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Development and Validation of Reverse Phase High Performance Liquid Chromatography with Fluorescence Detector (RP-HPLC-FL) Method for the Determination of Gemifloxacin in Pharmaceutical Dosage Forms

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ABSTRACT

Gemofloxacin (GEMI) is a potent antibiotic for the treatment of infection caused by gram-positive bacteria, especially streptococcus pneumonia. It is belonging to fluoroquinolone class of antibiotics and is the more active antibacterial agent among the agents in the same class. A reverse phase high performance liquid chromatography has been developed for the determination of GEMI in pharmaceutical dosage form. The separation was carried out using Hichrom packing Kromasil-100-C18 (250 mm×4.6 mm) with particle size of 5 µm and mobile phase consisting of acetonitrile: 10 mM phosphate buffer at pH 3 (15:85) at a flow rate of 1.0 mL/min. GEMI was monitored by fluorescence detector set at λex/λem 268 / 390 nm. The method was statistically validated with respect to linearity, limit of detection (LOD) and limit of quantification (LOQ), precision and accuracy. and the linearity. The method was found to be linear in concentration range of 10-150 ng/mL. The LOD and LOQ were measured to be 2.56 ng/mL and 7.77 ng/mL, respectively. The proposed method was successfully applied for the determination of GEMI in tablets formulation.

Keywords: Gemifloxacin Mesylate; RP-HPLC; Fluorescence Detector; Pharmaceutical Formulations

Introduction

Fluoroquinolones are gaining much interest since their inception in last decades of the previous century. They provide activity against both gram positive and gram-negative organisms by the virtue of their fluorine and piperazine moieties bearing little side effects [1,2]. GEMI is a light brown powder, slight soluble in water, chemically, it is 7-[(4Z)-3(aminomethyl)-4-methoxyimino-pyrrolidin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1, 4dihydro-1, 8-napththyridine-3-carboxylic acid (Figure 1). It is fourth generation FQs antibacterial agent having affinity towards bacterial topoisomerase IV [3]. GEMI was approved by the U.S. Food and Drug Administration for the treatment of the upper respiratory tract infections and demonstrate a broad-spectrum activity against many pathogenic grams negative and gram positive bacteria, including many of the so called atypical respiratory

pathogens [4]. A number of analytical methods have been reported for the determination of GEMI in pharmaceutical formulation and biological samples, including capillary electrophoresis [5,6], ion selective electrodes determination [7,8], spectrophotometric [9-16], spectrofluorometric [9,10,15-21], HPLC with UV detector [22-32], diode-array detector [33] and HPLC with triple quadruple mass detector [34]. HPLC method with Fluorescence detector (HPLC-FL) for the determination of GEMI in spiked human plasma was demonstrated by Al-Hadiya, et al. [35]. In this method a mobile phase consisted mainly of acetonitrile was used, and the limit of detection (LOD) and limit of quantification (LOQ) were found to be 10 and 30 ng/mL, respectively. To best of our knowledge HPLC-FL for the determination of GEMI in pharmaceutical formulation have not been reported. Therefore, this work was devoted to developing an inexpensive, simple, fast, and sensitive HPLC-FL method for the quantification of GEMI in pharmaceutical formulation.

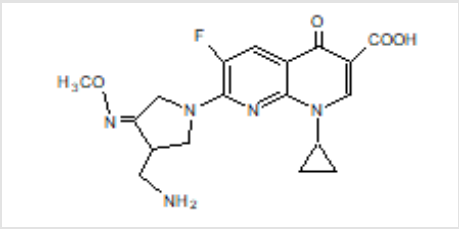

Figure 1: Chemical structure of Gemifloxacin (GEMI).

Experimental

Chemical and Reagent

Gemifloxacin (GEMI) (98%) was obtained from Bayer (AG, Leverkusen Germany); Factive tablets (320 mg GEMI per tablet) LG life science Ltd, Kore lisansiyla Abdilbrahim ilacsan. VeTic.A.S. Maslak /Istanbul 0.3.052007, 210/86. Deionized water was used to prepare all solutions in this study.

Instruments

High Performance Liquid Chromatography System (HPLC) Shimadzu Item LC-20AB, prominence Liquid pumps [Shimadzu-UFLC] DGU-20A5. Degasser prominence, CBM. 20A Communications Bus Module, Sil-20AC. Auto Sampler 20Mpa (max pressure) CTO-20AC Column oven, Fluorescence detector RF-10AXL Shimadzu, LC, solution software. The analytical column used was Hichrom packing Kromasil-100-C18 (250 mm×4.6 mm) with particle size of 5µm. The mobile phase containing 10 mM sodium dihydrogen orthophosphate, adjusted to pH 3 with orthophosphoric acid and acetonitrile in the ratio 85:15. The flow rate was 1.0 mL/min; injection volume 20 µL, column oven temperature was 25°C. The fluorescence detections were carried out at λex/λem 268 / 390 nm.

Preparation of Standard Stock Solution of GEMI

100 µg/mL stock standard solution of the drug was prepared by dissolving 10 mg of GEMI in 1.0 mL of NaOH (0.1 M), transferred into a 100 mL volumetric flask and diluted to the mark with deionized water. 1.0 mL from the stock solution of GEMI (100 µg/mL) was transferred into 100 mL volumetric flask and completed the volume with deionized water. The working solutions were made by dilute aliquot volume with the mobile phase.

Preparation of GEMI Sample Solution

10 tablets (Factive-320 mg of GEMI) were weighed, the average weight was determined, ground into a fine powder using mortar and mixed. An accurately weight of powder equivalent to 0.01g of GEMI was transferred into 100 mL volumetric flask, 1.0 mL of 0.01M sodium hydroxide was added and diluted to the mark with deionized water, to obtain 100 µg/mL of GEMI sample solution. The resulting solution was filtered through 0.22 µm nylon membrane filter and degassed by sonication.

Buffer Solutions

Buffer solution of pH 3.0 was prepared from 10 mM H3PO4 and 10 mM Na_2HPO_4 and adjusted by a pH meter.

Method Validation

The developed method was validated in terms of linearity, limit of detection (LOD) and limit of quantification (LOQ), precisions, and recovery. In order to obtain these validation data, calibration curve were constructed based on peak area obtained at Six different concentrations (10 ng/mL, 30 ng/mL, 50 ng/mL, 70 ng/mL,100 ng/mL and 150 ng/mL) of GEMI standards. LOD is defined as the lowest amount of analyte that can be detected whereas LOQ is defined as the lowest amount of analyte that can be quantified. The LOD and LOQ were calculated based on the approach of ''Standard Deviation of the Response and the Slope'' whereby the

$$LOD\ or\ LOQ = K.SD_a / b$$

where K=3.3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope of the calibration curve. Precision data was quantified by analyzing the peak areas of three replicates of three concentrations levels (10, 60, 150 ng/mL)) within the calibration curves and calculating the RSD. For recovery study, GEMI tablet solution at concentration of 10 ng/mL was spiked with GEMI standard at three concentration level (10, 50, 140 ng/mL).

Results and Discussions

Several systematic trials were performed to optimize the chromatographic conditions for developing a sensitive, precise and accurate HPLC method for simple analysis for GEMI in pharmaceutical dosage forms. In the present method mobile phase mainly consisted of buffer pH 3 and acetonitrile [85:15 (v/v)], was found to be optimum. This mobile phase was found to be suitable sine the as chromatographic peaks obtained were free from tailing. The excitation and emission wavelength of λex/λem 268 /396, were used. Under the above conditions GEMI was eluted in 4.8 min that was shorter than the reported method (6.5 min) [35].

Linearity

The calibration graph was generated using 20 µl injection loop. Six different concentrations of GEMI (10 ng/mL, 30 ng/mL, 50 ng/mL, 70 ng/mL,100 ng/mL and 150 ng/mL) were analyzed according to experimental conditions. Then the calibration curve was established according to the obtained response (peak area) and the concentrations of GEMI in standard solutions. The results show a good linear relationship. The calibration data was summarized in Table 1. The calibration curve and chromatogram of GEMI standard are shown in Figures 2 & 3, respectively.

Table 1: HPLC method parameters for determination of GEMI.

Parameter	Values
Mobile phase	Acetonitrile: phosphate buffer pH 3 15:85
Retention time (min)	4.8
Linear range (ng/mL)	10-150
LOD (ng /mL)	2.56
LOQ (ng /mL)	7.77
Slope	3773.8
Intercept	-5039.9
Correlation coefficient(r)	0.9998

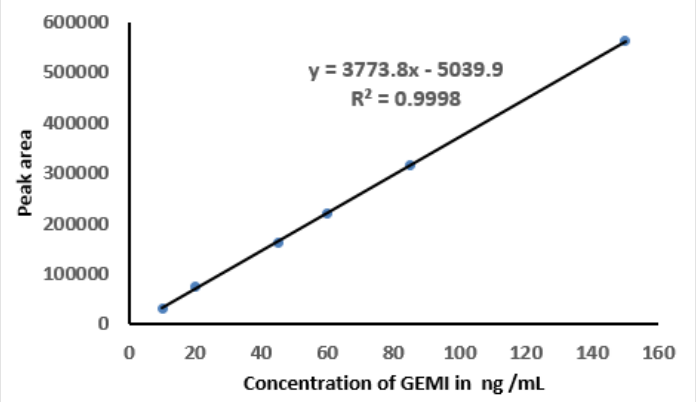

Figure 1: Calibration curve of GEMI.

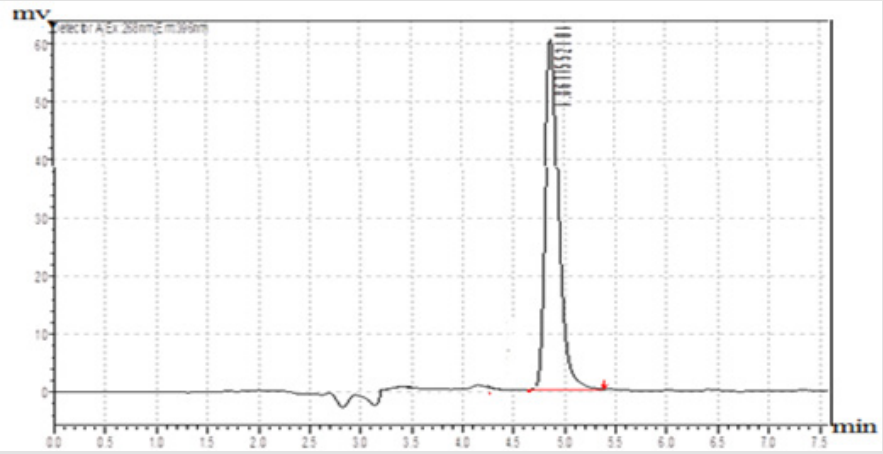

Figure 3: A Chromatogram for standard GEMI (150 ng/mL).

Limits of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were Determined Using the Formula:

$$LOD\ or\ LOQ = K.SD_a / b$$

where K=3.3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope. The values of LOD and LOQ were found to be 2.5 and 7.77 ng/mL respectively. These values are better that the reported in the literature [35] that were found to be 10 and 30 ng /mL for LOD and LOQ, respectively.

Precision for the Determination GEMI

Precision was measured in terms of repeatability of application and measurement. Repeatability was assessed by injecting in triplicate standard of GEMI at three different concentrations within the linear range of the calibration curve, at 10, 60 and 150 ng/mL, Table 2.

Table 2: Precision of the HPLC method.

Amount taken (ng/mL)	Amount Found (ng/mL)	% found ± RSD*
10	9.9	99.1 ±0.99
60	61	101.2 ±2.1
150	148.8	99.2±1.16

Note: *values are mean of 3 determinations.

Recovery for Determination of GEMI

The recovery test was studied by spiking GEMI tablet solution (10 ng/mL) with standard GEMI at three concentration levels (10, 50, 140 ng/mL). The percentage recoveries for method ranged from 98.7 % to 100.6% were obtained as shown in Table 3. These values almost in agreement with reported one [35].

Table 3: Recovery data of standard solutions added to tablet formulation.

GEMI tablet (ng/mL)	GEMI standard added (ng/mL)	Amount Found (ng/mL)	Recovery%±RSD*
10	10	19.7	98.7± 2.03
10	50	60.3	100.6± 0.14
10	140	148.8	99.2± 1.16

Note: *Recovery was calculated as the amount found/amount taken×100%

Values are means ± RSD for 3 determinations.

Analysis of Pharmaceutical Formulation

The proposed method was applied for the analysis GEMI in tablet formulation form. The method showed high accuracy for the determination of the studied drug. Chromatogram for GEMI tablet is shown in Figure 4. The proposed method has advantage of being virtually free from interferences by excipients, Table 4.

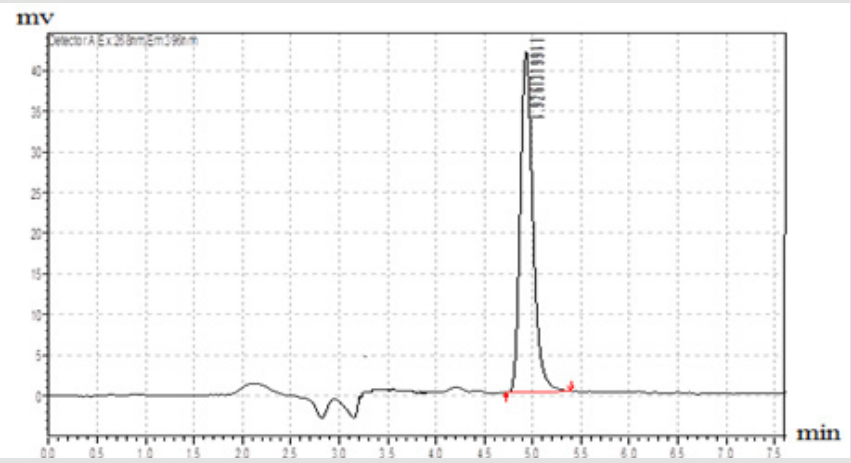

Figure 3: A Chromatogram for GEMI sample (100 ng/mL).

Table 4: Analysis of GEMI in tablet formulation.

Sample content (ng/mL)	Found (ng/mL)	% found ± RSD*
40	41.1	102.8± 0.46
100	102	102.3±0 .49

Note: (*values are means of 3 determinations).

Conclusion

A simple, accurate, precise HPLC method has been developed and validated for the quantification of GEMI in pharmaceutical formulation, according to the International Conference of Harmonization (ICH) guidelines for validation of analytical procedures [36,37]. The proposed HPLC methods have advantages over previously reported method [35] in terms of the simplicity, sensitivity, cost-effect. therefore, the method is practical and valuable for routine application in quality control laboratories for analysis of GEMI.

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