Original Article

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A Liquid Chromatographic Analysis of Gemifloxacin in Pharmaceutical Preparations Using 4-bromomethyl-7-methoxycoumarin Reagent

Gemifloksasinin 4-bromometil-7-metoksikumarin Belirteci Kullanılarak Farmasötik Preparatlarda HPLC ile Analizi

Cem ÖNAL[1,2]

[1]Cinnagen Medicine Company, Project Director, İstanbul, Turkey

[2]İstanbul Health and Technology University Faculty of Pharmacy, Department of Analytical Chemistry, İstanbul, Turkey

ABSTRACT

Objective: In this study, analysis of gemifloxacin in pharmaceutical preparations was performed in the presence of 4-bromomethyl-7-methoxycoumarin reagent and dibenzo-18-crown-6 ether catalyst, by high-performance liquid chromatography.

Methods: The excitation wavelength of the compound formed as a result of the derivatization process was found as λext. =325 nm and the emission wavelength as λem. =390 nm. Optimum reaction conditions were carefully studied. Chromatographic separations were performed in a 150 cm x4.6 mm, 5 µm I.D C18 column, and the mobile phase consisting of acetonitrile: 0.05 M aqueous ammonium acetate (pH=5.0) (70:30, v/v) under flow rate of 1.0 mL/min.

Results: The calibration curve was found to be linear in the range of 10-200 ng.mL^{-1}. Average recovery was 100.32% and relative standard deviation values were below 2%.

Conclusion: The method developed has been successfully applied in the analysis of the drug substance in pharmaceutical preparations.

Keywords: Gemifloxacine, liquid chromatography, fluorometric detection, 4-bromomethyl-7-methoxycoumarin, pharmaceutical preparation, validation

ÖZ

Amaç: Bu çalışmada gemifloksasinin 4-bromometil-7-metoksikumarin belirteci ve dibenz-18-taç-6 eter katalizör varlığında farmasötik preparatlarda yüksek performanslı sıvı kromatografisi ile analizi gerçekleştirilmiştir.

Yöntemler: Türevlendirme işlemi sonucu oluşan bileşiğin eksitasyon dalga boyu λext. =325 nm ve emisyon dalga boyu λem. =390 nm olarak bulunmuştur. Optimum reaksiyon şartları dikkatlice çalışıldı. Kromatografik ayırmalar, 250x4,6 mm, 5 µm I.D C18 kolonda, asetonitril-0,05 M sulu amonyum asetat (pH=5,0) (70:30, v/v) mobil fazında, 1 mL/dak akış hızında gerçekleştirilmiştir.

Bulgular: Kalibrasyon eğrisi 10-200 ng.mL^{-1} aralığında doğrusal bulunmuştur. Ortalama geri kazanım %100,32 ve bağıl standart sapma değerleri %2'nin altında bulunmuştur.

Sonuç: Geliştirilen metod, ilaç maddesin farmasötik preparatlardaki analizine başarıyla uygulanmştır.

Anahtar Sözcükler: Gemifloksasin, sıvı kromatografisi, florimetrik dedeksiyon, 4-bromometil-7-metoksikumarin, farmasötik preparat

Address for Correspondence: Cem ÖNAL, Cinnagen Medicine Company, Project Director, İstanbul, Turkey
E-mail: cemfox@yahoo.com **ORCID ID:** orcid.org/0000-0002-5840-7386

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Introduction

In addition to their gram-negative activity, quinolone antibiotics, which are formed by the addition of fluorine at the six position to the quinolone ring, are also effective in infections with gram-positive bacteria. The chemical formula of gemifloxacin (GMF) is [(R, S) -7- [3-aminomethyl-4-methoximino-1-pyrrolidinyl]-1-cyclopropyl-6-floro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid (1-4) (Figure 1).

Various methods such as spectrophotometric method (5,6), high performance liquid chromatographic method (HPLC) (7-11), voltammetric method (12) and capillary electrophoretic method (13) have been encountered in the literature for determination of GMF. As a result of literature research, no analysis of GMF using 4-bromomethyl-7-methoxycoumarin (BrMmC) reagent was found. HPLC analysis based on fluorescence measurement gains importance in order to increase the selectivity and sensitivity of the method. The BrMmC reagent is often used for derivatization of molecules containing carboxylic acid functional groups (14-16). The aim of this study was to conduct an HPLC analysis based on fluorimetric detection of GMF in pharmaceutical preparations by using 4-bromomethyl-7-methoxycoumarin reagent. This method developed has also been successfully applied in the analysis of pharmaceutical preparations of the drug substance.

Method

Devices

Chromatographic separations were performed with the Shimadzu LC 20A (Kyoto, Japan) liquid chromatographic system. The system consists of LC 20 AT Pump, SIL-20AC autosampler, CTO-10A column oven and RF-10AXL fluorescence detector. The excitation wavelength of the compound formed as a result of the derivatization process was found as λext. = 325 nm and the emission wavelength as λem. =390 nm. Chromatographic separations were performed in a 150 cm x 4.6 mm, 5μm I.D C18 column, and the mobile phase consisting of acetonitrile: 0.05 M aqueous ammonium acetate (pH 5.0) (70:30, v/v) under flow rate of 1.0 mL/min.

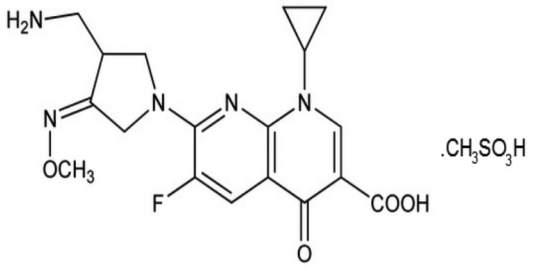

Figure 1. Chemical structure of gemifloxacin mesylate

Reagents and Solutions

The GMF and its pharmaceutical preparation (Factiva Film Tablet® 320 mg of GMF) were taken from Abdi Ibrahim Pharmaceuticals (Turkey), and BrMmC and dibenzyo-18-crown-6 ether catalyst were purchased from Sigma-Aldrich Chemie (Germany). All chemicals and reagents were used for analytical purity.

Stock Solutions

For the GMF stock solution, 133.33 mg of GMF mesylate was weighed exactly, dissolved in water in a 100 mL volumetric flask and made up to its volume (equivalent to 100 mg/mL GMF base). Standard solutions of GMF were taken from this solution and prepared with water. Stock and standard solutions are stable for about 1 week at +4 °C. The standard solution of BrMmC was freshly prepared daily in acetonitrile at a concentration of 100 μg.mL^{-1}. Dibenzo-18-crown-6 ether solution was prepared in acetonitrile to make up a volume of 1 μg.mL^{-1}.

General Analysis Method

Two hundred μL BrMmC, 50 μL dibenz-18-crown-6 ether solutions and 2 mg K_2CO_3 suspension were added to the substance solution (10-200 ng.mL^{-1}) containing different amounts of GMF in 100 μL volumes. The resulting mixture was stirred for 2 minutes and then reacted at 70 °C for 70 minutes. The blank trial was run with 100 μL of water as specified in this section. Volumes reached to 1000 μL with acetonitrile and then they were injected into the HPLC system.

Analysis Method for Tablets

The amount equivalent to 250 mg GMF was weighed and dissolved in 125 mL of water. Then, it was mixed in a mechanical stirrer for 20 minutes and in an ultrasonic bath for 20 minutes, and it was made up to 250 mL in volume and then filtered through filter paper. The filtrate was diluted with water and studied as specified in the "General Analysis Method". The amount of substance in the tablets was measured using the calibration chart and the corresponding regression equation.

Results

Determination of Chromatographic Conditions

Chromatographic conditions were studied in order to develop an HPLC method based on fluorimetric analysis of GMF with BrMmC reagent. For this purpose, columns such as C18, CN and C8 were tried to determine the most suitable column. Optimal conditions were provided with the following parameters: 250x4.6 mm, 5 μm ID C18 column, acetonitrile-0.05 M aqueous ammonium acetate (pH 5.0) (70:30, v/v) mobile phase, flow rate at 1 mL/min, λext. =325 nm wavelength and λem. =390 nm wavelength. The retention time of the GMF derivative was detected as approximately 3 min (Figure 2).

Optimization of Derivation Conditions

In this method developed, GMF was derivatized with 4-bromomethyl-7-methoxycoumarin reagent in the presence

of dibenz-18-crown-6 ether catalyst. In order to determine the optimum conditions, the effect of BrMmC concentration on derivative formation was examined first. It was observed that 200 µL of BrMmC solution (in the presence of 50 µL of Dibenzo-18-crown-6 ether and 2 mg of K_2CO_3 suspension) was sufficient for the derivatization reaction. When the effect of the presence of Dibenzo-18-crown-6 ether on the derivatization reaction was examined, it was observed that the efficiency of the derivative was increased. In order to determine the reaction temperature and reaction time, the reaction mixture was kept at 40, 50, 70 and 80 °C and for different periods. It was found that the most favorable results were obtained at 70 °C for 70 minutes (Figure 3). Conditions are given in Table 1.

Method Validation

The proposed analytical methods were validated according to the ICH guideline Q2 (R1) (17). A calibration curve was generated under the conditions stated above. According to the results obtained, it was observed that the calibration curve was linear in the range of 10-200 ng mL^{-1}. The equation of the measure curve was found as y=185.25 x +2146.2 (r2 = 0.9987), (x concentration is ng mL^{-1} and y is detector response).

The formula of LOD/LOQ = κSDa/b was used to calculate LOD or LOQ. Here the value of κ is 3 for LOD and 10 for LOQ. SDa indicates the standard deviation of the scale curve intersept and b is the slope. The LOD value was 0.0014 ng.mL^{-1} and the LOQ value was 0.0049 ng.mL^{-1}.

The precision values within day and between days were examined at 10, 100, and 200 ng.mL^{-1} for five consecutive days. The inter-day precision was 0.33-0.72% and the between-days precision was 0.48-0.93%. Results are given in Table 2.

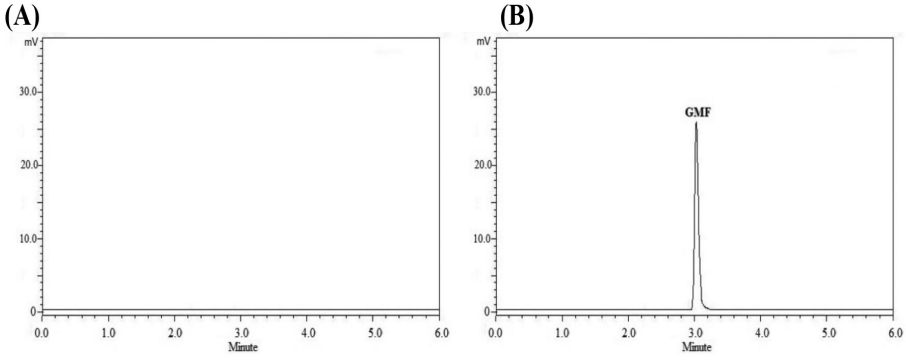

Figure 2. Chromatograms (A) Blank sample (B) GMF added sample (100 ng mL-1)

Table 1. Evaluation of derivatization parameters

Parameter	Range	Optimum value
Derivation time (minutes)	20-100	70
Temperature (°C)	40-80	70
µL of BrMmC	25-500	200
µL of Dibenzo-18-crown-6 ether	10-100	50
mg of K_2CO_3 compound	0.5-5	2

Table 2. Precision values of inter-day and between days

Concentration taken (ng.mL^{-1})	Concentration found (ng.mL^{-1}) ± SD	RSD %*
Intraday		
10.0	10.09±0.046	0.46
100.0	100.14±0.720	0.72
200.0	200.74±0.670	0.33
Between days		
10.0	10.11±0.046	0.67
100.0	100.49±0.930	0.93
200.0	200.98±0.970	0.48

*RSD: Relative standard deviation

The accuracy of the developed methods was examined using the standard addition technique. Standard solutions (10.0, 100.0, 150.0 ng.mL^{-1}) at 3 different concentration levels were added onto the pure analyte sample solution (10 ng.mL^{-1}), mixed and analyzed. The results obtained are presented in Table 2. The calculated average recovery percentage was found to be 100.32% on average. Results are shown in Table 3.

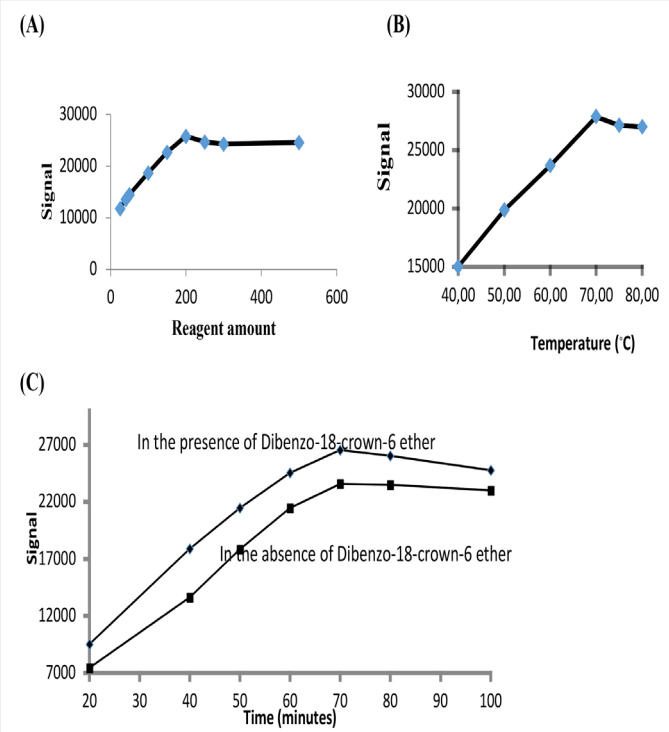

Figure 3. Effects of (A) reagent amount, (B) temperature, (C) reaction time and presence of Dibenzo-18-crown-6 ether on derivatization reaction

The developed method was also successfully applied in the analysis of the drug substance in pharmaceutical preparations and the results were compared with the spectrofluorimetric method recorded in the literature (5). The results were analyzed in terms of means and precision values using the t and f tests. According to these results, no interference was observed from additives and excipients. The results are given in Table 4.

Conclusion

In conclusion, in this study, the 4-bromomethyl-7-methoxycoumarin reagent used in the analysis of substances containing carboxyl groups was studied for the first time in GMF analysis and the developed HPLC analysis was successfully applied in pharmaceutical preparations of the drug substance. In the developed method, the analysis time was short (approximately 3 minutes) and the LOD and LOQ values were 0.0014 ng.mL^{-1} and 0.0049 ng.mL^{-1}, respectively. Since the developed method is more sensitive than other methods recorded in the literature, it is planned to be applied in the analysis of biological fluids in the later stages of the study.

Ethics

Peer-review: Externally peer reviewed.

Financial Disclosure: The authors declared that this study received no financial support.

Table 3. Recovery results

Concentration taken[a] (ng mL^{-1})	Added concentration (ng mL^{-1})	Concentration found[b] (ng.mL^{-1}) (mean ± SD[c])	Recovery (%)	RSD (%)
10.0	10.0	20.06±0.058	100.20	0.19
	100.0	110.16±0.61	99.33	0.80
	150.0	160.97±0.86	100.61	0.53

[a]Factive film tablets® (320 mg)
[b]n=5
[c]Standard deviation
RSD: Relative standard deviation, SD: Standard deviation

Table 4. Analysis of the tablet containing 320 mg GMF (n=5)

Amount stated on tablet[a] (mg/per tablet)	Reference method recovery (%) mean[b] ± SD[c]	RSD (%)	Recommended method recovery (%) mean[b] ± SD[c]	RSD (%)	t value	F value
320	100.15±0.14	0.45	100.18±021	0.67	1.942	1.075

[a]Factive film tablet® (320 mg)
[b]n=5
[c]Standard deviation
At the 95% confidence level, the t value is 2.78 and the F value is 6.39
RSD: Relative standard deviation, SD: Standard deviation

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