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Development and validation of spectroscopic methods for the estimation of Gefitinib in bulk and in tablet formulation

Vivekkumar K.Redasani*, Priyanka S.Patel, Sanjay J.Surana
R. C. Patel Institute of Pharmaceutical Education & Research, Shirpur, Dist: Dhule (MS), 425 405, (INDIA)
E-mail : vivek.redasani@gmail.com

ABSTRACT

Three simple, precise, specific and cost effective spectrophotometric methods have been developed for the estimation of Gefitinib in bulk and its tablet formulation. Gefitinib was estimated at 250 nm in UV-spectroscopy (Method A), 261 nm in first order derivative spectroscopy (Method B) and scanned at 254.78 - 260.390 nm in area under curve (Method C). The drug follows Beer-Lambert's law in the concentration range of 2 -12 µg/ml in all three methods. All the methods were validated by following the analytical performance parameters suggested by International Conference on Harmonization (ICH) guidelines. All validation parameters were within the acceptable range. The developed methods were successfully applied to estimate the amount of Gefitinib in bulk and in tablet formulation.

KEYWORDS

Gefitinib;
UV spectroscopy;
Derivative spectroscopy;
AUC;
Validation.

INTRODUCTION

Gefitinib (GFT, Figure 1) is chemically N-(3-chloro-4-fluorophenyl)-7-methoxy-6-[3-(morpholin-4-yl) propoxy] quinazolin-4-amine used in the treatment of certain types of cancer by selectively targeting the mutant proteins in malignant cells. It inhibits the intracellular phosphorylation of numerous tyrosine kinases associated with transmembrane cell surface receptors, including the epidermal growth factor receptor (EGFR-TK)[1-3].

Literature survey reveals various liquid chromatographic methods for development and validation of GFT in bulk and its pharmaceutical formulation which are concerned with biological fluids[4-8], enzyme linked immune assay[9] and spectrophotometric estimation in bulk drug and formulations[10].

To our knowledge, no article related to the UV-Visible spectrophotometric determination of GFT in pharmaceutical dosage forms has been reported. Unlike HPLC, consumption of mobile phase per sample basis is quite low. This saves cost per analysis and analysis time as well. The aim of current work is to develop accurate and repeatable, UV-Visible spectrophotometric methods for determination of GFT applicable for the routine analysis in tablet formulations as per ICH guidelines[11].

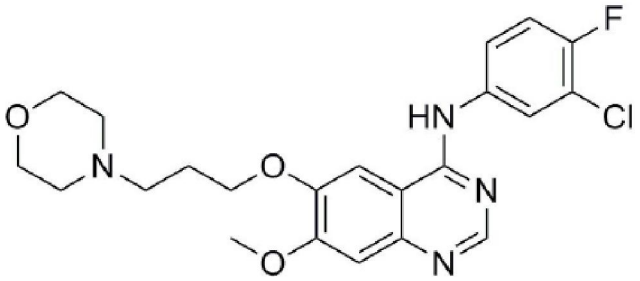

Figure 1 : Chemical Structure of Gefitinib

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EXPERIMENTAL

Reagents and chemicals

Gefitinib was supplied as a gift sample from Natco Pharma, Hyderabad (India). All chemicals and reagents used were of analytical grade and purchased from Merck Chemicals, India.

Instrument

A double beam UV-vis spectrophotometer (UV-2450, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe 2.21 with 10 mm quartz cells was used. The spectra were obtained with the instrumental parameters as: wavelength range: 200-400 nm; scan speed: medium; sampling interval: 1.0 nm; band width (Δλ):10.0 nm and spectral slit width: 1 nm. All weights were taken on electronic balance (Model Shimadzu AUX 120).

Preparation of standard stock solutions

An accurately weighed quantity of 10 mg GFT was transferred to 100 ml volumetric flask, dissolved in methanol and volume was made up to mark with the same solvent to obtain concentration 100 µg/ml. Then aliquots of standard stock solution were prepared by suitably diluting with same solvent to get the final concentrations of 2, 4, 6, 8, 10 and 12 µg/ml.

Method-A (Zero order derivative spectrophotometry)

From the stock solutions, appropriate volume of 1 ml was transferred to 10 ml volumetric flasks and the volume was adjusted to the mark with same solvent to obtain strength 10 µg/ml. The solution was scanned in the UV range 200-400 nm and DFP showed absorbance maximum at 250 nm (Figure 2a).

Method-B (First order derivative spectrophotometry)

The zero-order derivative spectra of concentration 10 µg/ml was derivatized into first order using UV-probe software of the spectrophotometer; amplitude of the trough was found at 261 nm (Figure 3a).

Method-C (area under curve)

The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra

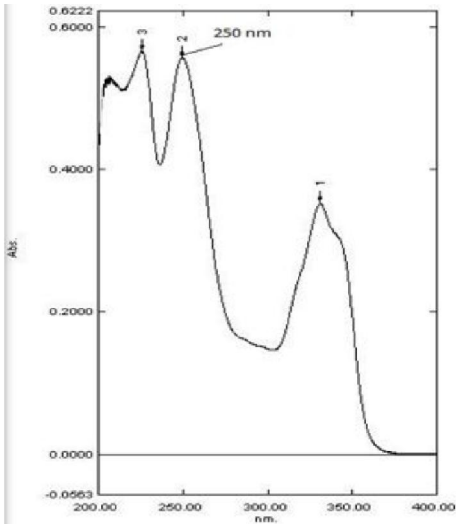
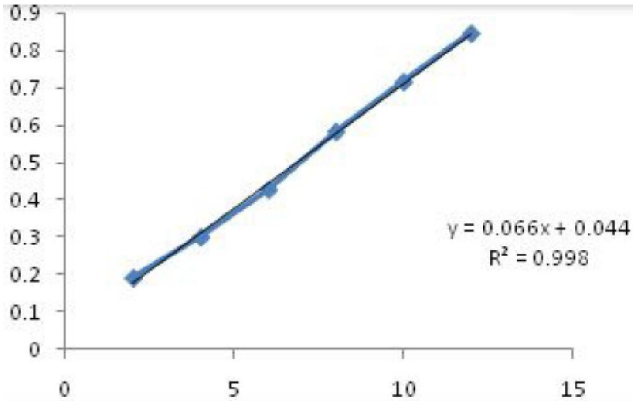


Figure 2a : UV Spectrum of GFT at 250nm



Y = 0.066 x + 0.044 Correlation coefficient = 0.998

Figure 2b : Calibration Curve of GFT

are obtained. It involves the calculation of integrated value of area with respect to the two selected wavelengths between 254.78 – 260.39. Area calculation processing item calculates the area bound by the curve and horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observations so as to get the linearity between AUC and concentration. The spectrum obtained of zero order derivative was used to calculate AUC (Figure 4a).

Analysis of in-house tablets

To determine the concentration of GFT in formulation, the contents of 20 tablets were weighed and finely powdered. A quantity equivalent to 10 mg of GFT was transferred to 100 ml volumetric flask and volume was made up to 50 ml with methanol followed by sonication for 30 min. The solution was filtered through Whatman

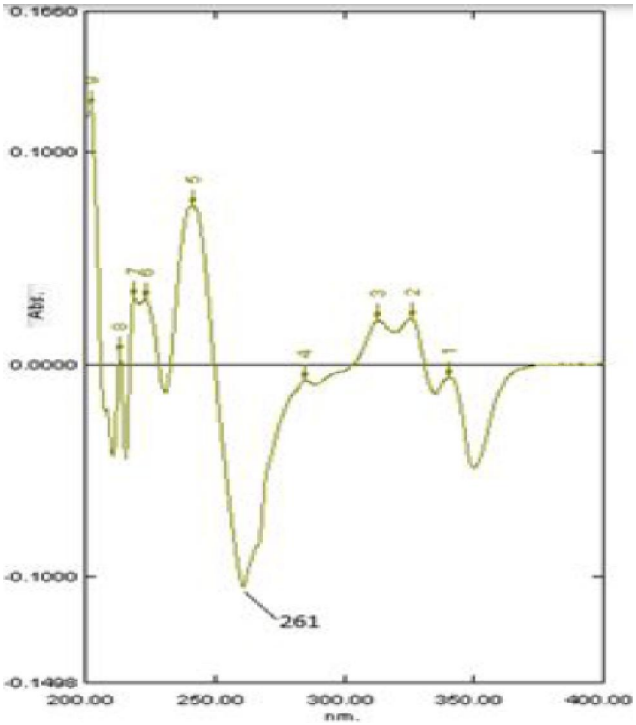
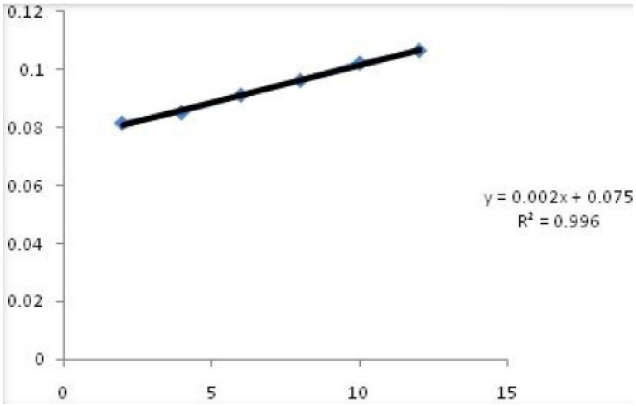


Figure 3a : First order derivative spectrum of GFT



Y = 0.002 x + 0.075 Correlation coefficient = 0.996

Figure 3b : Calibration Curve of GFT

filter paper and the residue was washed thoroughly with methanol. The filtrate and washings were combined and diluted up to the mark. From this solution 0.8 ml was transferred to 10 ml volumetric flask and diluted up to mark with same solvent. The resulting solution was analyzed for all the methods.

Validation of proposed method

Linearity

Different aliquots of GFT in range 0.2-1.2 ml were transferred into series of 10 ml volumetric flasks and the volume was made up to the mark with methanol to get concentrations 2, 4, 6, 8, 10 and 12 µg/ml, respectively. The solutions were scanned on spectrophotometer in the UV range 200-400 nm. Zero order spectrum of GFT obtained at wavelength 250nm by constructing the calibration plot as concentration *vs* absorbance (Figure 2b). First order derivative spectrum was obtained at 261 nm by constructing the calibration plot (Figure 3b) and concentration *vs* AUC (Figure 4b).

Precision

Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing the 4, 6 and 8 µg/ml of GFT solutions for three times in the same day. Inter-day precision was determined by analyzing daily for three consecutive days over a period of week using same concentrations.

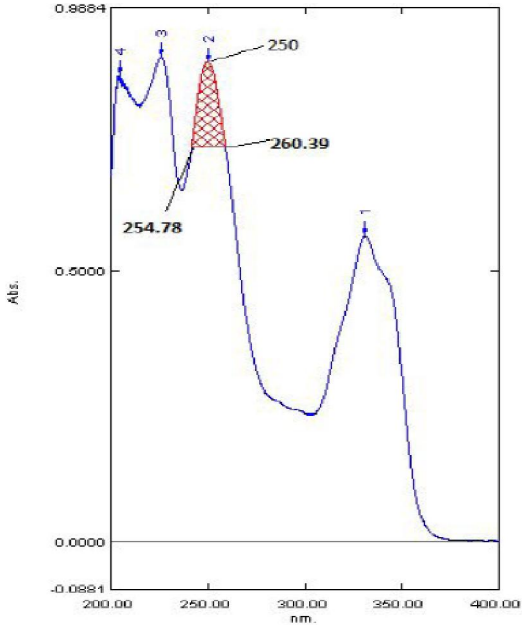
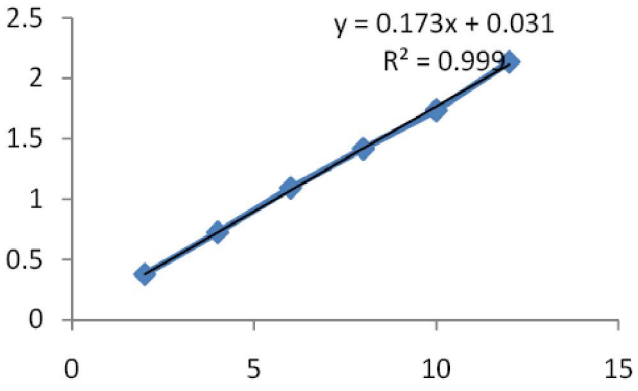


Figure 4a : UV spectrum of GFT showing AUC



Y = 0.173 x + 0.031 Coefficient of correlation = 0.999

Figure 4b : Calibration curve of GFT

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Recovery studies

To the pre-analyzed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80, 100 and 120 %. The solutions were reanalyzed by proposed method.

Repeatability

Repeatability was determined by analyzing 4 µg/ml concentration of GFT solution for six times. The absorbance of solutions was recorded and spectra were derivatized. AUC was measured for each derivative.

Ruggedness

Ruggedness of the proposed method was determined by analyzing aliquots from homogenous slot (4 µg/ml) in different laboratories by different analysts using similar operational and environmental conditions. The results are reported in terms of percent relative standard deviation (% RSD).

RESULTS AND DISCUSSION

The molecular structure of GFT is presented in Figure1. Methanol was selected as solvent because it provides highest solubility and AUC measurements. Three spectroscopic methods have been developed were GFT followed linearity in range of 2 - 12 µg/ml. The concentrations of the drug were calculated from linear regression equation. The amount of GFT estimated by methods was found to be within the acceptance criteria. Results obtained indicate that there is no interference of the excipients commonly used in tablets. These methods were validated for accuracy, precision, repeatability and ruggedness. The results are reported in terms of % RSD as shown in TABLE 1. Precision study at different time and day interval for each method, showed low standard deviation and % RSD less than 2, indicates that the proposed methods are precise. High recovery and low standard deviation confirmed that proposed methods are accurate for determination in pharmaceutical formulation. Also these methods were proved to be rugged as indicated by low values of % RSD.

TABLE 1 : Summary of validation parameters for proposed method

Parameters	Method A	Method B	Method C
Linearity (µg/mL)	2 - 12	2 – 12	2 – 12
Y = mx + C	Y= 0.066x + 0.044	Y= 0.002x + 0.075	Y= 0.173x + 0.031
Correlation coefficient	0.998	0.996	0.999
% Recovery*	98.94	99.10	99.60
%RSD	0.629 – 1.179	0.61 – 0.74	0.21 – 0.70
Precision (%RSD)			
Intra-Day*	1.229 – 1.513	0.55 – 0.73	0.40 -1.01
Inter-Day*	0.471 – 1.664	0.48-1.29	0.17 – 0.99
Repeatability#	1.152	1.62	0.23
Ruggedness (%RSD)#			
Analyst I	1.04	0.73	0.69
Analyst II	1.43	0.72	1.12

n = 3 #n = 6

CONCLUSION

The proposed methods were developed for the determination of Gefitinib based on UV-spectrophotometric and AUC. The developed methods can be used successfully for routine analysis of pharmaceutical dosage form of Gefitinib. However, these may not replace the presently known methods but can serve as an alternative where advanced instruments (e.g. HPLC) are not available for routine analysis.

ACKNOWLEDGEMENT

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REFERENCES

[1] Martindale; The Complete drug reference, 35th Ed., Council of Royal Pharmaceutical Society of Great Britain, **(2007)**.

[2] J.O.Maryadele; The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals', 14th Edition, Merck and Co. Inc.; Whitehouse Station, NJ, USA, **(2006)**.

[3] British Pharmacopoeia, London: The Stationary Office Medicinal and Pharmaceutical Substances (A-I), **1**, 106-108 **(2005)**.

[4] V.K.Kumar, N.A.Raju, S.Begum, J.S.Rao, T.Satyanarayana; Res. J. Pharm. and Tech., **2(2)**, 341-343 **(2009)**.

[5] P.V.V.Satyanarayana, M.Murali; Inter. J. Res. In Pharm. and Chem., **1(3)**, 338-341 **(2011)**.

[6] D.P.Bashaa, K.R.Shankar, G.V.N.Kiranmayi; Int. J. Chem. Sci., **10(1)**, 437-448 **(2012)**.

[7] N.A.Lankheet, M.J.Hillebrand, H.Rosing, J.H.Schellens, J.H.Beijnen, A.D.Huitema; Biomed. Chromatogr., **17**, **(2012)**.

[8] L.Faivre, C.Gomo, O.Mir, F.Taieb, A.Thomas, S.Ropert, M.Vidal, D.Dusser, A.Dauphin, F.Goldwasser, B.Blanchet; J. Chromatogr. B. Analyt. Technol. Biomed Life Sci., **879(23)**, 2345-2350 **(2011)**.

[9] T.Saita, H.Fujito, M.Mori; Pharma. Bulletin, **28**, 1833-1837 **(2005)**.

[10] P.P.Reddy, V.M.Balram, G.K.Mohan; Int. J. Chem. Res., **2(6)**, 1-8 **(2012)**.

[11] ICH Guidelines Q2 (R1), Validation of Analytical Procedures: Text and Methodology, **(2005)**.