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Note

Determination of danazol in pharmaceutical preparations by liquid chromatography

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Danazol [17α-pregna-2,4-dien-20-yno-(2,3*d*)-isoxazol-17-ol] is a synthetic androgen derived from ethisterone. It supresses the pituitary gonadotropins[1]. In this paper we report a high-performance liquid chromatographic (HPLC) method for the determination of danazol in three pharmaceutical preparations.

EXPERIMENTAL

Reagents and materials

Methanol and chloroform were of spectral grade. Deionized water was used to prepare the methanol–chloroform–water (70:15:15, v/v) mobile phase.

Three different capsule preparations of danazol, (A) 50, (B) 100 and (C) 200 mg per capsule, were studied.

Preparation of danazol solutions

A 7:3 (v/v) mixture of methanol and chloroform was used as a solvent and three solutions of danazol of concentrations (a) 0.5 mg/ml, (b) 1.0 mg/ml (c) 2.0 mg/ml were prepared.

Preparation of norethisterone acetate solution

Three solutions of norethisterone acetate of concentrations (d) 0.5 mg/ml, (e) 1.0 mg/ml and (f) 2.0 mg/ml were prepared in the same solvent.

Three working standard solutions were prepared as follows: 5 ml of danazol solution of concentrations (a), (b) and (c) + 10 ml of norethisterone acetate solution of concentrations (d), (e) and (f) were mixed separately and diluted to 50 ml with the solvent.

Preparation of the sample

Sixty capsules of the three danazol preparations (A, B and C) were weighed separately. Accurately weighed amounts of the powder equivalent to about 50 mg of danazol from A, 100 mg from B and 200 mg from C were transferred into three 100-ml beakers. A volume of 70 ml of the above solvent was added to each beaker and the solutions were filtered into three 100-ml volumetric flasks. After dilution to volume with the same solvent, the approximate concentrations of danazol in each flask were (g) 0.5 mg/ml, (h) 1.0 mg/ml and (i) 2.0 mg/ml.

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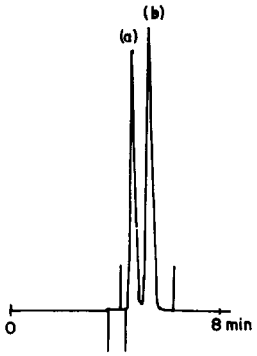

Fig. 1. Typical chromatogram showing separation of (a) danazol and (b) norethisterone acetate, using μBondapak C_{18}. UV detector (260 nm).

Three working sample solutions were prepared as follows: 5 ml of the sample solution of concentrations (g), (h) and (i) + 10 ml of norethisterone acetate solution of concentrations (d), (e) and (f) were mixed separately and diluted to 50 ml with the solvent.

Chromatography

A Waters Assoc. ALC/GPC 201 liquid chromatograph equipped with an M6000A solvent delivery system, a U6K universal injector, a Lamda-Max 481 UV–visible absorbance detector and an M-730 data module was used. Analyses were performed on a stainless-steel column (30 cm × 3.9 mm I.D.) packed with μBondapak C_{18} (Waters Assoc.).

Elution was carried out with methanol–chloroform–water (70:15:15, v/v) at a flow-rate of 1 ml/min. The detector wavelength was set at 260 nm and the solvent was pumped through the column to obtain a steady baseline. The working standard solutions, 200 μl for A, 100 μl for B and 30 μl for C, were injected separately. A typical chromatogram is shown in Fig. 1. A calibration graph was constructed in each instance.

In a similar way, the working sample solutions, 200 μl for A, 100 μl for B and 30 μl for C, were injected separately. The amount of danazol per capsule was determined by comparing the peak-area ratios of danazol and norethisterone acetate in the working standard and working sample solutions.

RESULTS

The proposed assay method was carried out by the internal standard ratioing method in order to eliminate any errors that may occur during injection. A calibration of the peak-area ratio of danazol to norethisterone acetate against the weight ratio is shown in Fig. 2. The regression equation is

$$Y = 0.0962 + 0.607X$$

where X is the weight ratio and Y the peak-area ratio. Beer's law was obeyed in the concentration range 0.05–0.8 mg/ml.

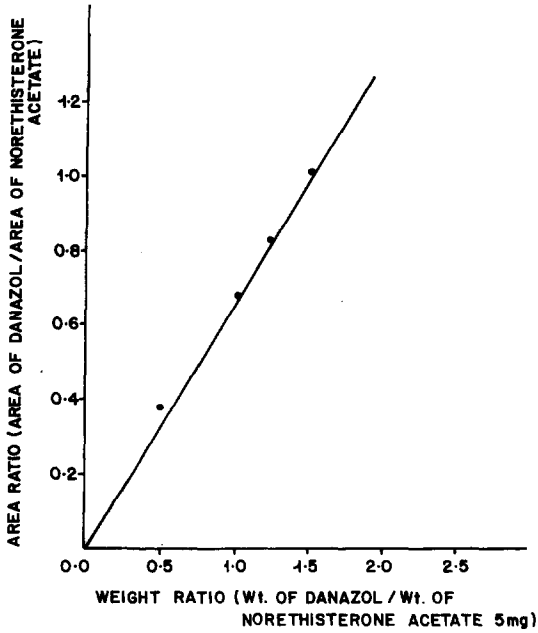

Fig. 2. Calibration graph for danazol using norethisterone acetate internal standard.

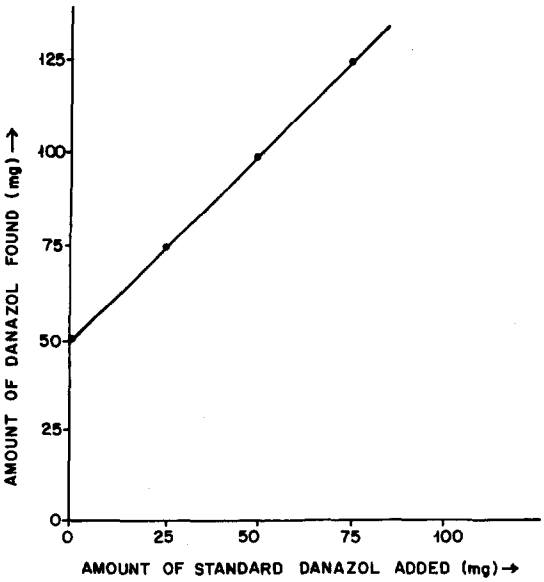

Fig. 3. Standard additions graph for danazol at four levels.

TABLE I

HPLC DETERMINATION OF DANAZOL IN PHARMACEUTICAL PREPARATIONS

Formulation	*Labelled amount (mg/capsule)*	*Amount found (mg/capsule)*	*Recovery (%)*	*Standard deviation (%)*	*Relative mean deviaton (%)*	*Coefficient of variation (%)*
A	50	49.73	100.0	1.09	1.16	1.39
B	100	99.44	99.9	1.54	0.78	0.95
C	200	199.31	99.9	1.42	0.34	0.44

The recovery of added danazol was studied at four different levels. From the amount of danazol found, the recovery was calculated using the equation

$$\text{Recovery (\%)} = \frac{N(\Sigma XY) - (\Sigma X)(\Sigma Y)}{N(\Sigma X^2) - (\Sigma X)^2} \cdot 100$$

where X is the amount of standard danazol added, Y the amount of danazol found by the proposed method and N the total number of observations. A plot of the amount of danazol found against the amount of standard danazol added is shown in Fig. 3. The intercept on the ordinate indicates the amount of danazol present per capsule.

To determine the reliability of the proposed assay procedure, statistical parameters such as the coefficient of variation and the relative mean deviation were calculated. The recovery, the amount of danazol (mg per capsule) found by the proposed method together with the statistical data for 50-mg, 100-mg and 200-mg capsules are summarized in Table I.

DISCUSSION

The isolation, synthesis and biological activity of five metabolites of danazol have been studied by a combination of column chromatography UV, IR, NMR and mass spectrometry[2]. The radioimmunoassay of danazol in human and monkey plasma has been reported[3,4]. Sharp *et al.*[5] studied danazol interference in testosterone by radioimmunoassay. The official method[6] for the assay of danazol employs UV measurements at 287 nm. The method proposed here is the first HPLC method for the determination of danazol in pharmaceutical formulations. Rees *et al.*[7] described the separation of sterols and of sterol acetates by reversed-phase chromatography on μBondapak C_{18} using methanol–chloroform–water (71:16:13) as the mobile phase. In this study it was found that the use of chloroform in the mobile phase improved the peak symmetry and gave a baseline resolution between the peaks. The amount of danazol found per capsule was calculated using the equation

$$\text{Amount per capsule} = A \cdot \frac{R_{\text{samp.}}}{R_{\text{std.}}} \cdot \frac{D}{W}$$

where A is the concentration of standard danazol (mg/ml), $R_{\text{samp.}}$ the peak-area ratio

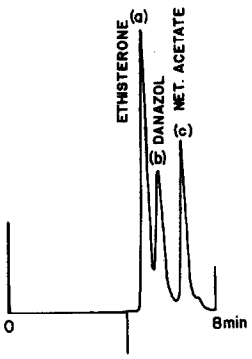

Fig. 4. Chromatogram showing the separation of (a) ethisterone, (b) danazol and (c) norethisterone acetate, using μBondapak C_{18}. UV detector (260 nm).

of danazol to norethisterone acetate in the sample, $R_{std.}$ the peak-area ratio of danazol to norethisterone acetate in the standard, D the dilution factor and W the weight of sample taken.

The recoveries of the added standard for the three formulations indicate that there is no interference from excipients present in the capsule. The coefficient of variation and the relative mean deviation are low, indicating that the proposed method is precise and reproducible.

Danazol is synthesized from Ethisterone B.P., which is present in trace amounts in bulk samples of danazol. It was found that ethisterone elutes before danazol (Fig. 4) under the experimental conditions used. It is also interesting that ethisterone is one of the metabolites of danazol in human plasma. It is therefore possible to detect and determine ethisterone impurity in danazol formulations and also to determine ethisterone in human plasma together with unmetabolised danazol using norethisterone acetate as an internal standard. These are the applications of the proposed method, in addition to the routine determination of danazol from pharmaceutical preparations.

ACKNOWLEDGEMENT

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