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Research Article

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Development and validation of UV spectrophotometric method for the determination of Nilotinib hydrochloride (An orphan drug)

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ABSTRACT

A simple, accurate, precise and sensitive UV spectrophotometric method was developed for the determination of Nilotinib hydrochloride in bulk and pharmaceutical dosage form. The solvent used is Methanol:Water (1:1) and the wavelength corresponding to the maximum absorbance of the drug was found at 263 nm. Beers law was observed in the concentration range of 7- 12µg/mL with correlation coefficent R^2 = 0.9984. The linear regression equation obtained by least square regression method were y = 0.1094x - 0.3008, where y is the absorbance and x is the concentration of the pure drug solution. The method was validated for several parameters like accuracy, precision as per ICH guidelines. The values of the relative standard deviation and % recovery were found to be satisfactory, indicating that the proposed method is precise and accurate and hence can be used for the routine analysis of Nilotinib hydrochloride in bulk and pharmaceutical formulation.

Keywords: Nilotinib hydrochloride;UV spectrophotomtery;ICH Validation;Capsules;Orphan drug;

INTRODUCTION

Nilotinib hydrochloride,4-methyl-*N*-[3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-3-[(4-pyridin-3-ylpyrimidin-2-yl)amino]benzamidesalt) in the form of the hydrochloride monohydrate salt with trade name Tasigna, is a tyrosine kinase inhibitor approved for the treatment of chronic myelogenous leukemia[1][2]. It is used to treat chronic myeloid leukaemia (CML) in people who have tested positive for Philadelphia chromosome.It is slightly yellow to slightly greenish yellow powder, slightly soluble in methanol and in dimethyl sulphoxide[3]. Molecular weight of Nilotinib Hydrochloride is 565.98[4] with empirical formula C28H22F3N7O.HCl. It was designated as an orphan drug by FDA for use in the treatment of CML in Europe,United States of America and Switzerland [5]

It is a tyrosine kinase inhibitor approved for the treatment of chronic myelogenous leukemia.It is used to treat chronic myeloid leukaemia (CML) in people who have tested positive for Philadelphia Chromosome. Philadelphia Chromosome is a genetic abnormality which is commonly found in people who have CML.Chronic myelogenous (or myeloid) leukemia (CML), also known as chronic granulocytic leukemia (CGL), is a cancer of the white blood cells[6]. FDA has approved a Risk Evaluation and Mitigation Strategy (REMS) for nilotinib[7].

The literature survey reveals that there are no LC methods were reported in major pharmacopoeias like USP, EP, JP and BP.There was one HPTLC, few RP-HPLC [8-13] and one stability indicating UPLC method for determination of related compounds of Nilotinib Hydrochloride[14].The purpose of the present research work is to develop a UV

spectrophotometric method for the determination of Nilotinib Hydrochloride in bulk and its marketed formulations followed by analytical method validation as per ICH recommended conditions.

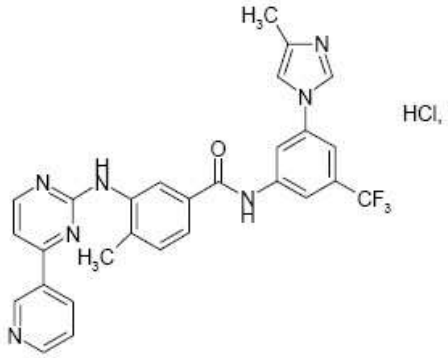

Figure.1 Structure of Nilotinib hydrochloride

EXPERIMENTAL SECTION

Instrumentation and software: Thermo Fischer Scientific UV/Visible double beam spectrophotometer UV10 with a spectral bandwidth of 2nm and wavelength accuracy of ± 0.2 nm was used for the study and 1.0 cm matched quartz cells were used for analytical method development and validation. VISIONlite version 5.0 installed on windows 7 operating system was used for data acquisition. Micropipette of Variable volume 10-1000 µL (Thermo Scientific) and analytical balance (Mettler Toledo) were used.

Reagents: Double distilled grade water was used for the study and all the reagents used in this study are of analytical grade.

Development and optimization of the spectrophotometric method:

Selection of solvent: Preliminary trails were made with the individual solvents and mixtures of double distilled grade water,AR grade methanol,AR grade acetonitrile,AR grade ethanol. However, adequate solubility and maximum sensitivity were observed when the solvent is 1:1 methanol and water. Hence the method is optimized using 1:1 methanol and water to produce reproducible assay sensitivities and spectral charaterstics.

Preparation of standard stock solution: 50 mg of Nilotinib Hydrochloride standard was accurately weighed and transferred to a 50 ml volumetric flask and the volume completed with equal proportions of methanol and water. The concentration of stock solution was 1mg/mL.

Preparation of sample solution: The contents of 20 capsules (Tasigna® 150mg, Novartis Pharma) were mixed and then powdered. The powdered contents equivalent to 200 mg of nilotinib in to a 100ml volumetric flask was taken. Initially 25 ml of solvent 1:1 methanol and water (25 ml) was added and the mixture was allowed to stand for 1 hr with intermittent sonication to ensure complete solubility of the drug, and then filtered through a 0.45m membrane filter, followed by adding1:1 methanol and water to obtain a stock solution of 2000µg/ml. Transfer for 5ml of this solution to a 50 ml of volumetric flask and made upto sufficient volume with mobile phase to give an concentration of 200µg/ml. The amount of nilotinib was computed by using the calibration curve equation. The solution was suitably diluted so as to obtain a concentration in the linearity range and absorbance was measured against blank at 263 nm. Result of analysis is shown in Table 9.

Selection of detection wavelength for maximum absorbance (λmax):

From the stock solution, 10ml was transferred to a 100ml volumetric flask and made up the volume with already selected solvent to give a 100µg/mL solution.From the above stock solution, pipette out 0.8 ml in to 10ml volumetric flask and finally made up the volume with 1:1 methanol and water solvent, to produce a concentration of 8µg/mL. The sample was then scanned in UV spectrophotometer from a range of 200-320 nm against above said solvent as blank and the wavelength corresponding to maximum absorbance in was found at 263 nm (figure.2).

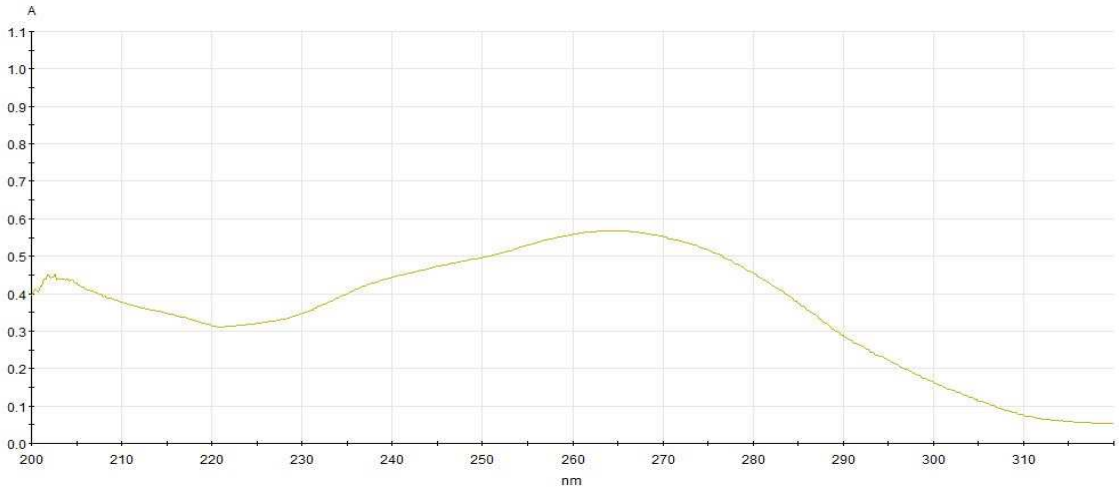

Figure.2 UV Spectrum of Nilotinib in solvent 1:1 methanol and water

Preparation of standard calibration curve

For the preparation of standard calibration curve, concentration of 7-12 µg/mL were prepared by pipetting out 0.7,0.8,0.9,1.0,1.1, 1.2 ml from the 100µg/mL solution in to a 10ml volumetric flask and made up the volume with above said solvent. The absorbance of each solution was measured at 263 nm against solvent as blank. Calibration curve of the drug was then plotted by taking the absorbance obtained on y-axis and the concentration of the solution on x-axis (Figure3).

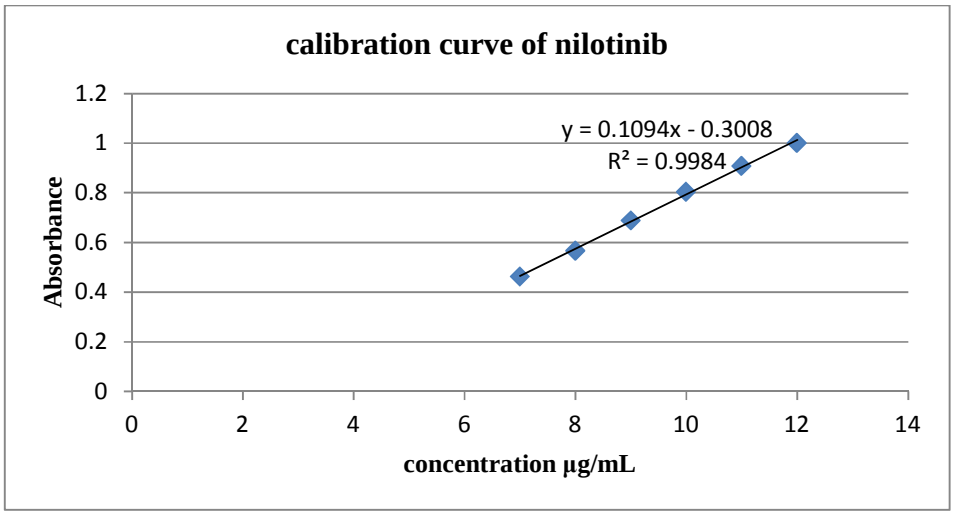

Figure.3 Calibration curve of Nilotinib

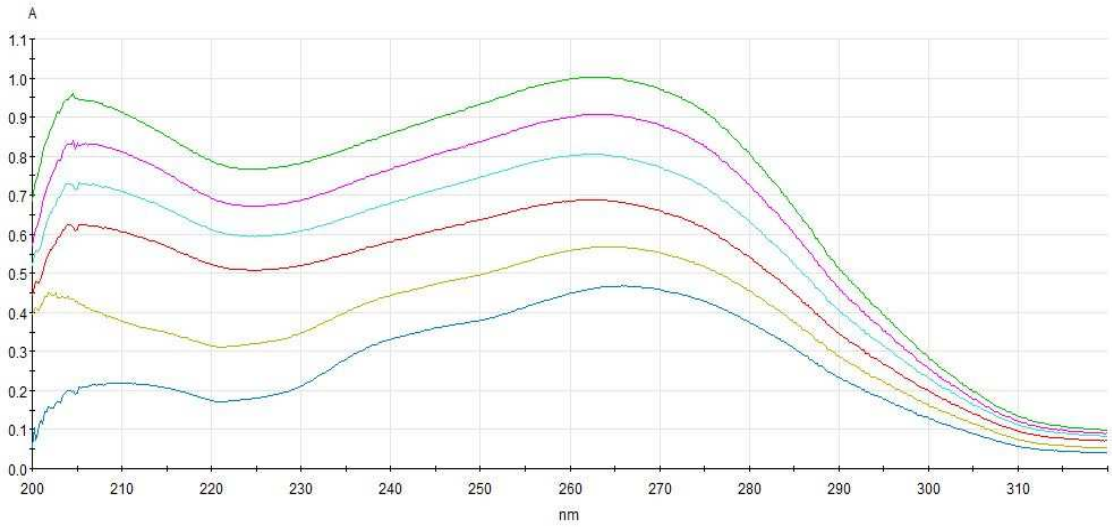

Figure.4 Overlay spectra of linearity for Nilotinib

Validation:

The method was validated for several parameters like linearity, accuracy, precision, Ruggedness, Robustness, Limit of detection(LOD), Limit of quantification(LOQ) according to ICH guidelines.

Linearity

The linearity of the analytical method was its ablity to elicit test results which are directly proportional to analyte concentration in samples within a given range. To establish the linearity of the proposed method, various aliquots of the standard solution of the drug were prepared from stock solution and analysed in triplicate. The drug showed linearity in the range of 7-12µg/mL with correlation coefficient 0.9984 shown in Table 1.

Table.1 Linearity table of Nilotinib

Concentartion (µg/mL)	Absorbance (AU)
7	0.463
8	0.567
9	0.688
10	0.804
11	0.908
12	1.001

Precision

Precision studies were carried out to check the reproducibility of the method. Repeatability was determined by preparing six replicates of same concentration of the sample and measuring absorbance. Intraday precision study was carried out by analyzing the prepared drug solutions at three different times in a day. The same procedure was followed for three different days to determine interday precision. The results were reported as %RSD. The precision result showed a good reproducibility (Table 2) with %RSD less than 2. The results of intraday and interday precision studies are shown in (Table 3 andTable 4).

Table.2 Repeatibility studies of Nilotinib

Concentartion (µg/mL)	Absorbance	Statistical Analysis
7	0.464	Mean: 0.463 SD: 0.00089 %RSD: 0.19
7	0.462	
7	0.463	
7	0.463	
7	0.464	
7	0.462	

Table.3 Intraday precision

Concentartion (µg/mL)	Absorbance 1	Absorbance 2	Absorbance 3	Average %RSD
7	0.464	0.465	0.463	
7	0.462	0.464	0.462	
7	0.463	0.464	0.462	
7	0.463	0.465	0.462	
7	0.464	0.463	0.463	0.16
7	0.462	0.465	0.462	
Mean	0.463	0.464	0.462	
SD	0.0008	0.0008	0.0004	
%RSD	0.19	0.18	0.11	

Table.4 Interday precision

Concentartion (µg/mL)	RSD			Average %RSD
	Day1	Day2	Day3	
7	0.25	0.39	0.47	0.37

Accuracy

Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by standard addition method adding different amounts (80%,100%,120%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the % recovery was calculated. The results are shown in Table 5.

Table.5 Accuracy studies of Nilotinib

Level of addition (%)	% Recovery	Statistical Analysis		
		Mean	SD	%RSD
80	99.80			
80	99.67	99.68	0.105	0.11
80	99.59			
100	100.02			
100	99.82	99.97	0.132	0.13
100	100.07			
120	100.89			
120	100.47	100.51	0.356	0.36
120	100.18			
Overall Mean Recovery,SD and %RSD		99.72	0.940	0.94

Ruggedness

Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD Table 6.

Table.6 Ruggudness studies of Nilotinib

Analyst 1		
Concentration (µg/mL)	Absorbance	Statsitical Analysis
10	0.804	
10	0.801	
10	0.799	Mean: 0.804 SD: 0.003 %RSD: 0.46
10	0.809	
10	0.804	
10	0.807	
Analyst 2		
10	0.807	
10	0.809	
10	0.801	Mean: 0.804 SD: 0.003 %RSD: 0.43
10	0.804	
10	0.800	
10	0.804	

Robustness

Analysis was carried out using medium concentraion 10 µg/mL standard at two different wavelengths, room temperature to determine the robustness of the method and the respective absorbance was measured. The results were indicated as %RSD in **Table7**

Table.7 Robustness studies of Nilotinib

Absorbance			
S.No.	262 nm	263 nm	264 nm
1	0.803	0.804	0.801
2	0.804	0.804	0.804
3	0.802	0.805	0.803
4	0.803	0.804	0.804
5	0.801	0.805	0.803
6	0.804	0.804	0.804
Mean	0.802	0.804	0.803
Total SD	0.001		
Total %RSD	0.12		

LOQ and LOD

Limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected. Limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined by suitable precision and accuracy. LOQ and LOD were determined by the following equation LOD=3.3 σ/s, LOQ =10 σ/s Where σ is standard deviation of y intercept of calibration curve and s is slope of regression equation. The LOD and LOQ values were found to be 0.28 µg/mL and 0.85 µg/mL respectively.

Quantification study in dosage form

Contents from twenty capsules were taken, accurately weighed and powdered. Tablet powder equivalent to 200 mg of nilotinib in to a 100ml volumetric flask. Initially 25 ml of solvent 1:1 methanol and water was added and the mixture was allowed to stand for 1 hr with intermittent sonication to ensure complete solubility of the drug, and then filtered through a 0.45m membrane filter, followed by adding1:1 methanol and water to obtain a stock solution of 2000µg/ml. Transfer for 5ml of this solution to a 50 ml of volumetric flask and made upto sufficient volume with solvent to give 200µg/ml solution and measure the absorbance against blank at 263 nm. The solution was suitably diluted so as to obtain a concentration in the linearity range of the method and result of analysis is shown in Table 9**.**

Table 9. Determinations of Active Ingredients in Capsules

Sample (n=3)	Label claim	Amount Found mg/Tab.	% Label Claim*
Nilotinib Hydrochloride Capsules	150 mg	148.81±0.126	99.20

RESULTS AND DISCUSSION

The proposed method is simple, accurate, economical and convinient method for the analysis of Nilotinib hydrochloride using UV spectrophotometry. The wavelength corresponding to maximum absorbance in 1:1 methanol and water was found at 263nm. Beers law was obeyed in the concentration range of 7-12 µg/mL with correlation coefficient 0.9984. Acurracy of the method was determined by the standard addition technique followed by recovery studies, a good percent recovery of 100.05% of the drug obtained indicate that the method is accurate. The method was found to be precise as %RSD values for interday and intraday was found to be less than 2. The method was also found to be rugged and robust as the % RSD values were found to be less than 2. The limit of detection and limit of quantification of the proposed method was found to be 0.28 and 0.85 µg/mL indicating that the method developed is sensitive. The results of assay obtained were found to be in good agreement with the labeled claim, indicating the absence of interference of the excipients. Whole Validation summary is tabulated below in Table 10.

Table.10 Validation summary of the UV spectrophotometric method

S.No	Parameter	Result
1.	Absorption Maxima (nm)	263
2.	Linearity Range (µg/mL)	7-12
3.	Standard Regression Equation	y = 0.1094x - 0.3008
4.	Correlation Coefficient (R^2)	0.9984
5.	Slope	0.1094
6.	Intercept	0.3008
5.	Accuracy(% Recovery ±SD)	100.05±0.421
6.	LOD (µg/mL)	0.28
7.	LOQ (µg/mL)	0.85

CONCLUSION

The developed method can be concluded to be simple, accurate, reliable and economical. The proposed method is specific without and interference of excepients and hence can be used for the routine analysis of Nilotinib Hydrochloride in bulk and in pharmaceutical formulation.

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