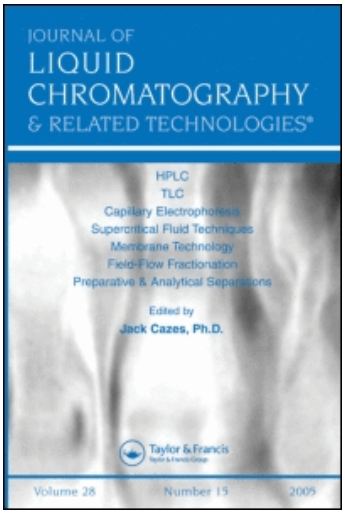

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PHOTODEGRADATION KINETIC STUDIES AND STABILITY-INDICATING ASSAY OF DILOXANIDE FUROATE IN DOSAGE FORMS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The effect of different conditions on the photostability of diloxanide furoate (DF) was investigated. To perform stability studies on DF, a specific stability-indicating high-performance liquid chromatographic (HPLC) method was established using an ultraviolet detector set at 258 nm. The accuracy, precision, and reliability of the method for the assay of DF in its tablets dosage form were established. Assay results were within RSD values less than 2%. The stability-indicating power of the HPLC method was validated by UV-degraded DF solution in a mixture of

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acetonitrile : water (55 : 45 v/v) contained in quartz cells and glass bottles. The resultant chromatogram indicated that the degradation products were well separated from the parent drug. Photostability of DF was studied under different light exposures, i.e., UV-irradiation, room artificial light, and sunlight. Using the HPLC method, the photodegradation kinetics of the drug was studied in both a quartz cell and glass bottle. In both cases the photodegradation process followed first-order kinetics. The effects of different solvents on the UV-irradiation of DF are also reported. One of the three major degradation products observed was identified.

Key Words: Diloxanide furoate; HPLC; UV irradiation; Sunlight; Analysis; Photodegradation; Kinetics

INTRODUCTION

Diloxanide furoate: (DF), 4-[*N*-methyl-2,2-dichloroacetamide] phenyl-2-furoate is a luminal amoebicide. The drug is used either alone for the treatment of asymptomatic cyst-passers, or co-formulated with some other antiprotozoal drugs (e.g. metronidazole) or with anthelmintics (e.g. mebendazole). The methods cited in the literature for the determination of DF in single,[1–9] or as co-formulated dosage forms,[10–24] covered spectroscopic, high performance liquid chromatography (HPLC), HPTLC, and GLC procedures. The comprehensive review by Al-Majed et al.[25] is a good reference for the work published for DF up to 1999. The only three HPLC methods reported[15,19,24] for the determination of DF in co-formulated dosage forms were not specified as stability-indicating. Furthermore, no previous work had been reported on the kinetic study of the stability of DF under the effect of light, though it is stated that the drug should be stored protected from light.[26]

This work covers the development of a stability-indicating HPLC method suitable for the assay of the drug in the presence of its photodegradation products and the use of the new procedure to study the extent of the photostability of DF solutions under different conditions and to shed a light on its photodegradation kinetics.

EXPERIMENTAL

Materials

Diloxanide furoate (DF) pure drug sample (Batch No. CDF/DF/175/97) was kindly provided by Eipico Pharmaceutical Company, Cairo, Egypt, and was used as received. Tablets, "Furamide", labeled to contain diloxanide furoate

BP 500 mg (Boots Pharmaceuticals) were obtained from local commercial sources; two different batches were analyzed: Lot No. 18j manufactured in Sep. 86, Exp. Sep. 89; and Lot No. 3W manufactured in Nov/1990 Exp. Nov/2001. Ammidin (internal standard) was obtained from Memphis Chem. Co. Cairo, Egypt. 2-Furan carboxylic acid (Furoic acid) was purchased from Merck, Darmstadt, Germany.

Other materials used are methanol and ethanol (BDH, Poole, England), acetonitrile (Hipersolv™, BDH, Poole, England), water (Chromosolv®, Riedel-De Haën, Germany), μ Bondapak C_{18} (10 μm) 150 × 3.9 mm (Waters), Spherisorb ODS2 (5 μm) 150 × 4.6 mm (Waters), Nova-Pak (4 μm) 150 × 3.9 mm (Waters), Lichrosphere 100 RP-18 (5 μm) 250 × 4.6 mm or 150 × 4.6 mm (Phase Separation Ltd.); and Ultrasphere (5 μm) ODS, 250 × 4.6 mm (Beckman).

Apparatus

A Waters liquid chromatograph consisting of a 600 E system controller, Rheodyne 7161 injector, tunable absorbance detector 486, and 746 data module was used. The column employed was 100 RP-18 Lichrosphere (5 μm) 150 × 4.6 mm i.d. The mobile phase used was water : acetonitrile (30 : 70 v/v) filtered through Millipore filter (0.22 μm) and degassed by bubbling helium gas (20 mL min^{-1}) into the solvent reservoir and was pumped isocratically at a flow rate of 1 mL min^{-1}. The UV detector was set at 258 nm. Attenuation was set at 16. Ultraviolet spectrophotometric studies were carried out using a Shimadzu UV 1601 PC Spectrophotometer (Kyoto, Japan).

The photodegradation process was carried out using a UV-lamp model UVGL-2 (Minerlight® Lamp multiband UV-254/366 nm-215–250 Volts. 50/60 Hz, 0.12 Amps®, San Gabriel, USA) fixed to a wooden cabinet in a horizontal position.

Preparation of Stock and Standard Solutions

All solutions of DF should be freshly prepared and protected from light. A stock solution of DF (200 μg mL^{-1}) in the mobile phase and a stock solution of the internal standard ammidin (300 μg mL^{-1}) in methanol were prepared and further diluted with the mobile phase, or methanol to give a final concentration of 20 μg mL^{-1} of DF and 60 μg mL^{-1} of ammidin.

Construction of Calibration Curve

One to 8 mL aliquots of DF solution (20 μg mL^{-1}) were transferred each into a 25 mL volumetric flask. To each aliquot, 2 mL of the internal standard

solution (60 μg mL^{-1}) in methanol was added before completion to volume with the mobile phase. Triplicate injections of each solution were applied to the HPLC column. The linearity of the curve was examined by plotting the peak area ratios of the drug to the internal standard vs. the corresponding concentrations and computing the regression equations. The slope consistency of the prepared standard was checked at different days. The reproducibility and precision of the method was tested by determining the relative deviations for three different concentrations, chosen within the linearity range in a study run within day ($n = 4$) and between days ($n = 16$).

Drug Analysis in Tablets

The average content of the drug in either of the two lots tested was calculated and an amount of the powdered tablets equivalent to 20 mg of DF was transferred into a 100 mL volumetric flask. The contents were shaken for 20 min with about 80 mL of the mobile phase. The volume was then completed with the mobile phase, mixed and filtered. Five milliliter of the filtrate were diluted to 50 mL with the mobile phase to give a final concentration of 20 μg mL^{-1} claimed amount. Three milli liter of this solution were transferred into a 25 mL volumetric flask, 2 mL of the internal standard solution were added and the volume was completed with the mobile phase. Six, 20 μL volumes were injected onto the column and the drug content was calculated by either referring to the calibration curve or by direct sample/equivalent standard matching. The following formula was adopted to calculate the drug content/tablet in mg:

$$\frac{A_u \times C_{\text{std}} \times D \times \text{average wt./tablet}}{A_{\text{std}} \times \text{wt. taken}}$$

where A_u and A_{std} are the peak ratios for the sample and standard, respectively; C_{std} is the concentration of the standard solution in mg% and D is the dilution factor for the sample.

Recovery Studies

To 2 mL of tablets solution (20 μg mL^{-1}), 2 mL of standard DF solution (20 μg mL^{-1}) and 2 mL of internal standard (60 μg mL^{-1}) were added, and the volume was completed to 25 mL with the mobile phase. Two milliliter volumes of either the sample or the standard solution were transferred to each of two separate 25 mL volumetric flasks. The internal standard solution (2 mL) was then introduced into each flask before completion to volume with the mobile phase.

Each of the above solutions was injected onto the column ($n = 6$) and the recovery was calculated from the following equation:

$$\frac{P_{(ad)} - P_{(sm)}}{P_{(st)}} \times 100$$

where $P_{(ad)}$ = peak ratio for added solution, $P_{(sm)}$ peak ratio for sample solution, and $P_{(st)}$ peak ratio for standard solution.

Photodegradation of Diloxanide Furoate in Quartz Cell

A solution of DF ($20\,\mu g\,mL^{-1}$) in methanol, or in the mobile phase contained in a quartz cell (LKB), was placed in the wooden cabinet at a distance of 3 cm apart from the light source, and irradiation was carried out at 254 nm at time intervals of 0, 30, 60, 90, 120, 150, 180, 210, 270, 330, and 390 seconds.

Another solution of DF containing $20\,\mu g\,mL^{-1}$ in 12% v/v methanol in water was exposed to the same light source and experimental conditions, but at time intervals of 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 min. The irradiation times were chosen based on the variation in the photostability of the drug in different solvents.

Photodegradation of Diloxanide Furoate in Glass Bottles

A solution of DF ($20\,\mu g\,mL^{-1}$) in the mobile phase contained in glass bottles (Supelco, Inc., Division of Rohm & Haab Co. Cat. No. 2-3297), was exposed to the same light source and experimental conditions as above, but at time intervals of 0, 1, 2, 3, 4, and 5 days. All the samples were analyzed by the HPLC method.

Determination of the Kinetic Parameters of the Photodegradation Reaction by High-Performance Liquid Chromatography

Twenty microliters of the above solutions irradiated in quartz cells or glass bottles were injected onto the column at the stated time intervals of irradiation at 254 nm. The area of the DF peak at zero time (AR_0) was taken as proportional to the initial concentration; the area of DF peak (AR_t) after irradiation was taken as proportional to the remaining concentration as a function of time (t). Regression analysis data of log (AR_t/AR_0) vs. time was then calculated.

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Effect of Sunlight on Diloxanide Furoate Solution

Equal volumes of DF solution ($20\,\mu g\,mL^{-1}$) in acetonitrile : water (55 : 45%) were contained in three identical glass bottles. One bottle was left exposed to sunlight and a second bottle was wrapped with a light insulating material before exposure to sunlight. The third bottle was left at room temperature exposed to the direct artificial room light. Duplicate (20 μL volumes) from each bottle were analyzed using the HPLC method at zero time and at different days intervals, up to two weeks.

Effect of Solvents on Photodegradation of Diloxanide Furoate

Three solutions of DF ($24\,\mu g\,mL^{-1}$) were prepared in methanol, ethanol (96% v/v), and water. Each of the three solutions in the quartz cell was UV-irradiated for 6 min. At the end of irradiation time, each solution was monitored by UV scanning and by the HPLC method with UV detector set at 258 nm. The percentage (w/w) of remaining drug was obtained relative to non-irradiated DF solutions.

UV Monitoring of the Photodegradation of Diloxanide Furoate

Changes in the UV absorption spectra of a solution of DF ($24\,\mu g\,mL^{-1}$) in methanol, occurring as a result of irradiation at time intervals of 0, 1, 2, 3, 4, 6, and 8 min, were followed up by scanning between 320–200 nm.

RESULTS AND DISCUSSION

A drug analyst is mainly concerned with the establishment of stability-indicating analytical methods, capable of assaying both the intact drug and its degradation products that may result from its oxidation, hydrolysis, photolysis, etc. Prior to the development of derivative spectroscopy, spectrophotometry, which suffers from interference from decay products, did not prove to be a useful tool in stability-indicating procedures. High performance liquid chromatography became one of the most frequently used methods in drug analyses and, due to its advantage as being a separating tool, presented the analyst with a procedure favorably suited to drug stability studies. Both isocratic and gradient elutions can be adopted in HPLC to develop stability-indicating methods; however, isocratic separation would be preferred as it is easy to conduct, and does not need much skill or sophisticated instruments. The availability of the broad spectrum

columns, makes isocratic separation more suitable for compounds that are separable only by gradient elution.

Preliminary trials were conducted to establish a stability-indicating method for the assay of DF using different columns [Lichrosphere 100RP-18, Ultrasphere, μ-Bondapak C_{18}, Nova-Pak, and Spherisorb ODS2] with mobile phases composed of acetonitrile : water mixtures at different compositions and flow rates. A successful isocratic method was obtained with each of the five columns, when used with mobile phases composed of a mixture of acetonitrile (50–70% v/v) and water (50–30% v/v) and flow rates of 1–1.6 mL min^{-1}, depending on the column's length. Optimal mobile phase composition with the Lichrosphere columns (used in this study) was acetonitrile : water (30 : 70 v/v) at a flow rate of 1.0 mL min^{-1} for the short column and 1.6 mL min^{-1} for the long column. Under the conditions described above, the drug peak was eluted at retention times of about 3–4 min, while the internal standard was eluted at about 4.5–6.0 min. Resolution of the drug from the internal standard was about 2.6–3.8; while the relative retention time of the internal standard peak to that of the drug was 1.5 in all cases. The retention time of the drug peak was $\simeq$ 3 min and that of the internal standard was $\simeq$4.6 min, using the Lichrosphere columns. The stability-indicating power of the optimized HPLC method was validated by the injection onto the column of a UV-degraded DF solution. The resultant chromatograms showed a decrease in the DF peak (RT$\simeq$3 min) and the appearance of photodegradates resolved at retention times of about 0.9–2.6 min.

Assay Method

A wide linearity range was obtained for the peak area ratio of diloxanide furoate to the internal standard (0.05 μg mL^{-1}–200 μg mL^{-1}). The drug concentration range used for the assay was 0.8 μg mL^{-1}–6.4 μg mL^{-1} giving the linear regression data of the following equation.

$$P = -3.21 \times 10^{-3} + 0.2436\ C \quad (r = 0.99978)$$

where P is the peak area ratio and C is the concentration in μg mL^{-1}. The detection limit ($S/N = 3$) at an attenuation of 4 was 0.015 μg mL^{-1} ($\sim 4.57 \times 10^{-8}$ M). The method was applied for the assay of two batches of DF in tablet form. The samples were selected to represent a batch with valid and another with invalid expiry dates. Both batches gave results of $\simeq$100%, thus, showing no signs of decomposition, thus, indicating high stability of the drug in the solid dosage form. The results were compared with those of the official B.P. method.[26] The statistical analysis of the results showed no significant

difference in accuracy and precision between the HPLC method and the official spectrophotometric method, as assessed by the values for t and F at 95% confidence level (Table 1). The results obtained for the added recovery studies ($100\% \pm 1.26$, $n = 6$) reflected the reliability of the HPLC method and the absence of interference by tablets excipients.

Ruggedness and Precision

Using three different concentrations within the linearity range, the assessment of the method was followed up by within-day, between-day data, and by follow up of the slope consistency for the data of the standard curve in each day, for four consecutive days. The results showed good reproducibility and precision, as reflected by the low RSD% values ($>2\%$).

Interferences Study

Diloxanide furoate is sometimes co-formulated with certain antiprotozoals and anthelmintics (e.g. metronidazole and mebendazole). The possible interference of the two drugs with the assay of DF using the HPLC method was studied. Neither metronidazole nor mebendazole interfered with the assay of DF by the HPLC method.

Table 1. Data for Assay Results of DF in Tablets as Compared to the Official BP Method

Preparation Furamide[a,b] Tablets	HPLC Method % $\pm$ SD[c]	Official Method % $\pm$ SD[d]	F	t
Lot 18 DOM Sep. 1986 Use by Sep. 1989	100.54 ± 0.84	99.90 ± 0.89	1.12 (5.79)	1.04 (2.36)
Lot 3W DOM Nov. 1998 Use by Nov. 2001	99.7 ± 0.63	99.93 ± 0.32	3.88 (19.3)	0.73 (2.36)

[a]Product of the Boots Company PLC, Nottingham, England.
The figures in parenthesis are the tabulated values of F and t at $P = 0.05$.
DOM: Date of manufacture.
[b]Diloxanide Furoate Tablets BP 500 mg.
[c]$n = 6$, 2.4 $\mu g\,mL^{-1}$.
[d]$n = 3$, 8 $\mu g\,mL^{-1}$.

Photodegradation

Some drug molecules undergo degradation upon exposure to light, which would necessitate special storage conditions and protection from light. The official monograph[26] directs that diloxanide furoate should be protected from light. Based on this direction, we attempted to conduct a study that may provide information on the stability of DF under defined conditions, using different light sources. Thus, accelerated photolysis of solutions of diloxanide furoate was investigated, utilizing the stability-indicating HPLC method and spectrophotometry, to follow any spectral alterations that may result from irradiations with UV light as a function of time. Figure 1 (a,b,c) is a specimen chromatogram of DF solution in mobile phase in a quartz cell, injected to the column before irradiation (a) and after irradiation at 254 nm for 120 and 390 seconds (b and c), respectively. The photodegradates were well resolved from the parent compound and were eluted before the drug peak, indicating higher polarity relative to the parent compound. The regression data obtained for the photodegradation of DF solutions in 12% v/v methanol in water, in pure methanol, and in the mobile phase in a quartz cell were, respectively:

$$\log \frac{AR_t}{AR_0} = 8.56 \times 10^3 - 0.0372\,t \ldots, \quad r = -0.9978, \quad t_{1/2} = 8.0\,\text{min}$$

$$\log \frac{AR_t}{AR_0} = -9.1 \times 10^{-3} - 0.1525\,t \ldots, \quad r = -0.9989, \quad t_{1/2} = 2.0\,\text{min}$$

$$\log \frac{AR_t}{AR_0} = 4.52 \times 10^{-3} - 0.1559\,t \ldots, \quad r = -0.9969, \quad t_{1/2} = 2.0\,\text{min}$$

Results obtained are an average of at least two determinations with RSD values $>5\%$.

The drug was destroyed during photolysis by an apparent first order reaction. The calculated $t_{1/2}$ values reflected the stability of DF in aqueous media. The regression data obtained for the photodecomposition of UV-irradiated DF solutions in the mobile phase contained in glass bottles were:

$$\log \frac{AR_t}{AR_0} = -0.0226 - 0.1168\,t \ldots, \qquad r = -0.9975, \; t_{1/2} = 2.48\,\text{days}.$$

Results obtained are an average of two determinations with RSD values $>10\%$.

This high stability of the drug solution in glass bottles, as compared to that in quartz cells, can be explained by the fact that glass bottles absorb UV light and, hence, transmit less energy to the drug solution they contain.

Figure 2 shows typical chromatograms for DF solutions in mobile phase used to study the effect of sunlight on DF solution contained in glass bottles. Figure 2a is the chromatogram of the DF solution protected from light by

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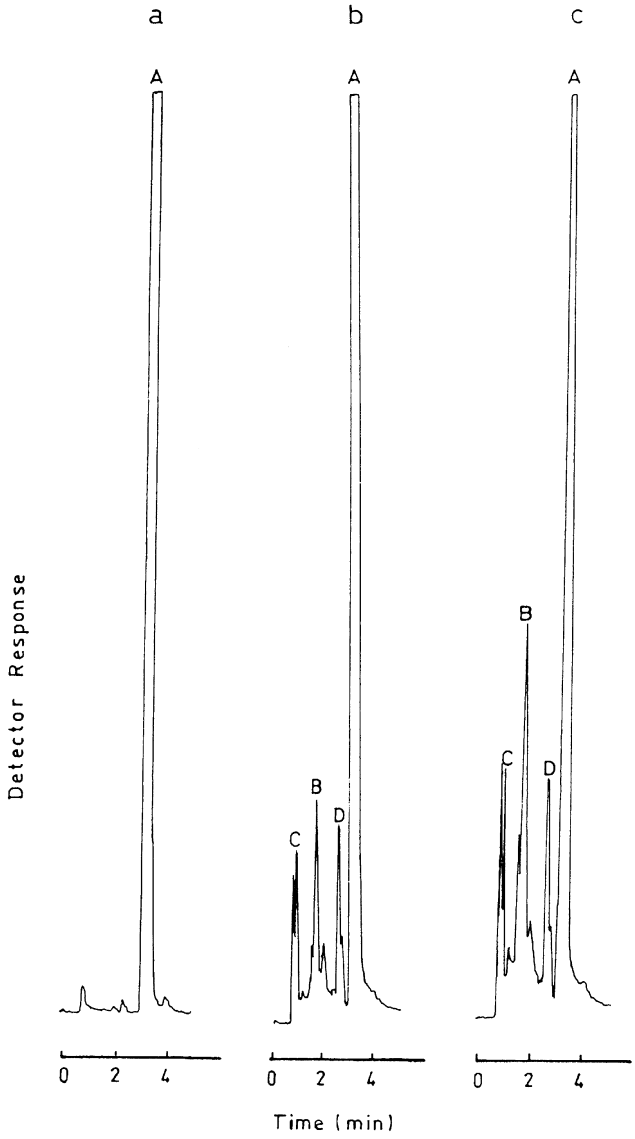

Figure 1. Typical chromatogram of irradiated DF solution (A) (quartz cell) in the presence of its photodegradation products. a: DF intact drug. b and c: DF after UV irradiation for 120 and 390 seconds, respectively, showing three major photodegradation products B, C, and D.

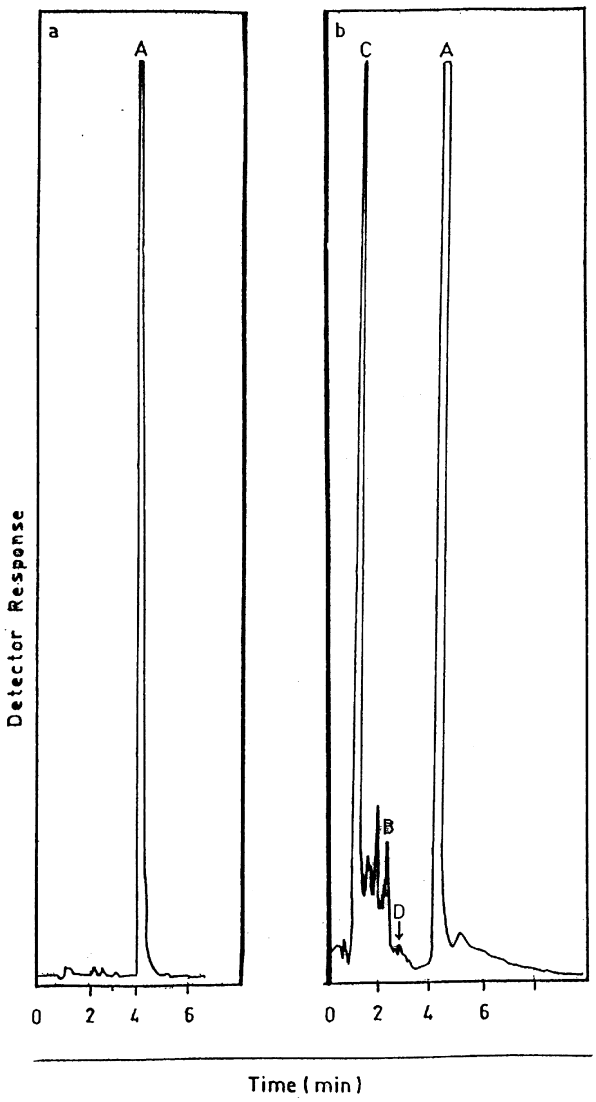

Figure 2. a: Typical chromatogram of DF solution in glass bottles wrapped with light insulting material before exposure to sunlight (blank). b: Typical chromatogram of DF solution after exposure to direct sunlight for two weeks. A: DF intact drug. B, C, and D: photodegradates.

covering the bottle with a light-insulating material and used as the blank where no photodecomposition occurred (similar to the blank left under artificial room light). Figure 2b shows that the DF solution was photodecomposed when exposed to direct sunlight for two weeks (surrounding temperature was about $40^{\circ}C \pm 2$). Peak C was identified as furoic acid. Peaks B and D, which were formed during the first days of exposure, were also observed to undergo further photodecomposition with time. Direct sunlight caused photodecomposition of DF solution in glass bottles, in a manner qualitatively similar to that caused by direct UV exposure at 254 nm for the same solution in glass bottles or quartz cells. To test the effect of heat on the process of decomposition, a solution of DF in the mobile phase was heated at 75°C for two hours before injecting onto the column. No decomposition of the drug was observed, indicating the heat stability of the drug under these conditions. The photodegradation study of DF solutions in quartz cells exposed to UV light at 254 nm and DF solutions in glass bottles exposed to direct sunlight or UV light, resulted in the formation of three major photodegrdates, one of which was identified as furoic acid (Figs. 1 and 2; peak C; $RT \simeq 1.0$ min), by injecting reference furoic acid and comparing the retention times and by spiking a photodegraded DF solution with the reference furoic acid and observing the increase in peak height of the coinciding peak. It is worth noting that the furoic acid peak formed in solutions irradiated in quartz cells seems to undergo decomposition as it is formed (Fig. 1b and 1c), while the furoic acid formed in the glass bottles was apparently stable (Fig. 2b). This observation can be explained by the fact that the physical properties of quartz cells allows the transmission of almost all the UV light passing through them and, hence, provide more energy to the solution in contrast to the glass bottles which absorb the UV light.

A study of the effect of diluting solvents (water, ethanol, and methanol) on photodegradation was conducted using the HPLC and the UV methods. The results of the HPLC method revealed that the rate of degradation of DF in water was 40% upon irradiation for 6 min as compared to 77% in ethanol (96% v/v) and about 82.5% in methanol (Table 2). These results provided another evidence for the stability of the drug in aqueous media. The lower stability in methanol and ethanol could be attributed to the possibility that these solvents can form free radicals, which may participate in the photodecomposition reaction. This instability of DF in methanol and ethanol (the solvents most frequently used in previous work,[2,4,12–16,19,24,26]) cautions against possible result misinterpretation, especially in developing derivative spectroscopic methods. The stability of DF can be improved by adding water to the organic solvents used to keep the drug in solution. The calculated percent recoveries obtained by the two analytical procedures, confirmed the stability-indicating property of the HPLC method. This property was found to be lacking in the UV method, as indicated by the high interferences observed in its results of the recovery studies (Table 2).

Table 2. Effect of Solvents on the Photodecomposition of DF as Monitored by the HPLC Method and the UV-Method (6 min Exposure—Conc. 24 μg mL^{-1})

	% w/w Remaining Drug[a]	
Solvent	HPLC Method	UV Method
12% Methanol in water	59.63	68.7
12% Methanol in ethanol	22.63	39.86
Methanol	17.34	37.87

[a]Results obtained are an average of at least two determinations with RSD values >2%.

An explanation for the high interference encountered with the UV method can be drawn from the results of Fig. 3, which show the spectral changes of the irradiated methanolic solution of DF at time intervals of 0, 1, 2, 3, 4, 6, and 8 min. The observed spectral changes reflect the decrease of DF peak at its λ_{max} at 258 nm and the subsequent formation of degradation product(s) having an absorption curve extending between 225 nm and 295 nm, the two λ_{max} observed for the degradation product(s). Therefore, the residual absorption of the product(s) at the λ_{max} of DF can lead to the interference observed in the results of the recovery studies (Table 2). Furthermore, the spectral changes seen in Fig. 3, confirm the photodegradation of DF solution by UV, since the decrease in the height of DF peak with the time of exposure to UV was accompanied with increased formation of the degradation product(s).

Effect of Artificial Room Light on the Stability of DF in Different Solvents

During our trials to optimize the conditions for the DF stability study, the reference drug, in different concentrations in methanol, was observed to undergo fast decomposition in artificial room light within a short period of time. To confirm this observation, a solution of DF (3.2 μg mL^{-1}) in methanol was prepared and injected onto the column on the first and the eighth day of its preparation, under the optimized conditions of the HPLC method. Figure 4a shows the chromatogram of DF solution (A) applied to the column on day one in the presence of the internal standard (S). Figure 4b shows the chromatogram of the same solution obtained after eight days. The fact that the stability of DF was affected by artificial room light is indicated by the reduction of the drug peak (A) and the appearance of the

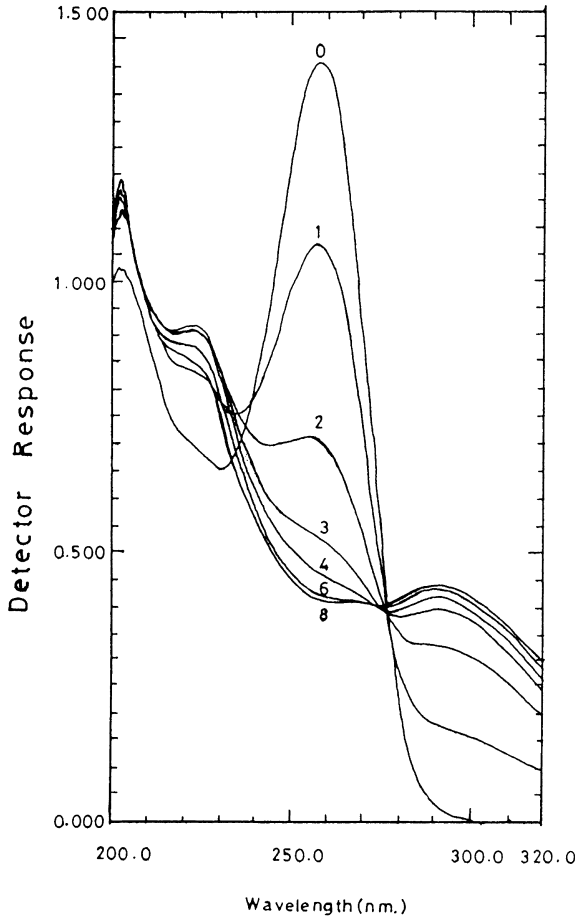

Figure 3. Spectral changes of the UV-irradiated methanolic solution of DF at different time intervals (0, 1, 2, 3, 4, 6, and 8 min) in quartz cells.

degradation products (B and C). This result urged us to study the effects of artificial room light on the stability of DF solutions in different solvents. Thus, solutions of DF in methanol, ethanol (96% v/v), or the mobile phase (water : acetonitrile, 30 : 70 v/v), at concentrations within those used in the standard curve, were exposed to artificial room light at room temperature for three days before being reassayed. The results of Table 3 indicate that methanol was the most active solvent participating in the instability of the drug. In a follow-up experiment, 0.8 $\mu g\,mL^{-1}$ of DF in 4% v/v methanol in the mobile phase and 8 $\mu g\,mL^{-1}$ of the drug in 40%

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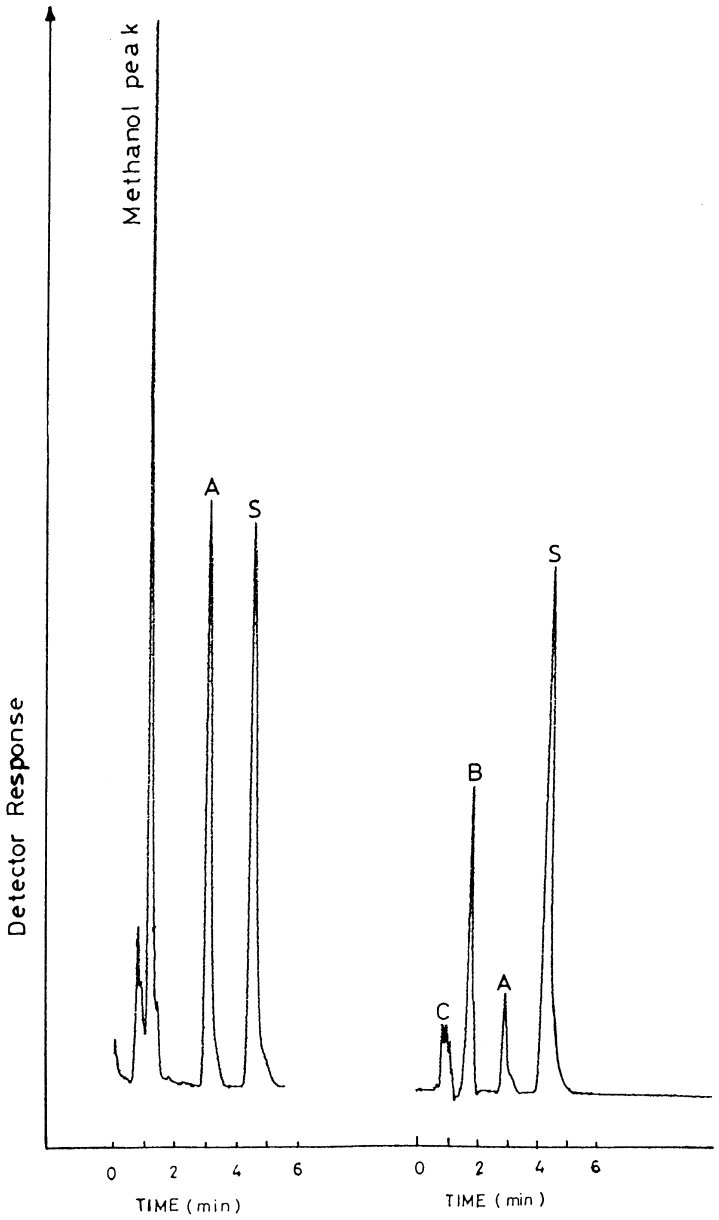

Figure 4. Left: Typical chromatogram of DF (A) in the presence of the internal standard (S) (Day 1). Right: Typical chromatogram of DF (A) in the presence of the internal standard (S) and the degradation products (B and C) (Day 8).

Table 3. Stability of DF Exposed to Artificial Room Light in Different Solvents

Diluting Solvent	Concentration ($\mu g\,mL^{-1}$)	% w/w Remaining After 3 Days[a]
Methanol	0.8	75.6
	2.4	76.62
	4.0	73.66
Ethanol	0.8	87.0
	2.4	84.0
	4.0	82.8
Mobile phase	0.8	100.5
	2.4	99.4
	4.0	99.13

[a]Results obtained are an average of at least two determinations with RSD values >2%.

v/v methanol in the mobile phase, were exposed to artificial room light as above, and the result was monitored at different days for eight days. The results obtained confirmed that the drug remains almost stable in solutions containing small amounts of methanol (4% v/v), while in the 40% v/v methanol solution in the mobile phase a considerable decrease in the concentration of the drug ($\simeq$10%) was observed. This decrease in concentration was accompanied by the appearance of decomposition products as monitored by the HPLC method. It is thus concluded, that while DF solutions in water showed considerable stability, the presence of methanol helps in destabilizing the drug.

Although, the calculated $t_{1/2}$ value for DF solution in the mobile phase irradiated at 254 nm, was the same as that obtained for DF solutions in methanol irradiated similarly, DF solutions in the mobile phase were proven to be very stable when exposed to artificial room light at room temperature, as reflected by the results shown in Table 3. Thus, for long term stability studies of DF, a mixture of acetonitrile : water was used as the solvent for DF. The DF solutions in methanol did not show any improvement in the stability of the drug when stored in amber colored bottles.

CONCLUSION

The HPLC method described in this work was validated as a stability-indicating assay procedure for the determination of diloxanide furoate in the

presence of its photodegradation products. The establishment of the HPLC method allowed the stability testing of DF in an attempt to provide evidence on how the quality of the drug may vary under the influence of different factors.

The studies on the effects of solvents on the photodegradation reaction, showed that aqueous solution of DF were more photostable than the drug solutions in methanol or ethanol.

The photodegradation kinetic results presented in this study, form the basis for future studies intended to cover other factors that can affect DF stability in its available, solid dosage form and may lead to the possibility of suggesting other formulations for the drug. Attempts in this direction are now underway, along with trials to isolate and characterize other photodegradates, and to identify the pathways for the photodegradation processes of the drug.

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