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Spectrophotometric and spectrofluorimetric estimation of ciprofloxacin and norfloxacin by ternary complex formation with eosin and palladium(II)

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Abstract

Spectrophotometric and spectrofluorimetric methods for the determination of two broad-spectrum fluoroquinolone antibacterials (ciprofloxacin and norfloxacin), either in pure form or in tablets, are described. Both methods are based on the formation of a ternary complex between palladium(II), eosin and the fluoroquinolone in the presence of methyl cellulose, as surfactant. Spectrophotometrically, under the optimum conditions, the ternary complexes showed an absorption maximum at 545 nm, with apparent molar absorptivities of 3.4×10^4 and 2.7×10^4 l mol^{-1} cm^{-1} and Sandell's sensitivities of 1.01×10^{-2} and 1.12×10^{-2} μg cm^{-2} for ciprofloxacin and norfloxacin, respectively. The solution of the ternary complex obeyed Beer's law in the concentration range 3–10 μg ml^{-1} for both quinolones. The proposed method was applied to the determination of the two drugs in pharmaceutical tablets. A fluorescence quenching method for the determination of both quinolones by forming this ternary complex was also investigated for the purpose of enhancing the sensitivity of the determination. The results obtained by the application of both procedures and the USP XXIII methods were in good agreement and statistical comparison by means of Student's *t*-test and the variance ratio *F*-test showed no significant differences between the three methods.

Keywords: Ciprofloxacin; Norfloxacin; Ternary complex; eosin; Palladium chloride; Spectrophotometry; Spectrofluorimetry

1. Introduction

The fluoroquinolones or pyridonecarboxylic acids (PCA) (e.g. ciprofloxacin hydrochloride (CI) and norfloxacin (NR)) are used as broad-spectrum antibacterial agents. Their mechanism of action and the antimicrobial spectra have been reviewed in detail [1]. The two antibacterials and their pharmaceutical tablets are official in the USP XXIII. The methods of analysis for the bulk drug are non-aqueous titration and a high-performance liquid chromatography (HPLC) procedure for NR and CI, respectively [2]. HPLC procedures, in the reversed-phase mode, have been reported for the quantitation of both antibacterials in tablets [2].

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A number of spectrophotometric methods for the determination of both antibacterials have been reported, including ion-pair formation with Bromothymol Blue [3], Supracene Violet and Tropaeolin OOO [4] and oxidative coupling with 3-methylbenzothiazolin-2-one hydrazone hydrochloride [5,6]. Many colorimetric methods based on the complexation of the quinolones with iron(III) have been reported [7–11]. Non-aqueous titration [12], polarography [13,14] and adsorptive stripping voltammetry [15] have also been used for their quantitation. HPLC has been used for the determination of both drugs in their pharmaceutical preparations or in biological fluids using either UV [16–23] or fluorescence detection [24,25]. A very comprehensive monograph on NR has been published [26].

This paper reports simple, sensitive and accurate spectrophotometric and fluorimetric methods for the determination of both antibiotics. The two methods are based on the chelate-forming ability of the carboxylic and carbonyl groups in these quinoline derivatives with palladium(II) with the subsequent formation of a ternary complex with eosin (sodium salt of 2,4,5,7-tetrabromofluorescein) in the presence of methyl cellulose. The optimum conditions (temperature, pH, etc.) were established before the application of the methods to the analysis of the drugs as bulk or in tablet form.

2. Experimental

2.1. Instrumentation

A Perkin-Elmer Model 550S double-beam spectrophotometer (Norwalk, CT, USA) with matched 10 mm quartz cells and attached to a Hitachi Model 561 recorder with a scan speed of 60 nm min^{-1} was employed for all absorbance measurements. A Perkin-Elmer Model 560-10S spectrophotometer equipped with a 150 W xenon lamp, excitation and emission grating monochromators and 1×1 cm quartz cell and attached to Perkin-Elmer Model 56 recorder was used. The spectrofluorimeter was a gift from the Alexander Von Humboldt foundation (Bonn, Germany) to Prof. A.M. Wahbi. A thermostated water-bath, accurate to ± 0.5°C, was utilized throughout.

2.2. Materials

Pharmaceutical-grade of CI and NR (Chem. Iberica, Barcelona, Spain) were kindly supplied by Pharco Pharmaceutical (Alexandria, Egypt) and were certified to contain 99.2% and 98.5%, respectively. They were used without further purification. Eosin (Merck, Darmstadt, Germany) was prepared as a 2×10^{-3} M solution in distilled water. Palladium(II) chloride solution was prepared as a 2×10^{-3} M solution by dissolving about 35.5 mg of palladium(II) chloride (Sigma, Milwaukee, Wl,USA) in 1 ml of concentrated hydrochloric acid, with the aid of heat, followed by the addition of 50 ml of boiled water and diluting to 100 ml with water in a volumetric flask. A 0.5% w/v solution of methyl cellulose (Prolabo, France, 1500 cP) was prepared by dissolving the appropriate amount in hot water (80°C) with stirring for 10 min, then chilling to 5°C for 30 min. Walpole's acetate buffer [27] was prepared by mixing different volumes of 0.2 M acetic acid with 0.2 M sodium acetate. The pH of the solutions should be adjusted by adding more of the 0.2 M acetic acid solution or the 0.2 M sodium acetate solution as required.

2.3. Standard solutions

Solutions of 0.25 mg ml^{-1} were prepared by dissolving 25 mg of CI or NR in distilled water in a 100 ml volumetric flask and diluting to volume. The solutions were stable for at least 1 week if they had been stored in a cool (<25°C) and dark place.

2.4. General procedures

2.4.1. Spectrophotometric method

An x ml volume of the working solution of the fluoroquinolone ($x = 0.3–1.0$ ml for both CI and NR) was pipetted into two sets of 25 ml volumetric flasks. A 1.5 ml volume of 0.5% methyl cellulose solution, 2 ml of the buffer solution of the appropriate pH (4 and 4.2 for CI and NR, respec-

tively), 0.75 ml of the eosin solution and 0.75 ml of palladium(II) chloride solution were added to the flasks, in this order. The mixture was diluted to volume with water, homogenized by shaking and immersed in a warm water-bath (60 ± 0.5°C) for 20 min. The solution was then cooled to room temperature (about 25°C) and its (solution A) absorbance was measured at 545 nm against a similarly prepared eosin palladium(II) chloride solution (solution B).

2.4.2. Fluorimetric procedure

Volumes of 0.5 ml of the above solutions were pipetted into two 100 ml volumetric flasks and diluted to volume with water. The difference in the relative fluorescence intensities between solutions A and B at a 540 nm emission wavelength with excitation at 310 nm was measured.

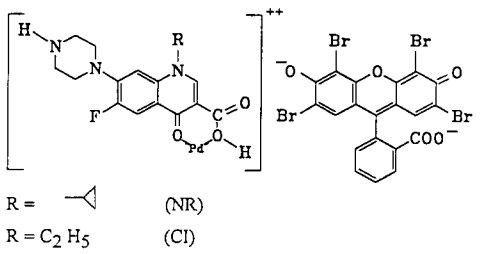
The concentration of CI or NR in the analyte solutions can be determined by reference to corresponding calibration graphs, which had been constructed previously according to the regression equations (Table 1).

2.5. Assay of pharmaceutical tablets

Twenty tablets were powdered and a quantity of the powder equivalent to 25 mg of the fluoroquinolone was extracted by shaking with 30 ml water, followed by another two extractions, each with 20 ml of water. The extracts were filtered through Whatman No. 41 filter-paper into a 100 ml volumetric flask and then diluted to volume with water. The assay for CI and NR content was completed as described in Section 2.4.

3. Results and discussion

The fact that the pyridonecarboxylic acid derivatives form very stable metal chelates [28] with different cations suggested the possibility of the utilization of this phenomenon for increasing the sensitivity of both spectrophotometric and fluorimetric measurements, through the formation of a stable ternary complex of acid–palladium(II)–eosin (Scheme 1). The absorption spectra of the binary Pd(II)–(CI) or (NR) and ternary



Scheme 1. Stable ternary complex of acid palladium(II)–eosin.

complexes drug–Pd(II)–eosin formed were scanned in the range 500–650 nm. It was found that, on addition of CI or NR to the eosin–Pd(II) solution (solution B), a difference in absorbance

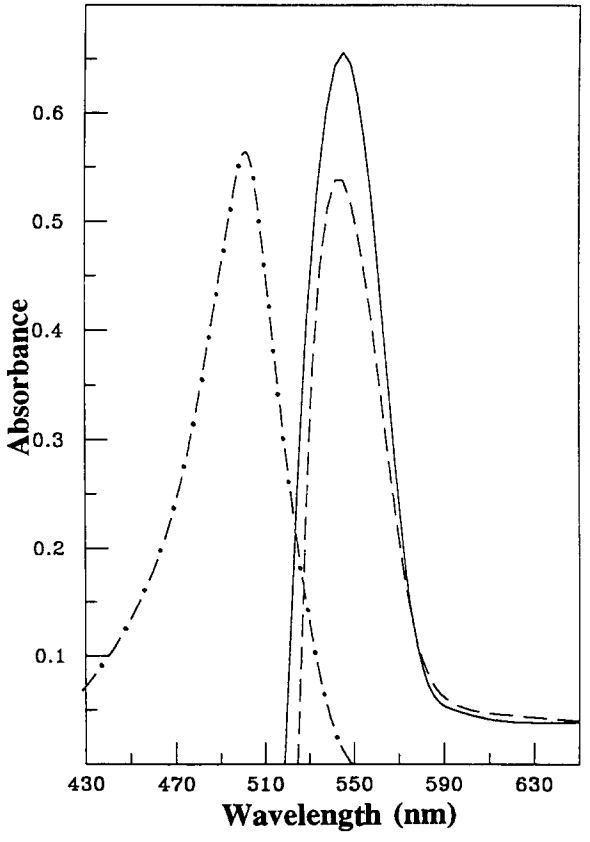


Fig. 1. Absorption spectra of the ternary complex of 7 μg ml^{-1} CI (—) and 6μg ml^{-1} NR (- - -) with eosin and Pd(II). Absorption spectrum of eosin and Pd(II) solution (- ·-).

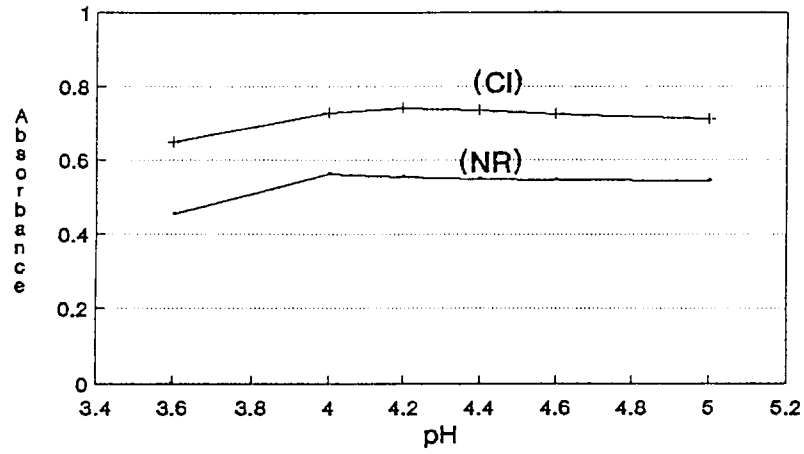


Fig. 2. Effect of pH on the absorbance of the drug–Pd(II)–eosin ternary complex. CI = 8 μg ml^{-1} and NR = 6 μg ml^{-1}.

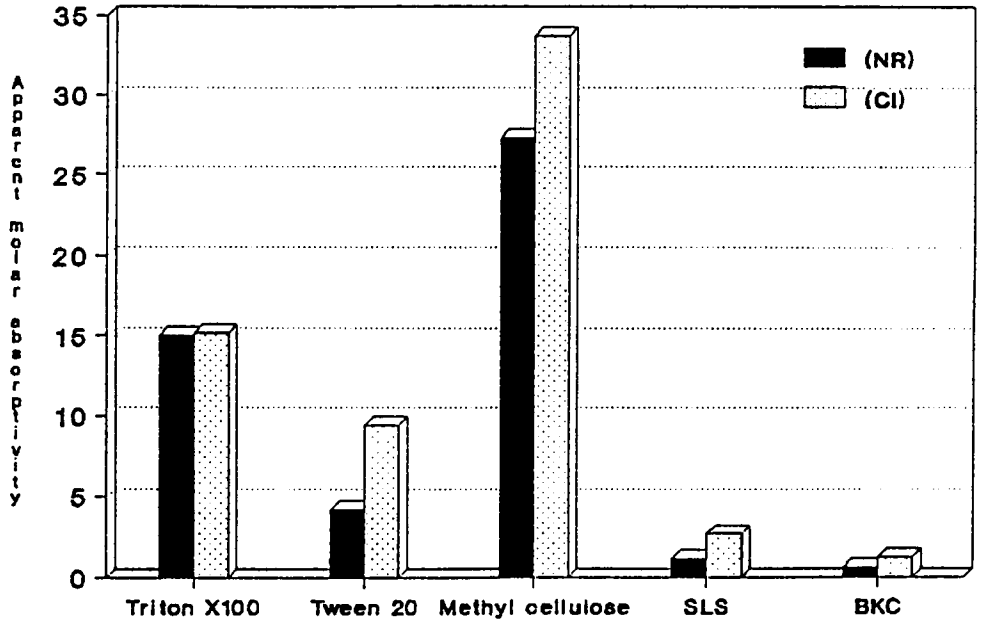


Fig. 3. Effect of different surfactants (1.5 ml of 0.5% w/v aqueous solution) on the apparent molar absorptivity of the CI/NR–Pd(II)–eosin ternary complex. SLS = sodium lauryl sulphate and BKC = benzalkonium chloride.

from eosin–Pd(II)–drug (solution A) and solution B was observed around 545 nm (Fig 1). Under the same experimental conditions, a solution containing only Pd(II) and the drug did not exhibit any absorption in that region. The absorption difference of solution B was proportional to the concentration of either CI or NR.

3.1. Assay parameters (spectrophotometric method)

To optimize the assay parameters, the effects of pH, reaction time at different temperatures, concentration of surfactant, eosin and palladium(II) chloride on the absorbance of the ternary complex formed were studied. The effect of pH on the

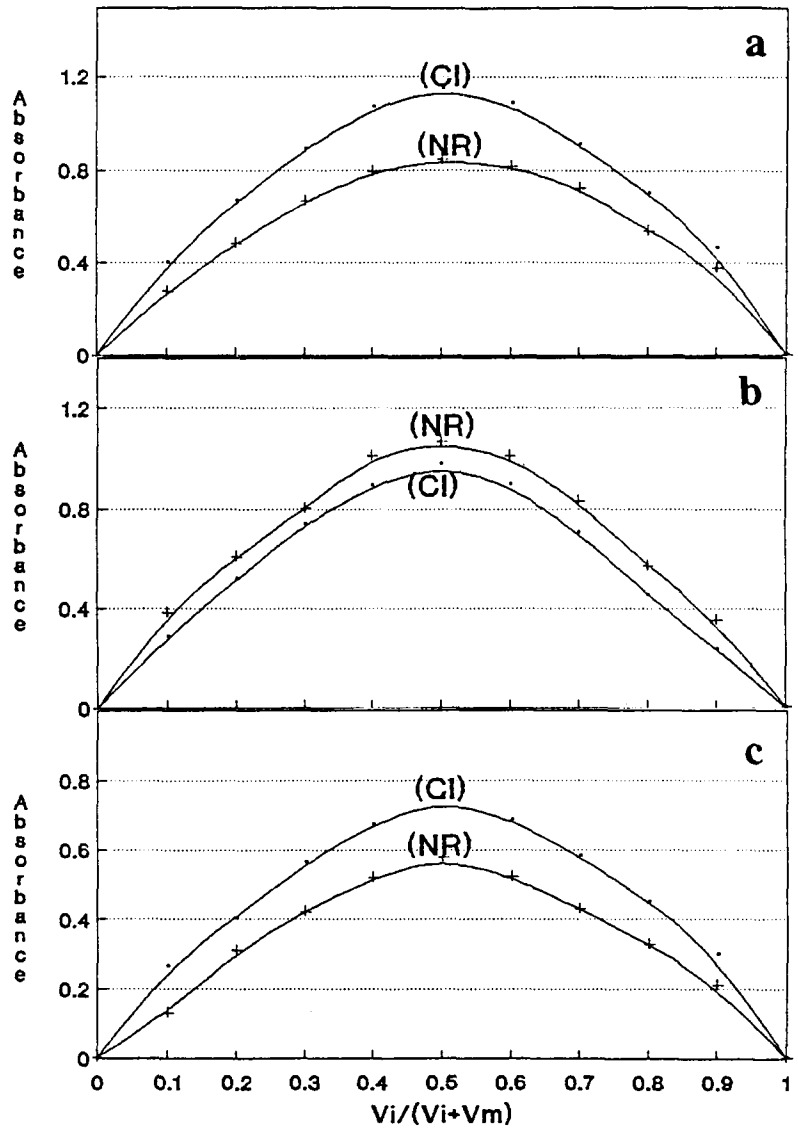


Fig. 4. (a) Continuous variation plots for drug:Pd(II) (5×10^{-3} M) complex ratio in the presence of excess eosin (1.5×10^{-3} M). V_i = drug; V_m = Pd(II). (b) Continuous variation plots for drug:eosin (5×10^{-3} M) complex ratio in the presence of excess Pd(II) (1.5×10^{-3} M). V_2 = drug; V_m = eosin. (c) Continuous variation plots for Pd:eosin (5×10^{-3} M) complex ratio in the presence of excess drug (2×10^{-3} M). V_i = eosin; V_m = Pd(II).

absorbance of the ternary complex was studied at 545 nm. The absorbance of the drug–Pd(II)–eosin complex solution was investigated over the pH range 3.6–5.0. The optimum absorbance was achieved at pH 4.2 and 4 for CI and NR, respectively, using 2 ml of acetate buffer (Fig. 2). In order to examine the effect of temperature and heating time on the formation rate and on the absorbance of the drug–Pd(II)–eosin ternary complex, the above-mentioned formation was carried out at different temperatures (room temperature, 40, 50, 60 and 70°C) using a thermostated water-bath for periods ranging from 10 to 40 min. Maximum and constant absorbance was obtained at 60°C after 20 min.

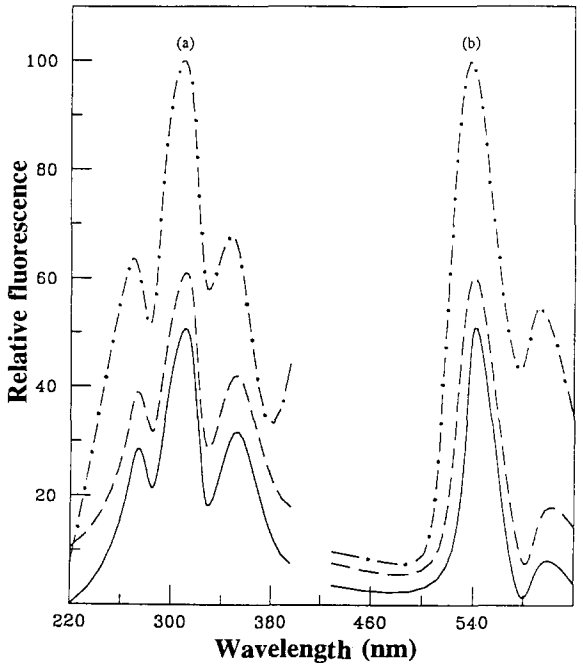


Fig. 5. (a) Excitation and (b) emission spectra of 0.07 μg ml^{-1} (CI) (—) and 0.05 μg ml^{-1} (NR) (– – –) after the formation of a ternary complex with eosin and Pd(II) solution (· · ·).

The effect of surfactants on the absorbance of the solution of the ternary complex was examined using various dispersing agents, such as benzalkonium chloride (BKC) (cationic), sodium lauryl sulphate (SLS) (anionic) and Triton-X 100, Tween 20 and methyl cellulose (non-ionic). Among the surfactants studied, best results were obtained in presence of methyl cellulose (1500 cP). Maximum and constant absorbance was obtained with 1.5 ml of 0.5% w/v methyl cellulose. The histogram in Fig. 3 shows the effect of each dispersing agent on the apparent molar absorptivity of the ternary complex solution examined.

The effect of the eosin and palladium(II) chloride concentrations on the absorbance of the ternary complex formed was studied, (i) keeping the concentration of the drug and palladium(II) chloride constant and varying the eosin concentration, and (ii) varying the palladium(II) chloride concentration while keeping the drug and eosin concentration constant. The optimum result was obtained using 0.75 ml of 2×10^{-3} M of both eosin and palladium(II) chloride solutions. The colour formed under the above-mentioned optimum conditions was stable for at least 1 h.

3.2. Constitution of the ternary complex

The nature of the ternary complex (drug–Pd(II)–eosin) was determined using Job's method of continuous variation [29]. The results of applying this method can be summarized as follows: the Pd(II):drug ratio in the presence of excess eosins was 1:1 (Fig. 4a), while the eosin:drug ratio in the presence of any excess but constant amount of palladium(II) chloride was 1:1 (Fig. 4b) and the Pd(II):eosin ratio in the presence of excess drug was 1:1 (Fig. 4c). Hence the composition of the ternary complex formed may be expressed as

Table 1
Optical characteristics and statistical data of the regression equations for ternary complex formation with ciprofloxacin hydrochloride (CI) and norfloxacin (NR)

Parameter	Ciprofloxacin hydrochloride		Norfloxacin	
	Spectrophotometry	Fluorimetry	Spectrophotometry	Fluorimetry
Beer's law range (μg ml^{-1})	3–10	0.035–0.070	3–10	0.025–0.050
Apparent molar absorptivity (l mol^{-1} cm^{-1})	3.37×10^4	–	2.73×10^4	–
Sandell's sensitivity (μg cm^{-2} per 0.001 A)	1.01×10^{-2}	–	1.13×10^{-2}	
Regression equation				
Intercept (a)	4.43×10^{-2}	−9.43	2.43×10^{-2}	8.28
Slope (b)	8.74×10^{-2}	835.89	8.56×10^{-2}	609.01
Correlation coefficient (r)	0.9999	0.09998	0.9999	0.9996
Variance (S_0^2)	1.42×10^{-5}	0.214	1.29×10^{-5}	3.57×10^{-2}

Table 2
Evaluation of the accuracy and precision of the two proposed procedures

Compared method	Added	Found ± SD[a]	RSD (%)	SAE[b]	Confidence limits[c]
Ciprofloxacin hydrochloride					
Spectrophotometry[d]	4.0	4.00 ± 0.038	0.950	0.017	4.00 ± 0.0472
	6.0	6.01 ± 0.063	1.039	0.028	6.01 ± 0.0777
	8.0	7.98 ± 0.037	0.465	0.017	7.98 ± 0.0472
			Mean: 0.818	0.021	
Fluorimetry[e]	4.5	4.50 ± 0.041	0.909	0.018	4.50 ± 0.0500
	5.5	5.50 ± 0.049	0.881	0.022	5.50 ± 0.0611
	6.5	6.50 ± 0.059	0.910	0.027	6.50 ± 0.0749
			Mean: 0.900	0.022	
Norfloxacin					
Spectrophotometry[d]	4.0	4.00 ± 0.022	0.548	0.010	4.00 ± 0.0278
	6.0	6.01 ± 0.037	0.607	0.016	6.01 ± 0.0444
	8.0	7.99 ± 0.041	0.519	0.019	7.99 ± 0.0527
			Mean: 0.558	0.015	
Fluorimetry	3.0	3.02 ± 0.040	1.31	0.018	3.02 ± 0.0500
	4.0	4.02 ± 0.036	0.89	0.016	4.02 ± 0.0444
	5.0	5.00 ± 0.047	0.93	0.021	5.00 ± 0.0583
			Mean: 1.04	0.018	

[a] Mean ± standard deviation for five determinations.
[b] SAE = standard analytical error.
[c] Confidence limits at $p = 0.95$ and four degrees of freedom.
[d] Concentration in $\mu g\ ml^{-1}$ (final concentration).
[e] The added concentrations are multiplied by 1×10^{-3}.

drug–Pd(II)–eosin (1:1:1) in the presence of methyl cellulose.

3.3. Study of the fluorimetric method

Because the formation of the ternary complex reduced the fluorescence of solution B, a fluorescence quenching method for the determination of CI and NR was developed. The uncorrected fluorescence spectra of solutions A and B are shown in Fig. 5. On addition of CI or NR to solution B, the relative fluorescence intensity of solution B decreased significantly, and the magnitude of the decrease was proportional to the concentration of the drug. In the development of the procedure for fluorimetric measurements, the same conditions as for the spectrophotometric method were adopted. The spectral and fluorimetric characteristics are summarized in Table 1.

3.4. Accuracy and precision of the methods

In order to determine the accuracy and precision of the methods, solutions containing three different concentrations of CI and NR were prepared and analysed in five replicates. The analytical results obtained from this investigation are summarized in Table 2. The mean relative standard deviation (RSD) and the mean standard analytical error (SAE) can be considered to be very satisfactory.

The proposed methods for the determination of ciprofloxacin and norfloxacin were applied to commercial tablets together with the official USP XXIII method. These determinations were carried out on the same batch of samples. The results obtained were compared statistically by Student's t-test and variance ratio F-test (Table 3). The experimental values did not exceed the theoretical values in either test, which indicates that there

Table 3
Determination of ciprofloxacin hydrochloride and norfloxacin in commercial tablets using the proposed procedures compared statistically with an official method

	Recovery ± SD (%)[b]		
	Proposed procedure		Official method[a]
Compound formulation	Spectrophotometry	Fluorimetry	
Ciprofloxacin hydrochloride			
Ciprobay tablets[e]	100.35	99.83 ± 0.77	100.15 ± 0.96
	$t^c = 0.36$	0.58	
	$F^d = 1.59$	1.55	
Cipro tablets[e]	99.55 ± 0.35	99.57 ± 0.25	99.75 ± 0.19
	$t = 1.12$	1.28	
	$F = 3.39$	1.73	
Ciprinol tablets[e]	98.93 ± 0.18	99.16 ± 0.39	99.01 ± 0.19
	$t = 0.68$	0.77	
	$F = 1.11$	4.21	
Mifoxin tablets[e]	98.02 ± 0.13	97.94 ± 0.16	97.93 ± 0.22
	$t = 0.79$	0.08	
	$F = 2.86$	1.89	
Norfloxacin			
Neofloxin tablets	97.37 ± 0.59	97.55 ± 0.46	97.60 ± 0.44
	$t = 0.70$	0.17	
	$F = 1.80$	1.09	
Noroxin tablets[f]	101.49 ± 0.47	101.01 ± 0.94	100.85 ± 0.97
	$t = 1.33$	0.26	
	$F = 4.20$	1.04	
Noracin tablets[f]	99.59 ± 0.65	99.29 ± 0.91	99.70 ± 1.40
	$t = 0.16$	0.55	
	$F = 4.68$	2.15	
Spectrama tablets[f]	99.35 ± 0.62	99.65 ± 0.98	99.55 ± 1.45
	$t = 0.28$	0.13	
	$F = 5.37$	2.19	
Neofloxin tablets[f]	97.37 ± 0.59	97.55 ± 0.46	97.60 ± 0.44
	$t = 0.70$	0.17	
	$F = 1.80$	1.09	

[a] USP XXIII [2].
[b] Mean ± standard deviation of five determinations.
[c] Tabulated t-value for $p = 0.05$ and eight degrees of freedom is 2.306.
[d] Tabulated F-value for $p = 0.05$ and $f_1 = f_2 = 4$ is 6.39.
[e] All the ciprofloxacin tablets were labelled to certain 250 mg of CI per tablet; Ciprobay tablets manufactured by Bayer, Leverkusen, Germany; Cipro tablets manufactured by Amryia Pharmaceutical Industries, Alexandria, Egypt; Ciprinol tablets manufactured by Rameda 6th of October City, Egypt, under licence from KRKA, Slovenia; Mifoxin tablets manufactured by Misr Pharmaceutical for Pharmaceutical Industries, Cairo, Egypt.
[f] All the norfloxacin tablets were labelled to contain 400 mg of norfloxacin per tablet; Noroxin tablets manufactured by EIPICO (Cairo, Egypt) under licence from Merck, Sharp & Dohme (New York, USA); Noracin tablets manufactured by Memphis Chemical (Cairo, Egypt); Spectrama tablets product of Amoun Pharmaceutical Industries (El Salam City, Egypt); Neofloxin tablets product of Alexandria Co. for Pharmaceutical and Chemical Industries (Alexandria, Egypt).

was no signficant difference between the methods compared.

4. Conclusion

The ternary complex formed under the above-mentioned conditions and measured either spectrophotometrically or spectrofluorimetrically can be regarded as an ion association complex between the Pd(II)–drug cation and the eosin anion. Compared with conventional methods using organic dyes or a metal ion alone the proposed methods, which do not require an extraction procedure, have the advantages of simplicity, sensitivity and reproducibility. The present methods should be useful for the determination of both CI and NR in tablet dosage form.

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