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Research Article

DEVELOPMENT AND VALIDATION OF METHOD FOR THE DETERMINATION OF NILOTINIB BY RP-HPLC IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A RP-HPLC method in Isocratic mode was developed for the estimation of Nilotinib in bulk and pharmaceutical dosage forms. The method was employed on C-18 column using Water and Acetonitrile in the ratio 50:50 v/v as mobile phase at a flow rate of 1mL/min.The UV detection wavelength selected was 254nm.The retention time for Nilotinib was found to be 3.874 min.The linearity for the method was observed in a concentration range of 5-250µg/mL with the correlation coefficient of 0.999.The developed method was validated as per ICH guidelines. The method was found to be simple, accurate and precise. The method has been applied for the determination of Nilotinib in pharmaceutical dosage forms.

KEY WORDS: RP-HPLC, Nilotinib, Method development, Validation.

INTRODUCTION

Nilotinib is a second generation oral tyrosine kinase inhibitor which is chemically 4-methyl-N-(3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl) phenyl)-3-(4-(pyridine-3-yl) pyramidin-2-ylamino) benzamide(Fig1).It is used in the treatment of chronic mylogeneous leukemia.Nilotinib[1] is a signal transduction inhibitor that selectively inhibits autophosphorylation of BCR-ABL which there by inhibits the cellular proliferation and migration. Literature study reveals that there is only one HPLC[2] method for the estimation of Nilotinib in capsules and few other analytical methods were developed for the estimation of Nilotinib in plasma[3-5]. As there are no other analytical methods available for the estimation of this drug in the bulk and pharmaceutical dosage forms, in the present work simple, accurate and precise RP-HPLC method has been developed and validated.

MATERIALS AND METHOD

Instrumentation

Chromatographic separation was achieved using a C-18 column (250mm x 4.6mm i.d.,5µm particle size) of Shimadzu model CBM-20A/20 Alite that is equipped with SPD M20A prominence photodiode array detector (PDA), maintained at 25^0C.

Materials Required

Nilotinib pure standard was obtained as a gift sample from Hetero drugs (India). Acetonitrile and Water of HPLC grade were purchased from Merck (India) and Qualigens (India) respectively. Nilotinib capsules available under the brand name Tasigna (150mg, Novartis Pharma) were purchased and used.

Optimized Conditions

The mobile phase with water and acetonitrile in the ratio of 50:50 %v/v was employed in isocratic mode at a flow rate of 1mL/min.The run time was 6 mins and 20µL of the sample was injected for every run into the column. The wavelength of the PDA detector was set at 254nm.

Preparation of Standard Stock Solution

Accurately about 5mg of Nilotinib was weighed and transferred to a 10mL volumetric flask.5mL of water was added to the flask and sonicated to dissolve it. The volume was then made up to the mark with water to get a standard solution of Nilotinib at a concentration of 500µg/mL

Preparation of Standard Solutions

Working solutions for HPLC injections were prepared on daily basis. Aliquots of the standard stock solution were taken and diluted with the mobile phase to get solutions in a concentration range of 5-250 µg/mL.

Assay of Formulation

Twenty capsules of Tasigna were purchased. They are weighed and then powdered. Aliquot of the powder equivalent to 50mg of Nilotinib was weighed and transferred to a 100mL volumetric flask.70mL of water was added to the flask and then sonicated to dissolve the powder completely. The volume was then made up to 100mL with water to get the stock solution of concentration 500µg/mL. It was then filtered with 0.45µm membrane filter.3mL of this solution was then transferred to a 10mL volumetric flask and then the solution was diluted with mobile phase to get a solution of 150µg/mL.20µL of this solution was then injected into the system. The chromatogram was depicted in Fig 3. The percentage assay of the drug was calculated and presented in table 1.

Method Validation

The method was validated as per ICH guidelines to demonstrate that it is suitable for the intended purpose. The method was validated for system suitability, linearity, accuracy, precision, limit of detection, limit of quantification and robustness[6].

System Suitability

System suitability parameters were studied to ensure that the instrument is suitable for the intended purpose. Retention time, tailing factor and theoretical plates were evaluated. The drug solution was injected five times into chromatographic system under the optimized conditions and the parameters were evaluated.

Linearity

Series of dilutions were prepared from the standard stock solution of Nilotinib in the concentration range of 5-250µg/mL.20µL of each of these solutions were then injected into the column and the chromatographic characteristics were studied under the optimized

conditions. The standard chromatogram of Nilotinib was shown in Fig 2. Calibration curves of peak area against concentration was found to be linear as shown in Fig 4.The linearity data were tabulated in table 2.

Accuracy

The recovery studies for the method were carried out by standard addition method. It was evaluated at three concentration levels (80,100 and 120%) and the percentage recoveries were calculated. The data is tabulated in table 3.

Precision

The precision of the method was determined by intra and inter day precision studies. This was evaluated by injecting three independent sample preparations of Nilotinib from a single formulation at three different concentration levels on the same day (Intra day) and on three different days (Inter day).The %RSD was then calculated. The data is represented in table 4.

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined based on the standard deviation of the response and the slope of the calibration curve. The sensitivity of the method was established by the LOD and the LOQ values.

Robustness

Robustness was established by introducing small changes in the HPLC optimized conditions which include the change in wavelength, flow rate and percentage of acetonitrile in mobile phase. This was studied using six replicates at a concentration level of 50µg/mL of Nilotinib.

Table 1: Report for assay

Drug	Amount present (mg/Capsule)	Amount found (mg/Capsule)	% label claim
Nilotinib	150mg	148.39	98.92

Table 2: Data for linearity

Conc ((µg/mL)	Peak area
5	160713
10	327700
20	638852
50	1597132
100	3391814
150	4688946
200	6291534
250	7863678

Table 3: Recovery studies of Nilotinib

%Spike level	Amount added (µg/mL)	Peak area	Amount found (µg/mL)	% Recovery	Mean % Recovery
80	18	592689	17.84	99.11	
80	18	598980	18.04	100.22	99.61
80	18	594932	17.91	99.5	
100	20	656845	19.88	99.4	
100	20	661309	20.03	101.5	100.21
100	20	659087	19.95	99.75	
120	22	716760	21.79	99.04	
120	22	720987	21.92	99.63	99.37
120	22	719530	21.88	99.46	

Table 4: Data for precision

S No	Conc (µg/mL)	Intraday precision		Inter day precision	
		Mean* ± SD	*%RSD	Mean* ± SD	8%RSD
1	10	328384± 948.68	0.288	325898.3 ±1619.65	0.49
2	50	1599168±7165.88	0.44	1584812 ±15405.7	0.97
3	100	3338938±33368.53	0.99	3356590±4042.56	1.20

*Mean of three replicates

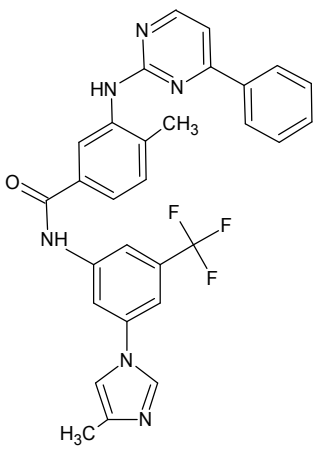

Figure 1: Chemical Structure of Nilotinib

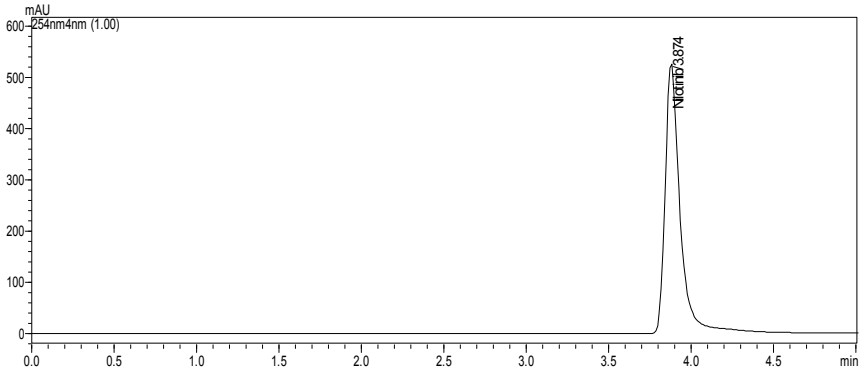

Figure 2: Standard Chromatogram of Nilotinib (100µg/mL)

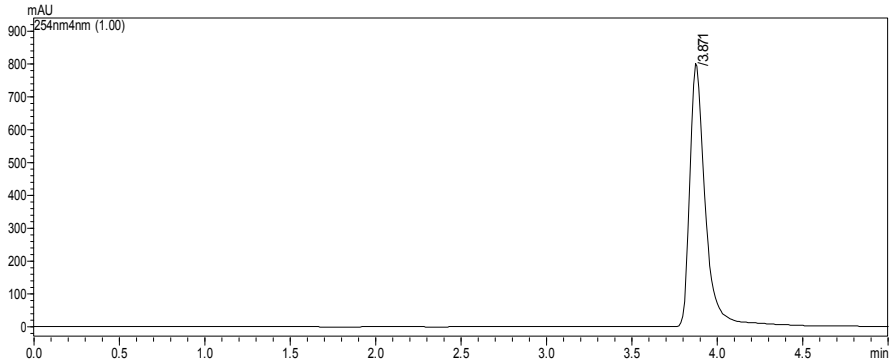

Figure 3: Sample Chromatogram of Nilotinib (150 µg/mL) Tasigna (Label claim 150mg)

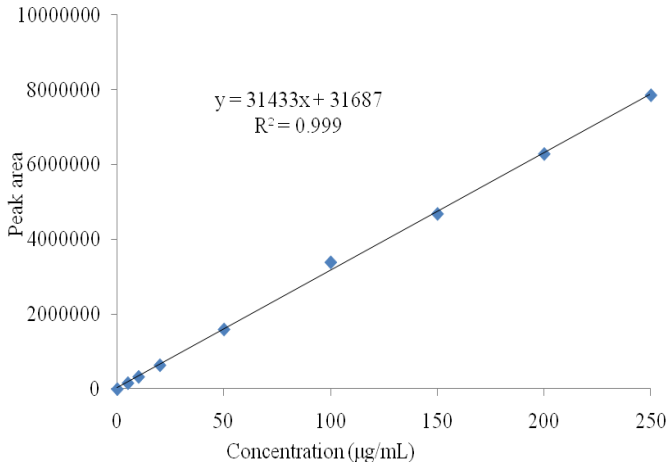

Figure 4: Calibration curve of Nilotinib

RESULTS AND DISCUSSION

The proposed method was found to be simple. Linearity was observed in the concentration range of 5-250µg/mL with the regression equation y= 31433x + 31687 and the correlation coefficient of 0.999.System suitability parameters indicates high column efficiency with large number of theoretical plates (>2000).The tailing factor was found to be 1.09 which is does not exceed the critical value (2).The average retention time was found to be 3.872.No interference was seen from any of the components of the pharmaceutical dosage form indicating the specificity of the method.The recovery studies were performed and the % RSD was found to be in the range 0.29-0.53.The % RSD was found to be 0.28-0.99 for intraday and 0.49-1.2 for inter day precision studies. Thus the method was found to be accurate and precise as the %RSD was not more than 2%.The limit of detection and limit of quantification for Nilotinib were found to be 0.26µg/mL and 0.79µg/mL respectively. The %RSD for the %assay of sample was calculated for each parameter in robustness and was found to be less than 2% confirming the robustness of the method.

CONCLUSION

A validated RP-HPLC method was developed for the determination of Nilotinib in bulk and pharmaceutical dosage forms. As the proposed method is simple, rapid, accurate, precise and specific it can be employed for the routine analysis of Nilotinib in pharmaceutical dosage forms.

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