Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Suppl 5, 2011

Research Article

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF DEFLAZACORT IN PHARMACEUTICAL DOSAGE FORM

SATISH A PATEL*

*** Department of Pharmaceutical Quality Assurance, Shree S.K. Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva, Mehsana 382711, Gujarat, India. Email: satishpatel_77@yahoo.com**

Received: 13 July 2011, Revised and Accepted: 3 Nov 2011

ABSTRACT

Three simple, sensitive, accurate, precise and economical spectrophotometric methods have been developed for the estimation of deflazacort in pharmaceutical dosage form. Method A is simple 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, rapid and economical for the routine quality control application in pharmaceutical formulations.

Keywords: Deflazacort, UV spectrophotometric method, First order derivative spectrophotometric method, Area under curve (AUC) method, Tablet formulation

INTRODUCTION

Deflazacort (Fig.1) is chemically (11b, 21-Dihydroxy-2'-methyl-5'bH-pregna-1, 4-dieno [17,16-d]oxazole-3,20 dione 21-acetate), 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].

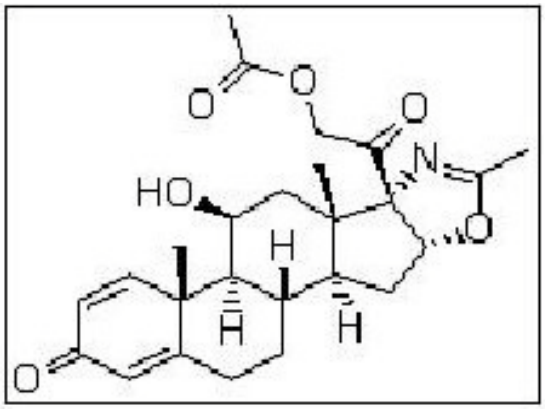

Fig. 1: Structure of deflazacort

The therapeutic importance of this drug has prompted the development of many methods for its assay. 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 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 reveals zero order[11], first order[12] and AUC[13] spectrophotometric methods for determination of other drug. Literature survey does not reveal any single UV spectroscopic method for determination of deflazacort using zero order, first order derivative spectroscopy and AUC method. Hence an attempt has been made to develop new UV methods for its estimation in pharmaceutical formulations with good accuracy, simplicity, precision and economy. Further, the study would embark upon the validation of the developed methodology as per the ICH guidelines[14].

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. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India)

Materials

Deflazacort bulk powder was kindly gifted by Zydus Cadila Healthcare, Changodar, India. The commercially available tablets of deflazacort were procured from local market labeled content 30 mg deflazacort. Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) and Whatman filter paper no. 41 (Whatman International Ltd., England) were used in the study.

Preparation of standard stock solution and working standard solution

An accurately weighed quantity of deflazacort (10 mg) was transferred to a 100 ml volumetric flask, dissolved, sonicated and diluted to the mark with methanol to obtain standard stock solution having concentration of deflazacort 100 µg/ml. Working standard solution (50 µg/ml) was prepared by appropriate dilution of stock solution in methanol.

Development of the methods

For selection of wavelengths, standard solution of deflazacort 10 µg/ml was prepared from working standard solution (50 µg/ml) in methanol for method A, B and C. The simple UV, first derivative and AUC spectra of solution was recorded in the scanning range of 200-400 nm.

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. Aliquots of

working standard solution (0.4 – 6.0 ml) were transferred into a series of 10 ml volumetric flask and diluted upto mark with methanol. The absorbences of the resulting solutions were measured at 243 nm against methanol as blank. Calibration curve was prepared by plotting absorbance versus concentration. The calibration curve was linear in concentration range of 2 – 30 µg/ml.

Method B is the 1st derivative spectrophotometric method. In this method the simple UV spectrum of deflazacort in methanol was obtained and derivatised to 1st order (n=1). Maxima occur at 228 nm and minima at 267 nm. Aliquots of working standard solution (0.4 – 6.0 ml) were transferred into a series of 10 ml volumetric flask and diluted upto mark with methanol. First derivative spectra were obtained which shows absorbance maxima at 228 nm and minima at 267 nm. A calibration curve was prepared by plotting the absorbance difference between maxima and minima versus concentration. The calibration curve was linear in concentration range of 2 – 30 µg/ml.

Method C is the AUC (Area Under Curve) method[11,13], which 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 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. Aliquots of working standard solution (0.4 – 6.0 ml) were transferred into a series of 10 ml volumetric flask, diluted upto mark with methanol and scanned in the spectrum mode from the wavelength range 200-400 nm. A calibration curve was prepared by plotting the area versus concentration. The calibration curve was linear in concentration range of 2 – 30 µg/ml.

Validation of the proposed methods

The methods were validated with respect to linearity, accuracy, precision (method precision, intermediate precision), limit of detection and limit of quantification according to the ICH guidelines[14].

Linearity (Calibration curve)

A calibration curve was plotted over concentration range of 2 – 30 µg/ml of deflazacort for method A, B and C. Accurately measured standard working solutions of deflazacort (0.4 – 6.0 ml) were transferred into a series of 10 ml volumetric flask and diluted upto mark with methanol. The absorbences of the resulting solutions were measured at 243 nm and was plotted versus concentration to obtain calibration curve and regression equation was calculated (Method A). First derivative curves of these solutions (Method B) were obtained, which shows maxima and minima at 228 and 267 nm, respectively. Area of the zero order spectra's were calculated between two selected wavelength 233 nm and 252 nm and the calibration curve of area against concentration was plotted (Method C).

Accuracy (% recovery)

The accuracy of the methods 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 deflazacort sample solutions of 10 µg/ml. The amount of deflazacort was estimated by applying obtained values to the respective regression equations.

Method precision (% repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of 10 µg/ml deflazacort standard solution (n = 6) without changing the parameters for the method. The repeatability was expressed in terms of relative standard deviation (% RSD).

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed methods were 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 (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines[14].

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

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

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Determination of deflazacort in pharmaceutical formulation (tablets)

Twenty tablets were accurately weighed and average weight was determined. The tablets were powdered in glass mortar. 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 and the volume was adjusted up to the mark with methanol. The solution was filtered through a Whatman filter paper No. 41. The resulting solution (5 ml) was diluted to 50 ml with methanol (10 µg/ml). Then this solution was analyzed by above three methods. The amount of deflazacort present in sample solution was determined by fitting the responses into the respective regression equations for deflazacort in all the methods.

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 (Fig. 2).

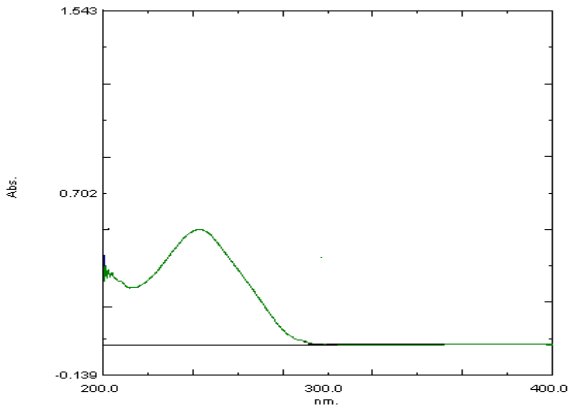

Fig. 2: Zero order spectrum of deflazacort in methanol (Method A)

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 (Fig 3).

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 deflazacort in methanol was obtained and area between two selected wavelengths measured. Area measured between 233 nm and 252 nm (Fig 4). 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 deflazacort in pharmaceutical formulations. The linearity ranges was found to be 2-30 µg/ml for all the methods. Accuracy was determined by calculating the recovery, and the mean was determined (Table 1).

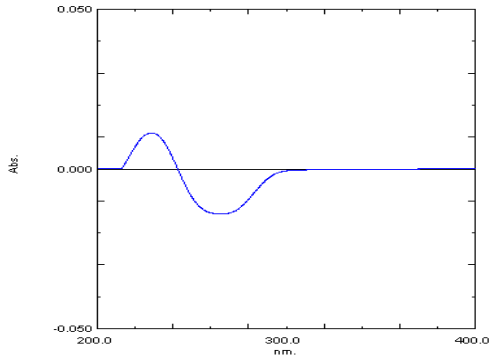

Fig. 3: First order derivative spectrum of deflazacort in methanol (n=1) (Method B)

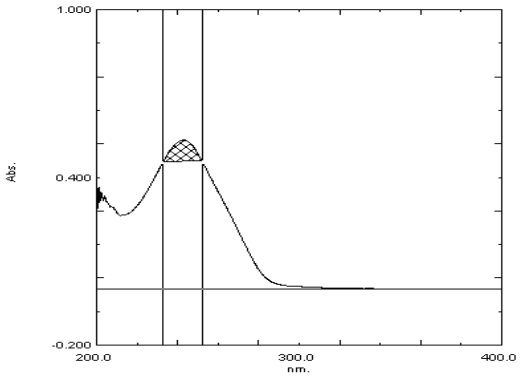

Fig. 4: AUC of deflazacort in methanol (Method C)

The methods were successfully used to determine the amounts of deflazacort present in tablets. The results obtained are in good agreement with the corresponding labeled amount (Table 2).

Characteristic parameters for regression equation and correlation are given in Table 3. Precision was calculated as repeatability (% RSD) and intra and inter day variation (% RSD) for deflazacort. The LOD and LOQ for deflazacort were found to be 0.30 and 0.90, 0.40 and 1.20, 0.55 and 1.65 µg/ml for method A, B and C, respectively indicates sensitivity of the proposed methods. By observing the validation parameters, the methods were found to be sensitive, accurate and precise (Table 3). Hence the methods can be employed for the routine analysis of deflazacort tablet formulations.

Table 1: Recovery data for proposed methods

Method	Level	Amount taken (µg/ml)	Amount added (%)	% Recovery ± S.D. (n = 3)
A	1	10	50	99.54 ± 1.14
	2	10	100	98.91 ± 0.76
	3	10	150	99.84 ± 0.50
B	1	10	50	99.21 ± 0.68
	2	10	100	99.61 ± 0.90
	3	10	150	99.61 ± 1.04
C	1	10	50	98.85 ± 0.69
	2	10	100	98.89 ± 0.51
	3	10	150	99.20 ± 0.46

Method A is the simple UV 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

Method	Tablet	Label Claim (mg)	Amount of drug estimated (mg/tab)	% Label Claim estimated (Mean ± S.D.) (n = 3)
A	T1	30	29.67	98.91 ± 0.41
	T2	30	29.65	98.84 ± 0.51
B	T1	30	29.68	98.92 ± 0.58
	T2	30	29.60	98.63 ± 0.48
C	T1	30	29.75	99.18 ± 0.62
	T2	30	29.60	98.68 ± 0.43

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

Table 3: Regression analysis data and summary of validation parameters for proposed methods

Parameters	Method A	Method B	Method C
Absorption maxima	243	228	233
Absorption minima	-	267	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
Repeatability (% RSD[a], n=6)	0.38	0.44	0.40
Precision, (% RSD[a]) (n = 3)	0.64-1.54	0.77-1.55	0.45-1.77
Interday	0.19-1.40	0.41-1.55	0.30-1.61
Intraday			
LOD[b] (µg/ml)	0.30	0.40	0.55
LOQ[c] (µg/ml)	0.90	1.20	1.65

[a]RSD = Relative standard deviation. [b]LOD = Limit of detection. [c]LOQ = Limit of quantification

CONCLUSION

The results of the analysis of pharmaceutical formulation by the proposed methods are highly reproducible and reliable and are in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of daflazacort. The methods can be used for the routine analysis of the daflazacort in tablet dosage form.

ACKNOWLEDGEMENT

The author is thankful to Zydus Cadila Healthcare, Changodar, Gujarat, India for providing gift sample of deflazacort for research work. The author is highly thankful to Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva, Gujarat, India for providing all the facilities to carry out the proposed work.

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