REDOX SPECTROPHOTOMETRIC DETERMINATION OF
FLUOROQUINOLONE ANTIBIOTICS DOSAGE FORMS

Hassan F. Askal

Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Assiut University, Assiut, Egypt

A general difference spectrophotometric procedure for the determination of seven antibacterial 7-piperazine fluoroquinolone derivatives in the range of 2-20 µg/ml is proposed. The procedure is based on the oxidation of these antibiotics using 1 ml of 0.5 mg/ml potassium permanganate in presence of 20 % sulphuric acid at room temperature (25 ± 5 °C) for 10 min. The decrease in permanganate color (ΔA) due to the interaction with such organic compounds is measured at 520 nm. Molar ratio determination indicated 1:3 (drug : permanganate). Assay precision (RSD) values were ±1.5 % or better for the bulk drugs and ranged from ±1.38 to ±1.2 % for tablets and ampoules. Drug recoveries were quantitative from the dosage forms tested. The proposed procedure is simple, rapid, accurate and readily applied to the determination of this new class of antibiotics in pharmaceutical products without interference from other ingredients or common additives.

INTRODUCTION

Recently, fluoroquinolones are considered to be the most active broad spectrum antibiotics effective against gram positive and gram negative pathogens that are resistant to other antimicrobials such as aminoglycosides, tetracyclines or β-lactams because of their special mechanism of action.¹³

A number of methods reported in literature for estimation of amifloxacin, difloxacin, norfloxacin, ofloxacin, ciprofloxacin, pefloxacin and lomefloxacin involve microbiological¹⁴⁵ chromatography,⁶¹³ electrometry,¹⁴²¹ fluorimetry²²,²³ and spectrophotometry.²⁴⁻⁻⁻²⁶ The determination of individual fluoroquinolones in pharmaceutical formulations are few using ferric ion,²⁶⁻⁻³⁰ charge-transfer,²⁹ and ion pair extraction.³¹,³²

Although microbiological and HPLC methods are more widely used in determination in biological fluids, they have their share of disadvantages, time-consuming and expensive. Potassium permanganate has been the work horse of redox analysis for more than a century. It is a strong oxidizing agent which quantitatively oxidize many of common reducing agents through titrimetric techniques.³³,³⁴

This paper describes for the first time a simple general redox spectrophotometric method...
for the rapid determination of seven amphoteric fluorquinolone antibacterials using acidified potassium permanganate solution. The chemical structures for these compounds are shown in Figure 1.

**EXPERIMENTAL**

**Instruments**
A Perkin-Elmer Lambda 3 B UV/VIS (Norwalk, CT, USA) and a Uvidec-320 (Tokyo, Japan) Spectrophotometer with matched 1 cm quartz cells were used. All volumetric measurements were made with standard glassware.

Infrared Spectrometer (IR-470 Shimadzu Corporation, Japan).

QCMP Program Catalogue Computational Chemistry software for IBM-PC and compatible computers, QCPE, Indiana University, Bloomington, IN., 1992.

**Reagents and Materials**
All solvents and reagents were of analytical reagent grade, De-ionized double distilled, high-purity water was used throughout.

Sulphuric acid solution, 20 %.

Potassium permanganate AR, stock solution, 0.5 mg/ml, prepared in double distilled water by heating to boiling and keeping it on the steam bath for an hour and then filtering the solution through a sintered glass filtering crucible (porosity No 4). Its solution should be freshly prepared and stored in a dark colored container.

Potassium bromate solution, 0.1 % prepared in distilled water.

Fluoroquinolone samples were supplied by different manufacturers and were used as working standards as certified by their corresponding supplier without further purification: Amifloxacin and Rosaxacin (Sterling Winthrop Inc., USA); Difloxacin hydrochloride (Abbott Laboratories, North Chicago, Illinois, USA); Norfloxacin anhydrous (EIPICO, Cairo, Egypt); Ofloxacin (Hoechst AG, Frankfurt, Germany); Ciprofloxacin hydrochloride. H₂O (Miles Inc. Pharmaceutical Division, West Haven, Germany); Pefloxacin mesylate (Rhône-Poulenc Rorer, Neuilly/Seine, France), Lomefloxacin hydrochloride (Searle, Illinois, USA) and Nalidixic acid (El-Naser Co, Cairo, Egypt).

**Dosage Forms**
The following available commercial preparations were analyzed: Spectrama tablets (Amoun Pharmaceutical Industries Co., Cairo, Egypt) labeled to contain 400 mg anhydrous norfloxacin per tablet; Neofloxacin tablets (The Alexandria Co. for Pharmaceuticals, Alexandria, Egypt) labeled to contain 400 mg anhydrous norfloxacin per tablet; Tarivid tablets (Hoechst Orient, Cairo, Egypt under licence of Hoechst AG, Frankfurt, Germany) labeled to contain 200 mg ofloxacin per film coated tablet; KIroll tablets (Amoun Pharmaceutical Industris Co., Cairo, Egypt) labeled to contain 200 mg ofloxacin per tablet; Mefoxin (Misr Co. for Pharmaceutical Industries, Cairo, Egypt) labeled to contain 250 mg ciprofloxacin hydrochloride monohydrate per tablet; and Peflacine ampoules (Rhône-Poulenc Rorer, Neuilly/Seine, France) labeled to contain 400 mg pefloxacin mesylate dihydrate and 15.3 mg sodium ascorbate per 5 ml ampoule.

**Preparation of drug standard solutions**
Stock solutions containing 1 mg/ml of the drug in 1 ml of 4 mol/L sulphuric acid and completing to 100 ml with double distilled water. Final working standard solutions containing 20-200 μg/ml were prepared daily by suitable dilution of the stock solutions with distilled water and stored away from direct sunlight.

**Recommended procedure**
In a 10-ml calibrated flask, 1 ml aliquots of the standard or sample solutions were placed followed by 1 ml of sulphuric acid solution 20 % and 1 ml potassium permanganate solution. Shaked and allowed to stand for 10 min at room temperature, diluted to the mark with distilled water and the decrease in absorbance (ΔA) at 520 nm was recorded against a reagent blank.
Fig. 1: Structural formulae of quinolones studied.
Tablets

An accurately weighed amount equivalent to 100 mg of each drug from composite of twenty powdered tablets were treated with 1 ml of 4 mol/L sulphuric acid in a 100-ml calibrated flask and completed to volume with distilled water, shaked for 10 min and filtered off through Whatman No. 41 to obtain solutions of 1 mg/ml. Further dilutions were made to obtain the final sample solutions.

Ampoules

A volume equivalent to 100 mg of peflucine mesylate dihydrate was transferred to a 100-ml calibrated flask and diluted to the mark with distilled water. Further dilutions were made to obtain the final sample solution.

Stoichiometry of the reaction

Job's method of continuous variation was employed under the working conditions of the reaction between fluoroquinolones and permanganate solution. Master equimolar solutions of permanganate and the different fluoroquinolones were prepared (1.266 x 10^{-3} M). A series of 5 ml volumes of mixtures containing master solutions in different complementary proportions (0:5 - 5:0) inclusive were prepared in 10-ml calibrated flasks. Add 1 ml 20 % sulphuric acid and complete with distilled water to 10 ml. After the flasks had been allowed to stand at 25±5°C for 10 min. The absorbance difference (ΔA) was measured at 520 nm against blank of potassium permanganate.

RESULTS AND DISCUSSION

The characteristic brilliant color of permanganate solution has always attracted attention. The absorption spectrum shows a series of well marked bands of which seven can be detected in the visible region, becoming less intense towards the ultraviolet. In a dilute aqueous solutions a well distinct band at 520 nm is detected, Figure 2. The solution, in the presence of dilute sulphuric acid, reacts with the investigated compounds quantitatively and it is used to estimate solutions of these substances spectrophotometrically at 520 nm without interference from the reaction products which are colorless. The reaction was governed by the electronegativity differences or ionization potential of permanganate and zwitterionic fluoroquinolones (pH 3-11) which provide a measure of the electron-affinity (force of electrostatic interaction).

![Absorption spectra of norfloxacin, 9.4 µg/ml (---), potassium permanganate 50 µg/ml (-----) and their reaction product (---).](image)

Fig. 2: Absorption spectra of norfloxacin, 9.4 µg/ml (---), potassium permanganate 50 µg/ml (-----) and their reaction product (---).

Interesting is that the redox process at first few seconds is very slow, nevertheless, succeeding portion of permanganate react more and more rapidly until the reaction becomes essentially instantaneous. This behavior is typical for an autocatalytic process, in which one of the reaction products functions as a catalyst for the next steps.

In solutions having hydrogen ion concentration of 0.5 M or greater, permanganate is reduced all the way to manganous ion.

These compounds are weaker acids than aromatic carboxylic acids, this may be due to intramolecular hydrogen bond formation stabilizing the protonated form of carboxylate group.

From a thermodynamic point of view, the increased acidity probably enhances the ease of protonation of such organic compounds and, therefore, the rate of its oxidation. The reaction was completed after 10 min in presence of 1 ml of 20 % sulphuric acid at room temperature.
The electromotive force for the overall reaction may be large in magnitude and positive in sign, but the mechanism of the redox process can be so complicated that the reaction may not occur at a convenient rate. The latter is often true if the redox reaction involves multiple electron transfer, or the formation or rupture of chemical bonds. 33

Under the above optimum conditions, the absorbance decrease, \( \Delta A \) versus concentration of fluoroquinolones was found to be linear over a wide range of drug concentrations with good correlation coefficients. Beer's law limits, regression parameters obtained by linear least square treatments of the results are given in Table 1.

Job's method of continuous variation was employed under the working conditions at room temperature to establish the reaction stoichiometry. It is evident from these obtained results and the typical plot in Figure 3, that the differences in structures of quinolones had no effect on the stoichiometry and the ratio of fluoroquinolones to permanganate was always 1:3.

![Graph showing absorbance vs concentration](image)

**Fig. 3:** Job’s plots for fluoroquinolones with KMnO₄ and 20% sulphuric acid.

<table>
<thead>
<tr>
<th>Compound (Batch No.)</th>
<th>Conc. range ( \mu g/ml )</th>
<th>Regression equations</th>
<th>Corr. Coeff. ( * )</th>
<th>( \times 10^4 ) L/mol.cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amifloxacin (AG 49375)</td>
<td>2-8</td>
<td>( \Delta A = -0.0235 + 0.0673 ) C</td>
<td>0.9988</td>
<td>2.17</td>
</tr>
<tr>
<td>Difloxacin HCl (117899-AX)</td>
<td>2-16</td>
<td>( \Delta A = 0.2520 + 0.0375 ) C</td>
<td>0.9983</td>
<td>1.60</td>
</tr>
<tr>
<td>Norfloxacin anhydr. (117899-AX)</td>
<td>2-14</td>
<td>( \Delta A = 0.0123 + 0.0457 ) C</td>
<td>0.9992</td>
<td>1.50</td>
</tr>
<tr>
<td>Ofloxacin (H-900)</td>
<td>8-20</td>
<td>( \Delta A = -0.1010 + 0.0380 ) C</td>
<td>0.9903</td>
<td>1.01</td>
</tr>
<tr>
<td>Ciprofloxacin HCl (3 ABW)</td>
<td>2-16</td>
<td>( \Delta A = 0.0081 + 0.0493 ) C</td>
<td>0.9992</td>
<td>1.66</td>
</tr>
<tr>
<td>Lomefloxacin HCl (COO 35)</td>
<td>4-20</td>
<td>( \Delta A = 0.0031 + 0.0330 ) C</td>
<td>0.9989</td>
<td>1.17</td>
</tr>
<tr>
<td>Péfloxacín mesylate. 2H₂O (8199340502)</td>
<td>4-20</td>
<td>( \Delta A = 0.0035 + 0.0355 ) C</td>
<td>0.9988</td>
<td>1.21</td>
</tr>
</tbody>
</table>

* Mean of five determinations.
Sensitivity, precision, accuracy and reproducibility

The apparent molar absorptivities of the reaction between permanganate and the studied antibiotics ranged from 1.01-2.17x10^4, Table 1. In order to study the precision, accuracy and reproducibility of the proposed procedure, standard solutions containing three different concentration levels of norfloxacin as a representative example of the seven studied fluoroquinolones were prepared and five absorbance measurements (ΔA) were made on each standard solution. The overall standard deviation (RSD) for fifteen measurements was 1.38 % while the standard analytical error (S.E.) did not exceed 0.054. The results of this study are given in Table 2.

Analysis of pharmaceutical formulations

To determine the recovery of the fluoroquinolones from the dosage forms, standard addition and recovery experiments were performed. The recovery ranged from 97.99-101.72 %. These findings, in combination with the established precision of the method, constitute quantitative recovery of the drug.

The proposed procedure was applied to the determination of the investigated compounds either alone or in the presence of sodium ascorbate (péflacine ampoules) dosage forms with good recovery, Table 3. The values obtained by the proposed method for different dosage forms were compared with their respective uv spectrophotometric procedures as under norfloxacin, and are in good agreement.

Table 2: Assessment of the precision, accuracy and reproducibility of the proposed procedure.

<table>
<thead>
<tr>
<th>Solution No.</th>
<th>Norfloxacin, µg/ml</th>
<th>± SD</th>
<th>RSD%*</th>
<th>Standard analytical error**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Added</td>
<td>Found</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.0</td>
<td>4.01</td>
<td>0.045</td>
<td>1.13</td>
</tr>
<tr>
<td>2</td>
<td>4.0</td>
<td>3.99</td>
<td>0.045</td>
<td>0.20</td>
</tr>
<tr>
<td>3</td>
<td>4.0</td>
<td>4.06</td>
<td>0.045</td>
<td>1.13</td>
</tr>
<tr>
<td>4</td>
<td>4.0</td>
<td>4.10</td>
<td>0.045</td>
<td>0.20</td>
</tr>
<tr>
<td>5</td>
<td>4.0</td>
<td>3.98</td>
<td>0.045</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>Mean 4.028</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>8.0</td>
<td>8.10</td>
<td>0.095</td>
<td>1.18</td>
</tr>
<tr>
<td>7</td>
<td>8.0</td>
<td>8.02</td>
<td>0.095</td>
<td>0.43</td>
</tr>
<tr>
<td>8</td>
<td>8.0</td>
<td>7.91</td>
<td>0.095</td>
<td>1.18</td>
</tr>
<tr>
<td>9</td>
<td>8.0</td>
<td>8.19</td>
<td>0.095</td>
<td>0.43</td>
</tr>
<tr>
<td>10</td>
<td>8.0</td>
<td>8.00</td>
<td>0.095</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>Mean 8.044</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>12.0</td>
<td>11.85</td>
<td>0.221</td>
<td>1.84</td>
</tr>
<tr>
<td>12</td>
<td>12.0</td>
<td>12.10</td>
<td>0.221</td>
<td>0.99</td>
</tr>
<tr>
<td>13</td>
<td>12.0</td>
<td>12.50</td>
<td>0.221</td>
<td>1.84</td>
</tr>
<tr>
<td>14</td>
<td>12.0</td>
<td>11.97</td>
<td>0.221</td>
<td>0.99</td>
</tr>
<tr>
<td>15</td>
<td>12.0</td>
<td>12.03</td>
<td>0.221</td>
<td>1.84</td>
</tr>
<tr>
<td></td>
<td>Mean 11.99</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean RSD = 1.38, ** Mean standard analytical error = 0.054
Table 3: Assay of some fluoroquinolone derivatives in some commercial dosage forms [maximum theoretical value for $t_-$ = 2.31 and for $F_-$ = 6.39 ($p = 0.05$)].

<table>
<thead>
<tr>
<th>Formulation* (Batch No.)</th>
<th>Claimed/ unit</th>
<th>Found, mg</th>
<th>Standard added, mg</th>
<th>% Recovery ± SD**</th>
<th>Ref. Method ± SD**</th>
<th>$t_-$</th>
<th>$F_-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neofloxacin® tab. (5055004)</td>
<td>400</td>
<td>403.10</td>
<td>400</td>
<td>100.75 ± 0.52</td>
<td>99.90 ± 0.65</td>
<td>2.28</td>
<td>1.56</td>
</tr>
<tr>
<td>Spectra tab. (401042)</td>
<td>400</td>
<td>399.00</td>
<td>400</td>
<td>99.79 ± 0.78</td>
<td>100.00 ± 0.50</td>
<td>0.51</td>
<td>2.43</td>
</tr>
<tr>
<td>Kiroll® tab. (374)</td>
<td>200</td>
<td>202.00</td>
<td>200</td>
<td>101.10 ± 0.56</td>
<td>101.40 ± 0.77</td>
<td>0.71</td>
<td>1.89</td>
</tr>
<tr>
<td>Tarivid® tab. (062)</td>
<td>200</td>
<td>203.50</td>
<td>200</td>
<td>101.72 ± 0.29</td>
<td>100.98 ± 0.38</td>
<td>3.46</td>
<td>1.72</td>
</tr>
<tr>
<td>Mefoxin tab. (168075)</td>
<td>250</td>
<td>245.00</td>
<td>250</td>
<td>97.99 ± 0.48</td>
<td>99.20 ± 0.91</td>
<td>2.63</td>
<td>3.59</td>
</tr>
<tr>
<td>Péflacin® amp. (5571718)</td>
<td>400</td>
<td>404.50</td>
<td>400</td>
<td>101.08 ± 0.42</td>
<td>100.86 ± 0.65</td>
<td>0.64</td>
<td>2.40</td>
</tr>
</tbody>
</table>

* Name of the drug and detailed composition in experimental section.
** Average of five determinations.

According to the $t$- and $F$-tests, there were no significant difference between the calculated and theoretical values at $p = 0.05$ indicating that the proposed method is as accurate and precise as the reported UV method.

Interference study

The proposed procedure has the advantage that the assay is performed at 520 nm in the visible range away from the uv absorbing interferents that might be coextracted from the dosage forms.

The interference caused from the presence of sodium ascorbate in péflaciné ampoules, glucose and lactose in tablets could be overcame by the use of mild oxidizing agent, 1 ml of 0.1% potassium bromate solution before adopting the proposed procedure which oxidizes it without affecting the drug. The commonly used tablet additives and excipients were found not to interfere with the analysis, under the very mild reaction conditions. Possible interference from foreign ions and substances was investigated, since it is likely that two or more of these substances would be coadministered e.g. antacids. These substances or additives cause no serious interference up to at least the concentration indicated except ascorbic acid which is not tolerated at all concentration levels. Starch and glucose were reported\textsuperscript{35} to react quantitatively with potassium permanganate at 95°C. The results are summarized in Table 4.

Suggested reaction mechanism

The course of degradation oxidative of quinoline and its derivatives is complex. Heterocyclic ring opening or disruption of both rings may occur on oxidation, preferably with acid permanganate to give oxalic acid, carbon dioxide and ammonia.\textsuperscript{36}

From the results obtained in the molar ratio, it is clear that each molecule of fluoroquinolone react with three molecules of permanganate. This would account for a ratio of 15 electrons for one molecule of fluoroquinolones.

Reviewing the literature in hand, no attempt was made to elucidate the reaction products in
Table 4: Analysis of certain fluoroquinolone derivatives in the presence of some co-formulated or co-prescribed ingredients.

<table>
<thead>
<tr>
<th>Substance added</th>
<th>Amount added (mg)</th>
<th>% Recovery of fluoroquinolones, ± SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>50</td>
<td>100.21 ± 0.360</td>
</tr>
<tr>
<td>Talc</td>
<td>25</td>
<td>100.18 ± 0.471</td>
</tr>
<tr>
<td>Sucrose</td>
<td>50</td>
<td>99.50 ± 0.315</td>
</tr>
<tr>
<td>Lactose</td>
<td>10</td>
<td>100.33 ± 0.426</td>
</tr>
<tr>
<td>Glucose</td>
<td>10</td>
<td>100.61 ± 0.581</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>10</td>
<td>99.85 ± 0.603</td>
</tr>
<tr>
<td>Gum acacia</td>
<td>10</td>
<td>100.00 ± 0.462</td>
</tr>
<tr>
<td>Gum tragacanth</td>
<td>10</td>
<td>100.25 ± 0.310</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>1</td>
<td>100.08 ± 0.511</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>1</td>
<td>100.00 ± 0.601</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>15</td>
<td>114.80 ± 0.286</td>
</tr>
<tr>
<td>Polyethylene glycol 4000</td>
<td>20</td>
<td>100.00 ± 0.468</td>
</tr>
<tr>
<td>Aluminum hydroxide</td>
<td>1800</td>
<td>98.98 ± 0.500</td>
</tr>
<tr>
<td>Magnesium oxide</td>
<td>3400</td>
<td>100.01 ± 0.602</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>400</td>
<td>100.00 ± 0.431</td>
</tr>
</tbody>
</table>

* Average of three determinations, * Added in mg per 400 mg of norfloxacin.

Other oxidimetric procedures for fluoroquinolones. Many oxidizable sites in the fluoroquinolone molecules would contribute to the reaction mechanism, both the carboxylic acid moiety and the piperazinyi nitrogen participates in the oxidation, decarboxylation and also the double bonds are site of attack and breakage. Although participation of ring nitrogen can not be discounted.

To sum up, it is believed that the molecule of fluoroquinolone, containing many oxidizable sites, the first and the most suitable site for oxidation is the terminal piperazine nitrogen. In another permanganometric method, but under very drastic conditions, with doxycycline and anticancer drugs the reaction products were found to be carbon dioxide and water. From the UV and IR data, of the reaction products where all the principle peaks at (3600-3250), 3050, 2950, 2830, (1725-1700), 1628, 1619, 1484, and 1250 cm⁻¹ corresponding to NH, OH, olefinic and aromatic CH, in methyl and methylene of ethyl and piperazine, carboxylic acid C=O, pyridone C=O and C=C, C-C and C-N of quinoline ring and C-F stretch have completely disappeared.

Correlation between log ε and some physicochemical parameters

The physicochemical characteristics of fluoroquinolones play an important role in their bioavailability and hence the antimicrobial activity. In other words, the different behavior of these quinolones, established from a physicochemical point of view, is clearly shown regarding pharmacokinetic properties. This fact again confirms the strong relationship between the physicochemical properties of drugs and their pharmacokinetic behavior.

Correlations between log ε and pK₁, pK₂, MIC (minimal inhibitory concentration), log Kᵦ (octanol partition coefficient), So (Intrinsic solubility), IP (Isoelectric point) were tried using linear regression equation. The obtained equations were:
log ε = -1.337 + 1.765 pK₁, \( r = 0.9351 \)
log ε = 23.089 - 3.538 pK₂, \( r = -0.5716 \)
log ε = 1.1640 - 0.2626 MIC \( r = -0.8156 \)
log ε = 23.0629 + 5.8852 log \( K_0 \), \( r = 0.6979 \)
log ε = 32.9091 + 3.000 So \( r = -0.8505 \)
log ε = 12.6044 - 1.2975 IP \( r = -0.5053 \)

From the data in Table 5, good correlations with pK₁ (carboxyl), moderate correlation with MIC, log \( K_0 \) and So while poor correlation with pK₂ (piperazine) and IP were found. The geometries of fluoroquinolones, permanganate and both together in all possible spatial positions were fully optimized at the full Self-Consistent Field (SCF) level, there was no correlation with heat of formation, Van der Wall forces or dipole moment (DM). The absolute values of DM for nalidixic acid and rosaxacin (3.46 and 3.42) which are nearly half that of other seven studied fluoroquinolones may account for the negative reaction response. Because the number of fluorines did not account for differences in the electron withdrawing effect due to the distance between the fluorine atom and the carboxylic acid site, the pK₃ was used in the regression analysis as an indicator of the electron density of the carboxylic acid moiety. Nalidixic acid and rosaxacin were not included in this evaluation because they did not react with permanganate under the present reaction conditions. The regression equation demonstrated that an increase in pK₃ value resulted in an increase in log ε. The change in the dissociation constant was an indicator of the electron density near the protonation site. As the electron density near the protonation site increased, the anionic form was less stable than the protonated form which resulted in an increase in the pK₃ value. An increase in the electron density would be expected to increase permanganate concentration for the drug molecule resulting in an increased oxidation.

The present investigation resulted in finding statistically significant relationships which may be useful in the prediction of pK₃ from the obtained molar absorptivity values.

Table 5: Correlation between log ε and some physico-chemical parameters of the studied quinolone antimicrobials.

<table>
<thead>
<tr>
<th>Quinolone derivatives</th>
<th>log ε</th>
<th>MIC, µg/ml</th>
<th>pK₁</th>
<th>pK₂</th>
<th>log ( K_0 )</th>
<th>IP</th>
<th>S⁺</th>
<th>DM⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amifloxacin</td>
<td>4.34</td>
<td>0.025</td>
<td>6.28</td>
<td>7.39</td>
<td>- 3.01</td>
<td>6.84</td>
<td>0.0621</td>
<td>6.15</td>
</tr>
<tr>
<td>Difloxacin</td>
<td>4.21</td>
<td>0.200</td>
<td>6.06</td>
<td>7.63</td>
<td>- 0.38</td>
<td>6.85</td>
<td>0.0609</td>
<td>5.51</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>4.18</td>
<td>0.100</td>
<td>6.20</td>
<td>8.70</td>
<td>- 2.00</td>
<td>7.34</td>
<td>0.3200</td>
<td>7.35</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>4.01</td>
<td>0.100</td>
<td>5.70</td>
<td>8.22</td>
<td>- 0.48</td>
<td>7.14</td>
<td>2.7500</td>
<td>6.41</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>4.22</td>
<td>0.0250</td>
<td>6.09</td>
<td>8.74</td>
<td>- 1.70</td>
<td>7.42</td>
<td>0.0792</td>
<td>6.95</td>
</tr>
<tr>
<td>Lomefloxacin</td>
<td>4.07</td>
<td>0.2500</td>
<td>5.83</td>
<td>9.30</td>
<td>- 1.36</td>
<td>7.56</td>
<td>1.0300</td>
<td>5.56</td>
</tr>
<tr>
<td>Péfloxacin</td>
<td>4.08</td>
<td>0.1000</td>
<td>6.100</td>
<td>7.70</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>6.90</td>
</tr>
</tbody>
</table>

MIC = minimal inhibitory concentration using E. Coli \( H_{50} \), \( K_0 \) = Octanol partition coefficient, IP = Isoelectric points, \( S^+ \) = Intrinsic solubility at 25°C in mg/ml, DM = Dipole moment.

* Computed by using MNDO/3, a general molecular orbital package implemented with molecular mechanics software MMX-PC.
Conclusion

A successful general procedure has been developed by which fluoroquinolones may be determined quantitatively by using permanganate in sulphuric acid medium. The proposed method is advantageous when compared to many of the reported methods in having higher sensitivity which permits the determination of up to 2 μg ml⁻¹, less time consuming and more accurate than the ion extraction and uv methods. It can be applied for the quality control analysis of fluoroquinolones containing dosage forms without interference. It must be considered non specific with regard to differentiation between them. These shortcomings do not affect the utility of the procedure in routine analysis and content uniformity determination of these drugs as they are singly prescribed or combined with other chromatographic procedures. A simple linear correlation between molar absorptivity and ionization constants, biological activity and partitioning of these series of quinolone has been provided.

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