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ORIGINAL ARTICLE

A Validated Reverse Phase-Ultra-Performance Liquid Chromatography Method for the Determination of Gemifloxacin Mesylate in Bulk and its Pharmaceutical Preparation

Gemifloksasin Mesilatın Bulk ve Farmasötik Preparatından Tayini için Valide Edilmiş Ters Faz-Ultra-Performans Sıvı Kromatografisi Metodu

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ABSTRACT

Objectives: Gemifloxacin Mesylate is a fourth generation fluoroquinolone antibacterial agent. A simple, accurate, and precise reversed phase (RP)-ultra performance liquid chromatography (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 an Acclaim^{TM} RSLC 120 C18 column 2.2 µm (2.1×100 mm) at 30°C by isocratic mobile phase at pH 3.0 composed of acetonitrile:phosphate buffer (25 mM) in a ratio of 75:25 (v/v). The column effluents were monitored at 276 nm using a photodiode array detector at a flow rate of 0.5 mL/min. The method was validated according to International Conference on Harmonization guidelines.

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

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

Key words: Gemifloxacin Mesylate, ultra performance liquid chromatography, method validation

ÖZ

Amaç: Gemifloksasin Mesilat dördüncü jenerasyon fluorokinolon antibakteriyel ajandır. Basit, doğru ve hassas bir ters fazlı (RP)-ultra performanslı sıvı kromatografisi (UPLC) yöntemi, Gemifloksasin Mesilatın, bulk ve farmasötik preparatında kısa sürede analizi için geliştirilmiş ve valide edilmiştir.

Gereç ve Yöntemler: Optimum ayırma, Acclaim^{TM} RSLC 120 C18, 2.2 µm (2.1×100 mm) kolonu kullanılarak, 30°C'de, 75:25 (h/h) asetonitril:fosfat tamponu (25 mM) içeren pH 3.0 izokratik hareketli faz ile 0.5±0.03 dk'da elde edildi. Kolon atığı 0.5 mL/dk'lık bir akış hızında fotodiyod detektörü kullanılarak 276 nm'de izlenmiştir. Yöntem Uluslararası Uyumlaştırma Konferansı kılavuzlarına göre valide edildi.

Bulgular: Kalibrasyon eğrisinin doğrusallığı 0.5 µg/mL ile 10 µg/mL arasındadır ve regresyon katsayısının karesi (r^2) 0.9991'dir. Gün içi kesinliğin % bağıl standart sapması (RSD) %0.081 ile %1.233 arasında değişirken, günler arası için %0.364 ile %1.018 arasında değişiyordu. Yöntem %93.71 ile %100.29 ve % RSD değeri 1.054 ile 2.722 arasında değişen % geri kazanım ile doğrudur. Saptama limiti ve tayin limiti sırasıyla 0.066 ve 0.2 µg/mL idi.

Sonuç: Valide edilmiş yöntem, Gemifloksasin Mesilatın bulk ve dozaj formundan tayininde kısa sürede (1 dakikadan az) tayinin olabileceğini kanıtlamıştır. Bildiğimiz kadarıyla, bu, Gemifloksasin Mesilatın belirlenmesi için ilk RP-UPLC yöntemidir.

Anahtar kelimeler: Gemifloksasin Mesilat, ultra performanslı sıvı kromatografisi, metod validasyonu

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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 by 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]

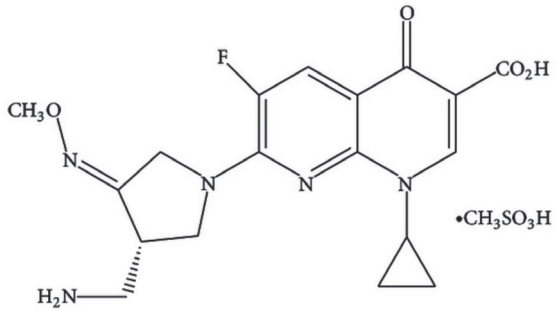

Figure 1. The chemical structure of Gemifloxacin Mesylate

The IUPAC name of Gemifloxacin Mesylate is 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 $C_{18}H_{20}FN_5O_4 \bullet CH_4O_3S$; its molecular weight is 485.49 g/mol.

In the literature, different analytical methods have been reported for its determination, including spectrophotometry,[5-7] spectrofluorimetry,[8-10] high performance thin layer chromatography (HPTLC),[11-14] high performance layer chromatography (HPLC)-ultraviolet (UV),[15-18] and liquid chromatography (LC)-mass spectrometry.[19,20]

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

The aim of the present research was the rapid and sensitive determination and quantification of Gemifloxacin Mesylate in its bulk and pharmaceutical preparation with lower consumption of solvents using reverse phase (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

Instruments and software

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

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

The mobile phase was composed of acetonitrile and 25 mM phosphate buffer (pH 3.00) (75:25, v/v). The mobile phase was mixed and then degassed using an 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 of deionized water, so that the final concentration was 1000 µg/mL. After that, serial dilutions (0.5-10 µg/mL) were accomplished to construct the calibration curve.

Sample preparation

Ten tablets of Quinabiotic® containing 320 mg of Gemifloxacin Mesylate equivalent to 320 mg of Gemifloxacin were accurately weighed and crushed into fine powder. A concentration of 1000 µg/mL was prepared by taking an equivalent amount of 25 mg of Gemifloxacin Mesylate and dissolving it in 25 mL of deionized water. The solution was sonicated for 15 min and then filtered using a 0.22-µm nylon syringe filter. After that a dilution equivalent to 1 µg/mL was prepared and it was then injected into the UPLC system.

Chromatographic conditions

The mobile phase was a mixture of acetonitrile and phosphate buffer (75:25, v/v) at a flow rate 0.5 mL/min. The temperature of the column oven was adjusted to 30˚C and the injection volume of the sample was 10 µL. The photodiode array (PDA) detector was maintained at a wavelength of 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 a response directly proportional to the sample concentration over a given range. The linearity of the analytical method was determined by preparing 7 serial dilutions ranging from 0.5 to 10 µg/mL. Each concentration was injected 3 times into the UPLC. After different peak areas were determined, 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 the 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 to 10, while for the limit of detection (LOD), it is equivalent to the concentration of the analyte in which S/N is equal to 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 the 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 % relative standard deviation (RSD). While inter-day precision was obtained by injecting four concentrations into the UPLC system, each concentration was injected 3 times on two consecutive days. The average peak between day one and day two was analyzed to calculate the standard deviation and, accordingly, % RSD was evaluated.

Accuracy is the closeness of the results obtained from a method to the true reference values. The accuracy of the method was determined by evaluating recovery studies on the pharmaceutical preparation. Three different solutions were prepared; each contained 1 µg/mL pharmaceutical preparation spiked with known concentrations of standard solution of 0.4 µg/mL, 0.8 µg/mL, and 1.2 µg/mL so that the final concentrations were 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 was determined.

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

System suitability test

System suitability was tested 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 shorter retention time were achieved using acetonitrile.

Both the buffer strength and pH were also studied. Although a higher concentration of buffer gave a shorter retention time and better peak shape, it led to an increase in pump pressure; accordingly, 25 mM was selected as the optimum buffer strength. For pH, a value greater than 3.00 showed a broader peak and lower pH did not improve the peak shape. As a consequence, pH 3.00 was chosen as the optimum buffer pH. This could be explained by the fact 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 achieve a short analysis time without increasing the pump pressure.

The optimum wavelength for detecting Gemifloxacin Mesylate was 276 nm using the PDA detector as shown in the spectrum (Figure 2). The flow rate was set at 0.5 mL/min. The optimum temperature for analysis was 30˚C and 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, the peak for Gemifloxacin Mesylate 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 reported in the literature. Accordingly, when compared to other HPLC-UV methods reported in the literature, it provided shorter analysis time and less consumption of solvents.

Next, 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 µg/mL. Using linear regression analysis, the slope, the intercept, and the regression coefficient

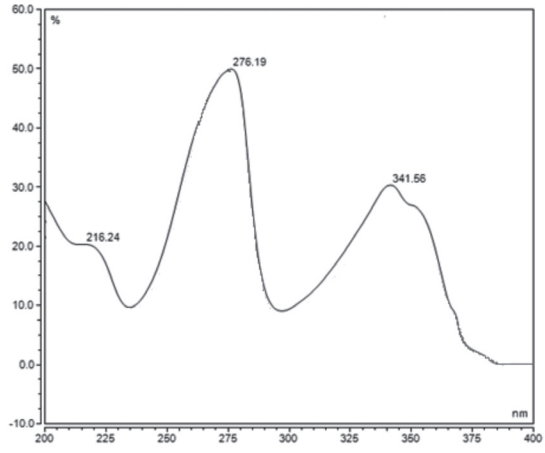

Figure 2. Ultraviolet spectrum of Gemifloxacin Mesylate

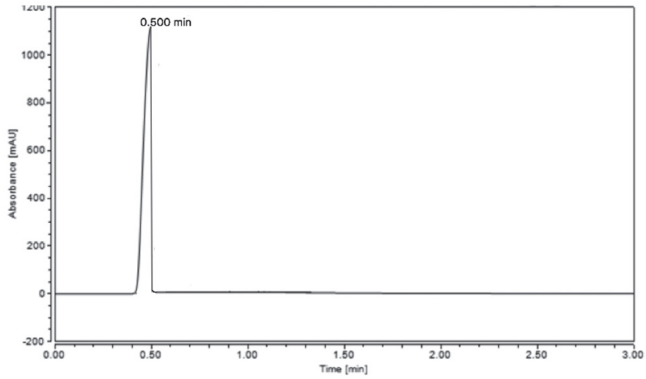

Figure 3. Chromatograms of 1 µg/mL Gemifloxacin Mesylate under the optimum condition, i.e., acetonitrile: 25 mM phosphate buffer, pH 3.00 (75:25; v/v), at a flow rate 0.5 mL/min and T 30°C

were determined from the regression equation y= 396.69x-6.8416. The regression coefficient [R^2] was equal to 0.9991. The slope was 396.69 and the intercept was 6.8416.

The LOD was 0.066 µg/mL, while the LOQ was 0.2 µg/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 are shown in Table 1.

Accuracy

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

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

RSD: Relative standard deviation, *Average of 3 times

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

RSD: Relative standard deviation, *Average of 3 repetitions

Robustness

To evaluate the robustness of the method, minor changes were made to the parameters intentionally. Hence, the % RSD was calculated and the results were as follows: 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 was tested 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 were assessed by the % RSD of both the peak area and the retention time. The data are presented in Table 3.

Table 3. System suitability data of the suggested UPLC method

Parameters	Gemifloxacin Mesylate
Number of theoretical plates (N)	3300
Asymmetric factor (A_s)	1.05
Capacity factor	5.25
% RSD (retention time)*	0.35
% RSD (peak area)*	0.634

UPLC: Ultra-performance liquid chromatography, RSD: Relative standard deviation, *Average of 4 repetitions

Application on pharmaceutical preparation

To determine the suitability of the method for the determination of Gemifloxacin Mesylate in its pharmaceutical preparations, Quinabiotic® 320 mg was purchased from the local market. Next, 10 tablets were weighed and crushed. Hence an equivalent amount of 25 mg was dissolved in 25 mL of 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

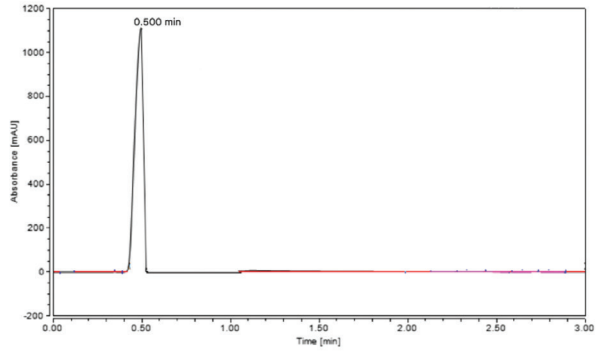

Figure 4. Chromatograms of 1 µg/mL Quinabiotic® under the optimum condition, i.e., acetonitrile: 25 mM phosphate buffer, pH 3.00 (75:25; v/v), at a flow rate 0.5 mL/min and T 30°C

1431. Accordingly, the method proved its ability to determine 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. It was investigated whether there are any significant differences between the two methods and to what extent 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.

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	

SD: Standard deviation

CONCLUSIONS

The newly developed RP-UPLC method for the short time analysis of Gemifloxacin Mesylate in its bulk and pharmaceutical preparation was rapid, simple, accurate, and precise. The method was validated as per ICH guidelines for linearity, accuracy, and precision. Linearity was determined at an acceptable range from 0.5 to 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 a lower retention time in comparison to other HPLC methods reported in the literature.

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

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