Forced Degradation Study and Validated Stability-Indicating RP–LC Method for Determination of Nilotinib in Bulk and Capsules

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Summary. A simple, selective, and precise stability-indicating reversed-phase liquid chromatographic method was developed and validated for the determination of nilotinib. Nilotinib was subjected to acid and alkali hydrolysis, oxidation, thermal, and photo-degradation. The degradation products were well separated from the pure drug. The method was based on isocratic elution of nilotinib and its degradation products on reversed phase C18 column (100 mm × 4.6 mm, 3.5 μm) — Zorbax Eclipse Plus using a mobile phase consisting of 10 mM KH₂PO₄:acetonitrile (54.5:45.5%, v/v) at a flow rate of 1 mL min⁻¹. Quantitation was achieved with UV detection at 265 nm. Linearity, accuracy and precision were found to be acceptable over the concentration range of 0.1–80 μg mL⁻¹. The drug was found to be susceptible to acid and base hydrolysis but resistant to oxidation, dry heat degradation, and photodegradation. The proposed method was successfully applied to the determination of nilotinib in bulk and in its pharmaceutical preparation.

Key Words: nilotinib, reversed-phase liquid chromatography, stability-indicating assay, capsules, anticancer drugs

Introduction

Nilotinib, 4-methyl-N-[3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-3-[(4-pyridin-3-ylpyrimidin-2-yl) amino]benzamide (Fig. 1) is a second-generation orally available signal transduction inhibitor of the Bcr-Abl kinase, c-kit, and platelet-derived growth factor (PDGF), all of which play a role in cell proliferation, cell migration, and angiogenesis [1,2]. Nilotinib has been approved in many countries since 2007 for the treatment of chronic myelogenous leukemia (CML) in cases of imatinib-acquired resistance and intolerance [3, 4].

Nilotinib has been determined in biological fluids by HPLC using UV detection either alone [5–7] or in mixture with imatinib and its main metabolite [8]. Besides, it has also been determined in cultured tumor cells using HPLC-UV method [9]. Moreover, LC–MS determination of nilotinib
has been described either alone in human plasma and serum [10] or in mixture with imatinib and dasatinib in human peripheral blood mononuclear cell [11] and in human plasma [12]. As it was found that there is no reported forced degradation study of nilotinib, a full study of the degradation behavior of nilotinib under acidic, basic, oxidative, thermal, and photolytic conditions was carried out. Moreover, a simple, sensitive, and selective LC method has been developed for the determination of nilotinib in its capsule dosage form and also in the presence of its degradation products. Optimization of LC conditions to separate the drug and its degradation products on a reversed phase C18 column method validation was discussed.

**Experimental**

**Instrumentation**

The HPLC (Agilent instrument 1260 series, Germany) system was equipped with vacuum degasser, mixer, autosampler, gradient quaternary pump, and UV/Vis detector. Separation and quantitation were made on Zorbax Eclipes Plus C18 rapid resolution column (100 mm × 4.6 mm, 3.5 μm). An Elma ultrasonic processor (Germany) was used.

A side-bench Ecocell 55 oven 250°C (München, Germany) was used to control the temperature during forced degradation studies. Jenway 3510 (Staffordshire, UK) was used to adjust the pH.
Reagents and Reference Samples

Standard nilotinib and Tasigna capsules (labeled as a nominal dose of 200 mg nilotinib as hydrochloride monohydrate per capsule) were supplied by Novartis Pharma, Switzerland. HPLC grade methanol, acetonitrile, and dibasic potassium phosphate were supplied by Sigma-Aldrich, Germany. Bi-distilled water was produced in-house (Aquatron Water Still, A4000D, UK). Membrane filters, 0.45 μm (Teknokroma, Barcelona, Spain), were used. All other chemicals and reagents used were of analytical grade unless indicated otherwise.

Chromatographic Conditions

Chromatographic separation was achieved on a Zorbax Eclipses C18 rapid resolution column (100 mm × 4.6 mm, 3.5 μm) applying isocratic elution based on a mobile phase consisting of 10 mM KH$_2$PO$_4$:acetonitrile (54.5:45.5%, v/v) at a flow rate of 1 mL min$^{-1}$. Analyses were performed at ambient temperature and detection was carried out at 265 nm. The injection volume was 20 μL.

Standard Stock Solution Preparation

Standard stock solution of nilotinib (1.0 mg mL$^{-1}$) was prepared by dissolving 100 mg of the drug in methanol, sonicated to dissolve, and completed to volume in a 100-mL volumetric flask. The required concentrations were prepared by serial dilutions.

Sample Preparation

The contents of 20 capsules were mixed and accurately weighed. A quantity of the powder equivalent to 200 mg nilotinib was sonicated for 15 min with 50 mL methanol, cooled, and filtered into a 200-mL volumetric flask. The solution was completed to volume with methanol. Two-milliliter aliquot was further diluted to 100 mL in a volumetric flask to obtain a concentration equivalent to 20 μg mL$^{-1}$. 

Procedure

Calibration Curve of Nilotinib

Accurately measured aliquots of stock standard solutions equivalent to 1–800 μg nilotinib were transferred into a series of 10 mL volumetric flasks. The solutions were completed to volume with methanol. A volume of 20 μL of each solution was injected in triplicates into the chromatograph. The conditions including the mobile phase at flow rate 1 mL min⁻¹ and detection at 265 nm were adjusted. A calibration curve was obtained by plotting area under the peak (AUP) against concentration (C).

Assay of Nilotinib in Bulk and Tasigna Capsules

The procedure mentioned in “Calibration Curve of Nilotinib” was repeated using concentrations equivalent to 15–50 μg mL⁻¹ nilotinib in bulk. For the determination of nilotinib in Tasigna capsules, the sample solution, prepared in “Sample Preparation”, was injected in triplicates. The concentrations of the examined drugs were calculated in reference to the working standard solution.

Forced Degradation of Nilotinib

Forced degradation studies of bulk drug included appropriate solid state and solution state stress conditions in accordance with the ICH regulatory guidance [13]. The stock solution was used for the forced degradation study to provide an indication of the stability-indicating property and specificity of proposed method. Prior to injection, samples were withdrawn at appropriate time, neutralized (in case of acid and alkali hydrolysis), and the solutions were diluted with methanol.

Acid- and Base-Induced Degradation

An appropriate volume of 5 M HCl was added to 5 mL of methanolic stock solution, and the mixture was diluted with 10 mL water to reach molarity of 0.1 M, 0.5 M, and 1 M HCl, separately. The mixtures were kept at room temperature for 8 h. Alkaline degradation studies were carried out in a similar manner with molarities of 0.1 M, 0.5 M, and 1 M NaOH for 8 h. These experiments were repeated in molarities 0.1 M, 0.5 M, 1 M, and 2 M at higher temperature of 75°C for 30 min and of 80°C for 90 min while keeping all other conditions constant. The forced degradation in acidic and basic media was performed in the dark in order to exclude the possible
degradative effect of light. Twenty microliters of the resultant solutions were injected onto column, and the chromatograms were run as described in “Chromatographic Conditions”.

**Hydrogen Peroxide-Induced Degradation**

Five milliliters of 6% \( (v/v) \) \( \text{H}_2\text{O}_2 \) and 30% \( (v/v) \) \( \text{H}_2\text{O}_2 \) were separately added to reach final concentrations of 3% and 15% \( (v/v) \) \( \text{H}_2\text{O}_2 \), respectively. The prepared mixtures were kept at room temperature for 12 and 5 h, respectively. Twenty microliters of the resultant solutions were injected onto column, and the chromatograms were run as described in “Chromatographic Conditions”.

**Thermal and Photolytic Degradation**

The dry powder of the drug was placed in oven at 55°C for 50 h to study dry heat degradation. The photochemical stability of the drug was also studied by exposing the dry powder to sunlight for 48 h. Powder was dissolved and diluted with methanol. Twenty microliters of the resultant solutions were injected onto column, and the chromatograms were run as described in “Chromatographic Conditions”.

**Results and Discussion**

**Method Development**

During the optimization cycle, several chromatographic conditions were attempted using Zorbx Eclipse C18 column (100 mm \( \times \) 4.6 mm, 3.5 μm). Chromatographic parameters, such as mobile phase composition and proportions, pH and flow rates, and detection wavelength, are all thoroughly studied and optimized in order to provide an excellent separation. Various mobile phase compositions, like methanol with water, or acetonitrile with water, in different proportions, were tried in an isocratic mode. Detection was carried out at 265 nm to obtain sufficient peak intensity for both drugs and degradants. During the development cycle, a mobile phase, consisting of 10 mM KH\(_2\)PO\(_4\):acetonitrile (54.5:45.5%, \( v/v \)) at a flow rate of 1.0 mL min\(^{-1} \) in an isocratic mode, gave a good separation of the drug and its degradation products. The retention time of nilotinib was found to be 6.1 min (Fig. 2).
System Suitability Tests

System suitability tests are an integral part of liquid chromatographic methods in the course of optimizing the conditions of the proposed method [14]. They are used to verify that the resolution and reproducibility were adequate for the analysis performed. The parameters of these tests are column efficiency (number of theoretical plates), tailing of chromatographic peak, repeatability as %RSD of peak area for six injections of a solution of 20 μg mL⁻¹ and reproducibility of retention as %RSD of retention time. The results of these tests for the proposed method are listed in Table I.

Table I. System suitability tests for the proposed LC method for the determination of nilotinib in bulk

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>4964</td>
</tr>
<tr>
<td>T</td>
<td>1.06</td>
</tr>
<tr>
<td>%RSD of 6 injections of Peak area</td>
<td>0.62</td>
</tr>
<tr>
<td>tR (min)</td>
<td>0.12</td>
</tr>
</tbody>
</table>

N: Number of theoretical plates; T: Tailing factor; %RSD: %Relative standard deviation; tR: retention time.

Method Validation

Linearity

In this study, eight concentrations were chosen. Each concentration was analyzed three times. Good linearity of the calibration curve was verified by the high correlation coefficient. The analytical data of the calibration curve including standard deviations for the slope and intercept (Sb, Sa) are summarized in Table II.
Table II. Results obtained by the proposed LC method for the determination of nilotinib

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>6.11</td>
</tr>
<tr>
<td>Wavelength of detection (nm)</td>
<td>265</td>
</tr>
<tr>
<td>Range of linearity (µg mL⁻¹)</td>
<td>0.1–80</td>
</tr>
<tr>
<td>Regression equation</td>
<td>Area = 98.099 C_µg.mL⁻¹ + 38.579</td>
</tr>
<tr>
<td>Regression coefficient (r²)</td>
<td>0.9999</td>
</tr>
<tr>
<td>LOD (µg mL⁻¹)</td>
<td>0.020</td>
</tr>
<tr>
<td>LOQ (µg mL⁻¹)</td>
<td>0.062</td>
</tr>
<tr>
<td>S_b</td>
<td>0.48</td>
</tr>
<tr>
<td>S_a</td>
<td>15.83</td>
</tr>
<tr>
<td>Confidence limit of the slope</td>
<td>98.099 ± 1.19</td>
</tr>
<tr>
<td>Confidence limit of the intercept</td>
<td>38.579 ± 38.75</td>
</tr>
<tr>
<td>Standard error of the estimation</td>
<td>35.84</td>
</tr>
<tr>
<td>Inter-day (%RSD)</td>
<td>1.45–1.82</td>
</tr>
<tr>
<td>Intra-day (%RSD)</td>
<td>0.60–0.73</td>
</tr>
<tr>
<td>Drug in bulk</td>
<td>99.63 ± 2.54</td>
</tr>
<tr>
<td>Drug in dosage form</td>
<td>101.35 ± 0.79</td>
</tr>
</tbody>
</table>

Accuracy

Accuracy of the results was calculated by % recovery of five different concentrations of nilotinib in bulk. The results obtained, including the mean of the recovery and standard deviation, are displayed in Table III.

Table III. Results obtained by the proposed LC method for the determination of nilotinib in bulk

<table>
<thead>
<tr>
<th>Theoretical concentration</th>
<th>AUP</th>
<th>Recovered concentration</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>519.848</td>
<td>4.91</td>
<td>98.13</td>
</tr>
<tr>
<td>15</td>
<td>1470.341</td>
<td>14.60</td>
<td>97.31</td>
</tr>
<tr>
<td>30</td>
<td>2922.416</td>
<td>29.40</td>
<td>98.00</td>
</tr>
<tr>
<td>50</td>
<td>5026.937</td>
<td>50.86</td>
<td>101.71</td>
</tr>
<tr>
<td>70</td>
<td>7109.486</td>
<td>72.09</td>
<td>102.98</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>99.63</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td>2.54</td>
</tr>
<tr>
<td>%RSD</td>
<td></td>
<td></td>
<td>2.55</td>
</tr>
</tbody>
</table>
Precision

The repeatability of the method was assessed by six determinations for each of the three concentrations (16–20–24 μg mL⁻¹). The repeatability of sample application and measurement of peak area for active compound were expressed in terms of percentage relative standard deviation (%RSD) and found to be less than 1% in three concentrations (Table I). Besides, intra-day and inter-day precisions (using three different concentrations in triplicates for three consecutive days) were carried out for nilotinib, and results are displayed in Table II.

Specificity

Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences. Good resolution and absence of interference from any of the degradation products are shown in Figs. 3 and 4. Besides, the chromatogram of the pharmaceutical formulation samples was checked for the appearance of any extra peaks. No chromatographic interference from any of the excipients was found at the retention time of the examined drug (Fig. 5). In addition, the chromatogram of nilotinib in the sample solution was found to be identical to the chromatogram obtained by the standard solution. Moreover, results close to 100% were obtained for the determination of nilotinib in dosage form (Table II). These results confirm the specificity of the proposed method.

Fig. 3. HPLC chromatogram of degraded nilotinib in (a) 0.1 N HCl at 80°C; (b) 0.5 N HCl at 80°C; (c) 1 N HCl at 80°C; (d) 2 N HCl at 80°C
Limit of Detection and Limit of Quantitation

Limit of detection (LOD), which represents the concentration of analyte at S/N ratio of 3, and limit of quantification (LOQ), at which S/N ratio is 10, were determined experimentally for the proposed methods, and results are given in Table II.

Robustness

Robustness was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 1.0 mL min⁻¹ to 0.8 mL min⁻¹ and 1.2 mL min⁻¹. The organic strength was varied by ±2%. These variations did not have a significant effect on chroma-
tographic resolution by the proposed LC method for nilotinib and its degradation products in alkali or acid conditions.

**Degradation Behavior**

**Acid- and Base-Induced Degradation**

The chromatograms of the alkaline-degraded samples for nilotinib, using different molarities of NaOH either on cold or by heating in 70°C for 30 min, showed no degradation of the intact drug. Increasing time to 90 min and temperature to 80°C led to the appearance of additional peaks. Using these drastic conditions in 2 M NaOH degraded about 35% of the intact drug, and a new peak appeared at a retention time of 2.2 (Fig. 4). On the other hand, exposing the intact drug to 2 M HCl, using the previous conditions, led to the appearance of three new peaks at retention times of 1.5, 1.8 and 3.8 min and 77% of the drug remained intact (Fig. 3). This reflects the partial stability of the amide linkage of nilotinib to acidic or alkaline hydrolysis. The peaks of degraded products were well resolved from the drug peak.

**Hydrogen Peroxide-Induced Degradation**

The sample degraded with 3% and 15% v/v hydrogen peroxide showed no additional peaks. This was confirmed by good percentage recovery of the intact drug.

**Thermal and Photolytic Degradation**

The samples degraded under dry heat conditions showed no additional peaks. The photodegraded sample showed no additional peak when drug solution was exposed to sunlight for 48 h.

From the aforementioned data, the drug was found to be susceptible to acid and base hydrolysis but resistant to oxidation, dry heat degradation, and photodegradation.

**Conclusion**

A validated stability-indicating LC method was developed to study the degradation behavior of nilotinib under acid, alkali, oxidation, thermal and photolysis conditions. The drug was found to be partially degraded in alkaline and acidic conditions due to the presence of amide linkage, which
is susceptible to hydrolysis but only in drastic conditions, and found to be stable to oxidation, thermal and photolysis conditions. The proposed LC method has the advantages of simplicity, precision, accuracy, and convenience for the separation and quantification of nilotinib either alone or in the presence of its degradation products. Hence, the proposed LC method can be used for the quality control of the cited drug.

References


Accepted by DA