Spectrophotometric Determination of Some Fluoroquinolone Antibacterials through Charge-transfer and Ion-pair Complexation Reactions

Amina Mohamed El-Brashy,* Mohamed El-Sayed Metwally, and Fawzi Abdallah El-Sepai

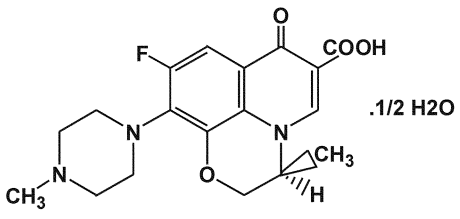
Analytical Chemistry Department, Faculty of Pharmacy, University of Mansoura, Mansoura 35516, Egypt
Received November 10, 2003

Two simple, rapid and sensitive spectrophotometric methods for the determination of three fluoroquinolones, namely levofloxacin, norfloxacin and ciprofloxacin have been performed either in pure form or in their tablets. In the first method, levofloxacin and norfloxacin are directly treated with bromocresol green (BCG) in dichloromethane while ciprofloxacin is allowed to react with the same dye in aqueous acidic buffer. Highly yellow colored complex species were formed instantaneously in case of levofloxacin and norfloxacin or after extraction into dichloromethane for ciprofloxacin. The formed complexes are quantified spectrophotometrically at their absorption maxima at 411 nm for levofloxacin and 412 nm for norfloxacin and ciprofloxacin. The second method involves the reaction of levofloxacin with ρ-chloranilic acid (ρ-CA) and norfloxacin with tetracyanoethylene (TCNE) in acetonitrile to give complexes with maximum absorbance at 521 and 333 nm for the two drugs, respectively. Adopting the first procedure, calibration graphs were linear over the range 1-20 μg mL^{-1} with mean percentage recoveries of 100.41 ± 0.72, 99.99 ± 0.54 and 100.23 ± 0.91 for the theree drugs, respectively. For the second procedure, the concentration ranges were 15-250 μg mL^{-1} for levofloxacin using ρ-CA and 0.8-16 μg mL^{-1} for norfloxacin using TCNE with mean percentage recoveries of 99.88 ± 0.45 and 100.26 ± 0.68 for the two drugs, respectively. The proposed methods were successfully applied to determine these drugs in their tablet formulations and the results compared favorably to that of reference methods. The proposed methods are recommended for quality control and routine analysis.

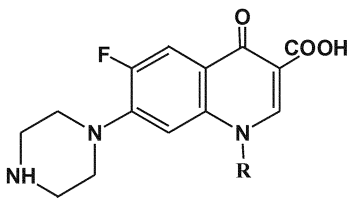
Key Words : Spectrophotometry, Organic dyes, Levofloxacin, Norfloxacin, Ciprofloxacin

Introduction

Levofloxacin [I] (−)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-benzoxazine-6-carboxylic acid hemihydrate (the S-(−) isomer which has twice the activity of the racemate ofloxacin), norfloxacin [II] and ciprofloxacin [III] are three broad-spectrum antimicrobial agents belonging to the fluoroquinolone group. They are commercially available for treatment of a wide range of infections showing good activity against gram-negative and gram-positive bacteria by inhibition of their DNA gyrase.[1,2]



Levofloxacin [I]



Norfloxacin [II] R= C_2H_5
Ciprofloxacin [III] R= $CH(CH_2)_2$

Several methods have been reviewed in the literature for the analysis of levofloxacin (either per se or as an isomer of the racemic ofloxacin), norfloxacin and ciprofloxacin. The USP[3] recommended liquid chromatographic method for determination of ciprofloxacin, while the BP[4] recommended non-aqueous titrimetric method with potentiometric detection of the end point for the analysis of norfloxacin and ciprofloxacin. Levofloxacin is not official in the USP nor in the BP, however, ofloxacin is official in the USP in which it is assayed by non-aqueous titrimetry. Spectrophotometric methods include oxidative coupling with 3-methyl-2-benzothiazolinonehydrazone hydrochloride (MBTH) and cerium (IV) ammonium sulfate[5,6] and ion-pair formation with acid-dye reagents such as supracene violet 3B, tropeolin 00, bromophenol blue, bromothymol blue or bromocresol purple.[7-9] Also, complexation with iron (III)[10,11] or with tris(o-phenanthroline) iron (II) and tris(bipyridyl) iron (II),[12] derivative spectrophotometry of their Cu(II) complexes,[13] charge-transfer complexation with π-acceptors such as 2,3-dichloro-5,6-dicyano-*p*-benzoquinone, 7,7,8,8-tetracyanoquinodimethane, *p*-chloranil, *p*-nitrophenol and tetracyanoethylene[14-18] and ternary complex formation with eosin and palladium[19] have been strongly reported. Other methods include titrimetry,[3,4,20] atomic absorption spectrometry,[21] flurimetry,[22-24] luminescence,[25,26] conductometry,[27] voltametry and polarography,[28,29] flow injection analysis,[30] HPTLC,[31] HPLC[3,32,33] and capillary electrophoresis.[34,35]

In the present study, two spectrophotometric methods for levofloxacin and norfloxacin and one for ciprofloxacin are proposed for their determination in bulk and pharmaceutical formulations. The methods are based upon direct charge-transfer complex formation of levofloxacin and norfloxacin with the acid-dye BCG in dichloromethane or with the π-acceptors ρ-CA and TCNE in acetonitrile and ion-pair complex formation between ciprofloxacin and BCG followed by subsequent extraction into dichloromethane. The methods are simple, rapid, sensitive and easy to apply. Furthermore, they don't need costly instrumentation required for published HPLC and capillary electrophoresis methods.

Experimental Section

Apparatus. A Shimadzu (Model 1601PC) UV-Visible spectrophotometer (Shimadzu, Kyoto, Japan), equipped with 1 cm matched quartz cells was used.

Materials and Reagents. All materials used were of analytical reagent grade. Levofloxacin, norfloxacin and ciprofloxacin were kindly provided by the Amriya Pharmaceutical Industries (Alexandria/Egypt) and their purities were established by applying the recommended official non-aqueous titrimetric methods.[3,4] Stock solutions of levofloxacin and norfloxacin were prepared as 20 mg % in dichloromethane for the acid-dye method or 100 mg % levofloxacin and 20 mg % norfloxacin in acetonitrile for the π-acceptor method. Ciprofloxacin was prepared as 20 mg % in 0.05 M HCl. BCG was prepared as 1×10^{-3} M in dichloromethane for levofloxacin and norfloxacin or in 0.05 M NaOH for ciprofloxacin, while ρ-CA (Merck) and TCNE (Merck) were prepared as 0.01 M in acetonitrile. Also, 5×10^{-5} M and 2.5×10^{-4} M solutions of levofloxacin and norfloxacin in dichloromethane and of ciprofloxacin in 0.05 M HCl and their equimolar solutions of BCG were prepared. In addition, 0.01 M and 5×10^{-3} M levofloxacin and 4×10^{-4} M and 2×10^{-4} M norfloxacin solutions and their equimolar solutions of ρ-CA and TCNE in acetonitrile were prepared. Acetate buffer, 0.4 M was prepared by mixing various volumes of 0.4 M acetic acid and 0.4 M sodium acetate solutions to the required pH value (2.5-6).

General Recommended Procedure.

Acid-dye method: *(a) For levofloxacin and norfloxacin:* Accurately measured aliquots of levofloxacin or norfloxacin in dichloromethane in the concentration range shown in Table 1, were transferred into a series of 10 mL volumetric flasks followed by 0.8 mL of 1×10^{-3} M BCG in dichloromethane. The mixture was shaken well, completed to volume with dichloromethane. The absorbance was measured at 411 or 412 nm for the two drugs, respectively against an appropriate blank prepared simultaneously.

(b) For ciprofloxacin: Into a series of 50 mL separating funnels, 4 mL 0.4 M acetate buffer pH 3 and 4 mL of 1×10^{-3} M BCG in 0.05 M NaOH were placed. Appropriate volume of ciprofloxacin in the concentration range shown in Table 1, was added to each funnel and mixed well then completed to 10 mL with distilled water. The funnels were shaken vigorously with 10 mL dichloromethane for 2 min and allowed to stand for clear separation of the two phases. The absorbance of the organic phase at 412 nm was measured against a reagent blank similarly prepared.

The π-acceptor method: *(a) ρ-CA:* Accurately measured aliquots of levofloxacin in acetonitrile in the concentration range shown in Table 1, were transferred into a series of 10 mL volumetric flasks followed by 2 mL of 0.01 M ρ-CA. The mixture was shaken, diluted to volume with acetonitrile and the absorbance was measured at 521 nm against an appropriate blank prepared simultaneously.

(b) TCNE: Accurately measured aliquots of norfloxacin in acetonitrile in the concentration range shown in Table 1, were transferred into a series of 10 mL volumetric flasks followed by 0.5 mL of 0.01 M TCNE, respectively. The mixture was diluted with 5 mL acetonitrile and heated in a water bath at 60 °C for 15 min then cooled and completed to volume with the same solvent. The absorbance was then measured at 333 nm against a reagent blank prepared simultaneously.

The concentration of the studied drugs was determined referring to the calibrated curves prepared previously by

Table 1. Collective performance data for the analysis of the studied drugs by the proposed methods

Method / Data	Bromocresol green			Chloranilic acid	TCNE
Drug	Levofloxacin	Norfloxacin	Ciprofloxacin	Levofloxacin	Norfloxacin
Solvent		Dichloromethane		Acetonitrile	Acetonitrile
λ_{max} (nm)	411	412	412	521	333
Reagent, milliliters	0.8	0.8	4	2	0.5
Working range (μg mL^{-1})	1-20	1-20	1-20	15-250	0.8-16
(ε)[a]	2.16×10^{-4}	2.27×10^{-4}	2.28×10^{-4}	1.20×10^{-3}	2.64×10^{-4}
Linear regression equation[b]	A = 0.0583 c + 0.0004	A = 0.0710 c + 0.0002	A = 0.0689 c + 0.0011	A = 0.0032 c – 0.0004	A = 0.0826 c + 0.0003
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999	0.9999
M.D.L.[c]	0.118	0.094	0.096	0.352	0.079

[a]Molar absorptivity (L·mole^{-1}·cm^{-1}). [b]With respect to A = bc + a, where c is the concentration in (μg mL^{-1}), A is the absorbance, a is the intercept and b is the slope. [c]Minimum detection limit (μg mL^{-1}).

plotting the absorbance against the concentration of each drug in μg mL^{-1}. Alternatively, the regression equation was derived.

Procedure for dosage forms: An accurately weighed quantity of the pulverized tablets equivalent to 100 mg of levofloxacin or 20 mg norfloxacin was extracted with dichloromethane for the acid-dye or acetonitrile for the π-acceptor method then sonicated for about 10 min. Another quantity equivalent to 20 mg of ciprofloxacin was extracted with 0.05 M HCl by shaking and sonication for 10 min. The extracts were filtered into 100 mL volumetric flasks and completed to volume with the same solvent. Aliquots of these solutions were analyzed as previously mentioned using BCG, ρ-CA or TCNE. The nominal content of the tablets was calculated either from a previously plotted calibration graph or using the regression equation.

Results and Discussion

The charge-transfer (CT) reaction has been widely studied recently. Many drugs are easy to be determined by spectrophotometry based on color CT complexes formed with electron acceptors.[36,37] BCG is an acid sulphonephthalein dyestuff. The color of such dyes is due to opening of lactoid ring and subsequent formation of quinoid group. It behaves as a strong electron acceptor due to the presence of the strong electron withdrawing sulphonic acid group conjugated with the aromatic ring system.[38,39] ρ-CA and TCNE are π-electron acceptors as a result of the strong electron withdrawing halo- and cyano- groups conjugated with the π-system.[39,40] The three acceptors have been widely utilized in the literature for determination of many drugs acting as electron donors according to CT and ion-pair mechanisms.[41-44]

The studied drugs exhibit absorption maxima in the ultraviolet region at 260-330 nm (Figure 1A, B & C). Owing to the presence of the F atom acting as an electron-withdrawing group, the benzene ring in the three drugs has lower electron density than the free terminal nitrogen atom in the piperazinyl moiety, so these drugs act as n-electron donors.[16] Therefore, these drugs react with electron acceptors to form CT complexes or radical anions according to the polarity of the solvent used.[45] For BCG, levofloxacin and norfloxacin in dichloromethane instantaneously form yellow colored reaction products with absorption maxima at 411 and 412 nm (Fig. 1A & B) for the two drugs, respectively. Interaction of levofloxacin with ρ-CA in acetonitrile instantaneously yields an intense purple color absorbing at a maximum of 521 nm (Fig. 2A) while norfloxacin reacts with TCNE in acetonitrile giving a product with an absorption maximum at 333 nm (Fig. 2B). The new absorption bands formed are the result of the formation of CT complexes.[39,40]

These complexes are formed through the lone pair of electrons donated by the levofloxacin or norfloxacin as *n*-donor and the charge transfer reagent as an electron acceptor in which a partial ionic bond (D^+ A^-) is assumed to be formed.

$$\underset{\text{Donor}}{D} + \underset{\text{Acceptor}}{A} \longrightarrow \underset{\text{Donor-acceptor complex}}{\left[D \longrightarrow A \right]} \longrightarrow \underset{\text{Radical anion}}{D^{+\bullet} + A^{\bullet -}}$$

This interaction was particularly strong on using ρ-CA and TCNE so that it involves a complete transfer of electronic charge with the formation of an acceptor radical anion ($A^{\bullet -}$). These radical anions formed were the predominant chromogens in those two reactions. The dissociation of the donor-acceptor complex in those reactions was promoted by the high ionizing power of the solvent, acetonitrile.[46]

Being insoluble in most organic solvents,[2] ciprofloxacin is allowed to react with BCG in aqueous acidic buffered medium to form dichloromethane-extractable ion-pair complex absorbing maximally at 412 nm (Fig. 1C). At pH 3, protonation of the terminal secondary amino group in ciprofloxacin

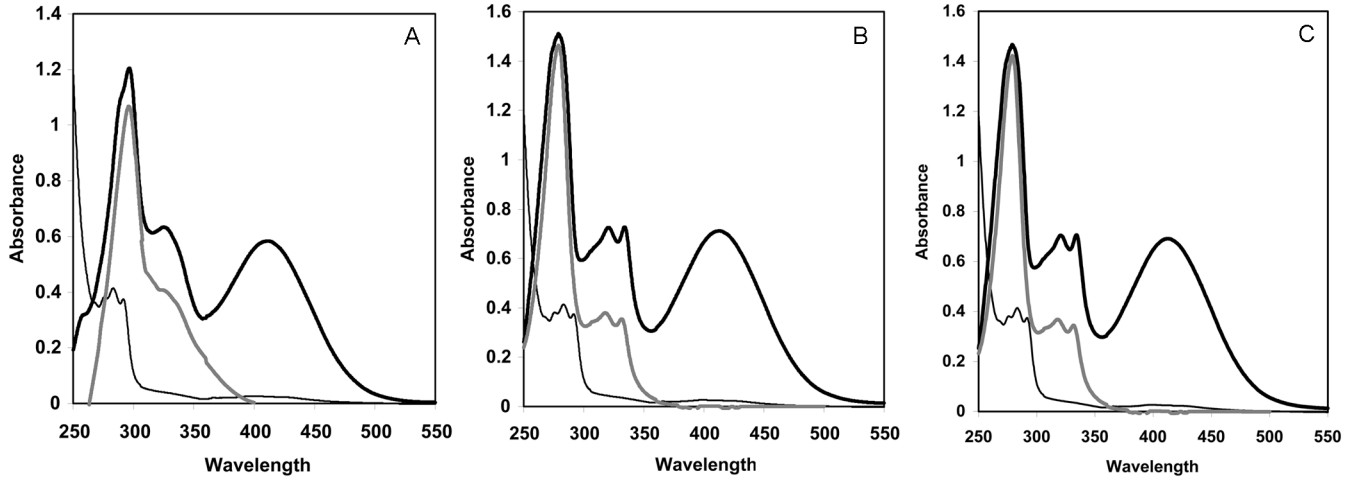


Figure 1. Absorption spectra of the formed charge-transfer complexes: A. Absorption spectra of: levofloxacin, 10 µg mL^{-1} (▬); bromocresol green, 8×10^{-5} M (———) and their CT reaction product (▬) in dichloromethane. B. Absorption spectra of: norfloxacin, 10 µg mL^{-1} (▬); bromocresol green, 8×10^{-5} M (———) and their CT reaction product (▬) in dichloromethane. C. Absorption spectra of: ciprofloxacin, 10 µg mL^{-1} (▬) in 5% water in acetonitrile; bromocresol green, 8×10^{-5} M (———) and their CT reaction product (▬) in dichloromethane.

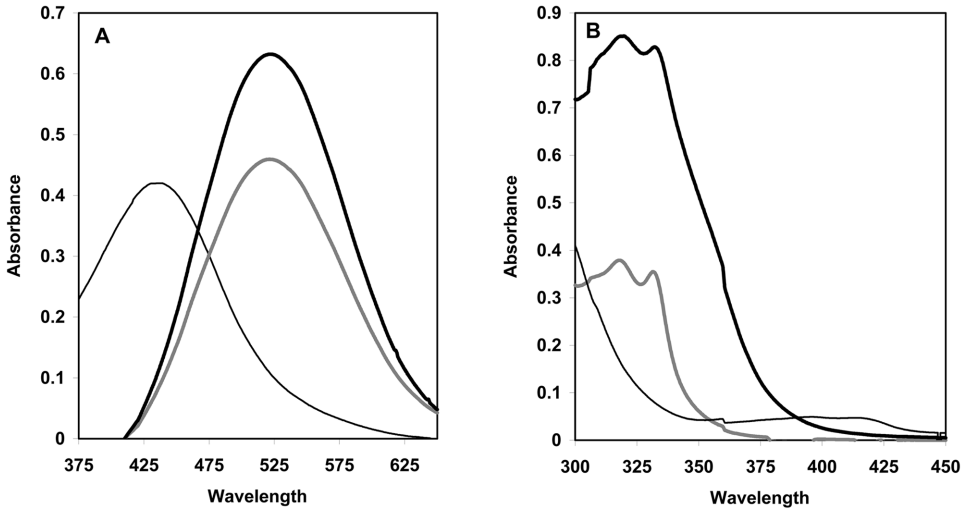


Figure 2. Absorption spectra of the formed charge-transfer complexes: A. Absorption spectra of: ρ-CA, 2×10^{-3} M (———) and its reaction products with levofloxacin, 140 μg mL^{-1} (▬▬) and 200 μg mL^{-1} (▬▬) in acetonitrile. B. Absorption spectra of: norfloxacin, 10 μg mL^{-1} (▬▬), tetracyanoethylene, 5×10^{-4} M (———) and their CT reaction product (▬▬) in acetonitrile.

molecule takes place in acid medium, which form ion-pair with the dissociated sulphonic acid group of BCG. The ion-pair complex is readily extracted into dichloromethane.

The different experimental parameters affecting the formation of the complexes were extensively studied to determine the optimal conditions for the assay procedure.

Effect of Time and Temperature. The optimum reaction time was determined by following the absorbance increment at the λ_{max} of the formed complexes. It was found that BCG and ρ-CA charge-transfer and ion-pair complexes were formed instantaneously at room temperature and remained stable for at least 24 h with the different solvents.

TCNE charge-transfer complex was completely developed after heating in a water bath at 60 °C for 15 min or at 80 °C for 10 min (Fig. 3). However, heating at 60 °C was chosen to construct the calibration curve as higher temperature was found to affect the linearity and reproducibility. The formed complex was stable for at least 24 h, thus permitting quantitative analysis to be carried out with good accuracy.

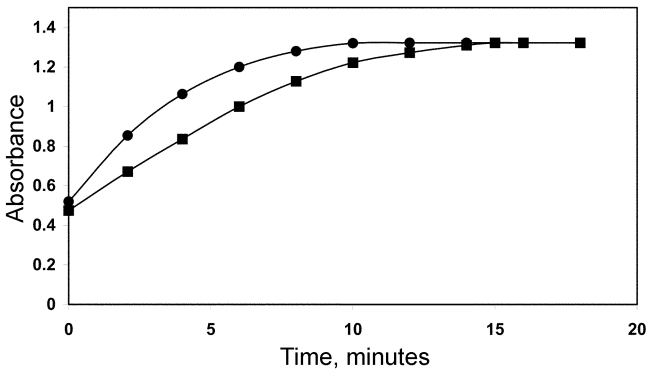


Figure 3. Effect of time and temperature on the development of the CT complex of norfloxacin (16 μg mL^{-1}) with TCNE in acetonitrile, λ = 333 nm. ■ , Temperature = 60 °C. ● , Temperature = 80 °C.

Effect of pH. The influence of pH on the ion-pair formation of ciprofloxacin with BCG has been studied using 0.4 M acetate buffer. The results are shown in Figure 4. It is clear that the absorbance was found to be constant within the pH range 2.5-3.5 at which the formed ciprofloxacin-BCG ion-pair was maximally stable and hence quantitatively extracted into dichloromethane. Thus, all the absorbance measurements were carried out at pH 3.

Effect of Reagent Concentration. The optimum reagent concentration was determined by adding various volumes of the acid-dye BCG to a fixed concentration of 20 μg mL^{-1} of

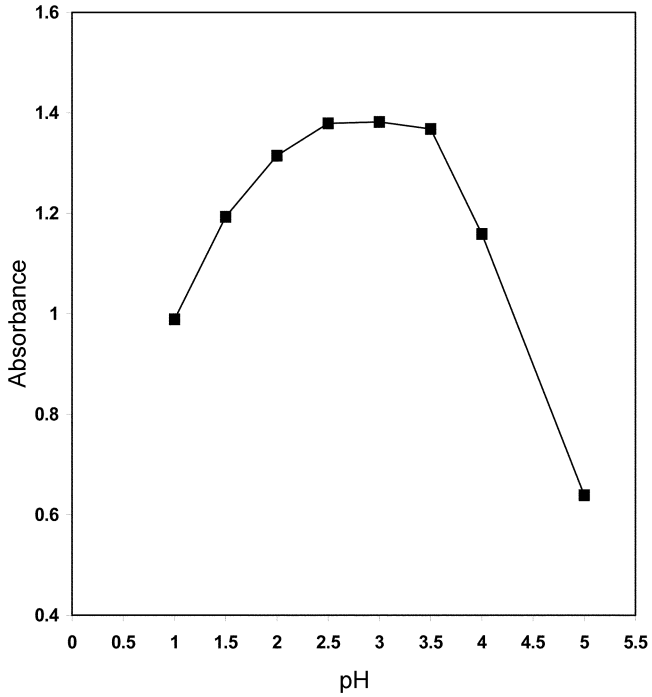


Figure 4. The influence of pH on the ion-pair formation of ciprofloxacin, 20 μg mL^{-1} with bromocresol green at 412 nm.

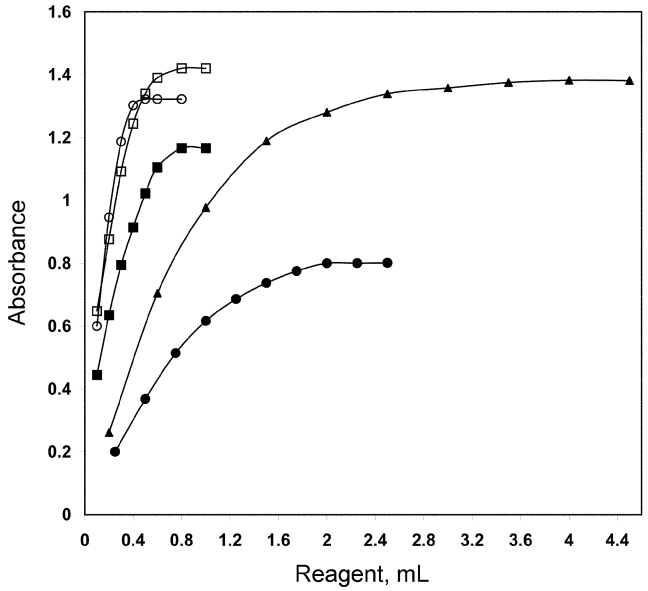


Figure 5. Effect of reagent volume on the absorption intensity of the CT or ion-pair complexes of: ■ , Levofloxacin (20 μg mL^{-1}) with BCG in dichloromethane; λ = 411 nm. □ , Norfloxacin (20 μg mL^{-1}) with BCG in dichloromethane; λ = 412 nm. ▲ , ciprofloxacin (20 μg mL^{-1}) with BCG in dichloromethane; λ = 412 nm. ● , Levofloxacin (250 μg mL^{-1}) with ρ-CA in acetonitrile; λ = 521 nm. ○ , Norfloxacin (16 μg mL^{-1}) with TCNE in acetonitrile; λ = 333 nm.

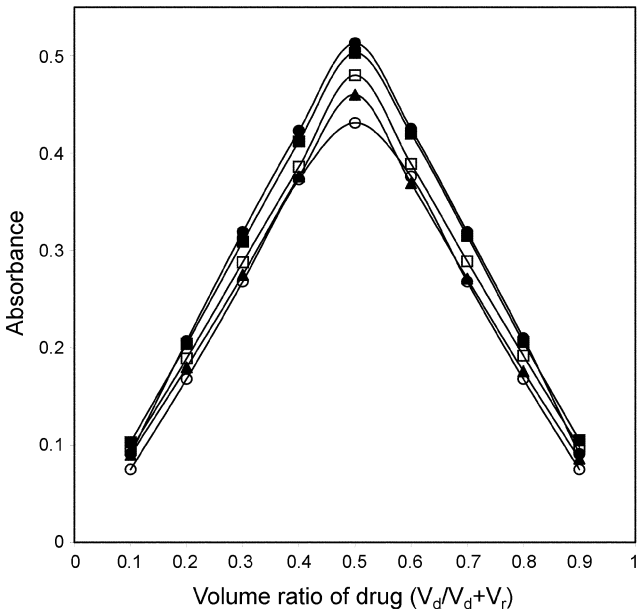


Figure 6. Continuous variation plots for the charge-transfer or ion-pair complexes of: ■ , Levofloxacin (5×10^{-4} M) with BCG in dichloromethane; λ = 411 nm. □ , Norfloxacin (5×10^{-4} M) with BCG in dichloromethane; λ = 412 nm. ▲ , Ciprofloxacin (5×10^{-4} M) with BCG in dichloromethane; λ = 412 nm. ● , Levofloxacin (0.01 M) with ρ-CA in acetonitrile; λ = 521 nm. ○ , Norfloxacin (4×10^{-4} M) with TCNE in acetonitrile; λ = 333 nm. Where, V_d and V_r are the volumes of added drug and reagent, respectively.

the drugs. It was found that 0.8 mL of 1×10^{-3} M BCG in dichloromethane for levofloxacin and norfloxacin and 4 mL of 1×10^{-3} M BCG in 0.05 M NaOH for ciprofloxacin were enough to develop the absorbance to its maximum intensity. For the π-acceptor method, various volumes of ρ-CA or TCNE solutions were added to a fixed concentration of 250 or 16 μg mL^{-1} of levofloxacin or norfloxacin, respectively. It was found that 2 and 0.5 mL of the reagent solutions, respectively, were optimum (Fig. 5).

Effect of Solvent. Different solvents have been tried in order to achieve maximum sensitivity and product stability. Dichloromethane, chloroform and dioxane were suitable solvents for BCG complexes, acetonitrile, dichloromethane, chloroform and dioxane had the same effect in case of ρ-CA complexes, while acetonitrile was the best solvent for TCNE complexes with regard to molar absorptivity and stability. However, dichloromethane for BCG and acetonitrile for ρ-CA and TCNE complexes were chosen for further studies in the present paper.

Stoichiometric Relationship. The molar ratio of the complexes formed between the studied drugs and the three reagents used was investigated applying the molar ratio[47] and continuous variation (Job's) methods[48] using equimolar solutions of the drug and reagent. The results indicated that all the complexes were formed in the ratio of 1 : 1 (Fig. 6 and 7). This finding supports that the interaction of the studied drugs and the reagents used takes place at only one site which was the more sterically free terminal basic aliphatic amino group.

Method Validation. Under the experimental conditions

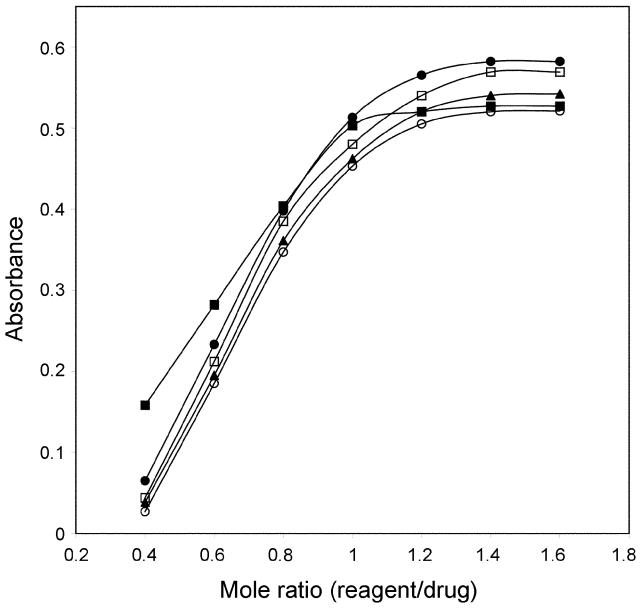


Figure 7. Mole ratio plots for the charge-transfer or ion-pair complexes of: ■ , Levofloxacin (2.5×10^{-4} M) with BCG in dichloromethane; λ = 411 nm. □ , Norfloxacin (2.5×10^{-4} M) with BCG in dichloromethane; λ = 412 nm. ▲ , Ciprofloxacin (2.5×10^{-4} M) with BCG in dichloromethane; λ = 412 nm. ● , Levofloxacin (5×10^{-3} M) with ρ-CA in acetonitrile; λ = 521 nm. ○ , Norfloxacin (2×10^{-4} M) with TCNE in acetonitrile; λ = 333 nm.

Table 2. Application of the proposed methods for the analysis of the studied drugs in pure form

Drug	Proposed method						Reference method	
	Acid-dye method			π- Acceptor method				
	Taken (μg/mL)	Found (μg/mL)	Recovery (%)[a]	Taken (μg/mL)	Found (μg/mL)	Recovery (%)[a]	Taken (μg/mL)	Recovery (%)[a]
Levofloxacin	2	2.02	100.83	20	19.90	99.48	2	101.22[8]
	4	4.01	100.15	40	39.68	99.19	4	100.02
	6	6.10	101.63	80	80.17	100.21	8	101.65
	8	8.05	100.66	100	99.95	99.95	10	100.39
	10	9.99	99.91	140	140.43	100.31	12	99.84
	15	14.91	99.41	200	200.70	100.36		
	20	20.02	100.11	250	249.23	99.69		
Mean ± SD		100.38 ± 0.72			99.88 ± 0.45		100.62 ± 0.78	
F-test		1.17 (4.53)[b]			3.00 (4.53)			
t-test		0.55 (2.23)			2.10 (2.23)			
Norfloxacin	2	2.00	99.88	1	1.00	100.11	4	98.78[16]
	4	4.01	100.31	2	2.03	101.52	8	101.15
	6	6.01	100.22	4	4.02	100.41	12	99.29
	8	7.92	98.94	8	8.04	100.46	16	98.36
	10	10.03	100.29	10	9.94	99.38	30	99.95
	15	15.09	100.58	12	11.96	99.67		
	20	19.95	99.74	15	15.05	100.32		
Mean ± SD		99.99 ± 0.54			100.26 ± 0.68		99.50 ± 1.09	
F-test		4.07 (4.53)			2.57 (4.53)			
t-test		1.03 (2.23)			1.49 (2.23)			
Ciprofloxacin	2	2.03	101.48				2	101.35[17]
	4	4.03	100.82				4	99.57
	6	6.01	100.12				8	100.64
	8	7.90	98.68				10	98.97
	10	10.07	100.72				15	100.28
	15	14.94	99.57					
	20	20.04	100.22					
Mean ± SD		100.23 ± 0.91					100.16 ± 0.92	
F-test		1.03 (4.53)						
t-test		0.13 (2.23)						

[a]The average of at least three determinations. [b]The values between brackets are the tabulated F- and t-values at $P = 0.05$ [49].

described above, the calibration graphs for the three drugs were constructed by plotting absorbance versus concentration in μg mL^{-1}. Conformity with Beer's law was evident in the concentration ranges cited in Table 1. Regression equations, intercepts, slopes and correlation coefficients for the calibration data were presented in Table 1.

The % recoveries of the pure drugs using the proposed methods compared with that given by the reference methods[8,16,17] are illustrated in Table 2. The reference methods recommended are spectrophotometric determination of levofloxacin by bromothymol blue[8] at 415 nm and norfloxacin and ciprofloxacin[16,17] by 7,7,8,8-tetracyanoquinodimethane at 743 and 843 nm for the two drugs, respectively. The validity of the proposed methods was evaluated by statistical analysis[49] between the results obtained and those of reference methods. Regarding the calculated student's t-test and variance ratio F-test (Table 2), there is no significant difference between the proposed and the reference methods regarding accuracy and precision.

The proposed methods were successfully applied to determine the three fluoroquinolones in their commercial tablets. The concentration of the studied drugs was calculated using the corresponding calibration equation shown in Table 1. The commonly used excipients and additives in the preparation of tablets were found not to interfere in the analysis. The % recoveries of the three drugs in their tablets compared with those of the reference methods[8,16,17] for the studied drugs, respectively, are given in Table 3.

Conclusion

The methods that are proposed in this work for the quantitation of levofloxacin, norfloxacin and ciprofloxacin are simple, direct and sensitive. Moreover, with the excep-

Table 3. Application of the proposed methods for the analysis of the studied drugs in their tablets

Pharmaceutical Preparations	Proposed method						Reference method	
	Acid-dye method			π-Acceptor method				
	Taken (μg/mL)	Found (μg/mL)	Recovery (%)*	Taken (μg/mL)	Found (μg/mL)	Recovery (%)*	Taken (μg/mL)	Recovery (%)*
Tarivid tablets[a] (ofloxcin, 200 mg/tablet)	2	1.98	99.15	20	19.81	99.06	2	101.22[8]
	4	4.04	101.03	40	40.44	101.10	4	99.13
	8	8.04	100.47	80	80.13	100.16	8	101.21
	12	11.91	99.29	140	140.44	100.31	10	100.74
	16	15.95	99.66	200	200.44	100.22	12	101.01
Mean ± SD		99.92 ± 0.81			100.17 ± 0.73			100.66 ± 0.88
Leaflox tablets[b] (levofloxacin, 500 mg/tablet)	2	2.03	101.71	20	20.13	100.63	2	100.19[8]
	4	4.01	100.17	40	39.81	99.53	4	100.53
	8	8.00	100.04	80	80.44	100.55	8	99.38
	12	11.93	99.43	140	139.45	99.64	10	98.70
	16	16.12	100.73	200	200.13	100.06	12	102.13
Mean ± SD		100.42 ± 0.86			100.08 ± 0.50			99.65 ± 0.88
Noroxin tablets[c] (norfloxcin, 400 mg/tablet)	2	2.01	100.61	1	0.99	98.91	4	99.95[16]
	4	3.96	98.92	2	1.99	99.70	8	97.90
	8	8.02	100.18	4	4.02	100.39	12	100.27
	12	12.03	100.25	8	8.06	100.74	16	98.11
	16	15.93	99.58	12	11.93	99.45	30	100.36
Mean ± SD		99.91 ± 0.67			99.84 ± 0.73			99.32 ± 1.21
Norbactin tablets[d] (norfloxcin, 400 mg/tablet)	2	2.00	99.90	1	1.00	100.12	4	100.19[16]
	4	3.99	99.62	2	2.02	100.91	8	100.53
	8	8.09	101.06	4	3.96	98.88	12	99.38
	12	11.95	99.55	8	8.02	100.29	16	98.70
	16	16.05	100.29	12	12.02	100.15	30	102.13
Mean ± SD		100.08 ± 0.62			100.07 ± 0.74			100.19 ± 1.3
Ciprobay tablets[e] (ciprofloxacin, 500 mg/tablet)	2	1.99	99.35				2	100.23[17]
	4	3.98	99.39				4	98.46
	8	7.92	99.04				8	100.91
	12	12.13	101.10				10	98.97
	16	16.12	100.77				15	100.43
Mean ± SD		99.93 ± 0.93						99.80 ± 1.04
Rancif tablets[f] (ciprofloxacin, 500 mg/tablet)	2	2.00	100.07				2	99.10[17]
	4	3.98	99.39				4	101.24
	8	8.08	101.03				8	99.25
	12	11.87	98.93				10	99.85
	16	16.14	100.86				15	101.02
Mean ± SD		100.06 ± 0.91						100.09 ± 0.99

*The average of at least three determinations. [a]Hoechst Orient S.A.E. Cairo, Egypt under license of Hoechst A g Frankfort, Germany. (Batch no. 11E04). [b]Pharaonia Pharmaceuticals. (Batch no. 3102). [c]Eipico (Egyptian Pharmaceutical Industries Co.) under license of Merck and Co. Inc RAHWAY. NJ. USA. (Batch no. 014079). [d]Cid Company, Giza, under licence from Ranbaxy. (Batch no. 117). [e]Alkan pharma under licence from Payer Leverkusen, Germany. (Batch no. 080). [f]Cid Company, Giza, under licence from Ranbaxy. (Batch no. 125).

tion of ciprofloxacin determination by the acid-dye method, they are less time consuming and do not require elaboration treatment and tedious extraction procedures required in chromatographic[3,32,33] and other traditional extractive spectrophotometric[7-9,36-38] methods. These, in addition to satisfactory sensitivity (1 μg mL^{-1}) and reproducibility compared to the official non-aqueous titrimetric methods, make the methods applicable for routine analysis of the three drugs both in pure form and in tablets. The formation of color probe using BCG and ρ-CA is instantaneous; thus, the methods could be readily adaptable to automated analysis such as flow injection analysis.

References

1. Wood, D. G.; Slack, R.; Peutherer, J. *Medical Microbiology,* 15th Ed.; Harcourt Brace and Company Limited: UK, 1998; p 52.
2. Martindale, *The Extra Pharmacopoeia,* 32nd ed.; The Pharmaceutical Press: London, 1999.
3. *United States Pharmacopoeia XXV; National Formulary XX*; US pharmacopoeial convention: Rockville, MD, 2002.
4. *The British pharmacopoeia*; HMSO: London, 1998.
5. Rizk, M.; Belal, F.; Ibrahim, F.; Ahmed, S. M.; El-Enany, N. M. *Sci. Pharm.* **2000**, *68*, 173.
6. Ramana-Rao, G.; Avadhanulu, A. B.; Vatsa, D. K. *Indian Drugs* **1990**, *27*, 532.
7. Suslu, I.; Tamer, A. *Anal. Lett.* **2003**, *36*, 1163.
8. Issa, Y. M.; Abdel-Gawad, F. M.; Abou-Table, M. A.; Hussein, H. M. *Anal. Lett.* **1997**, *30*, 2071.
9. Sastry, C. S. P.; Rama-Rao, K.; Prasad, D. S. *Talanta* **1995**, *42*, 311.
10. Djurdjevic, P.; Todorovic, M.; Stankov, M. J.; Odovic, J. *Anal. Lett.* **2000**, *33*, 657.
11. Suliman, F. E. O.; Sultan, S. M. *Talanta* **1996**, *43*, 559.
12. Nagaralli, B. S.; Seetharamappa, J.; Melwanki, M. B. *J. Pharm. Biomed. Anal.* **2002**, *29*, 859.
13. Rizk, M.; Belal, F.; Ibrahim, F.; Ahmed, S.; Sheribah, Z. A. *J. AOAC Int.* **2001**, *84*, 368.
14. Mostafa, S.; El-Sadek, M.; Alla, E. A. *J. Pharm. Biomed. Anal.* **2002**, *27*, 133.
15. Feng, Y.; Zhao, F. L.; Tong, S. Y. *Fenxi Kexue Xuebao* **2000**, *16*, 184.
16. Zhao, F. L.; Xu, B. Z.; Zhang, Z. Q.; Tong, S. Y. *J. Pharm. Biomed. Anal.* **1999**, *21*, 355.
17. Abdel-Gawad, F. M.; Issa, Y. M.; Fahmy, H. M.; Hussein, H. M. *Mikrochim Acta* **1998**, *130*, 35.
18. Xuan, C. S.; Wang, Z. Y.; Song, J. L. *Anal. Lett.* **1998**, *31*, 1185.
19. El-Walily, A. F. M.; Belal, S. F.; Bakry, R. S. *J. Pharm. Biomed. Anal.* **1996**, *14*, 561.
20. Kilic, E.; Koseoglu, F.; Akay, M. A. *J. Pharm. Biomed. Anal.* **1994**, *12*, 347.
21. El-Ansary, A. L.; El-Hawary, W. F.; Issa, Y. M.; Ahmed, A. F. *Anal. Lett.* **1999**, *32*, 2255.
22. Vilchez, J. L.; Ballesteros, O.; Taoufiki, J.; Sanchez-Palencia, G.; Navalon, A. *Anal. Chim. Acta* **2001**, *444*, 279.
23. Ocana-Gonzalez, J. A.; Callejon-Mochon, M.; Barragan-de-la-Rosa, F. L. *Talanta* **2000**, *52*, 1149.
24. Drakopoulos, A. L.; Ioannou, P. C. *Anal. Chim. Acta* **1997**, *354*, 197.
25. Aly, F. A.; Al-Tamimi, S. A.; Alwarthan, A. A. *Talanta* **2001**, *53*, 885.
26. Ocana, J. A.; Callejon, M.; Barragan, F. J. *Analyst* **2000**, *125*, 1851.
27. Belal, F.; Rizk, M.; Aly, F. A.; El-Enany, N. M. *Chem. Anal. (Warsaw)* **1999**, *44*, 763.
28. Ghoneim, M. M.; Radi, A.; Beltagi, A. M. *J. Pharm. Biomed. Anal.* **2001**, *25*, 205.
29. Rizk, M.; Belal, F.; Aly, F. A.; El-Enany, N. M. *Talanta* **1998**, *46*, 83.
30. Altiokka, G.; Atkosar, Z.; Can, N. O. *J. Pharm. Biomed. Anal.* **2002**, *30*, 881.
31. Argekar, A. P.; Sawant, J. G. *J. Planar Chromatogr.; Mod TLC* **1999**, *12*, 202.
32. Neckel, U.; Joukhadar, C.; Frossard, M.; Jaeger, W.; Mueller, M.; Mayer, B. X. *Anal. Chim. Acta* **2002**, *463*, 199.
33. Wong, F. A.; Juzwin, S. J.; Flor, S. C. *J. Pharm. Biomed. Anal.* **1997**, *15*, 765.
34. Wang, W.; Lu, J. D.; Fu, X. Y.; Chen, Y. Z. *Anal. Lett.* **2001**, *34*, 569.
35. Fierens, C.; Hillaert, S.; van-den-Bossche, W. *J. Pharm. Biomed. Anal.* **2000**, *22*, 763.
36. El-Sherif, Z. A.; Mohamed, A. O.; Walash, M. I.; Tarras, F. M. *J. Pharm. Biomed. Anal.* **2000**, *22*, 13.
37. Moustafa, A. A. M. *J. Pharm. Biomed. Anal.* **2000**, *22*, 45.
38. Rahman, N.; Hejaz-Azmi, S. N. *J. Pharm. Biomed. Anal.* **2000**, *24*, 33.
39. Görög, S. *Ultraviolet-Visible Spectrophotometry in Pharmaceutical Analysis*; CRC Press Inc.: Florida, 1995; p 188.
40. Foster, R. *Organic Charge-Transfer Complexes*; Academic Press: London, New York, 1969.
41. Khashaba, P. Y.; El-Shabouri, S. R.; Emara, K. M.; Mohamed, A. M. *J. Pharm. Biomed. Anal.* **2000**, *22*, 363.
42. Abdellatef, H. E. *J. Pharm. Biomed. Anal.* **1998**, *17*, 1267.
43. El-Ansary, A. L.; Issa, Y. M.; Selim, W. *Anal. Lett.* **1999**, *32*, 955.
44. Walash, M. I.; Rizk, M.; El-Brashy, A. *Pharm. Weekbl. Sci. Ed.* **1986**, *8*, 234.
45. Taha, A. M.; El-Rabbat, N. A.; Fattah, F. A. *J. Pharm. Belg.* **1980**, *35*, 437.
46. Askal, H. F.; Saleh, G. A.; Omar, N. M. *Analyst* **1991**, *116*, 387.
47. Job, P. *Spectrochemical Methods of Analysis*; Wiley Intersience: New York, 1971; p 346.
48. Rose, J. *Advanced Physico-chemical Experiments*; Pitman: London, 1964; p 54.
49. Caulcut, R.; Boddy, R. *Statistics for Analytical Chemists*; Chapmann & Hall: London, 1983.