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Il Farmaco 56 (2001) 677–681

IL FARMACO

# Spectrophotometric determination of diloxanide furoate in its dosage forms

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Received 20 November 2000; accepted 30 March 2001

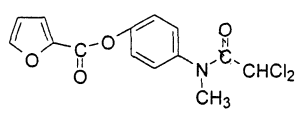
**Abstract**

A simple and sensitive spectrophotometric method has been developed for the determination of diloxanide furoate in its dosage forms. The method is based on the reaction of the drug with potassium permanganate in the presence of sodium hydroxide to produce a bluish green coloured species measurable at 610 nm. The absorbance–concentration plot is linear over the range 2.5–20 μg/ml with correlation coefficient ($n = 8$) of 0.9998 and minimum detectability of 0.2 μg/ml ($6.1 \times 10^{-7}$ M). The molar absorptivity was $1.1 \times 10^4$ l/mol cm. The different experimental parameters affecting the development and stability of the colour were carefully studied and optimised. The proposed method was applied successfully for the determination of diloxanide furoate in its tablet form. The results obtained were in good agreement with those obtained using the official method. The proposed method could be applied to the determination of diloxanide furoate in presence of some co-formulated drugs. The effect of sensitisers and surfactants on the performance of the proposed method was also studied. A proposal of the reaction pathway was presented. 

*Keywords:* Diloxanide furoate; Potassium permanganate; Pharmaceutical preparations; Spectroscopy; Pharmaceutical analysis

## 1. Introduction

Diloxanide furoate [2,2-dichloro-4-hydroxy-*N*-methylacetanilide-2-furoate] is a frequently described antiamoebic drug. It is considered as a safe and effective drug for the treatment of asymptomatic or mildly symptomatic persons who are passing cysts of *Entameba histolytica* [1,2]. It acts principally in the bowel lumen, and is used in the treatment of intestinal amoebiasis [3].




**Diloxanide Furoate**

The literature survey reveals few analytical methods for the determination of diloxanide furoate in pharmaceutical preparations. A good guide to the work published for diloxanide furoate is found in the comprehensive review written by Al-Majed et al. [4]. These methods are classified into spectrophotometry [5–7], HPLC [8,9], GLC [10,11] and HPTLC [12]. All these methods are either insufficiently sensitive [5–7] or tedious and require highly sophisticated instrumentation [8–11]. This led us to study the reaction of diloxanide furoate with $KMnO_4$ in an alkaline medium in an attempt to develop a simple and sensitive method for its determination in dosage forms. The results obtained were promising.

## 2. Experimental

### 2.1. Apparatus

Beckman Du-65 spectrophotometer (Fullerton, CA) with 1 cm quartz cells.

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0014-827X/01/$ - see front matter 
PII: S0014-827X(01)01110-7

### 2.2. Materials and reagents

Diloxanide furoate pure sample (Batch No. CDF/DF/175/97) was kindly provided by Eipico Pharmaceutical Company, Cairo, Egypt, and was used as received. Tablets containing the drug were obtained from the local market.

Potassium permanganate (Riedel-de Haen, Germany) 0.1 M aqueous solution was used.

Sodium hydroxide (Winlab., UK) 5 M stock solution was prepared.

Standard 0.1% (w/v) solution of diloxanide furoate was prepared in 0.1 M NaOH and was further diluted as appropriate.

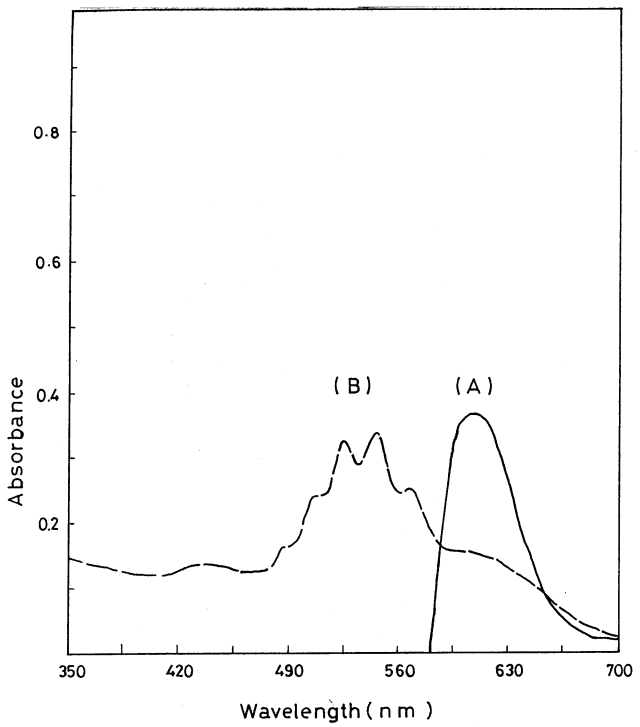



Fig. 1. Absorption spectra of the reaction product of diloxanide furoate (10 μg/ml) with the $KMnO_4$/NaOH system: (A) reaction product; and (B) $KMnO_4$ ($1 \times 10^{-4}$ M).

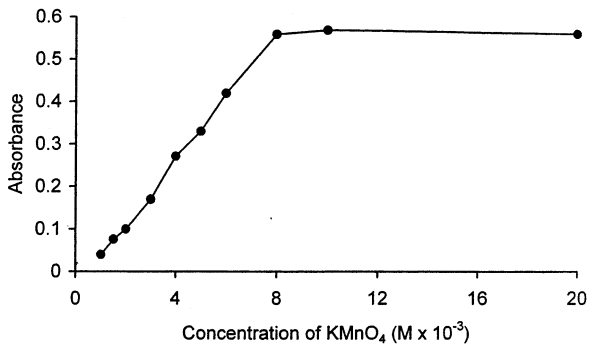



Fig. 2. Effect of concentration of $KMnO_4$ on the reaction of diloxanide furoate (15 μg/ml) with the $KMnO_4$/NaOH system.

### 2.3. Procedures

#### 2.3.1. Recommended analytical procedure

Transfer 1 ml of 0.1 M $KMnO_4$ and 0.2 ml of 5 M NaOH into a series of 10 ml measuring flasks. Add aliquot volumes of diloxanide furoate solution so that the final concentration is in the range of 2.5–20 μg/ml. Make up to volume of about 5 ml with distilled water, then leave at room temperature for about 15 min. Complete to the mark with distilled water. Prepare a blank solution containing the same amounts of $KMnO_4$ and NaOH. Measure the absorbance against the reagent blank at 610 nm. Plot the absorbance versus the final concentration to get the calibration graph. Alternatively derive the regression equation.

#### 2.3.2. Procedure for the tablets

Weigh and pulverize ten tablets. Transfer a weighed amount of the powder equivalent to 5.0 mg of diloxanide furoate into a small flask. Extract with $3 \times 10$ ml of water and filter into a 50-ml volumetric flask. Wash the residue and filter with few ml of water and pass the washings to the same flask. Complete to the mark with the same solvent. Transfer suitable aliquots of the solution into a 10-ml volumetric flask then proceed as described under Section 2.3.1. Determine the content of the tablet either from the calibration graph or using the regression equation.

## 3. Results and discussion

### 3.1. Optimisation of experimental parameters

Diloxanide furoate was found to react with $KMnO_4$ in alkaline medium producing a bluish green colour with absorption maximum at 610 nm (Fig. 1). Although $KMnO_4$ still has absorbance reading at 610 nm, the reagent blank will cancel this absorbance value. The various experimental factors affecting the development and stability of the reaction product were studied and optimised. Such factors were changed individually, while others remained constant, which include concentration of the reagents ($KMnO_4$ and NaOH), order of addition of reagents, time of reaction, buffers, sensitisers, surfactants, type of alkalies and co-formulated drugs. The influence of the concentration of $KMnO_4$ was studied using different concentrations ranging from $2.5 \times 10^{-4}$ to $2 \times 10^{-2}$ M final concentration. The highest result was obtained with $1 \times 10^{-4}$ M, Fig. 2. Complete reaction between $KMnO_4$ and diloxanide furoate takes place only in an alkaline medium. Different concentrations of NaOH ranging from 0.0125 to 0.175 M were tested. It was found that the reaction took place starting from 0.075 M upwards. However, to ensure a complete reaction, 0.1 M NaOH (final concen-

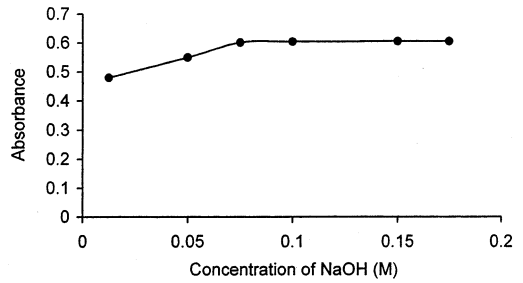



Fig. 3. Effect of concentration of NaOH on the reaction of diloxanide furoate (15 μg/ml) with the $KMnO_4$/NaOH system.

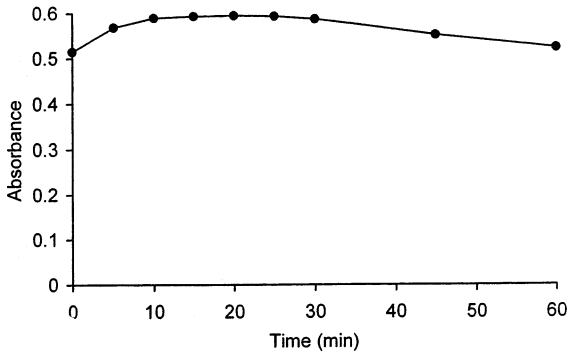



Fig. 4. Effect of time of reaction of diloxanide furoate (15 μg/ml) with the $KMnO_4$/NaOH system.

Table 1
Effect of co-formulated drugs on the determination of 10 μg/ml of diloxanide furoate at the optimum conditions

| Co-formulated drugs | Tolerance limit (μg/ml) |
|---|---|
| Metronidazole | 1.88 |
| Mebendazole | 3.0 |
| Tinidazole | 2.30 |
| Flubendazole | 1.74 |
| Oxamniquine | 1.30 |

tration) was chosen, as it gave the highest absorbance value Fig. 3. Other alkalies (KOH and $NH_4OH$) with the same concentration were also tested. However, their effect on colour development was less than that of NaOH; therefore, the latter was used throughout the study.

The order of addition of reagents is an essential part of the experiment. Addition orders, other than that described in the procedure, gave lower results. Complete colour formation was achieved by leaving the resulting solution at room temperature. Different time intervals were tested to ascertain the time after which the solution attains its highest absorbance. It was found out that the colour is developed immediately, reaches its maximum after 10 min and remains stable for 30 min; however, the colour should be measured after 15 min in order to avoid instability (Fig. 4).

Different sensitisers (quinine, rhodamine 6G and eosine), at concentrations of 5 μg/ml, were tested by adding them to the reactant mixture before allowing the mixture to stand for about 15 min. Excellent inhibitory effects were observed as these sensitisers reacted strongly with the $KMnO_4$/NaOH system. In the same manner, the effect of surfactants on colour development was studied. Different surfactants (cetrimide, gelatin and sodium lauryl sulphate) were tested by adding them to the reactant mixture before the mixture was allowed to stand. All tested surfactants reacted strongly with the $KMnO_4$/NaOH system, with inhibitory effects, as evident from the low absorbance readings. The effect of co-formulated drugs was studied by adding 5 μg/ml of each of the following compounds: metronidazole, flubendazole, mebendazole, oxamniquine and tinidazole. The apparent concentration of the drug in these samples was determined and the tolerance limit (concentration of interfering drug causing less than 3% relative error) were calculated (Table 1). Although the tolerance limits of the compounds are slightly low, the interference resulting from their presence can be avoided, as they are always present as the minor component, while diloxanide furoate is the major component (the medicinally recommended ratios are about 2:1 or 2:3, diloxanide furoate/other drugs), and show poor solubility in sodium hydroxide solution.

### 3.2. Analytical performance

The absorbance–concentration plot was found to be linear over the range 2.5–20 μg/ml. Linear regression analysis of the data ($n = 8$) gave the following equation:

$$A = 0.0055 + 0.0341C \quad (r = 0.9998)$$

where $A$ is the absorbance in a 1 cm cell and $C$ is the concentration of the drug (in μg/ml).

The apparent molar absorptivity was found to be $1.1 \times 10^4$ l/mol cm and $A^{1\%}_{1\,cm}$ was about 360. Statistical evaluation [13] of the regression line gave the following values: standard deviation of the residuals ($S_{y/x}$) is $3.66 \times 10^{-3}$, standard deviation of the intercept ($S_a$) is $2.40 \times 10^{-3}$, standard deviation of the slope ($S_b$) is $2.26 \times 10^{-4}$ while the percentage error is 0.39%. These small figures indicate the high precision of the proposed method.

The proposed method was applied for determining the pure sample of diloxanide furoate. The results obtained were compared with those given by a reference titrimetric method [14]. Statistical analysis [13] of the results obtained from both the methods revealed no significant difference between the performance of the two methods regarding accuracy and precision as revealed by Student's $t$-test and variance ratio, $F$-test, respectively (Table 2).

Table 2
Application of the proposed method and the official method to the determination of diloxanide furoate in pure sample

| Proposed method | | | Official method (14) | | |
|---|---|---|---|---|---|
| Amount taken (μg/ml) | Amount found (μg/ml) | Recovery (%) | Amount taken (mg) | Amount found (mg) | Recovery (%) |
| 5.0 | 5.028 | 100.56 | 200 | 197.14 | 98.57 |
| 7.5 | 7.449 | 99.32 | 300 | 297.72 | 99.24 |
| 10.0 | 10.086 | 100.86 | 400 | 397.44 | 99.36 |
| Mean ± SD | | 100.25 ± 0.62 | | 99.06 ± 0.35 | 99.40 ± 0.11 |
| t | | | 1.08 (2.78) | | |
| F | | | 3.14 (19.0) | | |

Each result is the average of three separate determinations; the figures in parenthesis are the tabulated values of $t$ and $F$ at $P = 0.05$.

Table 3
Application of the proposed method and the official method to the determination of diloxanide furoate in dosage form

| Preparation | Proposed method | | | Official method (14) | | |
|---|---|---|---|---|---|---|
| | Amount taken (μg/ml) | Amount found (μg/ml) | Recovery (%) | Amount taken (μg/ml) | Amount found (μg/ml) | Recovery (%) |
| Furamide [a] tablets | 5.0 | 5.11 | 102.2 | 6.0 | 5.84 | 97.33 |
| Diloxanide furoate (500 mg/tablet) | 10.0 | 9.85 | 98.50 | 8.0 | 7.81 | 97.60 |
| | 15.0 | 14.66 | 97.73 | 10.0 | 9.84 | 98.40 |
| Mean ± SD | | | 99.48 ± 1.82 | | | 97.78 ± 0.67 |
| $T$ | | | 1.60 (2.78) | | | |
| $F$ | | | 7.38 (19.0) | | | |

The figures in parenthesis are the tabulated values of $t$ and $F$ at $P = 0.05$; each result is the average of three separate determinations.
[a] Product of the Boots Company PLC, Nottingham, UK.

The proposed method was further applied to commercial tablets containing diloxanide furoate. The results obtained were compared favourably with those obtained with the official B.P. method [14]. Statistical analysis of the results obtained from both the methods (Table 3) revealed no significant difference between the performance of the two methods regarding accuracy and precision as revealed by the $t$-test and $F$-test, respectively [13].

### 3.3. Stability indication of the method

To assess the presence of phenol that might be present with diloxanide furoate, a calibration curve was constructed, at room temperature, simultaneously with the original calibration curve as shown in Fig. 6. It is obvious the amount of degradation product is negligible. Subtracting the absorbance of the degradation product gives the concentration of the intact drug.

### 3.4. Mechanism of the reaction

The stoichiometry of the reaction was studied adopting the limiting logarithmic method [15]. The absorbance of the reaction product was measured in the presence of both $KMnO_4$ and diloxanide furoate. A plot of log A versus log [$KMnO_4$] and [diloxanide furoate] gave straight lines. The values of the slopes were 0.93 and 1.0, respectively (Fig. 5). Hence, it is concluded that the molar reactivity of the reaction is 0.93:1, i.e. the reaction proceeds in a molar ratio of 1:1.

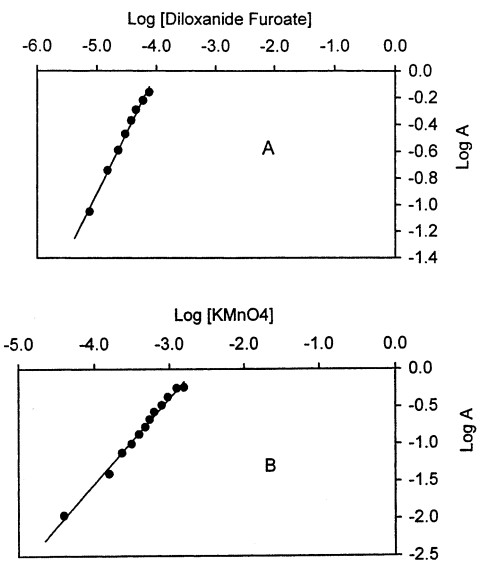



Fig. 5. Limiting logarithmic plots for the molar ratio: (A) log A vs log [diloxanide furoate]; and (B) log A vs log [$KMnO_4$].

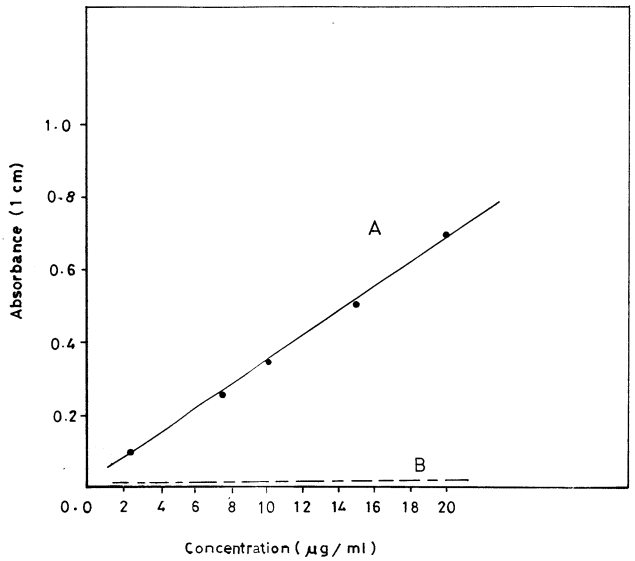
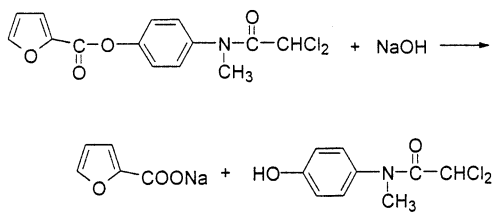



Fig. 6. Calibration curve of the reaction product of diloxanide furoate and the $KMnO_4$/NaOH system: (A) obtained under the described reaction conditions; and (B) obtained immediately at room temperature.

Therefore, the following pathway is proposed as the reaction mechanism. Diloxanide, being an ester of furoic acid, is hydrolysed upon treatment with NaOH as follows:




The phenolic group is oxidised by the $KMnO_4$/NaOH system whereby the permanganate is reduced to the manganate ion which is the coloured species.

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