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Research Article

STABILITY EVALUATION OF TOPICAL OINTMENT COMPRISING CALCIPOTRIOL AND PREDNICARBATE

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ABSTRACT

Stability studies ensuring the maintenance of product quality, safety and efficacy throughout the shelf life are considered as pre-requisite for the acceptance and approval of any pharmaceutical product. The objective of present investigation was to study the stability of a two-compound ointment containing Calcipotriol and Prednicarbate formulated using polyoxypropylene – 15-stearyl ether and Vitamin E Acetate when stored at different conditions for specific duration of time. Stability on storage is an important aspect which ensures the dosage form can exert the effects it is supposed to exert for the duration of storage. Appearance, drug content, pH and microbiological parameters are the parameters that have been assessed under different temperature to evaluate the stability of the ointment. The data obtained from the stability study showed that this formulation is stable for the 6 months period when stored at real time conditions.

Keywords: Calcipotriol, Prednicarbate, Polyoxypropylene-15 stearyl ether, Stability.

INTRODUCTION

Stability plays an important role in the drug development process. It explains several factors that affect the dating of drug products including physical and chemical stability. Stability of a drug can be defined as its ability in a specific container to remain within prescribed standards of physical, chemical, therapeutic and toxicological specifications to ensure the identity, strength, quality and purity of a formulation. Physico-chemical properties of pharmaceutical products may change during storage, but they are considered to be stable as long as their characteristics remain within the manufacturer's specifications. Stability testing is a key procedural component in the pharmaceutical development program for a new drug as well as new formulation[1]. Stability testing thus evaluates the effect of environmental factors on the quality of the a drug substance or a formulated product which is utilized for prediction of its shelf life, determine proper storage conditions and suggest labeling instructions to ensure that the medicine is safe and effective throughout its shelf life. Instability of the drug product may affect its purity, potency and safety which leads to unwelcome effects on patients. Stability testing therefore allows the establishment of recommended storage conditions, product shelf-life and expiry dating[2]. In general, the purpose of stability testing is to provide evidence of how the quality of drug product varies with time under the influence of some environmental factors like temperature, humidity and light that leading to set the shelf-life of pharmaceutical product. Shelf life is commonly estimated using two types of stability testing: real-time stability testing and accelerated stability testing[3-5]

Accelerated Stability Testing

Product is stored in proposed primary pack at elevated stress conditions i.e. temperature and humidity. The physicochemical and microbiological parameters are monitored throughout the study at predefined time intervals

Real-Time Stability Testing

Product is stored in proposed primary pack at recommended storage conditions. The physicochemical and microbiological parameters are monitored throughout the study at predefined time intervals.

MATERIALS AND METHODS

Calcipotriol Monohydrate was procured from D.K Pharmachem Pvt. Ltd., Prednicarbate was provided by Sun Pharma. Methanol (HPLC grade) and HPLC grade water was purchased from S. D. Fine chemicals.All microbiological medias were procured from Himedia Ltd. Nylon membrane filters (0.45 µm pore size) were purchased from Whatman International (UK). The apparatus and instruments were High performance liquid chromatography (HPLC- Waters), Centrifuge machine (REMI), pH meter (Electro lab).

Preparation of ointment formulation

In the topical treatment of psoriasis, often a combination treatment incorporating two or even more different pharmacologically active compounds is recommended. Thus, a topical pharmaceutical composition comprising of vitamin D analogue and topical corticosteroid would likely result in better patient compliance. Hence, an ointment containing Calcipotriol Monohydrate (equivalent to Calcipotriol) and Prednicarbate was formulated. Various trials were carried out using different solvents and antioxidants to achieve an ointment with optimized parameters as published in our earlier research work[6]. The optimized ointment formulation was prepared by dissolving Calcipotriol Monohydrate (equivalent to Calcipotriol) in solvent Polyoxypropylene -15-stearyl ether and then adding melted ointment base with antioxidant. Both phases were 70^0C. Prednicarbate was suspended in liquid paraffin and added to the mixture. The ointment was continuously stirred to ensure that both the drug substances were homogenously distributed. Batch was formulated and initial complete analysis was done. The ointment was filled in lacquered aluminum collapsible tubes and subjected to stability study.

Storage conditions for stability studies

The objective of this study was to evaluate stability of the drug product. The term stability refers to storage time allowed before any degradation product in dosage form achieves a sufficient level to represent a risk to the patient. Based on this time, the expiration date (shelf life) of a product is determined[7]. The active ingredients used in this formulation are Calcipotriol Monhydrate and Prednicarbate. Calcipotriol is sensitive to heat, light, acidic pH and oxidation[8]. Prednicarbate is light sensitive and may degrade at alkaline pH. The formulated ointment product was exposed to three different stability conditions to check the impact of different conditions on its characteristics as mentioned in Table 1.

Method of evaluation

Appearance

Appearance of ointment was evaluated by visually checking for clarity and texture.

pH

The pH of the ointment was determined by using digital pH meter.

Centrifuge Test

This test method is used to evaluate the accelerated deterioration of ointments. 6 g of ointment was filled in 10 ml-graduated centrifuge tubes. 6 tubes were subjected to spin at 4000 rpm for 10 minutes. The observations were recorded at each specified stability condition.

HPLC Analysis of the Drug contents

A reverse phase high-performance liquid chromatographic (RP-HPLC) method was developed for the analysis of calcipotriol and prednicarbate ointment. The concentrations were quantified by simultaneous estimation method using a Waters Alliance 2996 HPLC system with programmable auto sampler. The substances were separated on a Kromosil C18, (250 x 4.6 mm) 5 µ Isocratic illusion method using a detector wave length of 264 nm, injection volume of 50 µl and a flow rate of 1.0 ml/min. The mobile phase consisted of Water and Methanol (150:850 v/v). The samples were diluted with the mobile phase and filtered before drug content was determined. The retention time was around 5 minutes for prednicarbate and 7.5 minutes for calcipotriol as seen in the Figure1.

Preparation of standard solution (A)

25 mg of Prednicarbate working standard was accurately weighed and transferred into a 50 ml clean dry volumetric flask, about 20 ml of methanol was added and then the mixture was allowed to sonicate to dissolve the standard. Then the volume was adjusted with methanol.

Preparation of standard solution (B)

5 mg of Calcipotriol working standard was accurately weighed and transferred into a 100 ml clean dry volumetric flask, about 20 ml of methanol was added and then the mixture was allowed to sonicate to dissolve the standard. Then the volume was adjusted with methanol

Preparation of Mixed standard solution

5 ml of standard solution A and 1 ml of standard solution B was mixed in a 50 ml volumetric flask, and the volume was adjusted to 50 ml with methanol. This solution was used for chromatographic injection.

Sample Preparation

5 filled tubes of ointment were emptied in 100 ml beaker, mixed thoroughly with glass rod for 5 to 10 minutes. About 2 g sample was accurately weighed and transferred into 100 ml clean dry volumetric flask on water bath at around 50^0C to dissolve the ointment followed by sonication for 15 min. Then the mixture was allowed to cool at room temperature. Volume was adjusted with methanol. The solution was filtered through 0.45 µ membrane filter (Nylon) and used for chromatographic injection. Sample preparation and the standard preparation were injected separately and the responses for Calcipotriol and Prednicarbate were measured.

Microbial Evaluation

Total aerobic bacterial count

The test was performed by pour plate method on the optimized formulation. 10 g ointment was added to previously sterilized 90 ml of pH 7.0 buffered sodium chloride-peptone solution. 1.0 ml of above dilution was taken into two set (duplicate). 15-20 ml of sterile Casein soya bean digest agar which has been previously melted and cooled at 45^0C was added to two petri dishes in one set, for bacterial count. These plates were incubated at 30-35^0C and for 5 days with negative control. After incubation, the plates were observed and the number of colonies formed were counted and recorded[11,12].

Total Yeast and Moulds count

15-20 ml of sterile Sabouraud's Chloramphenicolagar (previously melted and cooled at 45^0C) was added to two petri dishes in one set, for yeast and moulds count. These plates were incubated at 20-25^0C for 5 days with negative control. After incubation, the plates were observed and the number of colonies formed were counted and recorded.

Test for pathogens

Test was performed to confirm the absence of pathogens i.e. *Escherichia coli, staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella* spp. in the ointment using the relevant medias.

Escherichia coli

To 10 g of ointment, 90 ml of buffered sodium chloridepeptone solution pH7.0 was added. 10 ml of this solution was inoculated with 100 ml of Casein Soyabean Digest Broth and incubated at 35-37^0C for 18-48 hours. The container was shaken, 1 ml was transferred to 100 ml of Mac-Conkey broth and incubated at 43-45^0C for 18-24 h. Sub culturing on plates of Mac-Conkey agar was done at 35- 37^0C for 18-72 hours. Growth of red, non-mucoid colonies of gram negative rods indicates possible presence of *E. coli*.

Staphylococcus aureus

To 10 g of ointment, 90 ml of buffered sodium chloride peptone solution pH 7.0 was added. 10 ml of this solution or the quantity corresponding to 1 g was inoculated with 100 ml of Casein Soya bean digest broth and incubated at 35- 37^0C for 18-48 h. Sub culturing on a plate of Baird –Parker agar was done and incubated at 35- 37^0C for 18-72 h. Black colonies of gram negative cocci surrounded by a clear zone indicate the presence of *S. aureus*.

Pseudomonas aeruginosa

To 10 g of ointment, 90 ml of buffered sodium chloride peptone solution pH 7.0 was added. 10 ml or the quantity corresponding to 1 g was to inoculated with 100 ml of Casein Soya bean digest broth and incubated at 35- 37^0C for 18-48 h. Sub culturing on a plate of Cetrimide Agar was done and incubated at 35- 37^0C for 18-72 h. If

no growth of micro organisms is detected, the product passes the test.

***Salmonella* Spp.**

To 10 g of ointment, 90 ml of Casein Soya bean digest broth was added and incubated 35- 37^{0}C for 18-24 h. 1 ml of the enrichment culture was transferred to 10 ml of Tetrathionate bile brilliant green broth and incubated at 41- 43^{0}C for 18-24 h. Sub culturing on deoxycholate citrate agar and xylose lycindeoxycholateagar was done and incubated at 35 -37^{0}C for 18 -72 h. The probable presence of *salmonella* was indicated by the growth of culture having the following appearance. Deoxycholate Citrate Agar: Well developed, colorless colonies. Xylose Lycin Deoxycholate Agar: Well developed red colored colonies with or without black centers.

RESULTS

Assay of both the drugs was performed in triplicate at all the stability intervals and the mean value was calculated. Standard deviation for assay of calcipotriol and prednicarbate is also shown in the chemical evaluation tables at respective stability conditions. No physical separation was observed during stability evaluation of the product at real time as well as accelerated stability conditions. The results of accelerated stability studies at 40^{0}C ± 2^{0}C/75 % RH ± 5 % RH through various stability parameters i.e. Physical and microbial are shown in Table 2. Physical evaluation parameters like pH, appearance, phase separation, were found to be acceptable at 1 month whereas the second month samples showed significant drop in the drug contents. Third month analysis was not performed as significant decrease in the drug content was observed during second month shown in Table 3. The result of 6 months real time stability studies at 25^{0}C ± 2^{0}C/60 % RH ± 5 % RH is mentioned in the Table 4. Physical evaluation parameters like pH, appearance, centrifugation, were found to be acceptable for a period of 6 months. The drug contents were also found to be within the acceptable limit shown in Table 5. Additional stability of the ointment was also studied at 5^{0}C ± 3^{0}C for 6 months. The physical, chemical and microbiological parameters of the ointment were found to be acceptable for a period of 6 months at 5^{0}C ± 3^{0}C as recorded in Table 6 and Table 7.

Table 1: Storage conditions for stability studies

Stability Conditions	Sampling Intervals			
40^0 C ± 2^{0}C/ 75 % RH ± 5 % RH	1 Month	2 Months	3 Months	6 Months
25^{0}C ± 2^{0}C/ 60 % RH ± 5 % RH	--	--	3 Months	6 Months
5^{0}C ± 3^{0}C	--	--	3 Months	6 Months

Table 2: Physical and microbiological evaluation of ointment stability at 40^{0}C ± 2^{0}C/ 75 % RH ± 5 % RH conditions

S. No.	Tests	Specification	Initial	1 Month	2 Month	3 Month	6 Month
1.	**Description**	White, translucent, homogenous, odorless ointments	Complies	Complies	Complies	Ointment turned slightly hazy.	ND*
2.	**Centrifuge Test.**	No separation	No separation	No separation	No separation	No separation	ND
3.	**pH**	4.5 – 6.5	5.54	5.86	6.31	ND	ND
4.	**Microbiological tests**						
a.	**Total aerobic bacterial count**	Not More Than 10^2/g	< 20 cfu/g	10 cfu/g	< 10 cfu/g	ND	ND
b.	**Total yeast and moulds count**	Not More Than 10^1 /g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	ND	ND
c.	**Pathogens**	Should be absent in 1 g	Absent	absent	Absent	ND	ND

*ND: Analysis not done

Table 3: Chemical evaluation of ointment stability at 40^{0}C ± 2^{0}C/ 75 % RH ± 5 % RH conditions

S. No	Time Interval	Calcipotriol		Prednicarbate	
		Mean Assay (%w/w)	**S.D****	**Mean Assay (%w/w)**	**S.D**
1.	**Initial**	99.87	0.305	101.10	0.458
2.	**1 Month**	94.70	0.556	96.90	0.436
3.	**2 Month**	92.07	0.404	94.53	0.208

**SD: Standard Deviation

Table 4: Physical and Microbiological Evaluation of Ointment Stability at 25^{0}C ± 2^{0}C/ 60 % RH ± 5 % RH Condition

S. No.	Tests	Specification	Initial	3 Month	6 Month
1.	**Description**	White, translucent, homogenous, odorless ointments	Complies	Complies	Complies
2.	**Centrifuge test**	No separation	No separation	No separation	No separation
3.	**pH**	4.5 – 6.5	5.54	5.68	5.75
4.	**Microbiological test**				
a.	**Total aerobic bacterial count**	Not More Than 10^2/g	< 20 cfu/g	< 10 cfu/g	< 10 cfu/g
b.	**Total yeast and moulds count**	Not More Than 10^1 /g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g
c.	**Pathogens**	Should be Absent in 1 g	absent	Absent	Absent

Table 5: Chemical evaluation of ointment stability at 25^{0}C ± 2^{0}C/ 60 % RH ± 5 % RH Condition

Time Interval	Calcipotriol		Prednicarbate	
	Mean Assay (%w/w)	**S.D**	**Mean Assay (%w/w)**	**S.D**
Initial	99.87	0.249	101.10	0.458
3 Month	97.73	0.208	97.03	0.153
6 Month	98.33	0.264	97.60	0.173

Table 6: Physical and microbiological evaluation of ointment stability at $5^0C \pm 3^0C$ Condition

S. No.	Tests	Specification	Initial	3 Month	6 Month
1.	Description	White, translucent, homogenous, odorless ointments	Complies	Complies	Complies
2.	Centrifuge test	No separation	No separation	No separation	No separation
3.	pH	4.5 – 6.5	5.54	5.63	5.68
4.	Microbiological test				
a.	Total aerobic bacterial count	Not More Than 10^2/g	< 20 cfu/g	< 10 cfu/g	< 10 cfu/g
b.	Total yeast and moulds count	Not More Than 10^1 /g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g
c.	Pathogens	Should be absent in 1 g	Absent	absent	Absent

Table 7: Chemical evaluation of ointment stability at $5^0C \pm 3^0C$ Condition

Time Interval	Calcipotriol		Prednicarbate	
	Mean Assay (%w/w)	S.D	Mean Assay (%w/w)	S.D
Initial	99.87	0.321	101.10	0.458
3 Month	98.57	0.208	99.60	0.264
6 Month	97.93	0.305	98.23	0.252

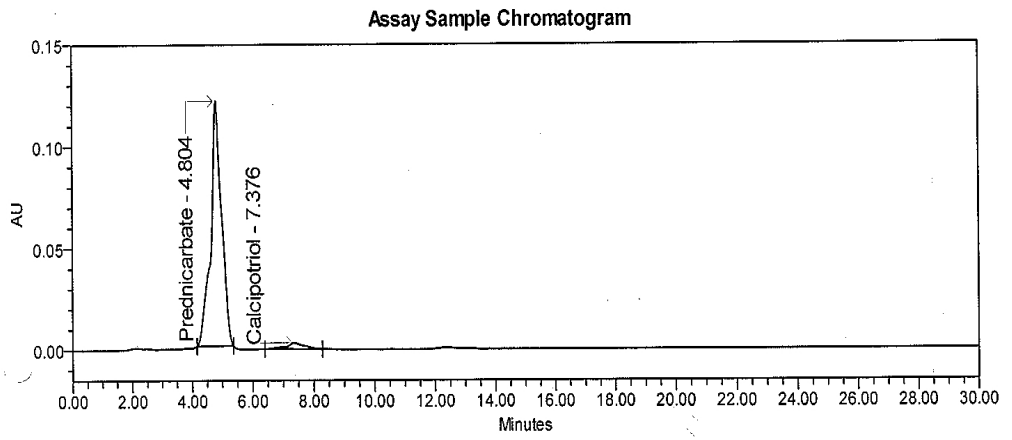

Figure 1: HPLC assay chromatogram showing the peaks of Calcipotriol and Prednicarbate

DISCUSSION

Stability studies are essential to yield quality product. The combination product comprising of Calcipotriol and Prednicarbate was exposed to different environmental conditions at $40^0C \pm 2^0C$/ 75 % RH ± 5 % RH, $25^0C \pm 2^0C$/ 60 % RH ± 5 % RH and $5^0C \pm 3^0C$ to evaluate the stability. The stability results of ointment were found satisfactory till 6 months at $25^0C \pm 2^0C$/ 60 % RH ± 5 % RH as well as at $5^0C \pm 3^0C$. At accelerated storage conditions, the physicochemical parameters were found to be satisfactory for one month whereas the drug contents were dropped significantly after two months of exposure. At third month, the appearance of the ointment turned slightly hazy when compared with the control sample hence product was not evaluated for the drug contents. The total aerobic microbial count (TAMC) is considered to be equal to the number of colony-forming units (cfu) found using soya bean digest agar. The total yeast and mould count (TYMC) is considered to be equal to the number of cfu found using Sabouraud Chloramphenicol agar. The total viable aerobic count is the sum of the bacterial count and yeast and mould count. The total aerobic microbial count was observed within the predefined limits throughout the stability. No pathogens were detected in the formulation at any stage of stability studies. The stability data also revealed that the ideal storage condition for the product should be below 25^0C, similar to the storage condition of commercially available combination product DAIVOBET containing Calcipotriol and Betamethasone (50 mcg and 0.5 mg/g).

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