International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.1, No.4, pp 1297-1304, Oct-Dec 2009

TRANSDERMAL PATCH FOR KETOTIFEN FUMARATE (KTF) AS ASTHMATIC DRUG

Hemangi J. Patel*, Jitendra S. Patel, Keyur D. Patel
***Pharmaceutics Dept,Shree H.N. Shukla Inst. of Pharmaceutical Edu. and Research,Amargadh, Bhichri, Rajkot.GUJARAT, INDIA.**

Corre. Author: jiturx@gmail.com, Ph: +91 9426362316

RUNNING TITLE: TRANSDERMAL PATCH FOR KETOTIFEN FUMARATE

ABSTRACT: Ketotifen fumarate is almost completely absorbed from the gastro-intestinal tract following oral administration, but bioavailability is reported to be only about 50% due to hepatic first-pass metabolism. The present study aims to prepare Transdermal patch for Ketotifen fumarate as asthmatic drug. Preparation of standard curve for Ketotifen fumarate in solution of 20% w/v PEG 400 in normal saline. Preparation of transdermal patches of Ketotifen fumarate using polymers : Eudragit L-100 and Ethyl cellulose in combination with Hydroxypropyl methyl cellulose, plasticized with polyethylene glycol 400. The patches were evaluated for various parameters like Thickness, Water-Vapor Permeability, Tensile Strength, Drug Content, Diffusion and Dissolution studies. Prepared patches exhibited Zero Order Kinetics and the permeation profile was matrix diffusion type.
Keywords: Ketotifen fumarate, Eudragit L-100, Ethyl cellulose, Hydroxypropyl methyl cellulose.

INTRODUCTION

Ketotifen fumarate has the properties of the anti-histamines in addition to a stabilizing action on mast cells analogous to that of sodium cromoglycate. It is given orally as the fumarate in the prophylactic management of asthma, and also used in the treatment of allergic conditions such as rhinitis and conjunctivitis. Ketotifen fumarate is taken by mouth in dose equivalent to 1mg of Ketotifen twice a daily with food.[1] The pharmacokinetics of Ketotifen fumarate has been studied. It is completely absorbed from the gastro-intestinal tract following oral administration, but bioavailability is reported to be only about 50% due to hepatic first pass metabolism. Most of the Ketotifen is eliminated in liver as metabolites and only 1% of the intact drug is excreted from the kidney. Therefore, Ketotifen fumarate might be designed as a suitable delivery system with long term effect and bypass the liver, such as transdermal delivery system, the delivery system may have constant drug delivery rate to the circulation system and convenient use for children.[2]

Transdermal drug delivery system [3,4] is a therapeutic system designed to transfer drugs through intact skin for systemic treatment. It offers controlled drug release pattern by a simple application to the skin's surface, eliminating the vagaries influencing the gastrointestinal absorption associated with oral administration and providing for more efficient drug utilization. The development of modern transdermal drug delivery system has its antecedents in ancient medical practice. The Egyptians applied ointments, ruminants, plasters and exotic injunctions to the skin till nineteenth century. Throughout first half of the twentieth century many advances were made with regard to understanding of topical drug delivery for local effect. The findings accumulated over the years and practically revolutionized the old theory of an impermeable skin barrier and motivated a number of researchers to develop rate controlled drug delivery system for controlling the transdermal administration of drugs to accomplish the objective of systemic medication.

Ketotifen fumarate (KTF) belongs to tricyclic compound of benzocyclohepatathiopene class, is a non-specific, oral mast cell stabilizer. The prominent biochemical, pharmacological activities are H_1 antagonism, phosphodiesterase inhibition and inhibition of calcium flux in smooth muscle preparation. This is useful in allergic asthma and rhinitis. The drugs have value in prophylaxis of atopic asthma and having 50% oral bioavailability due to hepatic first pass effect in the liver. The low dose therapeutic and substantial biotransformation of Ketotifen fumarate makes if an ideal candidate for Transdermal drug delivery system. Further over a prolonged period sustained blood level of the drug is required for control of allergic asthma and other allergic syndromes. The aim of the present study is to develop a matrix type monolithic transdermal system for Ketotifen fumarate.

MATERIALS AND METHOD

Ketotifen fumarate was gift sample from Sun Pharmaceuticals Ltd., Silvassa. Eudragit L100, Ethyl cellulose (18-20cps) and Hydroxypropyl methylcellulose (15cps) was supplied as gift by S.D. Fine chemicals Ltd, Mumbai .All other chemicals were of analytical and pharmacopoieal grade from commercial suppliers.

Calculation of dose to be incorporated in the Transdermal Therapeutic system :

The normal dose of Ketotifen fumarate is taken by mouth in dose equivalent to 1mg of Ketotifen twice a daily. Since Ketotifen fumarate undergoes extensive first pass hepatic metabolism, its bioavailability is about 50%. Transdermal system is meant to bypass the hepatic inactivation.

1.38mg of Ketotifen fumarate is equivalent to 1mg of Ketotifen. Circular cast film contain 28.0mg of Ketotifen fumarate is needed.

Preparation of Patches:

The transdermal patches of composition listed in Table 1 were prepared by solvent casting method. The polymer (EL100-HPMC) and (EC – HPMC) were dissolved in Ethanol at room temperature. Polyethylene glycol 400 and penetration enhancers (Dimethyl sulfoxide and propylene glycol) added concentration as shown in the Table 1, with continuous mixing at lower rpm initially and later at a higher speed. The drug was incorporated with continuing agitation and the volume was made up. The films were cast onto a suitably designed and fabricated glass mould and then dried in oven at 40^{o}C for six hours in an oven. The films were removed by using sharp blade by inserting along the edges of the film. The dried films were wrapped in butter paper and stored in a closed container away from light and in cool place. [5]

Standard Curve of Ketotifen Fumarate:

Table 2 shows the absorbance readings of Ketotifen fumarate in 20% w/v PEG 400 in normal saline. Fig 1 shows the standard calibration curve with slope of 0.0307. The calculation of in-vitro skin permeation studies are based on this calibration curve[6].

Physical Appearance:

All the transdermal patches were visually inspected for colour, clarity, flexibility and smoothness.[7]

Measurement of Thickness:

Patch thickness was measured by a dial caliper (mitotoyo). The average of the five observations was calculated.[7] Result was shown in Table 3.

Weight Uniformity:

The dried patches were weighed on digital balance (Afcosit). The average of five observations of each formulation was calculated.[9] Result was shown in Table 3.

Folding endurance:

The folding endurance is expressed as the number of folds (no. of times the film is folded at the same place) either to break the specimen or the develop visible cracks. This test is important to check the ability of sample to withstand folding. This also gives an indication of a brittleness, less folding endurance indicates more brittleness. Folding endurance of the film was determined by repeatedly folding a small strip of film (2cm x 2cm) at the same place till it broke. The number of times, the film could be folded at the same place, without breaking gave the valve of folding endurance.[10] Result was shown in Table 3.

Tensile strength and % Elongation[11,12,13]

The films were taken in rectangular containers using proportionate quantity of the solution calculated on the basis of area. The films were cut into strips of 1cm width and 15cm length. The films were fixed onto the Tensile strength apparatus in such a way that the length of film between the jaws was initially 10 cm. The trials where the breakage occurred at the jaw were invalid and the result was repeated on another strip. The Tensile strength was calculated by the formula,

Tensile strength = Break force [1 + change in length] / (width) (breadth) [initial length of the film]

The percent elongation was determined by noting the length just before the break point and substituting the formula

% Elongation = [Final length - Initial length] /Initial length * 100

Result was shown in Table 4.

Water vapor permeability[11,12,13]

Glass vials of 5 ml capacity were washed thoroughly and dried to a constant weight in an oven. About 1 gm of fused Calcium chloride was taken in the vials & the polymer films were fixed over the brim with the help of an adhesive tape. Then the vials were weighed and stored in a humidity chamber at 85 % RH condition for a period of 24 hours. The vials were weighed after 24 hr to note down the weight gain. Result was shown in Table 5.

In-vitro drug release[11,12,13]

Chien diffusion cell was used in our studies for in-vitro drug release. The cell consists of two chambers, the donor and the receptor. The donor compartment is open at the top and is exposed to the atmosphere. The receptor compartment is surrounded by a water jacket for maintaining the temperature at 37 ^{o}C ± 2^{o} C and is provided with a sampling port. The diffusion medium was 20% w/v PEG 400 in normal saline which was stirred with magnetic bead (operated by a magnetic stirrer). A semi-permeable parchment paper previously soaked overnight in 0.1N HCL was placed between the two chambers. Diffusion media was stirred to prevent the formation of concentrated drug solution just beneath the membrane. Samples from the receptor compartment were taken at various intervals of time over a period of 24 hours and the concentration of the drug was determined by UV Spectrophotometric method using the standard curve. Amount of drug diffused at various time intervals was calculated and plotted against time.

RESULTS AND DISCUSSION

Physical Appearance of the polymers (Eudragit L100, Ethyl cellulose, Hydroxypropyl methyl cellulose) used for the fabrication of transdermal systems showed good film forming properties. These systems were thin, flexible, smooth and translucent. The method adopted for casting the systems was found satisfactory.

Thickness of the Patch was almost uniform in all 11 formulations and it was found to vary from 241 ± 4.183 to 261 ± 4.183 microns with low standard deviations

The weights of all transdermal patches were found to be uniform with their low standard deviation values. For each formulation, the weight of five patches was taken on a digital balance. The minimum weight was found for F1 with mean weight of 0.0983 ± 1.641 x 10^{-3} gm and maximum weight was found to be 0.162±2.138 x 10^{-3}gm for F3 formulation.

The formulation F11 showed the maximum elongation whereas the least value was found with F6. To impact flexibility to a polymer, the plasticizer effect of propylene glycol and dimethylsulfoxide shows the significant increase in percentage of elongation. The percent elongation at break was found to vary between 15.833 ± 1.443 to 133.33±3.819%. The patch prepared from Eudragit L-100 – HPMC (9:1) show more percentage elongation than the patches prepared from Ethyl cellulose – HPMC (3:7). This may be due to cellulose derivative have the less film forming property than Eudragit. The tensile strength measures the ability of a patch to withstand rupture. Presence of propylene glycol and Dimethyl sulfoxide has shown good tensile strength. Both the combination show significant tensile strength. The mean value was found to vary between 2.322 ± 0.067 to 4.248 ± 0.044 kg/mm^2.

For the various formulations prepared drug content was found to vary between 1.375 ± 7.095 x 10^{-3} to 1.391 ± 4.619 x 10^{-3} mg. The cumulative percentage drug permeated and percentage drug retained by the individual patch in the in-vitro skin permeation studies were based on the mean amount of drug present in the respective patch.

Figure III & IV shows the plot of cumulative percentage of Ketotifen fumarate permeated as a function of time for EL-100-HPMC & EC-HPMC formulation respectively. The graph gives the comparison between formulation with and without permeation enhancers.

For EL-100 – HPMC, F1 was without permeation enhancer, whereas F2, F3, contained DMSO, F4, F5 contained propylene glycol and F11 contained DMSO and Propylene glycol. In case of F1 formulation, 44.739% of the drug was released and permeated through albino mice abdominal skin at the end of 24th hours. F2 & F3 containing DMSO as permeation enhancer of 7.5% w/w and 15% w/w respectively, show increase in permeation rate. 47.434% and 63.347% of drug was released for F2 & F3 respectively at 24th hour. Whereas, for F4 & F5 containing propylene glycol as permeation enhancer of 5% w/w and 10% w/w, permeation rate was 45.709% and 61.716% respectively at 24th hour. Formulation F11 contains both DMSO and Propylene glycol as permeation enhancer at concentration of 15% w/w and 10% w/w, and the permeation rate was found to be 71.636%.

The results of F2 to F5 show that there is an increase in permeation rate as compared to F1. It gives a permeation effect of DMSO and PG. In this DMSO has got more permeation than PG and increase in concentration, there is an increase in permeation of both. But because of more controlled release from EL-100 – HPMC patch the amount of drug permeation was non-significant, so for this an attempt was made to formulation (F11) contain both DMSO and PG, at 15% w/w and 10% w/w respectively. Above this concentration the patch was unable to be handled. From this formulation shows the significant increase in permeation rate of 71.636% due to synergistic effect of penetration enhancer.

For EC-HPMC, F6 was without enhancer whereas F7 & F8 contain DMSO at 7.5% w/w and 15% w/w concentration respectively. F9 & F10 contain PG at 5% w/w and 10% w/w concentration respectively. In case of F6, 72.109% of the drug was permeated at the end of 24th hour. F7 & F8 contains DMSO as enhancer, the permeation rate was 83.37% and 95.285% respectively whereas F9 & F10 contains PG as enhancer, permeation rate was 81.836% and 91.384% respectively.

The results of F7 to F10 shows an increase in permeation rate than F6. In this the patch contain DMSO gives more release than PG. But DMSO at 15% w/w and PG at 10% w/w concentration gives significant increase, that is, more than 90% of the drug at 24th hour from EC-HPMC patch.

From the above results, DMSO and PG, both belongs to class, organic solvents as penetration promoters, enhances the drug diffusivity through the skin by affecting the intracellular lipids or the intracellular proteins and increasing the partitioning of the drug into stratum corneum. And also miscibility and the solution properties of the solvent used could be responsible for the enhanced transdermal permeation of the drugs.

EL-100 – HPMC (9:1) patches: The lesser release from these patches than EC-HPMC patches may be attributed to the relatively hydrophobic nature of polymers which have less affinity to water. This results in decrease in the thermodynamic activity of drug in the patches and decreased release.

EC-HPMC patches (3:7): The EC-HPMC patches showed a higher rate and extent of release due to that is more than 90% was observed in F8 & F10 at 24th hour period of study. This may be due to the following reasons. Presence of higher portions of HPMC (7 parts) which is more permeable than Eudragit L-100. And high hydrophilic polymer (HPMC), which increases the thermodynamic activity of the drug in the patches, hence increases the release. Being highly hydrophilic, the polymer readily absorbs water and swell, resulting in

formation of large pores. This was confirmed by the water vapor transmission study.

Table 6 shows the skin permeation rate of Ketotifen fumarate through hairless mice abdominal skin. The skin permeation rate from F1 & F6 without permeation enhancer was 8.188 ± 0.465 μg/cm^2/hr and 13.215 ± 0.279μg/cm^2/hr respectively. Of the enhancer DMSO, used in 7.5 and 15% w/w concentration in F2, F7 and F3, F8, shows 8.667±0.097, 15.388±0.477 and 11.660±0.530, 17.487±0.441 μg/cm^2/hr respectively. In F4, F9 and F5, F10, propylene glycol was used as enhancer at 5% w/w and 10% w/w concentration gives permeation rate of 8.340±0.096, 16.819±0.242 and 11.301±0.339, 16.819±.242 μg/cm^2/hr respectively. In F11 contain both DMSO and PG as permeation enhancer at concentration 15% w/w and 10% w/w, show the permeation rate at 13.137 Figure V & VI shows the graphical representation of cumulative percentage drug retained as a function of time. These plots were found to be linear with correlation coefficient (r) values of –0.9999, 991, -0.9986, -0.9998, -0.9995, -0.9996, -0.9996, 0.9998, -0.9999 and 0.9992 for F1 to F11 patches respectively, as shown in Table 7. This linearity indicates that the permeation of Ketotifen fumarate from the patch followed Zero Order Kinetics. Negative values of correlation co-efficient indicates negative slope for the plot.

An attempt was made to see whether the drug release is by diffusion, by swelling or by erosion mechanism. When the cumulative percentage permeated was plotted against square root of time a good linearity was observed (Fig III & IV). These Higuchi's plots were found to be linear with correlation co-efficient (r) values of 0.9816, 0.9903, 0.9885, 0.9890, 0.9843, 0.9821, 0.9775, 0.9796, 0.9829, 0.9836, 0.9869 for F1 to F11 respectively, as shown in Table 7, which indicates that the mechanism of drug release was diffusion controlled Higuchi model.

CONCLUSION

From the results obtained so far it can be concluded that:- Eudragit L100: HPMC (9:1), EC: HPMC (3:7) with PEG 400 as plasticizer are promising controlled release transdermal drug delivery systems for Ketotifen fumarate. Incorporation of DMSO & PG as permeation enhancers into above said polymers patches enhanced the permeability of Ketotifen fumarate. Prepared patches exhibited Zero Order Kinetics and the permeation profile was matrix diffusion type. Results of in-vitro skin permeation of Ketotifen fumarate shows that patch of EC: HPMC is suitable for once a day drug delivery and Eudragit L100: HPMC patch show suitability for a prolonged regimen of controlled drug delivery for a period of more than a day. The result of the study show the feasibility of formulating rate-controlled transdermal films of Ketotifen fumarate for effective control and prophylaxis of allergic asthma. Further in-vivo investigations are required to correlate in-vitro permeation studies for the development of suitable transdermal system of Ketotifen fumarate.

Table:1 Formulation of Ketotifen fumarate containing different ratio of EL100,EC with HPMC.

Batch	EL100-HPMC (gm)		EC – HPMC (gm)		DMSO*	PG*
	9:1		3:7			
F1	1.35	0.15	-	-	-	-
F2	1.35	0.15	-	-	7.5%w/w 0.102ml	-
F3	1.35	0.15	-	-	15% w/w 0.204ml	-
F4	1.35	0.15	-	-	-	5% w/w 0.071ml
F5	1.35	0.15	-	-	-	10%w/w 0.142ml
F6	-	-	0.45	1.05	-	-
F7	-	-	0.45	1.05	7.5%w/w 0.102ml	-
F8	-	-	0.45	1.05	15%w/w 0.204ml	-
F9	-	-	0.45	1.05	-	5%w/w 0.071ml
F10	-	-	0.45	1.05	-	10%w/w 0.142ml
F11	1.35	0.15	-	-	15%w/w 0.204ml	10%w/w 0.142ml

Table 2 : Estimation of Ketotifen fumarate for standard calibration curve using UV-spectrophotometer 1201 at 301nm in 20% w/v peg 400 in normal saline.

Sl.No.	Ketotifen fumarate (µg/ml)	Absorbance 301nm			Mean (10^{-3})
		I	*II*	*III*	
1	2	0.52	0.050	0.050	0.051±1.15
2	4	0.124	0.122	0.122	0.123±1.153
3	6	0.180	184	184	0.182±2.309
4	8	0.243	0.244	0.241	0.243±1.528
5	10	0.312	0.316	0.314	0.314±2.000
6	12	0.373	0.372	0.372	0.372±5.77
7	14	0.436	0.432	0.433	0.434±2.081
8	16	0.490	0.492	0.492	0.491±1.15
9	18	0.554	0.552	0.553	0.553±1.000
10	20	0.626	0.624	0.625	0.625±1.000

Table 3 : Thickness, weight and Folding endurance of the patches

Patch	Mean Thickness (µm) n=5	Mean Weight (gm) (10^{-3}) n=5	Mean Folding Endurance n=5
F_1	241 ± 4.183	0.0983 ± 1.641	>150
F_2	252 ± 5.70	0.105 ± 6.612	>200
F_3	259 ± 4.183	0.1162 ± 2.138	>200
F_4	245 ± 7.906	0.102 ± 5.418	>200
F_5	253 ± 5.700	0.1054 ± 4.416	>200
F_6	245 ± 3.535	0.0977 ± 1.309	>200
F_7	250 ± 3.535	0.105 ± 4.48	>200
F8	259 ± 6.519	0.1094 ± 4.139	>200
F9	246 ± 4.180	0.1035 ± 1.454	>200
F10	254 ± 8.944	0.1072 ± 2.369	>200
F11	261 ± 4.183	0.1161 ± 5.395	>200

Table 4 : Percent elongation, Tensile strength and Drug content uniformity

Patch	Mean Percent Elongation at Break	Mean Tensile Strength (kg/mm^2)	Mean Drug Content Uniformity (n=3) (10^{-3})
F_1	26.666 ± 2.887	2.322 ± 0.067	1.379 ± 6.245
F_2	61.667 ± 1.443	2.887 ± 0.254	1.377 ± 5.508
F_3	116.667 ± 3.819	3.681 ± 0.0651	1.387 ± 7.204
F_4	54.167 ± 3.819	2.832 ± 0.070	1.375 ± 7.095
F_5	102.5 ± 2.500	3.602 ± 0.045	1.38 ± 5.196
F_6	15.833 ± 1443	3.546 ± 0.044	1.381 ± 1.156
F_7	36.667 ± 1.443	4.1 ± 0.043	1.391 ± 4.619
F8	50.833 ± 1.443	4.248 ± 0.044	1.383 ± 5.508
F9	38.333 ± 1.443	4.216 ± 0.044	1.380 ± 9.815
F10	46.667 ± 2.887	4.273 ± 0.084	1.387 ± 1.156
F11	133.333 ± 3.819	4.023 ± 0.066	1.382 ± 4.583

Table 5 : Water Vapor Transmission rate and Correlation coefficient.

Formulations	WVT rate (gm. μ/cm^2. 24 hr)	Correlation Coefficient
F1	2.021	0.9998
F2	3.794	0.9970
F3	4.140	0.9838
F4	2.585	9.9969
F5	3.833	0.9995
F6	9.304	0.9995
F7	10.533	0.9996
F8	13.774	0.9997
F9	9.999	0.9997
F10	10.858	0.9998
F11	8.807	0.9999

Table 6 : Skin permeation rate (μg/cm^2/hr) of Ketotifen fumarate through hairless mice abdominal skin.

Patch	I	II	III	Mean
F_1	8.214	8.639	7.710	8.188 ± 0.465
F_2	8.771	8.652	8.579	8.667 ± 0.097
F_3	12.142	11.744	11.093	11.660 ± 0.530
F_4	8.439	8.333	8.247	8.340 ± 0.096
F_5	10.974	11.651	11.279	11.301 ± 0.339
F_6	13.144	13.522	12.978	13.215 ± 0.279
F_7	15.817	14.875	15.472	15.388 ± 0.477
F8	17.052	17.476	17.934	17.487 ± 0.441
F9	14.922	14.703	15.326	14.984 ± 0.316
F10	17.098	16.693	16.667	16.819 ± 0.242
F11	12.779	13.442	13.190	13.137 ± 0.335

Figure 1 : Standard calibration curve for Ketotifen fumarate

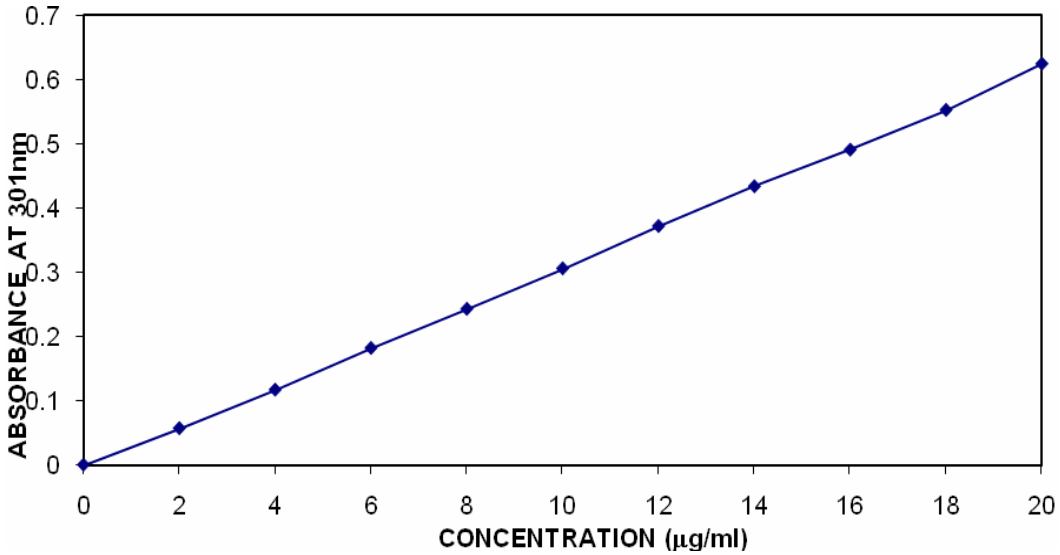

Table 7 : Values of Correlation coefficient (r), Slope of Ketotifen fumarate.

Patch	Cumulative Percent Drug Retained V/s Time		Cumulative Percent Drug Permeated V/s √Time	
	Correlation Coefficient (r)	*Slope*	*Correlation Coefficient (r)*	*Slope*
F_1	-0.9999	-1.695	0.9816	1.869
F_2	-0.9978	-2.109	0.9903	2.054
F_3	-0.9991	-2.765	0.9885	2.683
F_4	-0.9986	-1.931	0.9890	2.000
F_5	-0.9998	-2.552	0.9843	2.583
F_6	-0.9995	-3.188	0.9821	3.001
F_7	-0.9996	-3.362	0.9775	3.435
F8	-0.9996	-3.690	0.9796	3.860
F9	-0.9998	-3.364	0.9829	3.409
F10	-0.9999	-3.778	0.9836	3.893
F11	-0.9992	-3.241	0.9869	3.701

REFERENCES

1) Parafitt K., Martindale The Complete Drug Reference, 32nd edition, London : Royal Pharmaceutical Society, 1999, 755-756.
2) Lee Y. L., Ching C. H. and Chen J. L., In vitro and In-vivo percutaneous absorption studies of Ketotifen patches, Drug Dev and Ind Pharm., 1994, 20(19), 2965-2976.
3) Jain N. K., Advances in Transdermal Drug Delivery, Pharma Times, 2000, 21-22.
4) Jayaswal S. B. and Sood R., Transdermal Controlled Release Drug Administration, Eastern Pharmacist, 1987, 30(357), 47-52.
5) Kannikkannan N., Jayaswal S. B. and Singh J., Transdermal Delivery of Indomethacin : 1 Release Profile of Drug from Polymeric Patches, Indian Drugs, 1992, 30(9), 441-445.
6) Bhattacharya A. and Ghosal S. K., Effect of Hydrophobic Permeation Enhancers on the Release and Skin Permeation Kinetics from Matrix type Transdermal Drug Delivery System of Ketotifen fumarate, Eastern Pharmacist, 2000, 43 (507), 109-112.
7) Kulkarni R., Comparative Evaluation of Polymeric films for Transdermal application, Eastern Pharmacist, 2000, 43(516), 109-111.
8) Chakkapan S., Gandhi K., Thomas S., Katkam R. R., Puri C. P. and Shrivastava R., Studies in Transdermal Drug Delivery Systems for Estradiol, Indian J. of Pharma Sci., 1994, 56(4), 121-125.
9) Bhalla H. L. and Gadkari S. J., Transdermal Films of Isosorbide dinitrate, Indian Drugs, 1986, 24(6), 313-315.
10) Khanna R. Agrawal S. P. and Ahuja A., Preparation and evaluation of Muco-adhesive Buccal Films of Clotrimazole for oral Candida Infections, Indian J. of Pharma Sci., 1997, 59(6), 299-305.
11) Allen.D. J, De Marco J. D. and Kwan K. D., Free Films 1: Apparatus and preliminary evaluation, Indian J Pharm Sci., 1972, 61(1), 106.
12) Touitou E., Godin B. and Becker Y., Oleic acid a skin penetration enhancer affects Langerhans cells and corneocytes, J Control Release, 2000, 80, 1-7.
13) Tiwari A. K., Techniques for studying the molecular basis of Percutaneous Permeation enhancement, Indian drugs, 1999, 36(8), 492-496.

*****