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FORMULATION AND DEVELOPMENT OF ANTIFUNGAL NAIL LACQUER CONTAINING MICONAZOLE NITRATE USE IN TREATMENT OF ONYCHOMYCOSIS

Kanchan Yadav*[1], Jai Narayan Mishra[1], D. K. Vishwakarma[1]

[1*]Kailash Institute of Pharmacy and Management, Gorakhpur, Uttar Pradesh, India.

ABSTRACT

In this research paper the main aim is formulation and development of Anti-fungal nail lacquer which is used in treatment of onychomycosis. The main aim is formulation and evaluation of Anti-fungal nail lacquer containing miconazole nitrate which is used for the treatment in onychomycosis which is skin disorder cause by the pathogens include dermatophytes, candida and non-dermatophytes. Nail lacquer is also applicable in improvement clinical efficacy and also proper the patients compliance, Preparation of nail lacquer by simple mixing non-volatile, gloss, smoothness to flow, drug diffusion studies drug content compliance. Nail Lacquer is used on fingernail, toenail of the human beings. Which is protect the nail but, nail plate but most significant in maximize the beauty, gloss, impart color. Nail Lacquer is mostly applicable for those drug which have poor bioavailability in oral formulation this techniques is used in maximize the topical bioavailability of drug across the nail. In this formulation used different type of the use in this preparation which is 2 hydroxypropyl beta cyclodextrin, ethylcellulose, nitrocellulose, propylene glycol as well as drug formulate and obtain optimal release conclusion is success in this formulation.

KEYWORDS

Fungal infections, Nail lacquer and Onychomycosis.

Author for Correspondence:

Kanchan Yadav,
Kailash Institute of Pharmacy and Management,
Gorakhpur, Uttar Pradesh, India.

Email: navneet_its04@rediffmail.com

INTRODUCTON

Onychomycosis are the also called *tinea unguium* onycomycosis is a fungal infection of the nail Plate and nail bed. Approximately 50% of nail disease is the most common disorder in adults.

The most of the infection (90%-95%) are caused by the demotophytes. It caused by the yeast and molds. Among the superficial infection anychomycosis is the most difficult to manage it and to recure. Surgical avulsion are only treatment for

onycomycosis, and its procedure is the extremely painful and tranmaticanel it is replaced by systemic and topical therapy. The Antifungal oral drug like the ketoconazole, miconazole and itraconazole have been used in the treatment of onychomycosis. The side effect such as drug interaction and relapse. Oral therapy are the less accepted.

The conventional nail lacquer are used in cosmetics for long time beautification and protection of nail the nail lacquer has been used drug delivery system for the drug exhibited poor oral bioavailability.

The topical formulation used in dermatology like cream, oil based lotion, powders are the not specifically adopted to the nail since they are easily removed by the rubbing, whipping and washing. The medicated nail lacquers are used for formulation of transungual drug delivery system for maximal antifungal efficacy[1,2].

The present study was conducted to evaluate and formulae the terbinafine hydrochloride nail lacquer for the treatment of onychomycosis. Terbinafine was selected as a model drug. Terbinafine hydrochloride is a synthetic lipophilic antifungal agent and tends to accumulate in skin, nails, and fatty tissues. After oral administration, it is well absorbed (>70%), a peak plasma of $1\mu g/$mL after 2h with a single does of 250mg[3-5].

FUNGAL NAIL INFECTION

Fungal nail infection (onychomycosis [OM]) is a mycotic infection caused by fungal invasion of the nail structure and is one of the most common nail disorders, representing half of nail abnormalities in adults. Its prevalence in Europe is around 4.3% over all age groups and 15.52% of all nail dystrophies in children. Onychomycosis is more commonly diagnosed in men and older people, affecting 20-50% of people aged over 60 years. An increased incidence among older people may be attributed to multiple factors, including reduced peripheral circulation, diabetes, inactivity, relative immune suppression and reduced nail growth and quality. Toenails are affected more commonly than fingernails[6,7].

ONYCHOMYCOSIS INFECTION

Where Onychomycosis infects the area underneath the nail plate, the infection produces a thick hyperkeratotic nodule that contains clusters of branching filaments (hyphae) called dermatophytoma. Consequently, the nail becomes severely deformed and can cause nail lifting, brittleness and discoloration, which may result in acute plain. The abnormal thickness of the nail may lead to soft tissue breakdown and/or infection resulting in inflamed subcutaneous tissue (cellulitis), ulceration in the nail bed (subungual ulceration) and/or bone infection (osteomyelitis)[8].

CAUSES

- Dermatophytes
- Non-dermatophyte molds.
- Yeasts

CLASSIFICATION OF ONCYCHOMYCOSIS

Distal subungual onychomycosis

The more general form may growth in the toenails, fingernails or both, infection is normally caused by Trichophyton rubrum which attach in nail bed and the bottom of the nail plate, starting at migrating proximally done inherent nail matrix.

White super facial onychomycosis

Once 10% of cases which is caused by several fungus that direct attach the superficial layers of the nail plate and develop well represented opaque White Island on the plate the nail is rough, soft and friable. This several of disorder can be treated with topical antifungal drug alone.

Proximal subungual onychomycosis

It is fall out while infecting organism commonly attach the nail through proximal nail fold, penetrate the newer develop nail plate and then migrate distally.

Candida onychomycosis

It can category into three part

1. Infection starting as infection structure encompassing the nail known felon.
2. Chronicle for lower than 1% of disorder this position is seen in immune viamedia patients and attach direct of the nail plates.
3. While nail plate has removed from nail bed[9,10].

Diagnosis

Despite Onychomycosis having distinct clinical features, around half of nail dystrophy cases are caused by fungal infection, and therefore, clinical examination alone is rarely sufficient to diagnose Onchomycosis[11].

Treatment

- Topical Treatment
- Amorolfine
- Ticonazole
- Systemic therapy
- Combination therapy
- Photodynamic therapy
- Lifestyle advice[12,13]

Nail lacquer

Nail polish Lacquer is used to nail of finger and toe of the person to make up and/or defense against infection on nail surface. Conventional nail lacquers is applied as cosmetics for bigger duration to improve looks and give glossy to the surface of the nail of the person.

The external nail formulation like lacquers, enamel and varnisharean essential type glam or us substances in present life. It defense against in fection to the surface, but essential significant it is maximize their glossy and relate shade and sheen.

It is used toenail of the finger and the toe and/or protect to nail tops. Conventional applied as big interval for decoration as well as defense it.

Penetration of active object, the top tissue concentration are respect for the efficaciousness for the therapy of onychomycosis[13,14].

PREFORMULATIONS STUDIES

Recognition of Drug

Study of solubility

Saturated solubility of Miconazole nitrate was made by applying 10ml of distilled water/ethanol/acetone in 25ml volumetric flasks in thrice. Precaution was taken so that the drug dosage form stay in medium in spare. Then by using mechanical shaker, the flasks were shaken for 48 hours. The test sampling was done on 24th and 48th hour. The test sample is withdraw (1ml after filtration) was soluble with suited medium and analyzed by using UV spectrophotometer at 223nm.

Determination of the melting point

Melting point of drug determined by excellent measurement by fetching a few amount of drug in a capillary tube certain at once last and was attached in Thiel's melting point setup and temperature range at that the drug melted was presented. Mean of one of thrice readings was written.

Max determination

100mg of pure Miconazole nitrate was interpreted in a volumetric flask and soluble in a small few amount of phosphate buffer pH of 7.4 and volume made up to 100ml. 1ml of the trying firstly of dilution was taken and some diluted to 100ml. The trying test firstly solution scanned for excellent absorbance in double beam UV-Visible spectrophotometer in between the range of 400-200nm against phosphate buffer pH 7.4 as the clean Thrice reading were taken and mean was determined[15,16].

ANALYTICAL METHODS

Phosphate buffer solution preparation

0.2M Sodium hydroxide solution preparation

8gm of the sodium hydroxide was soluble in needful quantity of distilled H_2O in a 1000ml volumetric medium and volume made up to 1000ml with distilled H_2O.

0.2M potassium dihydrogen phosphate solution preparation

27.218gm of potassium dihydrogen orthophosphate was soluble in needful quantity of distilled H_2O in a 1000ml volumetric medium and volume was made up to 1000ml with distilled H_2O.

The pH of phosphate buffer solution preparation

50ml of potassium dihydrogen phosphate solution was taken in a 200ml volumetric flask and 39.1ml of 0.2M sodium hydroxide solution was mixed and made up to 200ml with distilled H_20.

Standard stock solution and Calibration curve of Miconazole nitrate preparation

Miconazole nitrate 100mg pure drug was right weighed and transfer into a 100ml volumetric flask of medium. And the volume was made up to 100ml with PBS of pH 7.4, to come into ownership

standard stock solution of 100mcg/ml concentration. According above solution of 2ml, 4ml, 6ml, 8ml, 10ml, was pipetted out into other 100ml volumetric flask and made up to 100ml with PSB of pH 7.4 come into ownership a concentration range of 20µg/ml, 40µg/ml, 80µg/ml and 100µg/ml solution. The analyzed of solution at 223nm by using UV-Visible spectrophotometer. The concentration versus absorbance was plotted on the graph. Drug constitute assessment and diffusion presented were aim on this calibration curve.

DRUG-POLYMER COMPATIBILITY DETERMINE

Pure drug FT-IR spectral analysis and polymer were portaged out singly and as composition. The compatibility between Miconazole nitrate, nitrocellulose, 2-HP-β-CD, propylene glycol and made development were carried out in the ratio 1:1. The test were located FT-IR window after mixing and triturating with potassium bromide[17,18].

FORMULATIONS STUDIES

Preparation of nail lacquer of Miconazole nitrate

Making of Nitrocellulose

Approximate 5gms of cellulose base (cotton) is mixed to 50ml concentrated sulfuric acid and 25ml 70% nitric acid mixture and chilled to 5-10^{o}C to give cellulose nitrate. Then cotton was separated and washed in chilled water and with $NaHCO_3$ Solution separated all acid remain.

It was then low at dried at room temperature.

Optimization of Nitrocellulose film former

4 different concentrations of nitrocellulose, 3%, 5%, 6%m 9%, were made applying 2 different plasticizers, Propylene glycol and glycerin at 10% concentration as per Table No.2. The optimal concentration for film formation was characterized by great determination by rating the thickness, tensile power, folding stress and H_2O opposition[19,15].

EVALUATION

Film thickness

The thickness of the flick was determined by applying screw gauge with a minimum count of 0.01 mm at many points of the films. The thickness was

Folding Endurance

Folding endurance of the films was measured by repeat foldaway a little strip of the film (approximately 2x2cm) at the same site till it brittle. The numerous of times film could be crimped at the same site, without brittle gives the factor of folding endurance[16].

FORMULATIONS STUDIES

Preparation of nail lacquer of Miconazole nitrate

Preparation of Nitrocellulose

Around 5gms of cellulose base (cotton) is mixed to 50ml concentrated sulfuric acid and 25ml 70% nitric acid mixture and chilled to 5-10^{o}C to give cellulose nitrate. Then cotton was separated and washed in chilled H_2O and with $NaHCO_3$ Solution to separate all acid remain. It was then easily slow dried at room temperature.

t=thickness of sample in cm.

Water resistance

This is determine of the resistance to the aqueous permeability of the layer. This was by applying a continuous layer on a plane and plunging it in water. This weight before and after submergence was written and maximize in weight was calculated. Larger the maximize in weight lesser the water resistance.

Development of nail lacquer

The Formulation was done according to formula shown. The Miconazole nitrate and Nitrocellulose was solubilize in Ethyl alcohol in the important substance used a magnetic stirrer at a various speed. To clear the solution important substance of 2-HP-β-CD, Salicylic acid and propylene glycol were mixed and volume to 100ml. The prepared nail lacquer was trans change to a narrow plastic screw capped glass bottle[17,18].

EVALUATION OF NAIL LACQUER

Nonvolatile content

10ml of preparation was take in a petri dish and firstly weighed were taken. This dish was put in the oven at 105^{o}C for 1hr, the petri dish was removed, cooled and weighed. This separated in weights was

taken. Mean of one of three cycle readings was reported.

Drying Time

A layer of formulation was used on a petri dish with the using by the brush. The time for make a dry-to-hard layer was noted use by stop watch.

Smoothness to flow

The preparation was dip from a heighted of 1.5 inches into a glass plate and dispersed on a glass plate and made to wave vertically and see obtaining for smoothness of layer.

Gloss

Development of nail lacquer was used on the nail and gloss needful and done with marketed cosmetic nail lacquer[19,20].

FORMULATIONS STUDIES

Development of nail lacquer of Miconazole nitrate

Viscosity

Using the brook field viscometer.

Adhesion

There are neither to amount of evaluation tools resultant to use the medicinal nail lacquer at this time of duration. The instruments is used of chemical balance applied in the general laboratory as showed. One pan of the balance was transfer with two stainless steel plates. In between the plates a film of 4 cm 2 was made and adhered. The poise of the balance was adjusted by mixing a weight to the right pan of balance. The force needful to pull away the plates determined and compared with a commercial cosmetic nail lacquer test sample[20,21].

Drug content appraisal

Nail lacquer equivalent to 200mg was soluble in 50ml phosphate buffer solution of pH 7.4. Then the solution was supersonic for 15 mints. Resultant solution was filtered, made up to 100ml with phosphate buffer solution of pH 7.4. From the above solution carried at 10ml and made up to 100ml with PBS of pH 7.4. Then the diluted solution was assessment spectro photometrically at wavelength of 223nm and determined the drug constituents.

Diffusion studies across artificial membrane

Diffusion studies were tested by Franz cell applying artificial membrane (cellophane) of 0.8µm. The membrane was loaded for 24hrs in solvent system and the solvent fill the receptor compartment.

Nail lacquer equivalent to 200mg was used evenly on the surface of the membrane.

The made membrane was assembled on the cell carefully to avoid entrapment of air bubbles in the membrane. The all weldment was maintained at 37^{o}C, and the speed of stirrings was kept constant for 20hrs. The 5ml aliquot of drug sample was taken at time intervals of 2hr, 4hr, 8hr, 10hr, 12hr, 16hr and 20hrs and was replaced by the fresh solvent. Samples were analyzed by double-beam UV spectrophotometer as per method mentioned in drug content appraisal. Each experiment was recurrent thrice.

***In vitro* permeation studies**

Hooves from freshly slaughter cattle, free of adhering tending to attach and cartilaginous tissue, were loaded in distilled water for 24hrs. Membranes of approximate 1mm thickness were cut form the distal part of hooves. *In vitro* permeation studies were tested by using from Franz diffusion cell, the hoof membrane was situated by paying attention on the surface of the nail membrane. The targeted receptor compartment was filled with solvent phosphate buffer solution of pH 7.4 and the all weldment was maintained at 37^{o}C with constant mixing for 48hrs. The 5ml factor of number of drug sample was taken after a time intervals of 2, 4, 6, 8, 10, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48hrs. Transferred by the fresh solvent. The drug analysis was done by using double-beam UV spectrophotometer at 223nm.

Determination of antimicrobial activity

Candida albicans were wages for testing antifungal act by the cup-plate method. The culture was take up on sobouraud's agar slants. 20ml of melted sabouraud's agar medium was confirm 72hrs. Old 0.2ml suspension of *Candida albicans* in the Petri dish and allowed to standard by conformity undisturbed for 15mints.

The cups (10mm diameter) were slugged in the Petri dish and filled with 0.05ml of a solution of the

sample. The plates were taken for diffusion at 40°C for 1hr, and followed by incubation at 30°C for 48 hrs. After done the incubation time the zone of suppression in millimeter were determined. On with test solution in every petri dish one cup was filled up with solvent, which play as control. The zone of suppression was noted and compared with control.

Stability study

Stability studies of nail lacquers were according ICH guidelines. Test samples were at temperature of 25±2°C/60 ±5% RH for 6 months and 40±2°C/75±5% RH for 1 month. Then the samples were analyzed for nonvolatile content, drying time, gloss, smooth of flow, drug content and diffusion across artificial membrane[22].

RESULTS AND DISCUSSION

Results for Analytical Study

Scanning of drug

Pure Miconazole nitrate sample was scanned using phosphate buffer solution (PBS) of pH 7.4 between 200nm to 400nm using UV visible spectrophotometer. The tallest peak of Miconazole nitrate was obtained at 223nm (Figure No.1) and thus the λmax of Miconazole nitrate was at 223nm and was used some spectrophotometric evaluations during the investigation.

Standard solutions of Miconazole nitrate in various concentrations (Table No.4) were made applying PBS pH 7.4 and their absorption was determined at 223nm. Drug concentration Vs. absorbance was plotted in Figure No.1, Table No.4.

PREFORMULATIONS STUDIES

Solubility studies of Miconazole nitrate

The result of solubility studies of pure Miconazole nitrate are given below:

From the data, solubility profile of Miconazole nitrate was insoluble in water, soluble in ethanol and acetone (Table No.5).

Melting point determination

The melting point was found to be 161°C ± 0.577 and as per the IP 2007 melting point of Miconazole nitrate was within the range of 159-160°C.

Drug excipient compatibility study

All the reference IR peaks of the pure drug Miconazole nitrate were also present in the spectra of mixture of drug-polymer and drug- permeation enhancer-excipients as mentioned in the above Table No.6.

So FTIR study showed that there is no interaction between drug and permeation enhancer. So the drug and permeation enhancer are compatible. The IR spectrums were given in the Figure No.3-9.

After spectral comparison it was confirmed that not compatibility reaction took place between drug and additives, as all main properties IR peaks of Miconazole nitrate are present in the physical mixture with individual additives and also in the final optimized formulation, All the additives peaks were obtained to be entire indicating nice compatibility.

Formulation development of Nail Lacquer

The aim of the present study was to furnish a preparation for conquer fungal developed on toe nails or finger nails so that the looks of the nails are valuable. Preparation consists a film former nitrocellulose, permeation enhancer such as 2-HP-β-CD, keratolytic agent like salicylic acid and an antifungal agent (Miconazole nitrate) and ethanol as solvent. Preparation is made by simple mixing method.

Optimization of nitrocellulose film former

Various concentration of film forming polymers were applied for film formation and then applied for optimization of film. Various concentrations were tried between 2-8%. From the conclusion, it was obtained that by maximizing the concentration polymer up to 6%, thickness and strength of film was coveted. While maximizing concentration more than 6%, sticky films were generated. Thus, 6% concentration of polymer was needful for some obtained of plasticizer. Plasticizer tried were Glycerin and Propylene glycol in 10% concentration each. Glycerin showed more sticky film which was unable to detach from surface. Thus, 6% nitrocellulose and 10% propylene glycol, due to its excellent film forming nature was choose for some optimization research.

Thickness

Unvarying thickness bespeak the unvarying of the preparation because of that suitableness of the executed procedure. Thickness of all the films determined by applying a micrometer screw gauge. Obtained result presented that thickness of all preparation varied from 55 to 59µm. The determined values were shown in the Table No.7. Data for film thickness was duplicate within the coveted range of thickness identified through review of literatures for films.

Folding endurance

Folding endurance bespeak the flexibility of the polymer film. In order to evaluate the flexibility, the made films were subjected to folding endurance research. The numerous of bend a film can sustain without interruption will dictate its folding endurance. The computed measured determined were above 125 in all of the generated layers and it was in the range of 126-178 for all the generated films. Regardless of polymer concentration applied, all the films presented nice folding endurance, bring out that the made films were having the capability to produce hold up the mechanical pressure along with nice flexibility. The folding endurance is a significant evaluation, which assure the flexibility of the generated films. Larger the folding endurance values better will be the flexibility of the films. 6% film (NF3) presented good folding endurance, because of that ensuring good flexibility.

Water Resistance

This is the determined of the opposition towards water permeability of the layer. This was done by applying uninterrupted layer on a surface and plunge it in water. The weight before and after immersion was noted and maximize in weight was determined. Large maximize in weight low the water opposition. Here Nitrocellulose Film of 6% (NF3) has relatively, low weight and has the better water resistance. The data were shown in Table No.8.

Having a base on above studies it was distinct that, NF3 formulation has the excellence properties needful for a nail lacquer and thence 6% w/v of nitrocellulose and 10% w/v of Propylene glycol was determined to be the optimum concentrations.

Evaluation of nail lacquer

All preparations presented coveted layer make, smoothness of flow was nice. Coveted quantity of nonvolatile substance (31-41%) was observed with complete evaporation of volatile matter leaving a thin layer; Conclusion were plotted in Table No.9. Drying time was obtained within 52-127 sec. Demur for F2, where it presented 127 sec, all formulation showed fast drying rate. That is less than 60 seconds. The numerous amount were shown in Table No.10.

Nonvolatile content

The non-volatile content of all formulation has been shown in the Table No.9, given below:-

Drying time

Smoothness of flow and Gloss

Both these parameters was obtained to be acceptable as can be received. The nail lacquer dipped onto the glass plate was obtained to dispersed and resultant in unvarying smooth layer. The gloss of the applied lacquer was worthy of comparison with marketed cosmetic test sample achieving the cosmetic credence.

Viscosity

The viscosity of the test sample ranged from 100 to 220 centipoise it was obtained that between 140 to 160 centipoise the product was clean and glossy. Furthermore this viscosity range furnished nice attachment and flow property. Viscosity outside this range generate translucence and minimize gloss which will not be cosmetically satisfactory (Table No.11).

Adhesive strength

The adhesive strength of the implied batch was shown to be worthy of comparison with marketed sample and thence can be arrived to exhibit equal adhesive strength on applied nail surface (Table No.12).

Percentage drug content determination

Percentage drug ingredients for all the lacquers were obtained to be satisfy and in between 86.25-99.01% which is shown in Table No.13. Largest % of drug constituents was obtained to be 99.01% (F11) and the smallest % of drug content was 86.25% (F3). Drug content more than 90% in the Preparation shows the large no. of quantity of drug

present in the Preparation, Confirming that the methods of preparation and the constituents choose are not poignant the stability of drug. Large drug constituents also show to confirm that, a nice curative result can be arrived.

Diffusion studies across artificial membrane

Diffusion research of all the preparations were obtained by artificial membrane (cellophane membrane -0.8µm) for 48 hrs. The diffusion studies were made on all formulations (Table No.14-17).

The top formulated batch F0 did not dwell of any permeation enhancers and *in vitro* diffusion revealed that only 27.10% drug released till 48 hrs. Thus trials were planned to incorporate a permeation enhancer. Salicylic acid at concentrations of 5% (F1), 10% (F2), 15% (F3) and 20% (F4) was tested out. The diffusion studies shown that only 64.18%, 65.10%, 68.34% and 69.10% respectively was obtained in 18 hours. It was clean that salicylic acid has valuable the drug permeation due to its keratoytic activity. But it was also determined that the drug permeation was not yet done and some maximize in salicylic acid concentration is not arrived to valuable permeation. Thence it was declared to choose 15% w/v of salicylic acid as the optimum concentration, to further improve drug diffusion it was decided to include 2-HP-β-CD in concentration of 5% (F5), 7.5% (F6) and 10% (F7) into formulations. The drug release and diffusion across membrane was found to improve in presence of 2-HP-β-CD. At concentration of 5%, 82.40% diffusion in 28% hour was observed. In case of F6, 89.0% diffusion as observed at 28th hours. It was also observed that as concentration of 2-HP-β-CD increased drug diffusion also improved drastically as clear from almost complete drug diffusion of 98.40% release in 20th hour with 7.5% concentration.

Though, inclusion of 2-HP-β-CD has improved drug diffusion to 98.40%, it was observed that the release was found to be complete within 20 hours. Therefore to sustain the drug release over an extended period it was decided to include a rate controlling polymer ethyl cellulose at concentration of 0.25% (F8), 0.5% (F9) and 0.75% (F10) and 1.0% (F11) into formulation. The result showed an extended and completed release of 96.80% at 28th hr. in F8 and 93.0% till 36th hour in F9. In F10, a drug diffusion of 97.20% was observed at 40th hr. And finally when the concentration of ethyl cellulose was increased to 1% in F11, a drug diffusion of 98.12 percent which sustained over a period of 48 hours was achieved.

***In vitro* ungual permeation studies**

To excite and constituting an imitation diffusion research with that of *in vivo* conditions, i.e. across nail plate, a diffusion study across hooves resultant form freshly slaughtered cattle was done. There was no importance difference and drug release data obtained across artificial hoof's membrane. This research achieve sureness which is nice *in vitro in vivo* correlation can be demur.

ANTI-MICROBIALSTUDY

The zone of inhibition for the many preparation was investigated, and it was obtained range from 17-22mm, which is allow to compare with that standard with 21mm. The show that all the formulations were sensitive to the microorganisms *Candida albicans*. Conclusion are shown in Table No.18.

Stability studies

Stability studies were applied to obtain the shelf life and storage condition of a product. In this determination F11 were subjected to speed upstabilitystudiesforasperdayof1month. Stability studies were performed in according to ICH guidelines with importance adjustments.

The studies were obtained to ascertain the changes in physical properties such as Non-volatile content, Drying time, % drug content, drug diffusion at three different conditions f higher temperature (40±2ºC) for 1 month. The conclusion are shown in Table No.19,20. The evaluation of formulation after stability study presented there was no important change with respect Non-volatile content, Drying time % drug content and drug diffusion with respect to result obtained before stability charging. Thence it was received that the formulation were obtained to acceptable stability compliance needful as per ICH guidelines[23,24].

Table No.1: Drug-Polymer compatibility study

S.No	Composition		Ratio	250 C +2
			/60 ºC RH	/75 ºC RH
1	Miconazole nitrate	100mg	6 Months	1 Month
2	Nitrocellulose	100mg	6 Months	1 Month
3	2-HP-β-CD	100mg	6 Months	1 Month
4	Propylene glycol	100mg	6 Months	1 Month
5	Miconazole + Nitrocellulose	1:1	6 Months	1 Month
6	Miconazole + 2-HP-β-CD	1:1	6 Months	1 Month
7	Final Formulation	NA	6 Months	1 Month

Table No.2: Optimization of nitrocellulose film former

S.No	Formulation code	Nitrocellulose (%w/v)	Plasticizers (% w/v)		Ethanol (ml)
			PG	Glycerin	
1	NF1	4	11	....	11
2	NF2	6	11	....	11
3	NF3	8	11	....	11
4	NF4	10	11	....	11
5	NF5	4	....	11	11b
6	NF6	6	....	11	11
7	NF7	8	....	11	11
8	NF8	10	....	11	11

Table No.3: Formulation Table

S.No	Ingredients (%)	F0	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
1	Miconazole nitrate	4	3	4	4	4	4	4	4	4	4	4	4
2	Nitrocellulose	8	8	8	8	8	8	8	8	8	8	8	8
3	Salicylic		7	12	17	22	17	17	17	17	17	17	17
4	2-HP-β-CD	...	...	...	...	...	6	7.10	10	10	10	10	10
5	Ethyl cellulose	...	...	...	...	...	...	...	...	0.28	0.53	0.8	1.10
6	Propylene Glycol	12	12	12	12	12	12	12	12	12	12	12	12
7	Ethanol q.s	100	100	100	100	100	100	100	100	100	100	100	100

Table No.4: Calibration Curve

S.No	Concentration(µg/ml)	Absorbance at 223nm
1	00	00
2	20	0.12
3	40	0.28
4	60	0.36
5	80	0.49

Table No.5: Solubility studies of Miconazole nitrate

S.No	Solvents	Solubility (mg/ml)
1	Ethanol	0.79
2	Water	0.045
3	Acetone	0.39

Table No.6: FTIR compatibility study interpretation

S.No	FTIR spectra of pure Miconazole nitrate		Miconazole nitrate Optimized Nail Lacquer Formulation (F11)	
	Wave number (cm^{-1})	Functional group	Functional group	Wave number (cm^{-1})
1	329.6	Imidazole C-N stretch	Imidazole C-N stretch	3179.79
2	3255.79	Aromatic CH stretch	Aromatic CH stretch	3109.43
3	2974.94	Aliphatic CH_2 stretch	Aliphatic CH_2 stretch	2959.96
4	2889.65	Aliphatic CH stretch	Aliphatic CH stretch	2901.10
5	1449.74	-CH_2- bending	-CH_2-bending	1774.97
6	141705	C-H bending (aliphatic)	C-H bending (aliphatic)	1411.95
7	1328.04	C-N stretch	C-N stretch	1321.74
8	1089.85	C-C stretch	C-C stretch	1089.48
9			C=C aromatic	1589.06
			C=C aromatic	1547.75
			C-H bending(aromatic)	721.66

Table No.7: Optimization of nitrocellulose film former

S.No	Nitrocellulose Concentration (%w/v)	1	2	3	4
1	Thickness	59 $\pm$ 0.04	60$\pm$ 0.05	56 $\pm$ 0.09	59 $\pm$ 0.010
2	Folding endurance	156	127	179	178
3	Tensile strength (Kg/cm^2)	2.57 $\pm$ 0.010	2.59 $\pm$ 0.010	2.61 $\pm$ 0.09	2.56 $\pm$ 0.010

Table No.8: Water (W) resistance of nail lacquers

S.No	Formulation code	W_1(g)	W_2(g)
1	NF1	6.89	6.99
2	NF2	6.80	6.99
3	NF3	6.80	6.99
4	NF4	6.99	7.19
5	NF5	6.84	6.96
6	NF6	6.88	6.97
7	NF7	6.96	6.98
8	NF8	6.96	7.09

Table No.9: Nonvolatile content of nail lacquers

S.No	Formulation code	Non-volatile content (%)	Formulation code	Non-volatile content (%)
1	F0	34 ± 0.40	F6	38 ± 0.80
2	F1	34 ± 0.40	F7	37 ± 0.78
3	F2	42 ± 0.80	F8	33 ± 0.48
4	F3	40 ± 0.46	F9	36 ± 0.49
5	F4	38 ± 0.80	F10	34± 1.29
6	F5	38 ± 0.70	F11	38 ± 0.89

Table No.10: Drying time of nail lacquers

S.No	Formulation code	Drying time (sec)	Formulation code	Drying time (sec)
1	F0	57	F6	59
2	F1	55	F7	65
3	F2	130	F8	58
4	F3	58	F9	66
5	F4	60	F10	60
6	F5	65	F11	59

Table No.11: Viscosity of nail lacquers

S.No	Formulation code	Viscosity	Formulation code	Viscosity
1	F1	101	F7	208
2	F2	112	F8	149
3	F3	124	F9	147
4	F4	137	F10	149
5	F5	189	F11	149
6	F6	199		

Table No.12: Adhesive strength of nail lacquers

S.No	Formulation Code	Force of Adhesion (N)	Adhesive strength (N/m^2)
1	F11	0.9	12.10
2	Market Samples	0.9	18

Table No.13: Percentage drug content

S.No	Formulation Code	Drug content (%)	Formulation code	Drug content (%)
1	F0	90.09	F6	89.39
2	F1	91.57	F7	90.14
3	F2	93.79	F8	98.06
4	F3	86.29	F9	98.27
5	F4	94.37	F10	97.59
6	F5	95.87	F11	99.07

Table No.14: Comparative study and optimization of salicylic acid concentration

S.No	Time (hr)	Percentage drug release (µg/ml)			
		F1	F2	F3	F4
1	0	0	0	0	0
2	2	9.89	11.29	13.55	15.30
3	4	10.27	12.10	14.10	16.99
4	6	13.30	14.40	16.40	17.88
5	8	16.47	17.40	18.89	20.10
6	10	26.99	28.99	32.88	30.38
7	12	32.88	36.39	40.29	36.19
8	16	43.19	42.39	48.48	42.99
9	20	48.29	49.88	51.89	50.16
10	24	49.69	50.88	52.68	54.39
11	28	52.58	54.99	56.88	58.48
12	32	56.29	58.70	59.309	60.29
13	36	58.100	59.977	61.29	63.47
14	40	60.89	62.199	63.99	66.25
15	44	62.100	63.88	65.99	68.86
16	48	64.66	65.100	68.100	69.19

Table No.15: Comparative study and optimization of 2-HP-β-CD concentration

S.No	Time (hr)	Percentage drug release		
		F5	F6	F7
1	0	0	0	0
2	2	26.26	32.13	39.32
3	4	32.366	43.56	49.89
4	6	38.522	52.83	59.69
5	8	46.55	61.66	67.75
6	10	48.28	69.36	76.499
7	12	56.37	76.26	85.09
8	16	65.16	80.03	92.19
9	20	76.44	83.36	98.45
10	24	79.93	88.99	96.29
11	28	82.44	89.00	94.28
12	32	80.27	86.355	93.19
13	36	79.45	84.19	91.86
14	40	77.38	82.177	90.098
15	44	76.68	80.89	89.19
16	48	74.79	78.29	88.99

Table No.16: Comparative study and optimization of Ethyl cellulose concentration

S.No	Time (hr)	Percentage drug release (µg/ml)			
		F8	F9	F10	F11
1	0	0	0	0	0
2	2	29.69	26.59	19.49	12.88
3	4	34.19	31.99	30.49	27.18
4	6	45.59	40.40	36.90	28.37
5	8	51.19	44.90	48.88	32.78
6	10	62.39	53.20	50.70	46.29
7	12	69.70	60.19	56.88	50.29
8	16	75.99	68.69	60.29	58.69
9	20	88.49	72.37	65.78	60.29
10	24	93.29	83.465	72.69	68.18
11	28	96.89	89.74	80.59	70.28
12	32	95.09	95.89	85.78	78.88
13	36	94.59	93.80	90.69	84.19
14	40	93.19	90.76	97.50	88.88
15	44	90.79	89.89	94.28	90.20
16	48	89.09	88.78	91.39	98.10

Table No.17: Comparison of drug diffusion across artificial membrane and hoof's membrane

S.No	Time	Percentage drug release (µg/ml)	
		Drug diffused through artificial membrane	% drug diffused through hoof's membrane
1	0	0	0
2	2	12.88	14.57
3	4	27.19	20.97
4	6	28.39	26.48
5	8	32.76	36.79
6	10	46.29	47.99
7	12	50.26	56.79
8	16	58.69	60.49
9	20	60.26	65.88
10	24	68.17	72.59
11	28	70.27	80.69
12	32	78.89	85.09
13	36	84.18	89.29
14	40	88.80	92.39
15	44	90.25	95.09
16	48	98.17	97.49

Table No.18: Zone of inhibition of Miconazole nitrate Nail lacquers

S.No	Formulation Code	Zone of Inhibition (mm)	Formulation code	Zone of Inhibition (mm)
1	F1	27	F7	20
2	F2	18	F8	29
3	F3	29	F9	19
4	F4	28	F10	29
5	F5	19	F11	29
6	F6	19	Standard	29

Table No.19: Stability studies data of F11

S.No	Parameter	Initial	After
1	Non content	36±0.89	35±0.38
2	Dry in tin (sec)	58	60
3	Drug content	99.07	98.50

Table No.20: *In vitro* Diffusion profile of F11 upon stability studies

S.No	Time	Percentage drug release (µg/ml)	
		Before stability	After stability
1	0	0	0
2	2	12.88	10.69
3	4	27.19	24.97
4	6	28.37	26.45
5	8	32.77	30.29
6	10	46.26	39.99
7	12	50.25	45.79
8	16	58.68	52.59
9	20	60.29	58.89
10	24	68.17	62.59
11	28	70.26	72.09
12	32	78.89	76.89
13	36	84.19	81.29
14	40	88.89	90.59
15	44	90.29	92.29
16	48	98.18	97.79

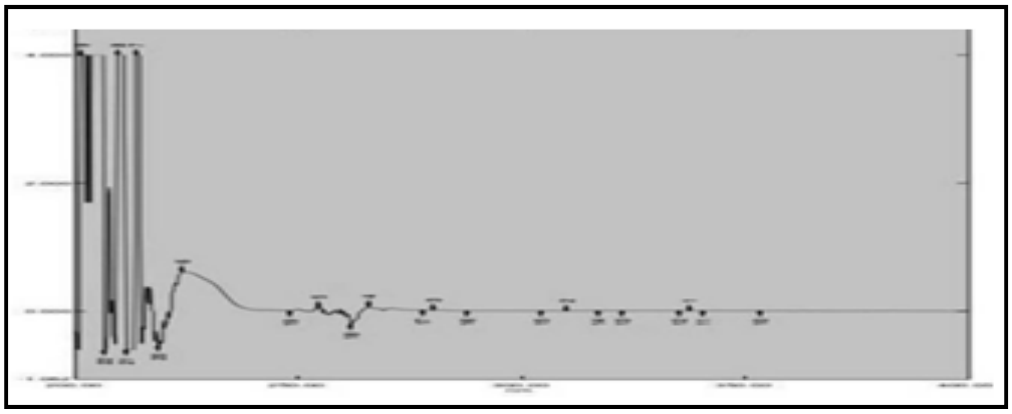

Figure No.1: UV spectrum of Miconazole nitrate in phosphate buffer solution of pH 7.4 Standard curve for Miconazole nitrate in phosphate buffer of pH 7.4

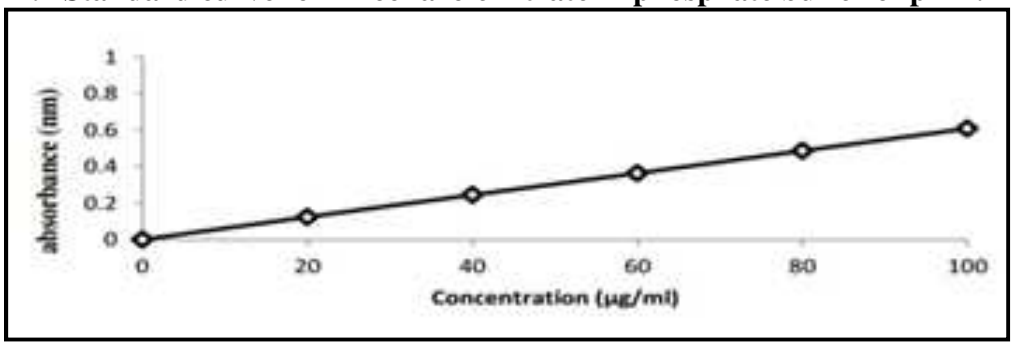

Figure No.2: Calibration Curve

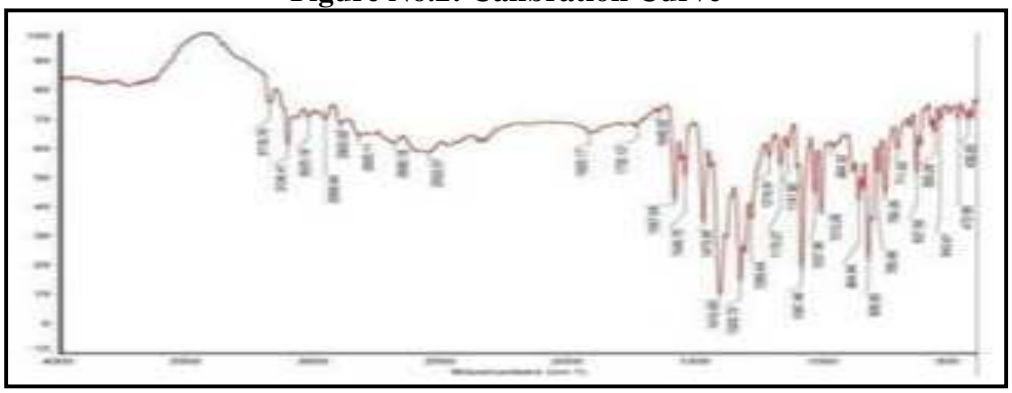

Figure No.3: IR spectra of miconazole nitrate

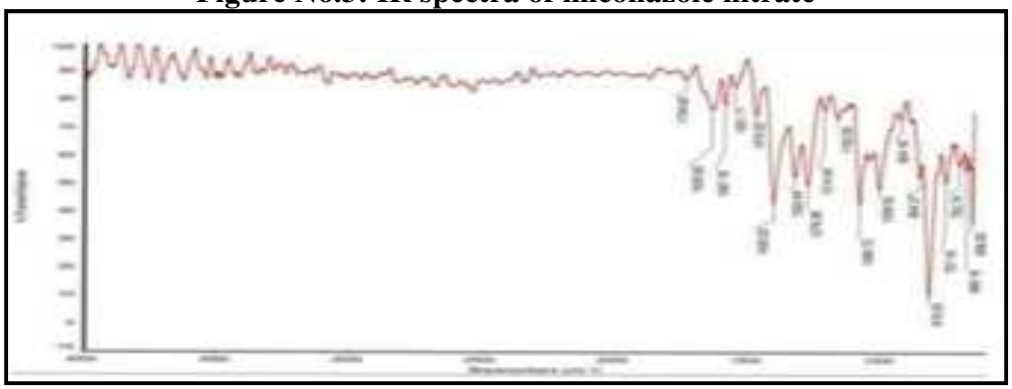

Figure No.4: IR spectra of nitrocellulose

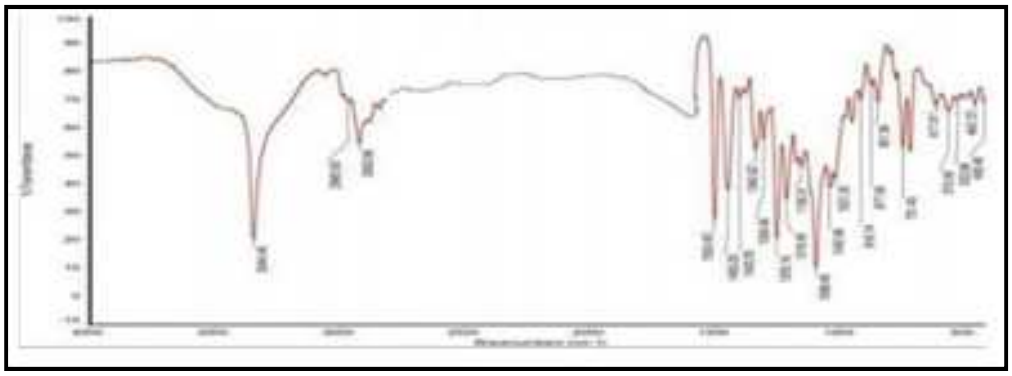

Figure No.5: IR spectra of ethyl cellulose

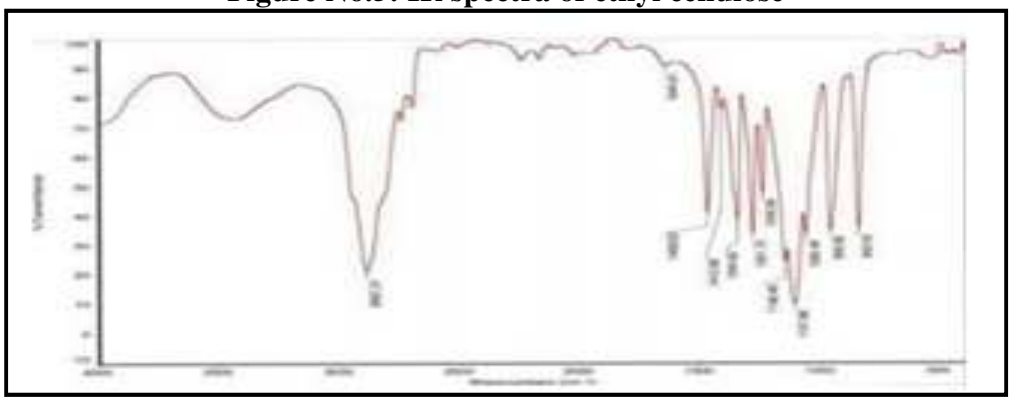

Figure No.6: IR Spectra of the beta hydroxyl propyl cellulose

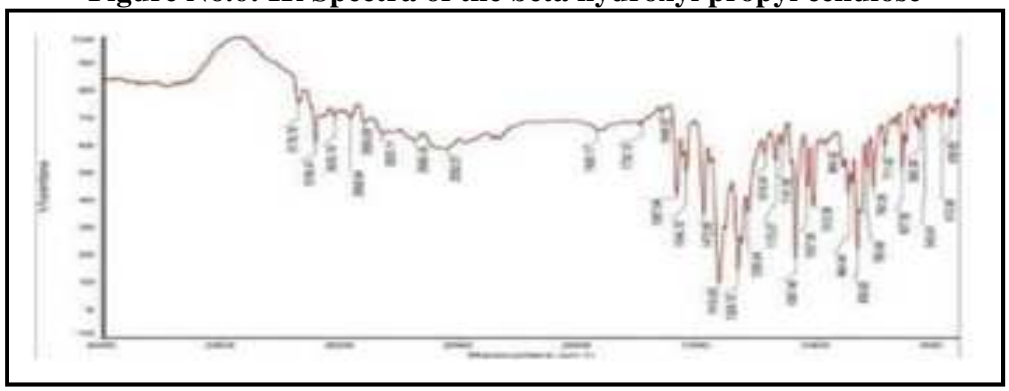

Figure No.7: IR Spectra of Miconazole nitrate and nitrocellulose

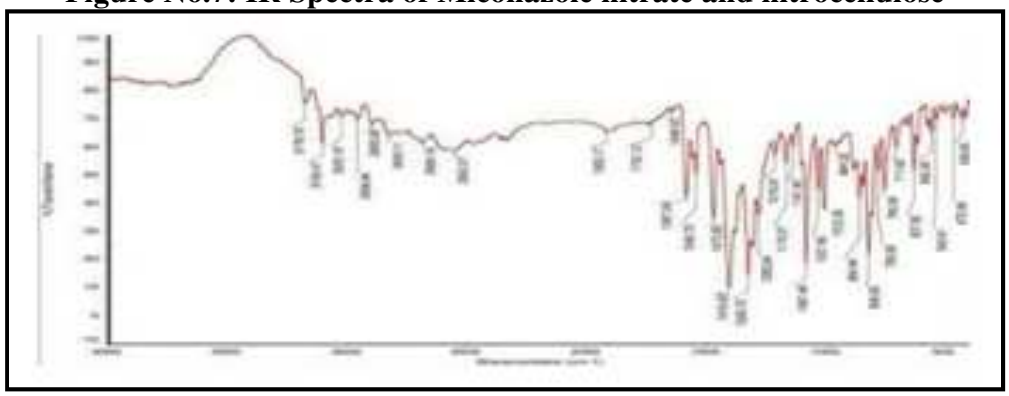

Figure No.8: IR Spectra of Miconazole nitrate and beta hydroxyl propyl cellulose

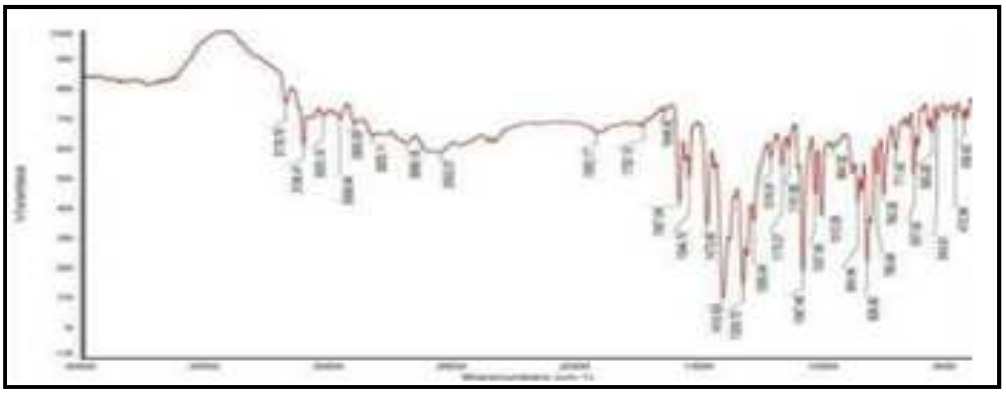

Figure No.9: IR Spectra of Miconazole nitrate optimized Nail lacquer

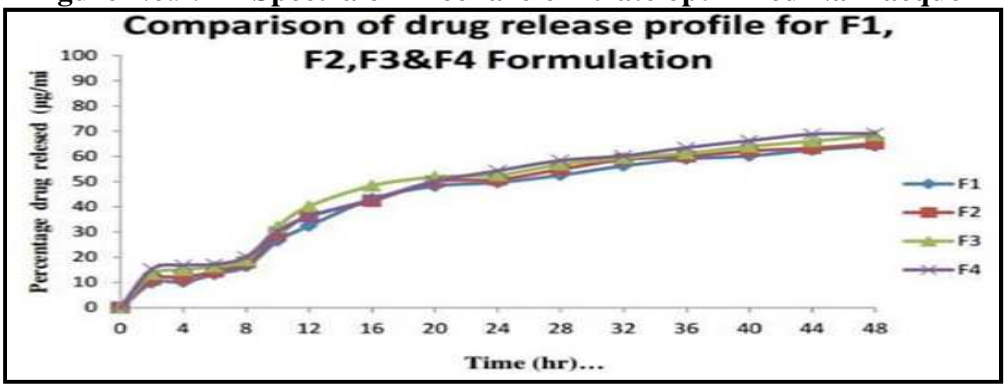

Figure No.10: Comparative Dissolution profile of F1 v/s F2 v/s F3 v/s F4

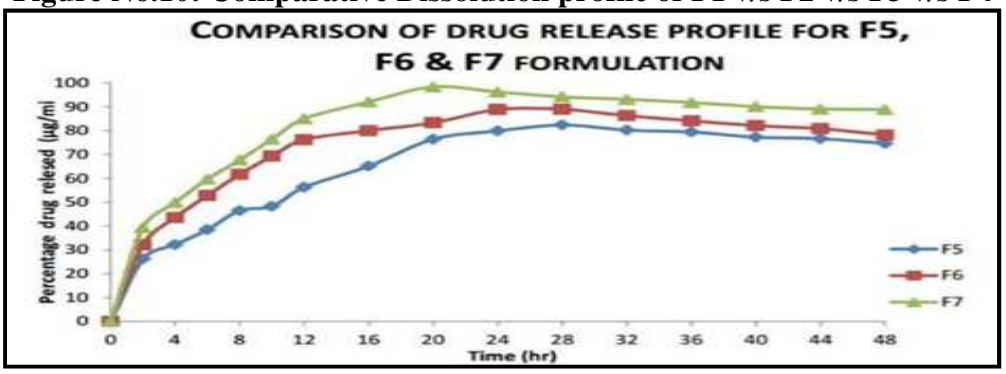

Figure No.11: Comparative Dissolution profile of F5 v/s F6 v/s F7

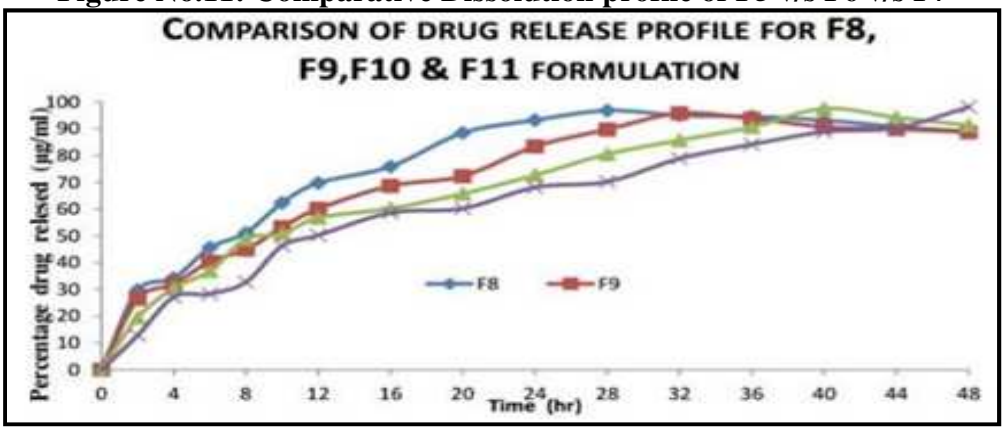

Figure No.12: Comparative Dissolution Profile of F8 v/s F9 v/s F10 v/s F11

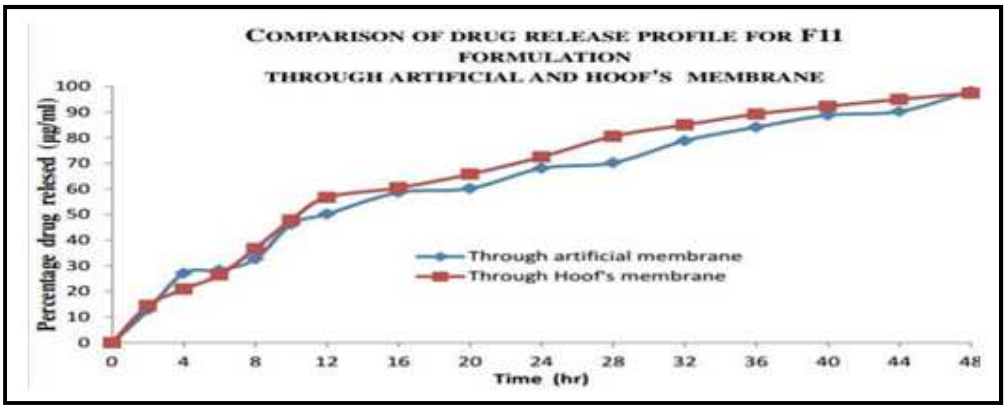

Figure No.13: Comparison of drug diffusion across artificial membrane and hoof's membrane

CONCLUSION

The selected medicament, the preparations were made with Salicylic acid. And by the FTIR research, resultant that the drug and the additives applied in the Preparation. Proved the formulations are sensitive to the required volatile contents by the microbial study.

The Preparation are sensitive to the microorganism *Candida albicans*. Confirmed by the microbial study. The F 11 was choose as the nail lacquer formulation based on optimization as well as drug diffusion studies. The preparation were survived at 40^{o} for 1 month confirmed by stability study. There was no more interchangeable in the values after stability test confirmed by the stability study. It was obtained that the preparations were achieved to confirmed stability compliance necessary as per ICH guidelines.

By research, obtained that medicated product shown, a nice base as a drug, which is applying in the treating of the nail infections, applied for glowing and glamorous of nails with easily and time consuming useful for applying which is improves patient compliance.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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