Estimation of nimodipine and piperine in pharmaceutical dosage forms by simultaneous equation method

Hardik P Patel1, Natvar J Patel1
1S.K.Patel College of Pharmaceutical Education and research, Ganpat University, Kherva, Mehsana, Gujarat, India.

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ABSTRACT

A new simultaneous equation method was developed and validated for the determination of Nimodipine (NIMO) and Piperine (PIPE) in lab prepared sustained release tablets. Calibration curves for Nimodipine and Piperine over concentration range of 1-20 and 1-12 µg/mL respectively were plotted and molar absorptivity for both the drugs were calculated at both the wavelengths of 237 nm (λ-max of Nimodipine) and 342 nm (λ-max of Piperine). The results of analysis have been validated statistically and by recovery studies. The value of standard deviation was satisfactory and recovery studies ranging from 100.57-101.07 % for Nimodipine and 98.12-99.16 for Piperine were indicative of the accuracy and precision of the proposed method. The results of the assay are in good agreement with the label amount. The method was found to be simple, rapid, and accurate and can be adopted in routine analysis of these drugs in formulations. Due to these attributes, the proposed method could be used for routine analysis of these drugs in combined dosage forms.

Key words: Nimodipine; Piperine; Simultaneous equation method.

INTRODUCTION


The alkaloid piperine is a major component of black pepper (Piper nigrum Linn) and long pepper (Piper longum Linn). Piperine has previously been shown to inhibit several cytochrome P450-mediated pathways in animal models (Atal et al., 1981). Accordingly, treatment of rodents with piperine resulted in increased plasma concentrations of several compounds such as theophylline, phenytoin, rifampin, and propranolol (Atal et al., 1981; Velpandian T., et al., 2001). NIMO is also drug which metabolized by cytochrome P4503A4 enzyme; this enzyme is inhibited by PIPE, therefore combination of NIMO and PIPE will increase the bioavailability of NIMO in human; hence the objective of the present study was to develop simple, rapid, accurate and specific UV spectrophotometric method for simultaneous determination of NIMO and PIPE in prepared pharmaceutical dosage forms.

A literature survey revealed that there is not reported any simultaneous equation method for analysis of NIMO and PIPE in pharmaceutical dosage formu-
diluted up to the mark with methanol then transfer 1mL of above solution into 10mL volumetric flask and diluted up to the mark with methanol to get a final concentration 3µg/mL of NIMO and 2 µg/mL of PIPE.

Validation studies:
Calibration curve (Linearity):
Calibration curves were plotted over a concentration range of 1-20µg/mL for NIMO and 1-12µg/mL for PIPE. Accurately measured standard stock solutions of NIMO (50, 125, 250, 375, 500, 625, 750, 875, 1000 µL) and standard stock solutions of PIPE (50, 100, 150, 200, 250, 300, 350, 400, 500, 600 µL) were transferred to separate series of 10mL volumetric flasks and diluted up to the mark with methanol. The absorbance of each solution was measured at both the wavelengths 237 nm and 342 nm. Calibration curves were constructed for NIMO & PIPE by plotting absorbance versus concentrations at both wavelengths. Each reading was average of six determinations.

Accuracy (% Recovery):
It is defined as closeness of agreement between the actual (true) value and analytical value and obtained by applying test method for a number of times. Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets (NIMO 3 µg/mL and PIPE 2µg/mL) with three different concentrations of standards (NIMO 1.5, 3, 4.5µg/mL and PIPE 1, 2, 3µg/mL).

Method precision:
Precision, repeatability of the results were evaluated by six replicate determinations of 5µg/mL concentration. Evaluation of intermediate precision, the results over the concentration range 1-20µg/mL for NIMO and 1-12µg/mL for PIPE was evaluated by six replicate determinations to estimate intraday variation and another six replicate determinations on different six days to estimate interday variation. The coefficients of variation (CV) values at these concentration levels were calculated.

Limit of Detection and Limit of Quantification:
The limit of detection (LOD) and the limit of quantification (LOQ) of the drugs were derived by calculating the signal-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations as per international conference on harmonization (ICH) guidelines.

LOD= 3.3 x s/S, LOQ= 10 x s/S
Where s = The standard deviation of the response and S = Slope of calibration curve.

Estimation of NIMO and PIPE from pharmaceutical dosage form:
The absorptivity coefficients of the two drugs were determined using calibration curve equation. The concentration of NIMO and PIPE were determined using the following simultaneous equations.

\[
\begin{align*}
C_1 & = \frac{A_1 a_1 y_1 - A_2 a_2 y_2}{a_1 y_1 - a_2 y_2} \quad (1) \\
C_2 & = \frac{A_1 a_2 x_1 - A_2 a_1 x_2}{a_2 x_1 - a_1 x_2} \quad (2)
\end{align*}
\]

C = Concentration of Nimodipine C_2 = Concentration of Piperine. A_1, A_2 = Absorbance of the mixture at 237nm and 342nm respectively, a_1, & a_2, = are denoted absorbance of Nimodipine at 237nm & 342nm respectively, a_1, y_1 & a_2, y_2 = are denoted absorbance of Piperine at 237nm & 342nm respectively.
Table 1: Data of recovery study of NIMO and PIPE

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount taken (µg/mL)</th>
<th>Amount added (µg/mL)</th>
<th>Amount found (µg/mL)</th>
<th>% Recovery ± S.D. (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIMO</td>
<td>3</td>
<td>1.5</td>
<td>4.52</td>
<td>100.57 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.0</td>
<td>6.05</td>
<td>100.99 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.5</td>
<td>7.58</td>
<td>101.07 ± 0.31</td>
</tr>
<tr>
<td>PIPE</td>
<td>2</td>
<td>1.5</td>
<td>2.97</td>
<td>99.16 ± 1.17</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.0</td>
<td>3.94</td>
<td>98.25 ± 1.25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.0</td>
<td>4.90</td>
<td>98.12 ± 1.33</td>
</tr>
</tbody>
</table>

Table 2: Optical and Regression characteristics and validation parameters of simultaneous equations method for analysis of NIMO and PIPE

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NIMO</th>
<th>PIPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer’s Law Limit (µg/mL)</td>
<td>1-20</td>
<td>1-20</td>
</tr>
<tr>
<td>Molar Absorptivity ([mol·cm⁻¹·µg⁻¹])</td>
<td>0.234×10⁶</td>
<td>0.538×10⁶</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg/mL·cm⁻¹)</td>
<td>0.0157</td>
<td>0.0164</td>
</tr>
<tr>
<td>Correlation Coefficient (r)</td>
<td>0.997</td>
<td>0.998</td>
</tr>
<tr>
<td>Standard Deviation (SD)</td>
<td>0.0036</td>
<td>0.0019</td>
</tr>
<tr>
<td>Precision</td>
<td>0.52±1.02</td>
<td>0.34±1.53</td>
</tr>
</tbody>
</table>

Table 3: Application of the proposed method to the pharmaceutical dosage forms

<table>
<thead>
<tr>
<th>Formulation</th>
<th>NIMO</th>
<th>PIPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount labeled (mg)</td>
<td>Amount found (mg)</td>
<td>% Found S.D. (n=3)</td>
</tr>
<tr>
<td>Batch-1</td>
<td>30</td>
<td>30.12</td>
</tr>
<tr>
<td>Batch-2</td>
<td>30</td>
<td>29.75</td>
</tr>
<tr>
<td>Batch-3</td>
<td>30</td>
<td>30.52</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION:
In this method the standard stock solutions of NIMO and PIPE were prepared in methanol. Calibration curves of NIMO and PIPE over concentration range of 1-20µg/mL and 1-12µg/mL respectively were plotted and molar absorptivity of both drugs were calculated at both the wavelengths 237nm (?-max of NIMO) and 342nm (?-max of PIPE). It is evident from the spectra of NIMO and PIPE that these drugs obey the Lambert-beer’s law at all the wavelength. Calibration curve of NIMO and PIPE at 237 nm are shown in Figure 4 and 6 respectively, while calibration curve at 342 nm are shown in Figure 5 and 7 respectively. The recovery study of NIMO and PIPE are mentioned in Table 1. The optical and regression characteristics and validation parameters are reported in Table 2.

Application to the pharmaceutical dosage form
The proposed validated method was successfully applied to determine NIMO and PIPE in bulk powder and in tablet dosage forms. Results shown in Table 3 indicate there is no interference of the excipients with the peaks of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of NIMO and PIPE in pharmaceutical dosage forms.

CONCLUSION:
All the factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive and rapid and can be applied successfully for the estimation of NIMO and PIPE in bulk and in pharmaceutical formulations without interference and with good sensitivity.

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REFERENCES:

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