Simultaneous Spectrophotometric Determination of Naproxen and Pantoprazole in Pharmaceutical Dosage Form

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Abstract
Two simple, but accurate, precise, reproducible, without separation and economical procedures for simultaneous estimation of naproxen and pantoprazole in combined capsule dosage form have been developed. One method employs solving of simultaneous equations using 262 nm and 289 nm as two analytical wavelengths for both drugs in methanol. The other method is Q- value analysis based on measurement of absorptivity at 262 nm and at isoabsorptive point 310 nm showing linearity in concentration range of 10.0- 50.0 $\mu g$ ml$^{-1}$ for naproxen and 8.0- 18.0 $\mu g$ ml$^{-1}$ for pantoprazole. The method was validated with respect to accuracy, precision, limit of detection and limit of quantitation. The proposed method is recommended for routine analysis since it is rapid, simple, accurate and also sensitive and without need to heat and organic solvent extraction.

Key words: Naproxen, Pantoprazole, Q- analysis, Simultaneous equation method.

Introduction
Naproxen [(+)-2-(6- methoxy-2 naphtyl) propionic acid], is a non- steroidal anti-inflammatory drug that also presents analgesic and antipyretic properties. Naproxen is extensively used in the treatment of many diseases e.g. Rheumatoid arthritis, acute gout and primary dysmenorrhea[1].Similar other non- steroidal, anti-inflammatory drugs, it inhibits the biosynthesis of prostaglandins. The United States Pharmacopeia [2] reports high performance liquid chromatography (HPLC) method for the determination of naproxen tablets. Several analytical methods have also been reported for the determination of naproxen in pharmaceutical preparations including UV-visible spectrophotometry [3-5] spectrofluorimetry [6-8], phosphorimetry [9,10], voltammetry [11], high-performance liquid chromatography [12-14], capillary
electrophoresis [15,16], coulometry[17] and oscilometric titration [18].
Pantoprazole sodium 5-(difuromethoxy)-2-[(3,4- dimethoxy-2- pyridinyl) methyl] sulfinyl]- 1H- benzimidazolesesquihydate, proton- pump inhibitor, inhibits gastric acid by blocking the H⁺ / K⁺ adenosine triphosphatase enzyme system of the gastric parietal cell. It is used for short- term treatment of erosion and ulceration of the esophagus. Different analytical methods are reported in the literature for the assay of pantoprazole sodium in dosage forms and in biological fluids including spectrophotometry [19-24], TLC [25], and HPTLC [26-28]. The present study involves the simultaneous estimation of naproxen and pantoprazole in combined dosage form.

Experimental
Instrumentation
UV- Visible spectrophotometer, model UV-1700 (Shimadzu, Japan) having cells with 1-cm light path. Shimadzu electronic balance was used for weighing the samples. Naproxen (96.7% purity) was provided by Strides Arcolab, Bangalore, India and Pantoprazole (98.5%) was supplied from Cresant therapeutics limited, Hyderabad, India. All other chemicals and solvents used were of analytical grade.

Preparation of standard stock solutions
A) Standard NAP stock solution (1mg/ml)
Pure form of naproxen powder (50.0mg) was accurately weighed and transferred into 50 ml volumetric flask and dissolved in methanol for further it diluted by 50 ml methanol (1000μg/ml). The stock solution is further diluted to get the required working standard solutions.
B) Standard PANTO stock solution (1 mg.ml⁻¹)
Pure form of pantoprazole powder (50.0mg) was accurately weighed and transferred to 50 ml volumetric flask and dissolved in methanol. Then the solution was diluted to 50 ml with methanol to prepare stock solution (1000 μg.ml⁻¹). The stock solution is then diluted with methanol to get the stock solution of concentration 10μg.ml⁻¹. This stock solution is used for preparing the working standard solutions.

Determination of Absorption Maxima
The working standard stock solutions of naproxen and pantoprazole were scanned in the range of 200- 400 nm against methanol as a blank. Naproxen and pantoprazole showed absorbance maxima at 262nm and 289nm respectively. The overlain spectra (λ_max) of both drugs was recorded (isoabsorptive point) at 310nm. When compared to other solvents, best results were found when methanol was used as solvent and very less interference was observed.

Calibration curves (linearity)
A) Calibration curve of naproxen
A calibration curve was plotted over a concentration range of 10.0 – 50.0 μg.ml⁻¹, using the stock solution of concentration 1000 μg/ml. Accurately measured standard stock solution of naproxen (0.1, 0.2, 0.3, 0.4 and
0.5 ml) were transferred to a separate series of 10.0 ml of volumetric flasks and diluted to the mark with methanol. The absorbance of each solution was measured at 262 nm. Calibration curve was constructed by plotting absorbance versus concentrations at 262 nm. Each reading is the average of three determinations.

B) Calibration curve of pantoprazole

curve was plotted over a concentration range of 8.0 – 18.0 µg/ml, using the stock solution of concentration 100 µg/ml. Accurately measured standard stock solution of pantoprazole (0.8, 1.0, 1.2, 1.4, 1.6 and 1.8 ml) were transferred to a separate series of 10.0 ml of volumetric flasks and diluted to the mark with methanol. The absorbance of each solution was measured at 289 nm. Calibration curve was constructed by plotting absorbance versus concentrations at 289 nm. Each reading is the average of three determinations.

Method I (Simultaneous equation method)

Two wavelengths selected for the method are 262 nm and 289 nm that provide absorption maxima of naproxen and pantoprazole respectively in methanol. The stock solutions of both drugs were measured at the selected wavelengths and absorptivities (a, 1%, 1 cm) for both drugs at both wavelengths were determined as mean of three independent determinations. Data are represented in Figure 1 and 2 respectively.

\[ \begin{align*}
CX &= A_2 a y_1 - A_1 a y_2 / a x_1 a y_1 - a x_1 a y_2 \quad \text{Eq (i)} \\
CY &= A_1 a x_2 - A v a x_1 / a x_1 a y_1 - a x_1 a y_2 \quad \text{Eq (ii)}
\end{align*} \]

Where, \( A_1 \) and \( A_2 \) are the absorbances of mixture at 262 nm and 289 nm respectively, \( a x_1 \) and \( a x_2 \) are absorptivities of naproxen at \( \lambda_1 \) and \( \lambda_2 \) respectively and \( a y_1 \) and \( a y_2 \) are absorptivities of pantoprazole at \( \lambda_1 \) and \( \lambda_2 \) respectively. \( CX \) and \( CY \) are concentrations of naproxen and pantoprazole respectively.

Method II (Absorption ratio or Q-analysis method)

From the overlain spectrum of naproxen and pantoprazole, two wavelengths were selected one at 310 nm which is the isoabsorptive point for both drugs and the other at 262 nm which
is $\lambda_{\text{max}}$ of naproxen (Figure 3).

**Figure 3.** The curve showing the isobestic point at 310 nm.

The absorbances of the sample solutions prepared in a similar manner as in the previous method were measured and the absorptivity values for both drugs at the selected wavelengths were also calculated and are represented in Figures 4 and 5 respectively.

**Figure 4.** Calibration graph of naproxen at $\lambda_{1}$ and isobestic point.

**Figure 5.** Calibration graph of pantoprazole at $\lambda_{1}$ and isobestic point.

The method employs $Q$ - values and the concentrations of drugs in sample solution were determined using the following formula:

For Naproxen:

$$C_1 = \frac{Q_0 - Q_2}{Q_1 - Q_2} \times \frac{a_1}{A}$$

For Pantoprazole:

$$C_2 = \frac{Q_0 - Q_1}{Q_2 - Q_1} \times \frac{a_2}{A}$$

Where,

$A$= Absorbance of sample at isoabsorptivity point, $a_1$ and $a_2$ = Absorptivies of naproxen and pantoprazole respectively at isoabsorptive point.

Application of proposed method for the determination of naproxen and pantoprazole in capsules

Ten capsules dosage form (Arthopan) manufactured by Cresant Therapeutics, Hyderabad, India were used. Each capsule contains naproxen 250 mg and pantoprazole 50 mg. The contents of the capsules were weighed and mixed thoroughly. For analysis of the drug, a standard addition method was used. To bring the two drugs in the linearity
range the quantity of powder equivalent to 5 mg of naproxen and 5mg of pantoprazole was weighed and dissolved in 30.0 ml of methanol and sonicated for 10 min. Then the solution was filtered through whatman filter paper no. 41 and then final volume of the solution was made up to 50.0 ml with methanol to get a stock solution containing 100 µg/ml of naproxen and 100 µg.ml\(^{-1}\) of pantoprazole. Appropriate aliquots of naproxen and pantoprazole within the Beer’s law limit were taken. In Method I, the concentration of both naproxen and pantoprazole were determined by measuring the absorbance of the sample at 262 nm and 289 nm. Values were substituted in the respective formula to obtain concentrations. Results of capsule analysis are shown in Table 1.

**Table 1.** Analysis of Capsule Formulation for naproxen by Method I and Method II.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Naproxen</th>
<th>METHOD I</th>
<th>Naproxen</th>
<th>METHOD II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/capsule</td>
<td>% Purity</td>
<td>mg/capsule</td>
<td>% Purity</td>
</tr>
<tr>
<td>1.</td>
<td>243.09</td>
<td>97.24</td>
<td>245.98</td>
<td>98.39</td>
</tr>
<tr>
<td>2.</td>
<td>240.05</td>
<td>96.02</td>
<td>248.09</td>
<td>99.2</td>
</tr>
<tr>
<td>3.</td>
<td>232.59</td>
<td>93.03</td>
<td>246.69</td>
<td>98.67</td>
</tr>
<tr>
<td>Mean</td>
<td>238.57±0.31193</td>
<td></td>
<td>246.69±0.6199</td>
<td></td>
</tr>
<tr>
<td>% RSD</td>
<td>2.26</td>
<td></td>
<td>0.43</td>
<td></td>
</tr>
</tbody>
</table>

RSD = Relative Standard Deviation

For Method II, the concentrations of both naproxen and pantoprazole were determined by measuring absorbance of the sample at 262 nm and 310 nm and values were substituted in the respective formula to obtain concentrations. Results of capsule analysis are shown in Table 2.

**Table 2.** Analysis of Capsule Formulation for pantoprazole by Method I and Method II.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Pantoprazole</th>
<th>METHOD I</th>
<th>Pantoprazole</th>
<th>METHOD II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/capsule</td>
<td>% Purity</td>
<td>mg/capsule</td>
<td>% Purity</td>
</tr>
<tr>
<td>1.</td>
<td>19.09</td>
<td>95.45</td>
<td>18.99</td>
<td>94.95</td>
</tr>
<tr>
<td>2.</td>
<td>19.49</td>
<td>97.45</td>
<td>19.01</td>
<td>95.5</td>
</tr>
<tr>
<td>3.</td>
<td>19.54</td>
<td>97.7</td>
<td>19.09</td>
<td>95.45</td>
</tr>
<tr>
<td>Mean</td>
<td>19.49</td>
<td>97.7</td>
<td>19.01</td>
<td></td>
</tr>
<tr>
<td>% RSD</td>
<td>1.27±0.1424</td>
<td></td>
<td>0.28± 0.03055</td>
<td></td>
</tr>
</tbody>
</table>

RSD = Relative Standard Deviation.

(Each capsule contains 250.0mg of naproxen and 20.0mg of pantoprazole)
**Method validation**

**Linearity**

The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation proportional to the concentration of analyte in samples within a given range. The range of analytical method is the interval between upper and lower level of analyte including levels that have been demonstrated to be determined with precision and accuracy using the method. The linear response of naproxen and pantoprazole were determined by analysing three independent levels of the calibration curve in the range of 10.0-50.0 $\mu$g.ml$^{-1}$ and 8.0 – 18.0 $\mu$g/ml$^{-1}$ respectively for naproxen and pantoprazole in triplicate.

**Precision**

The precision is the measure of either the degree of reproducibility or repeatability of analytical method. It provides an indication of random error. The precision of an analytical method is usually expressed as the standard deviation, relative standard deviation or coefficient of variance of a series of measurements.

**A) Repeatability (Precision on replication)**

It is a precision under a same condition (Same analyst, same apparatus, short interval of time and identical reagents) using same sample. Method precision of experiment was performed by preparing the standard solution of naproxen (10$\mu$g/ml$^{-1}$) and pantoprazole (10$\mu$g.ml$^{-1}$) for six times and analysed as per the proposed method. Percentage relative standard deviation (%RSD).

**B) Intermediate precision (Reproducibility)**

It expresses within the laboratory variations as on different days analysis or experiment within the laboratory. Variation of results within the same day is called intra-day precision and variation of results amongst days called inter-day precision. The intra-day precision (C.V) was determined for standard solutions of naproxen (10.0-50.0 $\mu$g/ml$^{-1}$) and pantoprazole (8.0-18.0 $\mu$g.ml$^{-1}$) for three times on the same day. The inter-day precision (C.V) was determined for standard solutions of naproxen and pantoprazole for three days.

**Accuracy (% Recovery)**

Accuracy of an analysis is determined by systemic error involved. It is defined as closeness of agreement between the actual (true) value and analytical value, 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 (Table 3). It is the measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by spiking precisely analysed samples of the capsules (naproxen 10$\mu$g. ml$^{-1}$and pantoprazole 8$\mu$g/ml$^{-1}$) with three different concentrations of standards (naproxen 10, 20,30 $\mu$g.ml$^{-1}$ and pantoprazole 8, 10, 12 $\mu$g.ml$^{-1}$).
Table 3. Recovery studies.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Naproxen</th>
<th>Pantoprazole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount Standard added (µg/ml)</td>
<td>Amount of Sample added (µg/ml)</td>
</tr>
<tr>
<td>1.</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>2.</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>3.</td>
<td>30</td>
<td>10</td>
</tr>
</tbody>
</table>

Limit of Detection

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantified under the stated experimental conditions. Limits of detection can be calculated using following equation as per ICH guidelines.

\[ \text{LOD} = 3.3 \times N/S \]

Where, \( N \) is the standard deviation of the peak areas of the drug and \( S \) is the slope of the corresponding calibration curve.

Results and Discussion

The overlain spectra of naproxen and pantoprazole exhibit \( \lambda_{max} \) at 262 nm and 289 nm respectively which are quite separated from each other. The optical characteristics are given in Table 4.

Additionally one isoabsorptive point was observed at 310 nm. This wavelength was selected for simultaneous estimation of naproxen and pantoprazole for Q-value analysis and it is assumed to be sensitive to wavelength. The optical characteristics are given in Table 5.

Table 4. Linear regression analysis data with their respective values for Method I.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Naproxen</td>
</tr>
<tr>
<td>Beer’s law limits (µg/ml)</td>
<td>262 nm</td>
</tr>
<tr>
<td>Molar absorptivity (L.mol(^{-1}).cm(^{-1}))</td>
<td>5.62 x 10(^3)</td>
</tr>
<tr>
<td>Correlation coefficient (R)</td>
<td>0.998</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg.cm(^{-2}))</td>
<td>0.040</td>
</tr>
</tbody>
</table>
Table 5. Linear regression analysis data with their respective values for Method II.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Naproxen</th>
<th>Pantoprazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer’s law limits (µg/ml)</td>
<td>10.0 -50.0</td>
<td>10.0 -50.0</td>
</tr>
<tr>
<td>Molar absorptivity (L.mol⁻¹.cm⁻¹)</td>
<td>5.62 x10⁻³</td>
<td>1.036 x 10⁻³</td>
</tr>
<tr>
<td>Correlation coefficient (R)</td>
<td>0.998</td>
<td>0.999</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg.cm⁻²)</td>
<td>0.040</td>
<td>0.22</td>
</tr>
<tr>
<td>Regression equation (Y)</td>
<td>Y = 0.020X + 0.016</td>
<td>Y = 0.009X - 0.000</td>
</tr>
<tr>
<td>Slope, b</td>
<td>0.020</td>
<td>0.009</td>
</tr>
<tr>
<td>Intercept, a</td>
<td>0.016</td>
<td>0.000</td>
</tr>
<tr>
<td>Relative standard deviation%</td>
<td>1.88</td>
<td>1.11</td>
</tr>
<tr>
<td>Limit of detection (µg/ml)</td>
<td>0.70</td>
<td>0.366</td>
</tr>
<tr>
<td>Limit of quantification (µg/ml)</td>
<td>2.1</td>
<td>1.098</td>
</tr>
</tbody>
</table>

Standard calibration curves for naproxen and pantoprazole were linear with correlation coefficients (r) values of 0.996-0.999 at all selected wavelengths and the values are the average of three readings with standard deviation in the range of 0.0030 – 0.0121. The calibration curves were repeated three times in a day and the average % RSD was found to be 1.11-2.71 % for naproxen and 2.43-0.77 % for pantoprazole at all the selected wavelengths, similarly the method was repeated for three days and the average % RSD was found to be 1.15 % for naproxen and 2.58 % for pantoprazole. The accuracy of the method was confirmed by recovery studies from capsule at three different levels of standard additions. Recovery in the range of 98.0 – 101.27 % justifies the accuracy of the method. The LOD values were found to be 0.7, 0.126, 0.366 and 0.83, 0.25, 0.47 for naproxen and pantoprazole respectively at 262 nm, 289 nm and 310nm. The LOQ values were found to be 2.1, 0.378, 1.098 and 2.475, 0.741, 1.41 for naproxen and pantoprazole respectively at 262 nm, 289 nm and 310nm. Based on various optical and validation parameters, method II is
more sensitive and reliable method compared to method I.

Conclusion
All these 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 naproxen and pantoprazole in pharmaceutical formulation without any interference.

Acknowledgement
The authors are thankful to Strides Arcolab for providing samples of pure drug of naproxen and Cresant therapeutics Ltd for pantoprazole. The authors are also thankful to Dr.H.G. Shivakumar, Principal of JSS College of Pharmacy for his encouragement to carry out this work.

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