Stability Indicating RP-LC Method for the Quantitation of Nicardipine Hydrochloride in Capsules

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ABSTRACT

A stability indicating reversed phase high performance liquid chromatographic method for the determination of nicardipine hydrochloride in its capsules is described. Chromatographic separation was achieved on a 250x4.6mm, 5µ, kromasil C18 column, in isocratic mode with mobile phase comprising of a mixture of acetonitrile and 0.03%v/v O-phosphoric acid (40:60v/v). The flow rate was 2.0mL/min, run time was 7min and PDA detection was carried out at 235nm. The retention time was found to be about 3.06min. The method was validated as per ICH guidelines and was demonstrated to be accurate, precise and specific. The forced degradation investigations for nicardipine hydrochloride formulation showed that it is resistant to base hydrolysis, thermal and humid conditions while degraded in acidic, oxidative and photolytic conditions. From the results it can be briefed that the method can be used as stability indicating assay of nicardipine hydrochloride in capsules.

Key words: Nicardipine HCl, isocratic mode, stability indicating assay, validation.

INTRODUCTION

Nicardipine HCl is chemically 2-[Benzyl(methyl)amino]ethyl methyl 1,4-dihydro-2,6-dimethyl-4-93-nitrophenyl)pyridine-3,5dicarboxylate mono hydrochloride.[1] It is official in Japanese Pharmacopeia and China pharmacopeia. It belongs to the class of 1,4-dihydropyridine calcium channel blockers. It is indicated for angina pectoris and hypertension.

ICH Q6A guidelines insists for the validated stability indicating assay methods for the quantification of drug substance and drug products for the products which are loaded for accelerated and longterm stability studies. The demonstration of specificity and the ability of the method to monitor a change in the chemical properties of the drug over time, invariably calls for a forced degradation study to be done on the drug substance and drug product. [2-5]

Many analytical methods for the assay of nicardipine in formulations and in pure were reported including spectrophotometry, [6-8] spectrofluorometric, [9] high performance liquid chromatography,[10,11] Investigations on forced degradation, degradation kinetics and photo stability of nicardipine in pure using drug solution were reported in literature. [12,13] Since there is a need for the characterization of drug – excipient, excipient substance related degradation products in drug products, the present study describes the development of a stability indicating assay method for the determination of Nicardipine hydrochloride in capsules as such.

MATERIALS AND METHODS:

Materials:

Equipment used:
The chromatographic separations were done using a modular HPLC system from Shimadzu, Kyoto, Japan. The modular system comprised of two LC-20ATvp, pumps, SPD M20A Photo diode array detector, SIL-20A auto sampler, CTO 10a, column oven, DGU 20A online degasser, system controller CBM 20A and data processed with LC solutions software. The second HPLC system used for precision studies was an integrated Shimadzu LC2010HT model equipped with quaternary pump system, UV-VIS detector, column oven, online degasser, auto injector systems and data processed with LC solutions software.

Chemicals and reagents used:
Nicardipine HCl working standard and nicardipine sustained release (SR) capsules, labeled claim 20mg were obtained from our F-R&D division. HPLC grade acetonitrile was obtained from Standard chemicals & reagents, Hyderabad, India. O-phosphoric acid, Hydrochloric acid 36%, Sodium hydroxide purified flakes, Hydrogen peroxide 30%, were obtained from Merck, Mumbai, India. 0.42µm, 0.2 µm membrane filters were obtained from pall life sciences, Mmbai, India; all the solutions were prepared in ultra-pure water obtained in- house from Milli Q water system (Millipore, NY, USA).

Chromatographic conditions:
Reversed phase C-18 columns of 250mmx4.6mm, 5µ were used of Kromasil, NY, USA, make. The detection was done at 235nm at a flow rate of 2mL/min with the injection volume of 100µL and ambient temperature maintained. The mobile phase consisted of acetonitrile- O-phosphoric acid 0.03%, mixture (40:60v/v) filtered through 0.42µm filter and degassed with vacuum spurge pump and the run time was set as 7min, column was equilibrated for 60min with mobile phase flowing through the system.

Experimental:
All the experiments were conducted under tungsten lamp avoiding UV light.

Preparation of mobile phase:
Dissolve 0.3ml of O-phosphoric acid in 1000ml of Milli Q water (0.03% H3PO4). Mix 600mL, 0.03% O-phosphoric and 400mL acetonitrile HPLC grade, to make mobile phase of 1000mL. Degas under vacuum.

Preparation of working standard solution:
Take about 50mg of Nicardipine HCl working standard into 100ml volumetric flask and dissolve in 25ml Mobile phase sonicate for 5min and makeup to the volume with mobile phase and shake. Filter through Whatman No 1
The assay results are indicated in table 1.

Preparation of sample solution:
Take sample powder of Nicardipine HCl, finely powdered pellets from about 5 capsules equivalent to 50mg of Nicardipine HCl into 100mL volumetric flask and dissolve in 25mL Mobile phase, sonicate for 20min and make up the volume with mobile phase and shake. Filter through 0.2µm filter; take 5ml of filtrate into 50mL volumetric flask and make up to the volume with mobile phase. Take 5 mL of the above solution into 50 mL volumetric flask and make up to the volume with mobile phase to attain a test concentration of about 2mcg/mL.

Preparation of matrix solution:
From the stock solutions of standard stock (10mg/mL, 1gm of Nicardipine HCl working standard in 100mL mobile phase) and excipient synthetic mixture (5gm of placebo pellets from capsules prepared at labeled claim concentration without the addition of the drug in 25mL acetonitrile and volume made up to 100mL with mobile phase, 5mg/mL), each 5mL additions are done to attain 50mg of drug and the excipient concentration 250mg.

System suitability parameters:
Six replicates of working standards at test concentration are injected and depending on the theoretical plate count and tailing factor, system suitability parameters were determined.

Analysis of sustained release capsules:
The proposed method was applied to the analysis of Nicardipine Hcl SR capsules. Labeled claim 20%w/w. The assay was calculated as

\[
\text{% Assay} = \frac{\text{Area(sample)}}{\text{Area(std.)}} \times \text{mg std/mg sample} \times \text{dil.factor} \times \text{std purity}
\]

The assay results are indicated in table 1.

Validation of the proposed method:
Validation of the method was done according to the ICH Q2 (R1) guidelines for specificity, accuracy, precision, linearity, and robustness.

Forced degradation studies in solid state:
In order to determine whether the method is stability indicating, forced degradation studies were conducted on Nicardipine HCl pellets taken from capsules. Initial assay was done for the formulation which was subjected to forced degradation studies. Each forced degradation samples were injected initially and at specified time periods 2hrs, 24th hr and 10th day by keeping the samples at 60°C, in dark and subjected to analysis by proposed method after the required dilutions. If degradation was sufficiently observed for a particular time period, the study was ceased at that specified time interval. The stress conditions were as follows

a)Acid degradation:
About 250mg (equivalent to 50mg of Nicardipine HCl) of Nicardipine HCl powder of finely powdered pellets from about 5capsules was taken into a 100mL volumetric flask and 5mL of 0.1N HCl was added and kept at 60°C for 2hrs in dark. After 2hrs the solution was cooled and neutralized with 0.1N NaOH and then diluted with mobile phase to attain the test concentration. Similarly degradation was performed with 1N and 5N strengths of HCl.

b)Base degradation:
About 250mg (equivalent to 50mg of Nicardipine HCl) of Nicardipine HCl powder, finely powdered pellets from about 5capsules was taken into a 100mL volumetric flask and 5mL of 0.1N NaOH was added and kept at 60°C for 2hrs in dark. After 2hrs the solution was cooled and neutralized with 0.1N HCl and then diluted with mobile phase to attain the test concentration. Similarly degradation was performed with 1N and 5N strengths of NaOH.

c)Oxidative degradation:
About 250mg (equivalent to 50mg of Nicardipine HCl) of Nicardipine HCl powder, finely powdered pellets from about 5capsules was taken into a 100mL volumetric flask and 5mL of 3% Hydrogen peroxide was added and kept at 60°C for the specified time periods in dark. After specified time periods, the solution was cooled then diluted with mobile phase to attain the test concentration. Similarly degradation was performed with 10% strengths of H$_2$O$_2$.

d)Photolytic degradation:
About 1g of Nicardipine HCl powder from finely powdered pellets from about 5capsules was taken in a petri dish and kept in UV chamber at 254&362nm for 24hrs and also another sample kept under fluorescent lamp for 24 hrs.

e)Thermal degradation:
About 1g of Nicardipine HCl powder from finely powdered pellets from about 5capsules was taken in a petri dish and kept in oven at 60°C for 24hrs and 10th day in dark condition.

RESULTS AND DISCUSSION:
The applied chromatographic conditions permitted good separation of
Nicardipine HCl. The method was simple since good separation was obtained without using the solid buffers and pH adjustment. Various trials were done using acetonitrile and water in different ratio and with 0.01% O-phosphoric acid and at specified conditions, good resolution was observed with the mobile phase and with a less run time of 7 min and the method validation parameters were as below.

System suitability:
The chromatographic separation was carried out to evaluate the chromatographic parameters like asymmetry of the peaks, resolution, theoretical plates. Figure 1 and 2 represents chromatograms of Nicardipine HCl Working standard and capsules. The resolution was obtained greater than 2, theoretical plates more than 2000, tailing factor less than 2 and the %RSD of the standard replicates is 0.21%. The results were shown in Table 2.

Table 2. System suitability parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>For Nicardipine HCl</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical plates</td>
<td>4025.5</td>
<td>&gt; 2000</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.5</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>Resolution</td>
<td>6.385</td>
<td>&gt; 2</td>
</tr>
<tr>
<td>%RSD of 5 replicates</td>
<td>0.21%</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>

Linearity and range:
The linearity of the method for Nicardipine HCl peak area response was examined at 6 points on a calibration curve in concentration ranges of 0.5-3 mcg/mL, shown in Figure 3. Linearity studies were conducted by three replicates of each concentration from matrix solution shown in Table 3. The regression equation was y = 148025x + 296.27. The correlation coefficient R² was 0.9997 indicating good linearity. The range of the method was 25-150% of the test concentration. The method was linear over the specified range.

![Image of calibration curve](image-url)

Figure 3. Calibration curve for the Nicardipine HCl

Table 3. Linearity of Nicardipine HCl

<table>
<thead>
<tr>
<th>% of Test concentration µg/mL</th>
<th>Concentration µg/mL</th>
<th>Average peak area*</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.5</td>
<td>75352</td>
<td>0.35</td>
</tr>
<tr>
<td>50</td>
<td>1</td>
<td>147866.7</td>
<td>0.26</td>
</tr>
<tr>
<td>75</td>
<td>1.5</td>
<td>222174.3</td>
<td>0.16</td>
</tr>
<tr>
<td>100</td>
<td>2</td>
<td>298259.7</td>
<td>0.12</td>
</tr>
<tr>
<td>125</td>
<td>2.5</td>
<td>365382.3</td>
<td>0.39</td>
</tr>
<tr>
<td>150</td>
<td>3</td>
<td>447296.7</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*Average of three replicates

Accuracy:
Accuracy was studied according to ICH guidelines, by spiking the known drug amounts to the synthetic mixture of the excipients used in the drug product. The accuracy was done over 50, 75, 100, 125 and 150% of the test concentration. The recoveries were calculated and listed in the Table 4. The recoveries were found be in the range of 98-102% which indicates the accuracy of the method.

<table>
<thead>
<tr>
<th>% of Concentration level</th>
<th>Average Recoveries*</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>101.23</td>
<td>0.25</td>
</tr>
<tr>
<td>75</td>
<td>101.41</td>
<td>0.16</td>
</tr>
<tr>
<td>100</td>
<td>101.6</td>
<td>0.12</td>
</tr>
<tr>
<td>125</td>
<td>101.06</td>
<td>0.61</td>
</tr>
<tr>
<td>150</td>
<td>101.7</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*Average of three replicates

Precision:

Repeatability:
Repeatability was performed by injecting six different sample solutions at 100% test concentration and the %RSD of the assays was found to be 0.73% as which indicates the method is repeatable.

Intermediate precision:
The precision was checked by assaying the samples on different days, different analysts, different equipment and different batches. The %RSD of intra-day precision was found to be 0.65%, which was done on same day at two point of times, same analyst and same equipment. The %RSD of the inter-day precision was found to be 0.46%, which was done on different days by two analysts, on different equipment and on different batches. The results indicated the preciseness of the method. The results are indicated in the Table 5.

Table 5. Precision

<table>
<thead>
<tr>
<th>Precision type</th>
<th>Average Assay*</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeatability</td>
<td>20.05</td>
<td>0.62</td>
</tr>
<tr>
<td>Intra day</td>
<td>20.2</td>
<td>0.65</td>
</tr>
<tr>
<td>Inter day</td>
<td>19.92</td>
<td>0.46</td>
</tr>
</tbody>
</table>

*Average of five replicates

Robustness:
Robustness was determined by varying the separation parameters: organic phase percentage (±2%), flow rate (±0.2 mL/min), wavelength (±2 nm). Three replicates of working standard solution are injected and % RSD of the peak areas is less than 1 which indicates the method is robust over the varied parameters and results are tabulated in the Table 6.

Table 6. Robustness

<table>
<thead>
<tr>
<th>Parameters changed</th>
<th>Variation</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>2.2 mL/min</td>
<td>0.43</td>
</tr>
<tr>
<td>Percent Organic phase</td>
<td>42%</td>
<td>0.086</td>
</tr>
<tr>
<td>Wave length</td>
<td>230 nm</td>
<td>0.21</td>
</tr>
</tbody>
</table>

*% RSD of three replicates

Specificity:
The specificity of the method was checked in the presence of excipients and degradation products from the stress studies. The drug peak was resolved from the excipients and degradation products indicating a resolution greater than 2, which indicates that the method is specific.

Degradation behavior:
All the injected samples were checked for peak purity index.

Acidic condition:
The drug was found to be liable to acid hydrolysis at higher strengths of acid 5N HCl. About 22% of degradation was seen at the end of the 2nd hr. No degradation was observed with lower strengths of 0.1N, 1N HCl at 2nd hr, since the degradation was observed with 5N, the study was stopped at this stage. The drug peak was well resolved from the other peaks with a resolution of 2.564. The results were given in the Table 7.

Table 7. Acid hyrolysis

<table>
<thead>
<tr>
<th>Strength of HCl</th>
<th>Initial assay mg</th>
<th>2ndhr assay mg</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1N HCl</td>
<td>20.15</td>
<td>20.21</td>
<td>No degradation</td>
</tr>
<tr>
<td>1N HCl</td>
<td>20.42</td>
<td>20.36</td>
<td>No degradation</td>
</tr>
<tr>
<td>5N HCl</td>
<td>19.85</td>
<td>16.2</td>
<td>Degradation observed</td>
</tr>
</tbody>
</table>

18% of degradation observed with 5N HCl

Base hydrolysis:
The drug was found to be resistant to base hydrolysis when it is studied in solid state. The study was performed till the 10th day with all the strengths, but no degradation was found to appear. This reveals that the drug was resistant to base hydrolysis when present in the solid state whereas it was reported that it degraded when in solution. The results were given in the Table 8.

Table 8. Base hydrolysis

<table>
<thead>
<tr>
<th>Strength of NaOH</th>
<th>Initial assay mg</th>
<th>2ndhr assay mg</th>
<th>24th hr assay mg</th>
<th>10th day assay mg</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1N NaOH</td>
<td>20.32</td>
<td>20.6</td>
<td>20.4</td>
<td>20.57</td>
<td>No degradation</td>
</tr>
<tr>
<td>1N NaOH</td>
<td>20.26</td>
<td>20.13</td>
<td>20.19</td>
<td>20.12</td>
<td>No degradation</td>
</tr>
<tr>
<td>5N NaOH</td>
<td>20.4</td>
<td>20.18</td>
<td>20.29</td>
<td>20.21</td>
<td>No degradation</td>
</tr>
</tbody>
</table>

No degradation observed in the alkaline medium when studied with pellets powder

Oxidative hydrolysis:
The drug found to be degraded initially and after 2hrs exposure with 10% Hydrogen peroxide. Initially there was about 2% degradation but after 2hr there was about 22% degradation. The drug did not degrade for lower concentration of 3% and since it degraded for 10% H₂O₂, the study was ceased at this point. The degradation peak was found at RT 1.24min and the drug peak is well resolved from the peaks with resolution greater than 2. The results were briefed in Table 9.

Table 9. Oxidative degradation

<table>
<thead>
<tr>
<th>Strength of H₂O₂</th>
<th>Initial assay mg</th>
<th>2ndhr assay mg</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3% H₂O₂</td>
<td>20.54</td>
<td>20.73</td>
<td>No degradation</td>
</tr>
<tr>
<td>10% H₂O₂</td>
<td>18.36</td>
<td>14.25</td>
<td>Degradation observed</td>
</tr>
</tbody>
</table>

2% of degradation was seen in initial injection when compared to initial assay of the product. 22% of degradation was observed after 2nd hr.

Photolytic degradation:
Nicardipine HCl was found to be degraded when exposed for UV light 256nm and 362nm for 24hrs. The peak purity index observed as low as 0.5 which indicated the degradation. So, all the experiments are to be done in dark conditions. The drug was found to be unaffected by the normal fluorescent light when exposed for 24hrs.

Thermal degradation:
Nicardipine HCl sample powder was unaffected by the heating process, since no degradation was found when the study was continued to 10th day.

Humid condition:
No degradation was found when the Nicardipine HCl pellets powder from about 2 capsules was exposed to higher humidity conditions like 80% RH. The results are tabulated in the Table 10.

Table 10. Photolytic, thermal and humid condition

<table>
<thead>
<tr>
<th>Parameter</th>
<th>24th hr</th>
<th>10th day</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV 254&amp;362nm</td>
<td>20.48</td>
<td>-</td>
<td>Degradation observed*</td>
</tr>
<tr>
<td>Fluorescent bulb</td>
<td>20.37</td>
<td>-</td>
<td>No degradation</td>
</tr>
<tr>
<td>Temperature of 60ºC</td>
<td>20.14</td>
<td>20.05</td>
<td>No degradation</td>
</tr>
<tr>
<td>80% RH</td>
<td>20.43</td>
<td>20.03</td>
<td>No degradation</td>
</tr>
</tbody>
</table>

* Peak purity index was found to be 0.5, with no change in peak area

CONCLUSION:
The study is an extension of the degradation behavior of Nicardipine HCl in formulation, the method is found to be specific for the drug in presence of excipients used in formulation and degradation products of the formulation it was simple since the runtime is less and no use of solid buffers. From the results it can be briefed that the method is accurate, linear and precise over the specified range. It can be concluded that the method can be routinely adopted for the assay of Nicardipine HCl in its capsules.

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