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COLORIMETRIC DETERMINATION OF SOME FLUOROQUINOLONE DERIVATIVES THROUGH ION PAIR FORMATION

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ABSTRACT

Two sensitive procedures were described for the determination of two fluoroquinolone containing compounds namely, norfloxacin and ofloxacin. In these procedures methyl orange and bromophenol blue were utilized in the determination of the concerned compounds by forming ion pair complex in buffered aqueous solution. The chloroform extract of the drug complexes were measured at λ max 422 and 414 nm with methyl orange and bromophenol blue, respectively. In procedure I, methyl orange was used at pH 3.5 for norfloxacin and pH 4 for ofloxacin. The proposed method was applied for determination of Noroxin and Tarivid tablets. In procedure II, bromophenol blue was used at pH 2.9 for ofloxacin and the procedure then applied for determination of Tarivid tablets with mean percentage recovery 99.84 ± 1.76. The results were compared with the official methods and showed no significant difference.

INTRODUCTION

Norfloxacin and ofloxacin are members of fluorinated, -4-quinolones, which are synthetic antibacterial agents related to nalidixic acid. The fluorine atom increases their penetration to target receptors and leads to broader spectrum of activity[1,2].

Different methods were reported for determination of these compounds including direct spectrophotometery or colorimetry after reaction with ferric salts or β-naphthol[3-5], as well as titrimetry[6].

The USP XXII described a non aqueous titration of norfloxacin by perchloric acid[7]. In addition fluorimetric methods after TLC separation were also described[8,9]. Also, reversed phase HPLC methods were described[10-14].

In the present work, attempts were made to determine norfloxacin and ofloxacin by forming extractable salts or ion pair between these positively charged nitrogen center at the proper pH and the negatively charged dyes as methyl orange and bromophenol blue in aqueous solution.

EXPERIMENTAL

Apparatus:

Shimadzu UV-visible recording spectrophotometer UV-260 and Chemcadet pH-meter, were used.

Chemical and reagents :

All chemicals and reagents were of analytical grade.

i-Methyl orange; 0.15 g% aqueous solution.

ii-Bromophenol blue; 0.1g % in aqueous ethanol.

iii-Buffer pH 4 was prepared by dissolving 125g KCl and 70g sodium acetate trihydrate in 700 ml distilled water and adjusting the pH by addition of glacial acetic acid (a bout 375ml) and brought to one liter with water.

iv-Buffer pH 3.5 was prepared as the aforementioned method using about 300 ml glacial acetic acid.

v-Buffer pH 2.9 was prepared as before using 50 g sodium acetate and about 300 ml glacial acetic acid.

Standard ofloxacin solution: 0.02g % aqueous acidic solution; was prepared by accurately weighing 20 mg of ofloxacin (from Hoechst orient, Egypt) and dissolved in 0.5 ml glacial acetic acid in 100 ml calibrated flask and completed to volume with distilled water (for procedure I).

Standard ofloxacin solution: 0.01g % (for procedure II)

Standard ofloxacin solution: 0.1g aqueous acidic solution (from Epico, Egypt) for procedure I.

Tarivid tablets, from Hoechst orient, Egypt (Batch No 054), labeled to contain 200 mg ofloxacin per each tablet.

Noroxin tablet, from Epico, Egypt (Batch No 942249), labeled to contain 400 mg norfloxacin per each tablet.

Procedure I:

A-Determination of authentic samples:

5 ml aliquot containing 0.1-1 mg or 1-9 mg of ofloxacin or norfloxacin, respectively, was transferred to separating funnels, followed by 4 ml buffer pH 4 for ofloxacin or pH 3.5 for norfloxacin and 5 ml methyl orange. The volume was brought to 50 ml with distilled water, extract with 20 ml chloroform added in three portions.

The collected extract was transferred into 25 ml calibrated flask, 1.5 ml ethanol was added and completed to volume with chloroform.

The yellow coloured chloroformic extract was measured at λ max 422 nm against colorless blank prepared in the same manner without the drug.

B-Determination of tablet dosage forms:

Twenty tablets of Tarivid or Noroxin were accurately weighed and the average weight of one tablet was determined. The tablets were triturated thoroughly and an amount equivalent to 20 mg of ofloxacin or 100 mg of norfloxacin was dissolved in 0.5 ml glacial acetic acid in 100 ml volumetric flask, completed to volume with distilled water, filtered and completed as mentioned before under procedure 1A.

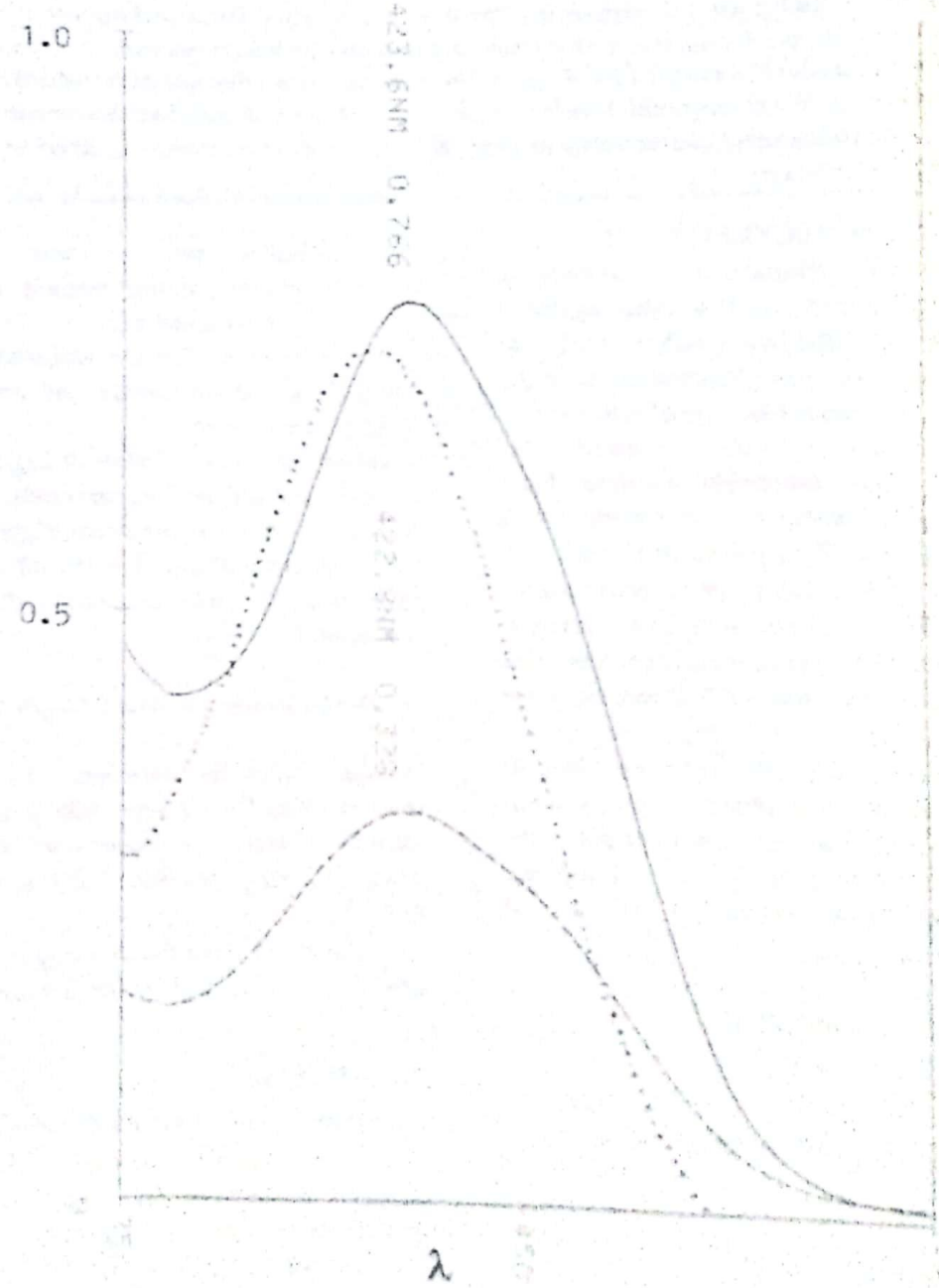

Fig. (1) : Absorption curves of ofloxacin-methyl orange (——), norfloxacin-methyl orange (- -) and ofloxacin-bromophenol blue (.....)

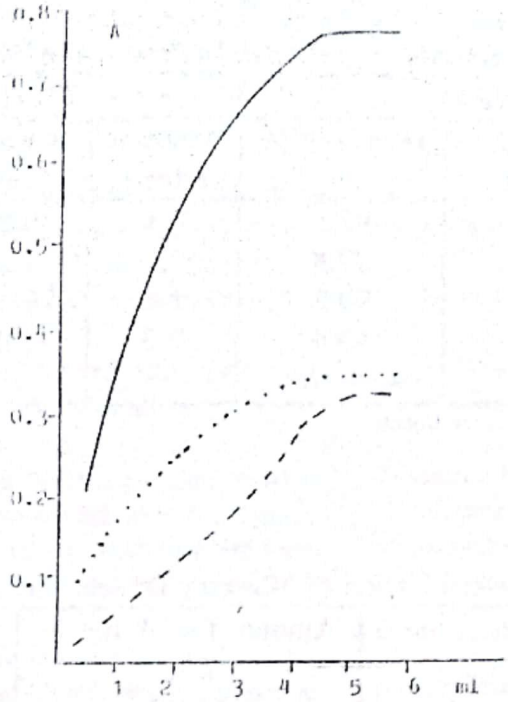

Fig. (2) : Effect of methyl orange volume on ofloxacin (——) and norfloxacin (– – –) and volume of bromophenol blue on ofloxacin (.....).

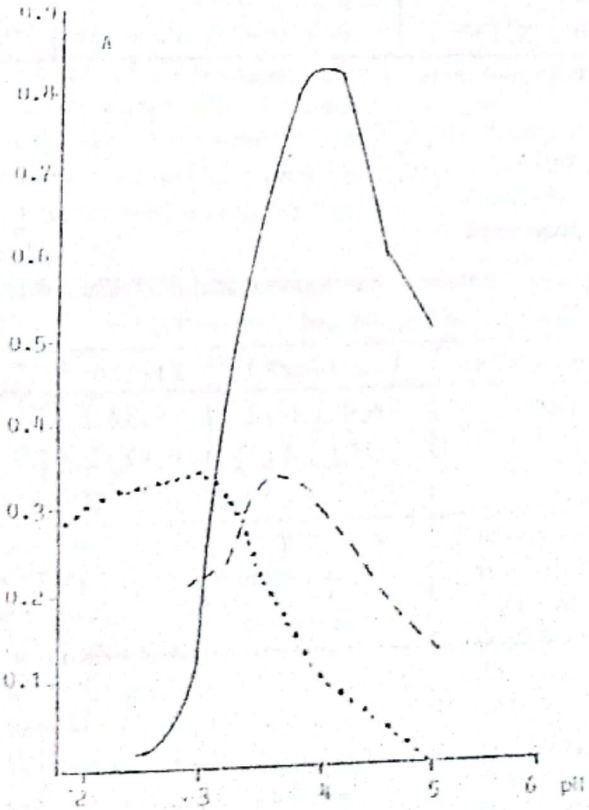

Fig. (3) : Effect of pH on ion pair complex of ofloxacin (——) and norfloxacin (– – –) with mehtyl orange and ofloxacin (.....) with bromophenol blue.

Table (1): Determination of Tarivied Tablets by the Proposed Methods.

Procedure I			Procedure II		
Amount added	Amount found	recovery %	Amount added	Amount found	recovery %
0.4	0.39	97.5	0.8	0.81	101.23
0.8	0.78	97.8	1.2	1.18	98.33
1.2	1.19	99.2	1.6	1.55	97.88
1.6	1.59	99.4	2.0	2.04	101.90
Mean ± SD		98.48± 0.96			99.84± 1.79

Each value is the mean of three experiments.

Table (2) : Determination of Noroxin Tablets Using Procedure I.

Amount claimed, mg%	Amount found, mg %	Recovery %
8	7.73	97.0
12	11.90	99.16
16	15.71	98.13
20	19.93	99.63
24	23.42	97.10
Mean ± SD		98.31 ± 1.04

Each value is the average of three experiments.

Table (3) : Comparison Between the Proposed and the Official Methods for the Determination of Tarivid and Noroxin Tablets.

	Preparation	Procedure I	Procedure II	Official
Mean ± SD Student's test F ratio	Tarivid	96.9 ± 1.12 1.25 (2.447) 2.57 (9.28)	99.84 ± 1.76 0.31 (2.447) 4.25 (9.28)	100.3 ± 1.8[6]
Mean ± SD Student's test F ratio	Noroxin	98.31 ± 1.04 1.29 (2.365) 2.089 (6.59)		99.4 ± 1.5[7]

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Procedure II:

A-Determination of authentic ofloxacin:

Aliquot (5 ml) containing 0.05-0.5 mg of ofloxacin was transferred to a separating funnel, followed by 4 ml buffer pH 2.9 and 5 ml of bromophenol blue, and completed to 25 ml with distilled water.

The mixture was extracted with 20 ml chloroform, added in three portions, and the collected extract was transferred into 25 ml calibrated flask. Then 2 ml ethanol was added and completed to volume with chloroform.

The yellow coloured chloroformic extract was measured against blank at λ max 414 nm.

B-For tablet dosage form:

Amount of the powdered tablets equivalent to 10 mg ofloxacin was taken and dissolved in 0.5 ml glacial acetic acid, completed to volume with distilled water, filtered and completed as mentioned under procedure II A.

RESULTS AND DISCUSSION

Norfloxacin and ofloxacin are amino compounds as they contain piperazine moieties, therefor they form yellow chloroformic extract with methyl orange at λ max 422 nm and at 414 nm with bromophenol blue as shown in Fig. (1).

The pH of the aqueous phase is critical for colour formation, so the optimum pH was studied for each drug. In case of methyl orange pH 3.4-3.6 and 3.9-4.1 were found to be the optimum for norfloxacin and ofloxacin, respectively, while in case of bromophenol blue the optimum pH was 2.8-3 for ofloxacin Fig. (2).

Acetic acid-sodium acetate buffer serves well in maintaining the proper pH.

KCl is included in these buffer merely as an aid in affecting complete separation of the organic phase and aqueous layer.

Bromophenol blue was unsuitable for quantitative determination of norfloxacin due to the lower solubility in chloroform, another extracting solvent as methyl chloride was tried and was also unsatisfactory for complete extraction.

The yellow colour was stable up to 48 h without any change in intensity or in the λ max.

The amount of the dyes should be sufficient and the excess has no effect on the colour intensity ,Fig.(3).

Addition of ethanol after extraction was necessary to prevent colour adsorption to the wall of the flask.

Calibration graphs were constructed by blotting the absoubance as a function of concentration using the methyl orange the relation was linear in the range of 3-36 mg and 0.44 mg % for norfloxacin and ofloxacin, respectively , while for ofloxacin - bromophenol blue the relation was linear in the range 0.2- 2 mg %.

Using the methods of least squares the calibration graphs were described by the following regression equations:

A= 0.006+0.0083 C r = 0.997 for norfloxacin-methyl orange.

A= 0.0342+0.1867 C r = 0.997 for norfloxacin-methyl orange.

A= 0.01+0.378 C r = 0.991 for ofloxacin-bromophenol blue

Where: c is concentration in mg % in final solution.

The validity of these regression equations was tested by analyzing the studied compounds in their pharmaceutical preparations by standard addition techniques and the results obtained are shown in tables (1.2) and compared with those obtained by the official or reported method as shown in table (3)

Statistical analysis of the results showed no significant difference as the calculated t and f values are less than the tabulated values and the proposed methods are equally precise and accurate as the official methods.

Moreover the suggested methods are simple, selective and no interference, form the tablet excipients or the coats.

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التقدير اللونى لبعض مشتقات الفلوروكينولون خلال تكوين أيون مزدوج

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تشمل هذه الدراسة طريقتين لتقدير إثنين من مشتقات الفلوروكينولون وهم نورفلوكساسين وأوفلوكساسين باستخدام الميثيل البرتقالي والبرموفينول الأزرق ، وقد تم قياس المستخلص الكلوروفورمى لمتراكب الأيون المزدوج لكل من المركبين عند طول موجه ٤٢٢ نم فى حالة الميثيل البرتقالى و ٤١٤ نم فى حالة البروموفينول الأزرق.

وقد تم استخدام الميثيل البرتقالى فى الطريقة الأولى عند أس أيون هيدروجينى ٣,٥ فى حالة نورفلوكساسين و ٤ للاوفلوكساسين.

أما فى الطريقة الثانية تم استخدام البروموفينول الأزرق عند أس هيدروجينى ٢,٩ فى حالة أوفولكساسينى وقد تم تطبيق الطريقتن على لمستحضرات الصيدلية التى تحتوى على هذه المواد أثبتت النتائج تطابقها مع الطرق الدستورية.