METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF MICONAZOLE AND ORNIDAZOLE IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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Abstract: The liposomes are concentric bilayered vesicle in which an aqueous volume is entirely enclosed by the membranous lipid bilayer mainly composed of the natural or synthetic phospholipids. The liposomes are one of unique drug delivery system which can be use in controlling & targeting drug delivery system. The liposomes are generally classified based upon the structure, method of preparation, composition and application, conventional liposome, and specialty liposome. The liposomes can be filled with drugs & used to deliver the drugs for cancer & other diseases. This review is focused on the various aspects of liposomes like classification, methods of preparation, characterization and applications of liposomes.

Keywords: Liposomes, Phospholipids, Targeting drug delivery system, Bilayered

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INTRODUCTION

Indian sub-continent, being Tropical region has high temperature and high humidity conditions, which are favourable for the growth of fungal and other infections. Presently, the treatment is the Azole group/antibiotics. With continuous efforts, the combination of Azole have become a preferred choice for the better response and therapeutics efficacy. To develop separate methods of quantification for two drugs requires more efforts for the chemist and less output in terms of number of samples analysed. In lieu of above, efforts are now being put in developing a common analytical method for multicomponent formulation analysis.

Thus, despite of various analytical methods developed, still no work has been done in developing Simultaneous method of Quantification of these drugs. As there is no Stability indicating HPLC method reported for these drugs in combined dosage forms.

Therefore it was wisely thought worthwhile to develop Stability indicating RP-HPLC method for simultaneous estimation of Micronazole and Ornidazole in the combined Pharmaceutical dosage form. Moreover, there are two methods develop for the simultaneous estimation of Ornidazole and Miconazole by HPLC, one is for cream formulation and the other has been developed for Tablet formulation. Moreover the run time for Chromatogram is higher, thus the method is superior, accurate and more reliable than the developed one.

Looking from the QA point of view, the method can be successfully adopted for routine quality analysis of Ornidazole and Miconazole in combined tablet dosage from without any interferences from common excipients and impurities. Also method can be transferred to utilize for routine Laboratory analysis and Assay of Ornidazole and Miconazole in their tablet dosage form.

DRUG PROFILE

1.4.1. MICONAZOLE

<table>
<thead>
<tr>
<th>INTRODUCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name</strong></td>
</tr>
<tr>
<td><strong>Official in</strong></td>
</tr>
<tr>
<td><strong>Description</strong></td>
</tr>
</tbody>
</table>
Structure

Chemical Formula \( \text{C}_{18}\text{H}_{14}\text{Cl}_4\text{N}_2\text{O} \)

Mol. Weight 416.13

IUPAC Name 1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]ethyl]-1-Himidazole

Categories
- Anti infective

Solubility
- Very slightly soluble in Water, freely soluble in Methanol, soluble in Ethanol (96 per cent)

PHARMACOLOGY

Classes Benzoids

Mechanism of action Miconazole interacts with 14α-demethylase, a cytochrome P450 enzyme necessary to convert lanosterol to ergosterol. As ergosterol is an essential component membrane, inhibition of its synthesis results in increased cellular permeability causing leakage of cellular contents.

PROPERTIES

State Solid.

CAS NO. 22916-47-8

Melting point 159-163°C

Experimental properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log P</td>
<td>5.86</td>
</tr>
<tr>
<td>PKa</td>
<td>6.77 (Basic)</td>
</tr>
</tbody>
</table>
1.4.2. ORNIDAZOLE

INTRODUCTION

<table>
<thead>
<tr>
<th>Name</th>
<th>Ornidazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Official in</td>
<td>IP 2010</td>
</tr>
<tr>
<td>Description</td>
<td>Ornidazole is a drug that cures some protozoan infections. It has been investigated for use in Crohn's disease after bowel resection</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemical Formula</th>
<th>C₇H₁₀ClN₃O₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mol.Wt</td>
<td>219.625 g/mol</td>
</tr>
<tr>
<td>IUPAC</td>
<td>1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol</td>
</tr>
<tr>
<td>Categories</td>
<td>Anti amoebiasis</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in chloroform and in methanol. Practically insoluble in water</td>
</tr>
</tbody>
</table>

PHARMACOLOGY

<table>
<thead>
<tr>
<th>Classes</th>
<th>Tissue amoebicides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanism of action</td>
<td>It causes the inhibition of DNA synthesis resulting in loss of helical structure.</td>
</tr>
</tbody>
</table>

PROPERTIES

<table>
<thead>
<tr>
<th>State</th>
<th>Solid.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS NO.</td>
<td>16773-42-5</td>
</tr>
<tr>
<td>Melting point</td>
<td>77-78°C</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experimental Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Property</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Log P</td>
</tr>
<tr>
<td>pKa</td>
</tr>
</tbody>
</table>
AIM: Literature review reveals that numbers of individual analytical methods available for estimation of Miconazole and Ornidazole in their individual dosage forms. But no method has been reported for simultaneous estimation of Miconazole and Ornidazole in combined pharmaceutical dosage form by RP-HPLC. So it is develop reverse phase high performance liquid chromatographic method for simultaneous estimation of Miconazole and Ornidazole in Combined Dosage Form. So Aim of present work is to develop simple, accurate, precise, rapid, specific, sensitive and selective Reverse Phase HPLC method for simultaneous estimation of Miconazole and Ornidazole.

3.2 OBJECTIVE

1) To develop HPLC method for simultaneous estimation of, Miconazole and Ornidazole in pharmaceutical dosage form.

2) Applying the newly developed, validated analytical method for the estimation of Miconazole and Ornidazole in formulations.

The Preliminary Analysis:

MICONAZOLE:

1) Description

The sample of Miconazole was observed for its color and texture.

2) Melting point

The sample of Miconazole was taken in capillary and place into the melting point apparatus. Observed the melting point and compared with the reference.

3) Identification Test

Potassium Bromide IR disc was prepared using 1mg of Miconazole on Hydraulic Pellet Press. This disc was scanned in the region of 4000–400cm⁻¹ in FTIR and obtained IR spectrum was compared with the reference spectrum of Miconazole.

4) Solubility

The sample of Miconazole was taken in test tubes and observed for solubility in various solvents like water, methanol, 0.1 N Hcl and 0.1 N NaOH.

ORNIDAZOLE:
1) Description

The sample of Ornidazole was observed for its and texture.

2) Melting point

The sample of Ornidazole was taken in capillary and place into the melting point apparatus. Observed the melting point and compared with the reference.

3) Identification Test

Potassium Bromide IR disc was prepared using 1mg of Ornidazole on Hydraulic Pellet Press. This disc was scanned in the region 0f 4000–400cm⁻¹ in FTIR and obtained IR spectrum was compared with the reference spectrum of Ornidazole.

4) Solubility

The sample of Ornidazole was taken in test tubes and observed for solubility in various solvents like water, methanol, 0.1 N Hcl and 0.1 N NaOH.

>Development and Validation for Simultaneous Estimation of Miconazole and Ornidazole By RP-HPLC.

**Apparatus and Instruments:**

Model: Shimadzu HPLC System

Pump:- LC-20 AT

Column: C₁₈ (250 mm × 4.6 mm i.d.,5µm)

Injector: 20µL fixed loop.

Detector: UV Detector

Software: Spinchrom

Analytical balance: Electronic Balance (Shimadzu AUX220)

**REAGENTS AND MATERIALS:**

✓ Miconazole was procured from Yash Pharma

✓ Ornidazole was procured from Yash Pharma

✓ Water
✓ Methanol
✓ Acetonitrile
✓ Hydrogen phosphate

Preparation of standard solutions:

(A) Ornidazole standard stock solution: (500 μg/mL)

A 50 mg of Ornidazole was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with methanol.

(B) Miconazole standard stock solution: (100 μg/mL)

A 10 mg of Miconazole was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with methanol.

(C) Preparation of standard solution of binary mixtures of Ornidazole (50 μg/mL) and Miconazole (10 μg/mL)

Take 1 mL from the Ornidazole stock solution and 1mL from Miconazole stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

Selection of wavelength:

Standard solution of Ornidazole (5 μg/mL) and Standard solution of Miconazole (10 μg/mL) were scanned between 200-400 nm using UV-visible spectrophotometer.

Both solutions were scanned between 200 - 400 nm.

Wavelength was selected from the overlay spectra of above solutions.

Chromatographic separation:

Standard solutions of 25-75 μg/ml of Ornidazole and 5-15 μg/ml of Miconazole were injected in column with 20 μl micro-syringes. The chromatogram was run for appropriate minutes with mobile phase Buffer(pH 5): Methanol (50:50)

The detection was carried out at wavelength 240 nm. The chromatogram was stopped after separation achieved completely. Data related to peak like area, height, retention time, resolution etc were recorded using software.
System suitability test:

It is an integral part of chromatographic method. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as a whole. System suitability testing provides assurance that the method will provide accurate and precise data for its intended use.

Chromatographic conditions:

- **Column**: C$_{18}$ (250 mm × 4.6 mm i.d., 5µm)
- **Mobile Phase**: Buffer (pH 5): Methanol(50:50)
- **Flow Rate**: 1.0 ml/min
- **Detection Wavelength**: 240 nm
- **Runtime**: 10 min
- **Injection volume**: 20.0 µl

Validation of Developed RP-HPLC method

**Linearity**

The linearity for Miconazole and Ornidazole were assessed by analysis of combined standard solution in range of 5-15 µg/ml and 25-75 µg/ml respectively,

5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of Ornidazole(500 µg/ml) and Miconazole(100 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 5,7.5,10,12.5,15 µg/ml and 25,37.5,50,62.5 and 75 µg/ml for Miconazole and Ornidazole respectively. In term of slope, intercept and correlation co-efficient value. The graph of peak area obtained verses respective concentration was plotted.

**Precision**: Results should be expressed as Relative standard deviation (RSD) or coefficient of variance.

**A. Repeatability**

Standard solution containing Ornidazole (50µg/ml) and Miconazole (10 µg/ml) was injected six times and areas of peaks were measured and % R.S.D. was calculated.
B. Intra-day precision

Standard solution containing (25, 50, 75 µg/ml) of Ornidazole and (5, 10, 15 µg/ml) of Miconazole were analyzed three times on the same day and % R.S.D was calculated.

C. Inter-day precision

Standard solution containing (25, 50, 75 µg/ml) of Ornidazole and (5, 10, 15 µg/ml) of Miconazole were analyzed three times on the different day and % R.S.D was calculated.

Accuracy

- For Miconazole

5 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 240 nm. The amount of Miconazole was calculated at each level and % recoveries were computed.

- For Ornidazole

25 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 240 nm. The amount of Ornidazole was calculated at each level and % recoveries were computed.

LOD and LOQ

The LOD was estimated from the set of 3 calibration curves used to determination method linearity. The LOD may be calculated as,

\[ LOD = 3.3 \times \frac{SD}{Slope} \]

Where,

SD= Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

The LOQ was estimated from the set of 3 calibration curves used to determine method linearity. The LOQ may be calculated as,

\[ LOQ = 10 \times \frac{SD}{Slope} \]
Where,

SD = Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

Robustness:

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.

2. pH of Mobile phase was changed (± 0.2) 5.2 and 4.8.

3. Ratio of Mobile phase was changed (±2) Buffer: Methanol (48:52) and Buffer: Methanol (52:48)

Analysis of formulation

Take Tablet Powder equivalent to 50 mg of Ornidazole and 10 mg of Miconazole was transferred to a 100 ml volumetric flask, add 60 ml of Mobile phase and shake well till 15 minutes and made up volume up to the mark with mobile phase. The solution was filtered through Whatman filter paper no. 42 and first few drops of filtrate were discarded. 1 ml of this solution was diluted to 10 ml with mobile phase. The solution was injected 20 µl. The areas of resulting peak were measured at 240 nm.

Identification of Drugs

❖ Determination of Solubility

<table>
<thead>
<tr>
<th>Drug</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miconazole</td>
<td>Slightly Soluble in Water &amp; Freely Soluble in Methanol</td>
</tr>
<tr>
<td>Ornidazole</td>
<td>Insoluble in water in Soluble Methanol</td>
</tr>
</tbody>
</table>

❖ Determination of Melting Point:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Melting Point (Observed)</th>
<th>Melting Point (Observed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miconazole</td>
<td>158-162°C</td>
<td>158-163°C</td>
</tr>
<tr>
<td>Ornidazole</td>
<td>76-78°C</td>
<td>76-78°C</td>
</tr>
</tbody>
</table>
Identification by IR

Fig: IR Spectra of Sample Ornidazole

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO₂ (Aromatic)</td>
<td>1500</td>
</tr>
<tr>
<td>C-N</td>
<td>1200</td>
</tr>
<tr>
<td>C=C</td>
<td>1250</td>
</tr>
<tr>
<td>C-Cl</td>
<td>750</td>
</tr>
</tbody>
</table>

Fig. : IR Spectra of sample Miconazole
### Functional group

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-O</td>
<td>1050</td>
</tr>
<tr>
<td>C-N</td>
<td>1300</td>
</tr>
<tr>
<td>C=C</td>
<td>1690</td>
</tr>
<tr>
<td>C-Cl</td>
<td>730</td>
</tr>
</tbody>
</table>

### Development of RP-HPLC Method

#### 1 Selection of wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study drug solutions of Miconazole (10 ppm) and Ornidazole (50 ppm) were prepared in Methanol. These drug solutions were than scanned in UV region of 200-400 nm and overlay spectrums were recorded.

![Figure: UV Spectra of Miconazole (10 ppm) and Ornidazole (50 ppm) in Methanol (Wavelength Taken 240 nm)](image)

#### 2 Selection of Mobile Phase

Trail contains various mobile phase which are considered of Methanol, Water and Acetonitrile in different proportions and different volumes at different flow rate were tried. On the basis of...
various trails the mixture of Buffer (pH 5.0): Methanol (40:60), at 1.0 mL/min flow rate, proved to be better than the other mixture in terms of peak shape, theoretical plate and asymmetry.

Trials are summarized in the following table.

**TABLE: List of Mobile Phase trials**

<table>
<thead>
<tr>
<th>Sr.</th>
<th>Mobile Phase</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water : Methanol (20:80)</td>
<td>Two Peaks Observed</td>
</tr>
<tr>
<td>2</td>
<td>Water : Methanol (20:80)</td>
<td>First peak was of Miconazole, Confirmed by injecting Miconazole (10ppm) individual</td>
</tr>
<tr>
<td>3</td>
<td>Water : Methanol (20:80)</td>
<td>Second peak was of Ornidazole, Confirmed by injecting Ornidazole (50ppm) individual</td>
</tr>
<tr>
<td>4</td>
<td>Water : Methanol (30:70)</td>
<td>Both Peak are merged and Peak shape of peak of Ornidazole is Irregular</td>
</tr>
<tr>
<td>5</td>
<td>Phosphate Buffer (pH 6.0): Methanol (50:50)</td>
<td>Peak Shape Became sharp but peak of Miconazole observed at Solvent peak time</td>
</tr>
<tr>
<td>6</td>
<td>Phosphate Buffer (pH 6.0): Methanol (60:40)</td>
<td>Retention time of Second peak increased but peak of Miconazole still observed at Solvent peak time</td>
</tr>
<tr>
<td>7</td>
<td>Phosphate Buffer (pH 6.0): Methanol (70:30)</td>
<td>Again Retention time of Peak of Ornidazole Increased by increasing the proportion of Buffer but Peak of Miconazole is as it is.</td>
</tr>
<tr>
<td>8</td>
<td>Phosphate Buffer (pH 5.0): Methanol (70:30)</td>
<td>Now Retention time of Peak of Miconazole increased,</td>
</tr>
<tr>
<td>9</td>
<td>Phosphate Buffer (pH 5.0): Methanol (60:40)</td>
<td>Run time decreased</td>
</tr>
<tr>
<td>10</td>
<td>Phosphate Buffer (pH 5.0): Methanol (50:50)</td>
<td>Again run time decreased</td>
</tr>
<tr>
<td>11</td>
<td>Phosphate Buffer (pH 5.0): Methanol (40:60)</td>
<td>First peak observed at solvent peak time, so above mobile phase is final</td>
</tr>
</tbody>
</table>
Figure: HPLC Chromatogram of Miconazole (10ppm) and Ornidazole (50ppm) in Water: Methanol (20:80)

Figure 6.5: HPLC Chromatogram of Miconazole (10ppm) in Water: Methanol (50:50)

Figure: HPLC Chromatogram of Ornidazole (50ppm) in Water: Methanol (50:50)
Figure: HPLC Chromatogram of Miconazole (10ppm) and Ornidazole (50ppm) in Water: Methanol (30:70)

Figure: HPLC Chromatogram of Miconazole (10ppm) and Ornidazole (50ppm) in Buffer (pH 6.0): Methanol (50:50)
Figure: HPLC Chromatogram of Miconazole (10ppm) and Ornidazole (50ppm) in Buffer (pH 6.0): Acetonitrile (60:40)

Figure: HPLC Chromatogram of Miconazole (10ppm) and Ornidazole (50ppm) in Buffer (pH 6.0): Methanol (70:30)
Figure: HPLC Chromatogram of Miconazole (10ppm) and Ornidazole (50ppm) in Buffer (pH 6.0): Methanol (70:30)

Figure: HPLC Chromatogram of Miconazole (10ppm) and Ornidazole (50ppm) in Buffer (pH 5.0): Methanol (60:40)
Mobile Phase was selected based on the review of literature. Various mobile phases were tried. Trial contains various mobile phases which consisted of Methanol, Water, Triethylamine in different proportions with various pH and different volumes at flow rate 1 ml/min were tried. On the basis of various trials the mixture of Buffer (pH 5.0):Methanol (50:50) was selected.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Miconazole</th>
<th>Ornidazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention Time</td>
<td>3.320</td>
<td>4.707</td>
</tr>
<tr>
<td>Theoretical Plates</td>
<td>7539</td>
<td>7262</td>
</tr>
</tbody>
</table>
Asymmetry | 1.350 | 1.400  
Resolution   | 7.418  

Optimization of flow rate

1ml/min flow rate, proved to be better than the other in terms of resolution, peak shape and shorter retention time.

Table: RP-HPLC optimized chromatographic conditions

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Chromatographic Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode of elution</td>
<td>Isocratic</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>Buffer (pH 5.0):Methanol (50:50)</td>
</tr>
<tr>
<td>Column</td>
<td>C18 (25cm x 0.46 cm) Hypersil BDS</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1ml/min</td>
</tr>
<tr>
<td>Runtime</td>
<td>6 min</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20 µL</td>
</tr>
<tr>
<td>Detection wavelength</td>
<td>240 nm</td>
</tr>
</tbody>
</table>

Validation of RP-HPLC method:

Specificity:

Fig. :- Chromatogram of Miconazole and Ornidazole std
The Chromatograms of Miconazole and Ornidazole standards and Miconazole and Ornidazole sample show no interference with the Chromatogram of Miconazole and Ornidazole Blank, so the Developed method is Specific.

**Linearity:**

The regression line equation for Ornidazole and Miconazole are as following:

For Ornidazole: \( y = 98.439x - 0.8074 \) and For Miconazole: \( y = 84.221x + 0.6068 \)

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Concentration(µg/ml)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>2517.111</td>
</tr>
<tr>
<td>2</td>
<td>37.5</td>
<td>3636.106</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>4923.711</td>
</tr>
<tr>
<td>4</td>
<td>62.5</td>
<td>6082.409</td>
</tr>
<tr>
<td>5</td>
<td>75</td>
<td>7446.404</td>
</tr>
</tbody>
</table>

Table: Linearity data for Ornidazole
<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Concentration(µg/ml)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>430.105</td>
</tr>
<tr>
<td>2</td>
<td>7.5</td>
<td>621.961</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>847.982</td>
</tr>
<tr>
<td>4</td>
<td>12.5</td>
<td>1040.391</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>1273.655</td>
</tr>
</tbody>
</table>

Table: Linearity data for Miconazole.

Fig.: Overlay chromatogram of different concentrations of mixtures of Ornidazole and Miconazole

Fig.: Calibration Curve of Miconazole (5-15μg/ml).
Fig. : Calibration Curve of Ornidazole (25-75 μg/ml)

Precision I. Repeatability

**Ornidazole**

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Conc (μg/ml)</th>
<th>Area</th>
<th>Mean ± S.D (n=6)</th>
<th>% R.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>50</td>
<td>4956.633</td>
<td>4962.454±26.202</td>
<td>0.528</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4951.608</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4986.296</td>
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<td></td>
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<td>4961.373</td>
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<td>4996.102</td>
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<td></td>
<td>4922.712</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table**: Repeatability data for Ornidazole.

**Miconazole**

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Conc (μg/ml)</th>
<th>Area</th>
<th>Mean ± S.D (n=6)</th>
<th>% R.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>10</td>
<td>847.956</td>
<td>849.973 ±3.179</td>
<td>0.374</td>
</tr>
<tr>
<td></td>
<td></td>
<td>847.155</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>853.119</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>848.838</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>854.802</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>847.970</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table**: repeatability data for Miconazole.
II. Intraday precision

<table>
<thead>
<tr>
<th>SR. NO.</th>
<th>Conc. (µg/ml)</th>
<th>Area Mean ± S.D. (n=3)</th>
<th>% R.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>2482.292 ± 3.734</td>
<td>0.150</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>5000.660 ± 28.846</td>
<td>0.577</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>7438.857 ± 30.997</td>
<td>0.417</td>
</tr>
</tbody>
</table>

Table: Intraday precision data for estimation of Ornidazole

<table>
<thead>
<tr>
<th>SR. NO.</th>
<th>Conc. (µg/ml)</th>
<th>Area Mean ± S.D. (n=3)</th>
<th>% R.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>424.703 ± 1.082</td>
<td>0.255</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>856.778 ± 3.470</td>
<td>0.405</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>1272.391 ± 5.272</td>
<td>0.414</td>
</tr>
</tbody>
</table>

Table: Intraday precision data for estimation of Miconazole

III. Interday precision

<table>
<thead>
<tr>
<th>SR. NO.</th>
<th>Conc. (µg/ml)</th>
<th>Area Mean ± S.D. (n=3)</th>
<th>% R.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>2460.137 ± 31.438</td>
<td>1.277</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>4955.970 ± 61.494</td>
<td>1.241</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>7470.511 ± 37.323</td>
<td>0.500</td>
</tr>
</tbody>
</table>

Table: Interday precision data for estimation of Ornidazole.

<table>
<thead>
<tr>
<th>SR. NO.</th>
<th>Conc. (µg/ml)</th>
<th>Area Mean ± S.D. (n=3)</th>
<th>% R.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>421.478 ± 3.597</td>
<td>1.049</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>849.944 ± 7.245</td>
<td>0.852</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>1278.500 ± 5.597</td>
<td>0.438</td>
</tr>
</tbody>
</table>

Table: Interday precision data for estimation of Miconazole.
## Accuracy:

<table>
<thead>
<tr>
<th>SR. NO.</th>
<th>Conc. Level (%)</th>
<th>Sample Amount (μg/ml)</th>
<th>Amount Added (μg/ml)</th>
<th>Amount recovered (μg/ml)</th>
<th>% Recovery</th>
<th>% Mean Recovery ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80 %</td>
<td>25</td>
<td>20</td>
<td>20.136</td>
<td>100.681</td>
<td>99.679 ± 1.726</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>25</td>
<td>20</td>
<td>19.537</td>
<td>97.686</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>25</td>
<td>20</td>
<td>20.134</td>
<td>100.670</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>100 %</td>
<td>25</td>
<td>25</td>
<td>24.970</td>
<td>99.880</td>
<td>100.539 ± 0.977</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>25</td>
<td>25</td>
<td>25.019</td>
<td>100.074</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>25</td>
<td>25</td>
<td>25.415</td>
<td>101.662</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>120 %</td>
<td>25</td>
<td>30</td>
<td>29.891</td>
<td>99.638</td>
<td>99.589 ± 0.799</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>25</td>
<td>30</td>
<td>29.630</td>
<td>98.766</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>25</td>
<td>30</td>
<td>30.109</td>
<td>100.362</td>
<td></td>
</tr>
</tbody>
</table>

### Table: Recovery data for Ornidazole

<table>
<thead>
<tr>
<th>SR. NO.</th>
<th>Conc. Level (%)</th>
<th>Sample Amount</th>
<th>Amount Added</th>
<th>Amount recovered (μg/ml)</th>
<th>% Recovery</th>
<th>% Mean Recovery ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80 %</td>
<td>5</td>
<td>4</td>
<td>4.038</td>
<td>100.945</td>
<td>100.572 ± 0.648</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>5</td>
<td>4</td>
<td>3.993</td>
<td>99.824</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>5</td>
<td>4</td>
<td>4.038</td>
<td>100.947</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>100 %</td>
<td>5</td>
<td>5</td>
<td>5.006</td>
<td>100.112</td>
<td>100.326 ± 0.218</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>5</td>
<td>5</td>
<td>5.016</td>
<td>100.318</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>5</td>
<td>5</td>
<td>5.027</td>
<td>100.547</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>120 %</td>
<td>5</td>
<td>6</td>
<td>5.991</td>
<td>99.848</td>
<td>99.986 ± 0.545</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>5</td>
<td>6</td>
<td>5.971</td>
<td>99.524</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>5</td>
<td>6</td>
<td>6.035</td>
<td>100.587</td>
<td></td>
</tr>
</tbody>
</table>

### Table: Recovery data for Miconazole
LOD and LOQ:

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

\[
\text{LOD} = 3.3 \times \frac{\text{SD}}{\text{slope of calibration curve}}
\]

\[
\text{LOQ} = 10 \times \frac{\text{SD}}{\text{slope of calibration curve}}
\]

Where, SD = Standard deviation of intercepts

**Limit of Detection:**

<table>
<thead>
<tr>
<th>Ornidazole</th>
<th>Miconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD = 3.3 x (SD / Slope)</td>
<td>LOD = 3.3 x (SD / Slope)</td>
</tr>
<tr>
<td>= 3.3 x (71.061/98.43)</td>
<td>= 3.3 x (12.469/84.22)</td>
</tr>
<tr>
<td>= 2.382µg/ml</td>
<td>= 0.489 µg/ml</td>
</tr>
</tbody>
</table>

*Table: Limit of Detection data for Miconazole and Ornidazole.*

**Limit of Quantitation:**

<table>
<thead>
<tr>
<th>Ornidazole</th>
<th>Miconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOQ = 10 x (SD / Slope)</td>
<td>LOQ = 10 x (SD / Slope)</td>
</tr>
<tr>
<td>= 10 x (71.061/98.43)</td>
<td>= 10 x (12.469/84.22)</td>
</tr>
<tr>
<td>= 7.219 µg/ml</td>
<td>= 1.481 µg/ml</td>
</tr>
</tbody>
</table>

*Table: Limit of Quantitation data for Miconazole and Ornidazole.*

**Robustness:**

<table>
<thead>
<tr>
<th>SR NO.</th>
<th>Area at Flow rate (-0.2 ml/min)</th>
<th>Area at Flow rate (+0.2 ml/min)</th>
<th>Area at pH (-0.2)</th>
<th>Area at pH (+0.2)</th>
<th>Area at Mobile phase(-2)</th>
<th>Area at Mobile phase(+2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5136.090</td>
<td>3333.630</td>
<td>5076.642</td>
<td>5001.410</td>
<td>5031.404</td>
<td>4912.148</td>
</tr>
<tr>
<td>2</td>
<td>5115.490</td>
<td>3282.563</td>
<td>5042.385</td>
<td>5001.071</td>
<td>5021.296</td>
<td>4909.360</td>
</tr>
<tr>
<td>3</td>
<td>5115.459</td>
<td>3374.725</td>
<td>5081.618</td>
<td>4991.104</td>
<td>5021.230</td>
<td>4966.243</td>
</tr>
<tr>
<td>% R.S.D</td>
<td>0.232</td>
<td>1.386</td>
<td>0.422</td>
<td>0.117</td>
<td>0.117</td>
<td>0.651</td>
</tr>
</tbody>
</table>

*Table: Robustness data for Ornidazole.*
### Table: Robustness data for Miconazole.

<table>
<thead>
<tr>
<th></th>
<th>Flow rate (-0.2 ml/min)</th>
<th>Flow rate (+0.2 ml/min)</th>
<th>pH (-0.2)</th>
<th>pH (+0.2)</th>
<th>Mobile phase (-2)</th>
<th>Mobile phase (+2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>878.606</td>
<td>2282.685</td>
<td>868.529</td>
<td>855.633</td>
<td>860.790</td>
<td>840.382</td>
</tr>
<tr>
<td>2</td>
<td>875.087</td>
<td>2354.415</td>
<td>868.557</td>
<td>859.950</td>
<td>848.874</td>
<td>843.762</td>
</tr>
<tr>
<td>3</td>
<td>872.713</td>
<td>2368.847</td>
<td>869.428</td>
<td>853.941</td>
<td>859.081</td>
<td>849.686</td>
</tr>
<tr>
<td>% R.S.D</td>
<td>0.339</td>
<td>1.976</td>
<td>0.059</td>
<td>0.362</td>
<td>0.753</td>
<td>0.558</td>
</tr>
</tbody>
</table>

### Analysis of marketed formulation by developed method

**Sample Stock Solution (Miconazole 100 μg/mL, and Ornidazole 500 μg/mL):**

Take Tablet Powder equivalent to 10 mg of Miconazole, and 50 mg of Ornidazole was transferred to a 100 ml volumetric flask, Add 60 ml Mobile phase and Shake for 15 min and make up volume with Mobile phase. The solution was filtered through Whatman filter paper no. 42.

**Working Sample Preparation (Miconazole 10 μg/mL, and Ornidazole 50 μg/mL):**

Take 1 mL from standard stock solution and transferred to 10 ml volumetric flask and made up volume up to the mark with the mobile phase.

Inject above Solution 20 μl for Assay Analysis.

### Table: Analysis on marketed formulation

<table>
<thead>
<tr>
<th>Tablet</th>
<th>Candifem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Label claim</td>
<td>Miconazole (100mg)</td>
</tr>
<tr>
<td>Assay (% of label claim*)</td>
<td>97.402±0.885</td>
</tr>
<tr>
<td>Mean ± S. D.</td>
<td></td>
</tr>
</tbody>
</table>

The assay results were comparable to labelled value of each drug in Combined dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

### SUMMARY AND CONCLUSION

- **RP-HPLC method was developed for simultaneous estimation Miconazole and Ornidazole.** In RP-HPLC method, good resolution and separation of two drugs was achieved. Buffer (pH 5.0):Methanol (50:50) was used as mobile phase. Retention time of Miconazole and Ornidazole were found to be 3.320 min and 4.707 min respectively with a flow rate of 1
ml/min. The proposed method was accurate and precise. Therefore proposed method can be used for routine analysis of Miconazole and Ornidazole in Combined Dosage form.

- Validation parameters like Linearity, Accuracy, Precision, Robustness, System suitability, Specificity were tested.

- Observation of all these parameters leads to the point that developed RP-HPLC method is linear, accurate, precise, specific and robust.

- It can be successfully adopted for routine quality control analysis of Miconazole And Ornidazole in combined Tablet dosage form without any interference from common excipients and impurity.

- This method can now transfer to utilize for routine laboratory analysis and assay of Miconazole And Ornidazole In Their Tablet Dosage Form.

CONCLUSION:

- RP-HPLC method was developed for simultaneous estimation Miconazole and Ornidazole. In RP-HPLC method, good resolution and separation of two drugs was achieved. Buffer (pH 5.0):Methanol (50:50) was used as mobile phase. Retention time of Miconazole and Ornidazole were found to be 3.320 min and 4.707 min respectively with a flow rate of 1 ml/min. The proposed method was accurate and precise. Therefore proposed method can be used for routine analysis of Miconazole and Ornidazole in Combined Dosage form.

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- It can be successfully adopted for routine quality control analysis of Miconazole And Ornidazole in combined Tablet dosage form without any interference from common excipients and impurity.

- This method can now transfer to utilize for routine laboratory analysis and assay of Miconazole And Ornidazole In Their Tablet Dosage Form.
COMPARISON WITH THE EXISTING METHOD:

<table>
<thead>
<tr>
<th>Developed Method</th>
<th>Existing Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile Phase: Phosphate Buffer (pH 5.0): Methanol 50:50</td>
<td>Mobile Phase: Acetonitrile: Methanol 80:20</td>
</tr>
<tr>
<td>Column: C\textsubscript{18} (250 mm × 4.6 mm i.d.,5µm)</td>
<td>Column:C\textsubscript{18} (250 mm × 4.6 mm i.d.,5µm)</td>
</tr>
<tr>
<td>Detection Wavelength: 240nm</td>
<td>Detection Wavelength: 219nm</td>
</tr>
<tr>
<td>Flow rate: 1ml/min</td>
<td>Flow rate: 1ml/min</td>
</tr>
<tr>
<td>Injection Volume: 20 µl</td>
<td>Injection Volume: 20 µl</td>
</tr>
<tr>
<td>Run time: 6min</td>
<td>Run time: 10min</td>
</tr>
</tbody>
</table>

REFERENCES:

1. “Introduction to Candidiasis”, September-2016,
7. Watson DG. A Text book for pharmacy student and pharmaceutical chemist; 2nd Edn;
8. Churchil Livingstone publication, UK, 1999, p 238-274
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