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Development and validation of UV spectrophotometric method for estimation of Deflazacort in bulk drug and Pharmaceutical formulation

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Abstract

An Uv spectrophotometric method for the quantitative determination of Deflazacort, having a highly potent anti-inflammatory and immunosuppressive action, in tablets was developed in present work. The parameters linearity, accuracy, precision, limit of detection limit of quantitation, range were studied according to International Conference on Harmonization guidelines. UV spectroscopic determination was carried out at an absorption maximum of 244 nm nm using methanol as solvent. Results of the analyses were validated statistically and by recovery studies. The proposed method is simple, rapid, precise and accurate and can be used for the reliable quantitation of deflazacort in pharmaceutical formulation. In the UV spectroscopic method linearity over the concentration range of deflazacort was found to be 1-9 ug/ml with a correlation coefficient 0.8979.

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Deflazacort, UV spectrophotometric method, linearity, Anti-inflammatory, immunosuppressive.

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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].

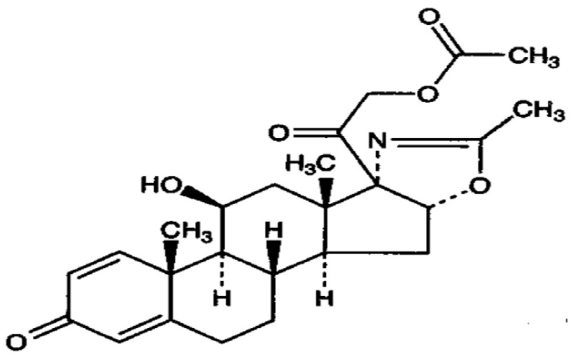

Fig: 1- Structure of deflozacort

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 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:

CHEMICALS AND REAGENTS:

Methanol was used throughout UVspectrophotometric method development and validation. Deflazacort bulk powder was kindly gifted by Next wave Pharma, India. The commercially available tablets of three different of deflazacort were procured from local market labeled content 50 mg deflazacort. Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) and Whatman filter paper no. 1 (Whatman International Ltd., England) were used in the study.

INSTRUMENTATION:

A shimadzu model 1800 (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).

PREPARATION OF STANDARD STOCK SOLUTION:

Deflazacort was accurately weighed (equivalent to 100 mg deflazacort) in 100 ml volumetric flask and diluent (100 ml) was added, shaken till dissolved and volume was made up to the mark with diluent and mixed well (1 mg/ml).From that solution 1ml was taken and dissolved upto 100 ml and that will give 10ug/ml concentration of solution.

PREPARATION OF WORKING STANDARD SOLUTION:

Deflazacort working standard solution was prepared by diluting standard stock solution to produce the concentration range of (1-9) ug/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 [10].

LINEARITY AND RANGE:

The linearity was determined by analyzing 9 independent levels of calibration curve in the range of 1-9µg/ml. Absorbance of each solution against methanol was recorded at 244nm The calibration curve of absorbance vs. concentration was plotted and correlation co-efficient and regression line equation for Deflazacort were determined.

PRECISION:

METHOD PRECISION (% REPEATABILITY):

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of 8 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 8different concentrations of standard solutions of deflazacort (2, 5 ,8 µg/ml). The results were reported in terms of relative standard deviation (% RSD).

SYSTEM PRECISION:

The precision of the system was checked by repeated scanning and measurement of the absorbance of 2 µg/ml, 5ug//ml, 8ug/ml deflazacort standard solution (n = 6) without changing the parameters for the method. The repeatability was expressed in terms of relative standard deviation (% RSD).

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 80%, 100% and 120% levels to pre quantified deflazacort sample solutions of 8 µg/ml. At each level of the amount 3 determinations were performed. The amount of deflazacort was estimated by applying obtained values to the respective regression equations and % recoveries were computed.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ):

The LOD and LOQ were estimated from the set of 3 calibration curves used to determine method linearity.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

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

DETERMINATION OF DEFLAZACORT IN PHARMACEUTICAL FORMULATION (TABLETS):

Twenty tablets of each brand were accurately weighed and average weight was determined. The tablets were powdered in glass mortar. A weight of the powder equivalent to 100 mg DFZ was transferred to a 100 ml volumetric flask containing 50 ml methanol and the mixture was sonicated for 30 min then diluted to 100 ml with methanol (1000 ug/ml). The solution was filtered and 1 ml of filtered solution was diluted hundredfold to furnish a concentration of 10µg/ml. From that solution the working sample dilute solution has been prepared.

RESULT AND DISCUSSION:

In experiment simple UV spectrophotometric instrument was used. From this 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 1-9 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 1-9 µg/ml for this 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 deflazacort. LOD value for deflazacort was found to be 0.90 ug/ml and LOQ value is 2.83 µg/ml respectively indicates sensitivity of the proposed method. The method was successfully used to determine the amounts of deflazacort present in tablets. The results obtained are in good agreement with the corresponding labelled amount (Table 2). 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 deflazacort in tablet formulations. Regression analysis data and summary of validation Parameters values for the proposed method is given on (Table 3).

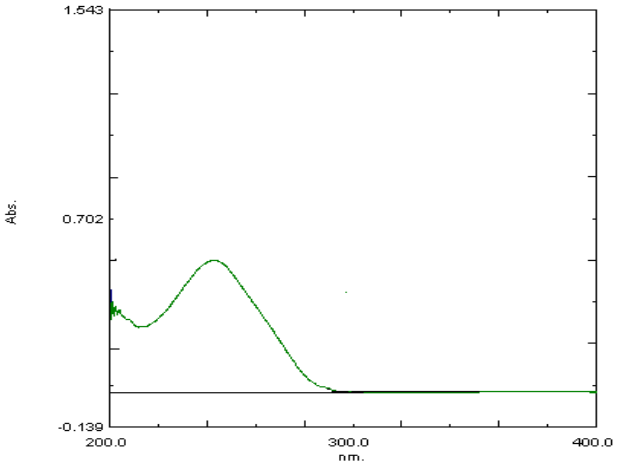

FIG. 1: UV SPECTRUM OF DEFLAZACORT IN METHANOL

Table 1: Recovery data of proposed method

	LEVEL	AMOUNT TAKEN(ug/ml)	AMOUNT ADDED	%RECOVERY±S.D
Method employed	I	8	80	99.35+0.14
	II	8	100	98.31+1.02
	III	8	120	99.21+0.34

TABLE 2: RESULTS OF ANALYSIS OF TABLET FORMULATIONS:

Tablet	Level Claim	Parameters	Amount Found
Brand A	50 mg	Mean	98.21
		S.D	0.52
Brand B	50 mg	Mean	98.45
		S.D	0.72
Brand C	50 mg	Mean	98.53
		S.D	0.93

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

Parameters	Value
λ_{max}	243 nm
Beer's-Lambert's range (µg/ml)	(1-9) ug/ml
Regression equation y =mx + c	Y=0.0132x+0.5743
Slope (m)	0.0132
Intercept (c)	0.5743
Correlation coefficient (r^2)	0.8979
Recovery + S. D. (n = 3)	99.41 + 0.13
LOD (µg/ml)	0.90
LOQ (µg/ml)	2.83
Intermediate Precision(%RSD) Interday (n = 3) Intraday (n = 3)	 0.7652 - 1.6549 0.1987 - 1.5043

CONCLUSION:

The proposed spectrophotometric method was 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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