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UTILITY OF DICHLOROMALEIMIDE DERIVATIVE AS A CHROMOGENIC REAGENT FOR THE SPECTROPHOTOMETRIC DETERMINATION OF CERTAIN FLUOROQUINOLONES AND RELATED COMPOUNDS

Sobhy M. El-Adl, Kamel A. Metwally, and Mansour E. Abou-Kull

Medicinal Chemistry Department, Faculty of Pharmacy, University of Zagazig, Zagazig, Egypt

ABSTRACT

A simple and sensitive spectrophotometric procedure is described for the quantitative determination of certain fluoroquinolones (ciprofloxacin, enoxacin, and norfloxacin) either in authentic samples or in their pharmaceutical formulations. The cited drugs are interacted with a synthetic reagent, [N-2,6-dimethylphenyl-2,3-dichloromaleimide] forming an intense colour which can be measured at λmax 514 nm. The optimum interaction conditions, molar ratio of the reactants, and calibration graphs have been studied. A comparative study with the official pharmacopoeial methods of assay of the cited drugs showed no significant difference between them. Furthermore, the proposed procedure was more sensitive and selective. Moreover, the reaction product was isolated and subjected to the structural study using IR and NMR spectroscopy.

INTRODUCTION

The fluoroquinolones form an important new class of oral synthetic broad spectrum antibacterial agents. The fluorine atom at the 6-position provides increased potency against gram-negative bacteria and covers gram-positive organisms while the piperazine moiety at the 7-position is responsible for antipseudomonal activity. Excellent therapeutic effects have been shown in the treatment of respiratory, biliary, and urinary tract infection (1). Chemically, ciprofloxacin is 1-cyclopropyl -6-fluoro-1,4- dihydro -4- oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid. Enoxacin is 1-ethyl- 6-fluoro -1,4-dihydro-4-oxo-7- (1-piperazinyl) -1,8- naphthyridine-3-carboxylic acid. Norfloxacin is 1-ethyl-6-fluoro-4-oxo-7-(1-piperazinyl)- 1,4- dihydro quinoline-3-carboxylic acid. Numerous methods were reported for the determination of these compounds in biological fluids as well as in pharmaceutical preparations including: titrimetric (2,3). spectrophotometric(4,5), fluorimetric(6), polarographic(7), voltametric, (8) and HPLC methods.(9-12) The reagent of dichloromaleimide derivative was previously utilized by the authors for determination of isoniazid(13). The aim of this work was developing a simple colorimetric procedure using dichloromaleimide derivative for the quantitative estimation of three widely used fluoroquinolones, namely, ciprofloxacin (I), enoxacin (II), and norfloxacin (III). The proposed method has been applied to the determination of these drugs in the bulk form and in their pharmaceutical formulations with satisfactory results. Furthermore, the structural investigation study of the reaction product was determined using IR and NMR spectroscopy.

EXPERIMENTAL

Instrumentation:

1-Shimadzu 260 UV, UV-Vis self-recording spectrophotometer, (Japan).

2-Infrared spectrophotometer, PU 9700 series, Philips, (England), using KBr disc.

3-Varian EM-390, 90MHz NMR spectrometer (USA) using DMSO-d_6 as solvent.

Materials and reagents:

All the reagents and solvents were of the analytical grade quality.

1-Standard drug solutions: each is prepared in concentration of 50 mg% in aqueous ethanol; ciprofloxacin powder is obtained from (Bayer Leverkusen, Germany), norfloxacin powder from (Merck & Co. Inc. Rahway, NJ, USA) and enoxacin powder from (Sigma Chem. Co., USA).

2-Dosage forms: tablets and injections were purchased from local market. Serviflox tablets are manufactured by Novartis Pharma S.A.E, Cairo; labeled to contain 250, 500, & 750 mg of ciprofloxacin HCl per each tablet. Ciprofloxacin infusion solution is produced by Amriya Pharm. Ind., Alexandria; labeled to contain 200 mg of ciprofloxacin per each 100 ml infusion solution. Noroxin tablets are manufactured by E.I.P.I.Co., Egypt; labeled to contain 400 mg of norfloxacin per each tablet. Neofloxin tablets are produced by Alex. Co. Pharm., Alexandria; labeled to contain 400 mg of anhydrous norfloxacin per each tablet.

3-Reagent: N - 2, 6- dimethylphenyl -2 ,3- dichloromaleimide ; was synthesized according to the reference procedure;(14) crystallized from n-hexane; m.p. 141±1°C, ^{1}H-NMR (δ ppm): 2.15 (s, 6H, $2CH_3$); 7.1-7.6 (m, 3H, aromatic protons). The reagent stock solution is prepared in concentration of 1 mg/ml^{-1} in ethanol.

Procedures:

Authentic powder:

Various aliquots of the standard drug solutions, ranging from 0.2-1.4 ml for ciprofloxacin, 0.3-1.8 ml for enoxacin, and 0.24-1.68 ml for norfloxacin, were transferred separately into series of 10 ml calibrated flasks. Exactly 4 ml of dichloromaleimide reagent solution was added and the mixture was heated on a water bath at 70 °C for 25 minutes. The solutions were cooled then diluted to the volume with ethanol and the absorbance was measured at λ max 514 nm against a reagent blank prepared in the same way but without the drug. All measurements were repeated three times at each concentration.

II-Application to the pharmaceutical preparations:

Tablets:

Twenty tablets were weighed and the average weight of one tablet was determined. The tablets were powdered, mixed, and an accurately weighed portion equivalent to 50 mg of the drug was quantitatively extracted with ethanol by the aid of sonication for 20 minutes. The solution was filtered into a 100 ml calibrated flask and completed to the volume with ethanol. Different aliquots from this solution were

treated with the working dichloromaleimide reagent and the method was continued as described under procedure(I).

Injections:

An accurately measured volume of ciprofloxacin infusion solution (25 ml) equivalent to 50 mg of ciprofloxacin was quantitatively transferred into a 100 ml calibrated flask. The solution was diluted to the volume with ethanol and different aliquots from this solution were treated with dichloromaleimide reagent. Then, the method was completed as described under procedure (I).

III-Procedure for product isolation and subsequent examination:

An equimolar solution of each of fluoroquinolone drugs and dichloromaleimide reagent were mixed together with 3 ml of acetic acid. The mixture was vigorously stirred, with the aid of electrical stirrer, and heated on a water bath at 70 °C for 25 minutes. Ethanol was allowed to be evaporated and the resulted precipitate was filtered, washed, dried, and subjected to the investigation by TLC, m.p, IR and NMR spectroscopy.

RESULTS AND DISCUSSION

The studied fluoroquinolones (ciprofloxacin I, enoxacin II, and norfloxacin III) contain a secondary amino group of piperazine moiety which interacts with the N-substituted dichloromaleimide reagent forming an intense colour. The course of the reaction between the cited drugs and dichloromaleimide is a nucleophilic substitution reaction with the liberation of HCl. On the other hand, when the analogous reaction was applied on fluoroquinolones with a tertiary amino group of piperazine such as ofloxacin (N-CH_3) and enrofloxacin (N-CH_2CH_3), the alternative ordinary colour was not produced, where quaternary ammonium compound was formed in this respect. This may be explained according to the following suggested reaction pathway, (as shown in scheme 1) which agree with reports on similar reactions in the literature[15,16].

The absorption spectra of dichloromaleimide reagent and the reaction products are shown in Fig. 1.

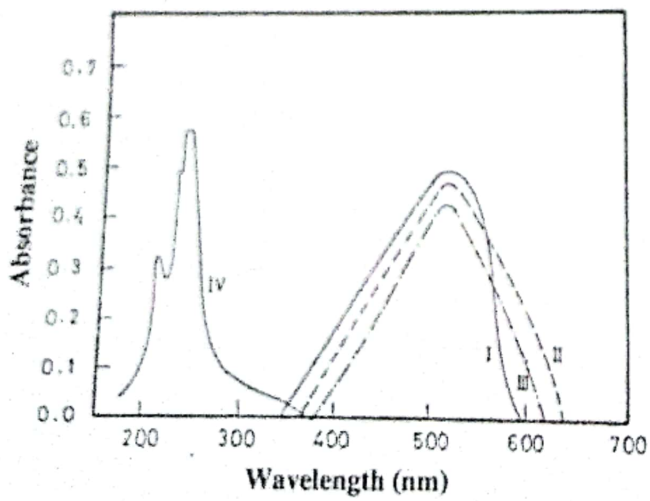

Fig. 1: Absorption spectra of fluoroquinolones-dichloromaleimide product. (I) ciprofloxacin, (II) enoxacin, (III) norfloxacin and (IV) the reagent blank.

The reagent exhibited maximum absorption at 237 nm; the fluoroquinolone drugs (I, II, III) showed λ max at 283, 274, and 281 nm, respectively. The reaction product of the cited drugs has λ max at 514 nm, while that of other fluoroquinolones containing tertiary amino group has λ max at 345 nm.

The reaction product structure was confirmed by melting point determination and by IR & NMR spectroscopy. Where the characteristic NH peak of piperazine moiety that observed at 3400-3200 cm^{-1} has disappeared. The other feature characteristic infrared band assignment has been constructed in Table 1. Also, the melting point values were 141±1, 228 ±1 & 213 ±1°C for the reagent, norfloxacin, and the product, respectively. In the NMR spectrum of the product, the characteristic peak of NH proton of drug itself that observed at 9.3 ppm has omitted. In addition, the liberated HCl in the interaction was tested by silver nitrate solution giving white precipitate.

Table 1: Fluoroquinolones infrared band assignments:

Wave number (cm^{-1})	Assignment
3500-3100	N-H and O-H stretch
3050	Olefinic and aromatic CH stretch.
2950-2850	Symmetric and asymmetric C-H stretch in methylene and piperazine groups.
2550-2500	Hydrogen bonded O-H stretch.
1720-1700	Carboxylic acid C=O stretch.
1630	Pyridone C=O stretch.
1620	Pyridone ring C=C stretch.
1470	Quinoline ring C-C and C-N stretch.
1250	C-F and carboxylic C-O stretch.

The intensity of the product absorption was independent of pH, but maximum stability was between pH 6-8 in the aqueous alcoholic medium. The absorbance increased considerably with increasing reagent concentration, 4 mls of dichloromaleimide was found to give maximal sensitivity. To accelerate the reaction colour formation, higher temperature was used. Heating on a water bath at 70°C for 25 minutes was essential to obtain maximal absorbance readings. There is no appreciable formation of the colored compound at room temperature. These experimental conditions were established by varying each parameter individually and observing its effect on absorbance of the colored species.

The stoicheiometric relationship of the reactants (drug : reagent) was studied using Job`s continuous variation method. The interaction between these two components has been carried out using an equimolar concentration, giving a 1:1 molar ratio between them as shown in Fig. 2. This finding was expected since the

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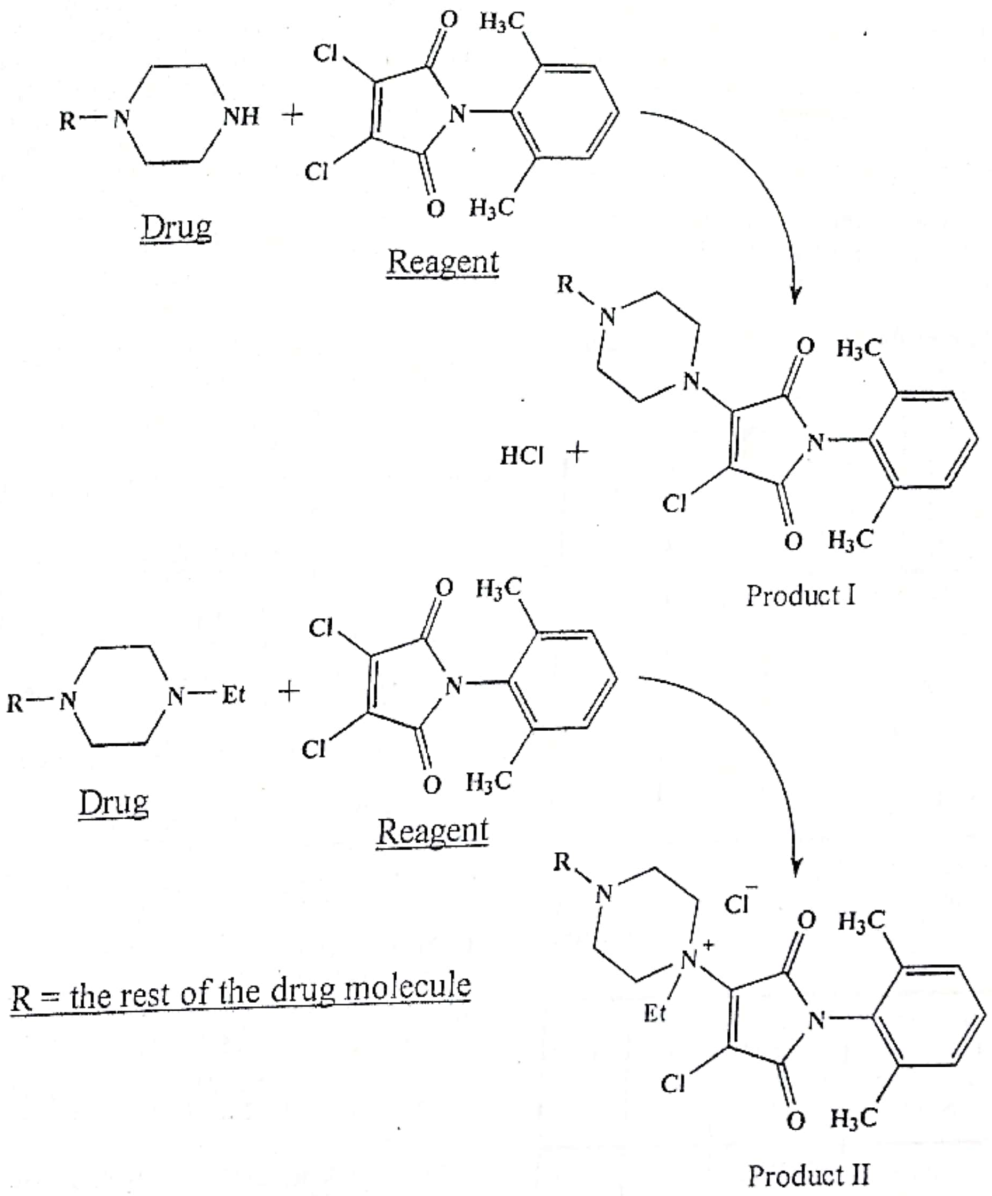

Scheme 1: Suggested reaction pathway of fluoroquinolones with dichloromaleimide reagent.

cited fluoroquinolones have only a single secondary amino group of piperazine moiety.

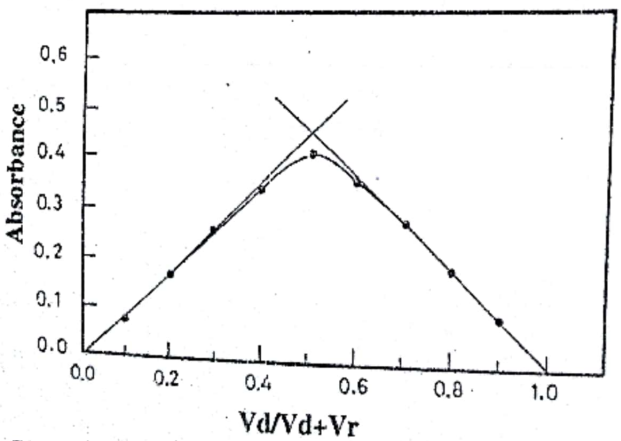

Fig. 2: Job's continuous variation plot of equimolar solutions of norfloxacin & dichloromaleimide reagent (3.2×10^{-4} M) in ethanol.

Under the experimental conditions described, the absorbance was a linear function of the concentration, over the range of 10-70; 15-90, and 12-84 µg ml^{-1} for ciprofloxacin, enoxacin, and norfloxacin, respectively. Using the method of least squares, regression analysis was carried out for intercept and slope. Good linearity was obtained as indicated from the correlation coefficients of 0.9998, 0.9997, & 0.9998, respectively. The regression equations describing the calibration graphs were as follows:

For ciprofloxacin	A = 0.007 + 0.121 C
For enoxacin	A = 0.006 + 0.068 C
For norfloxacin	A = 0.002 + 0.069 C

Where A is the absorbance and C is the concentration in mg% in the final measured solution.

The validity of the proposed procedure for the estimation of the studied compounds in their pure state and in their pharmaceutical forms was tested by analyzing these products with the official pharmacopoeial methods. [3,10] A standard addition

technique was used for the determination of the cited drugs in their dosage forms. The results in tables (2,3) indicate that the proposed method is fairly accurate and as precise as the official method, since the calculated t and F values do not exceed the tabulated values.[17] The measured mean percentage recovery, the standard deviation, relative standard deviation, the standard analytical error, and the confidence limits of the statistical analysis postulated the accuracy and precision of the proposed method.

Table 2: Determination of the three fluoroquinolones in bulk form using the proposed method compared with the titrimetric method.

Sample	Official method (mean ± S. D)	Proposed method (mean ± S. D)	t	F
Ciprofloxacin	99.20± 0.42	98.97 ± 0.70	0.676	2.72
Enoxacin	99.06 ± 0.59	98.93 ± 0.60	0.351	1.03
Norfloxacin	98.79 ± 0.64	99.00 ± 0.26	0.70	5.85

Table 3: Determination of the fluoroquinolones in their dosage forms using the proposed method compared with the non- aqueous titration method.

Sample	Non-aqu. titr method (mean ± S. D)	Proposed method (mean ± S. D)	t	F
Serviflox tablets	100.14 ± 0.68	99.94 ± 0.73	0.44	1.15
Ciprofloxacin infusion solution	100.12 ± 0.80	100.10 ± 0.93	0.04	1.36
Neofloxin tablets	100.60 ± 0.60	100.76 ± 0.66	0.40	1.22
Noroxin tablets	100.84 ± 0.47	100.62 ± 0.65	0.61	1.91

* t tabulated value at p = 0.05 is 2.31
* F tabulated value at p = 0.05 is 6.39
* Mean is the average of 5 experiments.

The official B.P. 1998 describes a non aqueous titration method for the assay of both norfloxacin and ciprofloxacin, that requires at least 300 mg of each drug for its determination. On the other hand, the official U.S.P. 1995 describes also a non aqueous titration method for assay of norfloxacin, that requires 460 mg of the drug for its estimation, while it describes HPLC method for the assay of ciprofloxacin or ciprofloxacin HCl. Enoxacin is not official drug in the B.P or U.S.P, and it is an isostere of fluoroquinolones.

The above study shows that the proposed method is much more sensitive than the official methods. Also, the proposed procedure is a selective one depending upon the presence of an intact molecule and not the hydrolytic products. Thus it can be used to analyze the different fluoroquinolones in the presence of their impurities.

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استخدام مشتقات الداى كلوروماليميد للتقدير الكمى لبعض مركبات الفلورو كينولون والمركبات المشابهة

صبحى محمد العدل ، كامل عبدالرحيم متولى ، منصور السيد أبو كل

قسم الكيمياء الطبية - كلية الصيدلة - جامعة الزقازيق - الزقازيق - مصر

فى هذا البحث تم تطوير طريقة طيفية جديدة للتقدير الكمى لبعض مركبات الفلوروكينولون (سبروفلوكساسين ، اينوكساسين ، نور فلوكساسين) والمركبات المشابهة وذلك باستخدام الكاشف المشيد كيميائياً لمشتقات الداى كلوروماليميد وتم تطبيق الطريقة المقترحة على المستحضرات الصيدلية . وتم دراسة أنسب الظروف للتفاعل الكيميائى ، وتعيين النسب المولارية بينهما .

وقد تم التعرف على الناتج باستخدام الأشعة تحت الحمراء والرنين النووى المغناطيسى وتم تقدير المركبات المذكورة فى وجود المركبات الصيدلية الأخرى دون أى تداخل. وقد تم تطبيق الطريقة المقترحة على الأقراص والحقن ، وتم مقارنة النتائج بالطريقة الدستورية الانجليزية التى تعتمد على المعايرة ، ولم يوجد فرق جوهرى بينهما ، إلا أن الطريقة المقترحة كانت أكثر دقة وحساسية وأختيارية.