**A validated RP-UPLC method for the determination of Gemifloxacin Mesylate in bulk and its pharmaceutical preparation.**

**Abstract:** ***Objectives:*** Gemifloxacin Mesylate is a fourth generation fluoroquinolone antibacterial agent. A simple, accurate and precise reversed phase UPLC method was developed and validated for short time analysis of Gemifloxacin Mesylate in its bulk and pharmaceutical preparation.

***Materials and Methods:*** The optimum separation was achieved at 0.5±0.03 min using Acclaim™ RSLC 120 C18 column 2.2µm (2.1 x 100 mm) at 30°C by isocratic mobile phase at pH= 3.0 composed of acetonitrile: phosphate buffer (25mM) with ratio 75:25 (v/v). The column effluents were monitored at 276 nm using photo diode array detector (PDA) at flow rate 0.5 mL/min. The method was validated according to ICH guidelines.

***Results:*** The linearity of the calibration curve ranged from 0.5**µ**g/mL to 10 **µ**g/mL and square of the regression coefficient ($r^2$) was 0.9991. The % RSD of inter-day precision ranged from 0.081% to 1.233%, while for intra-day ranged from 0.364 % to 1.018%. The method was found accurate with % recovery ranging from 93.71% to 100.29 % and % RSD ranged from 1.054 to 2.722. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.066 and 0.2 **µ**g/mL.

***Conclusion:*** The validated method proof its ability for the assay of Gemifloxacin mesylate in its bulk and dosage form in short time (less than 1 minute). To the best of our knowledge, this is the first RP-UPLC method for the determination of Gemifloxacin mesylate.

**Keywords:** Gemifloxacin Mesylate, Ultra performance liquid chromatography, Method validation.

## INTRODUCTION

Gemifloxacin Mesylate (Figure 1) is a synthetic broad-spectrum antibacterial agent for oral administration. It is a member of the fourth generation fluoroquinolone antibiotics. Its mechanism of action is the inhibition of both topoisomerase IV and DNA gyrase, which are essential for bacterial cell replication. It is characterized by its broad spectrum of activity against both Gram-positive and Gram-negative bacteria. It is used in the treatment of respiratory tract and urinary tract infections. [1-4]

IUPAC name of Gemifloxacin Mesylate 7-[(4Z)-3-(Aminomethyl)-4-methoxyiminopyrrolidin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,8 naphthyridine-3-carboxylic acid, Methanesulfonic acid. Its molecular formula is $C_{18}H_{20}FN_5O_4 \bullet CH_4O_3S$, molecular weight 485.49 g/mol

In literature, different analytical methods have been reported for its determination; including spectrophotometric [5-7], spectrofluorimetry [8-10], High performance thin layer chromatography (HPTLC) [11-14], HPLC-UV [15-18] and LC-MS [19-20].

Ultra-performance liquid chromatography (UPLC); introduced in 2004; proved to be more efficient than high performance liquid chromatography (HPLC) in many aspects such as resolution, sensitivity and consuming much smaller amount of solvents.

The aim of this research is the rapid and sensitive determination and quantification of Gemifloxacin Mesylate in its bulk and pharmaceutical preparation with lower solvents consumption using RP-UPLC- UV in addition to its validation with respect to International Conference on Harmonization (ICH) guidelines. To the best to our knowledge, this is the first RP-UPLC method for the determination of Gemifloxacin mesylate.

## MATERIALS AND METHODS

*Instrument and software*

The UPLC system employed was Thermo Fisher UHPLC Dionex Ultimate 3000 (Germering, Germany). The pump was ISO-3100SD, while, the autosampler was WPS 3000 SL, and the column thermostat was TCC-3000 SD. The detector was Diode Array (DAD- 3000 RS) (Germering, Germany). The software utilized for data acquisition was Chromeleon 6.8 (Germering, Germany). pH of buffer was measured using pH meter (A Jenway pH-meter 3310, Dunmow, Essex, United Kingdom). MiliQ water was produced in house from ultrapure water purification system (Thermo scientific Barnstead Smart2Pure 3 UV, Hungary). The separation was carried out using Acclaim™ RSLC 120 C18 column 2.2µm (2.1 x 100 mm), Thermo Fischer.

*Chemicals and Reagents*

Acetonitrile (HPLC grade), monobasic potassium phosphate and phosphoric acid (High grade) were purchased from Sigma-Aldrich, Germany. Gemifloxacin Mesylate standard was obtained from sigma pharmaceutical company (Cairo, Egypt). Gemifloxacin Mesylate pharmaceutical preparations (Quinabiotic®, Utopia) were purchased from the Egyptian market.

*Methods*

*Mobile phase preparation*

Mobile phase is composed of acetonitrile and 25 mM phosphate buffer (pH 3.00) (75:25, v/v). The mobile phase was mixed then degassed using ultrasonicator.

The phosphate buffer was prepared by mixing monobasic potassium phosphate and phosphoric acid, and then the pH was measured by the pH-meter and adjusted to 3.00.

*Standard Solution preparation and calibration curve plotting*

The standard stock solution was prepared by dissolving 25 mg of Gemifloxacin Mesylate standard in 25 mL deionized water, so that the final concentration is 1000 **µ**g/mL. After, serial dilutions (0.5-10 µg/mL) were accomplished to construct the calibration curve.

*Sample Preparation*

10 tablets of Quinabiotic® containing 320 mg Gemifloxacin Mesylate equivalent to 320mg Gemifloxacin was accurately weighed and crushed into fine powder. A concentration of 1000 **µ**g/mL was prepared by taking and equivalent amount of 25 mg Gemifloxacin Mesylate and dissolved in 25 mL deionized water. The solution was sonicated for 15 min, then filtered using 0.22**µ**m nylon syringe filter. After a dilution equivalent to 1 **µ**g/mL was prepared and then injected into the UPLC.

*Chromatographic conditions*

The Mobile phase was a mixture of Acetonitrile: phosphate buffer (75:25, v/v) at a flow rate 0.5 mL/min. The temperature of the column oven was adjusted at 30°C, the injection volume of the sample was 10µL. The photodiode array detector was maintained at wavelengths 276 nm.

*Method Validation*

Validation was performed as stated in the ICH guidelines with reference to the following parameters: Linearity, Limit of quantification and detection, precision (inter and intra-day) and accuracy.[21]

Linearity is the ability of a method to get the response directly proportional to sample concentration over a given range. The linearity of the analytical method was determined by preparing 7 serial dilutions ranging from 0.5 - 10 µg/mL. Each concentration was injected 3 times into the UPLC. After obtaining different peak areas, the average peak area was obtained for each concentration. Hence, concentrations against the average peak area were plotted accordingly in a calibration curve. Using linear regression analysis, the regression equation was determined along with the correlation coefficient. Linearity was evaluated using square of the regression coefficient ($r^2$).

For the limit of quantification (LOQ), it is equivalent to the concentration of the analyte in which S/N is equal 10. While for the limit of detection (LOD), it is equivalent to the concentration of the analyte in which S/N is equal 3.3.

Precision measures whether the method is able to generate reproducible results or not. The precision of the method was evaluated using intra-day (repeatability) and inter-day precision (intermediate precision). Intra-day precision was determined by injecting 4 different concentrations into UPLC each was injected three times on the same day. The average peak was obtained along with the standard deviation. The precision was evaluated with respect to %RSD. While inter-day precision was obtained by injecting four concentrations into the UPLC, each concentration was injected for 3 times on two consecutive days. The

average peak between day one and day two were analyzed to calculate the standard deviation and accordingly, %RSD was evaluated.

Accuracy is the closeness of the results obtained from a method to the reference true values. The accuracy of the method was determined by evaluating recovery studies on the pharmaceutical preparation. Three different solutions were prepared; each containing 1 µg/mL pharmaceutical preparation spiked with known concentration of standard solution of 0.4 µg /mL, 0.8 µg /mL and 1.2 µg /mL so that the final concentrations are 1.4 µg/mL, 1.8 µg/mL /mL and 2.2 µg/mL respectively. Each sample was injected three times on two consecutive days. Accuracy was evaluated by calculating percentage recovery and accordingly, %RSD is determined.

The robustness of the method was assessed by the ability of the method to remain unaffected by little deliberate changes in the following parameters[22]: wavelength, % acetonitrile and pH of the buffer.

*System Suitability Test*

System suitability test was determined by injecting a working solution of 1 µg/mL gemifloxacin mesylate under the optimum condition.

## RESULTS AND DISCUSSION

*Method Development*

In order to achieve the optimum condition, the analytical conditions including temperature, mobile phase composition, wavelength and flow rate, were optimized.

At the beginning, methanol was investigated as an organic solvent, instead of acetonitrile. Better peak shape and less retention time were achieved using acetonitrile.

Also, both the buffer strength and pH were studied. Although that higher concentration of buffer showed less retention time and better peak shape, but it led to increase in the pump pressure; accordingly 25 mM was selected as the optimum buffer strength. For the pH, greater pH than pH 3.00 showed broader peak and lower pH did improve neither the peak shape nor the peak shape. As a consequence, pH 3.00 was chosen as the optimum buffer pH. This could be explained that at pH 3.00, it was below the pKa of gemifloxacin mesylate ($pKa_1$=5.53 , $pKa_2$=9.53).

While for the temperature, reaching T 30°C, was enough to enhance the peak shape, higher temperature did not have a significant effect on the peak. For the flow rate, 0.5 mL/min was optimum to reach a short analysis time without increasing the pump pressure.

The optimum wavelength for detecting Gemifloxacin Mesylate was found to be at 276 nm using photodiode array detector (PDA) as shown in the spectrum (Figure 2). The flow rate was set at 0.5mL/min. the most optimum temperature for analysis was found out to be at 30 °C the pH for the phosphate buffer was 3.0. The mobile phase composition was Acetonitrile: 25 mM phosphate buffer, pH 3.00 (75:25 v/v). Under this condition, Gemifloxacin Mesylate peak appeared at tR 0.5±0.03 min (Figure 3). To the best to our knowledge, this is the first UPLC- PDA method for the analysis of Gemifloxacin Mesylate analysis reported in literature. Accordingly, when compared to other HPLC-UV method reported in literature; it provided shorter analysis time and less consumption of solvents.

After, the analytical method developed was evaluated and validated as per ICH guidelines.

*Validation of the developed method*

*Linearity*

The graphical representation calibration curve shows that the linearity ranged from 0.5 to 10 **µ**g/mL. Using linear regression analysis, the slope, the intercept and the regression coefficient were determined from the regression equation y = 396.69x-6.8416. The regression coefficient [$R^2$] was equal 0.9991. The slope was 396.69 and the intercept was 6.8416.

The limit of detection (LOD) was 0.066 **µ**g/mL, while the limit of quantification (LOQ) was 0.2 **µ**g/mL.

*Precision*

For the inter-day precision %RSD was determined and ranged from 0.0807% to 1.2326%, while for intra-day precision %RSD ranged from 0.1562 % to 1.0176%.

The results of both inter and intra-day precision is presented as shown in Table 1.

*Accuracy*

Accuracy of the method was evaluated. % Recovery was found to be ranging from 93.71% to 100.29% while % RSD ranged from 1.054 to 2.721 as shown in Table 2.

*Robustness*

To evaluate the robustness of the method, minor changes were brought to the parameters intentionally. Hence, the % RSD was calculated and the results were as follow: Wavelength 276 ± 3 nm with % RSD 1.73%, the % acetonitrile 75± 1% with % RSD 2.35% and pH of the buffer 3.00 ± 0.5 with % RSD 2.90%.

*System Suitability Test*

System suitability test was performed to demonstrate the adequacy of the analysis system. In order to accomplish that different parameters were verified. The column efficiency which can be evaluated by the plate number, the asymmetric factor to evaluate the peak symmetry and the reproducibility of the system assessed by the % RSD of both the peak area and the retention time. The data are presented in Table 3.

*Application on pharmaceutical preparation*

To determine the ability of the method to be applied for the determination of Gemifloxacin Mesylate in its pharmaceutical preparations, Quinabiotic® 320 mg, was purchased from the local market. After, 10 tablets were weighed and crushed. Hence an equivalent amount of 25 mg was dissolved in 25 mL deionized water, followed by sonication and filtration. Then the filtrate was diluted with deionized water to have a concentration equivalent to 1 µg/mL. As presented in Figure 4, the tR of Gemifloxacin Mesylate was 0.500 min and tablet excipients did not interfere with the analysis. The % recovery was 99.496 with % RSD 1.431. Accordingly, method proved its ability for the determination of gemifloxacin mesylate in its pharmaceutical preparations.

*Statistical Analysis*

To ensure the applicability of the newly suggested method, it was compared to a published reference method. In order to investigate if there are any significant differences between the two methods and to find the extent to which this difference can affect the applicability of the new method rather than an already used one.

Comparing the obtained F- and t-values with the tabulated ones, it is clear that the obtained values were lower than the theoretical tabulated values, i.e the methods suggested do not exhibit significant differences in comparison to those of the published methods which reflects the accuracy and precision of the suggested UPLC method. The results are shown in table 4.

**CONCLUSION**

The newly developed RP-UPLC method for short time analysis of Gemifloxacin Mesylate in its bulk and pharmaceutical preparation was found to be rapid, simple, accurate and precise. The method was validated as per ICH for linearity, accuracy and precision. Linearity was determined at an acceptable range from 0.5-10 µg/mL. Finally, the method was accepted for the analytical evaluation of the drug in its pharmaceutical preparations with respect to the satisfactory results obtained and it showed lowest retention time; in comparison to other HPLC methods reported in literature.

*Conflict of Interest: No conflict of interest was declared by the authors.*

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**Figure captions:**

**Figure 1:** The chemical structure of Gemifloxacin Mesylate.

**Figure 2:** UV-spectrum of Gemifloxacin Mesylate.

**Figure 3:** Chromatograms of 1 **µ**g/mL Gemifloxacin Mesylate under the optimum condition named: acetonitrile: 25 mM Phosphate buffer, pH 3.00 (75:25; v/v), at a flow rate 0.5 mL/min and T 30°C.

**Figure 4:** Chromatograms of 1 **µ**g/mL Quinabiotic® under the optimum condition named: acetonitrile: 25mM Phosphate buffer, pH 3.00 (75:25; v/v), at a flow rate 0.5 mL/min and T 30°C.

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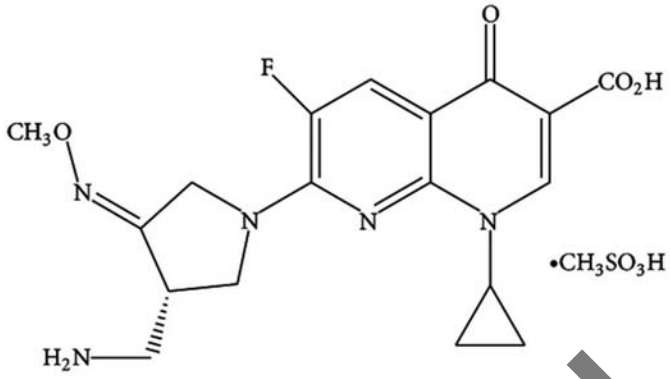
**Table 1:** Inter-day and Intra-day precision of Gemifloxacin Mesylate.

**Table 2:** Accuracy of Gemifloxacin Mesylate.

**Table 3:** System Suitability Data of the suggested UPLC method.

**Table 4:** Statistical comparison between the proposed method and reference methods.

**List of Figures**

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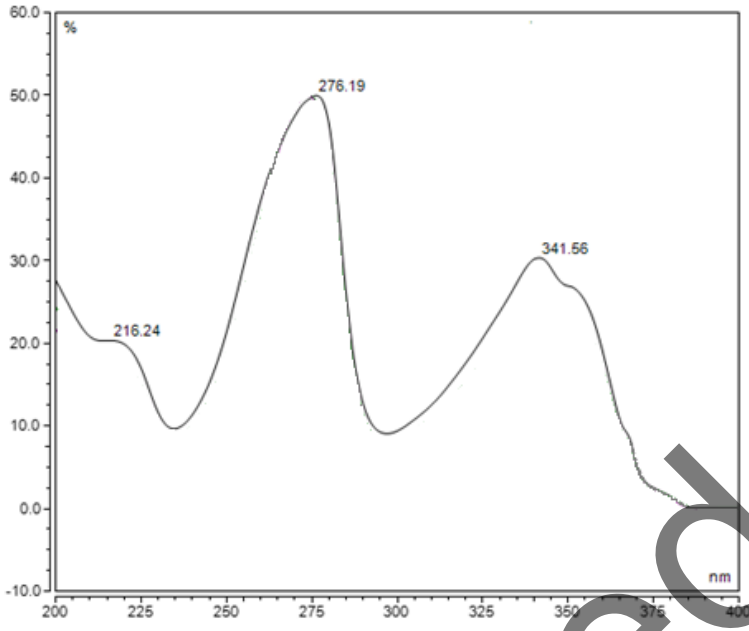


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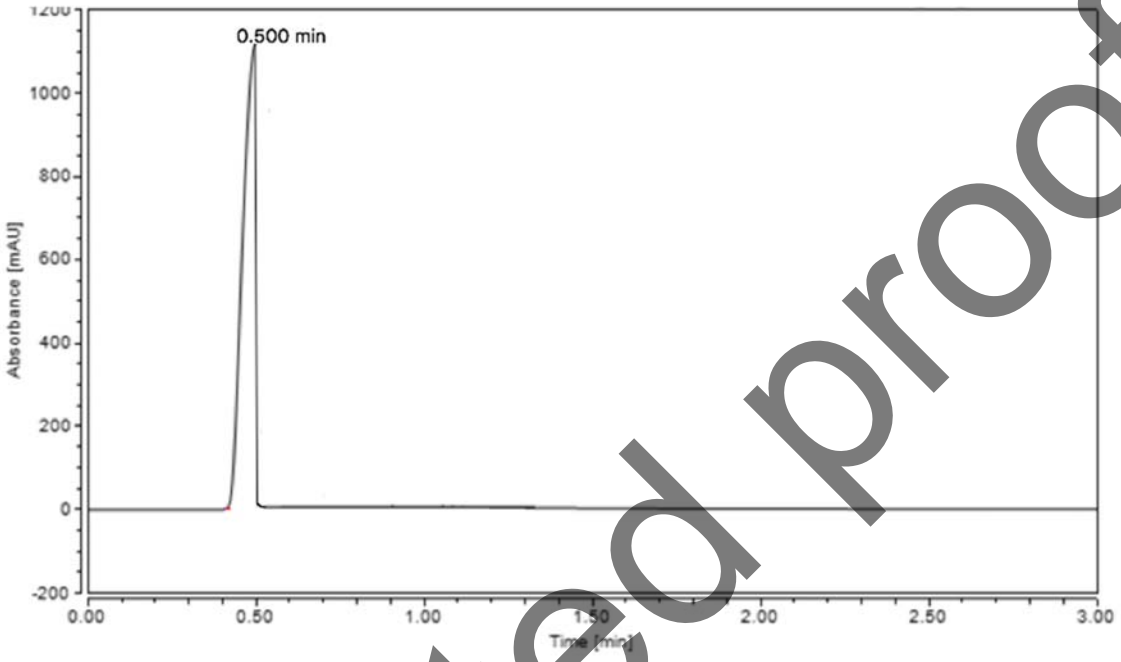


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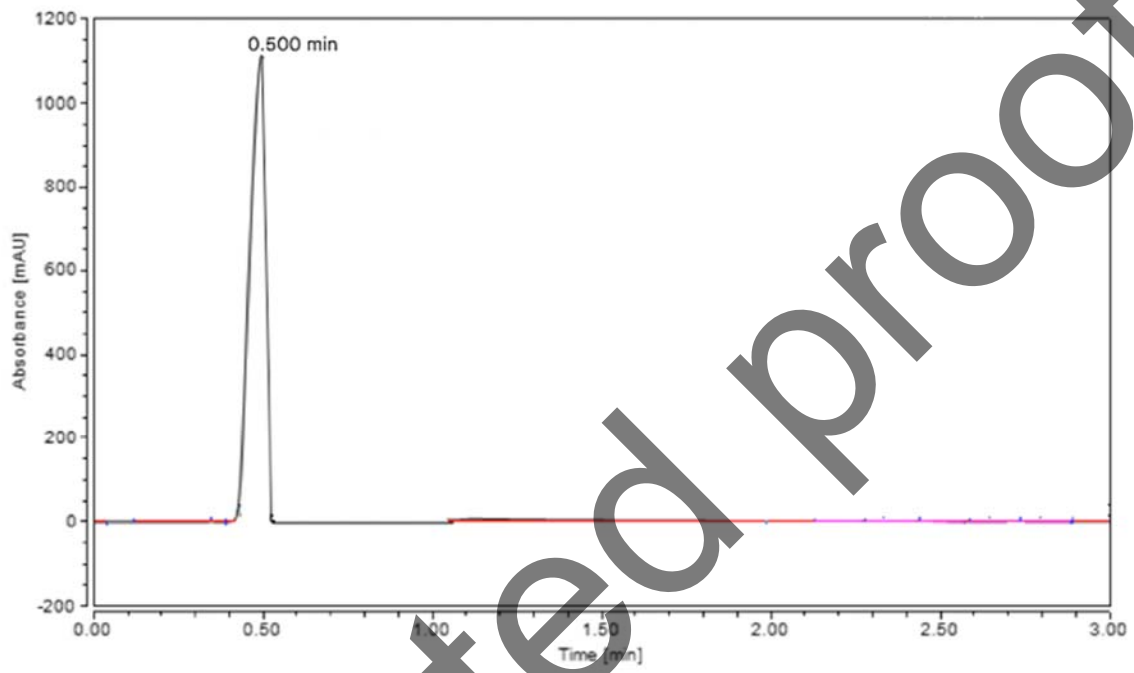


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## List of Tables

**Table 1: Inter-day and Intra-day precision of Gemifloxacin Mesylate**

| Concentration (µg/mL) | Inter-Day precision | | Intra-Day precision | |
|---|---|---|---|---|
| | Peak Area* | % RSD | Peak Area* | %RSD |
| 1 | 76.458 | 1.233 | 75.791 | 0.156 |
| 2 | 145.734 | 0.653 | 145.061 | 1.018 |
| 4 | 230.634 | 0.368 | 230.035 | 0.364 |
| 8 | 306.400 | 0.081 | 306.575 | 0.653 |

* Average of 3 repetitions.

**Table 2: Accuracy of Gemifloxacin Mesylate**

| Theoretical concentration (µg/mL) | Actual Concentration* (µg/mL) | %RSD | % Recovery* | %RSD |
|---|---|---|---|---|
| 1.4 | 1.374 | 1.197 | 98.19 | 2.722 |
| 1.8 | 1.687 | 0.632 | 93.71 | 1.054 |
| 2.2 | 2.206 | 1.217 | 100.29 | 1.723 |

* Average of 3 repetitions

**Table 3:** System Suitability Data of the suggested UPLC method.

| **Parameters** | **Gemifloxacin Mesylate** |
|---|---|
| **Number of Theoretical plates (N)** | 3300 |
| **Assymetric Factor($A_s$)** | 1.05 |
| **Capacity Factor** | 5.25 |
| **% RSD (Retention Time)*** | 0.35 |
| **% RSD (Peak Area)*** | 0.634 |

* Average of 4 repetitions

**Table 4:** Statistical comparison between the proposed method and reference methods.

| | Proposed Method | Reference Method |
|---|---|---|
| **Pure solution** | | |
| Mean ± SD | 100.06± 0.76 | 99.38± 0.51[23] |
| n | 7 | 6 |
| Student's T Test (Tabulated) | 1.91 (2.18) | |
| F Test (Tabulated) | 2.24 (4.95) | |
| Probability | <0.05 | |
| **Quinabiotic®** | | |
| Mean ± SD | 97.40± 1.83 | 98.48± 1.82[11] |
| n | 3 | 5 |
| Student's T Test (Tabulated) | 0.81 (2.37) | |
| F Test (Tabulated) | 1.01 (6.94) | |
| Probability | <0.05 | |