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Developing Novel Topical Formulations for Azole drugs

A Dissertation

Presented in Partial Fulfilment of Requirements for
The Doctor of Philosophy Degree in Pharmaceutical Sciences
with Emphasis in Pharmaceutics and Drug Delivery
The University of Mississippi

By

Praveen Kolimi

(May 2023)

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ABSTRACT

Itraconazole is a broad-spectrum triazole antifungal agent used to prevent and treat fungal infections. Itraconazole is commercially available as an oral and intravenous formulation; however, its administration via these routes is associated with various side effects, such as hepatotoxicity, peripheral neuropathy, cardiac dysrhythmia, and hearing loss. Therefore, in this research, stable non-aqueous topical creams and gels loaded with itraconazole were investigated with the intent to prolong drug contact, improve drug permeation and penetration into skin layers, and overcome the side effects associated with other routes of administration for improved therapeutic outcomes. Chapter 1 discusses the development and characterization strategies during the development of the itraconazole non-aqueous creams while chapter 2 reports the preparation and optimization of the itraconazole non-aqueous gels. The lead cream formulation was stable over three months (the last time-point tested) during refrigeration and room-temperature storage. The cream formulation developed with 15% w/w Transcutol® P improved itraconazole permeation and deposition through the skin by 8.4- and 8.2-fold, compared to the drug solution, respectively. All lead gel formulations were found to have desirable physicochemical characteristics and showed three-month stability (the last time-point tested) during refrigeration, room temperature, and accelerated storage conditions. The gel formulation developed with 0.5% w/w Carbopol® 940 P improved itraconazole permeation and deposition through the skin by 5.1- and 6.8-fold, compared to the drug solution, respectively. Chapter 3 discusses the development and validation of HPLC method for the antifungal drug efinaconazole for application to human nail permeation studies. The developed method was validated as per FDA guidelines, and the results met the

acceptance criteria. The method developed was specific, and the method was linear over the efinaconazole concentration range of 0.05 to 10 $\mu\text{g/mL}$ ($R^2 \geq 0.9981$). In conclusion, the itraconazole-loaded non-aqueous creams and gels can serve as alternative drug delivery platforms for the treatment of fungal skin infections over oral/parenteral itraconazole formulations. Moreover, the developed efinaconazole HPLC method can be applied for formulation evaluation and clinical studies.

DEDICATION

I would like to dedicate to my family members and friends

LIST OF ABBREVIATIONS

| | |
|-------|--|
| ITZ | Itraconazole |
| TEER | Trans-epidermal electrical resistance |
| SC | Stratum Corneum |
| PEG | Polyethylene Glycol |
| O/W | Oil-in-Water () |
| GMS | Glycerol monostearate |
| LOD | Limit Of Detection |
| OD | Octyl Dodecanol |
| CS | Cetostearyl |
| CSA | Cetostearyl alcohol |
| DSC | Differential scanning calorimetry |
| USFDA | United States Food and Drug Administration |
| HLB | Hydrophilic-Lipophilic Balance |
| RT | Retention Time |
| UV | Ultraviolet |
| PBS | Phosphate Buffer Saline |
| USP | United States Pharmacopeia |
| SA | Stearyl alcohol |
| AUFS | Absorbance Units Full Scale |
| HPLC | High-Performance Liquid Chromatography |
| UFLC | Ultra-Fast Liquid Chromatography |
| FTIR | Fourier Transform Infrared |
| UPLC | Ultra-Performance Liquid Chromatography |
| SD | Standard Deviation |
| WOA | Work of Adhesion |
| RH | Relative humidity |

| | |
|-----------------|--|
| HPTLC | High-Performance Thin-Layer Chromatography |
| IVRT | In Vitro Release Test |
| IVPT | In Vitro Permeation Test |
| MM | Malignant Melanoma |
| NMSC | Nonmelanoma Skin Cancer |
| SCC | Squamous Cell Carcinoma |
| BCC | Basal Cell Carcinoma |
| HP- β -CD | 2-Hydroxypropyl-B-Cyclodextrin |
| IS | Internal Standard |
| QC | Quality Control |
| EFN | Efinaconazole |
| LQC | Lower Quality Control |
| MQC | Medium Quality Control |
| HQC | Higher Quality Control |
| LLOQ | Lower Limit of Quantification. |

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Chapter 1 Development and characterization of itraconazole non-aqueous creams for the treatment of superficial fungal infections

1.1 Abstract

Itraconazole (ITZ) is a triazole broad-spectrum antifungal agent, used for the prevention and treatment of fungal infections worldwide. ITZ is available commercially as an oral and intravenous formulations; however, administration through these routes is associated with several side effects such as hepatotoxicity, peripheral neuropathy, cardiac dysrhythmia, and hearing loss. The objective of the current study was to prepare a stable non-aqueous cream loaded with ITZ for, prolonged drug contact, improved drug permeation and penetration into skin layers, and avoiding side effects associated with other routes of administration for improved therapeutic outcomes. The developed creams were characterized for drug content, pH, texture analysis, rheology behavior, fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), *in vitro* release, and *ex vivo* drug permeation through human cadaver skin, and stability studies. The lead formulations were stable over three-month (the last time-point tested) at refrigerated and room temperature storage. The cream formulation developed with 15% w/w Transcutol[®] P improved ITZ permeation and deposition through the skin by 8.4- and 8.2-fold, compared to drug solution, respectively. Thus, ITZ-loaded non-aqueous creams could serve as an alternative drug delivery platform for oral/parenteral ITZ formulations during the treatment of fungal skin infections.

Keywords: Itraconazole, Cream, Fungal, Transcutol® P, Polyethylene glycol, Topical, *In vitro*, *Ex-vivo*, Permeation, Deposition.

1.2 Introduction

Fungal infections are causing serious health problems in immunocompromised and non-immunocompromised patients and placing a huge burden on health care providers around the world. These infections can be superficial, invasive, or both [1]. Superficial fungal infections are considered to affect 20% to 25% of the current world population, and the incidence continues to rise annually [2]. Fungal infections are commonly caused by *Aspergillus*, *Candida*, *Coccidioides*, *Penicillium*, and *Cryptococcus* pathogens [3]. However, *Candida* alone is associated with one billion cases every year [3]. Most fungal infections invade the stratum corneum (SC) and penetrate further into deep layers of the skin, and finally develop serious cutaneous human candidiasis if left without proper treatment [4].

Itraconazole (ITZ) is a broad-spectrum triazole antifungal agent, which is active against many fungal species including *Aspergillus*, *Blastomyces*, *Cryptococcus*, *Candida*, and *Histoplasma capsulatum* var [5]. ITZ binds to fungal cytochrome P450 isozymes to inhibit the conversion of lanosterol to ergosterol with consequent inhibition of ergosterol synthesis, perturbation of membrane-bound enzyme activity, and membrane permeability [6]. ITZ is given orally with 65, 100, or 200 mg doses (tablets and capsules) on daily basis to treat superficial fungal infections [4]. However, ITZ has low gastrointestinal absorption due to its poor aqueous solubility [7]. In addition, oral and parenteral administration is associated with many side effects including hepatotoxicity, cardiac dysrhythmia, peripheral neuropathy, and hearing loss [8]. ITZ oral administration is also associated with nephrotoxicity but it is less when compared to other

antifungals [4]. Moreover, ITZ interferes with the metabolism of other drugs (e.g., simvastatin and omeprazole) through the induction of cytochrome P450 enzymes and ultimately affects their pharmacological action [7]. Furthermore, the extent of ITZ absorption from its solid dosage forms is affected by food intake [9]. Hence, the topical administration of drugs could minimize its undesired side effects and pharmacokinetic drug-drug and drug-food interactions. However, ITZ has a high molecular weight (705.64), poor aqueous solubility (1.0 ng/mL), and high lipophilicity ($\text{LogP} > 5.0$) [4,10]. In addition, the SC barrier function negatively affects the transdermal delivery of ITZ [4].

Recently, topical drug delivery is gaining popularity for treating superficial fungal infections. The onsite application of drugs avoids problems associated with gastrointestinal absorption due to aqueous solubility, pH, drug-food interactions, and presystemic metabolism [11]. It also reduces daily dosing, thus, improving patient compliance and convenience compared with systemic administration. Furthermore, it offers easy termination of drug effect by the removal of the drug delivery vehicle from the skin surface, and it is suitable for self-administration [11]. Many topical formulations such as liposomes, solid lipid nanoparticles, nanostructured lipid carriers, microemulsions, and nanoemulsions have been reported for topical application [12–16]. Although these nanocarriers have improved drug solubility and permeability through the skin to an extent, they are free-flowing, and this leads to a short contact time of the drug with the skin [12].

Creams are the most commonly used topical formulation for the treatment of many dermatological disorders [17]. Based on the poor aqueous solubility of ITZ, incorporating the drug into oil-in-water (O/W) cream could serve as an alternative option for the topical application. Moreover, polyethylene glycol (PEG) was recently used as excipient in creams, and demonstrated excellent stability and permeability results across the human cadaver skin [18]. Therefore, oil/PEG as non-

aqueous phase was replaced O/W cream in this study that could provide better stability due to water removal from the formulation along with improved drug solubility, and prolonged skin residence time, thus, enhanced permeability and deposition after topical application.

1.3 Materials and Methods

Materials:

ITZ was purchased from TCI[®] chemicals (Tokyo Chemical Industry, OR, USA). Transcutol[®] P was a generous gift from Gattefossé Corporation (Paramus, NJ, USA). PEG 200 was purchased from Sigma Aldrich (St. Louis, MO, USA). PEG 400, Kolliwax[®] GMS II (Glycerol monostearate 40-55 (type II)), Kolliwax[®] CSA 50 (Cetostearyl alcohol 50), Kolliwax[®] CSA 70 (Cetostearyl alcohol 70), Kolliwax[®] CA (Cetyl alcohol), Kolliwax[®] SA (Stearyl alcohol), Kollicream[®], Kollicream[®] 3C (Coco-caprylate/caprata), Kollicream[®] OD (Octyl dodecanol), Kolliphor[®] CS 20 (Macrogol Cetostearyl ether 20), and Kolliphor[®] CS 12 (Macrogol Cetostearyl ether 12) were gifted by BASF Corporation (Florham Park, NJ, USA). Tween[®] 80, Oleic acid, and high-performance liquid chromatography (HPLC) grade solvents were obtained from Fischer Scientific (Pittsburgh, PA, USA). Glassware such as scintillation vials, centrifuge tubes, and HPLC vials was acquired from Fischer Scientific (Hampton, NH, USA). Cryopreserved human cadaver skin devoid of any disease was obtained from New York firefighters skin bank (New York, NY, USA).

Methods:

1.3.1 Ultra-Fast Liquid Chromatography (UFLC)

Samples were analyzed for ITZ using a slightly modified reported HPLC method with partial validation [19]. A Shimadzu UFLC (Shimadzu Corporation, Nakagyo-Ku, Kyoto, Japan) system consisting of SIL-20AC autosampler connected with a UV/VIS PDA detector (SPD- M20) and

LC-20 AD solvent delivery module was used for the quantification of ITZ. Chromatographic separation was achieved using reversed-phase chromatography on Waters symmetry[®] C₁₈ column (150 mm × 4.6 mm, 5 μm, Waters, Milford, CA, USA). The mobile phase consisted of acetonitrile and phosphate buffer (10 mM, pH=3.0 adjusted with acetic acid) in a ratio of 80:20 % v/v. The mobile phase was pumped isocratically at a flow rate of 1.2 mL/min. Prior to use, the mobile phase was degassed and filtered through a 0.45 μm membrane filter (MF-Millipore[™], Saint-Quentin, Yvelines, France). The UV detection wavelength was set to 261 nm and the detector sensitivity was set at 1.00 AUFS (Absorbance Units Full Scale). The injection volume was adjusted at 40 μL. The analysis was carried out at 25°C with a run time set at 6.0 mins since the ITZ retention time was 3.5 mins. The method was found to be linear over the ITZ concentration range of 0.025-100 μg/mL.

1.3.2 Excipient's solubility studies

The solubility of ITZ in various lipid excipients was determined by the supersaturation method for the selection of cream excipients. The solubility was determined by dissolving excess amount of ITZ in one gram of different liquid lipids, surfactants, and melted solid lipids in glass vials under continuous shaking in a shaking water bath (Precision[™], Fisher Scientific, Waltham, MA, USA) set at 100 rpm and 60±1.0°C for 24 h. After removing samples from the shaking water bath, a measured volume (liquid lipid) or amount (solid lipid) was dissolved in methanol (extraction solvent) in Eppendorf tubes (1.5 mL). The samples were then centrifuged (AccuSpin 17R centrifuge, Fisher Scientific, Waltham, MA, USA) for 20 mins at 13,000 rpm. The supernatant was collected using an Eppendorf micropipette, and analyzed for ITZ content, using the UFLC method described above, following proper dilution. Excipients with high ITZ solubility values were selected.

1.3.3 Preparation of creams

Creams were prepared using the fusion method [18]. The aqueous phase and the oily phase were heated in separate beakers and maintained at the same temperature (60.0 ± 2.0 °C). ITZ was dissolved in the aqueous phase. Then, the aqueous phase was added to the oily phase, and the mixture was homogenized for 30 mins at 3000 rpm by Silverson Homogenizer L5M-A (Silverson Machines, East Longmeadow, MA, USA). Next, the hot mixtures were subjected to programmed cooling from 60°C to 25°C during 20 mins using a VWR chiller system (VWR International, Radnor, PA, USA).

Control formulation (0.5% w/v)

ITZ solution (ITZ-S) formulation was prepared by dissolving ITZ (0.5% w/v) in a solvent mixture of PEG 200 and PEG 400 (1:1).

1.3.4 Drug content

An accurately weighed amount (20 mg) of the prepared cream, removed from three different (top, middle, and bottom) locations of the cream container, was extracted in methanol (1 mL). The mixture was vortexed for 10 mins before being subjected to bath sonication for 10 mins. The mixtures were then centrifuged (Fisherbrand™, Waltham, MA, USA) at 13,000 rpm for 20 mins. The supernatant was analyzed for ITZ content, using the UFLC method described above, following appropriate dilution with the extracting solvent. Drug content was calculated according to the following equation (Eq. 1).

$$\% \text{ drug content} = \frac{\text{Actual amount}}{\text{Theoretical amount}} \times 100 \quad (1)$$

1.3.5 Rheological study

All selected cream formulations were studied for rheological properties using HR-2 Rheometer (Waters™, TA® Instruments, New Castle, DE, USA) at 32.0±1.0°C with a 20 mm parallel plate, 600-grit sandpaper, and solvent trap. The viscosity of the developed creams was measured at a shear rate of 0.01 sec⁻¹. All viscosity measurements were performed in triplicates.

1.3.6 Texture analysis

The texture analysis for selected formulations were performed on a texture analyzer (Model TA-XT2i, Texture Technologies Corp, Hamilton, MA, USA) using a 5.0 kg load cell. A cylindrical acrylic probe (TA-3, diameter: 1 inch) and a soft matter kit (TA-275) were used for this experiment. The cream was loaded into the soft matter fixture while the acrylic probe was attached to the arm of the texture analyzer. The probe was lowered at 0.50 mm/sec speed till it touched the surface of the cream sample. On touching the surface of the sample and identifying the trigger force (2.0 g), the cylindrical probe produced a deformation of up to 1 mm within the cream sample. The speed during probe removal from the sample was the same as the probe lowering speed (5.0 mm/sec) [20]. The base of the probe and the top of the soft matter fixture were cleaned properly before each measurement. Each cream formulation was measured in triplicate.

1.3.7 pH measurement

The pH of the prepared creams was measured using the Mettler Toledo pH meter (Mettler Toledo, Columbus, OH). The pH probe used for the measurement was the Mettler Toledo InLab® Microelectrode. The pH meter was calibrated using different standard buffers with known pH values of 4.01, 7.00, and 10.00, respectively before use. The pH measurements were carried out in triplicate.

1.3.8 *In vitro* release testing

1.3.8.1 ITZ stability in receiver medium

The drug should be stable in the receiver medium used for *in vitro* release and *ex vivo* permeation and deposition studies during the study period. The stability of ITZ in the receiver medium was evaluated at 100 µg/mL concentration for 6 and 48 h at 32.0±1.0°C. Samples (500 µL) were collected from the receiver medium at 6 (release) and 48 h (permeation) time points and added to 0.5 mL of methanol. The samples were vortexed for 5 mins before being centrifuged at 13,000 rpm for 15 mins. The supernatant was collected and analyzed for ITZ content using UFLC.

1.3.8.2 Membrane inertness

A membrane inertness experiment was conducted to evaluate the non-specific binding characteristics of ITZ to the diffusion membrane used for the *in vitro* release testing. The membrane selected for diffusion should offer free resistance for ITZ diffusion. The membrane inertness was tested with three different concentrations of ITZ (100, 200, and 400 µg/mL, n=3). The diffusion membrane was soaked in glass scintillation vials containing the three different concentrations individually and kept aside for 6 h. After 6 h, sample (500 µL) was collected from each vial and these samples were quantified using UFLC after proper dilution to determine the amount of ITZ adsorbed on the diffusion membrane [18].

1.3.8.3 Release study

Vertical Franz diffusion cell apparatus with an active diffusion area of 0.636 cm² (Logan Instruments, Somerset, NJ, USA) was used to conduct the *in vitro* release study. Cream samples (100 mg) were applied onto Whatman™ Nylon membrane filters (0.22 µm, Cytiva®, Marlborough, MA, USA), sandwiched between the two half-cells of the diffusion cell. The receiver compartment

consisted of 5 ml freshly prepared phosphate-buffered saline (PBS, pH 7.4) containing Tween[®] 80 (0.5% w/v). Tween[®] 80 was used as a solubilizer in the receiver compartment to maintain the sink conditions [21]. The receiver chambers were kept under continuous magnetic stirring at 32.0±0.5°C with the aid of a circulating water bath. The diffusion cells were equilibrated at the required temperature for one hour before the study. Samples (500 µL) were collected from the receiver compartment at predetermined time intervals (1.0, 1.5, 2.0, 3.0, 4.0, and 6.0 h) and replaced with the same volume of the freshly prepared release medium. The samples were then analyzed using the UFLC. The ITZ release profiles were calculated using Microsoft 365[®] office excel statistical functions (2019, Microsoft Corporation, Redmond, WA, USA).

1.3.9 *Ex vivo* permeation testing

The *ex vivo* skin permeation study was performed also using Franz diffusion cells with an effective diffusion area of 0.636 cm² and a receiver chamber volume of 5 mL. Prior to this study, human cadaver skin stored below –80°C in water-impermeable plastic bags was thawed for one hour at room temperature. The skin was mounted between the donor and receiver chamber with the stratum corneum (SC) side facing the donor compartment containing the test and control formulations. The skin barrier integrity was evaluated by measuring Trans-epidermal electrical resistance (TEER) at a frequency of 1 kHz and a low voltage of 100 mV. The skin having a TEER value ≥ of 20 k Ω cm² and without physical damage was only used for these permeation studies. The receiver chambers filled with freshly prepared PBS (pH 7.4) containing Tween[®] 80 (0.5% w/v) were kept under continuous magnetic stirring at 32.0±0.5°C with the aid of a circulating water bath. An infinite dose of the cream formulation was applied to the sandwiched skin using a syringe. Samples (500 µL) were removed at regular time points (0, 4, 8, 12, 16, 20, 24, 36, and 48 h) from

the receiver chambers and replenished with the same volume of the receiver medium. The amount of ITZ in samples was quantified using the UFLC method described above.

The cumulative amount of ITZ permeated (Q_n), cumulative amount permeated/area (Q_a), and steady-state flux (J_{max}) were calculated to study the *ex vivo* permeation of ITZ across the human cadaver skin. The analysis for all samples was conducted in triplicate.

Q_n was calculated using the following equation (Eq. 2):

$$Q_n = V_r C_{r(n)} + \sum_{x=1}^{x=n} V_s C_{r(x-1)} \quad (2)$$

Where n is the sampling time point; V_r is the volume in the receiver chamber (mL), V_s is the volume of the sample collected from the receiver chamber at the n th time point (mL) and $C_{r(n)}$ is the ITZ concentration in the receiver chamber at the n th time point. Skin permeation rate (dQ/dt) was calculated using the slope of the Q_n versus time plot.

Q_a was calculated using the following equation (Eq. 3):

$$Q_a = Q_n / A \quad (3)$$

Where A is the effective permeation area of the skin (0.636 cm²).

J_{max} was calculated using the following equation (Eq. 4):

$$J_{max} = (dQ/dt) / A \quad (4)$$

Where Q is the amount of ITZ transported through the skin.

1.3.10 Deposition of ITZ in skin tissue

Following the *ex vivo* permeation experiment, the skin was removed from the diffusion cell and washed with methanol-soaked cotton swabs (Q-tips[®]) to remove the remaining formulations. The effective skin diffusion area was weighed, cut into small pieces, and extracted in methanol (1 mL). The supernatant was then collected after centrifugation at 13,000 rpm for 15 min [22]. The

supernatant was injected into the UFLC to quantify ITZ within skin tissue homogenate by applying the following equation (Eq. 5):

$$Q_d = Q_s / W_s \quad (5)$$

Where Q_d is the amount of ITZ deposited within 1 mg of the skin, Q_s is the amount of ITZ quantified in the effective permeation area of the skin, and W_s is the weight of the effective permeation area of the skin.

1.3.11 Fourier Transform Infrared (FTIR) spectroscopy

FTIR analysis of pure ITZ, pure excipients, and placebo and drug-loaded creams was performed using an Agilent Technologies Cary 630 instrument (Santa Clara, CA, USA). The scanning range was set between 600 and 4000 cm^{-1} .

1.3.12 Differential scanning calorimetry (DSC)

The DSC thermograms of pure ITZ, pure excipients, and successful cream formulations were obtained using a differential scanning calorimeter instrument (DSC 25 series, Waters™, TA® Instruments, New Castle, DE, USA), calibrated with indium. Sample (5–8 mg) was loaded in hermetically sealed aluminum pans. DSC analysis was performed over the temperature range of 25–200°C at a heating rate of 20°C/min. Ultra-pure nitrogen was used as the purge gas at a flow rate of 50 mL/min. An empty aluminum pan was used as a reference. DSC data were analyzed by TRIOS software to examine the thermal behavior of ITZ in the developed formulations.

1.3.13 Stability studies

A stability study for selected creams was initiated under refrigerated ($4\pm 2^\circ\text{C}$) and room temperature ($25\pm 2^\circ\text{C}$) storage conditions. The formulation was evaluated for any change in physical appearance, pH, and drug content (%) at predetermined time intervals over three months.

1.3.14 Statistical analysis

Statistical comparison was performed using SPSS (IBM SPSS Statistics software, SPSS 28, USA).

Differences were considered statistically significant when the p value was < 0.05.

1.4 Results and Discussion

1.4.1 Screening of excipients

Drug solubility studies were initially performed for the proper selection of the lipids and surfactants to be used for ITZ-loaded cream development. This study is considered a critical experiment for obtaining better drug loading capacity in the final formulation. The results, in terms of the concentration of the saturated solution (mg/mL), are presented in **Table 1**. Among solid and liquid excipients, Kolliwax[®] CSA 70 (CSA-70), Kolliwax[®] CSA 50 (CSA-50), Kolliwax[®] CA (CA), Kolliwax[®] SA (SA), Kolliwax[®] GMS II (GMS II), Miglyol[®] 829 (MG), and oleic acid (OA) showed the highest solubilizing power towards ITZ. In addition, Transcutol[®] P surfactant showed a better solubilizing capacity than Tween[®] 80. Based on these results, many formulation trials were performed to obtain a cream with an acceptable consistency.

Table 1. Solubility of itraconazole in different excipients (n = 3).

| Liquid excipients | Solubility (mg/mL) | Solid excipients | Solubility (mg/mL) |
|--------------------------|---------------------------|---|---------------------------|
| Cottonseed oil | 0.03±0.01 | Kolliwax[®] CSA 70 (Cetostearyl alcohol 70) | 10.0±0.2 |

| | | | |
|------------------------------|------------------|---|-----------------|
| Sesame oil | 0.02±0.03 | Kolliwax® CSA 50 (Cetostearyl alcohol 50) | 5.0±0.1 |
| Castor oil | 0.14±0.09 | Kolliphor® CS 12 (Macrogol Cetostearyl ether 12) | Insoluble |
| Miglyol® 829 (MG) | 0.98±0.03 | Kolliphor® CS 20 (Macrogol Cetostearyl ether 20) | Insoluble |
| Oleic acid (OA) | 0.62±0.02 | Kolliwax® CA (Cetyl alcohol) | 5.0±0.2 |
| Kollicream® | 0.06±0.01 | Labrafil® M 2130 | Insoluble |
| Kollicream® 3C | 0.03±0.01 | Gelucire™ 48/16) | Insoluble |
| Kollicream® OD | 0.01±0.02 | Gelucire™ 43/01 | Insoluble |
| Kollisolv® MCT | 0.11±0.01 | Kolliwax® SA (Stearyl alcohol) | 10.0±0.2 |
| PEG 200 | 2.88±0.18 | Gelucire™ 44/14 | Insoluble |
| PEG 400 | 2.10±0.04 | Kolliwax® GMS II (Glycerol monostearate 40-55 (type II)) | 10.0±0.3 |
| Surfactants | | Gelucire™ 50/13 | Insoluble |
| Tween® 80 | 2.79±0.08 | | |
| Transcutol® P | 3.67±0.08 | | |

1.4.2 Preparation of ITZ loaded creams

All excipients were screened in concentrations within the inactive ingredients range, approved by the United States Food and Drug Administration (USFDA) for topical drug products (**Table 2**). All formulation trials are covered in detail in **Table 3**. Preliminary studies demonstrated that F1 and F2 creams demonstrated phase separation immediately after preparation. Creams containing <59.5% w/w of PEG (F1, F6, F7, F8, and F9) led to lesser than the desired consistency. The preliminary screening of F3-F5 and F10-F15 creams was conducted based on a visual examination after subjecting to three cycles of freeze-thaw stability. F10-F15 creams demonstrated phase separation of the dispersed phase from the continuous phase after changing the ratio of PEG 200: PEG 400 from 1:1 to 1:2, 1:3, 1:4, 4:1, 3:1, and 2:1. However, creams F3-F5 containing PEG 200: PEG400 (1:1) demonstrated an acceptable consistency and stability. Therefore, these three formulations were selected for further evaluation.

Table 2. Maximum potency per unit dose and maximum daily exposure for the excipients used in the preparation of itraconazole-loaded creams as per USFDA inactive ingredient database and the role of each excipient in the formulation.

| Inactive Ingredient | Route | Dosage Form | Maximum Potency per unit dose (% w/w) | Maximum Daily Exposure (MDE) | Reason for use |
|---|---------|-------------|---------------------------------------|------------------------------|---|
| Transcutol® P (Diethylene glycol monoethyl ether) | Topical | Cream | | 1290 mg | Solubilizer, surfactant, and permeation enhancer. |

| | | | | | |
|-----------------------|---------|----------|------|---|---|
| PEG 200 | Topical | Ointment | 39.0 | — | Solubilizer, co-emulsifier, humectant, and permeation enhancer. |
| PEG 400 | Topical | Cream | 7.5 | — | Solubilizer, emulsifier, emollient, and permeation enhancer. |
| | | Ointment | 65.0 | — | |
| Miglyol® 829 | NA | NA | NA | — | Solubilizer, emollient, and lubricant. |
| Oleic acid | Topical | Cream | 25.0 | — | Solubilizer and permeation enhancer |
| Cetostearyl alcohol | Topical | Cream | 12.0 | — | Stabilizer, co-emulsifier, stabilizer, viscosity increasing agent, and permeation enhancer. |
| Cetyl alcohol | Topical | Cream | 4.0 | — | Stabilizer, co-emulsifier, stabilizer, viscosity increasing agent, and permeation enhancer. |
| Glycerol monostearate | NA | NA | NA | — | Emulsifier and viscosity increasing agent. |

Table 3 Formulation trials during cream preparation (n = 3).

| Code | Aqueous phase (% w/w) | | | | Oily phase (% w/w) | | | | | | | Visual observations | Status (Pass/Fail) | The possible reason for formulation failure |
|------|-----------------------|---------|----|------------------|--------------------|----|--------|--------|----|----|--------|---------------------|--------------------|---|
| | PEG 200 | PEG 400 | *T | H ₂ O | MG | OA | CSA-70 | CSA-50 | CA | SA | GMS-II | | | |
| | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | | |
|-------|-------|-------|----|------|-----|----|---|---|---|---|---|---|---|------|--|
| ITZ-S | 49.75 | 49.75 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Clear solution. | NA | NA |
| F1 | 10 | 10 | 5 | 51.5 | 2.5 | 10 | 3 | 3 | 2 | 2 | 1 | 1 | - Red to brown color formation. - Phase separation. - Precipitation at the bottom of the container after preparation. | Fail | - Could be due to the chemical incompatibility of stearic acid with other cream excipients. - Low ITZ solubility in the aqueous phase of the formulation. |
| F2 | 35.75 | 35.75 | 5 | 0 | 2.5 | 10 | 3 | 3 | 2 | 2 | 1 | 1 | - Red to brown color formation. - Phase separation. | Fail | - Could be due to the chemical incompatibility of stearic acid with other cream excipients. |
| F3 | 34.75 | 34.75 | 5 | 0 | 2.5 | 10 | 4 | 4 | 3 | 0 | 2 | 2 | - Consistent Cream formed. - Stable after one week Freeze thaw stability studies. | Pass | |
| F4 | 32.25 | 32.25 | 10 | 0 | 2.5 | 10 | 4 | 4 | 3 | 0 | 2 | 2 | - Consistent Cream formed. - Stable after one week Freeze thaw stability studies. | Pass | |
| F5 | 29.75 | 29.75 | 15 | 0 | 2.5 | 10 | 4 | 4 | 3 | 0 | 2 | 2 | - Consistent Cream formed. - Stable after one week Freeze thaw | Pass | |

| | | | | | | | | | | | | | | | |
|-----|-------|-------|----|---|-----|----|---|---|---|---|---|--|--------------------|--|--|
| | | | | | | | | | | | | | stability studies. | | |
| F6 | 27.25 | 27.25 | 20 | 0 | 2.5 | 10 | 4 | 4 | 3 | 0 | 2 | The cream wasn't formed. | Fail | - Could be due to the lower emulsification power of Transcutol® P compared to PEG 200 and 400 for cream formation. | |
| F7 | 22.25 | 22.25 | 30 | 0 | 2.5 | 10 | 4 | 4 | 3 | 0 | 2 | The cream wasn't formed. | Fail | - Could be due to the lower emulsification power of Transcutol® P compared to PEG 200 and 400 for cream formation. | |
| F8 | 17.25 | 17.25 | 40 | 0 | 2.5 | 10 | 4 | 4 | 3 | 0 | 2 | The cream wasn't formed. | Fail | - Could be due to the lower emulsification power of Transcutol® P compared to PEG 200 and 400 for cream formation. | |
| F9 | 12.25 | 12.25 | 50 | 0 | 2.5 | 10 | 4 | 4 | 3 | 0 | 2 | The cream wasn't formed. | Fail | - Could be due to the lower emulsification power of Transcutol® P compared to PEG 200 and 400 for cream formation. | |
| F10 | 23.16 | 46.33 | 5 | 0 | 2.5 | 10 | 4 | 4 | 3 | 0 | 2 | - Consistent cream was formed. - Phase separation | Fail | | |

| | | | | | | | | | | | | | | | |
|-----|-------|-------|---|---|-----|----|---|---|---|---|---|---|--|--|--|
| | | | | | | | | | | | | | was observed after one-week Freeze-thaw stability studies. | | |
| F11 | 17.38 | 52.13 | 5 | 0 | 2.5 | 10 | 4 | 4 | 3 | 0 | 2 | - Consistent cream was formed. - Phase separation was observed after one-week Freeze-thaw stability studies. | Fail | | |
| F12 | 13.9 | 55.6 | 5 | 0 | 2.5 | 10 | 4 | 4 | 3 | 0 | 2 | - Consistent cream was formed. - Phase separation was observed after one-week Freeze-thaw stability studies. | Fail | | |
| F13 | 46.33 | 23.16 | 5 | 0 | 2.5 | 10 | 4 | 4 | 3 | 0 | 2 | - Consistent cream was formed. - Phase separation was observed after one-week Freeze-thaw stability studies. | Fail | | |

| | | | | | | | | | | | | | | |
|-----|-------|-------|---|---|-----|----|---|---|---|---|---|---|------|--|
| F14 | 52.13 | 17.38 | 5 | 0 | 2.5 | 10 | 4 | 4 | 3 | 0 | 2 | - Consistent cream was formed. - Phase separation was observed after one-week Freeze-thaw stability studies. | Fail | |
| F15 | 55.6 | 13.9 | 5 | 0 | 2.5 | 10 | 4 | 4 | 3 | 0 | 2 | - Consistent cream was formed. - Phase separation was observed after one-week Freeze-thaw stability studies. | Fail | |

All creams contain 0.5% w/w itraconazole.

*T= *Transcutol P*

1.4.3 Drug content

The analysis of drug content was based on three samples removed from three different (top, middle, and bottom) locations of each cream container. The drug content of the successful creams was within the acceptable limits defined by the USP (100.0 ± 10.0 % label claim). The ITZ content in F3, F4, and F5 formulations was 99.6 ± 1.5 , 101.1 ± 1.0 , and $98.7.1 \pm 1.9$ %, respectively. The low SD values (<2.0%) indicated good ITZ content uniformity within creams because drug content calculations were based on three samples removed from three different regions of each cream container.

1.4.4 Rheology study

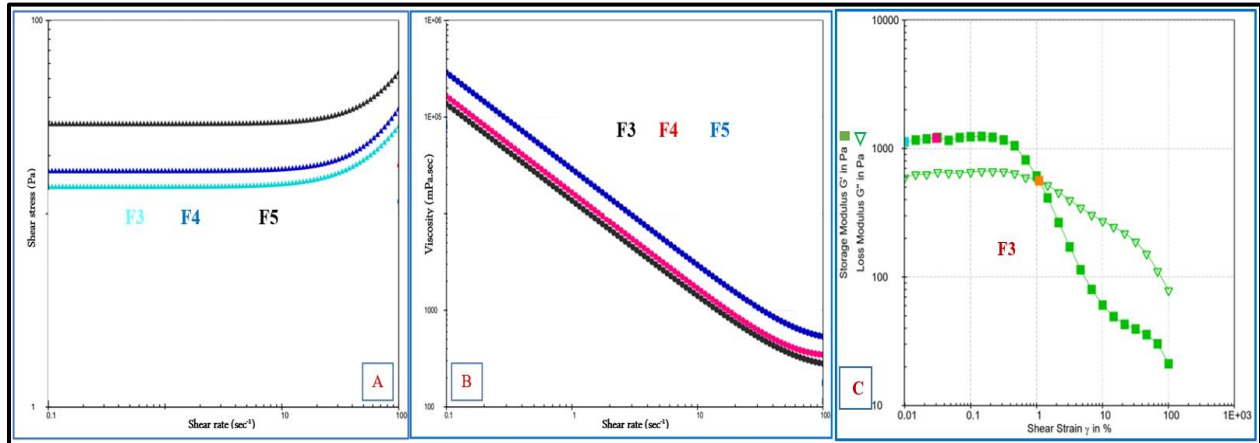
The rheology of semisolid products such as creams relies on the complex microstructure of drug products that could be affected by preparation conditions. Therefore, it is important to perform rheological studies to investigate the effects of processing conditions on the rheological properties. The variations in shear stress as a function of the shear rate applied to the cream samples at room temperatures (25°C) were determined, and the results are depicted in **Fig. 1A**. The highest value of the coefficient of determination ($R^2 \geq 0.999$) was observed for the Herschel-Bulkley model after fitting the experimental forward-flow data of rheological studies in terms of shear stress. The plotted rheology curves were nonlinear and followed non-Newtonian flow behavior, indicating the shear thinning of cream formulation within the range of 0.1-100 sec^{-1} , and the flow was converted to Newtonian behavior at shear rates above 100 sec^{-1} , as validated by the viscosity reaching a plateau.

This shear-thinning behavior generally arises from chain alignment with the flow path and consequently, the local drag is reduced. With increased shear rate, the alignment with the flow path becomes more complete, thus leading to a further decrease in viscosity [23]. In addition, **Fig. 1B** shows that the forward-flow experimental data fitted the Herschel-Bulkley model also in terms of apparent viscosity. The plot demonstrated that at low shear values, the viscosity of the products is high (high flow resistance), thereby providing good hardness to creams. As the shear values increase, the viscosity of the formulations decreases (low flow resistance) rapidly, thereby allowing the products to easily spread over a large surface area. The results were consistent with earlier published investigations [24].

Fig. 1C depicts the plot of storage modulus (G') and loss modulus (G'') against strain where G' represents the elasticity of the product and G'' shows the viscous behavior of the product. Based

on the graph, G' of the F3 formulation remained constant up to 0.7% strain (approximately), then, a decrease in G' was observed. The region where the G' remains constant with the increase in the strain values is termed the viscoelastic region in which the product can recover quickly upon any applied stress. It can also be observed that G' is significantly higher than G'' which indicates that the microstructure of the cream product is organized and is mainly controlled by the cohesive forces. Till the strain value of 0.7%, the cream behaved like a solid while with increasing the applied strain, both G' and G'' decrease while G'' exceeds G' and the product started to behave like a liquid [20,25].

Figure 1. Flow curves of cream formulations at room temperature, (A) Flow behavior modeling using the Herschel-Bulkley model, in terms of shear stress, (B) modeling in terms of apparent viscosity, and (C) modulus vs. strain (%) of F3.



1.4.5 pH measurement

Although this test is not included in the compendial monograph of each topical drug product, it should be a part of the manufacturer's specification for the drug product. It is well known that topically applied products should be acidified to keep the pH value of the formulation in the range of 4.0 to 6.0 [26]. The acidic pH of the skin surface maintains SC homeostasis and regulates barrier permeability [26]. The pH values of F3, F4, and F5 formulations were 4.6 ± 0.1 , 4.7 ± 0.2 , and 4.7 ± 0.1 , respectively which is favorable for topical application.

1.4.6 Texture analysis test

Textural features of cream formulations such as firmness, tackiness, stringiness, and work of adhesion (WOA) were investigated to provide more additional information about the characteristics of the cream such as its spreadability, mucoadhesion, and strength (Table 4). Firmness refers to the work required to remove the cream from the container for application to the desired area of the skin. Whereas tackiness is the property of being cohesive and sticky and it is defined as the work/force needed to overcome the attractive forces between the surface of the

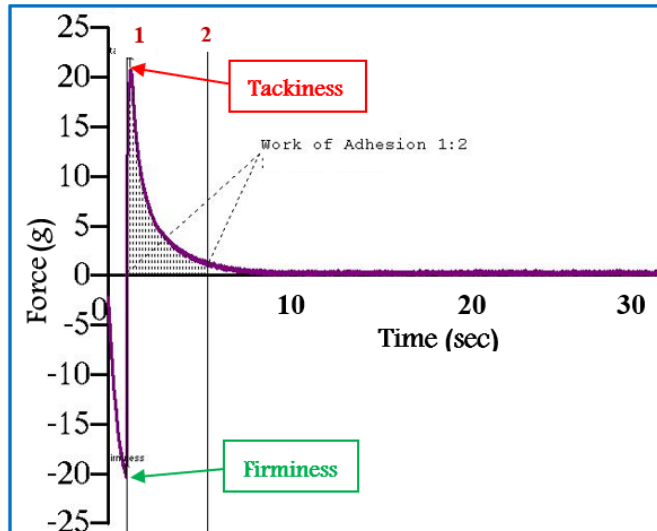
cream and the surface of the probe with which the formulation comes in contact. In addition, the cream must spread over the skin surface to provide close contact and a large surface area of contact for drug diffusion. Therefore, the WOA was measured to evaluate the spreadability of the cream formulations. The degree (distance) to which the cream hangs on the probe when the probe is removed (stringiness) was also measured. Stringiness is considered undesirable for the application of cream where a 'short' texture is required for quick and clean removal of fingers after topical application. This feature is correlated with the viscoelastic behavior of the product.

Table 4. Textural characteristics of successful cream formulations (mean \pm SD, n = 4).

| Cream | Firmness (g) | Tackiness (g) | Work of Adhesion (g.sec) | Stringiness (mm) |
|--------------|-------------------------|--------------------------|-------------------------------------|-----------------------------|
| F3 | 17.9 \pm 2.8 | 13.3 \pm 2.5 | 12.3 \pm 1.1 | 5.7 \pm 0.6 |
| F4 | 21.5 \pm 2.2 | 17.7 \pm 0.9 | 15.5 \pm 1.3 | 6.3 \pm 0.6 |
| F5 | 24.2 \pm 0.8 | 18.9 \pm 2.5 | 16.6 \pm 1.2 | 6.6 \pm 0.7 |

The force vs. distance profile for the F5 formulation is shown in Fig. 2. The firmness obtained for the cream formulation was 24.2 \pm 0.8 (g). WOA as per the force vs. distance profile is defined as the highest peak force obtained during the compression cycle [27]. The WOA represented the ratio of the area under the tackiness curve to the area under the firmness curve. WOA obtained for the cream was 16.6 \pm 1.2 (g.sec). Based on the tabulated results, all three formulations showed good results concerning spread ability, mucoadhesion, and strength.

Figure 2. Force vs. distance profile for F5 cream formulation.



1.4.6.1 Drug-stability in the receiver medium

Stability studies demonstrated that ITZ content in the receiver medium was 99.7 ± 2.7 and 98.2 ± 1.8 % at 6 h and 48 h, respectively. These results revealed that ITZ remained stable in the receiver media used for release, permeation, and deposition studies.

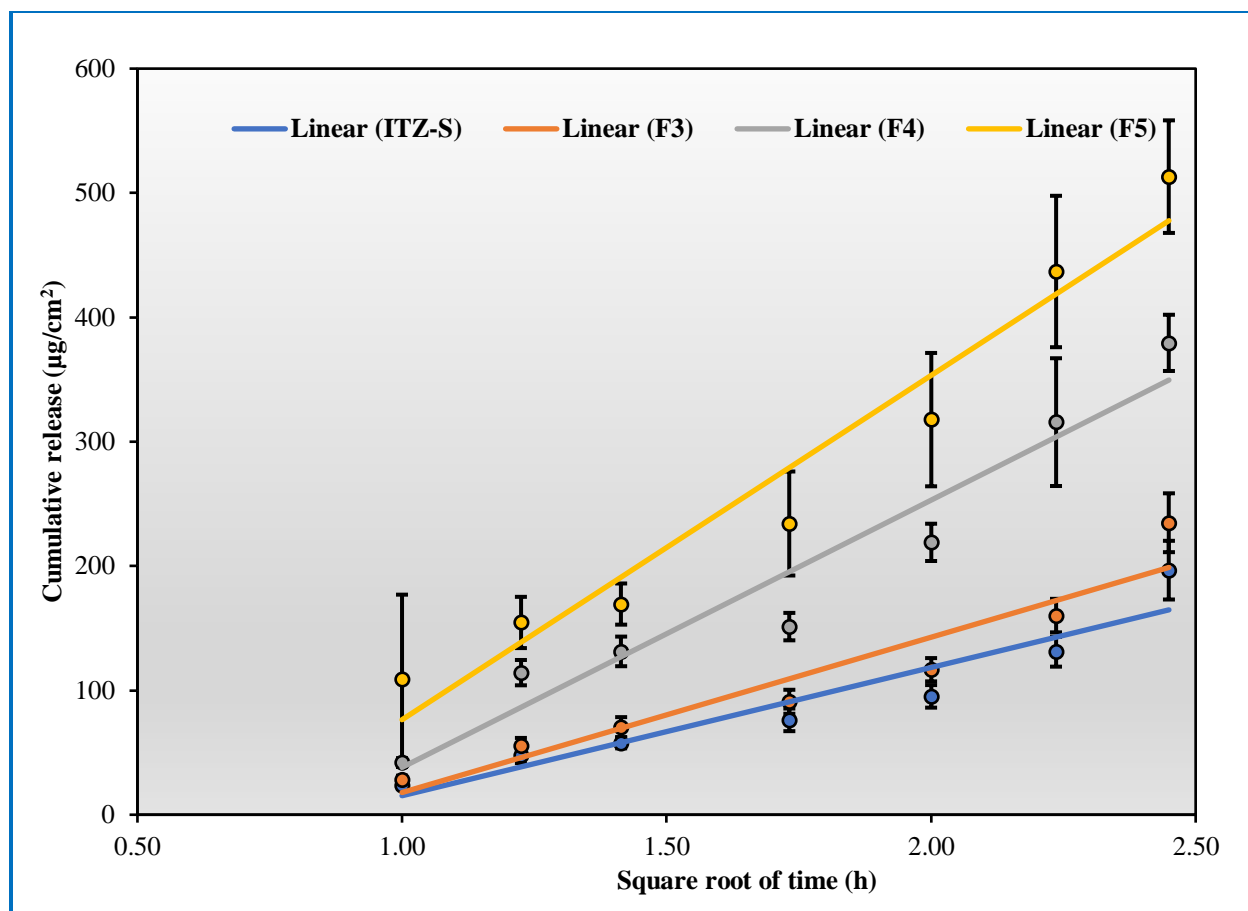
1.4.6.2 Membrane inertness test

The test demonstrated that there was negligible adsorption of ITZ to the diffusion membrane after exposure to the ITZ solution for 6 h. The amount of ITZ adsorbed on the membrane was 0.66 ± 1.22 , 0.76 ± 0.23 , and 0.12 ± 2.1 μg during the exposure of the diffusion membrane to 100, 200, and 400 $\mu\text{g}/\text{mL}$ ITZ solutions, respectively. Therefore, Nylon membranes did not act as a rate-controlling barrier and the ITZ release would be attributed to the formulation characteristics.

1.4.6.3 *In vitro* release test

The *in vitro* release profiles are shown in Fig. 3. The *in vitro* release rates of F3, F4, and F5 cream formulations (120.9 ± 6.9 , 219.4 ± 4.0 , 274.8 ± 7.3 $\mu\text{g}/\text{cm}^2/\text{h}$, respectively) were significantly ($p < 0.05$) higher compared to drug solution (103.9 ± 6.0 $\mu\text{g}/\text{cm}^2/\text{h}$). The results also showed that all tested cream formulations followed Higuchi's release profile. These observations indicate a direct dependence of the release rate on the diffusion coefficient. Among other factors, the diffusion coefficient is dependent on the solubility of the drug in the excipients of the formulation. ITZ release was inversely proportional to the amount of PEG mixtures within the formulations. Therefore, a lower release rate of the drug is expected when there is a high affinity of the drug for the excipients as in the case of the drug solution. The results came in accordance with earlier published investigations [28,29].

Figure 3. The *in vitro* drug release profiles from ITZ-S, F3, F4, and F5 formulations (mean \pm SD, n = 4)



1.4.7 *Ex vivo* skin permeation and deposition studies

Ex vivo skin permeation and deposition studies can be used to mimic and predict percutaneous absorption in humans [18,30]. The *ex vivo* permeation and deposition profiles and data are presented in Fig. 4 and Table 5. All cream formulation improved ITZ permeation and deposition significantly ($p < 0.05$) across human cadaver skin compared to drug solution. The transdermal flux and permeation of ITZ from different creams were in the following rank order: F5>F4>F3. This could be due to the following reasons; Transcutol[®] P is well established as an excellent solvent and permeation enhancer to improve the solubility, penetration, and percutaneous absorption of lipophilic drugs intended for topical application [31]. Transcutol[®] P may exert its role by the

modification of the barrier functions of the SC—increasing drug solubility/partitioning in SC, maintaining hydrated dynamics in SC, and intercellular lipid fluidization [21,31]. Therefore, the increase in Transcutol® P concentration in the formulation increased ITZ permeation and deposition simultaneously. The other possible reason is the interaction of other cream excipients with the skin. Based on the literature reports, fatty alcohols penetrate the skin lipids by disorganizing the lipid bilayers and act as a solvent for the actives to form a reservoir and permeate across the skin [21,32]. Furthermore, oleic acid has been reported to increase the transdermal permeability of both lipophilic and hydrophilic drugs through the perturbation of SC lipid bilayers and lacunae formation [33]. Moreover, PEG 200 and 400 have been reported to be excellent permeation enhancers through the interaction with keratin inside the cells, fluidize the SC and create channels for drug passage [34].

Figure 4. *Ex vivo* permeation and deposition studies of itraconazole formulations through human cadaver skin at infinite dosing (mean \pm SD, n = 4).

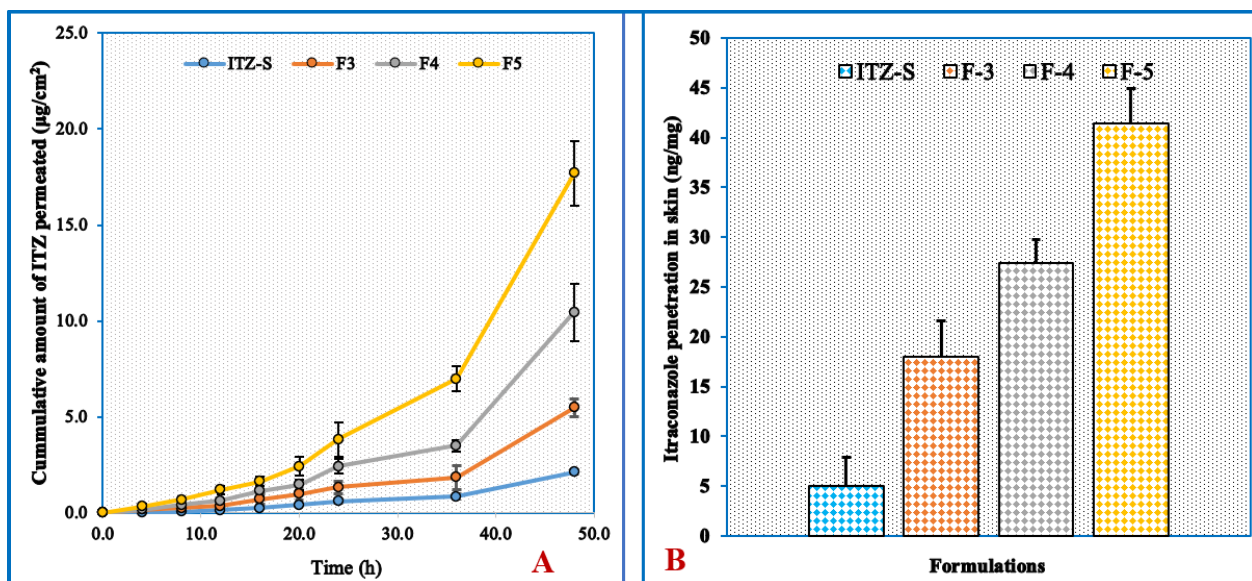


Table 5. The cumulative amount permeated and flux of itraconazole formulations at finite dosing (mean \pm SD, n = 4).

| Formulation | Cumulative amount permeated ($\mu\text{g}/\text{cm}^2$) | Flux ($\text{ng}/\text{h}/\text{cm}^2$) | Folds improvements in permeability | Folds improvements in Flux |
|--------------------|---|---|---|-----------------------------------|
| ITZ-S | 2.1 \pm 0.2 | 25.5 \pm 4.4 | NA | NA |
| F3 | 5.5 \pm 0.5* | 139.9 \pm 11.3* | 2.6 | 5.5 |
| F4 | 10.5 \pm 1.5*,# | 191.9 \pm 9.8*,# | 5.0 | 7.5 |
| F5 | 17.7 \pm 1.7*,#,& | 332.9 \pm 13.0*,#,& | 8.4 | 13.1 |

*Statistically significant at $p < 0.05$ in comparison to ITZ-S.

#Statistically significant at $p < 0.05$ compared with F3 formulation.

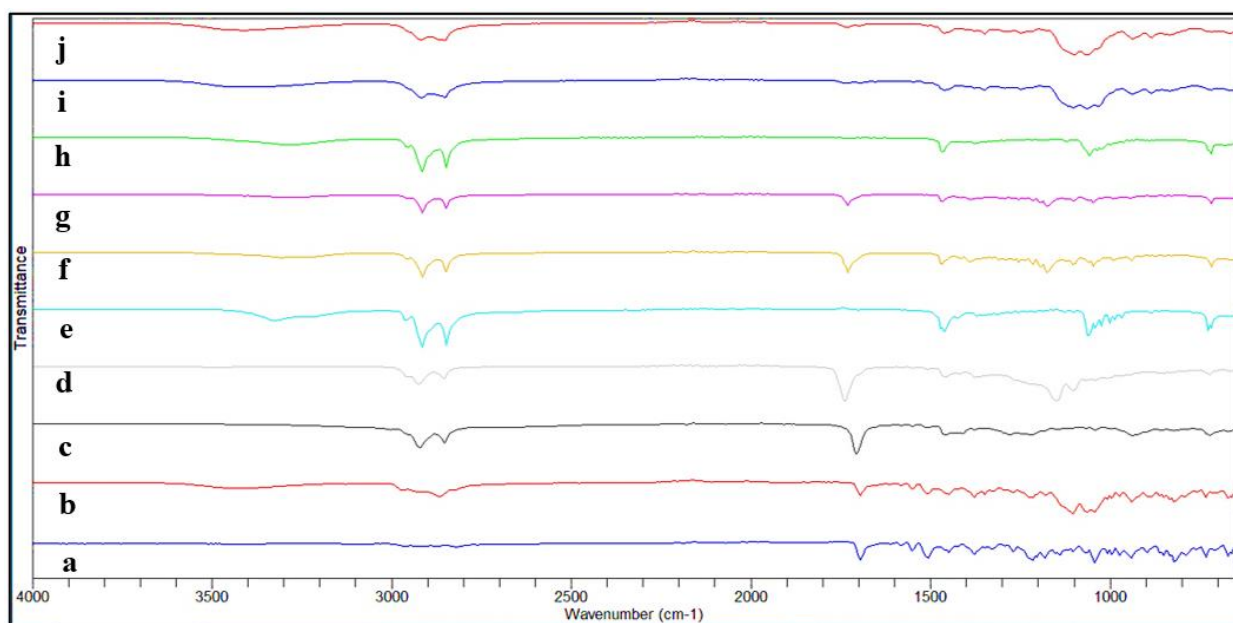
&Statistically significant at $p < 0.05$ compared with F4 formulation.

1.4.8 FTIR spectroscopy

The study of physicochemical interaction of actives and excipients in any dosage form is a fundamental part of preformulation studies during the development of drug delivery systems. Excipients can interact with actives chemically or physically (H-bonding); thus, they can affect drug product stability [18]. The FTIR spectrum of ITZ showed the characteristic bands including 1,699 cm^{-1} (C=O stretch), 1,454 cm^{-1} , and 1,512 cm^{-1} (aromatic C=C stretch), 1,045 cm^{-1} (aryl Cl), 1,224 cm^{-1} , and 1,046 cm^{-1} (C–O stretch), and 1,383 cm^{-1} (C–N stretch) as shown in Fig. 5 [35].

The FTIR did not show any characteristic peaks for ITZ in cream formulation due to the solubilization of ITZ within the semisolid matrix. In addition, the cream formulation did not show a major shifting of any peaks compared to ITZ and excipients spectra individually. The FTIR spectra demonstrated that there were no drug-excipient interactions in the prepared cream formulations.

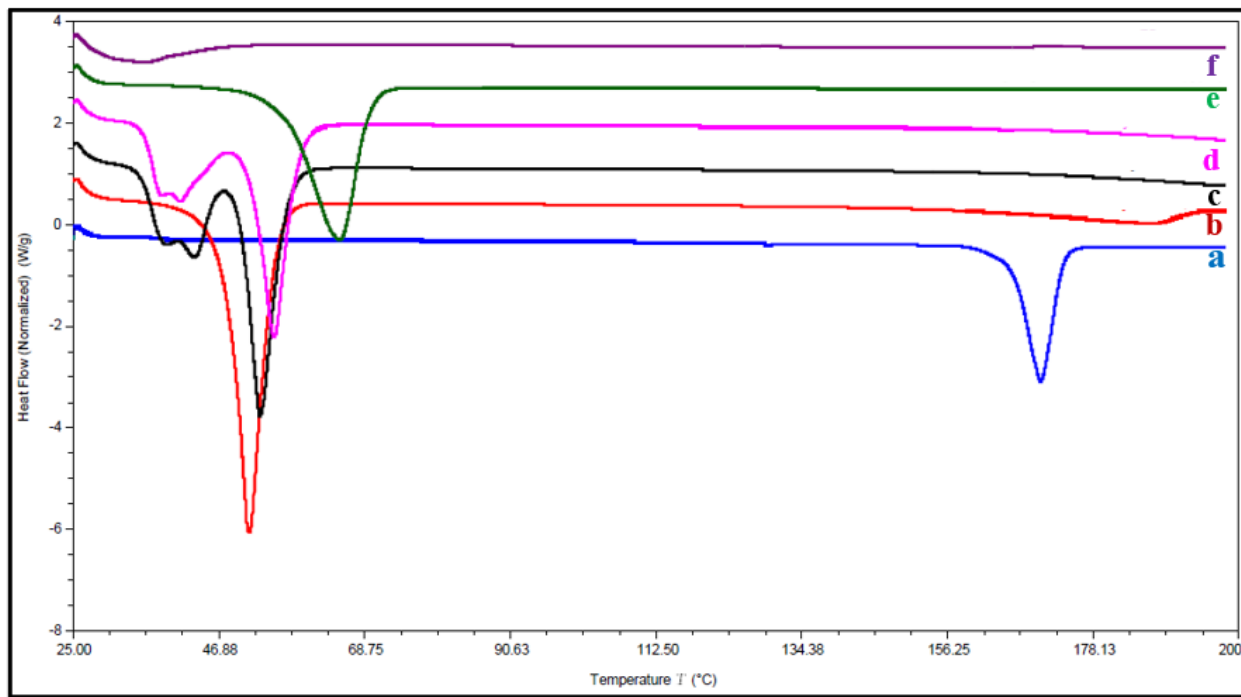
Figure 5. FTIR spectra of (a) itraconazole, (b) Transcutol® P, (c) oleic acid, (d) Miglyol® 829, (e) Kolliwax® CA, (f) Kolliwax® GMS II, (g) Kolliwax® CSA 50, (h) Kolliwax® CSA 70, (i) placebo F5 cream, and (j) F5 cream.



1.4.9 Differential scanning calorimetry (DSC)

The DSC thermogram of ITZ showed an endothermic peak at 167.8°C. However, the DSC thermogram of the tested cream formulation (F5) showed a complete disappearance of this drug peak (Fig. 6) suggesting the conversion of the drug from the crystalline form to the amorphous form. This observation could be due to the complete solubilization of the drug in cream excipients.

Figure 6. DSC thermograms of (a) itraconazole, (b) Kolliwax[®] CA, (c) Kolliwax[®] CSA 50, (d) Kolliwax[®] CSA 70, (e) Kolliwax[®] GMS II, and (f) F5 cream.



1.4.10 Stability studies

Stability study results demonstrated that there was no significant change ($p < 0.05$) observed with respect to physical appearance, pH, and drug content (%) of the tested cream formulation at the two storage conditions over three months as presented in [Table 6](#).

Table 6. Physical appearance, pH, and drug content (%) of F3, F4, and F5 cream formulations over three-month storage at 4 and 25°C (mean \pm SD, n = 3).

| F3 | | | | | | |
|-----|------------------|--------------|-------------|--------------|-------------|--------------|
| Day | Drug content (%) | | pH | | Appearance | |
| | 4 \pm 2°C | 25 \pm 2°C | 4 \pm 2°C | 25 \pm 2°C | 4 \pm 2°C | 25 \pm 2°C |
| | | | | | | |

| | | | | | | |
|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| 1 | 100.4±1.2 | 100.5±1.8 | 4.62±0.03 | 4.60±0.01 | No change | No change |
| 30 | 97.8±1.7 | 99.5±1.7 | 4.58±0.01 | 4.63±0.03 | No change | No change |
| 60 | 101.0±1.6 | 102.9±2.5 | 4.55±0.02 | 4.56±0.01 | No change | No change |
| 90 | 98.5±2.3 | 100.3±2.2 | 4.58±0.01 | 4.62±0.02 | No change | No change |
| F4 | | | | | | |
| 1 | 99.8±2.4 | 99.1±1.4 | 4.71±0.02 | 4.72±0.01 | No change | No change |
| 30 | 98.8±2.0 | 98.5±1.2 | 4.67±0.01 | 4.68±0.01 | No change | No change |
| 60 | 99.0±2.6 | 98.9±1.8 | 4.65±0.03 | 4.68±0.01 | No change | No change |
| 90 | 98.4±2.5 | 97.8±2.0 | 4.66±0.01 | 4.65±0.01 | No change | No change |
| F5 | | | | | | |
| 1 | 100.6±1.1 | 100.3±1.6 | 4.66±0.02 | 4.65±0.02 | No change | No change |
| 30 | 98.4±2.3 | 99.5±2.2 | 4.62±0.02 | 4.64±0.01 | No change | No change |
| 60 | 98.6±2.2 | 99.0±2.5 | 4.63±0.01 | 4.62±0.02 | No change | No change |
| 90 | 99.6±2.4 | 98.5±2.3 | 4.68±0.01 | 4.63±0.02 | No change | No change |

1.5 Conclusion

Itraconazole-loaded Oil/PEG (non-aqueous) creams were successfully prepared and evaluated for stability at two different storage conditions over a three-month stability study. The increase in Transcutol® P concentration in cream formulation resulted in simultaneous improvement in drug flux, permeation, and deposition across the human cadaver skin. The *in vitro* release testing revealed a sustained release profile with a Higuchi release pattern. Therefore, the current

investigation demonstrated that topically applied cream could serve as a better alternative for other itraconazole routes of administration for the treatment of fungal skin infections.

Chapter 2 Improved skin permeation and deposition of non-aqueous gels on Human cadaver skin using itraconazole as a model lipophilic drug

2.1 Introduction:

In the United States, 5.4 million cases of skin cancer are reported each year [36]. Skin cancer can be divided into two main categories, malignant melanoma (MM) and nonmelanoma skin cancer (NMSC) [37]. NMSC is further classified into squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) [37]. The incidence of BCC is high and represents 80% of NMSC [38]. It is well-established that the hedgehog pathway is involved in BCC and melanoma development [39]. Topical anti-cancer drug treatment would be a better and effective option for topical (skin) cancer treatments. Topical products offer patient compliance, which is of utmost importance, especially to cancer patients. Till now, 5-fluoro uracil and imiquimod are the only two topically (cream) applied drugs available for treating skin cancer [40]. Although these two drugs exhibited better therapeutic outcomes during skin cancer treatment, these drugs exhibited several adverse effects [40]. Erythema, pain, dermatitis, and pruritus are all common side effects for 5-fluoro uracil [40]. While mild to severe symptoms such as erythema, edema, weeping, pruritus, permanent hypopigmentation, crusting or scabbing or scaling, erosion, burning, and pain are all frequent side effects of imiquimod [40].

Drug repurposing is a cost-effective and timesaving identification of new pharmacological effects of conventional approved drugs. Nowadays, the repurposing of itraconazole (ITZ) for cancer treatment has been extensively investigated [41,42]. ITZ is an FDA-approved, triazole,

broad-spectrum antifungal agent used clinically for a wide range of superficial and deep fungal infections when administered orally once or twice daily [43]. An emerging *in vivo*, *in vitro*, and evidence-based clinical studies have confirmed that ITZ possesses anticancer activities, and it also has a synergistic action when combined with other antineoplastic agents [42]. ITZ has revealed its antineoplastic activity through several mechanisms, including inhibition of the Hedgehog pathway, decreased endothelial cell proliferation, prevention of angiogenesis, cell cycle arrest, and induction of auto-phagocytosis [42]. These several mechanisms enable ITZ to improve therapeutic efficacy and overcome drug resistance when used alone or in conjunction with other cytotoxic medicines [42]. Therefore, ITZ can be used as a therapeutic alternative for topical 5-fluoro uracil and imiquimod for skin cancer treatment.

However, ITZ has low oral bioavailability due to its poor aqueous solubility [7]. Moreover, the ITZ systemic absorption is associated with many side effects including hepatotoxicity, cardiac dysrhythmia, peripheral neuropathy, and hearing loss [8]. Furthermore, the absorption of ITZ from the oral dosage forms is highly affected by food intake [9]. Therefore, the topical application of ITZ could minimize its unwanted side effects and drug-food interactions. To the best of our knowledge, there is no effective topical formulation available for topical application of ITZ in skin cancer or fungal infection treatment, and therefore, it is a driving need to develop ITZ topical formulation. However, ITZ has a high molecular weight of 705.64, the poor aqueous solubility of 1 ng/mL, and high lipophilicity ($\log P > 5$) [4]. In addition, the barrier function of the stratum corneum (SC) could negatively affect the transdermal delivery of ITZ during topical application [4].

Among the different topical dosage forms, gels have demonstrated better therapeutic outcomes during topical application of different drugs including antifungal drugs [44–47]. Other additional

numerous advantages such as ease of preparation and scaling up, biocompatibility, and biodegradability of polymeric ingredients have also been reported for hydrogels [48]. However, it is difficult to incorporate lipophilic drugs into hydrogels [48]. It is also difficult to obtain a stable hydrogel without the addition of a surfactant [48].

Thus, this investigation aimed to develop a non-aqueous hydrophilic gel formulation using polyethylene glycol (PEG) as a drug delivery vehicle for ITZ for topical application. PEG is also used as a skin permeation enhancer and solubilizer in topical dosage forms. The non-aqueous gels prepared in this study could provide better stability due to water removal from the formulation along with improved drug solubility and prolonged skin contact time, thus, enhanced ITZ penetration during topical application.

2.2 Materials and Methods:

ITZ was procured from TCI[®] chemicals (Tokyo Chemical Industry, Portland, OR, USA). Gattefossé Corporation (Paramus, NJ, USA) gifted few oils and lipids such as Transcutol[®] P, Labrasol ALF, Labrafil M 2125 CS, Labrafil M 1944, Lauroglycol FCC and PluroOleique CC 497. Also, BASF Corporation (Florham Park, NJ, USA) provided Polyethylene glycol (PEG) 400, Kolliphor ELP, Kolicream OD and PEG 200 as gift samples. The Tween[®] 80, Oleic acid, and other HPLC grade reagents were purchased from Fischer Scientific (Pittsburgh, PA, USA). Cryopreserved human cadaver skin was procured from New York firefighters skin bank (New York, NY, USA).

2.3 Solubility studies in different vehicles

Labrasol ALF, Transcutol P, PEG 200, Caproyl 90, Kolliphor ELP, 20% 2-Hydroxypropyl- β -cyclodextrin (HP- β -CD), Labrafil M 2125 CS, Labrafil M 1944, Lauroglycol FCC, Propylene

Glycol, PluroOleique CC 497, Oleic Acid, Sesame oil and Kolicream OD were selected for the solubility of ITZ. In this study, 5 mL of each component was added separately to excess amount of ITZ in glass scintillation vials kept for mixing at $25 \pm 2^\circ \text{C}$ for 48 h at 1200 rpm in vortex. The amount of undissolved ITZ was separated by centrifugation and solubilized amount of ITZ (in the supernatant) was analysed by UFLC method.

2.4 Screening of penetration enhancers

The effect of excipients on permeation of ITZ across human cadaver skin was carried out using Franz diffusion cells [22]. A fresh human cadaver skin was placed between the donor and receptor compartments with 0.64 cm² exposure area. The stratum corneum surface was exposed to the donor chamber. The PBS pH 7.4 (contains 0.5% of Tween® 80) was placed in the receiver chamber. The samples with electric resistance $\geq 10 \text{ K}\Omega\cdot\text{cm}^2$ were used in the experiment. The receiver fluid was stirred continuously with the help of magnetic stirrer at $32.0 \pm 2.0^\circ \text{C}$. Further, 0.4 mL saturated solutions of ITZ in different excipients were added to the donor chamber. Then, 0.5 mL samples from receiver chambers were collected at different time intervals, such as 0, 2, 4, 6, 8, 12, 16, 20, 24, 36, and 48 h and replenished with 0.5 mL of fresh media. After terminal sampling the active diffusion area of skin was removed and washed. The skin was weighed, cut into small pieces, and homogenized. The ITZ content in skin homogenate samples were determined using UFLC method.

2.5 Optimization and formulation of non-aqueous gels for ITZ delivery

The various combinations of non-aqueous vehicles prepared and excess amount of ITZ was added (Table 7). The solubilized ITZ in non-aqueous vehicles were quantified by UFLC method. The various formulations of ITZ non-aqueous gel with different concentration of vehicles are listed in

Table 8. The specified amount of polymer was soaked in saturated ITZ (0.5 % w/v) non-aqueous solution for 3 h and dispersed using magnetic stirrer at 80 °C and neutralized with triethanolamine. The resulted gel was used for further studies.

Table 7 Selection of Non aqueous solvents

| Ratio | Non-Aqueous Vehicles | ITZ solubility |
|--------------|--|-------------------------|
| 1:1:1 | Transcutol P: Labrsol ALF: Caproyl 90 | Precipitation Formed |
| 1:1:1 | Transcutol P: Labrsol ALF: Kolliphor ELP | Precipitation Formed |
| 1:1:1 | Transcutol P: Labrsol ALF: Oleic acid | Less than 3.5 mg/mL |
| 1:1:1 | Transcutol P: Labrsol ALF: Oleic acid: PEG 200: PEG 400 | Less Than 4 mg/mL |
| 1:1:1 | Transcutol P: Labrsol ALF: PEG 200: PEG 400 | Less Than 4 mg/mL |
| 1:1:1 | Transcutol P: PEG 200: PEG 400 | Less than 4.5 mg/mL |
| 5:1:1 | Transcutol P: PEG 200: PEG 400 | 5 mg/mL |

Table 8 Optimization of Carbopol 940 P Gel in non-aqueous vehicle

| Formulations | ITZ (% w/v) | Transcutol P (% w/v) | PEG 200 (% w/v) | PEG 400 (% w/v) | Carbopol 940 P (% w/v) |
|---------------------|------------------------|---------------------------------|----------------------------|----------------------------|-----------------------------------|
| F-1 | - | 50 | 25 | 25 | - |

| | | | | | |
|------------|-----|----|----|----|-----|
| F-2 | 0.5 | - | 50 | 50 | - |
| F-3 | 0.5 | 50 | 25 | 25 | - |
| F-4 | 0.5 | 50 | 25 | 25 | 0.5 |
| F-5 | 0.5 | 50 | 25 | 25 | 1 |
| F-6 | 0.5 | 50 | 25 | 25 | 1.5 |

2.6 Characterization of Gel

2.6.1 pH analysis

The pH of 0.5 % w/v gel formulation was measured using Digital pH meter ((Mettler Instruments, Giessen, Germany). All the formulations were in triplicate (n=3).

2.6.2 Texture Analysis

The texture of the ITZ non-aqueous gels (F4, F5 and F6) was carried out using a TA.XT2i texture analyser, Texture Technologies Corp./Stable Micro Systems equipped with 5 Kg load, and TA-3 acrylic probe. The instrument was calibrated with 2 kg weight before running the test. The cavity of soft matter fixture was loaded with gel formulation and excess amount of gel was scraped out with the help of scrapper. Lastly, sample placed under the TA-3 probe and work of adhesion (WoA) was carried out using Texture Expert software. All the drug loaded formulations were performed in triplicate (n = 3).

2.6.3 Rheological analysis

The rheological analysis of non-aqueous formulations was performed using TA instrument DHR-2 rheometer. The parameters used for rheometer analysis were indicated in Table 9. The 1 gram of

gel was loaded onto the lower plate and the upper plate was dropped into the gel. The upward and downward flow curves for each non-aqueous formulation were measured over shear rates ranging from 10 to 900 s⁻¹ [49, 50] to analyze both elastic (G') and viscous (G'') material responses. All samples were carried out in the linear viscoelastic range.

Table 9 Parameters used for Rheometer analysis

| Parameters | Conditions |
|---------------------------------------|------------|
| Sample | 1 gram |
| Temperature | 32 ± 2°C |
| Plate diameter | 25 mm |
| Distance between upper and late plate | 550 µm |

2.6.4 Drug content

The ITZ gel formulations were dispersed in methanol using shaker at 1200 rpm. The resulted solution was centrifuged at 13000 rpm for 10 minutes, and supernatant was collected. Further, ITZ content in samples were quantified by using UFLC methods.

$$\text{Drug content} = [\text{Amount of ITZ in gel} / \text{Total weight of gel}] * 100$$

2.6.5 Stability

The non-aqueous gel formulation was placed in stability chamber under varied storage conditions (25 ± 2 °C with RH 60 ± 5%, 40 ± 2 °C with RH 75 ± 5%, and 4 ± 2 °C) for 3 months. After 3

months, the drug content of stored formulation was analysed using UFLC method. All the formulations were performed in triplicate (n = 3).

2.7 *In vitro* release test

In vitro release study of non-aqueous gels was carried out using the Franz diffusion cell (0.64 cm² exposure area) in PBS pH 7.4 with 0.5 % Tween 80. As presented in the above section 2.2, similar experimental conditions were employed for *in vitro* release study by replacing human cadaver skin with the artificial nylon membranes (pore diameter 0.2 µm), which is inserted between donor and receptor compartments with the help of a clamp. ITZ non-aqueous gels (1 gram) were placed in the donor compartment and sealed with paraffin film. The samples were withdrawn at pre-defined intervals (0, 0.5, 1, 2, 4, 6, 8 h) and the released amount of ITZ was quantified by using UFLC. All the samples were conducted in quadruplicate (n=4).

2.8 *Ex-vivo* Permeation and skin deposition studies:

The *Ex-vivo* permeation and deposition studies of ITZ formulations across the human cadaver skin was conducted using similar experimental conditions as presented in the above section 2.2. In this study, 1 gram of ITZ non-aqueous gels were load in the donor compartment and sealed with paraffin film. At different time intervals, such as 0, 4, 8, 12, 16, 20, 24, 36, 48 h, the samples were collected and the permeated amount of ITZ across the skin was quantified by using UFLC.

For the ITZ skin deposition study, the skin was removed from the cell and washed with PBS after termination of the experiment. Further, skin was sectioned into small pieces, and amount of ITZ deposited in skin homogenate samples was determined using UFLC. All the samples were conducted in quadruplicate (n=4).

2.9 Results and Discussion

2.9.1 Solubility of ITZ in different vehicles

The results of ITZ solubility studies were shown in Figure 7. Among various vehicles used in this study, the following ingredients showed highest solubilization of ITZ, including, Transcutol P ($3716.1 \pm 82.7 \mu\text{g/mL}$), Labrasol ALF ($1759.2 \pm 24.7 \mu\text{g/mL}$), Caproyl 90 ($1553.0 \pm 6.7 \mu\text{g/mL}$), PEG 200 ($1441.5 \pm 91.3 \mu\text{g/mL}$), Kolliphor ELP ($843.6 \pm 100.1 \mu\text{g/mL}$), Oleic acid ($611.0 \pm 26.8 \mu\text{g/mL}$) and 20% HP- β -CD ($597.3 \pm 12.6 \mu\text{g/mL}$). Therefore, the above vehicles were selected as for further screening of drug permeation studies. The other vehicles displayed low solubility of ITZ ($< 500 \mu\text{g/mL}$) and were discounted from this study, including Labrafil M 2125 CS, Labrafil M 1944, Lauroglycol FCC, Propylene Glycol, PluroOleique CC 497, Sesame oil, and Kollicream OD.

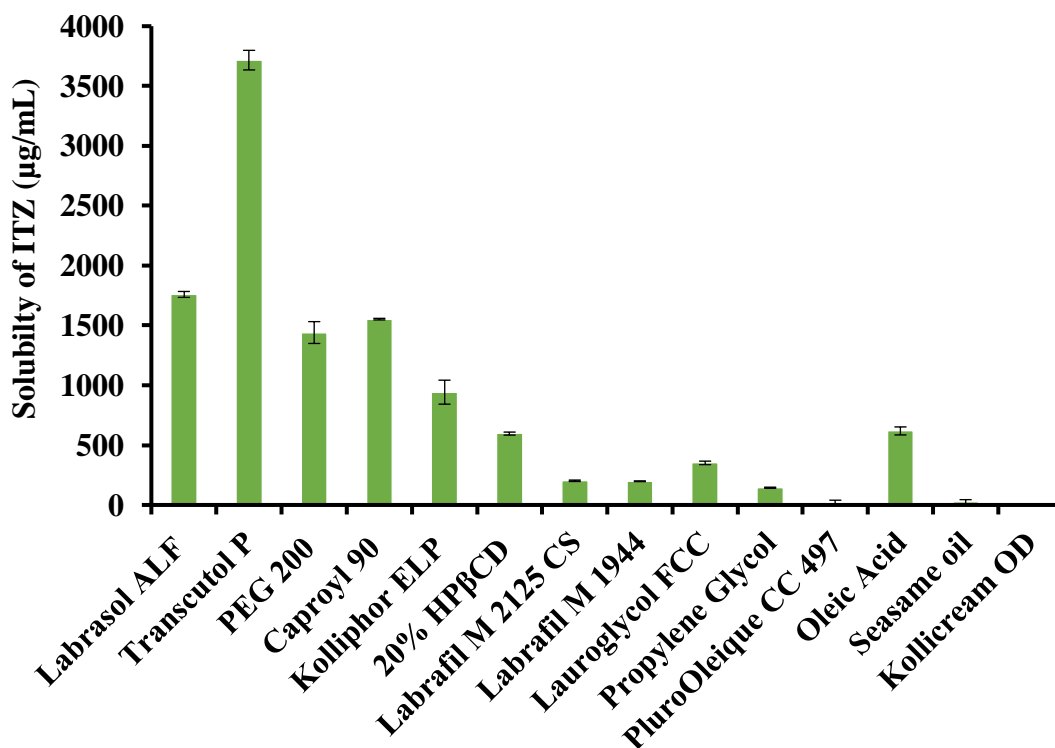


Figure 7 Solubility of ITZ in various vehicles (Data expressed as Mean \pm SD)

2.9.2 Selection of penetration enhancers for ITZ delivery

The effect of various vehicles on the penetration of ITZ were plotted and are shown in Figure 8. Among the screened vehicles, Transcutol P showed maximum penetration (35 ng/mg) of ITZ across human skin. The saturated ITZ solutions of Labrasol ALF, PEG 200, Caproyl 90, Kolliphor ELP and 20% HP- β -CD were showed low drug penetration (<5 ng/mg) across skin. The increased percutaneous penetration of ITZ was achieved due to change in the structure of stratum corneum and fluidization of the lipids [31]. Transcutol P is a widely used solubilizer and penetration enhancer in the development of BCS-II and IV drugs.

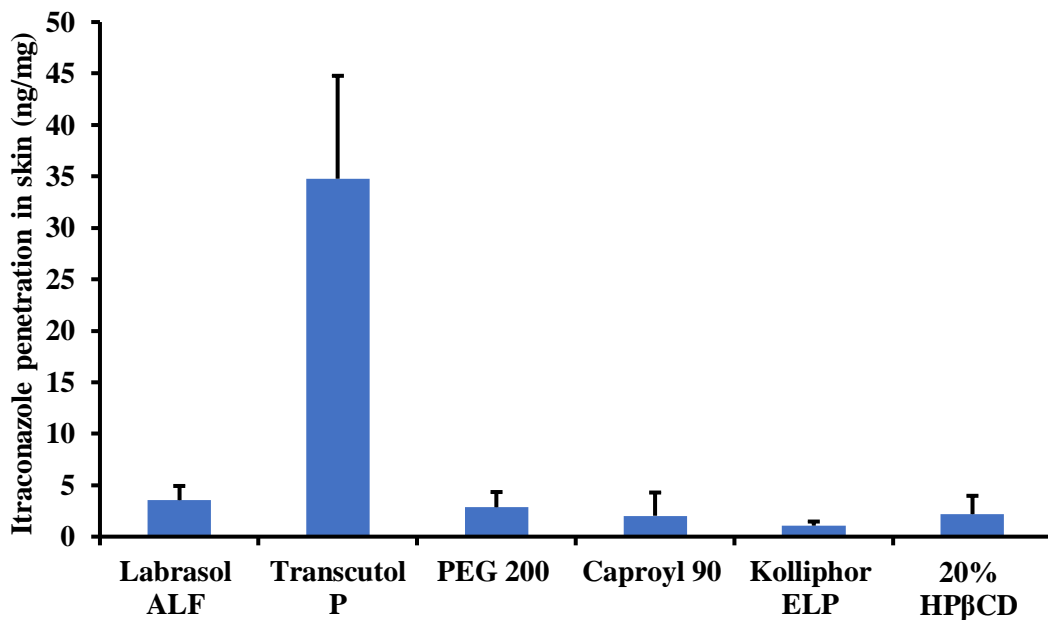


Figure 8 Itraconazole penetration with various vehicles across skin (Data expressed as Mean \pm SD)

2.9.3 Optimization and formulation of non-aqueous gels for ITZ delivery

The Transcutol P: PEG 200: PEG 400 (5:1:1) combination showed highest solubility (5 mg/mL) compared to other non-aqueous vehicles. This optimized non-aqueous vehicle combination was further used and performed preliminary studies to confirm the concentration of Carbopol 940P polymer, which are suitable to form a non-aqueous gel with a reasonable consistency.

2.9.4 Gel Characterization studies

2.9.4.1 pH Analysis

pH parameter is a very critical factor in the development of topical gels, must have equal human skin pH 4.5-6.5 to control skin irritation. All the formulations were displayed safe and acceptable pH range from 6.0-6.5.

2.9.4.2 Texture Analysis

Textural features of prepared gel formulations such as firmness, tackiness, stringiness, and work of adhesion (WOA) were calculated to provide more additional information about the characteristics of the gel formulations such as their spread ability, mucoadhesion, and strength. Firmness refers to the work required to remove the gel from the container for application to the desired area of the skin. Whereas tackiness is the property of being cohesive and sticky and it is defined as the work/force needed to overcome the attractive forces between the surface of the gel and the surface of the probe with which the formulation comes in contact. In addition, the gel must spread over the skin surface to provide a large surface area of contact for drug diffusion. Therefore, the WOA was measured to evaluate the spread ability of the gel formulations. The degree (distance) to which the gel hangs on the probe when the probe is removed (stringiness) was also

measured. Stringiness is considered undesirable for the application of gel where a 'short' texture is required for quick and clean removal of fingers after topical application to the skin. All these features are correlated with the viscoelastic behaviour of the product. Therefore, all textural features showed a simultaneous increase with increasing gel viscosity as shown in Table 10.

Table 10 Textural characteristics of prepared gel formulations (mean \pm SD, n = 4).

| Gel | Carbopol® 940 NF (% w/v) | Firmness (g) | Tackiness (g) | Work of Adhesion (g.sec) | Stringiness (mm) |
|------------|-------------------------------------|-------------------------|--------------------------|-------------------------------------|-----------------------------|
| F4 | 0.5 | 33.1 \pm 6.8 | 59.8 \pm 2.8 | 99.6 \pm 2.7 | 14.3 \pm 1.1 |
| F5 | 1.0 | 77.5 \pm 7.4 | 102.9 \pm 1.8 | 127.8 \pm 7.6 | 15.8 \pm 0.6 |
| F6 | 1.5 | 112.8 \pm 3.8 | 122.0 \pm 4.9 | 142.5 \pm 7.5 | 19.3 \pm 2.6 |

The force vs. distance plot for the F4 gel is depicted in Fig. 9. The firmness obtained for the gel formulation was 33.1 \pm 6.8 g. WOA as per the force vs. distance profile is defined as the highest peak force obtained during the compression cycle [27]. The WOA represented the ratio of the area under the tackiness curve to the area under the firmness curve. WOA obtained for the gel was 99.6 \pm 2.7 g.sec. Based on the tabulated results, all three formulations showed good results concerning spreadability, mucoadhesion, and strength based on comparison with published data for marketed gel formulation [20].

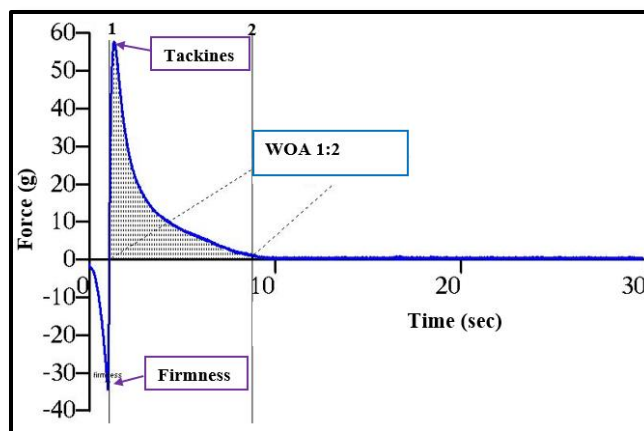


Figure 9 Force vs. distance profile for F4 gel formulation.

2.9.4.3 Rheometer analysis

The rheology of semisolid dosage forms such as gels relies on the formulation's microstructure and this fine structure could be affected by many process conditions. Therefore, a rheology study was performed to examine the effects of preparation conditions on the formulation rheology including viscoelastic behavior and the effect of shear rate on the product's viscosity. Increasing Carbopol concentration within the prepared gel resulted in a simultaneous increase in formulation viscosity; therefore, F6 (1.5 % w/v, 131290 m Pa.s) gel showed significantly higher viscosity than F4 gel (0.5 % w/v, 3675 m Pa.s). Fig. 10A shows that the forward- and backward-flow experimental data fitted to the Herschel-Bulkley model ($R^2 \geq 0.999$) in terms of apparent viscosity. The graphs showed that at low shear values, the viscosity of the products is high (high flow resistance), thereby providing good hardness to gels. As the shear values increase, the viscosity of the formulations decreases (low flow resistance) rapidly, thereby allowing the products to easily spread over a large skin surface area [24]. This shear-thinning (non-Newtonian flow) behaviour could be due to the fact that high molecular weight Carbopol polymers chains are entangled and randomly oriented at no shear (rest, high viscosity). At low shear rates, polymer chains start to

disentangle and align with the flow path, and consequently, the local drag is reduced. However, with increased shear rates, the alignment with the flow path becomes more complete, thus leading to a further decrease in viscosity [23].

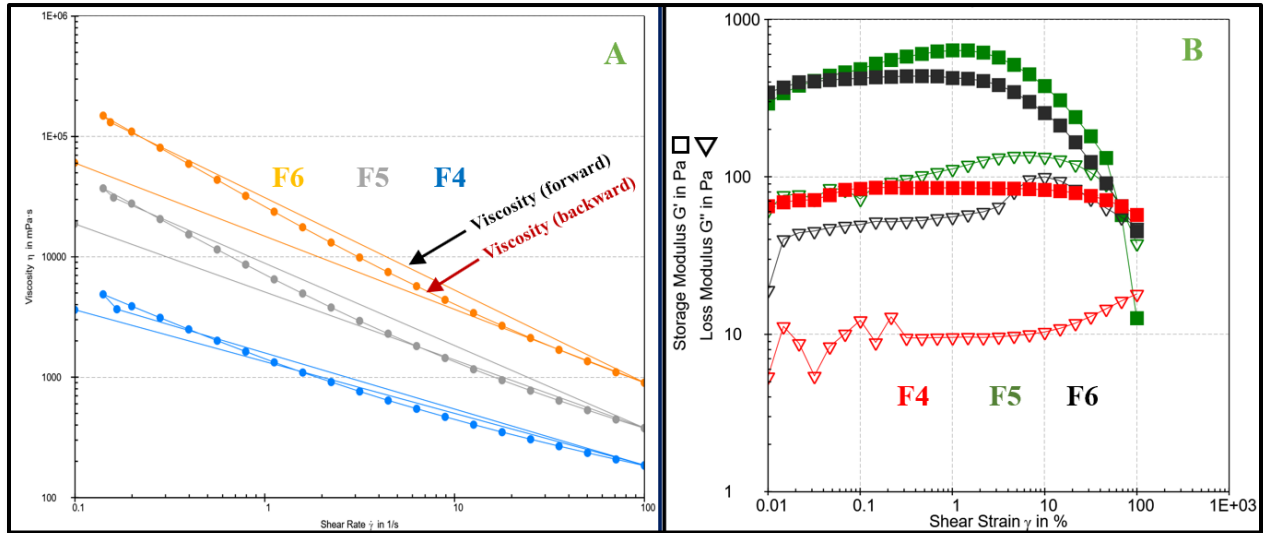


Figure 10 Rheological characterization plots of F4, F5, and F6 creams (A) viscosity vs. shear rate and (B) modulus vs.% strain (n=3).

Figs. 10B graphically illustrates the effect of strain (%) on the storage modulus (G' , elasticity) and loss modulus (G'' , viscous behaviour) of the gel. The figure shows that G' of all three gel formulations (F4, F5, and F6) remained constant up to ~1.0% strain (linear viscoelastic region), which indicates that the gel can recover quickly without any resistance within this region upon any applied stress. Then, a decrease in G' was observed. The figure shows also that G' is significantly higher than G'' which indicates that the microstructure of all gels is organized and is mainly controlled by cohesive forces between polymer chains. Up to 1.0% strain, the gels behaved like a solid. However, with increasing strain ($> 1.0\%$), both moduli decreased wherein G'' exceeds G' and the gel behaved like liquid dosage forms [20, 25].

2.9.4.4 ITZ content in formulations

Figure 11. represents the percentage of drug content in non-aqueous gel formulations. The drug content studies revealed that over 95% of desired ITZ observed in all the non-aqueous gel that indicates no significant degradation in the formulation.

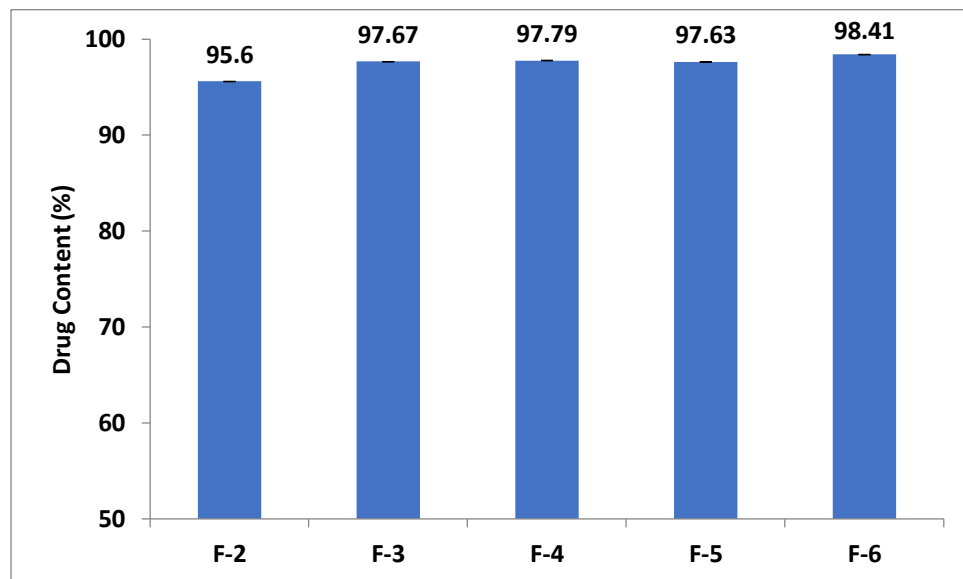


Figure 11 Drug content (%) in ITZ non-aqueous gel formulations

2.9.4.5 Stability

The stability of non-aqueous gels is expressed as drug (ITZ) content in the formulations. The content in all the formulations was found to be stable with maximum drug content (> 98 %, shown in Table 11) after 3 months storage at different conditions and there was no sign of physical instability of gels was observed upon visual observation.

Table 11 Stability of ITZ non-aqueous gels is expressed as drug content values

| Formulation | 25 ± 2 °C | 40 ± 2 °C | 4 ± 2 °C |
|-------------|-----------|-----------|----------|
| | | | |

| | with RH 60 ± 5% | with RH 75 ± 5% | |
|------------|-----------------|-----------------|---------------|
| F-2 | 99.05 ± 1.60 | 101.44 ± 1.64 | 100.89 ± 2.23 |
| F-3 | 98.68 ± 0.52 | 99.41 ± 0.28 | 99.20 ± 0.56 |
| F-4 | 98.34 ± 0.12 | 99.21 ± 0.24 | 98.98 ± 0.26 |
| F-5 | 99.37 ± 0.53 | 99.79 ± 0.77 | 99.02 ± 0.62 |
| F-6 | 99.00 ± 0.20 | 98.64 ± 0.18 | 98.20 ± 0.14 |

2.10 *In vitro* release test:

In vitro ITZ release kinetics is shown in Figure 12, that defines the cumulative released amount of ITZ from non-aqueous gel directly proportional to the square root of time. The F2, F3, F4, F5, and F6 formulations were exhibited the ITZ release rates 156.8 ± 60.9 , 643.5 ± 40.6 , 687.9 ± 41.6 , 468.3 ± 26.7 , and 538.4 ± 38.5 $\mu\text{g}/\text{cm}^2/\text{h}$, respectively. The F4 formulation showed highest ITZ release rate (687.9 ± 41.6 $\mu\text{g}/\text{cm}^2/\text{h}$) compared to other formulations. This behaviour is observed due to the low amount of Carbopol 940 P polymer used in F4 formulation, which resulted in fast gel matrix disentanglement and the speed dissolution of gel [51]. However, Pure ITZ could not permeate across the membrane.

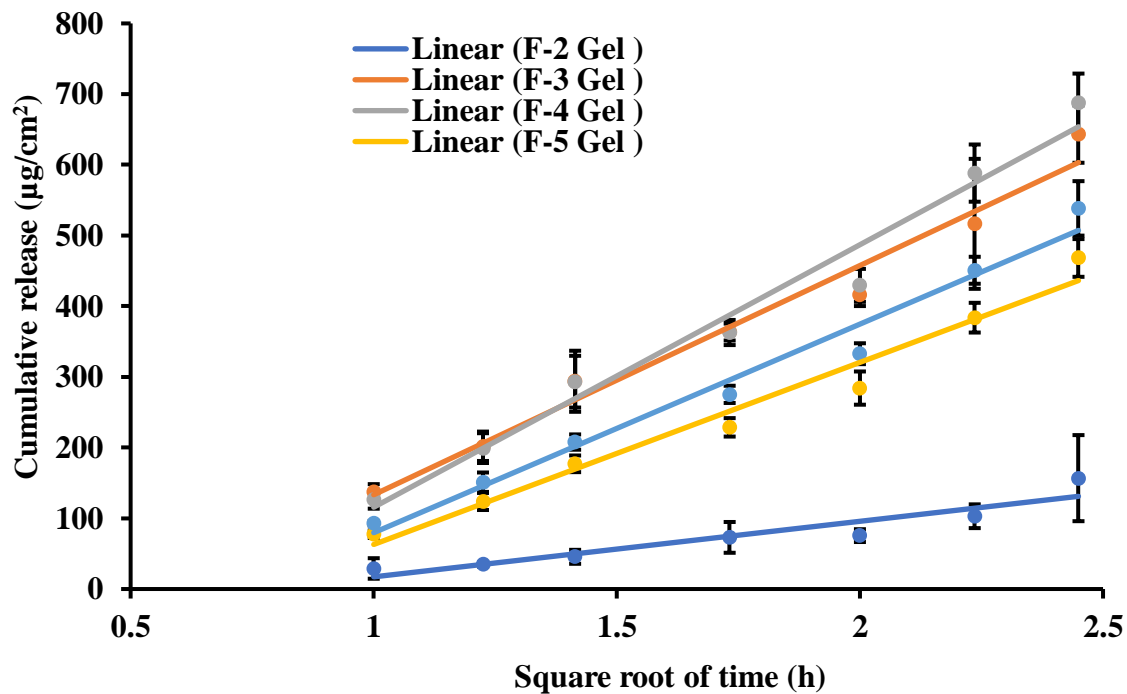
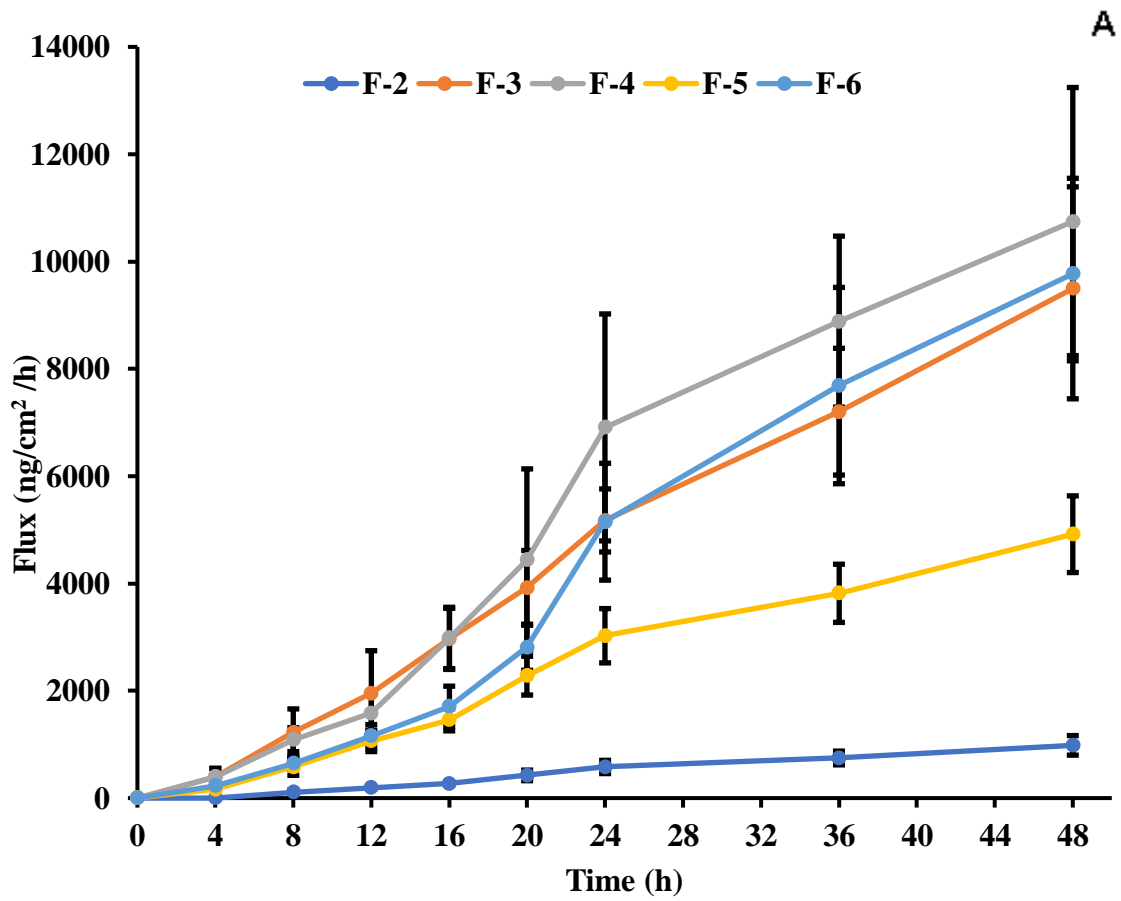


Figure 12 *In vitro* ITZ release from non-aqueous formulations, plotted as cumulative release vs square root of time.

2.11 *Ex-vivo* permeation and deposition of ITZ:

The results of *Ex-vivo* permeation and deposition of ITZ across human skin was presented in Figure 13. The F4 formulation was showed highest flux of $(1074.86 \pm 26.23 \text{ ng/cm}^2/\text{h})$. The F2, F3, F5, and F6, showed 982.32 ± 28.44 , 9495.59 ± 44.32 , 9770.72 ± 41.13 , 4917.67 ± 19.87 , $49770.72 \pm 34.14 \text{ ng/cm}^2/\text{h}$, shown in Figure 13A. Similarly, skin deposition ITZ was very high $(43.13 \pm 04.32 \text{ ng/mg})$ with formulation F4 compared to other formulations F2 $(01.06 \pm 0.78 \text{ ng/mg})$, F3 $(26.44 \pm 09.23 \text{ ng/mg})$, F5 $(08.61 \pm 01.44 \text{ ng/mg})$, and F6 $(06.07 \pm 02.33 \text{ ng/mg})$, shown in Figure 13B. Thus, increased *Ex-vivo* ITZ permeation and deposition of F4 formulation across the human skin is consistent with *in vitro* release rate. These results indicated that the low

amount of Carbopol polymer of gel significantly affected the transmembrane flux of each non-aqueous gel systems.



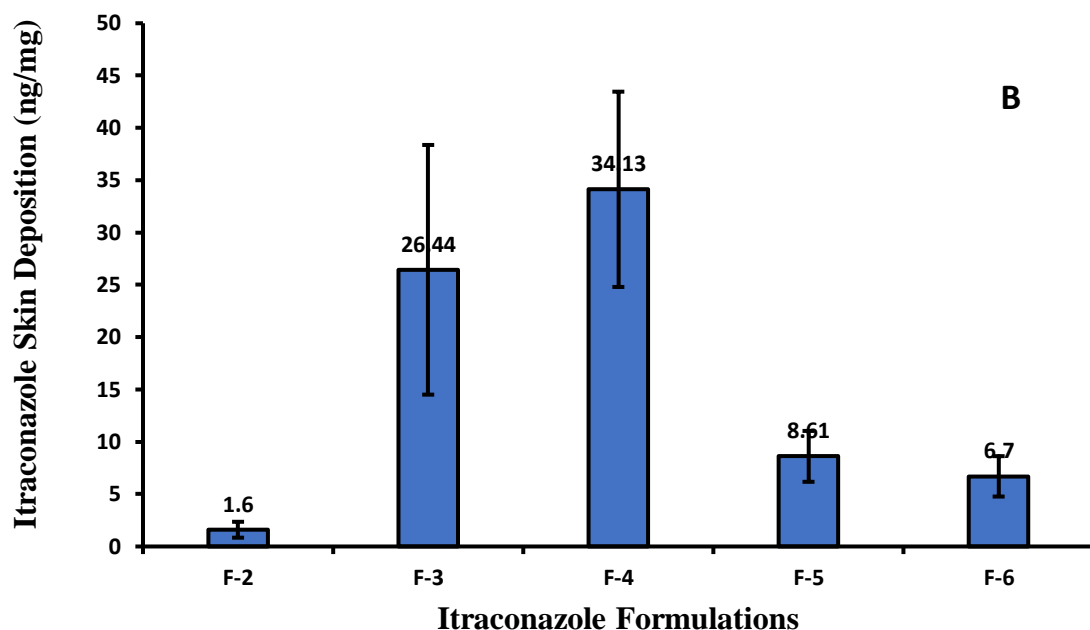


Figure 13 *Ex-vivo* ITZ permeation (A) and deposition (B) across human skin

2.12 Conclusion and future prospective

In this study, the ITZ based non-aqueous gel formulation was successfully developed using Carbopol polymer. The excipients used in the formulation showed enhanced solubility of ITZ that resulted in significant permeation and deposition across *Ex-vivo* human skin studies. Conclusively, the results indicate F4 non-aqueous gel formulation could be considered as effective and promising alternative for topical ITZ for fungal infection.

Chapter 3 Formulation Development and Validation of HPLC Method for Efinaconazole: Application to Human Nail Permeation Studies

3.1 Abstract

Efinaconazole is the first azole derivative approved by FDA for the topical treatment of onychomycosis. The objective of present study was to develop and validate HPLC method for estimation of efinaconazole in *ex vivo* human nail permeation study samples. The chromatographic analysis was performed on a HPLC system equipped with diode array detector. The efinaconazole and IS were extracted from the human nail samples by using the protein precipitation method. The samples were injected on to 5 μ m Polar C₁₈ 100Å, 4.6 mm X 150 mm column. The mobile phase consisted of 0.01 M potassium dihydrogen phosphate: acetonitrile (36:64) and eluent was monitored at 205 nm. The chromatographic separation of drug and analyte was achieved using isocratic elution at flow rate of 1 mL/min with a total run time of 15 min. The efinaconazole and internal standard (IS) were eluted at 6.4 ± 0.5 and 8.3 ± 0.5 min, respectively. The developed method was validated as per FDA guidelines, and the results met with acceptance criteria. The method developed was specific, and the analyte concentrations were linear at range of 50 to 10000 ng/mL ($R^2 \geq 0.9981$). The Validated HPLC method was applied for quantifying efinaconazole in human nail permeation study samples. The permeation of efinaconazole was increased by two folds with Labarfac CC (15135.4 ± 2233.9 ng/cm²) compared to formulations containing Transcutol P (6892.0 ± 557.6 ng/cm²) and Labrasol (7266.1 ± 790.6 ng/cm²). The study results

demonstrate developed efinaconazole HPLC method can employed for formulation evaluation and clinical studies.

Keywords: Onychomycosis, Efinaconazole, Method development and validation, HPLC, Nail permeation.

3.2 Introduction

Onychomycosis is a fungal infection of the nail, which can affect the integrity and structure of the nail which results in pain and hamper normal day-to-day activities [52,53]. Clinical symptoms of onychomycosis include nail discoloration, nail separation from the nail bed, brittleness, and nail thickening. Major cause of onychomycosis is dermatophytes and non-dermatophyte moulds [53–57]. The onychomycosis prevalence estimates up to 14% of North America population and prevalence increases with age. The treatment approaches of onychomycosis depend on the clinical type, severity and number of nails affected [58]. The oral antifungal terbinafine, itraconazole and griseofulvin are used for systemic treatment approaches. The combination of systemic and topical treatment approach increases the onychomycosis cure rate [59]. However, the systemic adverse events and drug interactions associated with use of oral antifungal agent's limits its clinical use in vulnerable patients [53,60].

The topical ciclopirox, efinaconazole and tavaborole antifungals are approved by Food and Drug Administration (FDA) for treatment of onychomycosis. The efinaconazole is most effective topical antifungal agent for treating onychomycosis [61]. The low keratin binding property of efinaconazole decreases resistance offered by keratin for delivery of drugs to the deeper layers of nails [60]. The efinaconazole is a chemically triazole derivatives acts mainly by inhibiting the 14 α demethylase enzyme, which disrupts the cell wall synthesis. The topical onychomycosis treatment

regimen involves application of 10% efinaconazole formulation to the toenails once daily for 48 weeks [60,62]. Hence, there is need of developing an effective formulation to deliver adequate levels of efinaconazole to decrease the time required for onychomycosis treatment. Sensitive analytical method is crucial for determination of efinaconazole in different stages of formulation development and evaluation of efinaconazole permeation across human nails to predict clinical efficacy of formulations.

High Performance Liquid Chromatography (HPLC) methods are developed and reported for analysis of efinaconazole in formulations. The lower limit of quantitation (LLOQ) of efinaconazole in reported HPLC methods are 28 and 50. $\mu\text{g/mL}$ [63,64]. Besides HPLC method, High-Performance Thin-Layer Chromatography (HPTLC) analytical method was developed and validated for quantification of efinaconazole [65]. All three existing analytical methods for efinaconazole estimation were developed and validated in accordance with regulatory guidelines. These analytical methods were developed and validated for determination of efinaconazole content in formulations for quality control purpose [63–65]. There is a need for development of sensitive and specific analytical method for estimation of efinaconazole in nail permeation study samples, to investigate the effect of formulation on permeation of drugs. Samples of nail permeation studies consists of many endogenous substances, which may elute with the analyte at the same or closer retention time (RT) and interfere in the analysis. Therefore, development of specific analytical method is important to prevent the interference of other molecules with drug of interest during biological sample analysis.

Hitherto no sensitive efinaconazole HPLC method has been reported for determination of analyte in human nail permeation studies. Therefore, the objective of the current study was to develop and

validate a HPLC method for the quantification of efinaconazole and to investigate its application for analysis of efinaconazole in *in vitro* human nail permeation study samples.

3.3 Materials and Methods

The efinaconazole was purchased from Sigma Aldrich (Saint Louis, Missouri, USA). The itraconazole was purchased from TCI America (Portland, Oregon, USA). HPLC grade solvents acetonitrile, ethanol, methanol, and Brij C20 were purchased from Thermo Fisher Scientific (Waltham, MA, USA). The Labrafac CC (Labrafac lipophile WL 1349), Transcutol P, and Labrasol were gifts samples obtained from Gattefosse Corporation (Paramus, NJ, USA). The Human toenails were purchased from Science Care, Inc. (Aurora, Colorado, USA). Other chemicals and reagents used were of research grade used without further purification.

3.3.1 Instrumentation and chromatographic conditions

The Shimadzu Ultra-Fast Liquid Chromatography (UFLC) system equipped with prominence SPD-M20A (Diode array detector) was used for the analysis of efinaconazole. To develop the efinaconazole HPLC method, the aqueous (0.1% formic acid, 0.01 M ammonium acetate, and 0.01 M potassium dihydrogen phosphate) solvents and organic (acetonitrile and methanol) solvents were investigated using isocratic and gradient elution profile. Different columns (C₁₈, C₈ and C₆) were scrutinized for optimizing the efinaconazole HPLC method. The absorption maximum wavelength for monitoring efinaconazole in eluent was determined by scan function in Diode array detector.

3.3.2 Sample preparation

A known quantity of analyte was weighed and dissolved in methanol for preparing primary stock solution of 1 mg/mL. The primary stock solution was further diluted to prepare working calibration standards. Similarly, an internal standard (IS) was prepared and used during the analysis along with the analyte. The primary stocks and calibration standard were stored at $-5\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$. Before analysis, all the samples were thawed and processed at room temperature.

3.3.3 Extraction procedure

The human nails were minced into small pieces, added phosphate buffer saline (PBS), and homogenized. To 500 μL of homogenate, 5 μL of itraconazole (internal standard) 5 $\mu\text{g/mL}$ was spiked. Drug and internal standard were extracted from homogenate by protein precipitation method using 495 μL acetonitrile. These samples were vortexed for 5 minutes and centrifuged at 14,000 rpm for 15 min. The supernatant was transferred into vials and subjected to HPLC analysis.

3.3.4 Method validation of efinaconazole

The developed efinaconazole HPLC method was validated in accordance with FDA guidelines[66–68].

3.3.5 Sensitivity

The standard deviation and slope of the calibration curve was considered for limit of detection (LOD) and LLOQ determination. The LOD and LLOQ of efinaconazole were determined with the formula $3.3\alpha/S$ and $10\alpha/S$, respectively. Where α is standard deviation of y-intercept and S is slope of calibration curve [67].

3.3.6 Selectivity

The selectivity of developed method was evaluated using nail homogenates of six different donors. The extracted samples of blank nail homogenate, analyte spiked to blank homogenate, and analyte and IS spiked to blank homogenate were analysed and results were compiled based on peak area and RT.

3.3.7 Calibration curve and quality controls

The analyte and IS peak area ratios were used for constructing calibration curve against the nominal concentration of calibration standards spiked in blank human nail homogenate. The calibration curve was prepared by spiking a working calibration solution in 500 μ L of blank nail homogenates to give final concentrations of 50, 100, 500, 2000, 4000, 6000, 8500, and 10000 ng/mL. The linear regression analysis was performed using the weighing factor $1/X^2$. The calibration curve should have a correlation coefficient $R^2 \geq 0.995$ [66–68]. The standard and quality control concentrations should be within the range of $\pm 15\%$ from the actual concentration except for LLOQ, which is $\pm 20\%$ [66–68].

3.3.8 Precision and accuracy

Inter and intraday precision and accuracy for efinaconazole were performed in human nail homogenate with six replicates at four different quality controls (QC) levels. The QC's were prepared by spiking working quality control solution to give final concentrations of 50, 150, 5000 and 7500 ng/mL for LLOQ, Lower Quality Control (LQC), Middle Quality Control (MQC), and Higher Quality Control (HQC), respectively. The accuracy acceptance criteria are $\pm 15\%$ of the actual concentrations, with the exception of LLOQ, where $\pm 20\%$ of actual concentrations. The

precision for all QC's shall be within ± 15 percent relative standard deviation (RSD) except for LLOQ, where it should not be surpassed more than ± 20 percent[66–68].

3.3.9 Recovery

Efinaconazole and IS extraction recovery were calculated by comparing the peak-area ratios of quality controls such as LLOQ, LQC, MQC, and HQC containing analytes in nail homogenates (n = 6) with the responses of LLOQ, LQC, MQC, and HQC analytes spiked in methanol. However, IS recovery was calculated at one concentration (5 $\mu\text{g/mL}$), which was used for method development and validation.

3.3.10 Stability studies

The stability of the drug was evaluated in nail homogenate at different conditions. The stability of efinaconazole was performed at bench-top for 8h at room temperature ($25 \pm 1^\circ\text{C}$), auto-sampler stability at 10°C for 48h, long term stability at $-80 \pm 10^\circ\text{C}$ for 90 days, and 3 cycles of freeze-thaw stability at $-80 \pm 10^\circ\text{C}$ (samples were frozen for 12 h and thawed at room temperature for 1 h and this cycle was repeated for three times). To determine the drug stability, LQC (150 ng/mL) and HQC (7500 ng/mL) controls were used. To consider analyte stable, the stability samples accuracy should be $\pm 15\%$ when compared with fresh samples prepared and precision $\pm 15\%$ RSD[66–68]. Every stability sample was prepared freshly at respective time points and compared with calibration standards, respectively.

3.3.11 Dilution effect

Dilution integrity was measured to confirm that the nail homogenate could be diluted with blank nail homogenate without showing any effect on final concentration determination. Six replicates

analyte 250 µg/mL concentration containing homogenate was diluted 100 and 50 times with blank nail homogenate. The accuracy should be ranged from 85-115% [66–68].

3.3.12 Efinaconazole formulation development

a) Solubility Studies: In current study, chemical permeation enhancers of different HLB values were used to study their effect on permeation of efinaconazole across nail plate. Labrasol (Caprylocaproyl Polyoxyl-8 glycerides), Transcutol P (diethylene glycol monoethyl ether) and Labrafac CC (Medium chain triglycerides) with a HLB value of 12, 4 and 1, respectively were used for human cadaver transungual studies. To prepare efinaconazole formulation, the solubility of efinaconazole in Labrafac, Transcutol P, and Labrasol were evaluated. Solubility studies were performed by adding excess of efinaconazole to Labrafac CC, Transcutol P and Labrasol. These samples were kept shaking at 1200rpm with 25 °C for 3 days and at equilibrium, samples were filtered using a Millipore (0.45 µm) syringe filter. The filtrate was analyzed using validated HPLC method. To evaluate the permeation enhancing potential of Labrafac CC, Transcutol P and Labrasol, efinaconazole formulations were prepared. The 10% w/v of efinaconazole was dissolved in ethanol containing 10% w/v of permeation enhancers. The composition of formulations prepared are presented in Table 12.

Table 12 The composition of efinaconazole formulation used for human cadaver nail permeation studies.

| Ingredients | F1 | F2 | F3 |
|----------------------|-----------|-----------|-----------|
| Labrafac CC (% w/v) | 10.0 | - | - |
| Transcutol P (% w/v) | - | 10.0 | - |
| Labrasol (% w/v) | - | - | 10.0 |

| | | | |
|-----------------------|------|------|------|
| Efinaconazole (% w/v) | 10.0 | 10.0 | 10.0 |
| Ethanol (% w/v) | QS | QS | QS |

b) Drug Content: The EFN formulations were dissolved in methanol and mixed for 24 hours at 1200-rpm speed by using vortexer. Then, the formulations were centrifuged (Fisherbrand™, Waltham, MA, USA) at 13000 rpm for 10 minutes and collected supernatant. Finally, EFN sample contents were quantified by using developed HPLC method. Drug content was measured by using below equation.

$$\text{Drug content} = [\text{Actual amount of EFN} / \text{Theoretical amount of EFN}] * 100.$$

c) In vitro release test: The EFN release study was conducted by using Vertical Franz diffusion cell apparatus with an active diffusion area of 0.636 cm² (Logan Instruments, Somerset, NJ, USA). The EFN formulations (500 uL) were placed on Whatman™ Nylon membrane filters (0.22 μm, Cytiva®, Marlborough, MA, USA), sandwiched between the two half-cells of the diffusion cell. The receiver chamber was consist of 5 mL of 1.5% Brij C20 in PBS (Receiver media) at 7.4 pH and temperature was maintained at 32 ± 0.5 °C with water circulator. At the same time, magnetic stir bar was placed in received medium and continuously stirred at 600rpm. The sample (0.5mL) volume from the receiver media were withdrawn at time points 1.0, 1.5, 2.0, 3.0, 4.0, and 6.0 h and replaced with freshly prepared receiver fluid. Then the resultant samples were subjected to HPLC for analyzing EFN drug concentrations.

3.3.13 *In vitro* human cadaver nail permeation studies

The human cadaver nail plates were taken from a deep freezer (-80 °C) and thawed at room temperature for overnight. Before performing permeation studies, human nails were hydrated with PBS for 3 hours, washed with water and dried. Then, human nails were mounted on a nail adapter with an active diffusive area of 0.2 cm². Nail adapters were placed in between donor and receiver chambers of Franz diffusion cell system (Logan Instruments Ltd, Somerset, NJ) and clamped. The receiver chamber was filled with 5 mL of 1.5% Brij C20 in PBS at 7.4 pH and temperature was maintained at 32 ± 0.5 °C with water circulator. The receiver media was continuously stirred at 600 rpm with a stir bar. After assembling, 100 µL of each formulation (Table 12) was loaded into donor chamber. At 0, 24, 48, 72, 96, 120, 144, and 168h time intervals 5 mL of receiver media was withdrawn and replaced with fresh receiver media. The efinaconazole was extracted and drug concentrations in receiver media were quantified by using validated HPLC method.

3.4 Results and Discussions

3.4.1 Chromatographic conditions

Itraconazole and efinaconazole are triazole derivative have similar physicochemical properties, such as solubility, lipophilicity, and absorbance maximum (205 nm). Because of these similarities, itraconazole was selected as an IS for efinaconazole method development and validation. The broad efinaconazole and IS peaks were observed when formic acid in water and methanol were used as mobile phases. The peaks of endogenous substances and IS interfered with analyte RT with the use of 0.01 mM ammonium acetate and methanol as mobile phase. The peak broadening and interference of analyte was improved with the use of 0.01 mM potassium dihydrogen phosphate and acetonitrile as mobile phase. The sensitivity of efinaconazole, and peak resolution

of analyte and IS was lower on C₈ and C₆ columns. The split peaks were observed on C₁₈ hydrophobic columns. These issues were resolved on using Luna Omega C₁₈ polar column.

The chromatographic separation of efinaconazole and IS were achieved using isocratic method 0.01 M potassium dihydrogen phosphate: Acetonitrile (36:64) with 1 mL/min flow rate and 60 µL injection volume. The Luna Omega 5 µm Polar C₁₈ 100Å, 4.6 mm X 150 mm column was used for elution of analyte with a run time of 15 min. The eluting efinaconazole and IS were monitored at 205nm (wavelength). The analytical method validation was performed as per FDA guidelines.

3.4.2 Efinaconazole method validation

3.4.3 Sensitivity

Existing HPLC methods have shown sensitivity of 28 and 50 µg/mL [63,64]. The current developed method was found to be more sensitive compared to previous reported methods. The LOD and LLOQ of efinaconazole determined using the present analytical method was found to be 10 and 50 ng/mL, respectively.

3.4.4 Selectivity

The selectivity of method was assessed to identify the possible interference of endogenous molecules in nail homogenate at RT of analyte and IS. The selectivity of method was evaluated by comparing the blank, blank with IS, blank with analyte. The samples analysed with chromatographic conditions mentioned in Section 3.1, there was no interference (no junk or ghost peaks) observed at the retention time of the analyte and IS (Figure 14). The analyte and IS were eluted at 6.4 ± 0.5 and 8.3 ± 0.5 min, respectively.

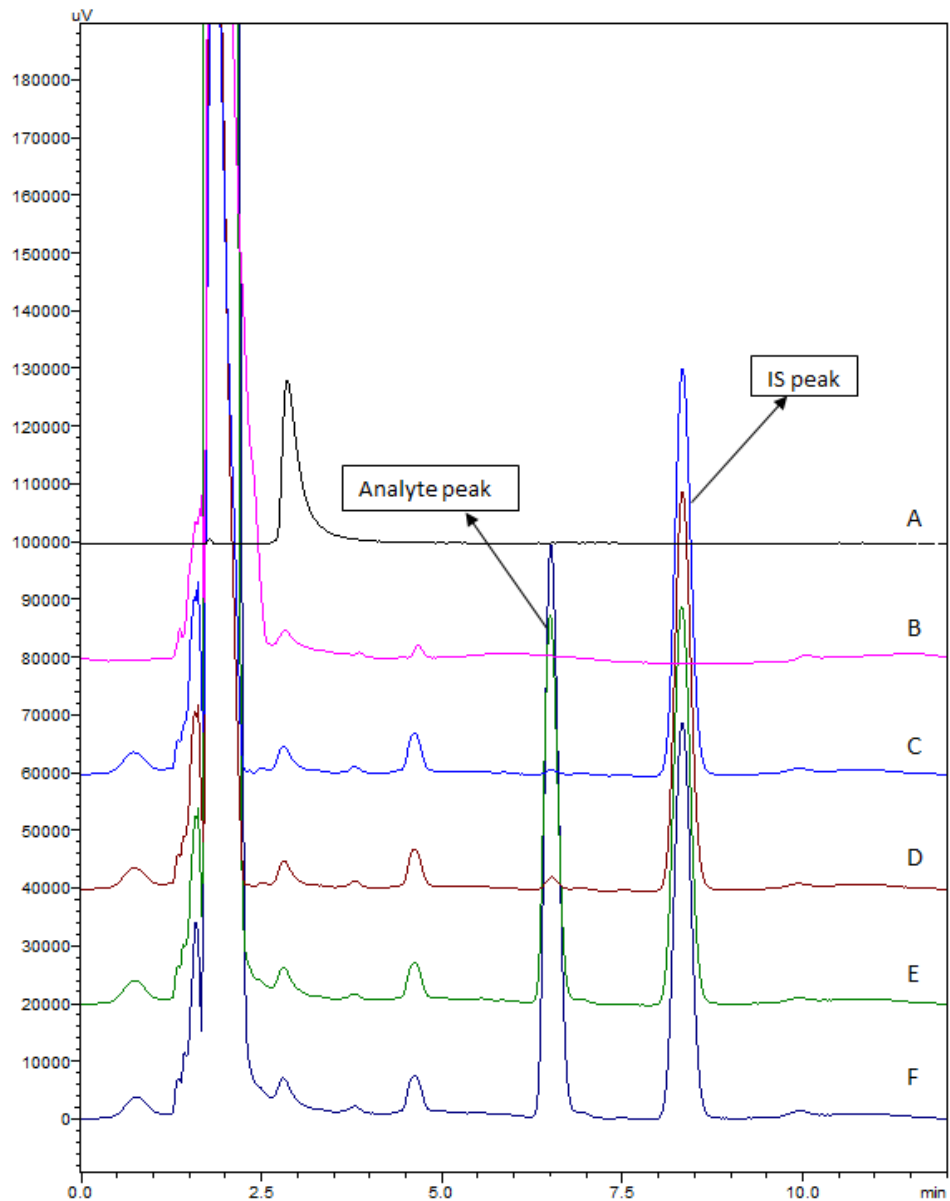


Figure 14 The HPLC chromatograms (Overlay) of mobile phase (A), human cadaver nail homogenate/blank (B), blank with IS (C), blank with analyte (LLOQ) (D), human cadaver nail permeation study sample at 144h (E) and blank with analyte (HQC) (F).

3.4.5 Calibration curve

The calibration curve was plotted in nail homogenate with eight calibration standards, and linearity ranged from 50 to 10000 ng/mL. The standard calibration curve was reliable and reproducible at

calibration range analysed. The regression value for the efinaconazole calibration curve (n=3) was found to be >0.9981. The linear equation for calibration curve was found to be $y = 0.000103x - 0.008980$. The percentage accuracy observed for the mean of back-calculated concentrations for four calibration curves for efinaconazole was within 86.2 to 111.1%. The precision, i.e., coefficient variation values for calibration curve of 3 different runs were ranged from 1.3 to 13.3.

3.4.6 Precision and accuracy

The precision and accuracy of intra- and inter-day analysis were calculated by using LLOQ, LQC, MQC, and HQC levels. To perform precision and accuracy, six replicates of QC's were analyzed on three separate days (Table 13 and 14). The percent RSD values of intra-day precision were ranged from 1.4 to 16.6%. However, one of the LLOQs out of six was crossed beyond the 15%, which met with acceptance criteria [66–68]. Inter-day precision percent RSD values were ranged from 0.3 to 6.2%. The back-calculated accuracy of inter and intra-day values were ranged from 85-115%, and precision values were within the 15-20% RSD, which met the acceptance criteria[66–68].

Table 13 The Intra-day precision of efinaconazole in human cadaver nail homogenate matrix at LLOQ, LQC, MQC and HQC levels (n=6).

| Day | Quality control | Mean ± SD (ng/mL) | RSD | %Accuracy ± %SD |
|-----|-----------------|----------------------|------|-----------------|
| 1 | LLOQ | 51.3 ± 6.3 | 12.4 | 102.7 ± 12.7 |
| 2 | | 57.0 ± 3.5 | 6.1 | 114.1 ± 7.0 |
| 3 | | 57.0 ± 9.4 | 16.6 | 114.0 ± 19.0 |

| | | | | |
|---|-----|----------------|-----|-------------|
| 1 | LQC | 159.0 ± 6.9 | 4.3 | 106.0 ± 4.6 |
| 2 | | 162.1 ± 9.7 | 6.0 | 108.1 ± 6.5 |
| 3 | | 150.0 ± 2.2 | 1.5 | 100.2 ± 1.5 |
| 1 | MQC | 4986.0 ± 88.3 | 1.8 | 99.7 ± 1.8 |
| 2 | | 5012.1 ± 106.6 | 2.1 | 100.2 ± 2.1 |
| 3 | | 5118.2 ± 110.6 | 2.2 | 102.4 ± 2.2 |
| 1 | HQC | 7587.0 ± 182.9 | 2.4 | 101.2 ± 2.4 |
| 2 | | 7546.7 ± 106.1 | 1.4 | 100.6 ± 1.4 |
| 3 | | 7748.3 ± 323.7 | 4.2 | 103.3 ± 4.3 |

SD: Standard deviation; RSD: Relative standard deviation

Table 14 The Inter-day precision of efinaconazole in human cadaver nail homogenate matrix at LLOQ, LQC, MQC and HQC levels (n=6).

| Quality control | Mean ± SD (ng/mL) | RSD | % Accuracy ± %SD |
|-----------------|-------------------|-----|------------------|
| LLOQ | 52.5 ± 3.3 | 6.2 | 104.9 ± 6.5 |
| LQC | 154.5 ± 2.8 | 1.8 | 103.0 ± 1.9 |
| MQC | 4984.6 ± 13.4 | 0.3 | 99.7 ± 0.3 |
| HQC | 7558.2 ± 46.3 | 0.6 | 100.8 ± 0.6 |

SD: Standard deviation; RSD: Relative standard deviation

3.4.7 Recovery

A simple protein precipitation method was used for the extraction of efinaconazole from nail homogenate. Organic solvents ethanol, methanol, and acetonitrile were investigated for increasing the recovery of the analyte from the biological matrix. Studies demonstrated that acetonitrile improves recovery efinaconazole from nail homogenate and provided good resolution from peaks of endogenous molecules in nail homogenate compared with other solvents used for extraction. An equal volume of acetonitrile: sample (1:1) were used for extracting analyte from the nail homogenate. The recovery was measured at LLOQ, LQC, MQC, and HQC levels, and the peak area ratio was considered for the calculations. The percentage recovery of drug analyte was 99.5 ± 11.1 , 102.3 ± 4.1 , 100.4 ± 1.4 and $101.4 \pm 1.4\%$ at LLOQ, LQC, MQC and HQC levels, respectively.

3.4.8 Stability studies

The stability studies were conducted to determine analyte stability under likely conditions of sample handling and storage. The stability of efinaconazole in nail homogenate at bench-top stability (8h), processed samples stability (autosampler stability for 48h at room temperature), freeze-thaw stability (3 cycles), and long term stability (90 days at -80 ± 10 °C) at LQC and HQC levels were within the acceptable limits of accuracy, i.e., ± 15 percent variability as compared to the fresh samples[66–68] (Table. 15).

Table 15 The bench-top, auto-sampler, long term, and freeze thaw stability of efinaconazole at LQC and HQC levels in human cadaver nail homogenate (n=6).

| SL No. | Stability test | LQC | | | HQC | | |
|--------|----------------|-----------------------|-----|----------------------|-----------------------|-----|----------------------|
| | | Mean \pm SD (ng/mL) | RSD | % Accuracy \pm %SD | Mean \pm SD (ng/mL) | RSD | % Accuracy \pm %SD |
| 1 | 0 hr | 150.4 \pm 2.2 | 1.5 | 100.2 \pm 1.5 | 7748.3 \pm 324.7 | 4.2 | 103.3 \pm 4.3 |
| 2 | Auto sampler | 153.3 \pm 7.3 | 4.8 | 102.2 \pm 4.9 | 7265.6 \pm 120.7 | 1.7 | 96.9 \pm 1.6 |
| 3 | Bench top | 136.3 \pm 9.0 | 6.6 | 90.9 \pm 6.0 | 7266.3 \pm 124.0 | 1.7 | 96.9 \pm 1.6 |
| 4 | Freeze thaw | 152.1 \pm 6.1 | 4.0 | 101.4 \pm 4.1 | 7617.4 \pm 56.6 | 4.1 | 101.6 \pm 0.8 |
| 5 | Long term | 169.6 \pm 14.7 | 8.7 | 113.0 \pm 9.8 | 7867.1 \pm 593.8 | 7.6 | 104.9 \pm 7.7 |

SD: Standard deviation; RSD: Relative standard deviation

3.4.9 Dilution integrity

A known higher concentration (250 $\mu\text{g/mL}$) of analyte was diluted to 100 times and 50 times in nail homogenate. The diluted samples in the biological matrix had shown accuracy from 95.1 to 96.6 % and 102.1 to 104.4 %, respectively. The accuracy of dilution integrity was within acceptance criteria (85-115%)[66–68]. Therefore, this study shows that it is possible to dilute and analyze the samples with concentrations greater than the upper limit of quantification to obtain reliable results.

3.4.10 EFN Formulation Development and *In vitro* human cadaver nail permeation studies

The saturation solubility of efinaconazole in Labrafac CC, Transcutol P and Labrasol were 759.2 ± 6.6 , 814.7 ± 5.4 and 599.3 ± 0.5 mg/mL, respectively. All the EFN formulations (Labrafac CC, Transcutol P and Labrasol) were showed more than 95 % drug recovery from the drug content studies. The drug content for all formulations were within the acceptable limits defined by the USP (100.0 ± 10.0 % label claim). Which clearly indicates that the EFN drug has uniform distribution all over the formulations.

The EFN formulations release patterns were shown in the below figure-15. The *invitro* release rate profile for in Labrafac CC, Transcutol P and Labrasol formulations were 452.3 ± 38.10 , 409.22 ± 16.90 & 195.90 ± 26.47 $\mu\text{g}/\text{cm}^2/\text{h}$, respectively. All formulations followed Higuchi's release profile. The results clearly showed that the release rate directly depends on diffusion coefficient. Among all three EFN formulations Labrafac CC (452.3 ± 38.10 $\mu\text{g}/\text{cm}^2/\text{h}$) showed the better results than in Transcutol P and Labrasol. This could be due to Labrafac CC excipient which has more lipophilicity (HLB value) and solubility than the other excipients such as Transcutol P and Labrasol.

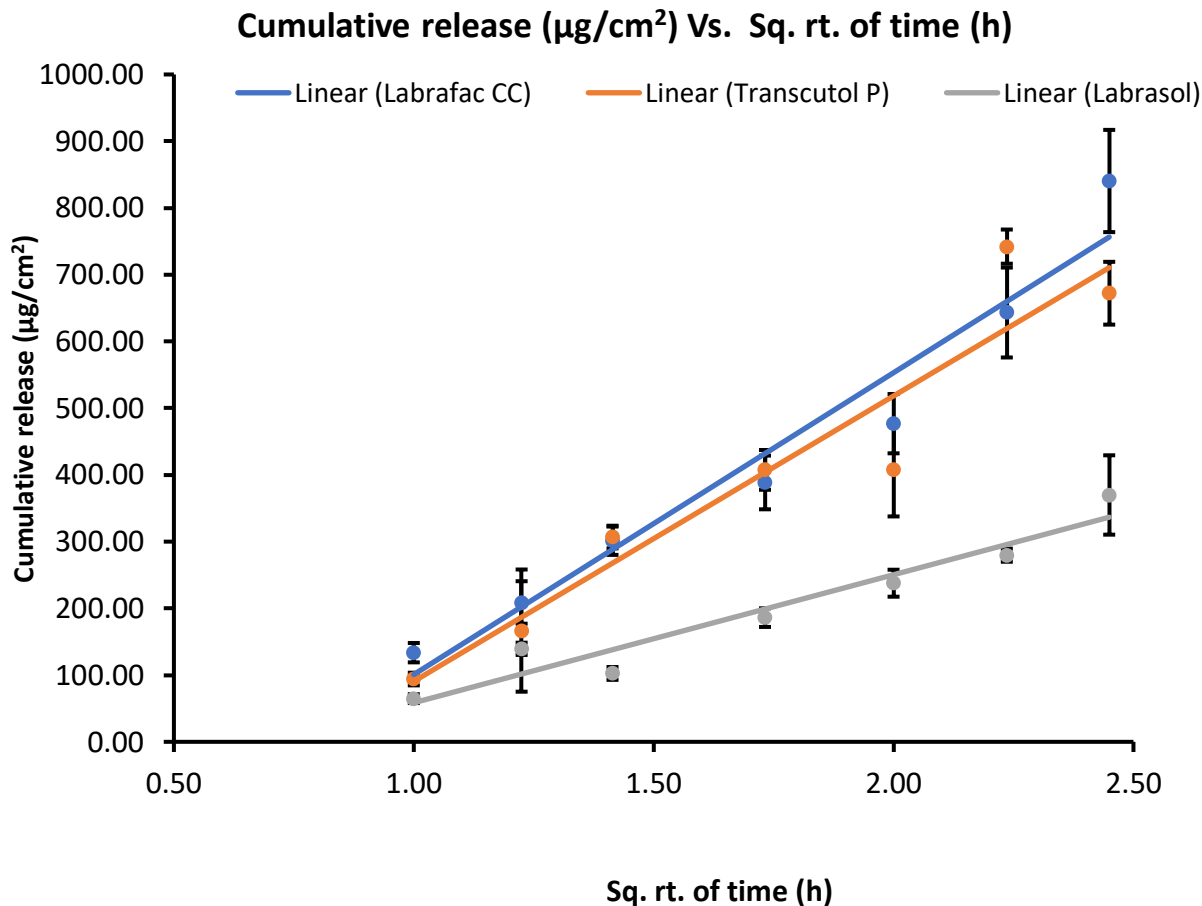


Figure 15 *In vitro* EFN release from the three formulations, plotted as cumulative release vs square root of time.

The validated efinaconazole analytical method was successfully applied for solubility determination and analysing *in vitro* permeation testing (IVPT) study samples. Previous studies have reported that Labrafac CC, Transcutol P, and Labrasol excipients increased permeation of drugs in nail permeation studies [69–71]. The efinaconazole formulations were prepared with these excipients to enhance permeation of efinaconazole across human cadaver nail plates. The permeation profile of efinaconazole across nail are shown in Figure 16. At 24h, the permeability of efinaconazole with Labrafac CC, Transcutol P, and Labrasol formulations were 320.4 ± 34.8 ng/cm^2 , 346.2 ± 2.7 ng/cm^2 , and 140.1 ± 140.0 ng/cm^2 , respectively. The cumulative amount of

efinaconazole permeated across the nail from all three formulations were similar up to 96h. However, at 120h the efinaconazole permeation from Labrafac CC formulation (8030.1 ± 598.6 ng/cm²) was increased up to two folds compared to permeation from Transcutol P (4580.3 ± 636.4 ng/cm²) and Labrasol (5448.7 ± 876.5 ng/cm²) formulations. At the end of the 7th day, the efinaconazole amount permeated from Labrafac CC (15135.4 ± 2233.9 ng/cm²) formulation was approximately two folds higher compared to Transcutol P (6892.0 ± 557.6 ng/cm²) and Labrasol (7266.1 ± 790.6 ng/cm²) formulations. Therefore, our results suggest that efinaconazole in Labrafac CC increases the permeation of drugs across nail compared to other two formulations.

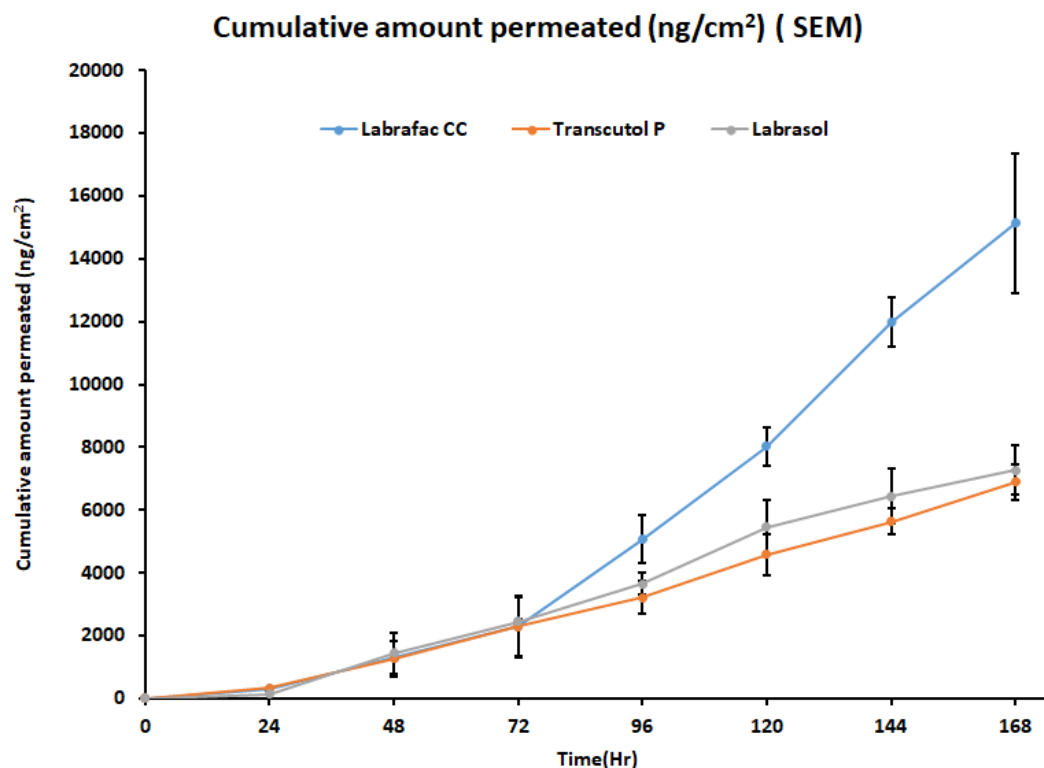


Figure 16 The effect of Labrafac CC, Transcutol P and Labrasol formulation on efinaconazole permeation across human cadaver nail.

3.5 Conclusion

The efinaconazole analytical method was successfully developed and validated in human nail homogenate in accordance with FDA guidelines. The method developed was simple, specific, sensitive, and reproducible for quantification of efinaconazole in nail homogenate. The developed method was employed for analysing *in vitro* human nail cadaver permeation study samples. The study results demonstrated that the developed method can be used for analysis of efinaconazole in formulation development stages. The nail permeation studies demonstrated that Labrafac CC would be potential nail permeation enhancer to deliver effective concentrations of efinaconazole to treat onychomycosis.

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