Available online at www.sciencedirect.com
ScienceDirect
Spectrochimica Acta Part A 67 (2007) 1306–1312
SPECTROCHIMICA ACTA PART A
www.elsevier.com/locate/saa

Spectrophotometric determination of gatifloxacin in pure form and in pharmaceutical formulation

Alaa S. Amin [a], Ayman Abou El-Fetouh Gouda [b,*], Ragaa El-Sheikh [b], Faten Zahran [b]

[a] *Chemistry Department, Faculty of Science, Benha University, Benha, Egypt*
[b] *Chemistry Department, Faculty of Science, Zagazig University, Zagazig, Egypt*

Received 19 July 2006; accepted 13 September 2006

Abstract

Simple, rapid, and extractive spectrophotometric methods were developed for the determination of gatifloxacin (GT) in bulk and pharmaceutical dosage form. These methods are based on the formation of yellow ion-pair complexes between the basic nitrogen of the drug and three sulphonphthalein acid dyes, namely; bromocresol green (BCG), bromocresol purple (BCP), bromophenol blue (BPB) and bromothymol blue (BTB) in phthalate buffer pH 3.0, 3.4 and 3.2, using BCG, BCP and (BPB or BTB), respectively. The formed complexes were extracted with chloroform and measured at 415, 417, 412 and 414 nm for BCG, BPB, BCP and BTB, respectively. The analytical parameters and their effects on the reported systems are investigated. The reactions were extremely rapid at room temperature and the absorbance values remains unchanged at 48 h for all reactions. Beer's law was obeyed in the ranges 2.0–20, 2.0–14 and 2.0–16 $\mu g\,mL^{-1}$ for BCG, BCP and (BPB or BTB), respectively. The composition of the ion pairs was found 1:1 by Job's method. Beer's law validation, accuracy, precision, limits of detection, limits of quantification. The proposed methods have been applied successfully for the analysis of the drug bulk form and its dosage form. The results were in good agreement with those obtained by the official and reported methods.

Keywords: Gatifloxacin; Spectrophotometry; Ion pair complex; Acid dyes

1. Introduction

Gatifloxacin (GT) is a fourth-generation synthetic broad-spectrum 8-methoxy fluoroquinolone antibacterial drug derivative. It is (1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid) (Fig. 1) offer several advantages over previous generation antibiotics. It has enhanced in vitro activity against clinically important pathogens and resistant strains (especially penicillin-resistant *Streptococcus pneumoniae*), with better pharmacokinetics.

Gatifloxacin is prescribed for the treatment of acute bacterial exacerbation of chronic bronchitis, acute sinusitis, community-acquired pneumonia, uncomplicated urinary tract infections (cystitis) and complicated urinary tract infections [1]. It acts intravenously by inhibiting topoisomerase II (DNA gyrase) or topoisomerase IV [2].

Gatifloxacin is not official in any pharmacopoeia. A survey of literature, reveals that gatifloxacin has been estimated in plasma by HPLC [3–8], LC–MS [9], HPTLC [10,11], LC [12], Spectrofluorimetric[13], capillary electrochromatography [14] and spectrophotometric [15–18] methods. No extractive spectrophotometric methods are cited in the literature. We report two simple and sensitive spectrophotometric methods for the analysis of gatifloxacin from pharmaceutical dosage forms.

Extractive spectrophotometric procedures are popular for their sensitivity in the assay of drugs; diltiazem HCl [19], maprotiline hydrochloride [20], some antiallergic drugs [21], sparfloxacin [22], ranitidine [23], ofloxacin and lomefloxacin [24], phenylephrine HCl and orphenadrine citrate [25], cetrizine HCl [26], trimethoprim [27], vitamin B_1 [28] and enrofloxacin and penfloxacin [29] and enoxacin [30]; therefore, ion-pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds.

In the present investigation, we report that the development of accurate, reproducible, and adequately sensitive four extractive

* Corresponding author. Tel.: +20 55 242 3346.
E-mail address: aymanchimca@yahoo.com (A.A. El-Fetouh Gouda).

1386-1425/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.saa.2006.09.041

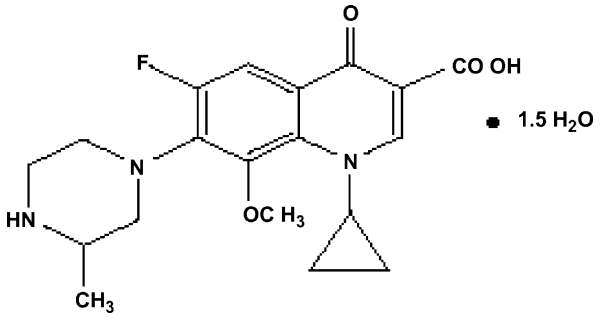

Fig. 1. The chemical structure of Gatifloxacin.

spectrophotometric methods based on the formation of chloroform soluble ion-pair complexes between gatifloxacin with anionic dye namely bromocresol green (BCG), bromocresol purple (BCP), bromophenol blue (BPB) and bromothymol blue (BTB). The proposed methods were applied to the determination of gatifloxacin in pharmaceutical formulation. No interference was observed in the assay of gatifloxacin from common excipients in levels found in pharmaceutical formulation. These methods are validated by the statistical data. The proposed methods are simple and suitable for routine determination of gatifloxacin. Also these methods provide economic procedures, less time consuming, and more sensitivity compared with other reported spectrophotometric methods.

2. Experimental

2.1. Apparatus

All absorption spectra were made using Kontron 930 (UV–visible) spectrophotometer (German) with a scanning speed of 200 nm/min and a band width of 2.0 nm, equipped with 10 mm matched quartz cells. An Orion research model 601A/digital ionalyzer was used for checking the pH of phthalate buffer solutions pH ranges from (2.0–7.0).

2.2. Materials and reagents

All chemicals and reagents were of analytical grade and water was always bidistilled water.

(1) Pure grade gatifloxacin is a sesquihydrate ($C_{19}H_{22}FN_3O_4.1.5\ H_2O$, Mw = 402.42) and its pharmaceutical dosage form (Tequin 400 mg gatifloxacin per capsule) were provided by Bristol Myers Squibb Company Egypt.
(2) A stock solutions (1×10^{-3} M) of gatifloxacin (40.24 mg/100 mL) and (100 μg mL^{-1}) (10 mg/100 mL) were prepared by dissolving pure drug in 100 mL bidistilled water and stored in a dark bottle at 4 °C. Diluted standard solutions were then prepared daily from the stock solution with bidistilled water.
(3) Bromocresol green, bromocresol purple, bromophenol blue or bromothymol blue were used without further purification (BDH Chemicals Ltd., Poole, England).
(4) A stock solution (1.0×10^{-3} M) was prepared by dissolving the appropriate weight of bromocresol green, bromocresol purple bromophenol blue or bromothymol blue in 10 mL 96% ethanol and diluted to 100 mL with bidistilled water.
(5) Series of buffer solutions of KCl–HCl (pH 1.5–4.2), NaOAc–HCl (pH = 1.99–4.92), NaOAc–AcOH (pH = 3.0–5.6) and potassium hydrogen phthalate–HCl (pH = 2.0–7.0) were prepared by following the standard methods [31].
(6) potassium hydrogen phthalate–HCl (pH = 2.0–7.0) was prepared by dissolving 1.020 g potassium hydrogen phthalate in water and completed to 50 mL with water and adjusting pH by addition of 0.1 M hydrochloric acid. Freshly prepared solutions were always employed.

2.3. Construction of calibration curves

Aliquots of (0.2–2.0 mL) the standard drug solutions (100 μg mL^{-1}) were transferred to 10 mL measuring flasks and added 3.0 mL potassium hydrogen phthalate buffers of pH 3.0, 3.4 and 3.2 using BCG, BCP and (BPB or BTB), respectively, then add 3.0 mL of BCG, BCP, BPB or BTB. The mixture was extracted twice with 5.0 mL chloroform by shaking for 2.0 min, then allowed to stand for clear separation of the two phases and the chloroform layer was passed through anhydrous sodium sulphate. The absorbance of the yellow colored complexes were measured at 415, 412, 417 and 414 nm for BCG, BCP, BPB and BTB, respectively, against corresponding reagent blank similarly prepared. All measurements were made at room temperature (25 ± 2 °C). The procedures were repeated for other analyte aliquots and calibration plots were drawn to calculate the amount of drug in unknown analyte samples.

2.4. Procedure for assay of pharmaceutical formulations

The contents of 10 tablets (Tequin 400 mg gatifloxacin per capsule) were weighed, ground into a fine powder and mixed. An accurately weighed portion of the powder equivalent to one tablet was transferred into a 50 mL volumetric flask. The volume was made up to the mark with water. After 30 min of mechanically shaking, the solution was filtrated in a 50 mL calibrated flask through Whatman No. 42 filter paper. Necessary amounts of filtrate were diluted to a 100 mL bidistilled water and the same procedure were applied as described under the procedure for bulk samples.

3. Results and discussion

The nitrogenous drugs are present in positively charged protonated forms and anionic dyes of sulphonpthalein group present mainly in anionic form at pH ≥ 3. So when treated with an acid dye at pH 3.0, 3.4 and 3.2 of potassium hydrogen phthalate buffer using BCG, BCP, (BPB or BTB), respectively, a yellow ion-pair complex which is extracted with chloroform is formed. The absorption spectra of the ion-pair complexes, which were formed between gatifloxacin and each of BCG, BCP, BPB and BTB were measured in the range 350–550 nm

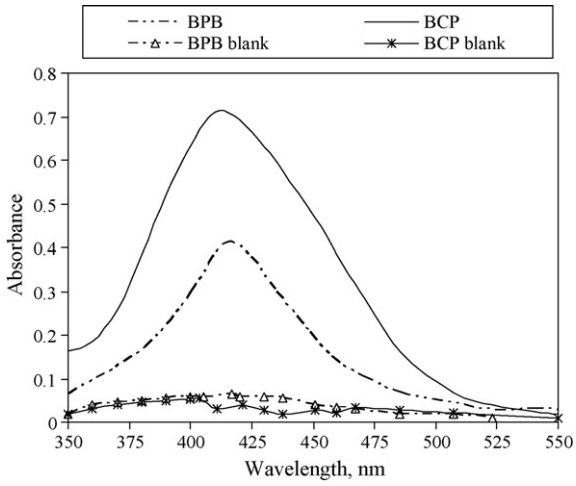

Fig. 2. Absorption spectrum of ion-associate complexes of GT–BPB and GT–BCP [GT] (10 μg mL^{-1}) against reagent blank.

against the blank solution and shown in (Figs. 2 and 3). The ion-pair complexes show maximum absorbance at 415, 412, 417 and 414 nm for BCG, BCP, BPB and BTB, respectively. The optimum reaction conditions for determination of the ion-pair complexes were established. Then linearity, accuracy, precision, sensitivity, and stability of proposed methods were described and these developed methods applied to pharmaceutical preparations as tablets and obtained results evaluated statistically.

3.1. Optimum reaction conditions for complex formation

The optimization of the methods was carefully studied to achieve complete reaction formation, highest sensitivity and maximum absorbance. Reaction conditions of the ion-pair complex were found by studying with preliminary experiments such as pH of buffer, type of organic solvent, volumes of the dye, and shaking time for the extraction of ion-pair complexes.

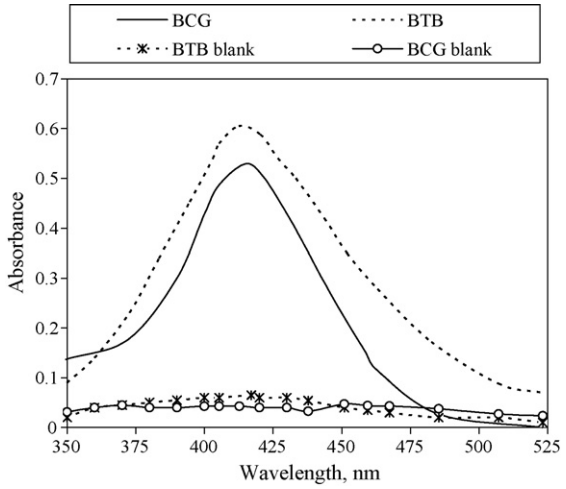

Fig. 3. Absorption spectrum of ion-associate complexes of GT–BCG and GT–BTB [GT] (10 μg mL^{-1}) against reagent blank.

3.1.1. Selecting of the extracting solvents

The effect of several organic solvents viz., chloroform, carbon tetrachloride, ethyl acetate, xylene, diethylether, butyl acetate, toluene, dichloromethane and chlorobenzene were tried for effective extraction of the colored species from aqueous phase. Chloroform was found to be the most suitable solvent for extraction of colored complex for all reagents, yielding maximum absorbance intensity and considerably lower extraction ability for the reagent blank and it was also observed that only double extraction was adequate to achieve a quantitative recovery of the complex and the shortest time to reach the equilibrium between both phases.

3.1.2. Effect of time and temperature

The optimum reaction time was investigated from 0.5 to 4.0 min by following the color development at ambient temperature (25 ± 2 °C). Complete color intensity was attained after 2.0 min of mixing for all complexes (Fig. 4). Raising the temperature up to 30 °C has no effect on the absorbance of the formed complexes, whereas above 30 °C, the absorbance start to decay. The absorbance remains stable for at least 24 h.

3.1.3. Effects of pH on the ion-pair formation

The effect of pH was studied by extracting the colored complexes in the presence of various buffers such as KCl–HCl (pH = 1.5–4.2), NaOAc–HCl (pH = 1.99–4.92), NaOAc–AcOH (pH = 3.0–5.6) and potassium hydrogen phthalate–HCl (pH = 2.0–7.0). It was noticed that the maximum color intensity and highest absorbance value were observed in potassium hydrogen phthalate–HCl buffer of pH 3.0, 3.4 and 3.2 for BCG, BCP and (BPB or BTB) in addition to the stability of the color without affecting the absorbance at pH 3.0, 3.4 and 3.2 for BCG, BCP and (BPB or BTB) methods, respectively (Fig. 5). Further, 3.0 mL potassium hydrogen phthalate buffers gave maximum absorbances and reproducible results.

3.1.4. Effects of reagent concentration

The effect of the reagents were studied by measuring the absorbances of solutions containing a fixed concentration of gatifloxacin (10 μg mL^{-1}) and varied amounts of the respective reagent. Maximum color intensity of the complex was achieved with 3.0 mL of 1 × 10^{-3} M of each dye. Although a larger vol-

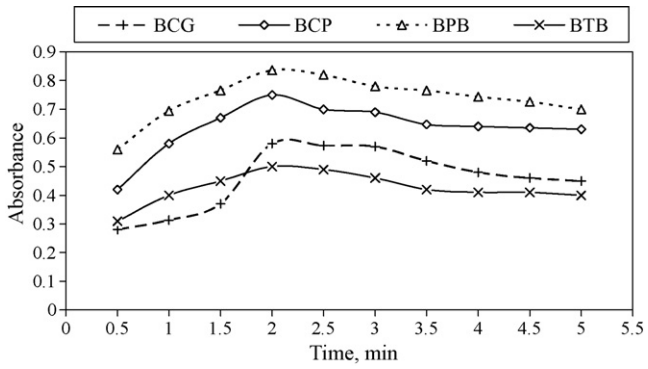

Fig. 4. Effect of shaking time on the ion-pair complexes.

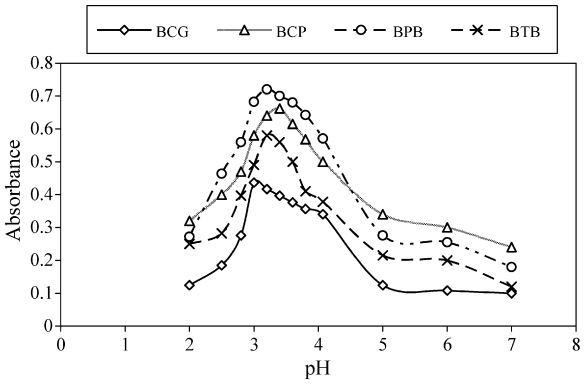

Fig. 5. Effect of pH of potassium hydrogen phthalate buffer solution on the ion-pair complexes.

ume of the reagent had no pronounced effect on the ion-pair complex formation, the absorbances increased slightly due to background of the colored reagent (Fig. 6).

3.1.5. Stoichiometric relationship

Job's method of continuous variation [32] of equimolar solutions was employed: a 1.0×10^{-3} M standard solution of drug base and 1.0×10^{-3} M solution of BCG, BCP BPB and BTB, respectively, were used. A series solutions was prepared in which the total volume of drug and reagent was kept at 10 mL for BCG, BCP BPB and BTB, respectively. The absorbance was measured at 415, 414, 412 and 417 nm for BCG, BPB, BCP and BTB, respectively. The molar ratio of the reagents (drug:dye) in the ion-pair complexes was determined by the method continuous variations (Job's method) (Fig. 7). The results indicate that 1:1 (drug:dye) ion-pairs are formed through the electrostatic attraction between positive protonated GT^+ and negative BCG^-, BCP^-, BPB^- and BTB^-. The extraction equilibrium can be represented as follows:

$$GT^+_{(aq)} + D^-_{(aq)} \leftrightarrow GT^+D^-_{(aq)} \leftrightarrow GT^+D^-_{(org)}$$

where GT^+ and D^- represent the protonated gatifloxacin and the anion of the dye, respectively, and the subcript (aq) and (org) refer to the aqueous and organic phases, respectively.

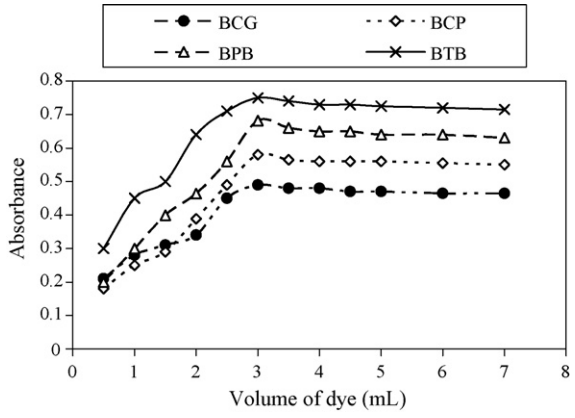

Fig. 6. Effect of reagent concentration on the reaction of (10 μg mL^{-1}) GT with BCG, BCP, BPB and BTB (1.0×10^{-3} M).

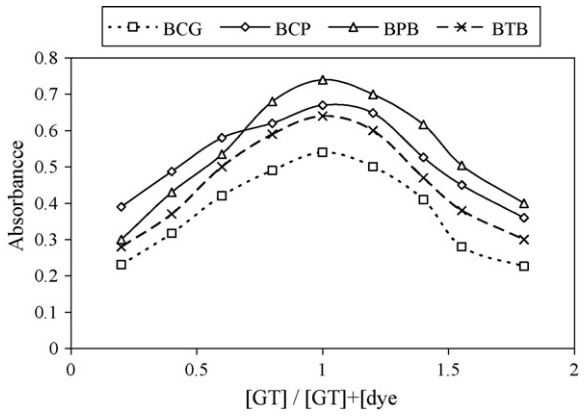

Fig. 7. Job's method of continuous variation graph for the reaction of gatifloxacin with acid-dyes BCG, BCP, BPB and BTB, [drug] = [dye] = 1×10^{-3} M.

3.1.6. Conditional stability constants (K_f) of the ion-pair complexes

The stability of the ion-pair complexes was evaluated. The formation of the ion-pairs were rapid and the yellow color extracts were stable 24 h for gatifloxacin-dye without any change in color intensity and the maximum absorbance at room temperature.

The conditional stability constants (K_f) of the ion-pair complexes for gatifloxacin were calculated from the continuous variation data using the following equation [31]:

$$K_f = \frac{A/A_m}{[1 - A/A_m]^{n+2} C_M (n)^n}$$

where A and A_m are the observed maximum absorbance and the absorbance value when all the drug present is associated, respectively. C_M is the mole concentration of drug at the maximum absorbance and n is the stoichiometry with which dye ion associates with drugs. The log K_f values for GT–BCG, GT–BCP GT–BPB and GT–BTB ion-pair associates were 5.55 ± 0.14, 3.72 ± 0.22, 4.21 ± 0.28 and 2.74 ± 0.34, respectively ($n = 5$).

3.2. Method validation

3.2.1. Linearity

At described experimental conditions for gatifloxacin determination, standard calibration curves for GT with BCG, BCP, BPB and BTB calibrations were constructed by plotting absorbances versus concentrations. The linear regression equations, standard deviation, slopes and intercepts, correlation coefficients, relative standard deviation of response factors, and linearity ranges were given in (Table 1) for each proposed spectrophotometric method. The molar absorptivities of each methods was calculated and these values showed that the molar absorptivity of BCG > BPB > BTB > BCP ion-pair complexes.

3.2.2. Sensitivity

The detection limit (LOD) for the proposed methods were calculated using the following equation [33]:

$$\mathrm{LOD} = \frac{3s}{k}$$

Table 1
Statistical analysis of calibration graphs and analytical data in the determination of gatifloxacin by BCG, BCP, BPB and BTB methods ($n = 6$)

Parameters	Proposed methods			
	BCG	BCP	BPB	BTB
Wavelengths λ_{max} (nm)	415	412	417	414
pH	3.0	3.4	3.2	3.2
Regression equation[a]				
Slope (b)	0.0923	0.0319	0.126	0.0256
Intercept (a)	−0.0043	−0.00585	−0.0091	0.0019
Correlation coefficient (r)	0.9997	0.9998	0.9994	0.9985
Beer's law limits ($\mu g\,mL^{-1}$)	2.0–20	2.0–14	2.0–16	2.0–16
Ringboom limits ($\mu g\,mL^{-1}$)	3.0–17.5	3.0–13	3.0–14.5	3.5–14
Molar absorptivity ε, ($L/mol^{-1}\,cm^{-1} \times 10^4$)	3.8267	1.683	3.7786	2.852
Sandell, s sensitivity ($ng\,cm^{-2}$)	10.52	23.91	10.65	14.11
LOD ($\mu g\,mL^{-1}$)	0.285	0.252	0.312	0.232
LOQ ($\mu g\,mL^{-1}$)	0.950	0.840	1.03	0.773
R.S.D.%	0.773	1.3655	0.5456	0.66
RE%	0.8117	1.4332	0.5727	1.26

LOD, limit of detection; LOQ, limit of quantification; ε, molar absorptivity coefficient.
[a] $A = a + bC$, where A is the absorbance, a is the intercept, b is the slope and C is the concentrationof drug in $\mu g\,mL^{-1}$.

where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits obtained for the absorbance were found to be $0.285\,\mu g\,mL^{-1}$ for GT–BCG, $0.252\,\mu g\,mL^{-1}$ for GT–BCP, $0.312\,\mu g\,mL^{-1}$ GT–BPB and $0.232\,\mu g\,mL^{-1}$ GT–BTB methods.

The limits of quantitation, LOQ, defined as [33];

$$\mathrm{LOQ} = \frac{10s}{k}$$

According to this equation, the limit of quantitation were found to be $0.950\,\mu g\,mL^{-1}$ for GT–BCG, $0.840\,\mu g\,mL^{-1}$ for GT–BCP, $1.03\,\mu g\,mL^{-1}$ GT–BPB and $0.773\,\mu g\,mL^{-1}$ GT–BTB methods.

3.2.3. Specificity, precision, and accuracy

Specificity of ion-pair reaction and selective determination of GT which was the basic nitrogenous compounds with sulphonphthalein dyes could be possible. Percentage relative standard deviation (R.S.D.%) as precision and percentage relative error (Er%) as accuracy of the suggested method were calculated. Precision was carried out by six determinations at four different concentrations in these spectrophotometric methods. The percentage relative error calculated using the following equation:

$$\mathrm{Er}\% = \left[\frac{\text{founded} - \text{added}}{\text{added}}\right] \times 100$$

The inter-day and intra-day precision and accuracy results are shown in (Table 2). These results of accuracy and precision show that the proposed methods have good repeatability and reproducibility.

3.2.4. Robustness and ruggedness

For the evaluation of the method robustness, some parameters were interchanged; pH, dye concentration, wavelength range, and shaking time. The capacity remain unaffected by small deliberate variations. Method ruggedness was expressed as R.S.D.% of the same procedure applied by two analysts and in two different instruments on different days. The results showed no statistical differences between different analysts and instruments suggesting that the developed methods were robust and rugged (Table 3).

3.3. Effects of interference

To assess the usefulness of the method, the effect of diluents, excipients and additives which often accompany GT in its dosage forms (starch, lactose, glucose, sugar, talc, sodium chloride, titanium dioxide, and magnesium stearate) was studied. The results indicated that there is no interference from the degradation, indicating a high selectivity for determining the studied GT in its dosage forms.

3.4. Analysis of pharmaceutical preparations

The proposed methods have been successfully applied to the determination of GT in commercial tablet. The results obtained are shown in (Table 4). The four suggested methods were applied successfully to the determination of GT in commercial tablet. Six replicate determinations were made. (Table 5) shows that satisfactory recovery data were obtained and the assay results were in a good agreement with the label claims. Moreover, to check the validity of the proposed methods, dosage form (Tequin 400 mg per tablet) were tested for possible interference with standard addition method. There was no significant difference between slopes of calibration curves and standard addition methods at four methods. Therefore it is concluded that the excipients in pharmaceutical dosage forms of GT such as starch, lactose, talc,

Table 2
The intra-day and inter-day precision and accuracy data for gatifloxacin obtained by the proposed methods ($n = 6$)

Method	Intra-day					Inter-day			
	Added ($\mu g\,mL^{-1}$)	Recovery (%)	Precision R.S.D.%[a]	Accuracy Er%	Confidence limit[b]	Recovery (%)	Precision R.S.D.%[a]	Accuracy Er%	Confidence limit[b]
BCG	4.0	100.1	0.84	0.1	4.004 ± 0.0353	100.75	0.46	0.75	4.03 ± 0.0193
	8.0	99.57	0.92	−0.425	7.966 ± 0.077	100.625	0.83	0.625	8.05 ± 0.070
	12	99.95	0.77	−0.05	11.994 ± 0.097	99.17	0.64	−0.833	11.90 ± 0.080
	16	100.2	1.08	0.2	16.032 ± 0.182	99.38	0.98	−0.625	15.90 ± 0.164
BCP	4.0	99.72	0.99	−0.275	3.989 ± 0.041	99.20	0.76	−0.80	3.968 ± 0.032
	8.0	99.15	0.85	−0.85	7.932 ± 0.071	100.2	0.57	0.2	8.016 ± 0.048
	12	98.94	1.12	−1.06	11.873 ± 0.140	99.90	0.66	−0.1	11.988 ± 0.083
	16	99.45	0.88	−0.55	15.912 ± 0.147	100.31	0.39	0.313	16.05 ± 0.0657
BPB	3.0	100.12	0.76	0.133	3.004 ± 0.024	99.70	1.08	−0.3	2.991 ± 0.0339
	6.0	99.85	0.79	−0.15	5.991 ± 0.050	98.92	0.54	−1.083	5.935 ± 0.0336
	9	99.92	0.81	−0.078	8.993 ± 0.076	99.30	0.45	−0.70	8.937 ± 0.0422
	12	100.15	1.23	0.15	12.018 ± 0.155	100.10	0.61	0.10	12.012 ± 0.0769
BTB	3.0	99.67	0.64	−0.33	2.99 ± 0.020	100.67	0.57	0.67	3.02 ± 0.0181
	6.0	101.17	0.58	1.17	6.07 ± 0.037	100.50	0.72	0.50	6.03 ± 0.0456
	9	100.11	0.73	0.11	9.01 ± 0.069	99.78	0.69	−0.22	8.98 ± 0.0650
	12	99.83	0.88	−0.17	11.98 ± 0.111	100.08	0.94	0.083	12.01 ± 0.1185

n, number of determination, R.S.D.%, percentage relative standard deviation; Er%, percentage relative error.
[a] Mean of five determination.
[b] Confidence limit at 95% confidence level and five degrees of freedom ($t = 2.571$).

Table 3
The results of analysis from pharmaceutical preparation and standard of gatifloxacin by two different analysts and instruments ($n = 6$)

	Different instrument			Different analyst		
	X	±S.D.	R.S.D.%	X	±S.D.	R.S.D.%
Gatifloxacin-BCG						
Pure gatifloxacin ($10\,\mu g\,mL^{-1}$)	9.98	0.17	1.703	10.02	0.24	2.30
Tequin (400 mg gatifloxacin per capsule)	397	0.867	0.218	399	0.975	0.195
Gatifloxacin-BCP						
Pure gatifloxacin ($8.0\,\mu g\,mL^{-1}$)	8.01	0.11	1.373	7.97	0.21	2.635
Tequin (400 mg gatifloxacin per capsule)	402	1.18	0.294	398.5	1.35	0.271
Gatifloxacin-BPB						
Pure gatifloxacin ($8.0\,\mu g\,mL^{-1}$)	7.96	0.095	1.193	7.99	0.19	2.378
Tequin (400 mg gatifloxacin per capsule)	399	0.822	0.206	394	1.448	0.293
Gatifloxacin-BTB						
Pure gatifloxacin ($8.0\,\mu g\,mL^{-1}$)	7.98	0.13	1.63	8.02	0.10	1.25
Tequin (400 mg gatifloxacin per capsule)	398	0.98	0.246	4.01	0.77	0.185

Table 4
Determination of gatifloxacin in pharmaceutical dosage form

Sample	Nominal value (mg)	Official HPLC method ($n = 5$)	Proposed methods		Recovery[a] ± S.D.%	
			BCG	BCP	BPB	BTB
Tequin capsules	400	99.60 ± 0.73	99.82 ± 0.76	99.25 ± 0.94	99.90 ± 0.86	99.95 ± 0.81
t			0.51	0.72	0.65	0.93
F			1.08	1.66	1.39	1.23

The theoretical values of t and F at $P = 0.05$ are 2.31 and 6.39, respectively.
[a] Average of six determinations.

Table 5
Determination of gatifloxacin in it is pharmaceutical dosage form applying the standard addition technique

Dosage forms	Taken ($\mu g\,mL^{-1}$)	Added ($\mu g\,mL^{-1}$)	Proposed methods		Recovery[a] (%)	
			BCG	BTB	BPB	BTB
Tequin capsules	5.0	3.0	99.86	99.50	99.80	99.54
		6.0	100.05	100.20	99.65	100.40
		9.0	99.80	99.80	100.15	100.30

[a] Average of six determinations.

stearic acid, titan dioxide, yellow iron oxide were not found any interference in the analysis of GT. At 95% confidence level the calculated *F*-value did not exceed the theoretical *F*-value indicating no significant difference between the four proposed methods and the reference HPLC method (Table 4).

4. Conclusion

Unlike the gas chromatographic and HPLC procedures, the spectrophotometer is simple and is not of high cost. The importance lies in the chemical reactions upon which the procedures are based rather than upon the sophistication of the instrument. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibility in the assay of a particular component in complex dosage formulations. The reagents utilized in the proposed methods are cheaper, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. The method is unaffected by slight variations in experimental conditions such as pH and reagent concentration. Moreover, the methods are accurate, reproducible, adequately sensitive and free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control is well established by the assay of gatifloxacin in pure form and in pharmaceutical preparations.

References

[1] S. Budavari (Ed.), The Merck Index, 13th ed., Merck and Co. Inc., Whitehouse Station, NJ, 2001, p. 777.
[2] C.M. Perry, B.J.A. Barman, H.M. Lamb, Drugs 58 (1999) 683.
[3] L. Sivasubramanian, A. Muthukumaran, Ind. J. Pharm. Sci. 67 (3) (2005) 367.
[4] S. Al-Dgither, S.N. Alvi, M.M. Hammami, J. Pharm. Biomed. Anal. 41 (1) (2006) 251.
[5] M.I. Santoro, N.M. Kassab, A.K. Singh, E.R. Kedor-Hackmam, J. Pharm. Biomed. Anal. 40 (1) (2006) 179.
[6] B.R. Overholser, M.B. Kays, K.M. Sowinski, J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci. 798 (1) (2003) 167.
[7] H.R. Salgado, C.C. Lopes, J. AOAC. Int. 89 (3) (2006) 642.
[8] H. Liang, M.B. Kays, K.M. Sowinski, J. Chromatogr. B. Anal. Technol. Biomed. Life Sci. 772 (2002) 53.
[9] K. Vishwanathan, M.G. Bartlett, J.T. Stewart, Rapid. Commun. Mass Spectrom. 15 (2001) 915.
[10] S.A. Shah, I.S. Rathod, B.N. Suhagia, M. Baldaniya, Ind. J. Pharm. Sci. 66 (3) (2004) 306.
[11] B.N. Suhagia, S.A. Shah, I.S. Rathod, H.M. Patel, D.R. Shah, B.P. Marolia, Anal. Sci. 22 (5) (2006) 743.
[12] H.A. Nguyen, J. Grellet, B.B. Ba, C. Quentin, M.C. Saux, J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci. 810 (1) (2004) 77.
[13] J.A. Ocana, F.J. Barragan, M. Callejon, J. Pharm. Biomed. Anal. 37 (2) (2005) 327.
[14] H. Lu, X. Wu, Z. Xie, X. Lin, L. Guo, C. Yan, G. Chen, J. Sep. Sci. (16) (2005) 2210.
[15] P.U. Patel, B.N. Suhagia, C.N. Patel, M.M. Patel, G.C. Patel, G.M. Patel, Ind. J. Pharm. Sci. 67 (3) (2005) 356.
[16] K. Ilango, P. Valentina, K.S. Lakshmi, C. Arvind, A.S. Rachel, R.V. Bhaskar, K.A. Kiran, Ind. J. Pharm. Sci. 68 (2) (2006) 273–275.
[17] K. Venugopal, R.N. Saha, IL Farmaco 60 (11–12) (2005) 906.
[18] H.R. Salgado, C.L. Oliveira, Pharmazie 60 (4) (2005) 263.
[19] N. Rahman, S.N. Hejaz-Azmi, J. Pharm. Biomed. Anal. 24 (2000) 33.
[20] A. Özkul, A. Öztunç, Pharmazie 55 (2000) 321.
[21] K.C. Ramesh, B.G. Gowda, M.B. Melwanki, J. Seetharamappa, J. Keshavayya, Anal. Sci. 17 (2001) 1101.
[22] H.R. Marona, E.E. Schapoval, J. Pharm. Biomed. Anal. 26 (2001) 501.
[23] T. Perez-Ruiz, C. Martinez-Lozano, V. Tomas, A. Sanz, E. Sahuquillo, J. Pharm. Biomed. Anal. 26 (2001) 609.
[24] Y.M. Issa, F.M. Abdel-Gawad, M.A. Abou Table, H.M. Hussein, Anal. Lett. 30 (1997) 2071.
[25] S.A. Shama, J. Pharm. Biomed. Anal. 30 (2002) 1385.
[26] B.G. Gowda, M.B. Melwanki, J. Seetharamappa, J. Pharm. Biomed. Anal. 25 (2001) 1021.
[27] A.L. EI-Ansary, Y.M. Issa, W. Selim, Anal. Lett. 32 (1999) 955.
[28] S. Liu, Z. Zhang, Q. Liu, H. Luo, W. Zheng, J. Pharm. Biomed. Anal. 30 (2002) 685.
[29] S. Mostafa, M. EI-Sadek, E.A. Alla, J. Pharm. Biomed. Anal. 28 (2002) 173.
[30] İ. Süslü, A. Tamer, J. Pharm. Biomed. Anal. 29 (2002) 545.
[31] H.T.S. Britton, Hydrogen Ions, fourth ed., Chapman and Hall, 1952.
[32] J. Inczedy, Analytical Applications of Complex Equilibria, Ellis Horwood Ltd., England, 1976, p. 137.
[33] J.C. Miller, J.N. Miller, Significance tests. In Statistics in Analytical Chemistry, third ed., Ellis Horwood, Chichester, 1993 (Chapter 3).