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Research Article

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Quantification of Betamethasone Dipropionate in Pharmaceutical Ointment by RP-HPLC

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Abstract A simple, specific, precise and accurate reverse phase HPLC method has been developed for the quantification of Betamethasone in pharmaceutical ointment. The chromatographic separation was achieved on Zorbax column, 150 mmx4.6 mm, 5 µm, using PDA detector. The mobile phase was 50% acetonitrile (v:v), at flow rate 1.0 mL/min. Active ingredient Betamethasone was detected at 230 nm, at 30ºC temperature for column. The retention time was 15.4 min. The method was validated according to the ICH guidelines with respect to specificity, linearity (r^2 =1), accuracy (99.5 to 100.1%), precision (RSD<2%).

Keywords Betamethasone Dipropionate; Ointment; RP- HPLC; Validation

Introduction

Betamethasone is one of the most significant topical corticosteroids. It belongs to synthetic corticosteroids. Its anti-inflammatory effect is 30 times greater than effect of cortisol. In dermatological preparations, it comes in various forms - as ester dipropionate, acetate, benzoate, valerate. In dermatological preparations, it comes alone or in combination with various antibiotics, antiseptics, keratolytics, keratoplastics, antifungals, antipsoriatics, chemotherapeutics, etc. When it comes alone , it is used in the treatment of diseases such as allergic skin diseases, namely acute, subacute and chronic forms of contact allergic dermatitis, occupational dermatitis, seborrheic dermatitis, atopic dermatitis (neurodermitis), diaper dermatitis, interring. It is also used in the treatment of acute and chronic non-allergic dermatitis, photodermatitis, x-ray dermatitis, psoriasis, pemphigus, lichen, and erythema and similar. In children, the absorption of a proportionally higher amount of topically administered betamethasone may result in a manifestation of systemic toxicity, which is why the use in children should be minimized. Topical administration of betamethasone on the skin can lead to a reduction in collagen content in the subcutaneous tissue and therefore cause atrophic changes in the skin, irreversible stretch marks, and prolonged therapy can lead to rashes, itching, local hirsutism, local hyperpigmentation as well as skin depigmentation, hair depigmentation and inhibition the function of the sebaceous glands, which calls into question the justification for their use [1, 2, 3].

United States Pharmacopoeia (USP) [4] describe HPLC method for estimation of betamethasone dipropinonate in pharmaceutical ointment, using internal standard.The accent of the experimental research to present and describes simple, sensitive, accurate and precise RP-HPLC method for determination of betamethasone.

Materials and Methods

Instruments

Instrumentation HPLC system (Agilent technology) consisting of gradient pump, Auto sampler, column oven and photodiode array detector (PDA, Agilent technology) was employed for analysis. Chromatographic data was acquired using chemstation software.

Reagents and Materials

Betamethasone dipropionate was supplied by USP. Acetonitrile (HPLC, Semikem), Milli-Q Water.

Chromatographic Condition

Zorbax column, 150 mmx4.6 mm, 5 µm was used as a stationary phase. The mobile phase consisting acetonitrile and Milli-Q Water in the ratio of 50:50 (v/v). The flow rate of the mobile phase was 1.0 mL/min. Detector signal was monitored at a wavelength of 230 nm. The column temperature was kept 30°C and injection volume was 20 µl.

Preparation of Standard Solutions

The standard stock solution was prepared in 0.08 mg/ml in 50% acetonitrile as solvent. From this solution, prepared are solutions for calibration curve from 0.008 mg/ml to 0.1 mg/ml in same solvent.

Preparation of Sample Solutions

Transfer a portion of ointment at about 0.75 g into capped 100 ml flask. Add 50 ml of solvent, heat in a water bath at 50°C, shaking intermittently until the sample melts. Then, remove from the bath and shake vigorously until the ointment has solidified. Pipette 5 ml of this solution into 10 ml flask and add solvent to the volume.

Validation of the Proposed Method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [5].

System Suitability

The system suitability test as per method should be performed and checked before performing any parameter (symmetry factor less than 2 and theoretical plates more than 1500).

Linearity and Range

A standard linearity solution was prepared to different concentration of 10%, 20%, 50%, 100% and 120% of the target concentration.

Method precision (Repeatability)

Five solutions were prepared from betamethasone dipropionate and they were injected by one analyst and analysed on same day.

Accuracy

The accuracy of the method was carried out at three levels in the range of 50%, 100% and 120% of the working concentration of sample.

Specificity

A blank preparation and standard preparation were prepared and injected.

Results

Separation was obtained by using Zorbax column, 150 mmx4.6 mm, 5 µm as a stationary phase at 30°C temperature and using a mobile phase 50% acetonitrile at a flow rate 1.0 ml/min and wavelength for detection was 230nm. Under these optimized conditions, the analyte peaks was well resolved and free from tailing. Symmetry is 0.90. The retention time is at about 15.4 minutes, and theoretical plates are 18063. Specificity of chromatograms shown in figure 1.

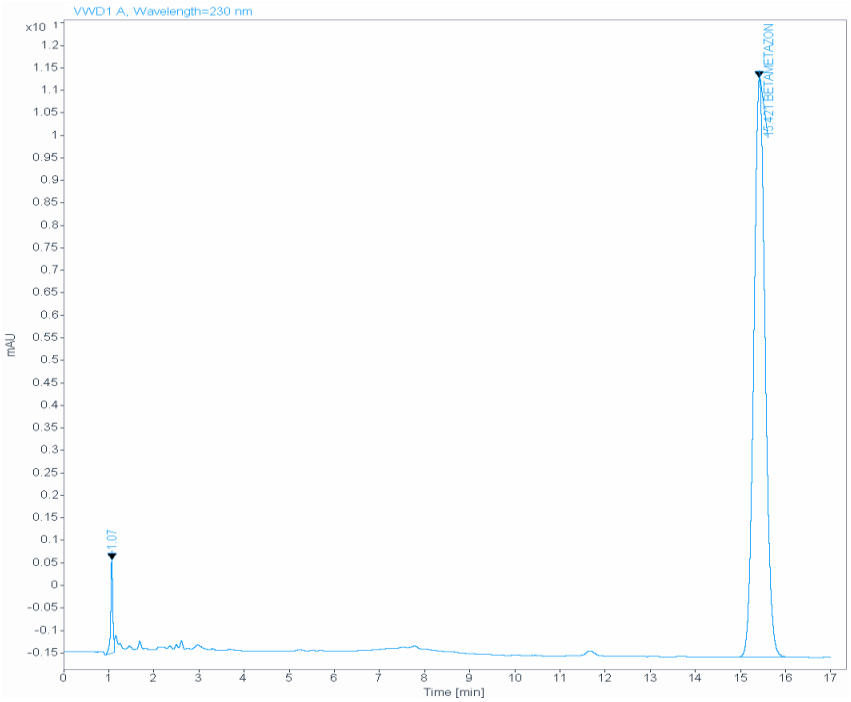

Figure 1: Chromatogram of Betamethasone peaks

Specificity of the chromatograms was checked for the appearance of any extra peaks. No chromatographic interference from ointment excipients was found (Figure 2).

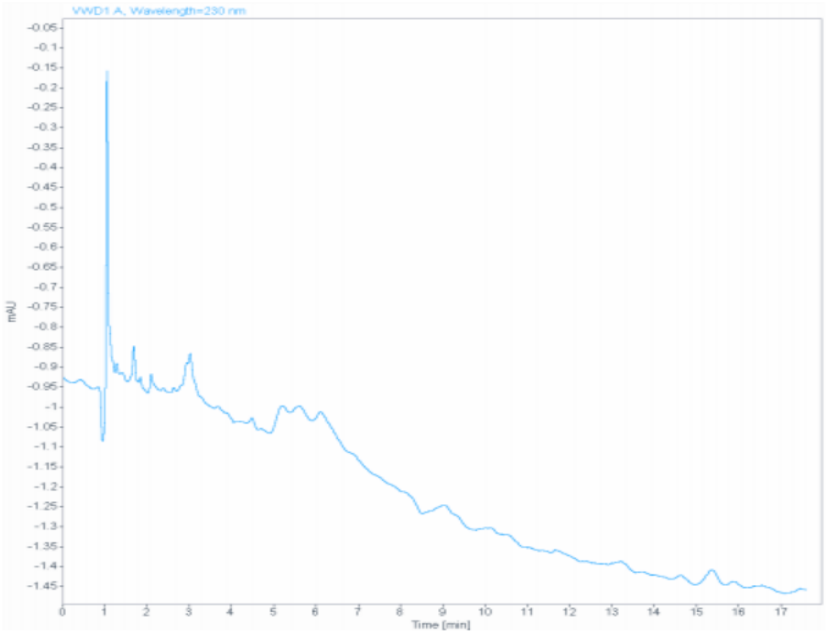

Figure 2: Chromatogram of solvent solution

Linearity was performed in the range of 0.008 –0.1mg/ml. The data for the peak area against the concentration were treated by linear regression analysis and the correlation coefficient value obtained was 1 (Figure 3).

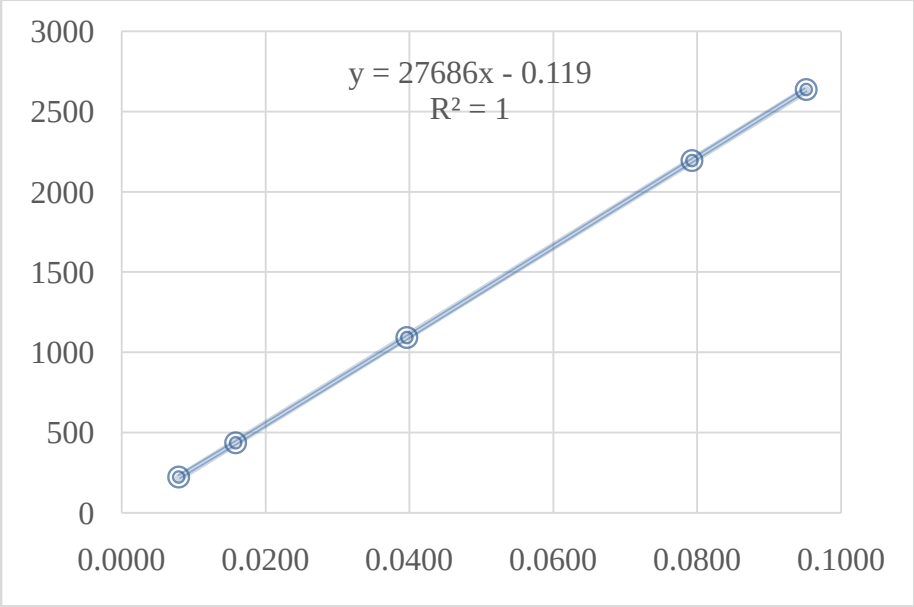

Figure 3: Linearity curve data

The accuracy was expressed as the percentage of analytes recovered by the assay method. It was confirmed from results that the method is accurate (Table 1).

Table 1: Accuracy data

%	**Betamethasone**
	Recovery (%)
50%	99.5
100%	100.0
120%	100.1
	99.9

In Precision, the relative standard deviations (RSD) for five replicate injections was 1.0%. Results for four samples are expressed as mg/g ointment and percentage and presented in table 3.

Table 2: Assay results

Sample No.	**Betamethasone**	
	mg/g ointment	**(%)**
Sample 1	0.53	106.9
Sample 2	0.45	90.0
Sample 3	0.52	104.9
Sample 4	0.49	98.5

Conclusion

Proposed RP-HPLC method is specific, accurate and precise for the quantification of betamethasone in pharmaceutical ointment. This method is simple and cost effective as it uses simple mobile phase. The method was validated as per ICH guidelines. All other parameters such as specificity, linearity, precision and accuracy, passes the criteria set by ICH guidelines. The described method is suitable for routine analysis of betamethasone in ointment.

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