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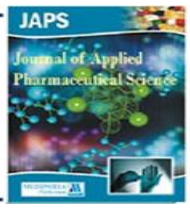

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Development and validation of spectrofluorimetric method for estimation of deflazacort in tablets

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ABSTRACT

A simple and sensitive spectrofluorimetric method has been developed for the determination of deflazacort in pharmaceutical tablet dosage forms. The method was based on the liebermann-burchard reaction, in which the chloroform extract of deflazacort is reacted with acetic anhydride and sulfuric acid to produce strong fluorescence. The resulting fluorophor exhibit excitation and emission wavelengths at 300 and 435 nm, respectively. Linear relationship for the fluorescence intensity was obtained in the concentration range of 0.5 - 10 µg/ml. The method was validated in terms of linearity (0.5-10 µg/ml), repeatability (RSD, 0.99 %), precision (intra-day variation, RSD, 0.239 to 1.287 % and inter-day variation, RSD, 0.360 to 1.830 %) and accuracy (99.12 to 100.28 %). The limit of detection and limit of quantification for deflazacort were found to be 0.15 and 0.45 85.70 µg/ml, respectively. The developed method was successfully used for the assay of deflazacort tablet formulation. The spectrofluorimetric method was found to be simple, sensitive, accurate, precise and economic and can be used for the routine quality control testing of deflazacort in tablet dosage form.

Key words: Deflazacort, spectrofluorimetric, liebermann-burchard reaction, fluorophor, validation, tablet.

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INTRODUCTION

The deflazacort is chemically (11β,16β)-21-(acetyloxy)-11-hydroxy-2′-methyl-5′H-pregna-1,4-dieno[17,16-d]oxazole-3,20-dione (Maryadele, 2006) is a glucocorticoid used as an anti-inflammatory and immunosuppressant (Sweetman, 2007). Deflazacort is not official in any pharmacopoeias; hence official method is not available for determination of deflazacort. Literature survey reveals high performance liquid chromatography (HPLC) (Cardoso, 2007; Santos-Montes et al., 1994, 1993 & 1999; Ozkan et al, 2003, Gonzalo-Lumbreras et al.,1997) , liquid chromatography-mass spectrometry (LC/MS) (Ifa et al, 2000) , LC-MS/MS with ESI (Mazzarino et al., 2008) for estimation of deflazacort in biological fluids as well as in pharmaceutical formulations. Literature survey does not reveal any simple spectrofluorimetric method for determination of deflazacort in tablet formulation. The present manuscript describes simple, sensitive, accurate, precise and economic spectrofluorimetric procedure based on the liebermann-burchard reaction for the determination of deflazacort in pharmaceutical tablet dosage forms.

MATERIALS AND METHODS

Apparatus

Fluorescence spectrophotometer with single quartz cell of 1cm path length (Shimadzu, RF 1501, Japan) was used to measure fluorescence intensity of resulting solutions. Analytical balance (CP224S, Sartorius, Gottingen, Germany), ultrasonic bath (Frontline FS 4, Mumbai, India)

and constant temperature water bath (Labtronic, India) were used in the study.

Reagents and Materials

Deflazacort active pharmaceutical ingredient (API) was kindly gifted by Cadila Healthcare Ltd, Gujarat (India), with 98.90 % purity. The commercial fixed dose product containing 30 mg deflazacort was procured from the local pharmacy. AR grade sulfuric acid, acetic anhydride and chloroform (S. D. Fine Chemical Ltd., Mumbai, India) and Whatman filter paper no. 41 (Whatman International Ltd., England) were used in the study.

Preparation of Diluent

Chloroform was used as diluent.

Preparation of standard stock solution of deflazacort

An accurately weighed quantity of about 50 mg deflazacort was transferred into 50 ml volumetric flask. About 25 ml of chloroform was added and sonicated to dissolve. The solution was cooled at room temperature and made up to volume with diluent to get final concentration of 1000 µg/ml.

Preparation of working standard solution of deflazacort

Deflazacort working standard solution was prepared by diluting standard stock solution (5.0 ml) to 50 ml with diluent to produce required concentration (100 µg/ml).

Preparation of sample solution

Twelve tablets were weighed and powdered. The quantity of the powder (equivalent to 10 mg of deflazacort) was transferred to a 100 ml volumetric flask, sonicated for 30 minutes with diluent (50 ml) to dissolve the drug as completely as possible. The solution was filtered into a 100 ml volumetric flask through a Whatman filter paper No. 41. The residue was washed with diluent (20 ml), and washes were added to the volumetric flask. The solution was diluted up to 100 ml with diluent (100 µg/ml). The resulting solution (5.0 ml) was diluted up to 50 ml with diluent to get final concentration of 10 µg/ml.

Methodology

Selection of excitation and emission wavelengths

The standard solution of deflazacort was scanned over the range of 200 nm to 400 nm wavelengths for selection of excitation wavelength. It showed highest florescence intensity at 300 nm. So, excitation wavelength 300 nm was selected to obtain emission. The standard solution of deflazacort was scanned over the range of 300 nm to 600 nm wavelengths for selection of emission wavelength by selecting 300 nm as an excitation wavelength. It showed highest florescence intensity at 435 nm. Hence, emission wavelength of 435 nm was selected for measurement of fluorescence intensity.

Optimization of reaction conditions

A series of experiments were conducted to establish the optimum analytical conditions for the reaction of deflazacort with acetic anhydride and sulfuric acid. The parameters optimizations were performed on throughout study of deflazacort by altering each variable in turn while keeping the others constant.

Selection of acid and optimization of acid concentration

Different acids as HNO_3, H_3PO_4 and H_2SO_4 were tested to determine which one is the most suitable for optimum reaction development. Sulphuric acid could be used which give high intensity fluorescence as compare to others. The effect of sulphuric acid concentration on the sensitivity of the method was studied by using different concentrations of sulphuric acid (25%, 50%, 75%, 90% and 98%). Highest fluorescence intensity was found in 98% H_2SO_4.

Optimization of volume of acid

By applying different volumes (0.1 - 1.5 ml) of the same concentration (98% H_2SO_4), a volume of 1.0 ml in a total volume of 10 ml was found to be quite enough for maximum fluorescence intensity.

Optimization of volume of acetic anhydride

By applying different volumes (1.0 - 4.0 ml) of acetic anhydride, a volume of 3.0 ml in a total volume of 10 ml was found to be quite enough for maximum fluorescence intensity.

Optimization of heating temperature and time

The influence of different heating temperatures and incubation time were studied. The effect of different heating temperatures and time period on the sensitivity of the method was studied by selecting 40°C, 70°C and 100°C temperatures with using 5 to 30 min heating time period. From study, the best temperature was found to be 100°C and complete reaction was attained by getting maximum fluorescence intensity at a period of 15 min.

Validation of the proposed method

The proposed method is validated according to the International Conference on Harmonization (ICH) guidelines (ICH, 2005).

Linearity (Calibration curve)

Calibration curves were plotted over a concentration range of 0.5 - 10 µg/ml for deflazacort. Accurately measured standard working solutions of deflazacort (0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml) were transferred to one set of a series of 10 ml volumetric flasks. Then 3.0 ml acetic anhydride and 1.0 ml of sulfuric acid was added to each flask and mix well. The flasks were heated at 100^0C for 15 minutes in water bath.

Cool the solutions at room temperature and volume was made up to mark with chloroform. The relative fluorescence intensity of all the resulting solutions were measured at an excitation wavelength of 300 nm and an emission wavelength of 435 nm. The calibration curve was prepared by plotting fluorescence intensity versus concentration (µg/ml) and regression

equation was calculated. The procedure was repeated three times for all the concentrations.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of deflazacort by the standard addition method. Known amounts of standard solutions of deflazacort were added at 50%, 100% and 150% levels to prequantified standard solutions of deflazacort (4 µg/ml). The amount of deflazacort was estimated by applying the obtained values to the regression equation of the calibration curve.

Method Precision (% Repeatability)

The precision of the instruments was checked by repeated spotting of same solution and repeated scanning of the same spot (n=6) of deflazacort without changing the parameters for the spectrofluorimetric method. The repeatability was reported in terms of relative standard deviation (% RSD).

Intermediate Precision (Reproducibility)

The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentration of standard solution of deflazacort (0.5, 1, 2, 4, 6, 8, and 10 µg/ml) for the proposed method. The results were reported in terms of relative standard deviation (% RSD).

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and the LOQ of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where σ = Standard deviation of the response

S = Slope of calibration curve

Estimation of deflazacort in pharmaceutical formulation (tablet)

Pharmaceutical formulation of deflazacort was purchased from local pharmacy. Sample solution was prepared as described earlier. This solution was then analyzed by described method. The amount of deflazacort present in the sample solution was determined by fitting fluorescence intensity values of deflazacort into the equation of line representing calibration curve of deflazacort. The potential interference from excipients was also examined.

RESULTS ANDDISCUSSION

The spectrofluorimetric method was based on the liebermann-burchard reaction, in which the chloroform extract of deflazacort is reacted with acetic anhydride and sulfuric acid to produce strong fluorescence which exhibit excitation and emission wavelengths at 300 and 435 nm, respectively (Figure 1 and 2).

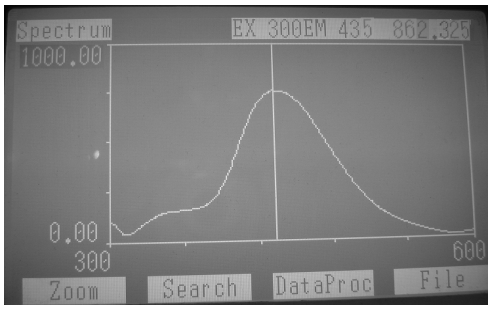

Fig. 1: Representative fluorescence spectra of standard deflazacort (10 µg/ml) showing λ_{em} at 435 nm.

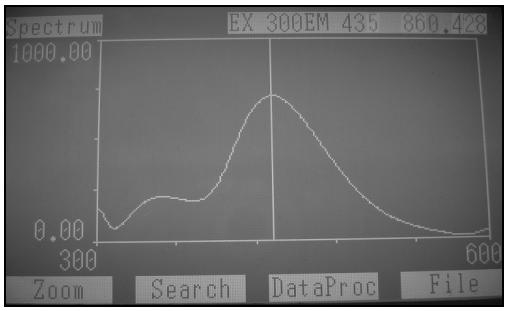

Fig. 2: Representative fluorescence spectra of sample deflazacort (10 µg/ml) showing λ_{em} at 435 nm.

The developed method was optimized using different parameters such as acid and reagent concentration and volume, heating temperature and time required for development of maximum fluorescence intensity and stability of developed fluorophor. Maximum fluorescence intensity was obtained with 1.0 ml 98% H_2SO_4 (Figure 3 and 4).

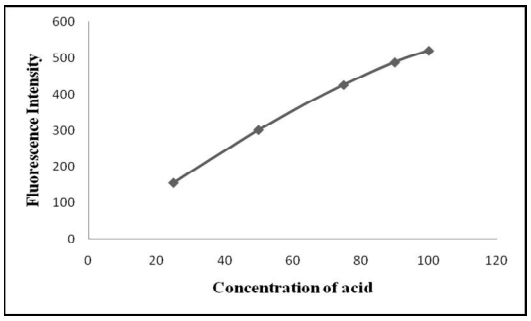

Fig. 3: Optimization of acid concentration.

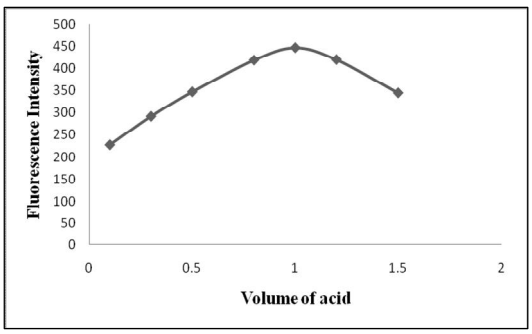

Fig. 4: Optimization of volume of acid.

The volume of acetic anhydride for maximum intensity was found to be 3.0 ml in a total volume of 10 ml. (Figure 5). The heating temperature and time required to get maximum fluorescence intensity was found to be 100^0C for 15min heating on boiling water bath (Figure 6). The developed fluorophor was stable up to 12 hr at room temperature (28 ± 1.0^0 C).

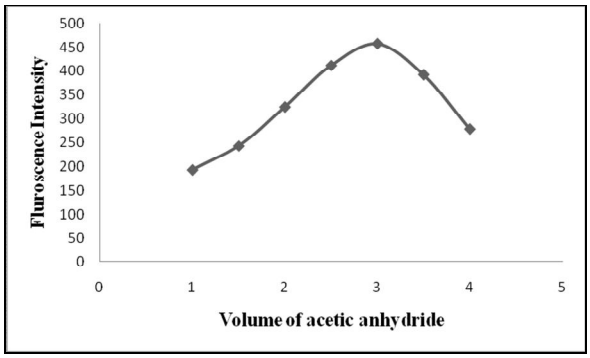

Fig. 5: Optimization of volume of acetic anhydride.

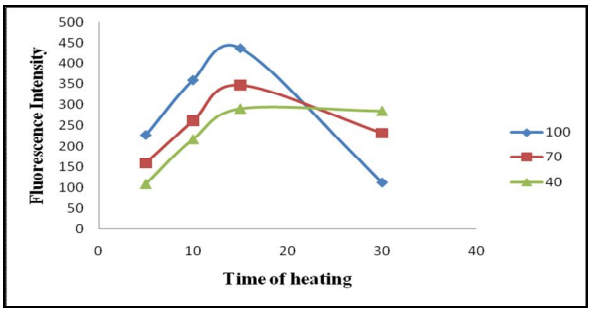

Fig.6: Effect of temperature and duration of heating time

Linearity range for deflazacort was found in the concentration range of 0.5 to 10 µg/ml, with a correlation coefficient of 0.9962. The average linear regression equation was represented as y = 76.936x + 110.79, where x = concentration of deflazacort in µg/ml and y = fluorescence intensity. The limit of detection and limit of quantification for deflazacort were found to be 0.15 and 0.45 µg/ml , respectively indicate sensitivity of the method. The intra-day precision and inter-day precision (% RSD) was calculated for standard deflazacort solutions (0.5 - 10 µg/ml) for 3 times on the same day and for 3 times over a period of one week, respectively. The intra-day and inter-day variation (% RSD) were found to be in the range of 0.239 to 1.287 % and 0.360 to 1.830 %, respectively. These values indicate that the method is precise. Precision of the instrument was checked by repeated scanning of the same concentration (4 µg/ml) of deflazacort six times without changing parameters for the method and % RSD for measurement of peak area was found to be 0.99 indicates repeatability of the proposed method. Different validation parameters for the proposed method for determining deflazacort content are summarized in Table 1. Accuracy of the method was evaluated by calculating recovery of deflazacort by standard addition method at 3 different levels of the calibration curve (n = 5). The mean recovery was found to be 99.62 ± 0.60 % ensuring that the method is accurate (Table 2).

Table 1: Summary of regression analysis and validation parameters of deflazacort by spectrofluorimetric method.

Parameters	Deflazacort Spectrofluorimetric method
Linearity (µg/ml)	0.5 – 10
Régression équation y=mx+c	y = 76.936x + 110.79
(a) Slope (m)	76.936
(b) Intercept (c)	110.79
(c) Corrélation coefficient (r^2)	0.9962
%Recovery ± SD, (n=3)	99.6235 ± 0.5995
Repeatability (%RSD, n=6)	0.9997
Intermediate Precision (a) Intraday precision (%RSD) (n = 3) at 7 level	0.2386-1.2867%
(b) Interday precision (%RSD) (n = 3) at 7 level	0.3597-1.8296%
LOD (µg/ml)	0.15
LOQ (µg/ml)	0.45
% Assay ± SD (n= 6)	**Brand 1:** 99.27 ± 0.52 **Brand 2:** 98.86 ± 0.64

Table 2: Recovery data for deflazacort by spectrofluorimetric method.

Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (%)	% Recovery	Mean % Recovery ± S. D.
Deflazacort	I	4	50 %	99.95 99.77 98.69	99.47 ± 0.68
	II	4	100 %	100.6 99.97 100.2	100.3 ± 0.33
	III	4	150 %	98.95 99.56 98.83	99.12 ± 0.39

Table 3: Analysis of marketed formulation of deflazacort by spectrofluorimetric method

Brand	Label Claim (mg/tab)	Amount Found (mg/tab)	% Label Claim (mg/tab)	Mean % label claim ± S. D.
Brand 1	30	29.64	98.80	99.27 ± 0.52
	30	29.85	99.51	
	30	29.83	99.42	
	30	29.54	98.47	
	30	29.93	99.78	
	30	29.90	99.66	
Brand 2	30	29.65	98.83	98.86 ± 0.64
	30	29.54	98.47	
	30	29.95	99.85	
	30	29.50	98.33	
	30	29.49	98.29	
	30	29.82	99.40	

This method was applied to determine the content of deflazacort in market sample of single component deflazacort tablet. The average percentage of deflazacort in market samples were found to be 99.27 ± 0.52 % for tablet brand 1 and 98.86 ± 0.64 % for tablet brand 2 (n = 6). The results are in agreement with

the labeled value of deflazacort in tablet dosage form (Table 3). The results indicate that the proposed method was found to be simple, sensitive, precise and accurate for the estimation of deflazacort in tablet formulations.

CONCLUSION

The results of the analysis of pharmaceutical formulation by the proposed method 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 deflazacort. The observations and results obtained from this study, including linearity and range, accuracy, precision (method precision as repeatability and intermediate precision as intra and inter day precision) are lie well within acceptable results. From the experimental studies it can be concluded that proposed method can be adopted for the routine analysis of deflazacort in tablets without interference of excipients.

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