

Original Article

DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF NILOTINIB HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: To develop a rapid, accurate, linear, sensitive and stability indicating RP-HPLC method for the determination of nilotinib in bulk and pharmaceutical dosage forms in the presence of its four related substances.

Methods: The RP-HPLC method was developed for the chromatographic separation of nilotinib and its impurities by using waters Xterra RP-18 (150*4.6 mm, 3.5 µm) column with a mobile phase combination of 10 mM ammonium formate with pH-3.5 and acetonitrile in gradient mode. An injection volume of 20 µl. Flow rate was 1.0 ml/min and detection was carried a wavelength of 250 nm. The method was validated as per ICH guidelines.

Results: The retention time for nilotinib and its four impurities were found to be 4.37, 7.40, 8.96, 10.21 and 10.87 min respectively. The linear regression analysis data for the calibration plots showed the good linear relationship in the concentration range of 0.04-3.0 ppm for the nilotinib impurities. The % recovery of nilotinib impurities was found to be 96.8-99.4% in the linearity range. The detection limit (LOD) values were about 0.014, 0.016, 0.005 and 0.03 ppm respectively and the quantification limit (LOQ) values were 0.042, 0.048, 0.014 and 0.09 ppm respectively. The % degradation at various stress conditions like acid, alkaline, oxidative, thermal and photolytic stress was found to be 8.92, 18.35, 5.63, 0.88 and 3.89 respectively.

Conclusion: The RP-HPLC method compatible with LC-MS was developed for the analysis of nilotinib and its four impurities. It was validated as per the ICH guidelines and found to be linear, robust, precise, accurate, sensitive, stability indicating and can be used for routine as well as stability analysis of capsule dosage forms as well as for drug substance.

Keywords: RP-HPLC, Nilotinib hydrochloride, Method development and validation

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INTRODUCTION

Nilotinib is a synthetic amino pyrimidine, a second generation tyrosine kinase inhibitor used for the treatment of imatinib-resistant chronic myelogenous leukemia in the form of nilotinib hydrochloride monohydrate salt. Nilotinib has around 20 times higher affinity over imatinib and has an *in vitro* activity against many imatinib-resistant mutants [1, 2]. It is used to treat people who have tested positive for Philadelphia chromosome, a common genetic abnormality found in people having chronic myeloid leukemia (CML). CML is also known as chronic granulocytic leukemia (CGL). It is a cancer of white blood cells that occurs due to the increased and unregulated growth of myeloid cells in bone marrow and their accumulation in the blood. CML is a clonal bone marrow stem cell disorder in which a proliferation of mature granulocytes and their precursors is found. CML is now largely treated and targeted with drugs like tyrosine kinase inhibitors like imatinib, nilotinib, and dasatinib that led to improved long-term survival rates since the introduction of first such agent.

The presence of impurities can have a significant impact on the product quality, safety and efficacy, hence the percentage level of impurities need to control in the drug substance as well as a drug product. An extensive literature survey reveals that there were few analytical methods available for the determination of nilotinib in plasma, biological fluids, bulk and in pharmaceutical dosage forms by liquid chromatography and liquid chromatography mass spectrometry (LC-MS) techniques [3-12]. The objective of the current work is to develop a simple, precise, accurate, robust and stability indicating LC-MS compatible HPLC method for the determination of nilotinib hydrochloride and its related impurities in bulk and pharmaceutical dosage forms, followed by method validation as per current International conference on harmonization (ICH) guidelines.

MATERIALS AND METHODS

Chemicals and reagents

Nilotinib hydrochloride drug substance was gifted by the synthetic division of Hetero laboratories Ltd, Hyderabad, India. Acetonitrile, methanol and ethanol of HPLC grade (Rankem chemicals, India). Ammonium formate, sodium hydroxide, hydrochloric acid and hydrogen peroxide (Merck chemicals, Darmstadt, Germany). HPLC grade water was obtained from milli-Q water purification system (Millipore, Milford, USA).

Instrument

The HPLC system used for the chromatographic method development, forced degradation and validation was Agilent-1260 quaternary pump separation module with a PDA detector. HPLC system consisted of quaternary pump G1311C and photodiode array detector G4212B. The signal output was monitored and processed using EZ chrome Elite software on a Lenovo computer. Chromatographic separation was achieved on waters Xterra RP-18 150*4.6 mm, with a particle size of 3.5 µm was used. pH of the mobile phase was adjusted on a microprocessor waterproof pH tester (pH tester 20, Eutech instruments, Oakton, USA). Thermal degradation study was carried out in a dry, hot air oven (Ultra Biotech, Bangalore, India) and photolytic degradation is carried out on photostability chamber. (Atlas suntester CPS+, Illinois, USA).

Physical properties

The chemical name of nilotinib hydrochloride monohydrate is 4-methyl-N-[3-[(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-3-[(4-pyridin-3-yl)pyrimidin-2-yl] amino] benzamide hydrochloride monohydrate fig. 1 with molecular formula

$C_{28}H_{22}F_3N_7O \cdot HCl \cdot H_2O$. It is white to slightly yellowish crystalline powder with melting range of 288-290 °C. The molecular mass of nilotinib is 529.52 while 583.99 for nilotinib hydrochloride monohydrate.

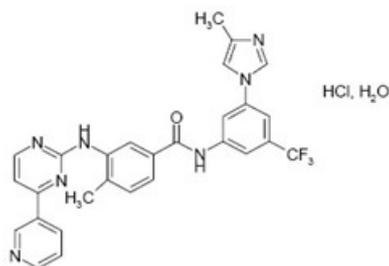


Fig. 1: Structure of nilotinib hydrochloride monohydrate

pKa and solubility

Nilotinib is a typical Biopharmaceutical classification system (BCS) a class-IV compound having poor solubility and poor permeability. Nilotinib solubility decreases with increase in aqueous pH indicating

pH dependent solubility. Nilotinib is practically insoluble in buffer solutions with pH-4.5 and higher pH values. It is slightly soluble at pH-1.0. The solubility was determined at 37 °C by using UV-visible spectrophotometer at 250 nm wavelength. UV spectrum of nilotinib in pH-2.0 buffer was plotted below in fig. 2. It clearly indicates nilotinib was having a maximum absorbance in the range of 250-270 nm. The solubility of nilotinib in water is around 0.024 mg/liter at 25 °C. Nilotinib is sparingly soluble in ethanol and methanol. pK_{a1} of nilotinib is 2.1 and pK_{a2} is 5.4.

Methods

Chromatographic conditions

The aim of the study was to develop a single and simple LC-MS compatible stability indicating HPLC method for the separation of related substances with possible lowering of retention time at a column temperature of 40 °C. Separation was achieved using gradient elution mode with 10 mM ammonium formate with pH-3.5 and acetonitrile as mobile phase with a run time of 14 min. The retention time of nilotinib was found to be around 8.0 min. Filtered the mobile phase through 0.22 μ m nylon membrane filter and degassed. Injection volume was 20 μ l with a mobile phase flow rate of 1.0 ml/min. The detection wavelength was 250 nm. Buffer: acetonitrile in the ratio 50:50 V/V was used as diluent.

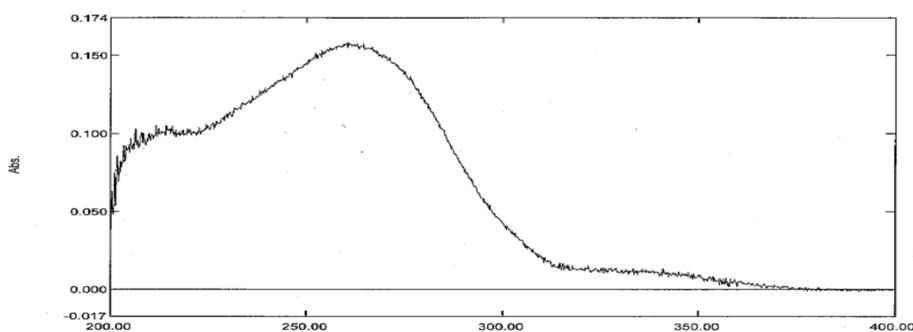


Fig. 2: UV Spectrum of nilotinib hydrochloride

LC-MS conditions

The LC-MS system used for the identification of related substances of nilotinib formed during the forced degradation study was agilent system equipped with mass lynx software. Waters xterra RP-18, 150*4.6 mm, 3.5 μ m HPLC column was used as stationary phase with a mobile phase containing a gradient of 10 mM ammonium formate (0.63 g of ammonium formate) in 1000 ml of milli q water and acetonitrile in the ratio 90:10 % V/V as solvent A and pH-3.5 buffer: acetonitrile in the ratio 10:90 % V/V as solvent B. The flow rate was 1.0 ml/min with a gradient programme of 0.0/10, 2.0/10, 8.0/80, 10.0/80, 12.0/10 and 14.0/10 (time in min/% B). The injection volume was 10 μ l. The analysis was performed on positive and negative modes of electron spray ionization modes with a mass scan range of 50-1000 m/z capillary voltage is 3700V, end plate offset-500V, charging voltage 2000V, the corona current as 10 nA. Nebulizer pressure as 0.3 bar. Source temperature and desolvation temperatures were 180 and 360 °C respectively. Cone gas flow was 4.0 L/min and desolvation gas flow was 10.0 L/min.

Buffer preparation

Dissolved accurately 0.63 g of ammonium formate in 1000 ml of milli-q water and mixed well to prepare 0.010 M solution of the solution, further adjusted the pH to 3.5 with formic acid. The buffer solution was filtered through 0.22 μ m membrane filter.

Standard preparation

A working standard solution stock of nilotinib hydrochloride was prepared by dissolving 50 mg of the standard into 100 ml volumetric flask, to this added 30 ml of diluent and sonicated for 10 min. Allowed the solution to attain room temperature and diluted to

the volume with diluent to have a solution with a concentration of 500 ppm.

Diluted standard preparation

Diluted 2 ml of the standard solution to 50 ml with diluent and mixed well. Further diluted 2 ml of the resulting solution to 100 ml with diluent and mixed well to a concentration of 0.4 ppm.

Sample preparation

Weighed 20 capsules and determined the average weight of the capsule. The capsules were emptied carefully and collected the content of 20 capsules. Transferred sample equivalent to 100 mg of nilotinib into 50 ml volumetric flask added 30 ml of diluent and sonicated for 10 min with intermediate shaking in sonicator at a temperature below 25 °C. The solution was cooled to room temperature and diluted with diluent to a concentration of 1000 ppm. The solution was filtered through 0.22 μ m nylon membrane filter.

Validation of the developed method

The optimized analytical method was validated according to the International conference on harmonization (ICH) guidelines for analytical procedures Q2 (R1) [13, 14].

System suitability

According to the United States pharmacopeia (USP), system suitability test should be performed prior to analysis. System suitability test was performed to ensure the system performance before initiating the experiment, by monitoring the parameters like % RSD, theoretical plates, resolution and asymmetry.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities [15].

Linearity

The linearity of the detector response was established for all the known impurities with concentration ranging from LOQ to 150 % of the specification level (0.10 %) with respect to test concentration. The samples were analyzed as per the described test method. A linearity graph was plotted between the area of impurity (Y-axis) versus actual concentration in ppm (X-axis) and determined the coefficient of correlation and Y-intercept at 100 % response.

Precision

The precision of the analytical method is the closeness agreement for a series of measurement from multiple samplings as mentioned in ICH Q2 (R1). As per the guidelines, method precision and intermediate precision were analyzed on the homogeneous sample and the % RSD of individual impurity for precision and intermediate precision was calculated and reported.

Accuracy

The accuracy of the analytical method is the closeness of agreement between the true value and experimental value. The accuracy of the four impurities was performed at levels ranging from LOQ to 150 % of the specification level of the impurity with respect to test concentration level. The % recovery was calculated by comparing the impurity level at each level of the spiked sample with as such sample.

LOD and LOQ

The detection limit (LOD) and quantification limit (LOQ) for all the four impurities were established by means of linearity method. The impurity solutions from concentration ranging from 0.01 % to 0.10 % with 5 different levels in duplicate were prepared and injected. Based on the impurity response and STEYX value, the least concentration of each impurity up to which it can be identified and quantified was calculated.

Robustness

The robustness of the method was evaluated to establish the capability of the method by changing the experimental conditions and studying its impact on the system suitability. Robustness was performed by changing the method parameters like mobile phase flow rate and column temperature.

RESULTS

Method development and optimization

As there was no stability indicating HPLC method available for the separation of nilotinib from its related substances, the objective of the method was to separate the analyte peak from the potential impurities arise during the forced degradation study. There was no LC-MS compatible HPLC method available for the identification of degradants during stability period and storage conditions. For the optimization of the HPLC method, forced degradation sample was taken as reference. There was spectral co-elution in the case of these impurities on different stationary phases like C8, C18 and phenyl with different buffer ratios and organic modifiers like methanol and acetonitrile. Nilotinib is having better solubility in the range of pH-2.0 to 4.0, hence buffers with varying concentrations were chosen in this pH range. Initial trials were taken on pH-2.4 phosphate buffer with acetonitrile as mobile phase and test concentration of 1000 ppm in mobile phase was injected in which there was no clear separation between the impurities and nilotinib. Further trials were taken by varying the pH value of the mobile phase buffer from 2.0 to 3.5. i.e., ammonium formate buffer was selected as a mobile phase buffer as it had the maximum buffering capacity at its pKa (pKa of ammonium formate is 3.5). Forced degradation sample was injected and was found that all the four impurities were spectrally pure with longer run time and broader peak shapes. In order to shorten the run time gradient separation mode was optimized with satisfactory separation. Optimal separation was achieved on waters xterra RP-18 150*4.6 mm, 3.5 μ m HPLC column maintained at 40 °C. Gradient elution was performed using the mixture of 10 mM ammonium formate buffer pH-3.5 (pH was adjusted with formic acid) and acetonitrile as mobile phase at a flow rate of 1.0 ml/min and UV detection at 250 nm. Fig. 3 shows separation of all the four impurities from nilotinib hydrochloride in the proposed method.

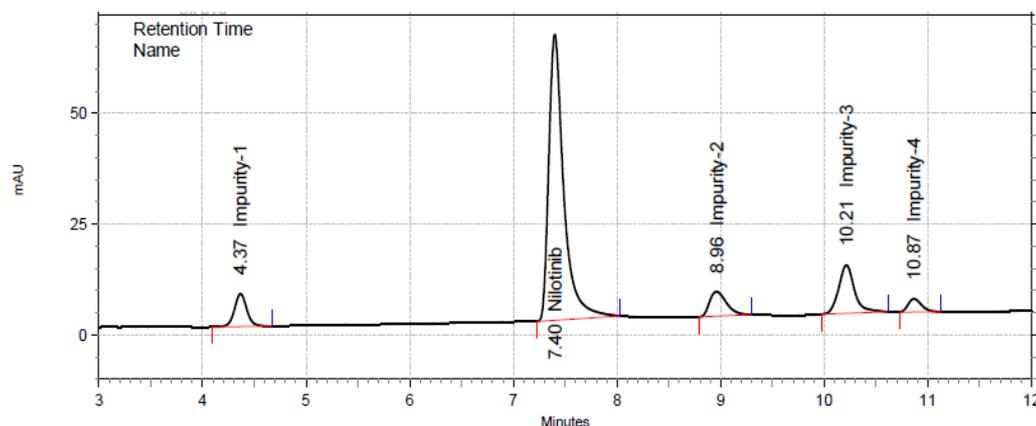


Fig. 3: A typical chromatogram showing separation of the four impurities along with nilotinib hydrochloride

Method validation

System suitability

System suitability was verified by replicate analysis of 6 injections of nilotinib diluted standard solution and the chromatogram obtained. The system suitability parameters such as asymmetry, theoretical plate count and reproducibility (% RSD) of analyte retention time (Rt) and area of the six replicates were calculated from the chromatogram. System suitability parameters were mentioned in table 1.

Specificity

The specificity of the method was evaluated for nilotinib hydrochloride in the presence of its blank, placebo, known impurities and other degradation products. Forced degradation studies were performed on nilotinib drug substance and drug product to verify the stability indicating property and specificity of the proposed method. Stress studies were performed under conditions of acidic, alkaline, hydrolytic, peroxide, thermal and photolytic as mentioned in ICH Q1A (R2). The forced degradation study was initiated based on the approach suggested by Blessy M

and Ruchi DP [16]. Four samples (Blank, drug substance, drug product and placebo) were generated for every stress condition and were compared against the unstress (as such) solutions for the net amount of degradation. Acidic degradation of nilotinib was conducted at 60 °C in 0.1 N HCl, alkaline degradation at 0.1 N NaOH at 60 °C for 4 h, water at 80 °C, peroxide degradation with 3 % H₂O₂ at room temperature for 1 hr at a drug concentration of 1000 ppm until sufficient degradation of the drug (~ 20 % of the initial amount) was achieved. Thermal stress was performed at 105 °C for drug substance, drug product, and placebo till 48 h. Photolytic stress

was performed by exposing the sample to ultraviolet radiation of an overall energy of 200-watt hours/m² and visible radiation of 1.2 million lux hours [17]. The peak purity of the stressed sample was evaluated by EZ chrome software such that the peak purity should be greater than 0.99, demonstrating the homogeneity of the peak. The forced degradation samples were analyzed on LC-MS to obtain the molecular masses of the potential degradant and the structure was further confirmed by analyzing the isolated sample by NMR. The impurities structures in fig.4, along with the mass numbers were tabulated below along with relative retention time (RRT) in table 2.

Table 1: System suitability report

Injection	Rt* (Min)	Area	Asymmetry	Theoretical plates
1	7.41	421531	1.12	7854
2	7.41	420145	1.15	7844
3	7.40	421935	1.18	7801
4	7.40	420889	1.11	7832
5	7.42	421008	1.13	7862
6	7.41	420045	1.11	7870
Average	7.41	420925.5	1.13	7844
SD	0.01	745.43	0.03	24.87
% RSD	0.10	0.18	2.41	0.32

*Rt-Retention time

Table 2: Impurities name, RRT and m/z value

S. No.	Name	Rt *(min)	RRT#	m/z value
1	Nilotinib(imp-I)	4.37	0.58	530.19
2	Nilotinib HCl	7.40	1.00	564.16
3	Acid impurity (imp-II)	8.96	1.21	307.13
4	Amide impurity (imp-III)	10.21	1.39	305.13
5	N-Oxide (imp-IV)	10.87	1.47	544.22

*Rt-Retention time; # RRT-Relative retention time

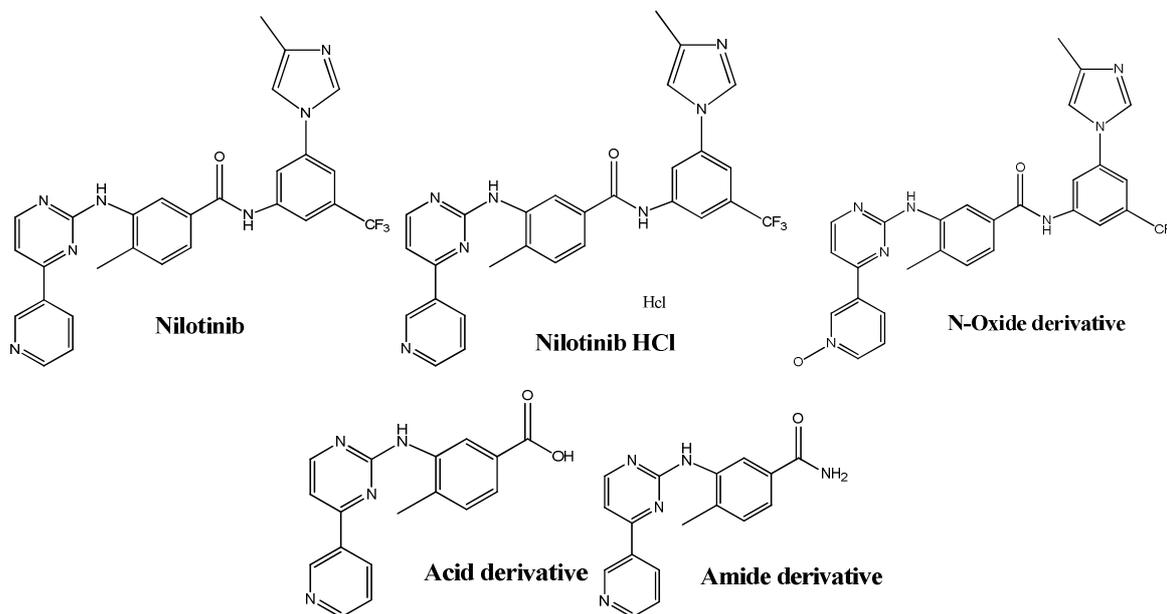


Fig. 4: Structures of nilotinib hydrochloride and its impurities

Precision

Method precision and intermediate precision analysis were performed on the homogeneous sample. The % RSD of individual impurity for precision and intermediate precision should be not more than 15.0 and was observed for the 12 samples around 4.0 % for each individual impurity.

Method precision

Six different test samples were prepared as per the test method by spiking nilotinib hydrochloride impurity-I, impurity-II, impurity-III and impurity-IV at 0.10 % level with respect to test concentration. The % impurity level for the six samples was calculated by the diluted standard method. The % impurity in the unspiked sample

was subtracted from spiked sample and reported the % impurity for each sample.

Intermediate precision

Six different samples were prepared by spiking the impurity at 0.10 % level with respect to test concentration and analyzed as per the

method on a different day, different HPLC system and different HPLC column of the same make or equivalent. The % impurity levels for the six samples were calculated by the diluted standard method and reported the % of each impurity and the cumulative % RSD of the twelve samples (method precision+intermediate precision) were calculated and the compilation was tabulated in table 3.

Table 3: Method precision and intermediate precision values of nilotinib impurities

S. No.	Sample ID	% Imp-1	% Imp-2	% Imp-3	% Imp-4	
1	un spiked sample	0.02	0.02	0.03	0.01	
2	Method precision	spiked sample-1	0.11	0.11	0.13	0.11
3		spiked sample-2	0.11	0.11	0.13	0.11
4		spiked sample-3	0.11	0.10	0.12	0.11
5		spiked sample-4	0.11	0.11	0.13	0.12
6		spiked sample-5	0.10	0.10	0.12	0.12
7		spiked sample-6	0.11	0.10	0.13	0.11
8	Intermediate precision	spiked sample-7	0.11	0.11	0.12	0.11
9		spiked sample-8	0.10	0.10	0.13	0.11
10		spiked sample-9	0.11	0.10	0.13	0.11
11		spiked sample-10	0.11	0.11	0.12	0.11
12		spiked sample-11	0.11	0.10	0.12	0.11
13		spiked sample-12	0.10	0.10	0.13	0.12
Average		0.11	0.10	0.13	0.11	
SD		0.00	0.01	0.01	0.00	
% RSD		4.2	4.9	4.1	4.0	

LOD and LOQ

The detection limit and quantification limit for all the four impurities were evaluated by linearity method. The diluted solutions of the impurity stock from the concentration of 0.01 % to 0.10 % with 5 different levels in duplicate. A graph was plotted between

concentration and area for all the impurities individually. STEYX, slope values, detection limit (LOD=3.3*STEYX/Slope) and quantification limit (LOQ=10*STEYX/Slope) for all the impurities were calculated and tabulated in table 4. Precision at LOQ level for all the impurities was verified by injecting the impurity mixture and the % RSD was found to be less than 10.0 and tabulated in table 5.

Table 4: Detection limit (LOD) and quantification limit (LOQ) establishment

S. No.	Level	Impurity-1		Impurity-2		Impurity-3		Impurity-4	
		Conc (ppm)	Area						
1	0.01	0.2	12450	0.2	17724	0.2	16243	0.2	11398
2	0.03	0.6	36728	0.6	51931	0.6	48242	0.6	33852
3	0.05	1.0	61628	1.0	87911	1.0	80565	1.0	55850
4	0.08	1.6	99102	1.6	140374	1.6	128969	1.6	90842
5	0.10	2.0	124002	2.0	175645	2.0	161293	2.0	113866
Slope		62076.8		87890.4		80619.6		56977.1	
STEYX		260.0		423.6		111.3		514.7	
LOD (PPM)		0.014		0.016		0.005		0.030	
LOQ (PPM)		0.042		0.048		0.014		0.090	

Table 5: Precision at LOQ level

S. No.	Impurity-1	Impurity-2	Impurity-3	Impurity-4
	0.042	0.048	0.014	0.09
1	2468	4395	3249	2280
2	2389	4320	3141	2276
3	2415	4368	3226	2265
4	2436	4356	3230	2259
5	2460	4316	3256	2284
6	2452	4309	3262	2276
Average Area	2436.67	4344.00	3227.33	2273.33
SD	30.01	34.37	44.62	9.46
% RSD	1.23	0.79	1.38	0.42

Accuracy

The accuracy of the analytical method is the closeness of agreement between the true value and experimental value. The accuracy of the impurities was performed at levels ranging from LOQ to 150 % of the specification level of the impurity with respect to test

concentration level. These accuracy samples were prepared by spiking the sample with impurity mixture at each level on the sample and % recovery was calculated. Accuracy was performed at 5 different levels in triplicate (6 preparations for the highest level and lowest level) and the % recovery was tabulated below in table 6.

Table 6: Accuracy of impurities

S. No.	Level (%)	Impurity-1		Impurity-2		Impurity-3		Impurity-4	
		Conc (ppm)	Avg % Recovery						
1	LOQ#	0.04	96.8	0.05	96.9	0.01	97.1	0.09	97.0
2	25	0.5	97.2	0.5	97.1	0.5	97.8	0.5	97.9
3	50	1.0	98.0	1.0	97.8	1.0	98.3	1.0	98.1
4	100	2.0	98.7	2.0	98.4	2.0	98.9	2.0	98.6
5	150#	3.0	99.4	3.0	99.1	3.0	99.2	3.0	99.3

#-Indicates six preparations; whereas other samples were prepared in triplicate

Linearity and range

The linearity of the detector response was established for all the known impurities with concentration ranging from LOQ to 150 % of the specification level (0.10 %) with respect to test concentration. The samples were analyzed as per the test

method. A linearity graph was plotted between the areas of impurity (Y-axis) versus concentration in ppm (X-axis) and determined the coefficient of correlation and Y-intercept at 100 % response. The correlation coefficient, slope, residual sum of squares and Y-intercept for all the impurities were tabulated in table 7.

Table 7: Linearity of impurities

S. No.	Impurity-1		Impurity-2		Impurity-3		Impurity-4	
	Conc (ppm)	Area						
1	0.04	2472	0.05	4395	0.04	3236	0.04	2262
2	0.1	6101	0.1	8175	0.1	8025	0.1	5610
3	0.5	30197	0.5	42192	0.5	39805	0.5	27993
4	1.0	61628	1.0	87901	1.0	80092	1.0	83138
5	2.0	124002	2.0	174923	2.0	156981	2.0	163783
6	3.0	178720	3.0	259308	3.0	238675	3.0	246089
Correlation coefficient		1.000	1.000		1.000		0.999	
Slope		60117	86826		79278		83681	
Y-Intercept		638	-83		42		-4489	
Residual sum of Squares		0.999	1.000		1.000		0.998	

Robustness

The robustness of the method was evaluated to establish the capability of the method by changing the experimental conditions and its impact was studied on the system suitability parameters. Flow rate was varied from 1.0 ml/min to 0.8 ml/min and 1.2 ml/min, column temperature varied to 35 °C and 45 °C from 40 °C. The system

suitability parameters were evaluated under these changed conditions and were compared with respect to the original conditions.

The system suitability results for the varied conditions didn't vary much from the actual conditions, indicates that the optimized chromatographic conditions for the HPLC method is robust. The results were compiled and tabulated in table 8.

Table 8: Robustness results for the varied and actual conditions

S. No.	System suitability parameter	Actual condition	Flow (ml/min)		Column temperature (°C)	
			0.8	1.2	35	45
1	Rt (Min)	7.41	8.23	7.15	7.52	7.16
2	Asymmetry	1.13	1.21	1.08	1.15	1.16
3	Theoretical plates	7844	6592	8564	7354	7852
4	% RSD of Area	0.18	0.23	0.21	0.25	0.22

Table 9: Forced degradation study data

Experiment	Stress condition	% Area	% impurity	% Assay	% Mass balance*
1	As such	99.85	0.15	99.46	99.61
2	0.1 N HCl at 60 °C for 4 h	91.08	8.92	89.95	98.87
3	0.1 N NaOH at 60 °C for 4 h	81.65	18.35	79.12	97.47
4	3 % H2O2 at 25 °C for 1 hr	94.37	5.63	91.86	97.49
5	105 °C for 48 h	99.12	0.88	98.19	99.07
6	Photolytic stress	96.11	3.89	95.23	99.12

*% Mass balance: Sum of % Assay and % impurity

Forced degradation studies

Decomposition ratio refers to the ratio between the assay value of the degradant in a stress condition and the unstressed drug with the same concentration is generally recommended to be within the range of 5-20 %. Appropriate decomposition ratios were measured

in the acidic (8.92 %), alkaline (18.35 %), oxidative (5.63 %), thermal (0.88 %) and ultraviolet (3.89 %) conditions. nilotinib was stable to ultraviolet while sensitive to acid, alkali, heat and oxidant. The presence of the degradant did not interfere with the separation of nilotinib and the impurities. Peak purity analysis was performed with the DAD detector. Purity angles for nilotinib were within the

purity threshold limits in all degradation conditions, confirming that the nilotinib peak was homogeneous without co-elution substances. The detection wavelength of 250 nm was chosen as it was close to the maximum absorption of nilotinib hydrochloride and its related substances. The content of each impurity was evaluated by calculating the mass balance of the drug against the standard at the

same concentration to that of the sample. Mass balance (% Assay of stressed sample+% of impurity during degradation) was calculated for all the stress samples and the amount of degradation in each condition were mentioned in table 9. Fig.5 shows forced degradation study chromatograms in thermal, acid, base and peroxide conditions.

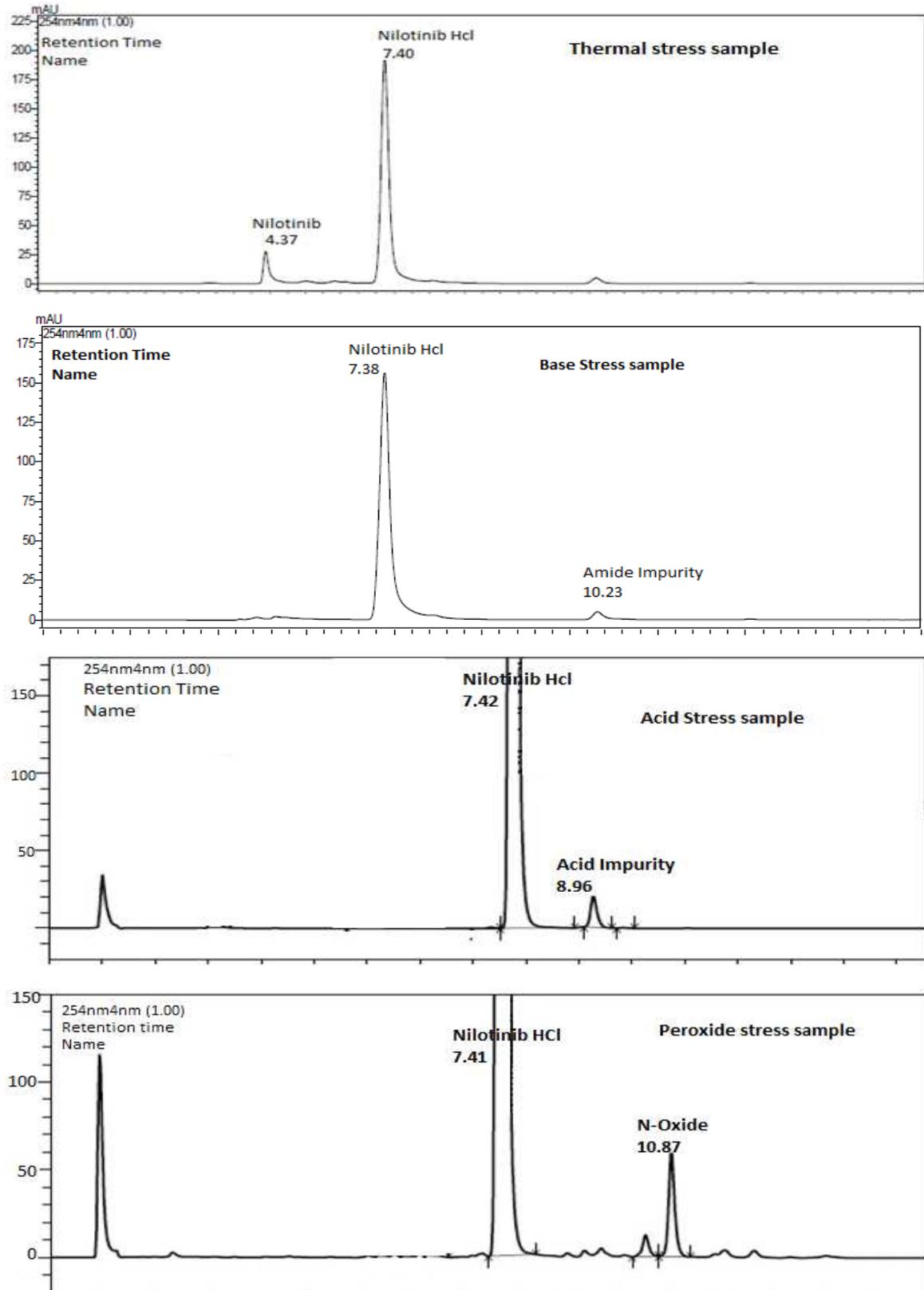


Fig. 5: Chromatograms of nilotinib hydrochloride in thermal stress, base stress, acid stress and peroxide stress samples respectively

Solution stability

The standard and sample solutions were prepared as per the test procedure and solution stability was established at room temperature and at controlled temperature (2-8 °C). These solutions were injected serially at respective time intervals and the stability of these solutions was evaluated till 24 h. The comparison of these solutions was evaluated against the freshly prepared sample and standard solution. Nilotinib is prone to photolytic degradation hence solution stability was established for light protected sample as well as unprotected sample. The mobile phase solution stability was established by injecting the standard solution after 24 h and 48 h. The standard and sample solutions were stable for 24 h when protected from light at 2-8 °C and for 4 h when stored at room temperature.

CONCLUSION

A simple and rapid stability indicating RP-HPLC method was developed for the determination of nilotinib in the presence of its degradation products. In this method, nilotinib was exposed to various stress conditions as stated in ICH guidelines and the results of stress degradation studies show that nilotinib is labile to acid, base, peroxide, thermal and photolytic conditions. Validation of the HPLC method as per the ICH guidelines demonstrates that the method is specific, linear, precise, accurate, stability indicating and robust. The developed HPLC method is LC-MS compatible for the identification of mass of the degradants if any generated during the stability and process optimization. The method is also cost effective with respect to solvent consumption. Thus the proposed method can be used to determine the impurities in bulk drug and finished formulations in quality control laboratories.

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CONFLICT OF INTERESTS

Declared none

REFERENCES

- Manley PW, Druce P, Fendrich G, Furet P, Liebetanz J, Martiny-Baron G, *et al.* Extended kinase profile and properties of the protein kinase inhibitor nilotinib. *Biochim Biophys Acta* 2010;1804:445-53.
- Weisberg E, Manley P, Mestan J, Cowan JS, Ray A, Griffin JD. AMN107 (nilotinib): a novel and selective inhibitor of BCR-ABL. *Br J Cancer* 2006;94:1765-9.
- Marek D, Marie-Christine W, Daniel BL, Thomas F, Heidemarie B. High-performance liquid chromatography with ultraviolet detection and protein precipitation as a way of quantitative determination of nilotinib with and without an internal standard. *J Liq Chromatogr Relat Technol* 2012;35:2503-10.
- Pursche S, Ottmann OG, Ehninger G, Schleyer E. High-performance liquid chromatography method with ultraviolet detection for the quantification of the BCR-ABL inhibitor nilotinib (AMN107) in plasma, urine, culture medium and cell preparations. *J Chromatogr B: Anal Technol Biomed Life Sci* 2007;852:208-16.
- Haouala A, Zanolari B, Rochat B, Montemurro M, Zaman K, Duchosal MA, *et al.* Therapeutic drug monitoring of the new targeted anticancer agents imatinib, nilotinib, dasatinib, sunitinib, sorafenib and lapatinib by LC tandem mass spectrometry. *J Chromatogr B: Anal Technol Biomed Life Sci* 2007;877:1982-96.
- Silvia DF, Antonio DA, Francesca DM, Elisa P, Lorena B, Marco S, *et al.* New HPLC-MS method for the simultaneous quantification of the antileukemia drugs imatinib, dasatinib and nilotinib in human plasma. *J Chromatogr B: Anal Technol Biomed Life Sci* 2007;877:1721-6.
- Masatomo M, Naoto Takahashi, Ken IS. High-performance liquid chromatography with solid-phase extraction for the quantitative determination of nilotinib in human plasma. *Biomed Chromatogr* 2010;24:789-93.
- Yuki M, Yamakawa Y, Uchida T, Nambu T, Kawaguchi T, Hamada A, *et al.* High-performance liquid chromatographic assay for the determination of nilotinib in human plasma. *Biol Pharm Bull* 2011;34:1126-8.
- Prenen H, Guetens G, de Boeck G, Debiec RM, Manly P, Schöffski P, *et al.* Cellular uptake of the tyrosine kinase inhibitors imatinib and AMN107 in gastrointestinal stromal tumor cell lines. *Pharmacology* 2006;77:11-6.
- Satyanarayana L, Naidu SV, Narasimha Rao M, Suma Latha R. The estimation of nilotinib in capsule dosage form by RP-HPLC. *Asian J Pharm Technol* 2011;1:82-4.
- Sudhakar BK, Venkataramanna M, Mohanareddy C, Narayanareddy P, Latha J. A validated stability indicative UPLC method for nilotinib hydrochloride for the determination of process related and degradation impurities. *J Chromatogr Sci* 2014;52:880-5.
- Harika M, Kumar GS. Development and validation of method for the determination of nilotinib by RP-HPLC in bulk and pharmaceutical dosage forms. *Int Res J Pharm* 2012;3:161-4.
- ICH guidance on method validation; Validation of analytical procedures Text and Methodology Q2; 2005.
- ICH harmonized tripartite guideline; Validation of analytical methods Definitions and Terminology EMEA; 1994.
- ICH harmonized tripartite guideline; Stability testing of new drug substances and products Q1A; 2003.
- Blessy M, Ruchi DP, Prajesh NP, Agarwal YK. Development of forced degradation and stability indicating studies of drugs. *J Pharm Anal* 2014;4:159-65.
- ICH harmonized tripartite guideline; Photostability testing of new drug substances and products Q1B; 1996.
- Sowjanya G, Annapurna MM, Sriram AV. Development and validation of a stability indicating RP-HPLC method for the determination of nilotinib (A tyrosine kinase inhibitor). *Indo Am J Pharm Res* 2013;3:4541-51.

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