Absorbance correction method for the simultaneous estimation of norfloxacin and tinidazole in API and combined tablet dosage formulation by UV spectrophotometry

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ABSTRACT

This research focuses on the estimation of the concentrations of Norfloxacin and Tinidazole simultaneously in API and combined tablet formulation using a very new, simple, accurate and precise method called ‘Absorption correction method’ using UV spectrophotometer. Beer’s law was obeyed for Norfloxacin in the range of 1-7µg/mL at 278nm and also for Tinidazole in the range of 4-247µg/mL at 316nm. From the accuracy, percentage recovery for Norfloxacin and Tinidazole was found to be in the range of 98.33- 101.68% and 98.52-101.44% respectively. %RSD values reported were less than 2. As all the validation parameters were found to be within the limits given by ICH guideline, the developed method was found to be linear, accurate, precise and rugged.

Keywords: Norfloxacin, Tinidazole, Absorption correction method, UV Spectrophotometer

INTRODUCTION

Now a day’s lot of drugs come in the combination form with other drugs. Therefore developing a method for those drugs has become a challenging task for the analyst. This research focuses onto one of the recent UV spectrometric method for estimation of Norfloxacin and Tinidazole in a combined tablet formulation.

Method
Absorption correction method is a new method for simultaneous detection of drugs. The condition for performing this method is only that one of the drugs must show zero absorbance at the lambda max of the other drug.

Drug Information
Norfloxacin is a first generation synthetic fluoroquinolone which is used as a synthetic chemotherapeutic antibacterial agent [1, 2]. It is used to treat common as well as complicated urinary tract infections [3].

IUPAC name of Norfloxacin is [1-ethyl-6-fluoro-1,4-dihydro-4-oxa-7-(piperazinyl)quinolone-3-carboxylic acid] [4].

Figure I: Structure of norfloxacin
Mechanism of action- It is a broad spectrum antibiotic showing activity against gram negative and gram positive bacteria. It inhibits bacterial cell division by inhibiting DNA gyrase, topoisomerase II and IV which are necessary enzymes for causing separation of bacterial DNA [5].

A number of methods like capillary electrophoresis [6], HPLC [7], TLC [8], LC [9], voltammetry [10], ISE [11], differential pulse polarography [12], fluorimetry [13] and potentiometric titration [14] have been widely used for the detection in pharmaceuticals.

Tinidazole is a derivative of 2-methylimidazole, which belongs to the class of nitroimidazole antibiotics. It is an anti-parasitic drug and used for a treatment of various amoebic and parasitic infections [15].

Tinidazole having IUPAC name as 1-[2-(ethylsulfonyl)ethyl]-2-methyl-5-nitroimidazole [16].

Mechanism of action- It penetrates through the cell membrane of both aerobic and anaerobic microorganisms and bind to DNA resulting in cell death by damaging DNA [17].

Various techniques like Proposed methods have mainly used in high performance liquid chromatography [18-24], high performance thin layer chromatography [25-28], gas liquid chromatography [29-33], packed column supercritcal fluid chromatography [34, 35], voltammetry [36, 37], polarography [38], capillary electrophoresis[39], flow injection analysis [40], UV-spectrophotometry [41- 44] and derivative UV-spectrophotometry [45- 52].

MATERIALS AND METHODS

Instrumentation
This research was carried out on Shimadzu- 1800 double beam UV- visible spectrophotometer with the help of the software ‘UV PROBE’. All the glass wares used were of ‘A’ grade.

Reagents and chemicals
The API of norfloxacin was obtained from CiplaPvt. Ltd. Vikhroli, Mumbai and tinidazole was obtained from Aarti Pharma, Mumbai as a gift sample. The tablet formulation used was of a brand name ‘Nor-TZ’ containing norfloxacin (400mg) and tinidazole (600mg). Double distilled water was used for the experiment. All other chemicals used were of analytical grade.

Experimental Conditions
Phosphate buffer of 0.05M concentration having pH=3 (adjusted using ortho-phosphoric acid) was used as a diluents to dissolve both the drugs. Therefore all the dilutions were prepared using phosphate buffer (pH=3). The analysis was completely carried out in controlled conditions of temperature, humidity and pressure.

Preparation of standard stock solution
400 mg of norfloxacin and 60 mg of tinidazole were weighed accurately and transferred into a 100ml volumetric flask. The stock solution was prepared by dissolving both the drugs in phosphate buffer (pH=3) by sonication technique and then made up to the mark with phosphate buffer (pH=3). The final concentrations obtained, were 400µg/ml and 600µg/ml for norfloxacin and tinidazole respectively.

Spectral studies
The stock solution of norfloxacin was diluted to make 4 µg/ml solution and tinidazole to 6 µg/ml. Phosphate buffer (pH=3) was used as a blank and the solutions were scanned in a UV Spectrophotometer in the range of 200 nm to 400 nm in 1 cm cell. The spectrum so obtained was recorded.
It was observed from the spectrum, that at 278 nm only norfloxacin has substantial absorbance, whereas at 316 nm, both tinidazole and norfloxacin showed substantial absorbance. Thus, estimation of norfloxacin was carried at 278 nm without any interference, as tinidazole has zero absorbance at 278 nm. At 316 nm the absorbance of norfloxacin was deducted from the total absorbance of the mixture which gave the corrected absorbance for tinidazole.

The concentrations of the solutions were calculated using the Beer-Lamberts Law:

\[ A = a \times b \times c \]

Where,

‘A’ is the absorbance of the solution, ‘a’ is the absorptivity, ‘b’ is the path length and ‘c’ is the concentration.

Thus, concentration at different wavelengths was found using the equation:

\[ C_x = \frac{A_x}{a_x} \]

\[ C_y = \frac{(A_{xy} - A_x)}{a_y} \]

Where,

\( C_x \) is the concentration of the drug ‘x’ at a wavelength, \( C_y \) is the concentration of the drug ‘y’ at a wavelength, \( A_x \) is the absorbance of drug ‘x’, \( A_{xy} \) is the absorbance of mixture of drugs ‘x’ and ‘y’. \( a_x \) is the absorptivity of drug ‘x’ at a wavelength, \( a_y \) is the absorptivity of drug ‘y’ at a wavelength.

From the standard stock solutions of norfloxacin and tinidazole, the aliquots were transferred into 10 ml calibrated volumetric flasks and diluted using phosphate buffer (pH=3), to make final concentrations of the range 1 to 7 µg/ml for norfloxacin and 4 to 24 µg/ml for tinidazole and the calibration curves were obtained at their respective wavelengths, 278 nm and 316 nm. Also, the calibration curve for norfloxacin was plotted at 316nm for the concentration range 1 to 7µg/ml.

### Analysis of synthetic mixture of norfloxacin and tinidazole

From the stock solutions of norfloxacin and tinidazole, different mixtures of the two drugs were prepared into 10 ml calibrated volumetric flaks and diluted using phosphate buffer (pH=3). All the mixtures prepared, were then measured for absorbance at wavelengths 278 nm and 316 nm and their concentrations were determined using absorbance correction method.

### Analysis of tablet formulation

The average weight of 20 tablets was determined accurately. The tablets were powdered into fine particles by triturating them using a mortar and pestle. Powder equivalent to 40mg of norfloxacin was weighed accurately and transferred into a 100 ml calibrated volumetric flask. A minimum quantity of phosphate buffer (pH=3) was added to the volumetric flask to dissolve the powder using sonication for about 20 minutes, after which the volume was made up to the mark using same solvent. The solution was then filtered using a 0.45 µ Whatmann filter paper using a vacuum pump. The filtrate in the form of a clear solution was then subjected to further dilutions to obtain 40 µg/ml solution of norfloxacin and 60 µg/ml solution of tinidazole theoretically. At all the selected wavelengths the absorbance of sample solution was accurately measured. The concentrations of norfloxacin and tinidazole in the tablet formulation were thus calculated. Six solutions using the same procedures were analyzed.

### Method Validation

It is the process of confirmation that the developed analytical procedure is specific for its intended use. There are 8 steps of method validation given by USP [53]

1. Accuracy
2. Precision
3. Specificity
4. Limit of detection
5. Limit of quantification
6. Linearity and range
7. Ruggedness
8. Robustness

According to ICH guidelines for the qualitative test or assay procedure LOD, LOQ parameters are not required [54].
Accuracy
It was calculated in terms of recovery which was performed to check the interferences at 3 different concentrations. From this the % recovery was calculated from the total amount of the drug found. This analysis was performed in triplicates for each concentration.

Precision
For checking precision of the method, repeatability and intermediate precision were performed. Six replicates of the same concentration were used for determining the repeatability. Inter-day and intra-day analysis was performed in triplicates for the same concentration, on the same day and for the successive three days for the determination of intermediate precision. Percentage relative standard deviation was obtained.

Ruggedness
It is the degree of reproducibility of results obtained after analysis of samples at different place, different time, by different analyst etc. Variations between the conditions and its effect on the results are determined by ruggedness. The determination of norfloxacin and tinidazole was thus carried out using different analyst and using different instrument. Percentage relative standard deviation was then calculated.

Linearity
As per ICH guidelines, for the establishment of linearity, a minimum of 5 concentrations is recommended. The concentration range for norfloxacin was 1-7 µg/ml and for tinidazole 4-24 µg/ml and were analysed at 278nm and 316nm respectively. Concentrations of norfloxacin at 316nm were also measured. From this analysis calibration curves between concentration and absorbances of drugs were plotted.

Sensitivity
Sensitivity was checked by determination of limit of detection (LOD) and limit of quantification (LOQ) using the following equations for six replicates:

\[ LOD = 3.3\sigma + s \]
\[ LOQ = 10\sigma + s \]

Where,

- \( \sigma \) is the standard deviation of y intercept of calibration curve and 's' is the slope of regression equation.

RESULTS AND DISCUSSION
The method is proposed to be rapid, accurate, sensitive, precise and economical for the simultaneous estimation of norfloxacin and tinidazole in API form and also tablet formulation. The method follows absorbance correction in which at one wavelength only one drug shows substantial absorbance and at other wavelength both the drugs shows absorbance. At 278nm only norfloxacin shows absorbance giving direct determination from absorbance, whereas at 316nm both the drugs norfloxacin and tinidazole shows absorbance. Here, where absorbance correction plays a role by deducting the absorbance of norfloxacin from the absorbance of mixture of norfloxacin and tinidazole. Figure III shows the absorbance spectra for both the drugs by using 0.05M phosphate buffer (pH=3) as a diluent.

Beer’s law was obeyed for norfloxacin at both the wavelengths 278nm and 316nm within the range of 1-7 µg/ml. The correlation coefficients from the linearity range of both the drugs were found to be above 0.999. LOD and LOQ were found to be 0.0293µg/ml and 0.0888µg/ml for norfloxacin and 0.0835µg/ml and 0.2531µg/ml for tinidazole. This proves that the method is sensitive.

Table I: Data for spectral and linearity characteristics

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Norfloxacin*</th>
<th>Tinidazole*</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{max} )</td>
<td>278 nm</td>
<td>316 nm</td>
</tr>
<tr>
<td>Linearity Range (µg/ml)</td>
<td>1-7</td>
<td>4-24</td>
</tr>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.9995</td>
<td>0.9995</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>0.1351</td>
<td>0.0316</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.0216</td>
<td>0.0512</td>
</tr>
<tr>
<td>Regression equation ( y = mx + c )</td>
<td>( y = 0.1351x +0.0216 )</td>
<td>( y = 0.0316x +0.0512 )</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.0293</td>
<td>0.0885</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>0.0888</td>
<td>0.2531</td>
</tr>
</tbody>
</table>

\*Mean of six observations
Synthetic mixture of drugs at various concentrations were analysed and the % recovery of norfloxacin was 100.9% to 102% and for tinidazole was 100.05% to 101.25% (Table 2). This indicates that there is no interference between these drugs.

<table>
<thead>
<tr>
<th>Concentration of Norfloxacin (µg/ml)</th>
<th>% Recovery (Norfloxacin)</th>
<th>Concentration of Tinidazole (µg/ml)</th>
<th>% Recovery (Tinidazole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical</td>
<td>Experimental*</td>
<td>Theoretical</td>
<td>Experimental*</td>
</tr>
<tr>
<td>1</td>
<td>1.009</td>
<td>100.9</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>2.035</td>
<td>101.75</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>3.028</td>
<td>100.96</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>4.06</td>
<td>101.5</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>5.10</td>
<td>102</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>6.09</td>
<td>101.5</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>7.11</td>
<td>101.57</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
<td>18.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>20.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22</td>
<td>22.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>24.18</td>
</tr>
</tbody>
</table>

*Mean of three replicates

The label claim present in the tablet formulation was found to be 101.18±0.1778 for Norfloxacin and 98.95±0.1300 for Tinidazole and their % RSD were 0.18% and 0.13% respectively (table III) which indicated that the method is precise as the values were in a good agreement with the label claim.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Norfloxacin</th>
<th>Tinidazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Label claim (mg)</td>
<td>400</td>
<td>600</td>
</tr>
<tr>
<td>% Assay*</td>
<td>101.18</td>
<td>98.95</td>
</tr>
<tr>
<td>SD</td>
<td>0.1778</td>
<td>0.1300</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.18%</td>
<td>0.13%</td>
</tr>
</tbody>
</table>

*Mean of six determinations

The precision of the method was also carried out using intraday and inter-day analysis. The values of % RSD for norfloxacin were 0.21% for intraday and 0.81% for inter-day and for tinidazole they were 0.32% for intraday and 0.85% for inter-day analysis which indicated that the method is precise. For the estimation of ruggedness of the method, the analysis was performed on different instruments and by different analyst. The values of % RSD were less than 2% which indicated that the method is rugged and can be suitably used for estimation of norfloxacin and tinidazole in combination and parameters like difference in days, instruments or analyst won’t affect the method.
Table IV: Results for intermediate precision and ruggedness of method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>% Label claim estimated (Mean ± % RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Norfloxacin</td>
</tr>
<tr>
<td>Intraday precision (n=3)</td>
<td>100.76±0.31</td>
</tr>
<tr>
<td>Inter-day precision (n=3)</td>
<td>100.32±0.56</td>
</tr>
<tr>
<td>Instrument I (n=3)</td>
<td>101.67±0.33</td>
</tr>
<tr>
<td>Instrument II (n=3)</td>
<td>101.93±0.26</td>
</tr>
<tr>
<td>Analyst I (n=3)</td>
<td>100.82±0.30</td>
</tr>
<tr>
<td>Analyst II (n=3)</td>
<td>99.98±0.21</td>
</tr>
</tbody>
</table>

n= number of replicates

The accuracy of the method was detected by performing % recovery at 3 different concentrations at 80%, 100% and 120% of the standard drug of norfloxacin and tinidazole. % recovery for norfloxacin was found to be in the range of 101.25% to 102 % and for tinidazole 100.83% to 102%. % RSD values obtained were 0.3694 and 0.5772 for norfloxacin and tinidazole respectively. Lower % RSD indicates that the drugs can be recovered from formulation and therefore shows accurate method.

Table V: Results of recovery studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount present (µg/ml)</th>
<th>Amount added (µg/ml)</th>
<th>Amount found (µg/ml)</th>
<th>Amount recovered (µg/ml)</th>
<th>% Recovery*</th>
<th>S.D</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOR</td>
<td>2.00</td>
<td>1.60</td>
<td>3.60</td>
<td>1.62</td>
<td>101.25</td>
<td>0.3755</td>
<td>0.3694</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>2.00</td>
<td>4.00</td>
<td>2.04</td>
<td>102.00</td>
<td>0.5853</td>
<td>0.5772</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>2.40</td>
<td>4.40</td>
<td>2.44</td>
<td>101.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TINI</td>
<td>3.00</td>
<td>3.00</td>
<td>6.00</td>
<td>3.06</td>
<td>102.00</td>
<td>0.5853</td>
<td>0.5772</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>3.60</td>
<td>6.60</td>
<td>3.65</td>
<td>101.38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean of three observations

The results obtained from above set of observations prove that the method is useful in determining the concentration of the drugs from the synthetic mixture and tablet formulation. The developed method is based on the use of very economical solvent and hence can be performed with ease. Simultaneous estimation of the drugs can be achieved by various other techniques like simultaneous equation method or absorbance ratio method [55], but absorbance correction method was found to be most important in the case of drugs which show zero absorbance at maximum absorption of another drug. Estimation of norfloxacin and tinidazole thus can be economically and simply done by absorption correction method.

CONCLUSION

The method was developed and validated which concluded that the method is sensitive, linear, simple, accurate, precise, rugged and also economical. Thus, the proposed method can be effectively applied for analysis of norfloxacin and tinidazole in bulk dosage forms as well as in combined tablet dosage form.

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