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Atomic absorption spectroscopic, conductometric and colorimetric methods for determination of fluoroquinolone antibiotics using ammonium reineckate ion-pair complex formation

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Abstract

Three accurate, rapid and simple atomic absorption spectrometric, conductometric and colorimetric methods were developed for the determination of norfloxacin (NRF), ciprofloxacin (CIP), ofloxacin (OFL) and enrofloxacin (ENF). The proposed methods depend upon the reaction of ammonium reineckate with the studied drugs to form stable precipitate of ion-pair complexes, which was dissolved in acetone. The pink coloured complexes were determined either by AAS or colorimetrically at λ_{max} 525 nm directly using the dissolved complex. Using conductometric titration, the studied drugs could be evaluated in 50% (v/v) acetone in the range 5.0–65, 4.0–48, 5.0–56 and 6.0–72 μg ml^{-1} of NRF, CPF, OFL and ENF, respectively. The optimizations of various experimental conditions were described. The results obtained showed good recoveries of 99.15 ± 1.15, 99.30 ± 1.40, 99.60 ± 1.50, and 99.00 ± 1.25% with relative standard deviations of 0.81, 1.06, 0.97, and 0.69% for NRF, CPF, OFL, and ENF, respectively. Applications of the proposed methods to representative pharmaceutical formulations are successfully presented.

Keywords: Norfloxacin, ciprofloxacin, ofloxacin and enrofloxacin determination; Ion-pair complex; Ammonium reineckate; Dosage forms

1. Introduction

Norfloxacin is a fluoroquinoline carboxylic acid, currently used as a broad spectrum antibacterial drug [1], against gram-positive and gram-negative aerobic pathogens and is considered to be the first commercially available member of the modern fluoroquinolones [2]. Norfloxacin is specifically prescribed for the treatment of complicated urinary tract infections. Its mode of action has advantages over other common antibiotics.

As the structural formula shows, norfloxacin is related to other quinolones including ofloxacin (6-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido (1,2,3 de)-1,4 -benzoxazine-6-carboxylic acid), ciprofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid), and enrofloxacin (1-cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolone carboxylic acid). These drugs have been recently introduced as powerful broad-spectrum antibacterial agents which are derivatives of quinolone carboxylic acid or quinolones. Their mode of action is thought to be through blocking bacterial DNA replication and transcription by inhibiting DNA gyrase, ultimately giving rise to cell lysis. Because of this special mechanism of action, they are considered to be the most active broad-spectrum antibiotics effective against gram-positive and gram-negative pathogens to combat infections caused by micro-organism that are resistant or multi-resistant to other antimicrobials, such as amino glycosides, tetracycline's of β-lactams.

A number of spectrophotometric methods for the determination of fluoroquinolones [3–10] were reported. The polarographic [11,12], stripping voltammetric [13] and chromatographic [14–18] methods involve multiple steps and sometimes utilize expensive and sophisticated instruments were also studied. The development of AAS,

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conductometric and colorimetric methods for these drugs is worthwhile.

Herein, three different techniques for the simple and accurate determination of these drugs mentioned above were investigated. Ammonium reineckate is used to form ion-pair complexes with the studied drugs with good chromophore. The purpose of the present investigation is to develop a simple, accurate and precise AAS, conductometric, and colorimetric methods for the determination of norfloxacin (NRF), ciprofloxacin (CIP), ofloxacin (OFL), and enrofloxacin (ENF) and to apply the procedures to various dosage forms.

2. Experimental

2.1. Apparatus

The pH values of solutions were measured using an Orion research model 601 A/digital ionalyzer pH-meter. The absorption spectra for all measurements were carried out using JASCO 530 V spectrophotometer equipped with 10 mm quartz cells. A YSI model 32 M conductance meter (Yellow Springs Instrument Co., Yellow Springs, DH, USA) was used. The measurement range was 1.0–10.0 μS with maximum error of ±0.2%. The YSI model 3417 dip-type cell was used with a cell constant, K_{cell}, of 1.0. The atomic absorption measurements for the determination of chromium ion were carried out using a Hitachi atomic absorption Z-6100 polarized Zeeman spectrometry. For AAS, the chromium was measured at λ_{max} 357.87 nm, slit width, 0.2 nm, relative noise, 1.0, detection limit, 0.01 μg ml^{-1}, linear dynamic range, 0.01–100 μg ml^{-1}, lamp current, 5.0 mA and integration time, 30 s, the flame used was the acetylene—air mixture.

2.2. Reagents

Analytical grade reagents and double distilled water was used to prepare all solution. NRF was obtained from the Egyptian International Pharmaceutical Industries Company (EIPICO), CIP was supplied by Amriya Pharmaceutical Industrial Company, Alexandria, Egypt, OFL was obtained from Hoechest Orient, and ENF was supplied by Pharma Swede-Egypt (AVICO). A stock standard solutions of 250 μg ml^{-1} was prepared by dissolving an exact weight (0.025 g) of the pure analytical-reagent grade drug in about 70 ml of water, to which 0.01 M hydrochloric acid was added, in a 100 ml measuring flask. The mixture was warmed at 50 °C in a water bath for 5.0 min, agitated by an electrical shaker for another 5.0 min, cooled to room temperature and diluted to volume with water.

Stock solution, 5×10^{-3} M ammonium reineckate (Aldrich product) solution was also prepared by dissolving appropriate weight in 100 ml double distilled water.

2.3. Formulations

The following commercial formulations were subjected to the analytical procedures: Neofloxin tablets (Alexandria Company for Pharmaceuticals and Chemical Industries, Alexandria, Egypt), containing 400 mg of norfloxacin per tablet, Noroxin tablets (EIPICO) containing 400 mg of norfloxacin per tablet, Spectrama tablets (Amoun Pharmaceutical Industries Company (APIC), Egypt) containing 400 mg norfloxacin per tablet. Tarivid tablets (Hoechst Orient, Cairo, Egypt) containing 200 mg ofloxacin per tablet. Oflicin eye drops (Memphis Company for Pharm & Chemical Ind., Cairo Egypt), containing 0.3 g per 100 ml, Ciprofloxacin tablets (Amriya Pharm. Ind. Co.) containing 250 or 500 mg ciprofloxacin per tablet. Rancil tablets (Chem. Ind. Develop. Co., CIDICO, Giza, Egypt) containing 250 mg per tablet and Avitryl injectable (AVICO, Egypt) containing 10% (v/v) enrofloxacin.

2.4. Working solutions

Weight accurately 100 mg from a composite of the mixed contents of 10 tablets, then follow the procedure as for stock standard solution of the studied drugs.

2.5. General procedures

2.5.1. Atomic absorption spectral procedure

An aliquot containing 1.00–18.0 mg of the investigated drug was transferred into a 10 ml calibrated flask, 4.0 ml of 5×10^{-3} M of ammonium reineckate and 1.0 ml of 0.01 M HCl were added successively. The mixture was left to stand for 10 min and then the precipitate was filtrated. The precipitate is separated and dissolves in least amount of acetone, and completed to the mark in a 100 ml calibrated flask with water. This solution is then aspirated directly in the atomic absorption spectrometer and measured the chromium ion concentration. Calculate the concentration of the tested drug from the relevant calibration graph.

2.5.2. Conductometric procedure

A volume containing 2.0–22 mg of drug was transferred to a 50 ml calibrated flask and made up to the mark with 50% (v/v) acetone–water mixture. The contents of the calibrated flask were transferred to a beaker and the conductivity cell was immersed. 5×10^{-3} M ammonium reineckate solution was then added from a microburette and the conductance was measured subsequent to each addition of reagent solution and after thorough stirring. The conductance reading, taken 2.0 min after each addition, was corrected for dilution [19] by means of the following equation, assuming that conductivity is a linear function of dilution.

$$\Omega^{-1}_{\text{correct}} = \Omega^{-1}_{\text{obs}} \left[\frac{v_1 + v_2}{v_1} \right]$$

where Ω_{obs}^{-1} is the observed electrolytic conductivity, v_1 the initial volume ad v_2 the volume of reagent added.

A graph of corrected conductivity versus the volume of added titrant was constructed and the end-point determined. 0.1 ml of 5×10^{-3} M ammonium reineckate is theoretically equivalent to 0.160, 0.176, 0.173, and 0.185 mg of NRF, CIP, OFL, and ENF, respectively. The procedure takes 15–30 min in all.

2.5.3. Colorimetric procedure

Proceed as above in AAS procedure "dissolved in acetone" and then completed to the mark in 10 ml calibrated flask. The absorbances of solutions were measured at 524 nm, against a reagent blank solution prepared in the same way without drug. The calibration graph was obtained by applying the procedure, using standard drug solutions.

3. Results and discussion

According to Babko [20], large number of analytically important complexes consists of the system metal ion—electronegative ligand—organic base. Most of these complexes are extractable in the usual organic solvents such as hydrocarbons and hologenated derivatives. NRF, CIP, OFL, and ENF are found to react with ammonium reineckate to form stable ion pair complexes. These complexes are sparingly soluble in aqueous solution, but are readily soluble in acetone.

Investigations were carried out to establish the most favourable conditions for the ion pair complex formation of NRF, CIP, OFL, and ENF with ammonium reineckate to achieve sharp end point and/or maximum colour development, in the determination of the drug. The influence of some variables on the reaction has been tested as follow:

3.1. Conditions for conductometric titrations

The optimum conditions for performing the titration in a quantitative manner were elucidated as described below.

Three different titrations were attempted: (i) aqueous drug solution with aqueous reagent solution, (ii) acetone drug solution with acetone reagent solution and (iii) drug solution with reagent solution, both in acetone–water (50% (v/v)) mixture. Preliminary experiments showed that procedure (iii) were the most suitable for successful results, because in procedures (i) and (ii) precipitates were formed which caused some errors. The reagent concentration in each titration must be not less than ten times that of the drug solution in order to minimize the dilution effect on the conductivity through the titration. The optimum concentration of ammonium reineckate was 5×10^{-3} M to achieve a constant and highly stable conductance reading after 2.0 min mixing. Concentrations less than these led to unstable readings and more time was needed to obtain constant conductance values. On raising the temperature to 40 °C, no change in the conductance reading was observed, whereas above which, the conductance value changed and so changed the shape of the titration curve.

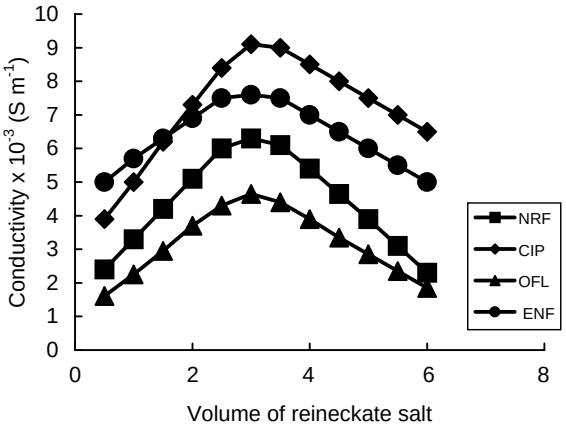

Fig. 1. Conductometric titration of 4.8, 5.19, 5.34, and 5.55 mg of NRF, OFL, CIP, and ENF using 5×10^{-3} M ammonium reineckate.

Representative titration curves are shown in Fig. 1. Two straight lines are obtained, intersecting at the end-point, the first branch ascending and the second descending. The increase of conductance may be attributed to the formation of ion-pair in solution as a result of the complexation reaction.

After the end-point, the titration curves indicate a continuous decrease of conductance, despite the excess of the reagent. This may be due to further ionic condensation, leading to species of lower mobility.

3.2. Conditions for colorimetric method

3.2.1. Effect of reagent concentration

Experiments was carried out in which the volume was kept constant at 10 ml while the concentration of reagent was increased; revealed that 4.0 ml of 5×10^{-3} M is the

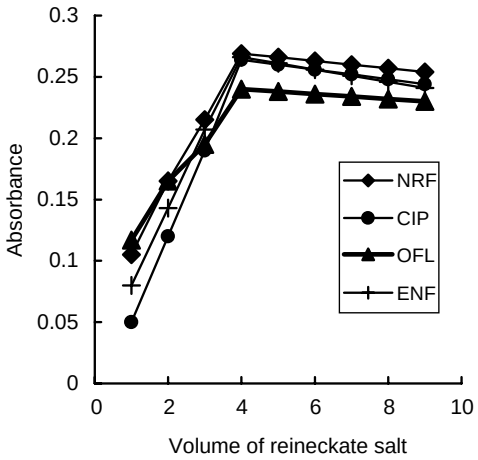

Fig. 2. Effect of 5×10^{-3} M ammonium reineckate on the absorbance of 0.7 mg ml^{-1} of the studied drugs.

optimum concentration (Fig. 2). The excess reagent used is probably as a result of dissociation in aqueous medium as fraction of the ion-pair formed.

3.2.2. Effect of acidity

Different acid media was used to increase the colour intensity of the formed precipitated ion-pair. Sulfuric, phosphoric, hydrochloric and acetic acid were tested. The optimum one was hydrochloric acid of 0.01 M concentration, since the results are highly concordant at this media. Moreover, the amount of 0.01 M HCl added to 10 ml was found to be 1.0 ml that gave marginally the best results.

3.2.3. Effect of solvent

Acetone was found to be the best solvent for dissolving the precipitated ion-pair formed in aqueous acidic media. On the other hand, dioxane and propanol are possible substitutes, but methanol, ethanol, benzene and chloroform were unsuitable owing to the limited solubility of ion-pair in these solvents.

3.3. Optimization of AAS measurements

It was not practical to aspirate the dissolved ion-pair in acetone to the atomic absorption spectrometer. It is better to dilute the formed ion-pair with water in a ratio 10% (v/v) acetone aqueous media, which can be aspired directly to the AAS.

3.4. Quantification

For AAS method, calibration graphs with good linearity were obtained as recorded in Table 1. The linear regression equations were also calculated. Correlation coefficient, intercept and slope values for the calibration data calculated using the least squares method. Detection and quantitation limits were also evaluated an recorded in Table 1.

Table 1
Analytical characteristic of the AAS procedure

Parameters	NRF	OFL	CIP	ENF
Range of determination ($\mu g\,ml^{-1}$)	5.0–130	10–150	5.0–140	10–180
Detection limit ($\mu g\,ml^{-1}$)	1.5	3.1	1.4	3.3
Quantification limit ($\mu g\,ml^{-1}$)	4.9	9.8	4.7	10.1
Range of errors (%)	±1.2	±1.6	±1.5	±1.9
Regression equation[a]				
Intercept	−0.007	0.009	0.013	−0.010
Slope	0.023	0.027	0.025	0.031
Correlation coefficient (r)	0.9996	0.9994	0.9988	0.9995
Relative standard deviation (%)	0.89	1.05	0.96	1.17
Calculated *t*-value (2.57)[b]	1.18	1.56	1.43	1.82
Calculated *F*-value (5.05)[b]	2.57	3.40	3.09	4.15

[a] $A = a + bC$, where C is the concentration in $\mu g\,ml^{-1}$.
[b] Theoretical values for five degrees of freedom and 95% confidence limits.

Table 2
Linear regression analysis for the studied drugs using reineckate salt

Parameters	NRF	OFL	CIP	ENF
Optimum concentration ($\mu g\,ml^{-1}$)	40–360	40–400	40–380	40–440
Shift or intercept of the regression line[a]	0.042	0.038	0.028	0.033
Slope of regression line	0.9980	0.9985	1.0025	0.9978
Relative standard deviation (%)	1.34	1.50	1.22	1.80
Student's *t*-test[a] (2.57)[b]	1.88	2.05	1.73	2.17
Variance *F*-test[a] (5.05)[b]	3.12	3.48	3.76	3.56

[a] Observed vs. theoretical.
[b] Tabulated values at 95% confidence limits (for slope).

The linear regression equation was applied to the result obtained from conductometric titration (Table 2) to establish whether the proposed method exhibits any fixed or proportional bias. Student's *t*-test (at 95% confidence level) was applied to the result obtained compared with that obtained when applying the official method [21]. The results showed that it did not differ significantly and there are no systematic differences between the proposed and official methods. The calculated standard deviations can be considered satisfactory, at least for the level of concentrations examined.

Beer's law is valid within the concentration range 0.10–1.50 $mg\,ml^{-1}$. For more accurate analysis, Ringbom optimum concentration range was calculated to be 0.25–1.25 $mg\,ml^{-1}$. The molar absorptivity, Sandell sensitivity, detection and quantification limits were calculated and recorder in Table 3.

The performance of the proposed methods was assessed by comparison with the official method (based on the titration of the drug dissolved in glacial acetic acid with 0.1 M perchloric acid using a suitable anhydrous electrode system). Mean values were obtained with a Student's *t*- and *F*-tests at 95% confidence limits for five degrees of freedom [22]. The results showed comparable accuracy (*t*-test) and precision (*F*-test), since the calculated values of *t*- and *F*-tests were less than the theoretical data.

The reproducibility of the proposed methods was assessed by running six replicate samples, each containing 80, 200 and 600 $\mu g\,ml^{-1}$ using AAS, conductometric, and colorimetric methods, respectively, of the studied drugs in the final assay solution. The relative standard deviations were calculated and recorded in Tables 1–3.

3.5. Analytical applications

The proposed procedures were applied to determine the studied drugs in their pharmaceutical formulations. The results in Table 4 indicate the high accuracy and precision. As can be seen from Table 4, the proposed method has the

Table 3
Spectral characteristics and precision data

Parameters	NRF	OFL	CIP	ENF
Beer's low limits ($mg\,ml^{-1}$)	0.10–1.25	0.15–1.40	0.15–1.15	0.20–1.50
Ringbom range ($mg\,ml^{-1}$)	0.25–1.10	0.25–1.20	0.25–1.00	0.30–1.25
Molar absorptivity ($l\,mol^{-1}\,cm^{-1}$)	1.181×10^3	9.66×10^2	1.03×10^3	1.08×10^3
Sandell sensitivity ($\mu g\,cm^{-2}$)	0.26	0.33	0.30	0.32
Detection limits ($\mu g\,ml^{-1}$)	31	48	45	65
Quantification limits ($\mu g\,ml^{-1}$)	97	149	146	197
Regression equation[a]				
Slope (a)	3.36×10^{-3}	3.00×10^{-3}	3.30×10^{-3}	3.33×10^{-3}
Intercept (b)	-2.16×10^{-2}	4.15×10^{-2}	6.7×10^{-2}	-4.1×10^{-2}
S.D. of slope	6.18×10^{-4}	5.22×10^{-4}	3.67×10^{-4}	2.58×10^{-4}
S.D. of intercept	9.85×10^{-3}	1.27×10^{-4}	8.43×10^{-3}	2.1×10^{-3}
Correlation coefficient (r)	0.9994	0.9988	0.9992	0.9990
Relative standard deviation (%)	1.72	1.56	1.85	1.60
Range of error (%)	±1.4	±1.1	±1.6	±1.5
Stability of ion-pair (h)	36	42	36	48
Students t-test[b] (2.57)[c]	1.48	1.67	1.83	1.56
Variance F-test[b] (5.05)[c]	3.27	4.15	4.52	3.98

[a] $A = a + bC$, where C is the concentration in $\mu g\,ml^{-1}$.
[b] Comparison with the official method [21].
[c] Values in parenthesis are the theoretical t- and F-values for five degree of freedom and 95% confidence limit.

advantages of being virtually free from interferences by excipients such as glucose, lactose, and starch or from common degradation products. The results obtained were compared statistically by the student's t-test (for accuracy) and the variance ratio F-test (for precision) with those obtained by the pharmacopoeial method [21] on samples of the same batch (Table 4). The values of t- and F-tests obtained at 95% confidence level and five degrees of freedom did not exceed

Table 4
Determination of NRF, CIP, OFL and ENF in various pharmaceutical formulations using ammonium reineckate

Method	Sample	Manufacturer	Content (mg per table)	Found[a] (mg)		t-test[b]	F-vaule[b]
				Proposed	Official		
Atomic absorption	Neofloxin	Alexandria	400	396	390	1.73	3.70
	Noroxin	EIPICO	400	403	408	1.18	2.45
	Spectrama	APIC	400	397	392	1.22	2.54
	Tarivid	Hoechest	200	201	195	1.64	3.44
	Oflicin	Memphis	$3.0\,mg\,ml^{-1}$	3.03	2.96	1.06	2.31
	Ciprofloxacin	Amriya	250	251	254	1.35	2.93
	Ranicif	RANBAXY	250	251	256	1.72	3.68
	Avitryl injectable	AVICO	10%	9.97%	9.88%	1.41	3.00
Conductometric	Neofloxin	Alexandria	400	390	390	1.88	4.06
	Noroxin	EIPICO	400	405	408	1.59	3.44
	Spectrama	APIC	400	394	392	1.62	1.67
	Tarivid	Hoechest	200	196	195	1.31	2.89
	Oflicin	Memphis	$3.0\,mg\,ml^{-1}$	2.99	3.03	1.77	3.70
	Ciprofloxacin	Amriya	250	247	245	1.45	3.11
	Ranicif	RANBAXY	250	248	255	1.09	2.38
	Avitryl injectable	AVICO	10%	10.02%	9.8%	1.29	2.66
Colorimetry	Neofloxin	Alexandria	400	393	390	1.47	3.15
	Noroxin	EIPICO	400	395	408	1.18	2.76
	Spectrama	APIC	400	402	392	1.07	2.43
	Tarivid	Hoechest	200	147	195	1.56	3.33
	Oflicin	Memphis	$3.0\,mg\,ml^{-1}$	2.98	2.92	1.82	3.57
	Ciprolfloxacin	Amriya	250	252	245	1.35	2.97
	Ranicif	RANBAXY	250	248	246	1.35	2.81
	Avitryl Injectable	AVICO	10%	9.95%	9.88%	1.63	3.45

[a] Average of six determinations.
[b] Tabulated values 95% confidence limits are (2.57) and (5.05) for t- and F-tested, respectively.

the theoretical tabulated value indicating no significant difference between the methods compared.

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