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Photodegradation kinetic study and stability-indicating assay of danazol using high-performance liquid chromatography

E.A. Gad Kariem *, M.A. Abounassif, M.E. Hagga, H.A. Al-Khamees

Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, PO Box 2457, Riyadh-11451, Saudi Arabia

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Abstract

A selective high-performance liquid chromatographic procedure for the stability-indicating determination of danazol in the presence of its photolytic degradation products is demonstrated. The photolysis was carried out in glass vials and quartz cell under UV light at 254 nm. Satisfactory results were obtained for the assay and recovery testing with RSD values less than 2%. Kinetic parameters evaluated comprise the order of reaction and the rate constants of the degradation of the danazol irradiated in glass vials or quartz cell. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Danazol; HPLC; UV irradiation; Determination; Photodegradation; Kinetics

1. Introduction

Danazol [Pregna-2,4-dien-20-ynol [2,3-d]-isoxazol-17-ol, (17α)- Fig. 1, is a synthetic androgen derived from ethisterone. It suppresses the pituitary gonadotropins [1].

A TLC and some HPLC methods have been described for identification and determination of danazol in bulk form [2,3], biological fluids [4–6] and in pharmaceutical formulations [7,8].

No evaluation has yet been demonstrated about the stability indicating characteristics of the cited HPLC methods for the determination of danazol in the presence of its photodegradates.

The effect of light is often considered an important factor in drug stability. Great stability problems arise by light of the shorter wavelength, in particular from the short visible and ultraviolet.

The photochemical degradation of danazol has not been the subject of any previous work although the official monograph [8] directs its storage in well closed containers protected from light. This work describes a photostability indicating HPLC method for determination of danazol in presence of its photodegradants and a preliminary photodegradation kinetics study of danazol irradiated at 254 nm in glass vials or quartz cell.

* Corresponding author.

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2. Materials and methods

2.1. Materials

Danazol was kindly donated through Dr Nader Erian, Banaja, Riyadh from Sanofi Winthrop, France.

Danol™ capsules containing 100 mg danazol were purchased from local pharmacies in Riyadh.

Ammidin (imperatorin) (internal standard) was kindly donated through Dr H. Zeinalabdin, General Director, the Memphis Chem. Co., Cairo, Egypt. Methanol (Spectrosol®, BDH, Poole, England). Acetonitrile (Hipersolv™, BDH, Poole, England). Water (Chromosolv®, Riedel-de Haën, Germany). μ Bondapak C18 (10 μm) 3.9 × 300 mm; resolve C18 (5 μm) 3.9 × 150 mm; Nova-pak phenyl (4 μm) 3.9 × 150 mm; Novapak C18 (4 μm) 3.9 × 150 mm, (all Waters); Lichrosphere 100 RP (5 μm) 4.0 × 125 mm (E. Merck) and Lichrosphere 100 RP (5 μm) 4.6 × 150 mm (Phase Separation Ltd.). All other chemicals were analytical grade reagents.

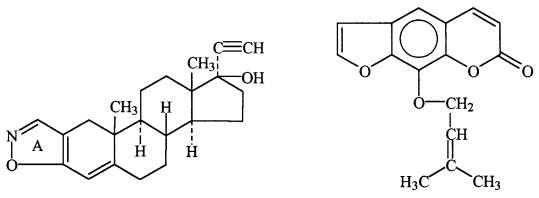
2.2. Photodegradation

2.2.1. Light source

UV lamp model UV GL-25 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.

2.2.2. Solution

Solution of danazol (0.0016%, w/v in CH_3CN: H_2O (70:30) contained in glass vials (Waters Assoc., M.A) or in quartz cell (LKB) was placed in the wooden cabinet at a distance of 5 cm from the light source and irradiation was carried out at 254 nm at different time intervals (0, 5, 10, 15, 20, 30, 45, 60, 90 and 120 min) for the danazol solution in glass vials and at time intervals (1, 2, 3, 5, 7, 9, 11 and 14 min) for solution in quartz cell. A part of the solution was placed in the dark and was considered as a blank. Photodegradation was followed by the developed HPLC method.



Danazol Ammidin (imperatorin)

Fig. 1.

2.3. Analytical procedures

HPLC was adopted to follow the kinetics of the degradation of danazol and as a stability indicating method for the assay of danazol in pharmaceutical formulation.

Waters Liquid Chromatograph consisted of a 600 E System Controller, U6K Injector, tunable absorbance detector 486 and 746 data module. The column was Lichrosphere 100 RP-18 (5 μm) 125 × 4.0 mm i.d. The mobile phase was acetonitrile: water (55:45 v/v) filtered through millipore filter (0.45 μm) and degassed by bubbling helium (20 ml/min) into the solvent reservoir. It was pumped isocratically at a flow rate of 1.3 ml min^{-1}. The UV detector was set at 287 nm.

2.3.1. Danazol assay in dosage formulation

2.3.1.1. Standard curve. All solutions of danazol should be freshly prepared and protected from light throughout the assay time. A stock solution of danazol 250 μg ml^{-1} in methanol and a stock solution of ammidin, the internal standard, 300 μg ml^{-1} in methanol, were diluted with the mobile phase to give 15 μg ml^{-1} of danazol and 30 μg ml^{-1} of ammidin as working solutions. 1–8 ml of danazol working solution (15 μg ml^{-1}) were transferred into 10 ml volumetric flasks; to each flask, 2 ml of the internal standard were added and volume completed with the mobile phase. Triplicate injections of each dilution were made and the curve, concentration μg ml^{-1} danazol versus detector response at 287 nm in terms of peak area ratio (drug/internal standard) was plotted.

2.3.1.2. Sample solution. Average content of 20 Danol™ capsules was calculated and an amount

of powder equivalent to 25 mg danazol was transferred into 100 ml volumetric flask, shaken for 10 min with about 80 ml methanol; volume completed with methanol, mixed and filtered. Three milliliters of the filtrate were diluted to 50 ml with the mobile phase to give 15 μg ml^{-1} claimed amount; 5 ml of this solution were transferred into 10 ml volumetric flask; 2 ml internal standard were added and volume completed with the mobile phase. Six 20 μl volumes were injected and 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 content/capsule in mg.

$$\frac{Au \times Cstd(mg\%) \times D \times AverageWt/Caps}{Astd \times Wt\ taken}$$

where *Au* is the peak ratio for sample; *Astd* is the peak ratio for std and *D* is the dilution factor for the sample.

2.4. Determination of the kinetic parameters of the photodegradation reaction by HPLC

Twenty microliters of the solution irradiated in glass vials or quartz cell were injected onto the column at the selected time intervals of irradiation at 254 nm. The area of the danazol peak at zero time (ARo) was taken as proportional to the initial concentration; the area of danazol peak (ARt) after irradiation was taken as proportional to the remaining concentration as a function of time (*t*). A plot of log (ARo/ARt) versus time was done.

3. Results and discussion

3.1. Assay method

HPLC has become the most used method in drug analysis due to its advantage in being a separating tool and hence is the most indicative of stability. In order to obtain a column/mobile phase system that would be an optimum condition for the separation and quantitation of danazol in presence of its photodegradation products, different columns including μ Bondapak C_{18}, Resolve C_{18}, Nova-Pak phenyl and two columns packed with the same stationary phase but with different column lengths and diameters i.e. Lichrosphere 150 × 4.6 mm i.d. (expected to have high resolving power due to its length and larger surface area) and Lichrosphere 125 × 4.0 mm i.d. (the narrower i.d. and shorter length can be useful for less solvent use and greater sensitivity) were investigated with different mobile phases composed of different percentage of organic modifier, pH and different flow rates. A 50% v/v mixture of acetonitrile-water or methanol-water was first investigated with these columns at a flow rate of 1 ml/min. Both systems showed promising results with the Lichrosphere columns and the phenyl column. This primary study also revealed that the mixture of CH_3CN/H_2O was more suitable to use with the Lichrosphere columns than the CH_3OH/H_2O mixture due to the elution of the danazol peak at a reasonable retention time with good peak symmetry compared to the late broad eluting danazol peak in the case of the CH_3OH/H_2O mixture. At 70% v/v CH_3OH:H_2O mixture the danazol peak eluted at a retention time of 16 min compared to a retention time of 4 min in case of 70% v/v CH_3CN:H_2O mixture at a flow rate of 1 ml min^{-1} for Lichrosphere 125 × 4.0 mm id. The optimal condition for the Lichrosphere column 125 mm × 4.0 mm id was CH_3CN:H_2O (55:45 v/v) at a flow rate of 1.3 ml/min. The retention time for danazol was 6 min (capacity factor 4.5). This same condition gave a retention time of $\simeq$ 10 min for danazol with Lichrosphere 150 × 4.6 mm id; (better resolution but longer analysis time); however, using CH_3CN:H_2O (70:30 v/v) at a flow rate of 1 ml min^{-1} reduced the retention time to $\simeq$ 5.8 min ($k' = 4.35$). Under these optimised conditions for the Lichrosphere columns, some degradation products eluted before and after the danazol peak.

For the phenyl column, the 50% v/v methanol-water mixture eluted the danazol at a reasonable retention time but with some peak tailing; increasing the methanol to 70% v/v at a flow rate of 1 ml min^{-1} improved both the danazol peak symmetry and capacity factor ($k' = 4.7$). The resolution of the degradants followed the same pattern as for the lichrosphere columns. The mixture of CH_3CN:H_2O was not suitable for the phenyl

Table 1
System suitability criteria and linearity data for the proposed HPLC method

Parameter	Danazol	Ammidin
Retention time (min)	6.46	4.60
Relative capacity factor (K_2/K_1)	1.40	
Resolution	5.20	
Tailing factor	$\cong 1.15$	$\cong 1.2$
Number of theoretical plates N m^{-1}	23 570	
HETP, cm	0.0043	
Slope consistency[a]	0.1700 ± 0.00528	
Intercept[b]	3.8×10^{-5}	
Correlation coefficient	0.9998	
Assay range	1.5–12 μg ml^{-1}	
Linearity range	0.5–200 μg ml^{-1}	

[a] Based on within day injection of the prepared standard ($n = 16$) and evaluation at 95% confidence limit.
[b] The intercept was confirmed not to be significantly different from zero by the t-test.

column due to early elution of danazol peak with poor resolution from the degradants even at lower percentage concentrations of the organic modifier ($k' \simeq 1.2$ for CH_3CN:H_2O (70:30, v/v) and $k' \simeq 2$ for CH_3CN:H_2O (55:45 v/v) at a flow rate of 1 ml min^{-1}. The use of ammonium carbonate (0.01 M, pH 8) and potassium dihydrogen phosphate (0.01 M, pH 2.2) instead of water for these optimized conditions, did not alter the capacity factors or the tailing factors of the eluting peaks. Lichrosphere 125×4.0 mm i.d. was used throughout in this study. An internal standard was used to minimize possible errors during injection [9]. Ammidin and diazepam were found possible to use with these systems. Ammidin was selected due to its stability to photoirradiation or on standing for long periods; however, it was not incorporated in the solution intended for the study of photodegradation so as to detect any peaks formed during danazol photodegradation by time.

Tables 1 and 2 summarises the results obtained for some of the system suitability parameters, linearity data, assay% ± S.D. and added recovery of the proposed HPLC method. The obtained results reflected good system suitability, accuracy and reliability of the assay method. The slope consistency was calculated at 95% confidence limit to give a more realistic result [10]. The relative retention time of danazol to the internal standard was 1.47. Under the experimental conditions mentioned before, tested linearity of the standard curve extended between 0.5 μg ml^{-1} to 200 μg ml^{-1} with a correlation coefficient $r = 0.9998$. The limit of determination evaluated at three times the detectability was 0.015 μg ml^{-1}. Table 3 shows data for the reproducibility and precision of the method as assessed by the follow up of within-day and between-day data for four concentrations within the linearity range. Good reproducibility and precision was reflected by the low

Table 2
Data for assay results% ± S.D. and added recovery

	Assay% ± S.D. (n)[a] RSD	Added recovery[b] $\bar{X}$ ± S.D. (n)[a] RSD
Danol capsules™	100.34 ± 0.81 (6) 0.81	99.91 ± 1.04 (6) 1.04

[a] n = number of replicates.
[b] Based on standard solution (3 μg ml^{-1}) added to claimed sample solution (6 μg ml^{-1}).

Table 3
Reproducibility and precision data as evaluated by RSD% for within-day and between-day study

Concentration (μg ml^{-1})	Within-day ($n = 5$) RSD%	Between-day ($n = 15$) RSD%
3	0.56	3.5
5	0.67	2.1
7.5	0.52	2.3
17.5	0.55	2.2

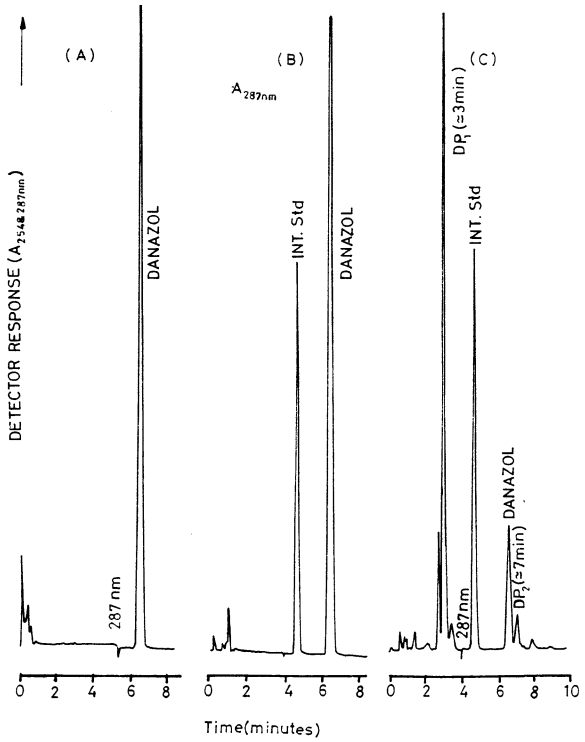


Fig. 2. Fresh danazol solution (16 μg ml^{-1}) (A); fresh danazol solution (16 μg ml^{-1}) and the internal standard (6 μg ml^{-1}) (B); UV irradiated danazol solution (16 μg ml^{-1}) in presence of internal standard (6 μg ml^{-1}) (C).

RSD% values for the within-day data; however, the increase in the RSD% values observed for the between-day data was due to the instability of the drug in solution even at daylight reflecting its high photosensitivity. This method being stability indicating, utilizing a simple mobile phase of high sensitivity is considered more useful than the HPLC method cited in the literature for the determination of danazol in capsules [7]. The USP Method [8] was evaluated for stability indicating characteristics; column Novapak C_{18}, 150 × 3.9 mm i.d. (4 μm) was used with mobile phase of acetonitrile: methanol: water (4:3:3 v/v) at a flow rate of 1.4 ml min^{-1} and UV detection at 270 nm. Good resolution and peak symmetries were obtained indicating excellent chromatographic characteristics; however, the internal standards indicated in this study were found to interfere with the eluting photodecomposition products for this system.

3.2. Photodegradation

The photodegradation study was intended to validate the proposed HPLC method as stability indicating method and to obtain useful information about the photodegradation of danazol which is known to be light sensitive [8]. This light sensitivity was observed while following the slope consistency of the prepared standard at different days which showed slight decrease of its value on the third day for solutions left at room temperature exposed to day light, with start of formation of a decomposition product having a retention time of about 7 min; after 7 days, the slope value dropped to about 93% of its initial value with clear formation of a photodegradation product referred to here as DP_2. This was further confirmed by the observed increase in RSD% value for the between-day results due to the slow formation

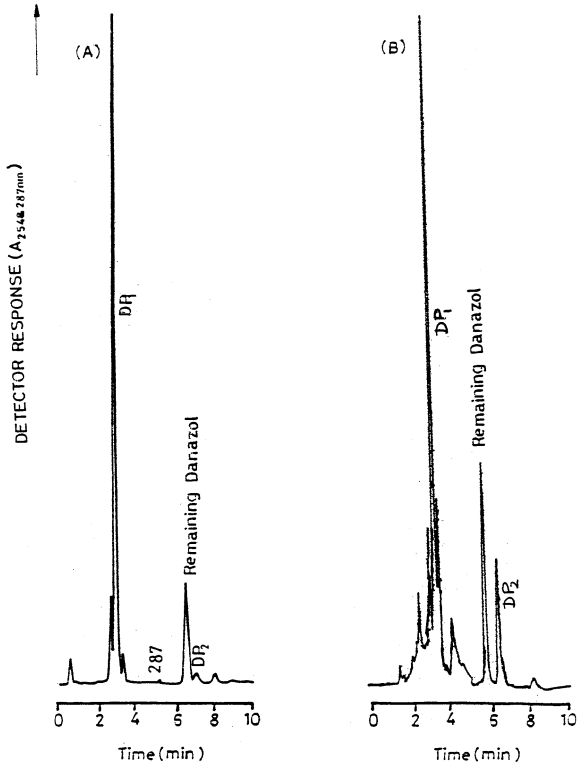


Fig. 3. A total of 45 min UV irradiated danazol solution (16 μg ml^{-1}) in glass vials (A); 9 min UV irradiated danazol solution (16 μg ml^{-1}) in quartz cell (B).

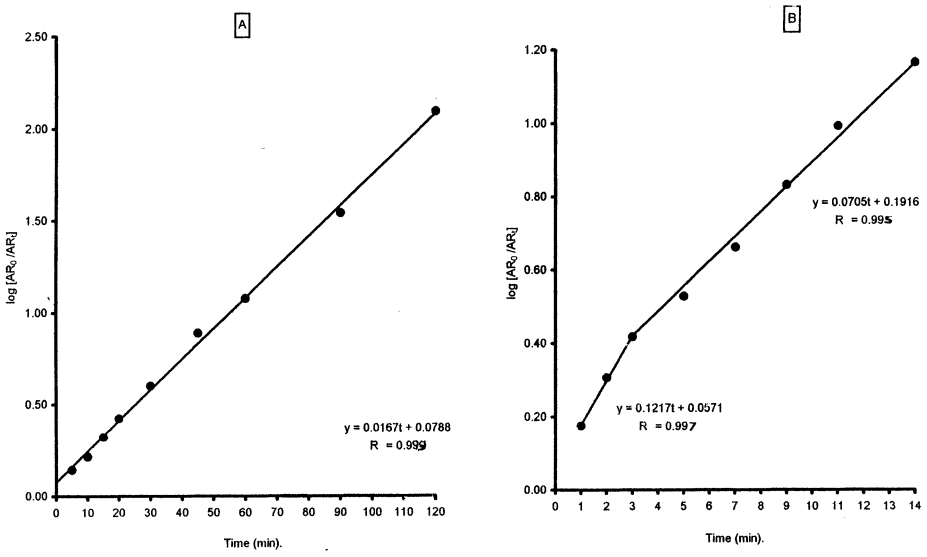


Fig. 4. First-order plots for decomposition rate of danazol (glass vials) (A) and (quartz cell) (B).

of DP_2 and the subsequent decrease in danazol concentration (Table 3). This indicated a high sensitivity to light and therefore solutions should be freshly prepared and protected from light.

When the irradiated solution was monitored at 287 nm, a reduction of the danazol peak height was observed without the appearance of drug degradation product; monitoring at 254 and 287 nm revealed a major degradation product referred to as (DP_1) among small other degradation products. This indicated that these photodegradation products resulting from irradiation of danazol solution at 254 nm seems to absorb maximally at about 254 nm and minimally at 287 nm. UV-scan of a photodegraded danazol solution (16 μg/ml) showed two peak maxima: one at 242 nm due to photodegradation products and another at 287 nm due to the remaining danazol. Trials were then carried out to optimise a wavelength, if possible, that would allow good sensing of all components without affecting the suitability and sensitivity of the described method. The detector response for the different eluting peaks after the injection of the photodegraded danazol solution (16 μg/ml) was studied at different wavelength settings for each separate injection and the obtained chromatograms were compared. Comparison for each peak response at the different wavelength settings was assessed in terms of peak area or peak height. At 242 nm the response for DP_1 was about 15% more than at 254 nm; some small adjacent peaks to DP_1 were slightly more absorbing at 254 nm than at 242 nm; the response to DP_1 and these adjacent peaks dropped greatly at 270 nm and even more at 287 nm, indicating that they can be detected at these wavelengths at high concentrations. Danazol and peaks eluting after it (including DP_2) absorb minimally at 242 and 254 nm. At 270 nm the response to danazol is about 35% less than that at 287 nm; the response to DP_2 and other adjacent peaks was about 20% more at 287 nm than at 270 nm. Since danazol in solution was observed to degrade at daylight to form DP_2 and the other degradants of k' values about 5, it was concluded that 287 nm was the suitable UV-detection setting for the HPLC-separation and quantitation (assay or kinetic study) for the sensitive tracing of danazol and its decay products that are formed at daylight. On the other hand, setting at 254 nm would be suitable for sensitive tracing of danazol photodegradants that are formed after irradiation at 254 nm (k' less than 2.5) if quantitative study for these products is to be done. For a simultaneous quantitation of these products as well as danazol, the wavelength can therefore, be set first at 254 nm or 242 nm for

5 min and then switched to 287 nm for the sensitive tracing of DP_1 and danazol in one run.

Fig. 2 demonstrates the typical chromatograms of pure danazol monitored at 254 nm and 287 nm (a), danazol and ammidin at 287 nm (b) and photoirradiated danazol in presence of ammidin at 254 nm and 287 nm (c).

Fig. 3 (a, b) show specimen chromatograms of a solution of danazol injected after 45 and 9 min of irradiation in glass vials and quartz cell, respectively.

The decomposition rate constant of danazol solution irradiated in glass vials (which absorb light and hence transmit less energy to the solution) was 38.55×10^{-4} min^{-1} ($t_{1/2} = 18$ min) (Fig. 4a). Fig. 4b shows two decomposition rate constants obtained for danazol solution irradiated in quartz cell (which transmitts almost all the UV light passing through it and hence provide more energy to the solution): one decomposition rate between 1–3 minutes irradiation time with k value $= 28 \times 10^{-3}$ min^{-1} ($t_{1/2} = 2.47$ min); the second rate between 3–14 minutes with k value $= 16.2 \times 10^{-4}$ min^{-1} ($t_{1/2} = 4.30$ min). Although no definite explanation can be stated for this apparent slowing of decomposition, however, it could be suggested that at this high energy a second component was formed in the system which may preferentially absorb light leading to photosensitization. This possibly formed compound can act as screening agent which protects the photosensitive danazol molecule [11]. Both plots show an apparent first-order reaction. Results obtained are an average of at least two determinations.

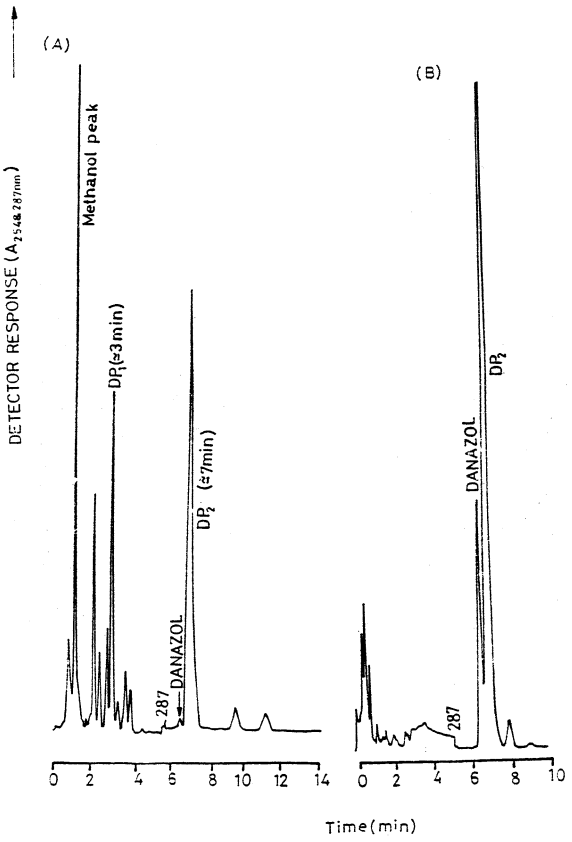


Fig. 5. Photoirradiated solution of danazol left under UV light for 24 h (A). DP_2 formation (6-month-old danazol solution) (B).

The photolysis of danazol in solution was observed to form two major products referred to here as DP_1 and DP_2 among other photodegradants. DP_1 was noticed to be formed quickly on exposing danazol solutions to UV; it is not formed in solutions left exposed to day light. A solution containing DP_1 on long exposure to UV light (24 h) tends to form secondary degradation products including DP_2 (Fig. 5a). DP_2 on the other hand, was formed by either long exposure of concentrated solutions of danazol to UV, or in solutions standing for long periods exposed to daylight. Fig. 5b shows a 6-month-old solution of danazol showing the formed DP_2.

All these kinetic results can be explained on the basis of destructive photolysis. This is well indicated by the formation of DP_1 with UV absorption at 242 nm compared to 287 nm for the parent compound i.e. a hypso chromic effect.

Most probably the destruction was on ring (A) (Fig. 1) forming a simple steriodal structure. DP_1 was suspected to be ethisterone, the precursor of danazol; however, DP_1 retention time was slightly different from that for ethisterone. On the other hand, the easy formation of DP_2 at daylight could be as a result of an attack at the less stable triple bond of the acetylinic group. However, it is not possible to postulate the pathways for the degradation process with certainty unless these products are isolated and identified.

No doubt an HPLC study in combination with UV diode array detection and on-line mass spectrometry (facilities which we lack in our laboratories) could greatly improve the quality of the results obtained; however, the photodegradation results obtained in this study are considered only preliminary results for future elaborated work in this line.

4. Conclusion

The HPLC method described in this work was validated as stability indicating method for the separation and quantiation of danazol in presence of its photodegradation products. Results obtained indicated the photosensitivity of danazol in solution to UV light. This proposed method is superior to the USP method in being more sensitive, incorporate an internal standard and utilizes only two solvent mixture. The method can be adopted for monitoring danazol in biological fluids due to its wide linearity range and low detectibility limit.

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