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METHOD DEVELOPMENT AND VALIDATION OF GEFITINIB IN BULK AND DOSAGE FORMS BY HPLC

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ABSTRACT

The aim of the present work was to develop and validate a simple, efficient, economical method for the estimation of Gefitinib in bulk and dosage forms by high pressure liquid chromatography. Chromatography was performed on with Agilent TC-C_{18} (2) 5 µm 4.6×250 mm, mobile phase containing Mobile phase – Acetonitrile, Methanol and Tetrahydrofuran (20:70:10) at a flow rate of 1 mL/min and eluents were monitored at 249 nm. The retention time of Gefitinib was 10.00 min showed a good linearity in the concentration range of 0.5-2.5 µg/mL for Gefitinib with a correlation coefficient of 0.997. The validation characteristics included specificity, linearity, and limit of detection, limit of quantification, precision, robustness and stability. The percent recoveries ranged between 85-115%, RSD < 2%. The method could be successfully used for the analysis of Gefitinib in bulk and dosage forms.

Key words: RP- HPLC, Gefitinib.

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INTRODUCTION

Gefitinib (*N*-(3-chloro-4-fluoro-phenyl)-7-methoxy-6-(3-morpholin-ylpropoxy) quinazolin-4-amine) is a drug used for certain breast, lung and other cancers. Gefitinib is an EGFR inhibitor, like erlotinib, which interrupts signaling through the epidermal growth factor receptor (EGFR) in target cells. Therefore, it is only effective in cancers with mutated and overactive EGFR. Gefitinib is the first selective inhibitor of *epidermal growth factor receptors* (EGFR*) tyrosine*

kinase domain. Thus gefitinib is an EGFR inhibitor. The target protein (EGFR) is a family of receptors which includes Her1(erb-B1), Her2(erb-B2), and Her 3(erb-B3). EGFR is overexpressed in the cells of certain types of human carcinomas - for example in lung and breast cancers. This leads to inappropriate activation of the anti-apoptotic Ras signalling cascade, eventually leading to uncontrolled cell proliferation. Research on gefitinib-sensitive non-small cell lung cancers has shown that a mutation in the EGFR tyrosine kinase domain is responsible for activating anti-apoptotic pathways (1, 2). The drug analysis plays an important role in the development, manufacture and therapeutic use of drugs. For the Method Development and Validation of new drug present in dosage forms UV- Spectrophotometer, HPLC and HPTLC methods are considered to be most suitable. Since these are powerful and rugged methods and also extremely precise, accurate, sensitive, specific, linear and rapid. Literature survey has revealed that various methods were reported for estimation of Gefitinib those are Colorimetry, UV Spectrophotometry, HPLC, UPLC, LC/MS and HPTLC (3-7). The objective of the proposed method is to develop simple and accurate method for the estimation of Gefitinib in pharmaceutical dosage forms by HPLC.

MATERIALS AND METHODS

Materials

Methanol, Acetonitrile, Purified water and K2H ortho phosphate from Merck Spl Pvt Ltd., Mumbai.

Methods

Standard Preparation

Accurately Weigh and transfer accurately 10mg of Gefitinib working Standard into a 10ml lean dry volumetric flask, and add about 10 ml of diluents, and sonicate to dissolve cool the solution to room temperature and dilute to volume with diluents and mix.

Sample preparation

Accurately weigh and transfer the sample equivalent to 10 mg of Gefitinib into a 10ml volumetric flask. Add about 10 ml of diluents, shake for 10 minutes on orbital shaker and sonicate for 07 minutes with occasional shakings. Cool the solution to room temperature and dilute to volume with diluents . filter the solution .

HPLC method was then validated to indicate that the analytical procedure used is suitable for intended use by using various parameters like specificity, linearity, precision, accuracy, system suitability (8).

RESULTS AND DISCUSSION

Good reproducibility was produced in optimized conditions (Fig-1). Optimized conditions are tabulated in Table-1

Table-1 Optimised conditions

S.No	Parameter	Condition
1	Column	Agilent TC-C_{18} (2) 5 µm 4.6×250 mm
2	Column Temperature	27°c
3	Wavelength	UV-249nm
5	Flow rate	1.0 ml/min
6	sample temperature	5^0C
7	Injection volume	10µL
8	Run time	10 minutes

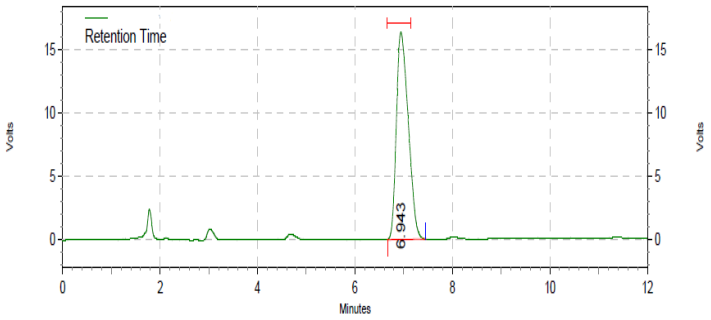

Fig -1 Chromatogram of Gefitinib

The system suitability studies were done with the 10mg of standard drug. The % of RSD values are below 2%, theoretical plate count is above 2000 and tailing factor is less than 2, indicating that the method is suitable.The linearity study was performed for the concentration of 50µg/ml to 250µg/ml level. Each level was injected into chromatographic system .The area of each level was used for calculation of correlation coefficient. Results are tabulated in Table-2 and fig-2. The linearity study was performed the correlation coefficient of Gefitinib was found to be 0.997 respectively

Table-2 showing results from linearity study

S.No	Linearity Level	Concentration (µg/ml)	Peak area
1	I	50	4655196
2	II	100	8761154
3	III	150	12474967
4	IV	200	17807373
5	V	250	20895262
Correlation Coefficient			**0.997**

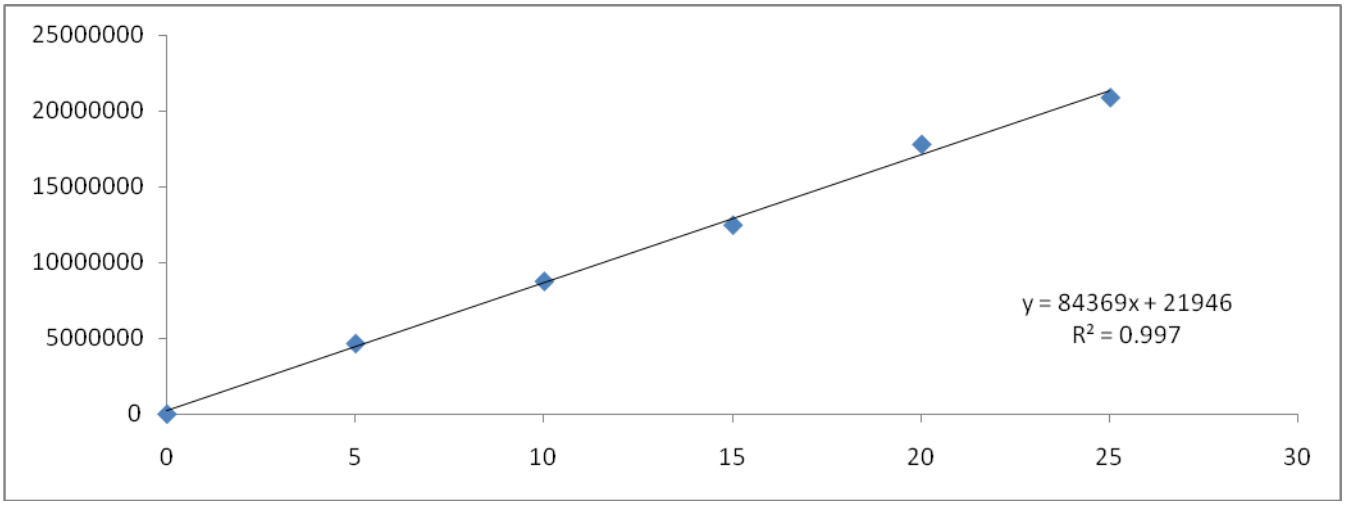

Fig-2 calibration curve of Gefitinib

The accuracy study was performed for 50%, 100% and 150% for Gefitinib. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery. The accuracy study was performed for % recovery. The % recovery was found to be 100.4 to 99.70% respectively (Table-3).

Table-3 Showing result from accuracy study

Level of % recovery	Amount of drug spiked(µg/ml)	Drug recovered	%Recovery	Mean	SD	%RSD
50	9.6	9.62	100.2	100.4	0.346	0.34
		9.62	100.2			
		9.68	100.8			
		9.62	100.2			
		9.68	100.8			
		9.62	100.2			
100	12	12.23	101.9	101.6	0.974	0.95
		12.08	100.6			
		12.31	102.5			
		12.23	101.9			
		12.31	100.6			
		12.08	102.5			
150	14.4	14.26	99.02	99.70	0.6451	0.64
		14.21	99.8			
		14.45	100.3			
		14.26	99.02			
		14.45	99.8			
		14.21	100.3			

The precision of method was determined by replicate injection of sample solution. The %RSD of area of intraday precision is 0.3%, 0.10% and 0.06%. %RSD of interday precision was found to be 0.3%, 0.09% and 0.07%. Precision results are within the limits.

CONCLUSION

From the overall results obtained it was concluded that the developed method was more accurate, precise, specific and robust with ±2º C in temperature, ±0.2 mL/min in flow rate, ±10% variation in organic phase. Hence, the developed chromatographic (HPLC) method for Gefitinib is said to be rapid, simple, precise, accurate, and cost effective that can be effectively applied for the routine analysis.

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