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DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE ESTIMATION OF LAPATINIB IN BULK DRUGS AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A new simple, precise and accurate Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for the estimation of Lapatinib in bulk drugs and pharmaceutical dosage formulations was developed and validated. The method was developed by using the solvent system methanol, potassium dihydrogen phosphate, and tetrahydrofuran in the ratio of 60:35:5 (v/v/v) at pH 6.2 was given high resolution chromatogram with low tailing factor (<2). The method was successfully applied for routine analysis of Lapatinib in bulk samples and its formulations.

Key words: Lapatinib, Estimation, RP-HPLC, Validation.

INTRODUCTION

Lapatinib(MF:$C_{29}H_{26}ClFN_4O_4S$) (Fig. 1), used in the form of lapatinib ditosylate, is an orally active drug for breast cancer (approved by U.S. FDA in 2007)and for some solid tumours[1]. It is a dual tyrosine kinase inhibitor[2] and is also used as an adjuvant therapy when patients have progressed on Herceptin[3]. It was marketed under the trade names Tykerb and Tyverb[4].

The drug lapatinib ditosylate is classified as a synthetic compound showing competitive inhibition of the natural product that is naturally derived substrate[5]. Like Sorafenib, lapatinib is a protein kinase inhibitor and it reduces the growth tumor-causing breast cancer stem cells[6]. Based on recent studies[7], GSK announced the approval of lapatinib in first-line therapy in triple positive (hormone receptor, EGFR, HER2) breast cancer patients[8]. The most common side effects caused by the drug are diarrhea, fatigue, nausea and rashes[2,9].

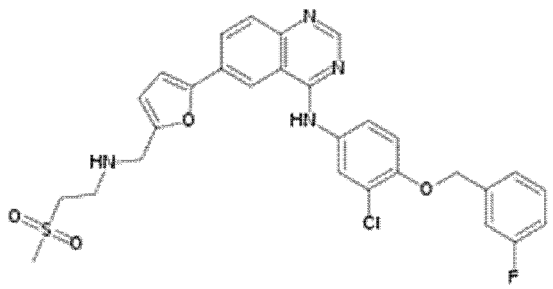


Fig. 1: Structure of Lapatinib{N-[3-chloro-4-[(3-fluorophenyl) methoxy] phenyl]-6-[5-[[[2-methylsulfonyl)ethyl] amino]methyl]-2-furanyl]-4-quinazolinamine}.

MATERIALS AND METHODS:

Equipment

Analysis was carried out using PEAK7000 isocratic HPLC with manual injection and the column used was Kromosil C_{18} (250×4.6mm). Electronic balance used was ELB300 and DIGISUN pH meter was used for all pH measurements.

Chemicals and Reagents

Lapatinib reference standard was a kind gift of V. V. MED Labs, Hyderabad and the tablet formulations used for testing the method were purchased from local market. All the solvents used were HPLC grade and employed as such supplied by Merck. The solutions and the mobile phase prepared were stored at room temperature.

Selection of suitable mobile phase

The mobile phase for the analysis of Lapatinib was set by injecting different ratio's of methanol (Make-MERCK. SF8SF80771), potassium dihydrogen phosphate and tetrahydrofuran. The selected mobile

phase ratio was Methanol: KH_2PO_4 (0.01M) and THF is 60:35:5 (v/v/v) and the p^H was found to be 6.2. The selected mobile phase has given a sharp peak with low tailing factor(1.94) i.e. <2.

Initial Chromatographic Conditions

Chromatographic analysis of the Lapatinib was done using a Kromasil C_{18} (250 x 4.6 mm, 5 µm) column. The mobile phase composition used was Methanol: KH_2PO4 (0.01M) and THF in ratio of 60:35:5 was filtered through 0.5µ nylon membrane filter before use and p^H was maintained at 6.2. The analysis was carried out in isocratic mode at a flow rate of 1ml/min, with column effluent being monitored at 253nm wavelength. Operating pressure is 3000psi at room temperature by injecting the volume 20 µl with run time 7min.

Preparation of Standard solutions

Pure standards of lapatinib were used as external standards in the analysis. Different concentrations of the standards were used based on the range required to plot a suitable calibration curve. About 20mg of the standard Lapatinib was accurately weighed and transferred in to 10ml volumetric flask and make up with sufficient mobile phase. Volumetric flask containing standard solution was sonicated for 10 minutes. The standard solution was then filtered with 0.45µm membrane filter paper. Different concentrations of these standards were analyzed using the same chromatographic conditions and a calibration curve was generated.

Sample Preparation

Tykerb(250mg) was used for testing the method. About 20mg of the commercial sample was taken in to 10ml volumetric flask and added mobile phase to get 2mg/ml sample solution. The sample solution was then filtered with 0.45µm membrane sample filter.

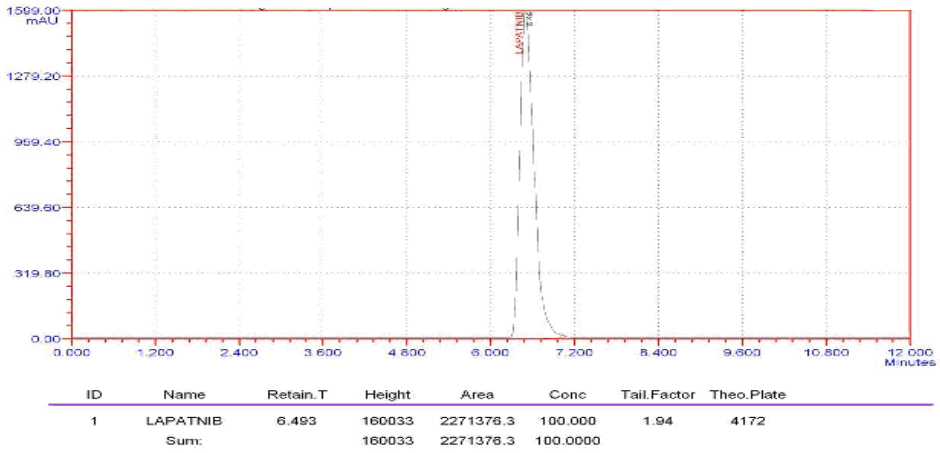
Procedure for analysis

With the optimized chromatographic conditions set for Lapatinib a study base line was recorded and stabilized for about 30 min. After the stabilization of base line successive aliquots of the sample solution were injected separately and the chromatograms were recorded, until the reproducibility of the peak areas were adequate. The same procedure was followed for the commercial sample used for testing the accuracy of the method. The sample was injected into the column at flow rate of 1ml/min.

RESULTS AND DISCUSSION

The titled drug was estimated by using different methods[10-11]. Feng Bai developed a sensitive method for the estimatimation of lapatinib in human plasma by using liquid chromatography electrospray tandem mass spectrometry (LC-ESI-MS/MS)[10]. Later in 2009 a selective liquid chromatographic tandem mass spectrometry (LC-MS/MS) method has been developed by Roche[11] to quantify cellular levels of the tyrosine kinase inhibitors (TKIs) dasatinib (Sprycel) and lapatinib (Tykerb, Tyverb). However, according

to the knowledge of the author no precise or accurate RP-HPLC method was developed for the estimation of lapatinib. In the present investigation an attempt was made to develop an accurate RP-HPLC method for the estimation of Lapatinib in bulk drugs and pharmaceutical dosage formulations. For this purpose different ratios of the mobile phase mentioned above were tested for the estimation of lapatinib. Finally Methanol: potassium dihydrogen phosphate (KH_2PO4, 0.01M) and tetra hydro furan (THF) in ratio of 60:35:5 (v/v/v) was selected as suitable mobile phase for the estimation of the drug. The selected mobile phase in the Kromosil C_{18} Column has given a sharp peak with tailing factor 1.94(<2) at rention time 6.5min and the chromatographic run time is 10min. The standard chromatogram obtained for lapatinib for the above said conditions was given in fig.2.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	LAPATNIB	6.493	160033	2271376.3	100.000	1.94	4172
	Sum:		160033	2271376.3	100.0000		

Fig. 2. Standard chromatogram of Lapatinib

Method validation

After the completion of HPLC method development the method was validated in terms of different parameters like specificity, linearity, precision, recovery, accuracy, LOD and LOQ.

Evaluation of linearity:

The linearity of measurement was evaluated by analyzing different concentrations of standard solutions of lapatinib. Solutions of 0.5mg/1ml, 0.25mg/1ml, 0.125mg/1ml, 0.0625mg/1ml and 0.03125mg/1ml with standard 100% pure lapatinib in the mobile phase was prepared and analyzed. After analysis the area of the peaks were recorded. The retention times and the corresponding peak areas are given in Table 1.

S.No.	Conc	Area	Rt.
1	0.5mg/1ml	6736610.2	6.894
2	0.25mg/1ml	532383.1	6.995
3	0.125mg/1ml	400396.6	7.110
4	0.0625mg/1ml	255463.1	7.313
5	0.03125mg/1ml	136646.0	7.645

Table-1: Linearity

With the above data a plot was drawn between concentration (on X-axis) and peak areas (on Y-axis). The plot was found to be a straight line satisfying linearity condition. The correlation coefficient (r^2 =0.9532, Fig. 3) of regression was found near to be 1.

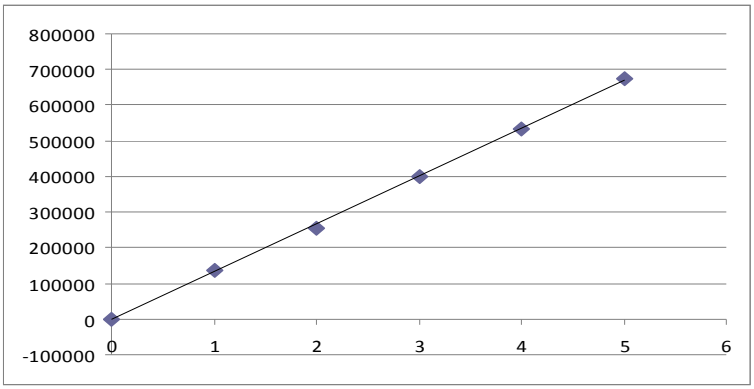


Fig.3: Linearity of Lapatinib

Accuracy (% Recovery):

To study of the reliability, suitability and accuracy of the method recovery experiments were carried out for lapatinib in two stages and the average recovery of the drug was calculated for both the sample and standard. For this, the recovery studies were performed using standard addition method i.e. a known quantity of pure drug was added to the pre-analysed sample formulation at the stage of one at 50% and another at 100%. The % recovery of the drug in each stage is calculated by using the formula given below. The average recovery of the lapatinib is 99.66% (Table 2).

% recovery = [(b-a)/c] X100

Where a- The amount of drug found before the addition of standard drug.

b- The amount of drug found after the addition of standard drug.

c- The amount of standard drug added.

The values obtained above are in good agreement in terms reliability, suitability and accuracy of the proposed method.

Si.No.	Conc.0.5mg/1ml	Area	T.P.	
1	Standard	673610.2	7041	**%** Of Accuracy = 99.66
2	Sample	673393.5	7001	

Table-2: Accuracy

Precision of Method:

A standard solution (2mg/ml) of drug substance was injected five times in two different days in a week and corresponding peak areas were recorded. The % RSD for day-1 and day-2 were found to be is 0.064 and 0.509 respectively and were less than 1% (Table 3). The values of %RSD prove that the method is precise.

Test-3	**Precission**			
	Concentration 2mg/ml			
	Injection	**Area**	**T.P.**	
DAY-1	1	2226032.9	4316	% R.S.D.=0.064
	2	2225618.4	4316	
	3	2224328.7	4320	
	4	2222775.5	4325	
	5	2223235.4	4328	
DAY-2	1	2243197.3	4223	%R.S.D = 0.5096
	2	2238172.7	4232	
	3	2237266.7	4234	
	4	2219056.4	4297	
	5	2219333.3	4296	

Table-3: Precision data for Day-1 and Day-2.

Specificity of the method

The specificity of the method was determined by observing any interference encountered from the ingredients present in the formulations. The test results obtained were compared with that of test results those obtained for standard drug. In the present study, it was shown that those ingredients are not interfering with the proposed method.

Ruggedness

Inter-day variations were performed by using five replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation.

Robustness and system suitability

Robustness of the method was carried out by varying two parameters slightly from the optimized chromatographic conditions, such as column temperature, flow rate and mobile phase. It was found that there were no appreciable changes in the chromatograms for column temperature variation, flow rate variation and mobile phase variation. The robustness limit for the above parameter variations was well within the acceptable limit and is less than 2%. This shows that the method is having good system suitability under the given set of conditions.

LOD and LOQ

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting increasingly low concentrations of the standard solutions by following the developed RP-HPLC method. The LOD is the smallest concentration of the analyte which gives a measurable response. The LOD for Lapatinib was found to be 10ng/ml. The LOQ is the smallest concentration of the analyte, which gives response that can be absolutely quantified. The LOQ for Lapatinib was found to be 35ng/ml (Table 4). The results of LOD and LOQ supported the sensitivity of the developed method.

Test-4	**L.O.D.**	10ng/ml
Test-5	**L.O.Q**	35ng/ml

Table-4: LOD and LOQ

CONCLUSION

A simple, rapid, precise and accurate RP-HPLC method was developed and validated for the estimation of lapatinib(an US FDA approved drug for breast cancer) in tablet dosage formulation and bulk drugs. Its chromatographic run time of 10min. allows the analysis of large number of samples in a short period of time. So, this method is suitable for the routine analysis of lapatinib in pharmaceutical dosage forms.

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