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Colourimetric and atomic absorption spectrometric determination of some fluoroquinolone derivatives

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Abstract

Three simple, accurate, sensitive and selective procedures for the determination of ten fluoroquinolones (amifloxacin, ciprofloxacin hydrochloride, difloxacin hydrochloride, enoxacin, enrofloxacin, lomefloxacin hydrochloride, lovefloxacin, norfloxacin, ofloxacin and pefloxacin mesylate) were described. Procedures I and II are based on the formation of ion-pair complexes between the drugs and ammonium reineckate reagent in an acidic medium at 25 $\pm 2^{o}$C and the formed precipitates are quantitatively determined either colourimetrically (procedure I) or by atomic absorption spectrometrically (procedure II). Procedure I is based on dissolving the formed precipitate with acetone, the volume was completed quantitatively and the absorbance of the solution was measured at 527 nm against pure solvent blank. The formed precipitates on the atomic absorption spectrometric procedure (procedure II) are quantitatively determined either directly or indirectly through the chromium precipitate formed or the residual un-reacted chromium in the filtrate at 358.6 nm and the optimum conditions for precipitation have been carefully studied. Procedure III is based on the reaction of the studied drugs with 2,2-diphenyl-1-picrylhydrazyl reagent (DPPH). The latter is employed to abstract a hydrogen atom from the drugs thereby promoting a process of radical coupling. This results in a reduction of the violet color of DPPH with the formation of the yellow colored 2,2-diphenyl-1-picrylhydrazine ($DPPH_2$).

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The decrease in the intensity of the violet color is used to measure the concentration of the drugs. All measurements are made at λ= 520 nm on methanolic solutions of the reagent and drugs. Beer's law is obeyed for the studied drugs in the range 2-36 $\mu g\ ml^{-1}$ with correlation coefficients not less than 0.9992. All procedures hold well accuracy and precision when applied to the analysis of the cited fluoroquinolones in different dosage forms with good recovery percent ranged from 98.88±0.40 to 100.99±0.44 without interference from additives.

Keywords: Fluoroquinolones; Reinecke's Salt ; 2,2-diphenyl-1-picrylhydrazyl; Colourimetrically ; Atomic Absorption Spectrometrically.

1. Introduction

Nalidixic acid was used primarily in the treatment of urinary tract infections but the problems of development of bacterial resistance or superinfection with inherently resistant species severely limited its use [1]. Recently, synthesis of the new quinolones with 6-fluoro and 7-piperazinyl groups resulted in enhanced activity against a wide range of gram negative and gram positive bacteria [2]. Many analytical procedures have been adopted for the determination of this class of compounds. The USP-XXIII and National Formulary-XVIII- (1995) specifies a non-aqueous titration method for norfloxacin bulk drug and an HPLC one for its dosage forms and for ciprofloxacin bulk drug and dosage forms [3], other methods are based on spectrophotometric [4-9], fluorometric [10-12] and polarographic [13] techniques. Many chromatographic methods [14-20] have been described. A number of visual spectrophotometric methods for the determination of individual compounds such as norfloxacin [10, 12], ciprofloxacin [15, 16], levofloxacin [9, 11, 13, 15] and enoxacin [8] are reported in the literature, few have been reported for other compounds. It is noteworthy to mention that, there is no general method for their determination as a large chemical group.

Reineck's salt is ammonium tetrathiocyanotodiamminochromate (III) monohydrate in which it can be used for quantitative determination of many pharmaceutical compounds applying gravimetric [21], titrimetric [22] and spectrophotometric [23] procedures.

2,2- Diphenyl-1-Picrylhydrazyl (DPPH) is an intense, violet-colored, stable, free radical which reacts as a chromogenic reagent [24,25] by extracting a hydrogen atom to form the yellow colored diphenylpicrylhydrazine ($DPPH_2$). This decrease in the intensity of the violet color is used as a measure of the quantity of the tested drugs. The radical DPPH was chosen for the present work because it does not dimerize and the problem of cage effect does not arise. In addition, it is highly colored and its concentration at any time can be estimated by its absorption in the visible range.

The purpose of the present work is to describe the development of two simple and accurate spectrophotometric procedures, as well as a selective and sensitive atomic absorption spectrometric procedure, for the analysis of the titled antibiotics in the pure form as well as in pharmaceutical preparations.

2. Experimental

2.1. Apparatus

- A Shimadzu UV1601, UV-Visible Spectrophotometer (Tokyo, Japan).
- A Shimadzu atomic absorption flame spectrophotometer model AA.640-13 with a chromium hollow cathode lamp under the following observation height above burner 1cm; single slot type burner; air flow-rate 21.51/ min; acetylene flow-rate 3.41/ min. operation conditions: lamp current 29 m A; slit width 0.7 nm; wavelength current 29 mA; slit width 0.7 nm; wavelength 358.6 nm.

2.2. Materials

All solvents and reagents were of analytical reagent grade, double distilled water was used for procedures I and II and methanol for procedure III throughout. Samples of fluoroquinolones were generously supplied by their respective manufactures: amifloxacin (Sterling Winthrop Inc., USA); difloxacin hydrochloride (Abbott Laboratories, North Chicago, USA); norfloxacin (Eipico, Cairo, Egypt); ofloxacin (Hoechst AG, Frankfurt, Germany); ciprofloxacin hydrochloride (Miles Inc. Pharmaceutical Division, West Haven, Germany); pefloxacin mesylate (Rhone-Poulenc Rorer, Neuilly/Seine, France); lomefloxacin hydrochloride (Searle, Illinois, USA); enoxacin, enrofloxacin and levofloxacin (Sigma Chem. Co., USA) and were used without further purification. Structures of the chosen fluoroquinolones are shown in Table 1.

2.3. Pharmaceutical preparations

The following available commercial preparations were analyzed: Spectrama® tablets, Batch No. 814 (Amoun Pharmaceutical Industries Co., Cairo, Egypt) labeled to contain 400 mg anhydrous norfloxacin per tablet; Neofloxacin® tablets, Batch No. 151 (Alexandria Co. for Pharmaceuticals, Alexandria, Egypt) labeled to contain 400 mg anhydrous norfloxacin per tablet; Norbactin® tablets, Batch No. 114 (Chem. Ind. Co., Giza, Egypt) labeled to contain 400 mg norfloxacin per tablet; Tarivid® tablets, Batch No. 12E06 (Hoechst Orient, Cairo, Egypt, under license of Hoechst AG, Frankfurt, Germany) labeled to contain 200 mg ofloxacin per tablet; Kirol® tablets, Batch No. 021269A (Amoun Pharmaceutical Industries Co., Cairo, Egypt) labeled to contain 200 mg ofloxacin per tablet; Mefoxin® tablets, Batch No. 2011 (Misr Co. for Pharmaceutical Industries, Cairo, Egypt) labeled to contain 250 mg ciprofloxacin hydrochloride monohydrate per tablet; Serviflox® tablets, Batch No. 950 (Under Licence from Biochemie Kundi Austria), labeled to contain 250 ciprofloxacin hydrochloride per tablet; Cipro® otic drops, Batch No. 1001111 (Chem. Indus. Develop. Co. ,Giza, Egypt)

labeled to contain 3.5 mg ciprofloxacin hydrochloride per each ml; Globacin® tablets, Batch No. 18501 (Global Napi Pharm. Egypt) labeled to contain 400 mg pefloxacin in the form of mesylate dihydrate per tablet; Peflacin® ampoules, Batch No. 102 (Rhone-Poulenc Rorer, Neuilly/Seine, France) labeled to contain 400 mg pefloxacin mesylate dihydrate and 15.3 mg sodium ascorbate per 5 ml ampoule; Tavanic® tablets, Batch No. 12E07R (Under Licence of Aventis Pharma Germany) labeled to contain 500 mg levofloxacin per tablet.

2.4. Reagents

2.4.1. Ammonium reineckate

Ammonium reineckate (Prolabo, Paris, France) were of purity not less than 99.9%. $3x10^{-3}$ M stock ammonium reineckate solutions were freshly prepared by dissolving in re-distilled de-ionized water.

2.4.2. DPPH

2,2-Diphenyl-1-picrylhydrazyl (Sigma, St. Louis, Mo, USA). A stock solution of 1.5 mg ml^{-1} was prepared by dissolving 0.15 g of DPPH in methanol and then diluted to 100 ml with the same solvent. Ten milliliter of this solution were diluted to 100 ml to give 0.15 mg ml^{-1} (working DPPH solution). The stock and working solutions were kept in a refrigerator and protected from light. The solution was found to be stable for at least one week at 4°C.

2.5. General assay procedures

2.5.1. Procedure I

In a 10 ml volumetric flask, 2 ml volume of sample drug solution, 0.5 ml of hydrochloric acid was added, 2.5 ml of fresh saturated reinecke's salt solution was added with agitation for 5 min. and complete to the volume with re-distilled de-ionized water. The formed precipitate was filtered through a sintered glass funnel (G_4) after one hour and washed thrice with 5 ml ice

water. Then the precipitate was dried in a vacuum desiccator. The formed precipitate in the crucible was then dissolved with acetone into a 25 ml volumetric flask together with the successive washings of the funnel and filtration device. The volume was completed quantitatively with acetone to the appropriated volume and the absorbance of the solution was measured at 527 nm against pure solvent as a blank.

2.5.2. Procedure II

2.5.2.1. Direct procedure

The above (drug-reineckate) precipitates were collected on a G_4 sintered glass crucible and washed with five 2 ml portions of ice water. The drugs reineckate precipitates were dissolved in 25 ml acetone. The solution was nebulized in an air-acetylene flame of AAS measurement of chromium at 358.6 nm. The absorbance was compared with a calibration graph prepared from the pure drug-reineckate solid complex under identical conditions.

2.5.2.2. *Indirect procedure*

The filtrate and washings from the direct procedure II (2.5.2.1) were collected in 100 ml volumetric flask and completed to volume with acetone. The resulting solution (2 ml) was diluted to 25 ml with acetone.

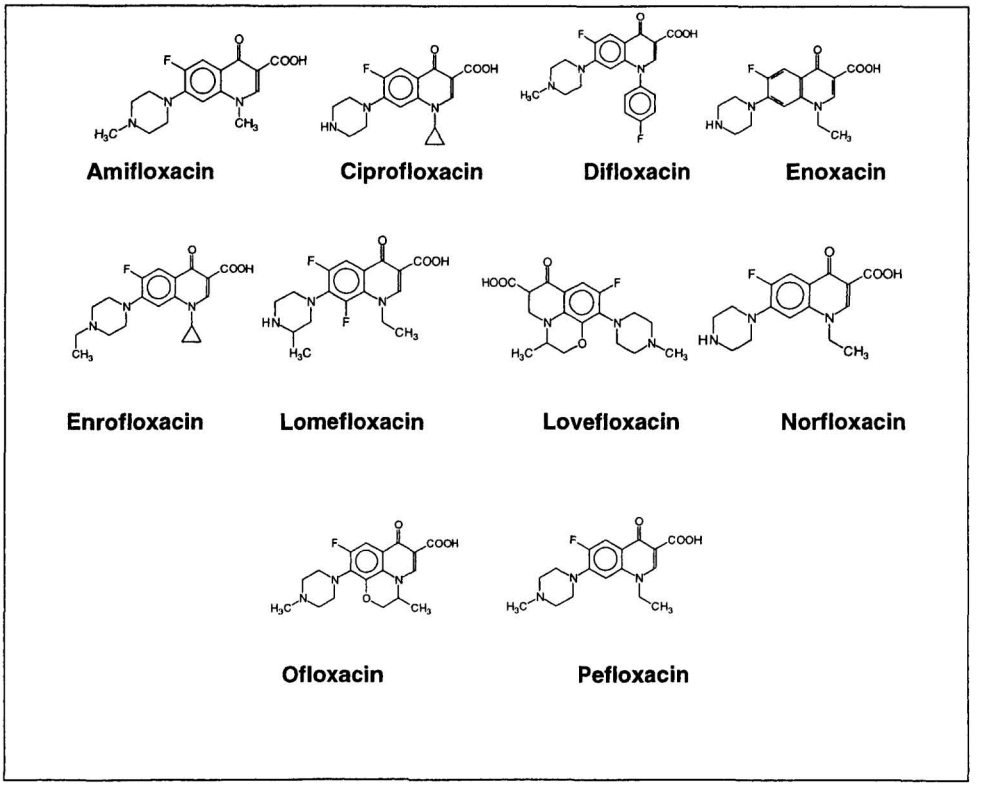
A blank (omitting addition of drugs) was prepared and absorbance was measured at the above flaming conditions. Chromium concentration was calculated from a calibration curve.

2.5.3. Procedure III

Transfer 1 ml of the investigated drug to a 10 ml volumetric flask, add 2 ml of DPPH solution. Mix well for enrofloxacin at 25°C, heat in water bath at 60 °C for 20 minutes for amifloxacin, difloxacin, enoxacin, lomefloxacin hydrochloride and pefloxacin mesylate and at 70 °C for 15 minutes for ciprofloxacin hydrochloride, lovefloxacin, norfloxacin and ofloxacin. Cool and

complete to the mark with methanol. Measure the absorbencies of the sample and a reagent blank against methanol at λ=520 nm. Calculate ΔA, i.e. the difference between the absorbance values of the blank and sample, which corresponds to the drug concentration.

Table 1. Structures of the investigated fluoroquinolones



2.6. Preparation of samples

2.6.1. Tablets

The contents of twenty tablets of the drug were thoroughly ground. A quantity equivalent to 50 mg drug was accurately weight into a 100 ml volumetric flask, completed to volume with the appropriated solvent, filtered, and the procedures were completed as under procedures I-III.

2.6.2. Vials

An accurately measured weight of vials equivalent to 50 mg of drug was dissolved in the appropriated solvent, (by shaking for 5 minutes), filtered if necessary. The solution was completed to 100 ml with re-distilled de-ionized water for procedures I and II and with methanol for procedure III. The procedures were completed as under procedures I-III.

2.6.3. Drops

Aliquot of Cipro® otic drops equivalent to 50 mg ciprofloxacin hydrochloride was transferred into a 100 ml volumetric flask and completed to volume with the appropriated solvent. The procedures were completed as under procedures I-III.

3. Results and Discussion

Mixing each aqueous solution of amifloxacin, ciprofloxacin hydrochloride, difloxacin hydrochloride, enoxacin, enrofloxacin, lomefloxacin hydrochloride, lovefloxacin, norfloxacin, ofloxacin and pefloxacin mesylate with ammonium reineckate in acidic medium at 25±2°C resulted in formation of red precipitate. It is based on the formation of ion-pair complexes between the drugs and ammonium reineckate (procedures I and II).

On the first colourimetric procedure, the absorption spectrum of the reaction products was measured at 527 nm.

On the atomic absorption spectrometric procedure, acidic solutions of the drugs gave purple coagulated precipitates with ammonium reineckate. These precipitates form the basis of the micro-quantitative determinations of the cited fluoroquinolones. The chromium ion content could be determined either directly in the precipitate or indirectly in the filtrate.

The ultraviolet-visible spectra of the assay solution of one of the tested drugs, DPPH and $DPPH_2$ (procedure III) are shown in Figure 1. The reaction is assumed to proceed via abstraction of hydrogen atoms from the drugs by DPPH [26]. This is accompanied by the change of violet color of DPPH to give

the yellow colored $DPPH_2$. Measure the absorbencies of the sample and a reagent blank against methanol at λ=520 nm.

The different variables that affect the determinations of all the studied fluoroquinolones with ammonium reineckate reagent (procedures I and II) and DPPH reagent (procedure III) were studied and optimized.

3.1. Optimization of the reaction conditions

3.1.1. Effect of pH

3.1.1.1. Reineckate methods

The cited fluoroquinolones reineckate salts were prepared starting with the same concentration of the drug and the ammonium reineckate while varying the type and amounts of the acid. The absorbencies of the final salt solutions in the appropriate solvent were taken as a measure of better precipitation.

Some 0.5 ml hydrochloric acid per 10 ml of the final solution mixture was the most suitable amount for complete precipitation of the cited fluoroquinolones.

3.1.1.2. DPPH method

The effect of pH on color reduction was studied for the investigated drugs. The results revealed that the reaction was independent on pH. Because of this independence of the reaction on pH, further investigations were not carried out to establish whether the constituents or pH range of the buffer solutions have not any effect on the interaction of DPPH with the investigated drugs.

3.1.2. Reagent concentration

3.1.2.1. Ammonium reineckate concentration

The general procedures (I and II) were applied using different concentrations of the reagent while the cited fluoroquinolones and acid

concentrations were constant. The absorbencies of the final salt solutions in the appropriate solvent were taken as a measure of better precipitation.

A total of 2.5 ml of 2% ammonium reineckate was sufficient for complete precipitation of fluoroquinolones-hydrochloric acid in the final solution.

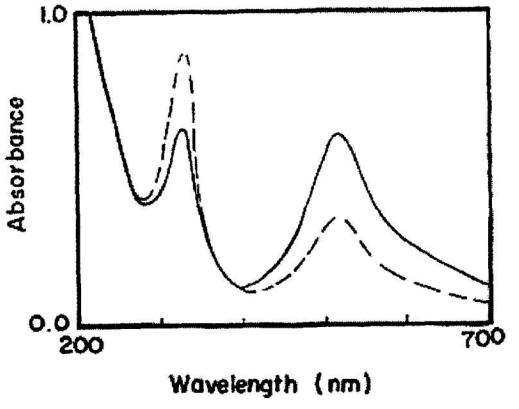


Figure 1. Absorption spectra of (—) DPPH, reaction mixture of DPPH-ofloxacin (-----)

3.1.2.2. DPPH concentration

DPPH is added in excess to that required to react with drugs to be analyzed. By measuring the excess reagent, the consumed DPPH would correspond to the amount of the drug.

The concentration of the reagent that gives the highest absorption value within the participle sensitivity range of absorbance was found to be 0.15 mg ml^{-1}. Two milliliters of this solution per 10 ml of the reaction mixture was used.

3.1.3. Reaction time and temperature

3.1.3.1. Reineckate methods

A series containing equal concentrations of the fluoroquinolones was analyzed using the corresponding standard procedure, but filtering the precipitate after various time intervals. The absorbance of the final

fluoroquinolones-reineckate solution in the appropriate solvent were taken as a measure for the best precipitation time.

One hour was found to be sufficient for complete precipitation of fluoroquinolones-reineckate on standing at 25 °C.

The effect of temperature on the formation of the coloured precipitate was investigated. The experiments were carried out at room temperature (25±2°C).

3.1.3.2. DPPH method

The reaction time was determined for the interaction of each studied drug with DPPH solution by following the color reduction at ambient temperature (25 ± 2 °C), 30, 40, 50, 60 and 70 °C. The optimum temperature and reaction time were recorded in table 2. The ΔA reached a constant level, at once and remained constant for at least 180 minutes.

Table 2. Reaction time and temperature of DPPH method

Drug	Temperature °C	Heating time Min.
Amifloxacin	60	20
Ciprofloxacin HCl	70	15
Difloxacin HCl	60	20
Enoxacin	60	20
Enrofloxacin	25	----
Lomefloxacin HCl	60	20
Lovefloxacin	70	15
Norfloxacin	70	15
Ofloxacin	70	15
Pefloxacin mesylate	60	20

3.1.4. Solubility and stability of the precipitated reineckates

Trials to find out the best solvent to dissolve fluoroquinolones-reineckate precipitate were performed using distilled water, acetone, dioxan,

methanol and ethanol. Then the stability of the produced colour in each solvent was examined periodically at different time intervals over 24 hours.

The colour of fluoroquinolones-reineckate acetone solution was stable for at least 24 hours.

3.1.5. Influence of solvents

3.1.5.1. Reineckate methods

The washing liquid of choice was ice water and acetone was the most suitable solvent.

3.1.5.2. DPPH method

The effect of dilution of the reaction product by different solvents namely, methanol, ethanol, n-propanol, isopropanol, n-butanol, acetone was studied. The results indicated that all solvents had no effect on the position of maximum absorption while the reactivity (ΔA value) was affected. Methanol was found to be the most suitable solvent.

3.2. Quantification

3.2.1. Beer's law

3.2.1.1. Reineckate methods

Standard curves were constructed by plotting the observed absorbencies readings versus the concentrations of fluoroquinolones in μg ml^{-1} of the final solution of the experiments. Conformance to Beer's law was evident. Plots showed good linearity with high correlation coefficients (Table 3).

3.2.1.2. DPPH method

Under the optimum parameters, Beer's law was obeyed for all studied drugs in the range shown in Table 3. The regression analysis of ΔA value

versus concentration were done for all the studied drugs according to the linear regression equation summarized in Table 3.

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3.2.2. Validation of the proposed procedures

Accuracy, precision, specificity, detection limit, quantitation limit and linearity range were tested (Tables 3 and 4).

Statistical analysis of the results obtained by the proposed procedures (I,II and III) compared with those of the official methods [27] for ciprofloxacin hydrochloride, norfloxacin and ofloxacin are given in table 3, where the remaining fluoroquinolones are not official, in which they are compared with reported methods [28-30]. The calculated t and F values do not exceed the tabulated ones, revealing equal precision and accuracy (Table 4).

3.2.3. Stoichiometric relationships

3.2.3.1. Reineckate methods

For the atomic absorption spectrometric method, the Job's method of continuous variation [38] indicated a molar ratio of 1:2 drug to reineckate (Figure 2).

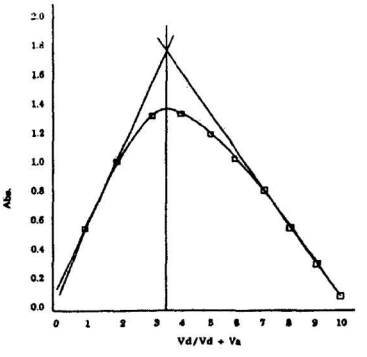


Figure 2. Job's plot of continuous variation between ofloxacin and reineck's salt.

Table 3. Parameters for calibration curves construction

Drug	Procedure	Linearity ($\mu g\ ml^{-1}$)	a	b	r	LOD	LOQ	C.V. (%)
Amifloxacin	Procedure I	8-32	0.0210	1.0230	0.9998	0.032	0.351	0.52
	Procedure II[a]	5-30	0.0025	0.1250	0.9999	0.352	0.251	0.30
	Procedure II[b]	5-30	0.0012	0.0361	0.9995	0.251	0.122	0.54
	Procedure III	2-12	0.0002	0.3015	0.9998	0.020	0.851	0.50
Ciprofloxacin hydrochloride	Procedure I	4-30	0.0098	0.0985	0.9994	0.142	0.351	0.53
	Procedure II[a]	5-30	0.0002	0.0258	0.9996	0.251	0.351	0.60
	Procedure II[b]	5-30	0.0054	0.0099	0.9999	0.119	0.333	0.60
	Procedure III	3-18	0.0259	0.0987	0.9994	0.250	0.251	0.55
Difloxacin Hydrochloride	Procedure	6-36	0.0369	0.0369	0.9996	0.021	0.259	0.52
	Procedure II[a]	5-30	0.0031	0.0753	0.9992	0.039	0.267	0.45
	Procedure II[b]	5-30	0.0811	0.0114	0.9999	0.084	0.301	0.45
	Procedure III	2-16	0.0038	0.0941	0.9999	0.091	0.364	0.49
Enoxacin	Procedure I	9-32	0.0100	0.0369	0.9999	0.021	0.412	0.62
	Procedure II[a]	5-30	0.0365	0.0963	0.9993	0.029	0.051	0.62
	Procedure II[b]	5-30	0.0257	0.1020	0.9995	0.088	0.192	0.63
	Procedure III	4-24	0.0036	0.0418	0.9997	0.120	0.521	0.59
Enrofloxacin	Procedure I	3-30	0.0098	0.0874	0.9992	0.021	0.315	0.63
	Procedure II[a]	5-30	0.0500	0.0974	0.9997	0.081	0.052	0.63
	Procedure II[b]	5-30	0.0213	0.1265	0.9999	0.028	0.011	0.59
	Procedure III	2-12	0.1590	1.0222	0.9992	0.014	0.021	0.59
Lomefloxacin hydrochloride	Procedure I	5-30	0.0009	1.0011	0.9993	0.024	0.022	0.55
	Procedure II[a]	5-30	0.0210	0.0032	0.9999	0.021	0.312	0.54
	Procedure II[b]	5-30	0.0365	0.1330	0.9998	0.052	0.154	0.49
	Procedure III	2-16	0.0084	0.9521	0.9996	0.001	0.364	0.51
Lovefloxacin	Procedure I	5-35	0.0009	0.0941	0.9995	0.110	0.241	0.63
	Procedure II[a]	4-24	0.1020	1.0031	0.9994	0.011	0.361	0.53
	Procedure II[b]	4-24	0.0099	0.9851	0.9899	0.102	0.367	0.52
	Procedure III	2-12	0.0051	0.0521	0.9999	0.100	0.651	0.57
Norfloxacin	Procedure I	9-30	0.0052	0.0350	0.9992	0.121	0.211	0.49
	Procedure II[a]	5-30	0.1230	1.2000	0.9993	0.047	0.371	0.53
	Procedure II[b]	5-30	0.0982	0.9871	0.9999	0.158	0.300	0.55
	Procedure III	5-25	0.0211	0.0201	0.9998	0.200	0.411	0.60
Ofloxacin	Procedure I	5-35	0.0036	0.0965	0.9997	0.365	0.330	0.61
	Procedure II[a]	5-30	0.1020	0.0278	0.9992	0.054	0.254	0.51
	Procedure II[b]	5-30	0.2150	0.0122	0.9996	0.014	0.320	0.59
	Procedure III	4-24	0.2591	0.0398	0.9992	0.351	0.214	0.63
Pefloxacin mesylate	Procedure I	5-32	0.0360	0.0248	0.9999	0.036	0.251	0.50
	Procedure II[a]	6-36	0.0321	0.0325	0.9993	0.214	0.264	0.49
	Procedure II[b]	6-36	0.0540	0.0952	0.9999	0.229	0.123	0.48
	Procedure III	2-12	0.0258	0.0364	0.9999	0.036	0.221	0.40

a: intercept; b: slope; r: correlation coefficient; LOD: limit of detection; LOQ; limit of quantitation; procedure II[a]: direct atomic absorption spectroscopic method; procedure II[b]: indirect atomic absorption spectroscopic method.

Table 4

Statistical analysis of the results obtained using the proposed procedures and reference methods for analysis of authentic samples

Drug		Proc. I	Proc. II Direct	Proc. II Indirect	Proc. III	Reference methods
Amifloxacin	$\bar{X}$ ±S.D.	99.20±0.52	98.98±0.30	99.35±0.54	99.35±0.50	99.22±0.50 [28]
	V	0.27	0.09	0.29	0.25	0.25
	t	0.06	0.92	0.35	0.37	
	F	1.08	2.78	1.16	1.00	
Ciprofloxacin hydrochloride	$\bar{X}$ ±S.D.	99.12±0.53	99.21±0.60	99.00±0.60	98.92±0.55	98.90±0.52 [27]
	V	0.28	0.36	0.36	0.30	
	t	0.59	0.76	0.24	0.05	
	F	1.02	1.33	1.33	1.11	
Difloxacin hydrochloride	$\bar{X}$ ±S.D.	100.01± 0.52	100.30±0.45	99.95±0.42	100.00±0.49	99.98±0.49 [28]
	V	0.27	0.20	0.18	0.24	0.24
	t	0.08	0.98	0.10	0.06	
	F	1.13	1.20	1.33	1.00	
Enoxacin	$\bar{X}$ ±S.D.	99.00±0.62	99.04± 0.62	99.01±0.63	98.96±0.59	98.93±0.60 [30]
	V	0.38	0.38	0.40	0.35	0.36
	t	0.16	0.25	0.18	0.07	
	F	1.05	1.06	1.11	1.03	
Enrofloxacin	$\bar{X}$ ±S.D.	99.96± 0.63	100.09±0.63	99.98±0.59	100.09±0.59	100.22±0.60 [28]
	V	0.40	0.40	0.35	0.35	0.56
	t	0.59	0.30	0.58	0.31	
	F	1.11	1.11	1.03	1.03	
Lomefloxacin hydrochloride	$\bar{X}$ ±S.D.	100.01±0.55	99.85±0.54	100.05±0.49	99.98±51	99.93±0.53 [28]
	V	0.30	0.26	0.24	0.26	0.28
	t	0.21	0.22	0.34	0.14	
	F	1.07	1.08	1.17	1.08	
Lovefloxacin	$\bar{X}$ ±S.D.	100.30±0.63	99.93±0.53	100.21±0.52	99.98±0.57	99.99±0.58 [28]
	V	0.40	0.28	0.27	0.32	0.34
	t	0.71	0.15	0.58	0.03	
	F	1.18	1.21	1.26	1.06	
Norfloxacin	$\bar{X}$ ±S.D.	100.09±0.49	100.00±0.53	99.98±0.55	99.95±0.60	100.02±0.52 [27]
	V	0.24	0.28	0.30	0.36	0.27
	t	0.20	0.05	0.10	0.17	
	F	1.13	1.04	1.11	1.33	
Ofloxacin	$\bar{X}$ ±S.D.	99.00±0.61	99.09±0.51	98.96±0.59	99.32±0.63	98.92±0.57 [27]
	V	0.37	0.26	0.35	0.40	0.32
	t	0.19	0.45	0.10	0.92	
	F	1.16	1.23	1.09	1.25	
Pefloxacin Mesylate	$\bar{X}$ ±S.D.	100.31±0.50	99.98±0.49	100.50±0.48	99.94±0.40	100.29±0.44 [29]
	V	0.25	0.24	0.23	0.16	0.19
	t	0.06	0.91	0.87	0.90	
	F	1.32	1.26	1.21	1.19	

Three and six determinations were used for the reported and the reference methods, respectively. The tabulated values of t and F at 95% confidence limit are t=2.23 and F=5.79.

3.2.3.2. DPPH method

The Job's method of continuous variation [31] indicated a molar ratio of 1:1 drug to DPPH reagent (Figure 3). Such results obtained have been indicating that one abstractable hydrogen is present in the drug molecule.

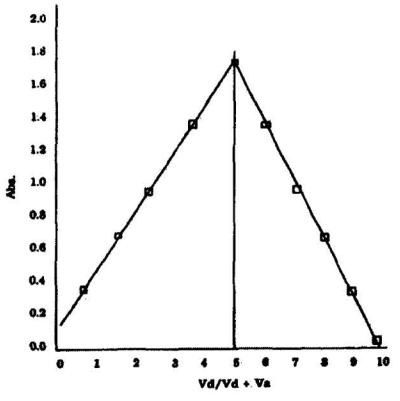


Figure 3. Job's plot of continuous variation between ofloxacin and DPPH reagent

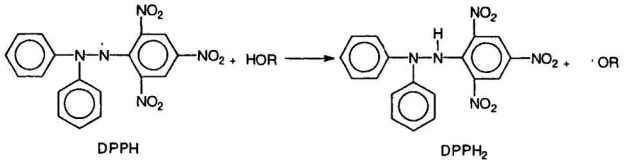
3.2.4. Reaction mechanism

3.2.4.1. Reineckate methods

Fluoroquinolones hydrochloride + ammonium reineckate = ammonium chloride + fluoroquinolones reineckate

3.2.4.2. DPPH method

The ultraviolet-visible spectra of the assay solution of one of the tested drugs, DPPH and $DPPH_2$ are shown in Figure 1. The reaction is assumed to proceed via abstraction of hydrogen atoms from the drugs by DPPH. This is accompanied by the change of violet color of DPPH to give the yellow colored $DPPH_2$ and the corresponding free radical of the drug (Scheme 1).



Scheme 1

3.2.5. Interferences

Before dealing with the analysis of the pharmaceutical preparations, the effect of common additives, adjuvants and excepients on the proposed methods were experimentally studied. The results obtained, revealed that hydroxypropyl methylcellulose, microcrystalline cellulose, sodium stearyl fumarate, titanium dioxide, talc, iron oxide red, iron oxide yellow, glucose, lactose, magnesium stearate and starch do not interfere.

3.2.6. Application

The applicability of the methods to various dosage forms were checked by analyzing synthetic mixtures containing the cited fluoroquinolones.

The results of the analysis of pharmaceutical preparations by the suggested procedures (I, II and III) are comparable to the official and reported methods and show good correlation and reveal good applicability without interference (Table 5).

The proposed methods are non-specific with regard to differentiation between the different fluoroquinolones. This shortcoming does not affect the utility of the method in routine analysis and content uniformity determination of these drugs as they singly prescribed. The enhanced sensitivity allows for additional advantage of handling without fear of increasing errors.

As conclusion, the developed methods are simpler, faster, more sensitive and accurate than the official titrimetric and chromatographic methods. In addition, the atomic absorption spectrometric method is selective and suitable for routine quality control.

Table 5

Statistical analysis of the results obtained using the proposed procedures and reference methods for analysis of fluoroquinolones in their pharmaceutical preparations.

Drug		Proc. I	Proc. II Direct	Proc. II Indirect	Proc. III	Reference methods
Spectrama® tablets	$\bar{X}$ ±S.D.	99.99±0.54	100.29±0.55	100.53±0.49	100.09± 0.55	100.60±0.51 [28]
	V	0.29	0.30	0.24	0.30	0.26
	t	1.63	0.81	0.20	1.34	
	F	1.12	1.15	1.08	1.15	
Neofloxacin® tablets	$\bar{X}$ ±S.D.	98.98±0.60	99.00±0.62	99.99±0.61	100.01±0.56	99.21±0.65 [29]
	V	0.36	0.38	0.37	0.31	0.42
	t	0.53	0.47	1.78	1.47	
	F	1.17	1.11	1.14	1.35	
Norbactin® tablets	$\bar{X}$ ±S.D.	100.40±0.53	100.05±0.63	100.00±0.63	99.97±0.58	99.98±0.60 [27]
	V	0.28	0.40	0.40	0.34	0.36
	t	1.08	0.16	0.05	0.02	
	F	1.29	1.11	1.11	1.06	
Tarivid® tablets	$\bar{X}$ ±S.D.	99.99±0.66	100.05±0.66	99.98±0.70	100.10±0.66	100.10±0.67 [29]
	V	0.44	0.44	0.49	0.44	0.45
	t	0.23	0.11	0.25	0.00	
	F	1.02	1.02	1.09	1.02	
Kirol® tablets	$\bar{X}$ ±S.D.	100.36±0.50	100.99±0.44	100.99±0.43	100.89±0.50	101.10±0.46 [29]
	V	0.25	0.19	0.18	0.25	0.21
	t	2.13	0.35	0.28	0.61	
	F	1.19	1.11	1.17	1.19	
Mefoxin® tablets	$\bar{X}$ ±S.D.	100.11±0.49	99.88±0.44	100.06±0.50	99.99±0.52	99.89±0.51 [27]
	V	0.24	0.19	0.25	0.27	0.26
	t	0.62	0.03	0.48	0.27	
	F	1.08	1.37	1.04	1.04	
Serviflox® tablets	$\bar{X}$ ±S.D.	100.09±0.45	99.59±0.40	99.99±0.51	100.03±0.41	99.94±0.44 [30]
	V	0.20	0.16	0.26	0.17	0.19
	t	0.48	1.21	0.14	0.30	
	F	1.05	1.19	1.37	1.12	
Cipro otic® Drops	$\bar{X}$ ±S.D.	100.08±0.55	99.89±0.50	100.04±0.49	100.15±0.51	100.01±0.49 [27]
	V	0.30	0.25	0.24	0.26	0.24
	t	0.19	0.34	0.09	0.40	
	F	1.25	1.04	1.00	1.08	
Globacin® tablets	$\bar{X}$ ±S.D.	99.01±0.44	99.00±0.45	98.88±0.40	99.50± 0.44	98.85±0.40 [28]
	V	0.19	0.20	0.16	0.19	0.16
	t	0.53	0.49	0.11	2.13	
	F	1.19	1.25	1.00	1.19	
Peflacin® ampoules	$\bar{X}$ ±S.D.	100.00±0.40	100.01±0.46	100.11±0.50	100.22±0.49	100.20±0.44 [29]
	V	0.16	0.21	0.25	0.24	0.19
	t	0.69	0.60	0.26	0.06	
	F	1.19	1.11	1.32	1.26	
Tavanic® tablets	$\bar{X}$ ±S.D.	99.52±0.60	98.97±0.55	99.54±0.51	100.02±0.54	99.29±0.52 [28]
	V	0.36	0.30	0.26	0.29	0.27
	t	0.56	0.87	0.69	1.95	
	F	1.33	1.11	1.04	1.07	

Three and six determinations were used for the reported and the reference methods, respectively. The tabulated values of t and F at 95% confidence limit are t=2.23 and F=5.79.

References

[1] Ross D L, Riley C M.
Antibacterial effect of nalidixic acid derivatives.
Int. J. Pharm. 1990;63:237-40.

[2] Desplaces N, Gutman L, Carlet J.
Synthesis of new quinolones resulted in enhanced activity against a wide range of bacteria.
J. Antimicrob. Chemotherap. 1986;17(a):25-30.

[3] United Sates Pharmacopeia XXIII, National Formulary XVIII, US Pharmacopeial Convention, Rockville, M. D.1995.

[4] Belal F, Al-Majd A A, Al-Obaid A M.
Methods of analysis of 4-quinolone antibacterials
Talanta 1999;50;4:765-86.

[5] Rizk M, Belal F, Ahamed S M, El-Enany N M.
A simple kinetic spectrophotometric method for the determination of certain 4-quinolones in drug formulations.
Sci. Pharm. 2000;68;2:173-88.

[6] Sauvaigo S, Douki T, Odin F, Caillat S, Ravanat J, Cadet J.
Analysis of fluoroquinolone photosensitization of 2-deoxyguanosine, calf thymus and cellular DNA.
Photochem. Photobiol. 2001;73;3:230-37.

[7] Park H, Oh C, Lee H, Lee J, Yang K, Bark K.
Spectroscopic properties of fluoroquinolone antibiotics in water-methanol and water acetonitrile mixed solvents.
Photochem. Photobiol. 2002;75;3:237-48.

[8] Altiokka G, Atkosar Z, Can N O.
The determination of levofloxacin and enoxacin by flow injection using UV detection in pharmaceutical preparations.
J. Pharm. Biomed. 2002;30;3:881-5.

[9] Incilay S, Ayla T.
Spectrophotometric determination of enoxacin as ion-pairs with bromophenol blue and bromocresol purple in bulk and pharmaceutical dosage form.
J. Pharm. Biomed. 2002;29;3:545-54.

[10] Cordoba-Diaz M, Cordoba-Borrego M, Cordoba-Diaz D.
Modification of fluorescent properties of norfloxacin in the presence of certain antacids.
J. Pharm. Biomed. 1998;18;4-5:565-71.

[11] Juan A O G, Manuel C M.
Spectrofluorometric determination of levofloxacin in tablets, human urine and serum.
Talanta 2000;52;6:1149-56.

[12] Wu S, Wujuan Z, Chen X, Hu Z Hooper M, Martin H, Beveley Z.
Fluorescence characteristic study of the ternary complex of fluoroquinolone antibiotics and cobalt (II) with ATP.
Spectrochim. Acta (A) 2001;57;6:1317-24.

[13] Atkosar Z, Altiokka G, Ergun B.
Pulse polargraphic determination of levofloxacin in tablets.
Pharmazie 2002;57;8:587-89.

[14] Bottcher S, Baum H, Hoppe-Tichy T, Benz C, Sonntag H.
An HPLC assay and a microbiological assay to determine levofloxacin in sot tissue, bone, bile and serum.
J. Pharm. Biomed. Anal. 2001;25;2:197-203.

[15] Antonio D C, Manuela N.
Liquid chromatographic-mass spectrometric methods for analyzing antibiotic and antibacterial agents in animal food products.
J. Chromatogr A 2002;974;1-2:53-89.

[16] Cheng F C, Tsai T R, Chen Y F, Hung L C, Tsai T H.
Pharmacokinetic study of levofloxacin and ciprofloxacin in rat blood and bile by microanalysis and high-performance liquid chromatography.
J. Chromatogr A 2002;961;1:131-36.

[17] Ulrike N, Christian J, Martin F, Walter J, Markus M, Bernhard X.
Simultaneous determination of levofloxacin and ciprofloxacin in in microdialysis and plasma by high-performance liquid chromatography.
Anal. Chimica. Acta 2002;463;2:199-206.

[18] Toussaint B, Bordin G, Janosi A, Rodriguez A R.
Validation of a liquid chromatography-tandem mass spectrometry method for the simultaneous quantification of 11 fluoroquinolone antibiotics in swine kidney.
J. Chromatogr A 2002;976;1-2:195-206.

[19] Cinquina A L, Roberti P, Giannetti L, Longo F, Draisci R, Fagiolo A, Brizioli N R.
Determination of enrofloxacin and its metabolite ciprofloxacin in goat milk by high-performance liquid chromatography with diode-array detection.
J. Chromatogr A 2003;987;1-2:221-6.

[20] Sinnaeve T N, Decaestecker I J, Claerhout P K, Jean-Paul R, Jan F.
Confirmation of ofloxacin precipitation in corneal deposits by microbore liquid chromatography-quadrupole time of flight tandem mass spectrometry.
Tecn. Biomed. Sci. 2003;1:193-6.

[21] Jerzy F.
Gravimetric method for determination of some pharmaceutical compounds.
Acta Pol. Pharm. 1975;32:603-7.

[22] Olech A.
Usage of reineck's salt for titrimetric determination of some compounds.
Acta Pol. Pharm. 1976;33:101-5.

[23] Salem H, Askal H.
Colourimetric and AAS determination of cephalosporins using reineck's salt.
J. Pharm. Biomed. Anal. 2002;29:347-54.

[24] Hosny E S.
Spectrophotometric determination of some antibiotics in pharmaceutical preparations.
Bull. Pharm. Sci. 1997;20;1:87-93.

[25] Salem H
Spectrophotometric determination of some pharmaceutical compounds using 2,2-diphenyl-1-picrylhydrazyl.
Sci. Pharm. 2000;68:403-19.

[26] Forrester A R, Hay J M.
Organic Chemistry of Stable Free Radicals, Academic Press, London. 1986.

[27] United Sates Pharmacopeia 24, National Formulary 19, US Pharmacopeial Convention, Rockville, M. D.2000.

[28] Glenn A L.
Vierordt's method for statistical analysis.
J. Pharm. Pharmacol 1960;12:595-608.

[29] Saleh G A.
Spectrophotometric determination of certain fluoroquinolones
Bull. Pharm. Assiut 1997;20:27-36.

[30] El-Adl S M, Metwally K A, Abou-Kull M E.
Determination of some fluoroquinolones colourimetrically.
Zag. J. Pharm. Sci. 1999;8;2:13-7.

[31] Rose j.
Advanced Physico-Chemical Experimental, Pittman, London. 1964;54.

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