DEVELOPMENT AND VALIDATION OF ZERO ORDER UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF PEFLOXACIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A new, simple, accurate, precise, reproducible and economical zero order UV spectrophotometric method for estimation of Pefloxacin Mesylate in bulk and tablet formulation has been developed. The method of single point standardization was used for quantitative estimation of pefloxacin mesylate and absorbance was determined at 288nm ($\lambda_{\text{max}}$ of pefloxacin mesylate) using methanol as solvent system. The solutions of pefloxacin mesylate obeyed Beer’s law in concentration range of 2-20 μg/ml. The method was validated for parameters such as linearity, accuracy, precision and limit of quantification, limit of detection, ruggedness and robustness as per ICH guidelines. The developed and validated method was used for determining the content of pefloxacin mesylate in commercial tablets containing 400mg of pefloxacin mesylate. The proposed method was thus found suitable for quantitative estimation of pefloxacin mesylate in bulk and tablet formulations without any interference of the excipients.

KEYWORDS: Pefloxacin Mesylate, Validation, Single point standardization, ICH guideline.

INTRODUCTION

Pefloxacin Mesylate, 1-Ethyl-6-fluoro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid mono methanesulfate dihydrate was introduced in 1985 as a new chemical entity (fig 1). It is a broad spectrum third generation fluoroquinolone antibiotic active against both gram positive and gram negative bacteria.\(^1\) It functions by inhibiting...
bacterial enzyme DNA gyrase, a type II and type IV topoisomerase enzymes, which are needed for the transcription and replication of bacterial DNA. The inhibition of the topoisomerases results in strand breakage, supercoiling and releasing of the bacterial chromosome. Therefore, DNA replication and transcription is inhibited. It is used in the treatment of various respiratory tract infection, urinary tract infection, sexually transmitted diseases and gastrointestinal infection. Pefloxacin Mesylate is official in United States Pharmacopoeia and British Pharmacopoeia but not in Indian Pharmacopoeia. The assay procedure for estimation of pefloxacin mesylate in these pharmacopoeias is by potentiometric titration method. Literature survey revealed a few spectrophotometric methods,[2-9] spectrofluorimetric,[10,11] atomic absorption spectrometric,[12] HPLC[13-15] and colorimetric method for its determination.[16] Thus an attempt was made to develop a simple, accurate and precise zero order method for determination of pefloxacin mesylate by UV spectrophotometry at its wavelength of maximum absorption. To the best of our knowledge no such method is yet reported for estimation of pefloxacin mesylate in bulk and its tablet formulation.

**Fig.1: Structure of Pefloxacin Mesylate**

**MATERIALS AND METHODS**

**Instrumentation**

A double beam LABINDIA 3000+ UV/Visible spectrophotometer with a pair of 1 cm matched quartz cells was used. Shimadzu digital balance (Model No: AUY220) was used for weighing the samples.

**Materials and reagents**

All chemicals and reagents were of analytical grade. Pefloxacin Mesylate was purchased from Yarrow Chemical Products, Dombivli, Mumbai. It was authenticated before use by
performing identification tests. Commercial tablet formulations were purchased from local pharmacy.

**Selection of solvent**
Selection of solvent was based on solubility and stability of drug in solvent system as well as extraction of drug from its formulation. Pefloxacin Mesylate is freely soluble in methanol; hence methanol was selected as a solvent for its UV spectrophotometric determination.

**Preparation of stock solution**
A standard stock solution of pefloxacin mesylate was prepared by transferring accurately weighed quantity of drug (25mg) to 25ml volumetric flask and dissolving it in methanol to get a concentration of 1000µg/ml.

**Preparation of working standard solution**
From the above stock solution of pefloxacin mesylate (concentration: 1000µg/ml), 1 ml was transferred to separate 50ml volumetric flask and volume was made up using methanol to get the final concentration as 20µg/ml of pefloxacin mesylate.

**Determination of wavelength**
The working standard solution of pefloxacin mesylate was scanned in UV range from 200-400nm. The \( \lambda \) max value was found to be 211 and 288nm (fig 2). However, at 211nm solution did not show linearity. Hence 288 nm was chosen as the wavelength for its estimation.

![Fig 2: UV spectrum of Pefloxacin Mesylate showing \( \lambda \) max at 288nm.](image-url)
Estimation of pefloxacin in bulk and tablet dosage form

Twenty tablets of brand PEFLOBID manufactured by CADILA (India) containing 400mg of Pefloxacin Mesylate were taken, their average weight was determined and crushed to fine powder. A quantity of tablet powder equivalent to 50 mg of pefloxacin mesylate was transferred to 50ml volumetric flask and dissolved in methanol and sonicated for 10 minutes. The volume was made up to 50 ml (1000µg/ml). The solution was filtered using Whatman paper No. 41. It was further diluted to get a solution containing 10µg/ml of Pefloxacin mesylate. This solution was analyzed at 288 nm and absorbance value obtained was substituted in equation for single point standardization to obtain the content of pefloxacin mesylate.[17]

\[ C_{test} = A_{test} \times C_{standard} \div A_{standard} \]

- \( C_{test} \): concentration in sample solution.
- \( C_{standard} \): concentration in standard solution.
- \( A_{test} \): absorbance of sample solution.
- \( A_{standard} \): absorbance of standard solution.

RESULTS AND DISCUSSION

Validation

The developed method was validated as per ICH guidelines for various parameters.[18, 19]

Linearity profile

From the stock solution of pefloxacin mesylate having concentration of 1000 µg/ml, various dilutions were made to obtain solutions containing 2-20 µg/ml of pefloxacin mesylate. Absorbance values of these solutions were measured at wavelength 288nm. Table 1 shows the linearity profile of pefloxacin mesylate. The calibration curve was generated as absorbance versus concentration (fig 3).

Table 1: Linearity profile of Pefloxacin Mesylate

<table>
<thead>
<tr>
<th>Statistical parameter</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>0.034</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.019</td>
</tr>
<tr>
<td>Regression coefficient</td>
<td>( Y = 0.034x + 0.019 )</td>
</tr>
<tr>
<td>Correlation coefficient ( R^2 )</td>
<td>0.994</td>
</tr>
<tr>
<td>Wavelength</td>
<td>288nm</td>
</tr>
<tr>
<td>Linearity</td>
<td>2-20 µg/ml</td>
</tr>
<tr>
<td>LOD µg/ml</td>
<td>0.097</td>
</tr>
<tr>
<td>LOQ µg/ml</td>
<td>0.294</td>
</tr>
</tbody>
</table>
Accuracy

Solutions were prepared in triplicate at level 80%, 100% and 120% of test concentration of pefloxacin mesylate using working standard solution of pefloxacin mesylate as per the test method. The absorbance of each solution was recorded in triplicate.

The recovery results (table 2) showed that the proposed method has an acceptable level of accuracy for pefloxacin mesylate which is from 80% - 120% of test concentration of pefloxacin mesylate.

Table 2: Accuracy

<table>
<thead>
<tr>
<th>Recovery level</th>
<th>Percent recovery</th>
<th>SD*</th>
<th>%RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>99.15</td>
<td>0.5054</td>
<td>0.5097</td>
</tr>
<tr>
<td>100%</td>
<td>99.12</td>
<td>0.6950</td>
<td>0.7011</td>
</tr>
<tr>
<td>120%</td>
<td>99.23</td>
<td>0.7788</td>
<td>0.7848</td>
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</tbody>
</table>

*Average of three replicates at each level of recovery, SD: standard deviation, %RSD: relative standard deviation

Precision

Precision of the method was demonstrated by repeatability, intraday and interday variation studies. For repeatability study, nine samples of fixed concentration (10µg/ml) of pefloxacin mesylate were taken and absorbance values were recorded. The mean, standard deviation and % RSD was calculated. Results are shown in table 3.

In intraday variation, nine different solutions of fixed concentration (10µg/ml) of pefloxacin mesylate were analyzed three times in a day i.e. morning, afternoon and evening and the absorbance values were noted. From the absorbance values, the parameters such as mean, standard deviation and %RSD were calculated. The acceptable limit for intraday variation

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Fig 3: Linearity of Pefloxacin mesylate

CALIBRATION CURVE

\[ y = 0.034x + 0.0195 \]

\[ R^2 = 0.9946 \]

PELOXACIN MESYLATE

<table>
<thead>
<tr>
<th>Absorbance</th>
<th>Concentration mcg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>0.2</td>
<td>5</td>
</tr>
<tr>
<td>0.3</td>
<td>10</td>
</tr>
<tr>
<td>0.4</td>
<td>15</td>
</tr>
<tr>
<td>0.5</td>
<td>20</td>
</tr>
<tr>
<td>0.6</td>
<td>25</td>
</tr>
</tbody>
</table>

---

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should be within 2% and results shown in table 4 indicate that the drug complied with the required limit.

In interday variation studies, solution of fixed concentration (10µg/ml) of Pefloxacin Mesylate was analyzed three times for three consecutive days and absorbance values were recorded. The mean, standard deviation and %RSD was calculated. Results are shown in table 5.

**Table 3: Repeatability**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Wavelength</th>
<th>Absorbance</th>
<th>Mean</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>10µg/ml</td>
<td>288nm</td>
<td>0.753</td>
<td>0.753</td>
<td>0.448</td>
<td>0.451</td>
</tr>
</tbody>
</table>

SD: standard deviation, %RSD: relative standard deviation

**Table 4: Intraday assay precision**

<table>
<thead>
<tr>
<th>Time</th>
<th>10 a.m.</th>
<th>1 p.m.</th>
<th>4 p.m.</th>
<th>Average % RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>% RSD (Pefloxacin 10 µg/ml)</td>
<td>0.442</td>
<td>0.451</td>
<td>0.509</td>
<td>0.467</td>
</tr>
</tbody>
</table>
RSD: relative standard deviation

**Table 5: Interday assay precision**

<table>
<thead>
<tr>
<th>Observation</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Average % RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>% RSD (Pefloxacin 10 µg/ml)</td>
<td>0.491</td>
<td>0.412</td>
<td>0.436</td>
<td>0.446</td>
</tr>
</tbody>
</table>
RSD: relative standard deviation

**Limit of detection**

The limit of detection (LOD) was determined by preparing different solutions of different concentration ranging from 0.2-1µg/ml. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value.

\[
LOD = 3.3 \times \frac{\sigma}{S}
\]

Where \(\sigma\) = standard deviation, \(S\) = slope

**Limit of quantification**

The limit of quantification (LOQ) was determined by preparing solutions of different concentration of 30%, 50%, and 80% (10µg/ml) of Pefloxacin, working standard and labeled
as LQC, MQC and HQC respectively. The absorbance was recorded in triplicates for each concentration and mean, standard deviation and %RSD was calculated.

$$LOQ = 10 \times \frac{\sigma}{S}$$

Where $\sigma =$ standard deviation, $S =$ slope

**Robustness**

Robustness of the method was determined by carrying out the analysis under different conditions of temperature such as room temperature, wavelength conditions and variation in concentrations. The respective absorbance was noted and the result is expressed as % RSD and shown in table 6.

Table 6: Robustness

<table>
<thead>
<tr>
<th>Temperature ($^\circ$C)</th>
<th>Wavelength (nm)</th>
<th>Concentration (µg/ml)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>22$^\circ$C</td>
<td>286</td>
<td>8µg/ml</td>
<td>0.459</td>
</tr>
<tr>
<td>25$^\circ$C</td>
<td>288</td>
<td>10µg/ml</td>
<td>0.506</td>
</tr>
<tr>
<td>28$^\circ$C</td>
<td>290</td>
<td>12µg/ml</td>
<td>0.492</td>
</tr>
</tbody>
</table>

**Ruggedness**

Ruggedness of the method was determined by carrying out the analysis by different analyst and the absorbance of 10µg/ml solution of pefloxacin mesylate was noted. The result is expressed as shown in table 7.

Table 7: Ruggedness

<table>
<thead>
<tr>
<th>Observation</th>
<th>Analyst 1 (Pefloxacin 10µg/ml)</th>
<th>Analyst 2 (Pefloxacin 10µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>0.398</td>
<td>0.372</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.400</td>
<td>0.376</td>
</tr>
</tbody>
</table>

SD: standard deviation, %RSD: relative standard deviation

**CONCLUSION**

The developed UV spectrophotometric method for the estimation of Pefloxacin Mesylate is simple, sensitive and economical. This method was also validated by checking the parameters such as accuracy, precision, linearity, robustness and ruggedness. The proposed method showed high level of precision as depicted by low values of standard deviation and relative standard deviation. Hence this method can be used routinely for rapid assay of Pefloxacin.
Mesylate in bulk and pharmaceutical formulations without interference of excipients and other additives.

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REFERENCES


