A VALIDATED SIMULTANEOUS ESTIMATION OF DOXYLAMINE SUCINNATE AND PYRIDOXINE HYDROCHLORIDE BY UV – SPECTROPHOTOMETRIC METHOD IN BULK AND FORMULATION

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ABSTRACT

A simple and rapid UV – Spectrophotometric method was developed and validated for the simultaneous estimation of Doxylamine succinate and Pyridoxine HCl in combined dosage forms. The said combination is used in treating morning sickness associated with pregnancy. For the simultaneous estimation of both drugs, 260 nm and 285 nm were selected as wavelength of analysis. The linearity range was taken 5-50μg/ml for Doxylamine and 2-18µg/ml for Pyridoxine which showed correlation coefficient of 0.9995 and 0.9998 respectively. The method was validated for accuracy and precision and % RSD was found to be within the acceptable limits. The percentage recoveries for both drugs were in the range of 95.5% – 98.33%. The LOD and LOQ of both drugs indicate that the method is sensitive. The assay of the method was in good agreement with the standards and enables the method to be adapted for routine analysis of the both drugs in combined dosage forms.

Keywords: Doxylamine succinate, Pyridoxine Hydrochloride, Simultaneous equations, Morning Sickness.

INTRODUCTION

Morning sickness is a common discomfort seen in most pregnant woman [1]. There was a need to treat this discomfort through a safe and effective medication so as to avoid birth defects. A combination of Doxylamine succinate, an anti – histamine in combination with Pyridoxine hydrochloride, Vitamin B6 was found to be safe for treating morning sickness during pregnancy as recommended by the guidelines of American college of Obstetricians and Gynecologists [2]. The chemical name for doxylamine succinate is ethanamine, N,N -dimethyl-2-[1phenyl-1-(2-pyridinyl)ethoxy]-, butanenedioate and that of pyridoxine is 3,4-pyridinedimethanol, 5-hydroxy-6methyl-, hydrochloride [3]. The chemical structures of Doxylamine and Pyridoxine are shown in Figure 1.

Doxylamine is an antihistamine derived from monoethanolamine possessing antimuscarinic action and pronounced sedative effects [4]. Pyridoxine is a precursor of pyridoxal, which functions in the metabolism of carbohydrates, proteins and fats. It is essential in Hb formation and GABA synthesis within the CNS. It also aids in the release of glycogen stored in the liver and muscles. Till date, very few analytical methods are reported for the simultaneous estimation of Doxylamine and Pyridoxine Hydrochloride in dosage forms. The methods so far reported utilize derivative spectrophotometry [5], [6], HPLC [7], ratio derivative spectrophotometry [8], HPTLC [9] and charge transfer complexation [10] for the estimation of both the drugs. Analytical methods that involve simultaneous estimation of doxylamine and pyridoxine in combination with other drugs [11], [12] were also reported. A method using simultaneous equations was also reported [13]. The present method aims at developing a simple and rapid UV - spectrophotometric method using simultaneous equations for the estimation of both drugs in dosage forms.
MATERIALS AND METHODS

Doxylamine succinate (99.94%) and Pyridoxine Hydrochloride (99.29%) were obtained Akums Drugs and Pharmaceuticals Limited, as gift samples. Methanol of AR grade was used as solvent. UV – Visible Double Beam Spectrophotometer – 2060, Analytical Technologies was used for measuring the absorbance.

Selection of Solvent

Solvents like methanol, acetonitrile were selected based on solubility studies for both the drugs. Similar concentrations of both drugs were prepared in both solvents and scanned over the wavelength range of 200 – 400 nm. It was observed that, solutions in methanol showed suitable conditions for the simultaneous estimation of both drugs - Doxylamine having the maximum absorbance at 260 nm and Pyridoxine having the maximum absorbance at 285 nm, which were selected as the wavelength of analysis (Fig 2 and Fig 3). Methanol was selected as the solvent for further study.

Preparation of Standard Solutions

The standard stock solutions of Doxylamine and Pyridoxine were prepared by dissolving 10 mg of pure drug in 10 ml of methanol so as to give a concentration of 1 mg/ml. The working standards were prepared from the stock solutions by appropriate dilutions to give a concentration range of 5 – 50 μg/ml for Doxylamine and 2 – 20 μg/ml for Pyridoxine.

Preparation of Calibration Curve

The absorbance of the working standards was measured at 260 nm for Doxylamine and 285 nm for Pyridoxine and was plotted against concentration to get a calibration curve.

Simultaneous Equations method

The two wavelengths selected 260 nm and 285 nm were the wavelengths of maximum absorption of Doxylamine succinate and Pyridoxine HCl. The absorbance and absorptivities of both drugs were measured at the said wavelengths and the concentrations of the drugs were calculated using the simultaneous equation [14] as follows

\[ C_4 = \left( A_2 a y_1 - A_1 a y_2 \right) / \left( a x_2 a y_1 - a x_1 a y_2 \right) \]
\[ C_5 = \left( A_1 a x_1 - A_2 a x_2 \right) / \left( a x_2 a y_1 - a x_1 a y_2 \right) \]

Where:
- \( C_4 \) = concentration of Doxylamine Succinate
- \( C_5 \) = concentration of Pyridoxine HCl
- \( A_1 \) = absorbance of samples at 260 nm
- \( A_2 \) = absorbance of samples at 285 nm
- \( a x_1 \) is the absorptivity of Doxylamine at 260 nm.
- \( a y_1 \) is the absorptivity of Doxylamine at 285 nm.
- \( a x_2 \) is the absorptivity of Pyridoxine at 260 nm.
- \( a y_2 \) is the absorptivity of Pyridoxine at 285 nm

Method Validation

The present method was validated according to ICH Q2 Guidelines [15].

Linearity

The calibration curves were constructed with concentrations ranging from 5-50μg/ml for Doxylamine and 2-18μg/ml for Pyridoxine. The absorbance of the drug was considered for plotting the graph. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision

The precision of the method can be determined through repeatability and Intermediate precision. The repeatability of the method was determined by taking six determinations of 100 % concentration (n = 6). The intermediate precision was determined by measuring the absorbance of the 100% concentration sample for two consecutive days.

Accuracy

The accuracy of the method was determined through percentage recovery studies. The pre analyzed sample was spiked with three different levels of reference standard solution and the absorbance was measured in triplicate. The percentage recovery was calculated individually for both drugs.

Limit of Detection and Quantitation

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated from standard deviation of response and slope.

Assay of Tablet Dosage Form

The present work was applied to estimate the drugs in tablet dosage forms. The test samples were prepared by weighing tablet powder equivalent to 10 mg of Doxylamine and Pyridoxine extracted with methanol and made the volume up to 10 ml with methanol. The working standard was prepared by appropriate dilution so as to get a concentration in the middle of linearity range. The absorbance was measured at 260 nm and 285 nm in triplicate. The results are shown in Table 6.

RESULTS AND DISCUSSION

Selection of Wavelength

The wavelengths of maximum absorbance for individual drugs were selected as the wavelength of analysis. The UV – Spectrum of both drugs is shown in Figure 2 and Figure 3.

Linearity

Standard solutions of Doxylamine and Pyridoxine were prepared and the absorbance was measured at 260 nm and 285 nm respectively. The linear regression parameters are summarized in Table 1.
Precision
The repeatability of the method is expressed as % RSD and is found to be 0.784 and 0.487 for Doxylamine and Pyridoxine respectively. The intermediate precision is expressed as % RSD and the inter day study is found to be 1.019 and 0.482 for Doxylamine and Pyridoxine respectively. The results are summarized in Table 2.

Accuracy
The accuracy of the method was determined by calculating percentage recovery of three levels in triplicate. The results are shown in Table 3 and Table 4.

LOD and LOQ
The limit of detection and limit of quantitation were calculated from standard deviation of response and slope and were found to be 0.1815 μg/ml and 0.551 μg/ml respectively for Doxylamine and 0.1941 μg/ml and 0.558 μg/ml respectively for Pyridoxine.

Assay of Tablet Dosage Form
The developed method was applied to tablet dosage form and the results obtained were in good agreement with the label claim for both drugs. The results are shown in Table 5.

Table 1. Linear Regression data of Doxylamine and Pyridoxine

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Doxylamine</th>
<th>Pyridoxine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Samples</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Wavelength (nm)</td>
<td>260</td>
<td>285</td>
</tr>
<tr>
<td>Linearity range (μg/ml)</td>
<td>5 - 50</td>
<td>2 - 20</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.9995</td>
<td>0.9998</td>
</tr>
<tr>
<td>Regression line equation</td>
<td>( y = 0.023x + 0.015 )</td>
<td>( y = 0.030x - 0.001 )</td>
</tr>
</tbody>
</table>

Table 2. Repeatability studies of Doxylamine and Pyridoxine

<table>
<thead>
<tr>
<th>Set No.</th>
<th>Absorbance of Doxylamine at 260 nm</th>
<th>Absorbance of Pyridoxine at 285 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.115</td>
<td>0.300</td>
</tr>
<tr>
<td>2</td>
<td>0.113</td>
<td>0.301</td>
</tr>
<tr>
<td>3</td>
<td>0.114</td>
<td>0.304</td>
</tr>
</tbody>
</table>

Figure 1. Chemical structures of Doxylamine and Pyridoxine

Figure 2. UV Spectrum of Doxylamine showing \( \lambda_{\text{max}} \) at 260 nm

Figure 3. UV Spectrum of Pyridoxine showing \( \lambda_{\text{max}} \) at 285 nm
Table 3. Accuracy of Doxylamine

<table>
<thead>
<tr>
<th>Recovery level</th>
<th>Amount added (μg/ml)</th>
<th>Amount found (μg/ml) Avg ± SD</th>
<th>% Recovery Avg ± SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 %</td>
<td>8</td>
<td>7.7 ± 0.02624</td>
<td>96.25 ± 0.282</td>
<td>0.293</td>
</tr>
<tr>
<td>100%</td>
<td>10</td>
<td>9.73 ± 0.119</td>
<td>97.3 ± 1.131</td>
<td>1.162</td>
</tr>
<tr>
<td>120%</td>
<td>12</td>
<td>11.80 ± 0.850</td>
<td>98.33 ± 0.452</td>
<td>0.4596</td>
</tr>
</tbody>
</table>

Table 4. Accuracy of Pyridoxine HCl

<table>
<thead>
<tr>
<th>Recovery level</th>
<th>Amount added (μg/ml)</th>
<th>Amount found (μg/ml) Avg ± SD</th>
<th>% Recovery Avg ± SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 %</td>
<td>8</td>
<td>7.64 ± 0.110</td>
<td>95.5 ± 0.848</td>
<td>0.8879</td>
</tr>
<tr>
<td>100%</td>
<td>10</td>
<td>9.69 ± 0.166</td>
<td>96.9 ± 1.414</td>
<td>1.459</td>
</tr>
<tr>
<td>120%</td>
<td>12</td>
<td>11.74 ± 0.199</td>
<td>97.83 ± 0.965</td>
<td>0.9864</td>
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</tbody>
</table>

Table 5. Assay of Tablet Formulation

<table>
<thead>
<tr>
<th>Sample</th>
<th>Label Claim</th>
<th>Amount Found</th>
<th>% Assay (Avg ± SD)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doximine tablets</td>
<td>Doxylamine 10mg</td>
<td>10.092 ± 0.0007</td>
<td>100.92 ± 0.701</td>
<td>0.6946</td>
</tr>
<tr>
<td></td>
<td>Pyridoxine 10mg</td>
<td>9.161 ± 0.0855</td>
<td>91.61 ± 0.855</td>
<td>0.9330</td>
</tr>
</tbody>
</table>

CONCLUSION

A simple, sensitive, economic and rapid technique for the estimation of Doxylamine Succinate and Pyridoxine Hydrochloride in dosage forms was developed. This method proved to be simple when compared to the other methods reported. The lower values Limit of Detection and Limit of Quantitation of when compared to the other spectrophotometric methods developed, proves the sensitivity of technique. The good agreement of validation parameters and the assay with standards enables the method to be adapted for routine analysis of both the drugs in dosage forms.

REFERENCES


