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Original Article

Development and validation of HPTLC method for determination of betamethasone valerate in API and pharmaceutical dosage form

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ABSTRACT

The aim of present work was to develop a simple and sensitive, HPTLC for the quantitative estimation of Betamethasone Valerate in its single component cream formulations (20 g). Betamethasone Valerate was chromatographed on silica Gel 60 F254 TLC plate using Ethyl Acetate: *n*- Heptane: Toluene: Ethanol (5.1:2.4:1.2:0.3 *v/v/v/v*) as mobile phase. Betamethasone Valerate in methanol scanned by Camag TLC scanner 4 with UV visible detector over wavelength range 200 to 400 nm, showed R_f value of 0.34 at wavelength 246 nm and selected for further studies. The method was validated in terms of linearity (1-9 µg/ml), precision (intra-day variation 1.40, inter-day variation 1.71), accuracy (84 to 96%) and specificity. The limit of detection and limit of quantification for Betamethasone Valerate were found to be 0.97 µg/spot and 2.96 µg/spot, respectively. It can be concluded from the results that the proposed method was accurate, precise and consistent the determination of Betamethasone Valerate in dosage form. This method was validated as per ICH guideline Q2 (R1). Results suggest that this method can be used for routine estimation of Betamethasone Valerate in bulk and pharmaceutical dosage forms.

Key Words: Betamethasone Valerate, Toluene, Ethanol, *n*-heptane, Ethyl acetate, HPTLC.

INTRODUCTION

Betamethasone Valerate is a corticosteroid. It works by modifying the body's immune response to various conditions and decreasing inflammation. Betamethasone Valerate is official in Indian Pharmacopoeia. Literature survey reveals that some methods have been developed for their determination by HPLC, HPTLC or spectrophotometry either alone or in combination. However, overall cost of analysis of reported HPTLC method is more. In this view, an economical HPTLC method has been developed for estimation of Bethamethasone Valerate in pharmaceutical dosage form.

MATERIALS AND METHODS

Bethamethasone Valerate standard was provided by GSK pharmaceuticals, Nasik, India. "Betnovate 20 g" skin cream were procured from local market. AR grade of solvents used for this study were purchased from Merck Pvt. Ltd, Mumbai.

Preparation of standard solution: A standard stock solution of Betamethasone Valerate was prepared by dissolving 10 mg of standard API in 10 ml of methanol to get concentration of 1000 µg/ml. This solution was further diluted to get 100 µg/ml solution of Bethamethasone Valerate as working standard.

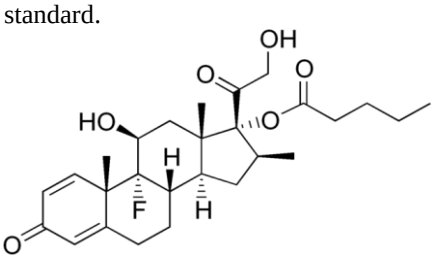

Fig. 1: Chemical Structure of Bethamethasone Valerate

Selection of wavelength for Detection: The working standard of Bethamethasone Valerate in methanol was scanned by Camag TLC scanner 4 with UV-visible detector over wavelength range 200 to 400 nm. Wavelength 246 nm was selected for further studies. (Figure 2).

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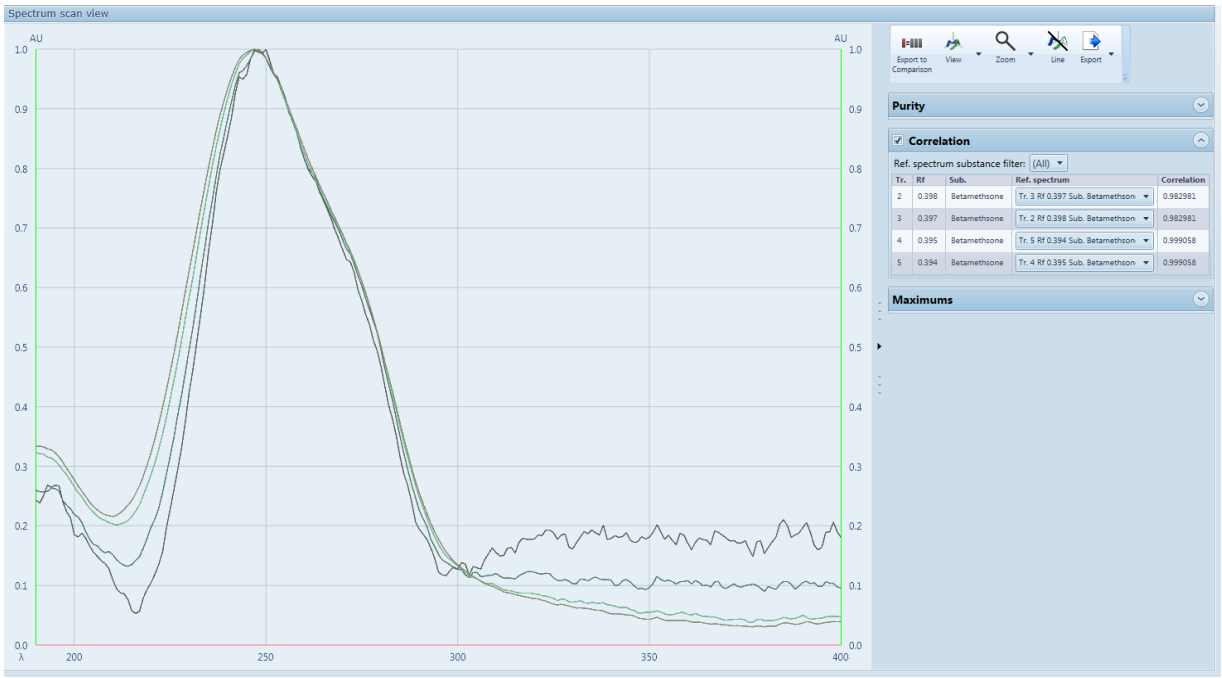

Fig. 2: The overlain UV spectra of 1000 µg/ml Bethamethasone valerate (API and sample) between 200 to 400 nm

Chromatographic Conditions: This analysis was performed on Camag HPTLC system (Switzerland). It is equipped with a Linomat-5 applicator, 100 µl sample syringe (Hamilton, Switzerland) and Camag TLC scanner 4. On the basis of trial and error method using different solvent system, following chromatographic conditions were chosen for analysis. Pre-coated silica gel 60 F254 TLC (E-Merck, Germany) plates (10x10 cm) were used as stationary phase. TLC plates were pre-washed with methanol and activated at 110°C for 10 min prior to application. The standard samples of Betamethasone Valerate were spotted on pre-coated TLC plates In the form of bands of length 4 mm using 100 µl sample syringe with a Linomat-5 applicator. The chromatographic development was carried using Ethyl Acetate: n- Heptane: Toluene: Ethanol (5.1:2.4:1.2:0.3v/v/v/v) as mobile phase with chamber saturation time of 20 minutes and the migration distance of 70 mm. Densitometric scanning was performed using Camag TLC scanner at 246 nm, operated by win CATS Software (Version 1.4.3, Camag).

Preparation of Calibration Curve: Different concentrations of the working standard solution were applied on the TLC plate, corresponding peak areas were recorded and linear regression was done between the peak absorbance *v/s* concentration. Finally, 1-9 µL range was selected for preparation of calibration curve and linear regression equation was obtained in this range (Figure 3).

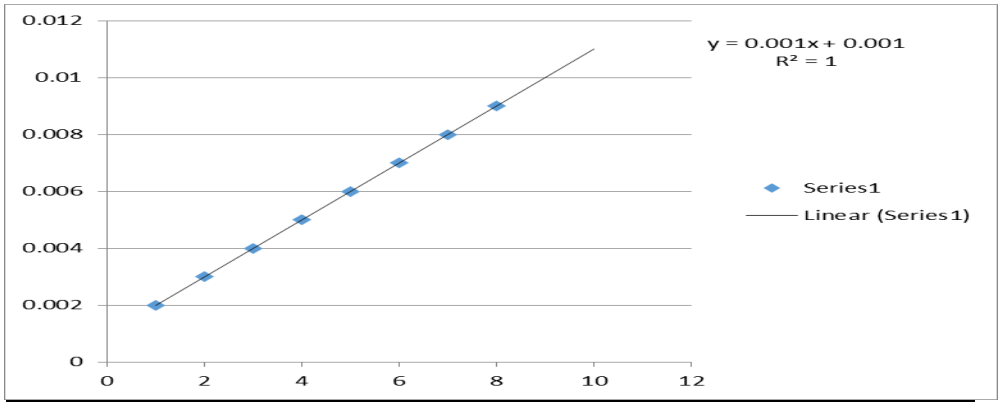

Fig 3. Calibration Curve of Betamethasone Valerate

METHOD VALIDATION

The objective of validation of an analytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity & Range, Precision, Limit of Detection (LOD) & Limit of Quantitation (LOQ) and Accuracy according to ICH Q2 (R1) guidelines.

Linearity and Range: The linearity was determined by using working standard solutions between 100-900 µg/spot. The spectra of these solutions were recorded at wavelength 246 nm. Calibration curve of peak absorbance *v/s* Concentration was plotted after suitable calculation and simple linear regression was performed. Regression equation and correlation coefficient were obtained. The range of solution has been decided according to statistical parameters of generated equation (Table 1).

Table 1. Linearity and Range of Betamethasone Valerate

Concentration µg/spot	Absorbance
100	0.0014
200	0.0025
300	0.0036
400	0.0047
500	0.0057
600	0.0066
700	0.0073
800	0.0083
900	0.0094

Precision

Repeatability: The precision of the method was checked by repeatedly injecting (n= 10) standard solutions of Betamethasone Valerate (500 µg/spot). Absorption of these solution was measured at 246 nm. Relative standard deviation (%RSD) was calculated (Table 2).

Reproducibility: The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days of same concentrations of 500 µg/spot of Betamethasone Valerate. The results were reported in terms of percentage relative standard deviation (%RSD). The results have been tabulated in (Table 3).

Limit of Detection (LOD) and Limit of Quantitation (LOQ): Nine sets of known concentrations (100-900 µg/spot) were prepared. Calibration curves were plotted for each set. LOD and LOQ were calculated using the regression equation (Table 5) and following formulae as;

LOD = 3.3 SD/S
LOQ = 10 SD/S

Where,
SD is standard deviation of y-intercept of the calibration curves

S is mean slope of five calibration curves

Table 2. Repeatability study of Betamethasone Valerate

Concentration (µg/spot)	Absorbance	Mean absorbance	%RSD
500	0.0039	0.00427	1.940
500	0.0042		
500	0.0042		
500	0.0042		
500	0.0041		
500	0.0041		
500	0.0042		
500	0.0045		
500	0.0045		
500	0.0048		

***n=10, % RSD = % Relative Standard Deviation.**

Table 3. Intraday and Interday Precision study of Betamethasone Valerate

Drug	Concentration (µg/spot)	% RSD	
		Intraday	Interday
Betamethasone Valerate	500	1.52	1.60
	500	1.16	1.87
	500	1.53	1.67

***n=3**

. **Table 4. LOD and LOQ of Bethamethasone valerate**

Drug	LOD	LOQ
Bethamethasone Valerate	0.97	2.96

Accuracy

Concentration taken in µg/spot (A)	Standard addition in µg/spot (B)	Total drug Concentration (µg/spot) (A+B)	Area	Average	% Recovery
200	160	360	5257	5372.667	96.06
			5530		
			5380		
200	200	400	5759	5622	85.89
			5583		
			5524		
200	240	440	6290	6290.333	84.23
			6337		
			6244		

Specificity: The specificity of the method was ascertained by analyzing standard drug and sample. The spot for drug in sample was confirmed by comparing the R_f and spectra of the spot with that of standard drug spot. The specificity of the method was also ascertained by peak purity profiling studies by analyzing the spectrum at peak start, middle and at peak end.

RESULTS AND DISCUSSION

The Calibration curve of Betamethasone Valerate was plotted as peak area *v/s* Concentration. The generated regression equation was y = 0.001x + 0.001 (R^2= 1). The R^2 value as 1 indicates that developed method was linear. The calibration curve was obtained in the range of 100-900 µg/spot. The proposed method was found to be precise as % R.S.D values for intraday as well interday precision were satisfactory. The drug at each of the 80%, 100% and 120% levels 96.06%, 85.89%, 84.23% showed good recoveries. Hence, it can be said that this method was accurate. The LOD and LOQ were calculated as 0.97 µg/spot and 2.96 µg/spot respectively. The result of the analysis of pharmaceutical formulation by the developed method was consistent with the label claim, highly reproducible and reliable. The method can be used for the routine analysis of the Betamethasone Valerate in formulation.

CONCLUSION

It can be concluded from the results that the proposed method was accurate, precise and consistent the determination of Betamethasone Valerate in formulation. This method was validated as per ICH guideline Q2 (R1). Results suggest that this method can be used for routine estimation of Betamethasone valerate in bulk and pharmaceutical dosage forms.

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