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Development and Validation of RP-HPLC Method for Quantitative Estimation of Corticosteroid in Bulk Drug and Pharmaceutical Formulation

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ABSTRACT

A simple, precise and stability-indicating HPLC method was developed and validated for the determination of Corticosteroid in bulk and pharmaceutical dosage forms for effective quality control. The drug was subjected to forced degradation under acidic, alkaline, neutral, oxidative, thermal and photolytic conditions, and was found to be stable under thermal and photolytic stress, while showing significant degradation under acidic, alkaline and oxidative conditions. Chromatographic separation was achieved on a Kromasil C18 column using an isocratic mobile phase of 20 mM potassium phosphate buffer (pH 6.2) and acetonitrile (50:50, v/v) with UV detection at 266 nm. The method showed linearity in the range of 20–100 µg ml^{-1} with a retention time of 5.232 min, and LOD and LOQ values of 4.52 and 14.12 µg ml^{-1}, respectively. The method was validated according to International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use Q2 (R1) guidelines and demonstrated acceptable analytical greenness with an AGREE score of 0.60.

Keywords: Validation, Corticosteroid, forced degradations, HPLC, stability indicating methods, ICH.

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1. INTRODUCTION

Corticosteroid Deflazacort (DFZ) widely used in the treatment of Duchenne muscular dystrophy (DMD), a genetic disorder caused by mutations in the dystrophin gene and characterized by progressive muscle weakness, respiratory complications and cardiac impairment. Compared with other steroid therapies, and is reported to produce fewer adverse effects on bone health and body weight, and it contributes to improved survival and delayed progression of muscle-related complications in affected children. A review of the literature indicates that several analytical approaches have been reported for the estimation of deflazacort in pharmaceutical dosage forms, including stability-indicating RP-HPLC methods, UPLC-based forced degradation studies, and advanced characterization of degradation products using FTIR, NMR and mass spectrometric techniques. These reported methods collectively highlight the importance of reliable and stability-indicating analytical procedures for quality control and stability assessment of in pharmaceutical formulations [1, 2].

The current study set out to create a straightforward, precise, accurate, and specific stability-indicating RP-HPLC method for estimating DFZ in tablet formulation along with the degradation products that are produced

2. EXPERIMENTAL WORK

MATERIALS:

Tablets used for analysis were procured from a local pharmacy and were manufactured by Macleods Pharmaceuticals. Ultra-pure water was obtained using a purification system from Millipore, and HPLC-grade methanol was purchased from

Merck (India) Limited, while all other reagents were of analytical-reagent grade [3]. Chromatographic analysis was performed using an HPLC system equipped with a UV–visible detector from Shimadzu (LC-2030 Plus). Separation was achieved on a Kromasil 100-5-C18 column (300 × 3.9 mm, 5 µm) with UV detection at 266 nm and an injection volume of 20 µL. An isocratic mobile phase consisting of acetonitrile and 20 mM potassium phosphate buffer (pH 6.2) in the ratio of 50:50 (v/v) was delivered at a flow rate of 1.0 mL min^{-1} with a total run time of 10 minutes. The mobile phase was filtered through a 0.4 µm membrane filter and degassed prior to use [4, 5].

Solvent system:

In order to achieve the needed concentration, 27.20 g of dissolved potassium dihydrogen phosphate was transferred to a 1000 mL volumetric flask, diluted with HPLC-grade water, and had its pH adjusted to 6.2 using 0.2 M sodium hydroxide. The buffer was then degassed. The mobile phase consisted of degassed acetonitrile (50:50 v/v) and transferred phosphate buffer pH 6.2. The mobile phase was then degassed by sonicating for up to fifteen minutes [6, 7].

Standard Solution:

About 10 mg of standard DFZ was precisely weighed, then put into a 10 ml volumetric flask. 5 ml of methanol were added, and the flask was placed in an ultrasonic bath to guarantee full solubilization. The volume was adjusted with methanol to provide a stock solution of 1000 µg/ml. After that, 0.5 ml of the stock solution was put into a 10 ml volumetric flask, and the mobile phase was added to bring it up to volume. This produced a solution with a concentration of 50µg/ml [8].

Method Validation:

The developed analytical method was validated for accuracy, precision, detection limit, quantitation limit, robustness and specificity in accordance with the guidelines of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use Q2 (R1). Accuracy and precision were evaluated by recovery studies at 80 %, 100 % and 120 % quality-control levels using spiked tablet samples, performed in triplicate over three consecutive days, where acceptable % recovery and low % RSD confirmed method reliability. The detection and quantitation limits were calculated from the calibration curve using the standard deviation of response and slope. Robustness was assessed by introducing small, deliberate variations in organic phase composition, flow rate and detection wavelength, and monitoring system suitability parameters. Specificity was demonstrated by complete separation of deflazacort from its degradation products and excipients, with no interfering peaks observed in blank chromatograms [9].

Stability indicating studies:

Stability studies of DFZ were performed at an initial concentration of 10 mg/10 mL with the objective of achieving 5–20 % degradation. Acidic degradation was carried out using 0.1 N HCl at room temperature for 8 h, followed by neutralization with 0.1 N NaOH, while alkaline degradation was conducted using 0.1 N NaOH for 4 h and subsequently neutralized with 0.1 N HCl. Oxidative stress was induced by treating the drug solution with 3 % hydrogen peroxide and maintaining it at room temperature for 12 h. Thermal and photolytic degradation studies were performed by exposing the powdered drug to 80 °C for 5 h in a hot air oven and to sunlight for 12 h, respectively. After completion of stress treatment, all samples were brought to room temperature, diluted with the mobile phase and analyzed. The purity of the drug peak obtained from stressed samples was assessed using a PDA detector to confirm the stability-indicating capability of the method [10].

3. RESULTS AND DISCUSSION

Development of chromatogram:

After trying a number of different mobile phases, it was discovered that a mobile phase consisting of methanol, 20 mM phosphate buffer pH 6.2, and acetonitrile (50:50 v/v) at a flow rate of 1 mL/min resolved DFZ. 266 nm was the ideal wavelength for detection. According to Figure 1, the DFZ retention time was 5.617 minutes. A system suitability test confirms that the chromatographic system's resolution and repeatability are sufficient for the analysis to be carried out. The USP acceptance criteria (Area % RSD < 2, asymmetry < 2, and No. of theoretical plates > 2000) were satisfied by all key parameters examined.

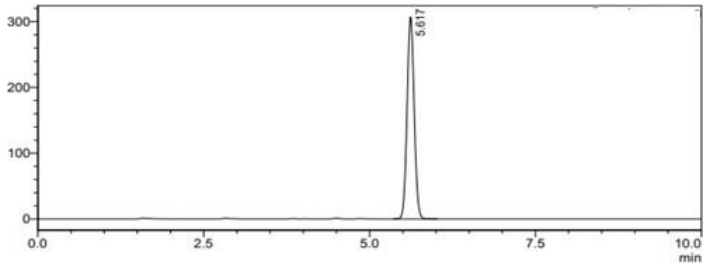

Fig. 1. Optimized chromatogram of DFZ

Stability indicating studies:

In the Stability indicating studies, it was discovered that DFZ degraded under oxidative degradation mediated by acids, alkalis, and peroxide. Under acidic, alkaline, and oxidative conditions, a decrease in the peak area of DFZ was noted along with the generation of breakdown products. However, after photolytic and humidity conditions were met, the peak area of DFZ with no degradation products decreased. The degradation behavior of DFZ under different forced degradation circumstances is summarized in Table 1 and shown in Figures 2, 3, and 4, respectively.

Table 1. Summary of Stability indicating studies

Sr. No.	Stress Conditions	Retention Time	% Degradation
1	Acid degradation - 0.1N HCl at RT for 8 days	2.412, 3.426, 5.534	12.44
2	Alkali degradation - 0.1N NaOH at RT for 4 days	3.322, 5.215, 5.457	19.52
3	Oxidative degradation -3% H_2O_2 at RT for 12 Hrs	2.234, 4.368, 5.275	10.28
4	Photolytic degradation exposed to 1.2 million lux hours of visible and 200 W*hr/m2 UV radiation.	5.462	No degradation
5	Dry heat- 80° C for 5 Hrs	5.462	No degradation
6	Wet heat degradation to 80°C for 5 Hrs	5.462	No degradation

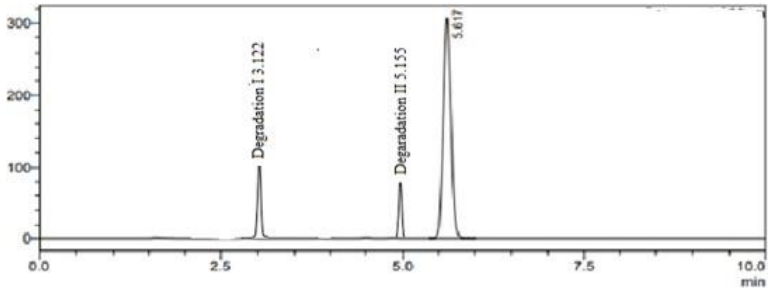

Fig. 2. Chromatogram of acid degradation of DFZ

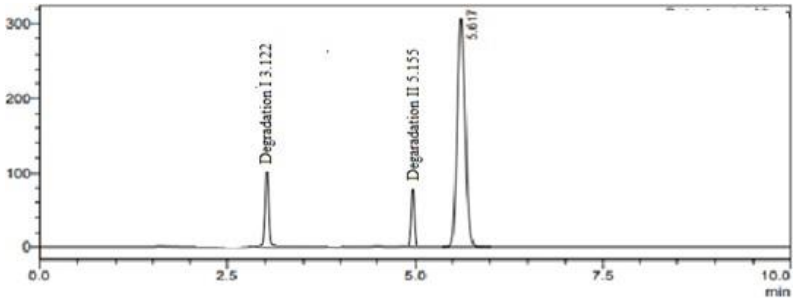

Fig. 3. Chromatogram of alkali degradation of DFZ

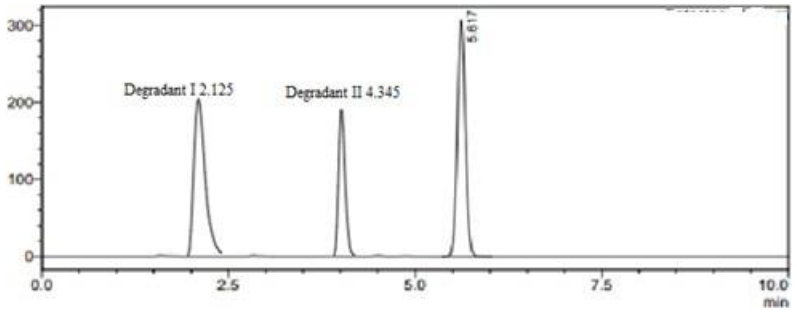

Fig. 4. Chromatogram of oxidative degradation of DFZ

Optimization and validation of method:

Following the ICH Q2 (R1) recommendations for the validation of analytical procedures for various validation parameters, the designed HPLC technique was validated. Limit of detection (DL), limit of quantitation (QL), linearity, accuracy, precision, and specificity were all evaluated.

Linearity study:

A series of working standard solutions of deflazacort in the concentration range of 20–100 µg/mL were prepared from the standard stock solution and injected (20 µL) into the chromatographic system at a flow rate of 1.0 mL min^{-1} with detection at 266 nm. The calibration curve was constructed by plotting peak area against the corresponding concentrations, which showed excellent linearity over the studied range with a correlation coefficient of 0.999, confirming the suitability of the method for quantitative analysis.

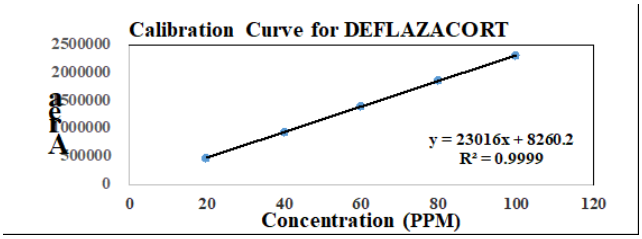

Fig. 5. Linearity curve of DFZ

Table 2. Linearity parameters for DFZ

Conc. (µg/ml)	Peak Area			Mean	SD	% RSD
	Inj-1	Inj-2	Inj-3			
20	466623	466578	466876	46669.3	18.00925	0.00%
40	922004	922194	922278	922165	140.50266	0.01%
60	1396543	1396678	1396739	1396643	2356.43650	0.16%
80	1855112	1852347	1853245	1852398	1282.39008	0.06%
100	2303543	2303546	2303578	2303583	395.910764	0.01%

Precision study:

In order to verify the method's repeatability, replicate injections of 50µg/mL of the solution were made six times on the same day as the intraday precision study of DFZ. Table 3 shows that the percentage RSD was 0.77%.

Table 3. Data of precision

Injections	Area of Standard
1	2114324
2	2125467
3	2117985
4	2158543
5	2136214
6	2115543
Mean	2123562.2
SD	17124.7261
% RSD	0.77%

Accuracy study:

Three concentration levels (80, 100, and 120 µg/mL) of deflazacort reference standards were precisely weighed and added to a mixture of the tablet excipients. Samples were prepared in triplicate at each level, and table 4 shows the recovery percentage.

Table 4. Accuracy parameters for Deflazacort

Level	Set	Amount added (µg/ml)	Amount found(µg/ml)	% Recovery	Mean	SD	% RSD
80%	1	48	48.20	100.22	100.54	0.082	0.08%
	2	48	48.24	100.43			
	3	48	48.28	100.36			
100%	1	60	59.02	99.84	99.92	0.042	0.04%
	2	60	59.94	99.93			
	3	60	59.92	99.91			
120%	1	72	72.62	100.82	100.91	0.08	0.07%
	2	72	72.74	101			
	3	72	72.66	100.932			

Specificity study:

In order to conduct the specificity studies, a placebo comprising all excipients except the medication was prepared. A sample solution was made in accordance with that. The chromatographic run's lack of peaks was seen as a sign of specificity.

Robustness study:

Three factors from the optimum chromatographic conditions—flow rate, wavelength, and mobile phase composition—were changed to assess the method's robustness. According to statistical analysis, there was no discernible difference between the results of the trials where other parameters were introduced and the findings obtained using the analytical conditions set for the procedure. Table 5 demonstrates the method's robustness.

Table 5. Robustness parameters for Deflazacort

Change in parameters	Area of standard	Mean	SD	% RSD	Area of sample	% Assay	Absolute difference in % assay
Lower wavelength (265nm)	2851245 2833456 2834538	2835642	9331.247	0.3324	2824513	99.42%	0.62
Higher wavelength (267 nm)	2624467 2627354 2635683	2656892	8462.324	0.23	2625981	99.94%	0.09
Low flow rate (0.9 ml)	2975467 2969012 2972692	2973032	2834.583	0.09	2967892	99.77%	0.22
Higher flow rate (1.1 ml)	3105723 3104343 3094368	3094783	1864.54	0.608	3086023	99.70%	0.26
Lower pH (pH 3.1)	3162524 3165674 3213426	3168420	28407.53	0.88	3147945	99.00%	0.96
Higher pH (pH 3.3)	3005432 3004895 2992364	3010584	7644.658	0.24	2954598	98.48%	1.52

Ruggedness study:

Three replicate injections of standard and sample solutions of concentrations, produced and examined by a different analyst on three separate days during a week, were used to perform inter-day variations. Table 6 illustrates, the approach proved to be robust.

Table 6. Ruggedness parameters for Deflazacort

Sr. No.	Area of Analyst -1	Area of Analyst – 2
1	2114538	2119456
2	2115342	2134263
3	2118646	2116424
Mean	2118426.320	2146542.543
SD	1868.846202	14648.42892
% RSD	0.089%	0.66%
Mean	**2124268**	
SD	**18236.22**	
% RSD	**0.86%**	

Limit of detection and Limit of Quantitation:

The LOD and LOQ of Deflazacort were found to be 4.72 µg/ml and 14.34 µg/ml respectively.

Greenness of method study:

AGREE: Analytical Greenness Calculator version 0.5, a software program, was used to determine the analytical greenness score of the suggested approach, which came out to be 0.60 (Figure 6).

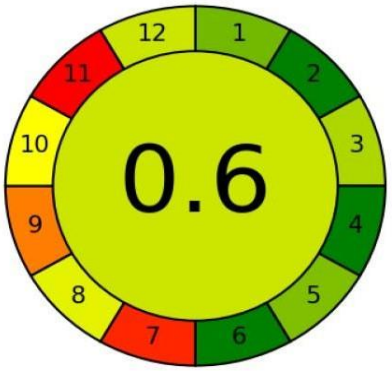

Fig. 6. Result of Greenness: AGREE analysis

4. CONCLUSION

A stability-indicating HPLC method for the estimation of DFZ in bulk drug and tablet dosage form was successfully developed and validated in accordance with the guidelines of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use. The method demonstrated excellent linearity, specificity, accuracy, precision and robustness over the studied concentration range. Stability indicating studies confirmed that all degradation products were well separated from the drug peak, indicating the stability-indicating capability of the method. The low % RSD values (<2.0) for repeatability and intermediate precision, along with low detection and quantitation limits, further confirmed the reliability and sensitivity of the method. In addition, the method was found to be rapid, cost-effective and suitable for routine quality control and stability testing of DFZ in bulk and finished pharmaceutical products.

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Conflict of Interests: There are no conflicts of interest.

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