RESEARCH ARTICLE

Design and Development of Analytical Method for Deflazacort Estimation: A Robust HPLC Approach

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ABSTRACT

The data show that the RP-HPLC method can accurately determine the amount of deflazacort (DEF) in compound. Validation of procedure followed ICH standards. The wavelength of estimation for deflazacort was 266 nm. Linearity was found to be 20 to 100 µg/mL for deflazacort. The %recovery for deflazacort was found to be 80 to 120%. Intraday precision of deflazacort was found to be 0.069 to 0.168% RSD. Interday precision found to be 0.0156 and 0.176% RSD .

Keywords: RP-HPLC, Deflazacort, Method development.

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INTRODUCTION

For the body to absorb a drug from an oral dosage form, the drug must be released from the product, dissolve or be solubilized under physiological conditions, and pass through the gastrointestinal tract.[1] Methyloxazoline deflazacort is a form of the steroid prednisolone. In addition to rheumatoid arthritis, it is also used to treat nephritic syndrome, organ transplant rejection, and juvenile chronic arthritis.[2] This chemical is poorly soluble in water and has a low oral bioavailability (about 70%). Despite its low mineral corticoid action, it was promoted as a glucocorticoid that is gentler on bones than others.[3] This research describes the development, evaluation of a straightforward dissolution test for deflazacort (DEF) tablets of 30 mg. Optimising the experiment for solubility and stability. However, The primary focus was a technical evaluation of how well 30 mg DEF tablets in our nation disintegrate.

MATERIAL AND METHODS

Each and every one of the compounds is of analytical and HPLC quality.

Method Development

UV spectroscopic method selection of solvent

Diluents: Water (HPLC Grade)

Preparation of deflazacort standard solution

To make standard solution 100 mg of DEF was weighed out, placed in a 100 mL volumetric flask. Total of 40 mL of water was then added to flask, and contents were shaken and sonicated to dissolve standard. More water was added to the diluted solution (20–100 µg/mL) to get the desired standard solution concentration.

Determination of λ_{max}

A UV-visible spectrophotometer scanned standard solution of DEF in wavelength of 200 to 800 nr. UV Spectra is shown in Figure 1.[4]

Identification by IR spectroscopy

Each 20 mg of DEF API and KBr was mixed properly then carefully triturated in a mortar pestle make thin plate, place in IR chamber and IR Spectrum was scanned as shown in Figure 2 and interpretation in Table 1.[5]

Method Development by RP-HPLC

Preparation of 50 mM phosphate buffer solution

Dissolved 6.8 g potassium dihydrogen phosphate in 1000 mL of water. Adjusted pH 3.2 ± 00.05 with orthophosphoric acid (OPA).

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Mobile phase

Prepared mixture of phosphate buffer pH 6.2 and acetonitrile in ratio of 50:50 v/v mixed well and degassed it.

Preparation of standard stock solution

For stock solution, 100 mg of the deflazacort working standard was weighed out into a 100 mL volumetric flask, 40 mL of water was added, flask was shaken and sonicated to dissolve contents, remaining volume was filled with water, and solution was filtered through a 0.45 micron membrane filter. (Concentration of Stock Solution: 1000 ppm).

Preparation of standard solution

The remaining 3 mL stock solution was transferred to 50 mL volumetric flask, diluted to the appropriate concentration with designated diluent, and thoroughly blended. (Conc. of Standard Solution: 60 ppm).

Preparation of sample solution

Total 20 tablets were carefully measured and ground to a powder in a mortar and pestle. A volumetric flask containing 100 mL was filled with deflazacort powder, which was carefully weighed to be 100 mg. Filtered via a 0.45-micron membrane filter after adding 40 mL of water, shaking for 5 minutes, and then sonicating for 30 minutes. From above solution, pipetted out 3 mL of filtrate to 50 mL volumetric flask, make up the volume with water, shake, collected sample after discarding the first few mL of solution.

Preparation of placebo solution

Accurately weighed powder of 100 mg was transferred to 100 mLvolumetric flask. Filtered via a 0.45 micron membrane filter after adding 40 mL of water, shaking for 5 minutes, and then sonicating for 30 minutes. After discarding the first few mL of solution, I transferred 3 mL of filtrate to a 50 mL volumetric flask, made up volume with water, shook, and collected the sample.[6]

Preparation solution for marketed tablets

Ten tablets were precisely measured and ground into a powder in a trituration. A volumetric flask containing 100 mL was filled with deflazacort powder, which was carefully weighed to be 100 mg. Filtered via a 0.45 micron membrane filter after adding 40 mL of water, shaking for 5 minutes, then sonicating for 30 minutes. The first few mL of the aforementioned solution were thrown away, and then 2 mL of filtrate were pipetted into a 50 mL volumetric flask, before the volume was filled up to the necessary level with water and the sample was collected.

System suitability

The sensitivity and consistency of the chromatographic system are evaluated to determine if the system suitability test meets the pharmacopeia requirements. Data was collected from a single blank (diluent) injection and five replicate standard solution injections into the chromatographic apparatus. System suitability studies' chromatogram is depicted in Figure 2, with findings tabulated in Table 2.[7]

Specificity

In cases when deflazacort was completely separated from the pill placebo, the HPLC approach demonstrated its specificity. The HPLC system was injected with blank (Diluent), placebo, standard, and sample solutions. There was no cross-contamination from the blank or placebo at the highest retention time for deflazacort. Figure 3 displays the chromatogram from the specificity studies, and Table 3 displays the outcomes.

Linearity

Injecting 20 to 100 mg of deflazacort in triplicate yielded linear results for this study. Slope, y-intercept, and correlation coefficient (r2-value) were calculated after a graph was drawn with concentration (in µg/mL) on the X-axis and peak area on the Y-axis, as shown in Figure 4 and 5. Chromatogram of linearity studies are shown in figures and results are shown in Tables 3 and 4. The Calibration curve is given in Figure 6.

Precision

Intraday Precision

Intraday precision study was performed by analyzing standard solution at 3 different concentrations 40, 60, and 80 µg/mL on the same day but in a different time. System suitability data for precision was shown in Table 5. The results are shown in Table 6.

Interday Precision

Interday precision study was performed by analyzing standard solution at 3 different concentrations of 40, 60, and 80 µg/mL on three consecutive days. Results are shown in Tables 7 and 8.

Ruggedness

Method's robustness was confirmed by analyzing a minimum of three standard solutions utilising a variety of analysts, instruments, and columns throughout multiple days. Roughness standard deviation (%RSD) was computed. Table 9 displays the findings.

Accuracy (Recovery)

Deflazacort's (60 ppm) accuracy was primarily assessed at 80, 100, and 120% of the working concentration. Every stage was made in triplicate for safety. Deflazacort concentration was determined using the standard test procedure. The percentage recovery was determined by adding the original amount to the one that was discovered. Tables 10 and 11 display the outcomes.

Formula of %Recovery

$$\%\text{Recovery} = \text{amount found} / \text{amount added}$$

Formula for amount added

$$\text{Amount added} = \text{weight taken}/\text{volume-1} \times \text{volume-2}/\text{volume-3} \times 1000$$

Formula for amount found

$$\text{Amount found} = \text{area of test}/\text{area of standard} \times \text{standard concentration}$$

Robustness

The robustness of approach was tested by systematically varying a number of experimental conditions, including the following: column temperature, ±5°C; flow rate, ±0.2 mL; min; wavelength, ±3 nm; mobile phase composition, organic component, ±5%; and so on. The approach underwent a change so that its impact could be gauged. Percent RSD and Assay were used to assess the obtained data for each individual case. You can see the outcomes in Table 12.

LoD and LoQ

Under specified experimental conditions, LoD is lowest concentration in a sample that can be identified. LoQ is the minimum detectable concentration of an analyte in a sample. signal-to-noise ratios of 3:1 and 10:1 were used to calculate these two values:

LOD=3.3× Standard deviation /Slop

LOQ=10× Standard deviation /Slop

Application of method to the marketed tablets

The discovered technique has been used to quantify deflazacort in commercially available tablet form. Here, we injected the system with a single blank, five replicates of the standard solution, and two copies of the sample solution. %assay of Deflazacort samples was calculated. The chromatogram of %assay of marketed tablets studies are shown in Figure 7 (s and t).

The method's robustness was validated by ensuring that the locations of peaks of interest were unaffected by minor adjustments to the analytical settings.

Accuracy determined by recovery study found entity recovery for 80 to 120% was in range of 98.72–101.64% and mean recovery was found to be 99.33–101.27%. [8-11]

RESULT AND DISCUSSION

Determination of λ_{max} (Selection of Wavelength)

Maximum absorbance (λ_{max}) was found to be 266 nm.

Identification by IR Spectroscopy

Each 20 mg of Deflazacort API and KBr was mixed properly then carefully triturated in a mortar pestle make thin plate, it was placed in IR chamber and IR Spectrum was scanned. Results showed specific IR Ranges.

System Suitability

When measuring the peak area response to deflazacort, there should not be more than a 2.0% relative standard variation between five independent injections of the standard solution. No more than a tailing factor of 2.0 should be used for the deflazacort peak in the typical solution. The theoretical plates number (N) must be greater than 2000 for the deflazacort peak in the standard solution. Based on the evidence, the system's suitability was determined to meet or exceed the acceptance criteria.[12]

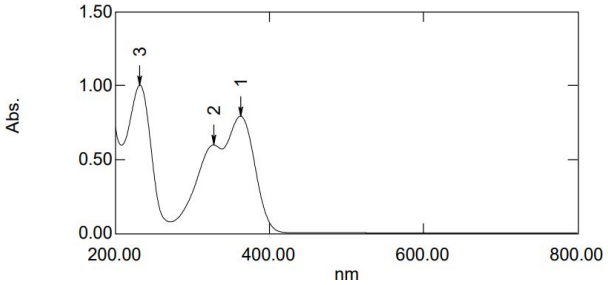

Figure 1: UV spectra of deflazacort

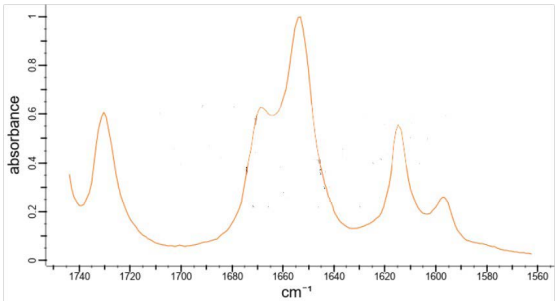

Figure 2: IR Spectra of Deflazacort

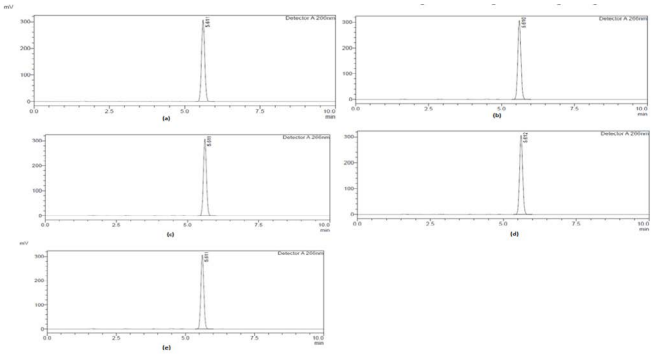

Figure 3: Chromatogram of STD for system suitability

Table 1: IR Range Value of Deflazacort API

Functional Group	*IR Range (cm^{-1}) Observed Frequency*
O-H Stretching	3600 – 2500
N-H Stretching	3500 – 3350
C=O Stretching	1725 – 1700
C-O Bending	1300 – 1000
C-N Bending	1350 – 1000
C=C Stretching	1600 – 1475

Specificity

All results should have the same retention rate. The retention time peak for deflazacort should not be observed in either the blank (Diluent) or the placebo samples. The results show that there is no blank/placebo interference at the deflazacort medication peak retention time. Standard peak is pure at the working concentration level.[13]

Linearity

System suitability criteria should be fulfilled. Response should be linear. The co-relation coefficient (r^2) should > 0.999.

Table 2: Data of system suitability test

S. No.	*Area*
01	2300228
02	2300252
03	2297940
04	2296584
05	2296946
Mean	2298450
Standard Deviation	1842.81
%RSD	0.08%
Retention Time(min)	5.611
Theoretical plates number(N)	1614

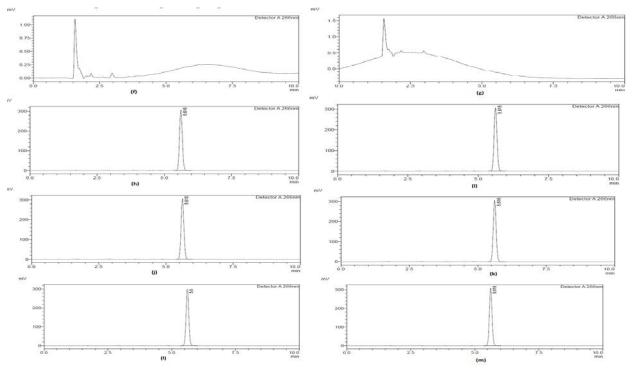

Figure 4: Chromatogram of (f)Blank, (g) Placebo, (h) STD 1, (i) STD2, (j) STD 3, (k) STD 4, (l) STD 5 and (m) sample for specificity

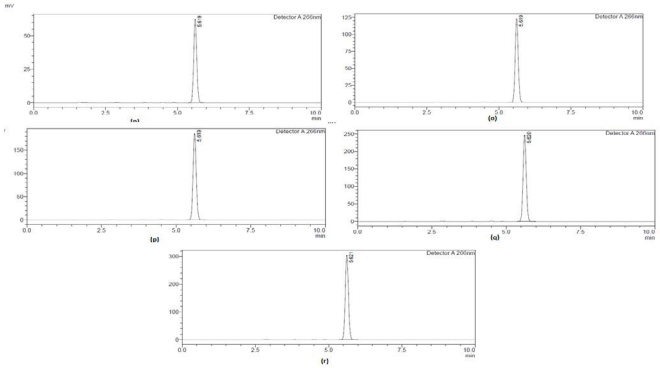

Figure 5: Chromatogram of Linearity at (n) 20 µg/mL (o) 40 µg/mL (p) 60 µg/mL (q) 80 µg/mL (r) 100 µg/mL.

The data shows that the system suitability criteria fulfilled. According to the results, the reaction was discovered to be linear. The observed value of r=0.99996 indicates that the technique is linear.[14]

Precision

Intraday Precision

System suitability criteria should be fulfilled. A standard solution should have RSD of no more than 2.0%. Based on evidence, the system meets all requirements. The results demonstrate that the percent RSD for the reference solution falls within the allowable range, indicating the accuracy of the approach.

Table 3: System suitability data for linearity

S. No.	*Area*
01	467779
02	923865
03	1395196
04	1855817
05	2303383
Mean	1389208
Standard Deviation	20605.89
%RSD	1.01%

Table 4: Result of linearity

Parameter	*Result*
Linearity Range(µg/mL)	20–100
Slope	23016
Intercept	8260
Correlation coefficient	0.9999

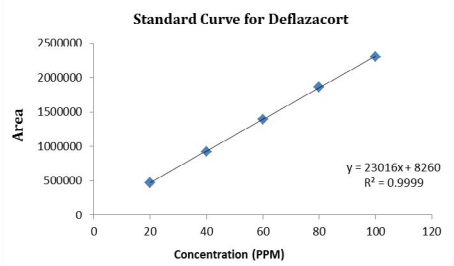

Figure 6: Calibration curve of deflazacort

Table 5: System suitability data for intraday precision

Sr. No.	*Area*
01	2296636
02	2292287
03	2293364
04	2293489
05	2296897
Mean	2294535
Standard Deviation	2163.26
%RSD	0.08%

Table 6: Data of intraday precision

Conc. (µg/mL)	*Area*			*Mean*	*SD*	*%RSD*
	10.10 am	*3.14 pm*	*6.44 pm*			
Lower (40 ppm)	924005	923866	923724	923865	140.502	0.0152
Middle (60 ppm)	1397437	1395412	1392739	1395196	2356.43	0.168
Upper (80 ppm)	1855199	1854960	1857291	1855817	1282.39	0.069

Interday Precision

System suitability criteria should be fulfilled. The %RSD for standard solution should > 2.0%.

Table 7: System suitability data for interday precision

S.No.	*Area*
01	2297185
02	2293597
03	2293624
04	2292257
05	2296137
Mean	2294560
SD	2030.076

Table 8: Data of interday precision

Conc. (µg/mL)	*Area*			*Mean*	*SD*	*%RSD*
	10.10 am	*3.14 pm*	*6.44 pm*			
Lower (40 ppm)	924015	923896	923727	923879	144.721	0.0156
Middle (60 ppm)	1397534	1395496	1392634	1395221	2461.52	0.176
Upper (80 ppm)	1855205	1854913	1857248	1855789	1272.22	0.0685

Table 9: Ruggedness data

Sr. No.	*Analyst 1 Area*	*Analyst 2 Area*
1	2297185	2297185
2	2293597	2293597
3	2293624	2293624
4	2292257	2292257
5	2296137	2296137
Mean	2294560	2294560
%RSD	0.0889	0.0889

Ruggedness

The ruggedness of study is as follows (Table 9).

Both the %RSD for the reference solution and the total %RSD should be less than 2%. The RSD value of % is consistent with an analytical approach that is sufficiently robust.

Accuracy (Recovery)

Result of recovery is shown in Table 10:

The requirements for a suitable system must be met. Individual recoveries from 80 to 120% should fall between the 97.0 to 103.0% range, whereas the mean recovery for this range should be between 98.0 and 102.0%. The requirements for a suitable system must be met. The data demonstrates that the mean and individual recoveries from 80 to 120% fall within the 98.0 to 102.0% range.

Table 10: Test preparation data of accuracy

Level	*Set*	*Area*
80%	1	1118795
	2	1120169
	3	1118599
100%	1	1392941
	2	1392014
	3	1391881
120%	1	1685690
	2	1688178
	3	1687126

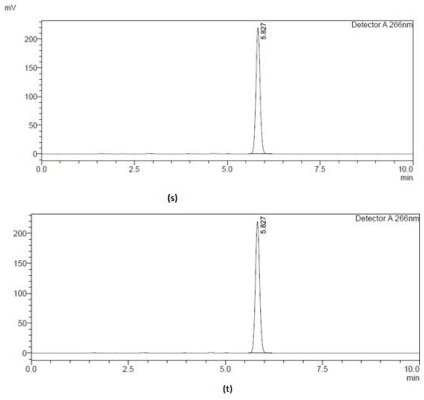

Figure 7: Chromatogram of STD (s) and sample (t) for %assay

Robustness

System suitability criteria should be fulfilled.

LoD and LoQ

SD and slope are taken from system suitability and linearity respectively. LoD and LoQ of DEF were found to be 1.36 and 4.13 µg/mL.

Application of Method to the Marketed Tablets

The %assay should be in range of 90 to 110%. System suitability criteria should be fulfilled. %assay of marketed

Table 11: Data of % recovery

Level	*Set*	*Quantity added (µg/mL)*	*Quantity found (µg/mL)*	*%Recovery*	*Mean*	*SD*	*%RSD*
80%	1	48	48.79	101.64	101.27	0.324	0.32
	2	48	48.41	100.85			
	3	48	48.64	101.33			
100%	1	60	60.09	100.15	99.54	0.498	0.50
	2	60	59.73	99.55			
	3	60	59.39	98.93			
120%	1	72	71.81	99.73	99.33	0.438	0.44
	2	72	71.67	99.54			
	3	72	71.08	98.72			

Table 12: Data of robustness

Change in Parameters	*Area of Standard*	*Mean*	*SD*	*%RSD*
Lower wavelength (266 nm)	1687125	1687222	95.04	0.005
	1687315			
	1687238			
Higher wavelength (264 nm)	1687015	1690498	5774.87	0.341
	1697164			
	1698725			
Low Flow Rate (0.9mL/min)	1684826	1692369	7023.88	0.415
	1693548			
	1698725			

formulation was found to be 99.06%. This shows that the results are closely matched to the label claim of tablets. Thus method is useful for content evaluation.[12-15]

CONCLUSION

The data show that the RP-HPLC method can be used to accurately determine the amount of deflazacort in the compound. ICH standards were used to validate the procedure.

The recovery testing results were positive, demonstrating that the tablet dosage form is free from excipient interference. The medicine is stable for several hours because of how easy and cheap the mobile phase is to make and how well the process works. The created approach also has a relatively quick run and retention time, hovering about 5.62 ± 0.25 minutes.

The present study demonstrates that the approach is reproducible; furthermore, the RP-HPLC method for analysing deflazacort is accurate, exact, specific, reproducible, sensitive, and cost effective.

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