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Research Article

Stability Indicating Rp-Hplc Method For The Estimation Of Drug In Marketed Formulation

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ABSTRACT

The aim and objective of the present work has been to develop new simple, sensitive and validated RP-HPLC method for the estimation of drugs in marketed formulation. Validation of developed Analytical methods according to ICH guidelines. Stress study was performed on Deflazacort and was found that it degrade sufficiently in acidic, alkaline and oxidative condition but less degradation was found in thermal and photolytic oxidation the % of degradation of active constituents was found to be 16.64%, 10.25 %and 8.67% respectively. The analysis was carried out on waters HPLC. It has 715 binary pumps, Rheodyne injector with a 20-microlitre loop, U.V. Vis. Detector, Thermo C-18 column (4.6 x 250mm, 5µ particle size) with data ace software. and the mobile Phase composition was water -methanol ratio 80:20 flow rate 1ml/min, methanol-water ratio 70:30 flow rate 1.2 ml/min, acetonitrile-water ratio 40:60 flow rate 1.2ml/ min and methanol - acetonitrile ratio 50:50 fl0w rate 1ml/min at room temperature and most suitable . The sample injection volume was 20 uL, and eluents of the isocratic elution mode were monitored at 241nm. The retention time was 5.25 min. The method was linear in the concentration range of 5-25 ug/ml with an 16.14 r2=0.999. The LOD and LOQ were found to 0.82ug/mL and 2.41ug/mL of Deflazacort.

Key words: Stability indicating, RP-HPLC, Method Validation, Deflazacort.

1. INTRODUCTION

Deflazacort chemically is a 1, 16β)-21-(acetyloxy)-11-hydroxy-2′-methyl-5′*H*-pregna-1,4-dieno[17,16-d]oxazole-3,20-dioneDeflazacort is anti inflammatory and a glucocorticoid used asaimmunosuppressant.[1] Deflazacort is a prodrug. Itspotency is around 70–90% that ofprednisone.Drug-interactions with liver enzyme inducers, are co-administered, e.g. rifampicin, rifabutin, carbamazepine, phenobarbitone, phenytoin, primidone and aminoglutethimide. For drugs, which inhibit liver enzymes, e.g. ketoconazole it may be possible to reduce the maintenance dose of deflazacort and diuretic drugs.[2]

RP-HPLC is a chromatography is a non-destructive procedure for resolving a multi-component mixture of trace, minor, or major constituents into its individual fractions. Different variations may be applied to solids, liquids, and gases. While chromatography may be applied both qualitatively and quantitatively, it is primarily a separation tool. Quantitative analysis can be carried out by measuring the area of the chromatographicpeak.[3]

Considerable advances have since been made and the method is used to separate colored as well as colorless substances. The column of calcium carbonate, used in Tswett's method, remains stationary and is therefore termed as the stationaryphase. The solution of vegetable extracts moves or flows

down the column and is therefore termed as the mobile phase. Chromatography may be regarded as a method of separation in which separation of solutes occur between a stationary phase and a mobile phase.[4]

MATERIALS AND METHODS

Analytical method development by HPLC[5]

Mobile Phase Selection:-

Initially to estimate Deflazacort number of mobile phase in different ratio were tried. Results were shown in Table no 3.1

Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Acetonitrile: methanol in the ratio of (50**:**50 v/v). The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0ml/min.

Selection of wavelength:-

100 mg of Deflazacort was weighed accurately and transferred to a 100 ml volumetric flask, and the volume was adjusted to the mark with the mobile Acetonitrile: methanol in the ratio of (50**:**50 v/v). From above solutions of 0.1 ml was transferred to 10 ml volumetric flasks, and make up the volume up to mark.

Resulting solution was scanned over UV range (200-400nm), maximum absorbance was found at Lambda max 242.0.

Selection of Separation Variable:-

Standard drug solution of Deflazacort was prepared in different mobile phase and chromatograph was recorded by using different column (5 and 10 µm) at different chromatographic condition like different flow rate and temperature. Considering the theoretical facts and after several trials separation variables were selected which were constant during whole experiment (Table no 3.2).

System SuitabilityParameters:-

Separation variables (Table no. 3.3) were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, three replicates of working standard of Deflazacort 10 µg/ml was injected separately. Peak report and column performance report were recorded for all chromatogram.

Preparation of Standard StockSolution:-

10mg of Deflazacort was weighed accurately and transferred to separate 10ml volumetric flask, and the volume was adjusted to the mark with the mobile phase Acetonitrile : methanol in the ratio of (50**:**50 v/v) to give a stock solution of 1000ppm.[6]

Preparation of Working StandardSolution:-

From stock solutions of Deflazacort 1 ml was taken and diluted up to 10 ml. from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 100 ml with mobile phase, gives standard drug solution of 5, 10, 15, 20, 25 µg/ ml concentration.

Preparation of the Calibration Curves of theDrug:-

Standard drug solutions were injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve. A typical chromatogram (figure 3.6) and the calibration curve are shown in (figure 3.5).

Analysis of tablet formulation:-

Assay of Tablet formulation:-

20 tablets were weighed and ground to a fine powder. Tablet powder equivalent to 10 mg Deflazacort was weighed and transferred to a 10 ml volumetric flask and volume was made up to 10 ml with Methanol to obtain concentration of 1000µg/ml. Resultant solution was filtered through Whatmann filter paper. 1 ml of filtrate was taken in 10 ml volumetric flask and volume was made up to 10 ml with diluents (Methanol) to obtain concentration of 100µg/ml. Further 1.0 ml of this solution was taken and diluted up to 10 ml obtain final concentration of 10µg/ml.

The amounts of Deflazacort in Tablet formulation was calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with Tablet formulation. Result is shown in (Table no. 3.7).

Validation

Linearity:-

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 5 to 25 µg/ ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure 3.8.1. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration. [7]

Accuracy&Precision:

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed (Table no. 3.8.2).

Repeatability

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis. (Table no.3.8.4.1). Standard dilutions

were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out.

Intermediate Precision

Analyst to Analyst

The intermediate precision expresses with in laboratories variation: different days, different analysts, different equipment etc. The standard dilution was prepared and three replicate of each dilution were analyzed by different analysts for all the developed methods. The statistical analysis method was carried out and the data is presented in (Table no3.8.4.2 -3.8.4.3).

Robustness:-

As per ICH norms, small, but deliberate variations, by altering the pH and / or concentration of the mobile phase were made to check the method capacity to remain unaffected. The effect of change in pH of mobile phase, flow rate, mobile phase ratio on the retention time, theoretical plates, area under curve and percentage content of deflazacort was studied. Results are shown in (Table no.3.8.5).

Forced degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were conducted on sofosbuvir powder and the analysis was carried out by HPLC with a U.V. detector. 20µl of each of forced degradation samples were injected.[8]

Acid degradation:

50 mg of deflazacort sample was taken into a 50 ml round bottom flask, 50 ml of 0.1 M HCl solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10µg/mlsubjected to HPLC and calculate the percentage degradation using calibration curve of deflazacort.

Alkalinehydrolysis:

50 mg of deflazacort sample was taken into a 50 ml round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of deflazacort.

Oxidativedegradation:

50 mg of deflazacort sample was taken into a 50 ml round bottom flask, 50 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of deflazacort.[9]

Thermal degradation:

50 mg of deflazacort sample was taken in to a petri dish and kept in oven at 50ºC for 4 weeks. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of deflazacort (table no 3.9).[10]

RESULTS AND DISCUSSION

Mobile Phase Selection:-

Table3.1: Mobile Phase selection

Mobile phase	Ratio	Flow rate	Conclusion
Water: Methanol	80:20	1.0ml/min	No peak found
Methanol : water	70:30	1.2ml/min	Peak Broadening
Acetonitrile: Water	40:60	1.2ml/min	No Peak found
MeOH: ACN: Water	70:20:10	1ml/min	Tailing
MeOH: CAN	50:50	1 ml/min	Most Suitable

Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Methanol**:** Acetonitrile in the ratio of 50**:**50. The mobile phase was filtered through 0.45 filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min

Selection of SeparationVariable:-

Table3.2 Selection of Separation Variable

Variable	Condition
Column	
Dimension.	250mm x 4.60mm
Particle Size	5□

Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	
Methanol	50
CAN	50
Flow rate	1ml/min
Temperature	Room temp.
Sample Size	20 □l
Detection wavelength	240.0 nm
Retention time Deflazacort	5.254 ±0.3 min

System SuitabilityParameters:-

Table3.3 Result of System Suitability Parameters for Deflazacort

System suitability Parameter	RT	AUC	Theoretical plates	Tailing factor
Rep-1	10.792	2575.221	3045	1.01
Rep-2	10.791	2576.332	3047	1.04
Rep-3	10.792	2575.221	3045	1.02
Mean	10.791	2575.591	3045.667	1.023
S.D.	0.0005	0.52373	0.942809	0.012472

Linearity and Calibration Curve:-

Table 3.4 Result of Linearity ofDeflazacort

Std. Conc. µg/ml	0	5	10	15	20	25
1	0	2575.221	5021.223	7434.225	10034.33	12523.2
2	0	2576.332	5023.225	7433.223	10035.44	12567.7
3	0	2575.221	5021.334	7435.221	10036.23	12565.3
Mean	0.00	2575.591	5021.927	7434.223	10035.33	12552.1
SD	0.000	0.641436	1.125182	0.999002	0.955588	25.0469
%RSD	0.000	0.024904	0.022405	0.013438	0.009522	0.19954

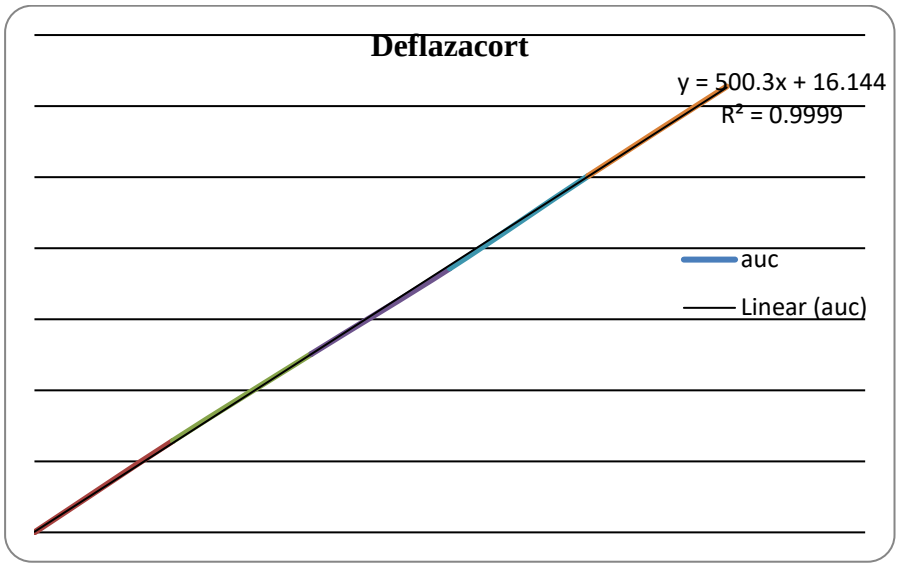

Figure 3.5:Calibration Graph of Deflazacort

Regression Equation

Y= mx +c,

Y= AUC

m= slope = 500.3

X= Conc. in µg/ml

c= Intercept = 16.14

r^2= 0.999

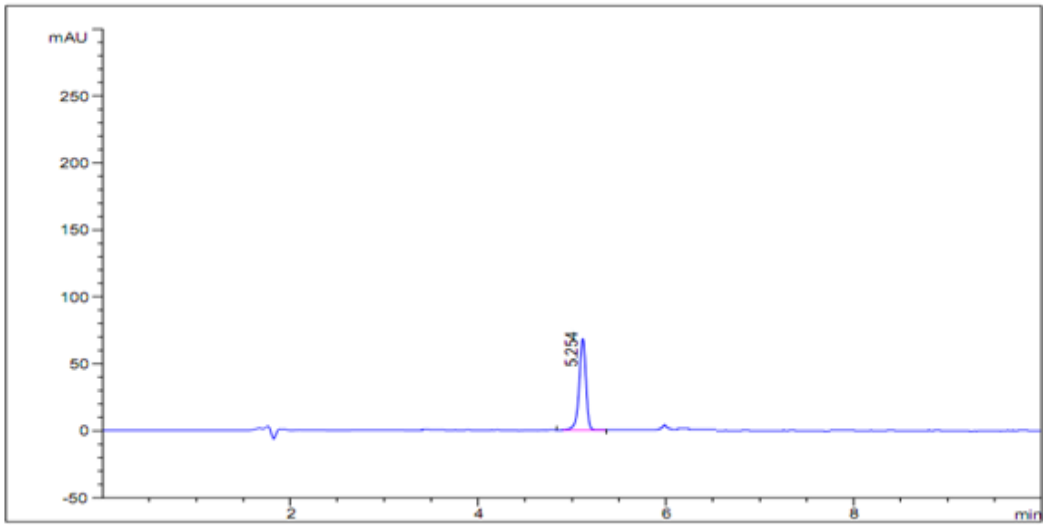

Figure 3.6 Chromatogram of Deflazacort

Assay of TabletFormulation:-

Table 3.7 Result of Analysis for Deflazacort in Tablet Formulation

Std Conc. µg/ml	DEFLAZACORT		
	10	15	20
Rep-1	10.37	15.50	20.80
Rep-2	10.25	14.90	20.94
Rep-3	10.12	15.20	20.50
% found *			
Rep-1	103.7	103.33	104.0
Rep-2	102.5	99.33	104.7
Rep-3	101.2	101.33	107.5
Mean	102.46	100.55	105.4
SD	1.250	1.072	1.852
% RSD	1.220	1.066	1.757

***Each reading is mean reading of three batch of formulation**

Validation of DevelopedMethod:-

Linearity:-

Table3.8.1 Response Ration Data for Linearity of Deflazacort

Replicates	Concentration (□g/ml)	Mean AUC	Response Ratio
Rep-1	5	2575.591	515.1182
Rep-2	10	5021.927	502.1927

Rep-3	15	7434.223	495.614
Rep-4	20	10035.34	501.767
Rep-5	25	12552.11	502.084
Mean			503.3552
S.D.			6.384107
R.S.D.			1.2683

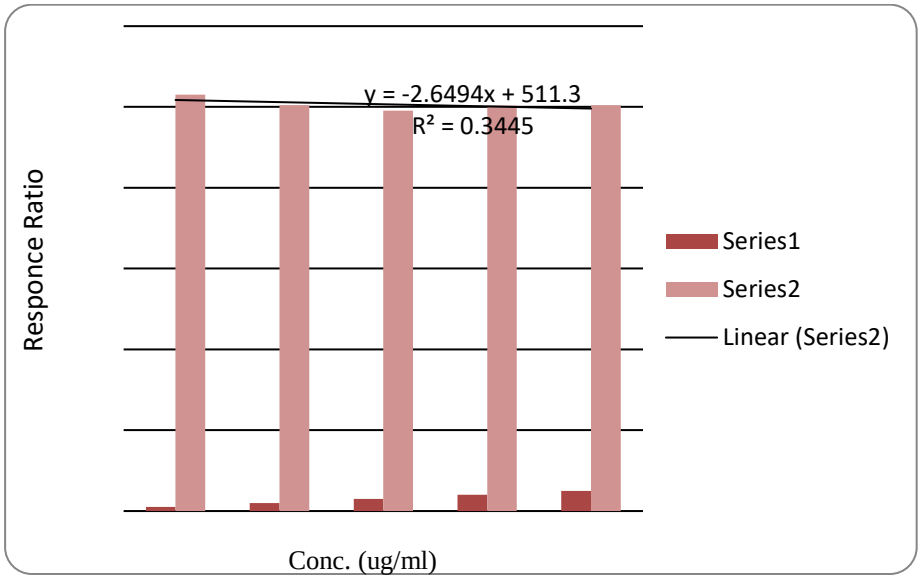

Figure3.8.1.1 Response Ratio Curve of Deflazacort

Result ofAccuarcy:-

Table3.8.2 Recovery Studies of Formulation

Level of Recovery (%)	**80**	**100**	**120**
	Deflazacort	**Deflazacort**	**Deflazacort**
Amount present	10	10	10
(mg)	10	10	10
	10	10	10
Amount of Std.	8	10	12
added	8	10	12
(mg)	8	10	12
Amount	8.1	9.9	12.0
recovered	8.0	10.1	12.1
(mg)	7.9	10.2	12.0
	101.25	99	100
% Recovery	100	101	100.8
	98.75	102	100

Table 3.8.3 Statistical Validation of Recovery Studies

Level of Recovery (%)	**Drug**	**% Recovery**	**Standard Deviation***	**% RSD**
80	**Deflazacort**	100.00	0.816	1.020
100	**Deflazacort**	100.66	1.247	1.234
120	**Deflazacort**	100.26	1.144	0.950

***Denotes average of three determinations**

Result of Precision:-

Repeatability:-

Table3.8.4 .1Results of analysis Data of Tablet Formulation

Drug	Label claim	Amount found*	Label claim (%)	S.D.	% RSD
Deflazacort	6 mg	5.98 mg	99.66	0.210	0.225

Intermediate Precision- (Inter-day and Intra-dayPrecision):-

Table 3.8.4.2. Intra-day and Inter-dayPrecision

Intra-day Precision		**Inter-day Precision**	
	% Label Claim		**% Label Claim**
	Deflazacort		**Deflazacort**
After 1hr	99.90	First day	98.00
After 2hr	99.50	Second day	97.50
After 3hr	99.20	Third day	97.00
After 4hr	99.00		
After 5hr	98.90		
After 6hr	98.30		
Mean	99.13	**Mean**	97.50
SD	0.546	**SD**	0.500
% RSD	0.551	**% RSD**	0.512

Analyst toAnalyst:-

Table3.8.4.3: Analyst to Analyst

Stability Indicating Rp-Hplc Method For The Estimation Of Drug In Marketed Formulation

Analyst	**Label claim**	**Amount found***	**Label claim (%)**	**S.D.**	**% RSD**
1	6 mg	5.98	99.60	0.110	0.158
2	6 mg	5.95	99.00	0.225	0.159

Result ofRobustness:-

Table3.8.5 Result of Robustness of Formulation

Compound	**% RSD in Normal**	**Changed Condition n= 6**	
Temperature		- 5 ºC	+ 5 ºC
Deflazacort	0.34	0.67	0.49
Flow rate		(-10%)	(+10%)
Deflazacort	0.49	0.70	0.97
Mobile phase ratio		- 2 %	+ 2 %
Deflazacort	0.34	0.88	0.25

Results of Forced Degradationstudies

Table3.9 Results of Forced degradation studies of Deflazacort

Stress conditions	**Drug recovered (%)**	**Drug decomposed (%)**

Standard drug	99.85	0
Acidic hydrolysis	83.26	16.64
Alkaline hydrolysis	89.65	10.25
Oxidative degradation	91.23	8.67

CONCLUSION

The result obtained shows the developed method to be precise, simple, rapid and accurate. Thus these can be used for routine analysis of Deflazacort in bulk drug and tablet dosage form. It was thus, concluded that the proposed methods is new, simple, accurate, safe, free form pollution, precise and can be successfully employed in the routine analysis.

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