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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF GEFITINIB IN BULK AS WELL AS IN PHARMACEUTICAL DOSAGE FORM BY USING PDA DETECTOR

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ABSTRACT

A simple, economic, accurate, sensitive, specific and precise stability indicating reverse phase high performance liquid chromatographic [RP- HPLC] method for the determination of Gefitinib in pure and tablet dosage form was developed and validated. The chromatographic separation was carried out using Shodex- C_{18} column [4.5 x 250 mm; 5 µm particle size] as a stationary phase, 0.02M potassium dihydrogen phosphate buffer [pH 6.5]: methanol: trifluro acetic acid [55:25:20 v/v/v] as a mobile phase, flow rate of 1 mL/min and the PDA detection was carried out at 254 nm. The retention time of Gefitinib was 3.247 minute. RP- HPLC method was developed with linearity range of 25-150 µg/mL of Gefitinib. The correlation coefficient [r^2] was found to be 0.9999. The assay results obtained in good agreement with the corresponding labeled amount by developed method within range of 100.69 ± 0.4397. Accuracy of the method was confirmed by recovery studies and the recoveries were found to be between 99.86 % to 100.712 % and the corresponding %RSD was found to be 0.4057, precision, LOD, LOQ, specificity, robustness and ruggedness were met all the acceptance criteria for the validation of analytical method as per ICH Q2 (R1) guideline. This method can be conveniently used to detect the possible degradation product in the dosage form of Gefitinib during stability studies (acidic, alkaline, oxidative, thermal and photolytic). The method proved to be effective on application to a stressed marketed tablet formulation.

Keywords: Gefitinib; RP-HPLC; stability indicating method; validation; ICH-guidelines

1. INTRODUCTION

Gefitinib [Fig, 1], chemically known as N-(3-chloro-4-fluoro-phenyl)-7-methoxy-6-(3-morpholin-4-yl propoxy) quinazolin-4-amine with empirical formula of $C_{22}H_{24}ClFN_4O_3$. It is used for the treatment of locally advanced or metastic non-small cell lung cancer (NSCLC) in patients who have previously received chemotherapy. It is currently being studied as a potential treatment option in multiple tumor types [1]. Gefitinib demonstrated to increase the overall survival of patients with metastatic colorectal cancer [2-3]. Geftistar is being approved with boxed warning altering patients and health care professionals that severe and fatal liver toxicity occurred in patients treated with Geftistar during clinical studies. The most common side effects reported in patients treated with Geftistar include weakness or fatigue, loss of appetite, hand-foot syndrome [also called palmar-

plantar erythrodysesthesia], diarrhea, mouth sores [mucositis], weight loss, infection, high blood pressure and change in voice volume or quality [dysphonia] [4].

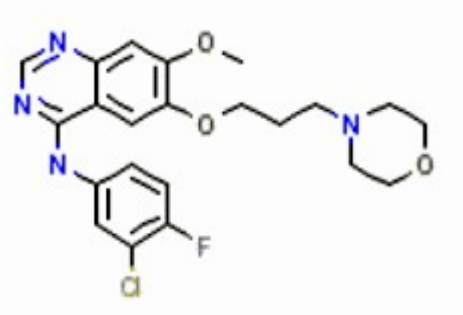

Figure 1. Structure of Gefitinib

Literature review revealed that several methods were reported for the estimation of Gefitinib [GEF] in tablets; it was estimated in bulk and in tablets by RP-HPLC [5-11], but to the best our knowledge, there is no stability-indicating method reported for GEF. The present work aims to develop a simple, precise, and accurate stability indicating RP-HPLC method for the estimation of GEF in pure and in its tablet formulation through stress studies under a variety of ICH recommended test conditions [12] and to develop a validated stability-indicating assay method [13-14].

2. EXPERIMENTAL

2.1. Chemical and reagents

GEF standard reference was purchased from Spectrum Labs Limited, Hyderabad, India. GEF tablets [Geftistar ® 250 mg, Lupin Laboratories, India] were purchased from a local retail pharmacy. HPLC grade methanol was purchased from E - Merck specialties Pvt. Ltd, Mumbai, Potassium dihydrogen phosphate (KH_2PO_4) Qualigens Fine Chemicals Ltd, Mumbai, Triethylamine was purchased from SD Fine Chemicals Ltd, Gujarat and Milli Q water [HPLC Grade] were used for the analysis. The analyses were performed on a Hitachi HPLC system containing D-2000 Elite HSM [English] software and PDA detector set at 254 nm with an isocratic elution at a flow rate of 1 mL/min on a Shodex- C_{18} column [4.5 x 250 mm; 5 µm]. Mobile phase composition of 0.02M potassium dihydrogen phosphate buffer (pH 6.5): methanol: trifluro acetic acid [55:25:20 v/v/v] was used.

2.2. Method Development

2.2.1. Chromatographic condition

Mobile phase	0.02M potassium dihydrogen phosphate buffer [pH 6.5]: methanol: trifluro acetic acid [55:25:20 v/v/v]
Diluent	Buffer: Methanol [70:30 v/v]
Column	Shodex- C_{18} column [4.5 x 250 mm; 5 µm].
Column temperature	30^0C
Detection wavelength	254 nm
Injection volume	10µL
Flow rate	1 mL/min
Run time	8 min

2.2.2. Preparation of Buffer Solution

Potassium dihydrogen phosphate (KH_2PO_4) 2.72 gm was accurately weighed and transferred in to a 1000 ml volumetric flask. About 900 ml of milli-Q water was added and the pH was adjusted to 6.5 with triethylamine.

2.2.3. Preparation of Diluent

The diluent was prepared by mixing 70 ml of buffer solution and 30 ml of methanol and the resulting solution was sonicated for 15 min and it was used as diluent.

2.2.4. Preparation of standard stock solution

Quantity of GEF equivalent to 250 mg was weighed and transferred in to a 100 mL volumetric flask, 30 mL of diluent was added and sonicated for 15 min and the volume was made up to the mark with diluent. From this solution further dilution was made to get the final concentration of 100 µg/mL. 10 µL of the final solutions were injected into the system and the chromatograms were recorded.

2.2.5. Preparation of sample stock solution

Tablet powder equivalent to 250 mg of GEF was weighed and transferred in to 100 mL volumetric flask, 30 mL of diluent was added and sonicated for 15 min and the volume was made up to the mark with diluent. From this solution further dilution was made to get the final concentration of 100 µg/mL. 10 µL of the final solution were injected into the system and the chromatograms were recorded.

2.3. Forced Degradation Studies of Gefitinib

2.3.1. Control Sample

A quantity tablet powder equivalent to 250 mg of GEF was accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of the diluent. The solution was sonicated for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 mL with diluent. Further pipette 10 mL of the above stock solution and transferred to 100 ml volumetric flask and made up to 100 ml with diluent. From the above resulting solution pipette out 4 mL and transferred to 10 mL volumetric flask and made up to 10 mL with diluent to get the final concentration of 100 µg/mL of GEF and 10 µL of the solutions were injected in to the system and the chromatograms were recorded.

2.3.2. Neutral Degradation Studies

A quantity tablet powder equivalent to 250 mg of GEF was accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of the diluent. The solution was sonicated for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 mL with diluent. Further pipette 10 mL of the above stock solution and transferred to 100 ml volumetric flask and made up to 100 ml with diluent. From the above resulting solution pipette out 4 mL and transferred to 10 mL volumetric flask and made up to 10 mL with diluent to get the final concentration of 100 µg/mL of GEF and the solution was refluxed in water bath for 30 minutes at 80^0C and 10 µL of the refluxed solutions were injected in to the system and the chromatograms were recorded.

2.3.3. Acid Degradation Studies

A quantity tablet powder equivalent to250 mg of GEF was accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of the diluent. The solution was sonicated for few minutes to dissolve the drug completely. Then it was filtered through 0.45 µ filter and the volume was made up to 100 mL with diluent, 10 mL of the above stock solution was transferred to

100 mL volumetric flask to that 10 mL of 2N hydrochloric acid was added and refluxed for 30 min at 80^0C. The resulting solution was diluted to 100 mL with diluent. From the above resulting solution pipette out 4 mL and made up to 10 mL with diluent to get the final concentration of 100 µg/mL of GEF and 10 µL of the refluxed solutions were injected in to the system and the chromatograms were recorded.

2.3.4. Alkaline Degradation Studies

A quantity tablet powder equivalent to 250 mg of GEF was accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of the diluent. The solution was sonicated for few minutes to dissolve the drug completely. Then it was filtered through 0.45 µ filter and the volume was made up to 100 mL with diluent, 10 mL of the above stock solution was transferred to 100 mL volumetric flask to that 10 mL of 2N sodium hydroxide was added and refluxed for 30 min at 80^0C. The resulting solution was diluted to 100 mL with diluent. From the above resulting solution pipette out 4 mL and made up to 10 mL with diluent to get the final concentration of 100 µg/mL of GEF and 10 µL of the refluxed solutions were injected in to the system and the chromatograms were recorded.

2.3.5. Oxidative Degradation Studies

A quantity tablet powder equivalent to 250 mg of GEF was accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of the diluent. The solution was sonicated for few minutes to dissolve the drug completely. Then it was filtered through 0.45 µ filter and the volume was made up to 100 mL with diluent, 10 mL of the above stock solution was transferred to 100 mL volumetric flask to that 10 mL of 3% hydrogen peroxide (H_2O_2) was added and refluxed for 30 min at 80^0C. The resulting solution was diluted to 100 mL with diluent. From the above resulting solution pipette out 4 mL and made up to 10 mL with diluent to get the final concentration of 100 µg/mL of GEF and 10 µL of the refluxed solutions were injected in to the system and the chromatograms were recorded.

2.3.6. Thermal Degradation Studies

A quantity tablet powder equivalent to 250 mg of GEF was accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of the diluent. The solution was sonicated for few minutes to dissolve the drug completely. Then it was filtered through 0.45 µ filter and the volume was made up to 100 mL with diluent. Further, pipette 10 mL of the above stock solution and transferred to 100 mL volumetric flask and made up to 100 mL with diluent. From the above resulting solution pipette out 4 mL and made up to 10 mL with diluent to get the final concentration of 100 µg/mL of GEF and the solution was placed in oven at 80^0C for 48 hours, 10 µL of the solutions were injected in to the system and the chromatograms were recorded.

2.3.7. Photolytic Degradation Studies

A quantity tablet powder equivalent to 250 mg of GEF was accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of the diluent. The solution was sonicated for few minutes to dissolve the drug completely. Then it was filtered through 0.45 µ filter and the volume was made up to 100 mL with diluent. Further, pipette 10 mL of the above stock solution and transferred to 100 mL volumetric flask and made up to 100 mL with diluent. From the above resulting solution pipette out 4 mL and made up to 10 mL with diluent to get the final concentration of 100 µg/ml of GEF and the solution was exposed to UV light by keeping the volumetric flask in UV chamber for 7 days, 10 µL of the solutions were injected in to the system and the chromatograms were recorded.

3. RESULTS AND DISCUSSION

3.1. Method Development and Optimization

In the present work, a stability indicating analytical method based on RP-HPLC using PDA detection was developed and validated for assay determination of GEF in tablet dosage formulation. The analytical conditions were selected, keeping in mind the chemical nature of GEF. The development trails were taken using different conditions. The column selection has been done on the basis of back pressure, peak shape, theoretical plates and day-to-day reproducibility of the retention time. After evaluating all these factors, Shodex- C_{18} column [4.5 X 250 mm; 5 µm] was found to be giving satisfactory results. The selection of buffer was done based on chemical structure of the drug. The pH range 6.5 was found suitable for solubility, stability, theoretical plates and peak shape of the drug. Best results were obtained with 0.02M potassium dihydrogen phosphate buffer [pH 6.5 adjusted with triethylamine]. For the selection of organic constituent of mobile phase, methanol and trifluro acetic acid was chosen to reduce the longer retention time and to attain good peak shape.

Best results were obtained with 0.02M potassium dihydrogen phosphate buffer [pH 6.5]: methanol: trifluro acetic acid [55:25:20 v/v/v]. The flow rate 1 mL/min and the injection volume was 10µL. The detection was carried out at 254 nm. The peak retention time of GEF was found to be 3.247 min with good baseline stability.

Typical chromatogram of GEF was shown in [Fig 2]. The calibration curve was plotted utilizing the peak area of GEF against concentration of the drug. The calibration curve showed linearity, over a concentration range of 25-150 µg/mL for the drug as showed in [Fig 3]. Regression coefficient [R^2] was found to be 0.9999. The number of theoretical plates obtained was 14554, which indicates the efficient performance of the column. Assay of GEF tablets [Geftistar ® 250 mg, Lupin Laboratories, India] using the developed method showed acceptable relative error values. The %RSD for assay of the drug was 0.4397. During injection of a standard solution, sample solution, the retention times were 3.233 min and 3.247 min respectively.

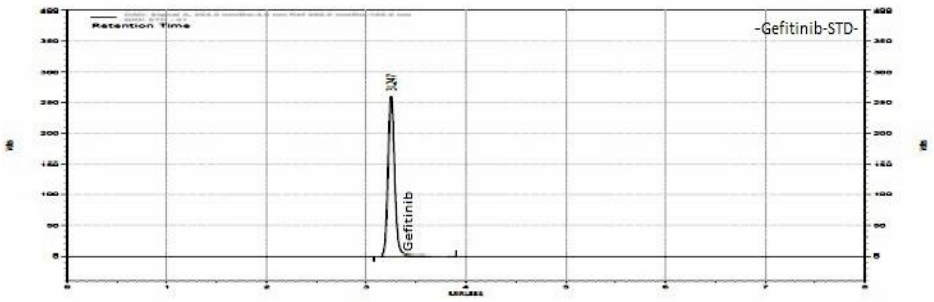

Figure 2. Typical chromatogram of GEF

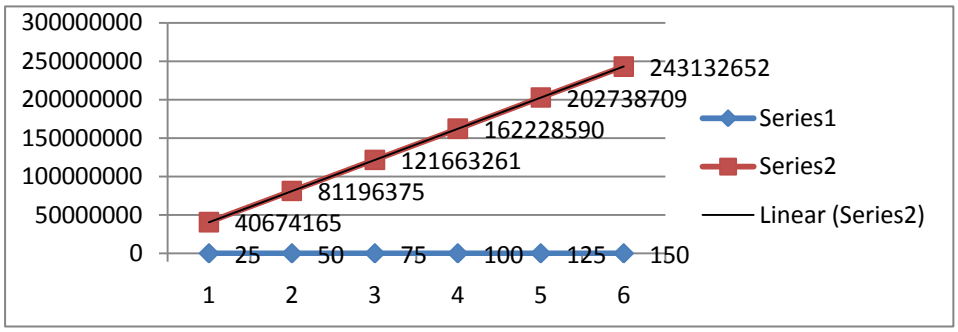

Figure 3. Calibration curve of GEF

3.1.1. Analysis of Pharmaceutical Formulation

Tablet powder equivalent to 250 mg of GEF was weighed and transferred in to 100 mL volumetric flask, 30 mL of diluent was added and sonicated for 15 min and the volume was made up to the mark with diluent. From this solution further dilution was made to get the final concentration of 100 µg/mL. 10 µL of the final solutions were injected into the system and the chromatograms were recorded. The retention time was found to be 3.247. The content of GEF in the tablets [Geftistar ® 250 mg, Lupin Laboratories, India], was computed by putting value of the peak areas in respective standard regression equation obtained from calibration curve. The results were shown in [Table 1].

Table 1. Estimation of GEF content in Tablets

Standard Area	Sample Area	*Label claim [mg/tab]	Amount Found [mg/tab]	% Assay
161760877	163294774	250	254.25	101.70
161046785	163088961		255.06	102.02
161970028	163379266		252.10	100.84
161994434	163097724		253.58	101.43
163057322	163542920		252.61	101.01
162569730	163219942		252.87	101.15
			Average	101.36
			SD	0.4457
			% RSD	0.4397
			SE	0.1819
			CI[Confidence Interval 99%]	100.69 – 102.02

* Geftistar ® 250 mg

3.2. Method Validation

3.2.1. Linearity

Linearity was demonstrated from the standard drug solution using six concentration levels for GEF. The peak areas were recorded and calibration plot was obtained by plotting peak area versus concentration of GEF. The correlation coefficient results revealed that developed analytical method having excellent linearity.

3.2.2. Accuracy

Accuracy was calculated by addition of standard drugs to preanalyzed sample at 3 different concentration levels and computing percentage recoveries. Standard limit of % recovery study is 98 - 102 % as per ICH guideline. From the studies it was concluded that % recovery study of GEF complies with standard limit of ICH guideline. The results were shown in [Table 2]. Average % recoveries obtained as 99.86% - 100.71%, which indicating that the method was accurate.

3.2.3. Precision

3.2.3.1.Repeatability

Six repeated injections of standard and sample solutions containing 100 µg / mL of GEF was prepared and the response factor of the drug peaks and %RSD were calculated. The results obtained were presented in [Table 3].

3.2.3.2.Intraday precision:

Solution containing 100 µg / mL of GEF was prepared from their respective standard stock solution. Analysis was replicated for 3 different times within same day. The results of intra-day

precision studies were shown in [Table 4]. They revealed that the %RSD of intra-day were within the permissible limits of 2%.

3.2.3.3.Interday precision:

Solution containing 100 µg / mL of GEF was prepared from their respective standard stock solution. Analysis was replicated for 3 different days. The results of inter-day precision studies were shown in [Table 5]. They revealed that the %RSD of inter-day were within the permissible limits of 2%.

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

The Limit of Detection and Limit of Quantification of the developed method were calculated from the standard deviation of the y-intercepts and slop of the calibration curve of GEF using the formula as given below.

Limit of Detection = 3.3 α / S

Limit of Quantitation = 10 α / S

Where α is the standard deviation of the y – intercepts and S is the slop of the calibration curve. The results of LOD and LOQ for GEF obtained were presented in [Table 6].

3.2.5. Robustness

Variation in the flow rate, mobile phase, nanometer and temperature has been made to the analytical method in order to evaluate and measure the capacity of the method to remain unaffected by such variations. The % RSD was found to be less than 2. The results were shown in [Table 7].

3.2.6. Ruggedness

Ruggedness of the method was confirmed by the analysis of formulation was done by the different analysts. The results were shown in [Table 8].

3.2.7. System suitability

System suitability was established to determine the adequate reproducibility of the proposed method. Parameters including asymmetry factor, theoretical plates, repeatability of peak area and retention time was calculated. The results were shown in [Table 9].

Table 2. Recovery Studies

Parameters	Amount Present [µg/ml]	Amount Added [µg/ml]	Peak Area	Amount Found [µg/ml]	Amount Recovered [µg/ml]	% Amount Recovered
80%	100	80	293289050	179.97	79.97	100.07
			293247801	180.67	80.67	100.95
			293279840	180.29	80.29	100.47
100%	100	100	325581660	199.78	99.78	99.88
			325583739	200.57	100.57	100.68
			325051785	199.82	99.82	99.93
120%	100	120	358139826	119.76	119.76	99.80
			358145820	220.63	120.63	100.63
			358138811	220.16	120.16	100.24
					Average	100.29
					SD	0.4069
					% RSD	0.4057
					SE	0.1356
					CI [Confidence Interval 99%]	99.86 - 100.71

Table 3. Precision

Standard solution		Sample solution	
No. of Injections	Peak area	No. of Injections	Peak area
1	161204218	1	163294774
2	160518004	2	163088961
3	161325213	3	163379266
4	161222677	4	163097724
5	161265954	5	163542920
6	162963422	6	163219942
Average	161416581	Average	163270599
SD	743179	SD	159161
%RSD	0.4604	%RSD	0.0974

SD: Standard deviation; RSD: Relative standard deviation

Table 4. Results of Intra-day Precision

Parameter	Concentration [µg / mL]	Peak area*	% Amount found*	SD	%RSD
0 Hours	100	163330555	100.67	0.1107	0.1100
3 Hours		163233336	100.61		
6 Hours		163270580	100.63		

*Mean of six determinations

Table 5. Results of Inter-day Precision

Parameter	Concentration [µg / mL]	Peak area*	% Amount found*	SD	%RSD
Day – I	100	163256821	100.62	0.6417	0.6376
Day – II		163279581	100.64		
Day – III		163053438	100.50		

*Mean of six determinations

Table 6. Results of LOD and LOQ

Slope		Y-Intercept
	1622075	2030.53
	1622391	-32884.35
	1619723	1653
	1622079	-31997.25
	1622200	1809.92
	1616322	-31562.35
Average	1620798	
SD		130364.9
LOD [µg / mL]		0.2654
LOQ [µg / mL]		0.8043

Table 7. Results of Robustness

Parameters	Retention time*	Peak area*	% Amount found*	SD	%RSD
Flow minus [0.8 mL/min]	4.360	163457773	100.75	0.1858	0.1845
Flow plus [1.2 mL/min]	3.567	162127579	99.93	0.9382	0.9388
nm plus [256 nm]	3.920	164446391	101.36	0.4539	0. 4478
nm minus [252 nm]	3.920	162650365	100.25	0.9396	0.9372
Temperature plus [32^0C]	3.993	163891554	101.02	0.3145	0.3113
Temperature minus [28^0C]	3.847	163472914	100.76	0.8102	0.8040
Methanol [20]	3.567	162584813	100.21	0.3248	0.3241
Methanol [30]	3.567	162291366	100.03	0.2086	0.2085
pH minus [- 0.2]	3.653	164657955	101.49	0.7035	0.6931
pH plus [+ 0.2]	4.160	161468007	99.53	0.5451	0.5476

*Mean of six determinations

Table 8. Results of Ruggedness

Parameter	Concentration [µg/ml]	Peak Area*	% Amount Found*	SD	% RSD
Analyst-1	100	163077598	100.52	0.4038	0.4017
Analyst-2		163468621	100.83	0.4082	0.4069

*Mean of six determinations

Table 9. Results of System suitability parameters

Parameters	Results
Theoretical plates [N]	14554
Asymmetry factor	1.1
Retention time	3.247
% RSD of peak area	0.4604
% RSD of retention time	0.4056

3.3. Forced Degradation Study

Forced degradation studies were performed to evaluate the stability indicating properties [Specificity] of the proposed method. GEF was subjected to neutral, acid, base, oxidation, thermal and photo degradation to ensure the effective separation of degradation peaks and main peak. From the degradation of these solutions under the stress condition gives us an idea about the origin of degrading products. Degradants did not show any interference with the elution of drug peaks. Hence, the method is stability indicating. The results of degradation studies were shown in Table 10.

Table 10. Results of Forced degradation study

Parameters	Degradation Time	Peak Area*	% Degradation	% of Active Drug Present After Degradation

Control sample	-	165442050	-	-
Neutral sample	30 min	164807352	0.88	99.61
Acidic degradation	30 min	162956045	2.03	98.46
Alkaline degradation	30 min	161201970	3.06	97.43
Oxidative degradation	30 min	152556260	8.28	92.21
Thermal degradation	48 hours	165401972	0.52	99.97
Photolytic degradation	7 days	164408441	1.12	99.37

3.4. CONCLUSION

A simple, sensitive, precise and accurate stability indicating RP-HPLC analytical method was developed for the estimation of GEF in pure and tablet dosage form. The method was successfully validated and proved as linear, precise, accurate and robust. Documented evidences of the present work suggesting that the developed method was an economical one in terms of lower methanol concentration for the estimation of the drug and can be successfully employed for routine analysis in quality control laboratories.

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