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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DEFLAZACORT USING UV SPECTROPHOTOMETRIC.

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Abstract: An UV spectrophotometric method for the quantitative determination of Deflazacort, having a highly potent anti-inflammatory and immunosuppressive action. The parameters linearity, accuracy, precision, limit of the detection limit of quantitation and range were studied according to International Conference on Harmonization guidelines. UV spectroscopic determination was carried out at an absorption maximum of 243 nm using methanol as solvent. The 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 bulk. In the UV spectroscopic method, linearity over the concentration range of deflazacort was found to be 4 - 20 µg/ml.

Key Word: - Deflazacort, UV spectrophotometric, Methanol.

INTRODUCTION:

Deflazacort is supplied as a crystalline soiled (11β, 16β)-21-(Acetyloxy)-11-hydroxy-2′-methyl-5′H-pregna-1, 4-dieno [17, 16-d] oxazole-3, 20-dione, as shown in Figure 1, is a prednisolone oxazoline derivative having anti-inflammatory and immunosuppressive action. It works by blocking the production of particular molecules that cause immunological and allergic reactions, which cause inflammation. It also reduces the number of white blood cells in the bloodstream. This, together with a reduction in inflammatory molecules, can help to avoid organ transplant rejection by preventing the body from fighting foreign tissue. It can be used to treat some forms of leukemia, Uveitis, Nephrotic syndrome, Rheumatoid Arthritis and Juvenile Chronic Arthritis, Pemphigus, Asthma, and other airway illnesses. This medication is not listed in any pharmacopeia. A review of the literature reveals no straightforward spectroscopic approach for determining deflazacort. The current paper presents easy and sensitive spectroscopic approaches for determining deflazacort.

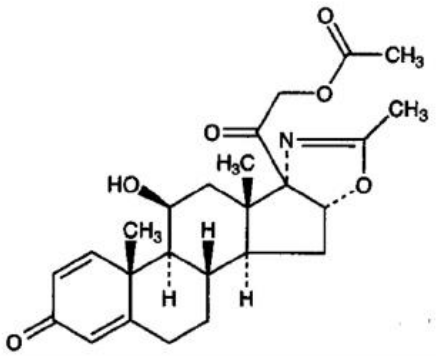

Figure 1: - STRUCTURE OF DEFLAZACORT.

CHEMICALS AND REAGENTS:

Methanol (HPLC Grade) was used throughout the UV spectrophotometric method development and validation. Deflazacort bulk powder was kindly gifted by, Dr. Nitin Deshmukh, Head, Glenmark plant, India.

INSTRUMENTATION:

To measure absorbance, a Shimadzu model 1800 (Japan) double beam UV/Visible spectrophotometer with a spectral width of 2 nm, wavelength precision of 0.5 nm, and a pair of 10 mm matched quartz cells was employed. The UV-Probe system software generated the spectra automatically.

SELECTION OF DETECTION WAVELENGTH:
The drug solution was scanned over the range of 200 – 400 nm.

PREPARATION OF STANDARD PLOT OF DEFLAZACORT:
Deflazacort was weighed 100mg and transferred into a 100ml volumetric flask and then 20ml methanol was added, shaken till dissolved, and volume was made up to the mark with methanol. From the solution, 10ml was withdrawn and dissolved up to 100ml volumetric flask with methanol. Pipette out 0.4, 0.8, 1.2, 1.6, and 2 ml and transfer into separate 10ml volumetric flasks and makeup with methanol to get solution of concentration of 4, 8, 12, 16, and 20 µg/ml. The absorbance was determined at 243nm.

PREPARATION OF STANDARD STOCK SOLUTION:
Deflazacort was accurately weighed 100mg and added into a 100ml volumetric flask and methanol was added, shaken till dissolved, and volume was made up to the mark with solvent (methanol) and mixed well (1000ppm). From the solution, 10ml was withdrawn and dissolved up to 100ml and which will give a 100ppm concentration of the solution.

PREPARATION OF WORKING STANDARD SOLUTION:
Deflazacort working standard solution was prepared by diluting standard stock solution to produce the concentration range of 4 - 20 µg/ml.

VALIDATION OF THE PROPOSED METHOD
According to the ICH recommendations, the techniques have been validated for linearity, accuracy, precision (method precision, intermediate precision), limit of detection, and limit of quantification.

LINEARITY AND RANGE:
The linearity was determined by analyzing 6 separate calibration curve levels ranging from 4 - 20 µg/ml. At 243nm, the absorbance of each solution against methanol was measured. The absorbance vs. concentration calibration curve was plotted, and the correlation coefficient and regression line equation for Deflazacort were determined.

PRECISION:
METHOD PRECISION (% REPEATABILITY):
The precision of the instrument was tested by repeatedly scanning and measuring the absorbance of 12ppm deflazacort reference solution (n = 3) without altering the procedure parameters. The repeatability was stated in terms of relative standard deviation (% RSD).

INTERMEDIATE PRECISION (REPRODUCIBILITY):
The intraday and interday precision of the proposed methods was determined by assessing the corresponding responses three times on the same day and three times on different days during a one-week period of 12 µg/ml concentrations of standard deflazacort solutions. The results were expressed as a percentage of the standard deviation (% RSD).

ACCURACY (% RECOVERY):
The accuracy of the methods was tested by estimating deflazacort recovery using the usual addition method. To pre-quantified deflazacort sample solutions of 12 µg/ml, known amounts of standard deflazacort solutions were added at 80%, 100%, and 120% levels. Three determinations were made at each level of the amount. The quantity of deflazacort was calculated by plugging the acquired values into the corresponding regression models and calculating the percentage recoveries.

DETECTION OF LIMIT:
The LOD and LOQ were calculated using the set of three calibration curves that were 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.

RESULT AND DISCUSSION
SELECTION OF DETECTION WAVELENGTH:
In the experiment, a basic UV spectrophotometric device was employed. This yielded a simple UV spectrum of deflazacort in methanol, with absorption maxima (λ max) at 243 nm as shown in Figure 2.

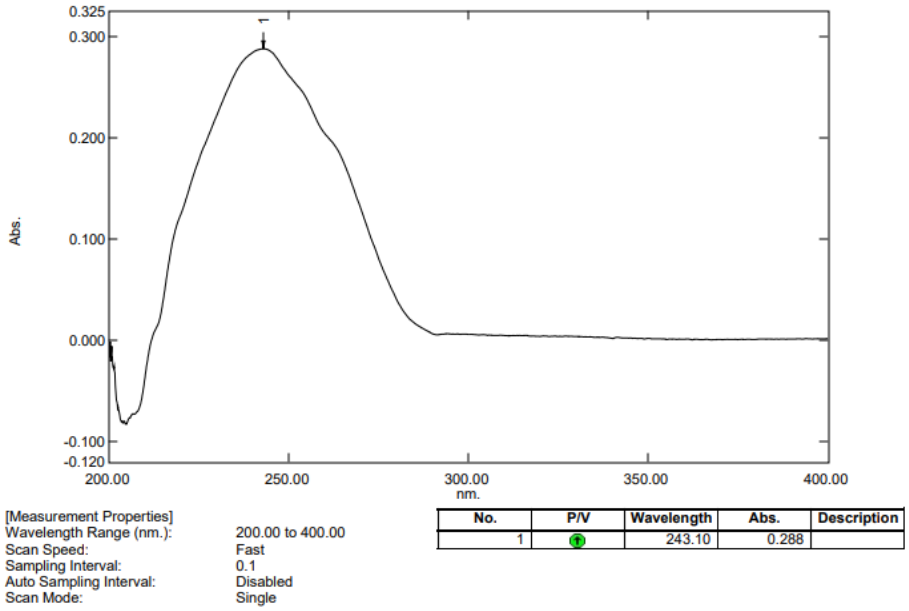

Figure. 2: - UV SPECTRUM OF DEFLAZACORT IN METHANOL

PREPARATION OF STANDARD PLOT OF DEFLAZACORT:

The calibration curve was linear in the concentration range of 4 - 20 µg/ml. the calibration curve was showed the linear equation as, y = 0.1694x - 0.1742, with a correlation coefficient, R^2 = 0.999 as shown in figure 3.

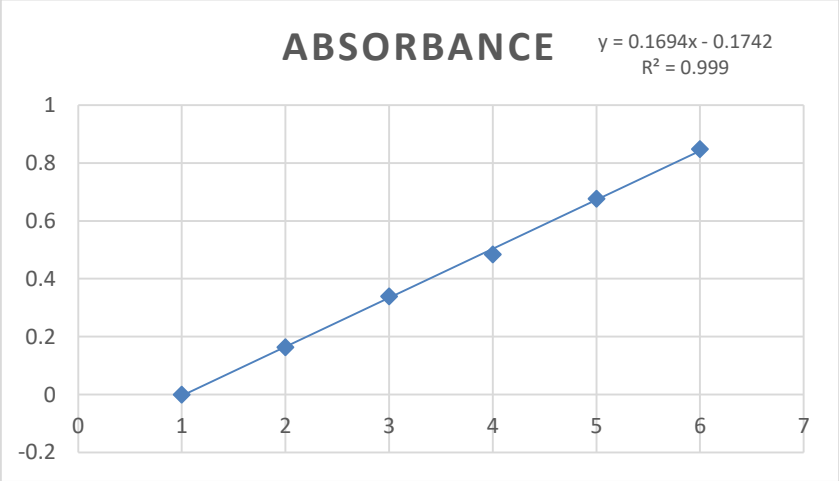

Figure. 3: CALIBRATION CURVE OF DEFLAZACORT.

LINEARITY AND RANGE:

The linearity ranges for deflazacort were found to be linear in the range of 4 - 20 µg/ml. The regression equation was found to be y = 0.1694x - 0.1742, R^2 = 0.999.

ACCURACY:

The accuracy of the analytical method for deflazacort was determined at 80%, 100%, and 120% levels of standard solution. Absorbance was determined at 243nm and results were expressed in terms of %recovery as shown in Table 1.

	Level	Amount Taken (µg/ml)	Amount Spiked	%Recovery±SD	%RSD
Method Employed	I	12	80%	96.70±0.475	0.492
	II	12	100%	100.58±1.106	1.099
	III	12	120%	99.206±0.279	0.282

Table 1: - RECOVERY DATA OF PROPOSED METHOD.

PRECISION:

The precision was assessed as repeatability and intra and inter-day variation results showed good reproducibility with the relative standard derivation (%RSD) below 2.0% as shown in Tables 2 and 3 respectively.

Sr. No	Time	Conc	Abs	Mean Abs	SD	%RSD
1	Morning	12ppm	0.448	0.450	0.00646	0.587945
			0.453			
			0.449			
2	Afternoon	12ppm	0.451	0.452	0.002646	0.585343
			0.455			
			0.450			
3	Evening	12ppm	0.453	0.454	0.001732	0.381509
			0.456			
			0.453			

Table 2: - RESULT OF INTRADAY PRECISION.

Sr. No	Time	Conc	Abs	Mean Abs	SD	%RSD
1	Day 1	12ppm	0.432	0.437	0.004583	1.048644
			0.438			
			0.441			
2	Day 2	12ppm	0.453	0.458333	0.005508	1.201652
			0.458			
			0.464			
3	Day 3	12ppm	0.529	0.529	0.002	0.378072
			0.531			
			0.527			

Table 3: - RESULT OF INTERDAY PRECISION.

DETECTION LIMIT:

The LOD value for deflazacort was found to be 0.403297 ug/ml and the LOQ value is 1.222113 µg/ml respectively indicating the sensitivity of the proposed method. LOD and LOQ indicate that the method was highly sensitive and fast.

CONCLUSION:

The method was validated and found to be simple, sensitive, accurate, and precise as per ICH guidelines. The % RSD for the validation parameters was found to be less than 2%. Hence proposed method may be used for routine analysis of these drugs in pharmaceutical dosage forms. The accuracy of the proposed method was confirmed by performing accuracy studies that showed the results within the range. The precision of the proposed UV method was confirmed by performing intra-day and inter-day precision. Results were well within acceptance criteria that indicate the excellent scope of the method for the determination of Deflazacort in bulk.

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