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Spectrofluorimetric, Atomic Absorption Spectrometric and Spectrophotometric Determination of Some Fluoroquinolones

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Abstract: Simple, accurate, sensitive and selective spectrofluorimetric, atomic absorption spectrometric and spectrophotometric methods are described for the quantitative determination of ten fluoroquinolones (amifloxacin, ciprofloxacin hydrochloride, difloxacin hydrochloride, enoxacin, enrofloxacin, lomefloxacin hydrochloride, levofloxacin, norfloxacin, ofloxacin and pefloxacin mesylate). The first method was a spectrofluorimetric method in which samples of the studied drugs in 0.1 N H_2SO_4 showed native fluorescence at 450 nm when excitation was at 290 nm. The calibration graph was rectilinear from 0.3-1.4 μg mL^{-1} (method I). Cobalt sulphate was used for precipitation of the ion associates formed from the reaction with the cited drugs. The formation and solubility of the solid complexes at the optimum conditions of pH and ionic strength values have been studied. The method depends on direct determination of the ions in the precipitate or indirect determination of the ions in the filtrate by atomic absorption spectroscopy. The optimum conditions for precipitation were carefully studied. Rectilinear calibration graphs were obtained in the range of 3-30 μg mL^{-1} for each of the investigated drugs. The molar ratios of the formed chelats were determined by Job's method and their association constants were also calculated (method II). Ammonium vanadate was used for the spectrophotometric determination of the selected fluoroquinolones by oxidation in sulphuric acid medium resulting in the development of a greenish blue colour measured at 766 nm which was attributed to the vanadium (IV) produced by reduction of vanadium (V) by the selected drugs. The optimum conditions for heating time, reagent concentration and sulphuric acid concentration were carefully studied. The accuracy and precision of the proposed method was confirmed by estimating five or six replicates within Beer's law limits were obtained in the range 10-40 μg mL^{-1} for each of the investigated drugs with correlation coefficients not less than 0.9994 for the investigated drugs (method III). The developed spectrofluorimetric, atomic absorption spectrometric and spectrophotometric methods were applied successfully for the determination of the studied drugs in their pharmaceutical dosage forms with a good precision and accuracy compared to official and reported methods as revealed by t-and F-tests. The first method was approximately ten times more sensitive than the second and third methods. Atomic absorption spectrometric method was also applied for the determination of studied drugs in spiked urine and plasma samples.

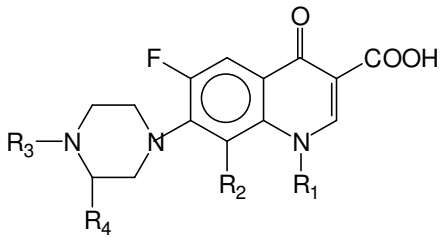
Key words: Fluoroquinolones, Spectrofluorimetry, 0.1N H_2SO_4, Atomic Absorption Spectrometry, Cobalt Suphate, Spectrophotometry, Ammonium Vanadate, Pharmaceutical Dosage Forms, Biological Fluids

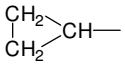
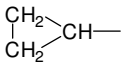
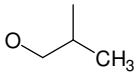
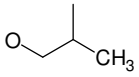
INTRODUCTION

Quinolones comprise an interesting group of antibacterials whose action is based on their anti-DNA activity. They all possess a carboxylic group in position 3 and a carbonyl group in position 4, hence they are often referred to as 4-quinolones. Their antibacterial activity is greatly increased by the addition of 6-fluoro and 7-piperazinyl groups to the molecule and named fluoroquinolones. They are the second-generation members of quinolones and are greatly effective against both gram-negative and gram-positive pathogens that are resistant to other antibacterials [1]. The structures of the investigated quinolones are given in Table 1.

Several methods have been reported for the determination of quinolones in pure form, in dosage forms and in biological fluids. Nalidixic acid, norfloxacin, ciprofloxacin and its hydrochloride are official in both USP XXIV [2] and BP 1998 [3], while ofloxacin is official in USP XXIV only. Both USP XXIV and BP 1998 recommend HPLC methods for determination of ciprofloxacin in raw material and in dosage forms. The USP XXIV recommends non-aqueous titration methods for determination of nalidixic acid, norfloxacin and ofloxacin in raw material, while HPLC methods are described for analysis of their dosage forms. The BP 1998 recommends a non-aqueous titration method for determination of nalidixic

Table 1: Structures of the Investigated Fluoroquinolones



Compound	R_1	R_2	R_3	R_4
Amifloxacin	$NHCH_3$	H	CH_3	H
Ciprofloxacin		H	H	H
Difloxacin	C_6H_4-F	H	CH_3	H
Enoxacin	C_2H_5	H	H	H
Enrofloxacin		H	C_2H_5	H
Lomefloxacin	C_2H_5	F	H	CH_3
Levofloxacin			CH_3	H
Norfloxacin	C_2H_5	H	H	H
Ofloxacin			CH_3	H
Pefloxacin	C_2H_5	H	CH_3	H

acid and norfloxacin in raw material and spectrophotometric method for determination of norfloxacin in dosage forms. Several methods for determination of quinolones including: titrimetric [4-6], spectrophotometric [7-16], spectrofluorometric [17-24], electrochemical [25-27], electrophoretic [28] and chromatographic methods [29-33] were proposed. Several methods including chelation of fluoroquinolones with Fe(III) [8,13], Cu(II) [18], Al(III) [16, 19, 22], Mg (II) [16, 19], Ca (II) [16] and Tb(III) [23] were reported. Most of the analytical methods employed for the determination of the studied drugs in biological fluids are HPLC methods which require complex and expensive equipment, provision for use and disposal of solvents, labour-intensive sample preparation procedure and personnel skilled in chromatographic techniques.

Although atomic absorption spectrometry is a rapid method and has very low detection limits which can not be reached by most of other methods, it has not been applied yet to the determination of these drugs. The present study includes new direct and indirect methods for determination of amifloxacin, ciprofloxacin hydrochloride, difloxacin hydrochloride, enoxacin, enrofloxacin, lomefloxacin hydrochloride, levofloxacin, norfloxacin, ofloxacin and pefloxacin mesylate. The present study represents the utilization of cobalt sulphate as reagent for the determination of the studied drugs by direct and indirect atomic absorption spectrometric measurements.

The spectrofluorimetric, atomic absorption spectrometric and spectrophotometric methods proved to be very sensitive and accurate for the determination of these compounds in bulk powders, in pharmaceutical dosage forms and in biological fluids.

MATERIALS AND METHODS

Apparatus: Shimadzu recording spectrofluorophotometer. Modl RF-540 (P/N204-0-2900). Calibrated before use and connected to a printing recorder.

Spectronic ™ Genesys™, UV/VIS spectrophotometer connected to an IBM computer loaded with the Winspec™ application software.

A Shimadzu atomic absorption flame spectrophotometer model AA.640-13. For AAS, cobalt was measured at wavelength 240.7 nm, slit width 0.2 nm, relative noise 1.0, detection limit 0.01 μgmL^{-1}, lamp current 10 mA and integration time 3 s. The flame used was the acetylene-air mixture.

The pH values of solutions were measured using an

Orion Research Model 601A digital pH-meter.
All calculations were carried out on IBM computer using Microsoft excel 2002 for windows ME. SMAC program [34] was used for all statistical methods.

Materials and Reagents: All solvents and reagents were of analytical reagent grade, double distilled water was used 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); pefloxacin mesylate (Rhone-Poulenc Rorer, Neuilly/Seine, France); ofloxacin (Hoechst AG, Frankfurt, Germany); ciprofloxacin hydrochloride (Miles Inc. Pharmaceutical Division, West Haven, Germany); lomefloxacin hydrochloride (Searle, Illinois, USA); enoxacin, enrofloxacin and levofloxacin (Sigma Chem. Co., USA) and were used without further purification. Ammonum vanadate, 5% w/v solution, prepared by dissolving 5 gm in boiling 50% v/v sulphuric acid and diluting to 100 mL with 50% v/v sulphuric acid and 0.01 M cobalt sulphate (0.2% w/v solution) were Aldrich products. Sulphuric acid (ADWIC), 0.1 N and 50% v/v prepared in de-ionized water.

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.

Standard Preparations: Stock solutions containing 3 μg mL^{-1} of each fluoroquinolone were prepared in ethanol. Working standard solutions for methods I, II and III were prepared by suitable dilution of the stock solutions with ethanol as shown in Table 2.

Table 2: Association Constants of the Formed Chelate

Drugs	Cobalt Ass. Const. x 10^{-7} (k)	Log (k)
Amifloxacin	125.5894	8.9635
Ciprofloxacin HCl	110.6514	9.0129
Difloxacin HCl	142.6511	9.0265
Enoxacin	135.824	8.9613
Enrofloxacin	157.6138	9.0589
Lomefloxacin HCl	108.9865	9.1688
Levofloxacin	90.9093	9.0019
Norfloxacin	198.0684	9.1688
Ofloxacin	106.9811	9.0029
Pefloxacin mesylate	117.4493	9.2500

Average of three determinations

Procedures

For Spectrofluorimetric Method (Method I): Into a 100 mL measuring flask, transfer 1 mL of fluoroquinolones stock solution and complete the volume with 0.1N H_2SO_4. Different portions of the previous solutions within the concentration range of 3-140 μg were diluted to 100 mL with 0.1N H_2SO_4. The fluorescence was recorded at 450 nm with excitation at 290 nm. A blank of 0.1N H_2SO_4 was measured and the calibration graph was rectilinear from 0.3-1.4 μg mL^{-1}.

For Atomic Absorption Spectrometric Method (Method II)

General Procedure: Aliquots of working standard drug solutions were quantitatively transferred into 25 mL measuring flasks. To each flask 1.0 mL of 10^{-2} M standard solution of cobalt sulphate is added and pH was adjusted to 8.1 using 1 mL of buffer solution. The solutions are shaken well and left to stand for 15 min and then filtered through Whatman P/S filter paper (12.5 cm). The precipitate was washed with redistilled deionized water until metal free.

Direct Method: The precipitates obtained above were dissolved in the least amount of dilute acetic acid and complete to 25 mL with redistilled deionized water. Two mL of the resulting solution was diluted to 25 mL with redistilled deionized water.

Indirect Method: The filtrates and washings were collected in 100 mL volumetric flasks and completed to

volume with redistilled deionized water. Ten millilitre of the resulting solution was diluted to 100 mL with redistilled deionized water.
A blank (omitting addition of drugs) was prepared and absorbance was measured at the flaming conditions. Metal concentrations were calculated from a calibration curves.

For Spectrophotometric Method (Method III): To different aliquots of standard drug solution containing 0.2-1.0 mg of the analyzed drugs, 3 mL of 5% w/v ammonium vanadate was added in a 10 mL volumetric flask followed by 2 mL of concentrated sulphuric acid. The mixture was mixed well and boiled gently for 20 min. in water bath, then cooled and diluted to volume with bidistilled water. The absorbance was measured at 766 nm against blank (omitting the addition of drug).

For Pharmaceutical Preparations

Procedures for Tablets: An accurately weighed amount, equivalent to 10 mg of each drug from composite of 20 powdered tablets, was transferred into a 100 mL calibrated flask and diluted to the mark with the appropriate solvent, sonicated for 20 min and filtered off to obtain solutions of 100 μg mL^{-1}. Further dilutions were made to obtain sample solution and then the general procedures were followed.

Procedure for Ampoules: A volume equivalent to 10 mg of each drug was transferred into 100 mL calibrated flask and diluted to the mark with the appropriate solvent to obtain solution of 100 μg mL^{-1}. Further dilutions were made to obtain sample solution and then the general procedures were followed.

Procedure for Drops: One milliliter of the drops was transferred into a 100 mL calibrated flask and diluted to the mark with the appropriate solvent to obtain a solution of 30 μg mL^{-1}.Further dilutions were made to obtain sample solution and then the general procedures were followed.

Procedure for Determination of Molar Ratio: Drug and cobalt solutions of equimolar concentrations ($1x10^{-4}$ M) were prepared. Aliquots of each solution were added in different ratios to a series of 10 mL calibrated flasks, so that the total volume of both is 5 mL. The pH is adjusted to 8.1 using 1 mL buffer solution and then the volume is completed with the appropriated solvent. The relative absorption intensity of each formed chelate is measured at its respective maxima.

Procedures for Biological Fluids

Urine Treatment: Urine samples were centrifuged at 4000 rpm for 5 min and then 1 mL of the clear supernatant was spiked with 1 mL of the drug stock solution.
Appropriate dilutions were made to obtain solutions in which the drug concentration is 100, 300 and 500 ng mL^{-1} then the atomic absorption spectrometric procedure was followed.

Plasma Treatment: Five milliliter plasma were deproteinized by the addition of 10 mL acetonitrile, centrifuged at 4000 rpm for 5 min. One milliliter of the clear supernatant was spiked with 1 mL of the drug stock solution.
The mixture was then extracted with 2 portions; each of 5 mL chloroform. The chloroformic extract was collected, evaporated on a boiling water bath, then appropriate dilutions were made to obtain drug solutions containing 100, 300 and 500 ng mL^{-1}, then the atomic absorption spectrometric procedure was followed.

RESULTS AND DISCUSSION

Method I: Fluoroquinolones show native fluorescence in water, alcohol, 0.1 N NaOH, 0.1 N HCl and 0.1 N H_2SO_4. Solutions of fluoroquinolones in 0.1 N H_2SO_4 exhibited the strongest fluorescence at 450 nm when excited at 290 nm. A linear correlation was obtained between the fluorescence intensity and concentration in the range 0.3-1.4 μg mL^{-1} and the correlation coefficient was not less than 0.999. The concentration of different samples of fluoroquinolones in bulk powder and pharmaceutical dosage forms were calculated from the following regression equation:

$$C = 0.01261\ F - 0.04213$$

Where, C is the concentration of drug in μg mL^{-1} and F is the intensity of fluorescence of drugs at 450 nm with excitation at 290 nm.

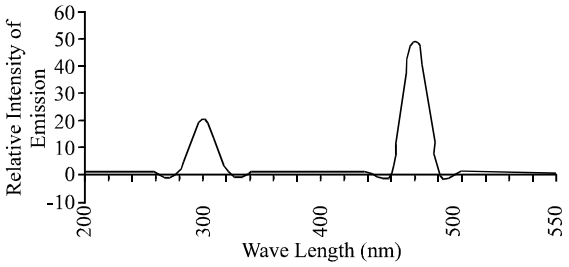
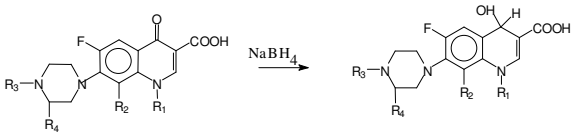


Fig. 1: Excitation and Emission Spectrum of Amifloxacin in 0.1 N H_2SO_4

The native fluorescence of fluoroquinolones is due to the high degree of conjugation found in the structure. Breaking this conjugation was done by adding sodium borohydride ($NaBH_4$) to fluoroquinolones aqueous solutions in a molar concentration of 3:1, in order to reduce the ketonic group in the pyridine ring. The fluorescence in this case decreased by about 50% (scheme I).



Scheme I

Method II: Also, slightly alkaline (pH 8.1) alcoholic solutions of amifloxacin, ciprofloxacin hydrochloride, difloxacin hydrochloride, enoxacin, enrofloxacin, lomefloxacin hydrochloride, levofloxacin, norfloxacin, ofloxacin and pefloxacin mesylate gave coagulated precipitates with cobalt sulphate. These precipitates form the basis of the micro-quantitative determinations of the cited acidic drugs. Co (II) contents can be determined either directly in the precipitate or indirectly in the filtrate by atomic absorption spectrometry.

Method III: The spectrophotometric method has been used for the quantitative determination of ten fluoroquinolones antibiotics by oxidation with ammonium vanadate in sulphuric acid medium resulting in the development of greenish blue colour at 766 nm which was attributed to the vanadium (IV) produced by reduction of vanadium (V) by the selected drugs (Fig. 2).

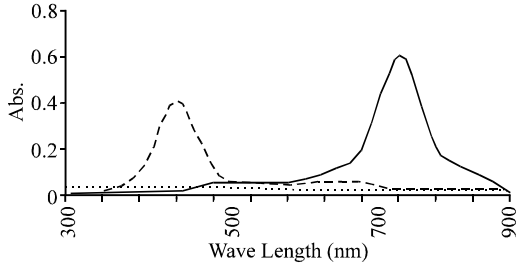


Fig. 2: Absorption Spectra of 0.3 mL of 1 mg mL^{-1} Amifloxacin (⋯), 3 mL of 5% w/v Ammonium Vanadate (- - -) and mixture of them (—)

Optimization of the Reaction Conditions
For Spectrofuorimetric Method (Method I)
Type and Concentration of Reagent: 0.1N H_2SO_4 exhibited the strongest fluorescence at 450 nm when excited at 290 nm.

For atomic Absorption Spectrometric Method
Type and Amount of Alcohol: Addition of the recommended amount of ethyl alcohol is to enhance the solubilization of the drugs and coagulation of the precipitates. Larger volumes of alcohol must be avoided to prevent solubilization of the formed precipitates.

Effect of pH: In order to study the effect of pH on precipitation, buffer solutions covering the acid to alkaline range were tried . Acid media have a solubilizing effect on the precipitate leading to lower results for the direct technique and higher ones for the indirect technique while higher alkali media precipitate the metal as its oxide or hydroxide leading to higher results for the direct technique. The optimum pH was found to be slightly alkaline (pH = 8.1).

Metal Concentration: Considering metal ion concentration effect on precipitation, 1 mL of the precipitating solution was found to be sufficient for complete precipitation.

Temperature: Regarding the temperature effect on precipitation, room temperature was found to be the most efficient. Higher temperature show solubilizing effect on the precipitate producing lower results for the direct technique and higher ones for the indirect technique.

Composition of the Formed Complex: Job's method of continuous variation [35] was used to study the molar ratios of the formed chelates. The method revealed 1:2 ratios for the metal ions (Co (II): quinolone chelates (Fig. 3)). This explains the use of the same optimum metal ion concentration for all the studied drugs.
The stability constants of the formed chelates were calculated using the following equations:

$$\beta = A/A_{ex}\, C_X \;\; / \;\; (C_M - A/A_{ex}\, C_X)\,(C_L - nA/A_{ex}\, C_X)^n$$

Where, β is the stability constant of the formed chelate, M indicates metal, L indicates ligand, n =X/(1-X) where X is the mole fraction of the ligand at the maximum of the continuous variation curve. A/A_{ex} is the ratio of the observed absorbance to that indicated by the tangent for the same wavelength. C_M and C_L are the concentrations of the metal and the ligand, respectively, $C_x = C_L/n = C_M$ [36].
The calculated stability constants for the formed chelates (Table 2) are ranging from 90.9093 x 10^7 to 198.0684 x 10^7 indicating good stability of the formed chelates.

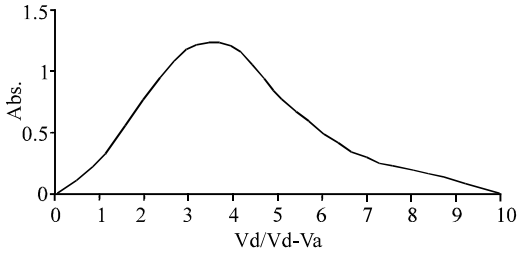


Fig. 3: Job's Curve of Equimolar Solutions of Amifloxacin with Cobalt at the Respective Maxima

For Spectrophotometric Method (Method III)

Effect of Heating Time: Gentle boiling for 20-30 min. was sufficient to produce maximum colour intensity.

Effect of Reagent Concentration: It was found that 1 mL of 5% w/v ammonium vanadate was the most suitable concentration for carrying out the assay.

Effect of Sulphuric Acid Concentration: Different concentrations (10, 30, 50, 70, 90 and 98% v/v) were tried and it was found that 2 mL of concentrated sulphuric acid (98%) gave best results.

Method Validation: The developed spectrofluorimetric, atomic absorption spectrometric and spectrophotometric methods were fully validated according to International Conference on Harmonization guidelines [37] and complied with USP XXIV validation guidelines. The methods were linear over the concentration ranges tested for the used metals. Calibration curves has correlation coefficients (r) higher than 0.999 and coefficients of determinations (r^2) higher than 0.998 (Table 3) indicating good linearity. Linearity was also checked by calculating the variance of the slope and t-test for the intercept (Table 3).

Table 3: Parameters for Calibration Curves Construction

Drugs	Method	Conc. range (μg mL^{-1})	a	b	r	r^2	LOD	LOQ	C.V. (%)
Amifloxacin	I	0.3-1.0	-0.025	0.853	0.9996	0.9992	0.125	0.322	0.58
	II a	5-25	0.041	0.265	0.9995	0.9990	0.024	0.251	0.49
	IIb	5-25	0.265	0.705	0.9997	0.9994	1.911	0.441	0.39
	III	15-40	-1.027	0.963	0.9996	0.9992	0.220	0.850	0.91
Ciprofloxacin	I	0.4-1.2	0.599	0.801	0.9994	0.9988	0.114	0.950	0.37
Hydrochloride	IIa	3-20	1.200	0.710	0.9995	0.9990	0.098	0.871	0.58
	IIb	3-20	0.911	0.521	0.9997	0.9994	0.211	0.747	0.80
	III	10-30	0.588	0.456	0.9996	0.9992	1.993	0.986	0.57
Difloxacin	I	0.5-1.4	0.635	0.574	0.9994	0.9988	0.236	0.698	0.84
Hydrochloride	IIa	6-30	0.666	0.158	0.9997	0.9994	0.124	0.901	0.49
	IIb	6-30	-0.058	0.207	0.9997	0.9994	0.952	0.499	0.51
	III	10-35	0.308	0.305	0.9997	0.9994	0.997	0.745	0.29
Enoxacin	I	0.2-1.2	-1.032	0.058	0.9995	0.9990	0.203	0.798	0.80
	IIa	5-25	0.870	0.157	0.9996	0.9992	0.997	0.984	0.56
	IIb	5-25	0.360	0.092	0.9996	0.9992	0.155	0.980	0.71
	III	10-30	1.201	0.795	0.9997	0.9994	0.201	0.379	0.55
Enrofloxacin	I	0.3-1.2	0.957	0.741	0.9995	0.9990	0.908	0.981	0.81
	IIa	3-20	1.039	0.850	0.9996	0.9992	0.951	0.690	0.58
	IIb	3-20	-0.520	0.128	0.9998	0.9996	0.195	0.920	0.61
	III	10-30	1.520	0.361	0.9998	0.9996	0.169	0.852	0.28
Lomefloxacin	I	0.5-1.4	0.369	0.571	0.9995	0.9990	0.2111	0.963	0.54
Hydrochloride	IIa	5-30	0.661	0.548	0.9996	0.9992	0.069	0.987	0.61
	IIb	5-30	0.368	0.471	0.9995	0.9990	0.101	0.802	0.67
	III	15-40	0.995	0.985	0.9998	0.9996	0.992	0.556	0.58
Levofloxacin	I	0.5-1.0	1.254	0.399	0.9997	0.9994	0.921	0.691	0.49
	IIa	5-25	0.369	0.250	0.9995	0.9990	0.120	4.631	0.51
	IIb	5-25	-0.058	0.668	0.9996	0.9992	0.025	0.398	0.45
	III	15-40	-0.998	0.269	0.9995	0.9990	0.260	0.490	0.90
Norfloxacin	I	0.3-1.4	0.336	0.487	0.9997	0.9994	0.000	0.588	0.89
	IIa	3-30	0.998	0.069	0.9996	0.9992	0.996	0.597	0.80
	IIb	3-30	0.036	0.258	0.9997	0.9994	0.030	0.459	0.60
	III	10-30	0.914	0.398	0.9995	0.9990	0.955	0.315	0.39
Ofloxacin	I	0.2-1.0	-1.064	0.984	0.9997	0.9994	0.998	0.783	0.81
	IIa	3-25	0.287	1.005	0.9995	0.9990	0.986	0.452	0.87
	IIb	3-25	0.954	0.821	0.9996	0.9992	0.175	0.425	0.80
	III	15-35	-0.985	0.871	0.9996	0.9992	0.125	0.851	0.49
Pefloxacin	I	0.3-1.4	1.284	0.745	0.9995	0.9990	0.126	4.940	0.68
Mesylate	IIa	3-30	0.782	0.489	0.9996	0.9992	0.098	0.759	0.60
	IIb	3-30	0.841	0.259	0.9998	0.9996	0.950	0.751	0.70
	III	10-40	0.561	0.486	0.9998	0.9996	0.049	0.763	0.81

a: intercept; b: slope; r: correlation coefficient; r^2: coefficient of determination; LOD: limit of detection; LOQ: limit of quantitation; IIa and IIb: Direct and Indirect AAS.

Table 4: Statistical Analysis of the Results Obtained Using the Proposed Procedures and Official or Reported Methods for Analysis of Authentic Samples

Drugs	Method I	Method II		Method III	Reference method
		Direct	Indirect		
Amifloxacin					
X±SD	99.84±0.68	99.15±0.59	99.09±0.36	99.82±0.56	99.20±0.52[a]
V	0.46	0.35	0.13	0.32	0.27
t	1.41	0.12	0.38	1.60	
F	1.70	1.30	2.08	1.19	
Ciprofloxacin hydrochloride					
X±SD	99.48±0.51	98.98±0.50	99.12±0.46	99.69±0.66	98.90±0. 52[b]
V	0.26	0.25	0.21	0.44	0.27
t	1.16	0.22	2.29	0.47	
F	1.04	1.08	1.29	1.63	
Difloxacin hydrochloride					
X±SD	99.50±0.46	99.98±0.53	99.09±0.48	98.99±0.61	100.0±0.52 [a]
V	0.21	0.28	0.23	0.37	0.27
t	1.47	0.05	0.29	0.02	
F	1.29	1.04	1.17	1.37	
Enoxacin					
X±SD	99.11±0.66	99.90±0.59	99.05±0.46	99.10±0.66	99.00±0.62 [a]
V	0.44	0.35	0.21	0.44	0.38
t	0.24	0.21	0.14	0.22	
F	1.16	1.09	1.81	1.16	
Enrofloxacin					
X±SD	99.96±0.58	99.99±0.59	99.99±0.66	99.97±0.69	99.96±0.63 [a]
V	0.34	0.35	0.44	0.4	0.40
t	00.00	0.07	0.07	0.02	
F	1.18	1.14	1.10	1.20	
Lomefloxacin hydrochloride					
X±SD	99.48±0.50	99.99±0.59	99.05±0.49	99.91±0.49	100.01±0.55 [a]
V	0.25	0.35	0.24	0.24	0.30
t	1.47	0.05	0.11	0.28	
F	1.20	0.05	0.11	0.28	
Levofloxacin					
X±SD	99.39±0.49	99.90±0.66	99.92±0.50	99.78±0.67	100.03±0.63 [a]
V	0.24	0.44	0.25	0.45	0.40
t	1.71	0.28	0.76	0.54	
F	1.67	1.10	1.60	1.13	
Norfloxacin					
X±SD	99.79±0.51	99.76±0.33	99.76±0.49	100.00±0.56	100.02±0.52 [b]
V	0.26	0.11	0.24	0.31	0.27
t	0.14	0.94	0.73	0.05	
F	1.04	2.45	1.13	1.15	
Ofloxacin					
X±SD	98.98±0.46	98.99±0.57	99.16±0.65	99.070.39	98.92±0.57[b]
V	0.21	0.32	0.42	0.15	0.32
t	0.17	0.17	0.54	0.47	
F	1.52	1.00	1.31	2.13	
Pefloxacin mesylate					
X±SD	99.97±0.51	100.00±0.60	99.89±0.51	99.98±0.46	100.31±0.50 [a]
V	0.26	0.36	0.26	0.21	0.25
t	0.94	0.77	1.16	0.99	
F	1.04	1.44	1.04	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. [a] USP XXIV, [b] Ref. [38]

Table 5: Assay of the Studied Drugs in Presence of Common Excipients

	Recovery % ± SD				
Drugs	Sucrose 10 mg*	Glucose 10 mg*	Starch 5 mg*	Talc 5 mg*	Magnesium stearate 10 mg*
Amifloxacin	98.8±1.2	99.3±0.96	99.4±1.0	99.3±0.8	99.6±0.75
Ciprofloxacin	98.6±0.9	100.5±1.0	98.6±0.8	99.1±0.6	98.6±1.4
Difloxacin	99.1±1.3	99.2±0.6	99.3±0.8	100.2±0.8	100.0±1.3
Enoxacin	99.7±0.9	99.7±1.3	98.7±0.6	100.1±1.2	99.8±1.1
Enrofloxacin	99.2±0.5	100.4±1.5	99.9±1.2	98.9±0.6	98.8±1.1
Lomefloxacin	99.6±0.2	99.9±0.9	99.7±1.0	98.6±0.7	98.9±1.0
Levofloxacin	98.9±0.6	99.8±1.1	99.3±0.4	99.2±0.9	100.8±0.8
Norfloxacin	99.5±0.9	98.7±0.4	99.1±0.8	98.5±0.2	99.9±0.8
Ofloxacin	99.6±0.7	98.9±0.4	99.8±0.7	98.9±0.9	99.8±0.9
Pefloxacin	98.9±1.2	99.7±1.3	99.8±0.9	99.7±1.0	98.7±0.8

* The amount of excipients added per 50 mg of drug

Table 6: Determination of Studied Drugs in Their Pharmaceutical Dosage Forms

Drugs	Method I	Method II		Method III	Reference method
		Direct	Indirect		
Spectrama® Tablets					
X±SD	97.00±0.66	98.00±0.70	96.87±0.62	97.01±0.75	96.99±0.69[a]
V	0.40	0.49	0.38	0.56	0.48
t	0.02	2.04	0.27	0.04	
F	1.20	1.02	1.26	1.17	
Neofloxacin® Tablets					
X±SD	99.11±0.59	99.20±0.61	99.20±0.59	98.88±0.61	98.98±0.60[b]
V	0.35	0.37	0.35	0.37	0.36
t	0.31	0.52	0.53	0.23	
F	1.03	1.03	1.03	1.03	
Norbactin® Tablets					
X±SD	99.00±0.90	98.98±0.91	98.21±0.91	98.97±0.78	98.39±0.85[a]
V	0.81	0.83	0.83	0.61	0.72
t	0.03	1.04	0.29	1.03	
F	1.13	1.15	1.15	1.18	
Tarivid® Tablets					
X±SD	100.01±0.70	99.98±0.68	99.96±0.65	99.98±0.62	99.99±0.66[b]
V	0.49	0.46	0.42	0.38	0.44
t	0.04	0.02	0.07	0.02	
F	1.11	1.05	1.05	1.16	
Kirol® Tablets					
X±SD	100.36±0.48	99.99±0.55	100.35±0.52	100.65±0.52	100.36±0.50[b]
V	0.23	0.30	0.27	0.27	0.25
t	00.0	0.83	0.03	0.80	
F	1.09	1.20	1.08	1.08	
Mefoxin® Tablets					
X±SD	99.98±0.54	100.69±0.48	99.98±0.56	100.00±0.55	100.11±0.49[b]
V	0.29	0.23	0.31	0.30	0.24
t	0.35	1.71	0.34	0.29	
F	1.21	1.04	1.29	1.25	
Serviflox® Tablets					
X±SD	101.12±0.88	102.00±0.90	101.98±0.85	101.98±0.88	102.00±0.87[b]
V	0.77	0.81	0.72	0.77	0.76
t	1.41	00.0	0.03	0.03	
F	1.01	1.07	1.06	1.01	
Cipro otic® Drops					
X±SD	100.01±0.90	99.95±0.89	99.98±0.94	100.12±0.88	99.88±0.89[a]
V	0.81	0.79	0.88	0.77	0.79
t	0.20	0.11	0.15	0.39	
F	1.02	1.00	1.11	1.03	

Table 6: Continued

Globacin® Tablets					
	X±SD	99.11±0.50	99.21±0.48	98.98±0.40	99.10±0.39	99.01±0.44[b]
	V	0.25	0.23	0.16	0.15	0.19
	t	0.29	0.60	0.10	0.32	
	F	1.32	1.21	1.19	1.27	
Peflacin® Ampoules						
	X±SD	99.99±0.39	100.01±0.33	99.96±0.42	99.98±0.42	100.00±0.40[b]
	V	0.15	0.11	0.18	0.18	0.16
	t	0.04	0.04	0.13	0.07	
	F	1.07	1.45	1.13	1.13	
Tavanic® Tablets						
	X±SD	99.80±0.50	99.25±0.49	99.52±0.63	99.52±0.54	99.52±0.60[b]
	V	0.25	0.24	0.40	0.29	0.36
	t	0.53	0.73	0.00	0.30	
	F	1.44	1.50	1.11	1.24	

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. [a] USP XXIV, [b] [38]

Table 7: Determination of the Studied Drugs in Spiked Urine and Plasma Samples Using Atomic Absorption Spectrometric Method

Drugs	Spiked amount (ng mL^{-1})	Recovery (%)±SD			
		Spiked urine sample		Spiked plasma sample	
		Direct	Indirect	Direct	Indirect
Amifloxacin	10	99.58±0.95	99.96±0.96	99.91±0.89	100.1±0.97
	30	100.56±0.92	99.98±0.93	100.95±0.89	99.98±1.02
	50	98.98±1.00	101.65±0.89	99.95±0.98	101.77±0.95
Ciprofloxacin	10	99.98±1.01	99.65±0.98	99.92±0.98	99.39±0.96
HCl	30	99.98±0.89	100.01±0.79	99.96±0.89	99.39±0.89
	50	100.32±0.99	98.96±0.90	99.09±1.02	100.2±1.01
Difloxacin	10	99.98±1.08	99.98±0.98	99.85±0.98	99.99±0.99
HCl	30	100.87±1.02	99.95±1.12	102.01±1.5	101.51±1.10
	50	100.9±0.59	99.98±0.84	98.99±0.94	98.89±1.02
Enoxacin	10	100.91±0.74	99.92±0.91	100.09±0.91	100.01±0.98
	30	99.45±0.87	99.85±1.00	99.85±1.30	99.98±1.00
	50	101.20±0.95	99.20±1.30	99.99±0.98	99.21±0.96
Enrofloxacin	10	100.1±0.29	99.95±0.39	100.90±0.67	99.01±0.56
	30	98.56±0.96	99.98±0.95	100.62±0.98	99.04±0.69
	50	101.75±0.69	99.98±0.75	100.05±0.95	99.95±1.05
Lomefloxacin	10	98.49±0.84	99.56±1.61	101.92±1.20	99.99±0.85
HCl	30	99.96±0.85	100.02±0.96	99.98±0.96	99.99±1.03
	50	100.20±1.10	99.99±0.98	100.23±0.98	99.00±0.39
Levofloxacin	10	99.98±0.39	100.97±0.99	99.98±0.69	100.98±0.98
	30	101.65±0.78	98.29±0.91	99.89±0.27	99.84±0.59
	50	100.65±0.98	99.26±1.20	100.96±0.59	100.45±0.69
Norfloxacin	10	101.26±0.85	100.95±1.30	99.99±0.90	99.69±0.89
	30	98.85±0.84	99.99±0.59	101.36±0.89	99.95±0.89
	50	99.95±0.89	101.98±0.99	100.99±1.25	99.95±0.99
Ofloxacin	10	98.29±0.98	100.58±0.52	98.87±0.59	100.89±0.95
	30	101.52±0.98	99.98±0.97	99.69±1.30	102.00±1.30
	50	99.89±0.59	99.89±0.75	99.19±0.96	98.28±0.95
Pefloxacin	10	99.32±0.78	99.31±1.00	99.89±0.98	99.20±0.89
Mesylate	30	100.3±1.90	98.89±0.98	99.45±1.41	99.89±0.90
	50	101.22±0.75	99.25±1.32	99.55±1.11	98.88±0.29

The accuracy of the methods were determined by investigating the recovery of each of the studied drugs at four concentration levels covering the specified range including 100% of the test concentration (three replicates of each concentration). The results showed excellent recoveries (Table 4). The complete set of validation assays was performed for each drug with the studied metals. The limit of detection (LOD) and limit of quantitation (LOQ) were also determined (Table 3) according to the JCH guidelines [37], the obtained values indicate the high sensitivity of the proposed methods.

The assay results were unaffected by the presence of excipients as shown by the excellent recoveries

obtained when analyzing the studied drugs in presence of commonly encountered excipients (Table 5). This fact indicates proper selectivity of the method for determination of the studied drugs in raw material and in their dosage forms. Different parameters affecting the procedures were studied to evaluate robustness; the analytical solutions were stable for at least 24 h showing reliability of the proposed methods.

Applications: The proposed procedures were applied successfully for determination of the studied drugs in their pharmaceutical dosage forms. Six replicate measurements were made in each case, the results obtained were validated by comparison with well established official and reported methods by means of t- and F-tests at 95% confidence level (Table 6) and no significant difference was found indicating good accuracy and precision.

No interference was observed from commonly used excipients such as starch, talc, glucose, sucrose and magnesium stearate (Table 5). Also, no interference was caused by the presence of sodium ascorbate with pefloxacin in the Pefloxacin® ampoules.

The high sensitivity attained by the atomic absorption spectrometric method allows the determination of the studied quinolones in biological fluids. Therefore, the proposed methods were applied for determination of the studied drugs in spiked samples of human urine and plasma and the recoveries were determined by calibration curve method. Excellent recoveries were obtained at three concentration levels of each drug in both urine and plasma samples (Table 7). The accuracy was assessed by investigating the recovery of each of the studied drugs at three concentration levels covering the specified range (three replicates of each concentration). The results showed excellent recoveries with S.D. less than 2.5% indicating both good accuracy and precision. Only plasma samples required deproteination and extraction steps while untreated urine samples are processed directly. This indicates that the proposed methods are selective enough to tolerate the presence of common excipients, other active constituents which may be found in different dosage forms (such as sodium ascorbate in Pefloxacin® ampoules) and matrices of biological fluids as urine and plasma. The proposed methods are advantageous than many of the reported spectrophotometric methods for determination of the studied drugs in pharmaceutical dosage forms and in biological fluids. They are also much simpler and less expensive and time consuming than represented HPLC methods.

CONCLUSION

The proposed spectrofluorimetric, atomic absorption spectrometric and spectrophotometric methods are simple, rapid, selective and highly sensitive. Therefore it is used for determination of the studied drugs either in bulk or in their corresponding dosage forms without interference from commonly used excipients and could be easily used in a quality control laboratory for their analysis.

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