Acta Pharmaceutica Sinica B 2011;1(4):248–253

Institute of Materia Medica, Chinese Academy of Medical Sciences
Chinese Pharmaceutical Association

Acta Pharmaceutica Sinica B

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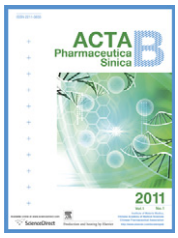

ORIGINAL ARTICLE

Spectrophotometric methods for the determination of gemifloxacin in pharmaceutical formulations

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Received 23 July 2011; revised 25 August 2011; accepted 17 October 2011

KEY WORDS

Gemifloxacin mesylate;
Spectrophotometry;
1,2-Naphthoquinone-4-sulphonate;
Pharmaceutical formulations

Abstract This paper describes two simple spectrophotometric methods for the determination of the antibiotic gemifloxacin mesylate (GFX) in pharmaceutical formulations. The first (A) is an indirect method in which oxidation of the drug with a known excess of cerium (IV) sulphate is followed by determination of the residual oxidant by adding excess methyl orange and measuring residual dye at 507 nm. The second (B) is a derivatisation method involving reaction of GFX with 1,2-naphthoquinone-4-sulphonate (NQS) in alkaline medium (pH 11) to form an orange-coloured product exhibiting maximum absorption (λ_{max}) at 411 nm. The methods were linear in the concentration ranges 2–9 and 5–30 μg/mL for methods A and B, respectively, with intra-day precision (as RSD) $<1.5\%$ for both. When applied to the determination of GFX in pharmaceutical tablets, the results were in good agreement with those obtained by capillary electrophoresis. The two methods are useful for routine analysis of GFX in quality control laboratories.

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Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.
doi:10.1016/j.apsb.2011.10.005

1. Introduction

Over the last twenty years, fluoroquinolones have emerged as one of the most important classes of antibiotics[1]. Gemifloxacin mesylate (GFX) [(*R*,*S*)-7-[(4*Z*)-3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid mesylate] is a fourth generation fluoroquinolone used for the treatment of pneumonia and bronchitis[2]. It is also currently under review by the U.S. Food and Drug Administration for the treatment of upper respiratory tract infections[3].

A number of analytical methods have been reported for the determination of GFX in pharmaceutical dosage forms including capillary electrophoresis[4], reversed phase high performance liquid chromatography (RP-HPLC) with UV and fluorescence detection, liquid chromatography–tandem mass spectrometry (LC–MS/MS), spectrofluorimetry and spectrophotometry[5–10]. The electrophoretic and chromatographic methods require sophisticated and/or expensive instruments and, although spectrofluorimetry is a simple technique, the only reported spectrofluorimetric method[8] involves an extraction step and heating to 80 °C.

Spectrophotometry is probably the most convenient analytical technique for routine analysis because of its inherent simplicity, low cost and wide availability in quality control laboratories. Two spectrophotometric methods have been previously reported for the determination of GFX[9,10]. One was based on the charge transfer complexation reaction of GFX with iodine and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone-7,7,8,8-tetracyanoquinodimethane (TCNQ) and tetracyanoethylene (TCNE)[9], and the other on ion-pair complex formation with safranin O and methylene blue in basic medium or napthol blue 12BR and azocaramine G in acidic medium[10]. The two methods are associated with major drawbacks such as the need for multiple extraction steps in the latter and for GFX free base in the former. In this paper, we report two new spectrophotometric methods for the determination of GFX in pharmaceutical tablets that overcome these drawbacks.

2. Materials and methods

2.1. Instrumentation

Absorbance was measured in 1 cm quartz cuvettes using a double beam UV-1800 ultraviolet–visible spectrophotometer (Shimadzu, Japan) with temperature maintained at 25 °C. pH was determined using a model pH211 pH meter (Hanna, Italy).

2.2. Materials

All chemicals used were of analytical reagent grade. Chemicals (suppliers) were as follows: Cerium (IV) sulphate (Loba-Chemie Indoaustranal Co., India); methyl orange (MO, Fluka Chemika Sigma-Aldrich); sulphuric acid (S. d. Fine Chem, Mumbai, India); sodium 1,2-naphthoquinone-4-sulphonate (NQS) (Aldrich Chemical Co., St. Louis, USA). Doubly distilled water was used to prepare all solutions.

2.3. Reagents

2.3.1. Cerium (IV) sulphate (250 μg/mL)

A 0.01 g/mL cerium (IV) sulphate solution was prepared by dissolving 0.5 g in 50 mL of 1.0 mol/L sulphuric acid. This stock solution was diluted with 1 mol/L sulphuric acid to produce a 250 μg/mL solution.

2.3.2. Methyl orange (50 μg/mL)

A 500 μg/mL solution was prepared by dissolving 50 mg in 100 mL water. After filtration, the solution was diluted 10-fold to obtain 50 μg/mL working solution.

2.3.3. Sulphuric acid (5 mol/L)

This was prepared by adding 274 mL concentrated sulphuric acid to 726 mL water with cooling.

2.3.4. NQS (0.3%, w/v)

This was prepared by adding 150 mg NQS in 50 mL water. The solution was freshly prepared and protected from light during use.

2.3.5. Buffer solution pH 11.0

This was prepared by adding 55 mL 0.2 mol/L NaOH and 35 mL 0.2 mol/L NaH_2PO_4 to 100 mL water and adjusting to pH 11.0. Other buffer solutions were also prepared according to literature methods.

2.4. Preparation of GFX stock and sample solutions

2.4.1. GFX stock solution

A stock solution (1 mg/mL) of GFX was prepared by dissolving 10 mg of pure drug in 10 mL water.

2.4.2. Sample solution

A sample of finely powdered tablet nominally equivalent to 100 mg GFX was dissolved in about 40 mL distilled water in a 100 mL volumetric flask. After shaking for 15 min, the contents were made up to volume with water, filtered (rejecting the first portion of the filtrate) and the filtrate diluted to obtain a suitable concentration for the analysis.

2.5. Assay procedures

2.5.1. Method A

Aliquots of the GFX stock solution were added to 10 mL volumetric flasks to give final concentrations of 2–9 μg/mL. Each flask was added 1 mL of 5 mol/L sulphuric acid and 1 mL of 250 μg/mL cerium (IV) sulphate solution. After mixing, flasks were allowed to stand at room temperature for 10 min with occasional swirling. Finally 1 mL of 50 μg/mL methyl orange solution was added and the solution diluted to the mark with water and mixed. After 5 min, the absorbance of each solution was measured at 507 nm against a reagent blank prepared in the same manner using 1 mL water instead of 1 mL methyl orange solution.

2.5.2. Method B

Aliquots of GFX solution were added to 10 mL volumetric flasks to give final concentrations of 5–30 μg/mL. Buffer solution (pH 11.0, 1 mL) was added followed by 1 mL NQS solution (0.3%, *w*/*v*). The reaction was allowed to proceed at room temperature for 15 min after which the reaction mixture was made up to the mark with water and the absorbance measured at 411 nm against a water blank similarly prepared.

2.6. Assay validation

Calibration curves were prepared and used to calculate the limit of detection (LOD) and limit of quantitation (LOQ) using the formula LOD or LOQ=kSD/b, where k is 3.3 for LOD and 10 for LOQ, SD is the standard deviation of the intercept and b is the slope. Concentrations of GFX in the tablet samples were determined from the calibration curves or from the respective regression equations. The accuracy (as relative error, RE) and intra-day precision (also called repeatability; as relative standard deviation, RSD) of the methods were evaluated by performing five replicate analyses of pure drug solutions at three different concentrations within the working ranges. The inter-day precision (also called reproducibility) was assessed by performing five replicate analyses of pure drug solutions at three concentrations over a period of five days using freshly prepared solutions on each day. The accuracy and precision of the method were further assessed by measuring recovery using powdered tablets spiked with GFX at three different concentrations. Each assay was performed in triplicate.

3. Results and discussion

3.1. Method A

The ability of cerium (IV) sulphate to oxidise GFX and interact with methyl orange is the basis of the indirect spectrophotometric method (A) developed here. In this method, excess cerium (IV) sulphate reacts with GFX in acid, the unreacted oxidising agent reacts with excess methyl orange and the residual methyl orange is determined by measurement of its absorbance at 507 nm. The absorbance was found to increase linearly with increasing concentration of GFX.

3.2. Method B

GFX exhibits maximum absorbance (λ_{max}) at 262 nm. Being in the ultraviolet, absorbance at this wavelength is susceptible to interference from co-extracted excipients in the tablet formulation. Accordingly, derivatization of GFX to produce a chromophore absorbing more in the visible region was appropriate. GFX contains a primary aliphatic amino group, which is suitable for derivatization by NQS, an analytical chromogenic reagent for the determination of primary and secondary amines[11–13]. GFX was found to react instantaneously with NQS under the experimental conditions to form an orange coloured product exhibiting λ_{max} at 411 nm (Fig. 1). Under the optimum reaction conditions, the absorbance was found to obey the Beer–Lambert law.

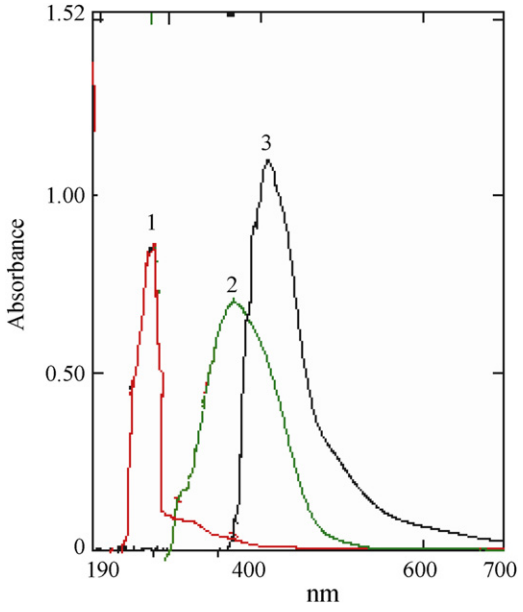


Figure 1 Absorption spectra of GFX (30 μg/mL) against water (1), NQS (0.3%, w/v) against water (2), and the reaction product of GFX (30 μg/mL) with NQS against reagent blank (3).

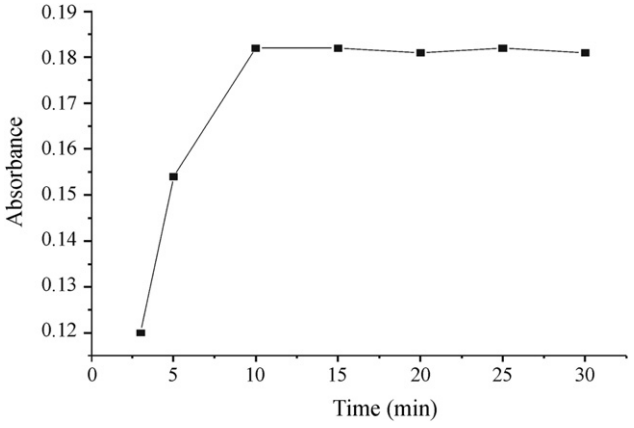


Figure 2 Effect of standing time on the reaction of GFX with $CeSO_4$. GFX (3 μg/mL): 1 mL; H_2SO_4 (5 mol/L): 1 mL; $CeSO_4$ (250 μg/mL): 1 mL; MO (50 μg/mL): 1 mL; temperature: 25 °C.

3.3. Optimisation of reaction variables

3.3.1. Method A

Preliminary experiments showed that the maximum concentration of methyl orange that could be determined spectrophotometrically was 5 μg/mL. A cerium (IV) sulphate concentration of 25 μg/mL was sufficient to extinguish the red colour of this methyl orange solution under acidic conditions. Hence, drug was reacted with 1 mL of 250 μg/mL oxidant solution before determining the residual cerium (IV) sulphate. For quantitative reaction between the drug and cerium (IV) sulphate, a contact time of 10 min was found to be sufficient (Fig. 2). A reaction time of 5 min was sufficient for the reaction between cerium (IV) sulphate and methyl orange after which the absorbance was stable for hours.

3.3.2. Method B

3.3.2.1. Effect of NQS concentration. The reaction was found to be dependent on NQS concentration with the absorbance of the reaction solution increasing as the NQS concentration increased. Maximum absorbance was attained at an NQS concentration of 0.3% (w/v) above which it decreased (Fig. 3).

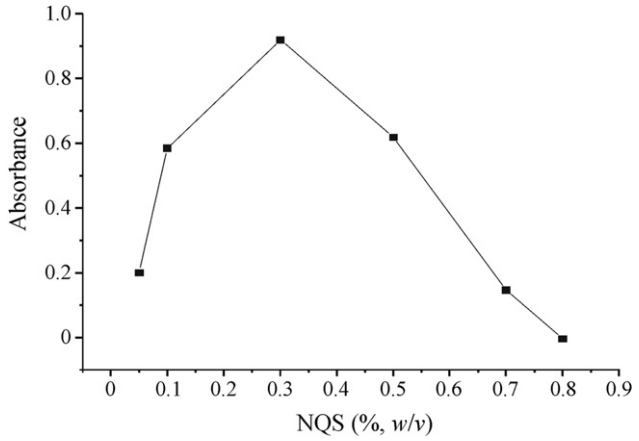


Figure 3 Effect of NQS concentrations on the reaction of GFX with NQS. GFX (30 μg/mL): 1 mL; NQS: 1 mL; buffer solution (pH 11.0): 1 mL; temperature: 25 °C; reaction time: 15 min.

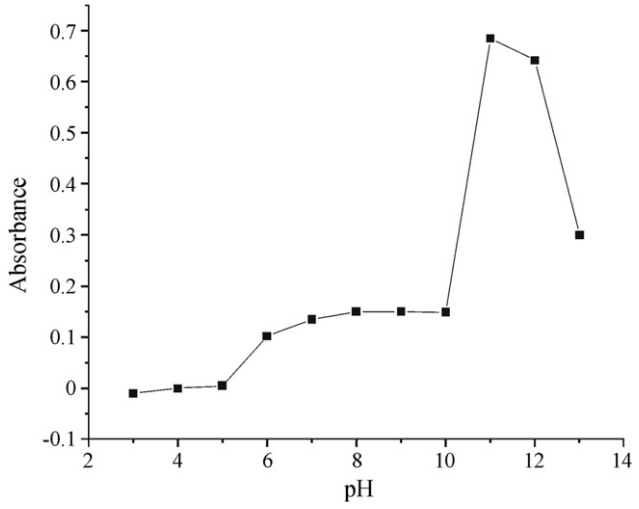


Figure 4 Effect of pH on the reaction of GFX with NQS. GFX (30 μg/mL): 1 mL; buffer solution of different pH values: 1 mL; NQS (0.3%, *w*/*v*): 1 mL; temperature: 25 °C; reaction time: 15 min.

3.3.2.2. Effect of pH. To generate the nucleophile from GFX requires an alkaline medium. It was found that at pH < 6.0 no GFX-NQS product was formed whereas at pH > 6.0 the absorbance due to the product increased rapidly with increasing pH. Maximum absorbance was attained at pH 11.0, and then decreased probably due to competition by hydroxide ion for NQS. On this basis, a pH of 11.0 was selected for the reaction (Fig. 4).

3.3.2.3. Effect of reaction time. By following the reaction for various lengths of time, it was found that the reaction went to completion over 15 min and a longer reaction time was not necessary (Fig. 5).

3.3.2.4. Stoichiometry of the reaction (Job's method). Stoichiometry of the reaction was established by Job's method of continuous variation[14]. Equimolar aqueous solutions of GFX and NQS (5×10^{-3} mol/L) were prepared in 10 mL volumetric flasks containing complementary proportions of the two compounds (0:10, 1:9, …, 9:1, 10:0, inclusive) and 1 mL of pH 11.0 buffer solution. The Job plot of absorption versus mole ratio was symmetrical and indicated that a 1:1 complex (Fig. 6) was formed in the reaction (Scheme 1).

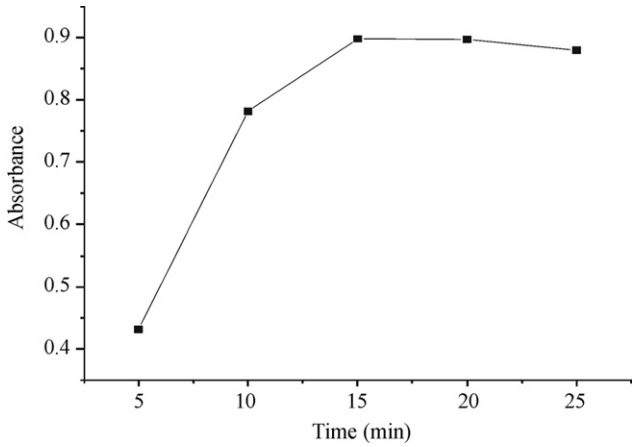


Figure 5 Effect of standing time on the reaction of GFX with NQS. GFX (30 μg/mL): 1 mL; buffer solution (pH 11.0): 1 mL; NQS (0.3%, *w*/*v*): 1 mL; temperature: 25 °C.

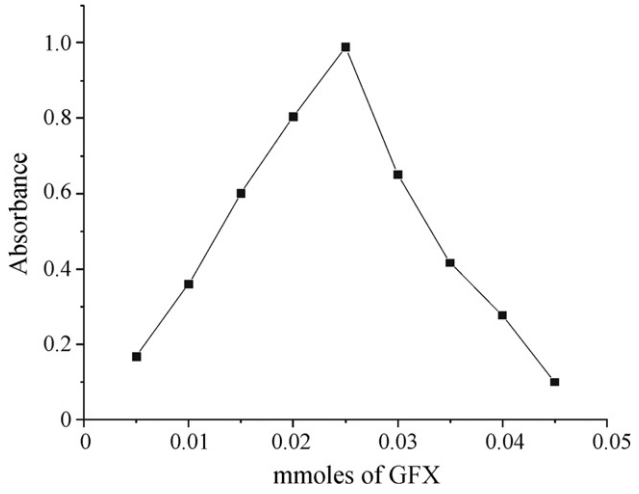
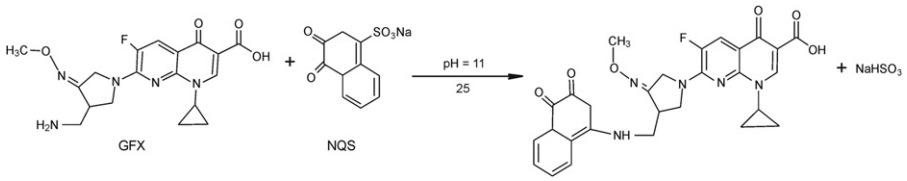


Figure 6 Job's method for NQS with GFX.

3.4. Assay validation

3.4.1. Linearity and sensitivity

Calibration curves for Methods A and B in the ranges 2–9 μg/mL and 5–30 μg/mL were linear with regression equations (correlation coefficients) of $Y = 0.01044 + 0.05199$ ($\pm 7.17881 \times 10^{-4}$) X ($r = 0.9994$) and $Y = -0.00357 + 0.01951$ ($\pm 3.15793 \times 10^{-4}$) X ($r = 0.9995$), respectively. The molar absorptivities (ε) at 507 nm and 411 nm for Methods A and B were 2.14×10^3 and 7.61×10^2 L/mol/cm, respectively. Values of LOD and LOQ were 0.27 and 0.82 μg/mL, respectively, for Method A and 1.04 and 3.15 μg/mL, respectively, for Method B. These parameters for the two methods are summarised in Table 1.



Scheme 1 Proposed reaction pathways between GFX and NQS.

Table 1 Parameters for the performance of the proposed method.

Parameter	Method A	Method B
Measurement wavelength, nm	507	411
Linear range, μg/mL	2–9	5–30
Molar absorptivity, L/mol/cm	2.117×10^4	7.523×10^3
Sandell sensitivity, μg/cm^2	0.018	0.052
Limit of detection, μg/mL	0.27	1.04
Limit of quantification, μg/mL	0.82	3.15
Regression equation, Y[a]		
Intercept (a)	0.01044	−0.00357
Standard deviation of intercept	0.00428	0.00615
Slope (b)	0.05199	0.01951
Standard deviation of slope ($\times 10^{-4}$)	7.17881	3.15793
Correlation coefficient (r)	0.99943	0.99948
Standard deviation	0.00465	0.00661

[a] $Y = a + bX$, where Y is the absorbance, a intercept, b slope and X concentration in μg/mL.

Table 2 Evaluation of accuracy and precision.

Method	Taken	Found	Range	Relative error (%)	SD	RSD (%)
Method A	4.5	4.51	0.010	0.24	0.004	1.44
	5.5	5.49	0.010	0.15	0.004	1.41
	6.5	6.49	0.007	0.02	0.003	0.92
Method B	17	17.04	0.012	0.02	0.005	1.48
	22	22.09	0.009	0.04	0.004	0.87
	27	26.99	0.014	0.03	0.005	1.04

GFX taken/found, range and SD are in μg/mL.
SD, standard deviation; RSD, relative standard deviation.
Mean value of five determinations.

3.4.2. *Accuracy and precision*

Accuracy (RE) was within 0.24% and 0.04% for Methods A and B, respectively, with corresponding intra-day precision (RSD) < 1.5% for the two methods. The results are compiled in Table 2. The inter-day precision (RSD) was < 3.5% reflecting the validity of the method for routine analysis in quality control laboratories.

3.4.3. *Recovery*

The percent recoveries of GFX were in the ranges of 99.4–100.4 and 99.9–100.7 for Methods A and B, respectively (Table 3). This shows the absence of interference from tablet excipients.

3.4.4. *Assay of tablets*

The content of tablets was found to be 99.94 ± 0.82% and 100.05 ± 0.83% of the label claim by Methods A and B, respectively (Table 4). Statistical analysis (t- and F-tests) showed there was no significant difference between these values at the 95% confidence level indicating the two methods have similar accuracy and precision.

4. Conclusions

The present study describes the successful development of two simple spectrophotometric methods for the determination of GFX in tablets that are superior to previously reported

Table 3 Results of recovery study by standard-addition method.

Method	Formulation studied	Amount of drug in formulation (μg)	Amount of pure drug added (μg)	Total found (μg)	% recovery of pure drug[a] ± SD	RSD%	Bias%
Method A	Factive tablets (320 mg)	3	2	4.99	99.84 ± 0.03	0.03	−0.20
		3	3	5.96	99.35 ± 0.02	0.02	−0.67
		3	4	7.03	100.43 ± 0.04	0.04	0.43
Method B	Factive tablets (320 mg)	10	5	14.98	99.89 ± 0.11	0.11	−0.13
		10	10	20.13	100.66 ± 0.07	0.07	0.65
		10	15	25.14	100.56 ± 0.10	0.10	0.56

[a]Mean value of three determinations.

Table 4 Application of the proposed and references methods for the analysis of dosage form containing GFX.

Method	Brand name[a] and dosage form	Label claim (mg/tablet)	Amount found	(% found ± RSD)[b]
Method A	Factive tablets	320	319.81	99.94 ± 0.82 (2.244, 0.288)
Method B	–	–	320.16	100.05 ± 0.83 (1.951, 0.281)
Reference method[4]	–	–	322.75	100.86 ± 0.44

Values in parenthesis are the calculated values of t and F; the tabulated values at 95% confidence limit are 2.776 and 6.26, respectively.
[a]Manufactured by Tabuk Pharmaceutical Mfg. Co., Tabuk, Saudi Arabia.
[b]Values are mean of five determinations.

spectrophotometric methods. All analytical reagents are inexpensive, stable and readily available in any analytical laboratory. The methods do not require complex procedures and are highly suitable for routine use in quality control laboratories.

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