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Formulation and Evaluation of Medicated Nail Lacquer for the Treatment of Onychomycosis

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Abstract

Aims: The objective of the research work was to study about medicated antifungal nail lacquer for the treatment of Onychomycosis. Medicated nail lacquers generally act on a principle that it will form a film on the application surface from which the drug is released at a controlled rate for an extended period. **Methods**: The purpose of the investigation was to formulate and evaluate the Luliconazole nail lacquer as an ungual drug delivery system for the treatment of onychomycosis. Luliconazole an antifungal class of a drug was chosen along with Eudragit RS-100, Ethyl cellulose, Butyl acetate, Urea, Dibutyl phthalate, Methanol. As an excipient Eudragit RS 100 and Ethyl cellulose was used as sustained drug release polymer. **Results**: Formulation of nail lacquer done with taking different % of excipients. Further, these lacquers were compared for drying time, nonvolatile content drug content, drug diffusion and anti -microbial studies. Out of all 5 formulations F2 was considered as the optimized formulation. The stability studies show that the formulation is stable at 40 C for 15 days. F2 Formulation showed 98.5% drug content and 45 mm zone of inhibition which is greater compared to other formulations. So, a good therapeutic outcome can be expected. Microbial study results proved that the formulations are sensitive to the Candida albicans. **Conclusion:** So, from the above studies it can be concluded that an nail lacquer can be a better tool for ungal drug delivery system for the treatment of nail infection, onychomycosis.

Keywords
Medicated nail lacquers, onychomycosis, Luliconazole, ungual drug delivery system.

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INTRODUCTION:

Over the years, the importance of nail permeability to topical therapeutics has been realized, primarily in relation to the treatment of onchomycosis; a fungal infection of fingernails and toenails which affects approximately 19% of the world population and is responsible for approximately 50% of all nail disorders. The human nail plate is a much more complex structure than it looks at the first sight. Nail unit comprise of various parts. Among them nail plate is the external most part which is rigid in nature. The rigidity of nail plate is formed by the keratin network present in nail plate. Major chemical constituents of nail plate are keratin which is found in combination with many disulphide link and less amount of lipid, due to which nail plate acts as hydrogel membrane, that acts as barrier. Nail unit consists of parts displayed below in fig.No.1, it is the topmost part of nail that is visible and is hard in nature.[2]

Nail Disease

Nail Clubbing, Hangnails, Ingrown Toe nail, Splitting or Peeling nails, Nail Psoriasis, Yellow Discoloured Nails, Beau's Lines, Nail Fungus, Onycholysis, Nail beds with a blue tinge, Onychomycosis.

Onychomycosis

Onychomycosis is also known as tinea unguium [5]. Onychomycosis accounts for one third of integumentary fungal infections and one half of all nail disease [6]. Onychomycosis is majorly caused due to dermatophytes such as *Trichophyton rubrum* [7] and non-dermatophytes such as *Candida albicans*. This infection is associated with various signs and symptoms including thickening of nail, nail discolouration, foul odour, breaking and cracking of nail and separation of nail from nail bed [8]. It can sometimes limit mobility; onychomycosis may indirectly decrease peripheral circulation, thereby worsening conditions such as venous stasis and diabetic foot ulcers. Fungal infections of the nails can also spread to other areas of the body and, perhaps, to other persons.

The causative pathogens of onychomycosis include dermatophytes, Candida, and non-dermatophytic molds. Dermatophytes are the fungi most commonly responsible for onychomycosis in the temperate western countries, while Candida and nondermatophytic molds are more frequently involved in the tropics and subtropics with hot and humid climate.

Risk factors for Onychomycosis include family history, increasing age, poor health, prior trauma, warm climate, participation in fitness activities, immunosuppression (e.g., HIV, drug induced), communal bathing, and occlusive footwear.

Classification of Onychomycosis

Distal and Lateral Subungual Onychomycosis
White Superficial Onychomycosis
Proximal Subungual Onychomycosis
Endonyx Onychomycosis
Total Dystrophic Onychomycosis [10]

Treatments of Onychomycosis

Onychomycosis is a term that encompasses all the nail pathologies caused by fungi and accounts for approximately 50% of all nail diseases [12]. The treatment of onychomycosis is a challenging task to patients and professionals as the infection is embedded within the nail. It may take a year or more to get cure as new nail growth must completely replace the old and infected one. Also because of the difficulty in attaining a definitive cure and the high recurrence rate. Patients greater than 55 years of age may have a higher rate of relapse.

- Oral therapy
- Topical therapy [10, 13, 14]

Nail Lacquer

Medicated nail lacquer is an excellent alternative for the treatment of fungal infection of nails and high efficacy of drug can be achieved [17]. Antifungal nail lacquers are available for treating onychomycosis and penetrate the nail better than creams and gels. Formulation of active objects, large tissue concentration for capacity for the treatment of nail fungal disease. [18]

The general nail lacquer contains of solvents, film forming polymer, resins which enable the film to in accordance with to nail plate and made known shinning to the film, colouring agent and suspending agents. [18, 21]

METHODS:

A. Preformulation Studies

I. Physicochemical Parameters

a. Solubility Profile

Solubility of drug is determined in 10ml of solvents (Distilled water, Ethanol, Methanol).

b. Melting Point Determination

Melting point of drug was determined by taking a small quantity of drug (luliconazole) in a capillary tube sealed at one end and was placed in Thiel's melting point apparatus and temperature range at which the drug melted was noted.

II. Analytical Method

a. UV-Spectrophotometric method

Preparation of Standard Stock Solution

Standard stock solution was prepared by transferring 10mg of accurately weighed LNZ to a 10ml volumetric flask and adding 1:1 methanol: water as solvent up to the mark to give 1000µg/ml solution. 1ml of 1000µg/ml stock solution was transferred to a 10ml volumetric flask and volume was made up to the mark to give 100µg/ml solution as standard working solution.

Preparation of Working Solution

A series of concentrations ranging from 4-18µg/ml. was prepared by pipetting out 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6 and 1.8ml of standard working stock solution to different 10ml volumetric flasks. 1:1 methanol: water was added up to the mark to give 4-18µg/ml working solutions of LNZ.

Determination of Absorption Maxima for LNZ in Distilled Water and Methanol (1:1)

The wavelength of maximum absorbance, λ max for LNZ in distilled water and methanol (1:1) was determined with the help of UV-Visible Spectrophotometer. Prepared solution of concentration 15µg/ml was scanned in the range of 200-400nm.

Preparation of Standard Plot in Distilled Water and Methanol (1:1)

Observed absorption maxima, λ max 296nm was used for further analysis of absorption for concentration ranging from 4 to 18µg/ml. The linear plot was constructed and correlation coefficient (r2) value was determined. The results were plotted with the help of error bars (Mean ±SD).

Preparation of Calibration curve

100mg of Luliconazole sample was weighed and transferred to 100ml volumetric flask and diluted up to the mark with methanol (1000µg/ml). 10ml of the above solution was pipetted out in a 10ml volumetric flask and diluted up to the mark. From this 1.5ml of the solution was pipetted out and transferred into a 10ml volumetric flask and diluted up to the mark with methanol to form 15µg/ml that was scanned in the range of 200-400nm using UV-visible Double Beam Spectrophotometer (Shimadzu 1800).

b. IR Spectroscopy

FTIR analysis for Luliconazole was done by Shimadzu corporation Japan. Each sample was mixed with potassium bromide in 1:100 and compressed to form pellets later observed at the range from 4000 to 400cm.

B. Formulation Studies

Luliconazole nail lacquer was prepared by simple mixing method.

Method of preparation

1. The polymers Eudragit RS 100 and ethyl cellulose were soaked and dissolved in methanol.
2. Luliconazole was dissolved in required amount of methanol.
3. The dissolved drug was added into the polymer solution.
4. Further, dibutyl phthalate, butyl acetate and urea were added in the desired amount and mixed properly using magnetic stirrer.
5. The formulation was then filled in the narrow mouth containers and sealed.

Formulation development

- Nail lacquer formulation was developed by considering polymers at different concentrations along with different concentrations of film former.
- Optimization of formulation was done by preparing 5 different formulations.
- The amount of Luliconazole (API) was kept constant at 2% in all formulations.
- Initial trials were taken with Eudragit RS100 polymer.
- As seen from Table No.1 Formulation F1 to F5 were prepared containing different concentrations of Eudragit RS100 from 16% - 20% and Ethyl Cellulose from 4% to 10%.
- Also, the different concentration of Urea was considered from 0 to 5%.
- The aim was to optimize the amount of permeation enhancers based on the drug permeability studies.
- Further trials focused on combination of two polymers, namely Eudragit RS100 and Ethyl Cellulose.
- Formulation F1 to F5 contained various concentrations of selected polymers along with an optimized amount of permeation enhancer.

C. EVALUATION OF STUDIES

1. Smoothness to flow

The sample was poured on a glass plate and was inclined vertically.

2. Gloss

The sample was uniformly applied over the nail. The gloss was visually compared with the marketed cosmetic nail lacquer.

3. Drying time

The optimized formulation was applied on a glass slide and was allowed to dry. The drying time was analysed until the film was dry to touch.

4. Non-Volatile Content

Sample measuring was poured into a glass petri plate and the weight was noted. The plate was then placed in the oven at 105 ˚C for 1 hour. The weight of the plate was noted after 1 hour.

$$\% \text{ Non-Volatile Content} = \frac{[\text{Initial Weight (W1)} - \text{Final Weight (W2)}]}{\text{Initial Weight (W1)}} \times 100$$

5. Viscosity

Viscosity of medicated nail lacquer was found out by Oswald Viscometer. The viscometer was cleaned with acetone and dried. Then the viscometer was mounted in vertical position on a suitable stand. In dry viscometer, water was filled up to mark 3. Time required in seconds was calculated for water to flow from mark 1 to mark 2. The viscometer was rinsed with medicated nail lacquer and then filled with liquid up to mark 3. The time required in seconds was calculated for the liquid to flow from mark 1 to mark 2 (t1). The mass and volume of liquids was calculated using gravity bottle and measuring cylinder

respectively. This was further used to calculate density of sample (medicated nail lacquer).

$$\text{Viscosity of sample} = \frac{\text{Density of sample x t1 x Viscosity of water}}{\text{Density of water x Density of sample}}$$

6. Drug content estimation

Nail lacquer equivalent to 200mg was dissolved in 100 ml of methanol then solution was ultra sonicated for 15 mins. The resulting solution was filtered and required dilution was made and drug content was analyzed using UV Spectrophotometer at 297nm.

7. Diffusion studies

The diffusion studies were conducted using Franz diffusion cell. Cellophane membrane was used as artificial membrane for diffusion studies. The membrane was soaked in acetate buffer pH 5.5: methanol (7:3) for 24 hrs. The formulation was applied on the cellophane membrane and was allowed to dry. The prepared membrane was placed on the cell carefully by avoiding air entrapment and 20ml of acetate buffer pH 5.5: methanol (7:3) was taken in receptor compartment. Set up was kept under at 37˚C. Aliquots measuring 5ml was withdrawn at intervals of 0, 30, 60, 90, 120 and 150 minutes.

8. Determination of Anti-fungal Activity

The antifungal activity was tested in using Candida albicans by the cup plate method. Nutrient agar plates containing Sabouraud's agar was sterilized by autoclaving. Sterilized agar measuring 20 ml was poured into pre sterilized glass petri plates and was inoculated with diluted fungal strain. The plates containing the agar were allowed to solidify. One well of 5 mm diameter were made in each plate using sterile cork borer. The Formulation measuring 0.2 ml was placed into the plates and allowed to diffuse. The plates were the. Incubated at 30°C for 48 hours. The zone of inhibition was evaluated after 48 hours.

9. Stability studies

Stability studies of nail lacquers were carried out as per ICH guidelines. The Formulation was stored at 40±2°C/75±5% RH for 0, 1, 3,7,15 days. Then the samples were analyzed for non -volatile content, drying time drug content, anti-microbial studies.

RESULTS

A. PREFORMULATION STUDIES

I. Results for Physicochemical Parameters

a. Solubility Profile of Luliconazole

Solubility of drug was found in methanol.

b. Melting Point Determination

The melting point was found to be 158°C.

II. Analytical Methods

a. UV-Spectrophotometric Methods

i. UV Scanning

Pure luliconazole drug sample was scanned using methanol between 200nm to 400nm using UV-Visible spectrophotometer. The highest peak was obtained at 296nm and thus λ_{max} was fixed at 296 nm shown in **Fig No.2**

i. Calibration Curve for Luliconazole

Standard solution of luliconazole was prepared in different concentrations using Methanol and their absorption was measured at 296nm. in Table no .3

b. IR Scanning

IR spectra of formulation and Drug shown in Table no.4 and 5 and Fig no. 3 &4.

B. FORMULATION-

Formulation was done by preparing 5 different formulations.

C. EVALUATION STUDIES

1. Smoothness to flow-This parameter was found to be satisfactory as can be observed from figure no. The nail lacquer poured on the glass plate was found to spread and result in a uniform smooth film.

2. Gloss-This parameter was found to show sufficient gloss as can be observed.

3. Drying Time-With increase in concentration of polymer, the drying time increased as shown in Table no .6

4. Non-Volatile Content- Percentage Non-Volatile Content as shown in Table no .7.

5. Viscosity

The viscosity of the medicated nail lacquer using Ostwald viscometer was found out to range from 120 to 200 centipoise. With increase in polymer concentration, the viscosity of the formulation increased.

6. Drug Content Estimation

The drug content of all 5 formulations was found to be between the ranges of 93% to 98.5% which was considered as accepted as given in table no 8.

7. Diffusion Study

Diffusion studies were conducted and a graph was plotted against % drug release and time in minutes for each formulation as shown in Fig no 5. It was found out that Formulation F1 gave only 19% of drug release. So, the addition of permeation enhancer was necessary to improve permeation of drug. In further trials Urea was incorporated as permeation enhancer.

8. Determination of Anti-fungal Activity

The zone of inhibition for the various formulations was determined as shown in table no. 9, and it was found to range from, which is comparable with that of standard with mm. This indicates that all the formulations were sensitive to the microorganism Candida albicans.

9. Stability Study

Stability Studies were conducted at accelerated temperature. In this investigation formulation F1 to F5 were subjected at accelerated temperature for the period of 15 days. There was no significant change in the stability.

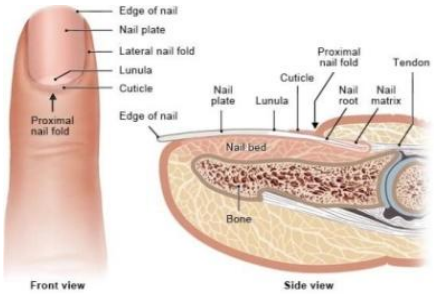

Fig No-1 Structure of nail [2]

Table No.1 Formulations

Sr. No.	Ingredients	F1	F2	F3	F4	F5
1.	Luliconazole	2%	2%	2%	2%	2%
2.	Eudragit RS100	16%	17%	18%	19%	20%
3.	Ethyl Cellulose	4%	5%	7%	9%	10%
4.	Dibutyl phthalate	4%	4%	4%	4%	4%
5.	Urea	0%	2.5%	2.5%	5%	5%
6.	Butyl acetate	23.5%	23.5%	23.5%	23.5%	0%
7.	Methanol q. s.	100%	100%	100%	100%	100%

Table no.2.Solubility result

Sr. No.	Solvent	Solubility Profile
1.	Methanol	Soluble
2.	Acetone	Insoluble
3.	Water	Insoluble

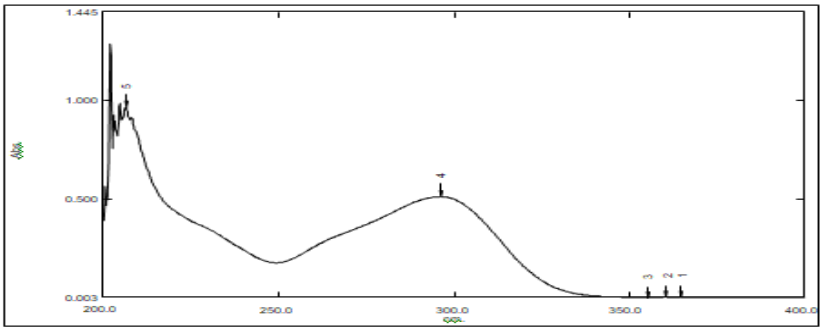

Fig.No.2. UV Scanning

Table no.3.Absorbance result for calibration curve

Concentration µg/ml	Absorbance at 296nm
0.2	0.108
0.4	0.202
0.6	0.333
0.8	0.402
1	0.504

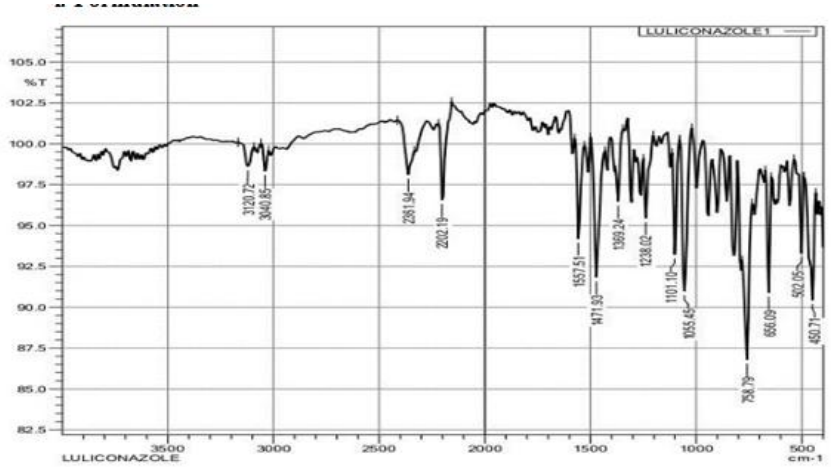

Fig.no.3.IR of Formulation

Table no.4 IR Spectra for Formulation

Frequency Range	Peak	Appearance	Functional Group
2000-1650	1728.22	Weak	C-H bending
1400-1000	1280.73	Strong	C-N Stretching
1600-1300	1450.47	Medium	C-H Bending
1400-1000	1134.14	Strong	C-O stretching
1000-650	748.83	Strong	C-C bending

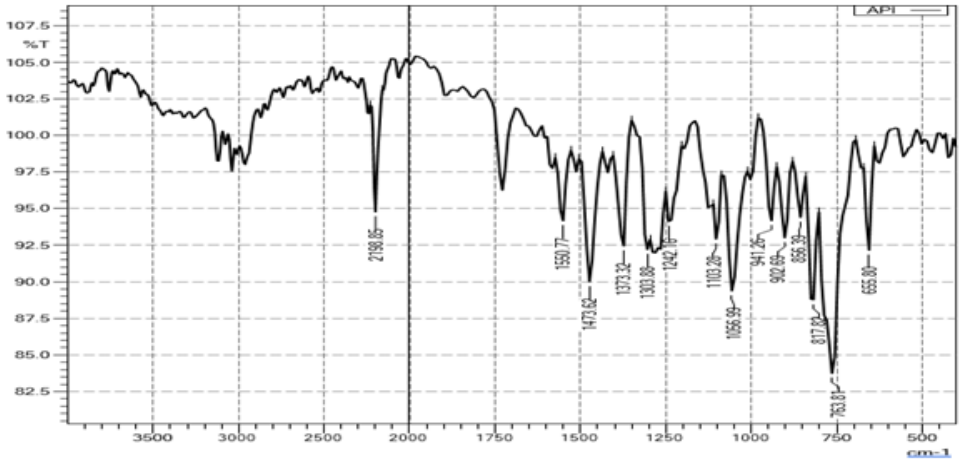

Fig.no.4. IR spectra of Luliconazole (API)

Table no.5 IR spectra of Luliconazole

Frequency Range	Peak	Appearance	Functional Group
2400-2000	2198.85	Weak	C=N stretching
900-700	763.81	Strong	C-H bending
900-700	817.82	Strong	C-H bending
1400-1000	1056	Strong	S=O stretching
900-700	902	Strong	C-H bending
1300-1600	1550	Strong	N-O stretching

Table no. 6. Drying Time

Formulation code	Drying time (in seconds)
Formula 1	40
Formula 2	45
Formula 3	56
Formula 4	64
Formula 5	78

Table no. 7. Non-Volatile Content

Formulation Code	Non-Volatile Content (%)
Formula 1	22.12
Formula 2	26.25
Formula 3	23.42
Formula 4	24.56
Formula 5	23.27

Table no. 8. Drug Content (%)

FORMULA CODE	DRUG CONTENT (%)
Formula 1	93%
Formula 2	98.5%
Formula 3	98%
Formula 4	92%
Formula 5	94%

Table no. 9. Zone Of Inhibition (in mm)

Formulation Code	Zone of Inhibition (In mm)
F1	35
F2	45
F3	35
F4	38
F5	40
Drug (API)	57

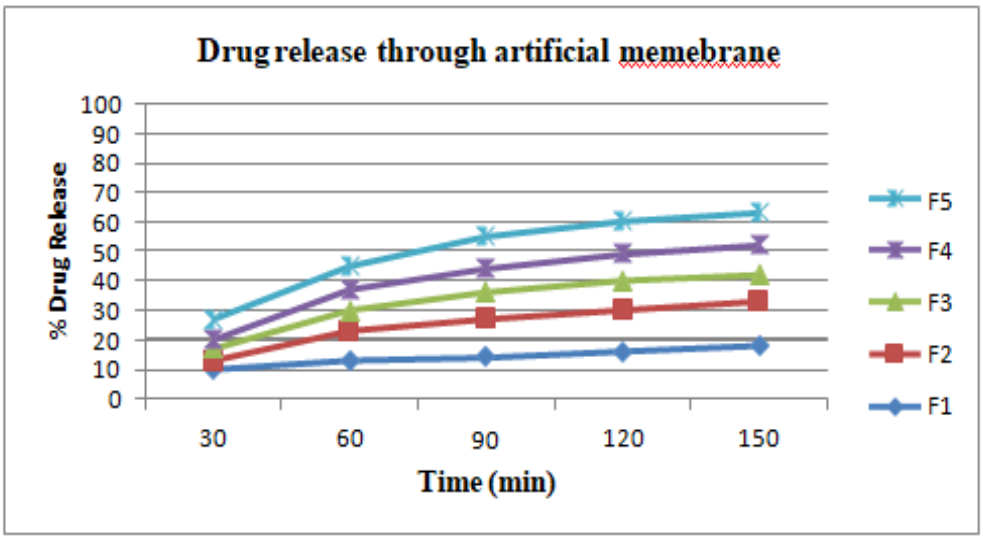

Fig. no 5. Diffusion Study

CONCLUSION:

The purpose of the research work was to study about medicated antifungal nail lacquer for Onychomycosis. Nail is formed by a compact network of keratin fibers which acts as a hydrogel membrane that acts as barrier for number of drugs. Although it's been found that numerous microbes affect the nail plate causing nail diseases. Dermatophytes are much more commonly known for the nail disease. Some of nail diseases namely onychomycosis, psoriasis, leuconychia etc. to treat such issues there are different formulations such as solution, cream, lotions, patches. But due to some drawback like unable to work properly after application, chance to get wipe or washed off, lower retention of drug at site; the formulations are comparatively tough to handle. Medicated nail lacquers generally act on a principle that it will form a film on the application surface from which the drug is released at a controlled rate for an extended period. So, we can say that nail lacquers are the best, cheap and it should have better patient compliance than other formulations or newer techniques that are employed for the enhancement of drug delivery over the nail plate.

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