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Formulation and evaluation of novel combined halobetasol propionate and fusidic acid ointment

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Abstract

Present investigation is to developed Novel ointment formulation in combination of Halobetasol propionate and Fusidic acid for the treatment of Atopic Dermatitis. Atopic Dermatitis is a noncontiguous skin disease, which can be prevent by administration of drugs through topical route having the ability to deliver a higher concentration of drug to the skin than would be possible with systemic therapy. Combination of Halobetasol propionate and Fusidic acid is good rational and novel for the treatment of Atopic Dermatitis, where inflammations reduce by Halobetasol propionate and secondary bacterial infection treated with Fusidic acid. Such formulation prepared by using multi-ingredients. To assess the efficacy of formulations assay, drug release, microbial activity, rheology, stability, spread ability, permeability and other physical characteristics were evaluated. Formulation containing water miscible base was found better than other formulation.

Key words: Ointment, Halobetasol Propionate (HP), Fusidic Acid (FA), Atopic dermatitis.

Introduction and Experimental

Eczematous diseases affect more than 10% of the general population and 15-25% of all dermatological patients suffer from Atopic Dermatitis. Children are more prone to the disease and a significant number of affected children continue to experience symptoms in their adulthood. Atopic Dermatitis is a chronic, relapsing noncontiguous disease and highly purities condition. Various complication e.g. keratosis, pilaris, itching vulgaris, chronic illness, bacterial, viral infection, erythrodomma, eye abnormalities occur due to integrity of the skin barrier compromised, lack of expression of antibacterial peptides on the skin and demonstration of increase adherence of microorganism to the skin patient

with Atopic Dermatitis[1,2]. Important treatment goals include providing moisture to the skin, preventing dryness and avoiding purities. As a result the occurrence of inflammation should concomitantly decline. While developing ideal topical dosage form must considered the factors like flux of drug across the skin, retention of dosage form on the skin surface, reservoir capacity of the dosage form and patients acceptability[3]. Although a large number of drugs are used for treating Atopic Dermatitis, there is either no scientific evidence to support their use or they have undesirable side effect. Thus combination in topical treatment with antibacterial and corticosteroid agent has been recommended. The different topical formulation developed using in combination of Halobetasol propionate and Fusidic acid as an ointment. The ointment formulation is generally applied to their weeping and oozing surface of skin. Antibacterial in combination with topical corticosteroid may produce more rapid decrease in S. aureus colonization than topical corticosteroids. Halobetasol propionate is a synthetic corticosteroid used to treat variety of skin condition e.g. eczema, dermatitis, allergies, rash in which reduces swelling, itching and redness. It is also acts as an anti-inflammatory and antipruritic agent[4]. Fusidic acid is bacteristatic antibiotics which treat of primary and secondary skin infection caused by sensitive strains of S. aureus, streptococci species and C.Minutissimum[5]. Acceptability and clinical efficacy of such preparations required them to posses optimal mechanical properties (ease of removal from the container, spreadability on the substrate), rheological properties (viscosity, elasticity, thixotropy, flowablity), and other desired property such as bioadhesion, desired drug release and absorption[6]. The objective of this research is to formulate a stable and good physical appearance ointment which is readily suitable for application.

Materials

Halobetasol propionate and Fusidic acid were received as a gift samples from M/s. Glenmark Research Center, Malegoan MIDC, Sinnar, Nashik, India. PEG 400, PEG 4000, Propylen glycol, White soft paraffin, white beeswax, Dehymuls (Dicocyl penta-erythrityl distearyl citrate, sorbiton sesquiolate and aluminum stearate) were obtained from M/s. S.D. Fine chemical Mumbai of pharmacopoeia quality. Bacterial Culture of Bacillus Subtilis, Staphylococcus aureus, Escherichia Coli and bacterioids fragilis, were obtained from Department of Microbiology B.K. College of Pharmacy, Sakoli.

Method for preparation of ointment

Formulation code-A

PEG 400, PEG 4000 and Propylene Glycol heat up to 70-72°C. Then dissolve Halobetasol propionate and Fusidic Acid in it under stirring and cool.

Formulation code-B

White Soft Paraffin, White Beeswax, Dehymuls E, Propylene Glycol heat up to 70-75°C to melt it completely. Then dissolve Halobetasol propionate and Fusidic Acid in it under stirring and cool.

Table No: 01

Formula for Ointment Formulation of Halobetasol Propionate and Fusidic Acid

Sr. No.	Name of ingredients	Formulation code	
		A (water miscible base)	B (Oleaginous base)
		% of formula	% of formula
1	Halobetasol Propionate	0.050	0.050
2	Fusidic Acid	2.00	2.00
3	White Soft Paraffin	--	86.950
4	White Beeswax	--	4.000
5	PEG 400	65.950	
6	PEG 4000	27.000	
7	Propylene Glycol	5.000	5.000
8	**Dehymuls E** (Dicocyl Pentaerythrityl distearyl citrate & Sorbitansesquioleate & Beeswax & Aluminum stearate).	---	2.000

Evaluation Parameters:

Description

Take about 1 gm of ointment in a clean glass dish and observe visually.

Identification

Halobetasol Propionate

Observe the retention time of the principle peak due to Halobetasol Propionate in sample preparation and standard preparation in assay[4].

Fusidic Acid

Observe the retention time of the principle peak due to Fusidic acid in sample preparation and standard preparation in assay[5].

Minimum Fill

Select a sample of 10 filled tubes, and remove any labeling that might be altered in Weight during the removal of the tube contents. Thoroughly cleanse and dry the outside of the tubes by a suitable means, and weigh individually. Quantitatively remove the contents from each tube, cutting the latter open and washing with a suitable solvent, if necessary, taking care to retain the closure and other parts of each tube. Dry and again weigh each empty tube together with its corresponding parts. The difference between the two weights is the net weight of the contents of the tube. Calculate the content of each tube and also calculate the average minimum fill[7].

Determination of drug content

The quantitative determination of Halobetasol Propionate and Fusidic acid was performed by High performance liquid chromatography (HPLC). A gradient HPLC (Shimadzu HPLC class VP Series) with LC-10 AT VP Pump, variable wavelength programmable UV/VISIBLE Detector SPD-10 A, VP,CTO-10AS Controller and RP-C18 Column Inertsil, ODS-3V, 250 x 4.6 mm, particle size 5 µ was used. Mobile phase was used a mixture of acetonitrile and buffer (containing 0.78% of sodium dihydrogen phosphate dehydrate pH adjusted to 5% orthophosporic acid) in ratio of 60:40 % v/v. The filtered mobile phase was pumped at a flow rate of 1.2 ml/min., column temperature was maintained at 40°C. The eluent was detected by UV detector at 238 nm and data were acquired, store and analyzed with software class ODS-3V[5,8].

Halobetasol Propionate stock solution

Weigh accurately about 25.0mg Halobetasol Propionate working standard and transfer into a 50mL volumetric flask. Add 35.0mL mobile phase and sonicate to dissolve. Dilute to the volume with mobile phase and mix. (Conc. of Halobetasol Propionate 500 µg/ml)

Preparation of Mixed Standard solution:

Weigh accurately about 40.0 mg Fusidic Acid working standard and transfer into a 100mL volumetric flask. Add 70.0mL mobile phase and sonicate to dissolve. Add 2ml of the above Halobetasol Propionate stock Solution. Dilute to the volume with mobile phase and mix

(Conc. of Halobetasol Propionate 10 µg/ml and Fusidic Acid 400 µg/ml)

Sample Preparation:

Weigh about 1.0 g ointment sample (Equivalent to 0.5mg of Halobetasol propionate drug and 20.0 mg of Fusidic acid drug) and transfer it into a 50mL volumetric flask. Add 35.0mL mobile phase. Sonicate it for 5-10 min. Heat the solution on boiling water bath at about 70° C for 50min or till the ointment melts completely. Cool to room temperature. Dilute to volume with mobile phase and mix. Chill the solution in ice bath for 15 minutes. Filter through 0.45µ nylon membrane filter.

(Conc. of Halobetasol Propionate 10 µg/ml and Fusidic Acid 400 µg/ml)

Consistency or hardness of an ointment:

The consistency or hardness of an ointment was measured by Penetrometer. Prepare the test samples by one of the following procedures:

- Carefully and completely fill three containers, without forming air bubbles. Level if necessary to obtain a flat surface. Store the samples at 25±0.5°C for 24 hrs, unless otherwise prescribed.

- Store three samples at 25±0.5°C for 24hrs. Apply a suitable shear to the samples for 5 min. carefully and completely fill three containers, without forming air bubbles, and level if necessary to obtain a flat surface.

- Melt three samples and carefully and completely fill three containers, without forming air bubbles. Store the samples at 25±0.5°C for 24hrs, unless otherwise prescribed.

Place the test sample on the base of the Penetrometer. Verify that its surface is perpendicular to the vertical axis of the penetrating object. Bring the temperature of the penetrating object to 25±0.5°C and then adjust its position such that its tip just touches the surface of the sample. Release the penetrating object and hold it free for 5sec. Clamp the penetrating object and measure the depth of penetration. Repeat the test with the two remaining containers[9].

Spreadability:

Spreadability of the formulation was determined by an apparatus suggested by Muttimer et al., which was suitably modified in the laboratory and used for the study. It consists of a wooden block, which was provided by a pulley at one end. A rectangular ground glass plate was fixed on this block. An excess of ointment (about 3 gm.) under study was placed on this ground plate. The ointment was then sandwiched between this plate and another glass plate having the dimension of fixed ground plate and provided with the hook. A 1 Kg. weight was placed on the top of the two plates for 5 minutes to expel air and to provide a uniform film of the ointment between the plates. Excess of the ointment was scrapped off from the edges. The top plate was then subjected to pull of 80 gms. With the help of string attached to the hook and the time (in seconds) required by the top plate to cover a distance of 10 cm. be noted. A shorter interval indicates better Spreadability[10].

Tube Extrudability:

It is a usual empirical test to measure the force required to extrude the material from tube. The method applied for determination of applied shear in the region of the rheogram corresponding to a shear rate exceeding the yield value and exhibiting consequent plug flow one such apparatus is described by Wood et al.

In the present study, the method adopted for evaluating ointment formulation for extrudability was based upon the quantity in percentage of ointment and ointment extruded from tube on application of finger pressure. More quantity extruded better was extrudability. The formulation under study was filled in a clean, lacquered aluminum collapsible tube with a nozzle tip of 5 mm opening and applies the pressure on tube by the help of finger. Tube extrudability was then determined by measuring the amount of ointment extruded through the tip when a pressure was applied on tube[11].

Viscosity of ointment and Rheological studies:

The viscosity of ointment was determined by CAP-2000 Brookfield viscometer. In a clean and dry 250ml beaker, take the test sample. Determine the viscosity of the test sample as per standard operating procedure of viscometer by using spindle nos. 1 to 4. Use each of the spindle's for finding out the viscosity of the sample at speeds of 0.3, 0.6, 1.5, 3, 6, 12, 30 and 60 r.p.m. respectively.

Record the dial reading and calculate the viscosity of test sample. Cream were tested for their rheological characteristics at 25°C using Brookfield viscometer (CAP-2000+ model).The measurement was Made over the whole range of speed setting from 10 rpm to 100 rpm with 30 second between two successive speeds and then in a descending order[12].

Figure-1: Rheogram for ointment formulation code-A

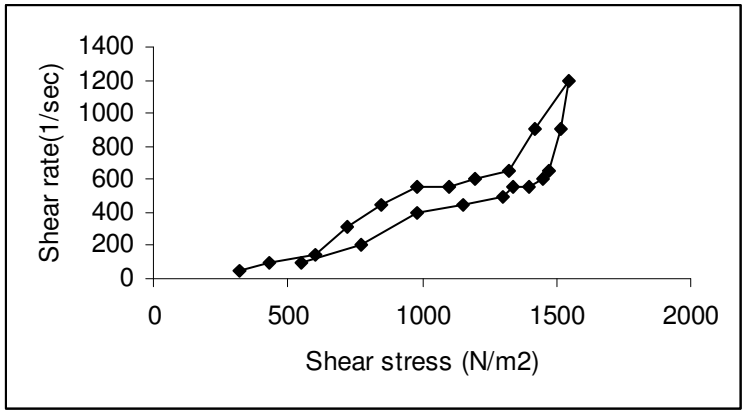
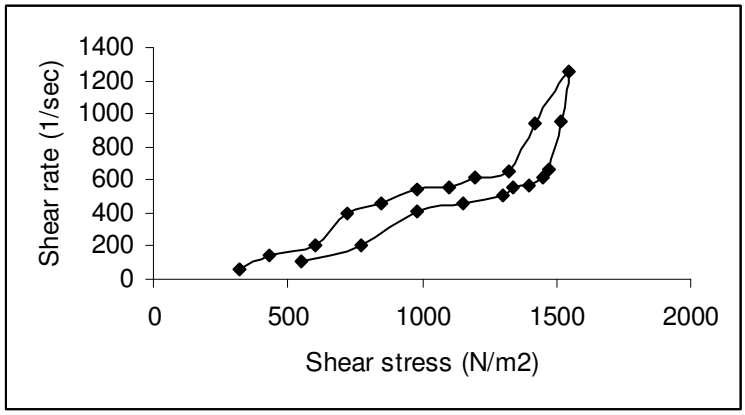


Figure-2: Rheogram for ointment formulation code-B



In-vitro diffusion study:

In modified kiescary chein diffusion cell, 2 gm of ointment kept in donor compartment. The entire surface of cellophane membrane was in contact with the receptor compartment containing 22 ml of phosphate buffer 2.5. The receptor compartment was continuously stirred (100rpm) using the magnetic stirrer. The temperature was maintained 37± 1°C. The surface area available for diffusion was calculated and found to be 3.14 cm^2. The study was carried out for 5 hrs and the sample was withdraw at 30 minute time interval and same volume was replaced with frees phosphate buffer. The content of Halobetasol propionate and Fusidic acid from withdrawn sample was measured after suitable dilution. The experiment was carried out in triplicate and average values are reported[13]**.**

Figure-3: Diffusion profile of Ointment formulation code-A

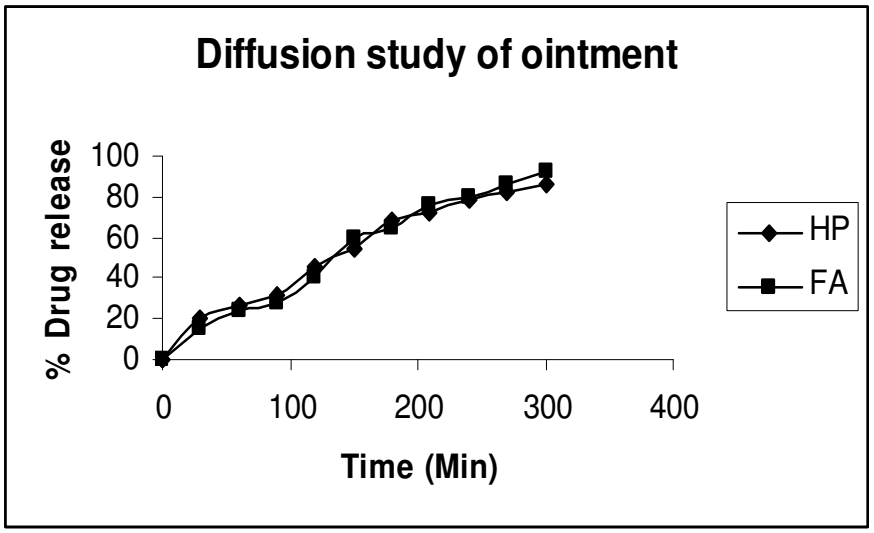
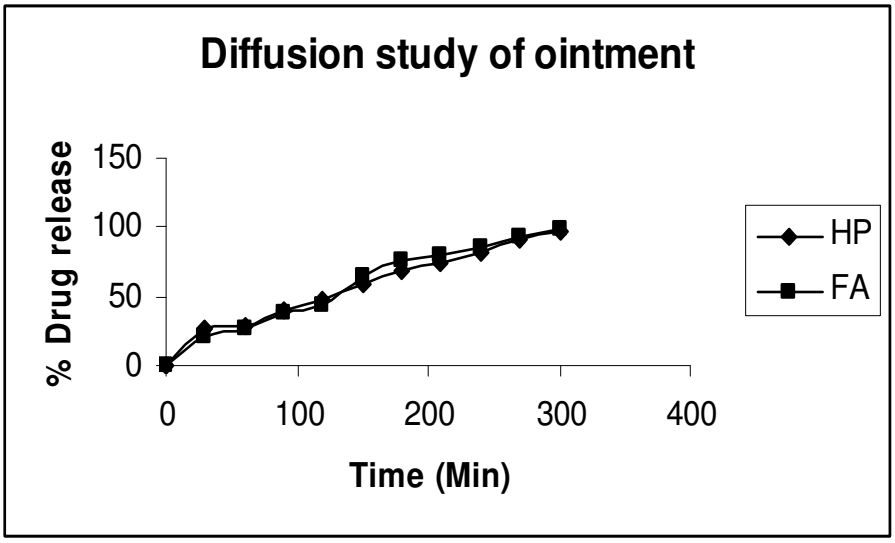


Figure-4: Diffusion profile of Ointment formulation code-B



Microbiological Studies:

The antibacterial activity of various ointment formulation of Halobetasol propionate and fusidic acid against various strain of anaerobic and aerobic microorganisms were evaluated by the standard cup plate method and the inhibition zone diameters were measured with the help of zone reader (Table no. 2). Bacillus subtilis, Staphylococcus aureus, Escherichia coli (aerobic organism) and bacterioids fragilis (anaerobic organism) were used for the testing of antibacterial activity. Nutrient agar media was used for aerobic bacterial culture and blood agar media was used for bacterial fragilis and incubated at Temperature of 37°C±2°C for 48 hrs[14].

Stability studies:

The International Conference on Harmonization (ICH) harmonized tripartite guidelines on stability testing of new drug substance and product was issued on October 27, 1993.

The formulated Halobetasol Propionate and Fusidic Acid ointments were filled in the collapsible tubes and stored at different temperature condition viz.25°C±2°C/60%RH±5%RH, 30°C±2°C/65%RH±5%RH, 40°C±2°C/75%RH±5%RH for a period of three months and studied for appearance, pH, viscosity, spreadability, extrudability and assay of drug[15].

Table No: 02

Antimicrobial activity of Halobetasol Propionate and Fusidic Acid Ointment

	Inhibition zone diameter , mm*			
Formulation code (Base)	Bacillus subtilis	Staphylococcus aureus	Escherichia coli	Becteroid fragilis
A (Oleaginous base)	31.54	31.33	31.11	30.15
B (Water soluble base)	36.42	34.24	32.21	35.12

- Values are average of three determinations

Result and Discussion

Management of Atopic dermatitis requires introducing treatment approaches that are effective and do not produce undesirable side effects. Atopic dermatitis manifested number of symptoms mainly purities, inflammation, redness and secondary bacterial infection. Therefore Formulation is one such exhibit combination therapy. In the present study combination Halobetasol Propionate and fusidic acid were used to asses the efficacy and favor to control of common symptoms of Atopic dermatitis. The composition of the ointment formulations were shown in Table no: 1. From the results, it is clearly evident that all formulations showed good extrudability, homogeneity and spreadability. Drug content of the formulations for Halobetasol propionate and Fusidic acid was well within the range between 102.18 % to 108.0 % and 99.76 % to 107.2 % respectively. The extrudability of ointment formulations from the collapsible tube, varies from 75.45% to 81.42 %, where as the results of spreadability varies from 5.03 to

6.71g.cm/sec.The viscosity of formulation ranged from 150cps to 226cps. The rheological behavior of all formulated ointment systems were studied and shown in figure-1to 2. Differences in concentration of base result in changes in occurring structure consistency. Shape of rheogram indicates are ease to apply. The release rate from cellophane membrane was observed. The amount of Halobetasol propionate and Fusidic acid release from all formulation studied and showed in figure-3 to 4. All the formulations were drug release in between 90 % to 97 % of both Halobetasol propionate and Fusidic acid up to the end of 5 hrs. Significant drugs release observed in formulation code-B up to end of 5 hrs and larger zone of inhibition observed in formulation code-B in comparison to other formulation

The developed ointment formulations were subjected to stability study as per ICH guidelines for the period of three months. The stability evaluation data were mentioned in Table no: 2. The physico-chemical characteristics and percent assay of drug in both the formulations were found to be satisfactory. From the data it is evident that, Halobetasol propionate and Fusidic acid ointment formulation containing water miscible base showed better in-vitro release profile and larger zone of inhibition comparison to oleaginous base. Formulation-B containing water miscible was found to be most satisfactory as comparable with formulation-A.

The conclusions were drawn from the results obtained in the present work of investigation, ointment formulation code-B exhibited stable and good physical properties.

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Table No: 03 : Stability evaluation data of Halobetasol Propionate and Fusidic Acid Ointment

Ointment	Formulation Code: A									
		25°C/60%RH			30°C/65%RH			40°C/75%RH		
	(0) Initial	1M	2M	3M	1M	2M	3M	1M	2M	3M
Appearance	White semisolid ointment	….	….	Complies	….	….	Complies	complies	complies	Complies
Consistency	226	….	….	224	….	….	224	225	224	224
Spreadability g.cm/sec	6.153	….	….	6.24	….	….	6.13	6.03	6.62	6.712
Extrudability (%)	79.45	….	….	79.23	….	….	79.14	80.10	81.26	81.42
% Assay Halobetasol Propionate Fusidic Acid (90-130%)	102.18 107.2	….	….	100.21 106.5	….	….	100.18 101.3	101.98 104.5	103.4 101.78	100.98 100.64

Ointment	Formulation code: B									
Appearance	White semisolid ointment	….	….	complies	….	….	complies	complies	complies	Complies
Consistency	150	….	….	152	….	….	163	168	171	176
Spreadability g.cm/sec	5.03	….	….	5.12	….	….	5.17	5.14	5.48	5.56
Extrudability (%)	76.60	….	….	75.45	….	….	75.61	76.13	76.65	76.78
% Assay Halobetasol Propionate Fusidic Acid (90-130%)	108.0 99.76	….	….	106.10 99.40	….	….	104.5 99.02	102.3 99.68	101.1 99.40	99.72 99.00

References

1. Larsen F.S. and Hanifin J.M., Secular change in the occurance of Atopic dermatitis, Acta Derm. Venerol suppl., 1992,176 ,7-12.
2. Larsen F.S., Atopic dermatitis; a genetic epidemiological study in a population based twin sample, J. Am Acad. Dermatol, 1993, 28, 719-723.
3. Lammintausta K, Kalimo K, Raitala R. And Forsten Y., Prognosis of atopic dermatitis, a prospective study in early adulthood, Int J Dermatol, 1991, 30, 563-568.
4. Halobetasol, MICROMEDEX, 2007.
5. British Pharmacopeia, volume-III, 2008, 2730.
6. Opaswong S., natural products in cosmetic, folk medicin, 2001, 265, 30-31.
7. Jones D. S., Woolfron A.D. and Brown A.F., texture, viscoelastic and mucoadhesive properties of pharmaceutical gels composed of cellulose polymers, Int. J. Pharm., 1997, 151, 223-231.
8. Indial Pharmacopeias, 1996, vol. 2, appendix 11.2,A-135.
9. Pharmacopeial Forum,The journal of standards development and official compendia revision, vol. 33, Number 6.
10. Wood J. H., Catacalos G. and Liberman S. V., Adaptation of commercial viscometers for special applications in pharmaceutical rheology-II, J. Pharm. Sci.,1963, 52, 375-378.
11. Shawn D. M., J Am Pharm Ass., 1949,38, 618-622
12. Kostenbauder H.B. and Martin A. N.,A rheological study of some pharmaceutical semisolid, J.Am. Pharm. Soc., 1954,43,401-407.
13. Vinod P. And Jeromes E., in-vitro release from corticosteroid ointment, JPS, 1995, 9, 84-87.
14. Bauer A.W., Kirby W .M ., Sherris J. C. And Truck M., antibiotic susceptibility testing by a standarized single disc method, Am J Clin pathol, 1966, 45(4), 493-496.
15. ICH Guidelines, Stability testing of Now Drug Substances and Products, 27th October 1993.

16. United States Pharmacopoeia, 24 NF (19), the United States Pharmacopoeia Convention Inc. USA, (2000).

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