New Improved RP-HPLC Method for Determination of Norfloxacin and Ornidazole in Their Combined Dosage Form

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ABSTRACT
A new rapid and improved method was developed and validated for the estimation of norfloxacin and ornidazole in their combined dosage form. The advantage of present method is faster elution time over the existing method available. The selected chromatographic column was PRONTOSIL AQ ODS, 250 x 4.6 mm (5 μm), Isocratic elution was performed at 294nm using mobile phase 50mM Sodium dihydrogen phosphate buffer : ACN : MeOH, (pH 2.5 adjusted orthophosphoric acid) 15:70:15 %v/v at a flow rate of 1ml/min with run time of 10 minutes. The method was found to be linear in the range of 4-20 µg/ml of norfloxacin and 5-25 µg/ml of ornidazole. The method was found to be accurate with % recovery of 99.06% – 101.74% for NOR and 99.36% – 101.11% for ORN respectively. The method was found to be precise with CV 0.46 – 0.72 for intraday (n=3) and CV 0.67 – 1.43 for interday (n=3) for NOR and CV 0.45 – 0.79 for intraday (n=3) and CV 0.63 – 0.77 for interday (n=3) for ORN respectively. The LOD for NOR and ORN was found to be 0.366 (µg/ml) and 0.649 (µg/ml) respectively. The method was also found to be specific as no interference observed when the drugs were estimated in presence of excipients. After the validation the method was successfully applied for the estimation of norfloxacin and ornidazole in their combined dosage form.

Key Words: Norfloxacin, Ornidazole, RP-HPLC, Validation.

INTRODUCTION
Norfloxacin is a synthetic quinolone (fluoroquinolones) with broad-spectrum antibacterial activity against most gram-negative and gram-positive bacteria. It is chemically 1-ethyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid1. Ornidazole to the class of imidazole derivative anti-infective and is used in the treatment of wide range of infections like gynecological infections, systemic infections, amebiasis and other protozoal infections. It is chemically 1-chloro-3-(2-methyl-5-nitroimidazol-1-yl) propan-2-ol2. The present combination is used in the treatment of severe amoebic dysentery and amoebic liver abscess. Now there are several methods are available for the estimation of NOR and ORN alone as well as in their combined dosage form like U.V.3,4,5,6, HPLC6.7,8,9,10,11,12 and HPTLC13.

But the present described method is having the advantage of faster elution time and hence less time consuming one. The developed method was subjected to validation. Various validation parameters like accuracy, precision (intraday and interday), reproducibility, robustness, and ruggedness, limit of detection (LOD) and limit of quantitation (LOQ). The system suitability parameters were also determined and were found to be satisfactory.

MATERIALS AND METHODS

Instruments and chemicals
The present method is developed on Perkin Elmer (Series 200), USA equipped with Diode array detector (UV-visible) with isocratic pump. The column used is PRONTISIL Q ODS-RP C18 column having dimensions of 250mm (length) × 4.6mm (i.d.). All the chemicals were of HPLC grade and were obtained from Lichrosolv- E. Merck (India) Ltd., Mumbai.
Selection and optimization of chromatographic conditions

Different mobile phases were tried initially for good separation. During the development of the method system suitability parameters were also determined. To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase pH, flow rate, and solvent ratio were studied. The resulting chromatograms were recorded and the chromatographic parameters such as capacity factor, asymmetric factor, resolution and column efficiency were calculated. The conditions that gave the best resolution, symmetry and capacity factor were selected for estimation.

Calibration curve for NOR and ORN

Calibration curve for the NOR (4-20µg/ml) and ORN (5-25µg/ml)

Appropriate volume of aliquots from NOR and ORN stock solutions (100 µg/ml for NOR and ORN) were transferred to same volumetric flasks of 10ml capacity. The volume was adjusted to the mark with mobile phase to give solutions containing NOR (4, 8, 12, 16 and 20µg/ml) and ORN (5, 10, 15, 20 and 25µg/ml). The mixed drug solutions were chromatographed for 10 minutes using mobile phase at a flow rate of 1.0ml/min. The graph was plotted for peak area vs. concentration for both the drugs.

Validation of developed and optimized method

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy, a synthetic mixture equivalent to marketed formulation was prepared and analysis of the same was carried out. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels taking into consideration percentage purity of added bulk drug samples.

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation (CV).

Repeatability

Standard mixture solutions of NOR (4, 8, 12, 16 and 20µg/ml) and ORN (5, 10, 15, 20 and 25µg/ml) were prepared and chromatograms were recorded. Area of the same concentration solution was measured six times and RSD was calculated.

Intra and inter day precision

Variation of results within the same day (intraday), variation of results between days (interday) was analyzed. Intraday precision was determined by analyzing NOR and ORN for three times in the same day. Interday precision was determined by analyzing both the drugs daily for three days.

Reproducibility

The peak areas of NOR (12µg/ml) and ORN (15µg/ml) were measured at different laboratory using another instrument by another analyst and the values obtained were evaluated using t-test to verify their reproducibility.

Linearity and Range

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

Specificity and selectivity

Specificity is a procedure to determine quantitatively the analyte in presence of component that may be expected to be present in the sample matrix. While selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. Commonly used excipients in Tablet preparation were spiked in a pre weighed quantity of drugs and then area was measured and calculations done to determine the quantity of the drugs.

Ruggedness

The solutions were prepared and then analyzed with change in a variety of conditions like different laboratory, different analyst, and different instrument that may vary but are within the specified parameters of assay.

Determination of NOR and ORN in their combined dosage form

Weigh accurately 20 tablets and powder it. The powder equivalent to 4mg NOR and 5mg ORN was accurately weighed and transferred to a 50ml volumetric flask containing methanol(25ml) and was sonicated for 20 mins. The flask was shaken and volume was made up to the mark with the same solvent. The above solution was filtered through Whatman filter paper (0.45µ). The aliquot (5ml) was taken and transferred to a 10ml volumetric flask and volume was made up to the mark with the same solvent. Further, 2ml of this solution was transferred to a 10ml volumetric flask and volume was made up to the mark with the
mobile phase to give a solution containing 8µg/ml NOR and 10µg/ml ORN. This solution (solution 1) was used for the estimation of NOR and ORN.

RESULT AND DISCUSSION

Selection and optimization of chromatographic conditions
Based on trial and error method, the mobile phase which give best possible separation and resolution was selected and retention time was also taken in to the consideration. Finally the system containing Acetonitrile: Methanol: 50mM Sodium dihydrogen phosphate buffer (pH 2.5, adjusted with orthophosphoric acid), 15:15:70 was found to be satisfactory and gave two well resolved peaks for NOR and ORN at 294nm. The retention time for NOR and ORN was 5.48 minutes and 3.52 minutes respectively (Figure 1). The optimized chromatographic conditions are shown in table 1. Using these optimized conditions for developed method best possible system suitability parameters were obtained which are shown in table 2.

Calibration curve for NOR and ORN
Various chromatograms were recorded for the prepared mixtures containing NOR and ORN. The calibration curves for NOR and ORN were prepared by plotting area vs. concentration (Figure 2-3). The result of calibration curve are shown in table 3.

Validation of developed and optimized method

Accuracy
Accuracy of the method was determined by spiking the sample at three different concentrations. The method was found to be accurate with % recovery of 99.06% – 101.74% for NOR and 99.36% – 101.11% for ORN respectively. Accuracy data obtained for the developed method are shown in table 4.

Precision
Precision was calculated as repeatability and intraday and interday variation for both the drugs. The method was found to be precise with CV 0.46 – 0.72 for intraday and CV 0.67 – 1.43 for interday for NOR and CV 0.45 – 0.79 for intraday and CV 0.63 – 0.77 for interday for ORN respectively. Precision data for the developed method are shown in table 5.

Specificity and selectivity
The method was also found to be specific as no interference observed when the drugs were estimated in presence of excipients.

Assay of marketed formulation
When the developed and validated method applied for the estimation of NOR and ORN from their combined dosage form, good percentage were obtained. Assay results are shown in table 6.

CONCLUSION
We can conclude from the obtained results that the present developed method offers several advantage over the available method in a way that it requires less time and hence less solvents. So this method is economical, simple and less time consuming. The present method is more sensitive over the other developed method in the terms of limit of detection which is in nanogram. So we can accurately determine the nanogram quantities of both the drugs present in the sample.

Table 1: Optimized chromatographic conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>Sodium buffer : ACN : MeOH = 15:70:15 %v/v.</td>
</tr>
<tr>
<td>Pump mode</td>
<td>Isocratic</td>
</tr>
<tr>
<td>Stationary phase</td>
<td>PRONTOSIL AQ ODS, 250 x 4.6 mm (5 µm)</td>
</tr>
<tr>
<td>Flow rate (ml/min)</td>
<td>1.0</td>
</tr>
<tr>
<td>Run time (min)</td>
<td>10.0</td>
</tr>
<tr>
<td>Volume of Injection (µl)</td>
<td>20.0</td>
</tr>
<tr>
<td>Detection wavelength (nm)</td>
<td>294</td>
</tr>
<tr>
<td>Retention time (min)</td>
<td>Norfloxacin 5.48</td>
</tr>
<tr>
<td></td>
<td>Ornidazole 3.52</td>
</tr>
</tbody>
</table>

Fig. 1: Chromatogram of mixture of NOR (8µg/ml) and ORN (10µg/ml)
Fig. 2: Calibration curve for NOR (4-20 µg/ml)

Fig. 3: Calibration curve for ORN (5-25 µg/ml)

Table 4: Accuracy studies for developed method

<table>
<thead>
<tr>
<th>Amount of Sample (µg/ml)</th>
<th>Amount of drug added ORN (µg/ml)</th>
<th>Amount Recovered NOR (µg/ml)</th>
<th>% Recovery NOR</th>
<th>% Recovery ORN</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>5</td>
<td>0</td>
<td>3.96</td>
<td>4.94</td>
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<tr>
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<td>5</td>
<td>2.5</td>
<td>6.03</td>
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<tr>
<td>4</td>
<td>5</td>
<td>4</td>
<td>7.92</td>
<td>9.99</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>6</td>
<td>9.98</td>
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Table 5: Precision data for NOR and ORN

<table>
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<tr>
<th>Conc. µg/ml</th>
<th>Intraday (n=3)</th>
<th>CV</th>
<th>Inter day (n=3)</th>
<th>CV</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>FOR NOR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.56</td>
<td>126977 ± 713.4753</td>
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<tr>
<td></td>
<td>12</td>
<td>0.46</td>
<td>195488 ± 908.5543</td>
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</tr>
<tr>
<td></td>
<td>16</td>
<td>0.72</td>
<td>258952.667 ± 1871.00116</td>
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</tr>
<tr>
<td></td>
<td>FOR ORN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.50</td>
<td>14360 ± 72.02083</td>
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</tr>
<tr>
<td></td>
<td>15</td>
<td>0.79</td>
<td>21636.33 ± 171.5294</td>
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</tr>
<tr>
<td></td>
<td>20</td>
<td>0.45</td>
<td>28771.33 ± 7.5</td>
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</table>

Table 6: Assay Result of marketed formulation

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Actual conc. (µg/ml)</th>
<th>Amount Obtained (µg/ml)</th>
<th>% NOR</th>
<th>% ORN</th>
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<tbody>
<tr>
<td>Tablet</td>
<td>8</td>
<td>10</td>
<td>7.96</td>
<td>10.05</td>
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REFERENCES