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Chromogenic Spectrophotometric Estimation Of Deflazacort In Bulk & Its Formulation Using 2, 2 Bi Pyridyl Reagent

Hafsa Sarwath, Hanaa Fatima Moin*, Sumaiya Tamkeen, Anupama Koneru, Imam Pasha.S

Sultan -Ul-Uloom college of Pharmacy, Banjara Hills, Road No.3, Hyderabad-500 034, Telangana, India.
Corresponding author: Hafsa Sarwath

Abstract:
Simple, precise, economic, less time consuming visible spectroscopic method for Deflazacort was developed by using 2, 2- Bipyridyl and Ferric Chloride. The absorption maximum of the chromogen was found to be 540 nm. The developed method was obeying Beer Lambert's law in the range of 50-250 µg/ml concentration. The method has also been statistically evaluated and the results were within the regulated limits. Molar absorptivity, Sand ell's sensitivity and correlation–co-efficient were found to be 1.406 x 10^3, 0.143 and 0.999 respectively and the method is free from the interferences of other additives present in the tablet formulation.

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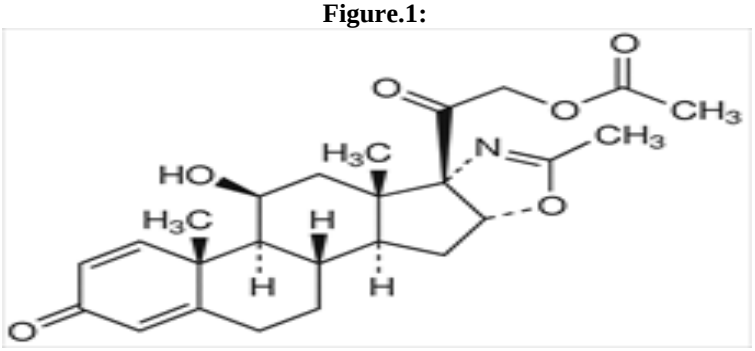
I. INTRODUCTION:

Deflazacort is chemically an oxazoline derivative of Prednisolone whose chemical names is 5'βH-Pregna-1, 4-dieno (17, 16-d) oxazole-3, 20-dione, 11β, 21-dihydroxy-2'-methyl- 21-acetate [1, 3]. Chemical Structure of Deflazacort is shown in the Figure 1. It is a glucocorticoid possessing potent anti-inflammatory and immunosuppressant actions [2, 4]. Deflazacort is an inactive prodrug and gets converted into its active metabolite, 21-desacetyl deflazacort which acts on the glucocorticoid receptors [8]. Only one UV-Spectroscopic method has been reported for the estimation of Deflazacort at a maximum wavelength of 244 nm using the reagent tetrazolium in alkaline medium [3]. The aim of the present work is to develop and validate rapid, economical and sensitive chromogenic spectrophotometric method for quantitative estimation of Deflazacort in bulk drug samples and formulations.

Figure.1:



II. EXPERIMENTAL

2.1 Instruments

SHIMADZU-1700 Ultraviolet Visible spectrophotometer (double beam) was used for all spectral measurements. Electronic balance of Scale tec (High precision balance) Model SAB-203 having sensitivity of 1 mg was used for weighing.

2.2 Chemicals and Reagents:

All the chemicals used were of analytical grade, were procured from A.R Chemicals and glasswares Pvt. Ltd.

Ferric Chloride (0.2% W/V**)**: was prepared by dissolving 0.2gms of Ferric chloride in 100 ml of distilled water.

2, 2 Bi pyridyl reagent (0.3%W/V): was prepared by dissolving 0.3gms of 2, 2 Bipyridyl in 100 ml of distilled water.

2.3 PROCEDURES:

2.3.1 Preparation of standard drug solution:

A standard drug solution of Deflazacort was prepared by dissolving 100 mg of drug in 100 ml of methanol in a standard volumetric flask to obtain a stock solution of 1 mg/mL

2.3.2 Bulk Drug Samples Estimation:

Aliquots of standard solution were taken for preparing 50-250µg/ml solutions. Suitable aliquots were transferred into respective labeled test tubes, to each of it 1ml of 0.2% W/V Ferric Chloride and 1ml of 0.3%W/V 2, 2 Bi pyridyl reagent were added. These solutions were heated at less than 70^{o}c for 10 minutes. The volume was made up to 10ml with methanol .The absorbance of the emerald green colored species was measured at 540 nm against reagent blank. The colored species was stable for more than 24hrs. The amount of Deflazacort present in the sample solution was computed from its calibration curve.

2.3.3 Estimation of Deflazacort in pharmaceutical tablets:

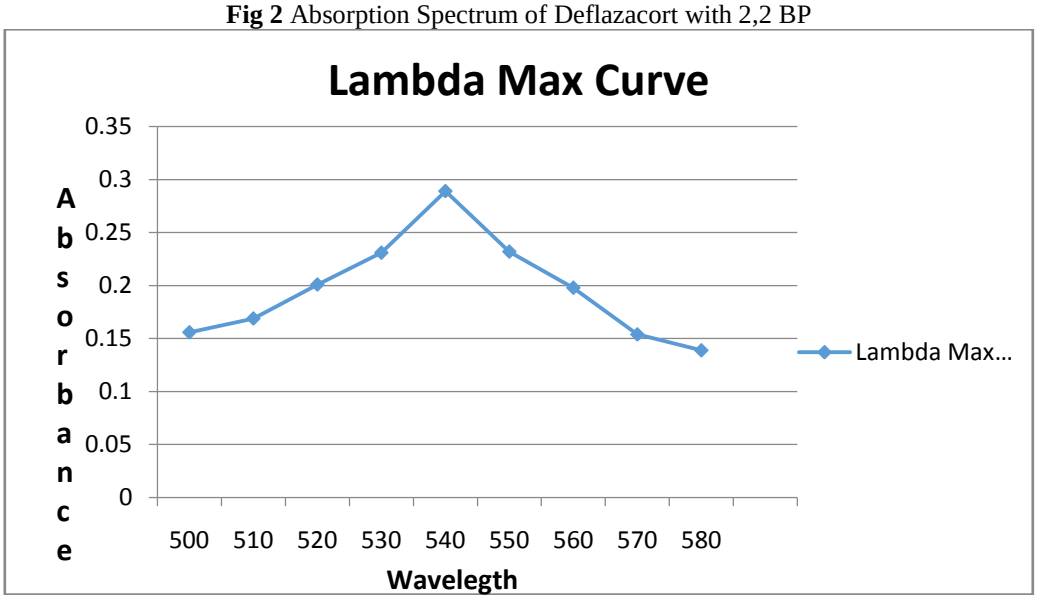
Sample solution was prepared by dissolving 100 mg equivalent of Deflazacort tablet powder in 100ml of methanol. The working standard solution of 1000 µg/ml is obtained. The prepared solution was kept in sonicator for sonication for 10 mins, same procedure applied to prepare, measure the absorbance as above for sample. Various formulations available and comparison with other method of estimation of same drug is described in Table 2 & 3.

III. RESULTS AND DISCUSSION

3.1 Optimization of parameters for Method:

The optimum conditions were established by changing one parameter while fixing the other parameters and noting the effect on absorbance of chromogen. Wave length maximum of drug with 2, 2,-BP is describe in the Fig 2. Deflazacort has hydroxyl, acetoxy group in the molecular structure making it possible to undergo reduction of ferric chloride , ultimately forming complex with 2,2 Bipyridyl . The effect of temperature of the reaction, quantity, concentration and order of addition of various reagents were studied, optimized after several experiments with respect to maximum sensitivity, color stability, adherence to Beer's law and other optimum conditions are incorporated in the procedure. Optical parameters of the method are described in Table 1.

Fig 2 Absorption Spectrum of Deflazacort with 2,2 BP

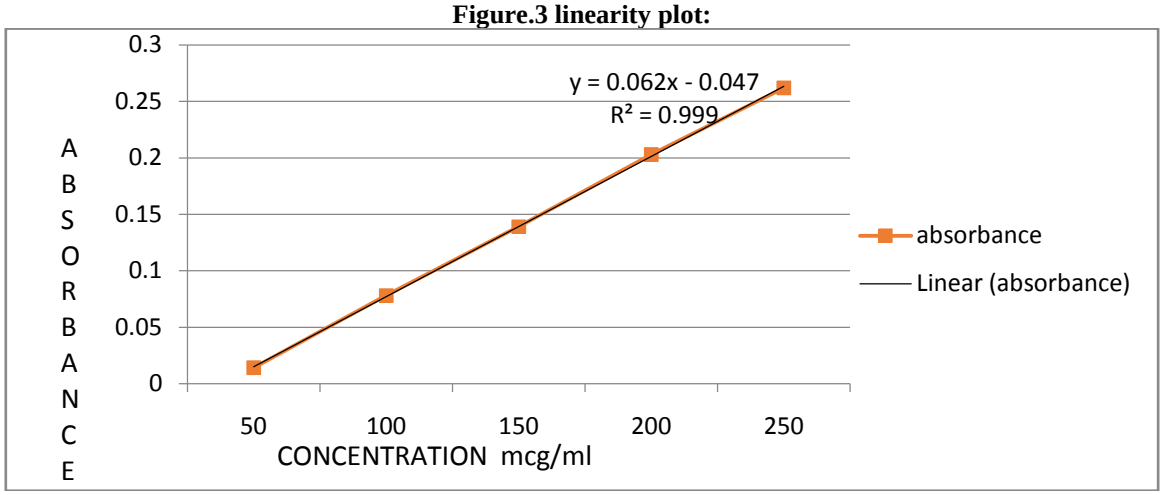


3.2. Optical Characteristics:

3.2.1 Linearity

By using the method of least squares regression analysis was performed to evaluate the slope (m), intercept (b) and correlation coefficient (r^2) was computed from various concentrations and the results are presented in Table 1.The graph showed negligible intercept as described by the regression equation y = mx + b where y is the absorbance and x is the concentration in µg/ml. Calibration curve was shown in Figure.3.

Figure.3 linearity plot:



The optical characteristics such as molar absorptivity, Beer's law limits, absorption maxima and Sandell's sensitivity are presented in Table 1

Table 1: Optical characteristics

01	λ_{max} (nm)	540
02	Beer's law range (µg/ml)	50-250
03	Molar extinction coefficient(L.mole^{-1} cm^{-1})	1.406 x 10^3
04	Sandell's sensitivity (µg/cm^2/0.001)	0.143
05	Regression equation (y = mx + c) * Slope (m) Intercept (c)	 0.062 -0.047
06	Correlation coefficient (r^2)	0.999
07	Precision (%Relative Standard Deviation)	0.357
08	Standard Error of Mean	0.474

3.2.2 Accuracy and Recovery

Commercially available tablets of Deflazacort (Table 2) were analyzed by the proposed method and as additional check on the accuracy of the method, recovery experiments were also conducted by spiking known amounts of pure drug in pre-analyzed formulation and the recovery was calculated in each of the case using the regression line equation developed under the Linearity experiment. Assay results of the proposed method was compared with that of reference method and statistically evaluated using one-way ANOVA with post-test followed by Dunnett multiple comparison test. The means of the proposed method are not significantly different from that of reference method (P > 0.05). The assay and accuracy results were presented in Table 3.The interference studies indicated the common additives and excipients present in formulations did not interfere with the proposed method.

Table 2: Commercially available formulations of Deflazacort.

S.no.	Brand name	Dosage form	Strengths available(mg)
1.	Defcort, Macleods Pharmaceuticals Ltd	Tablets	1, 6, 18, 24, 30
2.	Defza, Wallace Pharmaceuticals Pvt Ltd	Tablets	1, 6, 24, 30
3.	Cortimax, Zuventus Healthcare Ltd	Tablets	1, 6, 24, 30
4.	Mahacort DZ, Mankind Pharma Pvt Ltd	Tablets	1, 6, 24, 30
5.	Enzocort, Alkem Laboratories Pvt Ltd	Tablets	1, 6, 24, 30
6.	Dezacor, Aristo Pharmaceuticals Pvt Ltd	Tablets	1, 6, 18, 24, 30
7.	Defnalone, Lupin Pharmaceuticals Ltd	Tablets	1, 6, 24, 30
8.	DFZ, IPCA Laboratories	Tablets	1, 6, 24, 30
9.	Eticort, Franco Indian Pharmaceuticals Pvt Ltd	Tablets	1, 6, 24, 30

Table 3: Evaluation Of Deflazacort In Pharmaceutical Dosage Forms (N=6)

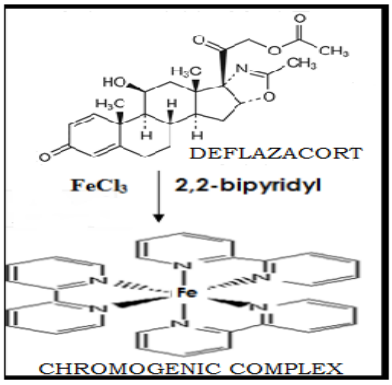
Sample[a]	Label Amount (mg)	Amount Obtained(mg) by Propose Method[b]	Amount Obtained(mg) by Reference Method	Percentage Recovered by Propose Method[c]
D_1	36	36.05±0.47	36.07±0.72	36.10±0.12
D_2	36	36.80±0.12	36.62±0.20	36.60±0.17

Reference method: reference no. [3]
a - D_1 and D_2 are the tablets from different batches
b – Mean ± SD of 6 determinations.
c – 10 mg of pure drug was added and recovered.
For the sample One-way ANOVA with post-test followed by Dunnett multiple comparison tests were performed. The results showed that $P > 0.05$ and the means of the proposed method are not significantly different from that of reference method.

IV. CHEMISTRY OF THE COLORED SPECIES FORMED

Deflazacort contains an hydroxyl, acetoxy functional group can be analysed using 2,2, Bipyridyl Reagent. The colour formation by 2,2, Bipyridyl Reagent may be explained in the following manner based on the analogy.Proposed Scheme of complex formation was described in the Fig 4.

Fig 4 Proposed Scheme of Chromogen complex formation:



V. CONCLUSION.

The proposed visible spectro-photometric method enables quantitative determination of Deflazacort in bulk drug samples and its tablets. Efficient visible spectrophotometric detection at the respective absorption maxima enabled determination with no interference from the excipients. The calibration curve was linear over the concentration range from 50-250 µg/ml for the proposed method. The relative standard deviation's (R.S.D.) was less than 10% and average recovery was above 99%. The proposed method is fast, sensitive, precise, accurate, and efficient and can be used in for analysis in quality control laboratories.

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