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A Novel Spectrofluorimetric Method for the Estimation of Gefitinib in Raw Material and Pharmaceutical Dosage Form

J. Ramesh[1*], Dr. N. Senthil Kumar[2]
[1]Asst.Professor, Department of Pharmaceutical Analysis, Swamy Vivekanandha College of Pharmacy, Elayampalayam, Tiruchengode, Namakkal (DT), Tamilnadu, India
[2]Department of Pharmaceutical Analysis, JKK Munirajah Medical Research Foundation's- Annai JKK Sampoorani Ammal College of Pharmacy, Komarapalayam, Namakkal -DT, Tamilnadu, India

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***Corresponding author**
J. Ramesh

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Abstract: A simple and sensitive spectrofluorimetric method has been developed for the estimation of Gefitinib in pure and pharmaceutical dosage form. Gefitinib exhibits maximum fluorescence intensity in ethanol and Beer's law was obeyed in the range of 1-3.5 µg/mL at an excitation wavelength (λ_{ex}) of 280 nm and an emission wavelength (λ_{em}) of 512 nm. Stability studies with respect to time and temperature were also carried out. The results obtained were in good agreement with the labeled amounts of the marketed formulations. This method has been statistically evaluated and found to be accurate and precise.

Keywords: Gefitinib, Spectrofluorimetry, Pharmaceutical formulations, Estimation.

INTRODUCTION

Gefitinib (GEF), chemically known as N-(3-chloro-4-fluoro-phenyl)-7- methoxy -6- (3-morpholin -4- yl propoxy) quinazolin-4-amine [Fig:1] 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]. GEF 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].

The analytical method, which has been reported, are very few for the determination of GEF in pure drug and in pharmaceutical dosage forms; it was estimated in bulk and tablet dosage forms by RP-HPLC [5-11], but to the best of our knowledge, there is no spectrofluorimetric method reported for the estimation of GEF. The present work deals with the development and validation of a novel, simple, rapid and reliable spectrofluorimetric method for the determination of GEF in bulk and tablet dosage forms. Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH) guidelines [12] for the determination of GEF in bulk and tablet dosage forms. GEF is soluble in ethanol, DMSO and dimethyl formamide (DMF), sparingly soluble in methanol and aqueous buffers. Its molecular weight is 446.902 g/mol.

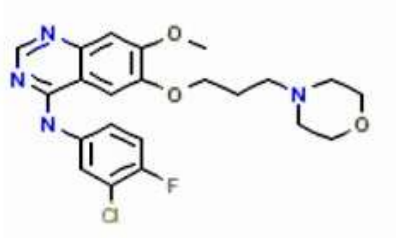

Fig-1: Structure of Gefitinib

Spectrofluorimetric Method

Spectrofluorimetry has assumed a major role in drug analysis because of its greater sensitivity and selectivity than absorbance spectrophotometry. Spectrophotometric technique rely upon the comparison of incident light (I_o) and transmitted light [13] (I_t) intensity. At very low concentrations of absorbing species, the difference becomes extremely difficult to detect and becomes the factor, which limits the sensitivity of this technique. In spectrofluorimetry, the emitted radiation is measured at right angle to the incident beam and at longer wavelength and as the concentration of the fluorescent species [14] decreases, so the intensity of the light emitted decreases.

The sensitivity arises the requirement that two wavelengths are involved the excitation wavelength and the fluorescence emission wavelength which discriminates [15] it form many compounds, which do not display significant fluorescence.

The emission of light by the molecules which are excited by the absorption of visible of UV radiation is the basis of fluorescence spectroscopy. Due to relatively low cost and high analytical sensitivity, this technique is widely employed in the quantitative analysis of drugs and metabolites and in the evaluation of these substances with biological macromolecules [16].

Molecular planarity and rigidity plays a significant role in the ability of a compound to fluoresce. A conjugated system of double bonds held in planar and rigid form that strongly absorbs in the 200-800 nm region of the electromagnetic spectrum is usually a good candidate for developing fluorescence.

MATERIALS AND METHODS

Instrument

Spectrofluorimetric analysis was performed using Jasco spectrofluorimeter model FB 8500 supported by Spectra manager software.

Chemicals

Gefitinib was obtained as gift sample from Spectrum Labs, Hyderabad and analytical grade ethanol (E - Merck Specialties Pvt. Ltd, Mumbai) were used for analysis. Geftistar tablets 250mg (Lupin Laboratories) were purchased from a local pharmacy.

METHOD

Preparation of Standard Drug Solution

About 100 mg of GEF was accurately weighed and dissolved in about 100 mL of ethanol to obtain a stock a stock solution of 1 mg/mL. This solution was further diluted with ethanol to obtain a working standard solution of 10 µg/mL.

Study of Fluorescence Spectral Characteristics of Gefitinib

The standard solution of GEF 10 µg/mL was prepared in different solvents like ethanol, methanol, DMSO and DMF and was scanned from 200-800 nm to find out the excitation and emission wavelength and also to find out the best solvent in which the drug exhibits maximum fluorescence. It was found that the drug exhibits maximum fluorescence in ethanol at an excitation wavelength of 280 nm and an emission wavelength of 512 nm.

Fixing the Excitation and Emission Bandwidth

GEF working standard solution10 µg/mL was taken and fluorescence intensity was measured at different bandwidth such as 5, 10, and 20 nm. Similarly it was done for fixing emission bandwidth. Both the excitation and emission bandwidth were fixed as 10 nm as the fluorescence intensity and fluorescence spectrum was found to be good.

Fixing the Response Time

After fixing the excitation and emission bandwidth as 10 nm, different response time was measured for the same solution. It found that GEF gave maximum fluorescence intensity at 0.1 sec (Fig-2).

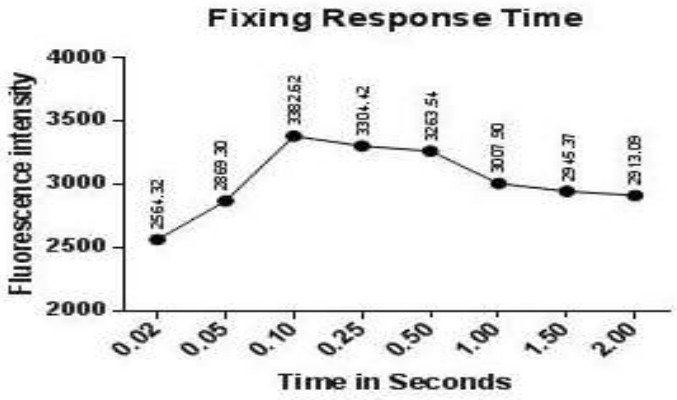

Fig-2: Fixing the Response Time on Fluorescence Intensity

Fixing the Sensitivity

By keeping the response time, excitation and emission bandwidth as constant, fluorescence intensity of 10 µg/mL of GEF solution was recorded by varying sensitivity as low, medium and high. Sensitivity was fixed as medium. Hence, the fluorescence intensity and fluorescence spectrum was found to be good and satisfactory.

Stability Profile With Respect to Time

GEF standard solution of 10 µg/mL was taken and set for time profile scan for 9 hours with excitation wavelength of 280 nm and emission wavelength of 512 nm. The fluorescence intensity was almost stable throughout the time up to 7 hours (Fig-3).

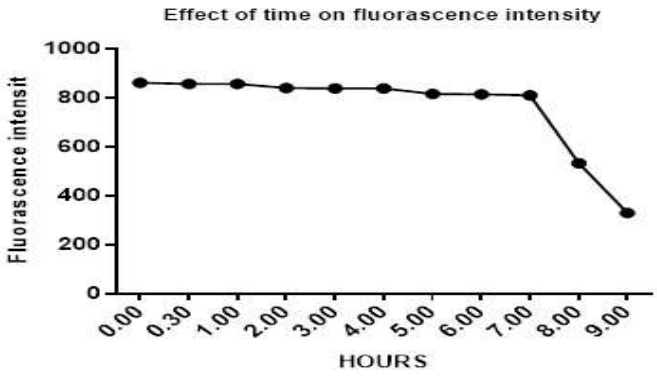

Fig-3: Effect of Time on Fluorescence Intensity

Effect of Temperature on Fluorescence Intensity

GEF standard solution of 10 µg/mL was taken and fluorescence intensity was taken at different temperature like 25^0C, 30^0C, 40^0C, 50^0C, 60^0C and 70^0C. The fluorescence intensity was found to decrease with increase in temperature (Fig-4). Hence an ambient temperature (30^0C) was employed during the measurement of the fluorescence intensity.

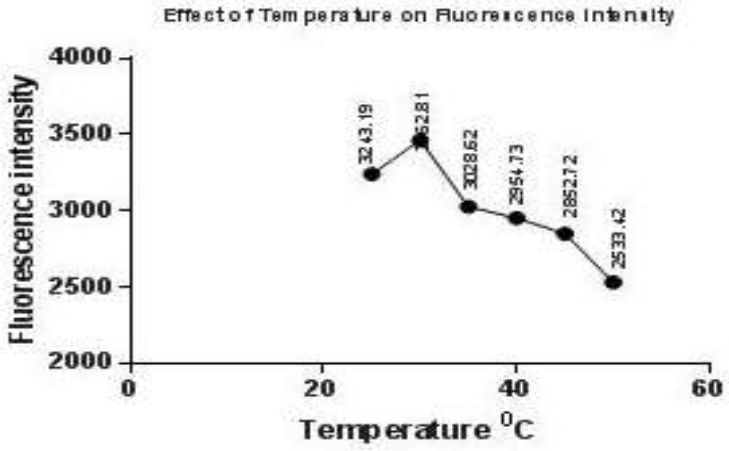

Fig-4: Effect of Temperature on Fluorescence Intensity

Effect of pH on Fluorescence Intensity

GEF standard solution of 10 µg/mL was taken in 7 different flasks and the pH was adjusted to 2, 3, 4, 5, 6,7 and 8 respectively and the fluorescence intensity was measured (Fig-5). This shows that GEF shows maximum fluorescence intensity at the pH 6. Hence pH 6 is employed for determination of the fluorescence intensity of GEF.

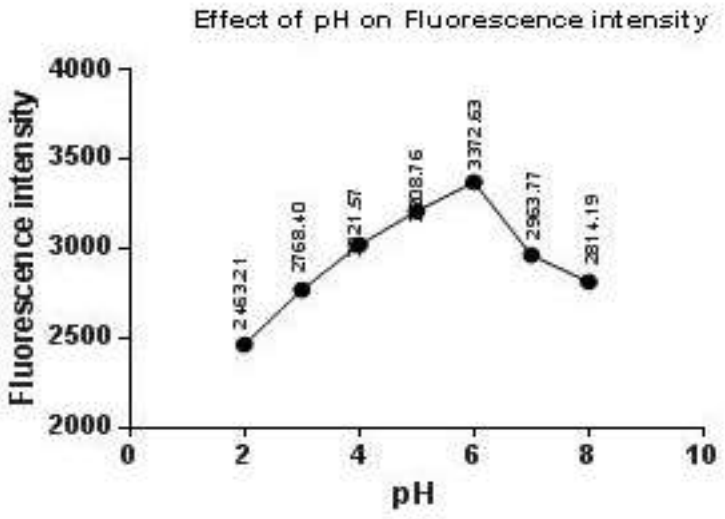

Fig-5: Effect of pH on Fluorescence Intensity

Table-1: Optimized Fixed Parameters for the Estimation of Gefitinib

Fixed Parameters	
Excitation Wavelength	280 nm
Emission Wavelength	512 nm
Excitation bandwidth	10 nm
Emission bandwidth	10 nm
Response time	0.1 seconds
Sensitivity	Medium
Temperature	30^0C
pH	6

Preparation of Calibration Graph

GEF working standard solution of 1 mg/mL was prepared and this solution was further diluted with ethanol to obtain a working standard solution of accurately 1, 1.5, 2.0, 2.5, 3.0, and 3.5 µg/mL. Fluorescence intensity was measured by setting the excitation wavelength at 280 nm and the emission wavelength at 512 nm. The calibration curve was prepared by plotting fluorescent intensity (I) *vs* concentration (µg/mL).

Estimation of Gefitinib in dosage forms

Twenty tablets of Geftistar each containing 250mg of Gefitinib were weighed accurately and made in to a fine powder. The tablet powdered equivalent to 10mg of Gefitinib was weighed accurately and transferred in to a 100 mL standard flask and it is dissolved in 50 mL of ethanol and was sonicated for a period of 20 min using ultrasonicator. The solution was filtered through a whatmann filter paper No.41. The volume was made up to the mark to get the concentration of 100 µg/mL. Further dilution was made to get the final concentration of 2.5 µg/mL. Standard solution was also prepared on same manner to get the final concentration of 2.5 µg/mL. The fluorescence intensity of the prepared solutions was measured at 512 nm under optimized experimental conditions. From the fluorescence intensity the amount of GEF present in the sample was calculated using single point standardization method .The assay values obtained were found to be within the limits.

Validation of the Developed Method

The developed method was validated for accuracy, precision, linearity, limit of detection and limit of quantitation as per ICH guidelines [12].

Accuracy

Accuracy of the developed method was established by recovery studies at three different levels 80, 100 and 120% of the sample in triplicate.

Precision

Intra-day precision was determined for calibration standards at three different time-points and inter-day precision on three different days.

Linearity

Linearity of the developed method was developed between 1-3.5 µg/mL. GEF working standard solution of 1 mg/mL was prepared and this solution was further diluted with ethanol to obtain a working standard solution and accurately 1, 1.5, 2.0, 2.5, 3.0, and 3.5µg/mL. Fluorescence intensity was measured by setting the excitation wavelength at 280 nm and the emission wavelength at 512 nm. The

calibration curve was prepared by plotting fluorescence intensity (I) *vs* concentration (µg/mL). The regression equation of the calibration curve was Y= mX+c.

Limit of Detection

Limit of Detection was determined on the basis of slope and standard deviation of the calibration curve.

$$LOD = 3.3\ \sigma/S$$

Where,

σ = standard deviation of Y intercept of regression lines.

S = slope of the calibration curve.

Limit of Quantitation

Limit of Quantitation was determined on the basis of slope and standard deviation of the calibration curve.

$$LOQ = 10\ \sigma/S$$

Where,

σ = standard deviation of Y intercept of regression lines.

S = slope of the calibration curve.

RESULTS AND DISCUSSION

The present study was focused on development of a new spectrofluorimetric method for the analysis of Gefitinib in bulk drug and tablet dosage form. Spectrofluorimetric analysis was performed using Jasco spectrofluorimeter model FB 8500 supported by Spectra manager software. For the method development suitable solvent, concentration of the drug and detection were studied and selected.

The solvent selected for the study was ethanol and the drug showed excitation wavelength at (λ_{ex}) of 280 nm and an emission wavelength (λ_{em}) of 512 nm (Fig-6). The concentration range of the drug selected for linearity was 1-3.5 µg/mL (Table-1) with correlation co-efficient value of 0.9995 indicating that good correlation exists between fluorescence intensity and the concentration (Fig-7).

To further assess the accuracy and reliability of the method, recovery experiments were performed by applying the standard-addition technique (80, 100 & 120% of the sample). The recovery was assessed by determining the agreement between the measured standard concentration and added known concentration to the sample. The results of recovery studies were 99.33-101.61, which indicate that the developed method was accurate (Table-2). High recovery values indicate that the developed method was free from interference of the excipients used in the tablet formulation.

The method was validated for intra-day and inter-day precision (Table-3). %RSD for inter-day and intra-day precision was less than 2, which indicates that the developed method was precise. The results of the assay were comparable with the corresponding labeled amounts (Table-4). Detection limit for Gefitinib was 0.0299 µg/mL and quantitation limit was 0.0906 µg/mL (Table-5) suggest that the developed method can be used for the estimation of Gefitinib even in micrograms accurately. Therefore, the proposed method is accurate and specific for the estimation of Gefitinib in bulk and tablet dosage form.

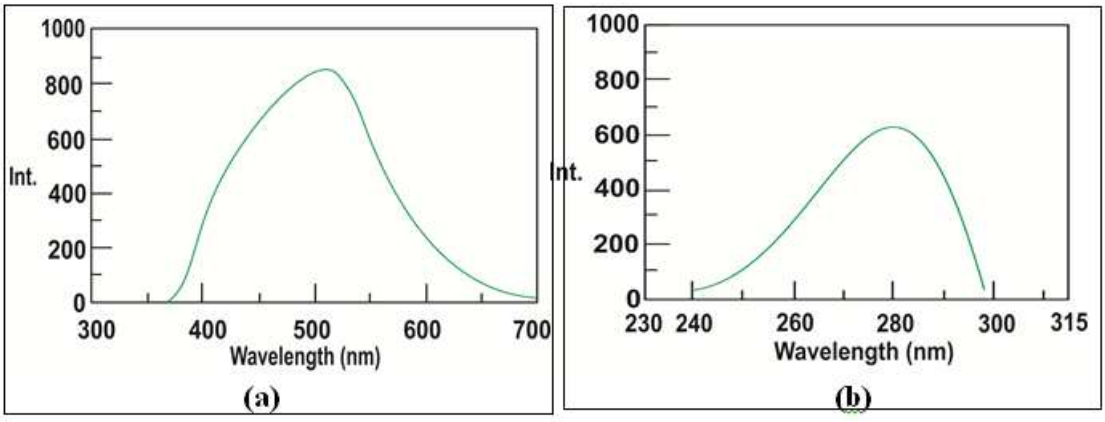

Fig-6: Emission Spectra (a) and Excitation Spectra (b) of Gefitinib

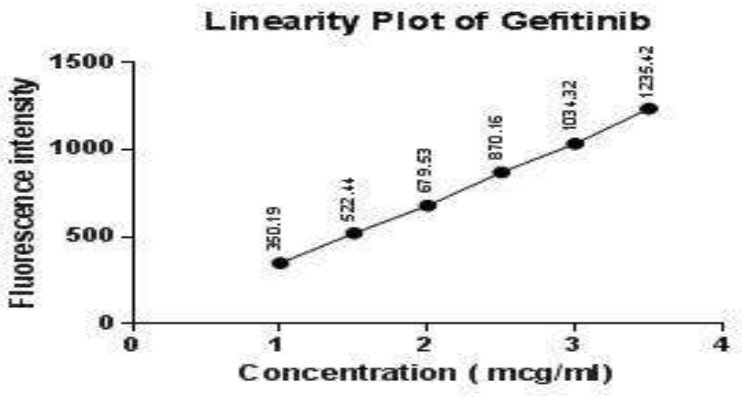

Fig-7: Linearity Plot of Gefitinib

Table-1: Linearity Data of Gefitinib

Concentration (µg/mL)	Mean Fluorescence intensity (I) (n = 6)
1.0	350.19
1.5	522.44
2.0	679.53
2.5	870.16
3.0	1034.32
3.5	1235.42
Slope	349.5740
y- intercept	- 3.8789
Correlation coefficient	0.9995

Table-2: Accuracy Data of Gefitinib

Parameters	Amount Present (µg/ml)	Amount Added (µg/ml)	Fluorescence Intensity (I)	Amount Found (µg/ml)	Amount Recovered (µg/ml)	% Amount Recovered
80%	2.5	2.0	1598.73	4.49	1.99	100.50
			1594.66	4.47	1.97	100.01
			1596.91	4.48	1.98	100.09
100%	2.5	2.5	1778.64	4.99	2.49	100.80
			1781.39	5.01	2.51	101.61
			1777.96	4.98	2.48	100.40
120%	2.5	3.0	1956.12	5.49	2.99	99.33
			1958.42	5.52	3.02	100.33
			1961.37	5.53	3.03	100.66
					Average	100.41
					SD	0.5866
					% RSD	0.5842
					SE	0.2074
					CI (Confidence Interval 99%)	99.75 – 101.06

Table- 3: Intra-day and Inter-day Precision Data of Gefitinib

Parameter	Intra-day			Parameter	Inter-day	
	Con (µg / ml)	Fluorescence Intensity (I) *	% Amount Found*		Fluorescence Intensity (I) *	% Amount Found*
0 Hours	2.5	858.36	98.63	Day – I	860.13	98.84
3 Hours		860.65	99.92	Day – II	859.50	99.77
6 Hours		859.90	98.89	Day – III	859.29	99.54
		SD	0.3541		SD	0.2534
		% RSD	0.3572		% RSD	0.2565

* Mean of six determinations

Table-4: Analysis of Gefitinib in Marketed Formulation

Fluorescence Intensity (I) of Standard	Fluorescence Intensity (I) of Sample	Label Claim (mg)	Amount Found (mg)	% Assay
859.54	860.22	250 mg	251.15	100.46
889.17	872.15		253.30	101.32
860.32	858.36		250.47	100.19
859.33	856.17		251.40	100.56
872.22	869.68		250.80	100.32
880.42	878.19		249.98	99.99
			Average	100.47
			SD	0.4207
			% RSD	0.4187
			SE	0.1881
			CI (Confidence Interval 99%)	99.77 – 101.16

Table-5: LOD and LOQ Data of Gefitinib

Slope		Y-Intercept
345.8720		-2.3632
352.9522		-3.9394
351.3106		-6.3248
351.8754		-8.4411
346.7285		0.5821
348.7053		-2.7878
Average	349.5740	
SD		3.1682
	LOD (µg/ml)	0.0299
	LOQ (µg/ml)	0.0906

CONCLUSION

The developed method was found to be simple, rapid, accurate, precise, economic, sensitive and easy to perform analysis. Hence, the method could be used in routine quality control of Gefitinib in raw material and tablet formulation.

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