Utility of some π-Acceptors for Spectrophotometric Determination of Gatifloxacin in Pure Form and in Pharmaceutical Preparations

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Four simple, quick and sensitive methods are described for the spectrophotometric determination of gatifloxacin. The methods are based on the reaction of gatifloxacin as n-electron donor with 7,7,8,8-tetracyanoquinodimethane (TCNQ); 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ); chloranilic acid (CLA) and *p*-chloranil (CL) as π-acceptors to give highly colored complex species. The colored products are quantitated spectrophotometrically at 460, 841, 530 and 545 nm for DDQ, TCNQ, CLA and CL, respectively. Optimization of the different experimental conditions is described. Beer's law is obeyed in the concentration ranges 5—60, 1.5—18, 30—360 and 20—240 $\mu g\,ml^{-1}$ of gatifloxacin, but for more accurate analysis, Ringbom optimum concentration range was found to be 7.5—55, 3—16, 35—350 and 25—230 $\mu g\,ml^{-1}$ of gatifloxacin for DDQ, TCNQ, CLA and CL, respectively. The limits of detection and quantification were calculated and the relative standard deviations for different concentrations of gatifloxacin using various acceptors were <1.28%. The association constants of 1 : 1 complexes and standard free energy changes using Benesi–Hildebrand plots were studied. The proposed methods were successfully applied to the determination of gatifloxacin in pharmaceutical dosage forms without interference from common additives encountered.

Key words gatifloxacin; charge transfer complex; spectrophotometry; pharmaceutical formulation

Gatifloxacin (GT) (1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid), (Fig. 1) is a fourth-generation a synthetic broad-spectrum 8-methoxy fluoroquinolone antibacterial drug derivative. Gatifloxacin offers 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 indicated 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]

A literature survey revealed that gatifloxacin has been estimated in plasma by non-aqueous titration using perchloric acid,[3] chromatographic,[4—13] spectrofluorimetric,[14] capillary electrochromatography,[15] electrochemical[16] and spectrophotometric[17—24] methods. No charge transfer spectrophotometric methods are cited in the literature. We report four simple and sensitive charge transfer spectrophotometric methods for the analysis of gatifloxacin from pharmaceutical dosage forms.

The molecular interactions between electron donors and acceptors are generally associated with the formation of intensely colored charge transfer complexes that absorb radiation in the visible region.[25] The photometric methods based on these interactions are usually simple and convenient because of the rapid formation of the complexes. Gatifloxacin is a good n-electron donor and will form charge transfer complexes with π-acceptors.

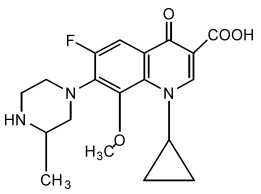

Fig. 1. Chemical Structure of Gatifloxacin

π-Acceptors such as 7,7,8,8-tetracyanoquinodimethane (TCNQ); 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ); chloranilic acid (CLA) and *p*-chloranil (CL) are known to yield charge transfer complexes and radical anions with a variety of electron donors.[26—36] This donor–acceptor interaction has been investigated with gatifloxacin as electron donor.

The present study describes direct, simpler, more sensitive and precise spectrophotometric methods than the existing UV and HPLC methods that are free from such experimental variables as extraction step, for the determination of gatifloxacin *via* charge transfer complex formation with the π-acceptors 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ); 7,7,8,8-tetracyanoquinodimethane (TCNQ); chloranilic acid (CLA); and *p*-chloranil (CL) in pharmaceutical formulations. No interference was observed in the assay of gatifloxacin from common excipients in levels found in pharmaceutical formulations. These methods rely on the use of simple and cheap chemicals and techniques but provide a sensitivity comparable to that achieved by sophisticated and expensive techniques such as HPLC and are validated by statistical data. The reaction conditions and application of the methods to determination of gatifloxacin in tablets have been established. In addition, the association constant, the stochiometric ratio of reactants and the standard free energy changes ($\Delta G°$) were determined.

Experimental

Apparatus All absorption spectra were made using Kontron 930 (UV–Visible) spectrophotometer (German) equipped with 10 mm matched quartz cells.

Materials and Reagents All reagents and solvents used were of analytical-reagent grade.

Pure grade gatifloxacin reference standard was provided by Bristol Myers

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Squibb Company Egypt, its potency was 99.60±0.70% by HPLC method.[7)]

The following commercial formulations were subjected to the analytical procedures: Tequin tablets (Bristol Myers Squibb Company, Egypt) labeled to contain 400 mg GTF/tablet. Floxin tablets (Global Napi Co, Egypt) labeled to contain 400 mg GTF/tablet. Gatilox tablets (EPCI, Egypt) labeled to contain 400 mg GTF/tablet.

Stock solutions of gatifloxacin were prepared by dissolving 50, 100 and 150 mg in 5.0 ml methanol and the volume was diluted to the mark in a 100-ml calibrated flask with acetonitrile to obtain stock solutions of 500, 1000 and 1500 $\mu g\,ml^{-1}$ of drug. In the same manner, a stock solution of 2×10^{-3} M of drug was also prepared. Such drug solutions are stable for a period of 3 d when refrigerated (4 °C).

7,7,8,8-Tetracyanoquinodimethane (TCNQ), Aldrich Chem. Co., Milwaukee, U.S.A.; 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ), Merck-Schuchardt, Munich, Germany; chloranilic acid (CLA) and chloranil (CL), Fluka, Switzerland, were freshly prepared as (5×10^{-3} M) solutions in acetonitrile; the solutions were stable for ≥1 week at 4 °C.

General Procedure. Method Using DDQ and TCNQ Into 10-ml calibrated flasks were placed 0.2—2.4 ml aliquots of 250 and 75 $\mu g\,ml^{-1}$ gatifloxacin in acetonitrile for the DDQ and TCNQ method, respectively, and 2 ml of DDQ or TCNQ reagent solution (5×10^{-3} M) was added. The reaction mixture was allowed to stand for 20 min at 25±2 °C for DDQ method and heated in a water bath at 60±2 °C for 15 min for TCNQ method. After cooling and diluting to volume ≤10 ml with acetonitrile the absorbance was measured at 460 and 841 nm for DDQ and TCNQ, respectively, against a reagent blank prepared in the same manner.

Method Using CLA and CL Into 10-ml calibrated flasks were placed 0.2—2.4 ml aliquots of 1500 and 1000 $\mu g\,ml^{-1}$ gatifloxacin in acetonitrile for the CLA and CL method, respectively and 3 ml of CLA or CL reagent solution (5×10^{-3} M) was added. The reaction mixture was heated in a water bath at 60±2 °C for 10 min for CLA or CL methods. After cooling and diluting to volume ≤10 ml with acetonitrile the absorbance was measured at 530 and 545 nm for CLA and CL, respectively, against a reagent blank prepared in the same manner.

Procedure for Dosage Forms An accurately weighed amount of finely powdered tablets equivalent to 100 mg of the drug was dissolved in about 20 ml of methanol and transferred into a 100-ml calibrated flask, and after 30 min of mechanical shaking was filtrated in a 100-ml calibrated flask through Whatman no. 41 filter paper. Necessary amounts of filtrate were diluted to 100 ml with acetonitrile, then the same procedure is followed as described above.

Stoichiometric Relationship Job's method of continuous variation[37)] was employed to establish the stoichiometry of the coloured products. A 2×10^{-3} M standard solution of gatifloxacin and a 2×10^{-3} M solution of DDQ, TCNQ, CLA and CL were used. A series of solutions was prepared in which the total volume of gatifloxacin and reagent was constant (2.0 ml). The reagents were mixed in various proportions and diluted in a 10-ml calibrated flask with acetonitrile solvent. The absorbance was measured after treating each reagent at best time and temperature against a reagent blank following the above-mentioned procedure.

Association Constant and Standard Free Energy Changes Serial volumes of 1.0—5.0 ml of 1×10^{-3} M gatifloxacin solution (in 1.0 ml steps) in acetonitrile were transferred into 10-ml calibrated flasks. To each flask, 1 ml of acceptor solution (1×10^{-3} M) was added. The absorbance of the complex was used to calculate the association constant and the standard free energy changes of complexation ($\Delta G°$) using the Benesi–Hildebrand Equation.

Results and Discussion

The interaction of gatifloxacin with DDQ, TCNQ, CLA and CL in acetonitrile yielded intense orange red colored chromogen for DDQ, a bluish-green colored chromogen for TCNQ, and red colored chromogen for CLA and CL, absorbing maximally at wavelengths 460, 841, 530 and 545 nm, respectively (Fig. 2), most probably due to the formation of charge-transfer complexes between gatifloxacin acting as n-donor (D) or Lewis base, and DDQ, TCNQ, CLA and CL as π-acceptors (A) or Lewis acids (Chart 1).

$$D^{\cdot\cdot}+A \rightleftharpoons [D^{\cdot\cdot}\rightarrow A] \xleftrightarrow{\text{Polar solvent}} D^{\cdot+}+A^{\cdot-} \text{ radical anion}$$

The dissociation of the DA complex was promoted by the high ionizing power (dielectric constant) of acetonitrile solvent.[38)] Further support for the assignment was provided by comparison of the absorption bands with those of the $DDQ^{\cdot-}$, $TCNQ^{\cdot-}$, $CLA^{\cdot-}$ and $CL^{\cdot-}$ radical anions produced

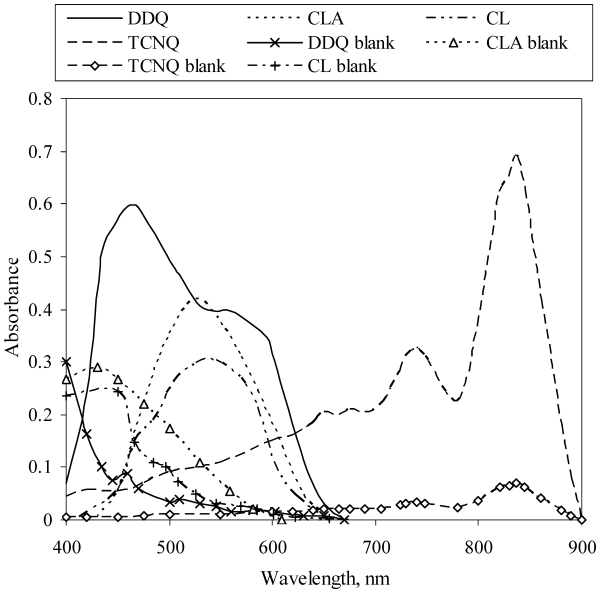

Fig. 2. Absorption Spectra of 30, 10, 200, 150 $\mu g\,ml^{-1}$ Gatifloxacin Complexes with (5×10^{-3} M) DDQ, TCNQ, CLA and CL, Respectively, Against Reagent Blank Solutions

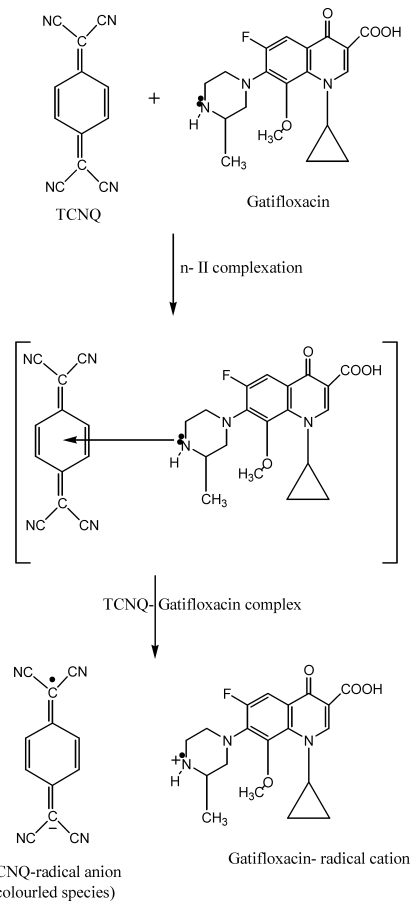

Chart 1. Suggested Structures of Gatifloxacin–TCNQ Charge Transfer Complexes

by the iodide reduction method in acetonitrile.[39,40]

The influence of different parameters on the color development was studied to determine optimum conditions for the assay procedures.

Choice of Solvent Although charge transfer complexes are probably formed in many solvents, the high cut-off points of some solvents obscured the scanning of the shorter wavelengths and therefore clear-cut spectroscopic evidence for charge transfer formation could not be ascertained. Also the low solubility of gatifloxacin in some other solvents restricted this.

Acetonitrile was found to be the best solvent for all the reagents (Table 1), because it has a high relative permitivity which ensures the maximum yield of $DDQ^{\cdot -}$, $TCNQ^{\cdot -}$, $CLA^{\cdot -}$ and $CL^{\cdot -}$ species. Other solvents (benzene, chloroform, methanol, methylene chloride and 1,4-dioxane) were also examined as possible substitutes (Table 1). Methylene chloride is a possible candidate, although it suffers from low boiling point which could result in fluctuations of concentration during handling and manipulation. Benzene was unsuitable due to the limited solubility of the reagents in this solvent. In 1,4-dioxane another orange product and not $TCNQ^{\cdot -}$ was obtained upon addition of the reagent. It is likely that the formation of this product also involves interaction with the solvent molecule.[41] The formation of $DDQ^{\cdot -}$, $TCNQ^{\cdot -}$, $CLA^{\cdot -}$ and $CL^{\cdot -}$ radicals was possible in methanol and ethanol; however, the color intensity was lower with those solvents than in acetonitrile. Due to the better solubility of the drug in methanol, the study revealed that the color development remained stable in ≤20% (v/v) methanol–acetonitrile.

Reagent Concentration When various concentrations of DDQ, TCNQ, CLA and CL were added to a fixed concentration of gatifloxacin, 2 ml of (5×10^{-3} M) solution of DDQ or TCNQ and 3 ml of (5×10^{-3} M) solution of CLA or CL were found to be sufficient for production of maximum and reproducible color intensity (Fig. 3). Higher concentrations of reagent did not affect the color intensity.

Reaction Time The optimum reaction time was determined by following the color development at room temperature (25±2 °C). Complete color development was attained after 20 min for DDQ, whereas for TCNQ, CLA and CL complete color development was not attained till 90 min; after heating on a water bath at 60±2 °C for 15 min for TCNQ or for 10 min for CLA and CL, complete color development was obtained. The colour remained stable for ≥42 h for all reagents.

Table 1. Effect of Solvent on the Absorption Intensity of Reaction Products of Gatifloxacin with DDQ, TCNQ, CLA and CL

Solvent	Absorbance			
	DDQ (460 nm)	TCNQ (841 nm)	CLA (530 nm)	CL (545 nm)
Acetonitrile	0.58	1.16	0.42	0.28
Benzene	0.04	0.31	0.07	0.09
Chloroform	0.09	0.46	0.11	0.08
Dichloromethane	0.13	0.61	0.14	0.12
1,2-Dichloroethane	0.16	0.88	0.09	0.11
1,4-Dioxane	0.07	0.08	0.07	0.05
Methanol	0.23	0.84	0.15	0.10

The relative sensitivity of the four reagents in analytical work can be compared by the apparent molar absorptivity values of the chromogens (Table 2). TCNQ exhibited the most intense band and was therefore selected for all further work. The most important spectral characteristics of the reaction of DDQ, TCNQ, CLA and CL with gatifloxacin investigated are presented in Table 2.

The *p*-chloranil method can only be used in aqueous buffer media. Comparison of the results obtained by the method using *p*-chloranil (CL) with those obtained using the same reagent in an aqueous buffer medium showed a wider range

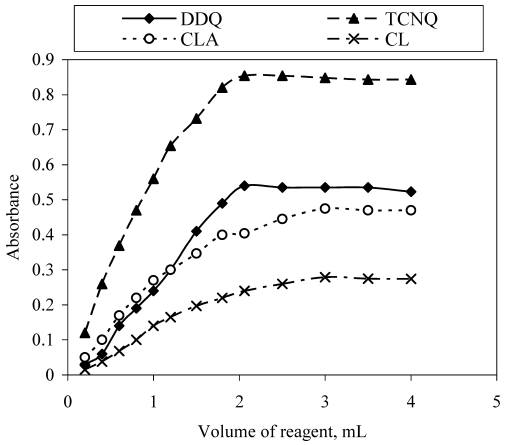

Fig. 3. Effect of Reangent Concentration (5×10^{-3} M) on the Formation of Gatifloxacin Complexes with DDQ, TCNQ, CLA and CL

Table 2. Statistical Analysis of Calibration Graphs and Analytical Data for the Complexation of Gatifloxacin with DDQ, TCNQ, CLA and CL Methods (n=6)

Parameters	Proposed methods			
	DDQ	TCNQ	CLA	CL
Wavelengths λ_{max} (nm)	460	841	530	545
Temperature/°C	25	60	60	60
Time/min	20	15	10	10
Beer's law limits (μg ml^{-1})	5—60	1.5—18	30—360	20—240
Ringbom limits (μg ml^{-1})	7.5—55	3—16	35—350	25—230
Molar absorptivity ε, (l/mol^{-1} cm^{-1})	4.028×10^3	2.169×10^4	5.74×10^2	6.165×10^2
Sandell's sensitivity (μg cm^{-2})	0.0999	0.0186	0.07	0.0653
Association constant log K	2.61	3.53	3.04	3.25
Standard free energy changes ($\Delta G°$)	−3.58	−5.41	−4.66	−4.98
Regression equation[a]				
Slope (b)	0.01	0.0521	0.0014	0.0015
Intercept (a)	0.0015	0.0066	0.0028	0.0034
Correlation coefficient (r)	0.9998	0.9996	0.9999	0.9998
Detection limits LOD (μg ml^{-1})	0.54	0.236	1.56	1.17
Quantification limits LOQ (μg ml^{-1})	1.80	0.787	5.20	3.90
RSD%	1.283	0.7384	0.5639	0.934
RE%	1.347	0.775	0.5919	0.981
Calculated t-value (2.57)[b]	0.711	0.184	0.054	2.35
Calculated F-value (5.05)[b]	2.286	1.301	2.219	1.197

a) $A=a+bC$, where A is the absorbance, a is the intercept, b is the slope and C is the concentration of drug in μg ml^{-1}. LOD, limit of detection; LOQ, limit of quantification; ε, molar absorptivity coefficient. *b*) The theoretical values of t (2.57) and F (5.05) at confidence limit at 95% confidence level and five degrees of freedom (p=0.05)

of determination, higher sensitivity and accuracy and less time consumption with the non-aqueous method proposed here.

Stoichiometry of The Reaction The stoichiometric ratio of the reactants (drug : reagent) in the charge-transfer complex was determined by the method of continuous variations (Job's method)[37] and found to be 1 : 1 for DDQ, TCNQ, CLA and CL (Fig. 4). This suggests that one nitrogen atom of the piperazine ring in gatifloxacin is involved in the reaction with DDQ, TCNQ, CLA and CL.

Association Constants and Standard Free Energy Changes A more detailed examination was made for the 1 : 1 complex of gatifloxacin–DDQ, gatifloxacin–TCNQ, gatifloxacin–CLA and gatifloxacin–CL. The absorbance of the complex was used to calculate the association constant using the Benesi–Hildebrand equation.[42]

$$\frac{[A_o]}{A_\lambda^{AD}} = \frac{1}{\varepsilon_\lambda^{AD}} + \frac{1}{K_c^{AD}\varepsilon_\lambda^{AD}} \times \frac{1}{[D_o]}$$

where $[A_o]$ and $[D_o]$ are the total concentrations of the interacting species, A_λ^{AD} and ε_λ^{AD} are the absorbance and molar absorptivity of the complex at 460, 841, 530 and 545 nm for DDQ, TCNQ, CLA and CL, respectively, and K_c^{AD} is the association constant of the complex. On plotting the values of $[A_o]/A_\lambda^{AD}$ *versus* $1/[D_o]$, a straight line was obtained (Fig. 5).

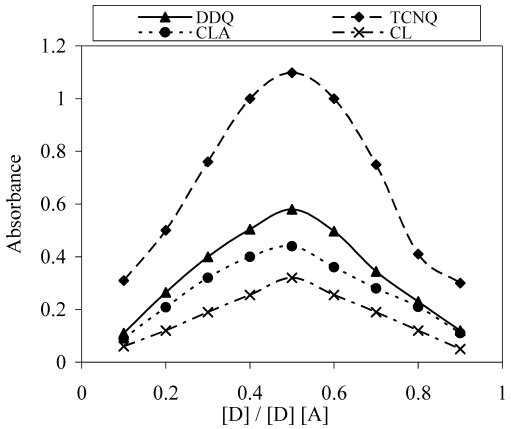

Fig. 4. Job's Method for Gatifloxacin Complexes with DDQ, TCNQ, CLA and CL; λ=460, 841, 530 and 545 nm, Respectively

Total molar concentration=4×10^{-4} M.

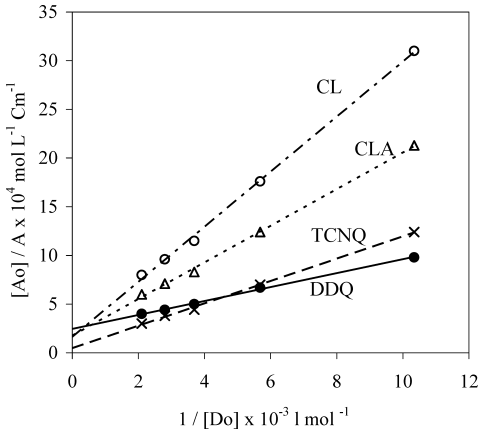

Fig. 5. Benesi–Hildebrand Plots for Gatifloxacin Complexes with DDQ, TCNQ, CLA and CL; λ=460, 841, 530 and 545 nm, Respectively

The intercept of this line with the ordinate is $(\varepsilon_\lambda^{AD})^{-1}$ and the slope equals $(\varepsilon_\lambda^{AD}K_c^{AD})^{-1}$. The calculated association constants are recorded in Table 2. The low values obtained for the association constants are common in these complexes due to the dissociation of the original donor–acceptor complex to the radical anion.

From the above $(\varepsilon_\lambda^{AD})^{-1}$ the molar absorptivities were 4.076×10^3, 2.132×10^4, 5.737×10^3 and 6.29×10^3 $1\,mol^{-1}\,cm^{-1}$ for DDQ, TCNQ, CLA and CL complexes, respectively, which are comparable with those obtained from the regression line equation of Beer's law (Table 2).

The standard free energy changes of complexation ($\Delta G°$) were calculated from the association constants (Table 2) by the following equation.[43]

$$\Delta G° = -2.303RT\log K_c$$

where ($\Delta G°$) is the free energy change of the complex ($kJ\,mol^{-1}$), R the gas constant ($1.987\,cal\,mol^{-1}\,deg^{-1}$), T the temperature in Kelvin (273+000 °C), and K_c the association constant of drug–acceptor complexes ($1\,mol^{-1}$).

Specificity and Interferences Regarding the interference of the excipients and additives usually presented in pharmaceutical formulations and interference due to the degradation products of gatifloxacin, the energy of the charge transfer (E_{CT}) depends on the ionization potential (I_P) of the donor and the electron affinity of the acceptor (E_A); hence the λ_{max} values of other π-donors mostly differ from those of the investigated acceptors if they are able to form CT complexes. This specificity of the charge-transfer reaction for gatifloxacin was attributed to its basic character, which allows the charge transfer, rather than the degradation products of gatifloxacin, which does not have sufficient basicity to achieve charge transfer. Potential interference by the excipients in the dosage forms was also studied. Samples were prepared by mixing a known amount (50 mg) of gatifloxacin with various amounts of common excipients such as glucose, lactose, talc powder, magnesium stearate and starch. The good percentage recoveries (Table 3) revealed that no interference was observed from any of these excipients with the proposed methods. The absence of interference from these excipients was attributed to the extraction with organic solvent prior to the analysis.

Quantification The reproducibility and accuracy of the suggested methods were assessed using different concentrations. The validity was checked occasionally during the work by running six replicate standard samples. Standard calibration curves for gatifloxacin were prepared by taking series of different concentrations and applying the suggested proce-

Table 3. Analysis of Gatifloxacin in the Presence of Common Excipients by Different Methods

Ingredient	Recovery[a] (%±S.D.)			
	DDQ	TCNQ	CLA	CL
Glucose (50)	98.76±0.59	99.81±0.54	98.52±1.05	99.58±1.23
Lactose (10)	99.46±0.46	100.22±0.85	100.25±0.58	99.62±1.05
Talc (10)	99.26±0.56	99.64±1.02	101.07±0.87	100.85±0.84
Magnesium stearate (10)	99.98±0.68	100.10±0.024	100.13±1.03	100.84±0.95
Starch (10)	100.05±1.01	98.65±1.05	98.47±1.10	98.98±1.24
Average±S.D.	99.50±0.53	99.68±0.62	99.69±1.15	99.97±0.83

a) Values are mean of three determinations.

Table 4. Between-Day Test of Precision and Accuracy for Determination of Gatifloxacin Using the Proposed Methods

Method	Taken (μg ml^{-1})	Recovery %±S.D.[a)]	Precision RSD %[a)]	Accuracy Er %	Standard error	Confidence limit[b)]
DDQ	15	100.25±0.21	1.40	0.04	0.086	15.04±0.033
	30	100.40±0.18	0.596	0.12	0.0735	30.12±0.189
	40	99.60±0.34	0.853	−0.16	0.139	39.84±0.357
	50	99.82±0.38	0.761	−0.09	0.155	49.91±0.399
			Mean 0.903			
TCNQ	4	99.90±0.06	1.50	−0.004	0.0245	3.996±0.063
	8	100.10±0.07	0.874	0.008	0.0286	8.008±0.0735
	12	99.96±0.11	0.917	−0.005	0.045	11.995±0.115
	16	100.05±0.13	0.812	0.008	0.053	16.008±0.0136
			Mean 1.03			
CLA	75	99.80±0.35	0.468	−0.15	0.143	74.85±0.367
	150	98.97±0.49	0.33	−1.54	0.2	148.46±0.514
	250	99.55±0.68	0.273	−1.12	0.278	248.88±0.714
	350	100.08±0.77	0.22	0.28	0.314	350.28±0.808
			Mean 0.323			
CL	50	100.15±0.41	0.819	0.08	0.167	50.08±0.43
	100	100.20±0.51	0.509	0.20	0.208	100.20±0.535
	150	99.85±0.64	0.427	−0.22	0.261	149.78±0.672
	200	100.38±0.82	0.408	0.76	0.335	200.76±0.861
			Mean 0.541			

a) Average of six determinations (n=6), RSD%, percentage relative standard deviation; Er%, percentage relative error. *b*) Confidence limit at 95% confidence level and five degrees of freedom (p=0.05).

dures with DDQ, TCNQ, CLA and CL acceptors. Beer's law is valid within the microgram concentration range of gatifloxacin (Table 2). The regression equations of these calibration graphs were utilized for determination of an unknown concentration of gatifloxacin in tablets. The mean molar absorptivity (ε) and Sandell sensitivity (Ss) as calculated from Beer's law are presented in Table 2. For more accurate analysis, Ringbom plots[44)] for optimum concentration ranges were obtained. The standard deviation of the absorbance measurements was obtained from a series of 13 blank solutions. The limits of detection (k=3) and of determination (k=10) of the methods were established according to IUPAC definition ($C_1=kS_0/s$ where C_1 is the limit of detection, S_0 is the standard deviation of blank determination, s is the slope of the standard curve and k is the constant related to the confidence interval[45,46)]) and the values were calculated and recorded (Table 2).

Accuracy and Precision To verify the validity, precision and applicability of the proposed method and the reproducibility of the results presented, six replicate experiments at four concentrations of gatifloxacin were carried out. Table 4 shows the values of between-day relative standard deviations for different concentrations of the drugs obtained from experiments carried out over a period of 4 d. It was found that the within-day relative standard deviations were <1.5%, which indicates that the proposed method is highly reproducible and that DDQ, TCNQ, CLA and CL were successfully applied to determine gatifloxacin via the formation of charge transfer complex.

The proposed methods are simpler, faster and much more sensitive and accurate than the official one in which the HPLC method[7)] was used for the determination of gatifloxacin. However, the principal advantage of the proposed methods is their suitability for routine quality control of the drug alone and in tablets without fear of interference caused by excipients expected to be present in tablets. Meanwhile, other drugs with basic centers are expected to give similar reactions with DDQ, TCNQ, CLA or CL reagent, so the methods are limited to the assay of single-drug formulations.

Table 5. Determination of Gatifloxacin in Pharmaceutical Dosage Forms Applying the Standard Addition Technique

Proposed method	Taken (μg ml^{-1})	Added (μg ml^{-1})	Proposed methods Recovery[a)] (%)		
			Tequin tablets	Floxin tablets	Gatilox tablets
DDQ	10	—	100.05	99.70	99.84
		10	99.86	100.10	99.75
		20	99.90	99.88	100.15
		30	100.13	99.95	99.55
		40	99.89	98.90	99.28
		50	100.08	99.60	100.14
Mean±S.D.			99.99±0.12	99.69±0.43	99.79±0.34
TCNQ	5	—	99.94	99.86	100.06
		2.5	99.89	100.10	99.79
		5	99.92	99.53	99.76
		7.5	100.15	99.30	100.12
		10	100.03	99.45	100.04
		12.5	99.70	99.91	99.80
Mean±S.D.			99.94±0.15	99.69±0.31	99.93±0.16
CLA	60	—	99.60	100.24	99.93
		60	100.30	99.75	99.85
		120	99.90	99.92	100.05
		180	100.08	100.70	99.83
		240	99.80	99.55	99.27
		300	100.20	99.84	99.65
Mean±S.D.			99.98±0.26	100.0±0.41	99.76±0.28
CL	40	—	100.10	99.97	99.95
		40	100.25	100.08	99.75
		80	99.91	100.14	100.27
		12	99.86	100.30	99.15
		160	99.78	99.94	99.85
		200	99.93	99.80	99.90
Mean±S.D.			99.97±0.17	100.04±0.17	99.81±0.37

a) Average of six determinations.

Analytical Applications Pharmaceutical preparations (Tequin, Floxin and Gatilox tablets) containing gatifloxacin were analyzed by the proposed methods and the accuracy was assessed by the standard additions method in which vari-

Table 6. Determination of Gatifloxacin in Pharmaceutical Dosage Forms

Sample	Recovery [a] ± S.D. %				
	Official method[7]	Proposed methods			
		DDQ	TCNQ	CLA	CL
Tequin tablets[b]	99.60±0.73	99.72±0.78	99.45±0.64	99.95±0.81	99.87±0.69
t[c]		0.25	0.35	0.72	0.60
F[c]		1.14	1.30	1.23	1.12
Floxin tablets[b]	99.85±0.45	99.50±0.35	99.45±0.68	99.60±0.59	100.05±0.61
t[c]		1.23	0.245	1.72	0.528
F[c]		1.65	1.10	0.75	1.84
Gatilox tablets[b]	99.75±0.63	99.94±0.71	99.26±0.57	99.90±0.53	99.89±0.67
t[c]		0.448	1.29	0.407	0.34
F[c]		1.27	1.22	1.41	1.13

a) Mean±standard deviation of six determinations. *b*) All gatifloxacin tablets containing 400 mg gatifloxacin per tablet; Tequin (Bristol Myers Squibb Company, Egypt); Floxin tablets (Global Napi, Egypt); Gatilox (EPCI, Egypt). *c*) The theoretical values of t (2.57) and F (5.05) at confidence limit at 95% confidence level and five degrees of freedom (p=0.05).

able amounts of pure drug were added to the previously analyzed portion of pharmaceutical preparations. The results are shown in Table 5 confirming that the proposed methods are not liable to interference by tablet fillers, excipients and additives usually formulated with pharmaceutical preparations. The proposed methods are highly sensitive; therefore they may be easily used for the routine analysis of gatifoxacin in pure form and in its pharmaceutical preparations.

The proposed methods were applied to determination of gatifloxacin in pharmaceutical preparations, the official HPLC method being used for comparative assay. The results are presented in Table 6. The performance of the methods was assessed by Student-t values and F-ratio tests. At a 95% confidence level, the calculated t- and F-values did not exceed the theoretical values, indicating that the proposed and the official methods are equally accurate.

Conclusion

The proposed methods are simple, less time consuming and more sensitive and validated than the reported UV and HPLC methods. The color development at room temperature required 20 min for DDQ, whereas for TCNQ, CLA and CL at room temperature complete color development was not attained till 90 min; however this can be shortened to 15 min for TCNQ and 10 min for CLA and CL by raising the temperature to 60±2 °C after heating on a water bath. The proposed method concerned with TCNQ was superior as compared with that of the other acceptors and the performance order of the proposed methods is TCNQ>DDQ>CL>CLA according to higher molar absorptivity and lower detection limits. The proposed methods are suitable for the determination of gatifloxacin in pharmaceutical formulations without interference from excipients such as starch and glucose, suggesting useful applications in bulk drug analysis.

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