Original Article

Ion-pair spectrophotometric estimation of gemifloxacin

Abstract

Introduction: The main objective was to develop and validate a simple, accurate, precise, and sensitive ion-pair spectrophotometric extraction method for the assay of gemifloxacin mesylate (GFX) in pure, tablets and spiked human urine. **Materials and Methods:** The method is based upon the reaction of gemifloxacin with methyl orange, forming a yellow-colored complex in acidic medium, which is extracted in chloroform and analyzed. The extracted complexes showed absorbance maxima (λ_{max}) found to be at 427 nm. **Results:** Beer's law was obeyed for a wide concentration range, i.e., 10–80 μg/mL as the extracted species seemed well defined and stable. The surface or an interphase adsorption phenomenon was not a problem. Optimization of the reaction was carried out with factors such as buffer strength, stability of complex, and molar ratio of drug: Dye and extraction time. The proposed method was validated as per the ICH guidelines. The recovery studies confirmed the accuracy and precision of the method. **Conclusion:** The above-mentioned method was a rapid tool for routine analysis of GFX in the bulk and pharmaceutical dosage forms.

Key words: Gemifloxacin mesylate, ion-pair, spectrophotometry, methyl orange, validation

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INTRODUCTION

Gemifloxacin is chemically known as 7-[(4*E*)-3-(aminomethyl)-4-methoxy iminopyrrolidin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1, 8-naphthyridine-3-carboxylic acid.[1] It is an oral broad-spectrum quinolone antibacterial agent used in the treatment of acute bacterial exacerbation of chronic bronchitis and mild-to-moderate pneumonia.[2,3] 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.[4,5] Gemifloxacin possesses a dual mechanism of action. It inhibits bacterial topoisomerase IV and gyrase enzymes, resulting in interruption of bacterial DNA synthesis,[6,7] this drug is not official in any pharmacopoeia. The literature survey revealed that analytical methods reported for the estimation of gemifloxacin mesylate (GFX) include rapid determination by HPLC–tandem mass spectrometry,[8] microchip electrophoresis in human plasma,[9,10] RP-HPLC and HPTLC,[11] RP-HPLC,[12] in human serum by RP-HPLC,[13] and simple UV spectrophotometric methods[14,15] for tablet formulation. Most of the spectrophotometric methods reported suffer from the disadvantages like narrow range of determination, require heating and long time for the reaction to be completed, use of non-aqueous systems, stability of the colored product formed, etc.

MATERIALS AND METHODS

Instrumentation

All spectral and absorbance measurements were made on a Systronic Model 117 digital spectrophotometer with 10mm matched quartz cells. A Metzer pH

meter and a Contech balance were used in the assay procedure.

Reagents

All chemicals used were of analytical grade and purchased from Qualigens Fine Chemicals, Mumbai, India. Gemifloxacin was obtained as gift from Sigma Aldrich, Mumbai, and double distilled water was used for preparing reagent solutions.

Preparation of acetate buffer

Acetate buffer pH 4 (I.P.-1997) was prepared by dissolving 2.86 mL of glacial acetic acid and 1 mL of 50% w/v solution of sodium hydroxide in a 100 mL volumetric flask, followed by addition of water to make up the volume. The pH was measured by a pH meter, and it was found to be 4.0.

Preparation of methyl orange solution (0.25%)

Methyl orange, 0.25 g, was weighed accurately and dissolved in distilled water, sonicated and the final volume was made up to 100 mL with distilled water.

Urine sample

Drug-free human urine was obtained from a healthy male aged about 28 years.

Standard stock solution

Ten (10) mg of GFX was accurately weighed and transferred into a 100 mL volumetric flask containing 40 mL of acetate buffer (pH 4) and sonicated for 4min. The final volume was made up to 100 mL with buffer in order to get a concentration of 100 μg/mL.

Construction of calibration curve

First, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 mL of standard stock solution were taken in eight different 10 mL volumetric flasks and diluted up to the mark with acetate buffer (pH 4) in order to get 10, 20, 30, 40, 50, 60, 70, and 80 μg/mL of drug concentration, respectively. Then the content of the volumetric flasks and 4 mL of methyl orange solution (0.25%) were transferred into eight different 125 mL separating funnels, and then 15 mL of chloroform were added into each separating funnel and shaken well for 5 min and kept aside for 5 min. The drug was extracted into the chloroform layer, and it was separated into eight different 25 mL volumetric flasks. The organic layer was then passed over anhydrous sodium sulfate, and the maximum absorbance was measured at 427 nm against the reagent blank. The blank solution was prepared by utilizing all the above reagents excluding the drug solution. The calibration curve was plotted using concentration *vs*. absorbance. The linear regression equation was found to be Y=242.5196x + 1.7897 and the correlation coefficient was calculated to be 0.9980. The linearity was observed in the concentration range of 10–80 μg/mL of GFX. The linearity data are given in Table 1. The calibration graph of the gemifloxacin is shown in Figure 1. The UV spectrum of gemifloxacin is shown in the Figure 2.

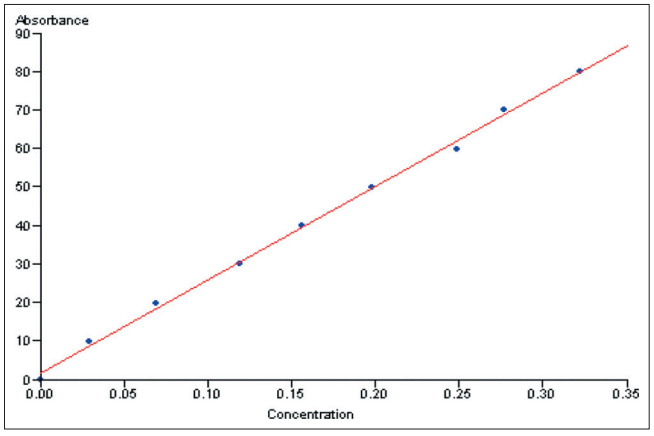

Figure 1: Calibration curve of gemifloxacin

Assay of tablets

Twenty tablets were weighed accurately and ground into a fine powder. An amount of powder equivalent to 10 mg of GFX was weighed into a 100 mL volumetric flask, about 40 mL of freshly prepared acetate buffer pH 4 was added and sonicated thoroughly for about 15 min, then the volume was made up to the mark with the acetate buffer, mixed well, and filtered using Whatman filter paper No. 42 and the first few milliliters of the filtrate were discarded. Then 0.3 mL of filtered tablet sample solutions were transferred into five different 10 mL volumetric flasks, and the

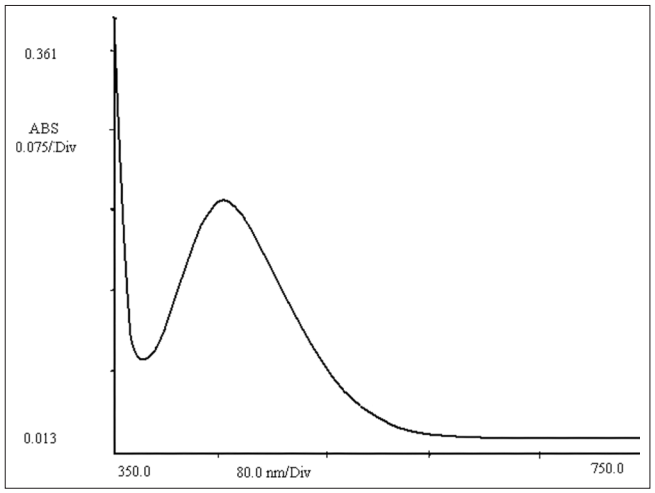

Figure 2: The UV spectrum of gemifloxacin

volume was made up to the mark with the buffer. The contents of the volumetric flasks were transferred into five different 125 mL separating funnels and 4 mL of methyl orange solution was added into each funnel. A 15 mL of chloroform was added into each separating funnel and shaken for 15 min and kept aside for 5 min. The chloroform layers were collected in the volumetric flasks and measured the absorbance at 427 nm. The concentration of the drug was calculated by employing the linear regression equation. The results of tablet analysis are shown in the Table 2.

Procedure for assay in spiked urine (pure drug)

In a 25 mL volumetric flask, 10 mL of urine, 5 mL of acetonitrile, and 10 mL of 30 μg mL^{-1} gemifloxacin solutions [in acetate buffer (pH 4)] were added. The resulting solution was filtered through a Whatman No. 42 filter paper and then transferred into a 125 mL separating funnel. Then, 4 mL of methyl orange solution (0.25%) was transferred into a separating funnel and 15 mL of chloroform were added into the separating funnel and shaken well for 5 min and kept aside for 5 min. The drug was extracted into the chloroform layer, and it was separated into 25 mL volumetric flasks. The organic layer was then passed over anhydrous sodium sulfate, and the maximum absorbance was measured at 427 nm against the reagent blank. The blank solution was prepared by utilizing all the above reagents excluding the drug solution. The concentration of GEM in urine was found by using the linear regression equation. The results are given in the Table 3.

Procedure for assay in spiked urine (formulation, i.e. tablet)

In a 25 mL volumetric flask, 10 mL of urine, 5 mL of acetonitrile, and 10 mL of 30 μg mL^{-1} tablet sample solution [in acetate buffer (pH 4)] were added. The resulting solution was filtered through a Whatman No. 42 filter paper, and then transferred into a 125 mL separating funnel. Then, 4 mL of methyl orange solution (0.25%) was transferred into a separating funnel and 15 mL of chloroform were added into the separating funnel and shaken well for 5 min and kept aside for 5 min. The drug was extracted into the chloroform layer, and it was separated into 25 mL volumetric flasks. The organic layer was then passed over anhydrous sodium sulfate, and the maximum absorbance was measured at 427 nm against the reagent blank. The blank solution was prepared by utilizing all the above reagents excluding the drug solution. The concentration of GEM in urine was found by using the linear regression equation. The results are given in the Table 4.

Table 1: Linearity data of gemifloxacin

Concentration (μg/mL)	Absorbance (427 nm)
0	0
10	0.029
20	0.069
30	0.119
40	0.156
50	0.198
60	0.248
70	0.277
80	0.322

Table 2: Analysis of commercial tablet (Gemez®) (n*=5)**

Formulation (μg/mL)	Label claim (mg/tab)	Amount found (mg/tab)	CI	SD	SE
30	320	321.026	100.320 ± 0.647	0.521	0.233

Validation

It was validated as per the ICH guide lines.[16-18]

Linearity

It was found that the selected drug shows linearity in the range 10–80 μg/mL.

Accuracy

It was found out by a recovery study using the standard addition method. Known amounts of standard gemifloxacin were added to pre-analyzed samples at a level from 80% up to 120% and then subjected to the proposed spectrophotometric method. The results of recovery studies are shown in Table 5.

Precision

Intra-day precision of the assay samples containing gemifloxacin (30 μg/mL) was analyzed at every half an hour interval of time in a day. Precision was calculated

Table 3: The results of pure drug in spiked urine

Pure drug (μg/mL)	Found[a] conc. (μg/mL)	CI	SD	SE
30	30.3175	101.0580 ± 2.306	1.449	0.7245

[a]Mean of four determinations

Table 4: The results of assay in spiked urine (formulation, i.e. tablet)

Formulation (μg/mL)	Label claim (mg/tab)	Amount found (mg/tab)[a]	CI	SD	SE
30	320	321.275	100.397 ± 0.673	0.424	0.212

[a]Mean of four determinations

Table 5: Recovery data of gemifloxacin

Analyte	% Level of recovery	Formulation (µg/mL)	Amount of standard drug added	Amount of standard drug found	CI	SD	SE
Gem	80	30	24	54.31	100.573 ± 1.631	1.025	0.512
	100	30	30	60.59	100.983 ± 2.037	1.280	0.640
	120	30	36	66.545	100.826 ± 2.016	1.265	0.632

SD: Standard deviation; SE: Standard error; CI, Confidence interval within which true value may be found at 95% confidence level=$R \pm ts/\sqrt{n}$; R, Mean percent result of analysis of recovery study (n=4)

as an intra-day coefficient of variation [% CV=(SD/mean) × 100] or % RSD as shown in the Table 6. The color complex was stable for 6 h.

Table 6: Intra-day precision data of gemifloxacin

Analyte	Concentration (µg/mL)	% RSD
Gemifloxacin	30	0.363

Sensitivity

The sensitivity of the method was determined with respect to LOD and LOQ. The LOD and LOQ were separately determined based on the standard calibration curve. LOD=(3.3 × SD/*S*), LOQ=(10 × SD/*S*), where SD is the standard deviation of the *y*-intercept of regression line and *S* is the average slope of the calibration curve. The lower limit of detection and the limit of quantitation were found to be 0.2563 µg/mL and 0.7767 µg/mL, respectively.

RESULTS AND DISCUSSION

In aqueous acidic medium, gemifloxacin reacts with methyl orange, forms a yellow-colored complex, which is extracted in chloroform and analyzed. The method was optimized with the following parameters:

a. *Buffer strength*: Various pH strengths of acetate buffer, i.e., 2.8, 3, 3.4, 3.7, and 4, were tried for the selection of buffer strength. The optimum buffer strength was found to be 4.0.
b. *Reaction time*: The optimization of reaction time was done by measuring the absorbance at an interval of 5min up to 60min. A minimum of 5min time was found to be sufficient to complete the reaction.
c. *Stability of complex*: Stability of the complex was observed, and it remained stable for 6 (six) h.
d. *Molar ratio of drug:dye*: The molar ratio of drug:dye was determined by Job's method and found to be 1:2.

The analytical wavelength for measuring absorption maximum for the gemifloxacin–methyl orange yellow complex was observed at 427 nm against the reagent blank. Absorption maximum at 412 nm observed for the reagent blank under identical experimental conditions was used. The extent of formation of complex is governed by the methyl orange concentration. The solute absorbances were plotted as a function of yellow concentration. The absorbance of the complexes initially increased in the concentration range of (0.02–0.25%) methyl orange and then attained practically a constant value in the concentration range of (0.25–0.28%) methyl orange. Thus, it was found that 0.25% concentration of methyl orange in the range of 3.0–5 mL and acetate buffer were necessary for the achievement of maximum color intensity. Hence 4.0 mL of methyl orange and acetate buffer pH 4 were selected. The effect of temperature on the product was studied at different temperatures. The colored product was stable in the temperature range of 0.0–35°C. At higher temperatures, the drug concentration is increased on prolonged heating due to volatile nature of chloroform. As a result, the absorbance value of the colored products was increased. However, the resultant product was stable for more than 6 h at 25 ± 5°C. The validity of the method for the assay of tablets was determined. The percentage recovery experiments revealed good accuracy of the data. There is no need for the separation of soluble excipients present in marketed tablets as the results were always reproducible equivalent to the labeled contents of the preparations. The recovery results of the proposed method were well agreed with the reported RP-HPLC method for gemifloxacin tablets.[12] The proposed method has been found to be new, accurate, simple, economic, sensitive, precise, and convenient and is suitable for routine analysis in a laboratory. It can be used in the determination of gemifloxacin in bulk drugs and its pharmaceutical preparations in a routine manner. The results were calculated and reported by utilizing the Smiths Statistical Package (SSP) software.

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