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FORMULATION AND EVALUATION OF TOPICAL GEL FOR ANDROGENIC ALOPECIA

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ABSTRACT

Simple topical gel of newer class dosage form is of Finasteride and Terbinafine prepared with polymer Carbapol 934-P combinations with HPMC-K100, solvants used are ethanol and propylene glycol and water. Triethanolamine used to adjust the pH, propyl paraben added as preservatives. These formulations prevent the spread of Tinea capities as well as Androgenic alopecia and increase the hair count. In preformulation studies UV absorption of both drugs carried out, the drug compatibility study was carried out by FTIR. The formulated gel was evaluated by stability parameters like spreadability, extrudability, viscosity, pH, homogeneity, drug content determination, skin irritation test, antifungal activity. *Ex-vivo* Bioadhesive strength measurement was done of topical gel as compaired to cellophane membrane drug release from fresh goat hairless skin. The *In-vitro* drug release of Finasteride and Terbinafine carried out from 0 to 8 hours All formulated gel shows proper stability study even flow, clear gel without entrapment of air bubble and adhere long time on the scalp. During UV absorption and drug and drug release study formulation-2 shows maximum absorption at both spectrum and maximum drug release. Hence formulation-2 was the excellent formulation.

KAYWORDS: Finasteride, Terbinafine, Androgenic alopecia, Tinea capities.

INTRODUCTION

The topical administration of drug in order to achieve optimal cutaneous or percutaneous drug delivery has recently gained an importance They can avoid gastrointestinal drug

absorption difficulties caused by gastrointestinal pH .Gel is defined as a substantially dilute cross-linked system, which exhibits no flow when in the steady-state. It is the crosslink within the fluid that gives a gel its structure and contributes to stickiness. Hair growth is cyclic, with phases of growth (anagen), involution (catagen), and rest (telogen) 1-3 the cycles of active growth and rest are regulated by complex messages between the epithelium and the dermis In a normal scalp, most follicles are growing 90 to 95 %, a few are undergoing involution (less than1%), and the remainder are resting 5 to 10% 4-6. At the end of telogen, hair is released and shed and the next cycle is initiated. The duration of anagen determines the length of hair, and the volume of the hair bulb determines the diameter. Each day, up to 100 hairs in telogen are shed from the head and about the same number of follicles enters anagen. Androgenetic Alopecia is a common hereditary thinning of hair induced by androgens in genetically susceptible men and women.In susceptible hair follicles of the scalp, dihydrotestosterone, the potent metabolite of testosterone, binds to the androgen receptor. The hormone-receptor complex then activates the genes responsible for the gradual and progressive transformation of large, terminal follicles to miniaturized follicles. Fungal infection in hair scalp caused by the superficial infection. Infected hairs are broken and shorter. Papular lesions around hair shafts spread and form typical patches of ring forms, as shown. Terbinafine hydrochloride is a synthetic alkylamine drug with broad spectrum of antifungal activity. Tinea capitis, the most contagious of all the tineas caused by dermatophytes,Treatment produces inhibition of the isoenzyme, resulting in a rapid reduction in scalp and serum DHT concentrations.

MATERIAL AND METHODS

Finasteride and Terbinafine were received as a gift samle from Dr.Reddy's lab, Haidrabad, and Marksan,Goa Respectively, HPMCK-100 received from Sigma Aldrich, Banglor, Carbopol 934P,from Wockhardt pharmaceuticals ,Aurangabad, and propylene glycol, triethanolamine, ethanol (Absolute) from Sd fine-chem ltd, Mumbai

Formulation and Development

A topical gel of Finasteride and Terbinafine was prepared from binary solvent system of ethanol and propylene glycol.The penetration of Finasteride and Terbinafine into and through the skin generally increased as ethanol fraction of binary solvent vehicle was increased due to one or more combinations. Ethanol evaporates quickly and concentrates the drug in the residue of formulation that remains on the skin. It alters the physical integrity of stratum

corneum barrier resulting in an increase in the ability of drug to penetrate the skin. The water solubility of Finesteride as well as Terbinafine gel are very low, thus, at the beginning of work, solubility had to be increased. After setting and the composition of different gel formulations is shown in. For formulation I, Terbinafine and Finasteride was dissolved in a solvent mixture (ethanol: propylene glycol: water). The pH of above mixture was adjusted to 7.4 with triethanolanime. The solution was finally gelled by adding carbopol 934-P and HPMC-K100 carefully with constant stirring (Magnetic stirrer with hot plate) at 900-1000 rpm for 15 min, followed by the required quantity of propyl paraben as a preservative. After stirring, the beaker containing the gel was allowed to stand in a water bath (25°) for 30 min. The gels were stored in wide mouthed bottles. Entrapped air bubbles were removed by keeping the gels in vacuum oven for 2 hours.

Preformulation Studies

Solubility

The sample was qualitatively tested for its solubility in various solvents. It was determined by shaking 2mg of drug sample in 5ml of solvent (i.e, water, methanol, ethanol, ether, chloroform, etc .) in small test tubes and noted down the time require to solubilise the sample completely.

Identification of drug

UV Spectrophotometric analysis of Drug: Ultravoilet absoraption in the range200nm to 400nm 10 mg of Finasteride and 50 mg Terbinafine dissolved in methanol and distilled water.

Perparation of Reagent and Buffers

Preparation of Potassium Dihydrogen Phosphate

Dissolve 27.218 g of potassium dihydrogen phosphate in water and dilute with water to 1000 ml.

Preparation of Sodium Hydroxide, 0.2 M

Dissolve 8.0 g of Sodium Hydroxide in water and dilute with water to 1000 ml. The Phosphate buffer pH 7.4 was prepared by taking 250.0 ml of 0.2 M potassium dihydrogen phosphate in a 1000-ml volumetric flask, added 195.5ml of 0.2 M sodium hydroxide and then added water to make up the volume. In the present study, a spectrophotometric method based on ultraviolet absorption at λmax 210 nm was selected for quantitative estimation of drug.

Preparation of standard Curve of Finasteride in PBS pH 7.4

Accurately weighed 10mg of Finasteride was transferred into a clean and dry 100ml stopper volumetric flask and was dissolved in minimum volume of methanol. The volume was made to 100ml with PBS pH 7.4 to produce stock solution of 100ug/ml. From this solution 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0ml, were withdrawn separately in different 10ml volumetric flasks and volume was made in each case up to 10ml with PBS pH 7.4 to produce the concentrations 2, 4,6,10, 12,14,16,18, and 20µg /ml. Absorbance of these solutions was recorded at λmax 210nm against PBS pH 7.4 as blank using Shimadzu (1800) U/V visible spectrophotometer.

Preparation of standard Curve of Terbinafine in PBS pH 7.4

A solution of Terbinafine Hydrochloride of concentration 1000mg/ml was prepared by dissolving 50mg of pure drug in 50 ml of methanol: glass distilled water (70:30). The solutions were scanned in the U. V. range; the absorbance was measured at 283nm against reagent blank.Different dilution of terbinafine hydrochloride in range 0.5-3ml transferred into the series of 10ml volumetric flask and the volume was made up to the mark with distilled water to get concentration 5,10,15,20,25,and 30 µg/ml respectively. The solutions were scanned on spectrophotometer in the UV range 200-400 nm. The spectrum was recorded at 283nm.

Procedure for Evaluation of Gel

Spreadibility

The spreadibility of the formulations was determined by an apparatus suggested by Mutimer et al,It consists of a wooden block which would provided by a pulley at one end. By this method, spreadability was measured on the basis of „Slip‟ and „Drag‟ characteristics of gels a rectangular ground glass plate was fixed on the block. An excess of gels (about 2g) under study placed on this ground plate. The gel was then sandwiched between that plate and another glass plate having the dimensions of the ground plate and provided with the hook. A 1kg weight was placed on the top of two plates for five minutes to expel air and provide a uniform film of the gel between the plates. Excess of gel was scrapped off from the edges. The top plate was then subjected to a pull of 30gm with the help of a string attached to the hook and the time (in sec) required by the top plate to cover a distance of 5cm was noted. The spreadibility was calculated.

S=ml/t

Where, S = Spreadibility,

m = weight tied to the upper end.

Extrudability

The method adopted for evaluating gel formulation extruded from lacquered aluminium collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of gel in 10 seconds. It consist of a wooden block inclined at an angle of 45° fitted with a thin, ling metal strip (tin) at one end. While the other end was free. The aluminium tube containing 10gm of gel was positioned on inclined surface of wooden block 30gm weight was place on free end of the aluminium strip and was just touched for 10 seconds. The quantity of gel extruded from each tube was noted by following formula:

Extrudability = Applied weight to extrude gel from tube (in gm) / Area (in cm2)

Viscosity

The removable sample holder of the Brookfield Digital Viscometer was filled with the sample, and then inserted into a flow jacket mounted on the viscometer. A small sample adapter (RV-7 spindle), rotated at a speed of 0,30,60,90,120 rpm respectively, was used to measure the viscosity of the preparations. The temperature of the sample was kept at 30°C by circulating water through the thermo stated water jacket. The sample was allowed to settle for 5 min prior to taking the reading. Gel viscosity measurements were evaluated using a Brookfield digital viscometer by applying increasing values of the shear rate, in order to reveal possible flow behaviour of the gels.

P^H

P^H of formulation determined by dispersing 0.5gm of gel in 50ml of water. Checked using digital pH meter at constant temperature prior to this, the PH meter was calibrated using buffer solution of pH 4.0, 7.0 and 9.2, and then electrode were washed with demineralised water. The electrode was then directly dipped in to gel formulation and constant reading as noted.

Homogeneity

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

Drug content

A 500mg of Finasteride and Terbinafine Topical gel was taken and dissolve in 50 ml of phosphate buffer pH7.4.The volumetric flask were kept for 2 hours and shaken to mix it properly. The solution was passed through the filter paper and filter paper and filtered. The drug content was measured spectrophotometrically at 210nm and 283nm against corresponding gel concentration as blanks.

Drug content = (Concentration×dilution factor×Volume taken) ×Conversion factor.

Skin irritation test

The albino mice of either sex weighing 20-22gms were used for this test. The intact skin was used. The hair was removed from the mice 3 days before the experiment. The animals were divided into two batches and each batch was again divided into two groups. The gel containing drug was used on test animal. A piece of cotton wool soaked in saturated drug solution was placed on the back of albino mouse taken as control. T he animal was treated daily upto seven days and finally the treated skin was examined visually for erythema and edema.

Anti-fungal activity

This was determined by potato dextrose agar employing cup plate technique using previously sterilized petridish. Solution of gel formulation and pure Terbinafine as a standard 1 mg/ml in 100% dimethyl sulfoxide was poured into cups bored of size 8 mm in to wells of potato dextrose agar plate previously seeded with test organism (Microsporum canis). After allowing diffusion of solution for 2 hrs, the plates were incubated at 27^{O}C for 48 hrs. The zone of inhibition measured around each cup was compared with that of the standard. Each experiment was performed in triplicate.

***Ex–vivo* Bioadhesive Strength Measurement of Topical Gel**

Fresh goat hairless skin was obtained from a local slaughter house. The membrane was washed with distilled water and then with 0.1 N NaOH Two pieces of skin were tied to the two glass slide separately from that one glass slide was fixed on the wooden piece and other piece was tied with the balance on right hand side. The right and left pans were balanced by adding extra weight on the left hand pan. 1 gm of topical gel was placed between these two slides containing hairless skin pieces, and extra weight 61from the left pan was removed to sandwich the two pieces of skin and some pressure was applied to remove the presence of air.

The balance was kept in this position for 5 minutes. Weight was added slowly at 200 mg/ min to the left – hand pan until the patch detached from the skin surface. The weight (gram force) required to detach the gel from the skin surface gave the measure of bioadhesive strength. They were calculated by formula. h = Weight required (in gms) / Area (cm^2)

***In-vitro* diffusion studies for topical gels**

The in-vitro release was carried out with the cellophane membrane. It was washed in running water for three hours in order to remove glycerin, which was included as humectants in the membrane. Then the membrane was soaked in 90% alcohol for 24 hours. For the removal of sulphur from the membrane, was treated with 0.3%w/v sodium sulphide at 800c for 2 minute. The membrane was washed with warm water 600 c for 2 minute followed by acidification with 0.2%v/v solution of sulphuric acid and rinsed with hot water to remove the acid.The that study gels was carried out by apparatus consist of which was opened at both the donor compartment 1gm of gel formulation equivalent to 10gm of drug was spread uniformly on the surface of cellophane membrane (previously soaked in water for overnight). Whole assembly was immersed in a beaker containing 100ml PH 7.4 phosphate buffer with methanol which was placed in water bath and maintained at 37 ± 2°C, the contents was stirred using magnetic stirrer at 50 rpm. The amount of drug release was determined by withdrawing 1ml of sample at specific time intervals. The volume withdrawn was replaced with equal volume of fresh and pre warm (37^0c) phosphate buffer media containing methanol. The absorbance of the withdrawing sample was measured at 210nm and 283nm.

***Ex-vivo* evaluation**

The abdominal hair was shaved using Depilatory after sacrificing with excess chloroform inhalation. The abdominal skin was surgically removed and adhering subcutaneous fat was carefully cleaned.Epidermis then soak in 2M NaBr solution for 6-8 hrs, skins were allowed to hydrate for1hour before being mounted on the Keshary-Chien diffusion cell with the stratum corneum(SC) facing the donor compartment. The gel sample was applied on the skin and then fixed in between donor and receptor compartment of K-fajan diffusion cell.

Accelerated stability studies

All the selected formulations were subjected to a stability testing for three months as per The International Conference on Harmonization (ICH) norms at a temperature of 40º ± 2º. All selected formulations were analyzed for the change in Homogenicity, Extrudability, pH or drug content by procedure stated earlier.

RESULT AND DISCUSSION

Solubility

Finasteride freely soluble in ethanol, methylene chloride, Chloroform and Terbinafine solubilised in anhydrous ethanol and methanol.

Finasteride

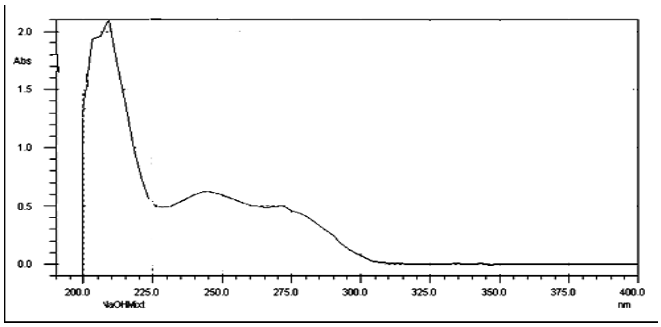

Fig. 1: UV Absorbance maxima of Finasteride.

Table 1: Concentration and Absorbance in PBS (λ max =210nm). Terbinafine.

Sr. No.	Drug Conc. (µg/ml)	Absorbance (observe)
1	2	0.0798
2	4	0.1042
3	6	0.239
4	8	0.3045
5	10	0.4103
6	12	0.48
7	14	0.558
8	16	0.6029
9	18	0.6788
10	20	0.754

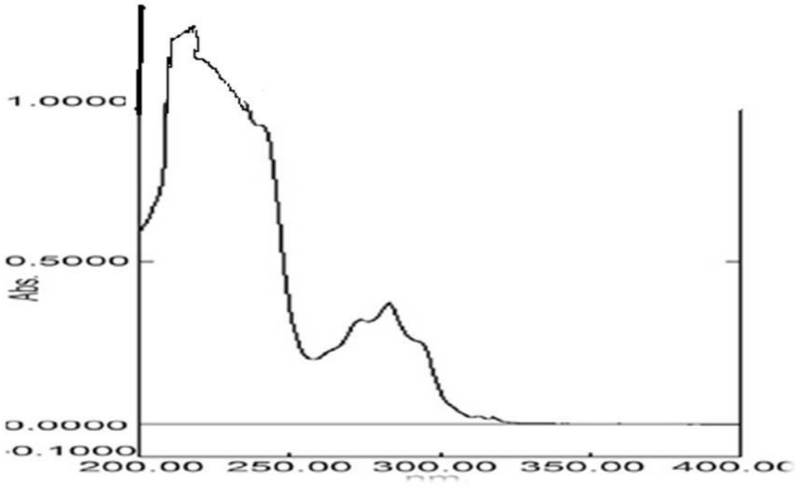

Fig. 2: UV absorption maxima of Terbinafine.

Table 2: Concentration and Absorption of PBS (λ max =210nm).

Sr .no.	Drug conc. (µg/ml)	Absorbance
1	0.5	0.001
2	10	0.027
3	15	0.036
4	20	0.056
5	25	0.065
6	30	0.086
7	35	0.102

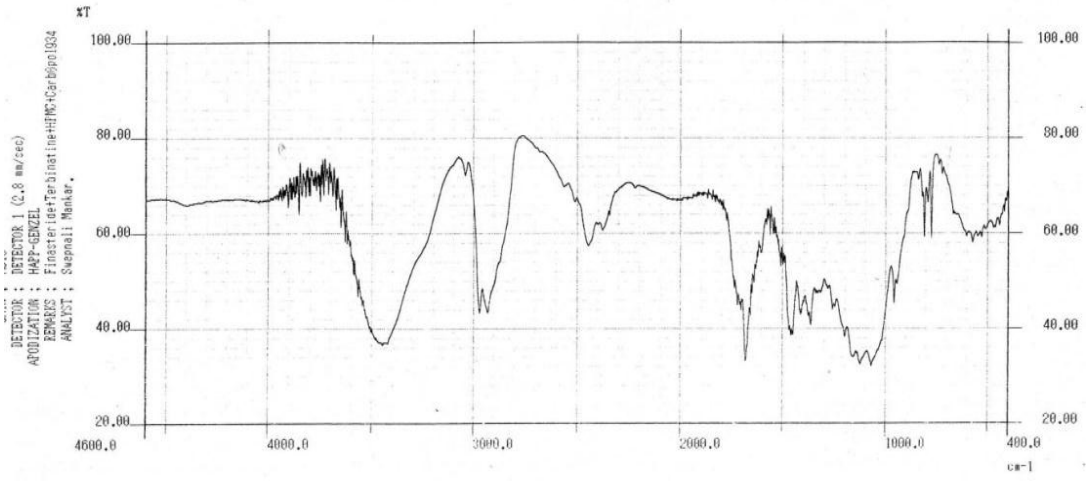

Fig 3: FTIR of Finasteride, Terbinafine, HPMC K-100 and Carbapol 934-P.

Compatibility Study: All substance is compatible with each other.

Spreadibility

The spreadability of simple topical gel was found to be S1= 11.5, S2 =15, S3 = 13.6, S4 = 10.7

Extrudability

The quantity of gel extruded from each tube was noted W1=0.60, W2=0.65, W3=0.63, W4=0.62.

P^H content

P^H checked by using digital pH meter at constant temperature. The P^H of F1, F2, F3 and F4 were found to be 7.5, 7.4, 7.6 and 7.5 respectively. All formulations show their P^H in between 7.3-7.8.

Homogeneity

By visual inspection all formulated gels should be clear, all drug uniform distribute

throughout the gel. They should not shown entrapment of air bubble inside it.

***Ex–vivo* Bioadhesive Strength**

From that formula the Bioadhesive strength of topical formulations were found to be F1=1.17gm, F2=1.85gm, F3=1.21gm, and F4=1.33gm.

Table 3: Result of evaluation Finasteride and Terbinafine topical gel Viscosity study.

Code	pH	Spreadability (gm/cm/sec.)	Extrudability (gm/cm^2)	Homogenicity	Bioadhesive Strength (gm)	Drug content (%)	
						210nm	283nm
F1	7.5	11.5	1.20	Clear	1.17	95	92
F2	**7.4**	**15**	**1.30**	**Clear**	**1.85**	**96.50**	**95**
F3	7.6	13.6	1.26	Clear	1.21	96	93.64
F4	7.5	10.7	1.24	Clear	1.33	92	92.70

Table 4: Viscosity study of Topical gel formulations.

Sr. no.	RPM	F1	F2	F3	F4
1	0	88.28	89.42	85.9	83.04
2	30	81.63	80.34	79.36	76.5
3	60	71.51	71.34	71.18	65.86
4	90	68.94	59.07	57.27	53.59
5	120	66.23	43.52	41.72	43.77
6	150	40.65	32.54	30.27	35.59
7	180	38.12	29.61	32.61	35.59
8	210	22.61	30.51	41.72	32.64

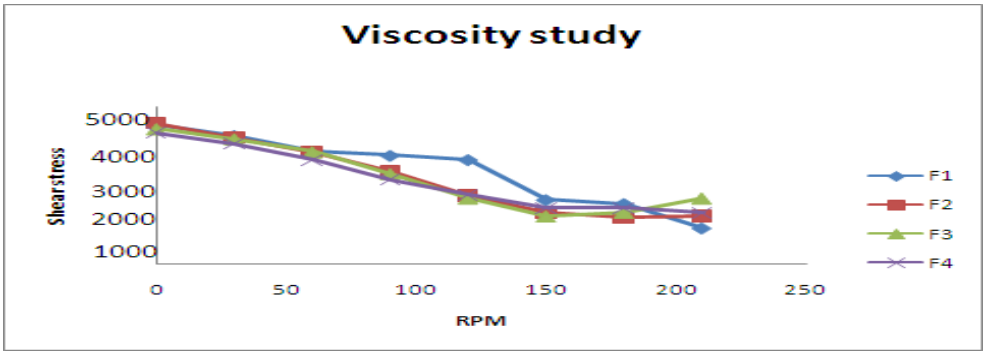

Fig. 4: Viscosity of Topical gel formulations.

In-vitro antifungal activity of terbinafine gel.

Table 5: Antifungal Activities.

Sr. No.	formulation Batches	Zone of Inhibition in cm (Mean±S.D. n=3)
1	Standard	2.25±0.2
2	F1	2.06±0.15
3	F2	2.10±0.05
4	F3	2.18±0.1
5	F4	2.21±0.2

Skin irritation test: The five albino mice should not shown inflammation, edema respectively.

***In-Vitro* drug release study**

Table 6: Observation of percentage drug release of topical gel.

Time	F1	F2	F3	F4
0	0.43	0.55	0.47	0.3
30	1.42	1.85	1.59	0.86
60	2.97	3.66	3.14	1.89
90	5.038	5.81	4.99	3.44
120	7.49	8.35	7.83	5.33
150	10.63	12.48	11.15	7.75
180	14.68	16.79	14.68	10.42
210	19.29	21.83	18.77	13.69
240	24.71	32.38	23.25	17.31
270	36.25	46.11	28.29	57.7
300	49.95	63.68	43.27	62.56
330	70.96	86.94	60.84	72.86

Table 7: Observation of percentage drug release of Terbinafine.

Time (min)	F1	F2	F3	F4
0	0.98	0.081	1.63	1.88
30	0.15	2.45	3.27	5.23
60	11.7	4.99	9.81	11.78
90	18.32	9.16	12.27	19.96
120	24.87	16.52	13.9	28.96
150	31.58	20.61	21.27	29.07
180	38.12	29.61	30.27	35.59
210	46.3	43.52	41.72	43.77
240	60.46	59.07	57.27	53.59
270	71.51	71.34	71.18	65.86
300	81.73	80.34	79.36	76.5

Table 8: Obsevation of *Ex-vivo* drug release Stability Study.

Time(hr)	F1	F2	F3	F4
0	0	0	0	0
1	18	21.3	20	12.13
2	21.04	37.13	31.84	21.59
3	36.12	45.06	42.04	30.64
4	49.5	51.74	46.23	39.89
5	63.85	62.04	56.89	52.1
6	76.41	76.58	61.2	61.56
7	81.03	84.12	75.31	74.01
8	83.12	92.98	79	77.39

***Ex- vivo* evaluation:** In Ex-vivo evaluation of topical gel the percentage drug release were found to be 83.12%, 92.98%, 79%, and 77.39%

Table 9: Stability study of various developed gel.

Sr.	Batches	Month	PH	Spreadability	Extrudability	Homogeneity
		1	7.2	12.9	1.23	Clear
		2	7.1	14.5	1.21	Clear
		3	7.2	11.6	1.10	Clear
		1	**7.4**	**15.24**	**1.29**	**Clear**
		2	**7.3**	**14.65**	**1.31**	**Clear**
		3	**7.4**	**16.47**	**1.30**	**Clear**
		1	7.1	11.64	1.22	Clear
		2	7.2	8.94	1.31	Clear
		3	7.6	15.6	0.95	Clear
		1	7.4	12.45	1.16	Clear
		2	7.6	10.336	1.18	Clear
		3	7.5	12.78	1.21	Clear

Table: 10 Stability study of Drug content present in Topical gel.

Sr.no.	Batches	Months	Drug content (%)	
			210nm	283nm
1	F1	0	83	86
		1	81	91
		2	83.40	90
		3	82.60	86
2	**F2**	**0**	**90**	**92**
		1	**84.60**	**91**
		2	**85.30**	**94**
		3	**85.40**	**89**
3	F3	0	82	85
		1	85	86
		2	79	83
		3	80	81.60
4	F4	0	81.60	82
		1	83.90	86
		2	82	85
		3	89	83