Analytical Method Development and Validation by RP-HPLC for Simultaneous Estimation of Cefixime and Ornidazole in Combined Tablet Dosage Form

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A B S T R A C T
A simple, Accurate, precise method was developed for the simultaneous estimation of the Cefixime and Ornidazole in Tablet dosage form. Chromatogram was run through ODS (250mm 4.6mm, 5μ). Mobile phase containing Buffer and Acetonitrile and methanol in the ratio of 45:45:10A was pumped through column at a flow rate of 1ml/min. Buffer used in this method was 0.01N KH₂PO₄ buffer at pH 4.6. Temperature was maintained at 30°C. Optimized wavelength for Cefixime and Ornidazole was 220nm. Retention time of Cefixime and Ornidazole were found to be 4.0 min and 2.8 min. %RSD of the Cefixime and Ornidazole were and found to be 0.66 and 0.61 respectively. %Recover was obtained as101.04% and 100.41% for Cefixime and Ornidazole respectively, LOD, LOQ values are obtained from regression equations of Cefixime and Ornidazole were 0.74ppm, 0.72ppm and 2.23ppm, 2.19ppm respectively. Regression equation of Cefixime is y = 2829.x + 711 and of Ornidazole is y = 1935x + 866.3.

Keywords: Cefixime, Ornidazole, RP-HPLC

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1. Introduction
Pharmaceutical Analysis is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter [1,2,3]. It is mainly involved in the qualitative...
identification or detection of compounds and quantitative measurements of the substances present in bulk and pharmaceutical preparation [4,5,6]. This combination of drugs was found to be more effective antibiotics. Cefixime is a third-generation cephalosporin and it is highly stable in the presence of beta-lactamase enzymes [7,8]. The antibacterial effect of cefixime results from inhibition of murepapside synthesis in the bacterial cell wall. Used in the treatment of uncomplicated urinary tract infections, otitis, gonorrhea [9]. Ornidazole is a nitro imidazole which has broad spectrum cidal activity against Protozoa and some anaerobic bacteria. Drug enters the cell by diffusion, the nitro group of drug is reduced by redox proteins present only in anaerobic organisms to reactive nitro radicals which exerts cytotoxic action by damaging DNA and other critical biomolecules. Then DNA helix destabilization &strand breakage has been observed. It is an anti-infective; used to treat selected protozoan infections. [10,11]

2. Materials and Methods

Materials:
Cefixime and Ornidazole, Combination Cefixime and Ornidazole tablets, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acetic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, tri ethyl amine, ortho-phosphoric acid etc.

Instrument:
HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special band width of 2nm and 10mm and matched quartz was be used for measuring absorbance for Cefixime and Ornidazole solutions [12, 13].

Methods:
Preparation of buffer:
Buffer: (0.01KH₂PO₄) Accurately weighed 2.72gm of Potassium di-hydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water and pH adjusted to 4.6 with dil. OPA Solution[14,15].

Standard Preparation:
Accurately Weighed and transferred 10mg of Cefixime and 25mg of Ornidazole working Standards into a 10ml clean dry volumetric flask, add 3/4⁰ volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation:
10 tablets was weighed, powdered and then the weight 2500 mg (equivalent to 500 mg of Cefixime and 1250mg of Ornidazole) was transferred into a 100mL volumetric flask, 75mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Linearity:
Linearity solutions are prepared such that 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml, 1.5ml from the Stock solutions of Cefixime and Ornidazole are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 25ppm, 50ppm, 75ppm, 100ppm, 125ppm, 150ppm of Cefixime and 62.5ppm, 125ppm, 187.5ppm 250ppm, 312.5ppm, 375ppm of Ornidazole.

Standard Preparation:
Accurately Weighed and transferred 10mg of Cefixime and 25mg of Ornidazole working Standards into a 10ml clean dry volumetric flask, add 3/4⁰ volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

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Accuracy:
Standard Preparation:
Accurately Weighed and transferred 10mg of Cefixime and 25mg of Ornidazole working Standards into a 10ml clean dry volumetric flask, add 3/4⁰ volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation:
10 tablets was weighed, powdered and then the weight 2500 mg (equivalent to 500 mg of Cefixime and 1250mg of Ornidazole) was transferred into a 100mL volumetric flask, 75mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered...
solution 0.2ml was pipeted out into a 10 ml volumetric flask and made up to 10ml with diluent.

50%: 10 tablets was weighed, powdered and then the weight 1250 mg was transferred into a 100mL volumetric flask, 75mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made up to 10ml with diluent.

100%: 10 tablets was weighed, powdered and then the weight 2500 mg was transferred into a 100mL volumetric flask, 75mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made up to 10ml with diluent.

150%: 10 tablets was weighed, powdered and then the weight 3750 mg was transferred into a 100mL volumetric flask, 75mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made up to 10ml with diluent.

3. Results and Discussion

**Figure 3:** Calibration curve of Cefixime

**Figure 4:** Calibration curve of Ornidazole

**Figure 5:** Chromatogram of blank

**Figure 6:** Typical chromatogram of Cefixime and Ornidazole

**Figure 7:** Linearity 50% Chromatogram of Cefixime and Ornidazole method

**Figure 8:** Linearity 100% Chromatogram of Cefixime and Ornidazole method

**Figure 9:** Linearity 150% Chromatogram of Cefixime and Ornidazole method
Figure 10: Repeatability Chromatogram of Cefixime and Ornidazole method

Figure 11: Inter Day precision Chromatogram of Cefixime and Ornidazole method

Figure 12: Accuracy 50% Chromatogram of Cefixime and Ornidazole method

Figure 13: Accuracy 100% Chromatogram of Cefixime and Ornidazole method

Figure 14: Accuracy 150% Chromatogram of Cefixime and Ornidazole method

Figure 15: LOD Chromatogram of Cefixime and Ornidazole method

Figure 16: LOQ Chromatogram of Cefixime and Ornidazole method

4. Conclusion
The method was found to be precise, accurate and linear over the linear concentration range. The method developed is unique in determining the impurities even at low levels than that of specifications. The analytical method validation of Cefixime and Ornidazole in tablet dosage form by RP-HPLC was found to be satisfactory and could be used for the routine pharmaceutical analysis of Cefixime and Ornidazole in tablet dosage form. Method was validated as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, forced degradation studies, ruggedness, robustness and solution stability. Therefore, this HPLC method can be used as a routine analysis of these drugs in pharmaceutical formulations.
Table 1: Calibration data of Cefixime and Ornidazole method

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration Cefixime (µg/ml)</th>
<th>Response</th>
<th>Concentration Ornidazole (µg/ml)</th>
<th>Response</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>75603</td>
<td>62.5</td>
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<tr>
<td>3</td>
<td>50</td>
<td>140100</td>
<td>125</td>
<td>243322</td>
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<td>4</td>
<td>75</td>
<td>213893</td>
<td>187.5</td>
<td>361563</td>
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<td>5</td>
<td>100</td>
<td>288926</td>
<td>250</td>
<td>489440</td>
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<tr>
<td>6</td>
<td>125</td>
<td>353978</td>
<td>312.5</td>
<td>607549</td>
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<tr>
<td>7</td>
<td>150</td>
<td>425093</td>
<td>375</td>
<td>722840</td>
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Table 2: Repeatability results for Cefixime and Ornidazole

<table>
<thead>
<tr>
<th>S. No</th>
<th>Cefixime</th>
<th>Ornidazole</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>294201</td>
<td>486989</td>
</tr>
<tr>
<td>2</td>
<td>294612</td>
<td>483539</td>
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<td>3</td>
<td>290375</td>
<td>478706</td>
</tr>
<tr>
<td>4</td>
<td>292645</td>
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<td>6</td>
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<tr>
<td>AVG</td>
<td>292573.7</td>
<td>484109.8</td>
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<tr>
<td>STDEV</td>
<td>1944.5</td>
<td>2961.135</td>
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<tr>
<td>%RSD</td>
<td>0.66</td>
<td>0.61</td>
</tr>
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</table>

*Average of six determinations

Table 3: Inter day precision results for Cefixime and Ornidazole

<table>
<thead>
<tr>
<th>S. No</th>
<th>Cefixime</th>
<th>Ornidazole</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>286674</td>
<td>477643</td>
</tr>
<tr>
<td>2</td>
<td>286121</td>
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<tr>
<td>3</td>
<td>285495</td>
<td>478876</td>
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<td>4</td>
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<td>5</td>
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<td>6</td>
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<td>AVG</td>
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<td>477944.5</td>
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<tr>
<td>STDEV</td>
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<td>581.7906</td>
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<tr>
<td>%RSD</td>
<td>0.50</td>
<td>0.12</td>
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Table 4: Accuracy results of Cefixime and Ornidazole

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount added (µg/ml)</th>
<th>Amount Recovered (µg/ml)</th>
<th>Recovery (%)</th>
<th>% RSD</th>
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<tbody>
<tr>
<td>Cefixime</td>
<td>50</td>
<td>49.82</td>
<td>99.64</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>99.15</td>
<td>99.15</td>
<td>0.08</td>
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<td></td>
<td>150</td>
<td>152.49</td>
<td>101.66</td>
<td>0.27</td>
</tr>
<tr>
<td>Ornidazole</td>
<td>125</td>
<td>124.12</td>
<td>99.30</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>248.60</td>
<td>99.44</td>
<td>0.04</td>
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<td></td>
<td>375</td>
<td>375.60</td>
<td>100.16</td>
<td>0.78</td>
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5. Acknowledgement
I gives my immense pleasure to express my sincere thanks to my guide Ramesh Dhani, M. Pharm, Ratnam Institute of Pharmacy, for giving guidance at all stages of my work.

6. References


