Available online at www.elixirpublishers.com (Elixir International Journal)

Pharmacy

Elixir Pharmacy 85 (2015) 34335-34339

ISSN: 2229-712X

Formulation Designing of Ungual Drug Delivery System of Fluconazole Nail Lacquer and Characterization

Patel Bansari Pankajkumar

Department of Pharmaceutics, NGSM Institute of Pharmaceutical Sciences, Paneer, Mangalore-575018, Karnataka, India.

ARTICLE INFO

Article history:

Received: 24 March 2015;

Received in revised form:

15 July 2015;

Accepted: 8 August 2015;

Keywords

Lacquer,

Drug,

Fluconazole.

ABSTRACT

The topical route of administration is more effective route as compared to the oral route in the treatment of fungal infections of nail. The formulation objective was to provide a sustained release of antifungal drug over extended period of time, so as to reduce frequency of administration, improve clinical efficacy and improve patient compliance. The purpose of the present study was to formulate Fluconazole nail lacquer containing two different penetration enhancers, for the treatment of onychomycosis and to find out which concentration of penetration enhancers gave better release of the drug. The formulation showed good non-volatile content, gloss, smoothness of flow, drug release, drug content estimation and antifungal activity. The in-vitro studies were carried out in Franz diffusion cell using phosphate buffer(pH 7.4) as medium. Whereas the permeation studies were carried out using hooves membrane. The percentage cumulative drug released was determined by UV spectrophotometer. The formulation containing ethyl cellulose (10%w/v) and Thioglycolic acid(3%v/v) and Dimethyl sulfoxide(3%v/v)showed highest release of drug. FTIR studies revealed that drug and excipients are compatible. Stability studies was done as per ICH guidelines for 1 month, which revealed no significant change with respect to the evaluations conducted before stability charging. The sensitivity of Fluconazole against *Candida albicans* was determined by measuring zone of inhibition by comparing with the standard drug for onychomycosis treatment.

Introduction

Drug delivery to the nail (ungual drug delivery) constitutes a major challenge, with the lack of understanding of both the barrier properties of the nail and formulations to achieve enhanced ungual delivery restricting the efficiency of topical treatments for nail disorders.[1] However, existing oral formulations typically contain large doses of active ingredients and also require long treatment periods, creating the potential for systemic toxicity especially in the liver. Thus, developing more effective methods for nail drug delivery is an important objective for the pharmaceutical industry. In order to successfully deliver active pharmaceutical ingredients (APIs) across the nail it is necessary to consider the anatomy and physiology of barriers. Using this information one can more effectively utilize drug delivery approaches to maximize the effectiveness of the API – getting the right amount to the right place at the right time.[2] Fluconazole is a broad spectrum antifungal drug. It is a triazole derivative, its chemical name is 2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,2-triazol-1-yl)propan-2-ol. Fluconazole acts by inhibiting 14 α-demethylase, a cytochrome P450 enzyme which convert lanosterol into ergosterol. Ergosterol is an essential component of fungal cell membrane, inhibition of it causes increase in the cellular permeability and causes leakage of cellular components.[3, 4]

The finger nail has a threelayer structure (outer to inner) – the dorsal, intermediate, and ventral layers, with a thickness ratio of approximately 3:5:2, respectively. The dorsal outer layer is dense and hard, consisting of cornified keratin only a few cells thick (approximately 200 μ).[5] The intermediate layer, in contrast to the dorsal layer, shows highly fibrous structure oriented in a direction perpendicular to the direction of nail growth and constitutes roughly 75% of the plate's thickness. The ventral layer is very thin and consists of a few layers of cells which connect the nail plate to the nail bed below. The growth rate of nails is highly variable among individuals, with average values of 3mm/month for finger nails and 1mm/month for toe nails. A normal finger nail grows out completely in about 6 months, whereas it takes a toe nail about 10-12 months.[6]

The present work investigated the amount of Fluconazole released from the different formulations containing different concentrations of Thioglycolic acid and Dimethyl sulfoxide for the treatment of Onychomycosis. The best formulation was evaluated for antifungal sensitivity test against the *Candida albicans.* Kinetic release studies as well as stability studies were carried out on the best formulation for evaluation of kinetic model for release of drug through the formulation and to check the stability of formulation.

Materials and Methods

Fluconazole nail lacquer(1%) was prepared by simple mixing method. The drug concentration(1 gm) was kept constant. 10 formulations were prepared as shown in table 1. Formulations contains different concentrations of Thioglycolic acid and Dimethyl sulfoxide(1%v/v to 3%v/v) in different ratios, ethyl cellulose(10% w/v and 11% w/v), propylene glycol(1% v/v), glycerine(1% v/v) and ethanol as the solvent.

Preformulation studies of Fluconazole

Spectrum Measurement: The standard solution of Fluconazole was prepared by dissolving 100mg in 10ml of methanol and make up the volume to 100ml with saline phosphate buffer (pH 7.4), further diluted to get 100μg and was scanned between 400-200nm in UV-Visible spectrophotometer (Jasco V-630 UV/Visible spectrophotometer), to obtain λ_{max}.[7]

Tele:

E-mail addresses: bunz.patel29@gmail.com

Construction of calibration curve: A stock solution of Fluconazole was prepared by dissolving 100mg in 10 ml of methanol and make up the volume to 100ml with saline phosphate buffer (pH 7.4). From this stock solution, suitable dilutions were prepared using the same solvent in the range of 100, 200, 300, 400, 500 and 600µg/ml. At λ_{max}, the absorbance of all the concentration solutions was measured against saline phosphate buffer (pH 7.4) as blank. Standard curve between concentration and absorbance was plotted and intercept (B) and slope (K) values were noted.[7]

Drug excipients compatibility studies: FTIR can be used to investigate and predict any physiochemical interaction between different excipients. IR spectra matching approach was used for detection of any possible chemical interaction between the drug and polymer. A physical mixture of drug, polymer and other excipients were prepared and mixed with suitable quantity of potassium bromide. It was scanned from 4000 to 400 cm^{-1} in a FTIR spectrophotometer (F.T.I.R, Shimadzu). The IR spectrum of the physical mixture was compared with those of pure drug and polymer and peak matching was done to detect any appearance or disappearance of peaks.[7]

Evaluation of nail lacquer[8]

Drug content: Drug content of nail lacquer was determined by dissolving accurately 1ml of nail lacquer in methanol. After suitable dilutions, absorbance was recorded by using UV- visible spectrophotometer (UV – 1700, Shimadzu, Japan) at 260 nm. Drug content was determined using slope of standard curve.

Non-volatile content: 1gm of sample was taken in a glass Petri dish of about 8cm in diameter. Samples were spread equally. The dish was placed in the oven at 105^0 C for 1 h the Petri dish was removed, cooled, and weighed. The difference in weight of sample after drying was determined that gives the volatile content present. The amount of volatile content was then subtracted from 1gm weight of nail lacquer

Drying time: A film of sample was applied on a glass Petri dish with the help of brush. The time to form a dry to touch film was noted using a stopwatch.

Smoothness of flow: The sample was poured on a glass slide on an area of 1.5 square inches and spread on a glass plate by making glass slide to rise vertically. And smoothness of flow was determined by comparing with standard marketed nail lacquer.

Gloss: Gloss of the film was visually seen, comparing it with a standard marketed nail lacquer.

Water resistance: This is the measure of the resistance towards water permeability of the film. This was done by applying a continuous film on a surface and drying then immersing it in water. The weight before and after immersion was noted and increase in weight was calculated. Higher the increases in weight lower the water resistance.

Diffusion studies across artificial membrane

Diffusion studies were performed using artificial membrane (cellophane).The membrane was soaked for 1hr in solvent system (saline phosphate buffer, pH 7.4), and the receptor compartment was filled with solvent. Test vehicle equivalent to 10mg was applied evenly on the surface of the membrane. The prepared membrane was mounted on the cell carefully to avoid entrapment of air bubbles under the membrane. The whole assembly was maintained at 37°C, and the speed of stirring was kept constant (600 rpm) for 24 h. The 5ml aliquot of drug sample was taken at time of 0.5, 1, 2, 4, 8, 16, 24 h and was replaced by the fresh solvent. Each experiment was replicated at least thrice. The drug analysis was done using double-beam UV spectrophotometer (U.V.1700 Shimadzu Corporation).

In vitro transungual permeation studies: In Hooves from freshly slaughtered cattle, free of adhering connective and cartilaginous tissue, were soaked in distilled water for 24h. Membranes of about 1 mm thickness were then cut from the distal part of hooves. In vitro permeation studies were carried out by using Franz diffusion cell, the hoof membrane was placed carefully on the cell, and the surface area available for permeation was 1.4 cm^2. Then the test vehicle equivalent 10mg was applied evenly on thesurface of the nail membrane. The receptor compartment was filled with solvent A (saline phosphate buffer, pH 7.4), and the whole assembly was maintained at 37°C with constant stirring for 30 h. The 5 ml aliquot of drug sample was taken after a time interval of 2 h and was replaced by the fresh solvent A. Each experiment was replicated at least thrice. The drug analysis was done by using double-beam UV spectrophotometer (Jasco Corporation, Japan).

Determination of zone of inhibition: Antifungal activity was checked by cup plate method. In this method a previously liquefied molten sabouraud dextrose agar media was inoculated with 0.2 ml of fungal suspension of *Candida albican*shaving a uniform turbidity at temperature of 4 to 8°C. 20 ml of culture medium was poured into the sterile petri dish having an internal diameter of8.5 cm. Care was taken for the uniform thickness of the layer of medium in different plates. After complete solidification of liquefied inoculated medium, the wells were made aseptically with cork borer having 6mm diameter. In one plate formulation (nail lacquer) and in another plate pure drug solution was placed carefully. Plates were kept for pre diffusion for 30 mins. After it normalized to room temperature; the plates were incubated at 22-27°C for 72 h. After incubation period was over, the zone of inhibition was measured with help of scale.

Stability studies: According to ICH guidelines at 40 ± 2^0C/75 ± 5% RH sample was stored in stability chamber for one month. The sample was evaluated for non-volatile content, drying time, gloss, smoothness of flow, water resistance, diffusion across artificial membrane and permeation studies.

Results and Discussion

In spectra measurement of Fluconazole λ_{max} was found to be 260 nm as shown in figure 1. The calibration curve of Fluconazole was obtained in range of 10–100 µg/ml at the wavelength of 260 nm using saline phosphate buffer (pH 7.4) as medium. It has shown good linearity with a regression coefficient of 0.998(r^2 value) as shown in figure 2.

FTIR studies revealed that there is no chemical interaction between the drug and polymer used, as seen in figure 3 and figure 4. All the characteristic IR peaks related to pure drug, Fluconazole also appeared in the IR spectrum of mixture of Fluconazole with ethyl cellulose, there was no chemical incompatibility between the drug and polymers.

Smoothness of flow for formulations F1, F2, F3, F4 was found to be good whereas for formulations F5, F6, F7, F8, F9, F10 was showed satisfactory flow property compared to marketed product.

Gloss of nail lacquer was evaluated by comparing with the marketed product. It was found to be satisfactory when compared to marketed product.

Non-volatile content results for formulation F1 to F10 is given in table 2. It was seen that as the polymer concentration increases from 10% to 11%w/v the non-volatile content increases. The formulation which had higher concentration of polymer showed higher non-volatile content as when compared to the formulation which contained lower concentrations of polymer. Non-volatile content depends and vary upon the concentration of polymer used.

Table 1. Formulation details of the nail lacquer

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Fluconazole(gms)	1	1	1	1	1	1	1	1	1	1
Ethyl cellulose (gms)	10	10	10	10	11	11	11	11	11	11
Propylene glycol (ml)	1	1	1	1	1	1	1	1	1	1
Glycerine (ml)	1	1	1	1	1	1	1	1	1	1
Thioglycolic acid (ml)	0	1	2	3	1	2	1	3	2	3
DMSO (ml)	0	1	2	3	2	1	3	1	3	2
Ethanol (ml)	100	100	100	100	100	100	100	100	100	100

Table 2. Non-volatile content of nail lacquer

Formulation	Non-volatile content (%)*	Formulation	Non-volatile content (%)*
F1	28.55	F6	31.49
F2	29.72	F7	30.35
F3	29.64	F8	33.63
F4	29.74	F9	33.68
F5	29.64	F10	33.56

Table 3. Water resistance test of nail lacquer

Formulation	W1(g)	W2(g)	Difference in weight (g)	Formulation	W1(g)	W2(g)	Difference in weight (g)
F1	6.30	6.33	0.03	F6	5.96	6.02	0.08
F2	6.36	6.42	0.06	F7	6.32	6.40	0.08
F3	6.40	6.47	0.07	F8	6.08	6.17	0.09
F4	6.27	6.35	0.08	F9	6.02	6.12	0.10
F5	6.13	6.21	0.08	F10	6.11	6.22	0.10

Table 4. In-vitro diffusion studies from F1 to F10

Time	f1	f2	f3	f4	f5	f6	f7	f8	f9	f10
0.5	5.99	5.928	6.712	7.425	6.600	6.099	6.587	6.113	6.910	6.612
1	12.37	12.21	14.65	15.76	13.16	13.86	14.66	13.79	15.86	14.46
2	21.34	22.86	23.86	27.60	24.01	23.95	24.22	24.89	25.85	25.91
4	31.63	32.69	35.60	39.85	35.67	34.71	35.69	36.42	39.07	37.71
8	44.08	45.96	50.95	54.45	49.09	49.47	50.99	50.36	52.95	52.67
16	58.83	61.31	65.97	78.79	65.86	64.75	66.74	65.66	68.16	70.11
24	78.12	81.01	85.22	95.34	85.19	83.59	86.31	86.65	88.43	91.90

Table 5. In-vitro permeation studies from F1 to F10

Time	f1	f2	f3	f4	f5	f6	f7	f8	f9	f10
2	14.2	15.18	15.47	15.24	14.86	14.66	14.32	15.28	14.86	15.56
4	21.6	23.03	23.95	24.18	22.53	22.17	21.92	23.10	22.69	23.7
6	29.04	31.33	32.28	33.32	30.38	29.86	29.5	31.4	30.56	32.26
8	36.66	38.97	40.76	42.74	38.3	37.63	37.21	39.89	38.43	40.71
10	44.36	47.27	49.45	51.97	46.41	45.8	45.07	48.34	46.60	49.31
12	52.12	55.81	58.14	61.31	54.47	54.38	53.12	57.43	54.93	58.03
16	60.12	64.73	66.88	70.69	62.71	63.21	61.34	66.61	63.17	67.25
20	68.16	73.49	76.09	80.26	71.09	71.74	69.62	75.56	71.55	76.34
24	76.38	82.83	85.47	89.9	78.8	80.98	78.00	84.87	80.04	85.61
30	84.55	92.23	95.93	98.07	87.41	89.84	86.54	94.25	88.64	94.8

Drying time for formulations F1 to F10 was found between 65 to 72 seconds. It was found that as the polymer concentration increases from 10%w/v to 11%w/v the drying time increases respectively. The time required for the solvent to evaporate from the more viscous solution is more than the less viscous solution.
From the water resistance test as shown in table 3, it can be seen as the polymer concentration increases the water resistance increases, as the concentration of polymer decreases the water resistance decreases. Formulations F1, F2, F3 and F4 showed lower water resistance when compared to F5, F6, F7, F8, F9 and F10.

The drug content for formulations F1 to F10 was found to be in range of 84.16% to 96.90%. From*in–vitro* diffusion studies for formulation F1 to F10 as shown in figure 5, it can be seen that formulation F4 containing lowest concentration of polymer i.e. 10%w/v and highest concentration of penetration enhancers i.e. 3%v/v of Thioglycolic acid and 3%v/v of DMSO showed the highest release of 94.26%. Whereas the formulation F5 containing the highest concentration of polymer i.e. 11%w/v and lowest concentration of penetration enhancers i.e. 1.00%v/v of Thioglycolic acid and 2%v/v of DMSO showed the most sustained release of 84.20% at the end of 10 h. The percentage cumulative drug released for all 10 formulations ranged between 80.74% to %94.26. It was found that as the polymer concentration decreases and penetration enhancers concentration increases the release of the drug increases. With decrease in the concentration of polymer more sustained release is obtained. In *in–vitro* permeation studies it was found that formulation F4 showed release of 98.07% at the end of 30 h, the release data of formulation F1 to F10 is shown in figure 6.

From *in–vitro* diffusion studies and *in–vitro* permeation studies it was found that thioglycolicacid was proved to a better penetration enhancer as compared to dimethyl sulfoxide. The effect of thioglycolic acid was attributed to its small molecular weight and damage caused on the keratin network and decrease in lipid content in the dorsal nail layer; this act which loosened the nail structure, allowing Fluconazole to penetrate easier. Form the data obtained by evaluation of nail lacquer, formulation F4 was found to be best formulation among all the 10 formulations.
The zone of inhibition for pure drug was found to be 26mm and for best formulation 25.1mm which can be seen in figure 7. It was found that best formulation F4 was effective as pure drug as the zone of inhibition of best formulation is closer to that of zone of inhibition for pure drug. The stability study data indicated that the medicated nail lacquer, showed good stability for 6 months when it was stored at temperature of 40±2^{0}C / 75±5% RH.

Conclusion

The prepared formulations were subjected to different evaluation parameters such as drying time, non–volatile content, water resistance, smoothness of flow, evaluation of gloss, drug content, *in-vitro* diffusion studies, *in–vitro* permeation studies, antifungal testing, drug release kinetic studies. From the evaluation data it was found that F4 formulation (10%w/v ethyl cellulose, 3% v/v Thioglycolic acid, 3% v/v Dimethyl sulfoxide) was best formulation. It was found that as the polymer concentration decreases and penetration enhancers concentration increases, percentage drug released also increases. From the *in–vitro* studies it was concluded that Thioglycolic acid was a better penetration enhancer as compared to DMSO. Short-term stability studies of optimized formulations indicate that there were no significant changes in the drying time, drug content and percentage drug release values after 60 days of storage at 40±2 ^{o}C with 75±5% RH.

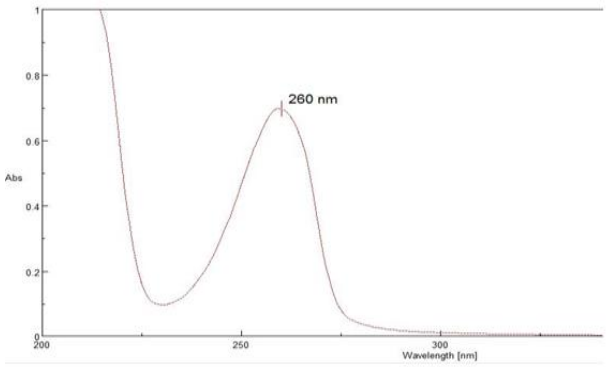

Figure 1. UV spectrum of fluconazole in saline phosphate buffer pH 7.4

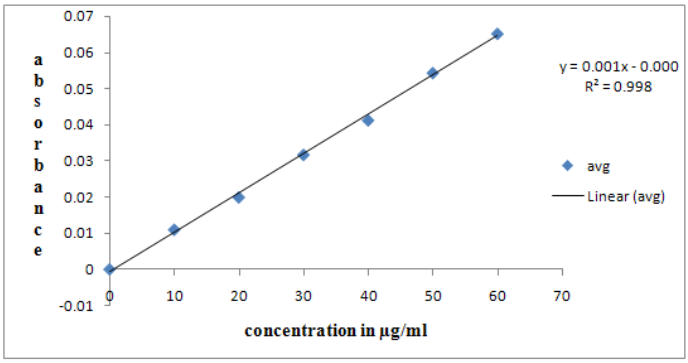

Figure 2. Standard calibration curve for Fluconazole

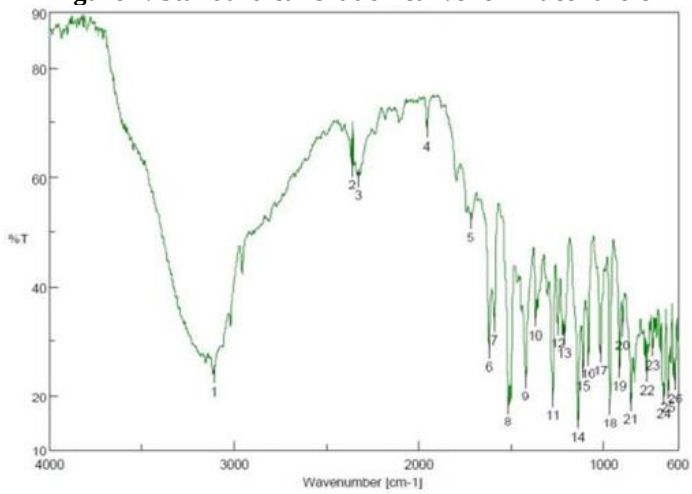

Figure 3. FTIR spectra of fluconazole (drug)

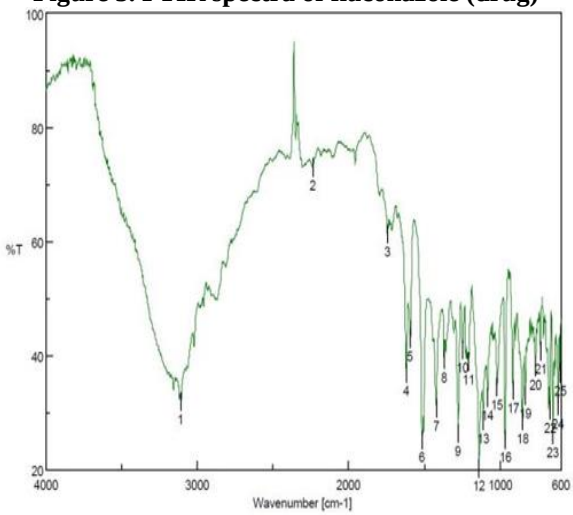

Figure 4. FTIR spectra of Fluconazole + Ethyl cellulose (drug + polymer)

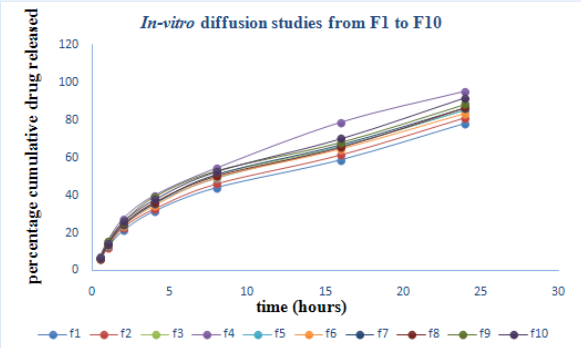

Figure 5. *In-vitro* diffusion studies of formulations F1 to F10

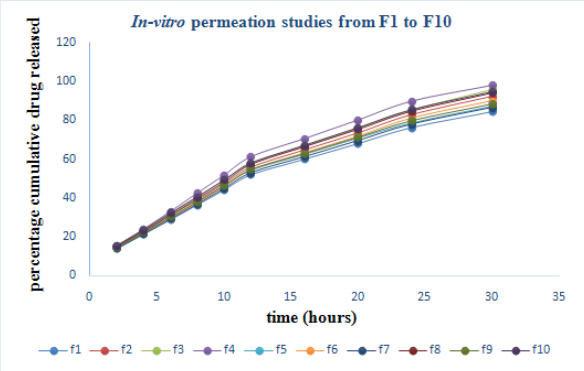

Figure 6. *In-vitro* permeation studies of formulations

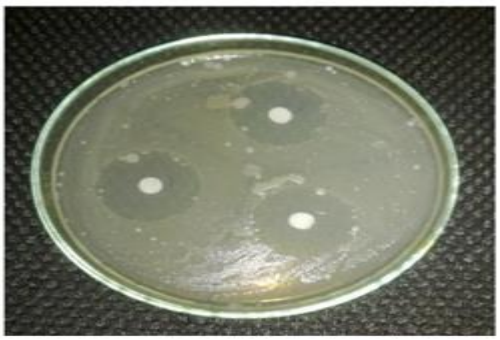

Figure 7. Zone of inhibition for antifungal activity

References

1. Forslind B. The structure of human nail: A scanning electron microscopy study. Int J Curr Pharm Res. 1975; 251:199.
2. Akomeah F, Nazir T, Martin G, Brown M. Effect of heat on percutaneous absorption and skin retension of three model penetrants. Eur J Pharm Sci. 2004; 21:337-45.
3. Martin VM.The use of Fluconazole and Itraconazole in the treatment of *Candida albicans* infection: a review. J Antimicrobial Chemotherapy.1999; 44:429–37.
4. Tripathi KD: Essentials of medical pharmacology. Jaypeebrothers medical publishers (p) LTD. 2007; 6: 763-64.
5. Dykyj JD. Anatomy of human nail. ClinPaed Med Surgery. 1987; 6(2): 521-28.
6. Baden HP, Goldsmith LA, Fleming GA. Comparative study of the physicochemical properties of human controlled release system of racemic propranolol. J Cont Rel. 2006; 113:43-56.
7. Basha NB, Kalyani P, Divakar G. Formulation and Evaluation of the Gel containing Fluconazole: Antifungal agent. Int J Drug Del Res. 2011; 3(4): 109–28.
8. Azharuddhin M, Suresh BH, Shabaraya AS, Prasad NGZ. Preparation and *in–vitro* evaluation of Antifungal Nail lacquer. Int J Uni PharmBio Sci. 2013; 2(4): 78–85.
9. Preethi M, Prathima S, Venkata RR, Sadanandam M. Formulation and evaluation of Duloxetine Hydrochloride delayed release enteric coated capsules.Int J Pharma Bio Sci. 2011; 2(3): 152–72.
10. Bramankar DM, Jaisawal SB. Biopharmaceutics and pharmacokinetics-A treatise. 2004; 347-48.