NEW SPECTROPHOTOMETRIC ESTIMATION OF NORFLOXACIN IN THE TABLETS USING MIXED SOLVENCY APPROACH

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ABSTRACT

Organic solvents are most frequently employed in spectrophotometric analyses. They may be sources of pollution. Some of them may be toxic while others may be costlier. Volatility may be a source of inaccuracy in spectrophotometric estimations. In the present investigation, it was proposed to solubilize Norfloxacin by use of mixed solvency concept. Norfloxacin shows maximum absorbance in the concentration range of 10-50 µg/ml at 323 nm. Method of analysis has been validated for different parameters like linearity, accuracy, precision, LOD and LOQ. The percent drug estimated in tablet formulation of Batch-I and of Batch-II were 98.13±0.650 and 98.78±0.565 respectively. The range of percent recoveries varied from 96.71±0.425 to 98.38±0.776. Sodium Benzoate, Niacinamide and sodium Caprylate do not interfere above 300 nm. The analytical method was found to be simple, safe (free from toxicity), economic and eco-friendly.

KEYWORDS: Norfloxacin, UV-Spectrophotometry, solid dosage formulation, mixed solvency concept.
INTRODUCTION
Increasing the aqueous solubility of Insoluble and slightly soluble drugs has been done by various methods to avoid the usage of organic solvents. Because of toxicity, volatility, and also high cost of organic solvents, an alternative method has been developed. Mixed solvency concept is one of the methods to enhance the aqueous solubility of less water soluble drugs. Mixed solvency concept may be a proper choice to preclude the use of organic solvents. So there is a broad scope for mixed solvency concept in quantitative estimation of other less water soluble drugs.

By application of this concept, innumerable solvent system can be developed. Maheshwari\cite{1-6} is one of the opinions that each substance possesses solubilizing power. He has given several ecofriendly methods in the area of drug estimations and formulations precluding the use of toxic organic solvents. The solubility of large number of poorly soluble drugs has been enhanced by mixed solvency concept.\cite{1-31}

The present research work also provides an ecofriendly method to estimate spectrophotometrically, the Norfloxacin drug in tablet formulations without the help of organic solvent.

Norfloxacin is chemically 1-ethyl-6fluro-4-oxo-7-piperazin-1-yl-1H-quinoline-3-carboxylic acid. White to light yellow crystalline powder with a slightly characteristic odour. Norfloxacin is chemotherapeutic antibacterial. The solubility of Norfloxacin in distilled water at room temperature was found to be 0.009%. Approximate solubility of Norfloxacin in blend (10% Sodium caprylate, 10% Sodium Benzoate and 10% Niacinamide) was more than 1.0%w/v.

EXPERIMENTAL
Chemicals and Reagents
Pharmaceutical grade Norfloxacin was a gift from Modern Laboratories Pvt. Ltd. Indore and its dosage formulation Norflox400 was purchased from local market. All other chemicals were of analytical grade.

Instrumentation
UV Visible spectrophotometer (Model 1800, Shimadzu, Japan) with 10 –mm path length connected to a computer was used for spectrophotometric analysis.
Calibration curve

Standard stock solution of Norfloxacin (5000µg/ml) was prepared by weighing 50 mg of Norfloxacin and transferred to a 10 ml volumetric flask and was dissolved in sufficient blend of 10% Sodium caprylate, 10% Sodium Benzoate and 10% Niacinamide. Then finally volume was made up to 10ml with the same blend to get a concentration of 5000 µg/ml. Appropriate volumes of this solution were further diluted with distilled water to obtain final concentrations in the range of 10-50 µg/ml. The absorbances of these standard solutions were noted at 323 nm against respective reagent blanks.

Table 1: Data of calibration curve.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration (µg/ml)</th>
<th>Stock Solution in (ml)</th>
<th>Final volume with distilled water (ml)</th>
<th>Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0.1</td>
<td>100</td>
<td>0.399</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0.2</td>
<td>100</td>
<td>0.780</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>0.3</td>
<td>100</td>
<td>1.197</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>0.4</td>
<td>100</td>
<td>1.592</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>0.5</td>
<td>100</td>
<td>1.995</td>
</tr>
</tbody>
</table>

Figure 1: Calibration curve of Norfloxacin.

Preliminary solubility studies

To determine the solubility of the drug in distilled water and mixed solvent blend (containing 10% Sodium caprylate, 10% Sodium Benzoate and 10% Niacinamide) at room temperature sufficient excess amount of the drug was added to a 25 ml capacity vial containing distilled water and the mixed solvent blend. After putting the vial cap and applying the aluminum seal, the vial was shaken mechanically for 12 hours at room temperature (27°C) in an orbital flask shaker. The solution was allowed to equilibrate for 24 hours undisturbed and then filtration
was done through Whatmann filter paper#41. The filtrate was appropriately diluted with distilled water to measure the absorbance at 323 nm against reagent blanks.

**Proposed method of analysis**

20 tablets of tablet formulation-I were accurately weighed and finely powdered. Amount of powder equivalent to 50 mg of bulk drug was transferred into 10 ml volumetric flask with 6 ml of blend (10% Sodium caprylate, 10% Sodium Benzoate and 10% Niacinamide) and the drug present in tablet powder was dissolved by sonication for 20 minutes. The flask was filled to the mark with the same blend and the resulting solution was filtered through Whatmann filter paper no.41. One ml of the above filtrate was diluted to 100 ml. Method was followed as described under analytical procedure and the absorbance was noted at 323 nm against the reagent blank. The drug content was calculated using the calibration curve. Same procedure was repeated for the tablet formulation II. The results of analysis are reported in Table-2. All analyses were performed thrice.

**Table 2: Analysis data of Norfloxacin tablet formulations with statistical evaluation (n=3).**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Batch</th>
<th>Label claim mg/tab</th>
<th>% Labeled claim estimated (mean ±SD)</th>
<th>Percent coefficient of variation</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norfloxacin I</td>
<td>400</td>
<td>98.13±0.650</td>
<td>0.662</td>
<td>0.375</td>
<td></td>
</tr>
<tr>
<td>Norfloxacin II</td>
<td>400</td>
<td>98.78±0.565</td>
<td>0.569</td>
<td>0.326</td>
<td></td>
</tr>
</tbody>
</table>

**Recovery studies**

To perform the recovery studies, standard Norfloxacin drug was added (40mg, 50mg and 60mg separately) to the pre-analyzed tablet powder equivalent to 50 mg of Norfloxacin and the drug content was determined by the proposed method. Results of analysis were reported in Table3.

**Table 3: Results of recovery studies with statistical evaluation. n=3.**

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Drug in Pre-Analyzed tablet powder(mg)</th>
<th>Amount of standard drug added(mg)</th>
<th>%Recovery estimated (mean±SD)</th>
<th>Percent coefficient of variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>50</td>
<td>40</td>
<td>97.56±0.300</td>
<td>0.305</td>
<td>0.227</td>
</tr>
<tr>
<td>I</td>
<td>50</td>
<td>50</td>
<td>97.88±0.572</td>
<td>0.585</td>
<td>0.330</td>
</tr>
<tr>
<td>I</td>
<td>50</td>
<td>60</td>
<td>96.71±0.425</td>
<td>0.439</td>
<td>0.245</td>
</tr>
<tr>
<td>II</td>
<td>50</td>
<td>40</td>
<td>97.15±0.572</td>
<td>0.588</td>
<td>0.330</td>
</tr>
<tr>
<td>II</td>
<td>50</td>
<td>50</td>
<td>97.46±0.400</td>
<td>0.410</td>
<td>0.230</td>
</tr>
<tr>
<td>II</td>
<td>50</td>
<td>60</td>
<td>98.38±0.776</td>
<td>0.780</td>
<td>0.448</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

The developed UV-spectrophotometric method was validated as per ICH guidelines in terms of linearity, and range, specificity, precision, sensitivity and accuracy.

In order to determine linearity range of developed method, a series of solutions were prepared using Norfloxacin stock solution at concentration range of 10-50µg/ml. The absorbances of the resultant solutions were measured at 323 nm against reagent blank. The calibration curves were constructed by plotting concentration on X axis and absorbance on Y axis. R^2 value not less than 0.999 was regarded as acceptance criteria (Figure 1).

Table 4: Developed UV method specification.

<table>
<thead>
<tr>
<th>Instrument and specification</th>
<th>UV-Spectrophotometer Shimadzu 1800</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scanning Range</td>
<td>200 nm to 400 nm</td>
</tr>
<tr>
<td>Solvent Used</td>
<td>Hydrotropic solvent</td>
</tr>
<tr>
<td>Strength of Solvent</td>
<td>10% Sodium caprylate, 10% Sodium Benzoate and 10% Niacinamide</td>
</tr>
<tr>
<td>Composition of Solvent</td>
<td>10% Sodium caprylate, 10% Sodium Benzoate and 10% Niacinamide</td>
</tr>
<tr>
<td>Wavelength Maxima of Norfloxacin</td>
<td>323 nm</td>
</tr>
</tbody>
</table>

Figure 2: UV-Spectrum of Norfloxacin.

Specificity was performed to exclude the possibilities of interference of solvent in the region of maximum absorbance peaks of Norfloxacin. The specificity of the method was tested under the normal conditions and results of the tests proved that the components other than Norfloxacin did not produce the deductible peaks at the maximum absorbance peaks of the drug.
Accuracy of the developed method was determined by recovery studies at three different levels. The pre analyzed samples were spiked with 80, 100 and 120% of mixed standard solution. The mixtures were analyzed and the recoveries were determined. The recovery study was carried out in triplicate. The mean % recovery of the Norfloxacin at each level should not be less than 98% and not more than 102% was considered as the acceptance criteria.

Precision was studied to find out intra-day and inter-day variations in the test method of Norfloxacin. Intra-day assay precision was found by analysis of standard drug thrice on the same day in different intervals of time. Inter-day assay precision was carried out on three different days and percentage relative standard deviation (%RSD) was calculated. The %RSD should not be more than 2.0%.

Sensitivity of proposed method was estimated in terms of limit of Detection (LOD) and Limit of quantification (LOQ). The LOD and LOQ of Norfloxacin by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3s/S and 10s/S respectively. Where S is the slope of calibration curve and s is standard deviation of response.

The solubility of Norfloxacin in distilled water at room temperature was found to be 0.009%. Approximate solubility of Norfloxacin in blend was more than 1.0% w/v.

It is evident from table-2 that the percent drug estimated in tablet formulation of Batch-I and of Batch-II were 98.13±0.650 and 98.78±0.565 respectively. The values are very close to 100, indicating the precision of the proposed analytical method. Further table-3 shows that the range of percent recoveries varied from 96.71±0.425 to 98.38±0.776 which are again very close to 100, indicating the accuracy of the proposed method. Proposed analytical method is further supported by significantly small values of statistical parameters viz. standard deviation, percent coefficient of variation and standard error (table3).

The limit of detection was found to be 0.453 µg/ml and the limit of quantification was found to be 1.353 µg/ml.

**CONCLUSION**

A rapid, simple, and non toxic UV spectrophotometric method has been developed for the determination and quantification of Norfloxacin. The present method also validated as per ICH guidelines for linearity, precision, accuracy. The results of all these parameter shows that
the present UV spectrophotometric methods found to be precise, linear, rapid, and accurate and can be used for routine quality control analysis of Norfloxacin in tablet dosage formulation in any laboratory.

REFERENCES


