DEVELOPMENT AND VALIDATION OF A DISSOLUTION TEST WITH SPECTROPHOTOMETRIC ANALYSIS FOR GEMIFLOXACIN IN TABLET DOSAGE FORM

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ABSTRACT
The aim of this work was to develop and validate a dissolution test for Gemifloxacin mesylate tablets using spectrophotometric method. The dissolution established conditions were: 900 mL of 0.01N HCl pH 2.0 as dissolution medium, using a paddle apparatus at a stirring rate of 50 rpm. The drug release was evaluated by UV spectrophotometric method at 271 nm. The method was validated to meet requirements for a global regulatory filing which includes linearity, specificity, precision, accuracy and ruggedness. In addition, filter suitability and drug stability in medium were demonstrated. The comparison of the obtained dissolution profiles of tablets, obtained from three different batches (A, B and C) of 320 mg Gemifloxacin mesylate was performed and the results showed no significant difference among the products.

Keywords: In vitro release, Stability, Antibacterial agent, Spectrophotometry, Validation.

INTRODUCTION
Gemifloxacin mesylate (Figure 1) is a potent, novel fluoroquinolone agent with a broad spectrum of antibacterial activity and it is used to treat respiratory and urinary tract infections that are proven or strongly suspected to be caused by susceptible gram-positive and gram-negative bacteria [¹]. Gemifloxacin mesylate is chemically (R, S)-7-[(4Z)-3-(amino methyl)-4-(methoxyimino)-1-pyrrolidinyl]-1cyclopropyl-6-fluoro-1, 4-dihydro-4-
oxo-1, 8-aphthyridine-3-carboxylic acid and its empirical formula is C18H20FN5O4•CH4O3S with molecular weight 485.49 \cite{2,3}.

Figure 1

Structure of Gemifloxacin mesylate

As a class, fluoroquinolones act by preventing deoxyribonucleic acid (DNA) synthesis through inhibition of the bacterial type II topoisomerase enzymes (DNA gyrase and topoisomerase IV), enzymes that are essential for bacterial growth \cite{4,5}. Gemifloxacin possesses a dual mechanism of action: it inhibits bacterial topoisomerase IV and gyrase enzymes, resulting in interruption of bacterial DNA synthesis \cite{6,7}. Drug absorption from a dosage form after oral administration depends on the release of the drug from the pharmaceutical formulation, the dissolution and/or its solubilization under physiological conditions, and the permeability across the gastrointestinal tract. Because of the critical nature of the first two of these steps, \textit{in vitro} dissolution may be relevant to the prediction of \textit{in vivo} performance \cite{8,9}. The dissolution test is a very important tool in drug development and quality control and the process of dissolution is shown in Figure 2. At present time there are no official monograph for Gemifloxacin raw material and tablets and no dissolution test has been described in literature for this drug. Few methods have been reported for Gemifloxacin determination in tablets by HPLC and HPTLC \cite{10–12}. Parameters to set up the dissolution test should be researched and defined for drugs that do not possess official monographs \cite{9}. For this reason, there is a crescent number of works describing the development of dissolution test for Citalopram, Rupatadine and Citrizine \cite{13–15}. The present paper describes the development and validation of dissolution test for quality control of Gemifloxacin in immediate release tablets. The best
dissolution conditions were used to evaluate development and validation of a dissolution method with dissolutions profile of three different batches of tablets.

![Figure 2](image)

**Figure 2**
Process of dissolution

**EXPERIMENTAL**

**Materials**

Gemifloxacin chemical reference substance (Ref no. GM WS/10/001, assigned purity-94.28%) and three batches of manufactured Gemifloxacin tablets containing 320 mg of Gemifloxacin mesylate each by Orchid HealthCare Ltd., India were used for the dissolution test. All reagents and solvents used were analytical grade. 0.01 M HCl of pH 2.0, pH 4.5 sodium acetate, pH 6.8 sodium phosphate and 2.1 simulated gastric fluid buffer solutions were prepared according to USP Pharmacopoeia [16].

**Instrumentation**

Dissolution test was performed in a Vankel (VK7025) auto sampler (VK8000) dissolution apparatus, multi-bath (n=6), in accordance to USP Pharmacopoeia [16] general method. The medium were vacuum degassed under in house vacuum and were maintained at 37.0 ± 0.5°C by using a thermostatic bath. A double-beam UV-Visible spectrophotometer (Model: CARY 50 CONC, Varian) with a fixed slit width (2 nm) using 1.0 cm quartz cell was used for all absorbance measurements. Elico pH analyzer (Model: Elico 11610) was used to determine the pH of all solutions.

**Solubility/stability determination and dissolution test optimization**

Gemifloxacin solubility was determined in 900 mL of Purified Water, 0.01M HCl, simulated gastric fluid (SGF) pH 2.1, sodium acetate buffer pH 4.5 and sodium phosphate buffer pH 6.8, using an amount of the drug equivalent a three times of dose in
the pharmaceutical formulation \cite{17}. Drug release tests were carried out according to conventional dissolution procedures recommended for single-entity products, using paddle (USP Apparatus II) at 25 and 50 rpm. Sampling aliquots of 10.0 mL were withdrawn at 0, 5, 10, 20, 30 and 45 minutes, and replaced with an equal volume of the fresh medium to maintain a constant total volume. At the end of each test time, samples aliquots were filtered and diluted with dissolution medium, when necessary, and quantified. The assay of the Gemifloxacin product was performed using previously validated spectrophotometric method \cite{18}, and the content results were used to calculate the percentage release on each time of dissolution profile. The cumulative percentage of drug released was plotted against time, in order to obtain the release profile and to calculate the in vitro dissolution data (n=12). The filtration procedure of Gemifloxacin and samples (tablets dissolved in dissolution medium, n=3) were evaluated using 0.1 μm, 0.2 μm, 0.45 μm cellulose acetate membrane filter (Phenomenex), and quantitative filter. The absorbance of filtered and unfiltered (centrifuged) solutions in dissolution medium was measured using concentration 14 μg/mL. To assess the stability of Gemifloxacin in dissolution medium, samples were diluted in 0.01 M HCl, and tested after 24 h at room temperature and also kept at 37 ± 0.5 °C for 2 h after dissolution. The stability of these solutions was studied by comparing values obtained with freshly prepared solutions.

**Method validation**

The UV spectrophotometric method used to analyze the Gemifloxacin samples in 0.01 M HCl dissolution medium was validated for specificity, linearity, precision, ruggedness and robustness according to USP Pharmacopoeia \cite{16} and ICH guideline \cite{17}. All absorbance were measured at 271 nm.

**RESULTS AND DISCUSSION**

**Development of dissolution test conditions**

The accomplishment of dissolution profile is recommended as support in the development and optimization of drug formulation as well as in the establishment of in vitro/in vivo correlation. When dissolution test is not defined in the monograph of dosage form or if the monograph is not available, comparison of drug dissolution profiles is recommended on three different dissolution media, in the pH range of 1–7.5. The dissolution test is widely used as in vitro test to determine the release rate of drug
products and to assure the quality of solid dosage forms by the pharmaceutical industry and regulatory agencies \textsuperscript{19}. The sink conditions are determined and expressed as percentage of drug released. Purified water, 0.01 M Hydrochloric acid, 2.1 SGF buffer, pH 4.5 acetate buffer, and pH 6.8 phosphate buffer are typical medium used in dissolution testing and these mediums are evaluated. The dissolution medium is selected based on the solubility data and screening study. The results showed that 0.01 M HCl was the best dissolution medium, since it provided highest drug release percent with greater stability and also ensured sink conditions. The dissolution profiles obtained with the different media tested are presented in Figure 3. The USP apparatus 2 (paddle) is chosen due to its acceptance as a standard procedure for tablets, and the stirring speed used at 25 or 50 rpm. Thus, significant difference was observed in the total drug released from the pharmaceutical formulation using 25 or 50 rpm, but based on the solubility the stirring rate of 50 rpm is selected as mild condition that allowed maximum discriminating power. The influence of rotation speed at 25 and 50 rpm is evaluated and the results are shown in Figure 4. Finally, the evaluation of 0.1 μm, 0.2 μm and 0.45 μm cellulose acetate membrane filters demonstrated that there is no interference in the analysis, giving values within 98-102% for the filtered samples compared to the centrifuged solutions. Based on these results, the selected conditions for dissolution test of Gemifloxacin in tablets are: 900 mL of 0.01 M HCl pH 2.0, using paddle apparatus at stirring rate of 50 rpm. In these conditions, typical acceptance criteria for the amount of drug dissolved are in the range of 70–80 % dissolved \textsuperscript{16}. In the present study, the % drug released for all three products are > 90% in 30 minutes (Figure 5), and the suggested acceptance criteria can be 85% in 30 minutes. The stability test of sample solutions showed that Gemifloxacin is stable in the dissolution medium when maintained at room temperature for 24 hour and at 37 ºC for 2 hour. The results are obtained from initial and final response factor and it is within the acceptable range, between 98-102% of the initial value, allowing the integrity of the drug during all the analysis and it is represented in Table 1.
Figure 3
Dissolution profiles in different media (DPDM) of Gemifloxacin tablets using 900 mL medium with paddle apparatus at stirring rate of 50 rpm

Figure 4
Dissolution profile in 0.01 M HCl at a stirring rate of 25 rpm and 50 rpm
Figure 5

Mean dissolution profiles (n=6) of three batches (A, B, and C) in 0.01 M HCl pH 2.0 at 37°C using paddle at 50 rpm

Table 1: Stability of Gemifloxacin tablet in different medium at two time points

<table>
<thead>
<tr>
<th>Dissolution Medium</th>
<th>Stability after 2 h at 37 ºC (%)</th>
<th>Stability after 24 h at room temp (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified water</td>
<td>99.3</td>
<td>99.5</td>
</tr>
<tr>
<td>0.01 M HCl</td>
<td>99.0</td>
<td>98.5</td>
</tr>
<tr>
<td>2.1 SGF</td>
<td>98.1</td>
<td>98.3</td>
</tr>
<tr>
<td>pH 4.5 Acetate buffer</td>
<td>98.4</td>
<td>99.1</td>
</tr>
<tr>
<td>pH 6.8 Phosphate buffer</td>
<td>101.2</td>
<td>102.4</td>
</tr>
</tbody>
</table>

Method validation

UV-VIS spectrophotometry and high performance liquid chromatography are the most frequently used analytical methods used for quantifying drug release in dissolution tests. The UV spectrophotometric method may be used if drug has a UV chromophore
and no UV interferences due to medium and excipients used in the formulation [19]. This method has the advantage of very rapid time of analysis and the relatively low cost for the routine quality control analysis. So the analytical method was validated for linearity, specificity, precision, accuracy, ruggedness and robustness in accordance with ICH guidelines.

**Linearity**

The linearity of Gemifloxacin response is evaluated from the range of 2.5–15.0 μg/mL and showed a good correlation coefficient (0.9996). To assess linearity, the standard curves of Gemifloxacin are constructed by plotting concentration (μg/mL) versus absorbance is shown in Figure 6. Linear regression is also calculated and the obtained equation is $y = 4.996x + 0.004$, where x is the concentration in μg/mL, y is amplitude for UV spectrophotometry.

![Figure 6](image)

**Figure 6**
Linearity or calibration curve of Gemifloxacin

**Specificity**

Specificity is examined by analyzing a solution of a placebo, which consisted of all the excipients of tablets without the active pharmaceutical ingredient. The absorption
spectrum of Gemifloxacin standard in dissolution medium shows maximum absorbance at 271 nm (Figure 7). At this wavelength, there is no interferences observed from the tablet excipients and medium, thus demonstrating the proposed method is specific for the analysis of Gemifloxacin.

![UV spectrum of Gemifloxacin standard in 0.01 M HCl showing $\lambda_{\text{max}}$ at 271 nm](image)

**Figure 7**
UV spectrum of Gemifloxacin standard in 0.01 M HCl showing $\lambda_{\text{max}}$ at 271 nm

**Precision**

The precision of the method is evaluated by measuring the repeatability in two different UV Vis spectrophotometers have shown RSD value of 0.2 and 0.1% respectively. The RSD values obtained during intermediate precision evaluation, on two different days were 0.1 and 0.2%, respectively. The values were submitted to statistical analysis using student’s $t$-test, showing non-significant difference ($p>0.05$), as shown in Table 2. These results demonstrated the good precision of the proposed method for dissolution test.
Table 2: Repeatability and intermediate precision of the dissolution method

<table>
<thead>
<tr>
<th>Samples</th>
<th>% Gemifloxacin released</th>
<th>Inter-day precision</th>
<th>Between-equipments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>UV A</td>
</tr>
<tr>
<td>1</td>
<td>98.3</td>
<td>97.2</td>
<td>97.2</td>
</tr>
<tr>
<td>2</td>
<td>97.9</td>
<td>99.4</td>
<td>99.4</td>
</tr>
<tr>
<td>3</td>
<td>98.4</td>
<td>99.4</td>
<td>99.4</td>
</tr>
<tr>
<td>4</td>
<td>98.9</td>
<td>100.2</td>
<td>100.2</td>
</tr>
<tr>
<td>5</td>
<td>99.6</td>
<td>99.8</td>
<td>99.8</td>
</tr>
<tr>
<td>6</td>
<td>100.3</td>
<td>100.6</td>
<td>100.6</td>
</tr>
<tr>
<td>Mean</td>
<td>98.9</td>
<td>99.4</td>
<td>99.4</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Student’s $t$-test

<table>
<thead>
<tr>
<th></th>
<th>$t$ calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inter-days</td>
<td>0.88</td>
</tr>
<tr>
<td>Between equipments</td>
<td>1.19</td>
</tr>
</tbody>
</table>

$RSD = \text{Relative standard deviation.} \quad t \text{ critic for } p = 0.05 (2.23).$

Accuracy

The accuracy is evaluated by applying proposed method to the analysis of in-house mixture of the tablet excipients with known amount of the Gemifloxacin working standard, corresponding to the concentrations of 50, 100 and 120%, which were subjected to dissolution test conditions described above. The accuracy was calculated as the percentage of the drug recovered from the formulation matrix. The accuracy was assessed from three replicate determinations of samples containing 11.2, 14.0 and 16.8 μg/mL of Gemifloxacin, giving concentrations respectively 11.28, 13.92 and 16.65 μg/mL. The recoveries obtained with a mean value of 99.73% and RSD lower than 0.52%,
demonstrated that the method is accurate for intended use. The percent recoveries obtained (Table 3) are considered acceptable \[^{[19]}\].

**Table 3: Results from accuracy as recovery studies \((n = 3)\) for Gemifloxacin tablets**

<table>
<thead>
<tr>
<th>Actual concentration (μg/mL)</th>
<th>Concentration found (μg/mL) ± a SD</th>
<th>b RSD (%)</th>
<th>c SE</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.2</td>
<td>11.28±0.06</td>
<td>0.58</td>
<td>0.02</td>
<td>100.7</td>
</tr>
<tr>
<td>14.0</td>
<td>13.92±0.09</td>
<td>0.47</td>
<td>0.03</td>
<td>99.4</td>
</tr>
<tr>
<td>16.8</td>
<td>16.65±0.12</td>
<td>0.52</td>
<td>0.07</td>
<td>99.1</td>
</tr>
</tbody>
</table>

\(^{a}\) Standard deviation  
\(^{b}\) Relative standard deviation  
\(^{c}\) Standard error

**Ruggedness**

Ruggedness of the method is determined by carrying out the analysis by two different analysts and the respective dissolution values are indicated by % RSD and statistical analysis was also done by using student’s t-test, showing non-significant difference \((p>0.05)\), as shown in Table 4.

**Table 4: Ruggedness of Gemifloxacin in tablet dosage form**

<table>
<thead>
<tr>
<th>Samples</th>
<th>% Gemifloxacin released</th>
<th>Analyst 1</th>
<th>Analyst 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>97.2</td>
<td>98.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>99.4</td>
<td>97.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>99.4</td>
<td>99.3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>100.2</td>
<td>97.9</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>99.8</td>
<td>98.8</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>100.6</td>
<td>99.2</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>99.4</td>
<td>98.6</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) % RSD  
\(^{b}\) Student’s t-test \(t=1.57\)

RSD = Relative standard deviation, \(t_{critic} for p = 0.05\) (2.23)
Robustness

The robustness of an analytical procedure refers to its ability to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability for the routine analysis. A portion of the test solutions are centrifuged and portions of test solutions are filtered through 0.1µm, 0.2 µm, 0.45 µm cellulose acetate membrane filters and Whatmann filter paper [20]. The absorbance of blank, standard, centrifuge and filtered solutions are measured at 271 nm. The percent of drug release for centrifuged and filtered test solutions are estimated and difference of % dissolution between centrifuge and filter samples are represented in Table 5.

Table 5: Robustness of Gemifloxacin in tablet dosage form

<table>
<thead>
<tr>
<th>Filters</th>
<th>% Dissolved</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1µm</td>
<td>99.4</td>
<td>2.3</td>
</tr>
<tr>
<td>0.2 µm</td>
<td>100.2</td>
<td>3.5</td>
</tr>
<tr>
<td>0.45 µm</td>
<td>99.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Whatmann filter paper</td>
<td>94.8</td>
<td>6.9</td>
</tr>
</tbody>
</table>

% dissolved for centrifuged sample is 101.7

CONCLUSION

A simple dissolution test developed and validated for Gemifloxacin tablets are considered satisfactory. The conditions that allowed the dissolution determination are 900 mL of 0.01 N HCl at 37.0 ± 0.5 ºC, paddle apparatus, 50 rpm stirring speed and filtration with 0.45 µ cellulose acetate membrane filters. In these conditions, the Gemifloxacin stability is good. The percent drug delivery is higher than 90% in 30 minutes for all evaluated products. Therefore, the proposed method is successfully applied and suggested for the quality control studies of Gemifloxacin pharmaceutical dosage forms contributing to assure the therapeutic efficacy of the drug.
REFERENCES


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