DEVELOPMENT AND VALIDATION OF METHOD FOR SIMULTANEOUS ESTIMATION OF PYRIDOXINE HYDROCHLORIDE AND DOXYLAMINE SUCCINATE IN TABLET DOSAGE FORM BY FIRST ORDER DERIVATIVE SPECTROSCOPY

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ABSTRACT

Pyridoxine Hydrochloride and Doxylamine succinate are used in combination for treatment of Morning Sickness.

Objective: To develop a simple spectrophotometric method for simultaneous estimation of Pyridoxine Hydrochloride (PYR) and Doxylamine succinate (DOX) in tablet dosage form.

Method: The method employed is first order derivative spectroscopy. Results: For determination of sampling wavelength 10 μg/ml of each of PYR and DOX were scanned in 200-400 nm range and sampling wavelengths were 231.8 nm for PYR where DOX showed zero crossing point and 253 nm for DOX where PYR showed zero crossing point in first order derivative spectroscopy. For this method linearity observed in 1-40 μg/ml for PYR and 2.5-80 μg/ml for DOX. LOD of DOX and PYR were 0.2826 and 0.3571 μg/ml respectively. LOQ of DOX and PYR were 0.4621 and 0.5918 μg/ml respectively.

Discussion: The recovery studies confirmed accuracy of proposed method and low values of standard deviation confirmed precision of method. The method is validated as per ICH guidelines.

Keywords: Pyridoxine Hydrochloride, Doxylamine succinate, first order derivative spectroscopy.

INTRODUCTION

Pyridoxine Hydrochloride is chemically described as 4,5-Bis(hydroxymethyl)-2-methylpyridine-3-ol Hydrochloride salt (¹) (Vitamin B₆) assists in the balancing of sodium and potassium as well as promoting red blood cell production and Doxylamine succinate is N,N-Dimethyl-2-[α-methyl-α-2-pyridinyl] benzyloxyethylamine Hydrogen Succinate is antihistamine, both in combination used for the treatment of Morning Sickness. Many analytical methods like HPLC (²-⁵), UV Spectroscopy (⁶-⁹) and charge transfer complexation (⁵) methods were reported for determination of PYR and DOX combination. In this communication we report a new UV spectrophotometric method using derivative spectroscopy.

Fig. 1: Structure of Pyridoxine Hydrochloride and Doxylamine succinate

MATERIALS AND METHODS

Instrument

Spectrophotometric analysis was carried out on a double beam UV-spectrophotometer (UV Lab India) using a 1 cm quartz cell. The instrument settings were zero order and first derivative mode and band width of 1.0 nm in the range of 200–400 nm.

Chemicals and reagents

The tablets of the said combination were purchased from a local pharmacy (The label claim contained 10mg of PYR and 10 mg of DOX). Distilled water was used as solvent to prepare all solutions.

Procedure (⁷-⁸)

Preparation of stock solution (1000μg/ml): Accurately weighed quantity of pure PYR (10mg) and pure DOX (10mg) were transferred into two separate 10mL volumetric flasks, dissolved in distilled water and made the volume to 10mL with the same solvent. The stock solution was sonicated for 2min.

Preparation of working standard solution (100μg/ml): From the above stock solution 1mL each of PYR and DOX was taken, transferred to separate 10mL volumetric flasks and the volume was made up to 10mL with distilled water.

Method

In this method, 10μg/ml solution of standard drug PYR and DOX were prepared using distilled water as solvent and scanned from 400nm to 200nm (Fig 2). The absorption spectra thus obtained were derivatized from first order to fourth order. First order derivative spectra were selected for analysis of both drugs. From the overlain spectra of both the drugs (Fig 3), wavelength selected for quantification were 231.8 nm for PYR (zero cross of DOX) and 253...
nm for DOX (zero cross of PYR). The calibration curve for PYR and DOX were plotted in the concentration range of 5-25 μg/ml at 231.8 nm and 253 nm respectively.

**Estimation of Tablet Dosage form**

For the estimation of PYR and DOX in commercial formulations, twenty tablets were weighed and average weight was calculated. The tablets were crushed and tablet powder and powder equivalent to 10 mg of PYR taken and added in 60 ml of Distilled water system sonicated for 10 min after sonication volume was made up to 100 ml. 1 ml of this stock solution was diluted to 10 ml to get concentration equal to 10 μg/ml of PYR and 10 μg/ml of DOX. This solution is scanned in range 200-400 nm taking distilled water as blank. The spectrum obtained was converted to first order derivative spectra, absorbances were noted and concentrations were determined from regression equations generated from calibration graph.

**RESULTS AND DISCUSSION**

Sampling wavelengths were determined from scanning individual drug samples in 200-400 nm range(Fig 2). Sampling wavelengths were 231.8 nm and 253 nm for PYR and DOX respectively in first order derivative mode(Fig 3).

![Fig. 2: Zero order overlain spectrum of PYR and DOX](image1)

![Fig. 3: First order overlain absorption spectrum of PYR and DOX](image2)

For this method equations generated were $Y = 0.004x - 0.013 \quad (r^2=0.985)$ and $Y = -0.00036x + 0.0004 \quad (0.987)$ for PYR and DOX respectively. Linearity of proposed method was found to be 1-40 μg/ml for PYR and 2.5-80 μg/ml for DOX. Limits of detection were found to be 0.2826 and 0.3571 μg/ml of PYR and DOX respectively. Limits of quantitation were found to be 0.4621 and 0.5918 μg/ml for PYR and DOX respectively. Results of tablet analysis were reported in table 1, result of precision studies and recovery study reported in table 2 and 3 respectively.
Table 1: Results of assay of tablet dosage form

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Label claim (mg/tab)</th>
<th>Amount found* (mg/tab)</th>
<th>% label claim*± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PYR</td>
<td>10</td>
<td>9.91</td>
<td>99.1%±0.873</td>
</tr>
<tr>
<td>DOX</td>
<td>10</td>
<td>10.14</td>
<td>101.4%±0.932</td>
</tr>
</tbody>
</table>

*Average of five determinations

Table 2: Results of Precision studies

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Label claim (mg/tab)</th>
<th>Amount found* (mg/tab)</th>
<th>% label claim*± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PYR</td>
<td>10</td>
<td>10.11</td>
<td>101.1%±0.962</td>
</tr>
<tr>
<td>DOX</td>
<td>10</td>
<td>10.18</td>
<td>101.8%±0.951</td>
</tr>
</tbody>
</table>

*Average of five determinations

Table 3: Recovery studies data showing average amount of drug recovered from sample solution

<table>
<thead>
<tr>
<th>Analyte</th>
<th>%Recovery (Mean±S.D.)*</th>
<th>RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PYR</td>
<td>99.5±0.8532</td>
<td>0.8575</td>
</tr>
<tr>
<td>DOX</td>
<td>100.13±0.916</td>
<td>0.9148</td>
</tr>
</tbody>
</table>

CONCLUSION

The method used is simple and rapid and does not involve the use of complex instrument, low value of Standard deviation showed that the method is precise and high percentage of recovery of as shown in table shows that the method is accurate.

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REFERENCES