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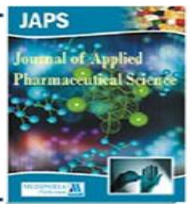

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High performance thin layer chromatographic method for estimation of deflazacort in tablet

Patel Satish A and Patel Natvarlal J.

ABSTRACT

A simple and sensitive high performance thin layer chromatography (HPTLC) method has been developed for the quantitative estimation of deflazacort in its single component tablet formulation (30 mg). Deflazacort was chromatographed on silica gel 60 F_{254} TLC plate using benzene: methanol: glacial acetic acid (7.5:2.0:0.5, v/v/v) as mobile phase. Deflazacort showed Rf value of 0.60 $\pm$ 0.02 and scanned at 243 nm using a camag TLC scanner 3. The method was validated in terms of linearity (100 – 800 ng/spot), precision (intra-day variation, 0.335 to 1.203% and inter-day variation, 0.231 to 1.471%), accuracy (98.87 to 99.77%) and specificity. The limit of detection and limit of quantification for deflazacort were found to be 25.97 ng/spot and 85.70 ng/spot, respectively. The developed method was successfully used for the assay of deflazacort tablet formulation. The method was found to be simple, sensitive, specific, accurate and precise and can be used for the routine quality control testing of deflazacort in tablet dosage form.

Key words: Deflazacort, HPTLC, validation, tablet.

Patel Satish A and Patel Natvarlal J.
Department of Pharmaceutical Quality Assurance, Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Kherva – 382711, Mehsana, Gujarat, India.

For Correspondence:
Patel Satish A,
Department of Pharmaceutical Quality Assurance, Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Kherva – 382711, Mehsana, Gujarat, India.
Phone & Fax No.: +91-2762-286082

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 et al., 2006) is a glucocorticoid used as an anti-inflammatory and immunosuppressant (Sweetman et al., 2007). Deflazacort is not official in any pharmacopoeias; hence official method is not available for determination of deflazacort. Literature survey reveals different liquid chromatographic method like reverse-phase HPLC method for estimation of deflazacort in pharmaceutical preparations (Cardoso et al., 2007), extractive HPLC method for deflazacort and its metabolite 21-hydroxydeflazacort (Santos-Montes et al., 1994), HPLC method for natural and synthetic corticosteroids (Santos-Montes et al., 1993), micellar liquid chromatography using sodium dodecyl sulphate for estimation of deflazacort in urine (Santos-Montes et al., 1999), RP-HPLC for dissolution studies and determination of deflazacort in pharmaceutical formulations and human serum samples (Ozkan et al., 2003), reversed-phase HPLC for corticoid alcohols and their derivatives (Gonzalo-Lumbreras et al., 1997), liquid chromatography-mass spectrometry method for determination of 21-hydroxydeflazacort in human plasma (LC/MS) (Ifa et al., 2000) and LC-MS/MS method based on class-characteristic fragmentation pathways for the detection of synthetic glucocorticosteroids in human urine (Mazzarino et al., 2008) for estimation of deflazacort. Literature survey does not reveal any simple HPTLC method for determination of deflazacort in tablet formulation. The present manuscript describes simple, sensitive, accurate, precise and specific HPTLC procedure for the determination of deflazacort in pharmaceutical tablet dosage forms.

MATERIALS AND METHODS

Apparatus

A Camag HPTLC system (Switzerland) comprising of Camag Linomat V semiautomatic sample applicator, Camag TLC Scanner 3, Camag (Muttenz, Switzerland) flat bottom and twin-trough developing chamber (10 × 10 cm), UV cabinet with dual wavelength UV lamp, Camag winCATS software, Hamilton syringe (100 µl), Sartorius CP224S analytical balance (Germany), Ultrasonic bath (Frontline FS-4, Mumbai, India) were used in the study.

Reagents and Materials

Deflazacort was kindly supplied by Zydus Cadila Healthcare, Changodar, India as a gift sample. The commercially available tablets of deflazacort were procured from local market labeled to contain 30 mg deflazacort. Silica Gel 60 F_{254} TLC plates (10 × 10 cm, layer thickness 0.2 mm, E. Merck, Germany) were used as stationary phase. Chloroform, toluene, benzene (AR grade, S.D. Fine Chemicals Ltd, Mumbai, India), methanol and glacial acetic acid (AR grade, Finar Chemicals Ltd, Ahmedabad, India) were used for mobile phase preparation and as solvents.

Preparation of standard solution

Deflazacort reference standard (100 mg) was accurately weighed and transferred into 100 ml volumetric flask. Methanol (50 ml) used as diluent was added and shake well until it dissolved. Volume was made up to mark with methanol and mixed (1 mg/ml). 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

Twenty 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 methanol (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 100 ml volumetric flask. The solution was diluted up to 100 ml with methanol (100 µg/ml). Two microlitres of this solution was applied to the HPTLC plate to get 200 ng/spot and followed by development.

Chromatographic conditions

The chromatographic estimations were performed using following condition; stationary phase, precoated Silica Gel 60 F_{254} aluminum sheets (10 × 10 cm) (pre-washed with methanol and dried in air); mobile phase, benzene: methanol: glacial acetic acid (7.5:2.0:0.5, v/v/v); chamber saturation time, 30 min; temperature, 25 ± 2^{o}, migration distance, 80 mm; wavelength of detection, 243 nm; slit dimensions, 5 × 0.45 mm; scanning speed, 10 mm/s. Following spotting parameter were used - band width, 6 mm; distance from the plate edge, 10 mm; space between two bands, 10 mm and spraying rate, 1 µl/s.

Chromatographic separation

Five microlitres of standard solution of deflazacort (100 µg/ml) was applied on TLC plate under nitrogen stream using semiautomatic spotter. The plate was dried in air and developed up to 80 mm at constant temperature using mixture of benzene: methanol: glacial acetic acid (7.5:2.0:0.5, v/v/v) as mobile phase in Camag twin-trough chamber previously saturated with mobile phase for 30 min. The plate was removed from the chamber and dried in air. Photometric measurements were performed at 243 nm in absorbance/reflectance mode with Camag TLC Scanner 3 using winCATS software incorporating the track optimization option.

Validation of the proposed method

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

Linearity (Calibration curve)

Aliquots of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 □l of standard deflazacort solution (100 µg/ml) were spotted on precoated TLC plate using semiautomatic spotter under nitrogen stream. The TLC plate was developed and photometrically analyzed as described under chromatographic separation. Each concentration was spotted five times on the TLC plate. The calibration curve was prepared by plotting peak area versus concentration (ng/spot) corresponding to each spot.

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 200 ng/spot standard solutions of deflazacort. 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 position of plate for the HPTLC 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 (200, 400 and 600 ng/spot) 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

Specificity

The specificity of the method was ascertained by analyzing standard drugs and the sample. The spots for deflazacort in the samples were confirmed by comparing the R_f and spectra of the spots with that of the standards.

Analysis of deflazacort in tablet

Five microlitres of sample solution was applied to the TLC plate to get 500 ng/spot and followed by development and scanning as described earlier. Analysis was carried out in triplicate, peak areas were measured at 243 nm and sample concentrations calculated. The amount of deflazacort present in the sample solution was determined by fitting area values of peak corresponding to deflazacort into the equation of line representing calibration curve of deflazacort. The potential interference from excipients was also examined.

RESULTS AND DISCUSSION

Deflazacort is soluble in methanol; therefore methanol was selected as solvent. Several mobile phases were tried to accomplish good separation of deflazacort. Using the mobile phase benzene: methanol: glacial acetic acid (7.5:2.0:0.5, v/v/v) and 10 × 10 cm silica gel $60F_{254}$ aluminum-backed plates, good separation was attained with retardation factor (R_f) values of 0.60 ± 0.02 for deflazacort (Figure 1 and 2).

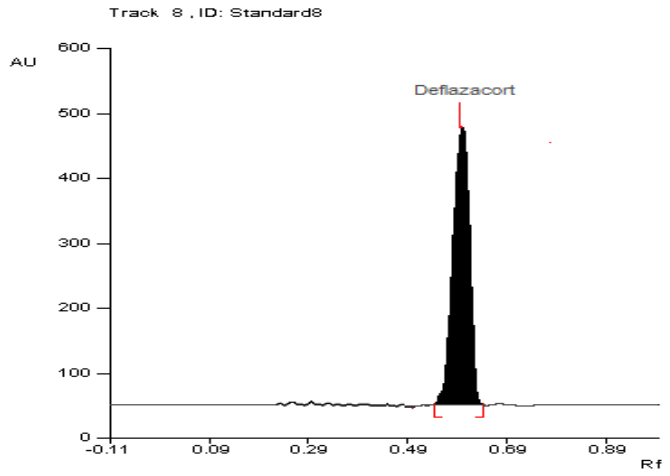

Fig. 1: HPTLC chromatogram of deflazacort with corresponding Rf value at 243 nm.

A wavelength of 243 nm was used for quantification of the drug. Even saturation of TLC chamber with mobile phase for 30 min assured better reproducibility and better resolution. Linearity range for deflazacort was found in the concentration range of 100 to 800 ng/spot, with a correlation coefficient of

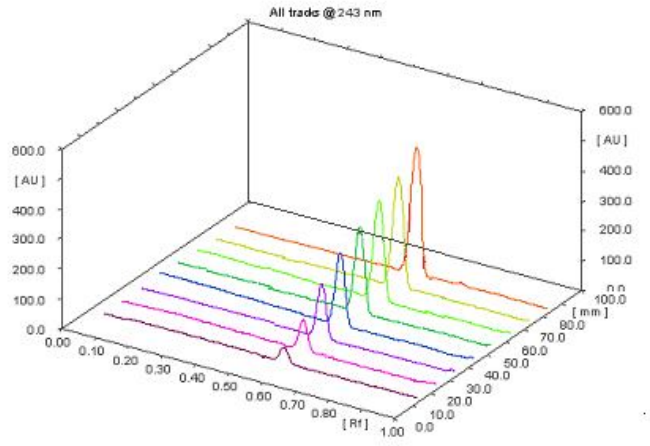

Fig. 2: 3-D Chromatogram showing peaks of deflazacort in different concentrations at 243 nm

0.9994. The average linear regression equation was represented as y = 3.5211x+89.893, where x = concentration of deflazacort in ng/spot and y = peak area. The limit of detection and limit of quantification for deflazacort were found to be 25.97 ng/spot and 85.70 ng/spot, respectively indicate sensitivity of the method.

The intra-day precision (%RSD) was calculated for standard deflazacort solutions (200, 400 and 600 ng/spot) for 3 times on the same day. The inter-day precision (% RSD) was calculated for standard deflazacort solutions (200, 400 and 600 ng/spot) for 3 times over a period of one week. The intra-day and inter-day variation (% RSD) were found to be in the range of 0.335 to 1.203 and 0.231 to 1.471, respectively. These values indicate that the method is precise.

Precision of the instrument was checked by repeated scanning of the same spot (200 ng/spot) of deflazacort six times without changing position of the plate and % RSD for measurement of peak area was found to be 0.81. The % RSD for measurement of peak area ensures proper functioning of HPTLC system indicates repeatability of the proposed method. Different validation parameters for the proposed HPTLC method for determining deflazacort content are summarized in Table 1.

Table 1: Regression analysis data and summary of validation parameters for the proposed HPTLC method

Parameters	**Results**
Linearity range (ng/spot)	10 - 800
Regression Equation (y = mx + c)	y = 3.5211x + 89.893
Slope	3.5211
Intercept	89.893
Correlation co-efficient (r^2)	0.9994
Precision (% RSD)	
Intra-day (n = 3)	0.335 - 1.203
Inter-day (n = 3)	0.231 - 1.471
Repeatability of peak area (% RSD) (n = 6)	0.81
Accuracy (% Recovery) (n = 3)	99.39 ± 0.46
Limit of detection (LOD) (ng/spot)	25.97
Limit of quantification (LOQ) (ng/spot)	85.70
Specificity	Specific

'n' is number of determination and RSD is relative standard deviation.

Table 2: Recovery data for the proposed method.

Drug	Level	Amount of sample taken (ng/spot)	Amount of standard spiked (%)	% Recovery	Mean % recovery ± S. D.*
Deflazacort	I	200	50 %	99.55 100.7 98.33	99.52 ± 1.17
	II	200	100 %	100.5 99.58 99.18	99.77 ± 0.71
	III	200	150%	99.40 98.85 98.37	98.87 ± 0.52

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

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 99.39 ± 0.46% ensuring that the method is accurate (Table 2).

The method was found to be specific for deflazacort. The specificity of the method was ascertained by analyzing standard drug and the samples. The spot for deflazacort in the sample was confirmed by comparing the Rf value and spectra of the spot with that of standard. The peak purity of deflazacort was assessed by comparing the spectra of standard at peak start, peak apex and peak end positions of spot. Good correlation was also found between standards and sample spectra (Figure 3). None of the formulation excipients were interferes in the quantification of deflazacort at this Rf value.

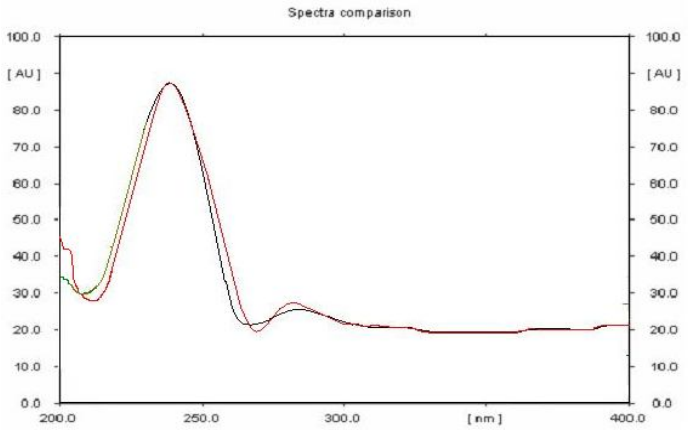

Fig. 3: Overlain UV absorption spectrum of standard and sample deflazacort.

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 sample was found to be 99.54 ± 0.26 % for tablet brand 1 and 99.10 ± 0.51 % 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 HPTLC method was found to be simple, sensitive, specific, precise and accurate for the estimation of deflazacort in tablet formulations.

Table 3: Analysis of tablet formulation of deflazacort by proposed HPTLC method (n = 6)

Tablet	Label claim (mg)	Parameters	% amount found (n = 6)
			HPTLC method
Brand A	30	Mean	99.54
		S. D.	0.26
Brand B	30	Mean	99.10
		S. D.	0.51

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

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 specificity, 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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