

DEVELOPMENT AND VALIDATION OF DEFLAZACORT DRUG IN PHARMACEUTICAL DOSAGE FORM

ABSTRACT

A Simple fast and precise reversed phase high performance liquid chromatographic method is developed for the Deflazacort drug. Chromatographic separation was performed a C₁₈ column (250×4.6 mm) as a stationary phase with the mobile phase of Acetonitrile, methanol and water (90:5:5 v/v). Flow rate is 1.0 ml/min the detection of wave length is 230 nm. This method was validated for linearity, accuracy, precision, LOD & LOQ as per the guidelines of ICH. The percentage of recovering was 98.8. The developed method was applied successfully for determination of Deflazacort in pharmaceutical dosage form.

KEYWORDS: High performance liquid Chromatographic method, Deflazacort, ICH, Stationary Phase

INTRODUCTION

Deflazacort¹⁻¹⁰ (DFZ) is an anti inflammatory drug and it is used for the treatment of rheumatoid arthritices and asthma it is chemically known (11β,16β)-21-(acetyloxy)-11-hydroxy-2'-methyl-5'H-Pregna-1,4-dieno[17,16-d]oxazole-3,20-dione. Chemical formula is C₂₅H₃₁NO₆. The chemical structural formula is (Figure 1). In literature survey, that there are several methods of Deflazacort. This proposed method is simple and accurate for determination of Deflazacort in pharmaceutical dosage form.

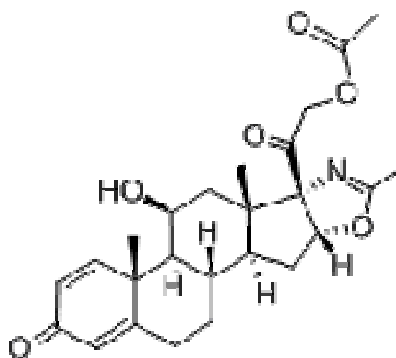


Figure1: Structural formula of Deflazacort

2.0 Experimental conditions:

2.1 Instrument: The high pressure liquid chromatographic system consisted of shimadzu HPLC model (VP series) containing LC-10 AT pump variable wave length programmable UV/Visible detector and rheodyne injector (7725 i) with 20 μ l fixed loop. Chromatographic analysis was performed using Intersil C-18 analytical column with 250 \times 4.6 mm.

2.2 Reagents and Materials: Methanol of HPLC grade, Acetonitrile and water is commercially available in the market was purchased from E Merck, Mumbai, India.

2.3 Chromatographic conditions: Chromatographic analysis was carried out at ambient temperature. Separation was achieved by gradient elution using mobile phase of Deflazacort drug is methanol, Acetonitrile and water (90:5:5 v/v). This mobile phase is filtered through 0.45 μ m nylon membrane. The injection volume was 20 μ l. The mobile phase flow rate is 1.0 ml/min. The analysis was carried out at 230 nm wave length. Chromatographic parameters and values are in (Table1).

Retention Time	6.0minutes
Column Length	25 cm
Theoretical Plates	3927 number
Tailing Factor	1.82

Table1: Chromatographic parameters and value

2.4 Preparation of Stock Solution: Stock Solution (10 μ g/ml) of DFZ standard drug accurately weighed 100 μ g and transferred in to 100 ml of volumetric flask containing 10 ml of mobile phase. The mixture solution was sonicated for 5 minutes and filtered through 0.45 μ m membrane the result solution concentration is 10 μ g/ml.

2.5. Preparation of sample solution: Sample solution was prepared from 0.2 ml of stock solution was taken in a 25 ml of volumetric flask and add 0.8 ml of mobile phase this mixture solution was sonicated for 3 minutes and filtered through 0.45 μ m membrane. Final concentration of resultant solution is 2 μ g/ml

3.0. Results and Discussion: In this work an analytical rp hplc¹¹⁻¹³ method for Deflazacort (DFZ) in a pharmaceutical formulation was developed and validated as per the guidelines of ICH. The UV spectra showed that the DFZ drug absorbs at 230 nm was selected as the detection wave length in liquid chromatography. Optomatimization of mobile phase was performed based on asymmetric factor and peak area obtained. Different mobile phases were tried for satisfactory separation, well resolved and good symmetrical peaks were and a Sharp Typical Chromatogram (Figure 2) obtained with the mobile phase of Acetonitrile, methanol and water in the ratio of

90:5:5 v/v respectively. The retention time of DFZ is 3.052 min and the number of theoretical plates is 9288. This indicates good base line.

HPLC REPORT

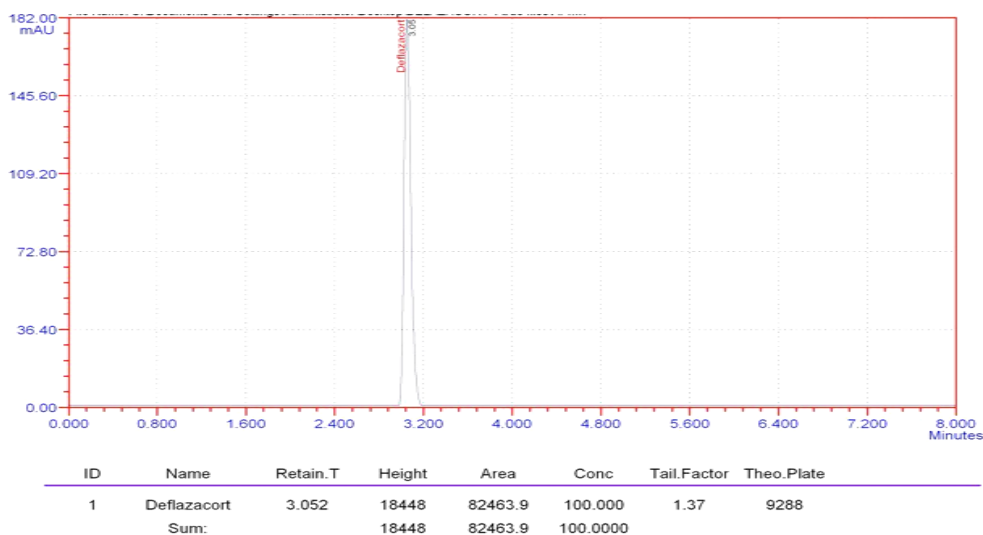


Figure 2: A Sharp Typical Chromatogram of Deflazacort.

3.1. Method Validation: The developed method was validated for linearity, LOD, LOQ, Precision, accuracy and robustness as stipulated by the ICH guidelines.¹⁴⁻¹⁶

3.2. Linearity: Linearity was evaluated by analysis of standard solution contains DFZ drug. Six standard concentration of DFZ ranging from 1 µg/ml to 5 µg/ml. A standard calibration curve of DFZ was constructed by plotting area versus concentration (Figure 3). The concentration and peak area of drug were subjected to regression analysis for calculating the slope, Intercept and correlation coefficient. The regression data are listed in (Table 2).

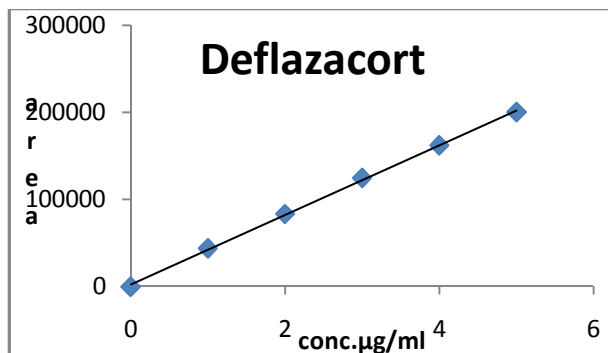


Figure 3: Calibration curve of Deflazacort

Table 2: Regression analysis of the calibration curve

Parameters	Values
Calibration range	1-5 µg/ml
Slope	39139.6
Intercept	5445.3
Correlation coefficient	0.999

3.3. Limit of Detection and Limit of Quantification: Limit of detection of Deflazacort drug is 35 ng and Limit of Quantification of Deflazacort is 125 ng.

3.4. Precision: The precision of the chromatographic analysis by measuring the repeatability (Intra- day precision) and the intermediate precision (Inter-day precision). The repeatability was evaluated by assay six samples at same concentration (2 µg/ml) on the same day and the intermediate precision was calculated on consecutive three days. The relative standard deviations (RSD) value was obtained less than 2 of each concentration.

3.5. Accuracy: The accuracy of the method evaluated by calculating recovery of DFZ. Sample solutions of DFZ drug with known concentration of reference standard. The recovery amount of DFZ was estimated by measuring the peak, area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times of the same concentration range and amount of DFZ was estimated. From the above estimation, percentage of DFZ drug recovery were calculated. The results of System suitability and validation parameters are given in (Table 3)

Parameters	Results
Theoretical plates (N)	9288
Retention (min)	8 minutes
LOD	35 ng
LOQ	125 ng
Accuracy (%)	98.84
RSD (%)	0.275

Table3: System suitability and validation parameters

CONCLUSIONS

The Developed method is simple, accurate, precise and specific assay for the analysis of Deflazacort in pharmaceutical dosage forms. This method was validation yields good results and presented good linearity, accuracy and precision of drug Deflazacort. The RSD values for all parameters were found to be less 2, which indicates the validity of method and results obtained by this method are in fair agreement. Finally this method can be used for better analysis and pharmaceutical formulations of Deflazacort.

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