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Validated Spectrophotometric Methods for Determination of Deflazacort in Tablet Dosage Form

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Abstract

Three simple, sensitive, precise and economical UV- spectrophotometric methods have been developed for the determination of deflazacort in tablet formulation. Method A is simple and direct UV spectrophotometric method and is based on determination of deflazacort in methanol at 243 nm. Linearity was obtained in the concentration range of 2 – 30 µg/ml. Method B is first order derivative spectrophotometric method and involved estimation of deflazacort in methanol using the first-order derivative technique at 228 nm as maxima and 267 nm as minima. Calibration curve was prepared by plotting the absorbance difference between maxima and minima versus concentration. Linearity was obtained in the concentration range of 2- 30 µg/ml. Method C is area under curve (AUC) method. The method involved calculation of integrated value of absorbance with respect to the wavelength between two selected wavelength 233 nm and 252 nm, respectively. Linearity was obtained in the concentration range of 2 - 30 µg/ml. These methods were successfully applied to pharmaceutical formulations because no interferences from tablet excipients were found. The suitability of these methods for the quantitative determination of deflazacort was proved by validation. The proposed methods were found to be simple, sensitive, accurate, precise, rapid and economical for the routine quality control application of daflazacort in pharmaceutical formulations.

Keywords: *Area under curve (AUC), first order derivative spectrophotometric, validation.*

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Introduction

The deflazacort is chemically, (11b, 21-Dihydroxy-2'-methyl-5'bH-pregna-1, 4-dieno [17, 16-d] oxazole-3,20 dione 21-acetate) (Figure 1), is an oxazoline derivative of prednisolone with anti-inflammatory and immunosuppressive activity. It acts by preventing the release of certain chemicals producing immune and allergic responses, resulting in inflammation. It also decreases the numbers of white blood cells circulating in the blood. This, along with the decrease in inflammatory chemicals, can prevent the rejection of organ transplants, as it prevents the body from attacking foreign tissue. It is useful for the treatment of certain types of leukemia, Uveitis, Nephrotic syndrome, Rheumatoid Arthritis & Juvenile Chronic Arthritis, Pemphigus, Asthma and other airway diseases [1]. This drug is not official in any pharmacopoeia. Several methods have been reported for the analysis of deflazacort in pharmaceutical dosage form as well as in the biological samples like

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serum and urine, *i.e*. high-performance liquid chromatography (HPLC)[2-7], liquid chromatography-mass spectrometry (LC/MS)[8], LC-MS/MS with ESI[9]. Literature survey does not reveal any simple spectroscopic method for determination of deflazacort. The present manuscript describes simple and sensitive spectroscopic procedures for the determination of deflazacort in pharmaceutical dosage forms.

Materials and Methods

Apparatus

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software (UV Probe version 2.10). A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India)

Reagents and Materials

Deflazacort was supplied by Zydus Cadila Healthcare, Changodar, India as a gift sample. The commercially available tablets of deflazacort were procured from local market labeled to contain 30 mg deflazacort. Methanol (AR Grade, S. D. Fine Chemicals Lts., Mumbai, India) and Whatman filter paper no. 41 (Whatman International Ltd., England) were used in the study.

Preparation of standard stock solution

Deflazacort was accurately weighed (equivalent to 100 mg deflazacort) in 100 ml volumetric flask and diluent (50 ml) was added, shaken till dissolved and volume was made up to the mark with diluent and mixed well (1 mg/ml).

Preparation of working standard solution

Deflazacort working standard solution was prepared by diluting standard stock solution (10.0 ml) to 100 ml with diluent to produce required concentration (100 µg/ml).

Preparation of sample solution

Twenty tablets were weighed and powdered. The quantity of the powder (equivalent to 10 mg of deflazacort) was transferred to a 100 ml volumetric flask, ultrasonicated for 30 minutes with methanol (50 ml) to dissolve the drug as completely as possible. Further diluted and make up the volume using diluent. The solution was filtered through a Whatman filter paper No. 41. The resulting solution (5 ml) was diluted to 50 ml with diluent (10 µg/ml).

Development of the methods

Method A: Zero order spectroscopic method

The solutions were scanned in the range from 400-200 nm, and the peak was observed and gives maximum absorbance at 243 nm. So, the wavelength selected for the analysis of the drug was 243 nm. The drug followed the Beer's-Lamberts law in the range of 2-30 µg/ml.

Method B: First order derivative spectroscopic method

The standard drug solution was diluted so as to get the final concentration in the range of 2-30 µg/ml and scanned in the first order derivative spectra. The first order derivative spectra at n = 1, showed a maxima and minima at 228 and 267 nm respectively. The amplitude of absorbance was measured at 228 nm (peak maxima) and at 267 nm (peak minima) and was plotted against concentration to give calibration curve, and regression equation was calculated. The amplitude was linear in the concentration range of 2-30 µg/ml.

Method C: Area under curve (AUC) method

The AUC method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelength 233 nm and 252 nm. Area calculation processing item

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calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. Suitable dilutions of standard stock solution (100 µg/ml) of the drug were prepared and scanned in the spectrum mode from the wavelength range 400-200 nm and the calibration curve was plotted.

Validation of the proposed methods

The proposed method is validated according to the International Conference on Harmonization (ICH) guidelines [10].

Linearity

Calibration curves for deflazacort were plotted over a concentration range of 2 – 30 µg/ml for all the methods. Accurately measured standard working solutions of deflazacort (0.2, 0.3, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml) were transferred to a series of 10 ml volumetric flasks and diluted up to the mark by diluent. Absorbance was measured at a wavelength 243 nm and was plotted absorbance versus concentration to give calibration curve for method A and from this curve regression equation was calculated. First derivative curves of different concentration solutions were obtained, which shows maxima at 228 nm and minima at 267 nm. The calibration curve of amplitude against concentration of the drug showed linearity for method B. Area of the zero order spectra's were calculated and the calibration curve of area against concentration was plotted for method C.

Accuracy (% Recovery)

The accuracy of the method was performed by calculating recovery of deflazacort by the standard addition method. Known amounts of standard solutions of deflazacort were added at 50, 100 and 150% levels to prequantified sample solutions of deflazacort (10 µg/ml). At each level of the amount 3 determinations were performed. The amount of deflazacort was estimated by applying obtained values to regression equation.

Method precision (% Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions (n = 6) of deflazacort (20 µg/ml) without changing the parameters for the methods. The repeatability was expressed in terms of relative standard deviation (% RSD).

Intermediate precision (Reproducibility)

The intraday and interday precision of the proposed method was performed by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 8 different concentrations of standard solutions of deflazacort (2, 3, 5, 10, 15, 20, 25 and 30 µg/ml). The results were reported in terms of relative standard deviation (% RSD).

Limit of detection and Limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by visually or by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using the following equations designated by International Conference on Harmonization (ICH) guidelines [10].

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response

S = slope of the calibration curve.

Estimation of deflazacort in pharmaceutical formulation

Pharmaceutical formulation of deflazacort was purchased from local pharmacy. Sample solutions were prepared as described earlier. Then this solution was analyzed by three methods. The nominal content of the tablets was determined

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either from the calibration curve or using the regression equation.

Results and Discussion

Method A is simple UV spectrophotometric method. In this method the simple UV spectrum of deflazacort in methanol was obtained which exhibits absorption maxima (λ max) at 243 nm (Figure 1). The calibration curve was linear in concentration range of 2 - 30 µg/ml. Method B is the 1st derivative spectrophotometric method. Maxima occur at 228 nm and minima at 267 nm (Figure 2). The calibration curve was linear in concentration range of 2 – 30 µg/ml. Method C is the area under curve method. In this method the simple UV spectrum of daflazacort in methanol was obtained and area between two selected wavelengths measured. Area measured between 233 nm and minima at 252 nm (Figure 3). The calibration curve was linear in concentration range of 2 – 30 µg/ml.

The proposed methods were found to be simple, sensitive, rapid, accurate, precise and economic for the routine analysis of daflazacort in pharmaceutical formulations. The linearity ranges was found to be 2-30 µg/ml for all the method. Accuracy was determined by calculating the recovery, and the mean was determined (Table 1). Precision was calculated as repeatability (relative standard deviation) and intra and inter day variation (% RSD) for daflazacort. LOD values for deflazacort were found to be 0.30, 0.40 and 0.55 µg/ml for method A, B and C, respectively. LOQ values for deflazacort were found to be 0.90, 1.20 and 1.65 µg/ml for method A, B and C, respectively indicates sensitivity of the proposed methods. The methods were successfully used to determine the amounts of daflazacort present in tablets. The results obtained are in good agreement with the corresponding labeled amount (Table 2). Characteristic parameters and summary of validation parameters for all the three methods are given in Table 3. By observing the validation parameters, the methods were found to be sensitive, accurate and precise. Hence the methods can be employed for the routine analysis of daflazacort in tablet formulations.

Conclusion

The proposed methods were compared using ANOVA test and the calculated value of F is found to be 2.03. Here $F_{calculated} = 2.03 < F_{tabulated} = 3.682$ (P= 0.05). So, it can be concluded that there is no significant difference among the above three methods. The proposed spectrophotometric methods were found to be, simple, sensitive, accurate and precise for determination of deflazacort in tablet dosage form. Hence it can be conveniently adopted for routine quality analysis of the drug in tablets.

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TABLE 1: RECOVERY DATA FOR PROPOSED METHODS

Method	Level	Amount taken (µg/ml)	Amount added (%)	% Recovery ± S. D. (n = 3)
A	I	10	50	99.54 + 1.14
	II	10	100	98.91 + 0.76
	III	10	150	99.84 + 0.50
B	I	10	50	99.21 + 0.68
	II	10	100	99.61 + 0.90
	III	10	150	99.61 + 1.04
C	I	10	50	98.85 + 0.69
	II	10	100	98.89 + 0.51
	III	10	150	99.20 + 0.46

Method A is the simple and direct UV spectrophotometric method, Method B is the first derivative method and Method C is Area under Curve method. n is number of determination and S. D. is standard deviation.

TABLE 2: RESULTS OF ANALYSIS OF TABLET FORMULATIONS

Tablet	Label claim (mg)	Parameters	% amount found (n = 3)		
			Method A	Method B	Method C
Brand A	30	Mean	98.91	98.92	99.18
		S. D.	0.41	0.58	0.62
Brand B	30	Mean	98.84	98.63	98.68
		S. D.	0.51	0.48	0.43

n is number of replicate and S. D. is standard deviation.

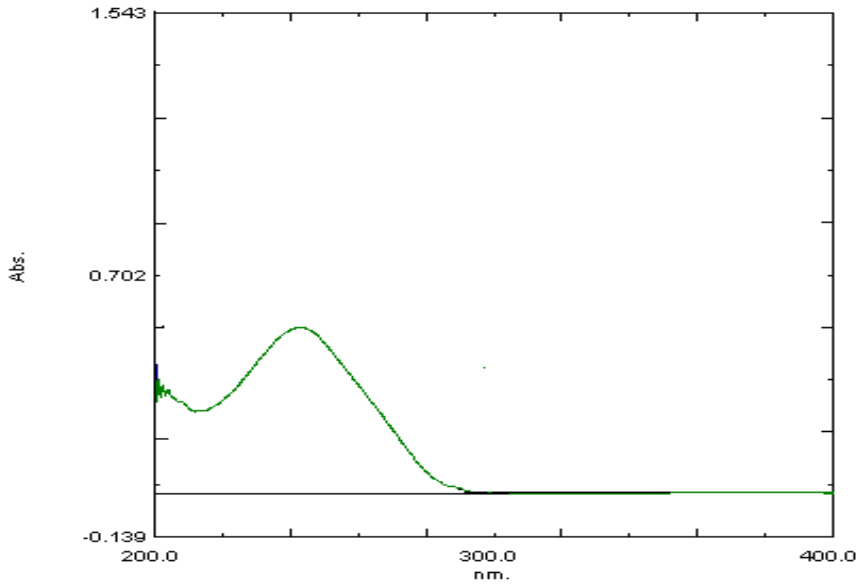

FIG. 1. SIMPLE UV SPECTRUM OF DEFLAZACORT IN METHANOL (METHOD A).

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TABLE 3: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETERS FOR THE PROPOSED METHODS

Parameters	Method A	Method B	Method C
λ_{max} (nm) & λ_{min} (nm)	λ_{max} = 243	λ_{max} = 228 λ_{min} = 267	233-252
Beer's-Lambert's range (µg/ml)	2-30	2-30	2-30
Regression equation y =mx + c	y = 0.035x+0.0078	y = 0.0017x-0.0002	y = 0.0603x+0.0063
Slope (m)	0.035	0.0017	0.0603
Intercept (c)	0.0078	-0.0002	0.0063
Correlation coefficient (r^2)	0.9998	0.9996	0.9994
Recovery + S. D.[a] (n = 3)	99.43 + 0.83	100.10 + 0.90	98.98 + 0.51
Repeatability (% RSD[b], n = 6)	0.4714	0.6839	0.5506
Intermediate precision (% RSD) Interday (n = 3) Intraday (n = 3)	0.6442 - 1.5390 0.1964 - 1.4085	0.7794 - 1.5503 0.4144 - 1.5503	0.4596 -1.7718 0.3077 - 1.6129
LOD[c] (µg/ml)	0.30	0.40	0.55
LOQ[d] (µg/ml)	0.90	1.20	1.65

[a]S. D. = Standard deviation. [b]RSD = Relative standard deviation. [c]LOD = Limit of detection. [d]LOQ = Limit of quantification. n is number of determinations.

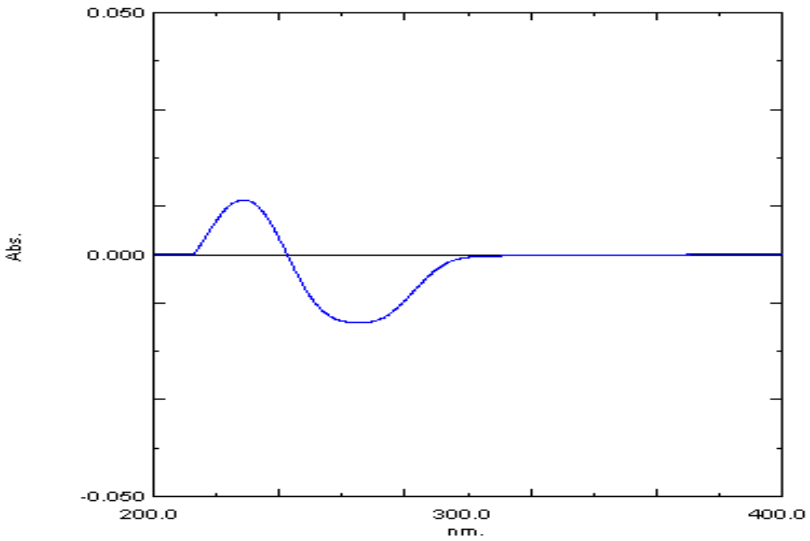

FIG. 2. FIRST DERIVATIVE SPECTRUM OF DEFLAZACORT IN METHANOL (METHOD B).

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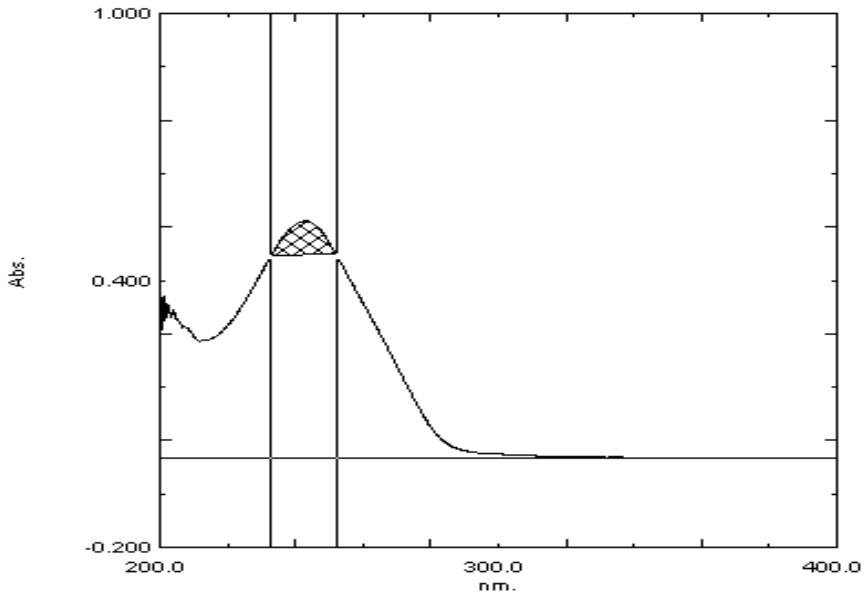

FIG. 3. AREA UNDER CURVE OF DEFLAZACORT IN METHANOL (METHOD C).

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