Asian Journal of Chemistry; Vol. 23, No. 11 (2011), 4827-4832

ASIAN JOURNAL OF CHEMISTRY

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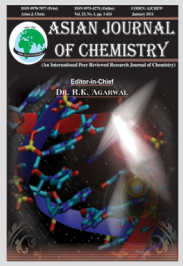

Spectrophotometric Determination of Lomefloxacin in its Pharmaceutical Dosage Forms

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(*Received*: 30 September 2010; *Accepted*: 15 July 2011) AJC-10160

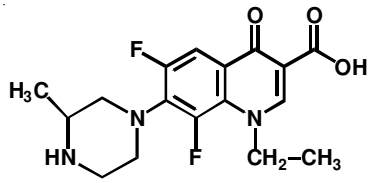
New highly selective reaction has been proposed as a basis for the spectrophotometric determination of lomefloxacin (LOM), a fluoroquinolone antimicrobial agent. This reaction involved the condensation of lomefloxacin with the oxidized catechol (OXC) and formation of a violet coloured reaction product that exhibited maximum absorption peak at 510 nm. The variables affecting the reaction were carefully investigated and the optimum conditions were established. The stoichiometry of the reaction was determined and the reaction pathway was postulated. The selectivity of the reaction for lomefloxacin among the other fluoroquinolones was evaluated and it was found to be highly selective for lomefloxacin. Under the optimum reaction conditions, Beer's law was obeyed in the range of 10-60 μg mL^{-1}. The assay limits of detection and quantitation were 2.37 and 7.90 μg mL^{-1}, respectively. The robustness and ruggedness of the method were satisfactory. The method was successfully applied to the analysis of lomefloxacin-containing pharmaceutical dosage forms and the results were statistically compared with those obtained by a pre-validated reference method. No significant differences were found between the two methods in terms of the accuracy and precision as revealed by the accepted values of *t*- and F-tests, respectively. The proposed method is superior to all the previously reported spectrophotometric methods in terms of its selectivity for lomefloxacin.

Key Words: Fluoroquinolones, Lomefloxacin, Oxidized catechol, Spectrophotometry, Pharmaceutical analysis.

INTRODUCTION

Lomefloxacin (LOM; 1-ethyl-6,8-difluoro-1,4-dihydro-7-[3-methyl-piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid, as hydrochloride salt) is a relatively new, second-generation synthetic fluoroquinolone antimicrobial agent with an expanded spectrum of activity against gram-positive and gram-negative bacteria[1]. It exerts its effect by inhibition of DNA gyrase (topoisomerase II) and topoisomerase IV, the bacterial enzymes which are responsible for the bacterial chromosome replication. Lomefloxacin has a broader spectrum of activity and more potent than the non-fluorinated quinolone antibacterials[2]. It contains a piperazine group at 7th position of the 4-quinolone nucleus, which results in anti-pseudomonal activity[3]. Lomefloxacin is used in wide range of infections such as uncomplicated and complicated urinary tract, respiratory and gastrointestinal tract, as well as in skin structure, otic and ocular infections[4].

Because of the therapeutic importance of lomefloxacin, there is a growing interest in the development of analytical methods for the purpose of its pharmaceutical quality control. The methods that have been reported for this purpose were the subject of many reviews[5-8]. Based on the number of reports cited in these reviews, spectrophotometry is considered the most widely used technique. This is attributed to its inherent simplicity, low cost and wide availability in most quality control laboratories.



Structure of lomefloxacin (LOM)

Most of the spectrophotometric methods that have been described for the determination of lomefloxacin utilized its common backbone structure. These methods were based on measuring its native UV-absorption[9] or employment of colour-developing reactions[10-15]. These reactions include chelation with metal ions *via* the quinolone C=O and carboxylic OH groups[10], charge-transfer reaction with various acceptors involving the tertiary piperazinly nitrogen[11], formation of ion-pair associates with various ion-pairing reagents[12-14] and

non-selective oxidation with an oxidizing agent[15]. All these methods lacked the selectivity as they were unable to discriminate between lomefloxacin and the other fluoroquinolones (FQs). Furthermore, many of these methods were not fully competent in their applications because of their procedures involved multiple tedious liquid-liquid extraction steps for the chromogens (*e.g.*, ion-pair formation-based methods). For these reasons, the development of more selective and simple spectrophotometric method for determination of lomefloxacin is necessary.

The present study describes, for the first time, a new highly selective reaction for lomefloxacin. This reaction involved the condensation of lomefloxacin, *via* its free piprazinyl -NH, with the oxidized catechol (OXC) and formation of a violet-coloured product exhibiting maximum absorption peak at 510 nm. The proposed reaction was adopted in the development of a highly selective and simple visible-spectrophotometric method for determination of lomefloxacin in its pharmaceutical dosage forms.

EXPERIMENTAL

An UV-1601 PC (Shimadzu, Japan) and a Lambda-3 B (Perkin-Elmer Corporation, Norwalk, USA) ultraviolet-visible spectrophotometers with matched 1 cm quartz cells were used for all measurements.

Chemicals and pharmaceutical dosage forms: The following authentic standards were used after confirming their purity and compliance with the pharmaceutical requirements. Lomefloxacin hydrochloride (Searle, Illinois, USA), amifloxacin (Sterling Winthrop Inc., USA), difloxacin hydrochloride (Abbott Laboratories, North Chicago, USA), enrofloxacin (Merck, Darmstadt, Germany), ofloxacin (Daiichi, Tokyo, Japan), pefloxacin methane sulphonate (Rhone-Poulenc Rorer, Neuilly/Seine, France), ciprofloxacin hydrochloride (Miles Inc. Pharmaceutical Division, West Haven, Germany), norfloxacin hydrochloride (Egyptian International Pharmaceutical Industries Co., Cairo, Egypt), gatifloxacin (Bristol-Myers Squibb S.A.E., Cairo, Egypt) and sparfloxacin (Global Napi Pharmaceuticals, Cairo, Egypt). Catechol (El-Nasr Chemical Co. Abo-Zaabal, Egypt). Silver oxide (Aldrich Chemical Co., Milwaukee, USA). All other chemicals and solvents used throughout this work were of analytical grade.

Lomoxin® tablets (Minapharm Egypt for Egyptian Group for Drug Trading, 10th Ramadan City, Egypt) are labeled to contain 400 mg of lomefloxacin per tablet. Orchacin® eye drops (Minapharm Egypt for Egyptian Group for Drug Trading, 10th Ramadan City, Egypt) are labeled to contain 3 mg of lomefloxacin per 1 mL of drops solution.

Preparation of standard lomefloxacin solution: An accurately weighed amount (50 mg) of lomefloxacin was quantitatively transferred into a 50 mL calibrated flask and dissolved in 10 mL of 0.01 M HCl. The solution was completed to volume with distilled water to produce a stock solution of 1 mg mL^{-1}. This solution was diluted with *n*-propanol to produce working solutions in the range of 100-600 μg mL^{-1}.

Preparation of sample solutions of lomefloxacin-containing dosage forms

Tablets: An accurately weighed amount, equivalent to 50 mg of lomefloxacin from composite of 20 powdered tablets, was transferred into a 100-mL calibrated flask and diluted to the mark with *n*-propanol, sonicated for 20 min and filtered off to obtain solutions of 0.5 mg mL^{-1}. Further dilutions were made to obtain sample solutions in the range of 100-600 mg mL^{-1}. These solutions were subjected to the analysis according to the recommended analytical procedures.

Eye drops: One mL of the drops was transferred into a 100-mL calibrated flask and diluted to the mark with *n*-propanaol to obtain solutions of 300 μg mL^{-1}. These solutions were subjected to the analysis according to the recommended analytical procedures.

Preparation of oxidized catechol reagent solution: Oxidized catechol solution (0.3 %, w/v) was freshly prepared by dissolving 0.3 g of catechol in 100 mL acetone containing 0.1 mg of silver oxide. The solution was filtered and the filtrate solution was used as a working oxidized catechol reagent.

Recommended analytical procedures: Accurately measured aliquots of lomefloxacin solution containing 150-600 μg mL^{-1} were transferred into separate 10-mL calibrated flasks. One mL of oxidized catechol solution (0.3 %, w/v) was added. The reaction was allowed to proceed for 40 min at room temperature (25 ± 5 ºC). The volume was then completed to the mark with *n*-propanol and the absorbance of the solution was measured at 510 nm against reagent blanks treated similarly.

Determination of molar ratio: The Job's method of continuous variation 16 was employed. Master equimolar solutions (1.4×10^{-2} M) of lomefloxacin and oxidized catechol reagent were prepared. Series of 10-mL portions of the master solutions of lomefloxacin and the reagents were made up comprising different complementary proportions (0:10, 1:9, …, 9:1, 10:0, inclusive) in 10 mL calibrated flasks. The reaction solutions were further manipulated according to the recommended analytical procedures.

RESULTS AND DISCUSSION

Involve reaction and strategy for its selection: The reaction involved in this study was based on the condensation of lomefloxacin, *via* its free piprazinyl -NH with the oxidized catechol reagent. This reaction yielded a violet-coloured reaction product which exhibits maximum absorption peak at 510 nm. The reaction pathway is given in Fig. 1 and the absorption spectra of lomefloxacin and its reaction product with oxidized catechol are given in Fig. 2. The present study describes, for the first time, the investigation of this reaction and its employment in the development of a new highly selective spectrophotometric method for the determination of lomefloxacin.

Since this reaction employed the free piprazinyl -NH of lomefloxacin, therefore, it is anticipated that all the fluoroquinolones containing the di-substituted piprazine will not interfere and ultimately a selective method has been developed. The oxidized catechol contains two electron-deficient positions in the *para* position for each oxo group (Fig. 1). These two positions are available for condensation with two molecules of lomefloxacin, thus a highly sensitive assay will be ultimately developed. oxidized catechol could be prepared by oxidation of catechol by any oxidant under mild conditions, however, in the present study, it was oxidized by silver oxide which, being

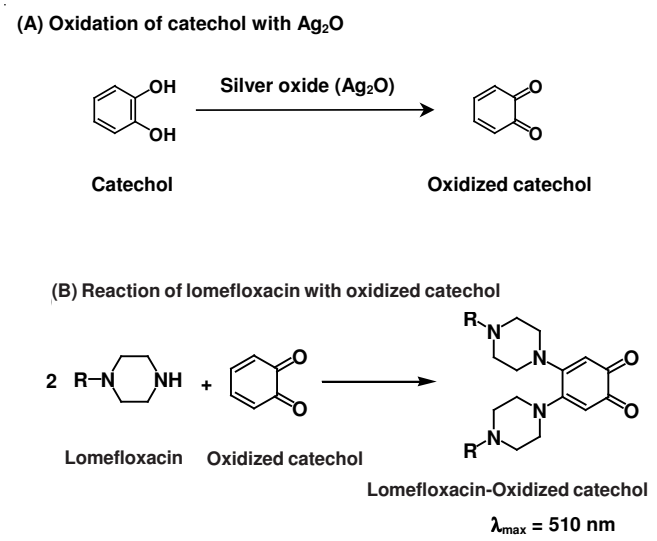


Fig. 1. Proposed pathways for the oxidation of catechol with Ag_2O (A) and the reaction of lomefloxacin with oxidized catechol (B)

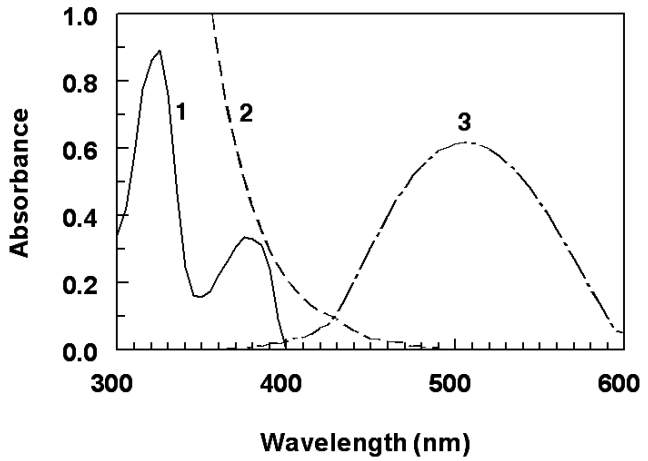


Fig. 2. Absorption spectra of (1): lomefloxacin (30 μg mL^{-1}), (2) oxidized catechol (0.3 %, w/v) and (3): lomefloxacin-oxidized catechol reaction product. The concentration of lomefloxacin in the reaction solution was 50 μg mL^{-1}

a solid oxidant, has the advantage of easily removing its excess from the oxidized catechol solution (Fig. 1).

The following paragraphs describe the conditions under which the reaction of lomefloxacin with oxidized catechol fulfills the requirements necessary for its employment as a basis for the spectrophotometric analysis of lomefloxacin.

Optimization of the reaction conditions: The effect of oxidized catechol concentration on the reaction was studied by carrying out the reaction at room temperature (25 ± 5 °C) using 1 mL of different oxidized catechol concentrations (0.1-0.5 %, w/v). The reaction was found to be dependent on the oxidized catechol concentration as the absorbances increased when oxidized catechol increased (Fig. 3). The highest absorbances were attained when the oxidized catechol concentrations were in the range of 0.25-0.5 % (w/v); further experiments were carried out using 0.3 %. Using this concentration, the effect of time on the formation of the reaction product was investigated by allowing the reaction to proceed for varying times. The absorbance values increased with the reaction time and leveled off after 0.5 h (Fig. 3). For high-precise readings,

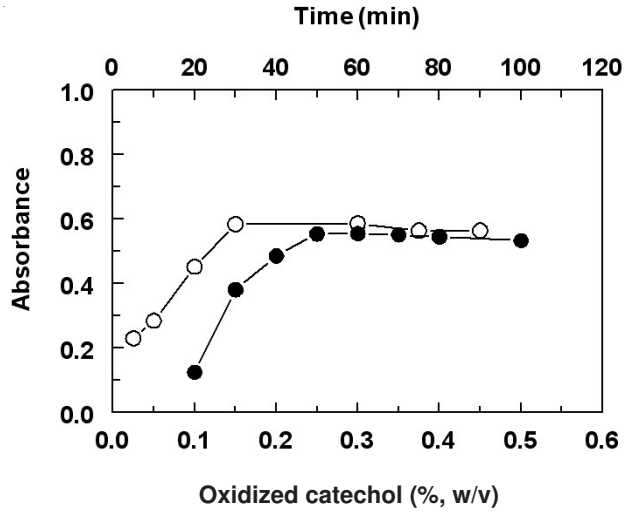


Fig. 3. Effect of oxidized catechol concentrations (●) and time (○) on the reaction of oxidized catechol with lomefloxacin (30 μg mL^{-1})

further experiments were carried out at 40 min. High temperature was found to enhance the reaction rate, however, it stimulated the evaporation of the solvent as well. This effect resulted in poor-precise readings, thus further experiments were carried out at room temperature. In order to select the most appropriate solvent for solubilization of the oxidized catechol as well as for diluting its reaction solution with lomefloxacin, different solvents (water, acetone, ethanol, *n*-propanol, isopropanol and propane-2-one) were tested. The best solubilization and highest readings were obtained when acetone and *n*-propanol were used, respectively.

A summary for the optimization of the conditions required for the reaction between lomefloxacin and oxidized catechol is given in Table-1.

TABLE-1
OPTIMIZATION OF VARIABLES AFFECTING THE REACTION OF LOMEFLOXACIN WITH OXIDIZED CATECHOL

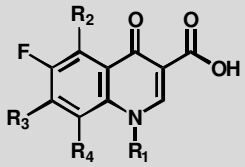
Variable	Studied range	Optimum condition
Oxidized catechol concentration (%, w/v)	0.1-0.5	0.3
Reaction time (min)	5-90	40
Temperature (ºC)	25-80	25 ± 5
Solvent for solublization of oxidized catechol	Different*	Acetone
Solvent for dilution of the chromogen	Different*	*n*-Propanol
Measuring wavelength (nm)	380-600	510

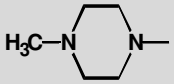
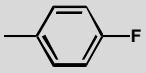
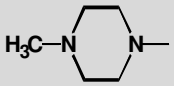
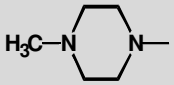
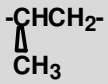
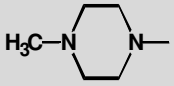
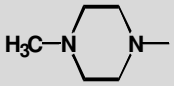
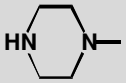
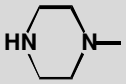
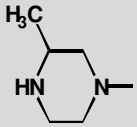
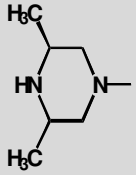
*Solvents tested: water, acetone, ethanol, *n*-propanol, isopropanol and propane-2-one.

Stoichiometry, mechanism and selectivity of the reaction: Under the optimum conditions, the stoichiometry of the reaction of lomefloxacin with oxidized catechol was investigated by Job's method[16]. The results indicated that the lomefloxacin:oxidized catechol ratio was 2:1 (graphical data is not shown). Based on this ratio and the presence of only one piperazinyl -NH in lomefloxacin molecule that is liable for condensation with oxidized catechol, the reaction was postulated to proceed according to the pathway given in Fig. 1.

The selectivity of the reaction for lomefloxacin was evaluated by applying the optimum reaction conditions on two

TABLE-2
CHEMICAL FORMULAE OF THE FLUOROQUINOLONES USED IN INVESTIGATING THE SELECTIVITY OF THE PROPOSED REACTION FOR LOMEFLOXACIN



Fluoroquinolones	R_1	R_2	R_3	R_4
Amifloxacin	-NCHCH$_3$	H		H
Difloxacin		H		H
Enrofloxacin		H		H
Ofloxacin		H		-OH
Pefloxacin	CH$_3$-CH$_2$–	H		H
Ciprofloxacin		H		H
Norfloxacin	CH$_3$-CH$_2$–	H		H
Gatifloxacin		H		-OCH$_3$
Sparfloxacin		-NH$_2$		F

groups of FQs available in our laboratory (Table-2). The first group contains di-substituted piprazinyl moiety (amifloxacin, difloxacin, enrofloxacin, ofloxacin and pefloxacin) and the second group contains mono-substituted piprazinyl moiety (ciprofloxacin, norfloxacin, gatifloxacin and sparfloxacin). oxidized catechol did not react with the fluoroquinolones that contain the di-substituted piprazine moiety confirming that the reaction of oxidized catechol with lomefloxacin proceeds *via* the condensation of oxidized catechol with the piprazinyl -NH of lomefloxacin. As well, oxidized catechol did not react with the free bases of gatifloxacin, norfloxacin and sparfloxacin, although they have free piprazinyl -NH in their structures. Meanwhile, it reacted with the hydrochloride salts of ciprofloxacin as well as lomefloxacin. The reactivity of the oxidized catechol with the HCl salts of ciprofloxacin and lomefloxacin, rather than the free bases of the other fluoroquinolones containing piprazinyl -NH (gatifloxacin, norfloxacin and sparfloxacin) could be attributed to the possible achievement of the optimum pH required for the reaction[17] by ciprofloxacin HCl and lomefloxacin, however this condition was not achieved with the free bases of the other fluoroquinolones. In conclusion: among all the fluoroquinolones tested in this study, only ciprofloxacin HCl cross-reacted with oxidized catechol. These data confirmed the high selectivity of the reaction involved in this study for lomefloxacin.

Validation of the method

Linearity and sensitivity: In the proposed method, linear plot (n = 3) with good correlation coefficients was obtained in the concentration range of 10-60 μg mL^{-1} (Table-3). The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the formula: LOD or LOQ = κSDa/b, where κ = 3.3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept and b is the slope[18]. The LOD and LOQ values were 2.37 and 7.90 μg mL^{-1}, respectively (Table-3).

Accuracy and reproducibility: The accuracy of the proposed method was evaluated by the recovery studies for added concentrations of lomefloxacin. The mean recovery

TABLE-3
STATISTICAL PARAMETERS FOR THE PROPOSED SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF LOMEFLOXACIN

Parameter	Value
Linear range (μg mL^{-1})	10-60
Intercept	0.0076
Slope	0.0128
Correlation coefficient	0.9978
Molar absorptivity (L mol^{-1} cm^{-1})	5584
LOD (μg mL^{-1})	2.37
LOQ (μg mL^{-1})	7.90

value was 100.0 ± 0.32, indicating the accuracy of the proposed method. The reproducibility of the method was determined by replicate analysis of six separate samples of the lomefloxacin working standard solution. The relative standard deviation (RSD) did not exceed 2 % indicating the high reproducibility of the proposed method.

Liability of interferences from excipient: The liability of interference from the common pharmaceutical excipients was evaluated for the proposed method. Samples were prepared by mixing known amount (30 mg) of lomefloxacin with various amounts of the excipients (sucrose, glucose, starch and magnesium stearate). These laboratory-prepared samples were analyzed by the proposed method applying the recommended procedure. The average recovery values were 99.3-99.8 ± 0.22-1.01 % (Table-4). These data confirmed the absence of interference from any of the common excipients with the determination of lomefloxacin by the proposed method.

TABLE-4
ANALYSIS OF L LOMEFLOXACIN IN PRESENCE OF SOME COMMON EXCIPIENTS BY THE PROPOSED METHOD

Excipient	Recovery (% ± RSD)**
Sucrose (10)*	99.8 ± 0.22
Glucose (10)	99.3 ± 0.96
Starch (5)	99.8 ± 1.01
Magnesium stearate (10)	99.3 ± 0.84

*Figures in parenthesis are the amounts of the excipients (mg) added to 30 mg lomefloxacin. **Values are mean of three determinations.

Robustness and ruggedness: Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, one parameter was changed whereas the others were kept unchanged and the recovery percentage was calculated each time. It was found that small variation in the method variables did not significantly affect the results; recovery values were 99.7-100.3 ± 0.65-0.95 % (Table-5). These acceptable values 18 indicated the robustness of the proposed method.

Ruggedness was also tested by applying the method to the analysis of lomefloxacin using the same operational conditions but using two different instruments at two different laboratories and different elapsed time. Results obtained from day-to-day and instrument-to-instrument variations were reproducible, as the RSD did not exceed 2 % (Table-5).

Application of the proposed method: It is evident from the above-mentioned results that the proposed method gave satisfactory results with lomefloxacin in its bulk. Thus its pharmaceutical dosage forms were subjected to the analysis

TABLE-5
ROBUSTNESS AND RUGGEDNESS OF THE PROPOSED SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF LOMEFLOXACIN

Parameter	Recovery (% ± RSD)*
Optimum conditions**	100.0 ± 0.32
Oxidized catechol conc. (%, w/v)	
0.25	99.7 ± 0.95
0.35	99.9 ± 0.65
Reaction time (min)	
38	99.7 ± 0.76
42	100.3 ± 0.87
Day-to-day	
Day-1	100.0 ± 0.76
Day-2	100.2 ± 0.27
Instrument-to-instrument	
UV-1601 PC (Shimadzu)	101.8 ± 1.54
Lambda-3 B (Perkin-Elmer)	98.6 ± 1.49

*Values are mean of three determinations ± RSD. **Described in the experimental section.

of their lomefloxacin contents by the proposed and a pre-validated reported[19] methods. The obtained recovery values (Table-6) were compared with those obtained from the reported method by statistical analysis with respect to the accuracy (by *t*-test) and precision (by F-test). No significant differences were found between the calculated and theoretical values of *t*- and F-tests at 95 % confidence level proving similar accuracy and precision in the determination of lomefloxacin by both methods.

TABLE-6
ANALYSIS OF LOMEFLOXACIN-CONTAINING DOSAGE FORMS BY THE PROPOSED METHOD

Dosage form	Recovery (% ± SD)*	*t*-Value**	F-Value**
Lomoxin® tablets	99.8 ± 1.38	1.4	4.6
Orchacin® eye drops	101.2 ± 1.23	2.1	5.1

*Values are mean of 6 determinations. **Tabulated values of *t*- and F- at 95 % confidence limit are 2.78 and 6.39, respectively.

Advantages of the proposed method over the previous spectrophotometric methods: The proposed method, because it involves measurements in visible region, is more selective than the previously reported spectrophotometric method that involved measurements in the ultraviolet region[9]. The previous visible spectrophotometric methods involved the backbone structure of lomefloxacin that is common in the other fluoroquinolones[10-15]. Therefore, these methods lacked the selectivity as they were unable to discriminate between lomefloxacin and the other fluoroquinolones. The proposed spectrophotometric methods involved the characteristic free piprazinyl -NH, rather than the common backbone structure. Furthermore, other fluoroquinolones containing the free piprazinyl -NH (except ciprofloxacin HCl) did not react with oxidized catechol under the conditions that have specified for lomefloxacin. For these reasons, the proposed method is superior in its selectivity to all the previous spectrophotometric methods.

The proposed method is superior to the previously reported visible spectrophotometric methods that involved the formation of ion-pair associates[12-14] in terms of the analytical procedures simplicity, as the proposed method does not require elaborate treatment of the samples, careful adjustment

of the critical optimum pH of the reaction medium and/or tedious liquid-liquid extraction for the chromophores. These advantages encourage the application of the proposed method in routine analysis of lomefloxacin in quality control laboratories, as an alternative for the existing methods.

Conclusion

New highly selective reaction has been proposed for lomefloxacin. This reaction involved the condensation of lomefloxacin with oxidized catechol and formation of a violet-coloured product that exhibited maximum absorption peak at 510 nm. This reaction was employed as a basis for the spectrophotometric determination of lomefloxacin in its pharmaceutical dosage forms. The proposed method is superior to all the previously reported spectrophotometric methods in terms of its selectivity and superior to many of the previous methods in terms of the procedures simplicity. These advantages strongly encourage the application of the proposed method in routine analysis of lomefloxacin in quality control laboratories, as an alternative for the existing methods.

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