



## DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR QUANTITATIVE ESTIMATION OF NILOTINIB CAPSULES 200 MG DOSAGE FORM

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### ABSTRACT

A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid chromatography assay method has been developed for estimation of Nilotinib in capsules dosage forms. The separation was achieved by using column YMC-Pack-ODS-AQ, (150 x 4.6 mm, 5 $\mu$ ) mobile phase consisted of 20 mmol/L ammonium acetate buffer and methanol in the ratio of (20:80 volume/volume). The flow rate was 1.0 mL/min. Nilotinib was detected using UV detector at the wavelength of 250 nm. Column temperature 40°C and sample temperature ambient and injection volume 10 $\mu$ L, run time 10 minutes. The retention time of Nilotinib was noted to be 4.50 min respectively. The proposed HPLC method was linear over the range of 10.05-30.016  $\mu$ g/mL, the correlation coefficient was found to be 0.999. Relative standard deviation for method precision was found to be 0.59. Recovery study results are found to be good in the range of 99.8-100.6 respectively. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

**KEYWORDS:** Liquid chromatography, Nilotinib and Validation.

### 1.0 INTRODUCTION

Nilotinib, sold under the brand name Tasigna, is a medication used to treat chronic myelogenous leukemia (CML) which has the Philadelphia chromosome.<sup>[1]</sup> It may be used both in initial cases of chronic phase CML as well as in accelerated and chronic phase CML that has not responded to imatinib.<sup>[2-3]</sup> IUPAC name is 4-methyl-N-[3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-3-[(4-pyridin-3-yl)pyrimidin-2-yl]amino benzamide. Molecular formula is C<sub>28</sub>H<sub>22</sub>F<sub>3</sub>N<sub>7</sub>O and Molecular weight 529.527 g/mol. It was designated as an orphan drug by FDA for use in the treatment of CML in Europe, United States of America and Switzerland. Chemical structure of Nilotinib is mentioned in **Figure 1**.

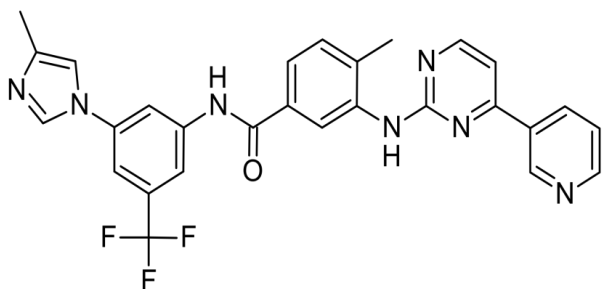


Figure 1: Chemical structure of Nilotinib.

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<sup>[4-9]</sup> and one stability indicating UPLC method for determination of related compounds of Nilotinib Hydrochloride.<sup>[10]</sup> Hence we tried to develop stability indicating HPLC method for Nilotinib. The present work describes a simple, stability indicating HPLC method for the determination of Nilotinib in Nilotinib capsules dosage form according to ICH guidelines.<sup>[11-12]</sup>

### 2.0 EXPERIMENTAL

#### 2.1. Chemicals and Reagents

Analytical-grade Ammonium acetate, Methanol and Water, both HPLC-grades, were from Merck Chemicals. Mumbai, India.

#### 2.2. Instrumentation

Agilent HPLC model:1260 with DAD, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Mettler Toledo Model) and Centrifuge Eppendorf 5810 were used in the present assay.

**Preparation of 20 mmol/L Ammonium acetate buffer solution**

Weighed accurately 1.54 g of Ammonium acetate into 1000 mL of water and mixed well. Filtered through 0.45 µm membrane filter and sonicate to degas.

**Preparation of Mobile Phase**

Prepared a mixture of 20 mmol/L ammonium acetate buffer solution and methanol in the ratio of 20:80 (%volume/volume). Filtered through 0.45 µm membrane filter and sonicate to degas.

**Preparation of Diluent**

Prepared a mixture of Water and methanol in the ratio of 20:80 (%volume/volume).

**Preparation of Standard solution**

Weighed and transferred 20 mg of Nilotinib working standard into a 100 mL volumetric flask and dissolved and made up the volume with diluent. Further diluted this solution 5 mL in to 50 mL volumetric flask and made up the volume with diluent and mixed well. (The concentration of the standard solution containing, Nilotinib 20 µg/mL).

**Preparation of Placebo solution**

Accurately weighed and transferred equivalent to 40 mg of Nilotinib Placebo powder into a 100 mL volumetric flask, dissolved and diluted to the volume with the diluent and mixed well. Transferred 5.0 mL of the above solution into a 100 mL volumetric flask, and diluted to the volume with the diluent. (The concentration of the sample solution containing, Nilotinib 20 µg/mL).

**Preparation of Test solution**

Accurately weighed and transferred equivalent to 40 mg of Nilotinib sample powder into a 100 mL volumetric flask, dissolved and diluted to the volume with the diluent, and mixed well. Transferred 5.0 mL of the above solution into a 100 mL volumetric flask, and diluted to the volume with the diluent. (The concentration of the sample solution containing, Nilotinib 20 µg/mL).

**Chromatographic conditions**

Chromatographic analysis was performed on YMC-Pack ODS-AQ, 150 x 4.6 mm, 5µ mobile phase consisted of ammonium acetate buffer and methanol in the ratio of (20:80 volume/volume). The flow rate was 1.0mL/min, column oven temperature 40°C and sampler cooler temperature 25°C, the injection volume was 10µL, and detection was performed at 250 nm using a photodiode array detector (PDA).

**3.0 METHOD DEVELOPMENT**

Spectroscopic analysis of compound Nilotinib showed that maximum UV absorbance ( $\lambda_{max}$ ) at 250 nm respectively. To develop a suitable and robust LC method for the determination of Nilotinib, different mobile phases were employed to achieve the best separation and resolution. The method development was

started with Inertsil ODS-3V, 150x4.6mm, 5µm with the following different mobile phase compositions like that 0.1% orthophosphoric acid buffer and acetonitrile in the ratio of 85:15 volume/volume. It was observed that when Nilotinib was injected, higher retention time, Peak Tailing, not satisfactory. Column stationary phase was not suitable for component. For next trial change the column from Inertsil ODS-3V to YMC pack-ODS AQ and the mobile phase consisted of ammonium acetate buffer and methanol in the ratio of 50:50 volume/volume respectively, flow rate 1.0 mL/min, column temperature 40°C and sampler cooler maintained ambient. UV detection as performed at 250nm. The compound Nilotinib was eluted at void volume 1.15 minutes and the peak shape was not good.

For next trial chromatographic conditions and mobile phase was same as above trial expect mobile phase composition, the mobile phase consisted of ammonium acetate buffer and methanol in the ratio of 20:80 volume/volume respectively, flow rate 1.0 mL/min, column temperature 40°C and sampler cooler maintained ambient. UV detection as performed at 250nm. The compound Nilotinib was eluted at void volume 4.50 minutes and the peak shape was found to be good. The chromatogram of Nilotinib standard using the proposed method is shown in **Figure: 2** system suitability results of the method are presented in **Table:1**.

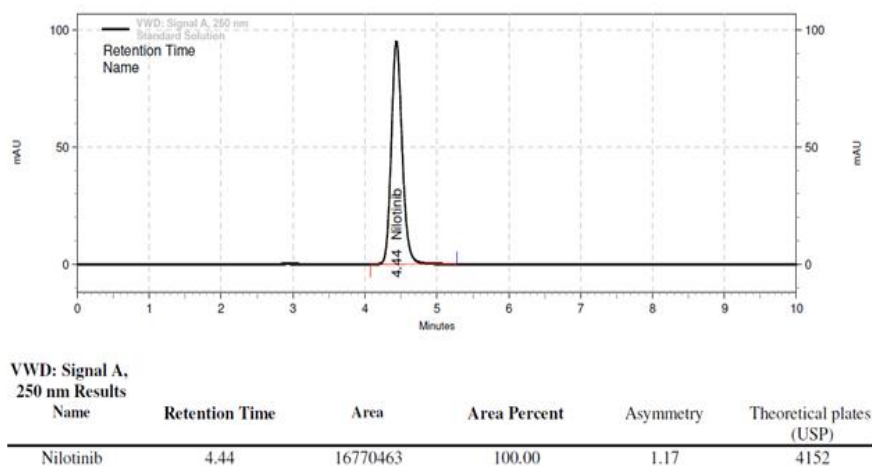


Figure 2: Typical chromatogram of Nilotinib standard.

#### 4.0 METHOD VALIDATION

The developed RP-HPLC method extensively validated for assay of Nilotinib in Nilotinib capsules formulation using the following parameters.

##### 4.1 Specificity & System suitability

###### Blank and Placebo interference

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms

were recorded. Chromatogram of blank solution **Figure:3** showed no peak at the retention time of Nilotinib peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Nilotinib in Nilotinib capsules formulation. Similarly chromatogram of placebo solution **Figure:4** showed no peaks at the retention time of Nilotinib peak. This indicates that the placebo used in sample preparation do not interfere in estimation of Nilotinib in Nilotinib capsules formulation.

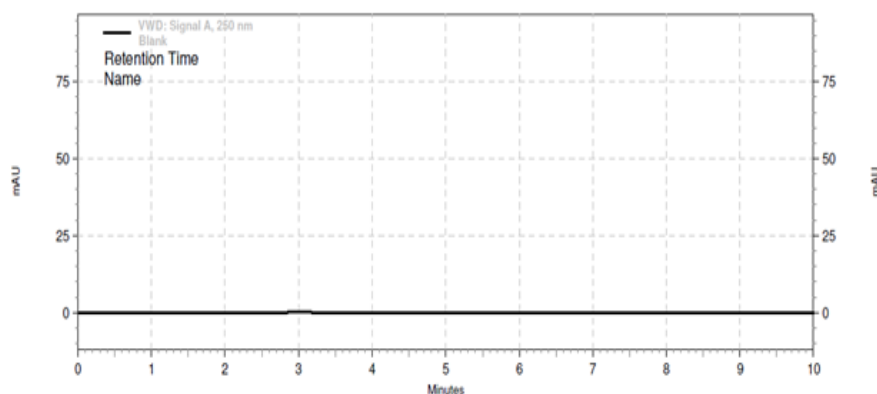


Figure: 3 Chromatogram showing the no interference of diluent for Nilotinib.

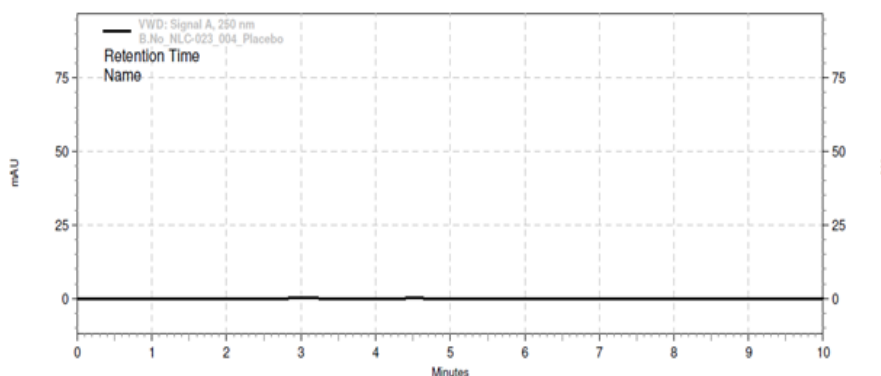


Figure: 4 Chromatogram showing the no interference of placebo for Nilotinib.

**Table: 1 Specificity results for Nilotinib.**

| S.No | Name              | Retention Time (min) |
|------|-------------------|----------------------|
| 1    | Blank             | ND                   |
| 2    | Placebo solution  | ND                   |
| 3    | Standard solution | 4.44                 |
| 4    | Sample solution   | 4.44                 |

**4.2 System precision**

The standard solution was prepared as per the test method, injected into the HPLC system for six times and evaluated the % RSD for the area responses. The data were shown in **Table:2**

**Table: 2 System precision data for Nilotinib.**

| S.No. | No.of injections | Area of Nilotinib |
|-------|------------------|-------------------|
| 1     | Inj-1            | 16770463          |
| 2     | Inj-2            | 16710546          |
| 3     | Inj-3            | 16799708          |
| 4     | Inj-4            | 16770854          |
| 5     | Inj-5            | 16724565          |
| 6     | Inj-6            | 16717410          |
|       | Average          | <b>16748924</b>   |
|       | SD               | <b>36285.62</b>   |
|       | %RSD             | <b>0.22</b>       |

- ❖ The relative standard deviation of six replicate standard solution results found to be within the specification limit i.e.0.22%.

**4.3 Method precision**

The precision of test method was evaluated by doing assay for six samples of Nilotinib capsules as per test method. The content in mg and % label claim for Nilotinib for each of the test preparation was calculated. The average content of the six preparations and % RSD for the six observations were calculated. The data were shown in **Table:3**

**Table 3: Method precision data for Nilotinib.**

| No. of Preparations | % Assay         |
|---------------------|-----------------|
| Preparation 1       | 101.8           |
| Preparation 2       | 101.9           |
| Preparation 3       | 101.7           |
| Preparation 4       | 101.1           |
| Preparation 5       | 100.4           |
| Preparation 6       | 100.9           |
| Average             | <b>101.3</b>    |
| SD                  | <b>0.596657</b> |
| %RSD                | <b>0.59</b>     |

- ❖ Overall and individual % of Assay are complies as per test method specification.
- ❖ The relative standard deviation of six assay preparations is 0.59.

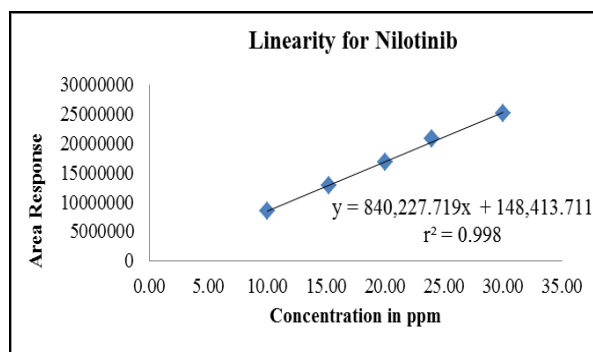
**4.4 Linearity of detector response**

The linearity of an analytical method is its ability to obtain test results which has a definite mathematical relation to the concentration of analyte. The linearity of

response for Nilotinib was determined in the range of 50 to 150 % (10.005- 30.016 µg/ml for Nilotinib). The calibration curve of analytical method was assessed by plotting concentration versus peak area and represented graphically. The correlation coefficient [r] was found to be 0.9990. Therefore the HPLC method was found to be linear standard curve were calculated and given in **Figure:5** to demonstrate the linearity of the proposed method. From the data obtained which given in **Table: 4** the method was found to be linear within the proposed range.

**Table 4: Linearity studies for Nilotinib by proposed method.**

| S.No                        | Linearity Level   | Concentration (ppm) | Area response        |
|-----------------------------|-------------------|---------------------|----------------------|
| 1                           | Linearity at 50%  | 10.005              | 8512797<br>8505036   |
| 2                           | Linearity at 75%  | 15.208              | 12862287<br>12845887 |
| 3                           | Linearity at 100% | 20.01               | 16848072<br>16828247 |
| 4                           | Linearity at 120% | 24.013              | 21357767<br>20368589 |
| 5                           | Linearity at 150% | 30.016              | 25053864<br>25090122 |
| Correlation coefficient (r) |                   |                     | 0.999                |
| $r^2$                       |                   |                     | 0.998                |
| Intercept                   |                   |                     | 148413.7112          |
| Slope                       |                   |                     | 840227.7191          |
| 100% Y-intercept            |                   |                     | 0.88                 |

**Figure 5: Calibration curve for Nilotinib.****4.5 Accuracy**

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in duplicate preparations on Nilotinib API spiked to placebo, analyzed as per the proposed method. The mean percentage recovery for 50%, 100%, 150% level. was found to be 100.60, 99.80 and 100.60 respectively. They are within the acceptance limits. Therefore, the HPLC method for the determination of assay of Nilotinib in formulation was found to be accurate. The data obtained which given in **Table:5** the method was found to be accurate.

**Table 5: Recovery studies for Nilotinib by proposed method.**

| Preparations        | Area Response | (mg) Recovered | (mg) Added | Recovery | Mean Recovery |
|---------------------|---------------|----------------|------------|----------|---------------|
| Accuracy at 50 %-1  | 8528538       | 20.4           | 20.1       | 101.5    | 100.6         |
|                     | 8534936       | 20.4           | 20.1       | 101.6    |               |
| Accuracy at 50 %-2  | 8412724       | 20.1           | 20.2       | 99.6     |               |
|                     | 8414718       | 20.1           | 20.2       | 99.6     |               |
| Accuracy at 100 %-1 | 16496724      | 39.4           | 40.0       | 98.4     | 99.8          |
|                     | 16556304      | 39.5           | 40.0       | 98.7     |               |
| Accuracy at 100 %-2 | 16951000      | 40.5           | 40.1       | 101.0    |               |
|                     | 16966943      | 40.5           | 40.1       | 101.0    |               |
| Accuracy at 150 %-1 | 25313212      | 60.4           | 60.2       | 100.3    | 100.6         |
|                     | 25343918      | 60.5           | 60.2       | 100.4    |               |
| Accuracy at 150 %-2 | 25406839      | 60.6           | 60.2       | 100.8    |               |
|                     | 25422085      | 60.7           | 60.2       | 100.9    |               |
| Overall Average     |               |                |            | 100.3    |               |
| Overall Std. Dev    |               |                |            | 1.039    |               |
| Overall %RSD        |               |                |            | 1.00     |               |

**4.6 Solution stability of analytical solutions**

Standard and sample solutions were kept for about 24 hrs at room temperature in transparent bottles in auto sampler and in refrigerator 2-8°C. The stability of standard and sample solutions was determined by comparison of “old” prepared standard solutions with freshly prepared standard solutions.

**Table 6: Results for solution stability of standard.**

| Time Interval | Similarity factor |              |
|---------------|-------------------|--------------|
|               | Room temperature  | Refrigerator |
| Initial       | NA                | NA           |
| 24hrs         | 0.99              | 1.01         |

**Table7: Results for solution stability of sample at room temperature.**

| Time Interval | %Assay | % of Assay difference |
|---------------|--------|-----------------------|
| Initial       | 101.8  | NA                    |
| 24hrs         | 102.1  | 0.3                   |

**Table 8: Results for solution stability of sample in Refrigerator.**

| Time Interval | %Assay | % of Assay difference |
|---------------|--------|-----------------------|
| Initial       | 101.8  | NA                    |
| 24hrs         | 102.2  | 0.4                   |

**4.7 Filter validation**

Performed the filter validation for sample solution, one portion of the solution was centrifuged and other portion of the solution was filtered through 0.45 µm PVDF and 0.45 µm Nylon filters:

**Table 9: Results for Filter validation of Nilotinib.**

| S.No. | Filter details                | Area Response | % Assay | Mean % Assay | Difference when compared to Centrifuged |
|-------|-------------------------------|---------------|---------|--------------|-----------------------------------------|
| 1     | Centrifuged Sample            | 16965214      | 101.0   | 101          | NA                                      |
|       |                               | 16987762      | 101.1   |              |                                         |
| 2     | 0.45 µm PVDF Filtered Sample  | 17099392      | 101.8   | 101.8        | -0.8                                    |
|       |                               | 17119143      | 101.9   |              |                                         |
| 3     | 0.45 µm Nylon Filtered Sample | 16870597      | 100.4   | 100.4        | 0.6                                     |
|       |                               | 16863102      | 100.4   |              |                                         |

**Observations**

- ❖ 0.45 µm PVDF filtered sample when compared with centrifuged sample was **-0.8**.
- ❖ 0.45 µm Nylon filtered sample when compared with centrifuged sample was **0.6**.

**Conclusion**

From the above results, it was concluded that both 0.45 µm PVDF and 0.45 µm Nylon filters were compatible.

**5.0 RESULTS AND DISCUSSION**

An RP-HPLC method for estimation of Nilotinib was developed and validated as per ICH guidelines. A

simple, accurate and reproducible reverse phase HPLC method was developed for the estimation of Nilotinib in Nilotinib capsules formulations. The optimized method consists of mobile phase ammonium acetate buffer and methanol in the ratio of 20:80%volume/volume with YMC Pack-ODS AQ (150 × 4.6mm, 5µ) column. The retention time of Nilotinib was found to be 4.50 minutes. The developed method was validated as per ICH Q2A (R1) guideline. The proposed HPLC method was linear over the range of 10.005- 30.016 µg/ml, the correlation coefficient was found to be 0.9990. Relative standard deviation for method precision was found to be 0.59%.

The accuracy studies were shown as % recovery for Nilotinib 50%, 100% and 150% level. The limit of % recovered shown is in the range of 98 and 102% and the results obtained were found to be within the limits. Hence the method was found to be accurate.

The solution stability of standard and samples are stable upto 24hrs on bench top and refrigerator (2-8°C). Performed the filter validation for sample solution both 0.45 µm PVDF and 0.45 µm Nylon filters were compatible.

## 6.0 CONCLUSION

We have developed a fast, simple and reliable analytical method for determination of Nilotinib in pharmaceutical preparation using RP-HPLC. As there is no interference of blank and placebo at the retention time of Nilotinib. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and Linearity. It allows reliably the analysis of Nilotinib in its different pharmaceutical dosage forms.

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## CONFLICT OF INTERESTS

The authors claim that there is no conflict of interest.

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