EVALUATION OF AN ANTIFUNGAL LULICONAZOLE GEL FORMULATION USING SEMI-AUTOMATIC DIFFUSION CELL APPARATUS AND APPLICATION OF MATHEMATICAL MODELS IN DRUG RELEASE KINETICS

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ABSTRACT:

Luliconazole, an FDA sanctioned novel azole antifungal drug that combats fungal contagions caused by Trichophyton rubrum and Epidermophyton floccosum, specifically tinea pedis, cruris and corporis. It is existing in the souk as topical cream 1%. Topical formulations possess diversified benefits for instance escaping of first pass metabolism, easiness of application, evades oscillation in drug planes, tranquil cessation when desirable, and amplified bioavailability. FDA advocates characterization parameters of luliconazole cream should include assessment of appearance, particle/globule size distribution, polymorphic forms, rheological behaviour, In-Vitro Release Test (IVRT), In-Vitro Permeation Test (IVPT) and In-vivo bioequivalence study for a generic product. FDA endorses usage of appropriate apparatus for IVRT technique as pronounced in USP General Chapter <1724>. These comprise diverse models of a vertical diffusion cell (VDC), an immersion cell, and a flow through cell used with USP Apparatus 4. The current research work addresses the evaluation of IVRT of two luliconazole cream formulation (Brands A and B) with that of lab made reference luliconazole gel using semi-automatic VDC apparatus through synthetic membrane. The study was done for 4 hours and analysed by UV-spectroscopy. The release kinetics was construed with various mathematical prototypes like zero order, first order, higuchi model and korsmeyer-peppas model. The outcomes showed that brand B of commercial cream formulations was found to be analogous to that of the reference formulation. The release kinetics of the formulations were found to be zero order that fits into Korsemeyar-peppas model. The variance between the in-vitro release rate of the two brands may be ascribed to diverse bases incorporated into the product. Zero-order indicates that the drug release is constant, independent of concentration. Fitting into Korsemeyer-peppas plot indicates that the release mechanism is diffusion controlled and follows Super case II transport as R^2 value is more than 0.89.

Keywords: Luliconazole, In-Vitro Release Test, Cream, Vertical Diffusion Cell, Release kinetics.

INTRODUCTION

Yeasts plus moulds nowadays flourishing amid the 10 finest recurrently secluded pathogens amongst patients in ICU. Roughly 7% of all delirious episodes that ensue during neutropenia can be accredited certainly to hostile fungal contagions. *Candida* has turn into the fourth foremost bloodstream quarantine in hospitals in the USA, bettering numerous factually

infamous bacterial contagions. Ever since the eighties, an amplified occurrence of hostile fungal contagions in patients who are not in an expiration stage of their causal disease was witnessed[1]. The great occurrence of apparent mycotic contagions displays that 20-25% of the biosphere's populace has skin mycoses, creating these one of the supreme recurrent forms of contagion. Pathogens responsible for skin mycoses are predominantly anthropophilic and zoophilic dermatophytes from the genres Trichophyton (T.), Microsporum (M.) and Epidermophyton (E.)[2]. Dermatophytes are fungi that assault and proliferate inside keratinized tissues (skin, hair, and nails) instigating contagion[3]. Dermatophytes can be categorized into three clusters: *Trichophyton* (which sources contagions on skin, hair, and nails), epidermophyton (which roots contagions on skin and nails) and *Microsporum* (which sources contagions on skin and hair) based upon their genera. On the basis of affected spot, these have been categorized into tinea capitis (head), tinea faciei (face), tinea barbae (beard), tinea corporis (body), tinea manus (hand), tinea cruris (groin), tinea pedis (foot), and tinea unguium (nail)[4].

Luliconazole is an imidazole antifungal API with an inimitable skeleton, as the imidazole moiety is assimilated into the ketene dithioacetate configuration. Luliconazole is the R-enantiomer specifically performing counter to dermatophytes and has extra powerful antifungal bustle than lanoconazole, which is a racemic assortment. The robust antifungal commotion of luliconazole is conceivably because of the blend of resilient *in-vitro* antifungal bustle and promising pharmacokinetic chattels in the skin. Solicitation of luliconazole 1% cream on one occasion every day is operative even in temporary use. It was former official in Japan in 2005 and far along sanctioned by FDA in November 2013[5]. Luliconazole is believed to hinder the enzyme lanosterol demethylase. Lanosterol demethylase is needed for the production of ergosterol, which is the main constituent of the fungus cell membranes. It is existing in the souk as topical cream 1% [6].

Topical drug distribution methods are confined drug distribution method for confined transfer of healing agents through skin to delight the cutaneous illness. These methods are typically beneficial for indigenous skin contagion[7]. Several topical complaints are healed with topical dermatologic formulations. Topical products occur in numerous forms for instance ointments, gels, creams, lotions, solutions, suspensions, foams and shampoos[8]. The benefits attributed to topical drug delivery systems are evasion of primary clearance metabolism, easiness of solicitation, ease of cessation of medicine, discerning location precise drug distribution, escaping of GI unsuitability, improved patient amenability and eludes flux in drug echelons. There are meagre shortcomings for instance skin exasperations and allergenic responses[9].

FDA advocates characterization constraints for Luliconazole cream 1% should comprise *in-vitro* checks such as appraisal of appearance, investigation of particle and/or globule size distribution, scrutiny of polymorphic form(s) of any undissolved luliconazole, examination of rheological behaviour, *In-Vitro* Release Test (IVRT), *In-Vitro* Permeation Test (IVPT) and *In-vivo* tests such as *in-vivo* bioequivalence study[10]. FDA proposes that the IVRT pivotal study relating the Luliconazole release rates between the test and RLD products should be accomplished in a style well-matched with the general procedures and statistical analysis method stated in the United States Pharmacopeia (USP) General Chapter <1724>, Semisolid Drug Products – Performance Tests. It endorses practice of apt apparatus for IVRT such as

varied prototypes of a vertical diffusion cell (VDC), an immersion cell, and a flow through cell used with USP Apparatus 4. It also articulates that the laboratory qualification of each diffusion cell should, at lowest, satisfy the diffusional area of the orifice in which the membrane is mounted, the volume of the receptor solution compartment in each diffusion cell, the control of a 32°C ± 1°C temperature (at the membrane), and the control of the rate of stirring or agitation, as valid[11]. This research paper chiefly emphases on *in-vitro* drug release study and determination of release kinetics of two brands of Luliconazole cream formulations (Brand A and B) in semi-automatic diffusion cell with that of prepared topical gel in the pharmaceutical laboratory.

MATERIALS AND METHODS
Materials:
Luliconazole has been received as a gift sample from Pfizer Healthcare India Pvt Ltd., Chennai. Two Indian brands of sold Luliconazole cream were collected from various pharmacies. These were arbitrarily coded as (A and B). Synthetic membrane (Durapore HVLP membrane) was bought from indigenous dealer.
Phosphate buffer pH 7.4, triethanolamine, parabens, carbopol 940, liquid paraffin, ethanol, span 20, tween 20 were acquired from appropriate dealer.
Equipment:
Diffusion cell apparatus (EDC-02) used in the present study was established from funded project **(EDC-02: Funded by Electrolab Pvt. Ltd., Mumbai).** Digital balance, UV-Visible spectrophotometer (UV-1700A Shimadzu corporation, Japan), pH meter, Magnetic stirrer, Water bath shaker (DQ-WB-01).

METHODOLOLOGY
Preparation of calibration curve of Luliconazole:
100mg of Luliconazole pure drug was liquefied in 10 ml of ethanol and made upto 100ml with phosphate buffer pH 7.4 in a volumetric flask. 20-50 µg/ml dilutions were primed with buffer from the stock solution. The absorbance's of the solutions were analysed at 296 nm[12]. A calibration curve was raised with concentrations of drug (µg/ml) against absorbance (nm) and the regression equation was intended as shown in fig. 1.
Preparation of Luliconazole reference cream:
Aqueous phase: Luliconazole pure drug was liquefied in ethanol. This ethanolic solution was transferred into china dish containing tween 20 and parabens dispersion in water.
Gel phase: The gel phase was prepared by dispersing carbopol 940 in purified water with constant stirring in a magnetic stirrer (400 rpm) for 1 hour. The pH of the gel of the formed gel was adjusted to pH 6.8 - 7 by adding triethanolamine.
Oil phase: Span 20 was dispersed in liquid paraffin in a separate china dish with slow stirring (200 rpm) in a magnetic stirrer.
The aqueous phase was added into gel phase. The oil phase was then transferred into aqueous gel phase and heated on a water bath at 70-80°C under continuous stirring for 10 minutes[13].
The formulated Luliconazole gel was examined visually for their colour, homogeneity, consistency and phase separation.

***In vitro* release test (IVRT):**

VDC system

The IVRT method for the two marketed brands of Luliconazole cream and reference product was performed with a VDC system **(EDC-02: Funded by Electrolab Pvt. Ltd., Mumbai).** It consists of two VDCs with the volume of 12.5 ± 0.1 ml and orifice of 15 mm. Each cell has a magnetic stirrer, circulating water bath to maintain the temperature, sample holder and reservoir. Each VDC comprise of two chambers, the donor (dosage) and the receptor chambers separated by a synthetic membrane. The formulation was applied onto the outer exterior of the

membrane in the donor (dosage) chamber. Samples (2 ml) from the 'sampling port' was taken out at appropriate time intervals and replaced with equal volume into the 'media replace port' with a 2ml syringe[14].

Membrane treatment

Durapore HVLP synthetic membrane was employed as the semi-permeable membrane. It was soaked in the medium for 10 to 15 minutes before the study[15].

Method

The receptor compartment of the two VDCs were filled with phosphate buffer pH 7.4. The temperature of 37 ± 2 °C and stirring speed of 500 rpm was fixed in the apparatus. Weighed quantity of formulation was sited over the pre-hydrated membrane and positioned into the donor compartment. The two compartments were then clinched together. All the openings in the donor and the receptor were then wrapped with Para film to prevent evaporation of the medium. Air bubbles are removed by tilting the cells. Once the temperature has been reached, the test was started. The test was carried out for 4 hours. The samples (2 ml) were withdrawn from the 'sampling port' at definite time intervals (15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 minutes) and replaced with an equal volume at the 'media replace port'. All the samples are analysed by UV-Spectrophotometry at 296 nm. The procedure was repeated in triplicate and the average drug release was calculated[16]. The time interval vs percentage drug release was summarized in table 1 and fig. 2.

Determination of release kinetics

The release kinetics of Brand A, B and that of reference formulation were determined by plotting zero order plot (time vs. cumulative % drug release), first order plot (time vs. log cumulative % drug release), Higuchi plot (square root of time vs. cumulative % drug release) and Korsmeyer-peppas plot (log time vs. log cumulative % drug release) as shown in fig. 3, 4, 5, 6 and table 2. Based on the "R^2" value, the best-fit model was selected[17].

RESULTS
Preparation of calibration curve of luliconazole
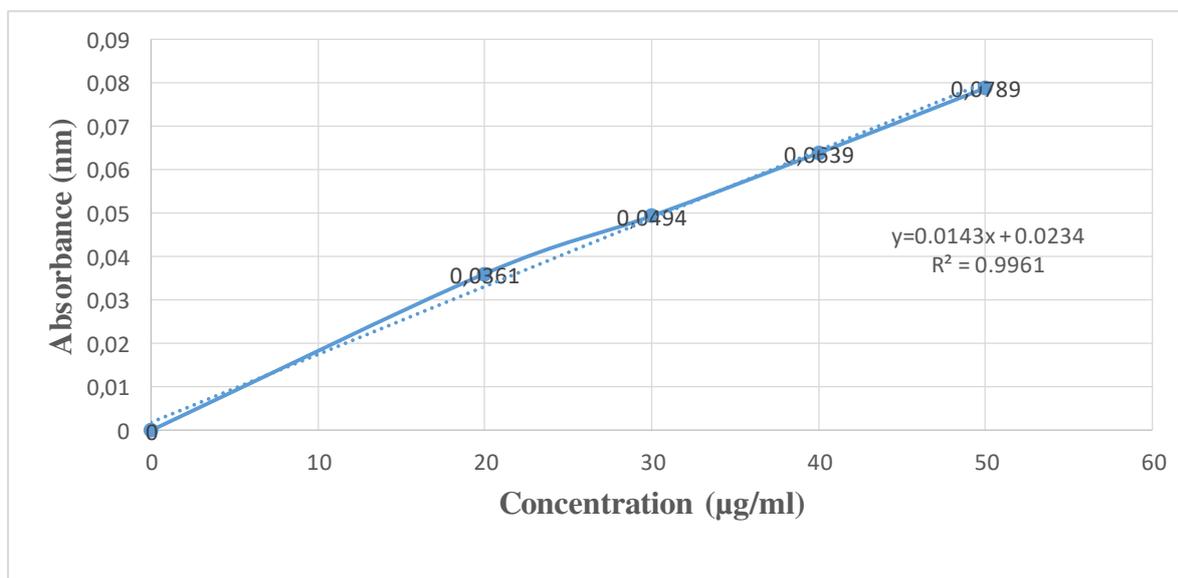
**Fig. 1: Calibration curve of luliconazole**

Figure 1 shows a plot of concentration vs absorbance which was found to be linear in the concentration range of 20-50 µg/ml.

In vitro release test (IVRT):

Table1: Comparative IVRT data of marketed and reference formulations in phosphate buffer pH 7.4.

Time (Minutes)	Cumulative drug release from formulations (%)		
	A	B	Reference
0	0	0	0
15	9.97	11.57	10.34
30	16.94	15.81	14.36
45	21.3	18.45	17.78
60	26.55	26.91	22.03
90	35.11	35.88	27.96
120	39.85	42.45	31.64
150	45.03	47.26	35.22
180	53.5	52.95	39.65
210	60.49	54.37	43.38
240	66.82	58.88	47.58

Table 1 shows the cumulative drug release data of brand A, B and reference formulations at predetermined time intervals.
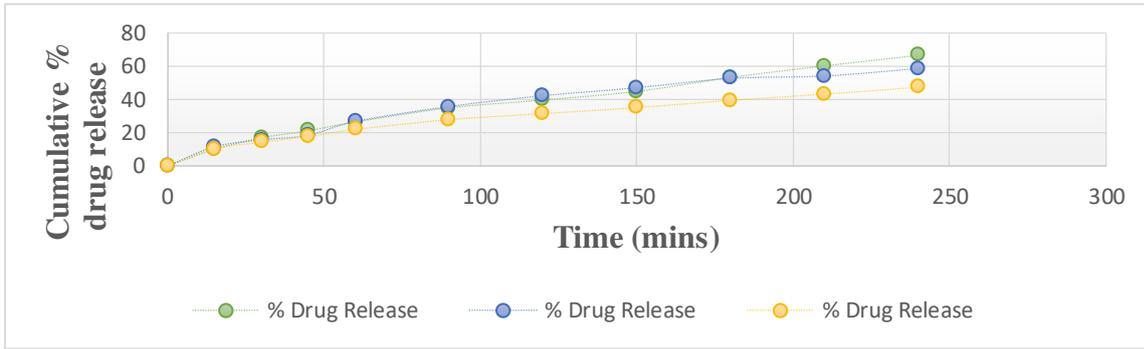
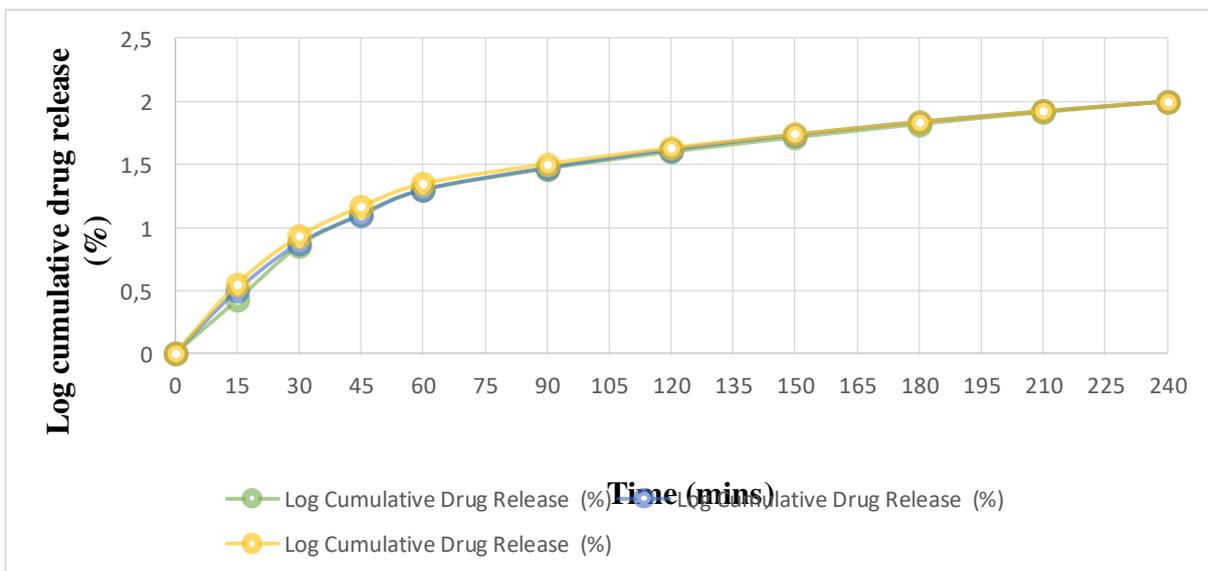**Fig. 2: Comparative drug release plots**

Figure 2 shows the cumulative % drug release plots of brand A, B and reference
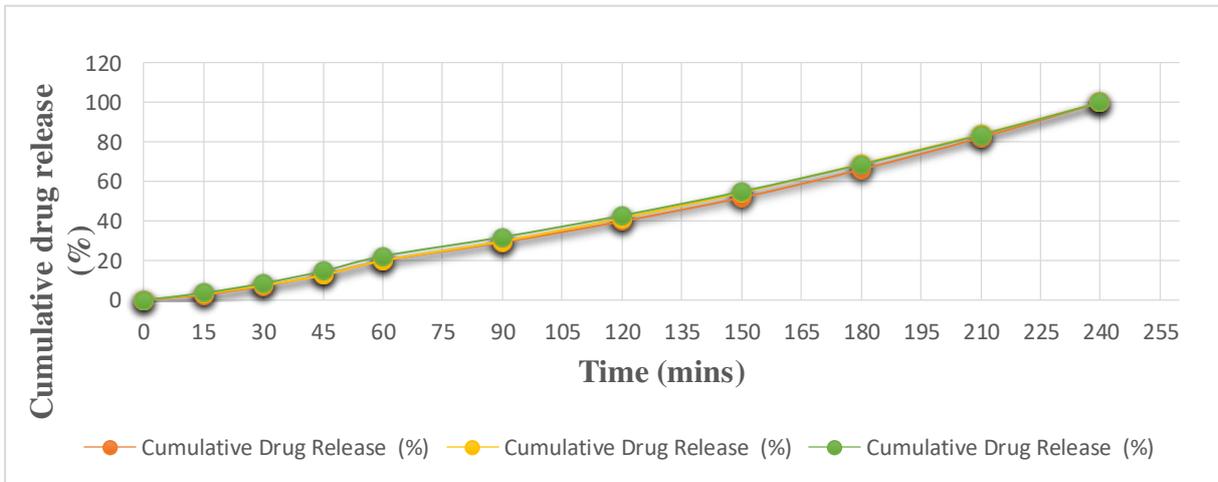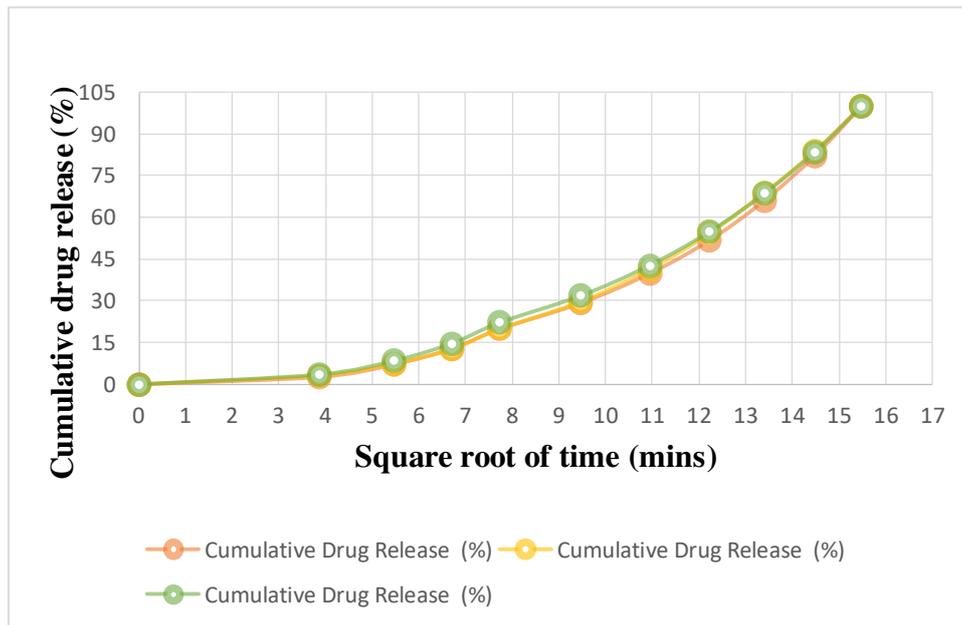

formulations.

Fig. 3: Zero order plot

Figure 3 shows the zero order plot of the two brands with that of the reference formulation of luliconazole.
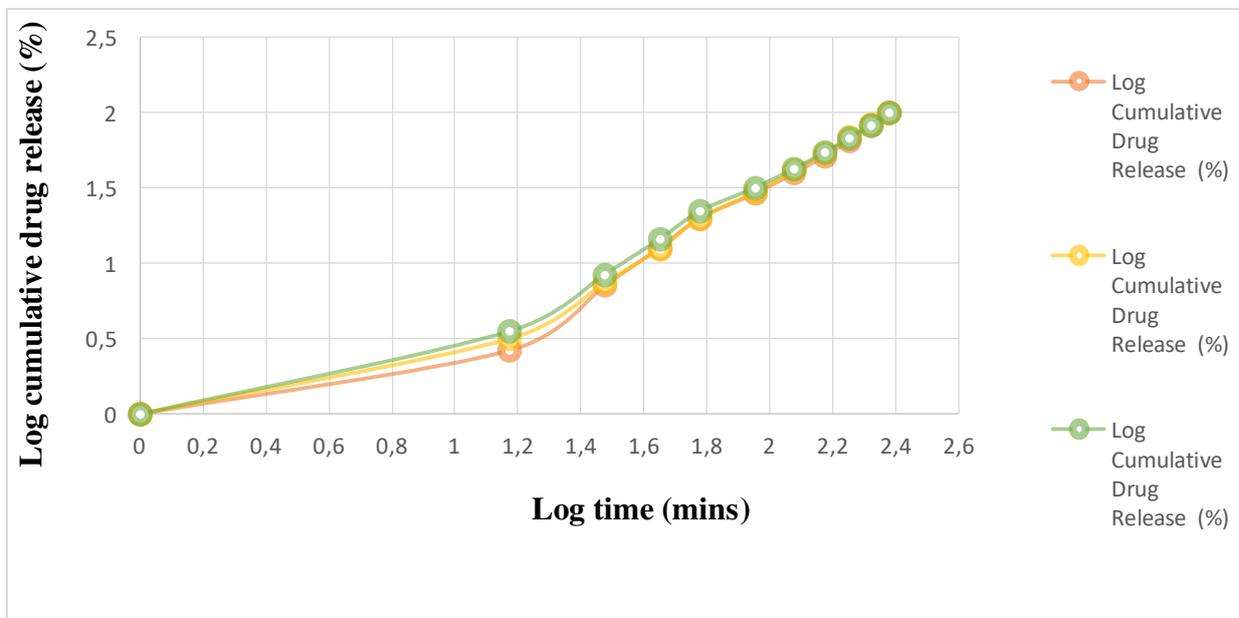

Fig. 4: First order plot

Figure 4 shows the first order plot of the two brands with that of the reference formulation of luliconazole.

Fig. 5: Higuchi plot

Figure 5 shows the Higuchi plot of the two brands with that of the reference formulation of luliconazole.

Fig. 6: Korsmeyer peppas plot

Figure 6 shows the Korsmeyer peppas plot of the two brands with that of the reference formulation of luliconazole.

Table 2: R^2 value for the drug release profile of two brands and reference formulation of luliconazole

R^2 Value	Brand A	Brand B	Reference
Zero Order	0.9889	0.9926	0.9944
First Order	0.8222	0.8235	0.7990
Higuchi	0.8734	0.8802	0.8919
Korsmeyer-peppas	0.9300	0.9407	0.9547

Table 2 shows the R^2 values of the four release kinetic models of Brand A, B and reference formulation.

DISCUSSION

Calibration curve of Luliconazole

Firstly, a stock solution was primed by liquefying 100 mg of Luliconazole pure drug in ethanol followed by making up the volume to 100 ml in a 100 ml standard flask. From the stock solution, 20, 30, 40 and 50 µg/ml dilutions were primed. The absorbances of the dilutions were studied at 296 nm. The calibration curve was found to be linear in the concentration range of 20-50 µg/ml with the linear equation of y=0.0143x + 0.0234 and R^2 value of 0.9993 according to fig. 1[18].

***In-vitro* release test (IVRT)**

The marketed brands A, B and reference formulation released the drug at pre-set time intervals according to table 1 and a plot of time (mins) vs cumulative drug release (%) was drawn as shown in fig. 2. After 1 hour of test, brand A has released 26.55 % and brand B has released 26.91 % of luliconazole. After 2 hour of test, brand A and reference formulation has released less than 40 % except that of brand B. We could comprehend that there is steady escalation of drug release in case of reference formulation than that of the marketed brands. Whereas, the slope of drug release is high for brand A compared to brand B. The cumulative drug release at the end of 4 hours was found to be 66.82 %, 58.88 % and 47.58 % for brand A, brand B and reference formulation respectively. From the outcomes attained, Brand B was found to be analogous to that of the reference formulation[19].

Determination of release kinetics

The outcomes of IVRT study was employed to decide the drug release kinetics of luliconazole cream by plotting four kinetic models such as zero order plot, first order plot, higuchi plot and korsemeyer peppas plot as shown in fig. 3, 4, 5 and 6. The R^2 value of all the four kinetic models for the Brand A, Brand B and reference formulation were determined in MS Excel worksheet and tabulated in table 2. From the upshots acquired, it was found that the release of luliconazole from the topical formulations was found to follow zero-order plot that fits into korsemeyer-peppas plot[20].

CONCLUSION

The great occurrence of apparent mycotic contagions displays that 20-25% of the biosphere's populace has skin mycoses, creating these one of the supreme recurrent forms of contagion. Dermatophytes are fungi that assault and proliferate inside keratinized tissues (skin, hair, and nails) instigating contagion. Luliconazole, an FDA sanctioned novel azole antifungal drug that

combats fungal contagions caused by Trichophyton rubrum and Epidermophyton floccosum, specifically tinea pedis, cruris, and corporis. It is existing in the souk as topical cream 1%. FDA advocates characterization parameters of luliconazole cream 1% should include assessment of appearance, particle/ globule size distribution, polymorphic forms, rheological behaviour, *In-Vitro* Release Test (IVRT), *In-Vitro* Permeation Test (IVPT) and *In-vivo* bioequivalence study for a generic product.

Hence, the contemporary research work was designed in the direction of the evaluation of IVRT of two luliconazole cream formulation (Brand A and B) with that of lab made reference luliconazole gel using semi-automatic VDC apparatus through synthetic membrane. From the IVRT study, it was found that Brand B was found to be analogous to that of the reference formulation. The fallouts achieved acme the influence of excipients on the enactment of topical products which in turn effect on the pharmacological activity[21]. Further, the drug release kinetics was found to follow zero-order plot that fits into korsemeyer-peppas plot. Zero-order plot indicates that drug releases at a steady rate independent of the concentration. The fitting of the R^2 value into Korsemeyer-peppas plot indicates that the mechanism of drug release is diffusion controlled and follows Super case II transport as the R^2 value is greater than 0.89[22].

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