STABILITY INDICATING UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ORNIDAZOLE AND DILOXANIDE FURUATE IN BULK DRUG AND COMBINED DOSAGE FORM

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Abstract: A simple, specific, accurate and precise stability indicating UV spectrophotometric method has been developed for the simultaneous determination of Ornidazole and Diloxanide furoate in bulk & tablet dosage form. The method was developed by simultaneous equation method using Methanol as solvent taking 311 nm as \( \lambda_{\text{max}} \) for Ornidazole and 258 nm as \( \lambda_{\text{max}} \) for Diloxanide furoate. The validation was carried according to ICH guidelines. Calibration curve was linear with correlation coefficient of 0.996 and 0.994 over a concentration range of 2.5 - 7.5 \( \mu \text{g/ml} \) and 3.25 - 11.25 \( \mu \text{g/ml} \) for Ornidazole and Diloxanide furoate respectively. The percent recovery was 99.79 and 98.38 for Ornidazole and Diloxanide furoate indicating accuracy and reliability of method. Forced degradation studies were carried out and spectra didn’t reveal any significant degradation products when subjected to stress conditions. So the developed stability indicating method can be applied for the simultaneous determination of Ornidazole and Diloxanide furoate in combined dosage forms.

Keywords: Ornidazole, Diloxanide furoate, Stability indicating, UV spectrophotometric.
INTRODUCTION

Ornidazole \(^2\) is chemically; 1-chloro-3-(2-methyl-5-nitro-1H-imidazole-1-yl) propane-2-ol is a 5-nitroimidazole (Fig. 1) derivative used as an anti-infective agent\(^1\). It is converted into reduction products that interact with DNA to cause destruction of helical DNA structure and strand leading to a protein synthesis inhibition. The free nitro radical generated as a result of this reduction is believed to be responsible for the antiprotozoal activity. It is suggested that the toxic free radicals covalently bind to DNA, causing DNA damage and leading to cell death. Ornidazole in the market, has gained increasing acceptance in diarrhoea, bacterial and protozoal infections.

Diloxanide Furoate \(^3\) is chemically 4-(2, 2-dichloro-N-methylacetamido) phenylfuran-2-carboxylate. (Fig. 2) It is a luminal amoebicide\(^1\). It is a dichloroacetamide derivative that principally acts in the bowel lumen and is used as intestinal amoebicide. It is the drug of choice in the treatment of asymptomatic intestinal Amoebiasis. Although both Ornidazole and Diloxanide furoate act as antiprotozoal drugs, however their combination has a synergistic effect and acts on both vegetative and cystic forms of Entamoeba histolytica.

Literature survey reveals that several methods were used for determination of Ornidazole and Diloxanide furoate by UV, HPLC, HPTLC \(^4\)-\(^{15}\) alone and in combination with other drugs but no stability indicating \(^20\) UV spectrophotometric method for the simultaneous estimation of Ornidazole and Diloxanide furoate in Pharmaceutical dosage forms have been reported so far. Hence the objective of this study is to develop a simple, economical, selective, accurate, precise and sensitive stability indicating UV spectrophotometric method for the simultaneous determination of Ornidazole and Diloxanide furoate in bulk and Pharmaceutical dosage forms and validate\(^19\) it making it suitable for routine quality control analysis.

MATERIALS AND METHODS

Chemicals and Reagents:

All reagents of analytical grade were used for the analysis. Pure drug samples of Ornidazole and Diloxanide furoate were obtained as gift samples from PEGASUS FARMACO PVT. INDIA LTD, Hyderabad. Fixed dose combination tablets (AMICLINE PLUS tablets) containing 250 mg Ornidazole and 375 mg Diloxanide furoate were procured from local market.

Instrumentation:

PG Instrumentations Ltd. Model no. 60 UV double beam spectrophotometer with a fixed slit width of 2 nm, 1 cm quartz cells was used. Electronic balance of Wensar weighing scales was used. Class ‘A’ volumetric glassware were used.
Preparation of standard stock solutions

a) Preparation of Ornidazole standard stock solution

25 mg of Ornidazole was weighed and transferred to a 10 ml volumetric flask. Mix with half quantity of Methanol, shake and volume was made up to the mark. Make further dilutions to give the working standard solution of ornidazole (25 μg/ml).

b) Preparation of Diloxanide furoate standard stock solution

37.5 mg of Diloxanide furoate was weighed and transferred to a 10 ml volumetric flask. Mix with half quantity of Methanol, shake and volume was made up to the mark. Make further dilutions to give the working standard solution of Diloxanide furoate (37.5 μg/ml).

Determination of wavelength for measurement

2 ml of working standard stock solution of Ornidazole (25 μg/ml) and Diloxanide furoate (37.5 μg/ml) were pipetted out into two separate 10 ml volumetric flask and volume was adjusted to the mark with Methanol to get 5 μg/ml of ORD and 7.5 μg/ml of DLX. Each solution was scanned between 200 - 400 nm against methanol as a reagent blank. From the spectra obtained 311 nm was selected as $\lambda_{\text{max}}$ of ornidazole and 258 nm was selected as $\lambda_{\text{max}}$ of diloxanide furoate as shown in fig. 3 and fig. 4.

Preparation of Calibration curves

Standard solutions of Ornidazole and Diloxanide furoate in the concentration range of 2.5 – 7.5 μg/ml and 3.75 – 11.25 μg/ml respectively were obtained by transferring 1, 1.5, 2.0, 2.5 and 3.0 ml from the working standard solution of Ornidazole (25 μg/ml) and Diloxanide furoate (37.5 μg/ml) into series of 10 ml volumetric flasks. The volume in each volumetric flask was made up with methanol as solvent. The absorbencies of the solutions were measured at both the wavelength 311 nm and 258 nm against the methanol as blank and absorptivity values are calculated. Calibration curve was plotted at both wavelengths and two equations were constructed using the absorptivity values obtained.

Method validation:

Linearity and Range

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 2. 5 – 7.5 μg/ml and 3.75 – 11.25 μg/ml for ORD and DLX respectively. The calibration curve of absorbance vs. respective concentration was plotted and correlation coefficient ($r^2$) and regression line equations for ORD and DLX were calculated.
Accuracy

To study the accuracy, % recoveries has to be calculated, recovery studies were carried out by standard addition method by adding the known amount of standard ORD and DLX to the pre-analyzed sample at three different concentration levels i.e. 50%, 100%, and 150% of assay concentration and percentage recoveries were calculated. The amount of ORD and DLX was calculated at each level (50%, 100%, 150%) and % recoveries were computed.

System Precision

Variation of results within the same day was analyzed. It was determined by measuring the standard mixture solution of ORD (25 μg/ml) and DLX (37.5 μg/ml) six times on the same day.

Method Precision

Variation of results within the same day was analyzed. It was determined by measuring the sample solution from a single batch containing ORD (25 μg/ml) and DLX (37.5 μg/ml) six times on the same day.

LOD and LOQ

The LOD and LOQ was estimated from the calibration curves used to determine method linearity. The LOD and LOQ was calculated as,

LOD = 3.3 * SD / Slope
LOQ = 10 * SD / Slope

Where,
S.D. = Standard deviation
Slope = slope of the calibration curves

Robustness

It was determined by analyzing the standard solution of ORD (25 μg/ml) and DLX (37.5 μg/ml) with small but deliberate change in λmax (max ± 1 nm).

Ruggedness

Ruggedness was evaluated by carrying out analysis of standard and sample solution containing (25μg/ml of ORD and 37.5μg/ml of DLX) on two different analysts using same operational and environmental conditions. The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two analysts.
System suitability study

It was determined by measuring the standard solutions of ORD (25 μg/ml) and DLX (37.5 μg/ml) at 311 nm and 258 nm five times and its absorbance is recorded and % RSD is calculated in order to check the reproducibility of system to give the results.

Specificity

In order to prove the specificity of the method, spectra of a sample solution and mixed standard solution of ORD 25µg/ml and DLX 37.5µg/ml is prepared and compared. Both the spectras were matched with each other to show two peaks at 311 nm and 258 nm of ORD and DLX respectively.

Simultaneous Estimation of Ornidaazole and Diloxanide furoate in Formulation

Twenty tablets were weighed and finely powdered and triturated well. An accurately weighed quantity of the powder equivalent to about 25mg of ORD and 37.5 mg of DLX was taken in 50 ml volumetric flask and dissolved in of methanol and sonicate for atleast 30 min further diluted upto the mark with same solvent. The solution was then filtered through the Whatmann filter paper No. 41. Necessary dilutions are made with methanol to give final concentration 25 μg/ml and 37.5 μg/ml of Ornidaazole and Diloxanide furoate respectively. The Absorbance of the resulting solution was measured at 311 nm and 258 nm against methanol. The concentration of ORD and DLX can be obtained by simultaneous equations. The concentration of two drugs in mixture was calculated by using following equations.

\[
C_x = \frac{(A_2 a y_1 - A_1 a y_2)}{(a x_2 a y_1 - a x_1 a y_2)}
\]

\[
C_y = \frac{(A_1 a x_2 - A_2 a x_1)}{(a x_2 a y_1 - a x_1 a y_2)}
\]

\[C_x = \text{Concentration of Ornidaazole in g/100 mL}\]

\[C_y = \text{Concentration of Diloxanide furoate in g/100 mL}\]

\[A_1 & A_2 = \text{Absorbance of sample mixture at 311 nm and 258 nm resp.}\]

\[a x_1 = \text{Absorptivity of Ornidaazole at 311 nm}\]

\[a x_2 = \text{Absorptivity of Ornidaazole at 258 nm}\]
$\alpha_1 =$ Absorptivity of Diloxanide furoate at 311 nm

$\alpha_2 =$ Absorptivity of Diloxanide furoate at 258 nm

**Forced degradation studies**

**a) Acid degradation**

2 ml of sample stock solution of mixture of Ornidazole and Diloxanide furoate containing 25 µg/ml and 37.5 µg/ml of Ornidazole and Diloxanide furoate respectively is taken, and 2 ml of 0.1 N HCl was added in 10 ml of volumetric flask and the volumetric flask was kept at room temperature. After 3 hours, solution was neutralized and diluted with methanol up to 10 ml and absorbance is measured at 311 nm and 258 nm.

**b) Alkaline degradation**

2 ml of sample stock solution of mixture of Ornidazole and Diloxanide furoate containing 25 µg/ml and 37.5 µg/ml of Ornidazole and Diloxanide furoate respectively is taken, and 2 ml of 0.1 N NaOH was added in 10 ml of volumetric flask and the volumetric flask was kept at room temperature. After 3 hours, solution was neutralized and diluted with methanol up to 10 ml and absorbance is measured at 311 nm and 258 nm.

**c) Oxidative degradation**

2 ml of sample stock solution of mixture of Ornidazole and Diloxanide furoate containing 25 µg/ml and 37.5 µg/ml of Ornidazole and Diloxanide furoate respectively is taken, and 2 ml of 3% $\text{H}_2\text{O}_2$ was added in 10 ml of volumetric flask and the volumetric flask was kept at room temperature. After 3 hours, solution was diluted with methanol up to 10 ml and absorbance is measured at 311 nm and 258 nm.

**d) Photolytic degradation**

Powdered sample was taken in a petriplate and exposed to a a UV light of 365 nm in UV chamber for 3 hrs. Weight of the sample equivalent to 25 mg Ornidazole and 37.5 mg diloxanide furoate was diluted with methanol up to 10 ml. From this solution, dilution was done to achieve the appropriate concentration and absorbance is measured at 311 nm and 258 nm.

**e) Thermal degradation**

Powdered sample was taken in a petriplate and and exposed to a temperature of 80 °C for 3 hours in an oven. for 3 hrs. weight of the sample equivalent to 25 mg Ornidazole and 37.5 mg diloxanide furoate was diluted with methanol up to 10 ml. From this solution, dilution was done to achieve the appropriate concentration and absorbance is measured at 311 nm and 258 nm.
Record the absorbance of stressed samples then compares it with absorbance of unstressed sample to determine the % degradation.

\[ \% \text{ degradation} = \frac{\text{Response of unstressed sample} - \text{response of stressed sample}}{\text{Response of unstressed sample}} \times 100 \]

**RESULTS AND DISCUSSIONS**

The proposed method for simultaneous estimation of Ornidazole and Diloxanide furoate utilizes the spectrum mode of analysis. The method utilizes 311 nm and 258 nm as analytical wavelength for estimation of Ornidazole and Diloxanide furoate respectively. A series of standard solutions were prepared for Ornidazole and Diloxanide furoate and absorbance’s of solutions were recorded at 311 nm and 258 nm to plot a calibration curve of absorbance versus concentration. The calibration curves were found to be linear in concentration range under study. Regression equation and Absorptivity values of Ornidazole and Diloxanide furoate were determined at selected wavelengths are presented in Tab. 1.

Substituting the values of \(ax_1, ax_2, ay_1\) and \(ay_2\) the equation could be rearranged as:

At 311 nm \[ A_1 = 560 C_x + 12 C_y \]
At 258 nm \[ A_2 = 98C_x + 772Cy \]

Where \(C_x\) and \(C_y\) are the concentration in g/100ml. The percentage of purity of Ornidazole and Diloxanide furoate in tablet dosage form is shown in Tab. 9

The calibration curve was linear over the concentration range of 2.5 – 7.5 \(\mu g/ml\) and 3.75 – 11.25 \(\mu g/ml\) for Ornidazole and Diloxanide furoate respectively. The relationship between the concentration and area of Ornidazole and Diloxanide furoate is linear in the range examined as shown in Tab. 2 since all points lie in a straight line and the correlation coefficient is well within limits as shown in fig. 5 – fig. 8. The % recovery in accuracy study of Ornidazole was found to be 99.8 % and % recovery of Diloxanide furoate was found to be 99.3 % as shown in Tab. 3. The %RSD in system precision study was found to be 0.0026 & 0.010 for Ornidazole at 311 nm and 258 nm respectively. The %RSD in system precision study was found to be 0.08 & 0.0019 for Diloxanide furoate at 311 nm and 258 nm respectively shown in Tab. 4. The %RSD in method precision study was found to be 0.0028 & 0.0023 for sample solution at 311 nm and 258 nm respectively as shown in Tab. 5. The value of LOD and LOQ were determined from standard deviation and slope values of calibration curve and the results obtained are shown in Tab. 6. Robustness was studied by change in wavelength and the results obtained showed %RSD values below 2% as shown in Tab. 7 and Ruggedness was studied by different analysts and the % RSD for assay by two different analyst was found to be below 2% as shown in Tab. 8. Forced
degradation studies were performed on the sample mixture and it was observed that
Ornidazole was unstable in alkaline degradation and Diloxanide in Photolytic degradation but
overall the net degradation was within the limits as shown in Tab. 10 and the corresponding UV
spectra of sample under different stress conditions are depicted in fig. 9 – fig. 14 . A system
suitability test of the spectrophotometric system was performed before each validation run and
%RSD was found to be 0.0026 and 0.0019 for Ornidazole and Diloxanide furoate respectively as
shown in Tab. 11. The specificity test showed there was no interference of excipient and sample
spectra matched with standard spectra.

**Tab. 1: Result of calibration curve**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ORD at 311nm</th>
<th>ORD at 258nm</th>
<th>DLX at 311nm</th>
<th>DLX at 258nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorptivity</td>
<td>560</td>
<td>98</td>
<td>12</td>
<td>772</td>
</tr>
<tr>
<td>Beer - Lambert’s Range</td>
<td>2.5 - 7.5 µg/ml</td>
<td>3.75 – 11.25 µg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>Slope</td>
<td>0.027</td>
<td>0.008</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>0.124</td>
<td>0.007</td>
<td>0.009</td>
</tr>
<tr>
<td>Equation</td>
<td>Correlation</td>
<td>R² = 0.996</td>
<td>R² = 0.991</td>
<td>R² = 0.996</td>
</tr>
</tbody>
</table>

**Tab. 2: Linearity data of Ornidazole and Diloxanide furoate**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Conc. (µg/ml)</th>
<th>Absorbance at λ₁ = 311 nm</th>
<th>Absorbance at λ₂ = 258 nm</th>
<th>Conc. (µg/ml)</th>
<th>Absorbance at λ₁ = 311 nm</th>
<th>Absorbance at λ₂ = 258 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2.50</td>
<td>0.191</td>
<td>0.028</td>
<td>3.75</td>
<td>0.001</td>
<td>0.288</td>
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<tr>
<td>2.</td>
<td>3.75</td>
<td>0.226</td>
<td>0.039</td>
<td>5.62</td>
<td>0.005</td>
<td>0.430</td>
</tr>
<tr>
<td>3.</td>
<td>5.00</td>
<td>0.267</td>
<td>0.047</td>
<td>7.50</td>
<td>0.009</td>
<td>0.574</td>
</tr>
<tr>
<td>4.</td>
<td>6.25</td>
<td>0.295</td>
<td>0.061</td>
<td>9.37</td>
<td>0.014</td>
<td>0.719</td>
</tr>
<tr>
<td>5</td>
<td>7.50</td>
<td>0.327</td>
<td>0.068</td>
<td>11.25</td>
<td>0.020</td>
<td>0.856</td>
</tr>
</tbody>
</table>
### Tab. 3: Result of Recovery Studies for Ornidazole and Diloxanide Furoate:

<table>
<thead>
<tr>
<th>Level</th>
<th>Amount of ORD in mixture (μg/ml)</th>
<th>Amount of Std ORD added (μg/ml)</th>
<th>Total amount of ORD (μg/ml)</th>
<th>Total amount of ORD found (μg/ml)</th>
<th>%Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 %</td>
<td>2.5</td>
<td>1.25</td>
<td>3.75</td>
<td>3.47</td>
<td>99.7</td>
</tr>
<tr>
<td>100 %</td>
<td>2.5</td>
<td>2.5</td>
<td>5</td>
<td>4.97</td>
<td>99.4</td>
</tr>
<tr>
<td>150 %</td>
<td>2.5</td>
<td>3.75</td>
<td>6.25</td>
<td>6.27</td>
<td>100.3</td>
</tr>
<tr>
<td>Avg</td>
<td></td>
<td></td>
<td>6.2</td>
<td>6.2</td>
<td>99.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Level</th>
<th>Amount of DLX in mixture (μg/ml)</th>
<th>Amount of Std DLX added (μg/ml)</th>
<th>Total amount of DLX (μg/ml)</th>
<th>Total amount of DLX found (μg/ml)</th>
<th>%Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 %</td>
<td>3.75</td>
<td>1.875</td>
<td>5.625</td>
<td>5.59</td>
<td>99.4</td>
</tr>
<tr>
<td>100 %</td>
<td>3.75</td>
<td>3.75</td>
<td>7.5</td>
<td>7.48</td>
<td>99.7</td>
</tr>
<tr>
<td>150 %</td>
<td>3.75</td>
<td>5.625</td>
<td>9.375</td>
<td>9.26</td>
<td>98.8</td>
</tr>
<tr>
<td>Avg</td>
<td></td>
<td></td>
<td>9.3</td>
<td>9.3</td>
<td>99.3</td>
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### Tab. 4: Results of system precision

<table>
<thead>
<tr>
<th></th>
<th>Absorbance at 311 nm</th>
<th>Absorbance at 258 nm</th>
<th></th>
<th>Absorbance at 311 nm</th>
<th>Absorbance at 258 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.No.</td>
<td></td>
<td></td>
<td>S.No.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>0.268</td>
<td>0.046</td>
<td>1.</td>
<td>0.008</td>
<td>0.566</td>
</tr>
<tr>
<td>2.</td>
<td>0.267</td>
<td>0.047</td>
<td>2.</td>
<td>0.010</td>
<td>0.567</td>
</tr>
<tr>
<td>3.</td>
<td>0.267</td>
<td>0.046</td>
<td>3.</td>
<td>0.009</td>
<td>0.567</td>
</tr>
<tr>
<td>4.</td>
<td>0.266</td>
<td>0.047</td>
<td>4.</td>
<td>0.009</td>
<td>0.567</td>
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<tr>
<td>5.</td>
<td>0.267</td>
<td>0.046</td>
<td>5.</td>
<td>0.010</td>
<td>0.566</td>
</tr>
<tr>
<td>6.</td>
<td>0.266</td>
<td>0.046</td>
<td>6.</td>
<td>0.009</td>
<td>0.564</td>
</tr>
</tbody>
</table>
Mean 0.266833 0.046333 Mean 0.009167 0.566167
SD 0.000753 0.000516 SD 0.000753 0.001169
%RSD 0.0026 0.010 %RSD 0.08 0.0019

Tab. 5: Results of Method precision

<table>
<thead>
<tr>
<th>S.No.</th>
<th>ORNIDAZOLE</th>
<th>DILOXANIDE FUROATE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absorbance at 311 nm</td>
<td>Absorbance at 258 nm</td>
</tr>
<tr>
<td>1.</td>
<td>0.287</td>
<td>0.574</td>
</tr>
<tr>
<td>2.</td>
<td>0.285</td>
<td>0.571</td>
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<tr>
<td>3.</td>
<td>0.287</td>
<td>0.575</td>
</tr>
<tr>
<td>4.</td>
<td>0.286</td>
<td>0.574</td>
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<tr>
<td>5.</td>
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<tr>
<td>6.</td>
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<tr>
<td>Mean</td>
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<td>0.573</td>
</tr>
<tr>
<td>SD</td>
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<td>0.00134371</td>
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<tr>
<td>%RSD</td>
<td>0.00285488</td>
<td>0.00234164</td>
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Tab. 6: Results of LOD and LOQ

<table>
<thead>
<tr>
<th>SD</th>
<th>ORD</th>
<th>0.0054</th>
<th>DLX</th>
<th>0.022</th>
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</thead>
<tbody>
<tr>
<td>Slope</td>
<td>ORD</td>
<td>0.27</td>
<td>DLX</td>
<td>0.76</td>
</tr>
<tr>
<td>LOD (μg/ml)</td>
<td>ORD</td>
<td>0.66</td>
<td>DLX</td>
<td>0.97</td>
</tr>
<tr>
<td>LOQ (μg/ml)</td>
<td>ORD</td>
<td>2</td>
<td>DLX</td>
<td>2.89</td>
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Tab. 7: Results of Robustness study

<table>
<thead>
<tr>
<th>ORNIDAZOLE (311 nm)</th>
<th>DILOXANIDE FUROATE (258 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>310 nm</td>
<td>312 nm</td>
</tr>
<tr>
<td>0.265</td>
<td>0.262</td>
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<tr>
<td>0.266</td>
<td>0.261</td>
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<tr>
<td>0.265</td>
<td>0.260</td>
</tr>
<tr>
<td>0.264</td>
<td>0.261</td>
</tr>
<tr>
<td>Avg = 0.265</td>
<td>Avg = 0.260</td>
</tr>
<tr>
<td>STD DEV = 0.0006</td>
<td>STD DEV = 0.0007</td>
</tr>
<tr>
<td>% RSD = 0.002</td>
<td>% RSD = 0.002</td>
</tr>
</tbody>
</table>

Tab. 8: Results of Ruggedness study

<table>
<thead>
<tr>
<th>ORNIDAZOLE</th>
<th>% Assay</th>
<th>DILOXANIDE FUROATE</th>
<th>% Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyst 01</td>
<td>99.41</td>
<td>Analyst 01</td>
<td>100.02</td>
</tr>
<tr>
<td>Analyst 02</td>
<td>100.02</td>
<td>Analyst 02</td>
<td>99.21</td>
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<tr>
<td>%RSD</td>
<td>0.45%</td>
<td>%RSD</td>
<td>0.311%</td>
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Tab. 9: Results of Simultaneous Estimation of Ornidazole and Diloxanide furoate in Combined Dosage Form

<table>
<thead>
<tr>
<th>Conc. of sample stock soln.</th>
<th>Absorbance at $\lambda_1 = 311$ nm ($A_1$)</th>
<th>Absorbance at $\lambda_2 = 258$ nm ($A_2$)</th>
<th>Conc. ORD obtained (µg/ml)</th>
<th>Conc. DLX obtained (µg/ml)</th>
<th>% Amt. of drug ORD</th>
<th>% Amt. of drug DLX</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 µg/mL ORD</td>
<td>0.286</td>
<td>0.628</td>
<td>4.94</td>
<td>7.48</td>
<td>98.8</td>
<td>99.7</td>
</tr>
<tr>
<td>7.5 µg/mL DLX</td>
<td>0.285</td>
<td>0.628</td>
<td>4.93</td>
<td>7.51</td>
<td>98.6</td>
<td>100.1</td>
</tr>
<tr>
<td></td>
<td>0.288</td>
<td>0.630</td>
<td>4.93</td>
<td>7.51</td>
<td>98.6</td>
<td>100.1</td>
</tr>
<tr>
<td></td>
<td>0.287</td>
<td>0.626</td>
<td>4.96</td>
<td>7.47</td>
<td>99.2</td>
<td>99.6</td>
</tr>
<tr>
<td></td>
<td>0.289</td>
<td>0.628</td>
<td>4.98</td>
<td>7.48</td>
<td>99.6</td>
<td>99.7</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>99.12</td>
<td>99.76</td>
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</table>

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Tab. 10: Results of Forced degradation studies

<table>
<thead>
<tr>
<th>Stress condition</th>
<th>Absorbance</th>
<th>%Degradation</th>
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<tbody>
<tr>
<td></td>
<td>ORD</td>
<td>DLX</td>
</tr>
<tr>
<td>Acid</td>
<td>0.242</td>
<td>0.565</td>
</tr>
<tr>
<td>Alkaline</td>
<td>0.224</td>
<td>0.569</td>
</tr>
<tr>
<td>Oxidation</td>
<td>0.248</td>
<td>0.554</td>
</tr>
<tr>
<td>Photolytic</td>
<td>0.244</td>
<td>0.508</td>
</tr>
<tr>
<td>Thermal</td>
<td>0.241</td>
<td>0.561</td>
</tr>
</tbody>
</table>

Tab 11: Results of system suitability study

<table>
<thead>
<tr>
<th>ORNIDAZOLE</th>
<th>DILOXANIDE FUROATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.No.</td>
<td>Absorbance at 311 nm</td>
</tr>
<tr>
<td>1.</td>
<td>0.268</td>
</tr>
<tr>
<td>2.</td>
<td>0.267</td>
</tr>
<tr>
<td>3.</td>
<td>0.267</td>
</tr>
<tr>
<td>4.</td>
<td>0.266</td>
</tr>
<tr>
<td>5.</td>
<td>0.267</td>
</tr>
<tr>
<td>Mean</td>
<td>0.266833</td>
</tr>
<tr>
<td>SD</td>
<td>0.000753</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.0026</td>
</tr>
</tbody>
</table>

Fig. 1: Structure of Ornidazole
Fig. 2: Structure of Diloxanide furoate

Fig. 3: UV spectrum showing λmax of Ornidazole

Fig. 4: UV spectrum showing λmax of diloxanide furoate

Fig. 5: Calib. Curve of ORD at 311 nm

\[ y = 0.027x + 0.124 \]
\[ R^2 = 0.996 \]

Fig. 6: Calib. Curve of ORD at 258 nm

\[ y = 0.008x + 0.007 \]
\[ R^2 = 0.991 \]
Fig. 7: Calib. Curve of DLX at 311 nm

Linearity of Diloxanide furoate at 311 nm

\[
y = 0.023x + 0.024
\]

\[
R^2 = 0.996
\]

Fig. 8: Calib. Curve of DLX at 258 nm

Linearity of Diloxanide furoate at 258 nm

\[
y = 0.078x - 0.013
\]

\[
R^2 = 0.994
\]

Fig. 9: UV Spectra of unstressed sample solution

Fig 10: UV spectra showing Acid Degradation

Fig 11: UV spectra showing Alkaline Degradation

y = 0.023x + 0.024

\[
R^2 = 0.996
\]
CONCLUSION

The observations and results obtained from this study including linearity, accuracy and precision lie well within acceptable limits. From the experimental studies it was concluded that proposed method was simple, sensitive, economic, precise, accurate and specific and can be adopted for the routine quality control analysis of both drugs in combined tablet formulation without interference of excipient. The statistical parameters and recovery studies were carried out and reported. The obtained results were found to be satisfactory as per ICH guidelines.

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