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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING RP-HPLC METHOD FOR ESTIMATION OF DEFLAZACORT IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: The objective of the method was to develop a simple, precise, rapid and stability indicating reverse phase high performance liquid chromatographic method (HPLC) for the quantitative determination of anti-inflammatory and immunosuppressive drug Deflazacort in its generic formulations.

Methods: In this method, a Hypersil Gold ODS C_{18}, 25cm 5µm, 4.6mm ID column (Thermo Scientific, Waltham, USA) with mobile phase consisting of acetonitrile and water in the ratio of 45:55 in an isocratic mode was used. The detection wavelength was 242nm and the flow rate 1ml/min.

Results: In the range of 1µg/mL-6µg/mL, the linearity of Deflazacort showed a regression coefficient of 0.999. The proposed method was sufficiently selective to distinguish the parent drug and the degradation products after hydrolysis, photolysis or chemical oxidation and from excipients. This developed method was validated by determining its sensitivity, accuracy and precision.

Conclusion: The developed method was simple, fast, accurate and precise and hence could be applied for routine quality control analysis of Deflazacort in solid dosage forms.

Keywords: Deflazacort, HPLC, Degradation, Validation.

INTRODUCTION

Deflazacort (DFZ) (Fig. 1) is chemically described as an oxazoline (1-(1, 16)-21-(acetyloxy)-11-hydroxyl-2-methyl-5H-pregna-1, 4-dieno [17, 16-d] oxazole-3, 20-dione) derivative of prednisolone. This medication is a glucocorticoid, prescribed for anti-inflammatory conditions, and used as an immunosuppressant. It is a prodrug [1-2]. It is a methyloxazoline derivative of prednisolone that is used in rheumatoid arthritis, nephritic syndrome, organ transplantation rejection and juvenile chronic arthritis, among other diseases [3, 4]. Deflazacort is not official in any pharmacopoeias; hence official method is not available for determination of deflazacort. Literature survey reveals different liquid chromatographic method like Spectrophotometric and reverse-phase HPLC [5-9], LC-MS [10], HPTLC [11], dissolution pattern for estimation of deflazacort have been reported [12-13]. Literature survey does not reveal any simple stability indicating HPLC method for determination of deflazacort in tablet formulation [14]. The present manuscript describes simple, sensitive, accurate, precise and specific stability indicating HPLC procedure for the determination of deflazacort in pharmaceutical tablet dosage forms.

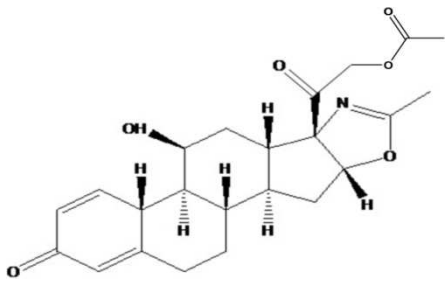

Fig. 1: Structure of Deflazacort

MATERIALS AND METHODS

Chemicals and Reagents

Working standards of pharmaceutical grade Deflazacort was obtained from Samex Overseas, India. Fixed dose Tablet **(**DAZACORT**)** containing 6 mg was purchased from local market, Pune, India. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India.

Instrumentation

The HPLC system (Jasco corporation, Tokyo, Japan) consisted of a Pump (model Jasco PU- 2080 Plus) along with manual injector sampler programmed at 20 µl capacity per injection was used. The detector consisted of UV/ VIS (model Jasco UV 2075). LC separations were performed on a Hypersil Gold C_{18}, 25cm 5µm, 4.6mm ID column (Thermo Scientific, Waltham, USA). Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. The mobile phase was consisted of a mixture of Acetonitrile: Water (45:55). The mobile phase was degassed and filtered by passing through a 0.45µm pore size membrane filter (Millipore, Milford, MA, USA) prior to use. The flow rate was 1 min/mL. All determinations were performed at ambient temperature with a detection wavelength of 242 nm.

Preparation of Standard Solution

Standard stock solution containing 1mg/mL of Deflazacort was prepared in acetonitrile. The working standard was prepared by diluting the above stock solution in mobile phase to reach a concentration range of 1-10 µg/mL.

Preparation of Sample Solutions

The pharmaceutical dosage form used in this study was DAZACORT, manufactured by Neclife (Nectar Life sciences Ltd, Chandigarh, India), labeled to contain 6mg of Deflazacort per tablet. From the powdered mass of 10 tablets, an amount equivalent to 6 mg of Deflazacort was weighed, transferred to a 50 mL volumetric flask, and dissolved in 25 mL of mobile phase. The solutions were sonicated for 30 min to enable dissolution of the active components from tablets and then diluted to volume with the mobile phase to obtain sample solutions containing 0.12mg/mL of DFZ. After mixing, the solutions were filtered through Whatman filter paper No. 0.45µm. The analysis was performed three times. The possibility of excipients interference with the analysis was examined.

Validation of The Method

Specificity

The specificity of the method was tested by comparing the chromatograms of standard DFZ with that of sample. Marketed formulation of DFZ was processed and analyzed under the same conditions, and the retention times of DFZ standard and DFZ from marketed formulation were detected.

Calibration curve

Six standard samples of DFZ (1-6 µg/mL) were prepared to generate the calibration curve. To avoid bias, standard curves were fitted using weighted least squares linear regression in the form of y = a + bx, where y represents the ratio of DFZ peak area and x represents the concentration of DFZ. Calibration curves were prepared and analyzed on three consecutive days to evaluate the linearity.

Accuracy, precision and limit of quantification

The accuracy and precision of the method were evaluated with 80, 100 and 120 % of the test concentration as per ICH guidelines (n = 3). Accuracy was obtained by calculating the ratios of the concentrations calculated with the calibration curves to the spiked values, and expressed as percentages. To validate the intraday and interday precision at three levels were freshly prepared and determined by quantitating three replicates on the same day and three consecutive days, respectively. The precision was calculated as the coefficient of variation (CV) of measurements.

The limit of quantification (LOQ) was estimated by analyzing the low concentrations of DFZ in the calibration curves, which could be quantitated accurately and the LOQ was verified by the analysis of three replicates.

Robustness

The robustness is a measure of method capacity to remain unaffected by small, but deliberate changes in chromatographic conditions. It was studied by testing influence of small change in flow rate (± 0.1mL/min), change in mobile phase composition acetonitrile ± 1 mL.

Degradation Studies

Acid and Base Induced Degradation

Acid decomposition studies were performed by exposing the solution of drug to 0.01N hydrochloric acid refluxed at 50°C for 15 mins. The studies in alkaline conditions were carried out in 0.01 N sodium hydroxide and the solution was kept at a room temperature. The resultant solutions were diluted in mobile phase and the sample solution was then injected separately under the optimized chromatographic conditions used for analysis of marketed formulation.

Hydrogen Peroxide Induced Degradation

To study hydrogen peroxide induced degradation, the sample was exposed to 3 % hydrogen peroxide at room temperature for a period of 2 hours. Resultant solution was diluted in mobile phase and then injected separately under the optimized chromatographic conditions used for the analysis of marketed formulation.

Photochemical Degradation

The photochemical stability of the drug was studied by exposing the stock solution (1000 µg/mL) to direct sunlight for 48 hrs. The resultant solution was diluted in mobile phase and injected separately under the optimized chromatographic conditions used for analysis of marketed formulation.

The drug solution was also kept in the Photostability chamber for 48 hours. Appropriate dilutions of pure drugs were prepared in mobile phase and then analyzed under the optimized chromatographic conditions.

RESULTS AND DISCUSSION

Optimization of HPLC Method

Chromatograms of Deflazacort at a concentration of 10 µg/mL are shown in Fig. 2. Significant interference from endogenous substances was not observed at the retention times of DFZ and marketed formulation, indicating the sample preparation method is effective. The retention time of DFZ was 7.9+0.2 min. The peaks were sharp and symmetrical with good baseline resolution and minimal tailing. UV spectrum of Deflazacort (Fig.3) showed maximum absorbance at 242nm and the same was selected as the scanning wavelength. The mobile phase was optimized by changing the composition of the mobile phase to achieve good resolution and symmetric peak shapes for analyte as well as a short run time. Finally, a mixture of acetonitrile: water (45:55) was adopted as the mobile phase.

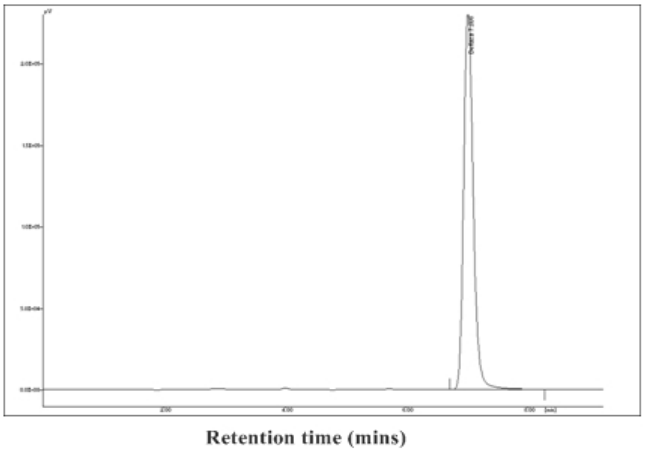

Fig. 2: Chromatogram of Deflazacort

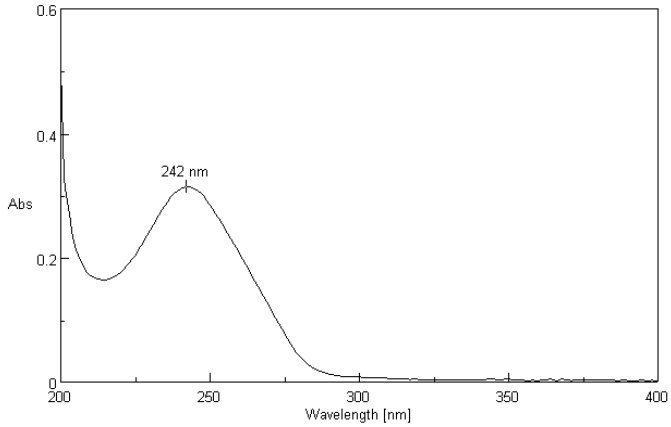

Fig. 3: UV spectra for Deflazacort

Validation of the developed method

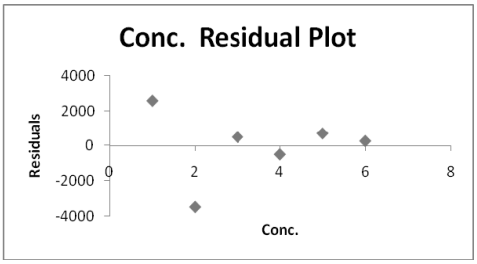

Fig. 4a: Concentration Residual Plot

Good linearity was obtained for DFZ in the range from 1–6 µg/mL. The regression equation of the calibration curve was as follows: y = 35879 x - 7924 (r^2=0.999) (Fig.4a), where y and x represented the peak area ratio and DFZ concentration, respectively (Table-1). No significance difference was observed in slopes of Standard curve. Residual analysis was done to ascertain linearity (Fig. 4b).

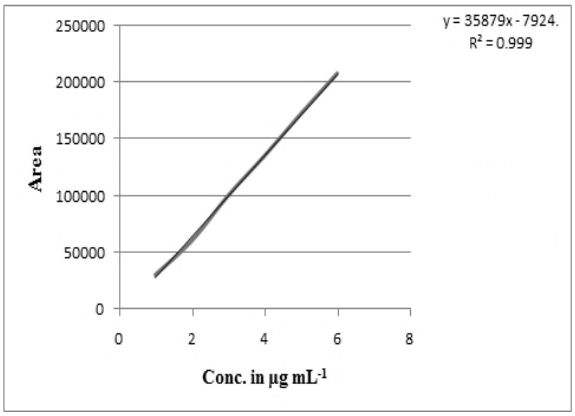

Fig. 4b: Calibration curve of Deflazacort

Table 1: Linearity Regression Data for Calibration Curve

Parameters	Deflazacort
Linearity range	1-6 µg mL^{-1}
r^2	0.999
Slope	35879 ± 530.1
Intercept	-7924 + 064
Confidence limit of slope [a]	34410 to 37350
Confidence limit of intercept [a]	13660 to 2194
Sy.x [b]	2218
P value [c]	< 0.0001

[a] 95% Confidence intervals

[b] Standard deviation of residuals from line

[c] P value is < 0.0001, considered extremely significant

LOD and LOQ were determined by visual detection method. LOD and LOQ of the drug was found to be 0.1 and 0.4 µg/mL respectively after studying a range of concentrations made from the stock solution. The precision of the method was determined by analysis of the marketed formulation. The repeatability of sample injection and intermediate precision, as RSD, were less than 2% for DFZ, indicating acceptable degree of intra-day and inter-day precision (Table 2).

Table 2: Intra-Day and Inter-Day Precision of Deflazacort

Conc. µg/mL	Intra-day precision (n=3)			Inter-day Precision (n=3)		
	Found conc. ± SD	RSD (%)	S.E.	Found conc. ± SD	RSD (%)	S.E.
2	1.92 ± 0.005	0.26	0.002	2.008 ± 0.003	1.49	0.0012
4	4.05 ± 0.012	0.29	0.004	4.105 ± 0.015	0.36	0.006
6	5.85 ± 0.09	1.53	0.036	5.95 ± 0.003	0.5	0.0012

To evaluate the robustness of the method, the optimized method parameters were varied at different levels. The results presented in Table 3 indicated that the developed method was unaffected by small variations in the optimized method parameters.

The accuracy was studied by measurement of recovery at three different levels (equivalent to 80, 100, and 120% of the amount originally present in the tablets). The mean % recovery was 100.06 % for DFZ (Table 4).

Table 3: Robustness of the method

Level	Factor	Retention Time	% RSD
Change in Conc. of Acetonitrile in Mobile Phase (n=3)			
-1	44	7.93	1.51
0	45	7.90	1.13
1	46	7.88	1.26
Change in Flow Rate (n=3)			
-1	0.9	7.86	0.63
0	1	7.90	1.01
1	1.1	7.92	0.88

Table 4: Accuracy study of the method

Drug	Label claim mg/tab	Amount added in mg (%)	Total amount (mg)	Amount Recovered (mg) ± SD	RSD (%)	Recovery (%)
Deflazacort	6	4.8 (80)	10.8	10.98±0.2	1.82	101.66
		6 (100)	12	11.78±0.09	0.76	98.16
		7.2 120)	13.2	13.25±0.22	1.66	100.37

The content of drug was analyzed in the tablets (DAZACORT). The possibility of excipients interference with the analysis was examined. The drug content observed from the result was 99.52% with no interference from the excipients.

Forced Degradation Studies

Acid and Base Induced Degradation

Optimized condition for acid degradation was achieved by exposing the solution to 0.01N hydrochloric acid refluxed at 50°C for 15 mins. The solution was then diluted with mobile phase and injected into the column. The following chromatogram was observed for degradation pattern. (Fig.5). The degradation peaks were observed at 2.092 mins and 3.808 mins.

The drug was found to undergo rapid degradation in alkaline conditions. Initially, refluxed with 0.01 N sodium hydroxide, it resulted in 90% degradation of the drug. Upon reducing the degradation conditions, it gave 50% degradation with 0.01N sodium hydroxide at room temperature in 15 mins. Thus it can be concluded that Deflazacort is unstable in alkaline conditions.

Hydrogen Peroxide Induced Degradation

The drug was found to be highly labile in oxidative conditions. When exposed to 3% H2O2 at room temperature for 2hrs it showed a degradation of 10% with two degradation products at 3.7 mins and 4 mins. (Fig. 6)

Photolytic Induced Degradation

The photochemical stability of the drug was studied by exposing the stock solution (1000 µg mL^{-1}) to direct sunlight for 48 hrs and the samples were collected over a time period of 1 hour. The resultant solution was diluted in mobile phase and injected into the Hypersil Gold C_{18} analytical column. The degradation was observed after 2hrs. (Fig. 7)

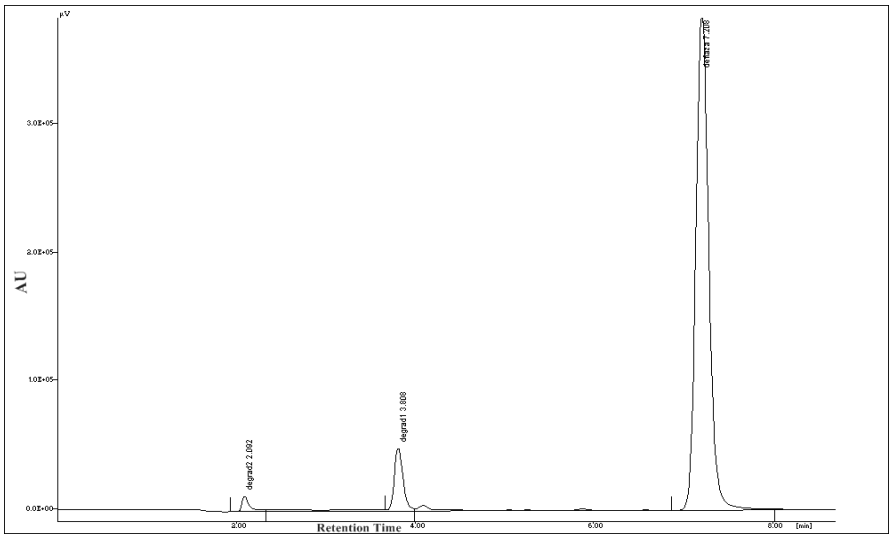

Fig. 5: Chromatogram for Acid Induced Degradation

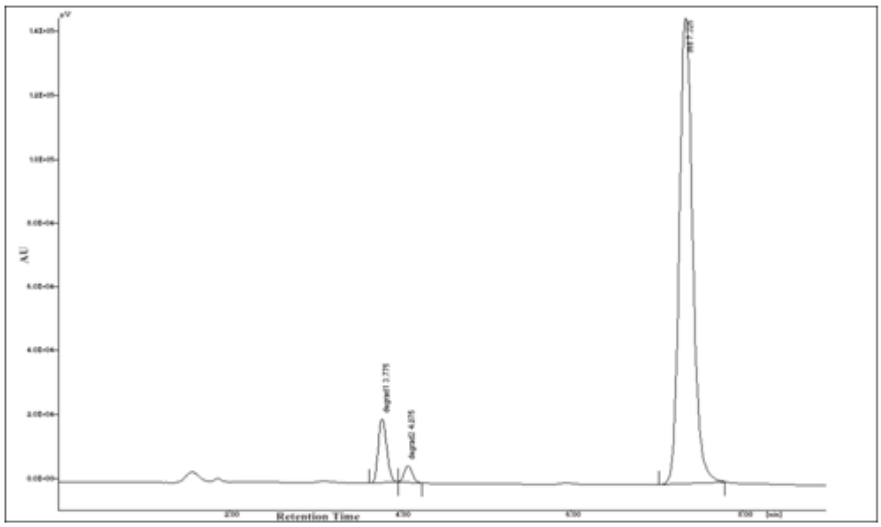

Fig. 6: Hydrogen Peroxide Induced Degradation

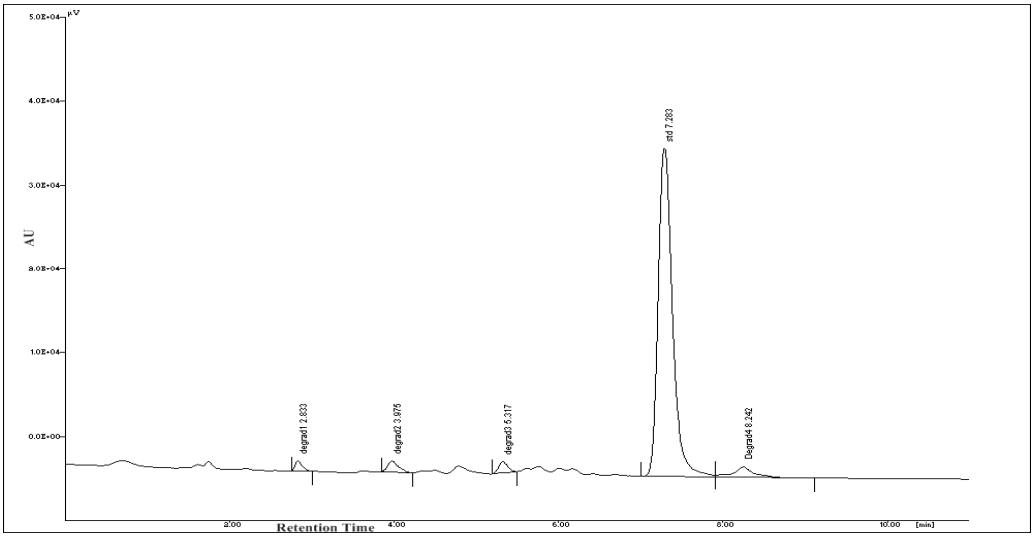

Fig. 7: Photolytic Degradation

On exposing the drug to sunlight it showed a degradation of 22% within 48hrs. (Fig. 8)

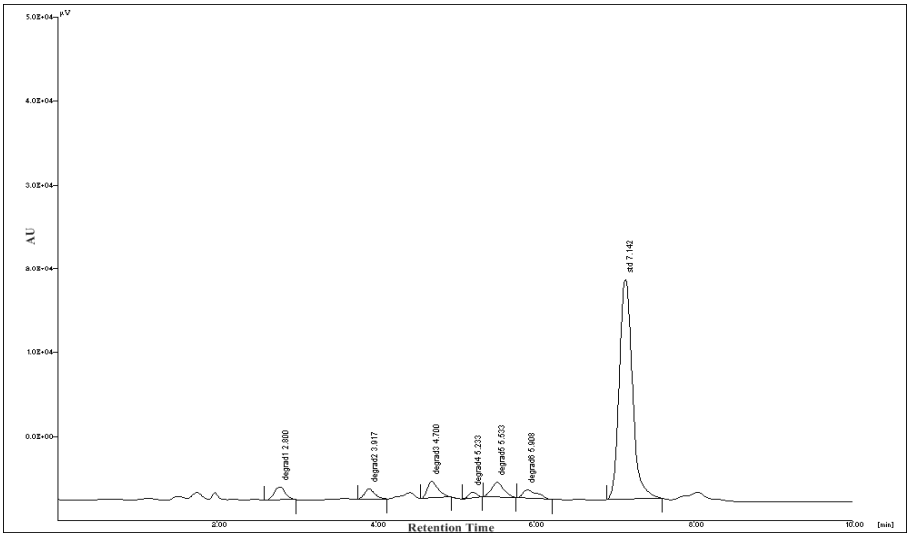

Fig. 8: Photolytic Degradation (Sunlight 48hrs)

Table 5: Summary of Degradation Studies

Conditions	Number of Peaks	Retention Time
Acidic (0.01N HCl)	Peak 1	2.092
	Peak 2	3.808
Oxidation (3% H_2O_2)	Peak 1	3.792
	Peak 2	4.092
Photolytic	Peak 1	2.833
	Peak 2	3.975
	Peak 3	5.317
	Peak 4	8.242
Photolytic (Sunlight)	Peak 1	2.800
	Peak 2	3.917
	Peak 3	4.700
	Peak 4	5.233
	Peak 5	5.533
	Peak 6	5.908

CONCLUSION

A HPLC method was successfully developed and validated for determination of DFZ. Deflazacort was found to be well resolved in a short run time of 7.9 ± 0.2 min. mins. No interference was found with the degradants and excipients. Method validation results have proved the method to be selective, precise, accurate, and robust and stability indicating. This method can be successfully applied for the routine analysis as well as for stability studies.

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