Research Article

Formulation, Development and *In-vitro* Evaluation of Terbinafine Hydrochloride Emulgel for Topical Fungal Infection

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ABSTRACT

The purpose of present research work was to develop an emulgel formulation of Terbinafine hydrochloride using carbopol 934 as a gelling agent for topical delivery with the aim to avoid hepatic first-pass metabolism, improve stability of emulsion, reduce dosage regimen and enhance residence time in the treatment of fungal infection. Emulgels have emerged as one of the most interesting topical drug delivery systems as it has dual release control i.e. emulsion and gel. The effect of concentration of the oil phase and emulsifying agent on the drug release was investigated using a 2^2 factorial design. The developed emulgels were evaluated for their physicochemical properties like color, homogeneity, consistency, spreadability, pH value, rheological behavior, drug content, drug release and stability. The Terbinafine hydrochloride emulgel formulation with the oil phase concentration 8%w/w and emulsifying agent concentration 3.5%w/w was the formula suggested as an optimized formulation by design expert software. Commercially available Terbinafine hydrochloride cream was used for comparison. All the prepared emulgels showed satisfactory physicochemical properties like color, homogeneity, consistency, spreadability, and pH value. The drug release was found to be higher for optimized formulation as compared to the marketed Terbinafine hydrochloride cream. The highest drug release was observed with E4, where the drug release showed 93.23% at 24hrs as compared to marketed Terbinafine hydrochloride cream was found 94.17% at 8hrs.The drug release from all the emulgels were found to follow diffusion-controlled mechanism. Stability studies indicated that the physical appearance, rheological properties, spreadability, drug release in all the prepared emulgels remained unchanged upon storage for 3 months.

Keywords: Emulgel, Emulsion, Factorial design, Gel, Terbinafine hydrochloride, Topical Drug Delivery.

INTRODUCTION

The field of pharmaceutical science has been developing steadily over the years, and has today become invaluable in helping to keep us healthy and prevent disease. An avenue of research that has progressed a great deal in the past few decades is the treatment of diseases via biomolecules such as drugs, proteins etc.[1] New techniques control the rate of delivery and duration of activity of the actives, thus reducing the frequency of dosing or application. They also help to target the delivery of an active to the tissue and control the undesirable effects of the actives. The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to promptly achieve and then maintain the desired drug concentration. Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. Skin is one of the most readily accessible organs on human body for topical administration and is main route of topical drug deliverysystem.[2]

Local actions include those at or on the surface of the skin, those that exert their actions on the stratum corneum, and those that modulate the function of the epidermis and/or the dermis. Approximately $2m^2$ and receives about one third of the blood circulating through the body. Topical drug delivery systems have been used for centuries for the treatment of local skin disorders, one side the topical applications of the drug offer the potential advantages of delivering the drug directly to the site of action and delivering the drug for extended period of time at the effected site that mainly acts at the related regions. On the other hand, topical delivery system increases the contact time and mean resident time of drug at the applied site leading to an increase in local drug concentration. When applied to diseased skin, topical drug products induce one or more therapeutic responses and the onset, duration and magnitude of these responses depend on the relative efficiency of three sequential processes namely, the release of the drug from the dosage form, Penetration of the drug through the skin barrier and generation of the desired pharmacological effect. As topical products deliver the drug directly to or near the intended site of action, measurement of the drug uptake into and drug elimination from the stratum corneum for the most part topical preparations are used for the localized effects at the site of their application by virtue of drug penetration into the underlying layers of skin or mucous membranes.[3] Both oil-in-water and water-in-oil emulsions are extensively used for their therapeutic properties and as vehicles to deliver various drugs to the skin. Emulsions possess a certain degree of elegance and are easily washed off whenever desired. They also have a high ability to penetrate the skin in addition, the formulator can control the viscosity, appearance, and degree of greasiness of cosmetic or dermatological emulsions.[4]

Oil-in-water emulsions are most useful as water washable drug bases and for general cosmetic purposes, while water-in-oil emulsions are employed more widely for the treatment of dry skin and emollient applications. Gels for dermatological use have several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non staining, compatible with several excipients, and water-soluble or miscible.[5]

Emulgels are emulsions, either of the oil-in-water or water- in- oil type, which are gelled by mixing with a gelling agent. They have a high patient acceptability since they possess the previously mentioned advantages of both emulsions and gels. Therefore, they have been recently used as vehicles to deliver various drugs to the skin. Emulgel is stable one and better vehicle for hydrophobic or water insoluble drugs.

Terbinafine is an allylamine which has a broad spectrum of antifungal activity in fungal infections of the hair and skin such as Pityriasis versicolor. It shows oral bioavailability is about 40% because of first pass hepatic metabolism.[6] So Terbinafine is increasingly administered by topical route may increase the bioavailability. Terbinafine is very slightly soluble in water so because of its hydrophobicity, emulsion can formulate. Emulsion is used both for hydrophilic and hydrophobic drug but stability is the major problem in case of emulsion. When gel incorporated in an emulsion can be overcome the stability problem of emulsion. Gel is having good absorption property along with greaseless, easily spreadability, easily removable, nonstaining and emollient but major limitation is delivering hydrophobic drug. The aim of present work was to develop an emulgel (combination of emulsion and gel) formulation of Terbinafine hydrochloride by using carbopol as a gelling agent. Emulgel has dual release mechanism due to emulsion & gel. The influence of concentration of both the oil phase and the emulsifying agent on the release of the drug from the prepared emulgels was investigated using 2^2 factorial design.

MATERIALS AND METHODS

Materials

The Terbinafine hydrochloride (B.P.) was received as a gift sample from ABIL chempharma Pvt. Ltd. Mumbai, India. Carbopol 934 (viscosity agent), Tween 20, Span 20, Methyl and Propyl Parabens, Liquid Paraffin, Propylene Glycol were purchased from Loba chemie, Mumbai, and alcohol purchased from Mahindra pure lab.Pune. All other reagents used were of A.R. grade.

Experimental Design

Optimization of Concentration of Gelling Agent, Oil Phase, and Emulsifying Agent

The concentration of gelling agent, oil phase and emulsifying agent are the independent variables and they were optimized by performing preliminary trial of emulgel. The carbopol concentration optimized by considering viscosity, pH and spreadability. From considering these parameter carbopol 1% shows good viscosity, spreadability and consistency, the optimized conc. are shown in table 1. The concentration of liquid paraffin were optimized by considering appearance & oil globule formation at the time of emulsion formation and also spreadability, viscosity considered after emulgel formulation. From considering these parameter, 6% & 8% conc. of oil phase shows oil globule formation, milkiness, good viscosity & Spreadability, the optimized conc. are shown in table 1. The various concentrations of span 20 & tween 20 i.e.1, 1.5, 2.0, 2.5, 3.0, 3.5% were optimized by considering appearance, phase separation & oil globule formation at the time of emulsion formation. From considering this parameter, 2.5% & 3.5% conc. of emulsifying agent shows good oil globule formation, milkiness, and longer phase separation, the optimized conc. are shown in table 1.

Table 1: Optimized concentration of carbopol, liquid paraffin, and span20: tween20

Parameter	Carbopol (%w/w)				Liquid paraffin (%w/w)				Span 20: Tween 20 (%w/w)					
	0.5	0.75	1.0	1.25	2	4	6	8	1	1.5	2.0	2.5	3.0	3.5
Spreadability (g.cm/s)	32.79	24.74	17.35	16.33	24.61	20.13	17.34	14.54	–	–	–	–	–	–
pH	6.12	6.14	6.17	6.25	_	_	_	_	_	_	_	_	_	_
Consistency	+	+	+++	++	_	_	_	_	_	_	_	_	_	_
Viscosity(cps) Max.100 rpm	3800	5000	7200	9600	5200	6400	7200	7600	_	_	_	_	_	_
Appearance (milkiness)	–	–	–	–	+	+	++	+++	_	+	_	++	_	+++
Oil Globule	_	_	_	_	No	No	Yes	Yes	No	No	No	Yes	No	Yes
Phase separation	–	–	–	–	10 min.	10 min.	20-25 Min.	40-45 Min.	10-15 min.	30-40 min.	10-20 min.	2hrs	25-30 min.	No up to 3hrs

{ (+)-Average, (++)-Good, (+++)-Excellent }

Factorial design

Various batches (E1-E4) of Terbinafine hydrochloride emulgel were prepared based on the 2^2 factorial design in which two independent factor i.e. Concentration of Liquid paraffin(X1), Span20: Tween 20(X2) and two level ie. High (+) & Low(-) which were optimized on the basis of preliminary trial. The concentration 6%(-)& 8%(+) w/w of Liquid paraffin whereas the concentration 2.5%(-) & 3.5%(+) w/w of span20: tween20 were taken as low and high level which are shown in table2. Also *In vitro* drug release (Y1) and spreadability (Y2) were taken as response parameters as the dependent variables.

Table 2: Quantitative composition of Terbinafine hydrochloride emulgel formulations (%w/w) as par 2^2 factorial design

Ingredients	Formulation code			
	E1	E2	E3	E4
Terbinafine hydrochloride B.P.	1.0	1.0	1.0	1.0
Carbopol 934	1.0	1.0	1.0	1.0
Liquid paraffin	**6.0**	**6.0**	**8.0**	**8.0**
Span 20	**2.0**	**1.5**	**1.5**	**2.5**
Tween 20	**1.5**	**1.0**	**1.0**	**1.0**
Propylene glycol	5.0	5.0	5.0	5.0
Ethanol	5.0	5.0	5.0	5.0
Methyl paraben	0.03	0.03	0.03	0.03
Propyl paraben	0.01	0.01	0.01	0.01
Triethanolamine	0.9	0.9	0.9	0.9
Purified water	q.s.	q.s.	q.s.	q.s.

Formulation of Terbinafine Hydrochloride Emulgel

The gel was prepared by dispersing Carbopol 934 in purified water with the help of magnetic stirrer and continues the stirring till the uniform solution was obtained. This uniform solution was neutralized at pH 6-6.5 with tri ethanolamine to form gel. The oil phase of the emulsion was prepared by dissolving Span 20 in liquid paraffin while the aqueous phase was prepared by dissolving Tween 20 in purified water. Methyl and propyl parabens were dissolved in propylene glycol whereas Terbinafine hydrochloride was dissolved in ethanol, and both solutions were mixed with the aqueous phase. Both the oily and aqueous phases were separately heated to 70 to 80°C then the oily phase was added to the aqueous phase with stirring continue for 15-20 minutes and cooled to room temperature. The obtained emulsion was mixed with the gel in 1:1 ratio with gentle stirring to obtain the emulgel.[7]

Solutions for Optimized Batch

After analysis of both independent variables and dependent variables by Design expert software (Design expert trial version 8.0.7 state Ease. Inc. Minneapolis USA) gave following solution (Table 3).

Composition of Optimized Terbinafine Hydrochloride Emulgel

The E4 emulgel formulation was optimized batch by suggested the design expert software in which Liquid paraffin (8% w/w) and Span20: Tween20 (3.5% w/w).

Characterization of Terbinafine Hydrochloride Emulgel

Physical Properties [8,9]

The prepared emulgel formulations containing Terbinafine hydrochloride were evaluated for various physicochemical parameters like color, homogeneity, consistency.

Measurement of pH [3]

The pH of developed emulgel formulations was determined using digital pH meter (Chemiline CL 180). 1g of emulgel was dissolved in 100 ml distilled water and kept aside for two hours. The measurement of pH of each formulation was done in triplicate and average values are calculated.

Spreadability [10]

It consists of a wooden block, which is attached to a pulley at one end. Spreading coefficient was measured on the basis of 'Slip' and 'Drag' characteristics of emulgels. A ground glass slide was fixed on the wooden block. An excess of emulgel (about 1 g) under study was placed on this ground slide. The emulgel preparation was then sandwiched between this slide and second glass slide having same dimension as that of the fixed ground slide. The second glass slide is provided with the hook. Weight of 100 g was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of the emulgel between the two slides. Measured quantity of weight (35g) was placed in the pan attached to the pulley with the help of hook. Time in seconds taken by two slides to slip off from emulgel and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, better the spreadability. It is calculated by using the formula.

$S = M. L / T$

Where, M = wt. tied to upper slide

L = length of glass slides

T = time taken to separate the slides

Rheological Study [10]

The viscosity of the developed emulgel formulations was determined by using a Brookfield viscometer (Brookfield Viscometer RVT) with spindle No.7.

Drug Content Determination

Terbinafine hydrochloride content in emulgel was measured by dissolving known quantity of emulgel in solvent (methanol) by Sonication. Absorbance was measured after suitable dilution at 283.2nm[11] using UV/VIS spectrophotometer (JASCO, V-630, Japan)

In-Vitro Drug Release Studies[4, 10]

The *In-vitro* drug release studies were carried out using a modified Franz diffusion cell. (With effective diffusion area 2.54 cm^2 and 20 ml cell volume) The formulation was applied on dialysis membrane (which was previously soaked in Acetate buffer pH 5.5 for 24 hours) which was sandwiched between donor and receptor compartment of the Franz diffusion cell. Acetate buffer pH 5.5 with 0.3% SLS was used as dissolution media. The temperature of the cell was maintained at 37±0.2°C by kept it in water bath. This whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead at 50 rpm. The samples (2ml aliquots) were withdrawn at suitable time interval and analyzed for drug content by UV visible spectrophotometer at 283.2 nm after appropriate dilutions.

Table 3: Solutions for Optimized Batch given by Design Expert Software

Liquid paraffin conc.(x1)	Emulsifying agent conc.(x2)	(%) Drug release(y1)	Spreadability (y2) (g.cm/s)	Desirability
8.00	3.5	92.54	12.75	0.799

Dissolution Model[13,14]

To study the release kinetics of in-vitro drug release, data was applied to kinetic models such as zero order, first order, Higuchi, Hixon-Crowell and Korsmeyer-Peppas.

Zero order

$$C = K_0 t \quad \text{..... (3.7.i)}$$

Where K0 is the zero-order rate constant expressed in units of concentration/time and t is the time in hrs.

First order

$$LogC = LogC_0 - K_t / 2.303 \quad \text{..... (3.7.ii)}$$

Where C_0 is the initial concentration of drug, K is the first order constant, and t is the time in hrs.

Hixson-Crowell

$$\sqrt[3]{Q_0} - \sqrt[3]{Q_t} = KHC \times t \quad \text{..... (3.7.iii)}$$

Where Qt is the amount of drug released in time t, Q_0 is the initial amount of the drug in the tablet, and KHC is the rate constant for the Hixson-Crowell rate equation as the cube root of the percentage of drug remaining in the matrix vs. time.

Higuchi

$$Q_t = K_t^{1/2} \quad \text{..... (3.7.iv)}$$

Where Q_t is amount of the release drug in time t, K is kinetic constant and t is time (h).

Korsmeyer Peppas

$$Mt / M\infty = Ktn \quad \text{..... (3.7.v)}$$

Microbiological Assay[4]

Ditch plate technique was used. It is a technique used for evaluation of bacteriostatic or fungistatic activity of a compound. It is mainly applied for semisolid formulations. Previously prepared Sabouraud's agar dried plates were used. Three grams of the emulgel are placed in a ditch cut in the plate. Freshly prepared culture loops are streaked across the agar at a rightangle from the ditch to the edge of the plate. After incubation for 18 to 24 hours at 25°C, the fungal growth was observed and the percentage inhibition was measured as follows

% inhibition = L2 / L1 × 100

Where L1 = total length of the streaked culture, and

L2 =length of inhibition.

Accelerated Stability Studies[12]

Stability studies were performed according to ICH guideline. The prepared emulgels (10 g) were packed in aluminum collapsible tubes and subjected to stored in hot air oven at 40°C/75% RH for a period of 3 months. Samples were withdrawn at 1 month time intervals and evaluated for physical appearance, pH, rheological properties, drug content and drug release.

RESULTS AND DISCUSSION

The present work aimed to increases stability of emulsion and to increases penetration through skin by formulating an emulgel with carbopol. The prepared formulations were characterized for physical appearance, pH, spreadability, viscosity, drug content and in- vitro drug release.

Measurement of pH

The pH values of all prepared formulations were ranged between 6.1 to 6.5 which are shown in Table 4.The formulation E3 & E4 which are considered acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 5.5.

Spreadability

The spreadability of various emulgel formulations is depicted in Table 4, from that it was concluded that E3 & E4 developed formulations showed satisfactory spreadability as compared to marketed cream.

Physical Appearance

The physical observation of prepared Terbinafine hydrochloride emulgel formulations are shown in Table 4.

Rheological Study

The measurement of viscosity of the prepared emulgel was done with Brookfield viscometer with spindle no.7. The highest viscosity was found in formulation E4 it may be due to high level of both the liquid paraffin concentration and emulsifying agent concentration. The

lowest viscosity was found in formulation E2 it may be due to low level of the liquid paraffin concentration and emulsifying agent. (Figure 1)

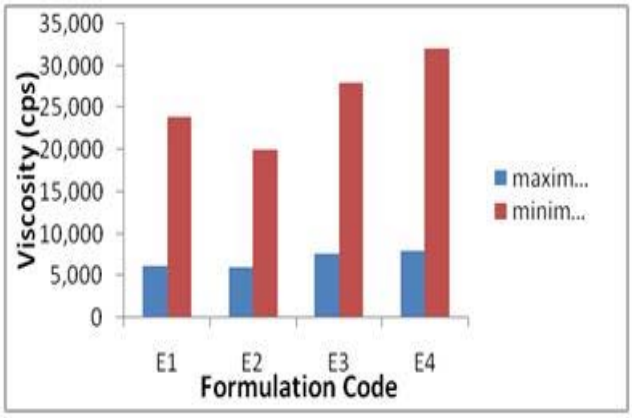

Figure 1: Viscosity of emulgel formulation

Drug Content Determination

Drug content was calculated using the equation, which was obtained by linear regression analysis of calibration curve of terbinafine hydrochloride in methanol. The drug content of all emulgel formulation is given in table 4.

In-Vitro Drug Release Studies

The *In-vitro* release profiles of Terbinafine hydrochloride from its various emulgel formulations is being depicted in Figure 2.It was observed that E4 batch got better release of the drug from all emulgel formulations. The release of the drugs from developed emulgel formulation can be ranked in the following descending order: E4 > E3 > E1> E2, Where the amounts of the drug release after 24hrs were 93.23%, 86.71%, 83.23%, 77.38% respectively. However 94.17 % of the drug was released from commercially available cream after 8hrs. Thus the higher drug release was observed with formulations E4, which was containing liquid paraffin in its high level and the emulsifying agent in its high level, this would be due to increase in the hydrophobicity of the emulgel, which in turn facilitates penetration of the release medium into the emulgel and diffusion of the drug from the emulgel.

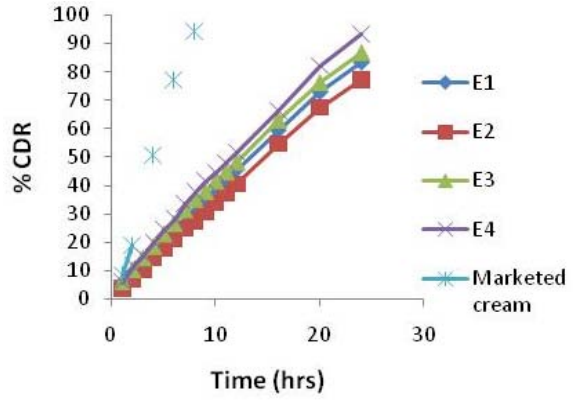

Figure 2: Release profile of Terbinafine hydrochloride from its emulgel formulations at 24hrs

Dissolution Model Study of Optimized Formulation

It was observed that all the formulation become liquefied and diluted at the end of the experiments, indicating water diffusion through the membrane. In the present study the release profile the value of R^2 is closer to 1 showing anomalous drug release, indicating that the drug release mechanism are diffusion follows the Peppas model.

Microbiological Assay

All emulgel formulations were microbiologically inert toward the tested *Candida albicans* strains showed by the use of control plates. The antifungal activity of Terbinafine hydrochloride in its different emulgel formulation as well as in its commercially available cream form shown in figure 3. Percentage inhibition activity was taken as a measure of the drug antifungal activity. The developed emulgel formulation can be ranked in the following descending order: E4 > E3 > E1> E2, Where the percentage inhibition found 45.67%, 42.81%, 39.25%, 36.28% respectively. However, 38.61 % of percentage inhibition was found in commercially available cream.

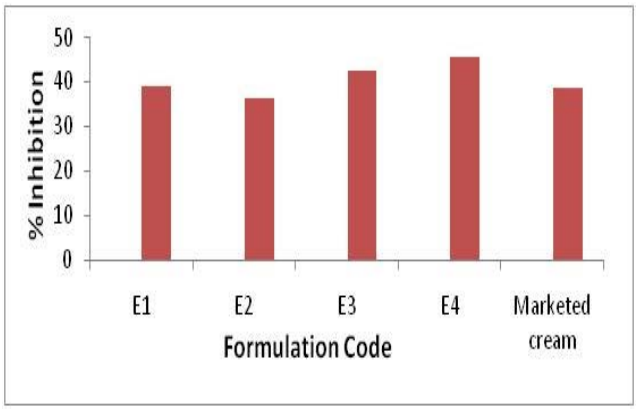

Figure 3: Percentage inhibition for the antifungal activity Terbinafine hydrochloride in its different Emulgel formulations compare with marketed cream.

Accelerated Stability Studies

Three stability batches of optimized formulations (E4) were evaluated at periodical intervals of time for 3 month accelerated storage conditions. The average pH (6.1 ± 0.O5), spreadability (14.25 ± 0.O9) remained relatively unchanged with no significant change in *In-vitro* drug release (93.35±0.72) after 3 months.

CONCLUSION

In the present study Terbinafine hydrochloride emulgel were prepared. Various variables such as the oil phase and the emulsifying agent were optimized by the factorial design. A 2^2 experimental design was employed to identify optimal formulation parameters for an emulgel preparation with the minimum value of spreadability and maximum value of *In-vitro* drug release. The optimized batch (E4) of emulgel with the liquid paraffin in its high level and the emulsifying agent in its high level proved to be the formula of choice, since it showed the highest drug

release, appropriate spreadability, good consistency and higher percentage inhibition. Also the prepared emulgel stable throughout during shelf life. Hence, the results of the present study clearly indicated promising potentials of emulgel as sustained release for delivering Terbinafine hydrochloride topically in the treatment of fungal infection and could be viewed as a potential alternative to conventional dosage forms.

Table 4: Physicochemical properties of emulgel formulations

Parameters	Emulgel Formulation				Marketed Terbinafine Hydrochloride cream
	E1	E2	E3	E4	
Color	White	White	White	White	White
Consistency	+	+	++	++	++
Homogeneity	+	+	++	++	++
pH	6.3	6.5	6.2	6.1	5.8
Spreadability(g.cm/s)	27.82	35.71	21.47	14.31	23.35
% Drug content	98.90	98.59	99.17	99.53	99.14

Table 5: Dissolution Model Study of Optimized Formulation

Optimized Formulation	R^2				
	Zero Order	First Order	Higuchi	Hixon Crowel	Korsmeyer peppas
E4	0.9964	0.8257	0.9801	0.7826	0.9996

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REFFERENCES

1. Bharadwaj S, Gupta G, Sharma V, Topical Gel: A Novel approach for drug delivery, Journal of Chemical, Biological and Physical Sciences, 2(6), 2012, 857.
2. Jain A, Gautam S, Gupta Y, Khambete H, Jain S, Development and characterization of Ketoconazole emulgel for topical drug delivery, Pelagia Research Library, 1(3), 2010, 222.
3. Jain A, Deveda P, Vyas N, Chauhan J, Development of Antifungal Emulsion Based Gel for topical fungal infection, International Journal of Pharma. Research and Development, 2(12), 2003, 18-19.
4. Khambete H, Deveda P, Jain A, Vyas A, Jain S, Emulsion for sustain delivery of Itraconazole for topical fungal disease, International Journal of Pharmacy and Pharmaceutical Sciences, 2, 2010, 105, 107.
5. Mohamed M, Optimization of Chlorphenesin Emulgel Formulation, AAPS PharmSciTech, 6(3), 2004, 1.
6. Dollery C, Therapeutic drugs, second edition, 2, 1999, T-41.
7. Panwar A, Upadhyay N, BairagiM, Gujar S, Darwhekar N, Jain D, EMULGEL: A REVIEW, Asian Journal of Pharmacy and Life Science, 1(3), 2011, 337- 338.
8. Bhanu P, Shanmugam V, Lakshmi P, Development And Optimization of Novel Diclofenac Emulgel For Topical Delivery, Pharmacie Globale International Journal of Comprehensive Pharmacy, 9(10), 2011, 1-4.
9. Bajaj A, Madan M, Amrutiya N, Formulation And *In Vitro* Evaluation Of Topical Emulgel Containing Combination of A Local Anaesthetic And An Anti-Inflammatory Drug, Indian Journal of Pharmaceutical Education and Research, 43(4), 2009, 352.
10. Khullar R, Kumar D, Seth N, Saini S, Formulation And Evaluation of Mefenamic Acid Emulgel For Topical Delivery, Saudi Pharmaceutical Journal, 2011, 2.
11. British Pharmacopoeia, London, 2, 2007, 1511.
12. International Conference on Hormonization (ICH) Guideline, Q1A (R_2), Stability testing of New drug substances and product.
13. Hamdy A, Ossama, YA, Hesham S, Formulation Of Controlled-Release Baclofen Matrix Tablets, Influence Of Some Hydrophilic Polymers on The Release Rate and *In Vitro* Evaluation, AAPS Pharm SciTech, 8(4), 2007, 1-11.
14. Hamid AM, Harris MS, Jaweria T, Rabia I, Once-Daily Tablet Formulation and *In Vitro* Release Evaluation of Cefpodoxime Using Hydroxypropyl Methylcellulose: A Technical Note, AAPS Pharm SciTech, 7(3), 2006, 4-5.

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