

Deanship of Graduate Studies  
Al-Quds University

Formulation and Analysis of  
Terbinafine Cream

Ayman Abdul-Majeed Kaddoumi  
M.Sc. Thesis  
Jerusalem- Palestine  
2005

Formulation and Analysis of  
Terbinafine Cream

By

Ayman Abdul-Majeed Kaddoumi

B.Sc.: Chemistry, Birzeit University, Palestine

Supervisor: Dr. Ibrahim Kayali

Co-Supervisor: Dr. Magdi Dakiky

A thesis submitted in partial fulfillment of requirements for the degree of  
master of applied and industrial technology

Department of Science and Technology

Al-Quds University

April 2005

Applied & Industrial Technology  
Department of Science Technology  
Deanship of Graduate Studies

Formulation and Analysis of  
Terbinafine Cream

By

Student Name: Ayman Abdul-Majeed Kaddoumi

Registration: No.: 9810982

Supervisor: Dr. Ibrahim Kayali

Co-Supervisor: Dr. Magdi Dakiky

Master thesis submitted accepted, Date: .....

The name and signatures of the examining committee members are as:

1-..... Head of Committee      Signature.....

2-.....Internal Examiner      Signature.....

3-.....External Examiner      Signature.....

Al-Quds University

2005

## DECLARATION

I certify that this thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledged, and that this (or any part of the same) has not been submitted for a higher degree to any university or institution.

Signed:.....

Ayman Abdul-Majeed Kaddoumi

Date:.....

## ACKNOWLEDGMENT

I would like to thank and show my appreciation to my supervisor; Dr. Ibrahim Kayali for his genuine guidance and invaluable remarks.

My true thanks also to the co-supervisor; Dr. Majdi Dakiky for his major and real assistance.

I appreciate the tireless effort of Al-Quds University that upgrades and promotes the students to develop the industry in the Palestinian companies.

Special thanks for Pharmacare-PLC for their financial and technical support in addition to the invaluable assistance to achieve this research, represented by Dr. Subhi Khoury, Dr. Bassim Khoury, Mr. Zuhdi Sawalhi, quality control & R&D staff.

Profound thanks for my dear daughter Majd for typing and general technical writing applied in the research.

Last but not the least, I am grateful to my soul mate; my wife, who inspired and encouraged me to explore the best in me. I thank her for dedication and patience.

## ABSTRACT

Terbinafine HCl, belonging to the allyl amine family, was formulated in a cream dosage forms to be equivalent quantitatively and qualitatively to the brand product; Lamisil cream. manufactured by Novartis, Pharma AG Basle, Switzerland. The active material is Terbinafine HCl. It is a broad-spectrum Antifungal agent. The assay method was developed and validated for accuracy, precision, selectivity, linearity, and ruggedness. The stability of the selected formula was studied. The prepared cream was compared with Lamisil cream penetration. Terbinafine HCl is slightly soluble in water. Triangular phase diagram was developed to point the Terbinafine HCl solubility regions in the diagram. The components of the diagram are water, terbinafine HCl, and tween 80.

Several trials were carried out to develop a stable, effective, pure, and safe cream. The process concentrated on two different paths; first: selecting the best kind and quantity of excipients specially the emulsifying agents and the preservatives. Second: changing in the mixing techniques. It is important to produce a cream having similar properties like the brand cream, such as pharmaceutical, chemical, microbiological, and physical properties. Many emulsifiers were tried separately or joint, such as Glycerol mono stearate (SE) Eumulgin B2 and cetostearyl alcohol so as to examine all their possible uses. At the same time, many trials were carried out to select the most effective and safest preservative(s). Change in the step-by-step procedure such as changing speed of mixing as to be 20, 80, 120, and 180 °c or changing the temperature before addition of the active material to the cream. No sources were available for Terbinafine HCl analysis in the pharmacopias (USP, BP &EP), therefore, it was necessary and essential to develop assay method for raw material and finished product. HPLC method was developed using RP18 as a stationary phase, methanol and acetate buffer (95: 5 respectively) were used as the mobile phase. The method was validated for the purpose of acquiring a high degree of assurance i.e. the applied/adopted method worked consistently to determine the product assay.

Five main validation parameters were examined in this thesis:

- Accuracy was determined by calculating the percent of recovery for three batches. Each batch was analyzed three times, where; five injections were carried out for each batch. The average values were determined and compared with the true value.
- The Precision was determined by calculating the variation coefficient for three different batches, each batch was analyzed three times, where, five injections were carried out for each batch.

- Selectivity was checked to ensure the absence of interference between the active and the excipients. It was tested by analyzing the cream with placebo sample.
- Ruggedness was also checked to verify that the method is working under a variety of normal testing conditions. Ruggedness was determined by keeping the prepared samples at different times (15 & 180 min), and at different temperatures (20 °c & 40 °c) for three batches, it was also determined by using two different columns having the same properties. The accuracy and precision were determined for three batches.
- Linearity was also tested to ensure a proportional between the analytical results. It was carried out by analyzing 5 different concentrations (50%, 75%, 100%, 125%, and 150% of method concentration) and plotting the results (in excel sheet) to calculate the correlation coefficient of the regression line.

The selected formula was checked for stability. An accelerated stability study method was applied. The cream was studied for a period of six months. The cream was kept under a temperature of 40 °c and a humidity of 75% for 1,2,3 and six months followed by complete analysis to ensure no chemical, physical, or microbiological changes may occur.

Penetration of the cream was compared with that for the brand product 'Lamisil' by using the Franz diffusion cell apparatus. It was found that the two formulas have approximately the same penetration profile.

Solubility of Terbinafine HCl was determined by addition of Terbinafine to a mixture of water and Tween 80. Many mixtures were prepared and plotted into a ternary phase. Wt of each sample was about 2.5 gm in 5 ml test tube. The test tube containing the final mixture was followed by proper mixing, sonication, and storage at 25 °c for 24 hours. The sample was allowed to settle and its clarity was observed. A mixture that showed cloudiness or had a precipitate was considered "two phases". At the same time regions of single-phase systems, liquid crystal systems and gels systems were determined for the ternary system used. After plotting the phase diagram, the solubility boundaries were determined for Terbinafine in the mentioned system. At the same time, regions of single-phase systems of micelles and inverse micelles in addition to hexagonal liquid crystal systems were determined for the ternary system used.

A good formula for Terbinafine cream was produced. It was equivalent to the Lamisil cream according to the chemical, physical, microbiological, and pharmaceutical properties. The selection of emulsifying agents was a mixture of Glycerel Mono Stearate (SE), Eumulgin B2 and Cetostearyl alcohol. The selected preservative was

chloro-cresol, which was active against the organisms mentioned in the USP. The other preservatives failed to do that or caused problems for the analysis. It was also found that the best speed of mixing was between 80 and 120 rpm to avoid air bubbles or phases separation. Addition of Terbinafine at 50, 60, or 70 °c did not show any difference, which means that Terbinafine HCl was stable under high temperatures 50 °c - 70 °c.

The developed method of analysis was found valid for all parameters tested.

The selected formula was stable under accelerated stability study at condition of 40 °c & 75 %RH, it can be given an expiry date of 2 years. The solubility of Terbinafine HCl was found is slightly soluble in water and Tween 80 (i.e. < 1 gm/100 solvent). The drawn phase diagram detected the soluble area of Terbinafine HCl in the mixtures that form the phase diagram. Simultaneously, the phase diagram showed the other areas of hexagonal liquid crystal, micelles, and inverse micelles in addition to the two phases regions.

## CONTENTS

1- Introduction	1
1-1- Formulation	1
1-2- Therapeutic Action	4
1-3- Analysis	6
1-4- Analytical Validation	7
1-5- Stability Study	10
1-6- Penetration	12
1-7- Phase Diagram	15
1-8- Specifications	16
1-8- Study Objectives	17
2- Experimental	19
2-1- Analysis of F. P & Determination of Brand Product Specification	19
2-1-1- Materials and consumables	19
2-1-2- Equipment	19
2-1-3- Tests:	20
2-2- Raw material analysis – (Terbinafine HCl)	25
2-3- Manufacturing Trials	28
2-3-1- O/W Method	29
2-3-1-1- Selection of Ingredients	30
2-3-1-2- Selection of Procedure	36
2-4- Analytical Validation	37
2-4-1- Trial Preparation	37
2-4-4- Parameters to be tested	37
2-4-5- Acceptance Criteria	40
2-4-6- Validation Report	40
2-5- Stability Study	40
2-5-1- Kind of Stability Study	40
2-5-2- Trial Preparation	40
2-5-3- Analysis Methods	40
2-5-4- Reference: Home method	40
2-5-5- Qty of samples	41
2-5-6- Tests to be monitored	41
2-5-7- Procedure	41
2-5-8- Stability Report	42
2-6- Penetration Test	42
2-7- Phase Diagram Preparation	43

2-7-1- Materials	43
2-7-2- Equipment & tools	43
2-7-3- Procedure	43
2-7-3-1-Preparation of water and tween 80 mixtures	43
2-7-3-2-qty of Terbinafine added to Tween 80 and water mixtures	44
3- Data, Calculations & Results	46
3-1- Analysis Results of Raw material (Terbinafine HCl)	46
3-1-1-Data	46
3-1-2-Calculation	46
3-1-3-Results	46
3-2-Brand product analysis (Lamisil Cream)	47
3-2-1-Result	47
3-3-Trials Results	50
3-3-1-Selection of Excipients	50
3-3-1-1-Selection of emulsifying agents	51
3-3-2- Selection of Preservative	59
3-3-3- Selection mixing procedure	62
3-3-3-1 Selection of Mixing Speed	61
3-3-3-2- Selection of Temperature for Active material Addition	64
3-3-4-Summary for Trials Results	65
3-4- Validation Results	67
3-4-1- Selectivity	67
3-4-2- Accuracy & Precision	69
3-4-3- Linearity	72
3-4-4- Ruggedness	74
3-5- Stability Study	82
3-5-1- Data	82
3-5-2- Results	83
3-5-3- Final Results	84
3-7- Penetration result	87
3-6- Phase Diagram Preparation	88
4- Conclusion	95
References	96
Abstract (in Arabic)	98

## List of Tables

<u>Table</u>	<u>Page</u>
Table [1-1]: Suggested excipients that are necessary for Terbinafine cream manufacturing	3
Table [1-2]: Summary for the most important tests should the cream pass	4
Table [1-3]: Storage condition for long term and accelerated stability study	11
Table [2-1]: Formulation of Terbinafine cream using GMS (SE) as emulsifier	30
Table [2-2]: Formulation of Terbinafine cream using Eumulgin B2 as emulsifier	31
Table [2-3]: Formulation of Terbinafine cream using cetostearyl alcohol as emulsifier	31
Table [2-4]: Formulation of Terbinafine cream using Cetostearyl alcohol and GMS (SE) as emulsifiers	32
Table [2-5]: Formulation of Terbinafine cream using Cetostearyl Alcohol and Eumulgin B2 as emulsifiers	32
Table [2-6]: Formulation of Terbinafine cream using GMS (SE) and Eumulgin B2 as emulsifiers	33
Table [2-7] Formulation of Terbinafine cream using Cetostearyl Alcohol, GMS (SE) and Eumulgin B2 as emulsifiers	33
Table [2-8]: Formulation of Terbinafine cream using Cetostearyl Alcohol, GMS (SE) and Eumulgin B2 as emulsifiers (change qty)	34
Table [2-9]: Selection of phenoxy ethanol as preservative for Terbinafine cream	35
Table [2-10]: Selection of chloro-m-cresol as preservative for Terbinafine cream	35
Table [2-11]: Selection of MP & PP as preservatives for Terbinafine cream	36
Table [2-14]: results of analysis format for stability study with tests limits	41
Table [2-15]: Preparation of water: tween 80 mixtures	44
Table [2-16]: Quantity of water needed to titrate ethanol-tween 80 mixtures	45
Table [3-1]: Data for Terbinafine HCl analysis as raw material	46

<u>Table</u>	<u>Page</u>
Table [3-2]: Result of Terbinafine HCl analysis as raw material	47
Table [3-3]: Analytical data for Lamisil cream (brand)	48
Table [3-4]: Analytical data for trials 1-10 analyzed by HPLC	48
Table [3-5]: Analytical data for trials 11-18 analyzed by HPLC	48
Table [3-6]: Analytical data for trial # 12 analyzed by HPLC for content uniformity test	49
Table [3-7]: Analytical data for trial # 13 analyzed by HPLC for content uniformity test	49
Table [3-8]: Analytical data for trial # 14 analyzed by HPLC for content uniformity test	49
Table [3-9]: Analytical data for trial # 15 analyzed by HPLC for content uniformity test	49
Table [3-10]: Analytical data for trials 11-18 analyzed by HPLC	50
Table [3-11]: Analysis results for Terbinafine cream using GMS (SE) as an emulsifying agent	51
Table [3-12]: Analysis results for Terbinafine cream using Eumulgin B2 as an emulsifying agent	52
Table [3-13]: Analysis results for Terbinafine cream using cetostearyl alcohol as an emulsifying agent	53
Table [3-14]: Analysis results for Terbinafine cream using GMS (SE) & cetostearyl alcohol as emulsifying agents	54
Table [3-15]: Analysis results for Terbinafine cream using Eumulgin B2 & cetostearyl alcohol as emulsifying agents	55
Table [3-16]: Analysis results for Terbinafine cream using GMS (SE) & Eumulgin B2 as emulsifying agents	56
Table [3-17]: Analysis results for Terbinafine cream using GMS (SE), Eumulgin B2 & Cetostearyl alcohol as emulsifying agents	57
Table: [3-18]: Analysis results for Terbinafine cream using GMS (SE), Eumulgin B2 & Cetostearyl alcohol as emulsifying agents (different qty)	58
Table [3-19]: Analysis results for Terbinafine cream using phenoxy ethanol, as a preservative	59
Table [3-20]: Analysis results for Terbinafine cream using chloro-m-cresol as a preservative	60
Table [3-21]: Analysis results for Terbinafine cream using MP & PP as preservatives	61
Table [3-22]: Analysis results for Terbinafine cream at mixing speed 20 rpm	62

<u>Table</u>	<u>Page</u>
Table [3-23]: Analysis results for Terbinafine cream at mixing speed 80 rpm	62
Table [3-24]: Analysis results for Terbinafine cream at mixing speed 120 rpm	63
Table [3-25]: Analysis results for Terbinafine cream at mixing speed 180 rpm	63
Table [3-26]: Analysis results for Terbinafine cream while active is added at different temperatures	64
Table [3-27]: Summary for all trials including the tests, results and reason for accepting or refusing the trial	65
Table [3-28]: Data for HPLC peak areas as a result of stability study for three batches through 6 months	82
Table [3-29]: stability study results of analysis for batch # 1	83
Table [3-30]: stability study results of analysis for batch # 2	84
Table [3-31]: stability study results of analysis for batch # 3	85
Table [3-28]: stability study HPLC peak areas data for three batches through 6 months	87

## List of Figures

<u>Figure/Scheme</u>	<u>Page</u>
Figure [1-1]: Terbinafine chemical structure	05
Figure [1-2]: Skin layers	13
Figure [1-3]: Stratum corneum layer composition	14
Figure [1-4]: France Diffusion Cell	15
Figure [3-1]: UV spectrum for Terbinafine HCl as standard RM	47
Figure [3-2]: UV spectrum for Terbinafine HCl as sample RM	47
Figure [3-3]: Selectivity results for sample, standard and placebo solutions	68
Figure [3-4]: HPLC Analysis results for Terbinafine stored under stress solutions	86
Figure [3-5]: Penetration of thesis cream and Lamisil cream using	87
Figure [3-6]: Phase diagram of Water- Terbinafine - and Tween 80	94

## LIST OF ABBREVIATIONS

w/o	Water in oil
o/w	Oil in water
cfu	Count formed units
Staphylococcus a.	Staphylococcus aureus
Pseudomonas a.	Pseudomonas aeruginosa
GMP	Good Manufacturing Practice
wt	Weight
USP	United State Pharmacopoeia
HPLC	High Performance Liquid Chromatography
UV	Ultra Violet
nm	Nano meter
st	Standard
sa	Sample
MP	Methyl Paraben
PP	Propyl Paraben
GMS (SE)	Glycerel Mono Stearate (self emulsifier)
TEA	Tr1-Ethanol-Amine
Rt	Retention Time
KF	Karl Fisher
Coeff.	Coefficient

# 1- INTRODUCTION

## 1-1 Formulation

Creams are semisolids emulsion systems. Like all other dosage forms, creams must be formulated, manufactured and packaged in a manner to ensure that they meet general standards of bioavailability, physical stability, chemical stability and freedom from contamination and elegance. These factors must essentially remain invariant and reproducible from batch to batch (1).

Formulation of drugs into transdermal dosage form requires interpretation and application of wide range of information from several study areas. The physical, chemical and pharmaceutical properties of drugs and additives need to be understood, the factors influencing drug absorption and the requirements of the disease also have to be taken into account when identifying potential delivery routes (2). The formulation requires evaluation of wide information such as the selected materials, analysis, equipment, in addition to the economic aspects.

Formulation of a generic new product requires following some important steps that are necessary to produce the required product. These steps can be summarized as the following:

- 1- Data collection for the brand product
- 2- Data collection for the active material used
- 3- Data collection for the additives may be used
- 4- Equipment selection
- 5- Selection of additives and preparation of suggested master formulas
- 6- Manufacturing of finished product (trials)
- 7- Development of analysis methods for the active material, brand product and the generic drug product.
- 8- Validation of analysis method (assay)
- 9- Stability study (accelerated and long term)
- 10- Improvement on the selected master formula (if necessary).

Data collection of the active material is a very important step. It includes the chemical, physical, pharmaceutical and general properties of substance. Effective information may lead to produce a stable, effective and safe dosage form. Data collection includes but is not limited to the following:

Organoleptic properties (color, odor, appearance...)

Particle size

Solubility characteristic in many solvents

pH and temperature stability profiles

Additives interactions

Manufacturer of the active material

Material cost and availability.

Data collection of the brand product is also a very vital step. It includes but is not limited to the following the chemical, physical and pharmaceutical properties of the product such as:

pH

Penetration

Smoothness

Stability

Viscosity

Color

Odor

Local irritation

Application site

Formulation additives

Analysis and analytical validation, and

Safety study.

The most time consumed for creating a new formula is selecting the product excipients and their specifications. Data collection should be done carefully. The following are some factors that must be taken into consideration before materials selection

- 1- Material effectiveness
- 2- material cost
- 3- ease of using
- 4- toxicity
- 5- ease of analysis
- 6- availability
- 7- purity, odor, color
- 8- incompatibility
- 9- particle size, and
- 10- stability.

There are many available excipients sharing the same function that can be used for Terbinafine cream formulation. Many trials are to be performed to select the appropriate materials. The following is a summary of these materials described in Table [1-1].

Table [1-1] Suggested excipients that are necessary for Terbinafine cream manufacturing

Function	Materials
Emulsifying agent	Glycerel mono stearate (SE), Eumulgin B2
Auxiliary Emulsifying agent	Cetostearyl alcohol
Emollient	Liquid paraffin, lanolin
Solvent	Alcohol, water
Alkalizing agent	TEA, NaOH,
Acidifying agent	Citric acid, HCl (0.2M)
Preservative	Mp, pp, chlorocresol, phenoxy ethanol
Base	Vaseline white
Continuous phase	Water

Emulsifying agent selection is restricted to the cream stability and the brand product viscosity. The alkalizing agent/acidifying agent type and quantity are restricted to the brand product pH. The base quantity is also confined to the viscosity and the cream stability. Preservative selection is critical to many factors such as; pH (cream), preservative concentration, solubility, sensitivity to the required micro-organisms, toxicity and environmental factors. All materials are restricted to their cost.

The best formula for producing a cream is the one that pass all the tests related to the semi-solids preparations. Table [1-2] shows the most important characteristics.

Equipment used in the manufacturing , processing, packing or holding of a drug product shall be of appropriate design and adequate size. Selection of equipment is an important subject. Many considerations should be taken before their selection such as criteria, availability of spares, maintenance, environmental issues, construction material and design, availability of process control, and the comprehensive cost (3).

For creams, there are two applied techniques, oil/water or water/oil depending on many factors (4) such as:

- a- The order of mixing may determine what type of emulsion forms.
- b- The nature of the emulsifier. If the emulsifier is more oil soluble, a W/O is more likely whereas a more water soluble one makes O/W emulsions more likely.
- c- The volume ratio of the oil and water.

- d- The phase in which you initially dissolve the emulsifying agent.
- e- The temperature of the system. if you increase the temperature on an emulsion made with nonionic surfactants, if you start with an O/W emulsion, it may well invert to a W/O.
- f- The amount of electrolyte, increased electrolyte decreases repulsion and makes the head groups less hydrophilic.

Table [1-2] Summary for the most important tests should the cream pass

Tests	Limit
Physical stability	No separation after centrifusion
Tackiness	Untacky cream after application
Greasiness	No Grease remaining on skin after application
Viscosity	$\pm 10\%$ of brand product viscosity
Appearance of applied film	Transparent after application
Skin allergies (irritation)	No irritation after application
Assay	90.0 – 110.0 % of labeled amount
pH	$\pm 0.2$ of brand product pH
Smoothness	Smooth on skin after application
Microbial contamination	Total count < 1000 cfu/g, free from Staphylococcus a., and Pseudomonas a.)
Organoleptic properties	Homogeneous white cream, free from air bubbles, the smell should be acceptable

Many trials may be carried out, The following are some ideas that aid the formulator to achieve his/her purpose in the shortest time

- Changing type and quantity of the emulsifying agent.
- Using more than emulsifying agent at the same time
- Addition of auxiliary emulsifying agent
- Changing type of preservative and using more than one preservative at the same time.
- Changing speed of mixing for oil phase with aqueous phase.
- Addition of active material at different temperatures (50 °c -70 c°).

## 1-2- Therapeutic Action

Terbinafine is the first member of a new class of antifungal agents, the allylamines, became available for the topical use (5). It composed of naphthalene group, allyl amine group, in addition to a triple bond between two carbon atoms as shown in figure [1-1].

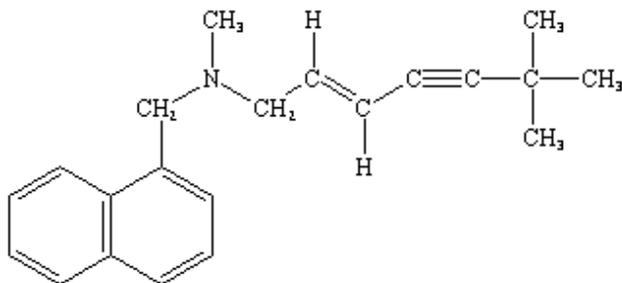


Figure [1-1] Chemical structure for Terbinafine

It has rapid responses in dermatophyte (tinea) infections, higher than the older topical antifungals such as nystatin and natamycin. The cure needs shorter period of treatment. It is very lipophilic, remaining in the stratum corneum for seven days after application, so it has advantages over many antifungals such as Imidazoles (Ticonazole, Econazole, Ketoconazole, Miconazole, Clotrimazole), Morphines Ciclopiroxolamine, and Thiocarbamate (5,6,7).

The antifungals agents act by blocking of some steps needed to build the fungus membrane (7). Terbinafine acts by inhibiting the fungal enzyme, squalene epoxidase that is necessary to build the cell membrane by stop making of the ergosterol (the main component of the cell wall). The accumulation of squalene within the cell is toxic to the organism (5,6,8).

Terbinafine is a broad-spectrum antifungal. It is used to treat fungal infections of the nails. In addition to the treating of dermatophyte that cause skin infections known as 'Ringworm', it is also used to treat yeasts infections of the skin which are another type of fungus. A common example of the yeasts is the candida albicans. Terbinafine has fungicidal effect by killing dermatophytes and killing or stopping the yeast growth to be as fungicidal and fungistatic at the same time depending on the species, for example; it is fungistatic against candida albicans (6,9,10, 11).

Antifungals agents do not produce instant results. So Terbinafine (as cream) does not act full effect before 2 – 6 weeks but the primary result can be taken after a week of application. The cream is used for ringworm of the foot (athlete's foot), ringworm of the groin (jock itch), and ringworm of the body (tinea corporis), tinea versicolor (sun fungus) and yeast infection of the skin (cutaneous candidiasis but for fungus of the toenail or finger nail the tablets are preferred (12).

Terbinafine can be used for onychomycosis. Onychomycosis refers to fungal infection of the nail caused by dermatophytes, yeasts, and nondermatophytic moulds. It accounts

for one third of fungal skin infections; at the same time it accounts for one-half of all nail disease. (13,14).

Terbinafine cream is applied gently twice daily for one week for Tinea Pedis treatment, one to two weeks for Tinea Corporis and Cutaneous Candidiasis and for two weeks for Pityriasis Versicolor treatment. Before applying of the cream, the affected area should be cleaned and dried well. It is possible to cover the infected area by using a suitable gauze after applying the cream specially at bed time. The hands should be washed well after using the cream. The cream is kept away from eyes, nose, mouth and other mucous membranes. (15,16). Another source of information (5) considers applying the cream once daily is enough, because the drug appears in high concentrations in the skin, and the half life is about 16-17 hours.

Terbinafine brand product is called Lamisil supplied as tablets, solution and cream manufactured by Novartis company.

### 1-3-Analysis

There are many tests for analyzing the drug. The type of the test applied depends on the drug dosage form. The aim of the analysis is to check GMP (Good Manufacturing Practice) compliance of the drug ie. 'effectiveness, purity and safety'

Analysis of creams , like other semisolids preparation, requires many necessary tests to ensure that the drug is safe, effective and pure. The following are the most important tests:

- Identification
- Color
- Assay & degradation
- Viscosity
- Content uniformity
- Stability of phases
- pH
- Grittiness
- Greasiness
- Skin allergies
- Tackiness
- Microbial contamination cfu/g (Total count, Staphylococcus a., and Pseudomonas a.).

Most of the modern analytical methods are mainly depending on two principles; chromatography and spectroscopy. The first is for separation aims but the second is for the detection, so they are used together in many instruments (17).

Many analytical methods are used to determine the assay of the active material (Terbinafine as in our case). The most appropriate method is the HPLC method which is a method of separation in which the stationary phase is contained in a column, one end of which is attached to a source of pressurized liquid eluent or mobile phase (18). HPLC can determine the amount of material in the dosage form, at the same time it can be used as an identification method for the active and the other product ingredients such as the preservatives. HPLC method can detect the materials at small quantities, so it can detect the degradation in the products especially for old batches.

The preferred detectors for most separations are the UV/VIS detectors of fixed and variable wavelengths. The current UV/VIS detectors are the diode array models that carry out detection continuously at a different wavelengths. For any time point in the chromatogram, an absorption spectrum can be displayed from the array storage (19).

Column selection is an important factor for best separation between the compounds. Reverse phase columns are useful for most compounds other than the normal phase for the drug analysis. For reverse phase, the mobile phase should be polar solvents to cause good separation (20).

The method of analysis should be good indicator for the stability studies (21). It must detect all impurities that may develop in the drug. At the same time the method of analysis should detect some inactive materials (excipients) such as the preservatives and the colors .

## 1-4- Analytical Validation

Validation is an establishing document evidence which provides a high degree of assurance that a specific method/process/machine will consistently work to determine the product specifications and quality attributes (22). In other words, validation is an operation intended to demonstrate that every process and procedure used for production, packing, or control of products does actually lead to the expected results (23).

Its importance appeared for the first time in 1971, when some people in the USA died as a result of an error in the sterilization process of some bottles of 5% dextrose infusion kept in the autoclave.

### 1-4-1-Validation Importance

There are many benefits that come up from the validation (24). Such benefits include but are not limited to the following

- 1-Manufacturers are required by law to conform to GMP regulation
- 2-Good business dictates that a manufacturer avoid the possibility of rejected or recalled batches
- 3-Validation helps to ensure product uniformity, quality and reproducibility.

#### 1-4-2-Validation Types

There are many types of validation (25) Such types include but not limited to the following:

- Analytical Methods
- Equipment
- Manufacturing process
- Cleaning
- Sterilization
- Environment

#### 1-4-3-Validation Options

There are three options for validation;

Prospective, retrospective, and revalidation (26).

##### 1-4-3-1- Prospective Validation

Validation done prior to manufacture and distribution (for new products at development stage) from an approved plan and protocol for validation with predefined acceptance criteria.

##### 1-4-3-2- Retrospective Validation

Validation based on review and statistical analysis of historical data for a analysis of a product over the two previous years at the same time, the total number of batches should be between 10 to 50.

##### 1-4-3-3- Re-Validation

There are many cases for re-validation of the method of analysis. Such cases include but not limited to the following

- Changes in a critical raw material used in the product
- Change or replacement in a critical piece of modular (capital) equipment.
- Sequential batches that fail to meet the product and process specification.

#### 1-4-4-Validation of Raw Materials

The validation process of a cream dosage form begins with a validation of the raw materials. Variation in raw materials (active or inactive excipients) is one of the major causes of product variation or deviation from specification. Each raw material should be validated by performing checks on several batches, preferably three. The batches chosen should be selected to represent the range of acceptable specifications both high and low. The most important parameters are particle size, microbial contamination, moisture content, color, pH, impurities and assay (28).

#### 1-4-5- Assay Validation

Unless a suitable analytical methods, or series of methods, are available to assess the quality and performance of cream dosage forms, the validation program will have limited validity.

##### 1-4-5-1- Analytical Parameters to be Validated

###### a-Accuracy

Accuracy is the closeness of the test results to the true value(27). It can be determined by calculating the percent of recovery on three different batches. Each batch is analyzed three times. 5 injections are carried out for each batch.

###### b-Precision

Precision is the degree of agreement between the test results (28). It can be determined by calculating the variation coefficient for three different batches. Each batch is analyzed three times. 5 injections are carried out for each batch

### c- Selectivity

Selectivity is the absence of interference between the analyte and the other components (27), and that the expected peak for the active (for HPLC methods) is true. It can be determined by comparing the analysis between the product containing the analyte and the product without the analyte (placebo).

### d-Linearity and Range

Linearity is a proportional relation between the analytical results and its concentration through a defined range (27). It can be determined by calculating the correlation coefficient of a regression line. At least 5 concentrations should be prepared and tested (50%-150%) of normal concentration of analysis method.

### e-Ruggedness

Ruggedness is the degree of reproducibility for the same samples under a variety of normal test conditions such as time, temperature, column used and others (27). It can be determined by analyzing samples kept at different times and temperatures or replacing the column with another two columns of the same type followed by measuring the precision of the samples kept under these conditions.

## 1-5- Stability Study

The term stability, with respect to the drug dosage form, refers to the chemical and physical integrity of the dosage unit and when appropriate, the ability of the dosage to maintain protection against microbiological contamination (29).

A stable cream is one in which the dispersed phase retains its initial character and remains uniformity distributed through out the continuous phase (30).

Although the stable cream is that retain their ingredients with no degradation for all components especially the active principle (31).

Stability studies are necessary for all drugs, specially the sem1-solids, because instability of the product can produce ineffective drugs by the loss in the assay of active principle or decreasing in its bioavailability. The drug product, therefore, has to satisfy stability criteria chemically, toxicologically, therapeutically and physically (32)

## Factors Affecting Product Stability

Stability parameters of a drug dosage form can be influenced by environmental conditions of storage such as temperature, light, air and humidity as well as the incompatibility between ingredients, manufacturing process, handling, dosage form, and packing components reactions (32). Incompatibility can be physical, chemical, and pharmaceutical. There are many routes for chemical reactions such as; Oxidation-reduction, hydrolysis, decarboxylation, racemization, photochemical and others (33).

## Modes of Stability Studies

There are many modes for stability study, but the most important modes are (33)

- 1- Long term stability study
- 2- Accelerated stability study

Table [1-3] shows the storage condition for both modes

Table [1-3] Storage condition for long term and accelerated stability study

Study mode	Condition	Period
Long term	$25 \pm 2 \text{ }^\circ\text{C}/60 \pm 5 \text{ RH}$	12 Months
Accelerated	$40 \pm 2 \text{ }^\circ\text{C}/75 \pm 5 \text{ RH}$	6 Months

Accelerated studies are designated to increase the rate of chemical degradation or physical changes of a drug product by using exaggerated storage conditions as part of the formal definitive storage program. Two factors are determined as a result of this study Storage conditions and the expiration date.

The expiration date for products is given upon the result of this study, which should not exceed two years, but can be extrapolated according to the results obtained from the long term stability studies.

For determination of storage condition; the samples are stored under condition (40 °c, 75% RH) for six months. This will lead to the following: if there is no change, two years expiration date is given, with label “store at room temperature”. Because 6 months equivalent to 2 years if the product is kept under 40 °c as calculated using Arrhenius equation. If significant change occurs in this condition, the formula must be changed (35,36).

Arrhenius equation (34)  $k=A*\exp^{(-E_a/R*T)}$

Where k is the rate coefficient, A is a constant,  $E_a$  is the activation energy, R is the universal gas constant, and T is the temperature (in degrees Kelvin).

To determine the incubation time necessary for stability claims using the Arrhenius equation

- a)  $Q_{10} = 2.0$  (conservative; “Rule of Two”. For every 10 degree increase in temperature the rate of degradation doubles)
- b) Room temperature = 20 °C (Controlled room temperature)
- c) Real Time = Acceleration Time \*  $Q_{10}^{(T2-T1/10)}$

T2 , T1, Temperatures at required storage condition and at room temperature respectively

$$\text{Real Time} = 6 * 2^{(40-20)/10} = 24 \text{ months} = 2 \text{ years}$$

From the chemical structure of Terbinafine (as shown in figure [1-1]), the substance composed of naphthalene group, allyl amine group, in addition to a triple bond between two carbon atoms. Allyl amine is relatively stable as a result of the resonance effect (conjugative effect) due to the overlap between the orbitals but it is responsible for the addition reaction by acidic substrates such as HCl, also for hydrolysis reaction, in addition, it is responsible for free radical reaction as a result of reaction with light. Naphthalene group is responsible for oxidation reduction reaction by reacting with oxidizing agent. The triple bond reacts with the basic materials such as NaOH because it is relatively acidic group.

All initial data should be collected and kept to compare results of analysis in the future such as assay, color, odor, degradation, pH, tackiness, greasiness, viscosity, skin allergies, microbial contamination and physical stability. A minimum of three batches are studied. The samples are kept in a cooled incubator.

## 1-6- Penetration

Transdermal and topical formulations permeation involve partitioning into and transport through the cutaneous layers, namely the stratum corneum, the viable epidermis, and the upper dermis. A topical product is designed to deliver the drug into the skin to treat dermal disorders and therefore skin is the target organ. The skin is a barrier to topically

administer drugs. Although, the outer layers provide resistance to the global permeation process (37).

With many drugs, the rate of permeation through the skin is extremely low without the use of some means to enhance the permeability of the skin. In order to increase the rate at which a drug penetrates through the skin, various approaches have been followed by using either a chemical penetration enhancer or a physical penetration enhancer. Physical enhancement includes electrophoretic techniques such as iontophoresis, in addition to the use of ultrasound. Chemical enhancers are administered along with the drug in order to increase the permeability of the stratum. An effective amount of a permeation enhancer is meant a non-toxic non-damaging non-irritating, but sufficient amount of the enhancer to provide the desired increase in skin permeability and, correspondingly, the desired depth of penetration, rate of administration and amount of drug deliver. Various compounds for enhancing the permeability of skin are known in the art and literature. Compounds that have been used to enhance skin permeability include solfoxides, ethers, surfactants, alcohols, fatty acids, fatty terpenes, alkanols, and organic acids (38). Chemical enhancer enables the Terbinafine bulky molecules to pass through the stratum corneum layer by expanding the paths of the liquid crystal layer of the stratum corneum layer. As shown in figure [1-1], the chemical structure of Terbinafine is composed of naphthalene group, allyl amine group and triple bond between two carbon atoms. All these groups are electron releasing, that means, it is difficult to lose a proton from the molecule, so the material is lipophilic. The material remains in the stratum corneum seven days because it is lipophilic (as the most composition of the stratum corneum).

The skin structure is composed of several layers (39) as shown in figure [1-2]. Epidermis is composed of several layers but the most important layer is “Stratum Corneum”. It is the limit step layer for the penetration process. It composed of protein, lipid and water layers (40), as shown in figure [1-3]

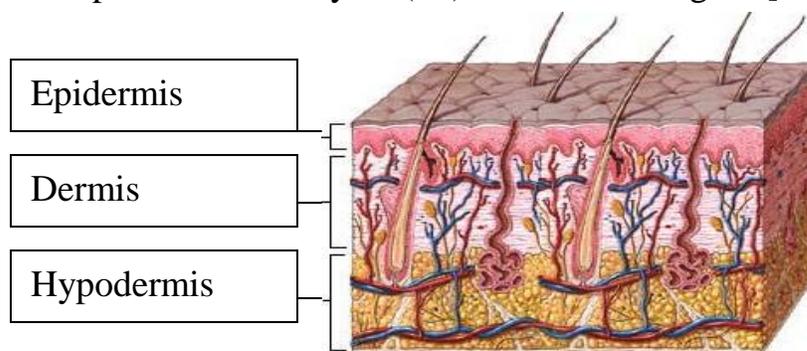
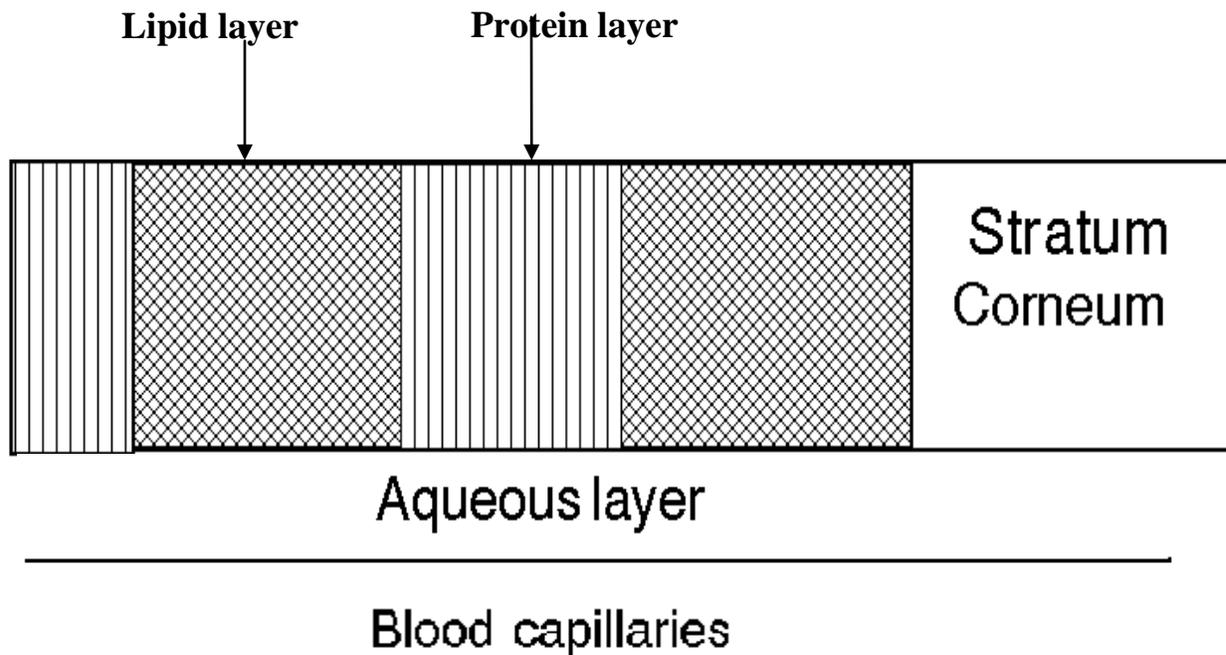
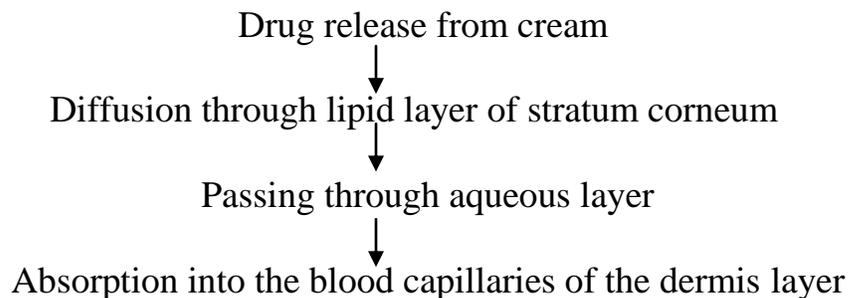


Figure [1-2] Skin layers



Figure[1-3] Stratum corneum layer composition

### Penetration Steps



The vehicle of the drug is important factor that it may affect permeability of the drug. In this thesis, the penetration of terbinafine HCl will be evaluated by comparing its penetration with that for Lamisil cream (the brand product), using Franze Diffusion Cell, as shown in figure [1-1], which is made of glass with a constant area of 1.35 cm<sup>2</sup>. It is consisted of a donor compartment (A) and a receptor compartment (B). The membrane (C) is mounted between the cell compartment and an O-ring (D). The two cell compartments are held together with a clamp. The system is kept at 32 °c by

circulating water through an external water jacket (E). The receptor solution is continuously stirred by means of a spinning bar magnet (F). The samples are withdrawn through the sampling port (G), (41).

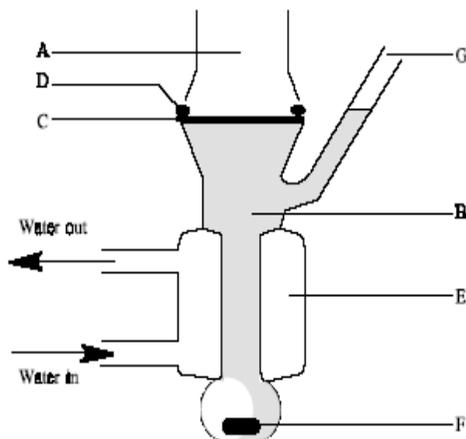


Figure [1-4] France Diffusion Cell.

A = donor compartment; B = receptor compartment; C = membrane; D = O-ring; E = water jacket; F = stirring bar; G = sampling port

## 1-7- Phase Diagram

An important aim to the thesis in addition to formulation and analysis is establishing a triangular phase diagram compositioning of terbinafine, water and tween 80 to determine the solubility of Terbinafine in the two mentioned solvents. Phase diagram is an essential tool for designing microemulsion system, liquid crystal systems, or two phases systems and in addition to the solubility of materials.

To find the soluble region of Terbinafine HCl in the phase diagram, a proportional amount of the substance is added to the two componets mixtures, followed by shaking, sonication and storage at 25 °c for 24 hours. The samples are centrifused at 5000 rpm for 15 minutes and their physical condition (clarity, ppt, and flowability) is determined. Mixtures that did not show any change in the meniscus after tilting to an angle of 90° are considered to be gels (42). Identification of phases can be done visually or by using polarized microscope, in addition to other identification methods.

Appearance of cloudness or precipitation showes beginning of insolubility of the substance in the mixtures.

Solubility of Terbinafine HCl in water and tween 80 can be determined to be compared with the substance solubility in the phase diagram mixtures. Terbinafine HCl is soluble in ethanol but slightly soluble in water and tween 80. Terbinafine HCl is soluble in alcohols such as ethanol. It is important to find other than alcohols to dissolve the material such as the mixtures of tween 80 and water. Alcohols are more expensive than water and tween 80, they have chemical activity that can affect product stability, in addition to the environmental issues.

Terbinafine HCl is added in excess to both solvents (water and tween 80) separately to calculate its solubility in these two solvents. The samples are stored at 25 c° for 24 hours, then filtered using 0.45 µm and analyzed using high performance liquid chromatography.

## 1-8- Specifications

### 1-8-1- Terbinafine Specification

Material name: Terbinafine HCl

Material Solubility: Terbinafine HCl dissolves in methanol, ethanol, and 0.1 N NaOH. It is slightly soluble in water.

Molecular wt: 327.9 g/mol

Material Source: A synthetic allylamine derivative.

Approved Supplier: Medifarma East

Safety Precautions: Wearing masks, gloves, caps, eyeglasses, the material is kept in well-closed containers.

Storage Conditions: It should be protected from heat, moisture and light.

Action And Use: Antifungal.

Tests to be done: Assay, identification, organoleptic properties (appearance, color, odor, ...), solubility, purity, and moisture content.

## 1-8-2- Finished Product Specification

### 1-8-2-1-Specification of brand product

- 1- Product name: Lamisil 1% cream
- 2- Therapeutic action: Broad spectrum antifungal agent.
- 3- Composition: 1% Terbinafine HCl
- 4- Pharmaceutical form: Cream
- 5- Manufacturer: Novartis, Switzerland
- 6- Expiration period: 5 years
- 7- How supplied: 15 g / tube
- 8- Color of cream: White
- 9- Tube Type: Aluminum with plastic cap
- 10- Other dosage forms for Lamisil line: Solution and tablets.
- 11- Storage: Store in a cool and dry place.

### 1-8-2-2-Specification of generic product

The specification of the generic product should be identical to the brand product according to the effectiveness, viscosity, composition, pH, color and others.

## General Specification

- 1- General appearance Homogeneous white and smooth cream, free from air bubbles.  
Its smell is acceptable.
- 2- How supplied 15 g / tube
- 3- Expiration period 2 years increased yearly up to 5 years.
- 4- Tube Type Aluminum with plastic cap.
- 5- Storage Store in a cool and dry place.

## 1-9- Study Objectives

This thesis has several objectives that can be condensed in the followings:

- 1- To develop a stable formula for Terbinafine cream followed by stability study to produce a cream equivalent to the brand product (Lamisil).
- 2- To develop an effective formula for Terbinafine cream and testing its penetration.
- 3- To learn more about the function of the excipients that might be used in the cream dosage form by carrying out different trials.

- 4- To develop a method of analysis followed by analytical validation.
- 5- To find out the best solubility system for Terbinafine HCl depending on a triangular phase diagram technique.

## 2- EXPERIMENTAL

### 2-1- Analysis of Finished Product & Determination of Brand Product Specification

#### 2-1-1-Materials and consumables

Soya bean casein digest agar from Difco Laboratories, Detroit, USA.  
Sodium chloride peptone solution pH 7.0 from Difco Laboratories, Detroit, USA.  
Fluid soybean casein digest medium from Difco Laboratories, Detroit, USA.  
Cetrimide agar medium obtained from Difco Laboratories, Detroit, USA.  
Baird Parker agar medium obtained from Difco Laboratories, Detroit, USA.  
Casein digest agar obtained from Difco Laboratories, Detroit, USA.  
Ammonium acetate obtained from Merck, Germany.  
Methanol HPLC obtained from Merck, Germany.  
Terbinafine HCl from Cross chem. International Co., Switzerland.  
0.1 N NaOH NaOH obtained from Merck, Germany.  
Milli-pore filter 0.45  $\mu\text{m}$   
Milli-pore filter 0.22  $\mu\text{m}$   
Sterile petri dishes (90 mm)  
Screw- cap sterile tubes  
Test tubes 5-15 ml

#### 2-1-2- Equipment

Balance, Precisa XT 220A  
Vortex, Gennie vortex, scientific industries, USA.  
HPLC Apparatus, Merck Hitachi  
Viscometer, Brockfield, Model DV-2+  
Centrifuge apparatus, Gemmy Industrial corp., serial No. 9104307/99  
Incubator, Ari J-Levy- Israel  
Autoclave (wet), Tuttnauer, Israel  
pH meter, Mettler Toledo, Mp230  
Sonicator, Elma – Transsonic T 780/H  
Karl fisher apparatus, 758 Titrino, Metrohm  
Hot plate with stirrer, Biby Stirilin Ltd, UK, Serial No. CB162/13583  
Spectrophotometer, Hithachi, U2000.  
Incubator, (for stability study) WTB Binder Labortechnik GmbH

## 2-1-3- Tests

### 2-1-3- 1- Assay

Method: HPLC

Materials Used: Methanol, 0.1M Ammonium acetate, water, Terbinafine HCl.

Mobile Phase: 95.0% Methanol 5.0%, 0.1M Ammonium acetate.

To prepare 0.1M ammonium acetate, 0.77 g of ammonium acetate is dissolved in 100 ml water.

Stationary Phase: RP18, 125\*4 mm, 5 $\mu$ m

UV-Wavelength ( $\lambda$ ): 223 nm

Flow rate: 1.0 ml/min.

Temperature: Room temperature

Vial volume: 20  $\mu$ l

#### Standard Preparation

250.0 mg of Terbinafine HCl is dissolved in about 60 ml mobile phase. The volume is completed to 100.0 ml. 1 ml is diluted into 100.0 ml using mobile phase.

#### Sample Preparation

0.250 gm of sample is shaken with about 60 ml mobile phase for 20 min. The sample is filtered through a Whatman filter paper No. 4. The filter paper is washed by about 30 ml mobile phase. The washings are added to the filtrate. The volume is completed to 100.0 ml using the same solvent. The sample is filtered through a 0.22  $\mu$ m Millipore filter paper.

#### Procedure

The vials for the sample and standard solutions are prepared. The automatic procedure is carried out for the pump, injection, detection, presentation, and calculation.

## Calculation

$$\% \text{ Material} = (\text{Sample Peak area} / \text{Standard Peak Area}) * 100$$

Limits  $90 \pm 110$  % of the labeled amount of material.

### 2-1-3- 2- Identification

The retention time of the sample and standard for the active material obtained in the assay under the same conditions must be the same or within the  $\pm 0.1$  minute range.

Limit Retention times for sample peak and standard peak should be identical.

### 2-1-3- 3-Impurities Determination

The same procedures used in the HPLC assay.

HPLC can detect the impurities accurately especially when the retention times are prolonged if necessary to aid the resolution by increase the difference of the polarity between the mobile phase and the stationary phase.

### 2-1-3- 4- pH Test

Instrument pH meter

#### Procedure

10 g of sample is mixed with 90 ml purified water, the sample is directly measured at normal temperature using calibrated pH meter

Limit  $\pm 0.2$  of the brand product

### 2-1-3- 5- Content Uniformity

#### Procedure

Three individual samples are tested for the assay; one injection from each sample is analyzed.

Limit 85.0% -115.0% of labeled amount.

### 2-1-3- 6- Phase Stability Test (Centrifusion Test)

Time: 15 minutes

Revolution: 5000 Rounds/minute

#### Procedure

A quantity of the cream is placed in two parallel centrifuge tubes and rotated at 5000 /minute for 15 minutes at room temperature.

Limit: No separation is allowed.

### 2-1-3- 7- Smoothness Test (Grittiness)

#### Procedure

A thin layer of the product is applied on the hand and checked for the smoothness and the absence of any particles.

Limit: The cream must be smooth.

### 2-1-3- 8- Organoleptic Properties

The color, odor, and general appearance of the cream are determined.

#### Limit

Odor acceptable and identical to the standard

Color white

General appearance no air bubbles, no impurities, homogeneity availability.

### 2-1-3- 9-Tackiness Test

A thin layer of the product is applied on the hand and checked for the absence of tackiness.

Limit: Untacky cream is required.

### 2-1-3- 10-Greasiness Test

A thin layer of the product is applied on the hand and checked for the absence of grease on skin.

Limit: No grease remains after cream application.

### 2-1-3- 11- Appearance of Applied Film Test

A thin layer of the product is applied on the hand and checked for the transparency of the applied film cream.

Limit: The applied film cream should be transparent to the skin.

### 2-1-3- 12- Skin Allergies Test

A thin layer of the product is applied on the hand and checked for the absence of an irritation on skin.

Limit: No irritation is allowed.

### 2-1-3- 13- Viscosity

About 50 g of the finished product are measured using Brookfield viscometer

Time: after 1 hour under the viscometer,

Spindle: T-bar spindle with length of 10.9 mm

Speed: 30 rpm,

Temperature: room temperature

Limit:  $\pm 10\%$  of brand product

## 2-1-3- 14- Microbial Analysis

Reference: USP 24

### a-Total Count

#### 1-Method Filtration method

#### 2-Media Preparation

Suspend 40 g of the Soya bean casein digest agar in 1L of purified water mix thoroughly, heat with frequent agitation, and boil for 1 min to dissolve the powder, autoclave at 121.0 c° for 15 min.

#### 3- Sample Preparation

Dissolve 1.0 g of the product in 10 ml buffered sodium chloride peptone solution pH 7.0.

#### 4-Procedure

- Use membrane filter having a nominal pore size of 0.45 µm.
- Pass the sample through the membrane filter.
- Wash the filter with 100 ml of buffered sodium chloride peptone pH 7.0 for 3 times.
- Transfer one membrane filter for Soya bean. Casein digest agar for the enumeration of bacteria and incubate at 35-37 c° for 48 hours.
- Calculate the no of cfu /gm.

5- Limit : Total viable aerobic count  $\leq$  1000 cfu /gm

### b- Staphylococcus aureus

Method: spread plate method

#### Procedure

- To 10 gm of sample add fluid soybean casein digest medium to make 100 ml, mix and incubate.

- Examine the medium for growth.
- If growth is present, use an inoculating loop to streak a portion of the medium on the surface of Baird Parker agar medium.
- Cover, invert, and incubate dishes at 35-37 °c for 48 hours.
- If upon examination, non-of the plates contains colonies, the tested sample meets the requirements for freedom from Staphylococcus aureus.

### *c- Pseudomonas aeruginosa*

#### Procedure

- To 10 gm cream add fluid soybean casein digest medium to make 100 ml, mix, and incubate.
- Examine the medium for growth, if growth is present, use an inoculating loop to streak a portion of the medium on the surface of cetrimide agar medium in a petridish.
- Cover, Invert and incubate the dish at 35-37 c° for 48 hours
- If upon examination, none of the plates contains greenish colonies, the test sample meets the requirements for freedom from Pseudomonas aeruginosa.

## 2-2- Raw material analysis – (Terbinafine HCl)

### 2-2-1- Identification Test

- a) It has two maximal UV absorbance at 283 nm and 223 nm in 0.1 M methanolic HCl for a solution of 2.0% mg/ml.
- b) The retention times of the sample and standard peaks should be identical for the chromatogram of assay 1.

### 2-2-2- Organoleptic properties

- The color of the powder should be white to off white crystalline powder.
- The odor of the powder is odorless.

### 2-2-3- Purity Test

Check visually the powder to detect any strange material between the granules.

### Limit

No strange matter should be found.

### 2-2-4- Moisture content

-Method: Karl Fisher method.

-Instrument: Karl Fisher apparatus

-Limit: Maximum 1.0%.

### 2-2-5-Melting Point

Limit: 169 – 172 °c

### 2-2-6- Assay

#### Assay 1

Reference: New developed method.

Method: HPLC.

Mobile Phase

95.0% Methanol 5.0%, 0.1M ammonium acetate.

To prepare 0.1M ammonium acetate, dissolve 0.77 g ammonium acetate in 100 ml water.

Stationary Phase: RP18, 125\*4 mm, 5µm.

UV-Wavelength ( $\lambda$ ): 223 nm

Flow rate: 1.0 ml/min.

Temperature: Room temperature

Vial volume: 20 µl.

## Standard/Sample Preparation

Dissolve 100.0 mg of Terbinafine HCl (st/sa) in about 60 ml methanol. Complete the volume to 100.0 ml by the same solvent. Dilute 1.25 ml in 50.0 ml using mobile phase.

## Procedure

Prepare the vials for the sample solution. Carry out the automatic procedure for the pump, injection, detection, presentation, and calculation. Measure the percent of the peaks other than the principal peak

## Calculation

$$\% \text{ Terbinafine} = (\text{Sample peak area} / \text{Standard peak area}) * 100$$

## Assay 2

Reference: Home method

Method: UV absorbance at 223 nm.

## Procedure:

Dissolve 100 mg of Terbinafine HCl in a 100 ml volumetric flask using about 60 ml of 0.1M methanolic HCl, dilute to the mark using the same solvent. Transfer 2 ml into a 100 ml volumetric flask containing about 60 ml of 0.1M methanolic HCl, dilute to the mark using the same solvent. Read the UV absorbance at 223 nm. Compare the result with a standard solution has the same concentration and solvent.

## Calculation

$$\% \text{ Terbinafine} = (\text{Sample absorbance} / \text{Standard absorbance}) * 100$$

Limit: 98.0% -102.0%

## Assay 3

Reference: Home method.

Method: Titration with 0.1 N NaOH

#### Procedure

Dissolve about 500 mg of Terbinafine HCl in 80 ml of methanol, add 10 ml of water and titrate with 0.1N NaOH using phenol red solution as indicator. Each ml of 0.1N NaOH is equivalent to 32.79 mg of substance.

% Terbinafine = (Sample titrant volume \* 32.79/sample wt) \* 100

Limit: 98.0% - 102.0%

#### 2-2-7- Degradation

Procedure Follow the same procedure mentioned in assay 1.

Calculation % Impurity = (Sample Peak area/Principal Peak Area ) \* 100

Limit Total of impurities areas  $\leq$  1.0 % of the principal peak area.

#### 2- 3- Manufacturing Trials

To create a new formula, many trials should be carried out. Many ideas can be tried using small trials of about 250 g cream. The best formula can be expanded to be one kilogram or more. The selected formulas are kept under accelerated stability study to check the chemical, physical, and microbial properties.

#### Materials

GMS (SE) Obtained from Cognis (Deutschland GmbH . D-40551 Dusseldorf), B.No. CA11510003)

Eumulgin B2 Obtained from Cognis (Deutschland GmbH . D-40551 Dusseldorf), B.No. CD20240003

Propyl Paraben Obtained from Clariant, (south wales Site, Mid Glamorgan CF38 2SN), B.No. 14917

Methyl Paraben Obtained from Clariant, (south wales Site, Mid Glamorgan CF38 2SN), B.No. GBGA003033

Chloro-cresole Obtained from Merck Schuchardt OHG, (D-85662 Hohenbrunn,) B.No. S34094

Cetostearyl alcohol Obtained from Ellis & Everard , B.No. 70-04072000

Liquid paraffin obtained from Compton B.V-Wezerstrast, B.No 450402

Phenoxy-ethanol, obtained from Merck, Germany.

Purified water obtained from Milli Q apparatus

Terbinafine HCl obtained from Cross chem. International Company, Switzerland, B.No 20041103

Vaseline White Obtained from Honeywell, (Eupen, Belgium), B.No. 21045

TEA Obtained from Merck, Germany , (Israel), B.No. 022005367

Ethanol absolute Obtained from Hayman, (Eastways Park Witham, England), B.No. 02/155C6

## Equipment and Tools

Beakers 500 ml

Balance, (sensitivity, 0.01 g) Precisa, XT 220A

Erweka mixer (14L): Erweka co., AR401 , Germany

Water bath: Julabo co., SW22.

In addition to all Analysis equipment mentioned above (See 2-1).

## 2-3-1- O/W Method

### General Manufacturing procedure

- Melt the hydrophobic materials by using water bath at temperature of 75 °c.
- At the same time, heat the water at the same temperature by using the water bath.
- Add the hydrophilic materials to the water phase while mixing.
- Add gradually while mixing, the oil phase to the water phase, both at 75 °c by using the mixer.
- Continue the mixing and cool the cream to 50 °c.
- Dissolve the active principle (Terbinafine HCl) by ethanol.
- When the cream reaches the temperature of 50 °c, add the active principle solution to the cream while mixing. Adjust the pH to  $4.6 \pm 0.2$  by drops of tri-ethanol amine.
- Continue the mixing and cool the cream to room temperature.
- Carry out all the important tests that employ the trial aim.
- Select the best trial that contains materials of low cost, available, pure, less toxicity, do not effect on analysis and that are used easily. The trial that complies with all requirements of the cream, such as good penetration, absence of tackiness, lower

greasiness, smoothness of cream on the skin, good viscosity, good pH, stable (physically and chemically), lower irritation, and good organoleptic properties.

## 2-3-1-1-Selection of Ingredients

### 1- Selection of Emulsifying Agents (Trials 1- 8)

See tables from [2-1] to [2-8]

a- Selected material: GMS (SE)

-Trial No. 1, (T1)

Table [2-1] Formulation of Terbinafine cream using GMS (SE) as emulsifier

Material	Function	Material Qty (g)
Terbinafine HCl	Active material	2.5
GMS (SE)	Emulsifying agent	20.0
Liquid paraffin	Emollient	20.0
Ethanol 96%	Solvent	5.0
Vaseline white	Base	10.0
TEA	Alkalizing agent	1.0
Water	Continuous phase	191.5
Total		250.0

b- Selected material: Eumulgin B2 (Cetearth 20)

Trial No. 2, (T2)

Table [2-2] Formulation of Terbinafine cream using Eumulgin B2 as emulsifier

Material	Function	Material Qty (g)
Terbinafine HCl	Active material	2.50
Eumulgin B2	Emulsifying agent	20.0
Liquid paraffin	Emollient	20.0
Ethanol 96%	Solvent	5.0
Vaseline white	Base	10.0
TEA	Alkalizing agent	1.0
Water	Continuous phase	191.5
Total		250.0

c- Selected material: Cetostearyl Alcohol

Trial No. 3, (T3)

Table [2-3] Formulation of Terbinafine cream using cetostearyl alcohol as emulsifier

Material	Function	Material Qty (g)
Terbinafine HCl	Active material	2.5
Cetostearyl Alcohol	Emulsifying agent	20.0
Liquid paraffin	Emollient	20.0
Ethanol 96%	Solvent	5.0
Vaseline white	Base	10.0
TEA	Alkalizing agent	1.0
Water	Continuous phase	191.5
Total		250.0

d- Selected materials: Cetostearyl Alcohol and GMS (SE)

Trial No. 4, (T4)

Table [2-4] Formulation of Terbinafine cream using Cetostearyl Alcohol and GMS (SE) as emulsifiers

Material	Function	Material Qty (g)
Terbinafine HCl	Active material	2.5
Cetostearyl Alcohol	Emulsifying agent (aux)	20.0
GMS (SE)	Emulsifying agent	20.0
Liquid paraffin	Emollient	20.0
Ethanol 96%	Solvent	5.0
Vaseline white	Base	10.0
TEA	Alkalizing agent	1.0
Water	Continuous phase	171.5
Total		250.0

e Cetostearyl Alcohol and Eumulgin B2

Trial No. 5, (T5)

Table [2-5] Formulation of Terbinafine cream using Cetostearyl Alcohol and Eumulgin B2 as emulsifiers

Material	Function	Material Qty (g)
Terbinafine HCl	Active material	2.5
Cetostearyl Alcohol	Emulsifying agent (aux)	20.0
Eumulgin B2	Emulsifying agent	20.0
Liquid paraffin	Emollient	20.0
TEA	Alkalizing agent, Enhancer	1.0
Ethanol 96%	Solvent, Enhancer	5.0
Vaseline white	Base	10.0
Water	Continuous phase	171.5
Total		250.0

f- GMS (SE) and Eumulgin B2

Trial No. 6, (T6)

Table [2-6] Formulation of Terbinafine cream using GMS (SE) and Eumulgin B2 as emulsifiers

Material	Function	Material Qty (g)
Terbinafine HCl	Active material	2.5
GMS (SE)	Emulsifying agent	20.0
Eumulgin B2	Emulsifying agent	20.0
Liquid paraffin	Emollient	20.0
Ethanol 96%	Solvent	5.0
TEA	Alkalizing agent	1.0
Vaseline white	Base	10.0
Water	Continuous phase	171.5
Total		250.0

g- Selected materials (Cetostearyl Alcohol, GMS (SE) and Eumulgin B2)

Trial No. 7, (T7)

Table [2-7] Formulation of Terbinafine cream using Cetostearyl Alcohol, GMS (SE) and Eumulgin B2 as emulsifiers

Material	Function	Material Qty (g)
Terbinafine HCl	Active material	2.5
Cetostearyl Alcohol	Emulsifying agent (aux)	20.0
Eumulgin B2	Emulsifying agent	20.0
GMS (SE)	Emulsifying agent	20.0
Liquid paraffin	Emollient	20.0
Ethanol 96%	Solvent	5.0
TEA	Base	1.0
Vaseline white	Alkalizing agent	10.0
Water	Continuous phase	151.5
Total		250.0

h- Selected materials: Cetostearyl Alcohol, GMS (SE) and Eumulgin B2

Trial No. 8, (T8)

Table [2-8] Formulation of Terbinafine cream using Cetostearyl Alcohol, GMS (SE) and Eumulgin B2 as emulsifiers (change qty)

Material	Function	Material Qty (g)
Terbinafine HCl	Active material	2.5
GMS (SE)	Emulsifying agent	20.0
Eumulgin B2	Emulsifying agent	10.0
Cetostearyl alcohol	Emulsifying agent (aux)	20.0
Liquid paraffin	Emollient	20.0
Ethanol 96%	Solvent	5.0
TEA	Alkalizing agent	1.0
Vaseline white	Base	10.0
Water	Continuous phase	161.5
Total		250.0

- Selection of Preservative, Trials 9 – 11

See tables from [2-9] to [2-11]

For topical preparation, the preservative should be sensitive to the pseudomonas aeruginosa and staphylococcus aureus.

There are many available preservatives such as phenoxy ethanol, chloro-m-cresol, methyl paraben, propyl paraben and others.

1- Selected Preservative: phenoxy-ethanol

Trial No. 9, (T9)

Table [2-9] Selection of phenoxy ethanol as preservative for Terbinafine cream

Material	Function	Material Qty (g)
Terbinafine HCl	Active material	2.5
GMS (SE)	Emulsifying agent	20.0
Eumulgin B2	Emulsifying agent	10.0
Cetostearyl alcohol	Emulsifying agent (aux)	20.0
Liquid paraffin	Emollient	20.0
Ethanol 96%	Solvent	5.0
TEA	Alkalizing agent	1.0
Vaseline white	Base	10.0
Phenoxy - Ethanol	Preservative	2.5
Water	Continuous phase	159.0
Total		250.0

## 2-Selected Preservative: Chloro-m-cresol

Trial No. 10, (T10)

Table [2-10] Selection of chloro-m-cresol as preservative for Terbinafine cream

Material	Function	Material Qty (g)
Terbinafine HCl	Active material	2.5
GMS (SE)	Emulsifying agent	20.0
Eumulgin B2	Emulsifying agent	10.0
Cetostearyl alcohol	Emulsifying agent (aux)	20.0
Liquid paraffin	Emollient	20.0
Ethanol 96%	Solvent	5.0
TEA	Alkalizing agent	1.0
Vaseline white	Base	10.0
Chloro-m-Cresol	Preservative	2.5
Water	Continuous phase	159.0
Total		250.0

## 3- Selected Preservatives MP & PP

Table # [2-11]

Trial No. 11 T11

Table [2-11] Selection of MP & PP as preservatives for Terbinafine cream

Material	Function	Material Qty (g)
Terbinafine HCl	Active material	2.5
GMS (SE)	Emulsifying agent	20.0
Eumulgin B2	Emulsifying agent	10.0
Cetostearyl alcohol	Emulsifying agent (aux)	20.0
Liquid paraffin	Emollient	20.0
Ethanol 96%	Solvent	5.0
TEA	Alkalizing agent	1.0
Vaseline white	Base	10.0
Methyl paraben	Preservative	2.5
Propyl paraben	Preservative	0.5
Water	Continuous phase	158.5
Total		250.0

## 2-3-1-2- Selection of Procedure

### -Change Speed of Mixing

Select the best formula from the trials (1-11) to start the following trials:

Trial No. 12, (T12) mixing speed 20 rpm

Trial No. 13, (T13) mixing speed 80 rpm

Trial No. 14, (T14) mixing speed 120 rpm

Trial No. 15, (T15) mixing speed 180 rpm

### -Change Temperature of Active material Addition

Select the best formula from the last four trials to continue the trials.

Trial No. 16, (T16) addition at 70 °c

Trial No. 17, (T17) addition at 60 °c

Trial No. 18, (T18) addition at 50 °c

## **2-4-Analytical Validation**

### 2-4-1- Trial Preparation

Carry out the tests on the best-selected trial as mentioned in the trial preparation.

2-4-2- Method: Use the HPLC assay method mentioned above (see 2.1.3.1)

2-4-3- Reference: New developed method.

2-4-4-Parameters to be tested

1-Selectivity, 2-accuracy, 3-precision, 4-ruggedness, and 5-linearity.

#### 2-4-4-1-Selectivity

Analyze the following solutions under the same conditions in order to determine that there is no interference between the active material peak and the other peaks

##### Standard Solution Preparation

Dissolve 250.0 mg of Terbinafine HCl in about 60 ml mobile phase. Complete the volume to 100.0 ml. Dilute 1 ml into 100.0 ml using mobile phase.

The final dilution concentration is 2.5% (mg/ml).

##### Sample Solution Preparation

Shake 0.25 gm of sample with about 60 ml of mobile phase for 20 min.. Filter through Whatman filter paper No. 4. Wash the filter paper by about 30 ml mobile phase. Add the washings to the filtrate. Complete the volume to 100.0 ml using the same solvent. Filter the sample through a 0.22  $\mu\text{m}$  Millipore filter paper.

##### Placebo Solution Preparation

Shake 0.25 gm of placebo cream with about 60 ml of mobile phase for 20 min.. Filter through Whatman filter paper No. 4. Wash the filter paper by about 30 ml mobile phase. Add the washings to the filtrate. Complete the volume to 100.0 ml using the same solvent. Filter the sample through a 0.22  $\mu\text{m}$  Millipore filter paper.

#### 2-4-4-2-Accuracy and Precision

Evaluate the accuracy and the precision of the method on three batches. Five samples are prepared from each batch. Each sample is injected three times.

Sample and standard solutions preparation Prepare the sample and the standard solutions as directed under Selectivity mentioned above.

#### 2-4-4-3-Ruggedness

Prepare a sample and a standard solution as directed under Selectivity, and analyze according to the following criteria

### 1- Different elapsed assay times

Analyze the same sample solution after 15 minutes, and after 3 hours of sample preparing. The samples are kept at room temperature. Inject each sample two times for three batches.

### 2- Different sample temperature

Analyze the same sample solution at temperature of 20 °c and another at 40 °c. Inject each sample two times for three batches.

### 3- Different columns

Analyze the same sample using three different columns but the same type. Inject each sample two times for three batches.

### 2-4-4-4-Linearity

#### Samples Preparation

##### -Preparation of stock solution

Shake 3.75 gm of sample with about 60 ml of methanol for 20 min.. Filter through Whatman filter paper No. 4. Wash the filter paper by about 30 ml methanol. Add the washings to the filtrate. Complete the volume to 100.0 ml using the same solvent. Filter the sample through a 0.22 µm Millipore filter paper.

##### Preparation of 3.75% solution

Dilute 5 ml of stock solution to 50 ml using methanol as a diluent.

##### Preparation of 2.5% solution

Dilute 3.33 ml of stock solution to 50 ml using ethanol as a diluent.

##### Preparation of 3.125% solution

Mix well 10.0 ml of 3.75.0% solution with 10.0 ml of 2.5% solution.

##### Preparation of 1.875% solution

Mix well 10.0 ml of 3.75% solution with 10.0 ml of methanol.

Preparation of 1.25% solution

Mix well 10.0 ml of 2.5.0% solution with 10.0 ml of methanol.

#### Procedure

Follow HPLC assay method mentioned above (see 2-1).

Inject each Sample preparation three times for three batches.

#### 2-4-5-Acceptance Criteria

Parameter	Statistical measure	Limit
Selectivity	Resolution (R)	No interference between the active material peak and other peaks ( $R > 1.5$ )
	Percent of Recovery (placebo)	Zero
Accuracy	Percent of Recovery	98.00 - 102.00
Precision	Coefficient of Variation	Maximum 1.50
Ruggedness	Coefficient of Variation.	Maximum 1.50
Linearity and Range	Correlation Coefficient	Minimum 0.99980

#### 2-4-6-Validation Report

At final, a validation report should be prepared. The report should contain the, data, results, conclusion, and recommendation.

## 2-5-Stability Study

2-5-1-Kind of Study: Accelerated.

2-5-2- Trial Preparation

Carry out the tests on the best selected trial as mentioned in the trial preparation.

2-5-3-Analysis Methods

Use the HPLC assay method mentioned above (see 2-1).

2-5-4-Reference: New developed method.

2-5-5-Qty of samples

Keep 8 tubes of 15 gm for each batch. Keep three batches. (Total = 24 tubes)

2-5-6-Tests to be monitored

Assay, pH, color, odor, degradation, tackiness, greasiness, viscosity, skin allergies, microbial contamination, contamination, and physical stability.

2-5-7-Procedure

The samples of three batches are stored under condition (40 °c, 75% RH) for six months in cooled incubator. They should be analyzing at zero time, 30 days, 60 days, 90 days and at 180 days. This will lead to one of the following

If a significant change occurs under the mentioned condition, the master formula must be modified.

Fill the results of analysis for each batch in the format mentioned in table [2-14]

Table [2-14] results of analysis format for stability study with tests limits

Test	Limit
Physical stability	Separation is prohibited after centrifusion
Tackiness	Tackiness is prohibited after application
Greasiness	Greasiness is prohibited after application
Viscosity	11250 - 13500 cp
Appearance of applied film	Transparent after application
Skin allergies (irritation)	Irritation is prohibited
Assay	90.0 – 110.0 %
Identification (active)	HPLC Rt is identical to standard
Cream properties	Homogeneous, white, and smooth cream, free from air bubbles. The smell should be acceptable
pH	4.4 - 4.8
Grittiness	Smooth cream after application
Microbial contamination Cfu/gm	Total count $\leq$ 1000 Pseudomonas a. = 0 Staphylococcus. a. = 0

### 2-5-8-Stability Report

At final, a stability study report should be prepared. The report should contain the data, results, and conclusion.

### 2-6- Penetration Test

The penetration test should be performed using Franz Diffusion Cell.

#### 2-6-1- materials and tools

Phosphate buffer 7.4 , octanol (Merck), methanol (Merck, HPLC grade), ammonium acetate (Merck), Lamisil cream, thesis cream, Franz Diffusion Cell, HPLC (Merck,hitachi), (dialysis membrane 32/32, size 8, MW CO- 12000-14000 Daltons), organic solvent resistant millipore filter (45 mm\* 0.22  $\mu$ m).

## 2-6-2- Procedure

### -Preparation of dialysis membrane 32/32

Soak 2 pieces of 3.0 x 3.0 cm membrane in a 200 ml beaker containing about 100 ml distilled water for 24 hours.

### - Preparation of organic millipore filter (0.22 $\mu\text{m}$ )

Soak 2 pieces of 45 mm organic millipore filter (0.22  $\mu\text{m}$ ) in a 200 ml beaker containing about 50 ml octanol for 24 hours.

### -Franz Diffusion Cell Set up

The cell is set up at constant temperature of 32 °c by circulating water at 32 °c through an external water jacket. A layer of dialysis membrane is put on the surface of the receptor compartment followed by the millipore filter. Another dialysis membrane is put over the filter. A sample of about 0.5 g is transferred from each cream to the surface of the dialysis membrane with good distribution. The donor compartment must be covered with parafilm. The receptor compartment must be filled with phosphate buffer pH 7.4 and stirred by means of a spinning bar magnet. Receptor solution samples, 2.0 ml aliquates, must be withdrawn through the sampling port of the receptor compartment at various time intervals. The cells must be refilled with receptor solution to substitute the sampled quantity. The experiments should run for 6 hours.

### - Samples analysis

Take a sample every 60 minutes from each tested cream (thesis sample & Lamisil sample).

Use the HPLC analysis method described above (see 2-1) to determine the dissolved quantity at each stage.

Plot the data obtained to get the penetration curves for both formulas.

## 2-7- Phase Diagram Preparation

### 2-7-1- Materials

Tween 80 obtained from Eignmann & Veronelli S.P.A, B.No 1040210114.

Terbinafine HCl obtained from cross chem. International company, Switzerland, B.No 20041103

Water (purified) obtained from Milli Q apparatus,

### 2-7-2- Equipment & Tools

Water bath Julabo SW22

Vortex, Gennie vortex, scientific industries, USA.

Analytical balance Precisa XT 220A

Test tubes, 5 ml

### 2-7-3- Procedure

#### 2-7-3-1-Preparation of different concentrations of water and tween 80 mixtures

Prepare the quantity described in table [2-15] in a suitable container of about 15 gm. Mix well manually and by vortex. The samples are kept in water bath for 24 hours at room temperature.

#### 2-7-3-2- Phase Diagram Preparation

Prepare the mixtures using the quantity described in table [2-16] in test tubes of 5 ml. Mix well manually, and by vortex. The samples are sonicated for one hour and kept in water bath for 24 hours at room temperature, and then the tubes are centrifuged at 5000 r/m for 15 min.

Table [2-15] Preparation of Water-Tween 80 mixtures

Set #	Water%	Tween 80%	Wt Water	Wt Tween 80
1	100.0	0.0	15.000	0.000
2	95.0	5.0	14.250	0.750
3	90.0	10.0	13.500	1.500
4	85.0	15.0	12.750	2.250
5	80.0	20.0	12.000	3.000
6	75.0	25.0	11.250	3.750
7	70.0	30.0	10.500	4.500
8	65.0	35.0	9.750	5.250
9	60.0	40.0	9.000	6.000
10	55.0	45.0	8.250	6.750
11	50.0	50.0	7.500	7.500
12	45.0	55.0	6.750	8.250
13	40.0	60.0	6.000	9.000
14	35.0	65.0	5.250	9.750
15	30.0	70.0	4.500	10.500
16	25.0	75.0	3.750	11.250
17	20.0	80.0	3.000	12.000
18	15.0	85.0	2.250	12.750
19	10.0	90.0	1.500	13.500
20	5.0	95.0	0.750	14.250
21	0.0	100.0	0	15.000

Table [2-16] Qty of Terbinafine needed to be added to Tween 80, and Water mixtures

Terbinafine%	*Mixture%	Wt Terbinafine (gm)	Wt Mixture
100	0.0	2.500	0
90	10.0	2.250	0.250
80	20.0	2.000	0.500
70	30.0	1.750	0.750
60	40.0	1.500	1.000
50	50.0	1.250	1.250
40	60.0	1.000	1.500
30	70.0	0.750	1.750
20	80.0	0.500	2.000
10	90.0	0.250	2.250
5	95.0	0.125	2.375
4	96.0	0.100	2.400
3	97.0	0.075	2.425
2	98.0	0.050	2.450
1	99.0	0.025	2.475
0	100.0	0	2.500

### 3- DATA, CALCULATIONS & RESULTS

#### 3-1- Analysis Results of Raw material (Terbinafine HCl)

##### 3-1-1-Data

The data are summarized in Table [3-1]

Table [3-1]-Data for Terbinafine HCl analysis as raw material

TEST	DATA
Identification UV HPLC	Two Maxima peaks at 283 nm & 223 nm The retention time of sa and standard are identical
Melting Point	171 °c,
Solubility	Soluble in methanol, methylene chloride, ethanol, and slightly soluble in water
Color	A white to off-white crystalline powder
Odor	Odorless
Moisure content (KF)	0.24%
Assay (UV)	St: 1.694 AU, sa: 1.690 AU
Assay (titration)	Wt (sa) 500.0 mg, Volume of NaOH: 15.2 ml
Assay (HPLC)	Area (sa): 4556408 & 4523377, Area (st): 4484335, 4504211

##### 3-1-2-Calculation

$$\% \text{Terbinafine (UV)} = 1.690/1.694 * 100 = 99.80$$

$$\% \text{Terbinafine (Tit.)} = 15.1 * 32.7/500 * 100 = 99.41$$

%Terbinafine (HPLC)

$$[(4556408+4523377)/2]/[(4484335+4504211)/2] * 100 = 101.06$$

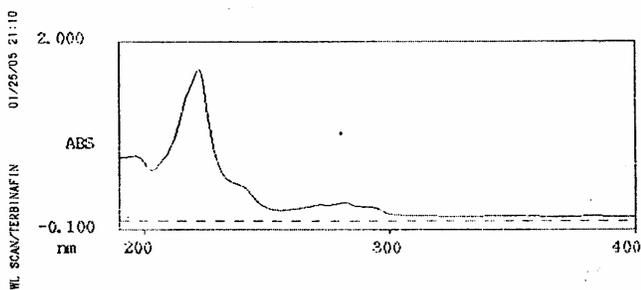
##### 3-1-3-Results (RM)

See Table [3-2]

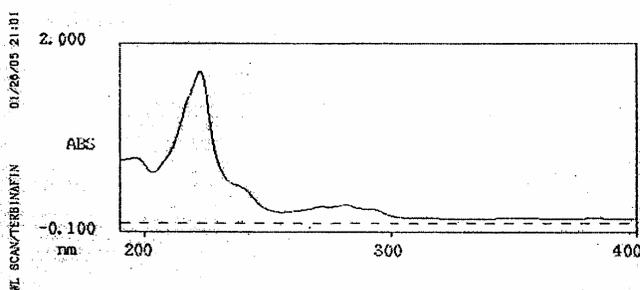
Table [3-2]-Result of Terbinafine HCl analysis as raw material

TEST	RESULT	LIMIT
Identification		
UV	Conform	Two Maxima peaks at 283 nm & 223 nm
HPLC	The retention times of sample and standard are identical	The retention times of sample and standard are identical
Melting Point	171 c°	169 - 172 c°
Solubility	Soluble in methanol	Soluble in methanol
Color	A white to offwhite crystalline powder	A white to offwhite crystalline powder
Odor	Odorless	odorless
Moisure content (KF)	0.24%	Max. 1.0%
Assay (UV)	99.76	98.0*102.0%
Assay (titration)	99.41%	98.0-102.0%
Degradation (HPLC)	Absent	Max 1.0%

Figure [3-1] and [3-2] UV spectrum for terbinafine HCl as raw material (standard and sample respectively)



NO.	Wavelength (nm)	Absorbance (ABS)
1	223.0	1.674
	203.0	0.369



NO.	Wavelength (nm)	Absorbance (ABS)
1	223.0	1.600
	203.5	0.564

### 3-2- Data & Result for Lamisil Cream & Trials

#### 3-2-1- Data & Result for Lamisil Cream Assay

B.No.: WB112

See Table [3-3]

Table [3-3]: Analytical data & results for Lamisil cream (brand) analysis by HPLC

Sample	Peak Area 1	Peak Area 2	Average	Result
Standard	4504211	4484335	4494273	101.17%
Lamisil	4547560	4546165	4546863	

#### 3-2-2- Data & Results for Trials 1-10 analysis (Assay)

Table [3-4]: Analytical data & results for trials 1-10 analyzed by HPLC

Sample	Peak Area	Result (Sa/St )
Standard	5094234	-
Trial 1	5113111	100.37
Trial 2	5245922	102.98
Trial 3	5130351	100.71
Trial 4	5170592	101.50
Trial 5	5133338	100.77
Trial 6	5189892	101.88
Trial 7	5162534	101.34
Trial 8	5137193	100.84
Trial 9	5172290	101.53
Trial 10	5177681	101.64

### 3-2-3- Data & Results for Trials 11-18 analysis (Assay)

Table [3-5]: Analytical data for the trials 11-18 analyzed by HPLC

Sample	Peak Area	Result (Sa/St)
Standard	4381545	—
Trial 11	4434701	101.21
Trial 12	4374760	99.85
Trial 13	4405891	100.56
Trial 14	4404382	100.52
Trial 15	4364742	99.62
Trial 16	4378750	99.94
Trial 17	4385059	100.08
Trial 18	4409452	100.64

### 3-2-4- Data & Results for Content Uniformity

#### Trial # 12: Uniformity of Content Data

Table [3-6]: Analytical data & results for trial # 12, analyzed by HPLC for content uniformity test

St	Sample A	% Av. Result sa A	Sample B	% Av Result sa B	Sample C	% Av Result sa C	Limit
4381545	4316891	99.09	4399268	99.66	4390239	99.87	85.00%-115.00%
4433106	4399268		4366658		4394429		

#### Trial # 13: Uniformity of Content Data

Table [3-7]: Analytical data & results for trial # 13 analyzed by HPLC for content uniformity test

St	Sample A	% Av Result sa A	Sample B	% Av Result sa B	Sample C	% Av Result sa C	Limit
4381545	4468538	101.42	4440330	100.83	4505771	101.96	85.00%-115.00%
4433106	4452419		4428224		4462840		

#### Trial # 14: Uniformity of Content Data

Table [3-8]: Analytical data & results for trial #14 analyzed by HPLC for content uniformity test

St	Sample A	% Av Result sa A	Sample B	% Av Result sa B	Sample C	% Av Result sa C	Limit
4381545	4450904	99.02	4444512	101.32	4462462	101.34	85.00%-115.00%
4433106	4258412		4467407		4451291		

## Trial # 15: Uniformity of Content Data

Table [3-9]: Analytical data & results for trial # 15 analyzed by HPLC for content uniformity test

St	Sample A	% Av Result sa A	Sample B	% Av Result sa B	Sample C	% Av Result sa C	Limit
4381545	4463765	101.51	4367212	99.96	4517198	102.67	85.00%-115.00%
4433106	4464526		4425422		4513245		

## Final Results (Content Uniformity)

From the data mentioned above, the content uniformity test is pass for all trials (12 -15).

## Results for Lamisil analysis (All tests)

Table [3-10]: Analytical result for Lamisil cream (brand)

TEST	RESULTS
Viscosity	12200 cps
Organoleptic properties Color Odor Appearance	White homogeneous cream Acceptable odor Free from air bubbles Free from dark impurities
Phase stability (centrifusion)	No separation
pH	4.65
Greasiness	No grease after application
Allergies	No irritation
Microbial contamination cfu/g Total count Pseudomonas a. Staphylococcus a.	<23 Absent Absent
Tackiness	Untacky cream after application
Grittiness	No grittiness after application
Degradation	Absent
Assay	101.17%
Analysis consideration	No analytical problems

According to the result of analysis for Lamisil cream, our generic cream should be conformable to the brand product especially for composition, pH ( $\pm 0.2$ ), also for the viscosity ( $\pm 10\%$ ), and the other tests mentioned in the certificate.

### 3-3- Trials Results

#### 3-3-1-Selection of Excipients

Tests: Viscosity, Colour, Odour, Centrifusion (phase separation), pH, Smoothness, Greasiness, Irritation, Microbial contamination, Degradation, Tackiness and Assay.

##### 3-3-1-1-Selection of Emulsifying Agents

1-: Using of Glyceryl Mono Stearate GMS (SE)

Trial # 1, See Table [3-11]

Table [3-11]: Analysis results for Terbinafine cream using GMS (SE) as an emulsifying agent

TEST	RESULTS
Viscosity	6212 cps
Organoleptic properties	
Color	White homogeneous cream
Odor	Acceptable odor
Appearance	Free from air bubbles and impurities
Phase stability (centrifusion)	Slight separation
pH	4.60
Greasiness	No grease after application
Skin allergies	No irritation
Microbial contamination cfu/g	
Total count	Total count: 350
Pseudomonas a.	Absent
Staphylococcus a.	Absent
Tackiness	Untacky cream after application
Grittiness	Untacky cream after application
Degradation	Absent
Assay	100.4%
Analysis consideration	No analytical problems

## 2- Using of Eumulgin B2

See Table [3-12]

Trial # 2

Table [3-12]: Analysis results for Terbinafine cream using Eumulgin B2 as an emulsifying agent

TEST	RESULTS
Viscosity	5863 cps
Organoleptic Properties Color Odor Appearance	White homogeneous cream Acceptable odor Free from air bubbles Free from dark impurities
Phase stability (centrifusion)	Slight separation
pH	4.54
Greasiness	No grease after application
Skin allergies	No irritation
Microbial contamination cfu/g Total count Pseudomonas a. Staphylococcus a.	Total count: 275 Absent Absent
Tackiness	Untacky cream after application
Grittiness	No greatness after application
Degradation	Absent
Assay	100.7%
Analysis consideration	No analytical problems

### 3-: Using of Cetostearyl Alcohol

See Table [3-13]

Trial # 3

Table [3-13]: Analysis results for Terbinafine cream using cetostearyl alcohol as an emulsifying agent

TEST	RESULTS
Viscosity	2902 cps
Organoleptic Properties Color Odor Appearance	White homogeneous cream Acceptable odor Free from air bubbles Free from dark impurities
Phase stability (centrifusion)	Separation of small phase
pH	4.75
Greasiness	No grease after application
Skin allergies	No irritation
Microbial contamination cfu/g Total count Pseudomonas a. Staphylococcus a.	Total count: 325 Absent Absent
Tackiness	Untacky cream after application
Grittiness	No Grittiness after application
Degradation	Absent
Assay	100.7%
Analysis consideration	No analytical problems

#### 4- Using of Cetostearyl Alcohol and GMS (SE)

See Table [3-14]

Trial # 4

Table [3-14]: Analysis results for Terbinafine cream using GMS (SE) & cetostearyl alcohol as emulsifying agents

TEST	RESULTS
Viscosity	7046 cps
Organoleptic Properties Color Odor Appearance	White homogeneous cream Acceptable odor Free from air bubbles Free from dark impurities
Phase stability (centrifusion)	No Separation
pH	4.51
Greasiness	No grease after application
Skin allergies	No irritation
Microbial contamination cfu/g Total count Pseudomonas a. Staphylococcus a.	Total count: 180 Absent Absent
Tackiness	Untacky cream after application
Grittiness	No Grittiness after application
Degradation	Absent
Assay	101.5%
Analysis consideration	No analytical problems

## 5-: Using of Cetostearyl Alcohol and Eumulgin B2

See Table [3-15]

Trial # 5

Table [3-15]: Analysis results for Terbinafine cream using emulgin B2 & cetostearyl alcohol as emulsifying agents

TEST	RESULTS
Viscosity	7810 cps
Organoleptic Properties Color Odor Appearance	White homogeneous cream Acceptable odor Free from air bubbles Free from dark impurities
Phase stability (centrifusion)	Separation of small phase
pH	4.70
Greasiness	No grease after application
Skin allergies	No irritation
Microbial contamination cfu/g Total count Pseudomonas a. Staphylococcus a.	Total count: 225 Absent Absent
Tackiness	Untacky cream after application
Grittiness	No Grittiness after application
Degradation	Absent
Assay	100.7%
Analysis consideration	No analytical problems

## 6-: Using of GMS (SE) and Eumulgin B2

See Table [3-16]

Trial # 6

Table [3-16]: Analysis results for Terbinafine cream using GMS (SE) & Eumulgin B2 as emulsifying agents

TEST	RESULTS
Viscosity	10734 cps
Organoleptic Properties Color Odor Appearance	White homogeneous cream Acceptable odor Free from air bubbles Free from dark impurities
Phase stability (centrifusion)	No separation
pH	4.58
Greasiness	No grease after application
Skin allergies	No irritation
Microbial contamination cfu/g Total count Pseudomonas a. Staphylococcus a.	Total count: 330 Absent Absent
Tackiness	Untacky cream after application
Grittiness	No Grittiness after application
Degradation	Absent
Assay	101.9%
Analysis consideration	No analytical problems

## 7- Using of Cetostearyl Alcohol, GMS (SE) and Eumulgin B2

See Table [3-17]

Trial # 7

Table [3-17]: Analysis results for Terbinafine cream using GMS (SE), Eumulgin B2 & Cetostearyl alcohol as emulsifying agents

TEST	RESULTS
Viscosity	14160 cps
Organoleptic Properties Color Odor Appearance	White homogeneous cream Acceptable odor Free from air bubbles Free from dark impurities
Phase stability (centrifusion)	No separation
pH	4.57
Greasiness	No grease after application
Skin allergies	No irritation
Microbial contamination cfu/g Total count Pseudomonas a. Staphylococcus a.	Total count: 385 Absent Absent
Tackiness	Untacky cream after application
Grittiness	No Grittiness after application
Degradation	Absent
Assay	101.3%
Analysis consideration	No analytical problems

## 8-: Using of Cetostearyl Alcohol, GMS (SE) and Eumulgin B2 (Change qty)

See Table [3-18]

Trial # 8

Table [3-18]:Analysis results for Terbinafine cream using GMS (SE), Eumulgin B2 & Cetostearyl alcohol as emulsifying agents (different qty)

TEST	RESULTS
Viscosity	12736 cps
Organoleptic Properties Color Odor Appearance	White homogeneous cream Benzyl alcohol odor Free from air bubbles Free from dark impurities
Phase stability (centrifusion)	No separation
pH	4.65
Greasiness	No grease after application
Skin allergies	No irritation
Microbial contamination cfu/g Total count Pseudomonas a. Staphylococcus a.	Total count: 362 Absent Absent
Tackiness	Untacky cream after application
Grittiness	No Grittiness after application
Assay	100.8%
Analysis consideration	No analytical problems

### 3-3-2- Selection of Preservative

#### a- Using of Phenoxy - Ethanol

See Table [3-19]

Trial # 9

Table [3-19]:Analysis results for Terbinafine cream using phenoxy ethanol, as a preservative

TEST	RESULTS
Viscosity	12912 cps
Organoleptic Properties Color Odor Appearance	White homogeneous cream Acceptable odor Free from air bubbles Free from dark impurities
Phase stability (centrifusion)	No separation
pH	4.71
Greasiness	No grease after application
Skin allergies	No irritation
Microbial contamination cfu/g Total count Pseudomonas a. Staphylococcus a.	Total count: 213 Absent Absent
Tackiness	Untacky cream after application
Grittiness	No Grittiness after application
Assay	101.5%
Analysis consideration	No analytical problems

b- Using of Chloro –M- Cresol

See Table [3-20]

Trial # 10

Table [3-20]:Analysis results for Terbinafine cream using chloro-m-cresol as a preservative

TEST	RESULTS
Viscosity	13001 cps
Organoleptic Properties Color Odor Appearance	White homogeneous cream Acceptable odor Free from air bubbles Free from dark impurities
Phase stability (centrifusion)	No separation
pH	4.60
Greasiness	No grease after application
Skin allergies	No irritation
Microbial contamination cfu/g Total count Pseudomonas a. Staphylococcus a.	Total count: <23 Absent Absent
Tackiness	Untacky cream after application
Grittiness	No Grittiness after application
Assay	101.6%
Analysis consideration	No analytical problems

c- Using of MP & PP

See Table [3-21]

Trial # 11

Table[3-21]: Analysis results for Terbinafine cream using MP & PP as preservatives

TEST	RESULTS
Viscosity	12759 cps
Organoleptic Properties Color Odor Appearance	White homogeneous cream Acceptable odor Free from air bubbles Free from dark impurities
Phase stability (centrifusion)	No separation
pH	4.69
Greasiness	No grease after application
Skin allergies	No irritation
Microbial contamination cfu/g Total count Pseudomonas a. Staphylococcus a.	Total count: < 23 3 Absent
Tackiness	Untacky cream after application
Grittiness	No Grittiness after application
Assay	101.2%
Analysis consideration	Bad resolution

3-3-3- Selection mixing procedure  
 3-3-3-1 Selection of Mixing Speed

1-Mixing at 20 r/m

See Table [3-22]

Trial # 12

Table[3-22]: Analysis results for Terbinafine cream at mixing speed 20 rpm

TEST	RESULTS
Viscosity	13111 cps
Organoleptic Properties	
Color	White homogeneous cream
Odor	Acceptable odor
Appearance	No air bubbles Free from dark impurities
Phase stability (centrifusion)	Slight separation
pH	4.70
Greasiness	No grease after application
Tackiness	Untacky cream after application
Grittiness	No Grittiness after application
Assay	102.1%
Uniformity of content	Conform

## 2- Mixing at 80 r/m

See Table [3-23]

Trial # 13

Table [3-23]: Analysis results for Terbinafine cream at mixing speed 80 rpm

TEST	RESULTS
Viscosity	12389 cps
Organoleptic Properties Color Odor Appearance	White homogeneous cream Acceptable odor No air bubbles Free from dark impurities
Phase stability (centrifusion)	No separation
pH	4.66
Greasiness	No grease after application
Tackiness	Untacky cream after application
Grittiness	No Grittiness after application
Assay	100.6%
Uniformity of content	Conform

### 3- Mixing at 120 r/m

See Table [3-24]

Trial # 14

Table [3-24]: Analysis results for Terbinafine cream at mixing speed 120 rpm

TEST	RESULTS
Viscosity	12444 cps
Organoleptic Properties Color Odor Appearance	White homogeneous cream Acceptable odor No air bubbles Free from dark impurities
Phase stability (centrifusion)	No separation
pH	4.66
Greasiness	No grease after application
Tackiness	Untacky cream after application
Grittiness	No Grittiness after application
Assay	100.5%
Uniformity of content	Conform

4- Mixing at 180 r/m

See Table [3-25]

Trial # 15

Table[3-25]: Analysis results for Terbinafine cream at mixing speed 180 rpm

TEST	RESULTS
Viscosity	11430 cps
Organoleptic Properties	
Color	White homogeneous cream
Odor	Acceptable odor
Appearance	No air bubbles, Free from dark impurities
Phase stability (centrifusion)	No separation
pH	4.60
Greasiness	No grease after application
Microbial contamination cfu/g	
Total count	Total count: 23
Pseudomonas a.	Absent
Staphylococcus a.	Absent
Tackiness	Untacky cream after application
Grittiness	No Grittiness after application
Assay	99.6%
Uniformity of content	Conform

### 3.3-3-2- Selection of Temperature for Active material Addition

Trial 16, 17 & 18, See Table [3-26]

Table [3-26]: Analysis results for Terbinafine cream while it is added at different temperatures

TRIAL NO.	TEMP.	ASSAY	DEGRADATION	ASSAY
T16	70 °c	99.9%	Absent	99.9%
T17	60 °c	100.1%	Absent	100.1%
T18	50 °c	100.6%	Absent	100.6%

### 3-3-4-Summary For Trials Results

A summary for all trials is described in Table [3-27] that shows the aim, test, result and accepting of refusing the trial.

Table [3-27]: Summary for all trials including the tests, results and reason for accepting or refusing the trial

T #	Aim	Test	Status of formula	Results
T1	GMS (SE) as emulsifier	Centrifusion	Fail, No Stability	Slight separation
T2	Eumulgin B2 as emulsifier	Centrifusion	Fail, No Stability	Slight separation
T3	Cetostearyl alcohol as emulsifier	Centrifusion	Fail, No Stability	Small separation
T4	Cetostearyl alcohol & GMS (SE) as emulsifiers	Viscosity	Fail, Lower than Lamisil [ by > 10%]	7046
T5	Cetostearyl alcohol & Emulgin B2 as emulsifiers	Centrifusion	Fail, Lower than Lamisil [ by > 10%]	7810
T6	Eumulgin B2 & GMS (SE) as emulsifiers	Centrifusion	Fail, Lower than Lamisil [ by > 10%]	11734
T7	Cetostearyl alcohol & GMS (SE) & Eumulgin B2 as emulsifiers	Centrifusion	Fail, higher than Lamisil [ by > 10%]	14160
T8	Cetostearyl alcohol & GMS (SE) & Eumulgin B2 as emulsifiers (different qty)	All tests	Good formula	12736
T9	Phenoxy ethanol as preservative	Microbial contamination	Fail, No good inhibition for Contam.	Some contamination
T12	Mixing speed at 20 r/m	Centrifusion	Separation	Fail, No Stability
T13	Mixing speed at 80 r/m	All tests	No problem	Good
T14	Mixing speed at 120 r/m	All tests	No problem	Good
T15	Mixing speed at 180 r/m	Appearance	Air bubbles	Appearance fail
T16	Addition active at 70 °c	Degradation	No problem	Good
T17	Addition active at 60 °c	Degradation	No problem	Good
T18	Addition active at 50 °c	Degradation	No problem	Good

### 3-4-Validation Result

#### 3-4-1-Selectivity

##### 3-4-1-1-Limit

Resolution factor:  $> 1.5$  , Recovery: 98.0-102.0  
Rt for st & sa should be identical  
so Placebo should have no peak at Terbinafine RT.

##### 3-4-1-2-Data, Calculation and Results

From the data shown in figure [3-3]

Resolution factor:  $2(2.13-1.24)/0.24-0.12 = 5.0$

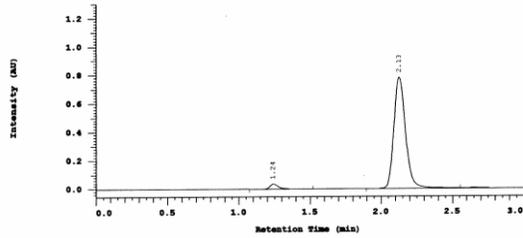
Rt (st): 2.13 min.,

Rt (st): 2.13 min., so they are identical

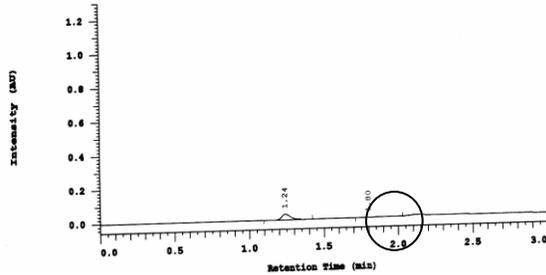
Assay: 101.0%

No overlapping, resolution factor  $\gg 1.5$ , Rt is identical, % Recovery: 101.0%, So the method is selective.

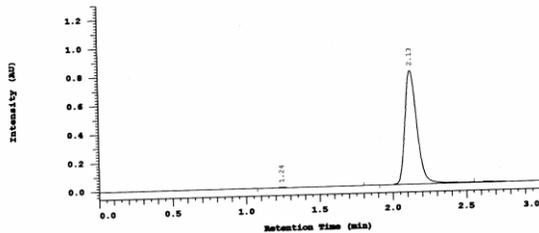
## Selectivity Result



Sample



Placebo (no peak near 2.13 min)



Standard

Rt (st): 2.13 min., Rt (st): 2.13 min

Resolution factor:  $2(2.13-1.24)/0.24-0.12=5.0$

Figure [3-3] selectivity results

### 3-4-2-Accuracy and Precision

#### 3-4-2-1- Data & Calculation

Sample	Peak Area
standard 1	4,615,110
standard 2	4,623,446
Average	4,619,278

#### Batch 1

Sample	Peak Area V1	% Assay	P. Area V2	Assay	Peak Area V3	Assay
sample 1	4,612,341	99.85%	4,623,574	100.093%	4,621,704	100.053%
sample 2	4,641,575	100.48%	4,627,452	100.177%	4,620,650	100.030%
sample 3	4,628,710	100.20%	4,629,748	100.227%	4,620,098	100.018%
sample 4	4,634,868	100.34%	4,615,634	99.921%	4,632,729	100.291%
sample 5	4,633,868	100.32%	4,615,638	99.921%	4,619,183	99.998%
Average	4,630,272	100.24%	4,622,409	100.068%	4,622,873	100.078%
Coeff. Of var.	0.2390		0.1420		0.1200	
Av. coef. of var.	0.1670					

#### Batch 2

Sample	Peak Area V1	Assay	P. Area V2	Assay	Peak Area V3	Assay
sample 1	4,616,346	99.937%	4,617,100	99.953%	4,626,778	100.162%
sample 2	4,621,628	100.05%	4,628,996	100.210%	4,630,868	100.251%
sample 3	4,618,458	99.982%	4,620,738	100.032%	4,627,052	100.168%
sample 4	4,636,725	100.38%	4,625,563	100.136%	4,629,589	100.223%
sample 5	4,636,330	100.37%	4,626,913	100.165%	4,627,052	100.168%
Average	4,625,897	100.14%	4,623,862	100.099%	4,628,268	100.195%
Coeff. Of var.	0.2140		0.0010		0.0004	
Av. coef. of var.	0.0718					

Batch 3

Sample	Peak Area V1	Assay	P. Area V2	Assay	Peak Area V3	Assay
sample 1	4,635,631	1.00	4,603,234	99.653%	4,595,080	99.476%
sample 2	4,634,600	100.33%	4,592,505	99.420%	4,629,714	100.226%
sample 3	4,594,949	99.47%	4,610,414	99.808%	4,623,889	100.100%
sample 4	4,634,029	100.32%	4,626,254	100.151%	4,619,517	100.005%
sample 5	4,732,844	102.46%	4,616,448	99.939%	4,609,687	99.792%
Average	4,646,411	100.59%	4,609,771	99.794%	4,615,577	99.920%
Coeff. Of var.	1.1000		0.0028		0.0029	
Av. coef. of var.	0.37					

3-4-2-2-Results

Accuracy

Batch	Batch 1	Batch 2	Batch 3	Limit
%Recovery	100.1	100.1	100.1	98.0%-102.0
Av.(% Recovery)	100.1			98.0%-102.0

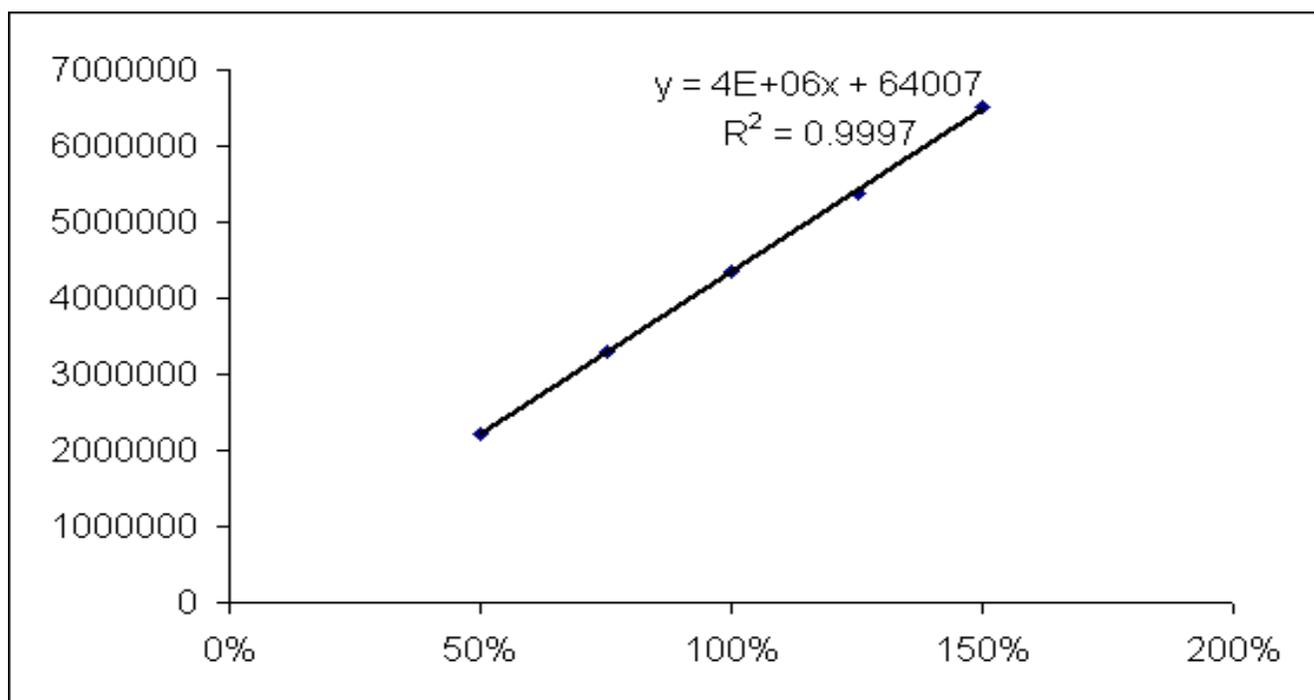
Precision

Batch	Batch 1	Batch 2	Batch 3	Limit
Coeff. of var.	0.167	0.072	0.369	Max 1.5
Av. coef. of var.	0.202			Max 1.5

### 3-4-3-Linearity

#### Data and Results for Batch 1

Conc.	P. Area sa 1	P. Area sa 2	P. Area sa 3	Peak Area Av.
50%	2227663	2192040	2199413	2206372
75%	3282383	3290950	3287233	3286855
100%	4341119	4338800	4342678	4340866
125%	5369006	5369621	5362946	5367191
150%	6525758	6501894	6518412	6515355
Average				4343328



#### Calculation

1-Correlation coefficient (correl)= square root of  $R^2$   
= square root of 0.9997 = 0.99985

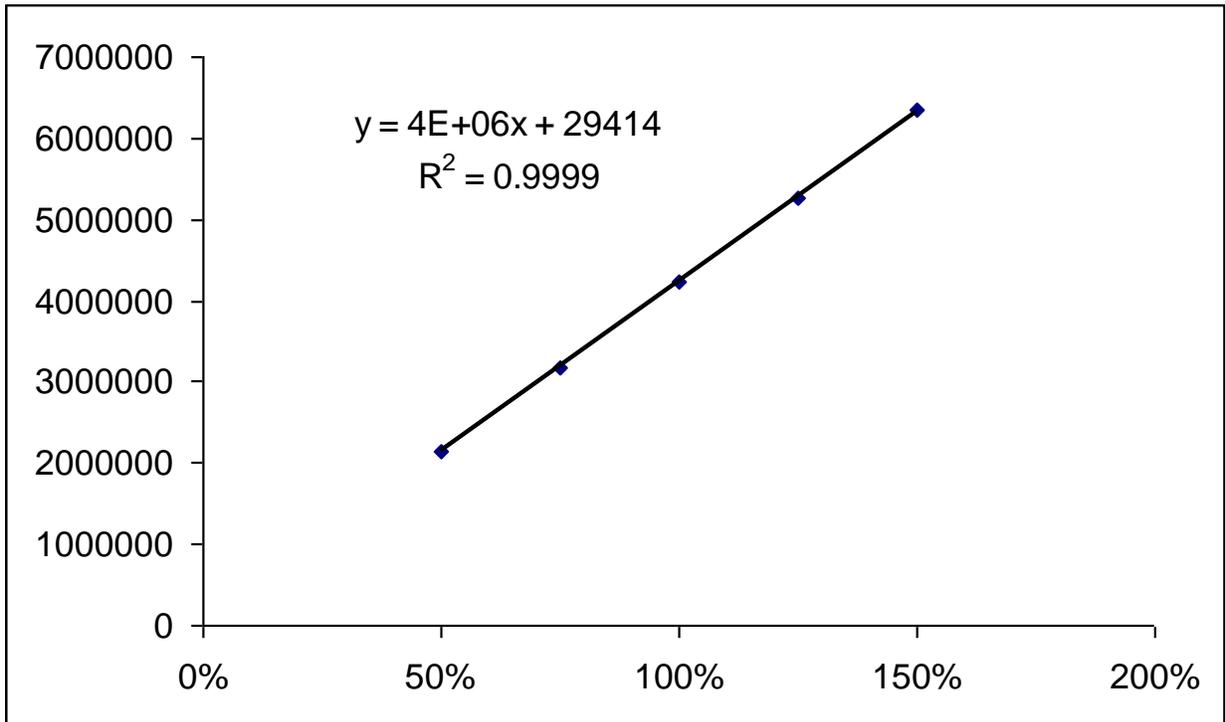
2- %Y-intercept =  $(64007/4343328) * 100 = 1.49$

Data and Results for Batch 2

Conc.	P. Area sa 1	P. Area sa 2	P. Area sa 3		Peak Ar
50%	2129675	2137955	2131978		2133
75%	3194978	3177527	3176264		3182
100%	4239913	4213709	4246936	4233519	
125%	5267740	5259401	5260527	5262556	
150%	6345254	6358644	6334777	6346225	

Average

4231685



Calculation

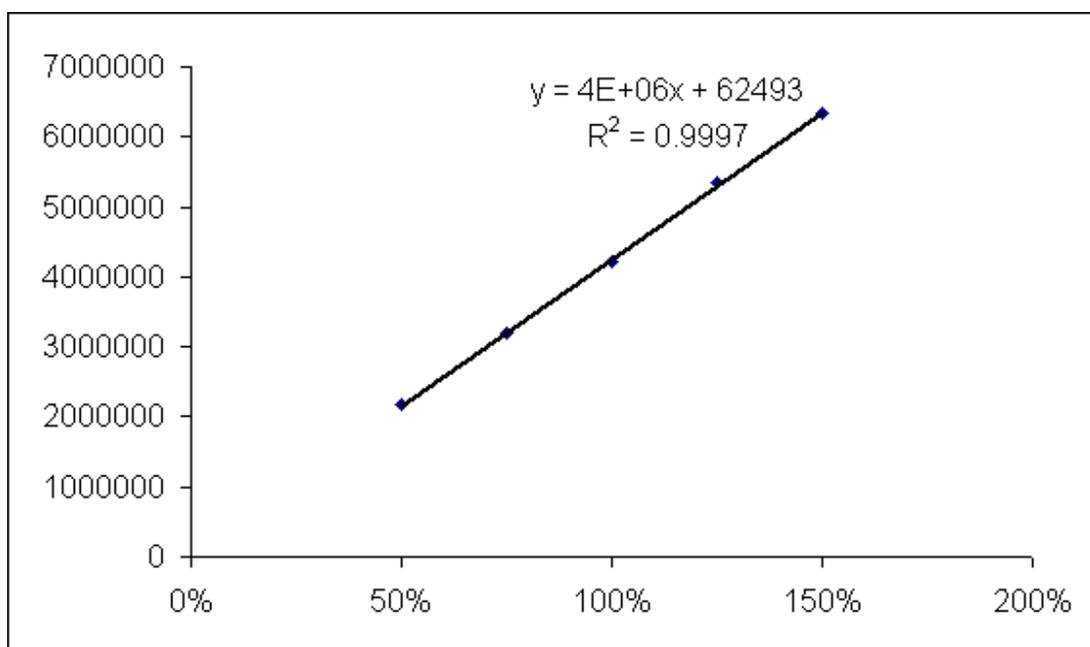
- 1- Correlation coefficient (correl) = square root of  $R^2$   
 = square root of 0.9999 = 0.99995
- 2- %Y-intercept =  $(29414/4231685) * 100 = 0.7$

### Data and Results for Batch 3

Conc	P. Area sa1	P. Area sa2	P. Area sa3	Peak Area Av.
50%	2167579	2178168	2167576	2171108
75%	3176905	3204378	3178163	3186482
100%	4195588	4225100	4247268	4222652
125%	5301964	5450066	5276885	5342972
150%	6298426	6310853	6372922	6327400

Average

4250123



### Calculation

1-Correlation coefficient (correl)= square root of 0.9997 = 0.99980

2- %Y-intercept =  $(62493/4250123) * 100 = 1.47$

### Calculation

Batch 1	Batch 2	Batch 3	Limit
0.99985	0.99995	0.99985	Min. 0.99980
1.49	0.70	1.47	Max. 2.00

From the above results for correlation coefficient and y intercept, the method is linear.

### 3-4-4-Ruggedness

#### 3-4-4-1- Data & Calculation

##### a-Sample preparation time

1- Keeping sample prepared for 15 minutes

##### Standard

Sample	Peak Area
st 1	4480064
st 2	4549655
Average	4514860

##### Batch 1

Sample	Peak Area	Assay
sa 1	4524730	100.22
sa 2	4510014	99.89
Average	4517372	100.06
Coeff. of variation	0.2300	

##### Batch 2

Sample	Peak Area	Assay
sa 1	4478768	99.20
sa 2	4502534	99.73
Average	4490651	99.46
Coeff. of variation	0.3700	

Batch 3

Sample	Peak Area	Assay
sa 1	4495224	99.57
sa 2	4473364	99.08
Average	4484294	99.32
Coeff. of variation	0.3400	

2- Keeping sample prepared for 180 minutes

Batch 1

Sample	Peak Area	Assay
sa 1	4501286	99.70
sa 2	4506154	99.81
Average	4503720	99.75
Coeff. of variation	0.0760	

Batch 2

Sample	Peak Area	Assay
sa 1	4496748	99.60
sa 2	4491729	99.49
Average	4494239	99.54
Coeff. of variation	0.0790	

Batch 3

Sample	Peak Area	Assay
sa 1	4514123	99.98
sa 2	4519068	100.09
Average	4516596	100.04
Coeff. of variation	0.0390	

## b- Sample Temperature

### 1- Sample Temperature at 20 °c

#### Batch 1

Sample	Peak Area	Assay
sa 1	4496286	99.59
sa 2	4512942	99.96
Average	4504614	99.77
Coeff. Of variation	0.2600	

#### Batch 2

Sample	Peak Area	Assay
sa 1	4513344	99.97
sa 2	4507508	99.84
Average	4510426	99.90
Coeff. of variation	0.0900	

#### Batch 3

Sample	Peak Area	Assay
sa 1	4542486	100.61
sa 2	4524722	100.22
Average	4533604	100.42
Coeff. of variation	0.2800	

2- Sample Temperature at 40 °c

Batch 1

Sample	Peak Area	Assay
sa 1	4525102	100.23
sa 2	4525015	100.22
Average	4525059	100.23
Coeff. Of variation	0.0010	

Batch 2

Sample	Peak Area	Assay
sa 1	4503700	99.75
sa 2	4528192	100.30
Average	4515946	100.02
Coeff. of variation	0.3800	

Batch 3

Sample	Peak Area	Assay
sa 1	4517373	100.06
sa 2	4507328	99.83
Average	4512351	99.94
Coeff. of variation	0.1600	

C- Using different columns

1- Column A

Batch 1

Sample	Peak Area	Assay
sa 1	4545279	100.67
sa 2	4515068	100.00
Average	4530174	100.34
Coeff. of variation	0.4700	

Batch 2

Sample	Peak Area	Assay
sa 1	4529186	100.32
sa 2	4496736	99.60
Average	4512961	99.96
Coeff. of variation	0.5100	

Batch 3

Sample	Peak Area	Assay
sa 1	4482638	99.29
sa 2	4524847	100.22
Average	4503743	99.75
Coeff. of variation	0.6600	

2- Column B

Batch 1

Sample	Peak Area	Assay
sa 1	4512393	99.95
sa 2	4498122	99.63
Average	4505258	99.79
Coeff. of variation	0.2200	

Batch 2

Sample	Peak Area	Assay
sa 1	4495395	99.57
sa 2	4480992	99.30
Average	4488194	99.44
Coeff. of variation	0.1900	

Batch 3

Sample	Peak Area	Assay
sa 1	4521899	100.16
sa 2	4509748	99.89
Average	4515824	100.02
Coeff. of variation	0.1900	

3-Column C

Batch 1

Sample	Peak Area	Assay
sa 1	4492732	99.51
sa 2	4506516	99.82
Average	4499624	99.66
Coeff. of variation	0.2160	

Batch 2

Sample	Peak Area	Assay
sa 1	4495811	99.58
sa 2	4486266	99.37
Average	4491039	99.47
Coeff. Of variation	0.1500	

Batch 3

Sample	Peak Area	Assay
sa 1	4482638	99.29
sa 2	4524847	100.22
Average	4503743	99.75
Coeff. of variation	0.6610	

### 3-4-4-2-Results

Sample preparation time 15 minutes

Batch #	% Assay	Limit	Coeff. Of variation	Limit
Batch 1	100.06	98.00-102.00	0.230	Max 1.500
Batch 2	99.46	98.00-102.00	0.370	Max 1.500
Batch 3	99.32	98.00-102.00	0.340	Max 1.500
Average	99.61	98.00-102.00	0.313	Max 1.500

Sample preparation time 180 minutes

Assay	%Result	Limit	Coeff. of variation	Limit
Batch 1	99.75	98.00-102.00	0.076	Max 1.500
Batch 2	99.54	98.00-102.00	0.079	Max 1.500
Batch 3	100.04	98.00-102.00	0.039	Max 1.500
Average	99.78	98.00-102.00	0.065	Max 1.500

Sample T: 20 °c

Assay	%Result	Limit	Coeff. of variation	Limit
Batch 1	99.77	98.00-102.00	0.260	Max 1.500
Batch 2	99.90	98.00-102.00	0.090	Max 1.500
Batch 3	100.42	98.00-102.00	0.280	Max 1.500
Average	100.03	98.00-102.00	0.210	Max 1.500

Sample T: 40 °c

Assay	%Result	Limit	Coeff. Of variation	Limit
Batch 1	100.23	98.00-102.00	0.001	Max 1.500
Batch 2	100.02	98.00-102.00	0.380	Max 1.500
Batch 3	99.94	98.00-102.00	0.160	Max 1.500
Average	100.06	98.00-102.00	0.180	Max 1.500

Using column A

Assay	%Result	Limit	Coeff. Of variation	Limit
Batch 1	100.34	98.00-102.00	0.470	Max 1.500
Batch 2	99.96	98.00-102.00	0.510	Max 1.500
Batch 3	99.75	98.00-102.00	0.660	Max 1.500
Average	100.02	98.00-102.00	0.547	Max 1.500

Using column B

Assay	%Result	Limit	Coeff. of variation	Limit
Batch 1	99.96	98.00-102.00	0.220	Max 1.500
Batch 2	99.75	98.00-102.00	0.190	Max 1.500
Batch 3	99.95	98.00-102.00	0.190	Max 1.500
Average	99.89	98.00-102.00	0.200	Max 1.500

Using column C

Assay	%Result	Limit	Coeff. of variation	Limit
Batch 1	99.75	98.00-102.00	0.216	Max 1.500
Batch 2	99.95	98.00-102.00	0.150	Max 1.500
Batch 3	99.57	98.00-102.00	0.661	Max 1.500
Average	99.76	98.00-102.00	0.342	Max 1.500

## Final Results for Ruggedness

From the data mentioned above, all cases (conditions) were pass, no effect for changing time of preparation between 15 min and 180 minutes, also elevation in the temperature of the prepared solution from 20 °c to 40 °c did not affect on the accuracy and precision. Replacing the column used by other two columns did not also affect on accuracy and precision. So the method is valid for all mentioned conditions.

### 3-5- STABILITY STUDY

The study was carried out on three batches ( 1, 2 & 3) using formula and mixing method of trial # 18. Also using method of analysis mentioned above. To ensure that the assay method is stability indicator, analysis for Terbinafine samples stored under stress condition was done. The stress conditions were; in solution of 4 N HCl, 2 N NaOH, 7.5% H<sub>2</sub>O<sub>2</sub> and in hot water solution, all were kept for an hour before analysis, see figure [3-4].

Each batch was injected two times. The study aim is to check the stability of the cream under accelerated stability study after 1,2,3 and 6 months of manufacturing date.

#### 3-5-1-Data (Assay)

See table [3-28]

Table [3-28] stability study HPLC peak areas data for three batches through 6 months

Peak Area	(0-time)	(30-days)	(60-days)	(90-days)	(180-days)
St (vial 1)	4351282	4371962	4403995	4260571	4205144
St (vial 2)	4319170	4316433	4421488	4268531	4211010
St (av.)	4335226	4344198	4412742	4264551	4208077
batch 1(vial 1)	4378993	4439125	4511796	4349326	4232168
batch 1(vial 2)	4382746	4371962	4521782	4274779	4203851
batch 1(av.)	4380870	4405544	4516789	4312053	4218010
batch 2 (vial 1)	4378390	4405544	4454690	4258817	4227573
batch 2 (vial 2)	4369315	4452438	4456569	4240869	4213924
batch 2 (av.)	4373853	4428991	4455630	4249843	4220749
batch 3 (vial 1)	4367079	4460986	4450727	4357321	4158508
batch 3 (vial 2)	4348370	4455884	4520069	4317960	4233381
batch 3 (av.)	4357725	4458435	4485398	4337641	4195945

### 3-5-2- Accelerated Stability Study Results

Batch (1)

See table [3-29]

Table [3-29] stability study results of analysis for batch # 1 after re-cooling to room temperature

Test	0-Time	30-Days	60-Days	90-Days	180-Days	Limit -
	24/10/2002	23/11/2002	22/12/2002	24/1/2003	24/4/2003	
Assay	101.05%	101.41%	102.36%	101.11%	100.24%	90.00-110.00%
pH	4.65	4.6	4.63	4.58	4.64	4.40-4.80
Greasiness	no grease					
Tackiness	no tackiness	no tackiness	no tackiness	no tackiness	no tackiness	no tackiness
Appearance	white cream					
Odor	conform	conform	conform	conform	conform	conform
Degradation	no degrades					
Viscosity	12367	12120	12209	12115	12015	11000-14000 cps
Phase Stability	no separation					
Smoothness	smooth cream					
Microbial Cont.	<23 c/g					

2- Batch (2)  
See Table [3-30]

Table [3-30] stability study results of analysis for batch # 2 after re-cooling to room temperature

Test	0-Time	30-Days	60-Days	90-Days	180-Days	Limit
	24/10/2002	23/11/2002	22/12/2002	24/1/2003	24/4/2003	
Assay	100.89%	101.95%	100.97%	99.66%	100.30%	90.00-110.00%
pH	4.69	4.67	4.67	4.62	4.63	4.40-4.80
Grittiness	no grease					
Tackiness	no tackiness	no tackiness	no tackiness	no tackiness	no tackiness	no tackiness
Appearance	white cream					
Odor	conform	conform	conform	conform	conform	conform
Degradation	no degrades					
Viscosity	12301	11912	11896	11972	12013	11000-14000 cps
Phase Stability	no separation					
Smoothness	smooth cream					
Microbial Contamination.	<23 c/g					

3- Batch (3)  
See Table [3-31]

Table [3-31] stability study results of analysis for batch # 3 after re-cooling to room temperature

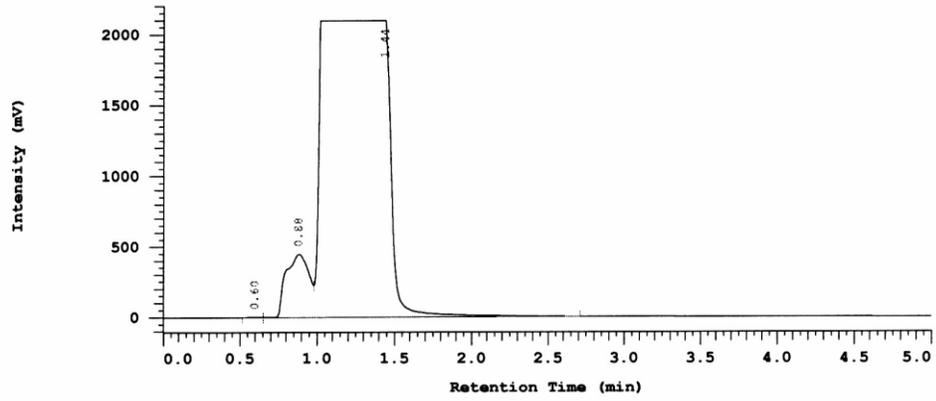
Test	0-Time	30-Days	60-Days	90-Days	180-Days	Limit
	24/10/2002	23/11/2002	22/12/2002	24/1/2003	24/4/2003	
Assay	100.52%	102.63%	101.65%	101.71%	99.71%	90.00-110.00%
pH	4.64	4.622	4.68	4.63	4.59	4.40-4.80
Grittiness	no grease					
Tackiness	no tackiness	no tackiness	no tackiness	no tackiness	no tackiness	no tackiness
Appearance	white cream					
Odor	conform	conform	conform	conform	conform	conform
Degradation	no degrades					
Viscosity	12910	11987	11885	12005	12112	11000-14000 cps
Phase Stability	no separation					
Smoothness	smooth cream					
Microbial Cont.	<23 c/g					

## Final Result for Stability Study

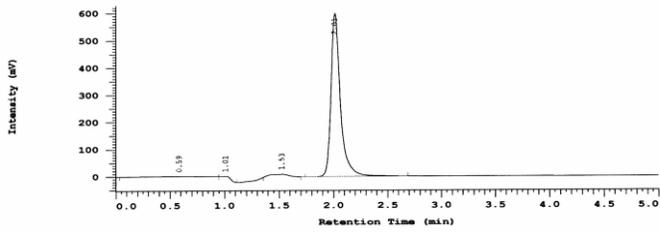
From the chromatograms shown in figure [3-4], there is a complete or partial destruction for Terbinafine HCl for all reagents react with. Therefore, the assay method is stability indicator. The selected formula (T18) is stable under accelerated stability study.

The product can be given two years as expiration time.

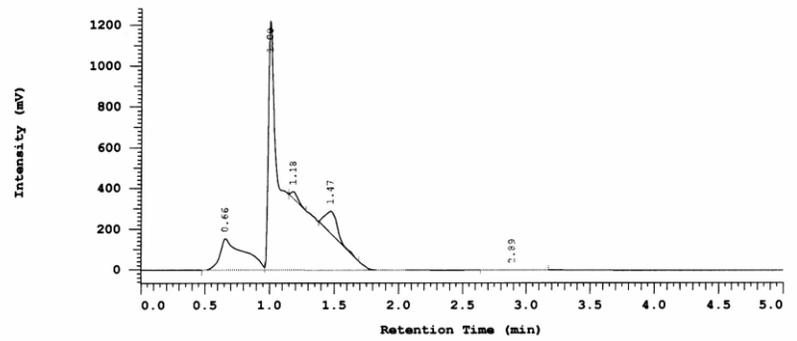
# Analysis of Terbinafine Stored Under Stress Conditions



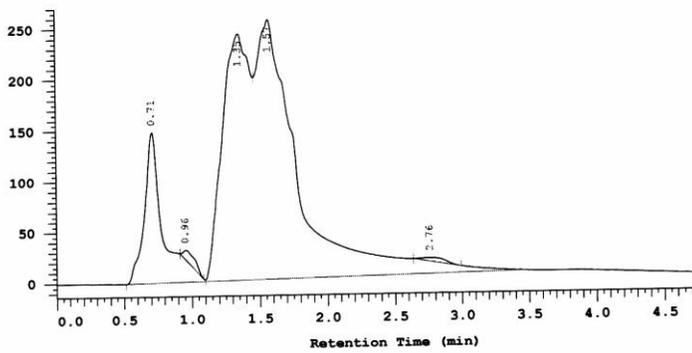
Terbinafine + 7.5% H<sub>2</sub>O<sub>2</sub>



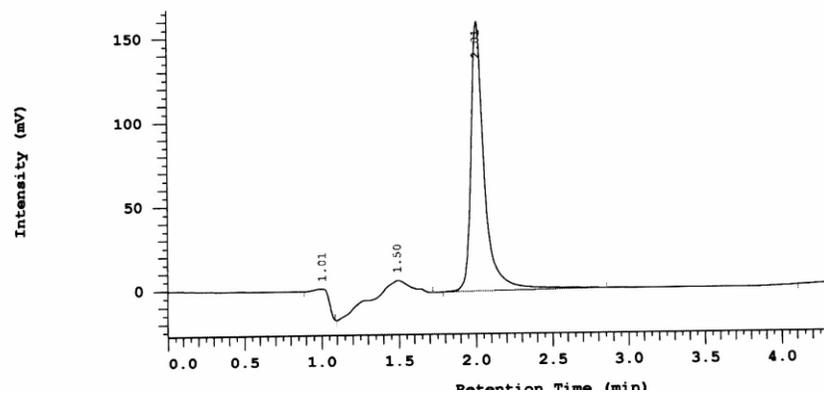
Terbinafine Standard



Terbinafine + 2 N NaOH



Terbinafine + 4 N HCl



Terbinafine + heat

Figure [3-4]: HPLC Analysis results for Terbinafine stored under stress conditions

### 3-6-Penetration Test

#### 3-6-1- Data

The penetration of generic cream was compared with penetration of Lamisil cream using Franz Diffusion Cell. Table [3-32] shows the penetration results for both creams.

Table [3-32]: HPLC Data and qty (ng) per cm<sup>2</sup> for penetration of generic cream and Lamisil cream

Time (min)	Generic Cream		Time (min)	Lamisil Cream	
	Peak Area	Qty (ng/cm <sup>2</sup> )		Peak Area	Qty (ng/cm <sup>2</sup> )
0	0	0	0	0	0
40	212048	7.709	60	206133	7.494
90	461287	16.770	90	545482	19.831
120	750474	27.284	150	825253	30.002
180	944504	34.338	218	997152	36.252
240	1038344	37.749	270	1122320	40.802
300	1119629	40.704	330	1217767	44.272
360	1216779	44.236	375	1217867	44.276

#### 3-6-2- Result

Figure [3-5] shows the comparison plot between penetration of generic cream and Lamisil cream. From the figure, we can conclude that there is a correspondence between the two creams.

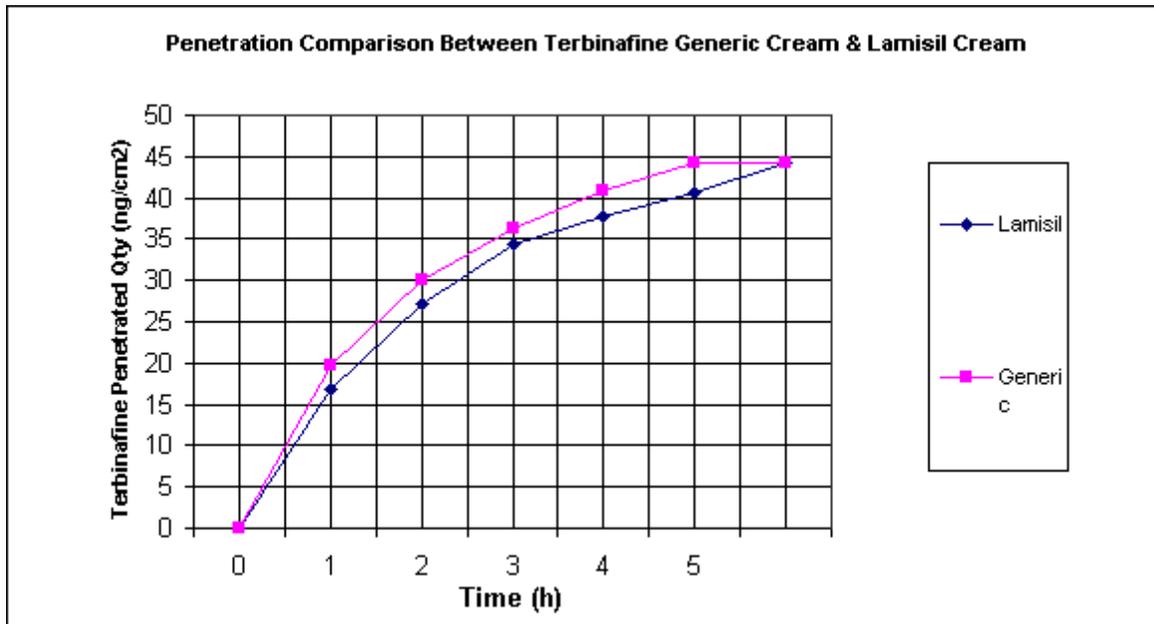


Figure [3-5]: Penetration of generic cream and Lamisil cream using Franz Diffusion Cell.

### 3-7- Phase Diagram data & Results

#### 3-7-1- DATA FOR PHASE DIAGRAM PREPARATION

Set #1 ( 100% water)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	0.0000	2.5000	0.0000	100.0000	0.0000	Single phase
2.4760	0.0256	0.0000	2.4760	1.0339	98.9767	0.0000	Two phases
2.4700	0.0542	0.0000	2.4700	2.1943	97.8528	0.0000	Two phases
2.4290	0.0760	0.0000	2.4290	3.1289	96.9661	0.0000	Two phases
2.4000	0.1023	0.0000	2.4000	4.2625	95.9118	0.0000	Two phases
2.3810	0.1250	0.0000	2.3810	5.2499	95.0120	0.0000	Two phases

Set #2 ( 95% water+ 05% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	0.1250	2.3750	0.0000	95.0000	5.0000	Micelles
2.4770	0.0254	0.1239	2.3532	1.0036	94.0357	4.9492	Two phases
2.4600	0.0540	0.1230	2.3370	2.1285	92.9594	4.8926	Two phases
2.4270	0.0770	0.1214	2.3057	3.0435	92.0787	4.8462	Two phases
2.4020	0.1030	0.1201	2.2819	4.0776	91.0938	4.7944	Two phases
2.3790	0.1240	0.1190	2.2601	5.2123	90.2936	4.7523	Two phases

Set #3 ( 90% Water+ 10% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	0.2500	2.2500	0.0000	90.0000	10.0000	Micelles
2.4756	0.0256	0.2476	2.2280	1.0127	89.0788	9.8976	Micelles
2.4643	0.0523	0.2464	2.2179	2.0583	88.1296	9.7922	Two phases
2.4322	0.0766	0.2432	2.1890	3.0234	87.2521	9.6947	Two phases
2.4010	0.1014	0.2401	2.1609	4.0128	86.3531	9.5948	Two phases
2.3780	0.1259	0.2378	2.1402	5.2944	85.4747	9.4972	Two phases

Set #4 ( 85% Water+ 15% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	0.3750	2.1250	0.0000	85.0000	15.0000	Micelles
2.4740	0.0256	0.3711	2.1029	1.0131	84.1295	14.8464	Micelles
2.4650	0.0530	0.3698	2.0953	2.0853	83.2109	14.6843	Two phases
2.4276	0.0766	0.3641	2.0635	3.0268	82.4000	14.5412	Two phases
2.4030	0.1031	0.3605	2.0426	4.0770	81.5031	14.3829	Two phases
2.3785	0.1258	0.3568	2.0217	5.2890	80.7301	14.2465	Two phases

Set #5 ( 80% Water+ 20% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	0.5000	2.0000	0.0000	80.0000	20.0000	Micelles
2.4758	0.0258	0.4952	1.9806	1.0207	79.1749	19.7937	Micelles
2.4600	0.0520	0.4920	1.9680	2.0505	78.3439	19.5860	Micelles
2.4270	0.0760	0.4854	1.9416	3.0063	77.5709	19.3927	Two phases
2.4020	0.1010	0.4804	1.9216	3.9953	76.7719	19.1930	Two phases
2.3790	0.1260	0.4758	1.9032	5.2963	75.9760	18.9940	Two phases

Set #6 ( 75% Water+ 25% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	0.6250	1.8750	0.0000	75.0000	25.0000	Micelles
2.4766	0.0256	0.6192	1.8575	1.0121	74.2327	24.7442	Micelles
2.4668	0.0527	0.6167	1.8501	2.0724	73.4312	24.4771	Micelles
2.4269	0.0762	0.6067	1.8202	3.0128	72.7168	24.2389	Two phases
2.4024	0.1023	0.6006	1.8018	4.0467	71.9368	23.9789	Two phases
2.3770	0.1256	0.5943	1.7828	5.2840	71.2359	23.7453	Two phases

Set #7 ( 70% Water+ 30% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	0.7500	1.7500	0.0000	70.0000	30.0000	Micelles
2.4746	0.0257	0.7424	1.7322	1.0171	69.2805	29.6916	Micelles
2.4650	0.0522	0.7395	1.7255	2.0541	68.5484	29.3779	Micelles
2.4276	0.0763	0.7283	1.6993	3.0165	67.8669	29.0858	Micelles
2.4023	0.1018	0.7207	1.6816	4.0258	67.1543	28.7804	Two phases
2.3792	0.1264	0.7138	1.6654	5.3127	66.4687	28.4866	Two phases

Set #8 ( 65% Water+ 35% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	0.8750	1.6250	0.0000	65.0000	35.0000	Micelles
2.4753	0.0255	0.8664	1.6089	1.0092	64.3372	34.6431	Micelles
2.4624	0.0515	0.8618	1.6006	2.0290	63.6684	34.2830	Micelles
2.4275	0.0758	0.8496	1.5779	2.9960	63.0318	33.9402	Two phases
2.4026	0.1025	0.8409	1.5617	4.0538	62.3404	33.5679	Two phases
2.3798	0.1259	0.8329	1.5469	5.2904	61.7340	33.2414	Two phases

Set #9 ( 60% Water+ 40% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	1.0000	1.5000	0.0000	60.0000	40.0000	Micelles
2.4759	0.0253	0.9904	1.4855	1.0000	59.3931	39.5954	Micelles
2.4658	0.0540	0.9863	1.4795	2.1253	58.7142	39.1428	Micelles
2.4275	0.0750	0.9710	1.4565	2.9668	58.2018	38.8012	Two phases
2.4026	0.1005	0.9610	1.4416	3.9737	57.5910	38.3940	Two phases
2.3791	0.1265	0.9516	1.4275	5.3171	56.9708	37.9805	Two phases

Set #10 (55% Water+ 45% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	1.1250	1.3750	0.0000	55.0000	45.0000	Micelles
2.4740	0.0257	1.1133	1.3607	1.0172	54.4345	44.5373	Micelles
2.4625	0.0526	1.1081	1.3544	2.0716	53.8497	44.0589	Micelles
2.4265	0.0766	1.0919	1.3346	3.0298	53.3169	43.6229	Two phases
2.4060	0.1017	1.0827	1.3233	4.0163	52.7695	43.1750	Two phases
2.3783	0.1262	1.0702	1.3081	5.3063	52.2286	42.7325	Two phases

Set #11 (52.5% Water+ 47.5% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	1.1875	1.3125	0.0000	52.5000	47.5000	Two phases
2.4770	0.0258	1.1766	1.3004	1.0200	51.9588	47.0103	Two phases
2.4620	0.0525	1.1695	1.2926	2.0679	51.4039	46.5083	Two phases
2.4273	0.0768	1.1530	1.2743	3.0367	50.8898	46.0432	Two phases
2.4080	0.1018	1.1438	1.2642	4.0163	50.3705	45.5734	Two phases
2.3793	0.1267	1.1302	1.2491	5.0559	49.8457	45.0985	Two phases

Set #12 (50% Water+ 50% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	1.2500	1.2500	0.0000	50.0000	50.0000	Two phases
2.4770	0.0258	1.2385	1.2385	1.0200	49.4846	49.4846	Two phases
2.4620	0.0525	1.2310	1.2310	2.0679	48.9561	48.9561	Two phases
2.4273	0.0768	1.2137	1.2137	3.0367	48.4665	48.4665	Two phases
2.4080	0.1018	1.2040	1.2040	4.0163	47.9719	47.9719	Two phases
2.3793	0.1267	1.1897	1.1897	5.3251	47.4721	47.4721	Two phases

Set #13 (47.5% Water+ 52.5% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	1.3125	1.1875	0.0000	47.5000	52.5000	gel (hexagonal)
2.4770	0.0258	1.3004	1.1766	1.0200	47.0103	51.9588	gel (hexagonal)
2.4620	0.0525	1.2926	1.1695	2.0679	46.5083	51.4039	gel (hexagonal)
2.4273	0.0768	1.2743	1.1530	3.0367	46.0432	50.8898	gel (hexagonal)
2.4080	0.1018	1.2642	1.1438	4.0163	45.5734	50.3705	Two phases
2.3793	0.1267	1.2491	1.1302	5.3251	45.0985	49.8457	Two phases

Set #14 (45% Water+ 55% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	1.3750	1.1250	0.0000	45.0000	55.0000	gel (hexagonal)
2.4765	0.0254	1.3621	1.1144	1.0043	44.5431	54.4416	gel (hexagonal)
2.4625	0.0526	1.3544	1.1081	2.0714	44.0589	53.8497	gel (hexagonal)
2.4273	0.0768	1.3350	1.0923	3.0369	43.6199	53.3132	Two phases
2.4027	0.1016	1.3215	1.0812	4.0180	43.1743	52.7686	Two phases
2.3792	0.1259	1.3086	1.0706	5.2917	42.7384	52.2358	Two phases

Set #15 (40% Water+ 60% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	1.5000	1.0000	0.0000	40.0000	60.0000	gel (hexagonal)
2.4760	0.0253	1.4856	0.9904	1.0006	39.5954	59.3931	gel (hexagonal)
2.4654	0.0524	1.4792	0.9862	2.0615	39.1675	58.7513	gel (hexagonal)
2.4273	0.0765	1.4564	0.9709	3.0251	38.7779	58.1668	Two phases
2.4023	0.1015	1.4414	0.9609	4.0151	38.3784	57.5676	Two phases
2.3796	0.1257	1.4278	0.9518	5.2824	37.9931	56.9896	Two phases

Set #16 (37.5% Water+ 62.5% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	1.5625	0.9375	0.0000	37.5000	62.5000	Two phases
2.4760	0.0253	1.5475	0.9285	1.0006	37.1207	61.8678	Two phases
2.4654	0.0524	1.5409	0.9245	2.0615	36.7196	61.1993	Two phases
2.4273	0.0765	1.5171	0.9103	3.0251	36.3543	60.5904	Two phases
2.4023	0.1015	1.5014	0.9008	4.0151	35.9798	59.9663	Two phases
2.3796	0.1257	1.4873	0.8924	5.1703	35.6185	59.3641	Two phases

Set #17 (35% Water+ 65% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	1.6250	0.8750	0.0000	35.0000	65.0000	Two phases
2.4740	0.0256	1.6081	0.8659	1.0131	34.6415	64.3343	Two phases
2.4629	0.0528	1.6009	0.8620	2.0791	34.2654	63.6358	Two phases
2.4272	0.0767	1.5777	0.8495	3.0328	33.9279	63.0089	Two phases
2.4026	0.1018	1.5617	0.8409	4.0253	33.5773	62.3579	Two phases
2.3799	0.1264	1.5469	0.8330	5.3111	33.2348	61.7219	Two phases

## Set #18 (32.5% Water+ 67.5% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	1.6875	0.8125	0.0000	32.5000	67.5000	Inverse micelles
2.4781	0.0254	1.6727	0.8054	1.0041	32.1703	66.8152	Inverse micelles
2.4697	0.0516	1.6670	0.8027	2.0273	31.8349	66.1186	Inverse micelles
2.4257	0.0756	1.6373	0.7884	2.9905	31.5177	65.4599	Two phases
2.4062	0.1023	1.6242	0.7820	4.0406	31.1746	64.7473	Two phases
2.3784	0.1256	1.6054	0.7730	5.2821	30.8694	64.1134	Two phases

## Set #19 (30% Water+ 70% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	1.7500	0.7500	0.0000	30.0000	70.0000	Inverse micelles
2.4781	0.0254	1.7347	0.7434	1.0041	29.6956	69.2898	Inverse micelles
2.4697	0.0516	1.7288	0.7409	2.0273	29.3860	68.5674	Inverse micelles
2.4257	0.0756	1.6980	0.7277	2.9905	29.0933	67.8843	Inverse micelles
2.4062	0.1023	1.6843	0.7219	4.0406	28.7766	67.1453	Two phases
2.3784	0.1256	1.6649	0.7135	5.2821	28.4949	66.4880	Two phases

## Set #20 (25% Water+ 75% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	1.8750	0.6250	0.0000	25.0000	75.0000	Inverse micelles
2.4490	0.0252	1.8368	0.6123	1.0065	24.7454	74.2361	Inverse micelles
2.4552	0.0548	1.8414	0.6138	2.1634	24.4542	73.3625	Inverse micelles
2.4273	0.0778	1.8205	0.6068	3.0740	24.2236	72.6708	Two phases
2.4063	0.1036	1.8047	0.6016	4.0917	23.9681	71.9043	Two phases
2.3768	0.1257	1.7826	0.5942	5.2874	23.7445	71.2336	Two phases

## Set #21 (20% Water+ 80% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	2.0000	0.5000	0.0000	20.0000	80.0000	Inverse micelles
2.4745	0.0256	1.9796	0.4949	1.0125	19.7952	79.1808	Inverse micelles
2.4544	0.0540	1.9635	0.4909	2.1337	19.5694	78.2778	Two phases
2.4243	0.0764	1.9394	0.4849	3.0235	19.3890	77.5559	Two phases
2.4025	0.1026	1.9220	0.4805	4.0587	19.1809	76.7235	Two phases
2.3798	0.1254	1.9038	0.4760	5.2694	18.9989	75.9955	Two phases

Set #22 (15% Water+ 85% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	2.1250	0.3750	0.0000	15.0000	85.0000	Inverse micelles
2.4745	0.0257	2.1033	0.3712	1.0162	14.8458	84.1263	Inverse micelles
2.4544	0.0546	2.0862	0.3682	2.1558	14.6738	83.1516	Two phases
2.4243	0.0765	2.0607	0.3636	3.0278	14.5413	82.4005	Two phases
2.4025	0.1016	2.0421	0.3604	4.0174	14.3914	81.5513	Two phases
2.3798	0.1265	2.0228	0.3570	5.3168	14.2427	80.7088	Two phases

Set #23 (10% Water+ 90% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	2.2500	0.2500	0.0000	10.0000	90.0000	Inverse micelles
2.4720	0.0254	2.2248	0.2472	1.0064	9.8983	89.0846	Inverse micelles
2.4530	0.0519	2.2077	0.2453	2.0525	9.7928	88.1353	Two phases
2.4262	0.0756	2.1836	0.2426	2.9886	9.6978	87.2804	Two phases
2.4062	0.1034	2.1656	0.2406	4.0847	9.5880	86.2918	Two phases
2.3777	0.1252	2.1399	0.2378	5.2656	9.4998	85.4980	Two phases

Set #24 (5% Water+ 95% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	2.3750	0.1250	0.0000	5.0000	95.0000	Inverse micelles
2.4570	0.0255	2.3342	0.1229	1.0161	4.9486	94.0242	Inverse micelles
2.4570	0.0526	2.3342	0.1229	2.0759	4.8952	93.0088	Two phases
2.4256	0.0768	2.3043	0.1213	3.0406	4.8465	92.0844	Two phases
2.4004	0.1002	2.2804	0.1200	3.9675	4.7996	91.1933	Two phases
2.3746	0.1251	2.2559	0.1187	5.2683	4.7498	90.2456	Two phases

Set #25 (0% Water+ 100% Tween 80)

Mixture (gm)	Terb. (gm)	Tween (gm)	Water (gm)	%Terb.	%Water	%Tween 80	Phase status
2.5000	0.0000	2.5000	0.0000	0.0000	0.0000	100.0000	Single phase
2.4740	0.0254	2.4740	0.0000	1.0061	0.0000	98.9838	Two phases
2.4605	0.0505	2.4605	0.0000	1.9912	0.0000	97.9888	Two phases
2.4283	0.0757	2.4283	0.0000	2.9910	0.0000	96.9768	Two phases
2.4018	0.1026	2.4018	0.0000	4.0592	0.0000	95.9032	Two phases
2.3787	0.1258	2.3787	0.0000	5.2886	0.0000	94.9770	Two phases

## PHASE DIAGRAM OF WATER, TWEEN 80 AND TERBINAFINE HCl

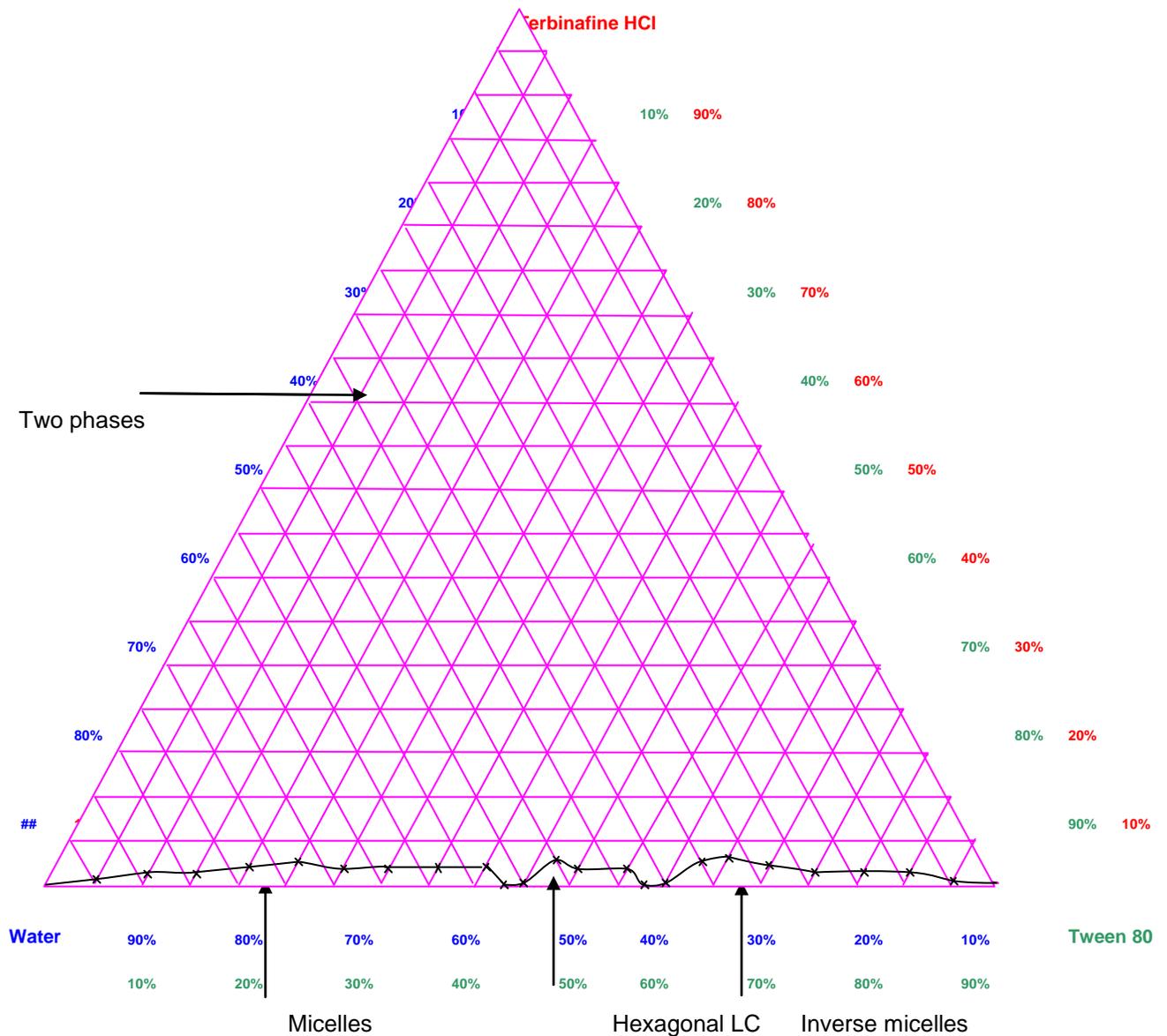


Figure [3-6]: Phase diagram of Water- Terbinafine - and Tween 80.

### 3-6-1-Phase Diagram Result

From the diagram shown above, it is clear the solubility regions of Terbinafine HCl. The solubility arrived up to 3.0% at three points ;  
 (68% water and 29% tween 80 at micelles region.  
 (46% water, 51% tween 80), at hexagonal region.  
 (29% water, 68% tween 80) at inverse micelles region  
 In addition to other soluble regions of 1% and 2% of Terbinafine HCl.

## 4-Conclusion

The Terbinafine HCl cream formula was developed, it was selected from about 18 formulas by using GMS (SE), cetostearyl alcohol and Eumulgin B2 as emulsifiers, chloro-cresol was used as the preservative, in addition to the emollient; paraffin oil, and the oil base; Vaseline. The pH is adjusted by TEA for about 4.6 to be equivalent to the brand product Lamisil. O/W technique was used to get less greasy product. The mixing speed was found 80 -120 rpm to get the best results using Erweka mixer. There was no effect for the addition temperature of terbinafine to the cream between 50 – 70 °c.

The formula was put under accelerated stability study for six months, no significant chemical, physical, microbial or pharmaceutical change occurred. The study condition was at temperature of 40 c° and a humidity of 75% for 1,2,3 and six months followed by complete analysis

The methods of analysis were developed and applied on the selected formula. It depended on the HPLC technique as the separation method using RP18 as a stationary phase and methanol and acetate buffer (95: 5 respectively) as the mobile phase, UV detector was used for detection purpose. The method of analysis (assay) passed the analytical validation process for all factors tried (accuracy, precision, linearity, and ruggedness). The method at the same time can be used for preservative potency determination in the product.

Penetration of the cream is compared with that for the brand product ‘Lamisil’ by using the Franz diffusion cell. The two formulas have approximately the same penetration profile.

Solubility of Terbinafine HCl was determined in water and tween 80 and in mixtures of the two solvents using phase diagram technique. It was found that it is slightly soluble in both solvents (water and tween 80), but soluble in some concentrations up to 3% at three different compositions (68% water:29% tween, 46% water: 51% tween, 29% water: 68% tween). The drawn phase diagram showed the Terbinafine soluble regions in addition to the other regions such as the micelles, hexagonal and inverse micelles regions.

## References

- 1- Gilbert S. Banker, Christopher T. Rhodes " Modern Pharmaceutics" 2nd edition. Marcel Dekker Inc, 1990. USA. Pages 310-311.
- 2- Michel E. Aulton "Pharmaceutics, The Science of Dosage Form Design" Churchill Livingstone, printed in Hong Kong 1988. Pages 410 - 411.
- 3- The Arab Union of the Manufacturers & Medical Appliances (AUPAM)." The Arab Good Manufacturing Practice Guidelines", 2nd Edition,, Amman, 1995. Page 17.
- 4- [http://www.olemiss.edu/courses/che545/notes/R\\_Chapter\\_8.html](http://www.olemiss.edu/courses/che545/notes/R_Chapter_8.html)
- 5- Roger Walker, Clive Editionward " Clinical Pharmacy and Therapuetics" 3rd Edition, Chrchill Hill Living Stone, 2003, Spain. Page 626.
- 6- H. P. Rang, M.M. Dale, J.M.Ritter " Pharmacology" 3rd Edition. Chrchill Hill Living Stone, 1995, USA.
- 7- <http://www.premec.org.nz/bulletins/38.htm>
- 8- <http://www.dermnet.org.nz/dna.fungi/terba.html>
- 9- <http://www.onlinepharmacy.co.nz/lamisil.html>
- 10- <http://home.intekom.com/pharm/novartis/lamisilc.html>
- 11- James E. F. Reynolds " Martendale, The Extended Pharmacopoeia" Thirtieth edition, The Pharmaceuticals Press. London,1993. Pages 331- 332.
- 12- <http://www.healthsquare.com/newrx/LAM1517.HTM>
- 13- <http://www.smartfoot.com/newsflash/mEditionscapeonycho.html>
- 14- <http://www.aafp.org/afp/20010215/663.html>
- 15- <http://www.lamisilinfo.com>
- 16- Joel G. Hardman, Perry B. Molinof " The Pharmacological Basic of Therapuetic" 9th Edition. McGraw-Hill book company, USA, 1996. Page 1187.
- 17- A. C. Moffat "Clark's Isolation and Identification of Drugs" 2nd ed. Pharmaceutical Press London 1986, page 203-204.
- 18- "The British Pharmacopoeia 2000,BP 2000", HMSO Publications Centre, London, Great Britain, 2000. Page A101.
- 19- Marvin C. McMaster " HPLC, A practical User's Guide", VCH Publishers, Inc. New York, 1994, Pages 112 -114.
- 20- Marvin C. McMaster " HPLC, A practical User's Guide", VCH Publishers, Inc. New York, 1994, Pages 85-90.
- 21- "The United State Pharmacopoeia, USP 24 ", The United State Pharmacopoeial Convention, Inc. USA, 2000, Page 1961.
- 22- Ira R. Berry & Robert A. Nash " Pharmaceutical Process Validation". 2nd Edition,1995, volume 57, printed in USA. Page 1982.
- 23- Professeur J. Dangoumau " Good Pharmaceutical Manufacturing Practice" 2nd. Edition. Journaux officiels, Paris,1982. Page 55.
- 24- Rebecca D. Fuller "Introduction to the Principles of Validation" Validation Technologies
- 25- Ira R. Berry & Robert A. Nash " Pharmaceutical Process Validation". 2nd Edition,1995, printed in USA. Page xv.

- 26- Ira R. Berry & Robert A. Nash "Pharmaceutical Process Validation". 2nd Edition, 1995, printed in USA. Pages xxxii-xxxvii
- 27- Ira R. Berry & Robert A. Nash "Pharmaceutical Process Validation". 2nd Edition, 1995, printed in USA. Pages 168 -171
- 28- "The United State Pharmacopoeia, USP 24 " 1995 The United State Pharmacopoeial Convention, Inc. USA, Page 1982.
- 29- "The United State Pharmacopoeia, USP 24 " 2000 The United State Pharmacopoeial Convention, Inc. USA, Page 1940.
- 30- Michel E. Aulton "Pharmaceutics, The Science of Dosage Form Design" 1988 Churchill Livingstone, printed in Hong Kong. Pages 294-296.
- 31- Glibert S. Banker, Christopher T. Rhodes " Modern Pharmaceutics" 2nd. Edition. 1990. Marcel Dekker Inc. USA, Pages 209-210.
- 32- Kenneth A. Connors, Gordon L. Amidon, Valantino J. Stella " Chemicals Stability of pharmaceuticals" 1986, A Wiley-interscience Publication-John Wiley & Sons, USA, Page 135.
- 33- Arther Osol, "Remington: The Science And Practice Of Pharmacy". 16th Edition. 1980. Mack publishing company, Easton, Pennsylvania. 1426-1430.
- 34- <http://www.gibraltarlabsine.com/stability.htm>
- 35- The Arab Union of the Manufacturers & Medical Appliances (AUPAM)." Arab Guidelines on Stability Testing of Pharmaceutical Products", 2nd. Edition, Amman, 1995.
- 36- "The United State Pharmacopoeia, USP 24 " 1995 The United State Pharmacopoeial Convention, Inc. USA, Pages 1959-1962.
- 37- <http://www.ijpe.org/Jul2002/Article05Page01.html>
- 38- <http://appft1.upsto.gov/netacgi/nph-arser?Sect1=PTO2&sect2=HITOFF&u=/netahtml/p...>
- 39- [www://.kamalsalon.com/structureofskin.htm](http://www.kamalsalon.com/structureofskin.htm)
- 40- [home.wxs.nl/~wtberge/skin...](http://home.wxs.nl/~wtberge/skin...) (Modelling dermal exposure and absorption through the skin)
- 41- <http://www.ualberta.ca/~csps/JPPS55%282%29/Y.Krishnaiah/nicardipine.htm>
- 42- <http://www.aapspharmscitech.org/view.asp?art=pt040110&pdf=yes>

## ملخص البحث

تيرينافين هايدروكلورايد الذي ينتمي إلى عائلة أليل أمين قد ركب في مستحضر كريم ليكون مكافئاً كيميائياً ونوعياً إلى المستحضر الأم (لاميسيل) الذي تنتجه شركة نوفارتيز السويسرية. المادة الفعالة هي تيرينافين هايدروكلورايد والتي هي مادة واسعة الفعالية ضد الفطريات. إن طريقة إيجاد كمية المادة الفعالة في المستحضر قد طورت وأجريت لها عملية التثبيت من خلال فحص مدى دقة الطريقة ومدى قرب كمية المادة الفعالة إلى الحقيقة. وكذلك الانتقائية ومدى الخطية ومدى مرونة الطريقة إلى بعض العوامل المؤثرة.

لقد درست ثباتية التركيبة المختارة وقورنت نفاذية الدواء مع نفاذية دواء لاميسيل. إن تيرينافين هايدروكلورايد قليل الذائبية في الماء، لذلك ذوبت المادة بخليط من الماء ومادة توين 80 من خلال بناء مثلث حالات المادة.

لقد أجريت عدة تجارب لتطوير كريم ذو تركيبة ثابتة وفعالة بالإضافة إلى نقاءه والأمان في استعماله. التجارب أجريت باتجاهين:

أولاً: اختيار المواد المناسبة كماً ونوعاً خصوصاً المستحلبات (التي تعمل على الجمع بين الزيوت والماء معاً) والمواد الحافظة.

ثانياً: التغيير في طريقة الخلط.

إنه من المهم إنتاج كريم له مواصفات مطابقة لمواصفات المستحضر الأم، من النواحي الصيدلانية والكيميائية والميكروبيولوجية، بالإضافة إلى النواحي الفيزيائية. كثير من المستحلبات قد جربت لوحدها أو بإضافة اثنين معاً أو أكثر مثل جلسريل مونوستيريت (ذاتي الفعالية) وأملمجن ب2 وسيتوستيريل الكحول، وذلك بتجربة كل الاحتمالات لاستعمالهم معاً. في نفس الوقت كثير من التجارب قد أجريت لاختيار أفضل وأمن مادة حافظة. التغيير أيضاً في خطوات العمل مثل التغيير في سرعة الخلط ليكون 20، 80، 120 و 180 دورة في الدقيقة. وكذلك التغيير في درجة حرارة الكريم قبل إضافة المادة الفعالة له (50°، 60°، 70°).

لا يوجد مصادر متوفرة لتحليل تيرينافين هايدروكلورايد في المراجع العالمية مثل المرجع البريطاني أو الأوروبي أو الأمريكي، لذلك إنه من الضروري والأساسي لتطوير طريقة التحليل للمادة الفعالة الخام وللمستحضر أيضاً.

لقد طورت طريقة تحليل تعتمد على الفصل الكروماتوغرافي، فيه العمود الذي يحمل المادة الثابتة يحتوي على مادة غير قطبية وفيه ايضاً المادة المتحركة 95% ميثانول و5% اسيتات من المحلول المتعادل.

لقد تم التثبت من طريقة التحليل للتأكد من أن الطريقة مضمونة وتعمل بشكل ثابت لإيجاد كمية المادة الفعالة في المستحضر. لقد فحصت خمسة من العوامل ذات العلاقة في التثبيت وهي:

- مدى قرب النتيجة إلى الحقيقة، وذلك من خلال حساب النتيجة لثلاث تشغيلات مختلفة. كل تشغيلة حللت ثلاث مرات، وفي كل مرة يتم تحليل خمس عينات، ثم يحسب المعدل للنتائج وتقارن مع القيمة الحقيقية.
- مدى الدقة في الطريقة قد فحصت أيضاً من خلال حساب معامل التغير لثلاث تشغيلات مختلفة، كل تشغيلة حللت ثلاث مرات، وفي كل مرة يتم تحليل خمس عينات.
- الانتقائية أيضاً حددت لضمان عدم تداخل مواد أخرى من مواد التركيبة في التحليل. وتم تحديد ذلك بإجراء الفحص بغياب المادة الفعالة في المستحضر.
- مدى مقاومة الطريقة للعوامل المؤثرة قد فحصت للتأكد أن الطريقة تعمل تحت ظروف مختلفة بنفس الكفاءة. تم حساب ذلك من خلال إبقاء المحاليل المحضرة على أوقات مختلفة ( 15 و 180 دقيقة) وعلى عدة درجات حرارة (20° و 40°) لثلاث تشغيلات مختلفة. وحدد أيضاً مدى تأثير استعمال عمودين مختلفين لهما نفس المواصفات. ثم تم حساب مدى الدقة ومدى قرب النتيجة من الحقيقة لكل العينات التي تم تحليلها.
- مدى خطية الطريقة قد حددت أيضاً للتأكد من أن النتيجة تزداد بنفس قيمة ازدياد تركيز العينة. لقد تم تحديد مدى الخطية من خلال تحليل خمسة تركيز مختلفة (50%، 75%، 100%، 125%، 150%) من تركيز العينة الجاهزة للفحص. وتم إجراء رسم بياني بالاعتماد على برنامج اكسل لحساب معامل الارتباط للخط المستقيم المرسوم.

لقد تم التأكد من ثباتية التركيبة النهائية المختارة للكريم، حيث تم الاعتماد على دراسة ثباتية مسرّعة لمدة ستة أشهر. تم حفظ الكريم على درجة حرارة 45 ° ورطوبة 75% لمدة شهر، شهرين، ثلاثة أشهر وستة أشهر، اتبعت بتحليل شاملة للتأكد من غياب أي تغير كيميائي، فيزيائي أو ميكروبيولوجي للمستحضر.

تم دراسة مدى نفاذية الدواء إلى الجلد وذلك من خلال مقارنة بينه وبين نفاذية الدواء الأم (لاميسيل) باستعمال "فرانس دفيوجن سل". وقد وجد أنه يوجد تطابق كبير بين نفاذية الدوائين.

تمت دراسة ذائبية تيرينافين هايدروكلورايد في خليط من الماء و توين 80 من خلال استخدام تكنيك مثلث حالة المادة وذلك بإضافة المادة الفعالة مع مزيج من الماء و توين 80. العديد من التجارب قد أجريت استخدم خلالها تراكيز مختلفة من المواد الثلاث ( تيرينافين، ماء، توين 80)، وتم تحضير العينات في أنابيب 5 مل، حضر في كل منها ما يقارب 2,5 غرام من الخليط، واتباع ذلك بخلط جيد ووضع على جهاز التراسونيك وحفظ على درجة حرارة 25 لمدة 24 ساعة. تركت العينات لتهدأ وتصفى وتم فحص مدى نفاذتها. المخلوط الذي فيه تعكير أو رواسب اعتبر غير نقي، وفي نفس الوقت المساحات في المثلث حددت مواقع المادة الفعالة الذائبة ومواقع الجل أيضاً وتبين جلياً حدود الذائبية للتيرينافين هايدروكلورايد في هذا النظام.

لقد تم إنتاج تركيبة جيدة للكريم وهي تكافئ مواصفات الدواء الأم (لاميسيل) بالنسبة للنواحي الكيميائية، الفيزيائية، الميكروبيولوجية والصيدلانية). تم اختيار ثلاثة مستحلبات معاً وهي جلسريل مونو ستيريت (ذاتي الفعالية)، امولجن ب 2، وسيتوستيريل الكحول، المادة الحافظة المستعملة هي كلوروكريسول التي كانت فعالة ضد الميكروبات الوارد فحصها في دستور الأدوية الأمريكي.

أما بقية المواد الحافظة فقد فشلت في فعاليتها أو أنها تسببت في بعض المشاكل في التحليل. ووجد أيضاً أن أفضل سرعة كانت بين 80 إلى 120 دورة في الدقيقة لمنع دخول الهواء أو حدوث فصل ما بين الطبقة المائية والطبقة الزيتية. إن إضافة المادة الفعالة على درجة حرارة 50 أو 60 أو 70 درجة مئوية لم يظهر أي فرق بين الحالات الثلاث، لذلك يمكن استنتاج أن تيرينافين هايدروكلورايد ثابت كيميائياً عند درجة حرارة 70 درجة مئوية.

إن طريقة التحليل وجدت ثابتة لكل العوامل التي تمت دراستها. ووجد أيضاً أن التركيبة ثابتة تحت الظروف القاسية التي تم دراستها عند درجة حرارة 40 و 75% الرطوبة النسبية. لذلك يمكن اعتبار المستحضر صالح لمدة سنتين.

لقد تم تحديد ذائبية تيرينافين في الماء و توين 80، وتبين أنه قليل الذائبية في كليهما أي أقل من 1غم/100 مل من المذيب. وتبين من خلال مثلث حالة المادة مواقع ذائبية تيرينافين في معظم المساحات التي يقل فيها تركيز المادة عن 3.0% بالإضافة إلى ظهور مواقع للجل وأخرى للعينات التي فيها راسب (أي مادتان منفصلتان في مزيج واحد) بالإضافة إلى مناطق أخرى مثل المايسلز والانفرز مايسلز.

