ABSTRACT

The main objective of present work is to develop simple, economic and reliable UV spectroscopic method for the routine analysis of Metronidazole and Norfloxacin in Syrup dosage form. There is no Literature found for estimation of Metronidazole and Norfloxacin in combined syrup dosage form by simultaneous UV spectroscopic method. So, Spectroscopic method was developed for simultaneous estimation of Metronidazole and Norfloxacin in syrup formulation by optimizing Acetic acid as a solvent for extraction. The developed method was validated as per ICH guidelines. The linearity range was found from 2-10μg/ml for both the drugs. Regression line equations were obtained Y=0.0892x+0.084 and Y=0.1839x+0.0377 for Metronidazole and Norfloxacin respectively. R² values were obtained 0.995 and 0.996 respectively for Metronidazole and Norfloxacin. Repeatability, Intrayday and inter day precision were carried out which gave %RSD<2. Accuracy was performed by spiking method. %RSD was obtained <2. Limit of detection for Metronidazole and Norfloxacin was obtained 0.068μg/ml and 0.014μg/ml respectively. Limit of quantification was obtained for Metronidazole and Norfloxacin was 0.229μg/ml and 0.047μg/ml respectively. From the results obtained from validation parameters we can conclude that developed method was linear, accurate and precise, so we can use this method for routine analysis of Metronidazole and Norfloxacin in syrup formulation.

KEYWORDS: - Analytical Method development, Validation, Metronidazole, Norfloxacin, Simultaneous Estimation Method.
INTRODUCTION

- Norfloxacin is a 1st generation fluoroquinolone antimicrobial agent & Metronidazole is a nitroimidazole drug used for a variety of anaerobic infections.\(^1\)

- Norfloxacin also used in the treatment of uncomplicated urinary tract infections, uncomplicated urethral and cervical gonorrhea. Metronidazole is also used in the treatment of amoebiasis, giardiasis, trichomoniasis, bacterial vaginosis, in symptomatic pelvic inflammatory disease in conjunction with other antibiotics such as Ofloxacin, Levofloxacin, or Ceftriaxone.\(^2\) Combination of Norfloxacin & Metronidazole is used in the treatment of diarrhea, amoebiasis, giardiasis, orodental infection.\(^3\)

- The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one.\(^4\) Some drugs are not official in Pharmacopoeia. Under these conditions, standards & analytical procedures for these drugs may not be available in pharmacopoeias. Thus it becomes necessary to develop newer analytical methods for such drugs and formulation.\(^5\)

- Various spectroscopic, Chromatographic, radiometric and Miscellaneous methods are available for analysis.

- Validation means finding or testing the truth of something.\(^6\) “The goal of validation is to establish documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.”\(^7\)

Table 1: Drug profile\(^8\)\(^9\)\(^10\)\(^11\)

<table>
<thead>
<tr>
<th>SR. NO.</th>
<th>PARAMETER</th>
<th>METRONIZOLE(^12)</th>
<th>NORFLOXACIN(^13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Structure</td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Norfloxacin" /></td>
</tr>
<tr>
<td>2.</td>
<td>Chemical Name</td>
<td>1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole</td>
<td>1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid</td>
</tr>
<tr>
<td>3.</td>
<td>Molecular formula</td>
<td>C(_6)H(_9)N(_3)O(_3)</td>
<td>C(<em>{16})H(</em>{18})FN(_3)O(_3)</td>
</tr>
<tr>
<td>4.</td>
<td>Molecular Weight</td>
<td>171.2 g/mole</td>
<td>319.34 g/mole</td>
</tr>
<tr>
<td>5.</td>
<td>CAS Registry No.</td>
<td>443-48-1</td>
<td>70458-96-7</td>
</tr>
<tr>
<td>8.</td>
<td>Solubility</td>
<td>Slightly soluble in water, in Ethanol (95%), in Acetone and Dichloromethane. Very slightly soluble in Ether.</td>
<td>Freely soluble in Acetic acid, Sparingy soluble in Chloroform, Slightly soluble in Acetone &amp; Ethanol (95%). Insoluble in Ether.</td>
</tr>
</tbody>
</table>
• From the literature survey it is revealed that there is spectroscopic method available for estimation of Metronidazole & Norfloxacin in combine dosage form as well as for single drug Metronidazole & Norfloxacin also, but mostly these methods are available for either solid dosage form or suspension. Other dosage forms are also available in the market, but for them, spectroscopic chromatographic method is not available. [8-11],[14-22]

• Here, our rational is to develop simple, economic & reliable method for routine analysis of Metronidazole & Norfloxacin in combined syrup dosage form.

• MATERIAL AND METHOD

Table 2: Material And Method

<table>
<thead>
<tr>
<th>Material</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole API</td>
<td>Nirlife</td>
</tr>
<tr>
<td>Norfloxacin API</td>
<td>Mahik Medicaments</td>
</tr>
<tr>
<td>Distilled water</td>
<td>In house</td>
</tr>
<tr>
<td>Ethanol(laboratory grade)</td>
<td>Merck Mumbai</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>Merck Mumbai</td>
</tr>
<tr>
<td>Infrared Spectroscopy</td>
<td>Brukner (Alpha)</td>
</tr>
<tr>
<td>Digital Balance</td>
<td>Shimadzu ATX224</td>
</tr>
<tr>
<td>UV-Visible Spectrophotometer</td>
<td>Shimadzu – 1800with matched quartz cells</td>
</tr>
<tr>
<td>Software</td>
<td>UV – Probe</td>
</tr>
<tr>
<td>Sonicator</td>
<td>Toshcon, Mumbai</td>
</tr>
<tr>
<td>Melting Point Apparatus</td>
<td>Janki, Mumbai</td>
</tr>
</tbody>
</table>

Formulation used was Nor metrogyl which is manufactured by Hema Laboratories.

Label claim: Each 5ml contains 100mg Metronidazole and 100mg Norfloxacin.
Simultaneous Estimation Method Development

Solubility trials

**METRONIDAZOLE**

Acetic Acid

Ethanol

Distilled Water

**NORFLOXACIN**

Acetic Acid

Ethanol

As shown in spectra of solubility of Metronidazole and Norfloxacin, it had been concluded that Acetic Acid can be common solvent for both the drugs.

Trials of solubility were carried out for Metronidazole and Norfloxacin in different concentration of Acetic Acid.

Metronidazole and Norfloxacin were dissolved in various concentrations like 0.1M, 0.2M, 0.3M, 0.4M, 0.5M and 10% of Acetic Acid.

These solutions were examined by UV spectrophotometer.

Overlay spectra were observed to optimize final concentration of Acetic Acid.

**Optimization of Acetic acid**

<table>
<thead>
<tr>
<th>Solvent used</th>
<th>Absorbance</th>
<th>Acetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>0.001</td>
<td>0.678</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.13</td>
<td>0.022</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.437</td>
<td></td>
</tr>
</tbody>
</table>

**Selection of $\lambda_{\text{max}}$**

- A specific concentration of solution of metronidazole & norfloxacin were prepared.
- Then these solutions were scanned in UV range of 200-400nm.
Spectrums were obtained.
The $\lambda_{\text{max}}$ obtained at a particular wave length, where absorbance was maximum.

Fig. 3: Solubility trials for Metronidazole

Table 5: Solubility trials of Metronidazole

<table>
<thead>
<tr>
<th>Concentration of Acetic Acid</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1M Acetic Acid</td>
<td>0.268</td>
</tr>
<tr>
<td>0.2M Acetic Acid</td>
<td>0.430</td>
</tr>
<tr>
<td>0.3M Acetic Acid</td>
<td>0.952</td>
</tr>
<tr>
<td>0.4M Acetic Acid</td>
<td>0.339</td>
</tr>
<tr>
<td>0.5M Acetic Acid</td>
<td>0.306</td>
</tr>
<tr>
<td>10% Acetic Acid</td>
<td>0.693</td>
</tr>
</tbody>
</table>
Fig. 4: Solubility trials for Norfloxacin

Table 6: Solubility trials for Norfloxacin

<table>
<thead>
<tr>
<th>Concentration of Acetic Acid</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1M Acetic Acid</td>
<td>0.452</td>
</tr>
<tr>
<td>0.2M Acetic Acid</td>
<td>0.798</td>
</tr>
<tr>
<td>0.3M Acetic Acid</td>
<td>1.323</td>
</tr>
<tr>
<td>10% Acetic Acid</td>
<td>1.006</td>
</tr>
</tbody>
</table>

Table 7: Selection of $\lambda_{\text{max}}$

<table>
<thead>
<tr>
<th>Drug</th>
<th>$\lambda_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>320nm</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>278nm</td>
</tr>
</tbody>
</table>

Fig. 5: spectra of formulation with 0.3m acetic acid

Calibration Curve for Metronidazole
- 25mg of Metronidazole was weighed dissolved in 0.3M acetic acid. (1000ppm)
- 10ml was withdrawn and diluted up to 100ml Distilled water (100ppm).
- From above solution 0.2, 0.4, 0.6, 0.8 and 1ml was withdrawn and diluted up to 10 ml Distilled water.
- 2, 4, 6, 8, 10 ppm solution were observed in UV spectrophotometer.
- Calibration curve was plotted in MS Excel. Slope, Intercept and Regression line was carried out.

![Fig. 6 Calibration curve for Metronidazole](image)

### Table 8: Calibration Curve for Metronidazole

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>Absorbance (320nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>0.062</td>
</tr>
<tr>
<td>0.4</td>
<td>0.138</td>
</tr>
<tr>
<td>0.6</td>
<td>0.230</td>
</tr>
<tr>
<td>0.8</td>
<td>0.313</td>
</tr>
<tr>
<td>1</td>
<td>0.394</td>
</tr>
</tbody>
</table>

![Fig. 7: Regression Line Equation](image)
Calibration Curve for Norfloxacin

- 25mg of Norfloxacin was weighed dissolved in 0.3M acetic acid. (1000ppm)
- 10ml was withdrawn and diluted up to 100ml Distilled water.(100ppm)
- From above solution 0.2, 0.4, 0.6, 0.8 and 1ml was withdrawn and diluted up to 10 ml Distilled water.
- 2, 4, 6,8,10 ppm solution were observed in UV spectrophotometer.
- Calibration curve was plotted in MS Excel. Slope, Intercept and Regression line was carried out.

![Fig 8: Calibration curve for Norfloxacin](image)

Table 9: Calibration curve for Norfloxacin

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Absorbance (320nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>0.298</td>
</tr>
<tr>
<td>0.4</td>
<td>0.645</td>
</tr>
<tr>
<td>0.6</td>
<td>0.993</td>
</tr>
<tr>
<td>0.8</td>
<td>1.440</td>
</tr>
<tr>
<td>1</td>
<td>1.844</td>
</tr>
</tbody>
</table>

![Fig 10: Calibration curve for Norfloxacin](image)
Table 10: Absorbance of Metronidazole and Norfloxacin in Formulation

<table>
<thead>
<tr>
<th>Drug</th>
<th>λ max</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>320nm</td>
<td>0.683</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>278nm</td>
<td>1.281</td>
</tr>
</tbody>
</table>

Table 11: Result Obtained From Method

<table>
<thead>
<tr>
<th>Cx Metronidazole 6µg/ml</th>
<th>Cy Norfloxacin 6µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1µg/ml</td>
<td>6.2 µg/ml</td>
</tr>
</tbody>
</table>

Validation of developed analytical method

Linearity

1. Sample solution was suitably diluted with 0.3M Acetic acid and further with Distilled water to obtain concentrations ranging from 2-10 µg/ml. Absorbance of these solutions was measured at 320nm(Metronidazole) and 278nm(Norfloxacin) for Method.

2. The calibration curve was plotted and slope, intercept and regression value (R²) was carried out.

3. The R² value should be less than 1 and near to 1, which indicates linear rise in the sults.
TABLE 12: Linearity

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>Absorbance</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Metronidazole (320nm)</td>
<td>Norfloxacin (278nm)</td>
<td></td>
</tr>
<tr>
<td>2+2</td>
<td>0.262</td>
<td>0.401</td>
<td></td>
</tr>
<tr>
<td>4+4</td>
<td>0.421</td>
<td>0.789</td>
<td></td>
</tr>
<tr>
<td>6+6</td>
<td>0.647</td>
<td>1.099</td>
<td></td>
</tr>
<tr>
<td>8+8</td>
<td>0.875</td>
<td>1.562</td>
<td></td>
</tr>
<tr>
<td>10+10</td>
<td>0.967</td>
<td>1.853</td>
<td></td>
</tr>
</tbody>
</table>

Fig.: 12: Linearity

Fig.: 13 Linearity (Metronidazole)

Fig.: 14 Linearity (Norfloxacin)
Precision

**Repeatability:** The precision of the instrument was checked by repeated scanning and measurement of the absorbance of 6 μg/ml Metronidazole and Norfloxacin sample solution (n = 6) without changing the parameters for the method.

**Table 13 Repeatability**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance</th>
<th>Concentration Obtained</th>
<th>Standard deviation</th>
<th>%Relative Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>320nm Metro</td>
<td>278nm Nor</td>
<td>Cx (μg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>0.559</td>
<td>1.109</td>
<td>6.18</td>
<td>6.61</td>
</tr>
<tr>
<td>2</td>
<td>0.558</td>
<td>1.107</td>
<td>5.97</td>
<td>6.53</td>
</tr>
<tr>
<td>3</td>
<td>0.559</td>
<td>1.109</td>
<td>6.18</td>
<td>6.61</td>
</tr>
<tr>
<td>4</td>
<td>0.557</td>
<td>1.107</td>
<td>5.91</td>
<td>6.53</td>
</tr>
<tr>
<td>5</td>
<td>0.557</td>
<td>1.107</td>
<td>5.91</td>
<td>6.53</td>
</tr>
<tr>
<td>6</td>
<td>0.558</td>
<td>1.108</td>
<td>5.97</td>
<td>6.53</td>
</tr>
</tbody>
</table>

**Intradays Precision:**: The intraday precision of the proposed method was performed by analyzing the corresponding responses 3 Times on the same day.

**Table 14: Intraday Precision**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance</th>
<th>Concentration Obtained</th>
<th>Standard deviation</th>
<th>%Relative Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>320nm Metro</td>
<td>278nm Nor</td>
<td>Cx (μg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>0.559</td>
<td>1.109</td>
<td>7.8</td>
<td>6.1</td>
</tr>
<tr>
<td>2</td>
<td>0.559</td>
<td>1.103</td>
<td>7.8</td>
<td>6.53</td>
</tr>
<tr>
<td>3</td>
<td>0.558</td>
<td>1.103</td>
<td>7.51</td>
<td>6.1</td>
</tr>
</tbody>
</table>

**Inter day Precision:** The inter day precision of the proposed method was performed by analysing the corresponding responses 3 times on different days.

**Table 15: Inter day Precision**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance</th>
<th>Concentration Obtained</th>
<th>Standard deviation</th>
<th>%Relative Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>320nm Metro</td>
<td>278nm Nor</td>
<td>Cx (μg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>0.513</td>
<td>1.044</td>
<td>6.9</td>
<td>5.77</td>
</tr>
<tr>
<td>2</td>
<td>0.512</td>
<td>1.045</td>
<td>6.76</td>
<td>5.71</td>
</tr>
<tr>
<td>3</td>
<td>0.511</td>
<td>1.044</td>
<td>6.8</td>
<td>5.77</td>
</tr>
</tbody>
</table>
Recovery studies

Recovery studies were carried out by adding known amount of standard solution, to the sample solution.

Table 16: Recovery Studies

<table>
<thead>
<tr>
<th>Recovery Level</th>
<th>Drug</th>
<th>Amount present (µg/ml)</th>
<th>Spiked drug (µg/ml)</th>
<th>Total Concentration (µg/ml)</th>
<th>Total amount recovered</th>
<th>% Recovery (n=3)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>Metronidazole</td>
<td>6</td>
<td>4</td>
<td>10</td>
<td>3.93</td>
<td>98.25%</td>
<td>0.72%</td>
</tr>
<tr>
<td>100%</td>
<td>Metronidazole</td>
<td>6</td>
<td>6</td>
<td>12</td>
<td>5.89</td>
<td>98.16%</td>
<td>0.83%</td>
</tr>
<tr>
<td>120%</td>
<td>Metronidazole</td>
<td>6</td>
<td>8</td>
<td>14</td>
<td>7.69</td>
<td>96.12%</td>
<td>1.13%</td>
</tr>
<tr>
<td>80%</td>
<td>Norfloxacin</td>
<td>6</td>
<td>4</td>
<td>10</td>
<td>3.86</td>
<td>96.5%</td>
<td>0.64%</td>
</tr>
<tr>
<td>100%</td>
<td>Norfloxacin</td>
<td>6</td>
<td>6</td>
<td>12</td>
<td>5.78</td>
<td>96.33%</td>
<td>0.59%</td>
</tr>
<tr>
<td>120%</td>
<td>Norfloxacin</td>
<td>6</td>
<td>8</td>
<td>14</td>
<td>7.78</td>
<td>97.25%</td>
<td>0.32%</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Table 17: Results

<table>
<thead>
<tr>
<th>Simultaneous Equation Method</th>
<th>Validation Parameters</th>
<th>Metronidazole</th>
<th>Norfloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Analytical Wavelength</td>
<td>320nm</td>
<td>278nm</td>
</tr>
<tr>
<td>Linearity &amp; Range</td>
<td>Beer’s range</td>
<td>2-10µg/ml</td>
<td>2-10µg/ml</td>
</tr>
<tr>
<td></td>
<td>Regression Equation</td>
<td>Y=0.0892x+0.084</td>
<td>Y=0.1839x+0.0377</td>
</tr>
<tr>
<td></td>
<td>Correlation Efficient</td>
<td>0.995</td>
<td>0.996</td>
</tr>
<tr>
<td>Precision</td>
<td>Repeatability (%RSD)</td>
<td>1.84</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>Intraday Precision (%RSD)</td>
<td>1.17</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>Inter Day Precision (%RSD)</td>
<td>1.057</td>
<td>1.38</td>
</tr>
<tr>
<td>Accuracy</td>
<td>80%</td>
<td>0.72</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>0.83</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>120%</td>
<td>1.13</td>
<td>0.32</td>
</tr>
<tr>
<td>LOD</td>
<td></td>
<td>0.068</td>
<td>0.014</td>
</tr>
<tr>
<td>LOQ</td>
<td></td>
<td>0.229</td>
<td>0.047</td>
</tr>
<tr>
<td>Sandell’s Sensitivity (µg/cm²/0.001absorbance unit)</td>
<td>0.0000046</td>
<td>0.0000039</td>
<td></td>
</tr>
</tbody>
</table>

ACKNOWLEDGEMENT

I would like to thank Dr. Dhananjay Meshram, Principal Pioneer Pharmacy Degree College for allowing me for my research work and providing me all premises and material requirements. I would like to thank all faculties of college specially Ms Minal Rohit for her
guidance. I would like to thank all Non-teaching staff for providing all materials whenever I needed.

CONCLUSION
Here, all the validation parameters are in expected range as per the ICH guidelines for newly developed UV Spectroscopic method.

REFERENCES
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8. Indian Pharmacopoeia Govt. of India, Ministry of health and family welfare, The controller of publication, 2010; 764,765, 841,842.