection-2	20390438	Injection-2	20664991
3	Injection-3	20332497	Injection-3	20716784
4	Injection-4	20547346	Injection-4	20397066
5	Injection-5	20474693	Injection-5	20412069
6	Injection-6	20444825	Injection-6	20210929
Average		20325168	**Average**	20719141
SD		282797.1	**SD**	204577.6
% RSD		1.406	**%RSD**	0.987

Table No.5: Inter day (intermediate precision) study results by HPLC

S.No	Day-1		Day-2	
	Injection	**Area**	**Injection**	**Area**
1	Injection-1	19761211	Injection-1	20456575
2	Injection-2	20390438	Injection-2	20934578
3	Injection-3	20332497	Injection-3	20582365
4	Injection-4	20547346	Injection-4	20222589
5	Injection-5	2474693	Injection-5	20456238
6	Injection-6	20444825	Injection-6	20589632
Average		20325168	**Average**	20540329
SD		285797.1	**SD**	23438
% RSD		1.407	**% RSD**	1.141

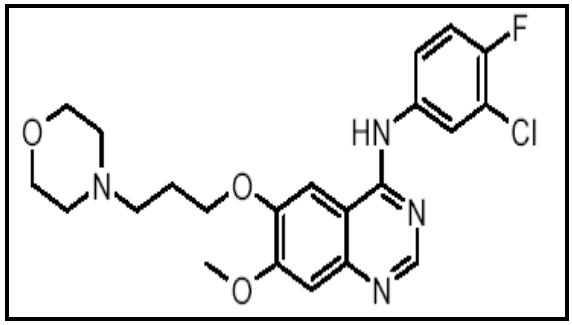

Figure No.1: Chemical Structure of Gefitinib

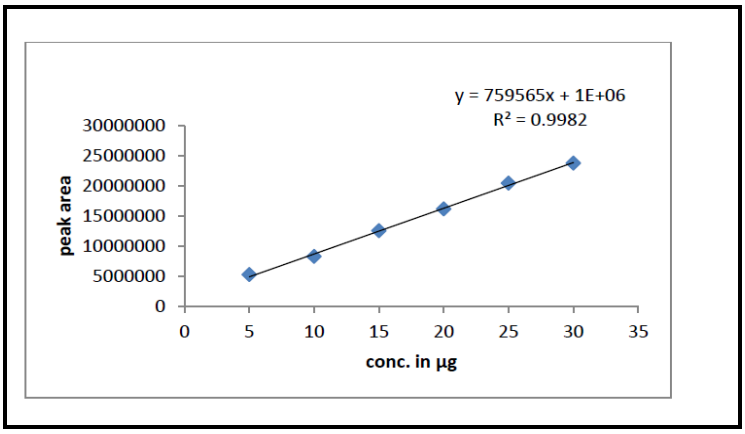

Figure No.2: Linearity curve of Gefitinib

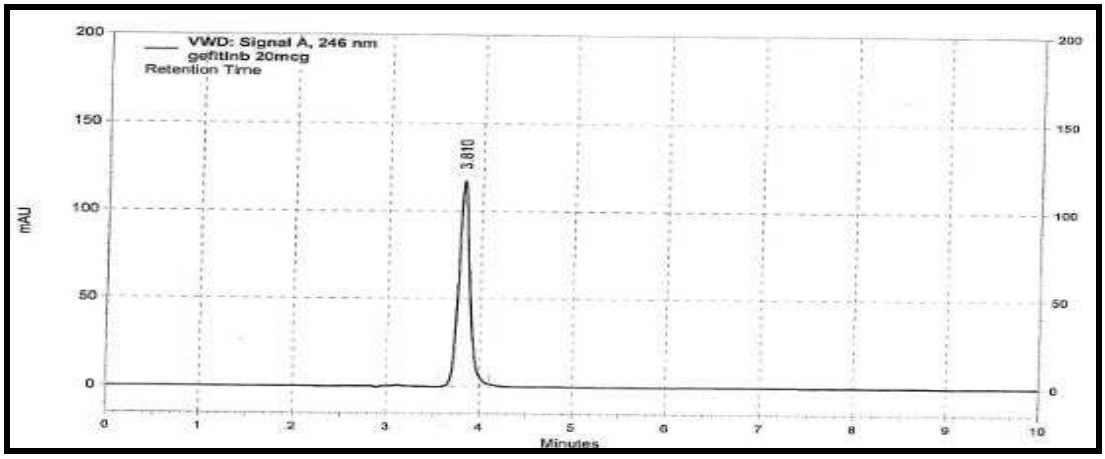

Figure No.3: Standard chromatogram of Gefitinib

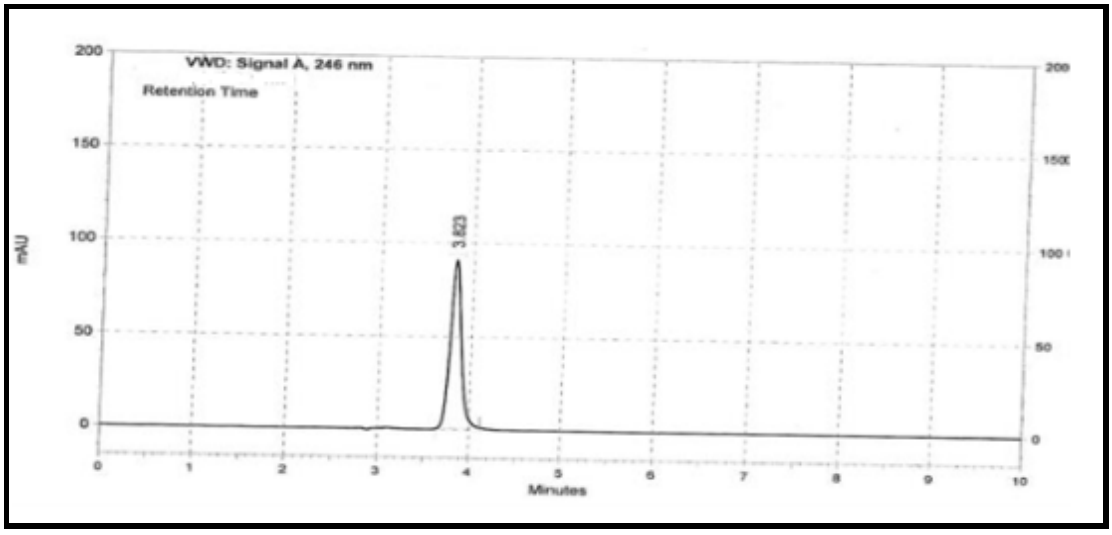

Figure No.4: Sample chromatogram of Gefitinib

CONCLUSION

In the present investigation, we have developed a simple, sensitive, precise and accurate RP-HPLC method for the quantitative estimation of Gefitinib in pharmaceutical formulations. The results expressed in Tables for HPLC method are promising. The HPLC method is more sensitive, precise and accurate compared to the spectrophotometric methods. This method can be used for the routine determination of Gefitinib in bulk drug and in Pharmaceutical formulations.

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