DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR ESTIMATION OF NIMODIPINE IN SOFT GELATIN CAPSULE

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ABSTRACT
Nimodipine is a calcium channel blocker, used in treatment of hypertension. A simple, precise, rapid and accurate isocratic reverse phase high performance liquid chromatography method was successfully developed and validated for estimation of nimodipine in soft gelatin capsule. Chromatographic separation was achieved on an Inertsil ODS-3V column (150 mm X 4.6 mm, 5 µm) using mobile phase containing methanol: tetrahydrofuran:water (30:20:50 v/v/v) at the flow rate 2.0 mL/min with UV detection at 235 nm. The column was kept at 40°C. A linear response was observed in the range of 60-180 µg/mL with correlation co-efficient of 0.9998. The mean percent recoveries of nimodipine in soft gelatin capsule were found to be in the range of 98.92-100.31%. The intraday and interday precision was found to be within limits. The proposed method has adequate specificity, sensitivity and reproducibility for quality control assay of nimodipine in soft gelatin capsule dosage form without any interference from excipients.

KEYWORDS: Nimodipine soft gelatin capsule, RP-HPLC, analytical method validation, calcium channel blocker.

INTRODUCTION
Nimodipine (3-O-(2-methoxyethyl) 5-O-propan-2-yl 2, 6-dimethyl-4-(3-nitrophenyl)-1, 4-dihydropyridine-3,5-dicarboxylate (Figure 1) is a 1,4-dihydropyridine calcium channel blocker. It has marked cerebrovascular dilating effects and lowers blood pressure, so used to treat hypertension [1, 2]. It is a yellow crystalline substance, practically insoluble in water [3].

Figure 1: Chemical Structure of nimodipine

Nimodipine is official in BP, EP and USP, but monograph of nimodipine soft gelatin capsule was not reported in all above pharmacopeias [4-7]. A survey of literature revealed that various
analytical methods like UV spectroscopic method, RP-HPLC, UPLC, LC-MS/MS, GC, and capillary electrophoresis have been reported for the estimation of Nimodipine in tablet, oral solution, infusion (IV), monkey plasma, serum, human plasma \[^8\text{-}32\]. Hence it was thought of interest to develop and validate RP-HPLC method for estimation of nimodipine in its soft gelatin capsule formulation.

**MATERIALS AND METHOD**

**Chemicals and Reagents**

Nimodipine API and soft gelatin capsule was gifted by Zydus Cadila Health Care, Moraiya, Ahmedabad, Gujarat, India. All other chemicals and solvents used were of HPLC grade.

**Instrument and Chromatographic Condition**

Quantitative HPLC was performed on high pressure liquid chromatography (Shimadzu LC2010® HPLC system, Japan), an Inertsil ODS-3V (150 mm X 4.6mm i.d., 5 µ particle size) and an auto injector with a 20 µL loop. The HPLC system was equipped with the software “Class-VP” (Shimadzu). The optimized mobile phase was consisting of methanol: tetrahydrofuran: water (30:20:50 v/v/v). It was filtered through a 0.45 µm PVDF filter. The mobile phase was degassed by ultra sonication for 15 min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 2.0 mL/min. The run time was set at 10 min and the column temperature was maintained at 40°C. The volume of injection loop was 20 µL. The diluent consisted of THF: IPA (50:50 v/v). The column was equilibrated for at least 30 min with the mobile phase flowing through the system, prior to injection of the solutions. The eluent was monitored at 235 nm and data were acquired, stored and analyzed with the software Class-VP.

**Preparation of standard solution**

Nimodipine (60 mg) was accurately weighed and transferred into 50mL volumetric flask. Diluent (20mL) was added and sonicated to dissolve and volume was made up to mark with methanol and aliquot 5 mL of stock solution into 50 mL volumetric flask and diluted up to mark with methanol (120µg/mL).

**Preparation of test solution**

Nimodipine soft gelatin capsule (10 capsules, labelled claim: 30 mg/capsule) was taken into 500mL volumetric flask. Distilled water (50 mL) was added and heated at 35°C temperature until it ruptured. Diluent (50 mL) was added and sonicated for 10 min and volume was made up to mark with methanol. It was centrifuge at 500RPM for 5 min and aliquot 5mL solution and
transferred into 25mL volumetric flask and diluted up to mark with methanol. This solution was filtered through 0.45µ PVDF filter (120µg/mL).

**Method Validation**
The develop method was validated as per ICH guideline for specificity, linearity, range, accuracy, precision, limit of detection, limit of quantitation and robustness parameter[33].

**System Suitability**
System suitability testing is an integral part of analytical procedures. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. System suitability test was performed by injecting five replicate injections of nimodipine solution (120µg/mL). The following parameters was determined: Number of theoretical plate, tailing factors, capacity factor and reproducibility.

**Specificity**
Specificity is the ability to assess unequivocally of the analyte in the presence of components which may be expected to be present. These might include impurities, degradants, matrix, etc. To perform the specificity blank, test solution and standard solution of nimodipine was injected in chromatographic system and chromatogram was recorded. The chromatograms of blank and test sample were compared with standard.

**Linearity and range**
The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample. The linearity of the method was determined at six concentration levels ranging from 60-180 µg/mL. Working standard solutions of nimodipine were prepared by suitable dilution of the standard stock solution with the diluent (methanol) to obtain concentration of nimodipine in the range of 60-180µg/mL. Each of these drug solutions (20 µL) were injected into the injector and the peak areas were recorded. The calibration curve was constructed by plotting amount injected (µg/mL) versus mean peak area.

**Precision**
It expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Intraday and interday precision were determined by analyzing samples of nimodipine at three different concentration level in triplicate within range. Intraday precision was performed by analyzing three different concentrations of nimodipine (60µg/mL, 120µg/mL, 180µg/mL) three times in a day and calculate percentage relative standard deviation (% RSD). Interday precision was
performed by analyzing three different concentrations (60µg/mL, 120µg/mL, 180 µg/mL) three times in different day and calculate percentage relative standard deviation (% RSD). Repeatability was performed by injecting nimodipine solution (120µg/mL) six times in chromatograph and calculate % RSD.

**Limit of detection (LOD) and Limit of Quantitation (LOQ):**
The lowest amount of analyte in a sample that can be detected, but not necessary quantify under the stated experimental conditions is known as LOD while the minimum amount of analyte in a sample that can be quantitated with suitable precision and accuracy is known as LOQ. The LOD and LOQ values were calculated by using calibration curve. The LOD and LOQ are calculated by using formula given in the ICH guideline Q2 (R1).

\[
\text{LOD} = \left( \frac{3.3 \times \sigma}{S} \right) /S \quad \text{and} \quad \text{LOQ} = \left( \frac{10 \times \sigma}{S} \right) /S
\]

Where, \( \sigma \) = Standard deviation of the Y intercept
\( S \) = Slope of the calibration curve equation

**Accuracy**
The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. To examine the accuracy of the developed method, recovery studies were carried out by standard addition method at three different concentration levels (80%, 100% 120%) in triplicate by spiking standard nimodipine solution in previously analyzed nimodipine soft gelatin capsule solution.

**Robustness**
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was studied by changing the flow rate (1.9 mL/min and 2.1 mL/min) and column oven temperature (38ºC and 42ºC).

**RESULT AND DISCUSSION**
The goal of the present study was to develop a rapid, precise, accurate and rapid RP-HPLC method for the analysis of nimodipine in its soft gelatin capsule formulation, using the C18 column with UV detection and validate the developed method as per ICH guideline. In order to obtain suitable peak shape, retention time and separation of nimodipine peak from excipients peak methanol, tetrahydrofuran and water were used in mobile phase. Satisfactory peak shape (tailing factor 0.96 and theoretical plate >4000) was obtained using the mobile phase methanol:
tetrahydrofuran: water (30:20:50 v/v/v) with a flow rate 2 mL/min. As nimodipine showed maximum absorption at 235 nm, the detector was set at 235 nm.

**TABLE NO: 1. RESULT OF SYSTEM SUITABILITY TEST**

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Parameters</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Retention time (min)</td>
<td>6.40±0.1</td>
</tr>
<tr>
<td>2</td>
<td>Capacity factor (k')</td>
<td>2.75</td>
</tr>
<tr>
<td>3</td>
<td>Tailing factor (T)</td>
<td>0.96</td>
</tr>
<tr>
<td>4</td>
<td>Injection Repeatability (% RSD)</td>
<td>0.10</td>
</tr>
<tr>
<td>5</td>
<td>No. of theoretical plates (N)</td>
<td>4243.2</td>
</tr>
</tbody>
</table>

Chromatogram of nimodipine standard and nimodipine soft gelatin capsule was shown in Figure 2 and Figure 3, respectively. It showed nimodipine peak around 6.40±0.1 min which is separated from the excipients peak. It indicates that the develop method successfully separate the drug and excipients peak. The method was found to be specific. There was no peak found in blank sample chromatogram at nimodipine peak retention time.

![Figure 2: Chromatogram of Standard solution](image2)

![Figure 3: Chromatogram of test solution](image3)
The developed method was linear in the concentration range from 60-180 µg/mL of nimodipine. The calibration curve was constructed by plotting concentration (X) versus the mean peak area of nimodipine (Y) (Figure 4). The correlation coefficient was found to be 0.9998. The regression equation was found as \( Y = 37167X - 45673 \). Where, Y is the peak area of nimodipine and X, the concentration of measured solution in µg/mL. The results showed excellent correlation between the peak area and the concentration of nimodipine in the range tested. The calculated LOD and LOQ values were found to be 0.38µg/mL and 1.17µg/ml, respectively.

![Figure 4: Calibration curve of Nimodipine](image)

The mean absolute recovery determined by spiking known amounts of nimodipine solution to the previously analyzed nimodipine soft gelatin solution in triplicate. The results were shown in Table 2. The average percent recoveries obtained as 98.92-100.31% indicate good accuracy of the method.

**TABLE NO 2: RESULT OF ACCURACY STUDY**

<table>
<thead>
<tr>
<th>Level</th>
<th>Pre-analysed sample (µg/mL)</th>
<th>Amount of nimodipine added (µg/mL)</th>
<th>Concentration (µg/mL)</th>
<th>Amount recovered Mean ± S.D(n=3)</th>
<th>(%) Recovery Mean±S.D(n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>60</td>
<td>36</td>
<td>96</td>
<td>94.96±0.06</td>
<td>98.92±0.06</td>
</tr>
<tr>
<td>100%</td>
<td>60</td>
<td>60</td>
<td>120</td>
<td>118.83±0.35</td>
<td>99.02±0.29</td>
</tr>
<tr>
<td>120%</td>
<td>60</td>
<td>84</td>
<td>144</td>
<td>144.05±1.00</td>
<td>100.31±0.69</td>
</tr>
</tbody>
</table>
This method was validated for intraday and interday precision. Intraday and interday precision were found to be 0.05-0.08% and 0.03-0.20% respectively, indicating good precision. Repeatability was found to be 0.11%. The results of precision were shown in Table 3.

**TABLE NO 3: RESULTS OF INTRADAY AND INTERDAY PRECISION STUDY**

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Intraday precision</th>
<th>Inter day precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak area</td>
<td>%RSD</td>
</tr>
<tr>
<td></td>
<td>Mean± S.D (n=3)</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>2188428.7±1790.397</td>
<td>0.08</td>
</tr>
<tr>
<td>100</td>
<td>4365331.7±2168.373</td>
<td>0.05</td>
</tr>
<tr>
<td>180</td>
<td>6623880.3±4141.453</td>
<td>0.06</td>
</tr>
</tbody>
</table>

The proposed method was found to be robust because change in flow rate and column oven temperature did not change system suitability criteria and assay deviate not more than 2.0%. In order to demonstrate the stability of both standard and sample solutions during analysis, both the solutions were analyzed over a period of 60 h. The results showed that retention time and peak area of nimodipine almost unchanged and no significant degradation was observed for 24 h.

The proposed method was successfully applied to the analysis of soft gelatin capsule of nimodipine (30 mg/capsule) and the results obtained are given in Table 4. The average drug content was found to be 100.04%.

**TABLE NO 4: ESTIMATION OF NIMODIPINE IN SOFT GELATIN CAPSULE BY HPLC METHOD**

<table>
<thead>
<tr>
<th>Label Claim (mg/capsule)</th>
<th>Peak Area Mean ± S.D. (n=3)</th>
<th>Amount recovered Mean ± S.D. (n=3)</th>
<th>% Assay Mean ± S.D. (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>4284174±23144.89</td>
<td>29.99±0.11</td>
<td>100.04±0.28</td>
</tr>
</tbody>
</table>

**CONCLUSION**

The results of the study showed that the proposed HPLC method is simple, rapid, precise, accurate, specific and sensitive for determination of nimodipine in its soft gelatin capsule dosage form without any interference from the excipients. Hence, this method is suitable for routine quality control assay of nimodipine in its soft gelatin capsule dosage form.
ACKNOWLEDGEMENT

The authors are thankful to L. M. College of Pharmacy, Navrangpura, Ahmadabad and Zydus Cadila Healthcare, moraiya, Ahmadabad for providing gift sample and research facility to complete this research work.

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