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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF NILOTINIB IN BULK AND TABLET DOSAGES FORM

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ABSTRACT

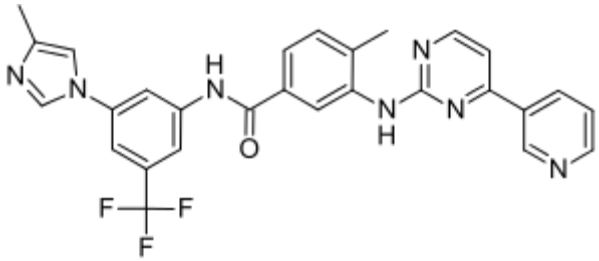
A simple and reproducible method was developed for nilotinib by Reverse Phase High Performance Liquid Chromatography (RP-HPLC). nilotinib was separated on C_{18} column [4.6x250mm, particle size 5µm] at the UV detection of 250nm. Methanol, Acetonitrile, Water (0.1%) was used as a mobile phase with various ratios and flow rates, eventually 30:20:50 v/v Methanol, Acetonitrile, Water (0.1%) was being set with the flow rate of 1.0mL/min. The statistical validation parameters such as linearity, accuracy, precision, inter-day and intra-day variation were checked, further the limit of detection and limit of quantification of nilotinib concentrations were found to be within the limits. Recovery and assay studies of nilotinib were within 99 to 102% indicating that the proposed method can be adoptable for quality control analysis of nilotinib.

KEYWORDS: Nilotinib, HPLC, Wavelength, Linearity, Assay.

INTRODUCTION

Nilotinib, 4- methyl- N –[3-) 4 Methyl- 1H imidazole- 1-yl-)- 5(trifluromethyl) phenyl]- 3-[(4- pyridine- 3-yl pyrimidin- 2-yl) amino] benzamide. It is an anti cancer drug. Nilotinib, in the form of the hydrochloride monohydrate salt, is a small molecule tyrosine kinase inhibitior approved for the treatment of imatinib resistant chronic mylogenous leukemia. Several analysis of Nilotinib such as an indirect as chromatographic, spectro-photometric survey revealed that no stability indicating RP-HPLC method is reported for determination of nilotinib in bulk drug and tablet dosage form.[1,2] The main objective of the proposed work was to develop a simple, accurate, precise and sensitive RP-HPLC method for the estimation

of nilotinib in bulk drug and tablet. The method was further optimized and validated in accordance with guidelines suggested by International Conference on Harmonization (ICH).[3]



NILOTINIB

MATERIAL AND METHODS

Quantitative HPLC was performed on a high performance liquid chromatography-Younglin HPLC system connected with PDA Detector 2998 and Empower 2 Software. The drug analysis data were acquired and processed using Empower 2 software running under Windows XP on a Pentium PC and Thermohypersil BDS C_{18} column of dimension [4.6x250mm, particle size 5µm]. In addition an analytical balance (DENVER 0.1mg sensitivity), Digital pH meter (Equiptronics pH 5-10), a sonicator (Unichrome associates UCA 701) were used in this study.

Standards and chemicals used

Pharmaceutical grade nilotinib was kindly supplied as a gift sample by Ujwal pharmaceuticals, Pune. Methanol and Acetonitrile were of HPLC grade and Purchased from Jinendra Scientifics, Jalgaon. Water HPLC grade was obtained from a Milli-QRO water purification system. Nilotinib tablets available in the market as Tasigna tablet of Million Health Pharmaceutical, Chennai, India.

Preparation of mobile phase

The combination of mobile phase is Methanol: Acetonitrile: Water (30:20:50) v/v and filtered through 0.45µ membrane filter and degassed by sonication.

Preparation of standard solution

Weigh accurately 10 mg of nilotinib and taken in a 100 ml standard flask. The volume was made up to 10 ml with the mobile phase and sonicated for 5 min. It consist of the 100

µgm/ml of nilotinib and filtered through 0.45µm membrane filter. Then the dilutions are made.

Preparation of stock solution

Weigh accurately 12.87 mg of nilotinib and taken in a 100 ml standard flask. The volume was made up to 10 ml with the mobile phase and sonicated for 10 min. It consists of 1000 µg/ml of Nilotinib and filtered through 0.45 µm membrane filter.

Quantification of nilotinib

Twenty tablets were finely powdered and an accurately weighed sample of powdered tablets equivalent to nilotinib (400 mg) were transferred to a 100 ml volumetric flask and dissolved in Mobile Phase. The solution was shaken well and allowed to stand for 10 min with intermittent sonication to ensure complete solubility of drug. The contents were made up to the mark with Mobile Phase and filtered through a 0.45µ membrane filter. From the filtrate, dilution was made in a 100 ml volumetric flask to get 12.87 µgm of nilotinib. The peak area measurements were done by injecting the sample for three times and the amount of nilotinib was calculated.

Chromatographic condition

For chromatographic analysis

Thermo Hypersil BDS C_{18} column [4.6x250mm, particle size 5µm] was used. The solvent system was a mixture of Methanol: Acetonitrile : Water (30:20:50) v/v. It was filtered under vacuum from 0.45 membrane filter and degassed in ultrasonic bath for 15 min before passing through the instrument. The flow rate was 1.0 ml/min. UV detection was carried out at 250nm.

Method Validation

Linearity

To establish linearity, the stock solutions were prepared (1000 µg/ml) of nilotinib using mobile phase as the solvent, again from the stock solution further dilutions were made to yield solutions in the concentration range of 1000 µg/ml of nilotinib. 1.0 ml of each solution was injected and records the chromatogram at 250 nm. The procedure was repeated for three times. The correlation coefficient was found to be 0.999.

Table no. 1: System Suitability Parameters.

Sr. NO.	Parameters	Values
1	Retention Time	4.633 min
2	Area %	100.00%
3	Theoretical Plate	5678.3
4	Tailing Factor	1.3500

Precision

Precision study of sample Nilotinib was carried out by estimating corresponding responses 6 times on the day for the 100% target conc. The percent relative standerd deviation (%RSD) is calculated which is within the acceptable criteria of not more then 2.0 in table 3.

Repeatability

Repeatability is the closeness of agreement between mutually independent test result obtain with the same method on identical test material on the same laboratory by the same operator using the same equipment within short interval of time in table 4.

Accuracy

The accuracy is the closeness of agreement between the true value and test result. Accuracy was determine by means of recovery experiments, by addition of active drug to placebo formations. It was calculated from the test result as the percentage of the analyte recorved by the assay.

Robustness

The robustness is evaluated by the analysis of Nilotinib under different experimental condition such as making small change in flow rate, λ max & mobile phase conc.

Ruggedness

The ruggedness was calculated by using two different analysts. An appropriate conc. 30µg/ml of nilotinib was analyzed and conc. was determine.

LOD & LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 0.0718 ∂/S and 0.2176∂/S, respectively as per ICH guidelines, where ∂ is the standared deviation of the response (y-intercept) and S is the slope of the calibration plot.

RESULT AND DISCUSSION

Linearity

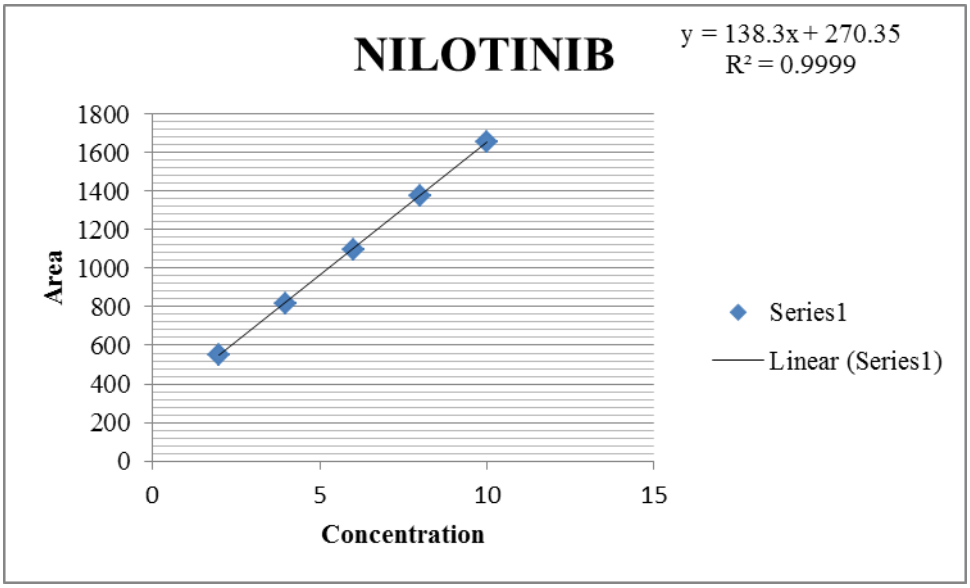


Fig no. 1: Linearity graph of Nilotinib.

Table no. 2: Linearity of Nilotinib.

Sr No.	Conc	Area-I	Area-II	Mean	SD	% RSD
1	2	552.46	550.69	551.575	1.25	0.23
2	4	817.35	821.94	819.645	3.25	0.40
3	6	1098.86	1103.56	1101.21	3.32	0.30
4	8	1375.83	1380.9	1378.365	3.59	0.26
5	10	1656.21	1661.34	1658.775	3.63	0.22

Precision

The precision of an analytical method is the closeness of replicate result obtained from Analysis of the same homogeneous sample. To study precision, three replicate standard Solution of Nilotinib (150 µgm/ml) were prepared and analyzed using the proposed Method. The percent relative standard deviation (%RSD) for peak responses was Calculated and it was found to be which is well within the acceptance criteria of not more than 2.0%. Result of system precision studies are shown in table no.3.

Table no. 3: Precision of Nilotinib.

Sr No.	Conc.	% RSD	
		INTRADAY	INTERDAY
1	2	0.64	1.19
2	6	0.61	0.62
3	10	0.23	0.56

Repeatability

Repeatability was ascertained by getting the sample analyzed by different analyst and Carrying out analysis for number of times. The results are shown in table no.4.

Table no. 4: Repeatability of Nilotinib.

Sr. No	Conc.	Area	I	II	Mean	AMT Found	% Amt Found	SD	%RSD
1	5	1368.12	1381.74	1370.82	1373.56	7.97	99.63	7.21	0.53

Accuracy

Accuracy of the method was tested by carrying out recovery studies at different Spiked levels. The estimation was carried out as describe earlier. At each level, Three determination were performed and result obtained. The amount recovered and the values of percent were calculated, result are shown in table no.5.

Table no. 5: Accuracy of Nilotinib.

80%						
Mg/Band	Amt added	Area		Amt found	Amt Rec	% Rec
5	4	1269.95	Mean	7.22	3.22	100.49
5	4	1265.92	SD	0.03	0.03	0.71
5	4	1273.52	% RSD	0.42	0.93	0.70
100%						
5	5	1385.59	Mean	8.03	20.50	100.67
5	5	1377.85	SD	0.03	0.03	0.76
5	5	1380.11	% RSD	0.38	0.15	0.76
120%						
5	6	1501.6	Mean	8.85	4.85	99.08
5	6	1495.32	S D	0.04	0.04	0.73
5	6	1490.84	% RSD	0.40	0.72	0.74

Ruggedness

The ruggedness was calculated by using two different analysts. An appropriate concentration 30 µg/ml of nilotinib was analyzed and concentration was determined.

Table no. 6: Ruggedness of Nilotinib.

Sr No.	Conc	Area	II	III	Mean	Amt Found	% Amt. Found	SD	% RSD
Analyst1	8	1371.54	1368.92	1378.23	1372.90	7.97	99.63	4.80	0.35
Analyst2	8	1380.04	1368.88	1374.25	1374.72	7.98	99.75	5.10	0.37

Robustness

The robustness of the method was assessed by altering the some experimental conditions such as, by changing the flow rate from 0.6 to 1.0 ml/min, wavelength and volume of the mobile phase.

Table No. 7: Robustness of nilotinib.

Parameters	Nilotinib	
Flow rate(ml/min)	0.98	1.02
% RSD	0.17	0.29
Wavelength	249	251
%RSD	0.70	0.61
Mobile Phase	19+19+52	31+21+48
%RSD	0.46	0.38

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 0.302 ∂/S and 1.009∂/S, respectively as per ICH guidelines, where ∂ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot in table no 8 as follows.

Table No. 8: LOD and LOQ of Nilotinib.

Parameters	Measured Value
LOD	0.0718
LOQ	0.2176

Assay for Formulation of Nilotinib

The validated method was applied for the determination of nilotinib in commercially available Tasigna tablets. The results of the assay (n=6) undertaken yielded 99.13% (%RSD = 0.37%) of label claim nilotinib. The mean retention time of nilotinib was 4.633 min. The results of the assay indicate that the method is selective for the analysis of nilotinib without interference from the excipients used to formulate and produce these tablets.

Table no 9: Assay for Formulation.

Drugs	Labelled amount % Lable claim	Amount taken(mg)	Amount found for assay (µg/mL) (mg)	%
Nilotinib	200	10	9.86+9.97	99.20

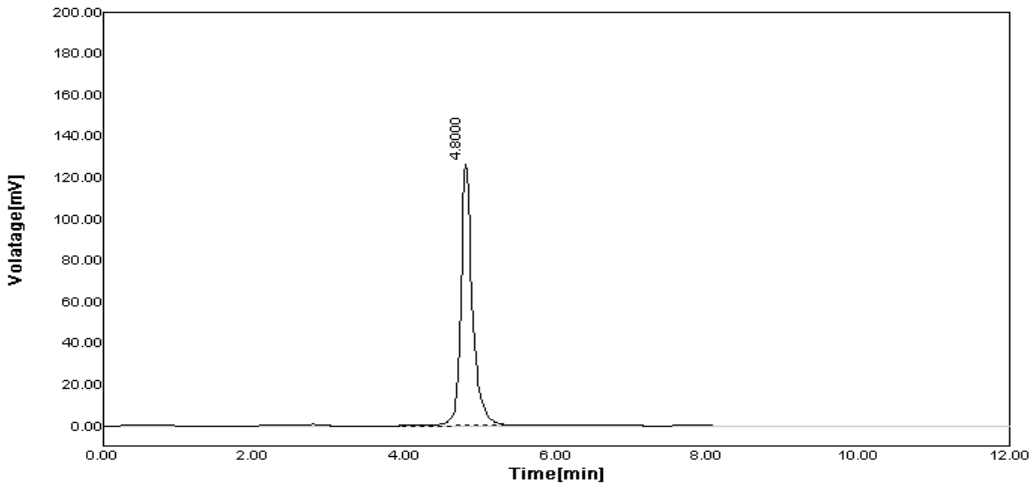


Fig No. 2: Optimized Chromatogam of Niotinib.

CONCLUSION

A validated RP-HPLC method was developed for the determination of Nilotinib. In the bulk and pharmaceutical dosage form. The method was validated for Linearity, precision, accuracy, ruggedness, robustness, LOD, LOQ, Repeatability The proposed RP-HPLC method can be successfully applied for the routine Control analysis of Nilotinib along with their dosage form.

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The authors would like to thanks our principal, beloved parents and all my well wishers, one and all who have helped me directly and indirectly in completing this project work.

LIST OF ABBREVATIONS

SR.NO	ABBREVATIONS
1	RP-HPLC- Reverse phase high performance liquid chromatography
2	HPLC- High Performance liquid chromatography
3	UV- Ultra violet
4	PDA- Photodiode Array
5	LOD- Limit of detection
6	LOQ- Limit of quantitation
7	RSD- Relative standard deviation
8	SD- Standard deviation
9	UCA- Unichrome associates
10	AMT FND- Amount found
11	AMT REC- Amount received
12	BDS- Base deactivated silica
13	ICH- International conference on Harmonization

LIST OF TABLES

TABLE NO	NAMES
1	System Suitability Parameter
2	Linearity of Nilotinib
3	Precision of Nilotinib
4	Repetability of Nilotinib
5	Accuracy of Nilotinib
6	Ruggedness of Nilotinib
7	Robustness of Nilotinib
8	LOD and LOQ
9	Assay for Formulation

LIST OF FIGURES

FIGURES	NAMES
Figure 1	Structure
Figure 2	Linearity Graph of Nilotinib
Figure 3	Optimize chromatogram of nilotinib

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