FORMULATION OF FILM FORMING SPRAY OF KETOCONAZOLE FOR THE TREATMENT OF FUNGAL INFECTION

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Abstract- The objective of this research was to create a new topical spray containing Ketoconazole as well as other non-toxic excipients by using LPG as a propellant. The work was intended to categorize and consider various formulations. The absence of chemical and physical incompatibility was discovered during a compatibility study, which disclosed that stimulant is consistent with container closures and excipients. The best methodology assessment from various formulations for a physicochemical tests, and performance test was discovered to be spray formulation k4. K4 was also found to be non-irritant and therapeutically more effective. According to the ICH guidelines, the stability research demonstrates that the optimized formulation was stable.

Keywords: Spray, Ketoconazole, Fungal infections, Polymers, Topical applications.

DOI Number: 10.48047/nq.2022.20.19.NQ99069 **NeuroQuantology 2022; 20(19):743-754**

Introduction

Fungal infection of the skin has been presently among the most severe dermal problems around the world. Pathogens were discovered to affect approximately 40 million individuals in underdeveloped and developed nations (Havlickova B. 2008, Selma M. 2004, and Ameen M. 2010). Dermatophytes are among the most likely reasons of onychomycosis and tinea. Candida species are also the greatest prevalent skin's surface fungal diseases (Akhtar N. 2015). Topical application is now an efficacious method for treating diseases both locally and systemically. Administration of drugs through the epidermis is an established treatment for local skin related maladies. It may consequence in better absorption into epidermis and hence improved absorption of the drug (Lee CM. 2006). Another of the most appropriate pathways for drug transport which initiate metabolism in the initial phase is topical delivery. It's usually effective against skin conditions (Deepshikha K. 2014) Due to the lesser adverse effects, steady delivery of drug, non-invasiveness, ease of its use, and comfort of discontinuation, medicinal delivery through skin is beneficial. However, topical administration of drugs is challenging because the skin serves as a barrier to delivery of drugs, and substance passage through epidermis seems to be a complicated system (Lee CM. 2006). Presently, ointments account for approximately 80% of such compositions. The utilisation of certain ointments towards the skin results in systemic behaviour that implies how absorption

takes place. Following that, systemic drug delivery via the topical delivery was accomplished with certain ointment and cream preparations for prevention and treatment. All of these formulations were not acceptable; the biggest limitation was varying drug uptake as a result of desired instructions to the anticipated coverage area.

Ketoconazole (cis-1-acetyl-4-[4-[2-(2,4-dichloro phenyl)-2-[1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine) is a lipophilic chiral imidazole antimycotic stimulant that is primarily prescribed as just a racemic (1:1) combination of Ketoconazole pharmaceutical formulations such as shampoo, cream, and tablet are used to effectively treat systemic fungal infectious diseases.

Experimental

Material and Methods

Ketoconazole was received as a gift sample from Ranbaxy Laboratories Ltd., (India Eudragit E100, Eudragit L 100, PVP k30, Klucel LF, Klucel EF Poly(methyl acrylate), Ethyl cellulose, Chitosan, (Sigma Aldrich, USA), all other chemicals were analytical grades.

Pre-formulation study of Ketoconazole

The pre-formulation survey gives useful information for creating sustainable dosage forms. The drug's organoleptic properties, such as color and odor, were tested physically. The melting point of the drug was calculated by using capillary rising model.

a. Spray containers, plasticizer, solvents, and film forming polymers- Screening

The solubility of a polymer, its capacity to produce clear films that are smooth and wrinkle-free after alcohol evaporates from skin, and if the stimulant as well as the solvent are consistent all affect the selection of polymer. Solubility is evaluated in various organic solvents, including isopropyl alcohol, ethanol, and acetone, and in various ratios. Plasticizers used are glycerine, PEG 400, castor oil, and propylene glycol. Different grades of Eudragit such as Eudragit L100, Eudragit E 100, Poly (methyl acrylate), Klucel LF, PVP K30 and Ethocel, were used as film forming polymers Baka, E. 2008, Ranade S. 2017)

b. Differential Scanning Calorimetry (DSC)

The Differential scanning test, 10 mg of a sample was added inside an aluminium pan and analysed at a scan speed of 10°C/min at temperature levels varying from 0 to 800°C in a nitrogen-free atmosphere (Manish K. 2018).

The MPEG-PCL2 and ketoconazole combination was heated up to dissipate in methylene chloride, forming a proper solution. To acquire a thin film, the organic solvent was removed from solution by using a rotary evaporator. To acquire a micellar solution, the film was heated up to 60 ° C and hydrated with water by using a vortex. The mixture was passed through a 0.22m filter membrane to eliminate any drug or even other precipitated content before being freeze dried.

Container compatibility study

Following the choice of spray containers, the product testing concentrate was placed inside the container and kept at

37° C for 7 days, permitting the liquid to make contact with the carton and the valve arrangement.

Corrosion, concentrate-dissolved coatings, dip tube discoloration, dip tube elongation, valve softness, valve cracking, and product concentrate discoloration were all observed.

In a systematic approach, PEG-400 (plasticizer) and EudragitS-100 (film formers) were dispersed in equal measures of menthol and camphor (eutectic mixture). Ketoconazole at 1% w/w was taken and dissolved in a solvent composed of 80% isopropyl alcohol and 20% acetone. The eutectic mixture of plasticizer and film formers was added gradually towards the drug solution and stirred for 30 min at 200-250 rpm before being sonicated. After that, the solution was transferred to the container assembly (Lachman L. 1991).

Table-1: preliminary selection of eutectic mixture

Batchcode	EutecticMixture (%W/V)	Eutectic Mixture	PolymerCon. (%W/V)	Drug(mg)	PEG-400 (%V/V)	Acetoneand IPA blend (q. s.)
K1	-	-	1%	200	1.4%	QS 20
K2	Menthol+Camphor (1%)	1:1	1%	200	1.4%	QS 20
K3	Menthol+Camphor (1%)	1:2	1%	200	1.4%	QS 20
K4	Menthol+Camphor (1%)	2:1	1%	200	1.4%	QS 20

Evaluation studies

a. Drying time- sprays have been ejected it into Petri dish, and the time it required for a film to establish was measured (Kashmira K. 2017).

b. Volume per spray- The average volume per spray was determined after sprays have been fired it into measuring cylinder.

c. pH- The pH of the formulation was determined using a calibrated digital pH metre (Bhadra S. 2016).

d. Stickiness- it is measured by lightly pressing cotton wool against dried film. It is graded based on the number of cotton fibres maintained by film: high if the film has a dense buildup of fibres upon that film, medium if the film has a thin fibre layer, and low when only variable or no fibre adhesion (Wangding L. 2016).

e. Drug Content- A solution equivalent of 10ml was positioned in a conical flask (100ml) and diluted with methanol (1000g/ml) to a required concentration. The mixture was filtered using Whatmann filter paper. A real outcome of 15g/ml was acquired by diluting with methanol. The absorption spectrum of this solution was taken at 257 nm (Mansi J. 2018).

f. Viscosity- At 25°C, a Brookfield viscometer was used to calculate viscosity (M.C. Gohel, 2009).

g. Drug release- The Franz diffusion cell was used to perform in-vitro drug release studies. To retain 0.5 g of film, a cellophane membrane was used. Diffusion tests were carried out at 37°C using a 250 mL phosphate buffer, pH 7.4 disintegrating medium. A 1 ml sample was taken every hour and was analysed by using UV spectrophotometer

Evaluations of containers

A. Pressure test-

Each box was fixed straight up at 25°C, and at least 3 - 4 aerosols were chosen. The actuator was pushed to eliminate fluid from valve. The pressure gauge was replaced, and the actuator was turned off. To activate the valve, the gauge was squished, and the pressure is exerted by propellant was evaluated using a pressure gauge for every container. The pressure gauge was turned down (United States of Pharmacoepia,32. National Fprmulary, 27).

B. Flammability and flame extension

To evaluate flame projection, the jar was filled with LPG gas inside the pressured gas system and examined for flammability. Flame projection (cm) was utilized as a channel to assess LPG gas flame flashback on paper, with a flame fuel and a spray proximity of 50 cm (Lachman L, 1991).

C. Test for leaks

Two kinds of leak studies were conducted, as described below.

Immediately conduct a leak test: After filling the containers with hot water (about 50°C), they were permitted to sink for around ten seconds. If you notice bubbles inside the jar, this suggests a leak.

Test for leaks with a lag: containers with exact weights were stored at room temperature for 2 months. The canisters are evaluated after 2 months. The weight change of a carton is used to detect vessel leakage ((United States of Pharmacoepia,32. National Fprmulary, 27).

D. Spray angle

The distance here between sheets and nozzle and was first determined. After spraying one actuation on with paper, the circle size was taken. The spray angle is derived as follows:

Spray angle (Θ) =$\tan^{-1}$(1/R)

Where, l and r are the paper's distance from the nozzle and average circle radius, respectively (United States of Pharmacoepia, 2000).

Solution volume delivered at each actuation- The following equation was used to measure how much solution is delivered at each actuation.

$$A_L = W_O - W_t / D$$

Where,

V_L — Solution volume supplied at each actuation,

W_t — Formulation weight after actuation,

W_o — Formulation initial weight before actuation,

D — Formulation density (Measuredusing a pycnometer)

E. Spray patterns

To create pH-sensitive filtrate, soak whatman filter paper in methyl red solution. The preparation was splashed upon paper (one actuation). The container's spacing from its location was fixed at 5 cm. The spray sequence then was assessed by sprinkling the concentrates both horizontally and vertically.

F. Short-term stability analysis

The planned batch's short-term stability was 25°C±2°C and 60% RH ±50% RH for 1 month. It was designed to demonstrate quality of a formula shifts over time as a consequence of environmental factors

like viscosity, pH, solution volume on actuation, spray angle, and that remained static all across the research (G. Maghraby, 2008. Mazzo D, 1999).

3. Result and Discussion

3.1 Preformulation Studies

Physical examination was performed to test Ketoconazole's organoleptic characteristics, including colour, appearance and odour.

a. Organoleptic Properties:

The procured drug was analysed on the basis of organoleptic properties such as colour, texture etc. The results were recorded.

Table-2: Organoleptic properties

S. No.	Parameters	Observations
1.	**Colour**	white colour
2.	**Appearance**	Crystalline powder
3.	**Clarity and colour of solution**	Clear Solution
4.	**Odour**	Odourless

b. Melting point:

The melting point of Ketoconazole was found to be 146 ˚C using capillary method.

c. Drug excipient compatbility

The physical mixture of polymer and drug DSC thermogram shows an exothermic peak correlating to the medication's melting point, and the combination is the same. DSC thermograms indicate that there is no incompatibility among polymer and drug. As a result, the correlating melting point apex remains constant, and the drug continues to remain crystalline.

d. FTIR Spectroscopy-

The FTIR spectroscopy of Ketoconazole was performed for identification analysis. The FT-IR spectrometer was used to track the ir spectrum of a drug. The specimens were prepared using the KBr disc technique and tested in transmission. Each spectrum was recorded in the 4000-400 cm^{-1} frequency range.

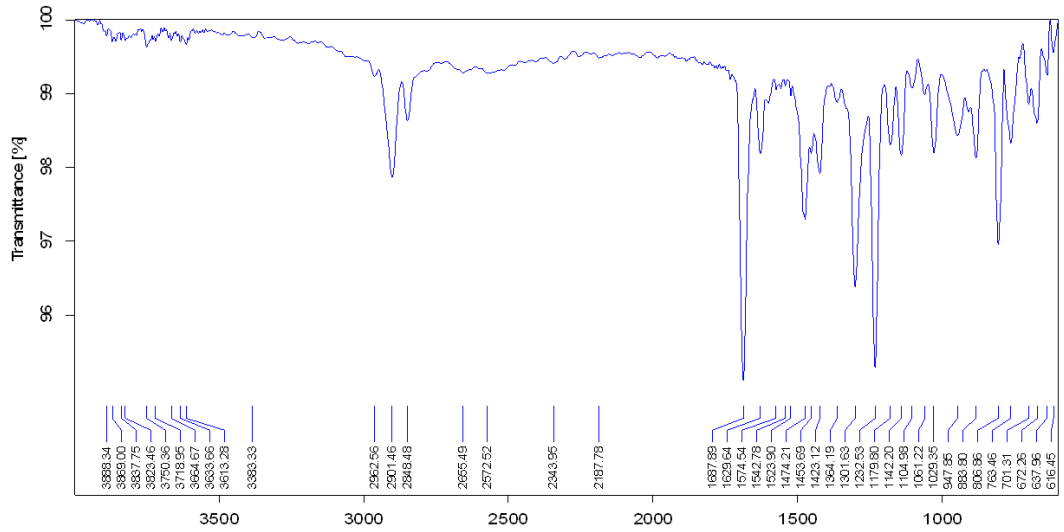

Figure- 1: FTIR spectroscopy of Ketoconazole

Primary screening of polymers

Table 3: observation table for the screening of polymer

Polymer	solvent	viscosity	Drying time(min)	Film formation	uniformity	appearance	stickiness	Color
Eudragit E100	Ethanol (10%w/w)	Low	4	Complete	uniform	Opaque	Non sticky	Off white
Eudragit	Ethanol + acetone (10%w/w)	Medium	3	Complete	uniform	opaque	Non sticky	Off white
Klucel LF	Ethanol (10%w/w)	Medium	4	Complete	uniform	transparent	Non sticky	colorless
Ethocel	Ethanol (10%w/w)	Medium	4	Complete	uniform	transparent	Non sticky	colorless
PVP K30	Ethanol +acetone (10%w/w)	Low	4	Complete	uniform	transparent	Non sticky	colorless
Poly(methyl acrylate)	Ethanol (10%w/w)	Medium	2	Complete	uniform	opaque	Non sticky	colorless

Primary screening of Plasticizers

Table 4: observation table for the screening of plasticizer

Polymer	**Clarity**	**Flexibility**	**Sprayability**	**Cosmetic Attractiveness**
Eudragit E100	Hazy & turbid	Brittle	Sprayable	Average
Eudragit L 100	Hazy & turbid	Brittle	sprayable	High
Klucel LF	Clear	Flexible	sprayable	High
Ethocel	Clear	Flexible	sprayable	High
PVP K30	Clear	Flexible	sprayable	high
Poly(methyl acrylate)	Clear	Brittle	sprayable	High

Screening of plasticizer

Table 5: observation table for the screening of plasticizer

Formulationcode	**Amt. of polymer (%w/w)**	**Name of plasticizer**	**Amt of plasticizer (%w/w)**	**Amt of ethanol**	**Quality of film**
F1	10	Glycerin	0.5	10	Good Peelable
F2	10	PEG 400	0.5	10	Poor Inflexible
F3	10	Propylene glycol	0.5	10	Good Rigid
F5	10	Castor oil	0.5	10	poor

Evaluation parameters related to the formulation

The pH was determined using a 25 ml solution spray. The pH among all samples at varying concentrations ranged from 4.2-5.5. The sprays developed had viscosities ranging from 22 cps-45 cps, providing an ideal balance among viscosity and spray strength to keep it skin with no leakage and disrupting till drying. Viscosity increased as the amount of Eudragit S 100 polymeric materials raised.

Analysis of drug content

1ml of solution was extracted and checked for drug content using a UV spectrophotometer. The content uniformity study results showed that the average product material per spray of constructed preparations was among 100.8±0.7 and 98.1±0.4, which is inside an accepted level.

Drying time

The test determined the solvents' effectiveness in drying the film, which has been discovered to be 30-60 seconds.Stickiness of the films by solvent evaporation

Stickiness

The sprays were neither tacky nor sticky since no cotton fibres were connected to the film

when dripped with cotton balls.

***In vitro* study**

The diffusion data for ketoconazole obtained for formulations K1 through K4 are shown in Table 5.7. The overall amount of Ketoconazole transferred in batches K1 to K4 varied from 96.54-75.99 percent and was observed over a 24-hour period at various time intervals. The start latency ejected by Ketoconazole stayed close in all instances, but still the time interval was changed.

Table-6: % drug release of optimized batches

Time(hr)	K1(%)	K2(%)	K3(%)	K4(%)
0	0	0	0	0
1	5.338	11.56	10.96	9.74
2	8.222	18.47	15.81	19.85
3	12.07	23.72	21.2	25.37
4	15.05	28.08	26.05	29.22
5	20.32	33.75	31.87	33.17
6	20.68	40.33	36.21	36.75
7	24.55	44.92	39.56	39.6
8	28.13	46.1	40.43	43.25
9	35.02	52.21	49.97	47.23
10	41.3	56.49	52.3	52.08
11	43.95	59.12	57.66	56.05
12	48.52	66.22	61.72	59.63
16	52.06	76.96	75.46	64.58
20	59.41	87.94	82.81	72.57
24	62.99	96.06	90.74	80.52

Menthol and camphor also combine to form hydrophobic eutectics. Menthol is a highly effective permeation enhancer. Camphor is quickly absorbed into the skin, generating a comparable scale of soothing to menthol while also acting as a mild local anesthesia and antimicrobial. They are both well-known subcutaneous permeation enhancers. They end up causing lipid leaching inside the skin, which causes the growth of pores. 1:1 Menthol: Camphor with the minimum viscosity and the highest dissolution.

Container-related evaluation variables

For optimized batch spray, tank-based parameters were tested.

a. Pressure examination

Because all preparations contain LPG as a propellant, the vapour pressure was discovered to be within the 5.0-6.0 kg / cm2 pressure gauge range. (1bar=14.51psig).

b. Test for flammability and flame extension

Distribution of spray was noted while the containers were kept 50 cm away from the

flame. Spray flammability was indicated by a flame extension of 65 cm from the flame. As a result, cases were instructed to keep canisters away from flames while splashing on to skin.

c. Container physical compatibility

The solution was retained inside the laboratory for a set period of time, and also no precipitation or crystallization was noticed.

Table-7: Compatibility study

Batchcode	Incompatibility (precipitation andCrystallization)				
	Time (Days)				
	3	6	9	12	15
K1	X	X	X	X	X
K2	√	√	√	√	**√**
K3	X	X	X	X	X
K4	X	X	X	X	X

d. Leakage test

Canister permeability was confirmed by moving the canisters through a 55°C water bath and observing weight variation. Selected samples were submitted to testing. The above test was taken in batch size.

Table- 8: Leakage results

Batches	Result	
	Immediate	Delayed
K2-	No leakage	No leakage

e. Spray angle and delivery amount upon each actuation

The solution volume provided at each motion and spray angle was approximately 0.14 ml and 560 hp, respectively. Two variables were found to be linked to concentration of polymer and viscosity. The spray angle was decided at random to be less than 850 for ease of use and coverage of the total surface area.

Table-9: Container Evaluation Parameters for OptimizingFormulation

Sr.No	Test parameter	Average results (Mean ± SD; n = 3)
1	Amount of delivered upon each actuation	0.13±0.04
2	Spray angle	55^0Θ ± 2.5
3	Leak test	No leak after feeling.
4	Film appearance	Complete, uniform,Transparent
5	Film flexibility	More value
6	Water wash ability	More value
7	Film formation time (exvivo)	30-50 S ± 2.08(s)

Short-term stability studies

The optimized preparation was kept at 30 ± 3°C away from light for 1 month.

Table-10: Short-term stability studies of optimized batch

Tests parameter	**Before stability (Mean ± SD; n = 3)**	**After stability (Mean ± SD; n = 3)**
Viscosity (cps)	33±0.07	35 ±0.19
Volume of solution delivered upon eachactuation	0.14 ± 0.01	0.13 ± 1.09
Spray angle	55^0Ɵ	60^0Ɵ
Leak test	No leak	No leak afterfeeling
Film appearance	Complete, uniform, Transparent	Complete, uniform, Transparent
Film flexibility	More value	More value
Film formationtime(s) *ex vivo*	35-50 ±0.05	27-46±1.04
pH	5.4±5.0	5.5±2.2
% drug release	96.06±1.87	97.45± 4.56

There were no substantial variations in the values of all parameters when compared to prior information. Thus, stability research published in accordance with International Conference on Harmonization (ICH) rules revealed that formulation K4 (optimized formulation) has been stable in terms of among all test parameters like physicochemical experiments, performance tests, and drug content release studies.

Conclusion

The topical spray could be utilized with a push button just at the site of application without infecting the residual material. The spray could well abide towards the skin and establish a clear and transparent thin layer wherever it is adapted, in addition, to raise penetrability and uptake. As a result, the substance is effectively conveyed just at action site while allowing no hurt or discomfort.

Conflict of Interest

The all authors declare no conflict of interest or otherwise.

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eISSN 1303-5150

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