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Development of Formulation and Method of Analysis for "Fluconazole 100 mg Tablets"

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Development of Formulation and Method of Analysis for "Fluconazole 100 mg Tablets"

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Al-Quds University
Jerusalem-Palestine

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 thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed:

Jamal Jamil Kort

Date:

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I would like to thank my supervisor Dr. Ibrahim Kayali for his supervision, patience and guidance during my work.

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Abstract

The aim of this thesis is to develop a stable generic formulation that is bioequivalent to the innovator product Diflucan capsule by Pfizer. Diflucan has different trade names; in the local market it is marketed as Trican. Diflucan is presented as a capsule form in the world, but as a tablet form in the USA. The goal is to develop a tablet form for the local market by using a simple and cost effective technology, and at the same time to retain the qualitative and quantitative properties of the innovator.

In developing the generic formulation, a comprehensive review for the available references was made on the drug. Official and authoritative references were used for the pharmaceutical and analytical aspects, as well as the different studies performed.

Fluconazole as an active ingredient is not described by the US or British Pharmacopoeias. Thus, an in-house method was developed for both the active as a substance and as a composition in the final product. The analysis is performed using an Merck-Hitachi HPLC systems consisting of an autosampler model L-7200, a pump model L-7100 and a diode array detector model L-7450A. The column used is LiCrospher RP-18 e, 5 μm, 250x 4mm, UV set at 261 nm and a mobile phase mixture of water: Methanol (45:55).

The method of analysis was validated. The method was found selective for the active to be analyzed. The precesion was found to be precise with a coefficient of variation of 0.87 and accurate with a percentage of recovery of 99.79. The method was also found linear over the range of 60% -140 % with a correlation coefficient of .99995 and y-intercept of 0.507. Robustness was tested with different analysts, different elapsed assay time, different sonicating time, different columns and instruments. All results were according to specifications.

A tablet form was chosen as it can be mass produced simply and quickly and thus the resultant cost is low. Direct compression method use the minimum machines of all other tablet forms, as no granulation is performed. The excepients used in the formulation are inert and widely used in direct compression tablets. Flowability, compressibility, disintegration and dissolution were tested on different types and percentages of excepients to select the suitable type and percentage.

The direct compression method was found very effective and the resultant tablet were within the predetermined specification.

A comparative dissolution profile was made for the formulated (Dican) tablet vs the brand product Trican capsule. The similarity factor calculation was applied. When calculating the similarity factor in the range 10 – 25 minutes, the result fails (f_2 = 44.1). This is due to the capsule shell of Trican, which delays the dissolution in the first 5 minutes. The similarity factor calculated in the range 15 – 25 minutes was found to be within limits (f_2=51.2).

The stability of the formulated tablet at accelerated conditions of T=40 ºC, RH= 75 and T=30 ºC, RH= 60 were studied for six months. The light effect was also studied for seven months. Samples were tested for appearance, assay, dissolution and hardness. The results indicate that no significant changes occurred and the product was stable at these conditions.

A bioequivalence study was performed for Dican tablets vs Diflucan capsules in Tanta University, Egypt. The peak plasma concentration (C_{max}) of Fluconazole following the administration of two Dican tablets ranged from 1.748 – 5.021 μg/ml with a mean value of 3.088 ± 0.796 μg/ml whereas, the C_{max} of Fluconazole following the administration of one Diflucan capsule (Pfizer) ranged from 1.636 – 5.707 μg/ml, with a mean value of 2.777 ± 0.999 μg/ml. The mean time to reach the peak concentration (t_{max}) was 1.979 ± 1.386 hr after administration of two Dican tablets and 3.395 ± 1.763 hr after administration of one Diflucan capsule. The area under the plasma concentration time curve ($AUC_{0\rightarrow\infty}$) after administration of two Dican tablet ranged from 78.09 – 226.05 μg.hr/ml, with a mean value of 142.5 ± 39.47 μg.hr/ml, while following administration of one Diflucan capsule, it ranged from 76.55 – 310.2 μg.hr/ml, with a mean value of 149.3 ± 60.333 μg.hr/ml. Relative Bioavailability = 95.44 % (as calculated from the AUC.

A potency test for Fluconazole was developed using the plate method using Candida Albicans as the test organism. The obtained results indicate that Dican tablet activity against Candida albicans is 103.1% compared to Trican capsule. The antimicrobial activity of Fluconazole was tested agaist 4 bacterial strains using MIC method. Salmonella, Staphylococcus Aureus, Pseudomonas Aeruginosa and Escherishia Coli were used. Fluconazole has activity against Staphylococcus Aureus using the MIC method. 250 μg/ml of Fluconazole was active against 4.4 x 10^3 microorganisms / ml of Staphylococcus Aureus in

tryptic soy broth (TSB). The results were compared from that obtained from Clarithromycin, an antibiotic indicated for Staphylococcus Aureus. 36.5 µg/ml Clarithromycin was active against 4.4×10^3 microorganisms / ml of Staphylococcus Aureus in tryptic soy broth (TSB). Thus, Fluconazole has an activity of 14.6 % as compared to Clarithromycin.

Fluconazole didn't show activity against Salmonella (up to 1000 µg/ml against 1.25×10^3 bacteria / ml), against Pseudomonas Aeruginosa (up to 1000 µg/ml against 4.3×10^3 bacteria / ml) and against Escherishia Coli (up to 1000 µg/ml against 2.9×10^3 bacteria / ml).

الخلاصة

ان الهدف من هذه الدراسة هو تطوير تركيبة دوائية ثابتة تكون مكافئة حيويا للمستحضر الاصلي ديفلوكان كبسولات لشركة بفايزر. لديفلوكان عدة اسماء تجارية في العالم، فهو يسوق في السوق المحلي تحت اسم ترايكان. و يسوق الديفلوكان في العالم على شكل كبسولات وفي الولايات المتحدة على شكل حبوب. الغاية هو تطوير تركيبة على شكل حبوب للسوق المحلي باستخدام تكنولوجيا بسيطة و قليلة التكاليف و في نفس الوقت تحافظ على الصفات الكمية و النوعية للمستحضر الاصلي.

اثناء تطوير هذه التركيبة تمت مراجعة شاملة للمراجع و المقالات المتوفرة عن المستحضر الام واستخدمت المراجع المصدقة و المعتمدة في مجالات الصيدلة و التحليل.

ان الفلوكانازول كمادة فعالة غير موصوف في دساتير الادوية الامريكية او البريطانية و بالتالي كان هناك الحاجة الى تطوير طريقة تحليل لها كم ادة خام و في المستحضر النهائي.باستخدام جهاز HPLC من نوع Merck Hitachi و المكون من اخذ عينات ذاتي نوع ل- 7200 ، مضخة من نوع ل- 7100 و جهاز قياس للاشعة الفوق بنفسجية متعدد الموجات نوع ل- 7450 أ. استخدم عمود طوله 250 ملم وقطره 4 ملم من نوع ليكروسفير معبأ بذرات السيليكا معدلة بسلسلة كربونية من 18 ذرة بقطر 5 مايكرون. طول الموجة التي استخدمت هي 261 و السائل المستخدم هو ماء و ميثانول بنسبة (55:45).

لقد تم التحقق من التثبتية لطريقة التحليل. فقد وجدت الطريقة انتقائية للمادة الفع الة المراد تحليلها. و قد وجدت النتائج متقاربة من بعضها وعامل اختلافها هو 0،87 كما ان النتائج كانت دقيقة و عامل الدقة فيها 99.97%. و قد وجدت الطريقة ذات علاقة خطية على مدى 60%-140% وعامل ترابط 0.99995 ونقطة تقاطع مع المحور العمودي ص 0.507. كما تم فحص قابلية الطريقة لمتغيرات عدة مثل المحلل، مدة تحليل مختلفة، مدة تحضير مختلفة، اعمدة و اجهزة مختلفة. كل النتائج كانت حسب المواصفات الموضوعة.

لقد اختيرت الحبوب كشكل صيدلاني لسهولة و سرعة انتاجه وبالتالي تكاليف اقل. ان طريقة الكبس المباشر في تصنيع الحبوب تستخدم اقل الاجهزة التصنيعية مقارنة بالطرق الاخرى حيث انه لا توجد عملية التحبب قبل عملية كبس الحبوب. لقد فحصت التدفقية و عامل الكبس والتفكك و التحلل باستخدام مواد مختلفة و نسب مختلفة. لقد وجدت هذه الطريقة فعالة و الحبوب الناتجة تقع ضمن المواصفات المحددة مسبقا.

لقد تمت دراسة منحنى التحلل المخبري للحبوب المنتجة مقارنة مع الكبسولات الاصلية. ان عامل تشابه منحنيي التحلل يساوي 44.1 في المدى بين 10-25 دقيقة و هو ادنى من

الحد المسموح به و هذا ناتج عن تاثير الكبسولة الفارغة في الترايكان كبسولات حيث انها تؤخر عملية التحلل في الخمسة دقائق الاولى. ان هذه النتيجة تتغير في المدى بين 15-25 دقيقة لتصبح 51.2 أي ضمن الحدود المسموح بها.

لقد اجريت دراسة الثباتية على الحبوب المنتجة بوضعها في ظروف الدراسة المتسارعة على حرارة 40 درجة مئوية و رطوبة نسبية 75 و في ظرف اخر على حرارة 30 درجة مئوية و رطوبة 60 لمدة ستة اشهر. و لقد تمت دراسة تاثير الضوء بتعريض الحبوب للضوء 24 ساعة يوميا لمدة سبعة شهور. ان النتائج تظهر عدم وجود تغيير يذكر في هذه الظروف على مواصفات المسحضر.

كما اجريت على المستحضر دراسة التكافؤ الحيوي في جامعة طنطا / كلية الصيدلة بجمهورية مصر مقارنة مع ديفلوكان كبسولات لشركة بفايزر. لقد وجدت C_{max} للفلوكونازول في الدم بعد تناول حبتين من الحبوب المنتجة تتراوح بين 1.748 - 5.021 مايكروغرام/ مل بمتوسط 0.796 ± 3.021 مايكروغرام/ مل بينما كانت بعد تناول كبسولة واحدة من ديفلوكان تتراوح بين 1.636 - 5.707 مايكروغرام / مل بمتوسط 0.999 ± 2.777 مايكروغرام/ مل. اما متوسط ال t_{max} للفلوكونازول في الدم بعد تناول حبتين من الحبوب فكانت 1.386 ± 1.979 ساعة بينما كانت بعد تناول كبسولة واحدة من ديفلوكان 1.763 ± 3.395 ساعة. اما المساحة تحت المنحنى AUC فكانت بعد تناول حبتين من الحبوب المنتجة تتراوح بين 226.05 - 78.09 مايكروغرام.ساعة/ مل بمتوسط 39.47 ± 142.5 مايكروغرام.ساعة / مل بينما كانت بعد تناول كبسولة واحدة من ديفلوكان تتراوح بين 76.55 - 310.02 مايكروغرام.ساعة / مل بمتوسط 60.333 ± 149.3 مايكروغرام.ساعة / مل. التكافؤ النسبي يساوي 95.44% عند حسابه من المساحة تحت المنحنى.

كما تم تطوير طريقة بيولوجية لحساب الفعالية للفلوكونازول تعتمد على طريقة الصحون (plate method) باستخدام (Candida Albicans) و تم مقارنة فعالية الحبوب المنتجة مع كبسولات ديفلوكان وكانت النتيجة 103.1%. وفي تجربة اخرى تم دراسة فعالية مادة الفلوكونازول ضد اربع انواع بكتيرية باستخدام طريقة (MIC). البكتيريا التي استعملت للدراسة هي: Pseudomonas Aeruginosa, Staphylococcus Aureus, Salmonella, E. Coli, لقد وجد ان للفلوكونازول فعالية ضد Staphylococcus Aureus حيث ان 250 مايكروغرام / مل منعت نمو 4.4 $10x^3$ من البكتيريا / مل باستخدام (TSB). و قد قورنت هذه النتيجة مع تلك التي حصل عليها باستخدام كلاريثرومايسين تحت نفس الظروف حيث ان 36.5 مايكروغرام / مل منعت نمو 4.4 $10x^3$ من البكتيريا / مل وبالتالي فان الفعالية للفلوكونازول مقارنة مع الكلاريثرومايسين هي حوالي 14.6%.

ان مادة الفلوكونازول لم تظهر فعالية ضد ال Salmonella (استخدمت تراكيز وصلت الى 1000 مايكروغرام / مل ضد 1.25 $10x^3$ من البكتيريا /مل)، اوضد ال Pseudomonas Aeruginosa (استخدمت تراكيز وصلت الى 1000 مايكروغرام /

مل ضد 4.3 $10x^3$ من البكتيريا / مل)، اوضد ال E. Coli (استخدمت تراكيز وصلت الى 1000 مايكروغرام / مل ضد 2.9 $10x^3$ من البكتيريا / مل).

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PART I INTRODUCTION

I-1 Mode of Action.[1,2,5,15]

Fluconazole is the first of a new subclass of synthetic triazole antifungal agents. It is designated chemically as 2,4-difluoro-a,a1-bis(1H-1,2,4-triazol-1-ylmethyl) benzyl alcohol with an empirical formula of $C_{13}H_{12}F_2N_6O$ and Molecular Weight 306.3. It is a white crystalline solid which is slightly soluble in water and saline.

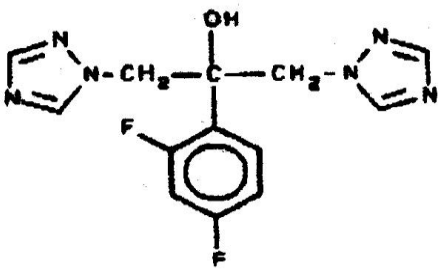

Figure I-1: Structure of Fluconazole

The only marketed product, active by oral route, similar to Fluconazole, is Ketoconazole. Fluconazole has the following advantages over Ketoconazole:

1. Therapeutic doses 4-8 times less (Fluconazole = 50 mg / day; Ketoconazole = 200 – 400 mg / day)
2. Fluconazole does not produce alternation in the hepatic enzymes production.
3. Fluconazole does not alter the hematic levels of testosterone.
4. Fluconazole presents an excellent penetration in the cerebrospinal fluid and this assures great possibilities for the SNC-micosis treatment.

Fluconazole is a highly selective inhibitor of fungal cytochrome P-450 sterol C-14 alpha-demethylation. Mammalian cell demethylation is much less sensitive to Fluconazole inhibition. The subsequent loss of normal sterols correlates with the accumulation of 14 alpha-methyl sterols in fungi and may be responsible for the fungistatic activity of Fluconazole.

I-2 Pharmacokinetics and Metabolism [1,5,15, 21]

The pharmacokinetic properties of Fluconazole are similar following administration by the intravenous or oral routes. In normal volunteers, the bioavailability of orally administered Fluconazole is over 90% compared with intravenous administration.
Peak plasma concentrations (C_{max}) in fasted normal volunteers occur between 1 and 2 hours with a terminal plasma elimination half-life of approximately 30 hours (range: 20-50 hours) after oral administration. In fasted normal volunteers, administration of a single oral 400 mg dose of Fluconazole leads to a mean Cmax of 6.72 µg/ml (range: 4.12 to 8.08 µg/ml) and after single oral doses of 50-400 mg, Fluconazole plasma concentrations and AUC (area under the plasma concentration-time curve) are dose proportional.
Steady-state concentrations are reached within 5-10 days following oral doses of 50-400 mg given once daily. Administration of a loading dose (on day 1) of twice the usual daily dose results in plasma concentrations close to steady-state by the second day. The apparent volume of distribution of Fluconazole approximates that of total body water. Plasma protein binding is low (11-12%). Following either single- or multiple-oral doses for up to 14 days, Fluconazole penetrates into all body fluids studied (see table (I-1)). In normal volunteers, saliva concentrations of Fluconazole were equal to or slightly greater than plasma concentrations regardless of dose, route, or duration of dosing.

Table I-1: Ratio of Fluconazole Tissue (Fluid)/Plasma Concentration

Tissue or Fluid	Ratio
Cerebrospinal fluid	0.5-0.9
Saliva	1
Sputum	1
Blister fluid	1
Urine	10
Normal skin	10
Nails	1
Blister skin	2
Vaginal tissue	1
Vaginal fluid	0.4-0.7

In normal volunteers, Fluconazole is cleared primarily by renal excretion, with approximately 80% of the administered dose

appearing in the urine as unchanged drug. About 11% of the dose is excreted in the urine as metabolites.

The pharmacokinetics of Fluconazole are markedly affected by reduction in renal function. There is an inverse relationship between the elimination half-life and creatinine clearance. The dose of Fluconazole may need to be reduced in patients with impaired renal function.

Fluconazole is presented as tablets, powder for oral suspension, or injections.

In Palestine, Fluconazole is presented as capsules.[14]

I-3 Microbiology [1,5,15, 21]

Fluconazole exhibits in-vitro activity against Cryptococcus neoformans and Candida spp. Fungistatic activity has also been demonstrated in normal and immunocompromised animal models for systemic and intracranial fungal infections due to Cryptococcus neoformans and for systemic infections due to Candida albicans.

Together with other azole antifungal agents, most fungi show a higher apparent sensitivity to Fluconazole in vivo than in vitro. Fluconazole administered orally and/or intravenously was active in a variety of animal models of fungal infection using standard laboratory strains of fungi.
Oral Fluconazole has shown to be active in an animal model of vaginal candidiasis.

I-4 Indications And Usage[1,5,15]

Fluconazole is indicated for the treatment of:
a- Vaginal candidiasis (vaginal yeast infections due to *Candida*).
b- Oropharyngeal and esophageal candidiasis. In open noncomparative studies of relatively small numbers of patients, Fluconazole was also effective for the treatment of Candidaurinary tract infections, peritonitis, and systemic Candida infections including candidemia, disseminated candidiasis, and pneumonia.
c- Cryptococcal meningitis.

I-5 Quantitative and Qualitative Determination [7,8,9,10]

Fluconazole is not listed in the United States Pharmacopoeia or in the British Pharmacopoeia. A development for a quantitative and qualitative method of analysis is necessary. The preferred method for analysis of Pharmaceutical preparations is the HPLC (High Performance Liquid Chromatography), as most pharmaceuticals can be separated by HPLC system. Most preparations have a UV maxima and so, a UV/Visible detector is mostly used in the HPLC analysis. HPLC is considered to be fast, reproducible, sensitive, easy to work with, cost effective and available in the market.

I-5-1 Quantitative Determination (Assay):

This is done by preparing a “Sample” and a “Standard”. They are then injected separately in the HPLC system. “Area under the Peak” is determined for each and the following formula is used to calculate the percentage of active material in the sample.

$$\% \text{ Active} = \frac{\text{Area of the Sample}}{\text{Area of the Standard}} \times 100$$

I-5-2 Qualitative Determination (Identification)

This is done by comparing the Retention Time t_R of the “Sample” and the “Standard”. They should be similar.

I-5-3 Important Parameters in Separation Process:

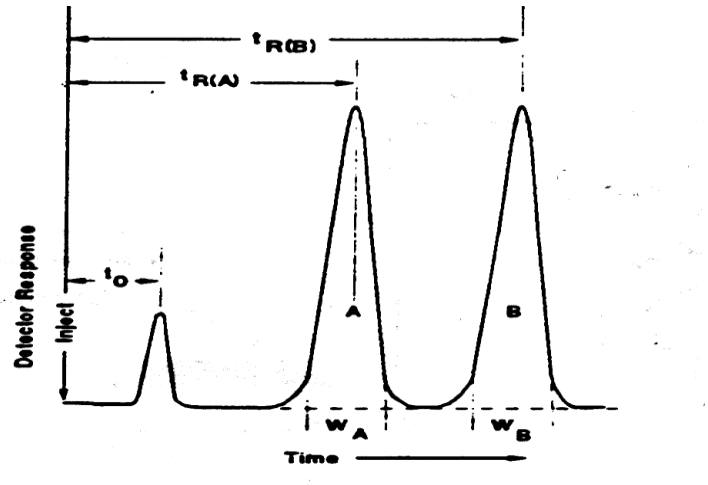

Figure I-2: Peak separation and identification

Figure (I-2) illustrates the most important parameters in identification and separation of different peaks.

t_0: Elution time of an unretained component.
t_R: Retention time of each molecule. At the same conditions and when separation occurs, each molecule has different retention time. Thus, retention time is used for the identification of the molecule.
W: Peak width at base.

a- Resolution (R)
The resolution is an indication for separation. For example:

Peak A with $t_{R(A)}$
Peak B with $t_{R(B)}$

The resolution R can be calculated by the following formula:

$$R = 2 \frac{t_{R(B)} - t_{R(A)}}{W_A + W_B}$$

Where:

$t_{R(A)}$ = Retention time of peak A
$t_{R(B)}$ = Retention time of peak B

W_A = Width of peak A
W_B = Width of peak B

For equal areas: R = 1.5 gives baseline separation

b- Peak Symmetry (S):

Another important parameter is the peak symmetry, which is calculated by the following formula:

$$S = \frac{A}{B}$$

Where A and B are illustrated in (figure I-3).
S = 1.0-1.05 gives the best symmetry.

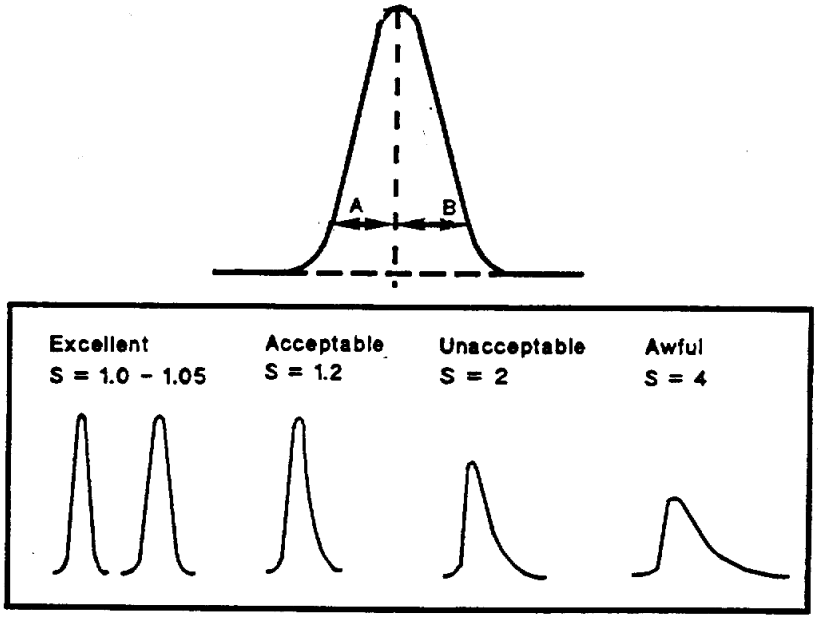

Figure I-3: Peak symmetry

I-6 Types of Tablets[3, 17]

A tablet form was chosen as it can be mass produced simply and quickly and thus the resultant cost is low. Direct compression method use the minimum machines of all other tablet methods, as no

granulation is performed and thus saving time, personnel and machines.

There are many types of tablet depending on their use:

- Those intended to be swallowed as a whole (coated and non-coated).
- Those intended to allow dispersion or dissolution in water prior to administration e.g. effervescent, dispersible.
- Chewable.
- Sublingual.
- Eextended Release.
- Enteric coated (delayed release).

As the pharmacokinetic data indicates, Fluconazole can be presented as tablets intended to be swallowed as a whole.

I-7 Dissolution of The Active Ingredient from the Dosage Form[7,8,11,12, 17]

Dissolution rate may be defined as the amount of drug substance that goes in solution per unit time under standardized conditions of liquid/solid interface, temperature and solvent composition.

Factors that affect the dissolution rate of dosage form can be classified into the following categories:

I-7-1 Physiochemical properties of the drug substance.

The best equation that describes the physiochemical properties is the modified Noyes-Whitney equation:

$$dc/dt = KA (Cs - Ct)$$

Where:

dc/dt: Rate of dissolution, where c is the mass of solute that has passed into solution in time t.

K : Dissolution constant,

A : Effective surface area of drug particle,

Cs: Saturated solubility of the drug in the diffusion layer

Ct: the concentration of drug at time t.

I-7-2 Choice of excepients

Diluents, binders, disintegrants, lubricants, surfactants and coating materials play all an important role in the dissolution rate.

I-7-3 The manufacturing process

In addition to the excepient effect, the manufacturing process plays also an important role. Method of granulation, granules size, moisture content and age of granules as well as the compression force utilized in the tabletting process, all affect the dissolution rate.

I-7-4 Dissolution apparatus and system

The most USP & BP official method for tablets is the rotating paddle method. It contains of approximately 1-in diameter X 1.375 in high. It is made from stainless steel, blade paddle rotates at a constant speed of between 25 rpm and 150 rpm (It is immersed in 900 (USP) or 1000 (BP) ml of dissolution medium in a cylindrical, glass flask of 1000 ml capacity. The medium in the flask is kept at a constant temperature of (37 $\pm$ 0.5) c° by means of a suitable water bath.

The standardized BP conditions for dissolution tests using the paddle are:

a- rotation speed: 50 rpm (paddle)
b- dissolution medium volume: 900 ml
c- dissolution medium composition: aqueous, commonly 0.1M HCl or phosphate buffers of pH 6.8 to 7.6
d- number of units tested: 6.

The standardized BP acceptance criteria for tests using either the basket or the paddle are that, for each unit tested, not less than 70% of the active ingredient or ingredients dissolves within 45 minutes. If one unit fails to meet this requirement, a retest may be carried out using the same number of units; all units in the retest must comply.

For developing formulations, an In-vitro dissolution profile is made by taking a sample at 10 minutes intervals or less. The dissolution profile of the developed formulation is compared with that of the innovative drug product at the same conditions, same sample method and using the same analysis method. The comparison of the two dissolution profile is calculated by the following formulas:

$$F1 = \{[\Sigma^{n}_{t=1} |R_t - T_t|] / [\Sigma^{n}_{t=1} R_t]\} \times 100$$

$$F2 = 50 \log \{[1+(1/n) \Sigma^{n}_{t=1} (R_t - T_t)^2]^{-0.5} \times 100\}$$

Where:
F1: The percentage difference between the two profiles at each sampling point.
F2: The similarity factor corresponds to the similarity measurement between two curves
n: number of samples
Rt: The percentage dissolved of the original brand at time t.
Tt: The percentage dissolved of the trial formulation at time t.

Limits:
F1 = 0 - 15
F2=50 - 100

I-8 Formulation [3,17]

Tablets may be defined as solid pharmaceutical dosage forms containing drug substances with or without suitable diluents and prepared either by compression or molding methods.

The formulation of Fluconazole tablet (Dican tablet) should be designed so that the final tablet will have all essential properties for a tablet. Each tablet must contain a known amount of drug and this must be checked by content uniformity tests. The tablets must also be uniform in weight, appearance and diameter. When they are swallowed whole, they should readily disintegrate in the stomach. They should be produced with sufficient strength to withstand the different stages of processing and packing, and yet be capable of rapid breakdown when administered in order to release the drug rapidly. The dissolution rate is critical as mentioned above. In addition, the tablets should be stable in its proposed packing, when

stored at its recommended storage conditions for a reasonable period of time. Finally, the tablets should be reasonably robust and be capable of withstanding normal patient handling and handling during transport.

Several parameters should be taken into consideration during the formulation process: dissolution rate, hardness and friability tests, weight uniformity and content uniformity to assure homogeneity of the drug substance in all tablets within a batch. The assay, identification and physical properties tests are important parameters to evaluate the resultant specifications. These tests are performed during the stability study as well. The results of the stability study are used to determine the ability of the packing to protect the product, the suitable storage condition and the suitable expiration date for the product. The method used for analysis should be validated to insure its linearity, selectivity, accuracy, precision, range and robustness.

In the formulation process, the majority of tablets are composed of the drug substance and other additives that make the powder system more compressible. There are two essential properties in the powder intended for compression into tablets; Powder Fluidity and Powder Compressibility.

Fluidity is required so that the material can be transported through the hopper of a tableting machine. It is also essential so that adequate filling of the dies occurs in the tableting machine to produce tablets of a consistent weight. Powder flow can be improved mechanically by the use of vibrators. However, the use of these devices can cause powder segregation. A better method to enhance powder fluidity is to incorporate a glidant into formulation. Materials such as fumed silicon dioxide (Aerosil) are excellent flow promoters even in concentrations of less than 0.01%. Another way to improve powder flow is to make the particles as spherical as possible. The most popular method is by granulation.

Compressibility is the property of forming a stable, intact compact mass when pressure is applied. Some materials compact is better than others depending on powder physics of each material. Nearly in all cases, granulation improves compressibility of powders.

I-8-1 Tablet Preparation Methods

The preparation of tablets can be divided into (a) dry methods and (b) wet methods. Dry methods include direct compression, slugging and roller compaction, and wet methods include wet granulation.

Direct compression is the most preferred of those methods employing liquids, since dry processes do not require the equipment and handling expenses required in wetting and drying procedures (cost effective) and can avoid hydrolysis of water-sensitive drugs (more stable). A direct compression vehicle is an inert substance which can be compacted with no difficulty and which may do so even when fairly large quantities of drugs are mixed with it. Materials currently available as direct compression diluents may be divided into three groups according to their disintegration properties and their flow characteristics:

1. Disintegration agents with poor flow, e.g. microcrystalline cellulose (Avicel), microfine cellulose and directly compressible starch
2. Free-flowing materials which do not disintegrate, e.g. dibasic calcium phosphate
3. Free-flowing powders which disintegrate by dissolution, e.g. spray-dried lactose, anhydrous lactose, spray-crystallized maltose, dextrose, sucrose, mannitol and amylose.

Microcrystalline cellulose (Avicel) is perhaps the most widely used direct compression excipient. It exhibits the highest compressibility of all known direct compression vehicles; however, it has relatively poor flow properties. Avicel is chemically an inert material and is compatible with most drugs.

I-8-2 Tablet Excipients

A tablet does not just contain the active ingredient but also include other substances, known as excipient, which have specific functions. Following are the main classes of excipients used:

a- Diluents

Diluents or 'bulking agents' are 'Inert' substances which are added to the active ingredient in sufficient quantity to make a reasonably sized

tablet. This agent may not be necessary if the dose of the drug per tablet is high. Generally, a tablet should weigh at least 50 mg and therefore very low dose drugs will invariably require a diluent to bring the overall tablet weight to at least 50 mg. The principal substance employed as a diluent is Lactose. It has a pleasant taste, rapidly dissolves in water, absorbs very little moisture and is fairly neutral in reaction. Its main disadvantage is that it is somewhat expensive and has poor flow characteristics. Lactose deforms easily under pressure and, as a result of this ductility, good tablets are normally produced. The spray-dried form of lactose flows much more readily and is used as a direct compression vehicle. Another very popular diluent is Microcrystalline Cellulose (Avicel). It is a nonfibrous form of cellulose and is obtained by spraying washed, acid-treated cellulose. It is water insoluble, but the material has the ability to draw fluid into the tablet by capillary action, thus it swells on contact and acts as a disintegrating agent. The material flows well and has a degree of self-lubricating qualities, thus requiring lower level of lubricant as compared to other excepients.

b- Binding agents (adhesives)

The direct compression method for preparing tablets requires not only a free flowing material, but also sufficiently cohesive to act as a binder. An example is Avicel, mentioned above, which is a special form of cellulose fibril in which the individual crystallites are held together largely by hydrogen bonding. It has an excellent compression properties.

c- Glidants

Glidants are materials which are added to tablet formulations in order to improve the flow properties of the granulations. They act by reducing interparticulate friction. The most commonly used and effective glidant is fumed (or colloidal) silica. Flow of granules can be dramatically improved by the addition of less than 0. 1% w/w of this material to powders and granules. Fumed silica is thought to act by lodging in the surface irregularities of the particles or granules, which effectively smoothes the particle surface.

d- Lubricants

These agents are required to prevent adherence of the granules to the punch faces and dies. They also ensure smooth ejection of the tablet from the die. Many lubricants also enhance the flow properties of the granules. Magnesium stearate is the most popular lubricant used and is normally effective on its own as both a die and a punch lubricant. It is incorporated by blending with the dry granules prior to compression, up to a concentration of about 5% w/w. A thin layer of magnesium stearate around the granule is just as effective as a thick layer from the lubrication point of view, but increased magnesium stearate quantities reduce the disintegration time, retard drug dissolution and also reduce the bonding forces between granules to produce soft tablets. The reduction in drug release properties is due to the hydrophobic nature of magnesium stearate preventing drug dissolution.

e- Disintegrating agents

Disintegrants are always added to tablets to promote breakup of the tablets when placed in an aqueous environment. The object of a disintegrant is to cause the tablet to disintegrate rapidly so as to increase the surface area of the tablet fragments and so promote rapid release of the drug.

Disintegrants can act by swelling in the presence of water to burst open the tablet. Starch is the commonest disintegrant in tablet formulation and is believed to act by swelling. However, other effective disintegrants do not swell in contact with water. Disintegrants that do not swell exert their disintegrating action by capillary action. Liquid is drawn up through capillary pathways within the tablet and ruptures the interparticulate bonds. This action serves to break the tablet apart.

The oldest and still most popular disintegrants are corn and potato starch. For rapid disintegration, an amount of 10 to 15% is needed.

A group of super disintegrants have gained in popularity as disintegrating agents, because of the low levels (2 to 4%) at which they are completely effective. In this group examples are croscarmelose or known also as Ac-Di-Sol (cross-linked cellulose), crospovidone (cross-linked polymer) and Sodium Starch Glycolate

(cross-linked starch). Croscaremelose is the most efficient disintegrating agent.

I-9 Stability Studies [4,6]

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as:

1. Temperature.
2. Humidity.
3. Light.

Stability testing enables:

1. To select adequate (from the view point of stability) formulations and container closure systems.
2. To determine shelf-life
3. Storage conditions.
4. To verify that no changes have been introduced in the formulation or manufacturing process that can adversely affect the stability of the product.

There are three types of stability testing performed:

I-9-1 Accelerated Testing

Studies designed to increase the rate of chemical degradation or physical change of an active drug substance or drug product by using exaggerated storage conditions as part of the formal, definitive, storage program.

These data, in addition to long term stability studies, may also be used to assess longer term chemical effects at non-accelerated conditions and to evaluate the impact of short term excursions outside the label storage conditions such as might occur during shipping.

Four climatic zones can be distinguished for the purpose of worldwide stability testing:

Zone I : Temperate.

Zone II : Sub-tropical with possible high humidity.
Zone III : Hot/dry.
Zone IV : Hot/humid.

The following table (I-2) summarize the Storage temperature, humidity and period for each climatic zone:

Table I-2: Accelerated stability parameters for each climatic zones

Zone	Storage Temp. [°C] ± 2°C	Humidity [%] ± 5%	Period [Months]
III, IV	40	75	6
I, II	40	75	3

I-9-2 Long Term (Real Time) Testing

Stability evaluation of the physical, chemical, biological and microbiological characteristics of a drug product and a drug substance, covering the expected duration of the shelf life and re-test period, which are claimed in the submission and will appear on the labeling.

The following table (I-3) summarize the Storage temperature and humidity for each climatic zone:

Table I-3: Long term stability parameters for each climatic zones

Zone	Storage Temp. [°C] ± 2°C	Humidity [%] ± 5%
I	21	45
II	25	60
III	30	35
IV	30	70

I-9-3 On-going Real Time Stability

It is the study in post-marketing carried out by the manufacturer on production batches according to pre-determined schedule in order to confirm the expected shelf life of the product.

Where 'significant change' occurs due to accelerated testing, additional testing at an intermediate condition e.g., 30 ºC ± 2 ºC/60 percent ± 5 percent RH should be conducted. 'Significant change' at the accelerated condition is defined as:

1. A 5 percent potency loss from the initial assay value of a batch;
2. Any specified degrades exceeding its specification limit;
3. The product exceeding its pH limits;
4. Dissolution exceeding the specification limits for 12 capsules or tablets.
5. Failure to meet specifications for appearance and physical properties e.g., color, phase separation, re-suspendibility, delivery per actuation, caking, hardness, etc.

The long term testing will be continued for a sufficient time beyond 12 months to cover shelf life at appropriate test periods.

After evaluation of the stability, the product may be labeled with the following storage conditions:

1. Store at normal storage conditions, at 15 ºC-30 ºC.
2. Store between 2 ºC– 8 ºC under refrigeration, no freezing.
3. Store below 8 ºC under refrigeration.
4. Store in freezer at (-)5 ºC– (-) 20 ºC.
5. Store below (-)18 ºC in a deep freezer.

General precautionary statements, such as “protect from light” and/or “store in dry place”, may be included, but should not be used to cover up stability problems.

If applicable, recommendations should also be given regarding the utilization period after opening and dilution, or reconstitution of a solutions and suspensions.

I-10 Validation of the Analytical Procedure[16, 21]

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. The analytical

procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc.

Validation of the analytical procedures is directed to the four most common types of analytical procedures:

1- Identification tests.
2- Quantitative tests for impurities' content.
3- Limit tests for the control of impurities.
4- Quantitative tests of the active substance or other selected component(s) in samples of substance or medicinal product.

The assay represents a quantitative measurement of the active or other selected components. Following are the typical validation characteristics which should be considered:

I-10-1 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

I-10-2 Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

I-10-3 Specificity (selectivity)

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

I-10-4 Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

I-10-5 Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

I-10-6 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Furthermore revalidation may be necessary in the following circumstances:

1- changes in the synthesis of the active substance;
2- changes in the composition of the medicinal product;
3- changes in the analytical procedure.

The degree of revalidation required depends on the nature of the changes. Certain other changes may require validation as well.

I-11 Bioequivalence Study[3,11,18,19]

Bioavailability is defined as a measure, relative to some standard, of the rate and amount of drug which reaches the systemic circulation unchanged following the administration of a suitable dosage form.

The gastrointestinal barrier is structurally a complex assemblage of lipids, proteins, lipoproteins and polysaccharides. Lipid-soluble substances penetrate the gastrointestinal boundary through passive diffusion by dissolving in the lipid like phase. Polar substances also penetrate the gastrointestinal boundary through passive diffusion if they are small enough to filter through microscopic pores in the membrane or the spaces between cells.

Passive diffusion can be described by Fick's law:

$$dm/dt = D\ A\ K_1\ Cg / h$$

Where:

dm/dt: Rate of appearance of drug in the blood at the site of absorption.

D: Effective diffusion coefficient of the drug in GI membrane,

A: Surface area of GI membrane,

K_1: Apparent partition coefficient of the drug between the GI membrane and the GI fluid

Cg: The concentration of drug in solution in the gastrointestinal fluid in the site of absorption.

h: The thickness of GI membrane.

Fluconazole is soluble in 0.1 N HCl, has lipophilic properties and has 90 % bioavailability versus the IV. Thus, a passive absorption is most likely occurs.

Few lipid-insoluble drugs are absorbed by Active Transport process. A carrier, which may be an enzyme or some other component of the cell membrane is responsible for effecting the transfer of drug. Active transport is a process whereby materials can be transported against a concentration gradient across a cell membrane. Therefore, active transport is an energy consuming process.

Facilitated diffusion or transport is also a carrier-mediated transport system which differs from active transport in that it cannot transport a substance against a concentration gradient of the substance. Therefore it does not require an energy input, but it does require a concentration gradient for its driving force.

In-Vivo bioequivalence is made to assure the similarity of the bioavailability between the developed formula and the original drug. This is done on 24 healthy volunteers according to FDA regulations. They are chosen all within the normal weight range. They are examined clinically for normal hematological value, renal and hepatic function values. Exclusion criteria included extreme weight ranges, anemia, liver or renal dysfunction, parasitic and other disease or conditions that is judged to affect the absorption, distribution and elimination of the drug substance.

The assessment of bioequivalence between the test and the reference product is based on the ratios of the mean pharmacokinetic parameters (C_{max}, t_{max}, AUC $_{0\rightarrow\infty}$) using ANOVA. Bioequivalence is concluded if either tail probability did not exceed the 90% confidence limit and was completely contained in the 0.80 – 1.25 range.

I-12 Biological evaluation of antifungal and antibacterial activity [3,7,8]

I-12-1 Antibacterial activity

To evaluate the antibacterial activity of a drug substance, the measurement of the minimum inhibitory concentration against particular test organism is performed. MIC is the lowest concentration of the drug substance found to inhibit the growth of the test organism. MIC is an absolute value and is not a comparison between standard and a sample. It is usually expressed in μg / ml. Other experimental details should be stated with the result such as the inoculum concentration and the culture medium. Fluconazole is an antifungal agent and its antibacterial effect will be evaluated against several pathogenic bacterial strains. British and US pharmacopoeias evaluate the

product cleanness by a specific total bacterial count and the absence of four pathogenic microorganisms: Salmonella, Staphylococcus Aureus, Pseudomonas Aeruginosa and Escherishia Coli. These microorganisms will be used in evaluation of the antibacterial effect of Fluconazole. To calculate the inoculum concentration used in the test, a plate count method is used. The method is done by preparing several dilutions of the inoculum solution used and spread a determined volume from it on a specific agar medium, and then incubate at 35-37 C for 24-48 hours. Each microorganism grows to form a colony. By calculating the number of colonies in a certain plate, and multiplying by the dilution factor of that plate, the original bacterial concentration is calculated.

I-12-2 Biological Assay comparison

There is no official monograph for calculating the biological assay potency of an antifungal. The available biological tests for the assay are done for antibiotics. The test compares the potency of a test sample with a reference one. There are two official methods for calculating the potency:

a- Cylinder Plate method
The test organism is inoculated in a specific agar medium. The agar is poured in a specific sterile plate. Wells or cylinders are placed on the surface of the plate at a specified distance. A specific volume of the antibacterial solution of known concentration is placed inside the well or cylinder. The solution diffuses through the agar forming zones, inhibiting the growth of the bacteria at this zone. The diameter of the zone depends on the concentration of the antibacterial used. A standard is prepared at the same concentrations and the zone diameters are compared together and calculation is done for the potency of the test sample.

b- Turbidimetric method
The test organism is inoculated in broth medium placed in sterile tubes. Different concentrations of the test solution are placed in the tubes. The transmittance of each tube is measured and compared to standard. The transmittance measure depends on the concentration of the antibacterial.

The plate method is preferred as standards and samples are found on the same plate, thus have the same conditions. For development of a method for Fluconazole, it is important to choose the suitable agar medium and test microorganism. Once a method of a biological assay is developed, Fluconazole tablet is evaluated using the original brand as the standard. The potency ratio may be calculated based on two point concentration assay using the following formula:

$$\text{Log } X = \text{LDR} \times \frac{(UH + UL) - (SH + SL)}{(SH - SL) + (UH - UL)} \qquad \text{.....(3)}$$

Where
X = the potency ratio
LDR = the logarithm of the dose ratio (ratio of the concentration of standard solutions)
UH = zone diameter for the higher concentration of the sample
UL = zone diameter for the lower concentration of the sample
SH = zone diameter for the higher concentration of the standard
SL = zone diameter for the lower concentration of the standard

PART II: EXPERIMENTAL

II-1 Specification and Test Method for the Active Substance "Fluconazole"

II-1-1 Specification (as per manufacturer, year 2002)

Empirical formula	$C_{13}H_{12}F_2N_6O$
Molecular weight	306.3
Description	Take 2 grams of the material and distribute it on a glass plate. Record the observations
Melting range	Charge the capillary glass rod of the melting range tester with the material to be analyzed. Record the temperature on which the material melts
Odor	Take 2 grams of the material and distribute it on a glass plate. Smell the material from a suitable distance. Record the odor.
Solubility	slightly soluble in water, 5% w/v solution in methanol is clear.
Loss on Drying at 105 ºC	Dry about 5 grams of the material at 105 C for 4 hours using the heat balance. Record the loss percentage.
Residue on Ignition	Weight 1.0 gm of the substance in a suitable crucible that previously has been ignited, cooled and weighed. Heat, gently at first until the substance is thoroughly charred, cool, moisten the residue with 1 ml of sulfuric acid, heat gently until white fumes no longer are evolved, and ignite at 800 ± 25°C, until the carbon is consumed . Cool in desiccators, weigh, and calculate the percentage of the residue. If the amount of the residue so obtained exceeds the limit specified , again moisten the residue with 1 *N* sulfuric acid, heat and ignite as before , and again calculate the percentage of the residue . Unless otherwise specified, continue ignition until constant weight is attained or until the percentage of the residue complies with the limits.
Heavy Metals	Standard Preparation: Into a 50-ml color-comparison tube pipet 1 ml of standard lead solution (10 µg of Pb), and dilute with water to 25 ml. Adjust with 1 *N* acetic acid or 6 *N* hydrochloric acid to pH of 3.5, dilute with water to 100 ml, and mix.

	Test Preparation: Transfer 1.0 gm of the substance to suitable crucible ,add sufficient sulfuric acid to wet the substance, and carefully ignite at low temperature until thoroughly charred, add to the carbonized mass 2 ml of nitric acid and 5 drops of sulfuric acid, and heat cautiously until white foam no longer are evolved. Ignite, preferably in a muffle furnace, at 500°C to 600°C, until the carbon is completely burned off.. Cool, add 4 ml of 6 *N* hydrochloric acid, cover, digest on a steam bath for 15 minutes, uncover, and slowly evaporate on steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 ml of hot water, and digest for 2 minutes. Add 6 *N* ammonium hydroxide dropwise, until the solution is just alkaline to litmus paper, dilute with water to 25 ml, and adjust with 1 *N* acetic acid to pH between 3.0 and 4.0, using short-range pH indicator paper as external indicator .Filter if necessary, rinse the crucible and filter with 10 ml of water, combine the filtrate and rinsing in 50-ml color-comparison tube dilute with water to 40 ml, then mix. Procedure: To each of the tubes containing standard preparation and test preparation , add 2ml of pH 3.5 acetate buffer, then add 1.2 ml of thioacetamide-glycerin base TS, dilute with water to 50 ml, mix, allow to stand for 2 minutes, and view downward over a white surface, the color of the solution from the test preparation is not darker than that of the solution from the standard preparation.
Related Substances by HPLC	Sum of impurities not more than 1.0%
Particle size:	100% < 180 µm
Bulk Density: Untapped	0.25 to 0.45 gm / ml
Tapped	0.35 to 0.55 gm / ml

II-1-2 Assay

a- Instrument

Merck-Hitachi HPLC systems consisting of an autosampler model L-7200, a pump model L-7100 and a diode array detector model L-7450A,

b- Mobile Phase

Mix 45% water and 55% Methanol.

c- Stationary Phase

LiCrospher RP-18 e, 5 μm, 250x 4mm.

d- Flow Rate

0.5 ml/minute.

e- UV Wavelength

261 nm.

f- Standard Preparation:

Dissolve 150 mg Fluconazole reference standard accurately weighed in a 100-ml volumetric flask, add 70 ml of mobile phase, shake and sonicate for 5 minutes, and complete the volume to the mark using the mobile phase. Dilute 5 ml in 50 ml mobile phase.

g- Sample Solution Preparation:

Dissolve 150 mg Fluconazole sample accurately weighed in a 100-ml volumetric flask, add 70 ml of mobile phase, shake and sonicate for 5 minutes, and complete the volume to the mark using the mobile phase. Dilute 5 ml in 50 ml mobile phase

h- Procedure

Let the mobile phase running until reaching a stable state of equilibrium. After filtration using a membrane filter of (0.20 μm), inject separately equal volumes (20 μL) of the prepared solutions triplicate as follows (Standard, Sample, Standard, Sample, Standard and Sample) into the chromatograph.

i- Calculation

$$\% \text{ Fluconazole} = \frac{\textbf{Sample} \text{ peak area}}{\textbf{Standard} \text{ peak area}} \times 100$$

II-2 Specification and Test Method for the Final Product Fluconazole Tablet

II-2-1 Specification

a- Shape: Triangular tablets

b- Unit Weight (None Coated): 251.0 mg ± 12.5 mg.

c- Color: Beige color.

d- Odor: Odorless.

e- Diameter: 9.0 ± 0.5 mm.

f- Hardness: Min. 4.0 K.

g- Friability: Max. 1.0% (100 rounds).

h- Description of Filling Material: a sachet made from Aluminum Foil (0.02 mm) on one side and colorless PVC (0.25 mm) on the other side.

II-2-2 Assay

a- Instrument
Merck-Hitachi HPLC systems consisting of an autosampler model L-7200, a pump model L-7100 and a diode array detector model L-7450A,

b- Mobile Phase
Mix 45% water and 55% Methanol.

c- Stationary Phase
LiCrospher RP-18 e, 5 μm, 250x 4mm.

d- Flow Rate

0.5 ml/minute.

e- UV Wavelength
261 nm.

f- Standard Preparation:

Dissolve 150 mg Fluconazole reference standard accurately weighed in a 100-ml volumetric flask, add 70 ml of mobile phase , shake and sonicate for 5 minutes, and complete the volume to the mark using the mobile phase. Dilute 5 ml in 50 ml mobile phase.

g- Sample Solution Preparation:

Weigh not less than ten tablets, determine the average weight, and powder them. Weigh in powder exactly the average weight of one and a half tablet and dissolve it in a 100-ml volumetric flask, add 70 ml of mobile phase , shake and sonicate for 5 minutes and complete the volume to the mark using the mobile phase. Dilute 5 ml in 50 ml mobile phase.

h- Procedure
Let the mobile phase running until reaching a stable state of equilibrium. After filtration using a membrane filter of (0.20 μm), inject separately equal volumes (20 μL) of the prepared solutions twice as follows (Standard, Sample, Standard and Sample) into the chromatograph.

i- Calculation

$$\text{Fluconazole} = \frac{\textbf{Sample}\ \text{peak area}}{\textbf{Standard}\ \text{peak area}} \times 100\%$$

II-2-3- Dissolution

a- Medium: 900 ml 0.1N HCl.

b- Apparatus: paddle, 50 round/min

c- Time: 45 min

d- Temperature: 37 ± 1 Cº

e- Standard preparation:

Transfer the weight of 11.1 mg of Fluconazole RS accurately weighed to 100 ml volumetric flask, add 15 ml of methanol, shake and sonicate for 5 min, add 70 ml of dissolution medium, sonicate for another 5 minutes, wait until cooling, then complete to the volume using dissolution medium.

f- Sample preparation:

After the dissolution time is over, directly and as soon as possible take and filter the needed samples from each dissolution vessels.

g- Procedure:

HPLC, the same settings as directed in the assay test.

h- Limits:

Not less than 80% of the active ingredient or ingredients dissolves within 45 minutes. If one unit fails to meet this requirement, a retest may be carried out using the same number of units; all units in the retest must comply.

II-2-4- Other tests:

a. Content uniformity

Assay 10 units individually as directed in the assay.

Limit:

If the average of the limit specified in the potency definition in the individual monograph is 100% or less: proceed as follows: the amount of the active ingredient in each 10 dosage lies within the range of 85.0 % to 115.0 % of the labeled claim and the relative standard deviation is ≤ 6.0%. If one dosage is out side the range 85.0 % to 115.0 % of the labeled claim and no unit is outside the (75.0% - 125.0%) or the RSD is greater than 6.0% or both, test additional 20 unit, the requirement are met if not more than 1 unit of the 30 units is outside the range of 85.0%to

115.0% of the labeled claim, and no unit is outside the range of 75.0% to 125.0% of the labeled claim and the RSD of the 30 dosage unite does not exceed 7.8%.

b. Hardness:

Take 10 tablets randomly and measure the hardness of each tablet.

Limit: Each tablet must lie in the range of Min. 4.0 K

c. Friability:

Take the weight (initial weight) of 20 tablets and place them in the friability device and rotate 100 rounds. Reweigh (final weight) the tablets and calculate the friability ratio.

Calculation:

$$\text{Friability ratio} = \frac{\text{initial weight - final weight}}{\text{initial weight}} \times 100$$

Limit: maximum 1.0%

II-2-5 Physical tests:

a. Purity:

Examine the material visually or by using a microscope for any strange bodies or black spots.

Limits: No strange matter should be found in the powder.

b. Odor:

Examine the material for the powder odor, compare its odor with a reference standard.

Limits: The granule odor should be identical to the reference standard.

c. Chipping and capping:

Examine the surface of tablets visually.

Limits: No chipping or capping should be found on the tablets surface.

II-3 Validation of the Test Method

II-3-1 Selectivity:

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

a. Standard Solution Preparation:

75 mg Fluconazole accurately weighed was transferred to 50-ml volumetric flask, 40 ml of mobile phase was added, sonicate for 5 minutes, allow to cool to room temperature, complete the volume to the mark using the same solvent. Pipette 5.0 ml of the prepared solution into a 50-ml volumetric flask and complete the volume to the mark using mobile phase *The final dilution concentration is 15.0 % (mg/ml)of Fluconazole .*

b. Sample Solution Preparation:

Transfer in powder exactly the weight of **one and half tablet** (375 mg) to a 100-ml volumetric flask, add 70 ml of the mobile phase, sonicate for 15 minutes and allow to cool to room temperature. Complete the volume to the mark using the same solvent. Allow any insoluble matter to settle, then transfer 5 ml of this solution to 50ml- volumetric flask, and complete to the volume using the same solvent. *The final dilution concentration is 15.0 % (mg/ml)of Fluconazole .*

c. Placebo Solution Preparation:

Transfer about 225 mg of a placebo Dican 100 tablet to 100-ml volumetric flask, add 70 ml of the mobile phase, sonicate for 15 minutes and cool to room temperature. Complete the volume to the mark using the same solvent [**Solution A**]. Pipette 5.0 ml of the prepared solution into a 50-ml volumetric flask and complete the volume to the mark using the mobile phase

The selectivity is also made for solution prepared under stress conditions. Solution samples of Fluconazole in the following solvents are prepared: 5N NaOH, 5N HCl, 30 % H2O2 and in water. The samples are kept for 48 hours. The water sample is boiled for 5 minutes and kept at 60°C for the same period. The assay, purity check are calculated. A check is also made for any peaks in the chromatograms.

II-3-2 Accuracy and Precision:

a. Evaluate the Accuracy and the Precision of the method on three trials (one trial per a day). Five sample solutions are prepared using a different trial for each day.
b. Sample and Standard Solutions: Prepare the sample and the standard solutions as directed under *Selectivity*.
c. Use the same batch of Fluconazole for the three trials.

III-3-3 Linearity:

a. Prepare five concentrations: 60%, 80%, 100%, 120%, and 140% of the final dilution concentration.
b. Use the same batch of the active material for all the solutions.
c. Prepare [Solution B] by dissolving 150 mg of Fluconazole in 100 ml mobile phase.
d. Prepare the concentration indicated in the following table:

Table II-1: Concentration preparation for Linearity

Concentration (%)	**[Solution A] (ml)**	**[Solution B] (ml)**	**Mobile Phase (ml)**
60	5	3.0	42
80	5	4.0	41
100	5	5.0	40
120	5	6.0	39
140	5	7.0	38

II-3-4 Robustness:

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

a. Two Different Analysts:

Each analyst must prepare the sample and the standard solutions to be analyzed using the same manner and under the same conditions. Each analyst must use the same prepared trial.

b. Two Different Instruments:
Use each of the Hitachi, (RI001) and the Hitachi, (RI002) HPLC systems.

c. Different elapsed assay times:

Preparation Time:
Analyze the same sample solution after 10 minutes and after 4 hours of preparing the sample solution.

Sonicating Time:

Sonicate each sample solution according to the following criteria:

Sample 1: sonicate for 10 minutes.
Sample 2: sonicate for 15 minutes.
Sample 3: sonicate for 20 minutes

d. Different columns:

Analyze the same sample solution using two different columns, which is of the same type and dimensions, (LiCrospher 100 RP-18, 5 µm, 250x4 mm).

II-3-5 Acceptance Criteria

Table II-2: Acceptance criteria for validation

Parameter	Statistical Measure	Limits
Selectivity	None	No interference between the active material peak and other peaks
Accuracy	Percent of Recovery	98.00 - 102.00
Precision	Coefficient of Variation	Maximum 1.50
Linearity	Correlation Coefficient Y-Intercept	Minimum 0.99980 ± 2.0 % of the average area
Ruggedness	Coefficient of Variation	Maximum 1.50

II-4 Formulation

II-4-1 Choice of Excipients

As a direct compression formula, the following excipients were selected for the trials:

Table II-3: Excipients used for trials in Fluconazole tablet formulation

	Material	Function
1	Fluconazole	Active substance
2	Avicel 102	Filler and Binder
3	Ac-Di-Sol	Disintegrant
4	Lactose spray dried	Filler
5	Lactose monohydrate	Filler
6	Aerosil	Glidant
7	Mg Stearate	Lubricant
8	Yellow iron oxide	Coloring Agent

All of the above materials are used for direct compression formulations.

II-4-2 Equipments and Machines

Trials are done on small batches (1 Kg). Mixing is done manually in PE bags and a single punch tableting machine is used. A pilot batch (30 Kg) is done for the best formula using the drum mixer and a rotary 16 punches tableting machine.

Drum mixer (50 Kg capacity)
Screen mesh 325 µm (mesh # 58)
Tableting machine, single punch.
Tableting machine, 16 punches.
Blistering machine.

II-4-3 Trials to be done

The trials are tested for the following parameters:
Flowability
Hardness
Disintegration time
Weight variation
Color homogeneity (when color is used)

a- Effect of filler type (Lactose monohydrate vs Lactose spray dried).

Table II-4: Composition of trials #1 vs # 2

	Trial # 1		**Trial # 2**	
Material	**mg**	**%**	**mg**	**%**
Fluconazole	100	40%	100	40%
Avicel 102	62.5	25%	62.5	25%
Lactose	77.5	31%	******	******
Lactose Spray Dried	******	******	77.5	31%
Aerosil	2.5	1%	2.5	1%
Mg-Stearate	2.5	1%	2.5	1%
Ac-di-sol	5.0	2%	5.0	2%
Total weight	250		250	

b- Effect of binder concentration (Avicel 102) on the hardness and disintegration:

Table II-5: Composition of trials #2 vs # 3

	Trial # 2		**Trial # 3**	
Material	**mg**	**%**	**mg**	**%**
Fluconazole	100	40%	100.0	40%
Avicel 102	62.5	25%	45.0	18%
Lactose Spray Dried	77.5	31%	95.0	38%
Aerosil	2.5	1%	2.5	1%
Mg-Stearate	2.5	1%	2.5	1%
Ac-di-sol	5.0	2%	5.0	2%
Total weight	**250**		**250**	

c- Effect of the presence of the disintegrant (Ac-Di-Sol) in the formula:

Table II-6: Composition of trials #3 vs # 7

	Trial # 2		**Trial # 7**	
Material	**mg**	**%**	**mg**	**%**
Fluconazole	100	40%	100.0	40%
Avicel 102	62.5	25%	62.5	25%
Lactose Spray Dried	77.5	31%	82.5	33%
Aerosil	2.5	1%	2.5	1%
Mg-Stearate	2.5	1%	2.5	1%
Ac-di-sol	5.0	2%	0	0
Total weight	**250**		**250**	

d- Effect of low concentration of the disintegrant (Ac-Di-Sol) on the disintegration time:

Table II-7: Composition of trials #3 vs # 4

	Trial # 3		**Trial # 4**	
Material	**mg**	**%**	**mg**	**%**
Fluconazole	100	40%	100.0	40%
Avicel 102	62.5	25%	62.5	25%
Lactose Spray Dried	77.5	31%	80.0	32%
Aerosil	2.5	1%	2.5	1%
Mg-Stearate	2.5	1%	2.5	1%
Ac-di-sol	5.0	2%	2.5	1%
Total weight	**250**		**250**	

e- Effect of the coloring material Yellow iron oxide:

The color is mixed with the other excipients in the first stage.

Table II-8: Composition of trial #5

	Trial # 5	
Material	**mg**	**%**
Fluconazole	100	40%
Avicel 102	62.5	25%
Lactose Spray Dried	76.2	30.5%
Aerosil	2.5	1%
Mg-Stearate	2.5	1%
Ac-di-sol	5.0	2%
Yellow Iron Oxide	1.3	0.5%
Total weight	**250**	

f- Effect of the hardness on disintegration time:

Five hardness ranges to be tested on trial # 2:

Table II-9: Different hardness ranges to be tested

Range	**Limits**
A	4-6
B	6-8
C	8-10
D	10-12
E	12-14

All formulas to be prepared by the following direct compression method (Figure II-1):

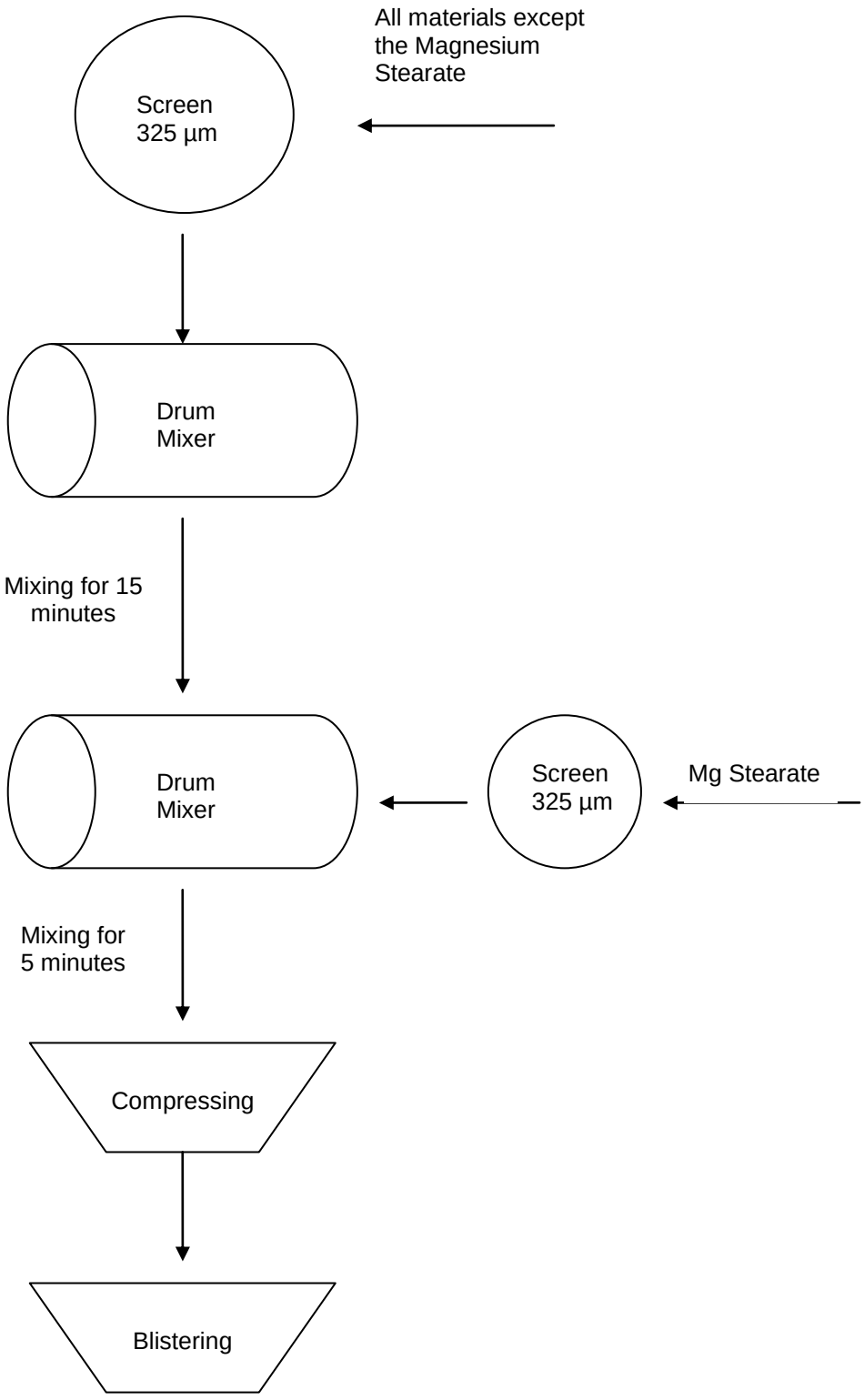

Figure II-1: Step by Step production scheme

II-5 Dissolution Profile:

Six tablets of the trial batch to be tested and six capsules of the original brand (Trican by Pfizer) to be compared using the dissolution test. The samples of each trial is taken every 10, 15, 20, 25, 35 and 45 minutes. The samples are injected in the HPLC system (using the dissolution test method). A comparative graphs are drawn. The comparison of the dissolution profile is calculated by the following formulas:

$$F1 = \{[\Sigma^{n}_{t=1} |R_t - T_t|] / [\Sigma^{n}_{t=1} R_t]\} \times 100$$

$$F2 = 50 \log \{[1+(1/n) \Sigma^{n}_{t=1} (R_t - T_t)^2]^{-0.5} \times 100\}$$

Where:

F1: The percentage difference between the two profiles at each sampling point.

F2: The similarity factor corresponds to the similarity measurement between two curves

n: number of samples

Rt: The percentage dissolved of the original brand at time t.

Tt: The percentage dissolved of the trial formulation at time t.

Limits:

F1 = 0 - 15

F2=50 - 100

II-6 Accelerated Stability Protocol on the final product:

II-6-1 Effect of accelerated conditions:

a. Composition:
Each tablet contains 100 mg Fluconazole.

b. Formula:

Table II-10: Formula used in the accelerated stability testing

RM	g	%
Fluconazole	400.00	40.0
Avicel 102	248.00	24.8
Lactose Spray Dried	310.00	31.0
Aerosil	10.00	1.0
Mg-Stearate	10.00	1.0
Ac-di-sol	20.00	2.0
Yellow Iorn Oxide	2.00	0.2
Total	**1000.00**	**100.0**

c. Batch Numbers:
R3G02 (pilot batch 30 Kg)
R4G02 (lab batch 1 Kg)
R5G02 (lab batch 1 Kg)

d. Packing:
Blisters of aluminum (0.02 mm) and colorless PVC (0.25 mm).

e. Storage date:
8/08/2002.

f. Storage Period:
Six months.

g. Testing intervals:
Initial, 1 month, 3 months and 6 months.

h. Storage conditions:
Temperature 40 ^{o}C, humidity 75 % R.H.
Temperature 30 ^{o}C, humidity 60 % RH

i. No. Of sample:
100 Tablets for each condition. 30 tablet for each test interval (not including initial) + 10 additional.

j. Acceptance criteria:

Table II-10: Acceptance criteria for accelerated stability testing

Testing parameters	Limits
Identification (RT min)	Complies
Assay	(90.0-110.0)%
Dissolution	Min 80.0% in 45 minutes
Appearance tests	Beige
Hardness	Min 4.0 K
Odor	Odorless

II-6-2 Effect of light on final products:

1. Batch Number:
R1G02

2. Packing:
Blisters of aluminum (0.02 mm) and colorless PVC (0.25 mm).

3. Storage date:
8/08/2002.

4. Period of storage:
7 months.

5. Storage condition:
The blisters are exposed to light (day light during the day and Neon light during night) at room temperature.

6. No. Of sample:
30 Tablets for each testing interval.

7. Acceptance criteria:

Table II-11: Acceptance criteria for long term stability testing

Testing parameters	Limits
Identification (RT min)	Complies
Assay	(90.0-110.0)%
Dissolution	Min 80.0%
Appearance tests	Beige
Hardness	Min 4.0 K
Odor	Odorless

II-7 Bioequivalence of the formulated tablet versus the innovator product Diflucan for Pfizer

The formula used for the study is indicated in Table II-10.

The study is done on 24 healthy volunteers. The subjects are asked to abstain from taking drugs and alcohol for at least 3 days prior to the experiment and throughout the study period. On the night before the experiment the volunteers are instructed to fast at least 10 hours before drug administration.
The volunteers are arbitrary divided into two equal groups each of 12 subjects. The first group was given the Trican capsule by Pfizer and the second is given Dican tablet with a crossover after washout period of two weeks. Blood samples are collected before and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 48, 72 and 96 hours post administration. Analysis of plasma sample concentrations is done by means of a validated HPLC assay method.

Statistical analysis is performed using Minitab Statistical Package version 13 on IBM PC. The assessment of bioequivalence between the test and the reference product is based on the ratios of the mean pharmacokinetic parameters (C_{max}, t_{max}, AUC $_{0\rightarrow\infty}$) using ANOVA. Bioequivalence was concluded if either tail probability did not exceed the 90% confidence limit and was completely contained in the 0.80 – 1.25 range.

Limits of acceptance are as follows:

AUC $_{0\rightarrow\infty}$: 80 to 125%
C_{max} : 80 to 125%

II-8 Microbial comparison between the Dican and Trican (Diflucan) for Pfizer.

II-8-1 Method used: Plate method

II-8-2 Test organism: Candida Albicans

II-8-3 Materials and equipment's

Petri dishes: plastic Petri dishes having 200 mm diameter and 10 mm height.
Autoclave
Incubator
Laminar flow

II-8-4 Media and diluent

a- Buffer No. 10, 0.2 M, pH 10.5: Dissolve 35.0 g of dibasic potassium phosphate in 1000 ml of water, and add 2 ml of 10 N potassium hydroxide. Adjust the pH with 18 N phosphoric acid or 10 N potassium hydroxide to 10.5 ± 0.1.

b- Antibiotic media No. 3 with agar.
Composition per 1 liter:

Beef extract	1.50 g
Yeast extract	1.50 g
Peptone	5.00 g
Dextrose	1.00 g
NaCl	3.50 g
Dipotassium hydrogen Phosphate	3.68 g
Potassium Dihydrogen Phosphate	1.32 g
Agar	1.32 g

Final pH = 7.0

c- Tryptic Soy Broth (TSB).

Composition per 1 liter:

Peptone from Caseine	17.00 g
Peptone from Soy meal	3.00 g
D (+) - Glucose	2.50 g
NaCl	5.00 g
Dipotassium hydrogen Phosphate	2.50 g

II-8-5 Preparation of inoculum

a- From the stock inoculum, inoculate a loop full of the organism in about 25 ml of (TSB). Stir well using the vortex.
b- Incubate at T = 32-35 °C for 24 hours
c- Measure the transmittance at = 580 nm
f- Adjust transmittance to 25% against the TSB as the blank. Use TSB for dilution if necessary.

II-8-6 Standard preparation

a. Prepare the stock solution by dissolving (use sonication) Trican 150 mg capsule in 150 ml buffer solution # 10 (pH=10.5).
b. Pipette 10 ml of this stock solution into a 50 ml volumetric flask and fill up to volume with buffer solution (SH).
c. Dilute 15 ml of this solution with 30 ml with buffer solution. (SL).

II-8-7 Sample preparation

a. Weigh not less than ten tablets, determine the average weight, and powder them. Prepare the stock solution by dissolving (use sonication) the average of 1 tablet accurately weighed in buffer solution # 10 (pH=10.5) in 100-ml volumetric flask and fill up to volume with the same solvent.
b. Pipette10 ml of this stock solution into a 50 ml volumetric flask and fill up to volume with buffer solution (UH).
c. Dilute 15 ml of this solution with 30 ml with buffer solution. (UL).

II-8-8 Procedure

a. Keep the medium in the water bath at 45-50 degree until use.
b. Prepare the first layer by placing 13 ml of the antibiotic medium in each plate and wait till solidification.
c. 1 ml of the 25 % bacterial suspension is added to 50 ml of the antibiotic medium prepared. Mix well.
d. Prepare the second layer by placing 5 ml of the inoculated medium, tilt the plate pack and forth to spread the media and let it to harden at room temperature
e. Using a 6 mm SS cylinder tube, make 4 wells in each plate. Make sure to make even space at a radius of 2.8 cm apart.
f. Cover the plates to avoid contamination.
g. Fill each well with 75 µl of the prepared dilutions of sample and standard as distributed
h. Incubate the plates at 35-37 °C for 24 hours
i. Measure the diameter of each zone of inhibition formed to the nearest 0.1 mm

II-8-9 Calculations

Use the following equation to calculate the potency ratio:

$$\text{Log } X = \text{LDR} \times \frac{(UH + UL) - (SH + SL)}{(SH - SL) + (UH - UL)}$$

II-9 Anti Microbial Activity of Fluconazole.

II-9-1 Materials

a. Sterile TSB*.*
b. Sterile Saline (0.9 % NaCl).
c. Tryptic Soy Agar

Composition per 1 liter:

Peptone from Caseine	15.00 g
Peptone from Soy meal	5.00 g
NaCl	5.00 g
Agar	15.00 g

d. Buffer No. 3, 0.1 M, pH 8.0 — Dissolve 16.73 g of dibasic potassium phosphate and 0.523 g of monobasic potassium phosphate in 1000 mL of water. djust the pH with 18 N phosphoric acid or 10 N potassium hydroxide to 8.0 ± 0.1.
e. Buffer No. 10 as described in II-8-4.

II-9-2 Test Organisms:

a. Salmonella
b. Staphylococcus Aureus
c. Pseudomonas Aeruginosa and
d. Escherishia Coli

II-9-3 Preparation of the Test Organism

a. Prepare a fresh broth of the tested organism by adding a loop full from the organism in 25ml of sterile TSB. Stir well using the vortex.
b. Incubate for 24h at T = 32-35 °C.
c. Take 10 µl of this incubated organism and dilute in 20 ml 0.9 % saline.

II-9-4 Preparation of the antimicrobial chemical dilutions

a. Prepare 10 tubes, each have 3 ml of sterile TSB.
b. Prepare 1mg/ml concentration of the antimicrobial chemical by dissolving 100 mg of Fluconazole in 100 ml Buffer number 10 described in II-8-4.
c. Transfer 3 ml of the 1mg/ml concentration of the antimicrobial chemical to the first tube to have a concentration of 500 µg/ml.
d. Transfer 3 ml from the first tube to the second tube to have a concentration of 250 µg/ml and repeat till reaches the tube number 10.

e. Take 50 µl of the final concentration of the tested organism that was prepared in step II-9-2 and add it to each of the 10 tubes (containing 3ml of sterile TSB and 3 ml of the antimicrobial chemical).
f. Incubate for 24-48 h at T = 32-35 °C and observe the growth of the organism.

II-9-5 Plate count for the inoculum.

a. Prepare 3 tubes, each have 9 ml of sterile 0.9 % Saline.
b. Transfer 1 ml of the inoculum prepared in II-9-3 in tube #1. Mix well.
c. Transfer 1 ml of the resultant solution from b in tube # 2. Mix well.
d. Transfer 1 ml of the resultant solution from c in tube # 3. Mix well.
e. Prepare 3 plates having 15 ml of Tryptic Soy agar having numbers 1, 2 and 3.
f. Transfer 0.1 ml of each test tube into the plate having the same number. Spread the solution all over the plate using a glass rod.
g. Incubate for 24 hours.
h. Choose the plate that have 50-200 colonies and count the number of colonies.
i. Calculate the original number of bacteria / ml by multiplying with the dilution factor.

II-9-6 Comparison with another antimicrobial.

The activity of Fluconazole is compared with the activity of Clarithromycin against the Staphylococcus Aureus. The same procedure is used with the following modifications:

a- Buffer # 3 pH=8 is used in the preparation of antimicrobial solution.
b- The concentration of Clarithromycin solution used is 0.1 mg / ml.

The activity of Fluconazole and Clarithromycin are compared by calculating the effective concentration of each against a fixed bacterial concentration.

PART III: RESULTS AND DISCUSSION

III-1 The Active Substance Specification

The active ingredient was analyzed by the manufacturer according to the procedure described in II-1. The results are summarized in the following table (III-1). All the results were according to the specifications.

Table III-1: Analysis results for the active substance Fluconazole

Test	Result	Specification
Description	White to off-white crystalline powder	White to off-white crystalline powder
Melting range	138-140 °C	136°C-140°C
Odor	Odorless	Odorless or almost odorless
Solubility	5% w/v solution in methanol is clear.	5% w/v solution in methanol is clear.
Loss on Drying at 105 ºC	0.2 %	Not more than 0.5% w/w
Residue on Ignition	0.21 %	Not more than 0.5% w/w
Heavy Metals	< 0.002 %	Not more than 0.002% w/w
Iron	< 0.002 %	Not more than 0.002% w/w
Related Substances by HPLC	0.59 %	Sum of impurities not more than 1.0%
Particle size:	Complies	100% < 180 µm
Bulk Density: Untapped	 0.31 g / ml	 0.25 to 0.45 g / ml
Tapped	0.42 g / ml	0.35 to 0.55 g / ml

III-2 Formulation summary of results

All trials were done with a total weight of 1 Kg

III-2-1 Effect of filler type

The effect of Lactose monohydrate vs Lactose spray dried was studied. The flowability was a lot improved by using the dried form of Lactose as indicate in the following table (III-2)

Table III-2: Effect of Lactose spray dries vs Lactose Monohydrate

Test	Trial # 1	Trial # 2
Flowability	Poor	Excellent
Hardness range	4-14	4-14
Disintegration time	Within 2 min.	Within 2 min.
Weight uniformity	Average wt ± 5%	Average wt ± 5%

III-2-2 Effect of binder concentration (Avicel 102)

Two concentrations of Avicel 102 were used (25 % in trial #2 and 18 % in trial #3). Disintegration was tested on a wide range of hardness it was not affected by this variation. In addition, the hardness was achieved easily between 4 and 14 K (kilopond) with both concentrations. The results are summarized in the following table (III-3)

Table III-3: Effect of Lactose spray dries vs Lactose Monohydrate

Test	Trial # 2	Trial # 3
Flowability	Excellent	Excellent
Hardness range	4-14	4-14
Disintegration time	Within 2 min.	Within 2 min.
Weight variation	Average wt ± 5%	Average wt ± 5%

III-2-3- Effect of Ac-Di-Sol on disintgration:

Two trials were done to study the effect of the disintegrant Ac-Di-Sol on disintegration. 2% of Ac-Di-Sol was used in trial #3, while no

disintegrant was used at all in trial # 7. There was no complete disintegration within 30 minutes in Trial # 7. The disintegration was completed within 2 minutes in Trial # 3. The other tests were not affected. The results are shown in the following table (III-4).

Table III-4: Effect of Ac-Di-Sol on disintegration

Test	Trial # 2	Trial # 7
Flowability	Excellent	Excellent
Hardness range	4-14	4-14
Disintegration time	Within 2 min.	No complete disintegration in 30 min.
Weight variation	Average wt ± 5%	Average wt ± 5%

III-2-4- Effect of different concentrations of the disintegrant (Ac-Di-Sol) in the formula:

Two trials were done to study the effect of the concentration of the disintegrant Ac-Di-Sol on disintegration time. 2% of Ac-Di-Sol was used in trial #3, while 1% of the disintegrant was used at all in trial # 4. A complete disintegration occurred within 15 minutes for the 1%, while it is within 2 minutes for the 2%. The results are shown in the following table (III-5).

Table III-5: Effect of Ac-Di-Sol concentration on disintegration

Test	Trial # 3	Trial # 4
Flowability	Excellent	Excellent
Hardness range	4-14	4-14
Disintegration time	Within 2 min.	15 minutes
Weight variation	Average wt ± 5%	Average wt ± 5%

III-2-5- Effect of the coloring material Yellow iron oxide

Trial # 5 contains the coloring agent Yellow Iron Oxide. The tests showed no effect of the coloring agent on the flowability, hardness, disintegrating time or weight variation. The results are shown in table (III-6).

Table III-6: Effect of Yellow Iron Oxide

Test	Trial # 5
Flowability	Excellent
Hardness range	4-14
Disintegration time	Within 2 min.
Weight variation	Average wt ± 5%

III-2-6- Effect of hardness on disintegration time

The effect of a range of hardness on the disintegration time on trial #2. The disintegration was not affected by hardness on the range of 4-14 K. The results were summarized in the following table (III-7)

Table III-7: Effect of hardness on disintegration time on trial # 2

Hardness Range (K)	Disintegration time
A (4-6)	Within 2 min
B (6-8)	Within 2 min
C (8-10)	Within 2 min.
D (10-12)	Within 2 min
E (12-14)	Within 2 min

III-2-7- Best Master Formula

According to the results, the best master formula is summarized in the following table (III-8)

Table III-8: Best Master formula

RM	g	%
Fluconazole	50.00	40.0
Avicel 102	31.00	24.8
Lactose Spray Dried	38.75	31.0
Aerosil	1.25	1.0
Mg-Stearate	1.25	1.0
Ac-di-sol	2.50	2.0
Yellow Iorn Oxide	0.25	0.2
Total	**125.00**	**100.0**

III-2-8- Production Step by step

a- Machines used

Drum Mixer PM21
Tableting Machine PM15
Blistering Machine PM01

b- Mixing Procedure

1. Into a the drum mixer, add all materials except the Magnesium Stearate after passing them through 325 µm , and mix for 15 minutes.

2. On the above mixture add Magnesium Stearate after passing through 325 µm, and mix for 5 minutes.

c- Compression

Compress the prepared mixture using the Tableting machine

Unit Weight: 250.0 mg
Max. Wt.: 262.5 mg
Min. Wt: 237.5 mg

d- Blistering
Blister the tablets in a colorless PVC – Aluminum foil blisters using the blistering machine.

III-3 Dissolution Profile

The dissolution Profile of the best formula of Fluconazole tablet (Dican tablet), B.N R3G02 and manufacturing date 07/2002, was tested in vitro against the Trican capsule, B.N 257044 and expiry 03/2006 of Pfizer using the dissolution method described in II-2-3. The similarity factor between the two curves were calculated using the equation described in II-5.

Table III-9: Dissolution values for 6 Dican tablets

Minutes	sa#1	sa#2	sa#3	sa#4	sa#5	sa#6	Average
10	95.50	90.48	80.20	88.53	80.01	75.45	85.03
15	99.88	95.97	86.84	94.35	98.20	98.96	95.70
20	99.78	97.59	91.12	96.75	99.88	99.34	97.41
25	101.20	98.28	96.15	97.35	100.28	100.31	98.93
30	101.43	98.64	99.21	98.10	101.54	101.68	100.10
40	100.98	100.75	98.62	97.81	100.19	101.07	99.90
45	101.21	97.19	98.41	95.94	98.15	96.62	97.92

Table III-10: Dissolution values for 6 Trican capsules

Minutes	sa#1	sa#2	sa#3	sa#4	sa#5	sa#6	Average
10	41.65	86.27	49.74	49.1	42	75.1	57.31
15	56.77	96.59	68.86	70.20	64.7	91.00	74.69
20	71.99	106.51	87.18	85.00	84.00	99.36	89.01
25	86.58	106.05	96.38	89.80	89.00	100.20	94.67
30	97.72	109.36	103.92	98.00	92.10	102.70	100.63
40	101.88	103.48	101.66	100.80	93.00	102.00	100.47
45	101.63	107.03	103.05	100.80	93.30	101.00	101.13

The average of the dissolution points for both the test and the reference samples is calculated and the F2 is then calculated. The first 25 minutes data only were put in calculation (5 minutes above the time in which 85 % dissolution occurs). The two profile are summarized in (figure III-1).

Table III-11: Average dissolution values for Trican capsules and Dican tablets

	10 min.	15 min	20 min	25 min	30 min.	40 min.	45 min.
Dican	85.03	95.70	97.41	98.93	100.10	99.90	97.92
Trican	57.31	74.69	89.01	94.67	100.63	100.47	101.13

$F2 = 50 \log \{[1+(1/n)\ \Sigma n\ t=1(Rt-Tt)^2]^{-0.5} \times 100\} = 44.1$

Limits: 50 – 100

Result:
When calculating the similarity factor in the range 10 – 25 minutes, the result fails (f_2 = 44.1). This is due to the capsule shell of Trican capsules, which delays the dissolution in the first 5 minutes.

The similarity factor calculated in the range 15 – 25 minutes was found to be within limits (f_2=51.2).

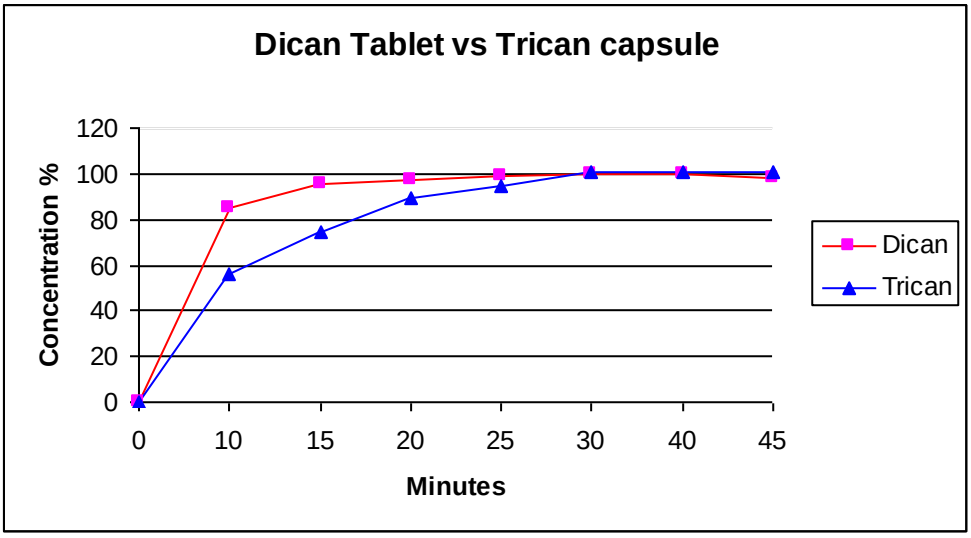

Figure III-1: Average dissolution profiles of Dican tablet and Trican capsule

III-4 The Final Product Specification

The final product was analyzed according to the procedure described in II-2. The results are summarized in the following table (III-12). All the results were according to the specifications.

Table III-12: Analysis results for Dican tablet B.N R3G02

Test	Result	Specification
Shape	Triangular tablets	Triangular tablets
Unit weight	256	251 ± 12.5 mg
Color	Beige color	Beige color
Odor	Odorless	Odorless
Diameter	9.0 mm	9.0 ± 0.5 mm
Average Hardness	11.5 K	Min. 4.0 K
Friability	< 0.1%	Max. 1.0 %
Assay	96.3 %	90.0 – 110 %
Dissolution	99.6 %	Min. 80.0 % in 45 minutes
Weight uniformity	Average wt ± 5%	Average wt ± 5%
Content uniformity	Conforms	Conforms
Purity	Pure	Pure
Chipping and capping	No capping	No capping

III-5 Validation of the Test Method

III-5-1 Selectivity

A standard, sample and placebo solutions were analyzed under the same conditions as described in II-3-1. The chromatograms shows Figures (III-2, III-3, III-4) that there is only the active ingredient peak in the chromatograms. The placebo solution chromatogram showed no peak for any of the inactives in it. The same is for the selectivity under stress condition. Thus the procedure is selective for the proposed formula.

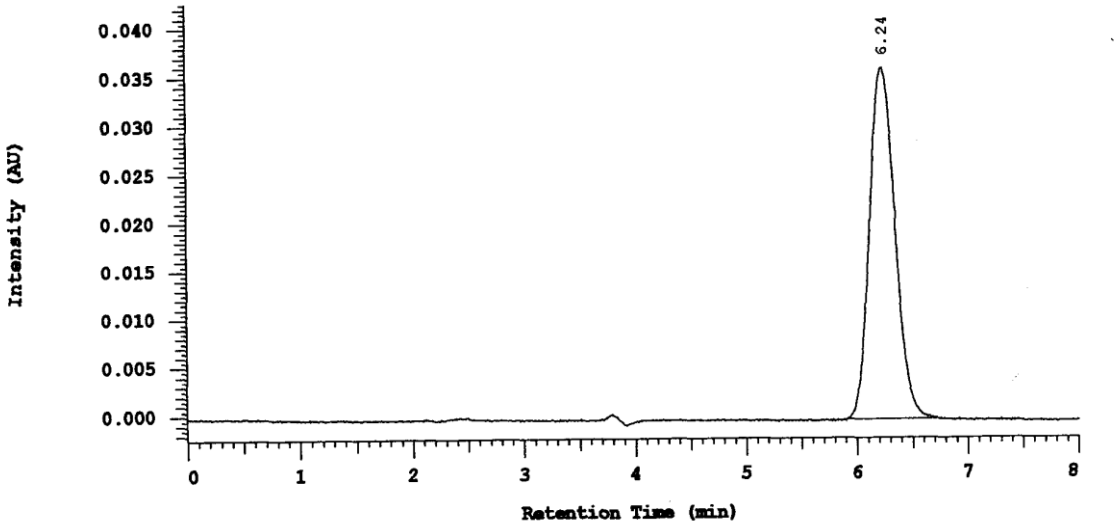

Figure III-2: Chromatogram for the standard solution

Peak information:
Purity: 0.9980
Asymmetry: 0.9454
Theoretical plates (EUP): 1946

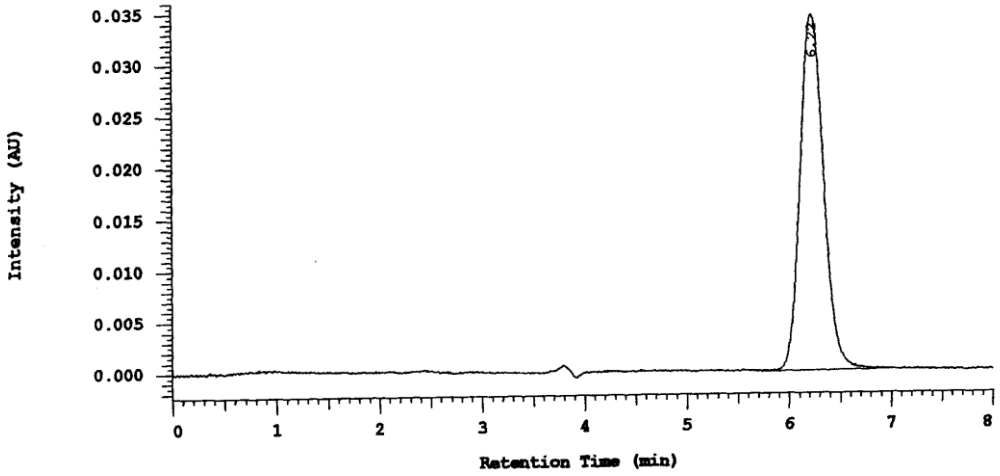

Figure III-3: Chromatogram for the sample solution

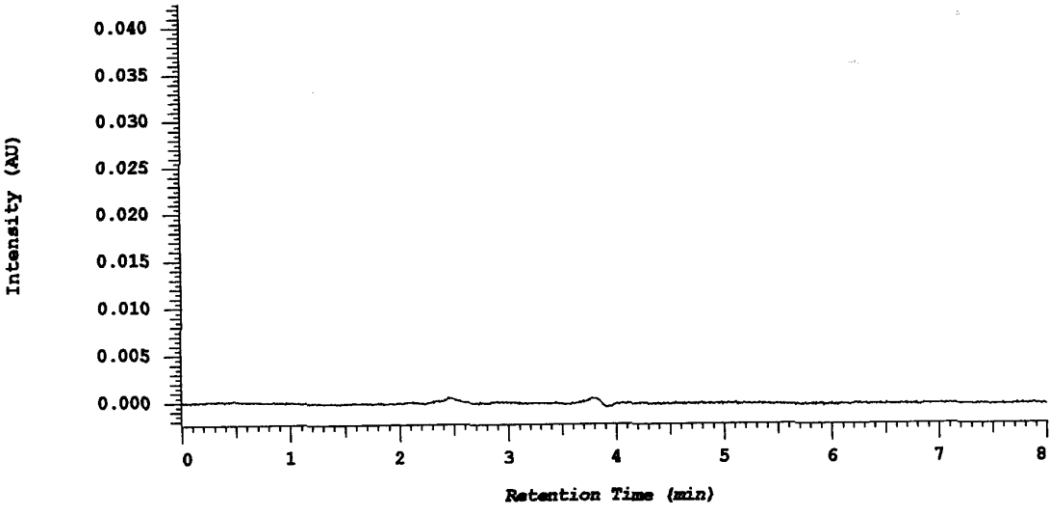

Figure III-4: Chromatogram for the placebo solution

Table III-13: Selectivity after stress conditions after 48 hours

Solvent	Assay at 0 time	Assay after 48 hours	Other Peaks than Fluconazole	Purity Check
5N HCl	100.0 %	100.0 %	None	0.9969
5N NaOH	100.0 %	102.5 %	Yes	0.9977
30 % H2O2	100.0 %	97.2 %	None	0.9994
Boiling in H2O	100.0 %	100.0%	None	0.9966

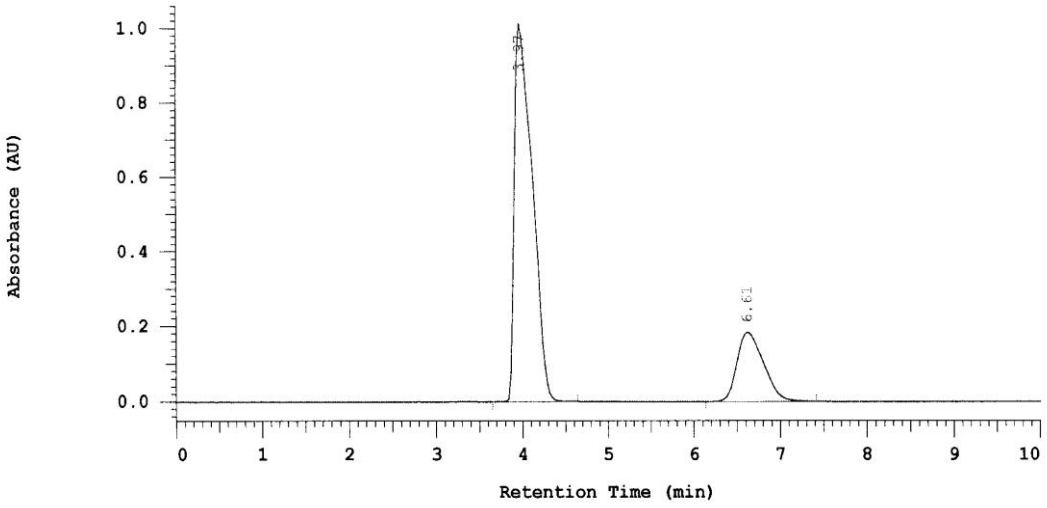

Figure III-5: Chromatogram for the HCl and Fluconazole solution

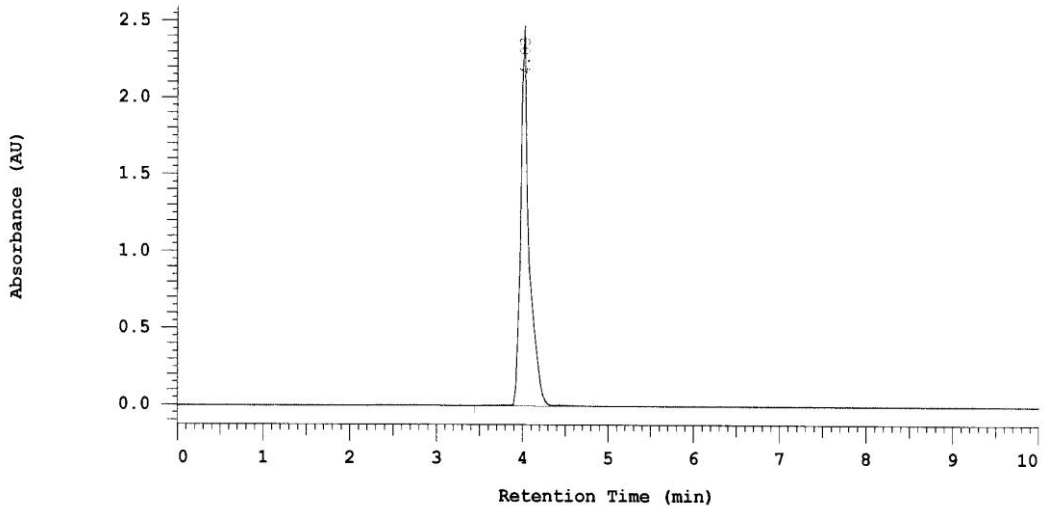

Figure III-6: Chromatogram for the HCl solution

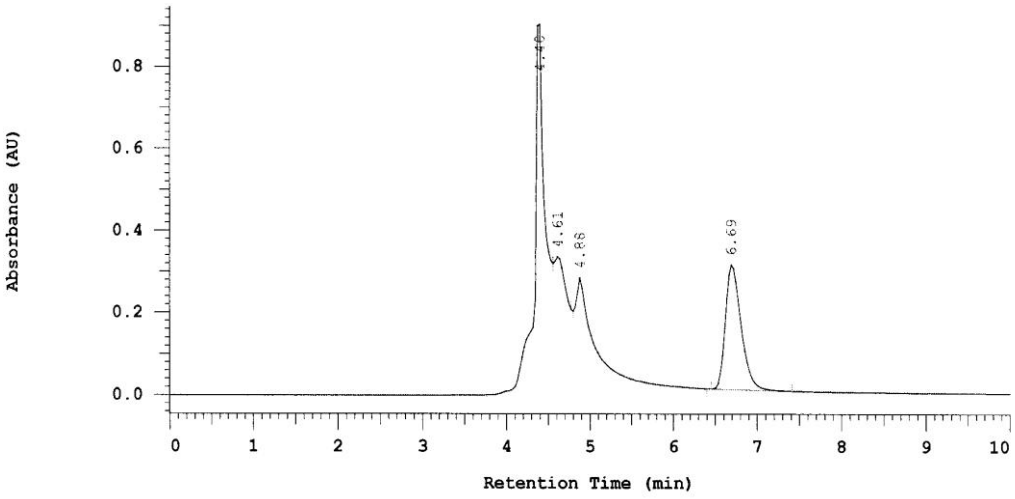

Figure III-7: Chromatogram for the NaOH and Fluconazole solution

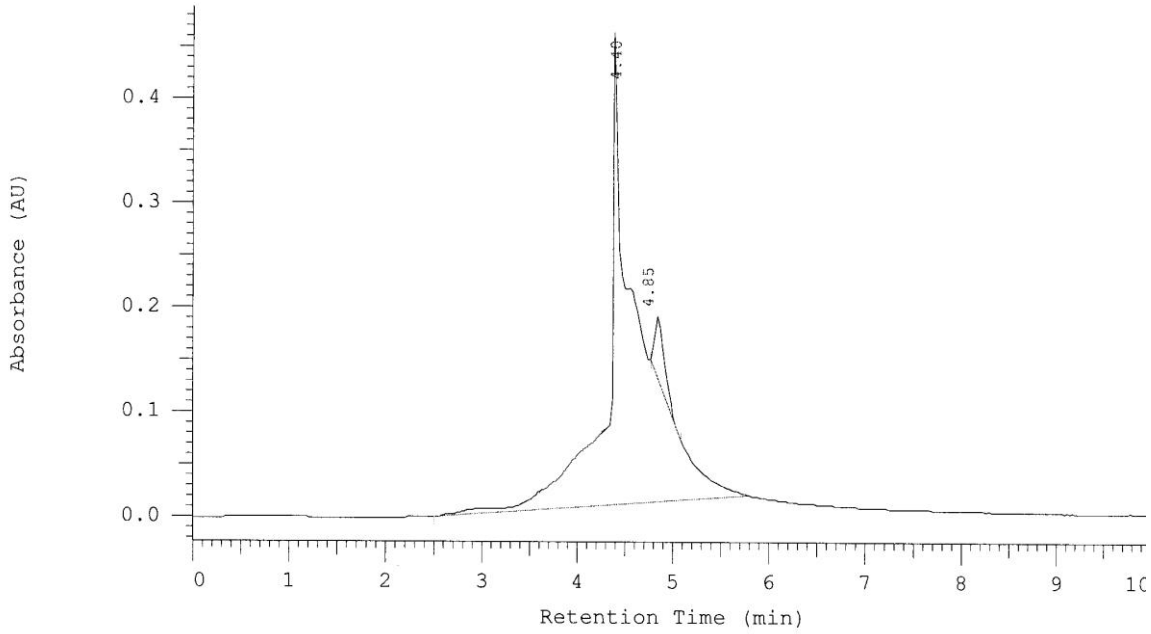

Figure III-8: Chromatogram for the NaOH solution

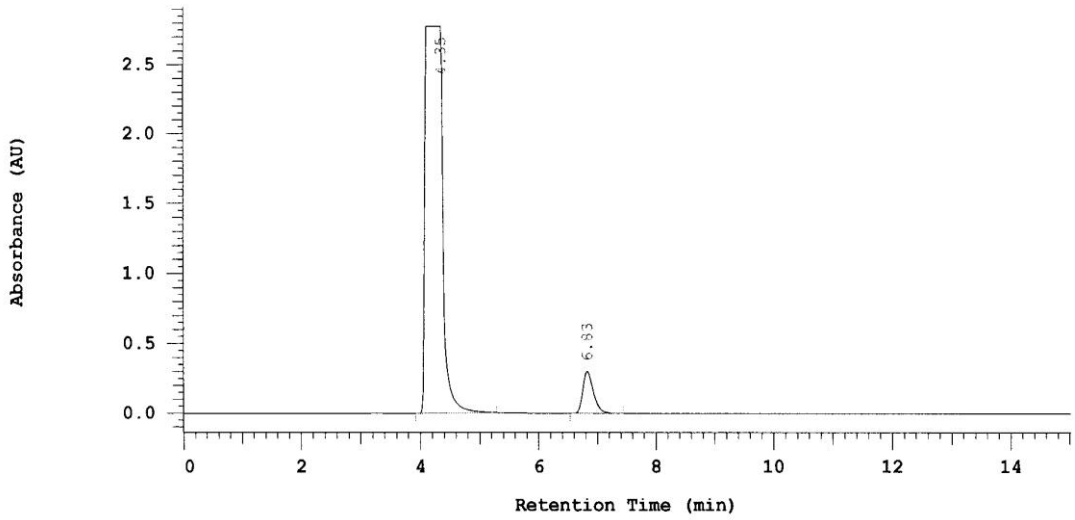

Figure III-9: Chromatogram for the H_2O_2 and Fluconazole solution

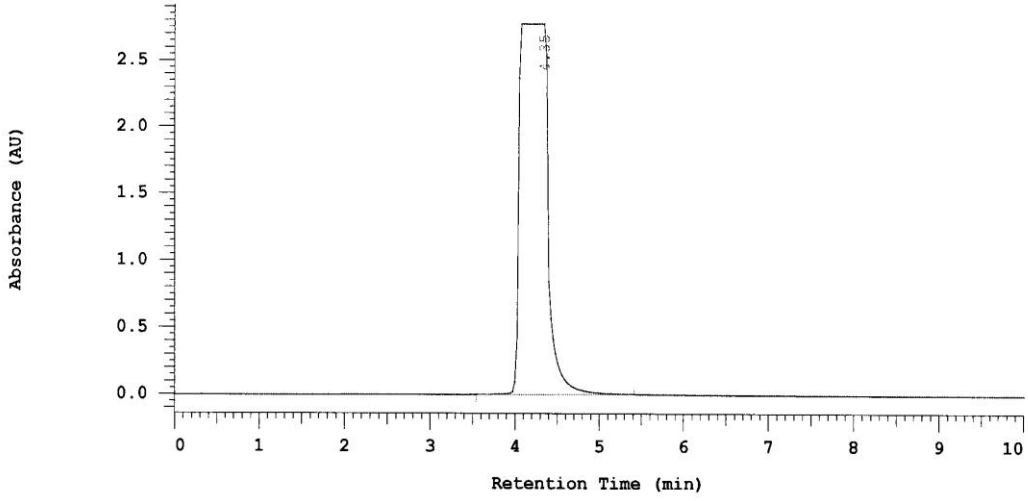

Figure III-10: Chromatogram for the H_2O_2 solution

III-5-2 Accuracy and Precision

Five samples were prepared and analyzed three times, one time per day. Two injections of each sample were made. The active percentage was calculated for each sample and the average for the five samples was calculated for each day. Standard deviation (ST. DEV.), Coeffecient of variation (COEF. VAR.) and % of recovery (% RECOV.) were calculated for each day. The results were compared to the specification and the results were obtained for each day. The three day average of the coefficient variation (Precision) and the percentage of recovery (Accuracy) were calculated and compared with the limits.

All data obtained for each day and the average data were with in specification specified. Thus, the method is considered accurate and precise.

The data is shown in the following tables (III-14, III-15, III-16, III-17)

Table III-14: Accuracy and precision analysis results for the first day

Sample No.	Standard Peak Area			Sample Peak Area			% Assay
	INJ. No. 1	INJ. No. 2	Average	INJ. No. 1	INJ. No. 2	Average	
I	291.41	292.33	291.87	286.50	286.00	286.25	98.07
II	287.05	286.91	286.98	281.85	281.06	281.45	98.07
III	289.71	292.37	291.04	290.00	290.30	290.15	99.69
IV	289.79	292.52	291.16	292.65	294.39	293.52	100.81
V	291.07	291.88	291.48	292.28	291.52	291.90	100.15
Coefficient of Variation		**1.25**	**Max 1.50**		**Pass**		
% Recovery		**99.36**	**98.00 -102.00**		**Pass**		

Table III-15: Accuracy and precision analysis results for the second day

Sample No.	Standard Peak Area			Sample Peak Area			% Assay
	INJ. No. 1	INJ. No. 2	Average	INJ. No. 1	INJ. No. 2	Average	
I	293.14	291.04	292.09	294.92	292.79	293.86	100.60
II	293.04	294.38	293.71	294.65	294.14	294.40	100.23
III	289.45	291.24	290.35	295.22	294.24	294.73	101.51
IV	294.34	293.31	293.83	297.08	295.08	296.08	100.77
V	293.22	295.08	294.15	290.41	293.00	291.71	99.17
Coefficient of Variation		**0.85**	**Max 1.50**		**Pass**		
% Recovery		**100.46**	**98.00 -102.00**		**Pass**		

Table III-16: Accuracy and precision analysis results for the third day

Sample No.	Standard Peak Area			Sample Peak Area			% Assay
	INJ. No. 1	INJ. No. 2	Average	INJ. No. 1	INJ. No. 2	Average	
I	291.76	292.84	292.30	291.75	291.64	291.70	99.79
II	292.81	290.11	291.46	291.33	292.46	291.90	100.15
III	294.11	292.99	293.55	291.77	292.33	292.05	99.49
IV	293.34	295.86	294.60	292.65	293.85	293.25	99.54
V	295.65	293..63	295.65	293.63	290.58	292.11	98.80
Coefficient of Variation		**0.50**	**Max 1.50**		**Pass**		
% Recovery		**99.55**	**98.00 -102.00**		**Pass**		

Table III-17: 3 Days average of the accuracy and precision.

Parameter	Statistical Measure	Result	Limit	Result
Precision	Coefficient of Variation	**0.87**	Max 1.50	**Pass**
Accuracy	% of Recovery	**99.79**	98.00 -102.00	**Pass**

III-5-3 Linearity

Five samples were prepared with concentrations between 60 % and 140 % from the assay concentration (15 mg / ml). Two injections were made for each concentration. Calculation was made for the coefficient of variation and y-intercept.

All results were according to the specification. Thus we can conclude that the method is linear at the range of 60 – 140 % from the assay concentration.

The data for linearity are represented in table (III-18) and Figure (III-11).

Table III-18: Linearity analysis results.

	Sample Peak Area		
%Conc. (mg/ml)	**Inj.1**	**Inj.2**	**Average**
9.00	174.29	175.93	175.11
12.00	233.98	234.23	234.11
15.00	294.18	293.84	294.01
18.00	350.57	352.77	351.67
21.00	408.51	408.49	408.50
Average Area			**292.68**

Correlation Coefficient	**0.99995**	**Pass**	**Min. 0.9998**
Y-Intercept	**0.507**	**Pass**	**± 5.85 (± 2% of the average area)**

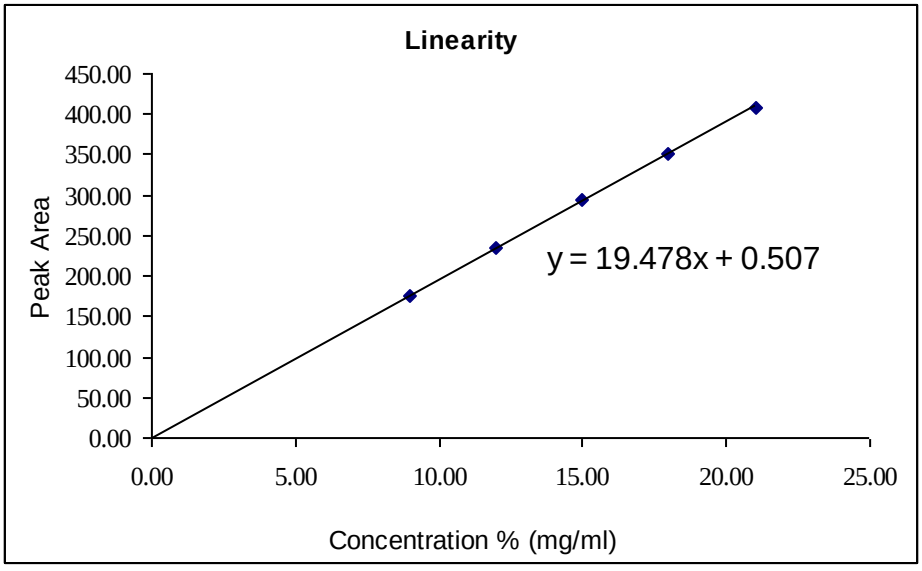

Figure III-11: Linearity curve

III-5-4 Range

According to the linearity data, the method is linear on the range of 60 % - 140 %

III-5-5 Robustness

1- Two Different Analysts

Table III-19: Robustness-two different analysts.

Analyst	Standard Peak Area			Sample Peak Area			% Assay
	Inj. No. 1	Inj. No. 2	Average	Inj. No. 1	Inj. No. 2	Average	
Analyst 1	294.24	292.21	293.23	294.94	295.30	295.12	100.65
Analyst 2	294.28	290.88	292.58	289.70	293.90	291.80	99.73
Coefficient of Variation	**0.64**	**Max 1.50**	**Pass**				

2- Different Elapsed Assay Time

Table III-20: Robustness-different elapsed assay time.

Time	Standard Peak Area			Sample Peak Area			% Assay
	Inj. No. 1	Inj. No. 2	Average	Inj. No. 1	Inj. No. 2	Average	
10 min.	294.28	290.88	292.58	289.70	293.90	291.80	99.73
4 hours	292.81	292.23	292.52	292.59	290.25	291.42	99.62
Coefficient of Variation	**0.08**	**Max 1.50**	**Pass**				

3- Different Sonicating Time

Table III-21: Robustness-different sonicating time

Time	Standard Peak Area			Sample Peak Area			% Assay
	Inj. No. 1	Inj. No. 2	Average	Inj. No. 1	Inj. No. 2	Average	
10 min.	293.90	290.14	292.02	291.05	293.09	292.07	100.02
15 min.	289.67	293.95	291.81	292.26	293.76	293.01	100.41
20 min.	292.45	293.83	293.14	293.61	290.29	291.95	99.59
Coefficient of Variation	**0.28**	**Max 1.50**	**Pass**				

4- Different Columns

Table III-22: Robustness-different columns

Column	Standard Peak Area			Sample Peak Area			% Assay
	Inj. No. 1	Inj. No. 2	Average	Inj. No. 1	Inj. No. 2	Average	
# 1	289.67	293.95	291.81	292.26	293.76	293.01	100.41
# 2	289.93	290.02	289.98	291.84	294.78	293.31	101.15
Coefficient of Variation	0.52	Max 1.50	Pass				

5- Different Instruments

Table III-23: Robustness-different instruments

Instrument	Standard Peak Area			Sample Peak Area			% Assay
	Inj. No. 1	Inj. No. 2	Average	Inj. No. 1	Inj. No. 2	Average	
RI002	281.70	279.12	280.41	278.71	278.84	278.78	99.42
RI001	294.29	290.88	292.59	289.70	293.09	291.40	99.59
Coefficient of Variation	0.13	Max 1.50	Pass				

III-5-6 Conclusion:

As the above data indicates, the method of analysis for Dican tablet is valid and pass all the criteria predetermined.

III-6 Stability summary of data and results

III-6-1Effect of accelerated condition (T=40±2 ºC, RH= 75±5)

Three batches were studied under accelerated conditions (T=40 ºC, RH= 75. The samples were packed in PVC-Aluminum blisters and incubated for six months. Analysis were performed at 0, 1, 3 and 6 months. The results were compared with the specifications predetermined. The results of each batch are summarized in tables (III-24, III-25 and III-26).

Table III-24: Accelerated stability (40 ºC, 75 RH) analysis data for B.N. R3G02

Testing parameters	Initial Result	After 1 Months	After 3 Months	After 6 Months	Limits
Identification (RT min)	Complies	Complies	Complies	Complies	Complies
Assay	96.3 %	95.6 %	93.8 %	95.9 %	(90.0-110.0)%
Dissolution	91.2 %	95.6 %	88.8 %	93.2 %	Min 80.0% in 45 minutes
Appearance tests	Complies	Complies	Complies	Complies	Beige color, Pure
Hardness	11.5 K	13.0 K	14.0 K	14.0 K	Min 4.0 K
Odor	Odorless	Odorless	Odorless	Odorless	Odorless

Table III-25: Accelerated stability (40 ºC, 75 RH) analysis data for B.N. R4G02

Testing parameters	Initial Result	After 1 Months	After 3 Months	After 6 Months	Limits
Identification (RT min)	Complies	Complies	Complies	Complies	Complies
Assay	98.1 %	94.0 %	94.6 %	94.0 %	(90.0-110.0)%
Dissolution	91.3 %	93.8 %	103.0 %	95.6 %	Min 80.0% in 45 minutes
Appearance tests	Complies	Complies	Complies	Complies	Beige color, Pure
Hardness	11.0 K	13.0 K	14.1 K	14.0 K	Min 4.0 K
Odor	Odorless	Odorless	Odorless	Odorless	Odorless

Table III-26: Accelerated stability (40 ºC, 75 RH) analysis data for B.N. R5G02

Testing parameters	Initial Result	After 1 Months	After 3 Months	After 6 Months	Limits
Identification (RT min)	Complies	Complies	Complies	Complies	Complies
Assay	98.9 %	96.3 %	99.5 %	101.2 %	(90.0-110.0)%
Dissolution	91.3 %	96.4 %	100.5 %	98.8 %	Min 80.0% in 45 minutes
Appearance tests	Complies	Complies	Complies	Complies	Beige color, Pure
Hardness	11.0 K	11.5 K	13.5 K	13.5 K	Min 4.0 K
Odor	Odorless	Odorless	Odorless	Odorless	Odorless

As the tables indicate, all results fit with in specifications with no significant changes.

III-6-2 Effect of intermediate condition (T=30 ±2 ºC, RH= 60±5)

Three batches were studied under intermediate conditions (T=30 ºC, RH= 60. The samples were packed in PVC-Aluminum blisters for six months. Analysis were performed at 0, 1, 3 and 6 months. The results were compared with the specifications predetermined. The results of each batch are summarized in tables (III-27, III-28 and III-29).

Table III-27: Accelerated stability (30 ºC, 60 RH) analysis data for B.N. R3G02

Testing parameters	Initial Result	After 1 Months	After 3 Months	After 6 Months	Limits
Identification (RT min)	Complies	Complies	Complies	Complies	Complies
Assay	96.3 %	95.1 %	94.5 %	96.8 %	(90.0-110.0)%
Dissolution	91.2 %	95.8 %	100.4 %	99.0 %	Min 80.0% in 45 minutes
Appearance tests	Complies	Complies	Complies	Complies	Beige color, Pure
Hardness	11.5 K	12.0 K	10.5 K	14.0 K	Min 4.0 K
Odor	Odorless	Odorless	Odorless	Odorless	Odorless

Table III-28: Accelerated stability (30 ºC, 60 RH) analysis data for B.N. R4G02

Testing parameters	Initial Result	After 1 Months	After 3 Months	After 6 Months	Limits
Identification (RT min)	Complies	Complies	Complies	Complies	Complies
Assay	98.1 %	93.5 %	92.9 %	94.3 %	(90.0-110.0)%
Dissolution	91.3 %	95.5 %	91.4 %	92.4 %	Min 80.0% in 45 minutes
Appearance tests	Complies	Complies	Complies	Complies	Beige color, Pure
Hardness	11.0 K	11.0 K	10.6 K	11.0 K	Min 4.0 K
Odor	Odorless	Odorless	Odorless	Odorless	Odorless

Table III-29: Accelerated stability (30 ºC, 60 RH) analysis data for B.N. R5G02

Testing parameters	Initial Result	After 1 Months	After 3 Months	After 6 Months	Limits
Identification (RT min)	Complies	Complies	Complies	Complies	Complies
Assay	98.9 %	94.5 %	102.2 %	99.3 %	(90.0-110.0)%
Dissolution	91.3 %	100.2 %	101.8 %	100.4 %	Min 80.0% in 45 minutes
Appearance tests	Complies	Complies	Complies	Complies	Beige color, Pure
Hardness	11.0 K	11.7 K	12.0 K	13.0 K	Min 4.0 K
Odor	Odorless	Odorless	Odorless	Odorless	Odorless

As the tables indicate, all results fit with in specifications with no significant changes.

III-6-3 Effect of Light

One batch was kept under direct light for 7 months, 24 hours per day. The samples were re-analyzed and compared with specification. The color was removed from the formula of this batch analyzed to eliminate any possible protection of the coloring agent used in the formula. The results are indicated in table (III-30)

Table III-30: Effect of light analysis data for B.N. R1G02

Testing Parameter	Initial Result	After 7 months	Limits
Identification	Complies	Complies	Complies
Assay	98.1 %	97.4 %	(90.0-110.0)%
Dissolution	93.6 %	94.3 %	Min 80.0% in 45 minutes
Appearance tests	White, Pure	White, Pure	White, Pure
Hardness	11.0 K	13.0 K	Min 4.0 K

As the table indicates, all results fit with in specifications with no significant changes.

III-6-4 Conclusion

According to the above data, there is no significant change in the chemical and physical properties of Dican 100 tablet when exposed to different accelerated conditions and to the direct light. As a result, the formula is stable and can be given two year of expiration date at normal conditions (15-30ºC) when packed in PVC-Aluminum blisters.

III-7 Bioequivalence Data

The bioequivalence study was done in Tanta University, Egypt. The summary of the results were as follows:

III-7-1 Pharmacokinetic results

The peak plasma concentration (C_{max}) of Fluconazole following the administration of two Dican tablets ranged from 1.748 – 5.021 μg/ml with a mean value of 3.088 ± 0.796 μg/ml whereas, the C_{max} of Fluconazole following the administration of one Diflucan capsule (Pfizer) ranged from 1.636 – 5.707 μg/ml, with a mean value of 2.777 ± 0.999 μg/ml. The mean time to reach the peak concentration (t_{max}) was 1.979 ± 1.386 hr after administration of two Dican tablets and 3.395 ± 1.763 hr after administration of one Diflucan capsule. The area under the plasma concentration time curve ($AUC_{0\rightarrow\infty}$) after administration of two Dican tablet ranged from 78.09 – 226.05 μg.hr/ml, with a mean value of 142.5 ± 39.47 μg.hr/ml, while following administration of one Diflucan capsule, it ranged from 76.55 – 310.2 μg.hr/ml, with a mean value of 149.3 ± 60.333 μg.hr/ml. The results obtained after administration of two Dican tablets were corrected by a factor of 0.75.

Table III-31: In-vivo pharmacokinetic results for Dican vs Diflucan

Parameters	**Dican (Pharmacare) (Mean ±S.D)**	**Diflucan (Pfizer) (Mean ±S.D)**
C_{max} (μg/ml)	3.088 ± 0.796	2.777 ± 0.999
t_{max} (hrs)	1.979 ± 1.386	3.395 ± 1.763
$AUC_{0\rightarrow\infty}$ (μg.hr/ml)	142.5 ± 39.47	149.3 ± 60.33

Relative Bioavailability = 95.44 % (as calculated from the AUC)

Tables (III-32 and III-33) Illustrates the detailed pharmacokinetic parameters calculated after administration of two Dican tablets and one Diflucan capsules respectively.

Figure III-12 illustrates the mean plasma concentrations after oral administration.

Table III-32: Pharmacokinetic parameters calculated after a single oral dose administration of two Dican tablets (each contains 100 mg Fluconazole) to 24 healthy male volunteers.

Volunteer No.	C_{max} (μg/ml)	t_{max} (hr)	κ (hr^{-1})	t ½ (hr)	$AUC_{0\rightarrow t}$ (μg.hr/ml)	$AUC_{t\rightarrow\infty}$ (μg.hr/ml)	Total $AUC_{0\rightarrow\infty}$. (μg.hr/ml)
1	2.964	3.0	0.015	44.88	98.81	27.68	126.49
2	4.220	0.5	0.018	38.33	122.1	28.29	150.39
3	3.017	0.5	0.022	30.48	101.5	12.82	114.32
4	1.748	2.0	0.015	44.40	91.61	29.40	121.01
5	1.957	0.5	0.013	52.33	86.63	32.65	119.28
6	3.805	1.5	0.024	28.79	126.3	13.38	139.68
7	2.920	2.5	0.021	31.91	133.2	21.63	154.83
8	2.199	0.5	0.025	26.67	72.45	11.58	84.03
9	2.307	0.5	0.019	36.14	83.41	13.03	96.44
10	2.886	3.0	0.021	32.97	97.97	15.72	113.69
11	2.959	2.0	0.016	41.88	167.4	51.95	219.35
12	2.468	1.5	0.027	25.24	67.19	10.90	78.09
13	2.729	2.5	0.013	50.00	111.3	54.46	165.76
14	2.097	4.0	0.020	33.60	116.0	23.01	139.01
15	3.506	1.5	0.016	41.79	129.7	28.85	158.55
16	2.719	6.0	0.020	34.61	105.8	19.97	125.77
17	5.021	2.5	0.012	55.01	141.9	56.45	198.35
18	3.021	2.0	0.020	33.59	110.2	17.39	127.59
19	3.892	1.0	0.016	41.33	155.2	42.48	197.68
20	3.687	4.0	0.018	37.99	91.77	22.79	114.56
21	2.810	1.0	0.021	32.13	109.1	17.93	127.03
22	3.481	0.5	0.013	49.81	104.4	36.05	140.45
23	3.526	1.5	0.016	41.16	143.1	40.04	183.14
24	4.193	3.0	0.015	46.08	169.7	56.35	226.05
Mean	**3.088**	**1.979**	**0.018**	**38.79**	**114.0**	**28.53**	**142.5**
S.D.	**0.796**	**1.386**	**0.004**	**8.209**	**27.63**	**14.85**	**39.47**
S.E.	**0.162**	**0.283**	**0.0008**	**1.676**	**5.641**	**3.033**	**8.058**
MAX	**5.021**	**6.000**	**0.027**	**55.01**	**169.7**	**56.45**	**226.05**
MIN	**1.748**	**0.500**	**0.012**	**25.24**	**67.19**	**10.90**	**78.09**

Table III-33: Pharmacokinetic parameters calculated after a single oral dose administration of one Diflucan capsule (each contains 150 mg Fluconazole) to 24 healthy male volunteers

Volunteer No.	C_{max} (μg/ml)	t_{max} (hr)	κ (hr^{-1})	t ½ (hr)	$AUC_{0\rightarrow t}$ (μg.hr/ml)	$AUC_{t\rightarrow\infty}$ (μg.hr/ml)	Total $AUC_{0\rightarrow\infty}$. (μg.hr/ml)
1	2.073	6.0	0.018	38.05	102.7	26.97	129.67
2	2.992	1.5	0.018	37.48	119.5	48.18	167.68
3	2.423	2.5	0.014	48.14	100.4	37.76	138.16
4	1.750	2.5	0.023	29.82	78.78	11.10	89.88
5	2.098	1.5	0.019	36.02	58.75	17.80	76.55
6	3.191	3.0	0.021	31.62	134.4	17.63	152.03
7	2.193	4.0	0.020	33.52	80.01	12.09	92.10
8	2.708	2.5	0.028	24.36	115.7	11.27	126.97
9	2.325	4.0	0.031	21.70	82.53	10.97	93.50
10	3.598	2.5	0.018	37.94	147.3	31.34	178.64
11	2.048	8.0	0.019	35.58	104.9	22.28	127.18
12	2.052	4.0	0.012	53.38	85.96	27.44	113.4
13	2.453	3.0	0.025	27.32	104.2	11.85	116.05
14	2.186	2.0	0.013	52.04	120.9	51.42	172.32
15	4.457	2.5	0.013	52.00	205.6	104.6	310.2
16	1.866	3.0	0.018	37.91	84.17	34.55	118.72
17	2.415	8.0	0.013	52.53	123.2	84.57	207.77
18	5.707	1.5	0.018	38.47	198.9	46.54	245.44
19	4.501	2.5	0.02	34.07	204.8	40.06	244.86
20	2.437	4.0	0.033	20.84	76.63	7.520	84.15
21	1.636	4.0	0.018	38.38	68.05	13.84	81.89
22	2.732	3.0	0.015	44.77	109.6	39.27	148.87
23	3.004	2.0	0.027	24.88	133.7	11.53	145.23
24	3.811	4.0	0.015	44.16	171.3	51.97	223.27
Mean	**2.777**	**3.395**	**0.019**	**37.29**	**117.1**	**32.18**	**149.3**
S.D.	**0.999**	**1.763**	**0.005**	**9.783**	**42.27**	**24.18**	**60.33**
S.E.	**0.204**	**0.359**	**0.001**	**1.997**	**8.630**	**4.936**	**12.31**
MAX	**5.707**	**8.000**	**0.033**	**53.38**	**205.6**	**104.6**	**310.2**
MIN	**1.636**	**1.500**	**0.012**	**20.84**	**58.75**	**7.520**	**76.55**

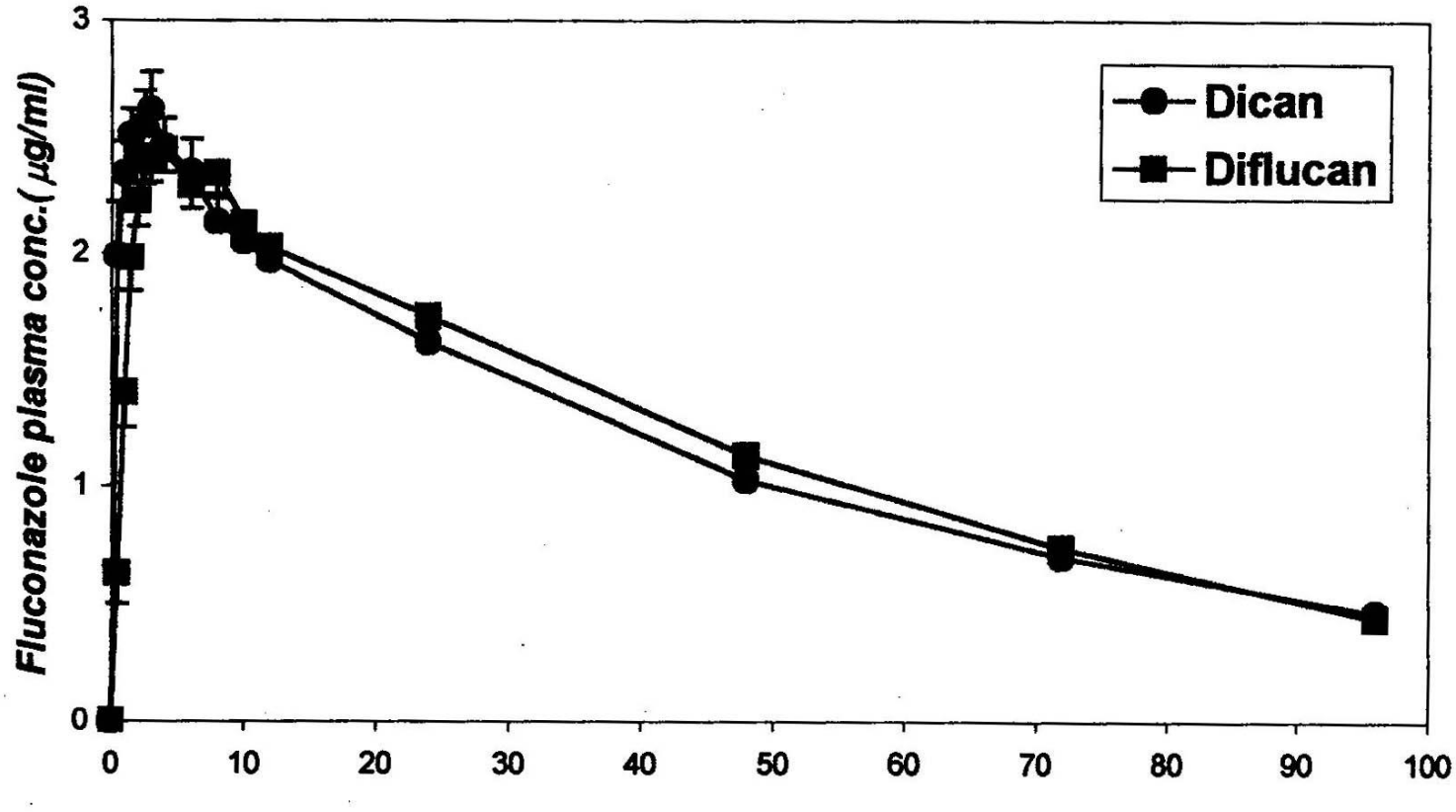

Figure III-12: Mean plasma concentration of Fluconazole following a single oral dose administration of two Dican tablets and one Diflucan capsule (Mean ± S.E.M)

III-7-2 Assesment of bioequivalence

The relative bioavailability of Fluconazole from Dican tablets compared to Diflucan capsules (Pfizer) was found to be 95.44 % as determined from the ratio of the $AUC_{0\rightarrow\infty}$ of both products.

It can be concluded that oral Dican tablets and oral Diflucan capsules are bioequivalent, since both preparations have equivalent rate and extent of Fluconazole absorption.

III-8Microbial comparison between the Dican and Trican (Diflucan) for Pfizer.

A potency test for Fluconazole was developed using the plate method using Candida Albicans as the test organism. Several media and solvents were tested for the best clear zones. Tables (III-34 and (III-35) summarizes the different media and solvents with the zone rate of clearance.

Table III-34: Zone clearance using different agar media

Agar Media	Zone Clearance
Antibiotic medium # 19	+
Antibiotic medium # 3	+++
Antibiotic medium # 1	+
YGC	++

Table III-35: Zone clearance using different solvents

Buffer type	Zone Clearance
Buffer I	+
0.1 N HCl	+
Buffer 10	++
Methanol	++

The standard and test solution concentrations were as follows:

Conc. Low: 66.6 (μg/ml)
Conc. High: 200.0 (μg/ml)

5 plates were prepared as in the procedure (II-8). Each plate contains 4 wells with the following samples (UL, UH, SL and SH). The plates were incubated at 35°C for 24 hours. The zone diameters were measured and the results were recorded, table (III-36).

Table III-36: Zone diameters for each plate (mm)

	UH	**SH**	**UL**	**SL**
Plate # 1	31	30	27	27
Plate # 2	29	30	26	25
Plate # 3	31	30	28	26
Plate # 4	30	31	27	27
Plate # 5	31	32	28	29
Average	30.4	30.6	27.2	26.8

Calculation:

$$\text{Log } X = \text{LDR} \times \frac{(UH + UL) - (SH + SL)}{(SH - SL) + (UH - UL)}$$

Dose ratio = 20.0 / 6.66 = 3
LDR = 0.477

Log X = 0.477 x (57.6 – 57.4) / (3.8 + 3.4) = 0.0131

X= 1.031

Conclusion:
From the results obtained, Dican tablet (B.N. R3G02 and manufacturing date 07/2002) activity against Candida albicans is 103.1 % compared to Trican capsule (B.N 257044 and Expiry 03/2006).

III-9 Anti-Microbial Activity of Fluconazole.

III-9-1 MIC results for Fluconazole:

The antimicrobial activity of Fluconazole was tested agaist 4 bacterial strains using the procedure in II-9. The results were as follows:

a. Salmonella: no inhibition for concentrations up to 1000 µg/ml.

b. Staphylococcus Aureus: inhibition in tubes # 2, in which the concentration of Fluconazole is 250 µg/ml.

c. Pseudomonas Aeruginosa: no inhibition for concentrations up to 1000 µg/ml

d. Escherishia Coli: no inhibition for concentrations up to 1000 µg/ml

III-9-2 MIC results for Clarithromycin:

Staphylococcus Aureus: inhibition in tube # 4, in which the concentration of Clarithromycin is 36.5 µg/ml.

III-9-1 Plate count

Salmonella: 150 colonies in plate #2 = 1.25 x 10^3 bacteria / ml in the MIC test tubes.

Staphylococcus Aureus: 53 colonies in plate #3= 4.4 x 10^3 bacteria / ml in the MIC test tubes.

Pseudomonas Aeruginosa: 52 colonies in plate # 3 = 4.3 x 10^3 bacteria / ml in the MIC test tubes.

Escherishia Coli: 35 in plate # 3 = 2.9 x 10^3 bacteria / ml in the MIC test tubes.

III-9-3 Calculation and Results

The concentration of the broth solution of a microorganism inoculum's equals (Conc. (bacteria / ml))=

Plate count x $10^{\text{No of plates}}$ / inoculum volume on plate (ml)

The final dilution in each test tube after inoculation with 50 µl in 6 ml is calculated as follows=

Conc. (bacteria/ml) x inoculum's volume (ml)/ total volume (ml)

The Final dilution of every bacterial strain is thus calculated as follows:

1- Salmonella

Conc. (bacteria / ml) = 150 x 10^2 / 0.1 ml = 15 x 10^4 bacteria/ml

The final dilution = (15 x 10^4 bacteria / ml) x (0.050 ml) / (6 ml)
= 1.25 x 10^3 bacteria / ml

2- Staphylococcus Aureus

Conc. (bacteria / ml) = 53 x 10^3 / 0.1 ml = 53 x 10^4 bacteria / ml

The final dilution = (53 x 10^4 bacteria / ml) x (0.050 ml) / (6 ml)
= 4.4 x 10^3 bacteria / ml

3- Pseudomonas Aeruginosa:

Conc. (bacteria / ml) = 52 x 10^3 / 0.1 ml = 52 x 10^4 bacteria / ml

The final dilution = (52 x 10^4 bacteria / ml) x (0.050 ml) / (6 ml)
= 4.3 x 10^3 bacteria / ml

4- Escherishia Coli

Conc. (bacteria / ml) = 35×10^3 / 0.1 ml = 35×10^4 bacteria / ml

The final dilution = (35×10^4 bacteria / ml) x (0.050 ml) / (6 ml)
= 2.9×10^3 bacteria / ml

III-9-4 Discussion and conclusion

Fluconazole has activity against Staphylococcus Aureus with a MIC of = 250 µg/ml against 4.4×10^3 microorganisms / ml in tryptic soy broth (TSB).

Clarithromycin has activity against Staphylococcus Aureus with a MIC of 36.5 µg/ml against 4.4×10^3 microorganisms / ml in tryptic soy broth (TSB).

Compared to Clarithromycin, which is indicated for S. Aureus, Fluconazole is about 14.6 % as active as Clarithromycin.

PART IV: CONCLUSION

New generic formulation for Fluconazole 100 mg tablet was developed using the direct compression method. A complete qualitative and quantitative analysis was performed for the resultant tablets and found to be within specification predetermined.

An HPLC method of analysis has been developed using a reversed phase stationary phase and a UV-detector. The method was validated and found to be selective, accurate, precise, and linear and withstand for the possible variations in instruments, tools and personnel.

A comparative dissolution profile was made for the formulated (Dican) tablet vs the brand product Trican capsule. The similarity factor calculation was applied. When calculating the similarity factor in the range 10 – 25 minutes, the result fails (f_2 = 44.1). This is due to the capsule shell of Trican, which delays the dissolution in the first 5 minutes. The similarity factor calculated in the range 15 – 25 minutes was found to be within limits (f_2=51.2).

Stability testing was conducted at stress conditions. A study for the effect of light was also performed. All results were within specifications with no significant changes occurred.

A bioequivalence study was performed for the tablets vs Diflucan capsule in the University of Tanta, Egypt. The formula proved to be bioequivalent to the brand product.

A potency plate method for measuring the potency activity of Fluconazole was developed. A biological comparison between the tablet and Trican capsule was conducted using the developed method. The tablet proved to be similar in activity compared to the brand product.

A test for antibacterial activity was performed using the minimum inhibitory concentration (MIC) method. The test was conducted against four bacterial strains. An activity was found against Staphylococcus Aureus. The results were compared with that obtained from MIC for Clarithromycin. The activity was about 14.6 % compared to Clarithromycin.

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