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# Development and Validation of RP-HPLC Method for the Estimation of Nilotinib in Bulk and Pharmaceutical Dosage form

**Archana Barla*, Kiran Kumar Buralla**
Department of Pharmacy, Viswanadha Institute of Pharmaceutical Sciences, Jawaharlal Nehru Technological University (JNTUK), Visakhapatnam, Andhra Pradesh, INDIA.

## ABSTRACT

**Background:** This paper describes the development of a simple, accurate, sensitive, precise and rapid method for analysis and quantification of Nilotinib by reverse phase high performance liquid chromatography (RP-HPLC) was developed and validated. The main objective was to identify the robust chromatographic conditions where an adequate separation of the components with quality peaks, within acceptable run time can be achieved. Nilotinib in bulk and formulations were analyzed and quantification. **Methods:** Nilotinib in bulk and Pharmaceutical dosage form were analyzed on Phenomenex enable $C_{18}$ column (15x4.6mm, 5µm particle size) as stationary phase. Mobile phase was composed of acetonitrile and phosphate buffer (pH 5) in the ratio of 60:40 %v/v at a flow rate of 1ml/min. The elution was analyzed using PDA detector at a detection wavelength of 260nm. The proposed method was validated by International Council for Harmonization (ICH) guidelines. **Results:** In this study, the chromatographic peaks of Nilotinib showed good resolution with retention time of 5.401min. Nilotinib showed an excellent linearity with 0.999 of correlation coefficient. The LOD was about 10.43 ng/ml and LOQ were about 31.63 ng/ml. Other validation parameters including precision, specificity, accuracy and robustness demonstrated good reliability in the quantification of Nilotinib. **Conclusion:** Thus the newly developed and validated method can be conveniently used for the quantification of Nilotinib in bulk and Pharmaceutical dosage form. Retention times were decreased and that run time was also decreased so the method developed was simple and economical that can be adopted in regular quality control test in industries.

**Key words:** Nilotinib, Tyrosine kinase inhibitor, PDA, Validation, RP-HPLC.

**Correspondence**

**Ms. Archana Barla,**
M Pharmacy, Department of Pharmacy, Viswanadha Institute of Pharmaceutical Sciences, JNTUK, Vizag, Andhra Pradesh, INDIA.
Phone no: +91 7995262801
Email: archana2721995@gmail.com

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## INTRODUCTION

Nilotinib is a second generation tyrosine kinase inhibitor (TKI) and the chemical name is 4-methyl-N-[3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino] benzamide, mono hydro chloride (Figure 1), monohydrate is a white to slightly yellowish to slightly greenish yellow powder with molecular formula $C_{28}H_{22}F_3N_7O.HCl.H_2O$ and molecular weight 584.[1,2] Rational design of novel inhibitors exhibiting effectiveness against imatinib-resistant mutants of BCR-ABL protein was carried out by researchers based upon the crystal structure of the imatinib-ABL complex and Nilotinib is a novel, selective BCR-ABL inhibitor so designed to fit into the ATP-binding site of the BCR-ABL protein with higher affinity than imatinib.[3] Literature survey revealed that Nilotinib was determined in pharmaceutical dosage forms by RP-HPLC[4,5] as well as in biological fluids using liquid chromatography[6-11] and liquid chromatography-mass spectrometric[12,13] methods. In the present work the authors have developed a simple, rapid, precise, accurate and robust stability indicating liquid chromatographic method for the determination of Nilotinib in capsules as per ICH guidelines.

## MATERIALS AND METHODS

### Materials

### Chemicals and reagents

Nilotinib references sample was a gift sample from Hetro Labs Ltd., Hyderabad, India. HPLC grade chemicals and reagents acetonitrile, potassium dihydrogen ortho-phosphate buffer and orthophosphoric acid of AR grade was obtained from Sd Fine-Chem Ltd and Milli Q Water (Merk). Membrane filter (Ultipor $^{®}N_{66}$ Nylon 6,6 membrane, 0.45µm, PALL Life Sciences). Nilotinib is industrially available as TASIGNA-200mg.

### Instrumentation

The HPLC analysis was done on Shimadzu HPLC system (Tokyo, Japan) with two LC-20AD separation modules AND SPD-m20A PDA detector, a Rheodyne injector (model 7125, USA). The chromatographic and included information were recorded utilizing LC solution data acquisition software. Absorbance spectra were recorded utilizing an UV-VIS spectrophotometer (Systronics 2202 model UV-1601PC, Japan) employ a quartz cell of 1 cm of path length.

### Chromatographic Conditions

The composition of mobile phase was acetonitrile and phosphate buffer (pH 5) at the proportion of 60:40 %v/v was used in isocratic mode at a flow rate of 1ml/min. The mobile phase was filtered through 0.45 µm Nylon membrane filter and sonicated for 20min before use. Injection volume was 20µl and detection was performed at 260nm at ambient temperature (Table 1).

### Selection of detection wavelength

For RP-HPLC method analytical wavelength was determined from UV-spectra of Nilotinib recorded by using UV-VIS spectrophotometer. Solutions of the drugs were scanned in the UV range between 200 to

400nm against blank. Nilotinib showed significant absorbance at 260nm using PDA detector (Figure 2).

### Preparation of phosphate buffer pH 5

Accurately weighed 0.68gm of phosphate buffer (potassium dihydrogen ortho phosphate) and transferred into a 500ml volumetric flask. Added 400ml of Milli Q water, dissolved by sonication and the final volume was made up to 500ml using Milli Q water. The pH of the buffer solution was adjusted to 5 ± 0.5 using orthophosphoric acid (dilute). Filtered by using membrane filter (Ultipor $^{®}N_{66}$ Nylon 6,6 membrane, 0.45µm).

### Preparation of Standard Solution of Nilotinib

Stock standard solution of Nilotinib was prepared in the mobile phase. It was stored at 4°C ± 0.05 and protected from light. Working standard solution of Nilotinib was freshly prepared by diluting the stock solution with mobile phase before analysis. Calibration curves revealing peak area ratios of Nilotinib were prepared at the range of 2, 4, 6, 8 and 10µg/ml.

### Preparation of Sample

Ten capsules were procured from local market and the contents were finely powdered. Powder equivalent to 100mg Nilotinib was accurately weighed and transferred into a 10ml of volumetric flask and added 8ml of mobile phase and sonicated for not less than 15min. The volume made up to 10ml with mobile phase and mixed. Filter the solution through the 0.45µm membrane filter.

## METHODS

The developed RP-HPLC method was validated as per International Council for Harmonization (ICH) guidelines.

### Linearity

Stock solution of Nilotinib (1mg/ml) was suitably diluted with acetonitrile to get concentration in the linearity range of 2 to 10µg/ml. A sample volume of 20µl was injected onto the column in triplicate, for each solution. Chromatograms, peak area and retention times of each solution were recorded. Calibration curve of Nilotinib was prepared by selecting concentration (µg/ml) on x-axis and average peak areas on y-axis (Figure 3 and Table 2). The calibration curve data was further subjected to statically analysis to find out the slope intercept and correlation of coefficient. $R^2$ for Nilotinib was found to be 0.998 (Tab.3). Chromatogram of Nilotinib (10µg/ml) is shown in Figure 4.

### Accuracy

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 at three levels (80, 100 and 120% of the assay concentration). The experimental was performed in triplicates. The % recoveries were calculated for all the concentrations. Results are summarized in Table 3.

### Precision

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 (2, 4 and 6µg/ml) 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. Precision results are expressed in Table 3.

### Sensitivity

Sensitivity of the method was determined from Limit of Detection (LOD) and Limit of Quantification (LOQ). The LOD and LOQ were determined using the calibration curve and results are summarized in Tab. 2.

LOD= 3.3xD/S and LOQ= 10xD/S,

Where,

D= standard deviation of Y intercept of regression line

S= slope of the calibration curve

### System suitability tests

The test was carried out by making six replicate injections of a standard solution containing 10µg/ml of Nilotinib and analyzing each solute for their peak area, theoretical plates (N), tailing factor (T) and asymmetric factors (As).

### Robustness

Robustness of the method was studied to evaluate the effect of small but deliberate variation of the chromatographic conditions on the method parameters. Robustness was determined by changing individually the flow rate (1±0.1 ml/min.), organic solvent (60±0.5 %v/v) and ionic strength of buffer (5±0.2).

**Table 1: Chromatographic conditions.**

| Parameters | Methods |
|---|---|
| Stationary phase | Phenomenex enable $C_{18}$ column |
| Mobile phase | Actonitrile: Phosphate buffer pH 5 (60:40 %v/v) |
| Flow rate | 1ml/min |
| Run time (minutes) | 8 |
| Column temperature | Ambient |
| Volume of injection | 20µl |
| Detector | PDA |
| Detection of wavelength | 260nm |
| Retention time (tR) | 5.401min |

**Table 2: Calibration curve of Nilotinib.**

| Concentration (µg/ml) | Peak area |
|---|---|
| 2 | 26485 |
| 4 | 50912 |
| 6 | 78764 |
| 8 | 106449 |
| 10 | 133134 |
| Parameters | Results for Nilotinib |
| Linearity | 2-10µg/ml |
| Regression equation | Y=13422x-1341 |
| Slope | 13422 |
| Intercept | 1341 |
| Correlation coefficient | 0.998 |
| LOD | 10.43ng/ml |
| LOQ | 31.63ng/ml |

# RESULTS

## Method development and optimization

In this experimental work, firstly, the ultraviolet absorption spectrum was obtained and the maximum absorption peak was found at 260 nm as 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 $C_{18}$ column and acetonitrile (ACN) and potassium dihydrogen ortho-phosphate ($KH_2PO_4$) as mobile phase. Figure 4 shows representative chromatogram generated over 8 min showing peaks of the drug. These initial trials were used as a basis to decide experimental ranges of three Critical Method Attributes, pH, flow rate and % acetonitrile in the mobile phase (Table 1).

## Validation

Linear regression analysis obtained the $R^2$ values as 0.999 for Nilotinib (Table 2 and Figure 3), confirming the linear relationship between the peak area and the concentration of the drug. In accuracy percent recoveries were found to be in the range of 99.65 to 100.65 for Nilotinib (Table 3). Both intraday precision measured in terms of %RSD was less than 2% over the chosen range of both the drugs (Table 3).

**Table 3: Accuracy and precision study for Nilotinib.**

| | Percentage | Nilotinib | SDV | %RSD | %Recovery |
|---|---|---|---|---|---|
| Accuracy | 80% | 231527<br>233286<br>235045 | 0.0326 | 1.1386 | 99.65 |
| | 100% | 261165<br>262924<br>264683 | 0.0246 | 0.7746 | 99.78 |
| | 120% | 287433<br>289192<br>290951 | 0.0192 | 0.5434 | 100.65 |
| Precision | Conc. | Drug Area | SDV | %RSD | |
| | 2 | 26485<br>26068<br>26399 | 220.16 | 0.838 | |
| | 4 | 51329<br>50912<br>50497 | 416.00 | 0.817 | |
| | 6 | 79591<br>78764<br>78347 | 633.16 | 0.803 | |

**Table 4: Assay of formulation.**

| Brand name | Available form | Label claim | Amount found | Assay |
|---|---|---|---|---|
| Tasigna | Capsule | 200mg | 199.9mg | 99.78% |

## Application of the developed method

The developed RP-HPLC method is sensitive and specific for the quantitative assurance of Nilotinib. The technique was approved for various parameters and, consequently, has been applied for the estimation of the drug in pharmaceutical dosage forms, such as capsules. Each capsule was analyzed in triplicate. The recovered amount of Nilotinib was 99.78% (Table 4). None of the ingredients of capsule interfered with the analyte peak.

The measured signal was shown to be precise, accurate and linear over the concentration range tested with a retention time of 5.401 min and made the method economical due to lower solvent consumption. The % RSD for all parameters was observed under 2, which shows the validity of technique and assay results obtained by this method are in reasonable agreement. Chromatogram of Nilotinib is given in Figure 4.

# DISCUSSION

In this study, the main objective was to identify the robust chromatographic conditions where an adequate separation of the components with quality peaks, within acceptable run time can be achieved. Target Analytical Profile was defined and systematic risk analysis was carried out to identify Critical Method Attributes having an impact on Critical Quality Attributes. Critical Quality Attributes was identified as capacity, resolution, separation factor and retention time. On the basis of risk priority number, mobile phase parameters were found to be most critical for the given analysis. Therefore, three parameters, % acetonitrile, pH and flow rate in the mobile phase were selected as critical method attributes.

In this experimental work, firstly, the ultraviolet absorption spectrum was obtained and the maximum absorption peak was found at 260 nm. The identified point is characterized by the specific critical method attributes combination as mobile phase consisting of acetonitrile and phosphate buffer (pH 5) in the ratio of 60:40 %v/v at a flow rate of 1ml/min. Elutes were analyzed using PDA detector at a detection wavelength of 260nm. The design space presents the operable method region where the changes will not affect the quality of analysis. The proposed method was validated by International Council for Harmonization (ICH) guidelines, Validation of Analytical Procedures: Text and Methodology $Q_2$ (R1).

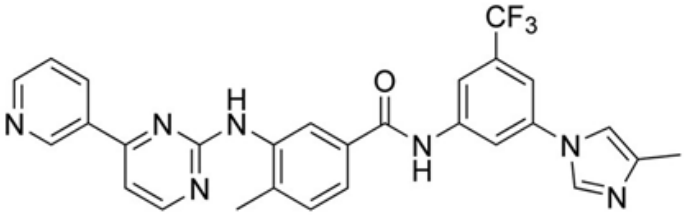



**Figure 1:** Chemical structure of Nilotinib.

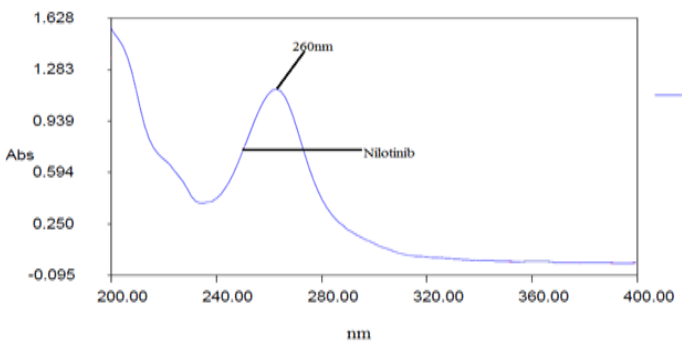



**Figure 2:** UV spectra of Nilotinib.

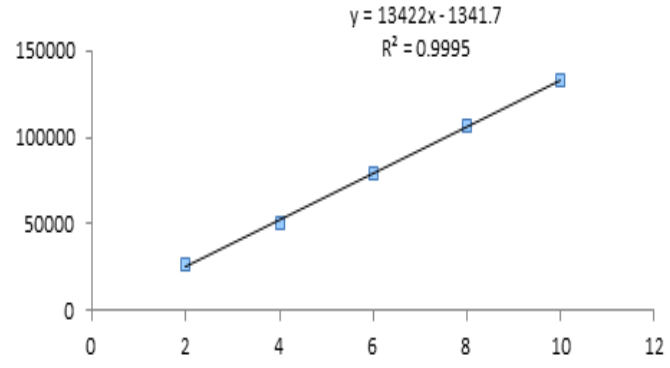
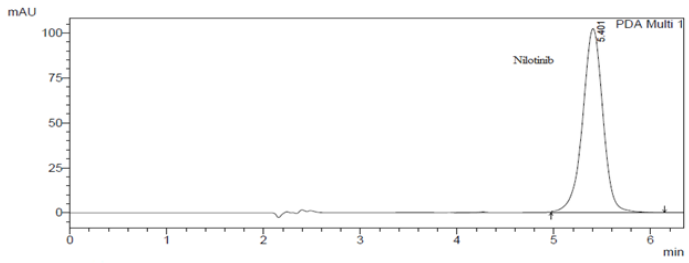
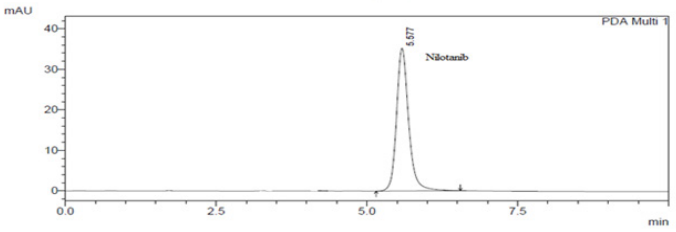



**Figure 3:** Calibration curve for Nilotinib.




A) Pure Drug (Nilotinib)




B) Formulation (Tasigna-200mg)

**Figure 4 (A & B):** Chromatograms corresponding to Bulk and formulation of Nilotinib.

Specificity was assessed by percent recovery of the drug when analyzed in combination. Percent recoveries of Nilotinib were within statistical limits. It was observed that the peak of the drug was well separated. The estimated limit of detection and limit of quantification values confirmed that the methods are sufficiently sensitive. Moreover, percent recovery of the drug was found to be acceptable. Hence, the developed method can be suitable, utilized for concurrent, quantitative analysis of Nilotinib.

The method was validated for linearity, precision, accuracy, sensitivity, system suitability as well as robustness. The developed method is convenient and effective for quality control as well as routine analysis of Nilotinib in pharmaceutical dosage form.

## CONCLUSION

An efficient isocratic reversed-phase high-performance liquid chromatography method was developed, which was optimized and validated for the simultaneous evaluation of Nilotinib in bulk and pharmaceutical formulations. The validation study supported the determination of the assay conditions by confirm that the assay was specific, precise, linear, accurate and robust.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. The article does not contain any studies with animals or human participants performed by any of the authors.

## ABBREVIATIONS

**RP-HPLC:** Reverse Phase High Performance Liquid Chromatography; **TAP:** Target Analytical Profile; **CMA:** Critical Method Attributes; **CQA:** Critical Quality Attributes; **ACN:** Acetonitrile; **TKI:** Tyrosine Kinase Inhibitor; **ICH:** International Council for Harmonisation; **QbD:** Quality by Design; **ATP:** Analytical Target Profile; **DOE:** Design of Expert; **LOD/DL:** Limit of Detection; **LOQ/QL:** Limit of Quantification.

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