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New Extractive Spectrophotometric Method Development and Validation for the Estimation of Danazol in Pharmaceutical Formulations

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ABSTRACT

A simple, sensitive, selective, accurate and economical spectrophotometric method has been described in the present work for the determination of danazol in bulk drug and pharmaceutical formulations (tablets). Method is based on the formation of yellow coloured ion-association complex between danazol and alizarine red S in acid medium followed by its extraction with chloroform, exhibiting absorption maximum at 430 nm, and obeying Beer′s law in the concentration range of 5-30 µg/mL. Statistical analysis of the results of the proposed method reveals high accuracy and good precision. The proposed method could be successfully extended to the commercial pharmaceutical formulations containing danazol.

KEYWORDS

Danazol, Alizarine red S, Spectrophotometry.

INTRODUCTION

Danazol is a synthetic steroid derived from ethisterone. It is a white to pale yellow crystalline powder, practically insoluble in water and sparingly soluble in alcohol. Chemically, Danazol is 17α-pregna-2,4-dien-20-yno[2,3-*d*]isoxazol-17-ol (Fig. 1). Danazol is a medication which is used in the treatment of endometriosis, fibrocystic breast disease, hereditary angioedema, and other conditions [1]. Literature survey revealed that few analytical methods have been developed for the estimation of danazol in formaceutical formulations [2-6].

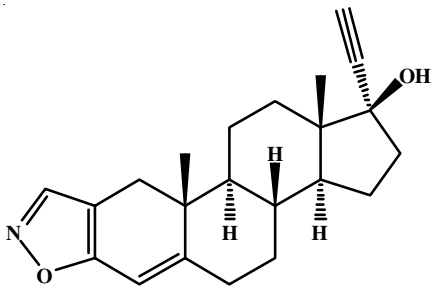

Fig. 1. Structure of danazol

QC laboratories in developing countries frequently prefer visible spectrophotometry as a quantitative method for determination of pharmaceutical drugs. In view of the suitability,

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sensitivity and simplicity, ion association complex formation [7-12] and oxidation [13,14] are the popular reactions among various existing techniques used for estimation of pharmaceutical drugs by using visible spectrophotometry. Lone pair of electrons present in hetero atom (like nitrogen) of pharmaceutical drugs aids in formation of ion association complex. Cations are yielded due the attraction of proton(s) by these lone pair of electrons.

Alizarin Red S is a water soluble dye. Anion is formed by the hydrolysis of alizarin red S in aqueous medium. Ion association complex is generated from these oppositely charged ions [10]. Spectrophotometric measurement of organic phase absorbance can be done after extracting the developed ion association complex in organic solvent [9].

One more added advantage of ion association complex is its applicability for exact measurement of targeted API′s in the presence of many other formulation constituents. Taken into the consideration of the above mentioned advantages, current method helps to develop a method which is based on the development of a soluble ion-pair complex between drug cation and anionic form of alizarin red S dye, an anionic dye. The formed ion-association complex was extractable in an organic solvent like chloroform.

EXPERIMENTAL

Teccomp UV-2301 double beam UV-visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Standard cuvettes of 10 mm path length are used for analysis. Sonicator (1.3 L) Ultrasonicator was used for sonicating the standard and formulation sample. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234).

Working standard sample danazol was obtained from Hetero Labs Pvt. Ltd., Hyderabad, India, formulation sample was purchased from local pharmacy. All the chemicals and solvents were of AR grade and purchased from Fine Chem Industries, Mumbai, India.

Identification of suitable solvent: The drug danazol was soluble in methanol, water and partially soluble in acetonitrile. By solubilizing the drug in different solvents in different concentrations like HCl, NaOH, all the solvents were scanned in the UV region and identified the absorption maxima for all the soluble solvents. It was found that at a solvent ratio of methanol and acetonitrile in the ratio of 75:25 were found to be most suitable solvent.

Preparation of standard drug solution: Danazol (100 mg) was accurately weighed and dissolved in 5 mL diluent then transferred to a 10 mL volumetric flask sonicate it for 5 min, finally volume was made up to the mark with same solvent to make 1000 µg/mL stock solution. From this 1 mL was again diluted to 10 mL to get a concentration of 100 µg/mL solution. This solution was used as standard stock solution.

Preparation of sample solution: Ten tablets were weighed and powdered. The amounts of tablet powder equivalent to 100 mg of danazol was weighed accurately and transferred to 5 mL solvent and kept for 15 min in sonicator and volume was made up to mark with same diluent in 10 mL volumetric flask. The solution was then filtered through Whatmann filter paper # 41. This filtrate was diluted suitably with diluent to get the solution of 1000 µg/mL concentration. Then from the serial dilutions to prepare a concentration of 10 µg/mL. The absorbance was measured against blank. The drug content of the preparation was calculated using standard calibration curve.

Preparation of alizarin red S reagent: Alizarin red S (200 mg) was weighed accurately and dissolved in 100 mL of hot distilled water.

Hydrochloric acid solution: Concentrated hydrochloric acid (8.6 mL) was measured accurately and taken in 1000 mL volumetric flask containing 500 mL of water. Then the final volume in volumetric flask was made upto the mark with water.

Method development: Standard drug (100 µg/mL) was taken in a 250 mL separating funnel, 6 mL of hydrochloric acid solution and 2 mL of alizarin red S were added. The content in the separating funnel was made upto 15 mL with water. The colour complex formed in the aqueous layer was extracted with 10 mL chloroform. The colour extracted in chloroform layer was compared with the similar black solution prepared without drug. Then the absorbance′s of formed colour was scanned in the visible region *i.e*. 800-400 nm against a reagent blank and identify the wavelength maxima for developed colour.

RESULTS AND DISCUSSION

Danazol is a synthetic steroid derived from ethisterone and prescribed for the treatment of endometriosis, fibrocystic breast disease, hereditary angioedema and other conditions. Spectrophotometry is considered as the most convenient analytical technique in pharmaceutical analysis because of its inherent simplicity and availability in most quality control and clinical laboratories. However, danazol not possess chromophore in its molecule, which is the essential requirement for the direct or indirect spectrophotometric analysis.

Based on the structure of drug danazol, A colour conversion method was applied for the estimation of drug in visible region. The drug shows positive reaction with alizarin red S dye solution. Then the absorbance′s of formed colour was scanned in the visible region *i.e*. 800-400 nm against a reagent blank and identified the wavelength maxima for the developed colour. The wavelength scan spectrum is shown in Fig. 2. The developed colour for the formation of colour complex with dye and drug shows maximum absorbance at 457 nm. Hence, further studies

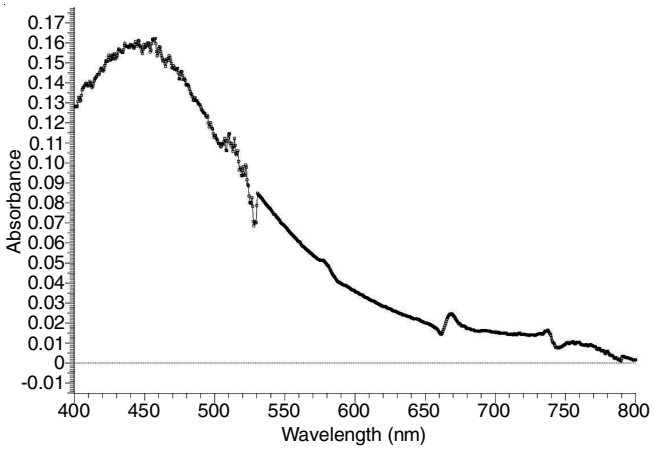

Fig. 2. Wavelength scan spectra in visible method

for the method developed for the analysis of danazol were carried at suitable wavelength of 457 nm.

Linearity: The absorbance of all the formed colour was measured and calibration curve was constructed with concentration against absorbance. Good linearity was observed within the concentrations under the study. Linearity range (Fig. 3) was found to be 5-30 µg/mL and linear equation was found to be y = 0.029x + 0.059. The results are shown in Table-1.

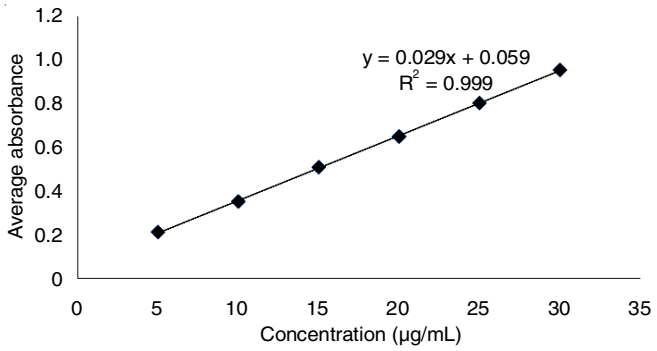

Fig. 3. Calibration curve in visible method for danazol

TABLE-1
LINEARITY RESULTS IN UV METHOD

S. No.	Concentration (µg/mL)	Absorbance
1	5	0.213
2	10	0.353
3	15	0.511
4	20	0.652
5	25	0.806
6	30	0.958

Precision: Precision of the method was determined by repeatability (intraday precision) and intermediate precision (interday precision) of standard solution. Precision was determined in six replicates of standard solution for the developed procedure. Then the resultant absorbance was measured using spectrophotometer at the proposed wavelength for the corresponding methods. Same procedure was followed for intraday precision also. %RSD of both precisions (intra and interday precisions) in the proposed methods were calculated and found to be within the acceptance limit of 0.576 for intraday precision and 0.963 for interday precision. Results of the precision are shown in Tables 2 and 3 for intra and interday precision, respectively.

Accuracy: The accuracy of a method is expressed as the closeness of agreement between the found value and reference value. It is determined by calculating the percentage relative error between the measured and added concentrations of danazol.

TABLE-2
INTRADAY PRECISION RESULTS

Sample	Concentration	Trial No.	Absorbance	RSD
Danazol	20 µg/mL	1	0.652	0.576 (acceptance criteria ≤ 2.0 %)
		2	0.651	
		3	0.655	
		4	0.659	
		5	0.652	
		6	0.648	

TABLE-3
INTERDAY PRECISION RESULTS

Sample	Concentration	Trial No.	Absorbance	RSD
Danazol	20 µg/mL	1	0.659	0.963 (acceptance criteria ≤ 2.0 %)
		2	0.651	
		3	0.655	
		4	0.643	
		5	0.648	
		6	0.644	

Results of the recovery are shown in Table-4. The % recovery was found to be in the range of 98.55 to 101.18 % in 50, 100 and 150 % spiked levels confirmed that the method is accurate.

TABLE-4
RESULTS OF THE RECOVERY

Recovery (%)	Accuracy				Assay (%)
	Target conc. (µg/mL)	Spiked conc. (µg/mL)	Final conc. (µg/mL)	Absorbance found	
50	10	5	15	0.251	98.82
	10	5	15	0.256	100.79
	10	5	15	0.257	101.18
100	10	10	20	0.324	98.48
	10	10	20	0.327	99.39
	10	10	20	0.331	100.61
150	10	15	25	0.415	100.24
	10	15	25	0.411	99.28
	10	15	25	0.408	98.55

Ruggedness: For ruggedness test of danazol, analysis was performed by different analyst. Only one parameter was changed in each experiment. Each deliberate small change was analyzed six independent series containing 20 µg/mL danazol. These results were compared statistically and no difference between results was observed. Therefore, the method is rugged to the small changes in experimental conditions. The results are shown Table-5.

TABLE-5
RESULTS OF THE RUGGEDNESS

Sample	Concentration	Trial No.	Absorbance	RSD
Danazol	20 µg/mL	1	0.661	1.116 (acceptance criteria ≤ 2.0 %)
		2	0.658	
		3	0.663	
		4	0.655	
		5	0.643	
		6	0.661	

Limit of detection and limit of quantification: LOD was obtained with 0.03 µg/mL for danazol. Limit of quantification (LOQ) is generally determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision. LOQ was found to be 0.10 mg/mL for danazol.

Formulation assay: Danazol in six different dosage forms was analyzed through the procedure as explained in the tablet solution. Analysis was performed under optimum conditions.

Each prepared solution was analyzed for seven independent determinations and each series were analyzed thrice. From the absorbance values, % assay was calculated and found to be more than 98 % assay for the developed method. This indicates that the method can be successfully applied for the estimation of drug in bulk and pharmaceutical dosage forms. The results of formulation assay is given in Table-6.

TABLE-6
FORMULATION ASSAY RESULTS OF DANAZOL

Available form	Label claim	Concentration (µg/mL)	Amount found (µg/mL)	Assay (%)
Tablet	100 mg	100	99.05	99.05

Conclusion

The proposed visible spectrophotometric method was validated and found to be simple, sensitive, accurate, reproducible, precise, rugged and relatively inexpensive. The developed method can be easily applied for the routine quality control analysis of danazol in bulk and pharmaceutical preparations.

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