

Available online on 15.01.2018 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

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Research Article

COMPARATIVE *IN VITRO* EVALUATION OF BRANDS OF CLOTRIMAZOLE CREAM FORMULATIONS MARKETED IN ETHIOPIA

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ABSTRACT

The aim of present work was to undertake comparative *in vitro* quality evaluation of six marketed clotrimazole cream formulations in Ethiopia with respect to physico-chemical properties like viscosity, spreadability, extrudability, pH and drug content. *In vitro* clotrimazole release from cream formulations was also studied using synthetic cellulose acetate membrane at 37 °C in a solvent containing methanol and PBS 7.4 in the ratio of 75:25 as receiver medium. The cumulative amounts of the drug released over 12 h ($\mu\text{g mm}^{-2}$) were analyzed. All clotrimazole cream formulations showed good and smooth homogeneous appearance with white color. The pH of clotrimazole cream formulations ranged from 4-7, which is a physiologically acceptable pH range and in principle devoid of any skin irritation. Clotrimazole content ranged from 90-110%, ensuring the uniformity of the drug content in all formulations. The increase in diameter of clotrimazole cream formulations following the spreadability test was found to range from 4-6 cm. Cream formulation D (Clotri-Denk) exhibited highest viscosity values than other formulations, whereas formulation E (Chinese Clotrimazole BP) showed lowest viscosity value. Cream formulation F (Mycoril) showed better extrudability and spreadability as compared to other formulations. Drug release from all formulations was slow in the first 6 hrs. After the 6th hr, steady drug release continued for formulation D and E. Fast drug release was observed in formulations A (Candid) and B (Candigen), whereas for the formulations C (Canesten), D and E, steady drug release pattern was observed after the 6th hr. It can be concluded that all clotrimazole cream formulations fulfilled the quality criteria of in-house and pharmacopeias specifications.

Key words: *In Vitro* Evaluation, Clotrimazole, Cream, Spreadability, Extrudability, Ethiopia

Article Info: Received 11 Nov, 2017; Review Completed 21 Dec, 2017; Accepted 22 Dec, 2017; Available online 15 Jan, 2018



Cite this article as:

Debebe D, Gabriel T, Brhane Y, Temesgen A, Nigatu M, Marew T, Comparative *in vitro* evaluation of brands of clotrimazole cream formulations marketed in ethiopia, Journal of Drug Delivery and Therapeutics. 2018; 8(1):17-22

DOI: <http://dx.doi.org/10.22270/jddt.v8i1.1546>

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INTRODUCTION

In vitro testing of semisolid dosage forms has become important tool and it has been suggested that there should be standard physicochemical testing parameters. In the manufacturing of semisolids, the *in vitro* release test can show changes in formulation composition and/or manufacturing processes¹.

Antifungal agents inhibit/impair membrane barrier function (Polyenes), macromolecule synthesis (Flucytosine), interfere with microtubules (Griseofulvin), or inhibit ergosterol synthesis (azole derivatives, morpholines). Azoles include two types: triazoles (Itraconazole, Voriconazole, Posaconazole) and

the imidazoles (Clotrimazole). Azoles interfere with an enzyme required for the production of ergosterol, an important component of the fungal cytoplasmic membrane^{2,3}.

Clotrimazole is a white or pale yellow, tasteless, crystalline powder which is practically insoluble in water. Clotrimazole has pH dependent solubility where solubility increases with decreasing pH due to presence of ionizable imidazole group present in its structure^{4,5}.

Clotrimazole was introduced as a topical agent in 1969. It is used for the treatment of superficial candidiasis, and in the skin infections: pityriasis versicolor and dermatophytosis. Clotrimazole may also be used

occasionally for symptomatic relief of the protozoal infection trichomoniasis when other drugs are contraindicated. It is also effective for prophylaxis in patients undergoing chemotherapy, myeloablative treatment, and transplant recipients and for patients with solid malignant neoplasms^{6,7}.

Clotrimazole is safer (especially in pregnant women) than other antifungals currently listed on the WHO Essential Drugs List. It is available without prescription in most countries and this regulatory approval status indicates that the drug is generally recognized as safe⁸.

Clotrimazole is one of the most prescribed drugs for the treatment of fungal infection in many health institutions in Ethiopia. The aim of this work was, therefore, to compare different brands of clotrimazole cream formulations available in Ethiopia.

MATERIALS AND METHODS

Chemicals and solvents

The reference/working standard Clotrimazole (99.4%) pure was received as a gift sample from FMHACA and EPHARM. Potassium dihydrogen phosphate (KH_2PO_4) (Fizmerk chemicals, India) and NaOH (Scharlau Chemie

SA, European Union) were obtained from industrial laboratory. Distilled water was used throughout the study.

Cream formulations

Six different Clotrimazole cream containing commercial formulations have been purchased from pharmacy outlets in Ethiopia as indicated in table 1, i.e. **Clotrimazole** BP 1% cream labelled to contain 14 gm of clotrimazole (SHANGHAI GENERAL PHARMACEUTICALS CO.,LTD. CHINA), **Canesten** 1% cream labelled to contain 20gm of clotrimazole (Bayer PLC., Germany), **Candid** cream labeled to contain 20gm of clotrimazole (GLENMARK PHARMACEUTICALS LTD, INDIA & MAHARASHTRA), **Mycoril** 1% cream labeled to contain 20 gm of clotrimazole (Remedica Ltd, Limassol industrial state, Limassol, Cyprus, EU), **Candigen** 1% cream labeled to contain 20 gm of clotrimazole (Galentic pharma Pvt.Ltd, India) and **Clotri-Denk** 1% cream labeled to contain 20 gm of clotrimazole (DENK PARMA GmbH & CO. KG, GERMANY).

Table 1: Description of the six clotrimazole cream formulations marketed in Ethiopia

Product	Brand name	%(w/w)	Labeled Weight (g)	Cost (Birr)	Mfg. date	Expiry date	Manufacturer's Name	Country of origin
A	Candid	1	20	24.20	Dec, 2015	Nov, 2019	GLENMARK Pvt Ltd	India
B	Candigen	1	20	18.10	Jul, 2016	Jun, 2019	Galentic Pharma Pvt.Ltd	India
C	Canesten	1	20	117.05	—	06 /2018	Bayer Plc.	Germany
D	Clotri-Denk	1	20	68.00	Sept, 2016	Aug, 2019	DenkPharma GmbH& Co.KG	Germany
E	Clotrimazole BP	1	14	12.00	May, 2016	May, 2019	Shanghai General pharmaceutical CO.,LTD	China
F	Mycoril	1	20	41.70	Nov, 16	Nov, 19	Remedica Ltd	Cyprus-EU

Methods

UV calibration curve

Stock solution was prepared by dissolving 100 mg of clotrimazole in 100 ml solvent containing methanol and PBS 7.4 in the ratio of 75:25. From this stock solution, eight different concentrations (10, 20, 30, 40, 50, 60, 70 and 80, ug/ml) were prepared. The UV absorbance readings of these solutions were measured at 270 nm using UV/Visible spectrophotometer (CECIL, 1021, 1000 series, England). Methanol was used as a blank. The values of absorbance were recorded and a standard curve was constructed by plotting absorbance against concentration. From this curve a regression line equation was obtained in MS Excel format. The absorbance versus concentration of solutions was plotted and a calibration curve with a linear regression equation of: $Y = 0.0156X - 0.0345$ (where, Y is the absorbance and X

is the concentration in ug/ml) and correlation coefficient of 0.9991 was obtained (Figure 1).

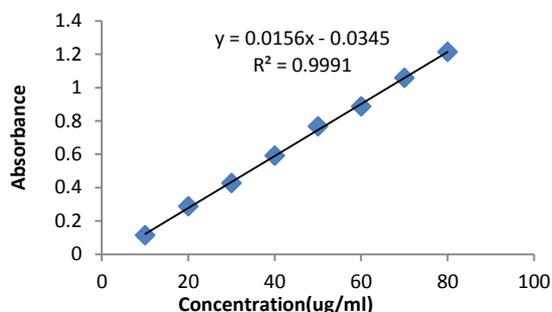


Figure 1: The UV absorption calibration curve of clotrimazole in methanol and PBS (pH 7.4) at 270 nm with 95% confidence bands for mean, ($r^2 = 0.9991$).

Physical appearance and homogeneity

The physical appearance and homogeneity of the marketed creams were tested by visual observations after the formulations were set in containers. All the marketed cream formulations were physically inspected for clarity/transparency, color, scent, texture, consistency, and homogeneity. The creams were also evaluated for the presence of any gritty particles^{7,9}.

Measurement of pH

The pH of various formulations was determined by using digital pH meter (model PH-210, HANNA instruments, Portugal) which was calibrated prior to use with standard buffered solutions of pH 4, 7 and 10. The samples were in contact with the pH electrode until the reading stabilized. The electrode was thoroughly washed between each determination to remove all traces of the previous sample. The electrode was then rinsed with DW and dried before use. The average of three readings was recorded as pH value.

Viscosity study

The measurement of viscosity of each cream formulation was determined by rotational viscometer (KINEMATICA, AG, Type Viscostar plus L, Switzerland) using different spindle numbers L3 and L4 at shear rate of 100 rpm at room temperature. The cream formulations were placed in the sample holder and the suitable spindle selected was lowered perpendicularly into the sample taking care in such a way that spindle does not touch the bottom of the container. The spindle was attached to the viscometer and then it was allowed to rotate at a defined speed at room temperature. The experiment was carried out in triplicate for each sample¹⁰.

Spreadability of formulations

The spreadability of each formulation was determined using the following technique with slight modifications: concentric circles of different radii were drawn on graph paper and a 400 cm² glass plate was fixed onto it. One gram cream formulation was placed within a circle of 2.4 cm diameter pre-marked on a glass plate. Another 400 cm² glass plate of 123g was placed gently on the cream and a standardized weight of 1Kg was allowed to rest on the upper glass plate for 3 min. The increase in the diameter due to spreading of the formulation was recorded. The spreadability (g.cm.min⁻¹) was calculated by using the formula:

$$S = m. l/t \dots\dots\dots \text{Eq. 1}$$

Where: S is spreadability, m is the weight of the upper plate and standard weight rested on it (g), l is the diameter of the spreading cream (cm), and t is the time taken (min)¹¹⁻¹³.

Extrudability of formulations

The method adopted for evaluating cream formulation for extrudability was based on the quantity in percentage of cream extruded from tube on application of certain load. The tubes were pressed to extrude the cream through 0.5 cm opening in 10 seconds and the extrudability of formulations was checked. It was then

placed between two glass slides and was clamped. Extrudability was determined by weighing the amount of cream extruded through the tip when a constant load of 1 Kg was placed on the slides. The percentage of cream extruded was calculated and grades were allotted (++++ excellent, i.e., for > 90% of cream extruded; +++ very good, i.e., for 80-89.9%; ++ good, i.e., for 70-79.9%; + fair, i.e., for 50-69.9% and 0 for poor, i.e., for < 50% of cream extruded)^{14,15}.

Drug content determinations

Hundred milligrams of each formulation was taken and dissolved in 100ml methanol and 10ml aliquot was diluted in 25 ml volumetric flask. The volumetric flask containing the cream solution was vigorously mixed in order to get complete solubility of the formulation drug. This solution was filtered and drug content was then estimated spectrophotometrically at 270 nm using methanol as blank¹⁶. Clotrimazole content was computed as the ratio of actual amount of clotrimazole in the sample to theoretical amount of clotrimazole in the sample, expressed as a percentage¹⁷.

In vitro release study of marketed clotrimazole cream preparations

In vitro release study was conducted using a glass tube with 100 mm height, 12.9 mm outer diameter and 12.1 mm inner diameter (effective release area of approximately 115 mm²) filled with 1 g of the cream, covered with a cellulose acetate membrane (0.45 μm) (which was previously soaked in phosphate buffer of pH 7.4 for about 12 hr), sealed with a rubber band (to make water tight), and inverted under the surface^{18,19} of 500 ml of phosphate buffer of pH 7.4 at 37 ± 0.5 °C in a United States Pharmacopeia (USP) dissolution tester apparatus II, with stirring speed of 50 rpm¹⁰. Samples of 5 ml was withdrawn at 5, 15, 30, 60, 120, 180, 240, 300, 360, 480, 600 and 720 min and replaced with an equivalent amount of fresh receptor medium²⁰. The cumulative percent drug release was determined in with validated UV spectrophotometer at 270 nm and the amount of clotrimazole released from the cream formulations was calculated based on the established standard calibration curve. Each sample was tested six times and mean values taken²¹⁻²⁴.

RESULTS AND DISCUSSIONS

Physicochemical evaluation of the cream formulations

Physical appearance

Color of all cream formulations was examined against black background. From the results, it is clearly evident that all the cream formulations showed good and smooth homogeneous appearance with white color.

Measurement of pH

The pH values of all marketed formulations ranged from 4.0 to 7.0, among the result which are considered there is no significant difference of the pH value. However, for formulation A, the pH was altered and in neutral range so it is not affecting the stability of the preparations as well as the skin. Thus, it can be assumed that the

formulation is acceptable for topical applications. The other clotrimazole cream formulations, namely Candid (B), Candigen (C) and Canesten (D) and F cream formulations had similar pH range but Formulation E lowest pH value as compared the other.

Viscosity

Viscosity plays a vital role in the dispensing and formulation of topical formulations. It is related to mechanical and physical properties such as spreadability, consistency and hardness of the preparation which in turn are related to ease of product removal from container, ease of application on the target surface and product feel on the application site²⁵.

As shown in Table 2, formulations B, C and D have nearly similar viscosity ranges and formulations A and F too are with similar range but with lower viscosity values from the previous three formulations. However Formulation E was the lowest viscosity value from the other all formulation.

Extrudability

The extrusion of every formulation from the tube is an important during its application and in patient acceptance. Results from extrudability indicated that cream formulations B, C and D had better extrudability than formulation A, E and F when they were extruded from aluminum collapsible tubes. Due to these results, formulations A, E and F had not extruded quickly from the tubes, whereas, formulations B, C and D extruded quickly from aluminum collapsible tube.

Spreadability

Spreadability is an essential attribute of topical dosage forms that can affect patient acceptance in terms of ease of application and clinical efficacy. One of the essential criteria for cream is that it should possess good spreadability. Spreadability is an important factor in therapy and it is shown as index of ease of application. The efficacy of topical therapy depends on the patient spreading the formulation in an even layer to deliver a standard dose. The optimum consistency of such a formulation helps ensure that a suitable dose is applied or delivered to the target site. This is particularly

important with formulations of potent drugs. A reduced dose would not deliver the desired effect, and an excessive dose may lead to undesirable side effects. The delivery of the correct dose of the drug depends highly on the spreadability of the formulation²⁶. The important factors to consider, during evaluating the spreadability of a formulation, include the rate and time of shear produced upon smearing and the temperature of the target site. The parallel-plate method is the most widely used method for determining and quantifying the spreadability of semisolid preparation. It provides accurate, reproducible, and statistically relevant data. The advantages of the method are simplicity and relative lack of expense. On the other hand, the method is not very precise and sensitive, and data which it generates must be manually interpreted and presented^{11, 26}.

Spreadability is commonly measured by the sample spread diameter or area that is formed upon application of the weight over the sample for a predetermined time period. Spreading of topically applied products was reported to affect drug delivery as reflected by dermatopharmacokinetic outcome such as drug concentration in the stratum corneum (SC). Formulations with higher spreadability values allow ease of application and thereby increased surface area available for drug permeation. A good cream takes less time to spread and will have high spreadability^{5, 27, 28}.

Results of spreadability as shown in Table 3.1 showed that cream F gave better spreadability from other clotrimazole cream but formulation C gave lowest spreadability value from the other formulation. The remaining formulation which is medium viscosity value as compare to the other above two formulations.

Drug content

The clotrimazole content was found to be in the range of 90%-110% in the cream formulations. Therefore, it may be assumed that these formulations were applicable for topical application. Formulation A had highest and formulation E had lowest drug content value from the results obtained. The rank order of drug content was: formulation A > B > D > C > F > E.

Table 2: Physico-chemical and drug content evaluation of the six clotrimazole cream formulations marketed in Ethiopia

Brand name	Labeled weight (g)	Net weight (g) (Mean±SD)	Physical appearance	pH (Mean±SD)	Viscosity (cP) at 100 rpm (Mean±SD)	Extrudability	Spreadability (g.Cm/min) (Mean±SD)	Drug Content (%) (Mean±SD)
Candid	20	24.06±0.01	White	6.68±0.09	8114±23.6	+	1773±93	108.52±0.04
Candigen	20	23.82±0.06	White	6.32±0.1	8153±11.4	+++	2146±95	106.16±1.83
Canesten	20	24.84±0.03	White	6.09±0.1	8152±15.3	+++	1304±65	95.67±0.03
Clotri-Denk	20	23.73±0.01	White	6.00±0.07	8157±27.73	+++	1497±84	100.38±0.99
Clotrimazol	14	17.24±0	White	4.45±0.21	7661±127.6	++	1398±98	90.82±0.05
Mycoril	20	24.55±0.03	White	5.94±0.18	8050±31.2	++	3057±1543	91.47±1.72

In vitro release study of marketed clotrimazole cream preparations

In vitro dissolution methods are developed to evaluate the potential *in vivo* performance of a semisolid dosage forms, and as quality control tests demonstrating the appropriate performance of drugs products. In recent years, the convergence of the increased understanding of the physiological environment and processes of absorption, critical deconstruction of the mechanisms of release from formulations, and improved computational tools has led to a more sophisticated discussion of the role of dissolution testing in drug product design and control. It is clear that meaningful results and interpretation of dissolution data can be achieved only when the biopharmaceutical and physical properties of the drug products are well understood, and that test methods are properly established through studies during formulation and manufacturing process design and clinical development¹⁸.

It is expected that drug release might be influenced by cream viscosity. As the viscosity of the cream increased, the release of the drug was expected to be slower. Complete drug release (100%) was not achieved at the 12th hr for all marketed formulation. Cumulative amount of drug released in dissolution medium showed extremely significant difference ($P \leq 0.0005$) which could be because of the differences in surfactant concentration. This effect may be strengthened by the ability of these emulsifying agents to lower the interfacial tension between oily and aqueous layer in the dispersion medium, increasing the hydrophilicity of the cream which in turn increases penetration of dissolution medium into the clotrimazole structure and then increasing the amount of drug released¹⁸.

Drug release from all formulations was slow in the first 6 hrs. After the 6th hr, steady drug release continued for formulation D and E. Fast drug release was observed in formulations A (Candid) and B (Candigen), whereas for the formulations C (Canesten), D and E, steady drug release pattern was observed after the 6th hr.

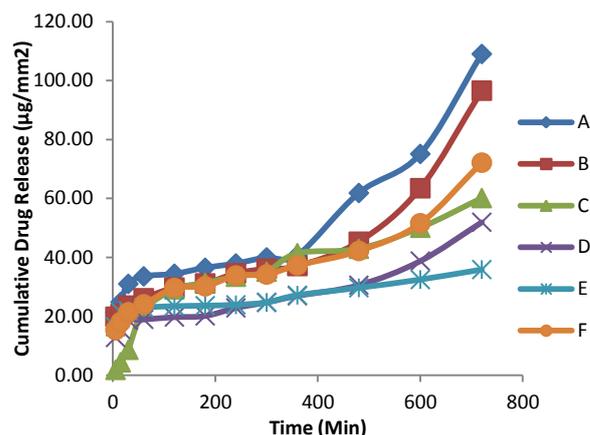


Figure 2: *In vitro* release evaluation of six clotrimazole cream formulations marketed in Ethiopia

CONCLUSIONS

The study results indicated that marketed clotrimazole creams included in this study seem to have showed good and smooth homogeneous appearance of white color without any gritty particles. Cream formulation B, C and D showed better extrudability as compared to the other formulations. Cream formulation D exhibited higher viscosity values than other formulations, whereas formulation E showed lowest viscosity value. Fast drug release was observed in formulation A but formulation E showed slowest drug release. Finally, it can be concluded that all clotrimazole cream formulations fulfilled the quality criteria of in-house and pharmacopeias specifications.

ACKNOWLEDGEMENTS

The authors are grateful for the opportunity provided by Addis Ababa University. Food, Medicine and Health Care Administration and Control Authority (FMHACA) and Ethiopian Pharmaceutical Manufacturing (EPHARM SH CO) are acknowledged for providing us clotrimazole reference/working standard. However, this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Abbreviations

BP	British Pharmacopeia
CYP	Cytochrome P450
DW	Distilled Water
EPHARM SH CO	Ethiopian Pharmaceutical Manufacturing
FMHACA	Food, Medicine and Health Care Administration and Control Authority
MPO	Myeloperoxidase
PBS	Phosphate buffered solution
USA	United State of America
USP	United States Pharmacopeia
USP-NF	United States Pharmacopoeia-National Formulary
UV	Ultraviolet
WHO	World Health Organization

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