



## DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION DANAZOL IN API AND COMBINED DOSAGE FORM

Prathibha K., Jagya and Dr. A. Yasodha\*

Department of Pharmaceutics, Dhanvanthri College of Pharmaceutical Sciences, Mahabubnagar- 509002, Telanagana, India.

\*Corresponding Author: Dr. A. Yasodha

Department of Pharmaceutics, Dhanvanthri College of Pharmaceutical Sciences, Mahabubnagar- 509002, Telanagana, India.

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### ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Danazol, in its pure form as well as in tablet dosage form. Chromatography was carried out on ODS C18 (4.6 X250mm, 5 $\mu$ m) column using a mixture of Methonol (100% v/v) as the mobile phase at a flow rate of 1.0 ml/min, the detection was carried out at 274nm. The retention time of the Danazol was 2.252  $\pm$  0.02min. The method produce linear responses in the concentration range of 5-25 $\mu$ m/ml of Danazol. The method precision for the determination of assay was below 2.0% RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

**KEYWORDS:** Danazol, RP-HPLC, Validation.

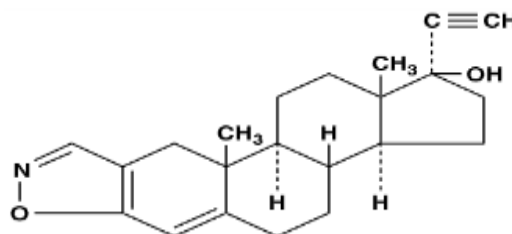
### INTRODUCTION

Danazol is a derivative of the synthetic steroid ethisterone, a modified testosterone. It was approved by the U.S. Food and Drug Administration (FDA) as the first drug to specifically treat endometriosis, but its role as a treatment for endometriosis has been largely replaced by the gonadotropin-releasing hormone (GnRH) agonists.<sup>[1]</sup> Danazol has antigonadotropic and anti-estrogenic method for the estimation of Danazol as per ICH guidelines 19. Moreover this method also find advantage and Even though analytical methods have been developed, a simple, rapid and sensitive method when compared with existing method.<sup>[2,3]</sup>

Danazol acts as an anterior pituitary suppressant by inhibiting the pituitary output of gonadotropins. Chromatography in which separation is based mainly on difference between the adsorption affinities of the sample components for the surface of an active solid. The analyte interact with solid stationary surface and are displaced with eluent for active sites on surface.<sup>[4-7]</sup> It possess some androgenic properties. To develop new simple, sensitive, accurate and economical analytical method for estimation of danazol. The utility of the developed method to determine the content of drug in commercial formulation was also demonstrated.<sup>[8]</sup>

Validation of the method was done in accordance with USP and ICH guideline for the assay of active

ingredient. The method was validated for parameters like system suitability, linearity, precision, accuracy, specificity, ruggedness, robustness, limit of detection and limit of quantification. The method provides means to quantify the component.<sup>[9]</sup> The proposed method was suitable for the analysis of Pharmaceutical dosage forms estimation of Danazol in bulk and pharmaceutical formulations.<sup>[10,11]</sup> Hence we developed a simple, rapid, sensitive and economical RP (Reverse Phase)-HPLC (High Performance Liquid Chromatography) The results obtained were promising.



### MATERIALS AND METHOD

#### Chemicals used

Standard were purchased from sura labs, water and methanol for HPLC from LICHROSOLV(MERCK), Acetonitrile for HPLC Merck.

**Equipments**

HPLC (Waters Alliance 2695 Separation Module, Software: Empower 2, 486 PDA Detector), pH meter (Labindia), Weighing machine (Sartorius).

**Preparation of standard solution**

Accurately weigh and transfer 10 mg of Danazol working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

**Preparation of mobile phase**

Accurately measured 1000 ml (100%) of HPLC Methanol in a 1000ml volumetric flask.

**Chromatographic conditions**

Waters ODS C18 column having dimensions 4.6 X 150mm, 5 $\mu$ m particle size. Mobile phase used was methanol (100% v/v), injection volume 10 $\mu$ l. UV detector wavelength used was 274nm and flow 1ml/min.

**Method validation**

The developed method was validation as per the ICH guidance. The method was validated for specificity, selectivity, linearity, sensitivity, accuracy, precision, robustness and ruggedness.

**Specificity and selectivity**

Specificity of the developed method was carried out by comparing the standard and the sample retention time with six injections and the lack of interference peak were studied. The selected chromatographic condition should be ideal and should be free from impurities, matrix, degradants, excipients, preservatives and related impurities on selected standard retention time and peak.

**Linearity and Sensitivity**

Linearity was carried by plotting a calibration graph of analyte peak area (y axis) versus concentration (x axis) ranging from 10.0-100.0  $\mu$ g/ml. Linear regression analysis was followed and the correlation of coefficient ( $r^2$ ) was used as measure of linearity. Limit of detection (LOD) and limit of quantitation (LOQ) were reported as 3 and 10 times the noise level obtained from three replicate injections of blank samples respectively.

**Accuracy**

Accuracy was evaluated to determine recovery of the standard solution from the sample solutions. Mean percentage (%) recovery of analytes was used as a measure of accuracy. Each sample concentration was spiked with the standard concentration and injected in triplicate and the percentage recovery was determined. Accuracy was evaluated to determine recovery of the standard solution from the sample solutions. Mean percentage (%) recovery of analytes was used as a measure of accuracy.

**Precision and repeatability**

Intermediate precision (intra- and interday) and repeatability were carried out on the analytes sample. Percentage relative standard deviation (% RSD) was considered as a measure of precision and repeatability. The analytes sample were prepared at three different concentration of low, middle and high concentration of linearity sample and injected six time on the same day and on three consecutive day for intra- and interday precision respectively.

**Robustness and Ruggedness**

The developed method was evaluated for robustness and ruggedness by slight changing the parameters such as instruments and operators, slight change in the optimized chromatographic conditions such as column temperature, flow rate and pH.

**RESULTS AND DISCUSSION**

Optimization of the chromatographic condition was carried out based on the sharp peak, ideal separation, stability and repeatability of the standards as the results are shown in the Table 1&2. Numbers of trials were carried out to achieve the above criteria by changing the mobile phase parameters such as buffer concentration, buffer strength, organic concentration, pH ratio and standard phase such as different column as C8 and C18. Based on the trial and error method the developed method was optimized at the mobile phase concentration of 25mM of methanol and acetonitrile at the ratio of 50: 50 v/v with the pH of 4 respectively.

Among the different solvent acetonitrile showed maximum solubility. Stationary phase used was C18 column (250 x 4.6 mm, i.d. 5 $\mu$ m). Detection of the optimized chromatograph was carried out at 274 nm as shown in the Table 3. The developed method for danazol showed good separation at the retention time of 8 min respectively. Broadening of the peak was observed when decrease in the concentration of organic phase while shifting of peak was observed towards the void volume side due to the high solubility.

Similarly in aqueous phase when the concentration increase no peak was observed due to the low solubility where else decrease in the concentration of aqueous phase broadening of peak was observed. Change in the pH shows that peak splitting. Increase or decrease of the detection wavelength shows decrease in the response.

Specificity of the developed method was carried out by comparing the standard and the sample retention time with six injections and the lack of interference peak were studied as shown in the Table 4. The selected chromatographic condition was free from impurities, matrix, degradants, excipients, preservatives and related impurities on selected standard retention time and peak. Hence the selected method was specific.

Linearity was carried by plotting a calibration graph of analyte peak area (y axis) versus concentration (x axis) ranging from 10.0-100.0 µg/mL was found to be linear in range as shown in the fig 1. Linear regression analysis was followed and the correlation of coefficient (r<sup>2</sup>) was used as measure of linearity. Regression equation for the linearity and the range was found to be 0.994 respectively. Accuracy was carried out by recovery studies by spiking the known standard concentration to the sample solution as shown in the fig 2 and found to be consistent at all the levels as shown in the Table 5.

Precision study was carried out at two different levels of intra- and interday levels. Six injection of three different

concentration of low, middle and high concentration of linearity such as 20, 60 and 100 µg/mL were injected and the percentage RSD and standard deviation were calculated and results are well within the limits as shown in the Table 6.

The developed method showed no change in the chromatographic condition when evaluated for the robustness and ruggedness and found to be rugged and robust. Limit of detection and limit of quantification for danazol were studied base on the signal to noise ratio and found to be 50 ng/mL and 200ng/ml.

**Table 1: Results of system suitability for Danazol.**

Sl. No.	Peak name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Danazol	2.277	1419842	198640	6291	0.85
2	Danazol	2.277	1429006	170366	6346	0.85
3	Danazol	2.267	1418422	169256	6985	0.86
4	Danazol	2.265	1408754	1532657	6584	0.85
5	Danazol	2.277	1425462	180021	6325	0.86
Mean			1420297			
Std. Dev.			7737.69			
% RSD			0.54			

**Table 2: Peak results for assay standard.**

Sl. No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Danazol	.265	1417834	167270	0.85	6291	1
2	Danazol	2.267	1421060	168871	0.85	6549	2
3	Danazol	2.267	1419161	168999	0.85	6982	3

**Table 3: Chromatographic data for linearity.**

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	5	478475
66	10	1001129
100	15	1416507
133	20	1841573
166	25	2283778

**Table 4: Results for repeatability for Danazol.**

Sl. No.	Peak name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Danazol	2.293	1413527	173368	6413	0.85
2	Danazol	2.276	1401699	175290	6214	0.85
3	Danazol	2.286	1411309	175314	6528	0.85
4	Danazol	2.277	1425886	176294	6624	0.85
5	Danazol	2.280	1412465	178661	6982	0.85
Mean			1412977			
Std. Dev.			8619.49			
% RSD			0.61			

Table 5: Results of intermediate precision analyst for Danazol.

Sl. No.	Peak name	RT	Area ( $\mu\text{V}\cdot\text{sec}$ )	Height ( $\mu\text{V}$ )	USP Plate Count	USP Tailing
1	Danazol	2.277	1405886	176294	6463	0.85
2	Danazol	2.255	1406800	178900	6251	0.85
3	Danazol	2.265	1413406	182073	6985	0.85
4	Danazol	2.255	1418482	181769	6235	0.85
5	Danazol	2.253	1412766	181366	6598	0.85
6	Danazol	2.252	1409515	359346	6458	0.85
Mean			1411143			
Std. Dev.			4706.682			
%RSD			0.33			

Table 6: Results of Accuracy for Danazol.

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean recovery
50%	708738.3	7.5	7.48	99.7%	99.3%
100%	1409436	15	14.82	98.8%	
150%	2076623	22.5	22.41	99.6%	

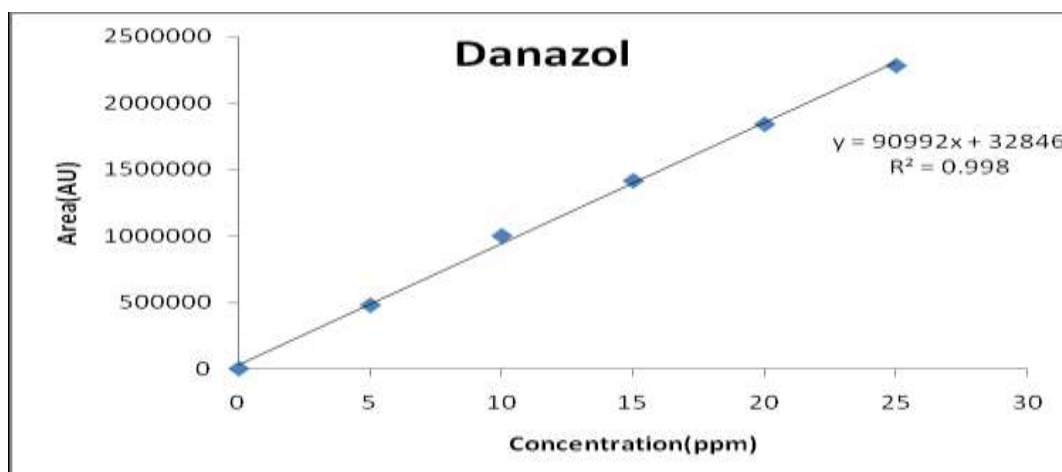


Fig. 1: Calibration curve.

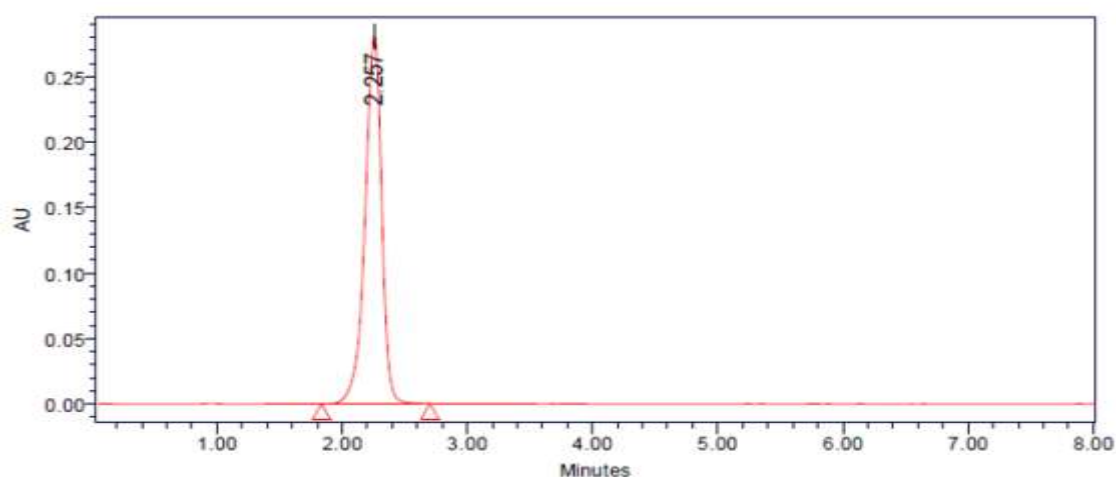


Fig. 2: Chromatogram showing accuracy-50% injection.

## CONCLUSION

A simple, rapid, cost effective and sensitive method was developed for the danazol drug and validated as per the ICH guidelines such as specific, sensitive, accurate,

precise, reproducible, robust, LOD and LOQ. The developed method for the danazol can be used for the quantitative and qualitative estimation of bulk and finished formulations.

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