



## RP-HPLC Method Development and Validation for Estimation of Nilotinib in Bulk and Formulation

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### KEYWORDS

HPLC, Nilotinib, Tyrosine kinase inhibitor, myelogenous leukemia, Retention time, Acetonitrile, Accuracy, Precision. Method development and validation, ICH guidelines.

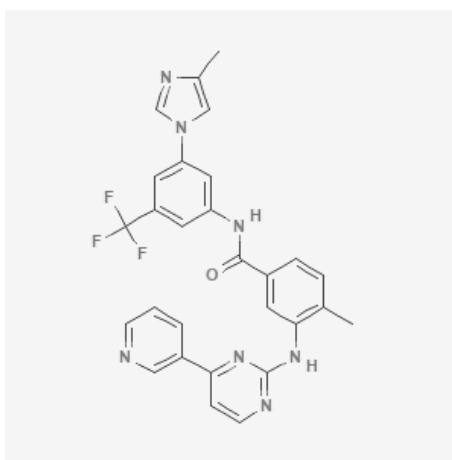
### ABSTRACT:

Nilotinib is an anticancer agent used in the treatment of chronic myelogenous leukemia. A simple, precise, accurate, stable, and economical High-Performance Liquid Chromatography (HPLC) method for the determination of Nilotinib in the capsule dosage form was developed. The chromatographic separation was achieved on Thermo Scientific C18 column (250mm x 4.6mm i.d. 5 $\mu$ ). The mobile phase selected was 0.1% trifluoroacetic buffer: acetonitrile in the ratio of 65:35 v/v. The flow rate was 1.0ml/min with 40°C column temperature and 20 $\mu$ l injection volume. The detection was carried out at 260nm. Retention time was observed at 5.888min. The method was linear over the range of 5 to 50  $\mu$ g/ml with a linear regression coefficient ( $r^2$ ) of 0.999. The HPLC method was validated as per ICH guidelines. This developed method is better for robustness specificity, rapidity, reproducibility, and superior system suitability parameter. Hence the method can be used for the routine analysis of nilotinib in bulk and formulation.

## 1. INTRODUCTION

Nilotinib is an orally available signal transduction inhibitor of the BCR-ABL kinase, c-kit and platelet derived growth factor (PDGF), all of which plays a role in cell proliferation, cell migration and angiogenesis. Nilotinib is a second generation tyrosine kinase inhibitor. It is used in treatment for chronic myelogenous

leukemia. Nilotinib is novel, selective BCR-ABL inhibitor so designed to fit into the ATP binding site of the BCR-ABL protein with higher affinity than imatinib. It is chemically 4-methyl-N-[3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide<sup>1,2</sup> as shown in figure 1. Nilotinib is available in market as conventional capsule with a trade name of Tasigna.



**Fig 1: Structure of Nilotinib**

Literature survey revealed that nilotinib was determined in pharmaceutical dosage forms by RP-HPLC as well as in biological fluids using liquid chromatography and liquid chromatography-mass spectrometric methods. In the present work the authors have developed a simple, rapid, precise, accurate and robust liquid chromatographic method for the determination of nilotinib in capsules as per ICH guidelines.<sup>3-13</sup>

## 2. MATERIALS AND METHODS

### 2.1 Chemicals and reagents:

Nilotinib working standard was procured from Central Drug Testing Laboratory, Mumbai central, Mumbai with claimed potency [97.5 % as is basis]. Tasigna (150 mg) Nilotinib capsule were received as a gift sample from Assistant Drugs Controller Office, Air Cargo, Mumbai. HPLC grade acetonitrile was procured from Rankem Laboratory, 0.1% trifluoroacetic acid from Molychem, and water-milli-Q Grade were used for the analysis. All reagents and solvents used were of analytical and HPLC grade.

### 2.2 Instrumentation:

The chromatographic analysis was carried out using Thermo Scientific Dionex ultimate 3000. It is a chromatographic method where a sample mixture or analyte was separated by a column with packing material at the high-

pressure pump with solvent passing through. HPLC system was equipped with UV Win Lab software and it was used in all the spectrophotometric measurements.

Thermo scientific Dionex ultimate 3000 is associated using software data system 7.2.6 with LC instrument attached to UV detector. The column used was Hemochrom 25cm × 4.6 mm & 5 μ.

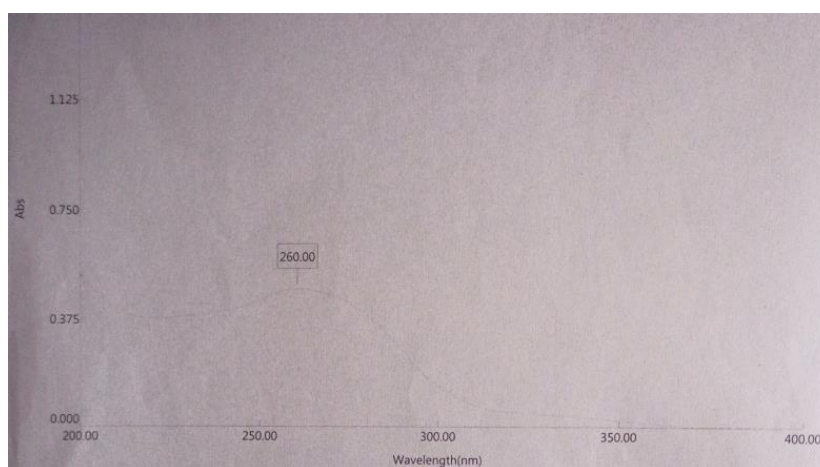
### 2.3 Selection of solvent (diluent):

Based on the solubility and chemical nature of Nilotinib, the mixture of water and Acetonitrile (50: 50) was selected as a diluent for the preparation of standard and sample solutions.

### 2.4 Selection of detection wavelength:

For RP-HPLC method analytical wavelength was determined from UV-spectra of Nilotinib recorded by using UV-VIS spectrophotometer. Accurately weighed quantity of Nilotinib (10 mg) was taken in a 100 ml volumetric flask and the volume was made up to the mark with diluent (100ppm) and from that stock solution was pipetted out 15ml into 10ml volumetric flask (15ppm).

Solution of the drug were scanned in the UV range between 200 to 400nm against blank. Nilotinib showed significant absorbance at 260nm. Figure 2 shows the UV spectrum of Nilotinib.



**Fig. no. 2: UV Spectra of Nilotinib**

### 2.5 Preparation of standard drug solution:

10 mg of Nilotinib standard drug was weighed accurately and transferred in 100ml of volumetric flask and was dissolved by sonication with sufficient amount of diluent. The volume was made up to mark (100  $\mu$ g/ ml). Then 1.5ml was pipetted out from the above stock solution and was diluted up to 10ml with diluent (15 $\mu$ g/ ml).

### 2.6 Preparation of sample solution:

A sample of the powdered capsules, equivalent to 150mg of the active ingredient was weighed and dissolved in diluent and the volume was made up to 100ml with diluent (1500 $\mu$ g/ ml). 1ml from the above solution was pipetted out in a 100ml volumetric flask and the volume was made up with diluent (15 $\mu$ g/ ml).

### 2.7 Method optimization:

In this experimental work, firstly, the ultraviolet absorption spectrum was obtained and the maximum absorption peak was found at 260 nm as shown in Figure 2. Therefore the detection wavelength of the detector was set at this wavelength for further analysis. Various

solvent systems have been reported in the literature for the chromatographic analysis of Nilotinib. By taking into account the nature of drugs under study, the method development trials were initiated by using 0.1% trifluoroacetic acid and acetonitrile with 60:40 ratio on Inert sustain column but no proper peak shape was found. The next trials were done on PERKIN by using different ratio of mobile phase 0.1% trifluoroacetic acid and acetonitrile (70:30) using Thermo column C18 but theoretical plates were found too less and the final method optimized after some trials by using mobile phase as 0.1% trifluoroacetic acid and acetonitrile at the ratio of 65:35 with column Hemochrom 25cm  $\times$  4.6 mm & 5  $\mu$ . The column oven temperature was kept at 40 $^{\circ}$ C, the flow rate was 1 ml/min which gives a good peak shape at the retention time of 5.88 min and the UV detector was set at 260nm wavelength and system suitability test results were obtained as per limits.

Table no 1, figure 2 and figure 3 shows the optimized chromatographic condition & chromatogram of of the Nilotinib standard and sample solution respectively.

**Table no.1 Optimized chromatographic conditions of Nilotinib.**

Parameters	Chromatographic conditions.
HPLC System	Thermo ultimate 3000
Column	Hemochrom 25cm $\times$ 4.6 mm & 5 $\mu$
Mobile phase	0.1% trifluoroacetic acid: Acetonitrile (65:35)
Diluent	Water: Acetonitrile (50:50)



Standard solution concentration	Nilotinib stock solution (15ppm)
Flow rate	1.0ml/min.
Run time	10 min.
Wavelength	260nm
Injection volume	20 $\mu$ l
Temperature	40 <sup>o</sup> C
Retention time	5.88 min.
Detector	UV-VIS

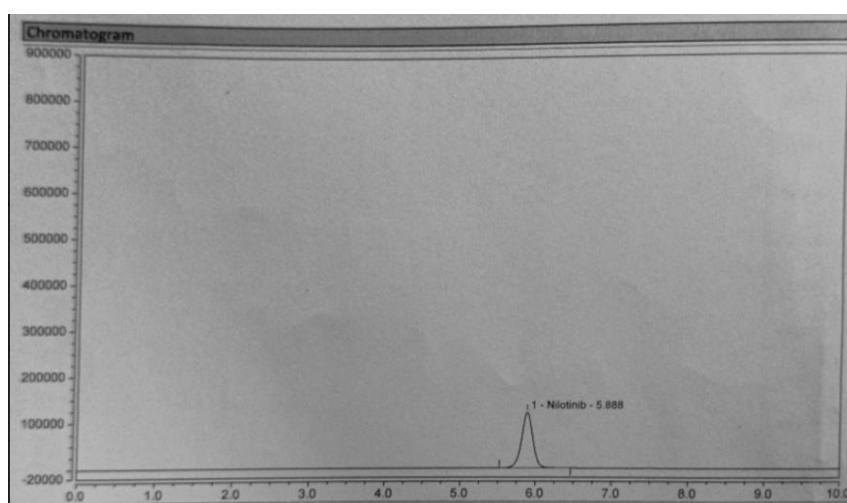


Fig. no. 2: Chromatogram of Nilotinib standard solution

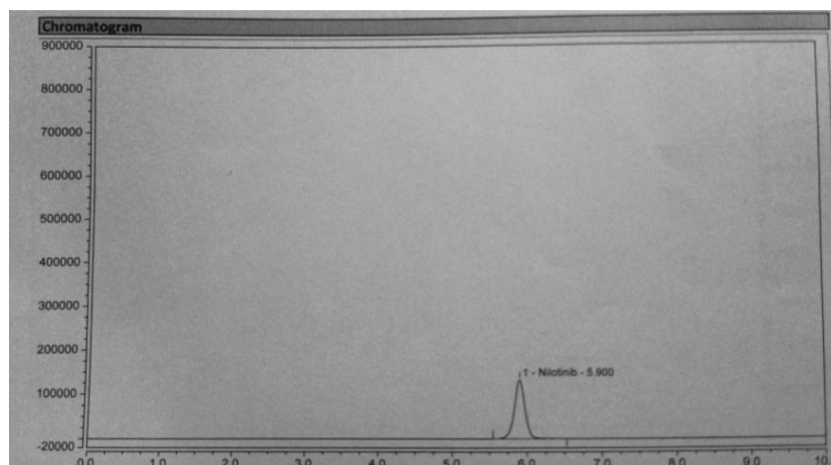


Fig. no.3: Chromatogram of Nilotinib sample solution

### 3. VALIDATION OF METHOD

Validation of developed method on HPLC was done as per ICH Q2 (R1) guidelines with respect to various parameters such as system suitability, specificity, precision, linearity, accuracy, assay, and robustness. Results of different tests were compared with these

standard guidelines of ICH Q2 (R1) to get accurate results<sup>14</sup>.

#### 3.1 Specificity:

The specificity was assessed by studying the blank, standard, and sample solutions accordingly. The blank chromatogram shows that the peak of blank does not hinder the



standard & sample solution chromatogram and their results. The peak purity of Nilotinib in capsule dosage forms was found within the limit which proved that there was no

interference of the blank peaks and excipient peaks at the retention time of Nilotinib as shown in figure 4, 5 and 6.

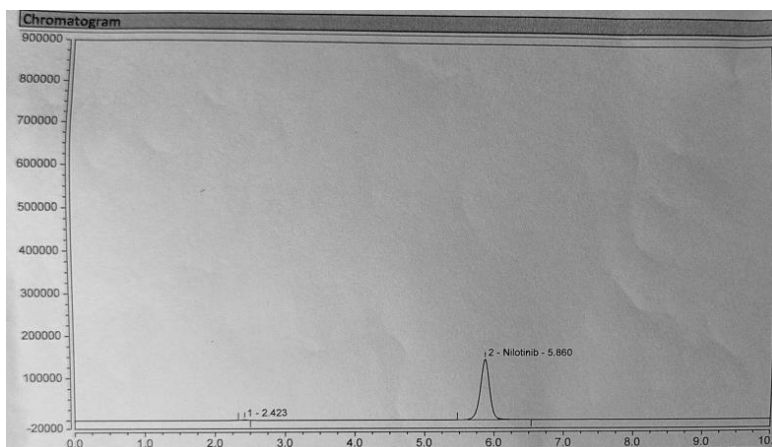


Fig no.4. Chromatogram of Nilotinib Standard Solution (pure drug)

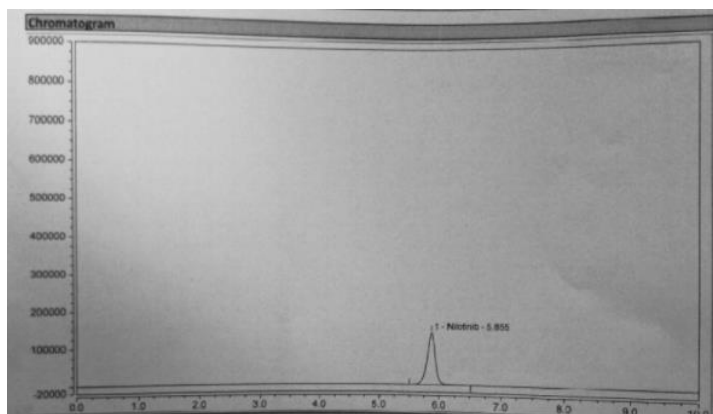


Fig no.5. Chromatogram of Nilotinib Sample Solution (Formulation)

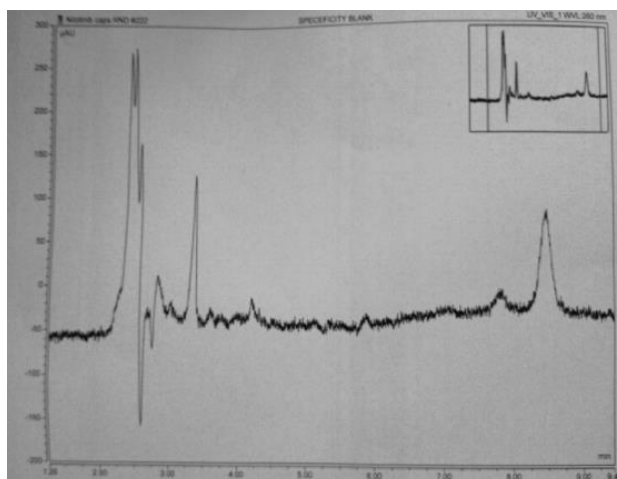


Fig no. 6: Chromatogram of Blank Solution





### 3.2 Linearity

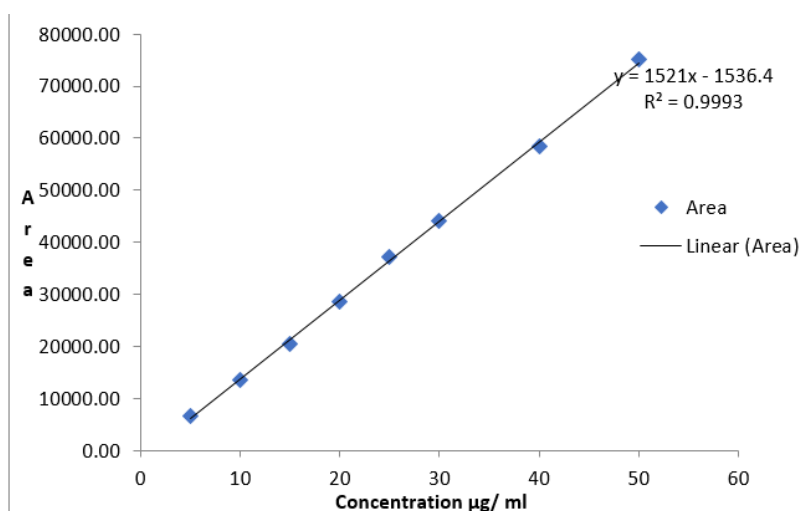
1. From the standard solution of Nilotinib aliquots were prepared in the concentration range of 5-50ppm. A graph was constructed by plotting concentration vs area obtained from the response which reveals the

linearity of the solution as shown in figure 7 and as per table no 2.

2. The linear calibration plot was constructed by analyzing the concentrations over the selected range. The response for the drug was linear in the concentration range between 5-50 µg/ml.

**Table no. 2: Linearity data of Nilotinib.**

Linearity Level	Concentration	Area
1	5	6679.82
2	10	13553.85
3	15	20498.66
4	20	28602.03
5	25	37262.82
6	30	44220.04
7	40	58407.91
8	50	75072.87



**Fig no.7: Calibration curve for Nilotinib**

### 3.3 Accuracy (Standard Addition Method):

Accuracy, which is the measure of closeness of the experimental value to the true value, was determined by standard addition method. To a pre-analyzed sample formulation a known

quantity of standard was added in the percentage of 110%, 120% & 130% as shown in table no 3. The percent recovery was evaluated as mean recovery, standard deviation and % relative standard deviation. Accuracy data of Nilotinib is shown in table no 3.

**Table no.3: Accuracy data of Standard Solution of Nilotinib.**

% level	Amount Spiked (ppm)	Amount recovered (ppm)	%Recovery	Mean recovery %	SD	%RSD
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100	15.00	148.50	99.00	99.83	0.9142	0.9157
100	15.00	151.22	100.81			
100	15.00	149.53	99.69			
110	16.50	162.05	98.21	96.84	2.2429	2.3160
110	16.50	161.80	98.06			
110	16.50	155.52	94.26			
120	18.00	178.44	99.14	99.60	1.0713	1.0756
120	18.00	177.92	98.84			
120	18.00	181.49	100.83			
130	19.50	198.99	102.04	100.98	0.9225	0.9136
130	19.50	195.75	100.39			
130	19.50	195.97	100.50			

### 3.4 Precision:

Precision expresses the degree of reproducibility of responses of repeated measurements.

Method precision was determined in terms of repeatability (intra-day) and intermediate precision (inter-day) studies by measuring the peak area and retention time of three different concentrations of Nilotinib. Repeatability was performed by repeated injection of three different concentrations from single batch under the same experimental conditions on the

same day. From the results, RSD values for retention time were less than 2%, while RSD values for peak area were less than 2% for the intra-day assay precision.

#### 3.4.1 System precision:

In system precision six injections of standard solution (15ppm) were given and their responses were obtained. Further from standard solution precision was determined. From the below table no 4, %RSD was found to be not more than 2.0%, concluded to be within the acceptable limits.

**Table no. 4: System Precision of Nilotinib Standard Solution(15ppm)**

System Precision (Standard)	
Injection no.	Area at 260 nm
1	20872.90
2	20784.95
3	20828.99
4	20756.97
5	20746.84
6	20777.00
Avg	20794.61
SD	47.78
% RSD (Limit NMT 2%)	0.23

#### 3.4.2 Method precision:

In this, sample solution of 15ppm was prepared and 6 injections of the sample were studied as

shown in table no 5. The % RSD depicting to be not more than 2.0%.



Method Precision (sample)	
Injection no.	Assay
1	99.83
2	101.30
3	101.52
4	99.98
5	103.35
6	98.74
Average	100.79
SD	1.62
%RSD (Limit NMT 2%)	1.61

**Table no. 5: Method Precision of Sample Solution (15ppm)**

**Intraday precision:** It was carried out by preparing the fresh solution and injecting on different time intervals i.e., at 10.AM, 1.00PM

& 4.00PM. Mean, S.D. & %R.S.D were evaluated respectively as given in table no 6.

**Table no. 6: Intraday precision of sample and standard solution of Nilotinib.**

Intraday Precision			
Sr No.	10:00 AM	01:00 PM	4:00PM
	% assay	% assay	% assay
1	98.16	98.12	98.17
2	97.97	98.09	98.34
3	98.07	98.12	98.35
4	98.10	98.23	98.38
5	98.98	99.02	97.92
Mean	98.252	98.318	98.232
SD	0.4102208	0.395524	0.190621
RSD	0.4175182	0.402292	0.194051
Limit	NMT 2.0%	NMT 2.0%	NMT 2.0%

**Inter day/ intermediate day precision:** Freshly prepared standard solution injected on two different days by two different analyst to

check their reproducibility on 2 different days. The average % mean, S.D, %R.S.D are given in table no 7 as follows:

**Table no.7: Interday precision**

Interday precision		
Sr. no.	Day 1	Day 2
	Analyst A	Analyst B
1	100.73	99.33
2	103.59	99.42
3	104.16	99.05
4	100.83	99.41





5	103.79	99.25
MEAN	102.61838	99.2926
SD	1.6926766	0.150611
%RSD	1.6494868	0.151684
Limit	NMT 2.0%	NMT 2.0%

**ROBUSTNESS:**

- The terms robustness and ruggedness refer to the ability of an analytical method to remain unaffected by small variations in the method parameters (mobile phase composition, column temperature, etc.) and influential environmental factors (room temperature, air humidity, etc.) and characterize its reliability during normal usage.
- Deliberate changes were made in temperature, flow rate, wavelength, and mobile phase in the estimation of Nilotinib solution. It indicates the reliability of the method. In this, the mean, S.D, and %R.S.D were detected.
- Table no 8 gives the resulted data of the robustness of Nilotinib. Findings indicates the reliability of the method.

**Table no.8: Robustness of Nilotinib**

Parameters	Change in parameter	% Estimation	Mean	SD	% RSD	Limit
Wavelength	258	98.88	98.57	1.35	1.37	NMT 2.0%
	260	99.74				
	262	97.09				
Flow rate	0.8	99.37	100.48	1.26	1.25	NMT 2.0%
	1.0	101.85				
	1.2	100.22				
Temperature	38	99.88	101.25	0.72	0.71	NMT 2.0%
	40	99.06				
	42	100.50				
Mobile Phase Ratio	30:70	101.48	100.04	1.53	1.53	NMT 2.0%
	35:65	100.19				
	40:60	98.44				

**SYSTEM SUITABILITY TEST (SST):**

- As system suitability test was an integral part of chromatographic methods development and it was carried out under ICH (Q2) guidelines. After the HPLC method of was optimized it is required to check their suitability and stability of the optimized method.
- The injections of blank (1injection) and standard Nilotinib (6 injection replicates) were given at a working concentration of 15ppm.
- The chromatograms obtained by which peak area, retention time, and theoretical plates and tailing factor of standard solution were determined and mentioned in table no 9 as follows.

**Table no.9: System Suitability data for Nilotinib.**

Sr. No.	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
1	20837.696	5.877	8049	0.99
2	20783.392	5.888	8074	0.99
3	20858.972	5.877	8101	0.99



4	20788.857	5.880	8079	1.00
5	20798.738	5.882	8258	1.00
6	20792.184	5.880	8266	1.01
Average	20809.973	5.881	8138	1.00
S.D.	30.87	0.004	97.6226	0.01
%R.S.D.	0.15	0.069	1.1996	0.82
Limits	NMT 2.0%	NMT 1.0%	NLT 2000	NMT 2.0%

**ASSAY:**

Six sample preparations of Nilotinib of 15ppm were prepared and injected into the chromatographic system. The mean, standard

deviation, and % RSD of the assay percentage of Nilotinib in the sample solution were calculated as shown in the table no 10.

**Table no.10: Assay of Nilotinib**

Sr. No.	Sample Weight (Equivalent to 150 mg)	Area of sample at 260nm	Assay
1	1 Capsule	20872.90	99.83
2	1 Capsule	20784.95	101.30
3	1 Capsule	20828.99	101.52
4	1 Capsule	20756.97	99.98
5	1 Capsule	20746.84	103.35
6	1 Capsule	20777.00	98.74
Average		20794.608	100.79
SD		47.78	1.62
%RSD (Limit NMT 2%)		0.23	1.61

**4. RESULTS AND DISCUSSION**

- A reproducible and simple method was developed for the determination of Nilotinib capsule dosage form. The results were obtained based on chromatographic conditions, including the use of a column tailored to the molecule's nature, the packing material, column dimensions, composition, and flow rate of the mobile phase, detection wavelength, and injection volume.
- After optimizing the method according to specific criteria, validation parameters were initiated. System suitability, assay, and precision tests were conducted. System suitability involved determining the tailing factor, theoretical plates, and retention time, all meeting the specifications outlined in the ICH guidelines.
- Linearity tests were performed, plotting different concentration samples versus area. The obtained results followed the

equation  $y = 1521x - 1536$  with an  $r^2$  value of 0.999, meeting the specified requirements. Subsequently, precision tests were conducted, including inter-day, intra-day, system precision, and method precision, all showing a relative standard deviation (R.S.D.) of not more than 2.00%.

- Accuracy tests were performed to determine the closeness of values and % mean recovery. The % recovery was found to be 99.69 with an R.S.D. not more than 2.00%. Additionally, assay tests determined the Nilotinib sample content, resulting in % assay values between 98% to 105%, and the %R.S.D. was not more than 2.00%.
- Robustness tests were performed by deliberately altering parameters such as wavelength, flow, and temperature. These tests confirmed the method's reliability, with % R.S.D. values not exceeding 2.00%. Specificity assessments were carried out by analyzing sample, standard, and blank



chromatograms, confirming that other peaks did not interfere with the actual sample and standard peaks.

## 5. CONCLUSION

The developed HPLC method is simple, specific, accurate, and precise for estimation of Nilotinib in a capsule dosage form. It was successfully validated in terms of linearity, accuracy, precision, specificity, and robustness in accordance with ICH guidelines. Also, the developed method is better than earlier published articles with respect to superior System Suitability Parameter such as Theoretical plates, tailing factor. Thus, the described method is suitable for routine analysis and quality control of Nilotinib in capsule dosage forms.

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