



Formulation, Optimization and *In-Vitro* Evaluation of Ketoconazole Cream

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Abstract

Variations in the physico-chemical properties of ketoconazole have been studied, following polymorphic changes caused by fusion-cooling processes or by recrystallization in solvents commonly used in the pharmaceutical industry. Changes in physico-chemical properties were measured by differential scanning calorimetry (DSC), infrared, X-rays and HPLC. Results revealed changes in the peak temperature of the different recrystallization and changes in their X-ray diffraction patterns. Infrared spectra of the samples indicated no changes in the chemical structure of ketoconazole. HPLC results indicated a decrease in the solubility except in one case. No degradation products were detected. The validated methods were applied for quantitative determination of ketoconazole in commercial and simulated emulsion formulations. Quantitative first derivative UV spectrometric determinations were made using the zero – crossing method at 257 nm, with methanol as background solvent.

Keywords: ketoconazole, recrystallization, polymorphic, physico-chemical properties.

Introduction

Balwada RP, et al., reported a Double-blind Comparison of 2% Ketoconazole and 1% Clotrimazole in the Treatment of Pityriasis Versicolor.[1] Forty adult patients of pityriasis versicolor were treated with either topical 2% ketoconazole cream (20patients) or topical 1% clotrimazole cream (20 patients). In global assessment of treatment after 2 weeks, IS (85%) out of 20 patients treated with ketoconazole cream were cured while 2 cases had considerable residual lesions. Li clotrimazole treated group, 17 (85%) out of 20 patients were cured and 3 still had considerable lesions. No significant difference was observed in response rates in the two groups. No side effects were reported in either group. Application of topical ketoconazole cream appears safe and effective for treating onychomycosis. The dosage of 150 mg once weekly for 6 months was recommended, considering both effectiveness and economy. Craig E. Poncelet et al, reported Ketoconazole delivery to human skin by topical

and oral administration in vitro and in vivo. Both in vitro and in vivo model systems were used to quantitatively measure the disposition of antifungal drug, ketoconazole within human skin after topical or oral administration. The concentration gradient of ketoconazole in human skin followed a pattern of stratum corneum > epidermis ~ dermis regardless of topical or oral drug administration. Oral administration of ketoconazole in vivo and in vitro produces similar concentrations in the three different skin layers. Topical administrations in both model systems also show similar drug concentrations within the stratum corneum and epidermis.

Materials and Methods

Ketoconazole, Propylene Glycol, Cetyl Alcohol, Steryl alcohol, Iso propyl myristate, Sorbitan Monostearate, Poly Sorbat 60, Polysorbat 80, Mannitol, Disodium EDTA, Water. **Chemicals:** Methanol, Ethanol, Tetrahydrofuran, Phosphate buffer, Chloroform, Diethyl ether, and water. UV spectroscopy Shimadzu 1601, Japan, HPLC Spectroscopy Azilent 1100 series, IR Spectroscopy FTIR 8001 Shimadzu Japan, Brookfield Viscometer Brookfield Engineering Laboratory USA, Digital pH Meter Elco Pvt. Ltd., Stability chamber Nutronic, Mumbai, Franz diffusion apparatus Franz diffusion apparatus, Laminar airflow Spectrum Pvt. Ltd., Autoclave Spectrum Pvt. Ltd., Stirrer Remi Electronics, India, Digital Weighing balance Scal Tech, Mumbai

Formulation of Ketoconazole Cream

Preparation of Solid Dispersion: Solid dispersion containing ketoconazole Mannitol in the proportion 1:1, 1:2, 1:4, 1:6, 1:8, were prepared by melting and melt solvent method.[2, 3]

Melting method Solid dispersion were prepared by melting the physical mixture ketoconazole and mannitol in a Sand bath to about 160°C, the molten mixture was immediately cooled and solidified in an ice bath with vigorous stirring the solid mass thus obtained was scrapped, crushed, pulverized and passes through 60/80 mesh. **Melt solvent method** Ketoconazole was dissolved in chloroform, and the solution was incorporated

in to the melt of Mannitol by pouring slowly into hot melt with vigorous stirring. The melt was cooled immediately and the mass was kept under vacuum in desiccators for 24 h. The solidified mass was scrapped, crushed, pulverized and passed through 60/80 mesh.

Preparation of Cream Base: The base was prepared to contain propylene glycol 10%, Cetyl alcohol 5%, Steryl alcohol 2%, Isopropyl myristate 2%, Sorbitan Monostearate 2%, Polysorbate 60, 2% polysorbate 80, 2%, disodium EDTA 0.005% in water QS – by emulsification method. Cetyl alcohol, Steryl alcohol, Isopropyl myristate, Sorbitan monostearate, were melted together at 60-70°C. The aqueous phase contains propylene glycol, polysorbate 60, polysorbate 80, disodium EDTA, and water was heated to the same temperature. The oil phase was added to water phase, slowly with constant stirring and stirring was continued until cooled. The active constituent ketoconazole was incorporated as solid dispersion into the cream base by trituration. The concentration of Ketoconazole was maintained at 2% of the cream. The creams were filled in collapsible tube and stored for 24 hrs before evaluation.

Preparation of Cream: Cream was prepared by incorporating the Solid Dispersion into cream base.[4, 5, and 6].

Evaluation of Topical Ketoconazole Cream:

Physico Chemical Evaluation. Solubility: The solubility of Ketoconazole was determined by using the solvent like water alcohol and ether etc. Statement of solubility was indicated by descriptive phrase and was intended to apply at 20° to 30°. The following table indicates the

meaning of the terms used INS statement of approximate solubility's. [7, 8] **Loss on drying:** This is employed in IP and USP. Although the loss in weight, in the sample so tested, principally is due to water small amount of other volatile material will be contribute the weight loss. The moisture balance combines both the drying process and weigh recording. It is suitable were large number of sample are handle and where a continuous records of loss I weight with time is required.[8] Determine on 1.000 g by drying in an oven at 100° C to 105° C for 3 hours. Mix and accurately weigh the substance to be tested .if the sample is in the form of large crystals, reduce the particle size it about 2 mm by quickly crushing .Tare a glass stopper ,shallow weighing bottle that has been dried for 30 minutes under the same condition to be employed in the determination. The difference between successive weights should not be more than 0.5 mg the loss on drying is calculated by formula.

$$\% \text{ LOD} = \frac{(W2 - W3)}{(W2 - W1)} \times 100$$

Where, W1 = Weight of empty weighing bottle W2 = Weight of weighing bottle + sample
W3 = Weight of weighing bottle + dried sample

Determination of pH: The pH of cream was checked by using a digital pH meter at constant temperature. Prior to this, the pH meter was calibrated by using buffer solution of pH 3.99, 7.0 and 9.2 and then the electrode was washed with demineralised water. The electrode was then directly dipped into cream formulation and constant reading was noted.

Viscosity determinations by Brookfield analog viscometer: Sample were incubated as 25° for at least 16 horus in incubator after blinking set by manually. Then run on brookfield analog viscometer using different rpm. Spindle were chosen to maintain a torque between 10% to 30%. Sample were incubated at 25° C for at least 16 hr in a incubator and then run on Brookfield viscometer using different rpm 0.5, 1, 2.5, and 5 rpm. Spindle were chosen to maintain a torque between 10% and 90% .the RV Spindle give viscosity at single immersion point in the sample .the helix path T bar spindle were rotated down and up in the sample giving viscosity at a number of points programmed over the run time .two readings taken over a period . The viscosity was calibrated using Brookfield viscosity standard 5000 (100% poly dimethyl siloxane) [9, 10, 11]

Spread ability: Spread ability of the formulation was determined by an apparatus, which was suitably modified in the laboratory and used for the study. It consists of a wooden block and provided with a pulley at one end. A rectangular ground glass plate was fixed on the wooden block. Excess of cream (about 2gm) under study was placed on this ground plate, and then the cream was sandwiched between this plate and another glass plate having the dimensions of the ground plate attached with a hook. A 300gm weight was placed on the top of the two plates for 5 minutes to expel air and to provide a uniform film of the cream between the plates. Excess of the cream was scrapped off from the edges. The top plate was then subjected to a pull of 30gm with the help of a string attached to the hook and the time (in seconds) required by the top plate to cover a distance of 10 cm was noted. The spreadability was calculated using the formula: [12, 13]

$$\text{Formula} = S = \frac{ML}{T}$$

S = Spreadability

M = Weight tied to upper glass slide l = length of glass slide T = Time taken in second

Extrudability: The apparatus used for extrudability was suitably fabricated in the laboratory. It consist of a wooden block inclined at an angle of 45 fitted with a thin long metal strip (tin) at one end, while the other end was free. The aluminum tube containing 15 gm of cream was position on inclined surfactant wooden block. 1 kg weigh was placed on free end of aluminum strip and was just touched for 30 sec. The quantity of cream extruded from each tube was noted.[13]

Table 1: Formulation of Ketoconazole cream

Ingredient	Quantity / gm % w/w											
	Batch I	Batch II					Batch III					Batch IV
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Ketoconazole	2	2	2	2	2	2	2	2	2	2	2	-
Mannitol	-	2	4	8	12	16	2	4	3	12	16	-
Prophylenglycol	10	10	10	10	10	10	10	10	10	10	10	10
Cetylalcohol	5	5	5	5	5	5	5	5	5	5	5	5
Steryl alcohol	2	2	2	2	2	2	2	2	2	2	2	2
IPM	2	2	2	2	2	2	2	2	2	2	2	2
Sorbitan monosterate	2	2	2	2	2	2	2	2	2	2	2	2
Polysorbate 60	2	2	2	2	2	2	2	2	2	2	2	2
Polysorbate 80	2	2	2	2	2	2	2	2	2	2	2	2
Disodium EDTA	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Purified water	72.99	70.99	68.99	64.99	60.99	56.99	70.99	68.99	64.99	60.99	56.99	74.99

Drug Content Uniformity: Drug content uniformity were performed according to the USP requirement for the cream formulation uniformity for content check Assay method by HPLC in the filled tube sample was taken from upper, middle and end portion and analysis by HPLC.[14]

Calculation

$$\frac{\text{Test area} \times \text{weight of STD.}}{\text{Standard area} \times 100} \times \text{Dilution factor} \times \frac{100}{\text{weight of test}} \times \text{Potency}$$

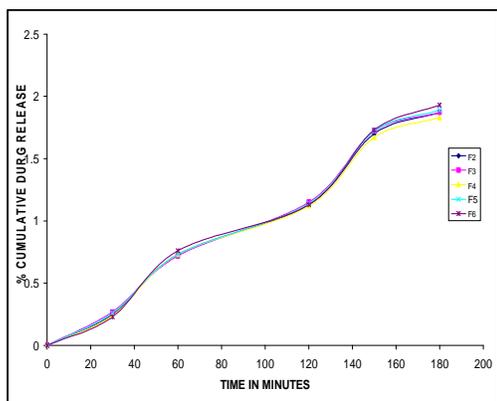
Drug Excipient Interaction Study:

Preparation of mobile phase: Ammonium acetate solution-Take 1 gm of ammonium acetate in 200 ml volumetric flask .make up volume with methanol and sonicate. Preparation of triethylamine- take 1 ml of triethylamine in 500 ml volumetric flask, and volume make up with methanol. And sonicate. Mobile phase- the mobile phase was constituted of mixture of a triethylamine in methanol (1:500 v/v) and ammonium acetate solution in water (1:200w/v), 75:25 v/v. [15, 16]

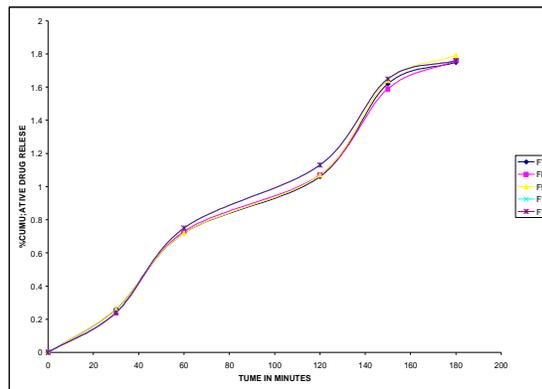
FTIR Studies: Ketoconazole were analyzed by IR spectral studies by using KBR pellet technique. In this method the Ketoconazole and KBr were mixed at the ratio 1:100. Then this mixture was pressed into pellet. The IR spectrum of was taken by using Fourier transform Ketoconazole IR (FTIR Nexus – 670).

Preparation of Ketoconazole Cream Test a) Transfer an accurately weighed quantity of cream, equivalent to 20.0 mg of Ketoconazole to a 100 ml volumetric flask and t volume mack up with methanol. mix and filter.(solution A) b) Transfer 2.0 ml of this solution to a 10 ml volumetric flask volume makeup with methanol , mix and filter.

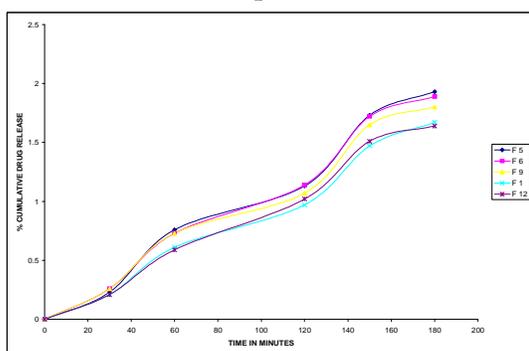
Graphs



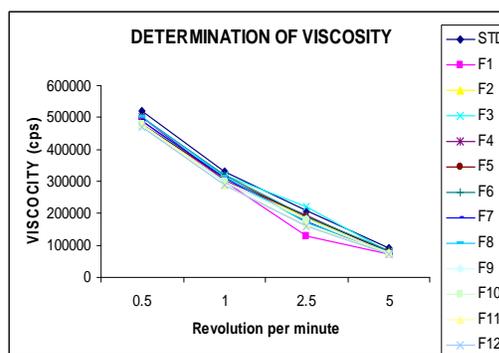
Graph: 1



Graph: 2



Graph: 3



Graph: 4

Chromatographic condition: Column Length 150 X 4.6 mm, Type Hipersil C – 18 Particle size – 5 micrometer Coloum temp. – Ambient, Detector : UV variable set at 225 nm Flow rate : 1.0 ml/min Pressure : (Maximum 300 bar) Injection volume : 25 microlitre Calculation based on : peak area

In-Vitro Drug Release Study [17,18,19] Drug Release by Franz Diffusion Cells: Medium : pH 7.4 Phosphate Buffer : Method : Franz Diffusion Cells rpm : 400 rpm. Drug release by Franz diffusion apparatus the *in-vitro* study was done by Hanson Frnaz diffusion apparatus. The apparatus consists of 6 glass tube with dissolution cell volume 7.00 ml which was opened at one end 1 gm of cream formulation equivalent to 20 mg of ketoconazole was spread uniformly on the surface of tyffryn diffusion membrane (previously soaked in water overnight) and was fixed to upper end of the tube. 7.4 pH phosphate buffer contained in beaker which was placed in water bath and maintained at $37 \pm 2^\circ\text{C}$ was continuously circulated on glass tubes. The cellophane membrane acts as a barrier between the cream phase and the phosphate buffer, the quantity of sample was withdrawn automatically from receptor fluid at different time intervals. The release of drug was estimate by using shiatsu UV visible spectrophotometer at 257 nm.

Franz Cell Membrane Diffusion Apparatus: Diffusion membrane: taffy (polysulfide) 0.45 μM , 25 MM, PALL PART #66221: UV spectrophotometer (1 cm cuvettes) Franz diffusion cell parameters: perform on 5 individual samples at a time. Receptor medium:pH 7.4 buffer medium temperature: $32^\circ\text{C} \pm 0.5^\circ\text{C}$ use the following parameters: diffusion membrane tuffryn (polysulfone) 0.45 μm , 25 mm, pall part #66221 rinse volume:2.0 ml sample collected volume0.5 ml sampling time: 30 min, 1 hour, 2hours, 4 hours and 6 hours (5 time points) stirring speed : 400 rpm dissolution cell volume: 7.0 ml

Stability studies: the storage condition used for stability studies were $25^\circ\text{C} \pm 2^\circ\text{C}$, 65% RH , $40^\circ\text{C} \pm 2^\circ\text{C}$, 75% RH. Stability studies were carried out on the selected batch of different formulation. Cream were stored at the above mentioned storage condition for three month and mentioned storage condition for three month and cream were evaluated for physical property and percentage assay of drug content.. **Stability studies:** the purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factor such as temperature, humidity, and light and to establish retest period for the drug substance or self life for the drug product and recommend storage condition. The storage condition used for stability studies were $25^\circ\text{C} \pm 2^\circ\text{C}$, 65% RH, $40^\circ\text{C} \pm 2^\circ\text{C}$, 75% RH.

Results and Discussion

The topical ketoconazole cream for fungal therapy were prepared by using solid dispersion method and evaluate by increase the solubility of insoluble drug.

Physicochemical Evaluation: Solubility in Water Practically Insoluble and in Toluene, Pet Ether, Acetone soluble, Ethanol, Methanol Freely Soluble.

Loss on Drying: was 0.026% w/w that is not more than 0.5% Specification / limit.

pH of the Cream: for all formulation was in between 5.5 to 6.0 that were in limit.

Viscosity: of all formulations were at different resolution (0.5, 1, 2.5, and 5) for F6 (500000, 320000, 190000, 84000) that were within limit.

Spreadability: for F6 were 6.13.

Extrudability: for F6 were 0.648.

Drug Content uniformity: Formulation F6 for upper, middle and lower part of tube was 98.61%, 98.68% and 98.78% respectively.

Drug Interaction Study: of drug with various excipients were conducted and found that there is no interaction with each other.

Drug excipient interaction study by IR Spectroscopy: Drug excipient interaction study was done by IR spectroscopy. The IR spectrum of pure drug Ketoconazole and Mannitol and solid dispersion of ketoconazole and Mannitol were shown. There was no interaction between the ketoconazole and Mannitol present in the ketoconazole + Mannitol solid dispersion Ketoconazole shown Peak (cm^{-1}) 1460, 1583, 1245, 1222, 1053, 1035, 1647, and 1047 while Mannitol 3402, 2948 and 3274.

Assay of Creams by U.V Spectroscopy: for F6 was 98.07%.

In vitro Drug Release: for F6 at the end of 180 min was 96.5%, while the % drug release from marketed product NIZRAL was only 94.5%.

Stability Studies: The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factor such as temperature, humidity, and light and to establish retest period for the drug substance or self life for the drug product and recommend storage condition. The storage condition used for stability studies were $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$, 65% RH, $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$, 75% RH.

Conclusion

In the present study, attempts were made to formulate solid dispersion ketoconazole creams. The result of this study demonstrates that solid dispersion represents a highly effective, carrier for topical cream preparations, where improved solubility and desired bioavailability. Solid dispersion of ketoconazole was prepared by melt method and melt solvent method. In vitro drug release study showed formulation F-6 given maximum release of drug.

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