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High Performance Liquid Chromatographic Estimation of Gefitinib in Pharmaceutical Dosage Forms

D. GOWRI SANKAR*, A. RAJESWARI, A. NAGESH BABU,
K. VANISHA DEVI and M. VAMSI KRISHNA
Department of Pharmaceutical Analysis and Quality Assurance, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530 003, India
E-mail: gowrisankar97@rediffmail.com

A reversed phase high performance liquid chromatographic method has been described for the estimation of gefitinib in its pharmaceutical formulations using C-18 column. The mobile phase consisted of pH 6.5 buffer (prepared by dissolving 1.5 g of potassium di-hydrogen phosphate in 550 mL of milli Q water (0.02 M) and adjusted the pH to 6.5 with triethylamine) and acetonitrile HPLC grade (Merck) in the ratio of 55:45 (v/v). The detection was carried out at 220 nm and the linearity was found to be in the range of 10-60 µg/mL. The method is simple, precise, specific, less time consuming and accurate for the estimation of gefitinib in pharmaceutical dosage forms.

Key Words: Gefitinib, RP-HPLC, Tablets.

INTRODUCTION

Gefitinib[1] (Fig. 1) is chemically N-(3-chloro-4-fluoro-phenyl)-7-methoxy-6-(3-morpholin-4-ylpropoxy)quinazolin-4-amine. It is used for the treatment of locally advanced or metastic non-small cell lung cancer (NSCLC) in patients who have previously received chemotherapy. Literature survey reveals that few LC-MS[2-5] methods for estimation of gefitinib were reported. The proposed method was simple, fast, accurate and precise for estimation of gefitinib in tablets.

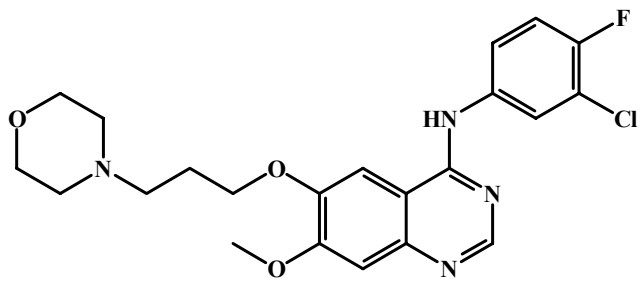

Fig. 1. Structure of gefitinib

EXPERIMENTAL

An isocratic high performance liquid chromatograph (Waters 2695) variable wavelength programmable UV detector Waters 2695, EMPOWER software and RP C-18 column (250 mm × 4.6 mm i.d., 5 µm particle size) was used.

Chromatographic conditions: The chromatographic column used was a 250 mm × 4.6 mm i.d., Inertsil-ODS-3v, C-18 column with 5 µm particles. The mobile phase consists of pH 6.5 buffer (prepared by dissolving 1.5 g of potassium di-hydrogen phosphate in 550 mL of milli Q water (0.02 M) and adjusted the pH to 6.5 with triethylamine) and acetonitrile HPLC grade (Merck) in the ratio of 55:45 (v/v).The mobile phase was mixed thoroughly and filtered through 0.45 µm membrane filter and sonicated before use. The flow rate of mobile phase was maintained at 1 mL/min. The column was maintained at ambient temperature and the detection was carried out by UV detector at 220 nm. The injection volume was 20 µL. The column performance parameters for the method have been summarized in Table-1.

TABLE-1
COLUMN PERFORMANCE PARAMETERS

Parameters	Results
Retention time (min)	17.17
Column length (cm)	25
Theoretical plates (n)	9072
Theoretical plates per meter (N)	36288
Ht equivalent to Theoretical. Plates (HETP) (mm)	0.0275
Tailing factor	0.750

Procedure: About 25 mg of pure sample of gefitinib was weighed accurately and transferred to 50 mL volumetric flask and dissolved in 25 mL of mobile phase. The solution was sonicated for 5 min and then made up the volume to 50 mL with mobile phase. 5 mL of this solution was diluted to 50 mL with mobile phase to get 50 µg/mL solution. Subsequent dilutions of this solution ranging from 10-60 µg/mL were made in 20 mL volumetric flasks. The solutions were filtered through 0.45 µm membrane filter and then 20 µL of filtrate was injected each time into the column at flow rate of 1 mL/min. Evaluation of the drug was performed with UV detector at 220 nm. Peak area was recorded for all peaks. A plot of peak area *versus* the respective concentration gives the calibration curve. The regression of drug concentration over the peak area was computed. The regression equation was used to estimate the amount of gefitinib in tablets.

Estimation of gefitinib in tablet dosage forms: Tablet powder equivalent to 125 mg was taken in 100 mL volumetric flask and 50 mL of mobile phase was added. The solution was sonicated for complete solubility of the drug, made up to the mark with the mobile phase. From this 2 mL of the solution was taken and diluted to 50 mL with mobile phase and filtered through a 0.45 µm membrane filter. From the filtrate, different aliquots were taken in separate 20 mL volumetric

flasks. The contents of the flask were made up to volume with mobile phase and mixed well. Each of the solutions (20 µL) was then injected five times into the column. From the peak areas, the drug content in tablets was quantified using the regression equation obtained from pure sample.

RESULTS AND DISCUSSION

A typical chromatogram of gefitinib was shown in Fig. 2. The retention time for gefitinib was 17.17 min. The peak areas from such different concentrations set up above were calculated and are shown in Table-2. A good linear relationship (r = 0.9999) was observed between the concentration of gefitinib and the respective peak area. The regression curve was constructed by linear regression fitting and its mathematical expression was y = 71800x + 5714 (where y is peak area and x is the concentration of gefitinib). The intra-day and inter-day variations of the method were determined using three replicate injections of four different concentrations, which were prepared and analyzed on the same day and three different days over a period of two weeks, a low coefficient of variation was observed (Table-3). This shows that the present HPLC method is highly precise.

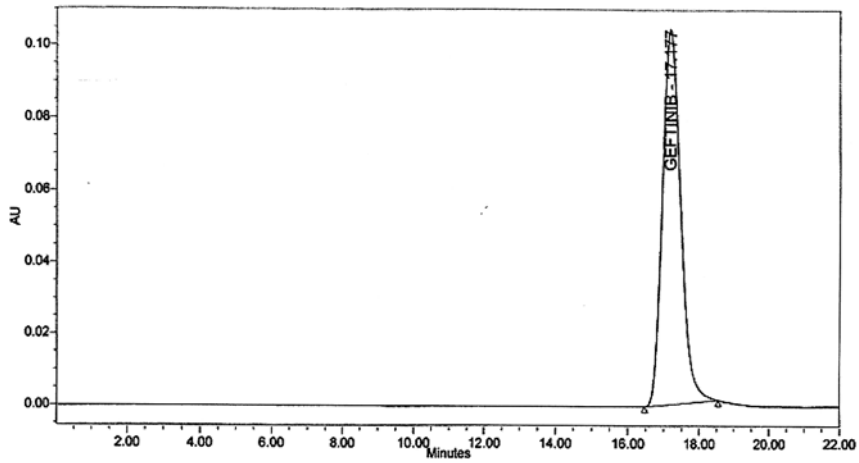

Fig. 2. Representative chromatogram of gefitinib

TABLE-2
CALIBRATION OF THE PROPOSED METHOD

Drug concentration (µg/mL)	Peak area
10	725146
20	1440293
30	2155439
40	2890586
50	3615733
60	4290879

Regression equation from 10-60 µg/mL
Y = 71800X + 5714 (r = 0.9999)

TABLE-3
PRECISION OF THE PROPOSED METHOD

Concentration of gefitinib (µg/mL)	Observed concentration of gefitinib (µg/mL)			
	Intra-day		Inter-day	
	Mean (n = 3)	CV (%)	Mean (n = 3)	CV (%)
10	10.02	0.906	9.98	0.758
20	20.01	0.284	20.07	0.612
30	30.01	0.201	30.00	0.222
40	39.98	0.180	40.05	0.240

TABLE-4
RESULTS OF THE RECOVERY STUDY

Amount of drug added (µg)	Recovery from drug solution		Recovery from tablet formulation	
	Mean amount found (n=3)	Mean % recovery	Mean amount found (n=3)	Mean % recovery
10	10.00	100.00	10.00	100.00
20	20.04	100.22	19.97	99.85
30	29.98	99.92	30.03	100.11

To ensure reliability and accuracy of the method, recovery studies were carried out mixing a known quantity of drug with preanalyzed sample and the contents were reanalyzed by the proposed method. About 99.85 % of gefitinib could be recovered from the preanalyzed samples indicating the high accuracy of the proposed HPLC method.

The drug content in tablets was quantified using the proposed analytical method and the results are shown in Table-5. The tablets were found to contain 99.99-100.00 % of the drug. It can be concluded that the proposed method was suitable for the estimation of gefitinib in routine quality control analysis.

TABLE-5
ASSAY OF GEFITINIB IN TABLET DOSAGE FORMS

S. No.	Labeled amount of drug (mg)	Mean (±SD) amount (mg) found by the (n = 5)		Mean (±SD)% labeled amount (n=5)
		Proposed method	Reference method[6]	
Tablet-1	250	249.99 ± 0.054	249.80	99.99 ± 0.021
Tablet-2	250	250.02 ± 0.078	251.03	100.00 ± 0.034

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