COMPARITIVE STABILITY STUDIES ON RIFAMPICIN IN FIXED DOSE COMBINATIONS BETWEEN BLISTER AND STRIP PACKAGED MARKETED PRODUCTS

A Dissertation submitted to

THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY
Chennai-600032

In partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY
IN
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Submitted by
REG. NO: 26105402

Under the Guidance of

Dr. N. N. RAJENDRAN, M. Pharm., Ph.D.,

DEPARTMENT OF PHARMACEUTICS

SWAMY VIVEKANANDHA COLLEGE OF PHARMACY

ELAYAMPALAYAM

TIRUCHENGODE-637205

TAMILNADU.

MAY-2012

CERTIFICATES

SWAMY VIVEKANANDHA COLLEGE OF PHARMACY
Elayampalaym, Tiruchengode, 637205
Namakkal (DT), Tamilnadu.
Phone: 04288-234417 (8lines)
Fax: 04288-234417

Dr. M. P. NARMADHA, M. Pharm., Ph.D.,
Principal

CERTIFICATE

This is to certify that the Dissertation entitled **"COMPARITIVE STABILITY STUDIES ON RIFAMPICIN IN FIXED DOSE COMBINATIONS BETWEEN BLISTER AND STRIP PACKAGED MARKETED PRODUCTS"** submitted to The Tamilnadu Dr. M.G.R Medical University, Chennai, is a bonafide project work of **Reg. No: 26105402,** in the Department of Pharmaceutics, Swamy Vivekanandha College of Pharmacy, Tiruchengode for the partial fulfillment for the degree of Master of Pharmacy under the guidance of **Dr. N. N. RAJENDRAN, M. Pharm., Ph.D.,** Swamy Vivekanandha College of Pharmacy, Tiruchengode.

Signature of the Principal
Dr. M. P. NARMADHA, M. Pharm., Ph.D.

SWAMY VIVEKANANDHA COLLEGE OF PHARMACY
Elayampalaym, Tiruchengode, 637205
Namakkal (DT), Tamilnadu.
Phone: 04288-2344178lines)
Fax: 04288-234417

Dr. N. N. RAJENDRAN, M. Pharm., Ph.D.,
Director of P.G Studies and Research

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Signature of Director of P.G. studies
Dr. N. N. RAJENDRAN, M. Pharm., Ph.D.

SWAMY VIVEKANANDHA COLLEGE OF PHARMACY
Elayampalaym, Tiruchengode, 637205
Namakkal (DT), Tamilnadu.
Phone: 04288-234417(8lines)
Fax: 04288-234417

R. NATARAJAN, M .Pharm., (Ph.D).,
Head, Department of Pharmaceutics

CERTIFICATE

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This work is original and has not been submitted earlier for the award of any other degree or diploma of this or any other university.

Signature of Head, Department of Pharmaceutics

R. NATARAJAN, M. Pharm., (Ph.D.)

SWAMY VIVEKANANDHA COLLEGE OF PHARMACY
Elayampalaym, Tiruchengode, 637205
Namakkal (DT), Tamilnadu.
Phone: 04288-234417(8lines)
Fax: 04288-234417

Dr. N. N. RAJENDRAN, M. Pharm., Ph.D.,
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Signature of guide

Dr. N. N. RAJENDRAN, M. Pharm., Ph.D.,

DEDICATED TO

MY PARENTS

BROTHER

AND

FRIENDS

ACKNOWLEDGEMENT

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A. Brahmini

Reg.No:26105402

CONTENTS

INTRODUCTION

1. INTRODUCTION

Tuberculosis is a ubiquitous, highly contagious chronic granulomatous bacterial infection caused by the Mycobacterium tuberculosis. Mycobacterium tuberculosis is rod shaped bacteria called as Koch's bacillus. Tuberculosis is the world's commonest cause for death after HIV/AIDS. According to WHO about 1/3rd of world population are infected with TB. More than 8 million people are commonly infected with TB annually in developing countries like sub- Saharan Africa and Asia.[1] The prevalence of TB in India accounts for 30% of global burden and when combined with cases from china constitute 40% of all cases globally. Approximately 10% of the infected people develop active TB.

TB spreads through droplets of secretions such as sputum or aerosols released by coughing from the infected persons. Its eradication requires prevention, early diagnosis and effective treatment of the infection. A vaccine called BCG is administered in many parts of the world where TB is common.

WHO and IUALTD recommended use of fixed dose combination of anti- T.B drugs because FDC reduce the number of tablets to be consumed and thereby increase the patient compliance.[2] Thus FDC plays a major role in preventing emergence of drug resistance.

Widely used FDC for treatment of TB is rifampicin, isoniazid, ethambutol and pyrazinamide.Treatment of TB involves administration of combination of rifampicin, isoniazid, pyrazinamide and ethambutol for initial 2 months followed by rifampicin and isoniazid for 4 months.[3] Isoniazid and rifampicin are the most potent anti-TB drugs kills more than 99% tubercular bacilli within 2 months of initiation therapy.[4]

Rifampicin is a critical component in the therapeutic armamentarium for tuberculosis Rifampicin is a semi synthetic derivative of macro cyclic antibiotic derived from *Streptomyces mediterranei*. Rifampicin act by inhibiting DNA dependent RNA polymerase. The bioavailability of rifampicin in FDC may be reduced owing to chemical reaction with isoniazid in acidic gastric environment; pyrazinamide and ethambutol catalyses the reaction.[5]

Rifampicin in the presence of isoniazid as FDC may undergo greater decomposition in the acidic conditions of the stomach, as compared to when rifampicin is administered alone. Thus less rifampicin will be available for absorption from FDC as compared to rifampicin administered as separate formulation.[6]

Rifampicin gets absorbed rapidly upon oral administration on empty stomach. Food and some antacids decreases oral absorption of Rifampicin. Rifampicin hydrolyses to 3-formyl rifamycin in acidic medium and hydrolysis is accelerated in the presence of isoniazid. Two major problems are reported with rifampicin and isoniazid FDC that includes the impaired and fall in bioavailability of rifampicin from FDC formulations with isoniazid and other problem is poor stability of rifampicin containing FDC[7]. The factors responsible for variation in bioavailability include changes in crystalline form, drug absorption by excipients, moisture content and particle size. Stability problems include changes in drug strength and increase in degradation product and gain in moisture.[8]

Many tropical countries have adverse environmental conditions, including high temperature, humidity and intense light. Products usually sold in secondary packages in shops that do not have air conditioning. [9] Hence the question arises: "should pharmaceutical products in tropical countries be tested for stability using the combination of temperature and humidity?"

Packaging also plays a role in affecting the stability of rifampicin. The primary role of packaging is to protect the dosage form from the moisture and oxygen present in the atmosphere. There are many types of packaging materials such as glass, plastic, rubber, metal and paper. Plastic has become the most popular materials for packaging pharmaceuticals because it is strong, light weight and reasonably inert. Solid dosage form is popularly packed in blister pack and strip pack.[10] Blister is a multidose container consisting two layers, of which one is shaped to contain the individual doses and strip is a multi-dose container consisting of two layers, usually provided with perforations suitable for containing single dose of solid.

Previously studies were carried out to determine the stability of FDC anti-tuberculosis products in commercial packages under ICH/WHO accelerated conditions (40^0C/75% RH) and suggested barrier packaging to prevent catalytic role in the interaction between isoniazid and rifampicin.[3] In another study conducted in similar conditions, it has been reported that strip products are more stable while blister products showed both physical and chemical changes.[11] Though marketed products of FDC anti-TB drugs in strip or blister packages are considered stable based on the data obtained from the official guide lines that recommend accelerated stability studies and ICH/WHO accelerated conditions (40^0C/75% RH), in actual package the storage of these products in the retail outlet at recommended conditions of storage are often overlooked and as such bioavailability of these products particularly rifampicin is questionable. It is necessary to examine whether these products in their original package are stable at varied temperature and humidity conditions.

To ensure their bioavailability as claimed by pharmaceutical manufacturer, therefore the present study aimed to investigate the stability of rifampicin from FDC marketed products available in strip and blister packages by exposing them 40^0C, 40^0C/75% RH and to room temperature at $30^0\pm 2^0$C for 60 days. The study may help to understand the influence of packages on the stability of rifampicin from FDC products when storage guidelines are over looked.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Tuberculosis is one of the most chronic and infectious disease occurring world wide ranging from developing countries to developed countries. Tuberculosis infection is caused by Mycobacterium tuberculii. Mycobacterium tuberculii is gram positive aerobic rod shaped acid fast bacillus. This Mycobacterium tuberculii was discovered by Robert Koch in 24th march in 1882 and named it as Koch's bacillus. [12]Primary infection is usually asymptomatic or latent with the development into lungs.

CLASSIFICATION OF ANTI-TUBERCULOSIS DRUGS:

Anti-Tuberculosis drugs are classified as:

1 .FIRST LINE GENERATION : First line class of drugs includes rifampicin, Pyrazinamide, ethambutol and isoniazid.

2. SECOND LINE GENERATION : Second line class of drugs includes amikacin and ethionamide

3. THIRD LINE GENERATION : Third line class include thioacetone, arginine, macrolide and vitamin D

GENERALLY TWO TB RELATED CONDITIONS EXIST:

LATENT TUBERCULOSIS

People with latent TB are not sick because the TB germs in the body are not active. The often prescribed medicine to prevent them from being infected by TB is giving isoniazid for 9 months.

ACTIVE TUBERCULOSIS

This kind of TB occurs when the immune system is not capable of defending the infection. When Mycobacterium tuberculii are active, it is called active tuberculosis. Rifampicin, isoniazid, ethambutol and pyrazinamide are the preferred dosage regimen for this kind of Tuberculosis.

2.1 EPIDEMOLOGY OF TUBERCULOSIS

Tuberculosis is the major cause of mortality and morbidity in many undeveloped countries like Latin America, Asia and Africa. National institutes of health of United States reported that about 17 billion people of world population are infected with TB annually. Of all these people infected with mycobacterium tuberculosis, about 5% will develop active TB disease and other 95% people will develop a latent infection that may later progress to cause disease depending upon the status of immune system.[13]

There are several reasons for increasing incidence of tuberculosis with current increase in cases of HIV. Part of reason is the development of multi drug resistant tuberculosis mutants. Globally South East Asia accounts for the maximum of 33% incidence of TB. This burden is increased by human immunodeficiency virus (HIV) infection, which impairs the immune system and allows large numbers of people already infected with tuberculosis to progress to active disease.

In South Africa around 10,000 people are infected with tuberculosis annually. In a view of severity and spread of disease in 1993 W.H.O declared TB to be a global emergency. In 2002 World Health Organization (W.H.O) notified 49,656 patients in Thailand (WHO Report 2004) and 6,906 deaths (Health information Group, 2003). 250,000 deaths were due to TB/ HIV co-infection. WHO estimates 460,000 multi drug resistant-TB cases occur each year.

The most populated countries of Asia have the largest number of cases: India, China, Indonesia, Bangladesh and Pakistan together accounts for more than half of global burden. There were 22 high burden countries, including Thailand that W.H.O particularly noticed. It is feared that by 2020 about 200 million individuals will become sick and 70 million people will die in the developed countries.[14]

In a view to control TB W.H.O and IUALTD recommended a strategy for TB control and named it as DOTS (Directly Observed Therapy). Every year W.H.O spends billions of dollars in the issue of TB control. According to South African National Tubercular Association (SANTA) DOTS spend 130 million US dollars in South Africa for curing TB in 1998. BY the proper use of DOTS 500 million U.S dollars will be saved in South Africa annually.[15] DOTS have been introduced in Thailand since 1996, the patient rate reduced after by applying DOTS therapy.

2.2 FIXED DOSE COMBINATIONS

Combination therapy refers to treatment with two or more drugs administered at one time to ensure patient compliance to combine the requisite drugs physically into one preparation is known as fixed dose combination. [16]

ADVANTAGES:

- Better patient compliance.
- Less chances to develop drug resistance.
- Simplicity of treatment with minimal prescription errors

DISADVANTAGES:

- Young children may receive higher dose than required.
- FDC is more expensive than individual components in terms of cost per tablet.

Fixed dose combinations from the who model list of essential medicines

Table 1: Fixed dose combinations from the WHO model list of essential medicines

Drug	Dose form	Strength for Daily use	Strength for intermittent use 3 times per week
rifampicin + isoniazid [RH]	Tablet	150 mg + 75 mg 300 mg + 150 mg	150 mg + 150 mg
	Tablet or pack of granules*	60 mg + 30 mg	60 mg + 60 mg
ethambutol + isoniazid [EH]	Tablet	400 mg + 150 mg	-
isoniazid + thioacetazone [HT]**	Tablet	100 mg + 50 mg 300 mg + 150 mg	- -
rifampicin + isoniazid + pyrazinamide [RHZ]	Tablet Tablet or pack of granules*	150 mg + 75 mg + 400 mg 60 mg + 30 mg + 150 mg	150 mg + 150 mg + 500 mg -
rifampicin + isoniazid + pyrazinamide + ethambutol [RHZE]	Tablet	150 mg + 75 mg + 400 mg + 275 mg	- -

* For paediatric use
** Although used in some programmes, WHO does not recommend the use of thioacetazone (T) because of the risk of severe toxicity, particularly in HIV infected individuals. In general, thioacetazone should be replaced by ethambutol.

W.H.O and IUALTD recommend use of fixed dose combinations. Anti-TB drugs are generally given in the form of fixed dose combinations. Anti-TB FDC formulations combine two or more first line anti-TB drugs like rifampicin, isoniazid, ethambutol and pyrazinamide of fixed proportion in a single dosage form.[2] The recommended strength for fixed dose combinations by WHO is rifampicin 150mg, isoniazid 75mg, pyrazinamide 400mg and ethambutol 275mg.

Treatment of TB involves rifampicin, isoniazid, ethambutol and pyrazinamide for initial 2 months followed by administration of rifampicin and isoniazid for 4 months which act on mycobacterium tuberculii by varying methods including sterilization, bacteriostatic and bactericidal.[17] Rifampicin and isoniazid are the most powerful

bactericidal drugs against all strains of TB bacilli. Ethambutol is used in combination with powerful drugs to prevent emergence of resistant to bacilli. Rifampicin act by inhibiting DNA – dependent RNA polymerase by blocking RNA transcription. The problem involved in the FDC is poor bioavailability of rifampicin. The reason for poor bioavailability of rifampicin is change in crystalline form of rifampicin, drug adsorption by excipients and formulation factors. [8]

Isoniazid is a synthetic antimycobacterial and bactericidal agent for both extracellular and intracellular organism and act by interfering with cell wall mycolic acid synthesis.Pyrazinamide exhibits invitro bactericidal activity. [18]

2.3 DEGRADATION OF RIFAMPICIN:

Degradation of rifampicin is PH dependent. In acidic medium rifampicin hydrolyses to 3-formylrifamysin and it undergoes air oxidation in alkaline medium to form inactive quinine derivative and rifampinquinone. 3-formylrifamysin (3FRSV) precipitates in acidic conditions and the formation of 3- formylrifamysin in the acidic environment of stomach is the important factor affecting bioavailability.[7] An elegant mechanism to explain the increased degradation of rifampicin in presence of isoniazid is [19]

Rifampicin + H_20 ⟷ 3 FRSV + 1- amino 4-methyl piperazine

Isoniazid + 3- FRSV ⟷ Isonicotyl hydrazone + H_20

Rifampicin hydrolyses to 3 FRSV. Isoniazid reacts with 3-FRSV in a reversible manner where the forward reaction is faster second order reaction and backward reaction is slower first order reaction.

The overall reaction is favored towards formation of hydrazone and thus an overall increase in degradation of rifampicin to 3- FRSV is observed the same time, hydrazone are known to hydrolyze in the acidic medium resulting in regeneration of isoniazid and 3-FRSV.[20]

The decomposition of rifampicin in acidic conditions in absence of isoniazid stop the formation of 3-formylrifamysin but in the presence of isoniazid, the reaction is proceeded to form hydrazone between 3- formylrifamysin and isoniazid resulting recovery of isoniazid but eventually causing loss of rifampicin. This indicates that isoniazid remains unaffected and plays a role of a catalyst in the degradation of rifampicin in acidic conditions to 3-FRSV. [4, 21] This explains the reason why the bioavailability problem is confined to rifampicin alone but not to isoniazid.

In acidic medium 12.4% of rifampicin alone is degraded to 3-formyl rifamycin within 1 hour and in the presence of isoniazid degradation of rifampicin is increased to 21.5%. This indicate that degradation of rifampicin to 3-formylrifamysin is almost twice and two times faster in the presence of isoniazid than that of rifampicin alone.

2.4 METHODS ADOPTEDTO PREVENT DEGRADATION OF RIFAMPICIN:

Approaches to prevent degradation of rifampicin include enteric coating of solid formulations or drug granules, use of alkaliniser at the time of administration of FDC formulations, exploitation of formulation factors including addition of additives and segregation of delivery of rifampicin and isoniazid.

The following approaches seem plausible. (i) Enteric coating of solid formulations or drug granules. (ii) Use of alkaliniser at the time of administration of FDC formulations. (iii) Exploitation of formulation factors, including addition of additives.[8] A novel formulation comprising of rifampicin and sodium lauryl sulphate prepared by co-grinding method, proved effective to minimize the degradation of rifampicin in acidic environment by its retarded release pattern. This segregation release pattern of rifampicin in alkaline environment and isoniazid in acidic environment of GI tract prevents degradation of rifampicin alone and its interaction with isoniazid.[22]

2.5 STABILITY STUDIES:

Stability is the capacity of drug to remain within specifications established to ensure its identity, strength, quality and purity. The purpose of pharmaceutical stability testing is to provide evidence on how the drug substance or drug product varies with time under the influence of variety of environmental factors such as temperature, humidity and light.

Stability testing includes long term stability studies, intermediate stability studies and accelerated stability studies. In long term stability study the product is stored at 25^0 C ± 2^0C/60% ± 5 RH for 12 months. In intermediate stability study the product is stored at 30^0C ± 2^0/65% ± 5% RH for 6 months and in accelerated stability studies the product is stored at 40^0C ± 2^0/75% ± 5% RH for 6 months. [23]

Table 2: The main objectives of stability testing are shown in the table below

Objective	Type of study	For use in
To select adequate (from the view-point of stability) formulations and container closure systems	Accelerated	Development of product
To determine shelf-life and storage conditions	Accelerated and/or long term	Development of product and registration dossier
To substantiate the claimed shelf-life	Long term	Registration dossier
To verify that no changes have been introduced in the formulation or manufacturing process that can adversely affect the stability of the product	Accelerated and/or long term	Post approval changes and quality assurance in general, including quality control

Accelerated testing

Studies designed to increase the rate of chemical degradation or physical change of a drug substance or drug product by using exaggerated storage conditions as part of the formal stability studies. Data from these studies, in addition to long term stability studies, can be used to assess longer term chemical effects at non-accelerated conditions and to evaluate the effect of short term excursions outside the label storage conditions such as might occur during shipping .[24]

Stability problems include change in drug strength, increase in degradation product, gain in moisture and chemical stability.[8] Chemical stability is generally expressed in terms of rate constant (k) representing either product formation or drug degradation. For any mechanism the rate of reaction (k) can be described by general rate equation. [25]

$$d\alpha /dt = kf(\alpha)$$

α = conversion of reaction

$f(\alpha)$ = the conversion function

For zero order reaction the reaction rate is independent of drug concentration while for the first order reaction the rate depends linearly on drug concentration.

Zero order $d\alpha/dt = k$

First order $d\alpha/dt = k(\alpha)$

Temperature dependence on the rate constant, k is usually expressed by Arrhenius equation

$$K = A_k e^{-E\alpha/RT}$$

A = Pre exponential factor

Ea = Activation energy (cal/mole)

T= absolute temperature

R = gas constant

Chemical instability of fixed dose combination is found to occur due to two reasons. One is direct interaction of rifampicin and isoniazid; the mechanism which involves interaction of imine group of rifampicin with amino group of isoniazid to yield hydrazone in solid formulation environment. The other reason is creation of an acidic hydrolytic environment upon moisture gain by ethambutol hydrochloride. The two co drugs present usually in fixed dose combinations accelerate the reaction between rifampicin and isoniazid.[5]

HUMIDITY:

Humidity can have on effect on solid drug substances. Pharmaceutical solid forms may contact with moisture during manufacturing process and storage at high relative humidity. The amount of moisture that is sorbed is dependent on the chemical properties, temperature, relative humidity and porosity of the packaging material. The excipients used in solid dosage form affect the stability of the formulation. The more amorphous is used in formulation the more water is sorbed. [26]

PACKAGING:

The primary role of packaging is to protect the dosage form from the moisture and oxygen present in the atmosphere. There are many types of packaging materials such as glass, plastic, rubber, metal and paper. Plastic has become the most popular materials for packaging pharmaceuticals because it is strong, light weight and reasonably inert. Solid dosage form is popularly packed in blister pack and aluminum foil.

Two major factors which affect packaging are leachable impurities and permeability of moisture and oxygen. Two blister packs one with polyvinylchloride and another with polyvinyl chloride and laminate of polymonochlorotrifluroethylene were backed with impermeable foil and used for packing. It was found that polyvinyl chloride laminated with polymonochlorotrifluroethylene blister pack could protect solid dosage form better than the other.[10]

2.6 TB DRUGS IN DEVELPOMENT:

The recent discovery of diarylquinone is a promising TB drug that shortens therapy. Andries *et.al* identified diarylquinoline compound which is highly active against Mycobacterium tuberculosis.

Flouroquinolines

These are broad spectrum of antibiotics currently used as second line drugs in TB therapy. Moxifloxacin and Gatifloxacin are fluroquinolines.[27] that has longer half life and more active against mycobacterium tuberculosis than ofloxacin. Moxifloxacin has been shown to kill a sub population of tubercle bacilli that has not been killed by rifampicin. Moxifloxacin in combination with rifampicin and pyrazinamide kills bacteria more effectively than standard regimen of INH +RIF + PZA

Rifampin derivative:

Rifampin derivatives include rifapentine [28], rifabutin and rifalazil. Rifalazil is highly active against a range of intracellular bacteria including mycobacterium tuberculosis.

Oxazolidones:

These are active against gram positive bacteria. This oxazolidones inhibit protein synthesis at an early stage by binding to 23s rRNA of the 50s ribosomal unit.[29]

Nitroimidazopyran:

It acts by inhibiting mycolic acid which is the main component of cell wall and protein synthesis.[30]

H.Bhutani *et.al,* [3] (2003) carried out a study to determine the physical and chemical stability of anti- tuberculosis fixed dose combination products under accelerated climatic conditions. In this study fixed dose combinations of rifampicin, isoniazid, pyrazinamide and ethambutol products were stored for 3 months under ICH/WHO accelerated conditions (40/75% RH) with and without original packaging in the presence and absence

of light. The unpackaged products underwent both physical and chemical changes. A significant finding is that pyrazinamide and ethambutol play a catalytic role in the interaction between isoniazid and rifampicin. This study suggested that unless fixed dose combinations are packed in barrier packaging, anti-tuberculosis fixed dose formulations are considered as unstable and consideration should be given to their development, packaging and stability testing.

S.Singh *et.al,* [31] (2003) conducted study on pilot stability study on four fixed dose combination anti tubercular products at 40^0C and 75% RH. The strip products were stable, while blister products showed both physical and chemical changes. The products in unpacked conditions showed severe (60) decomposition of rifampicin and the main decomposition product is isonicotylhydrazine of 3- formylrifamysin. This study suggested that attention should be paid to the detection and quantitation of the product in marketed formulations. The packaging material used in manufacturing of fixed dose combination products should also be of highest quality.

Saranjit Singh, *et.al* [8] 2001has given critical review of probable reasons for the poor/ variable bioavailability of rifampicin from anti-tuberculosis fixed dose combination products and likely solutions to the problems. Unfortunately the origin and cause of the problem is not clearly understood though the GMP and crystalline changes are cited as the principal reasons. The enhanced decomposition of rifampicin in the presence of isoniazid in stomach after ingestion is indicated as the key factor behind the problem.

C.J Shishoo *et.al,* [6] (2001) conducted study on impaired bioavailability of rifampicin in the presence of isoniazid from fixed dose combination. Bioavailability of rifampicin after administration of single component rifampicin (450 mg) capsule and rifampicin-isoniazid (RIF-INH) (450+300mg) single dose is noted. Cross-over test were conducted on six healthy male volunteers and HPTLC method was developed to know the amount of Rifampicin and its metabolite ,25-Desacetylrifampicin in urine. Significant decrease in bioavailability of rifampicin from fixed dose combination capsules was observed. This bioavailability study confirmed that stability of rifampicin in the presence of isoniazid in

acidic environment of stomach is the main factor for reduced bioavailability of rifampicin from RIF-INH combination formulations. This study underlines the fact that there is a urgent need to reconsider the formulation of fixed dose combination products in order to minimize or avoid the decomposition of rifampicin in gastrointestinal tract

C.J Shishoo *et.al*, [7] (1999) conducted study on stability of rifampicin in dissolution medium in presence of isoniazid. Rifampicin (RIF) hydrolyzes in acidic medium to form insoluble and poorly absorbed 3-Formyl rifamycin SV (3-FRSV). This study describes development of two principally different methods, Dual Wavelength UV–Vis.spectrophotometry (DW spectrophotometer) and HPTLC, to determine 3-FRSV in presence of RIF Using DWspectrophotometry, RIF was estimated by using wavelengths 475.0 and 507.0 nm and 3-FRSV was estimated using 457.0 and 492.0 nm. Both the methods were found to be specific, accurate and reproducible. The proposed methods were successfully applied to determine the rate of degradation of RIF to 3-FRSV in dissolution medium (0.1 N HCl) and also two times stability of RIF in market formulations of RIF and RIF with INH in dissolution in presence of isoniazid (INH). The rate of degradation of RIF in presence of INH was almost two times more than that of rifampicin alone medium. It has more than that of RIF alone. These methods were utilized to study the stability of rifampicin in market formulations of rifampicin and rifampicin with isoniazid.

It has been observed that RIF degrades by 12.4% to form 3-FRSV (RIF formulations) while in presence of INH the degradation is catalyzed to about 21.5% (RIF_INH formulations), in 45 min. Thus, lower concentration of RIF may be available for absorption leading to poor bioavailability of RIF from combination dosage forms (RIF_INH) as compared to formulations containing only RIF. It is proposed that specific analytical method should be used to measure RIF in presence of dissolution medium.

Hemant Bhutani *et.a,l*[5] (2005) conducted a study to know the Mechanistic explanation to the catalysis by pyrazinamide and ethambutol in the reaction between rifampicin and isoniazid in anti-TB FDCs. Rifampicin and isoniazid are known to interact with each other in solid formulation environment to yield isonicotinyl hydrazone (HYD). In earlier

studies, this reaction was indicated to be catalyzed by pyrazinamide and ethambutol hydrochloride, the two other co-drugs present in anti-tuberculosis fixed-dose combination (FDC) formulations. The present study was carried out to understand the catalytic role of pyrazinamide and ethambutol hydrochloride on the reaction between rifampicin and isoniazid. Organic bases and amides similar in structure to pyrazinamide and ethambutol hydrochloride were combined individually with rifampicin and isoniazid. The compounds employed were pyrazine, piperdine, pyrollidine, pyridine, triethylamine, diisopropylethylamine, picolinamide, benzamide, ethylenediamine, ethanolamine, diethanolamine, and triethanolamine. An additional study was carried out in the presence of free base of ethambutol. These mixtures were exposed to accelerated stability test condition of 40^0 C/75% RH for 15 days. The drugs showed different extent of degradation, yielding HYD, and in some cases degradation products of rifampicin. The results confirmed the catalytic role of pyrazinamide and ethambutol hydrochloride. The catalysis is postulated to involve intra molecular proton transfer during transhydrazone formation process, entailing a tetrahedral mechanism.

Shrutidevi Agrawal *et.al,* [32] (2004) conducted a study on Dissolution test as a surrogate for quality evaluation of rifampicin containing fixed dose combination formulations. Six FDC formulations were used in this study, of which four had passed bioequivalence while two failed. Formulations showed variable dissolution at different conditions and dissolution at 50 rpm was most sensitive and differentiated the release profiles of rifampicin under various pH conditions. It was possible to predict in vivo performance of rifampicin from FDCs when in vitro rate and extent of release at various pH was correlated with site, pH and concentration dependent absorption of rifampicin along with gastric emptying time. It was also seen that dissolution conditions recommended in USP for different types of FDCs were insensitive for the formulation changes. Based on this comprehensive evaluation, a decision tree was proposed which will act as a guideline for quality evaluation of FDC products and also provide a fundamental knowledge for optimization of formulations failing in dissolution studies.

Satish Balkrishna Bhise *et.al,* [22] (2007) formulated and evaluated novel fixed dose combination of anti-tuberculosis drugs. The solid mixtures of rifampicin and sodium

lauryl sulphate were prepared at molar ratio of 1:1 by co-grinding method. In vitro dissolution studies were carried out in 0.1N HCl and phosphate buffer pH 6.8. Solid mixtures prepared by co-grinding method were found to be useful in delaying the dissolution of rifampicin in acidic medium .The release of rifampicin from novel fixed dose combination formulation was 0% in 0.1N HCl up to two hours and faster release within 15 minutes in alkaline pH was achieved, while Isoniazid was released completely within 20 minutes in 0.1 N HCl. A novel formulation comprising of rifampicin and sodium lauryl sulphate, prepared by co-grinding method proved effective to minimize the degradation of rifampicin in acidic environment by its retarded release pattern. Thus this approach is beneficial for the segregation of release pattern of rifampicin in alkaline environment and isoniazid in the acidic environment of the GI tract, which will lead to prevent the degradation of rifampicin alone & its interaction with isoniazid.

Mukesh C. Gohel *et.al,*[33] (2007) developed a novel solid dosage Form of Rifampicin and Isoniazid With improved functionality to minimize degradation of rifampicin in acidic medium and to modulate the release of rifampicin in the stomach and isoniazid in the intestine. Gastro retentive tablets of rifampicin (150 mg) were prepared by the wet granulation method. Hard gelatin capsules (size 4) containing a compacted mass of isoniazid (150 mg) and dicalcium phosphate (75 mg) were enteric coated. Two tablets of rifampicin and 1 capsule (size 4) of isoniazid were put into a hard gelatin capsule. The in vitro drug release and in vitro drug degradation studies were performed. Rifampicin was released over 4 hours by zero-order kinetics from the novel dosage form. More than 90% of isoniazid was released in alkaline medium in 30 minutes. The results of dissolution studies revealed that a substantial amount of rifampicin was degraded from the immediate release capsule containing rifampicin and isoniazid powder owing to drug accumulation in the dissolution vessel and also to the presence of isoniazid. The degradation of rifampicin to 3-formyl rifampicin SV (3FRSV) was arrested (3.6%-4.8% degradation of rifampicin at 4 hours) because of the minimization of physical contact between the 2 drugs and controlled release of rifampicin in acidic medium. This study concludes that the problem of rifampicin degradation can be alleviated to a certain extent by this novel dosage formulation

Y. Ashokraj *et.al,* [34] (2006) conducted a study on Quality control of anti-tuberculosis FDC formulations in the global market. Accelerated stability studies were perform to determine the quality and performance of rifampicin containing fixed dose combination formulations with respect to physical, chemical and dissolution properties at (40^0C / 75% RH). All the formulations were found to be stable where extent of dissolution was within 10% of that of initial volume and all formulations passed the pharmacoepial limits for assay and content uniformity. The study revealed that good quality of rifampicin containing FDC that remain stable after 6 months accelerated stability testing are available in market place.

2.7 BACK GROUND OF REVIEW:

This study reveals the importance of carrying out the accelerated stability studies on marketed products of anti-tubercular FDC products containing rifampicin, isoniazid, pyrazinamide and ethambutol. From the previous study it was found that unpackaged FDC products undergo degradation more when compared to packaged FDC products and it also revealed that type of packaging also has influence on the stability and concluded that blister packs undergo more degradation when compared to strip packaging.[31] By considering all these factors the present study was focused to carry out the accelerated stability study on marketed products of anti-tubercular FDC containing rifampicin, isoniazid, ethambutol and pyrazinamide at different temperatures like 4^0C, 25^0C and at 40^0C with 75% RH for 60days and to determine the amount of rifampicin that gets degraded from marketed FDC products containing rifampicin, isoniazid, ethambutol and pyrazinamide.

AIM & OBJECTIVE

3. AIM AND OBJECTIVE

The aim of the present work is to carry out the accelerated stability study on four marketed products of anti-tubercular fixed dose combinations containing same dose of rifampicin, isoniazid, ethambutol and pyrazinamide at different temperatures like 4^0C, 25^0C and at 40^0C with 75% RH for 60days and to determine the amount of rifampicin that get degraded from four marketed fixed dose combination products.

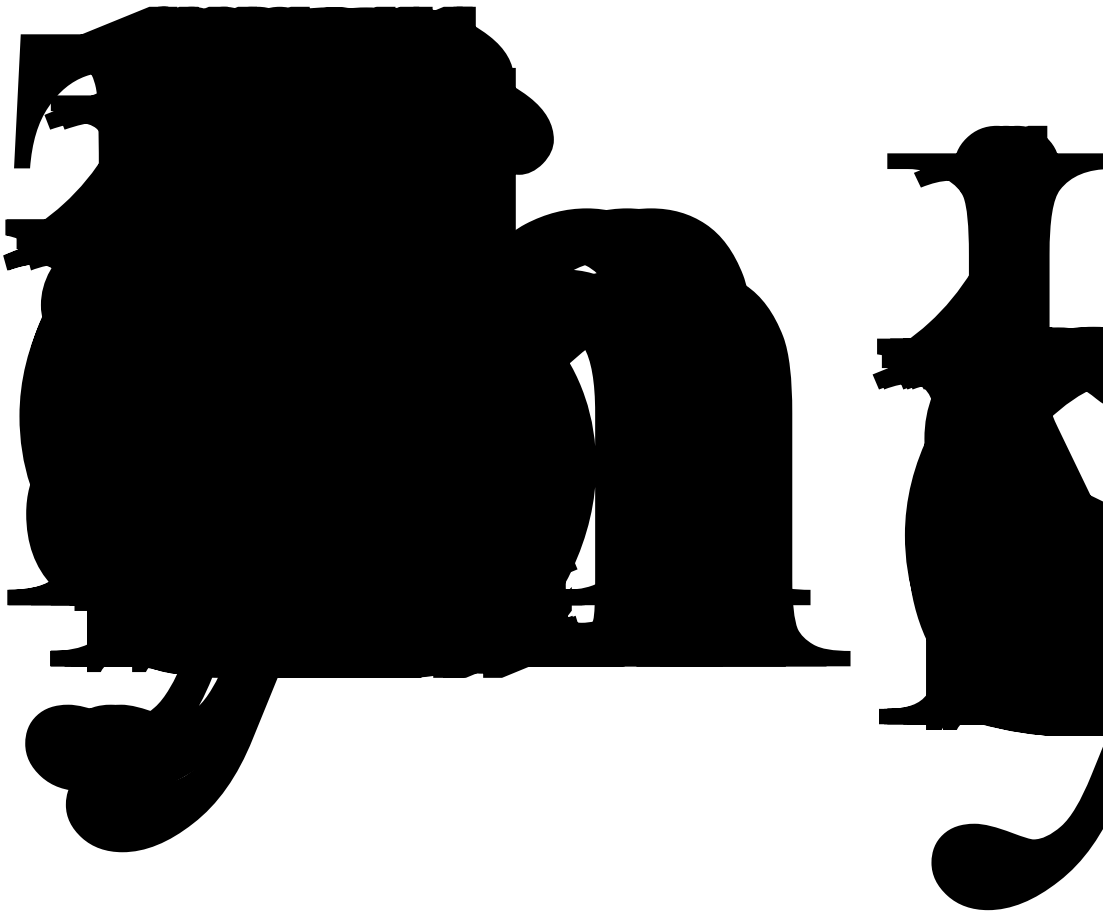
OBJECTIVE:

The objective of the present study is as follows

- To know the amount of rifampicin that gets degraded from marketed fixed dose combination product
- To select a better package for anti-tubercular fixed dose combination products that minimize degradation
- To choose a better formulation from the four different marketed fixed dose combination products.

PLAN OF WORK

4. PLAN OF WORK



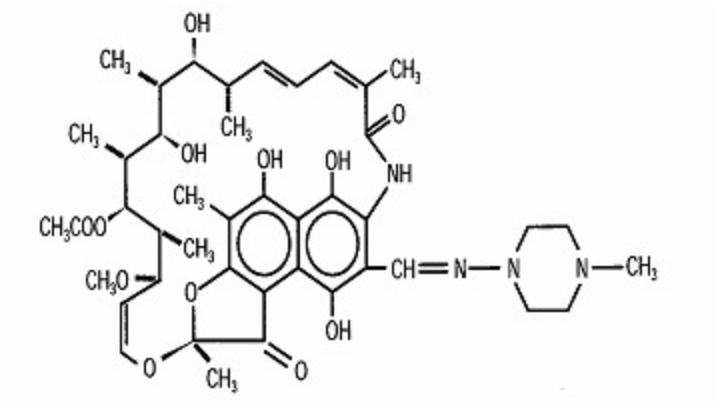
PROFILES

5. DRUG PROFILE

5.1 RIFAMPICIN:

Rifampicin is a semi synthetic antibiotic derivative of rifamycin group. Compound is derived from Amycolatopsis *rifamycinica.*

Structure :



Rifampicin

Empirical Formula : $C_{43}H_{58}N_4O_{12}$

Molecular weight : 822.94

Chemical name

3-[[(4-Methyl-1-piperazinyl)imino]methyl]rifamycin or 5,6,9,17,19,21-hexahydroxy - 23 - methoxy - 2,4,12,16,20,22 - heptamethyl - 8 - [N - (4 - methyl - 1 -piper-azinyl)formimidoyl] - 2,7 - (epoxypentadeca - [1,11,13]trienimino)naphtho[2,1 - b]furan - 1,11(2H) - dione 21-acetate

Physical and chemical properties

Appearance : Red brown powder

Melting point : 183 – 188^{0}C

Dose : 10mg/kg body weight in daily treatment

Solubility

Slightly soluble in water, soluble in ethylacetate and methanol and freely soluble in chloroform. [35]

Mechanism of action [36]

Rifampicin act by inhibiting DNA dependent RNA polymerase activity in susceptible cells. Rifampicin interacts with bacterial RNA polymerase but doesn't inhibit mammalian enzyme. At therapeutic levels, rifampin has bacterial activity against both intracellular and extracellular mycobacterium tuberculosis. Bacterial resistance to rifampin is caused by mutations leading to change in the structure of β subunit of RNA polymerase.

PHARMACOKINETICS

Absorption

It is well absorbed from the gastrointestinal tract. Peak plasma concentration is attained within 1.5 to 4 hours after oral administration. Absorption is reduced to 30% when the drug is ingested with food.

Distribution

Rifampicin is widely distributed in to all most all body tissues and fluids including cerebrospinal fluid barrier. About 90% of rifampicin binds to plasma proteins. [37] Rifampicin has high degree of placental transfer with a foetal to maternal serum level ratio of 0.3.

Volume of distribution	**:**	1.6 Liter / kg
Biological half life	**:**	3 to 5 hours

Metabolism

It is metabolized by liver microsomal enzymes its active metabolite is deacetylrifampicin. Formyl rifampicin is urinary metabolite that forms in urine.

Elimination

Rifampicin gets rapidly eliminated in bile and 30% of dose gets eliminated in urine in unchanged form and around 60% of oral dose is excreted in faeces.

Drug interactions [38]

1. Antacids containing aluminum hydroxide reduce the bioavailability of rifampicin

2. Isoniazid and rifampicin interaction has lead to hepatotoxicity
3. Presence of food decreases the absorption of rifampicin
4. Barbiturates and salicylates decrease the activity of rifampicin
5. Para- amino salicylic acid granules delay rifampicin absorption

Adverse effects

1. Acute haemolytic anemia, hypersensitivity

2. Diarrhoea , peripheral neuritis and vomiting

3. Severe gastrointestinal side effects, rash , chills and fever

4 .Edema, dermatitis

5. Opthalamic use of rifampicin causes irritation to eyes and ocular pain

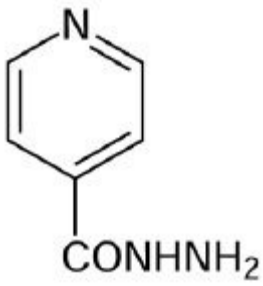
Use **:** Used in the treatment of tuberculosis

Storage **:** Stored in well closed container

5.2 ISONIAZID

Isoniazid was synthesized in 1912 at the German University of Prague by Meyer and Mally[39]

STRUCTURE



Isoniazid

Empirical formulae **:** C_6H_7N

Molecular weight **:** 137.14

Chemical name **:** Isonicotinic acid hydrazide

Physical and chemical properties

Appearance **:** White crystalline powder

Melting point **:** 170 - 174

Dose **:** 5mgh/kg body weight daily and 10 mg/kg body weight in thrice

Weakly treatment

Solubility **:** Freely soluble in water and sparingly soluble in alcohol [40]

Mechanism of action

Isoniazid kills actively growing tuberculii bacilli by inhibiting the biosynthesis of mycolic acid which is the major component of cell wall of mycobacterium tuberculosis. [41] At therapeutic levels isoniazid is bacterial against actively growing intracellular and extracellular mycobacterium tuberculosis.

PHARMACOKONETICS

Absorption

90% of drug gets absorbed upon oral administration and the presence of food reduces the absorption. Time to attain peak plasma concentration is about 1 to 2 hours

Distribution

Isoniazid is widely distributed to all fluids and tissues including cerebrospinal fluid, pleural and ascetic fluids, skin, sputum, muscles and lungs. It crosses the placenta and distributed in to breast and milk. Protein binding is very low about 10%.

Volume of distribution **:** 0.57 to 0.76 L/kg

Biological Half life **:** 1-5hours

Metabolism

Metabolism occurs by liver, isoniazid is acetylated by liver in to active metabolites which are excreted in urine. Acetyl isoniazid is further hydrolyzed to isonicotinic acid and acetyl hydrazine. Non acetylated isoniazid is excreted unchanged in urine.

Elimination

5 to 30% of drugs get excreted by renal excretion. Slow acetylaters excrete 25% to 66% of dose in urine as isoniazid and rapid acetylaters excrete 5 to 37% of dose in urine.

Drug interactions [42]

1. Concomitant use of acetaminophen and isoniazid cause nephrotoxicity

2. Alaprozolam administration with isoniazid cause elevated plasma concentrations of alaprozolam

3. Concomitant isoniazid therapy with BCG vaccine may inhibit efficacy of bcg vaccine

4. Antacids should not be administered with isoniazid

5. Administration of isoniazid with cycloserine cause increased CNS adverse effects

Adverse drug reactions

1. Peripheral neuropathy [(43)], seizures

2. Psychosis, optic neuropathy [(44)] and metabolic acidosis

3. Hypocalcemia, scaling and eczema

4. Memory loss, gynecomastia and vitamin B6 deficiency

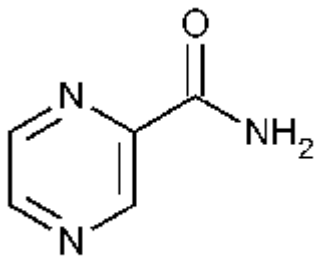
Use **:** Used in treatment of tuberculosis

Storage **:** Should protect from moist and light. Stored at 20^0C to 25^0C

5.3 Pyrazinamide

The synthesis of pyrazinoic acid, the active metabolite of pyrazinamide

STRUCTURE :



Pyrazinamide

Empirical formulae : $C_5H_5N_30$

Molecular weight : 123.11

Chemical name : Pyrazine -2- carboximide

Physical and chemical properties [45]

Appearance : White crystalline powder

Melting point : 190^0C

Dose : Orally 15 to 30 mg/kg once daily

Solubility : Sparingly soluble in water

Mechanism of action

Pyrazinamide is a synthetic purine analog of nicotinamide and exhibits in vitro bactericidal activity only at acidic PH. [46] Pyrazinamide is quite active against intracellular bacilli in the acidic environment of macrophages. Because of its action against intracellular bacilli, the organisms most likely to be responsible for relapse, it may play an important role in decreasing relapses. Within tuberculous lesions, it has been hypothesized that there may be 4 different populations of tubercle bacilli. Due to variables in their environments within the body, these 4 populations may differ in their metabolism and susceptibility to the ant tuberculosis drugs. One group of bacilli is felt to be metabolically active (rapidly and continuously growing). This group of organisms is believed to be killed readily by isoniazid, Rifampin, and streptomycin when used in bactericidal doses. The second group of bacilli is thought to have intermittent spurts of metabolic activity, during which time Rifampin is most capable of killing them. A third group of bacilli is thought to be found in acidic environments, such as within macrophages. Pyrazinamide appears to be especially effective against this particular group. Pyrazinamide should be used only in combination with other ant tubercular drugs in the treatment of M tuberculosis; resistance develops rapidly (within 6 to 8 weeks) when pyrazinamide is used alone.

PHARMACOKINETICS

Absorption

When given orally drug is completely absorbed from gastrointestinal tract, absorption is not influenced by food intake. After oral intake of 1500mg of pyrazinamide, a peak level is obtained; the time taken to reach peak serum concentration is decreased by antacids concentration.

Distribution

Pyrazinamide has excellent penetration in to cerebrospinal fluid ranging from 87 to 105% of corresponding serum concentration. Drug is distributed to all fluids, bile, kidney, liver and lungs. 31% of drug binds to plasma proteins.

Volume of distribution : 0.57 to 0.74L/kg

Biological Half life : 9 to 10 hours

Metabolism

Pyrazinamide is hydrolised in liver to its major active metabolite , pyrazonoic acid which further hydroxlated to main excretory product 5- hydroxypyrazinoic acid .Approximately 1% to 14% of the drug is excreted as unchanged pyrazinamide, with the remainder excreted as metabolites (Pyrazinoic acid, and 5-hydroxypyrazinoic acid).

Elimination

About 1 to 14% of drug excreted as unchanged pyrazinamide in urine, remaining excreted as metabolites.

DRUG INTERACTIONS

1. Allopurinol increases plasma concentration of pyrazoic acid which is directly responsible for renal urate secretion.
2. Pyrazinamide might antagonistically effect the action of medications that have uricosuric effect such as acetylsalicylic acid and probencid.
3. A potentially serious interaction exist with zidovudine in combination therapy.

Adverse drug reactions

1. Pellagra, thrombocytopenia and prophyria

2. Interference of metabolism of purine occurs

3. Arthralgia, hepatotoxicity[47]

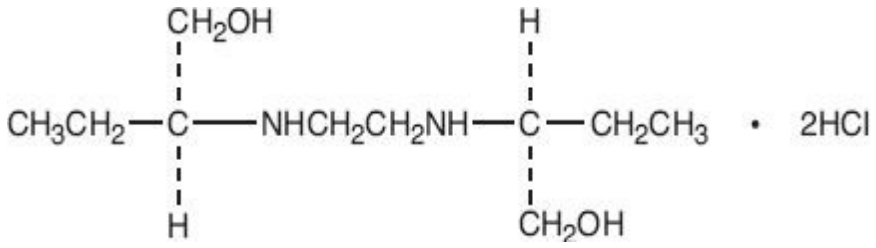
Use : Used in combination with anti- tubercular drug for the treatment of tuberculosis

Storage : Stored in well closed container at controlled room temperature at 15-30^0

5.4 ETHAMBUTOL

It is oral chemotherapeutic agent specifically active against actively growing micro organisms

Structure:



Ethambutol

Empirical formulae : $C_{10}H_{24}N_2O_2$

Molecular weight : 277.23l

Chemical Name : 2, 2 (ethylene di amino) di-1-butanol di hydro chloride.

Physical and chemical properties [48]

Appearance : White crystalline powder

Melting point : 199 - 204^{0}C

Solubility : Soluble in water and alcohol and slightly soluble in chloroform

Dose : 15mg/kg body weight

Mechanism of action

Ethambutol diffuses in to actively growing mycobacterium tuberculosis such as tubercle bacilli. Ethambutol appears to inhibit the synthesis of one or more metabolites [49], thus causing impairment of cell metabolism, arrest multiplication and cause cell death.

PHARMACOKINETICS

Absorption [50]

Absorption is rapid. Food doesn't show any effect of absorption, following a dosage of 25mg/kg body weight, a peak serum concentration of 4to 5mg/L is achieved with in 2-4 hrs after administration.

Distribution

Ethambutol is distributed to tissues and body fluids except cerebrospinal fluid. Ethambutol does not penetrate intact meninges, but 10 to 50% may penetrate the meninges of patients with TB meningitis. About 30% of drug binds to plasma proteins. Time taken to attain peak plasma concentration is about 2to 4 hours.

Volume of distribution : 1.6 lit/kg

Biological Half life : 3 to 4 hours

Elimination

Ethambutol gets eliminated by kidney 50 to 90% of drug is excreted as unchanged form in urine. And 20 to 22 % get excreted in feaces. 80% of ethambutol is eliminated by glomerular filtration and tubular secretion.

Drug interactions

1. Magnesium antacid reduces ethambutol resorption and lowers and delays respectively Cmax and Tmax.

2. Ethionamide and isoniazid in combination increases ethambutol occular toxicity

Adverse drug reactions

1. Aplastic anaemia[51], occular toxicity

2. Hallucination, loss of apetite

3. Dark urine, yellowing of skin

Use : Used in combination with anti-tubercular drug for the treatment of tuberculosis.

Storage : Stored at 15 - 30^{0}C in well closed container.

MATERIALS

6. MATERIALS AND INSTRUMENTS

6.1 MATERIALS

Four fixed dose formulations manufactured by licensed firms and different combinations of anti-tuberculosis drugs containing rifampicin, isoniazid, ethambutol and pyrazinamide were purchased from chemist shops which are packed in blister and strip packaging. The products are purchased in sufficient quantity to fulfill the study storage plan.

Storage of samples:

Three blister (F1, F2, F3) and one strip (F4) packaged FDC products of equal strength were procured and were investigated without cutting to avoid damage to packaging material or channel formation during cutting. One strip/blister of each type was kept in every storage condition and a minimum of three tablets were taken from the same package were analyzed. One set of each package was stored under ambient conditions at 25^0C, freezer at 4^0 C and stability chamber at 40^0C with 75% RH.

Table-2 **Formulation code for different type of packages**

Type of package	**Formulation code**	**Dose**
Blister	F1	Rifampicin-150mg,Isoniazid-75mg, Pyrazinamide-400mg Ethambutol- 275mg.
Blister	F2	Rifampicin-150mg,Isoniazid-75mg, Pyrazinamide-400mg Ethambutol- 275mg
Blister	F3	Rifampicin-150mg,Isoniazid-75mg, Pyrazinamide-400mg Ethambutol-275mg
Strip	F4	Rifampicin-150mg,Isoniazid-75mg, Pyrazinamide-400mg Ethambutol- 275mg

Equipment:

Table 3- **The following equipments were used in the study**

Instrument	**Manufacturer**
Hardness tester	Erweka GmbH Heusenstamm, Germany
Dissolution tester	Electro lab, Mumbai, India
UV-VIS spectrophotometer	Beckman 640i, Fullerton, CA, USA
Stability chamber	WTC Binder , Tuttlingen, Germany

METHODOLOGY

7. METHODOLOGY

7.1 Physical evaluation parameters: Marketed tablets containing fixed dose combinations of rifampicin, isoniazid, pyrazinamide and ethambutol were evaluated for physical parameters like hardness, weight variation and drug content.

Hardness test: For each formulation, the hardness of 3 tablets was determined by using Monsanto hardness tester and standard deviations were calculated.

Weight variation test: To study weight variation, of tablet of each formulation were weighed using an electronic balance and the test was performed according to the USP official limits of percentage deviation of tablet are presented in the table 4.

%Maximum positive deviation= (W_H-A/A) ×100

%Minimum negative deviation= (A-W_L/A) ×100

Where,

W_H= Highest weight in mg

W_L= Lowest weight in mg

A= Average weight of tablet in mg

Table 4: **USP official limits of weight variation test**

Average weight of tablet(mg)	Maximum percentage difference allowed
130 or less	10
130-324	7.5
More than 324	5

Drug content uniformity

Standard preparation

An accurately weighed amount of pure rifampicin (100 mg) taken and transferred into 100 ml volumetric flask. It was dissolved and made up to volume with pH 1.2 and absorbance was measured at 476 nm.

Sample preparation

Tablets were weighed individually then placed in a mortar and powdered with a pestle. An amount of powdered rifampicin (100 mg) was extracted in 0.1 N Hcl. The absorbance was measured at 476 nm after suitable dilution.

Calculation

The amount of rifampicin present in tablet can be calculated using the formula

$$\mathbf{A_t/A_sXS_w/100X100/S_tXA_v}$$

Where,

A_t= Absorbance of sample preparation

A_s= Absorbance of standard preparation

S_w= Weight of rifampicin working standard

S_t = Weight of rifampicin tablet (mg)

A_v = Average weight of tablet (mg)

7.2 Construction of standard curve for rifampicin:

Rifampicin is estimated spectrophotometrically at 475 nm.

Preparation of 0.1 N HCl [52]

Dissolve 8.5 ml of concentrated HCl in 1000 ml of distilled water.

Preparation of standard drug solution

Stock solution

100 mg of was dissolved in 100 ml of 0.1 N HCl, to get a solution of 1000 µg/ml concentration.

Standard solution

10 ml of stock solution was made to 100 ml with 0.1 N HCl thus giving a concentration of 100 µg/ml. Aliquot of standard drug solution ranging from 0.5 ml, 1 ml, 1.5 ml, 2 ml and 2.5 ml were transferred into 10 ml volumetric flask and were diluted up to the mark with 0.1 N HCl. Thus the final concentration ranges from 5-25 µg/ml. Absorbance of each solution was measured at 475 nm against 0.1 N HCl as a blank. A plot of concentrations of drug versus absorbance was plotted.

7.3In- vitro Dissolution study

Dissolution studies were performed for the fixed dose combination tablets by using dissolution medium of 0.1N (HCl), 900 ml in USP dissolution apparatus II at 50 rpm and $37^0 \pm 0.5^0$C Tablets were weighed individually and subjected to dissolution testing. 5ml sample was withdrawn at regular intervals (15, 30, 45 and 60 minutes) and diluted to 1ml with dissolution medium and drug content was determined by using Uv-visible spectrophotometer at 475nm.[53]An equal volume of fresh medium was replaced to maintain the dissolution medium and the percentage degradation was calculated by using the given formulae [5]

$$Percentage\ Degradation = \frac{Initial\ concentration - Final\ concentration}{Initial\ concentration} \times 100$$

RESULTS

8. RESULTS

8.1 Physical evaluation parameters: Marketed tablets containing fixed dose combinations of rifampicin, isoniazid, pyrazinamide and ethambutol were evaluated for physical parameters like hardness, weight variation and drug content.

Hardness:

Hardness was performed for the marketed fixed dose combination tablets and the results are given in the following table. All tablets passed hardness test and all the tablets was found to be within pharmacoepial limits.

At 4^0c:

The value of F1 ranges between 4.6 ± 0.39 to 3.8 ± 0.61(kg/cm^2) ($P < 0.001$), value of F2 ranges from 4.5 ± 0.43 to 3.9 ± 0.25 (kg/cm^2) ($P < 0.01$), value of F3 ranges from 4.5 ± 0.25 to 4.1 ± 0.32(kg/cm^2) ($P < 0.001$) and the value of F4 ranges from 4.6 ± 0.33 to 4.2 ± 0.26 (kg/cm^2) ($P < 0.001$).

Table.4- Hardness of tablets stored at 4^0C

Formulation code	Initial (kg/cm^2)	After 15 days(kg/cm^2)	After 30 days(kg/cm^2)	After 45 days(kg/cm^2)	After 60 days(kg/cm^2)
F1	4.6 ± 0.39	4.6 ± 0.54	4.4 ± 0.36	4.1 ± 0.56	3.8 ± 0.61
F2	4.5 ± 0.43	4.5 ± 0.39	4.3 ± 0.42	4.1 ± 0.37	3.9 ± 0.25
F3	4.5 ± 0.25	4.5 ± 0.63	4.4 ± 0.27	4.3 ± 0.54	4.1 ± 0.32
F4	4.6 ± 0.33	4.6 ± 0.31	4.5 ± 0.37	4.4 ± 0.81	4.2 ± 0.26

At 30^0C:

The value of F1 ranges between 4.6 ± 0.39 to 3.7 ± 0.29(kg/cm^2) ($P < 0.001$), value of F2 ranges from 4.5 ± 0.53 to 3.8 ± 0.36(kg/cm^2) ($P < 0.01$), value of F3 ranges from 4.5 ± 0.44 to 3.8 ± 0.31(kg/cm^2) ($P < 0.001$), the value of F4 ranges from 4.6 ± 0.36 to 3.9 ± 0.66 (kg/cm^2) ($P < 0.01$).

Table.5- Hardness of tablets stored at 30^0C

Formulation code	Initial (kg/cm^2)	After 15 days(kg/cm^2)	After 30 days(kg/cm^2)	After 45 days(kg/cm^2)	After 60 days(kg/cm^2)
F1	4.6 ± 0.62	4.4 ± 0.87	4.1 ± 0.58	3.9 ± 0.39	3.7 ± 0.29
F2	4.5 ± 0.53	4.3 ± 0.48	4.2 ± 0.29	4.1 ± 0.73	3.8 ± 0.36
F3	4.5 ± 0.44	4.4 ± 0.36	4.3 ± 0.77	4.0 ± 0.29	3.8 ± 0.31
F4	4.6 ± 0.36	4.5 ± 0.39	4.2 ± 0.23	4.2 ± 0.37	3.9 ± 0.66

AT 40^0C:

The value of F1 ranges between 4.6 ± 0.39 to 3.1 ± 0.22(kg/cm^2) ($P < 0.01$), value of F2 ranges from 4.5 ± 0.53 to 3.3 ± 0.39(kg/cm^2) ($P < 0.001$) value of F3 ranges from 4.5 ± 0.44 to 3.4 ± 0.43(kg/cm^2), ($P < 0.01$), the value of F4 ranges from 4.6 ± 0.36 to 3.7 ± 0.64(kg/cm^2) ($P < 0.01$).

Table.6- Hardness of tablets stored at 40^0C

Formulation code	Initial (kg/cm^2)	After 15 days(kg/cm^2)	After 30 days(kg/cm^2)	After 45 days(kg/cm^2)	After 60 days(kg/cm^2)
F1	4.6 ± 0.23	4.0 ± 0.33	3.7 ± 0.29	3.4 ± 0.65	3.1 ± 0.22
F2	4.5 ± 0.49	4.1 ± 0.57	3.8 ± 0.36	3.6 ± 0.40	3.3 ± 0.39
F3	4.5 ± 0.56	4.2 ± 0.42	3.9 ± 0.49	3.7 ± 0.58	3.4 ± 0.43
F4	4.6 ± 0.72	4.3 ± 0.73	4.1 ± 0.59	3.9 ± 0.39	3.7 ± 0.64

WEIGHT VARIATION:

Weight variation was performed for the marketed fixed dose combination tablets and the results are given in the following table. The weight variation of all tablets was found to be within the pharmacoepial limits of ± 5%.

AT 4^0C:

The value of F1 ranges between 1230 ± 36.05 to 1243 ± 20.81(mg) ($P < 0.001$), value of F2 ranges between 1078 ± 25.65 to 1201 ± 38.18(mg) ($P < 0.001$), value of F3 ranges

from 1146 ± 45.09 to 1253 ± 30.50(mg) (P < 0.001), the value of F4 ranges from 1230 ± 36.05 to 1186 ±15.27(mg) (P < 0.001).

Table.7-Weight variation of tablets stored at 4^0C

Formulation code	Initial(mg)	After 15 days(mg)	After 30 days(mg)	After 45 days(mg)	After 60 days(mg)
FI	1230 ± 36.05	1230 ± 36.05	1246 ± 15.27	1246 ± 25.16	1243 ± 20.81
F2	1078 ± 25.65	1130 ± 75.49	1090 ± 26.45	1163 ± 65.06	1201 ± 38.18
F3	1146 ± 45.09	1150 ± 30.00	1150 ± 30.00	1126 ± 94.51	1253 ± 30.50
F4	1230 ± 36.05	1140 ± 90.00	1143 ± 15.27	1193 ± 32.14	1186 ±15.27

At 30^0C:

The value of F1 ranges between 1230 ± 36.05 to 1275 ± 51.00(mg) (P < 0.01) value of F2 ranges between 1078 ± 25.65 to 1201 ± 38.18 (mg) (P < 0.01) value of F3 ranges from 1146 ± 45.09 to 1241 ± 41.00(mg) (P < 0.001) the value of F4 ranges from 1230 ± 36.05 to 1148 ± 18.93(mg) (P < 0.01).

Table.8-Weight variation of tablets stored at 30^0C

Formulation code	Initial(mg)	After 15 days(mg)	After 30 days(mg)	After 45 days(mg)	After 60 days(mg)
FI	1230 ± 36.05	1236 ±25.16	1240 ± 36.05	1266 ± 30.55	1275 ± 51.00
F2	1078 ± 25.65	1140 ±55.67	1180 ± 55.67	1146 ± 41.63	1213 ± 35.11
F3	1146 ± 45.09	1193 ±25.16	1240 ± 30.00	1160 ± 26.45	1241 ± 41.00
F4	1230 ± 36.05	1173 ±25.16	1180 ± 26.45	1156 ± 51.31	1148 ± 18.93

AT 40^0C:

The value of F1 ranges between 1230 ± 36.05 to 1180 ± 18.53 (mg) (P < 0.001) value of F2 ranges between 1078 ± 25.65 to 1230 ± 26.45(mg) (P < 0.001) value of F3 ranges

from 1146 ± 45.09 to 1330 ± 39.34(mg) (P < 0.001) the value of F4 ranges from 1230 ± 36.05 to 1220 ± 20.00(mg) (P < 0.001).

Table.9-Weight variation of tablets stored at 40^0C

Formulation code	Initial(mg)	After 15 days(mg)	After 30 days(mg)	After 45 days(mg)	After 60 days(mg)
FI	1230 ± 36.05	1245 ± 15.0	1196 ± 25.16	1181 ± 26.45	1180 ± 18.53
F2	1078 ± 25.65	1178 ± 20.20	1230 ± 20.00	1251 ± 17.51	1230 ± 26.45
F3	1146 ± 45.09	1243 ± 15.27	1093 ± 40.41	1146 ± 25.09	1330 ± 39.34
F4	1230 ± 36.05	1143 ± 25.16	1153 ± 45.09	1203 ± 25.16	1220 ± 20.00

DRUG CONTENT:

Drug content was performed for the marketed fixed dose combination tablets and the results are given in the following table. The drug content of all the tablets was found to be within the range of (80-110) %.

AT 4^0C:

The value of F1 ranges between 118 ± 0.375 to 96.3 ± 0.132(%) (P < 0.001) value of F2 ranges between 103 ± 0.195 to 97.5 ± 0.242(%) (P < 0.001) value of F3 ranges from 110 ± 0.069 to 96.9 ± 0.139(%) (P < 0.001) the value of F4 ranges from 107 ± 0.129 to 91.2 ± 0.299(%) (P < 0.001).

Table.10- Drug content of tablets stored at 4^0C

Formulation code	Initial (%)	After 15 days (%)	After 30 days (%)	After 45days (%)	After 60 days (%)
FI	118 ± 0.375	110.4 ± 0.286	109.7 ± 0.692	102.4±0.236	96.3 ± 0.132
F2	103 ± 0.195	100.7 ± 0.199	99.0 ± 0.329	98.1 ± 0.329	97.5 ± 0.242
F3	110 ± 0.069	103.2 ± 0.174	98.6 ± 0.192	96.9 ± 0.232	96.9 ± 0.139
F4	107 ± 0.129	101.9 ± 0.261	97.4 ± 0.229	93.8 ± 0.189	91.2 ± 0.299

At 30^0C

The value of F1 ranges between 118 ± 0.375 to 91.2 ± 0.329(%) ($P < 0.001$) value of F2 ranges between 103 ± 0.242 to 90.8 ± 0.234(%) ($P < 0.01$) value of F3 ranges from 110 ± 0.329 to 91.7 ± 0.256 (%) ($P < 0.001$) the value of F4 ranges from 107 ± 0.295 to 90.6 ± 0.136 (%) ($P < 0.01$).

Table.11- Drug content of tablets stored at 30^0C

Formulation code	Initial (%)	After 15 days (%)	After 30 days (%)	After 45 days (%)	After 60 days (%)
F1	118 ± 0.141	105 ± 0.163	99.3 ± 0.124	95.4 ± 0.149	91.2 ± 0.329
F2	103 ± 0.242	99.6 ± 0.121	96.7 ± 0.392	93.2 ± 0.126	90.8 ± 0.234
F3	110 ± 0.329	100.8 ± 0.321	94.8 ± 0.369	94.8 ± 0.023	91.7 ± 0.256
F4	107 ± 0.295	98.9 ± 0.331	92.7 ± 0.135	92.7 ± 0.235	90.6 ± 0.136

AT 40^0C

The value of F1 ranges between 118 ± 0.375 to 87.6 ± 0.026(%) ($P < 0.001$) value of F2 ranges between 103 ± 0.234 to 85.4 ± 0.321 (%) ($P < 0.001$) value of F3 ranges from 110 ± 0.322 to 88.3 ± 0.125(%) ($P < 0.01$) the value of F4 ranges from 107 ± 0.069 to 83.7 ± 0.312 ($P < 0.01$)**.**

Formulation code	Initial (%)	After 15 days (%)	After 30 days (%)	After 45 days (%)	After 60 days (%)
F1	118 ± 0.141	101 ± 0.163	97.8 ± 0.124	91.2 ± 0.062	87.6 ± 0.026
F2	103 ± 0.234	97 ± 0.223	94.1 ± 0.234	89.9 ± 0.139	85.4 ± 0.321
F3	110 ± 0.322	104.3± 0.121	98.8 ± 0.369	92.7 ± 0.392	88.3 ± 0.125
F4	107 ± 0.069	100.4 ±0.095	96.5 ± 0.251	80.3 ± 0.523	83.7 ± 0.312

Table.12- Drug content of tablets stored at 40^0 C

IN-VITRO DRUG RELEASE FOR MARKETED FIXED DOSE COMBINATION TABLETS

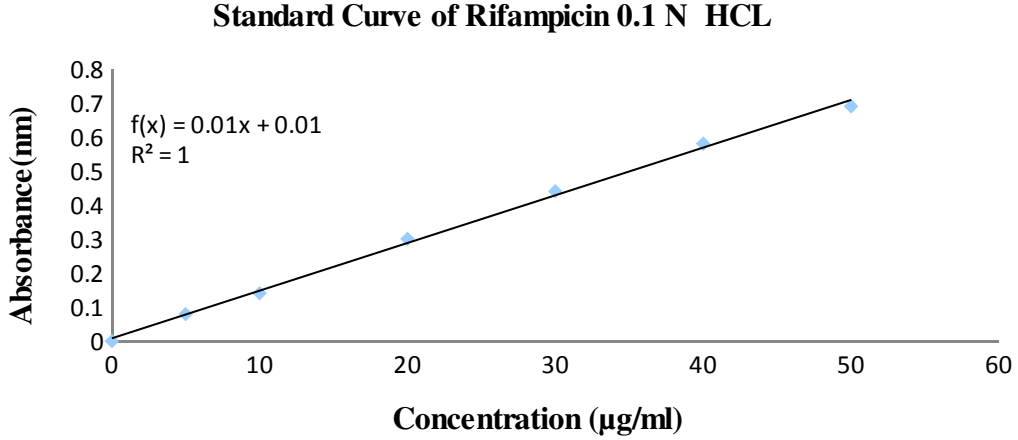
Determination of standard curve for rifampicin in pH 0.1N HCL medium

Standard curve of rifampicin was determined by using UV- spectrophotometer at 476nm.Graph was plotted by taking absorbance (nm) on X axis versus concentration (µg/ml) on Y-axis the results were shown in the table

Table.13 Standard curve of rifampicin in pH 0.1N HCL medium

Concentration (µg/ml)	Absorbance (nm)
5	0.079
10	0.14
20	0.3
30	0.44
40	0.58
50	0.69

Standard curve of rifampicin in 0.1N HCL



8.2 INVITRO DRUG RELEASE PROFILE BEFORE STORAGE

The stability of rifampicin was ascertained from the % release of drug at 60 minutes. As rifampicin was absorbed maximum within an hour from acidic Environment of stomach so the release is confined to 60 minutes.

Table.14 Invitro drug release profile for formulation F1 before storage

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.052	3.77	34.00	22.67 ± 0.012
30	0.08	5.72	51.49	34.33 ± 0.009
45	0.115	8.27	74.50	49.67 ± 0.816
60	0.148	10.62	95.64	63.76 ± 1.724
				**P<0.001

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.058	4.21	37.95	25.33 ± 0.062
30	0.084	6.05	54.48	36.32 ± 0.011
45	0.117	8.37	75.37	50.25 ± 0.016
60	0.149	10.71	96.40	64.27 ± 0.571
				**P<0.01

Table.16 Invitro drug release profile for formulation F2 before storage

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.064	4.58	41.29	27.53 ± 0.429
30	0.090	6.43	57.88	38.59 ± 0.436
45	0.119	8.57	77.19	51.46 ± 0.149
60	0.15	10.89	98.01	65.34 ± 0.277
				**P<0.01

Table.17 Invitro drug release profile for formulation F4 before storage

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.07	5.01	45.13	30.09 ± 0.530
30	0.096	6.92	62.31	41.84 ± 0.072
45	0.125	8.96	80.65	53.77 ± 0.294
60	0.16	11.46	103.2	68.8 ± 0.140
				**P<0.01

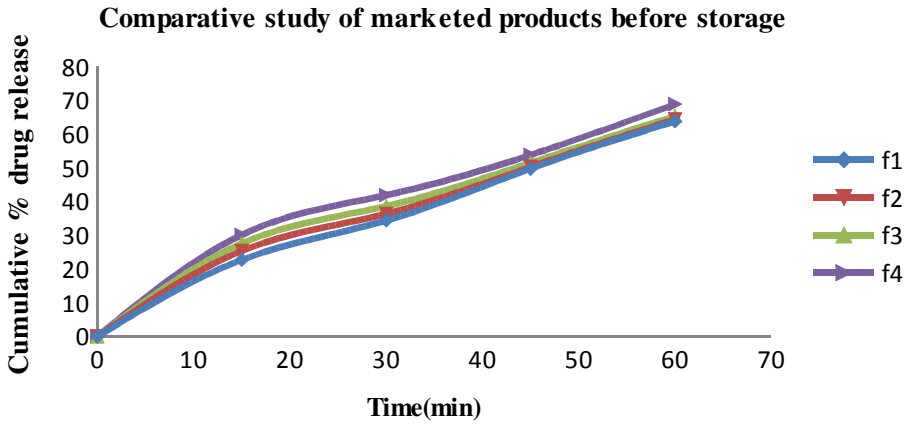


Fig: 2

8.3 IN VITRO DRUG RELEASE PROFILE AT 4^0C

Table.18 In- vitro drug release profile for formulation F1 after 15 days at 4^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.04	3.50	31.56	21.04 ± 0.394
30	0.075	5.42	48.79	32.53 ± 0.135
45	0.12	8.61	77.56	51.71 ± 0.179
60	0.145	10.41	93.76	62.51 ± 0.355
				**P<0.001

Table.19 In vitro drug release profile for formulation F2 after 15 days at 4^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.053	3.82	34.41	22.94 ± 0.140
30	0.079	5.65	50.92	33.95 ± 0.392
45	0.112	8.06	72.61	48.41 ± 0.364
60	0.147	10.5	94.56	63.04 ± 0.475
				**P<0.01

Table.20 In vitro drug release profile for formulation F3 after 15 days at 4^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.06	4.33	39.02	26.06 ± 0.129
30	0.08	6.2	55.86	37.24 ± 0.281
45	0.118	8.49	76.44	50.96 ± 0.156
60	0.149	10.7	96.34	64.23 ± 0.387
				**P<0.01

Table.21 In vitro drug release profile for formulation F4 after 15 days at 4^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.065	4.66	42.01	28.01 ± 0.125
30	0.094	6.76	60.84	40.56 ± 0.128
45	0.119	8.53	76.84	51.23 ± 0.162
60	0.131	11.38	102.42	68.28 ± 0.152
				**P<0.01

The percentage drug release of rifampicin in 0.1N HCL before storage was found to be 68 % and after storage for 15 days at 4^0C there was no change in the percentage of drug release this indicates that there was no degradation for after 15 days.

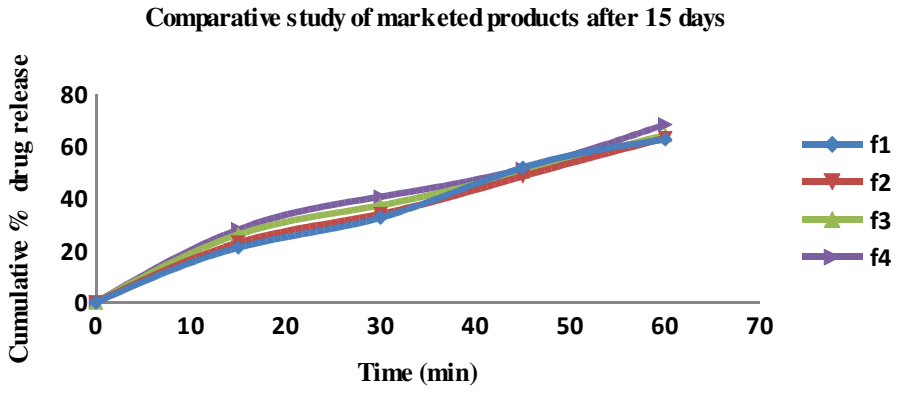


Fig.3

INVITRO DRUG RELEASE PROFILE AFTER 30 DAYS AT 4^0C

Table.22 In vitro drug release profile for formulation F1 after 30 days at 4^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.046	3.34	30.1	20.07 ± 0.212
30	0.075	5.18	46.02	31.08 ± 0.240
45	0.117	8.42	75.85	50.57 ± 0.183
60	0.144	10.34	93.06	62.04 ± 0.201
				**P<0.01

Table.23 In vitro drug release profile for formulation F2 after 30 days at 4^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.049	3.5	31.54	21.03 ± 0.181
30	0.074	5.34	48.09	32.06 ± 0.103
45	0.119	8.5	76.53	51.02 ± 0.201
60	0.145	10.42	93.78	62.52 ± 0.278
				**P<0.01

Table.24 In vitro drug release profile for formulation F3 after 30 days at 4^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.017	4.08	36.76	24.51 ± 0.013
30	0.081	5.84	52.56	35.04 ± 0.008
45	0.118	8.49	76.42	50.95 ± 0.140
60	0.148	10.58	95.22	63.48 ± 0.026
				**P<0.01

Table.25 In vitro drug release profile for formulation F4 after 30 days at 4^0C

Time(min)	Absorbance (nm)	Concentration(µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.065	4.65	41.89	27.93 ± 0.411
30	0.091	6.53	58.83	39.22 ± 0.132
45	0.116	8.34	75.12	50.08 ± 0.067
60	0.157	11.23	101.07	67.38 ± 0.430
				**P<0.001

The percentage drug release of rifampicin after storage for 15 days at 4^0C was found to be 68% and after 30 days the drug release was decreased to 63% due to degradation.

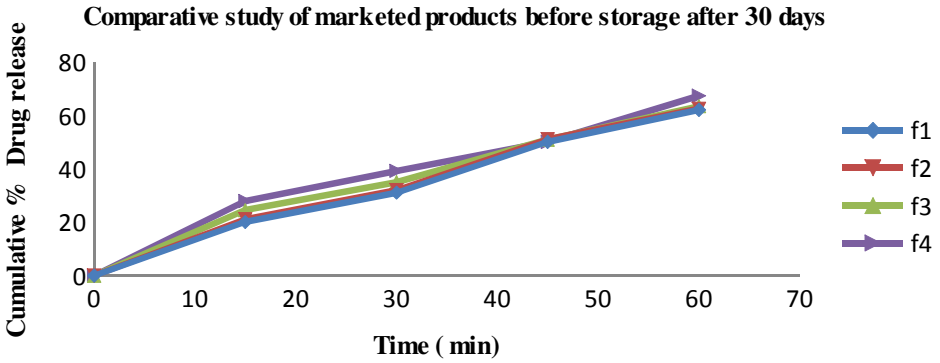


Fig. 4

INVITRO DRUG RELEASE PROFILE AFTER 45 DAYS AT 40C

Table.26 In vitro drug release profile for formulation F1 after 45 days at 4^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.051	3.7	33.37	22.25 ± 0.291
30	0.074	5.31	47.79	31.86 ± 0.121
45	0.12	8.59	77.38	51.59 ± 0.045
60	0.143	10.28	92.52	61.68 ± 0.406
				**P<0.01

Table.27 In vitro drug release profile for formulation F1 after 45 days at 4^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.054	3.86	34.77	23.18 ± 0.196
30	0.076	5.45	49.11	32.74 ± 0.134
45	0.121	8.71	78.45	52.30 ± 0.121
60	0.144	10.31	92.79	61.86 ± 0.172
				**P<0.01

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Table.28 In vitro drug release profile for formulation F3 after 45 days at 4^0C

Time(min)	Absorbance (nm)	Concentration(µg/ml)	Amount Of drug release(mg)	Cumulative %Drug Release
15	0.056	4.03	37.27	24.18 ± 0.111
30	0.077	5.52	49.71	33.14 ± 0.286
45	0.124	8.87	79.90	53.27 ± 0.244
60	0.146	10.49	94.49	62.94 ±0.143

				*** P< 0.001

Table.29 In vitro drug release profile for formulation F3 after 45 days at 4^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.062	4.48	43.38	26.92 ± 0.081
30	0.09	6.44	58.02	38.68 ± 0.092
45	0.114	8.21	73.89	49.32 ± 0.084
60	0.165	11.18	100.62	67.08 ± 0.054
				**P<0.01

The percentage drug release of rifampicin after storage for 45 days at 4^0C was reduced further due to the formation of degradation.

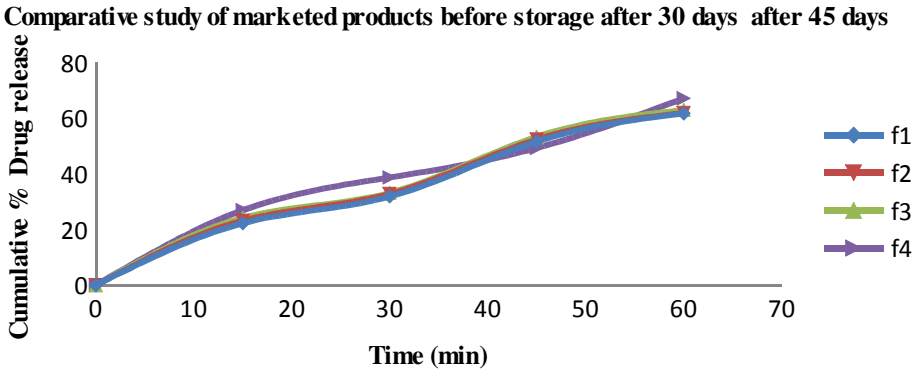

Fig-5

INVITRO DRUG RELEASE PROFILE AFTER 60 DAYS AT 4^0C

Table.30 In vitro drug release profile for formulation F1 after 60 days at 4^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.045	3.26	29.37	19.58 ± 0.115
30	0.082	5.87	52.90	30.27 ±0.0.121
45	0.115	8.25	74.31	49.54 ±0.0.216
60	0.142	10.17	91.53	61.02 ± 0.0.134
				**P<0.01

Table.31 In vitro drug release profile for formulation F2 after 60 days at 4^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.049	3.55	31.95	21.34 ±0.126
30	0.071	5.12	46.15	30.77 ± 0.315
45	0.118	8.43	75.91	50.64 ± 0.268
60	0.143	10.22	91.98	61.32 ± 0.427

				**P<0.001

Table.32 In vitro drug release profile for formulation F3 after 60 days at 4^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.055	3.93	35.37	23.58 ± 0.109
30	0.088	6.30	56.74	37.83 ± 0.542
45	0.12	8.62	77.44	51.63 ± 0.136
60	0.145	10.36	93.24	62.16 ± 0.251
				**P<0.01

Table.33 In vitro drug release profile for formulation F4 after 60 days at 4^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.063	4.56	41.1	27.40 ± 0.109
30	0.091	6.52	58.74	39.16 ± 0.458
45	0.117	8.39	75.51	50.34 ± 0.136
60	0.155	11.09	99.89	66.54 ± 0.251
				**P<0.001

When compared to F1, F2, F3 andF4 formulations stored at 4^0c the percentage drug release of rifampicin was found to be reduced more after 60 days and in F4 formulation less amount of rifampicin get released due to less degradation because F4 formulation is strip pack formulation.

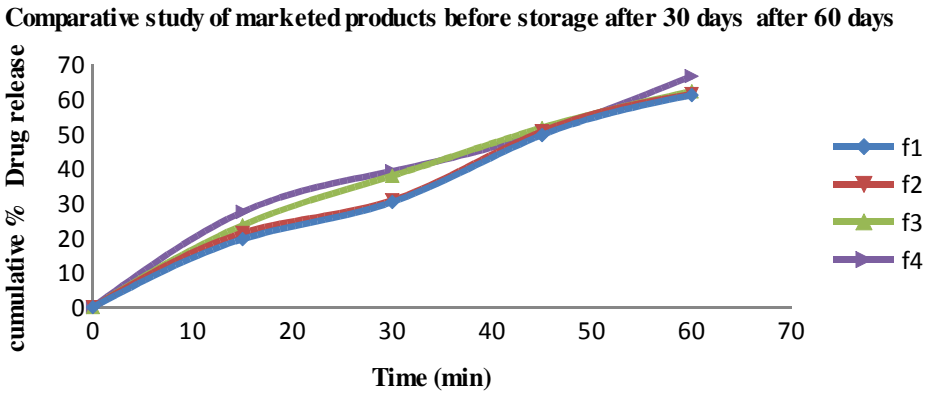

Fig-6

8.4 IN VITRO DRUG RELEASE PROFILE 30° C

Table.34 In vitro drug release profile for formulation F1 after 15 days at 30^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.046	3.31	29.80	19.87 ± 0.213
30	0.072	5.15	46.41	30.94 ± 0.174
45	0.115	8.28	74.56	49.71 ± 0.571
60	0.144	10.32	92.88	61.92 ± 0.482

				**P<0.01

Table.35 In vitro drug release profile for formulation F2 after 15 days at 30^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.049	3.5	31.5	21.04 ± 1.07
30	0.073	5.28	47.99	31.73 ± 0.954
45	0.117	8.28	75.48	50.32 ± 0.472
60	0.145	10.42	93.78	62.52 ± 0.218
				**P<0.01

Table.36 In vitro drug release profile for formulation F3 after 15 days at 30^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.055	3.97	35.77	23.85 ± 0.189
30	0.081	5.79	52.11	34.74 ± 0.361

45	0.115	8.28	74.5	49.68 ± 0.437
60	0.147	10.52	94.68	63.12 ± 0.207
				**P<0.01

Table.37 In vitro drug release profile for formulation F4 after 15 days at 30^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.064	4.59	41.31	27.54 ± 0.115
30	0.092	6.58	59.29	39.53 ± 0.089
45	0.116	8.34	98.55	50.05 ± 0.673
60	0.157	11.23	101.07	67.38 ± 0.954
				**P<0.01

The percentage drug release of rifampicin after storage for 15 days at 4^0C is found to be 68% and the percentage drug release after storage for 15 days at 30^0C is found to be 67%.

Fig-7

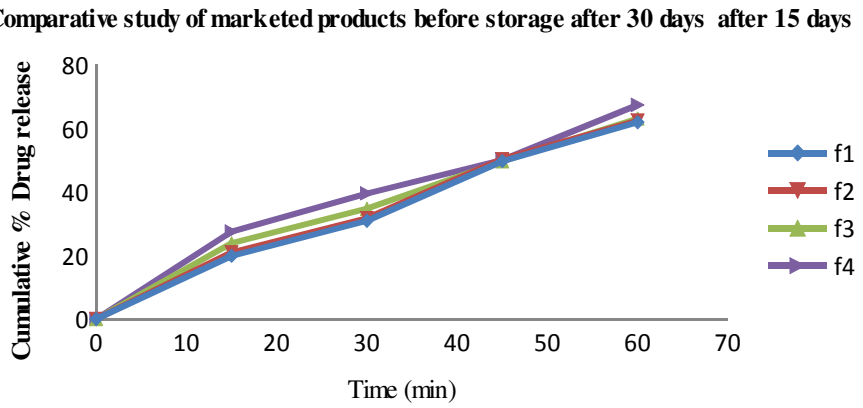

Table.38 In vitro drug release profile for formulation F1 after 45 days at 30^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.039	2.82	25.41	16.94 ± 0.231
30	0.066	4.77	42.97	28.65 ± 0.135
45	0.111	7.99	71.95	47.97 ± 0.189
60	0.14	10.06	90.54	60.36 ± 0.159
				**P<0.01

Table.39 In vitro drug release profile for formulation F2 after 45 days at 30^0C

Time(min)	Absorbance (nm)	Concentration(µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.04	3.08	27.78	18.52 ± 0.243
30	0.067	4.96	44.68	29.79 ± 0.296
45	0.113	8.07	72.70	48.47 ± 1.59
60	0.142	10.13	91.17	60.78 ± 0.087
				**P<0.001

Table.40 In vitro drug release profile for formulation F3 after 45 days at 30^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.045	3.22	21.04	19.36 ± 0.026
30	0.071	5.12	46.11	30.74 ± 0.061
45	0.119	8.20	73.84	49.23 ± 0.765
60	0.146	10.31	92.79	61.86 ± 1.609
				**P<0.01

Table.41 **In vitro drug release profile for formulation F4 after 45 days at 30^0C**

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.065	4.611	41.5	27.67 ± 2.18
30	0.088	6.35	57.18	38.12 ±1.609
45	0.118	8.46	76.2	50.8 ± 0.066
60	0.154	11	99	66.1 ± 1.327
				**P<0.001

The percentage drug release of rifampicin after 30 days was found to be 66%

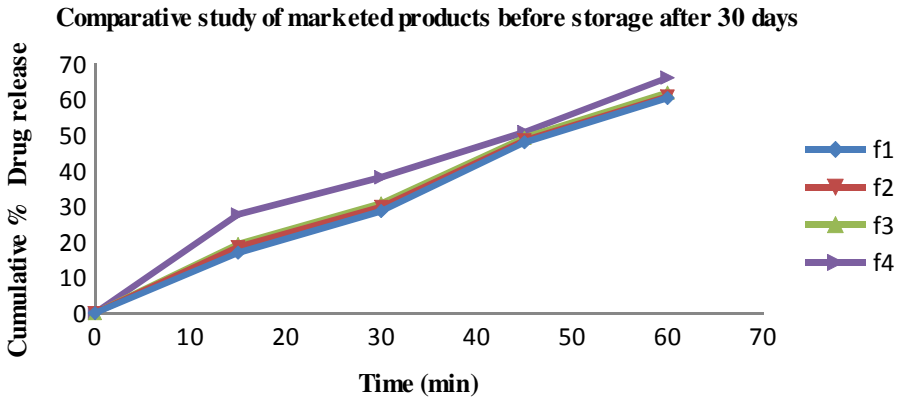

Fig-8

Table.42 In vitro drug release profile for formulation F1 after 45 days at 30^{0}C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release

15	0.051	3.7	33.37	22.25 ± 0.273
30	0.074	5.31	47.79	31.86 ± 0.791
45	0.12	8.59	77.38	51.59 ± 0.198
60	0.143	10.28	92.52	61.68 ± 0.111
				**P<0.01

Table.43 In vitro drug release profile for formulation F2 after 15 days at 30^0C

Time(min)	Absorbance (nm)	Concentration(µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.054	3.86	34.77	23.18 ± 0.176
30	0.076	5.45	49.11	32.74 ± 0.329
45	0.121	8.71	78.45	52.30 ± 0.980
60	0.144	10.31	92.79	61.86 ± 0.876
				**P<0.001

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Table.44 In vitro drug release profile for formulation F3 after 45 days at 30^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.056	4.03	37.27	24.18 ± 0.299
30	0.077	5.52	49.71	33.14 ± 0.351
45	0.124	8.87	79.90	53.27 ± 1.125
60	0.146	10.49	94.49	62.94 ± 1.596
				**P<0.01

Table.45 In vitro drug release profile for formulation F4 after 45 days at 30^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.062	4.48	43.38	26.92 ±0.242
30	0.09	6.44	58.02	38.68 ± 1.29
45	0.114	8.21	73.89	49.26 ± 1.53
60	0.165	11.18	100.62	67.08 ± 2.98
				**P<0.01

When compared to percentage drug release for 30 days and 45 days there was no change in the percentage of drug release.

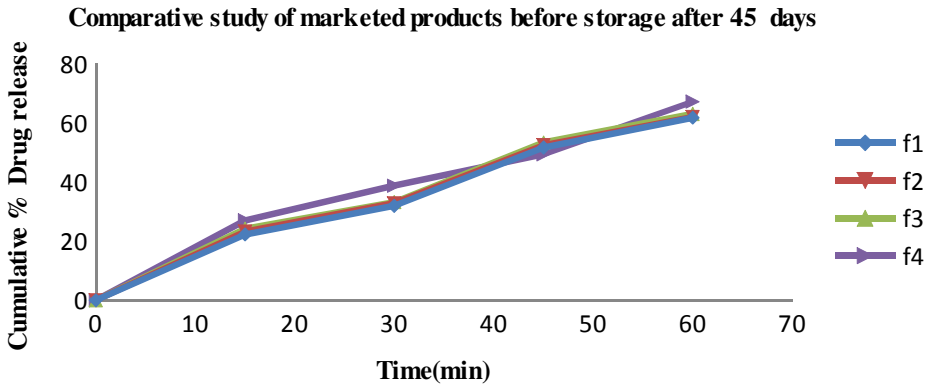

Fig-9

Table.46 In vitro drug release profile for formulation F1 after 60 days at 30^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.046	3.32	29.91	19.94 ± 0.242
30	0.066	4.72	42.48	28.32 ± 2.98
45	0.11	7.86	70.77	47.18 ± 0.257
60	0.139	9.91	89.19	59.46 ± 1.29
				**P<0.001

Table.47 In vitro drug release profile for formulation F2 after 60 days at 30^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.044	3.15	28.38	18.92 ± 0.181
30	0.064	4.62	41.59	27.73 ± 0.255
45	0.108	7.78	70.06	46.79 ± 0.462
60	0.138	9.86	88.74	59.16 ± 0.921
				**P<0.01

Table.48 In vitro drug release profile for formulation F3 after 60 days at 30^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.047	3.40	30.61	20.41 ± 0.293
30	0.068	4.89	44.05	29.37 ± 0.114
45	0.113	8.10	72.93	48.62 ± 0.525
60	0.140	10.01	90.09	60.06 ± 0.139
				**P<0.01

Table.49 In vitro drug release profile for formulation F4 after 60 days at 30^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.057	4.08	36.73	24.49 ± 0.035
30	0.078	5.62	50.62	33.76 ± 0.352
45	0.121	8.71	78.45	52.30 ± 0.305
60	0.146	10.46	94.14	62.76 ± 0.237
				**P<0.001

The percentage of drug release of rifampicin after 60 days was found to be 62% due to more amount drug get degraded at 30^0C.When compared to 4^0C and 30^0C the degradation was found to be more in 30^0C.

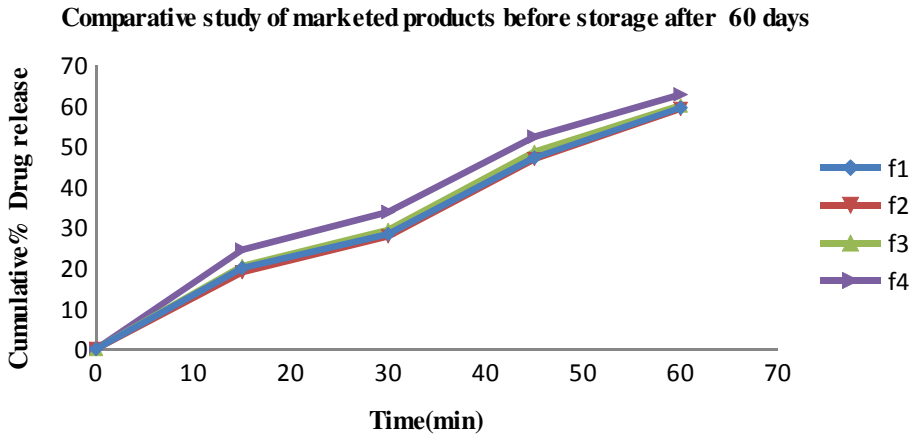

Fig-10

8.5 IN VITRO DRUG RELEASE PROFILE AT 40^0C

Table.50 In vitro drug release profile for formulation F1 after 15 days at 40^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.042	3.04	27.43	18.29 ± 0.092
30	0.070	5.04	45.36	30.24 ± 0.304
45	0.114	8.19	73.77	49.18 ± 0.275
60	0.141	10.13	91.17	60.78 ± 0.076
				**P<0.001

Table.51 In vitro drug release profile for formulation F2 after 15 days at 40^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.040	2.91	26.25	17.5 ± 0.038
30	0.064	4.93	44.41	29.61 ± 0.045
45	0.112	8	72.04	48.03 ± 0.083
60	0.141	10.11	90.99	60.66 ± 0.070
				**P<0.01

Table.52 In vitro drug release profile for formulation F3 after 15 days at 40^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.034	2.46	31.15	20.77 ± 0.048
30	0.073	5.28	47.53	31.69 ± 0.039
45	0.116	8.34	75.09	50.06 ± 0.051
60	0.144	10.34	93.06	62.04 ± 0.042
				**P<0.01

Table.53 In vitro drug release profile for formulation F4 after 15 days at 40^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.066	4.78	43.02	28.68 ± 0.048
30	0.092	6.62	59.59	39.73 ± 0.348
45	0.121	8.71	78.39	52.26 ± 0.266
60	0.154	11	99	66.04 ± 0.362
				**P<0.01

The percentage drug release of rifampicin after 15 days at 40^{0}C is found to be 66%.

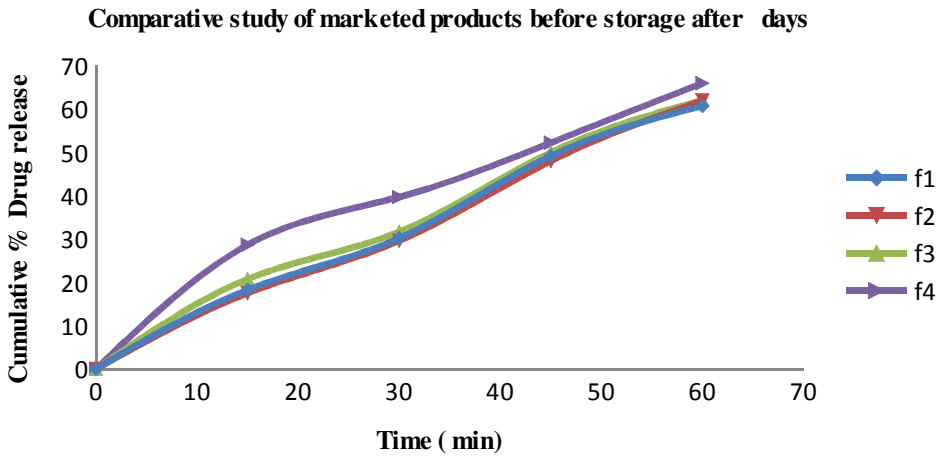

Fig-11

Table.54 In vitro drug release profile for formulation F1 after 30 days at 40^{0}C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.040	2.88	25.96	17.39 ± 0.908
30	0.067	4.79	43.17	28.78 ± 0.168
45	0.111	7.96	71.76	47.84 ± 0.754
60	0.139	9.94	89.46	59.64 ± 0.958
				**P<0.001

Table.55 In vitro drug release profile for formulation F2 after 30 days at 40^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.043	3.09	27.82	18.55 ± 0.121
30	0.069	4.94	44.46	19.64 ± 1.154
45	0.112	8.03	72.34	48.23 ± 0.329
60	0.14	9.98	89.82	59.88 ± 0.258
				**P<0.01

Table.56 In vitro drug release profile for formulation F3 after 30 days at 40^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.04	3.2	28.83	19.22 ± 0.707
30	0.071	5.13	46.18	30.79 ± 0.383
45	0.115	8.28	74.56	49.71 ± 1.603
60	0.141	10.09	90.81	60.54 ± 0.923
				**P<0.001

Table.57 In vitro drug release profile for formulation F3 after 30 days at 40^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.064	4.58	41.25	27.5 ± 0.837
30	0.088	6.35	57.22	38.15 ± 1.39
45	0.12	8.61	77.5	51.67 ± 0.083
60	0.153	10.94	98.46	65.64 ± 0.057
				**P<0.001

The percentage drug release of rifampicin after 30 days was found to be 65%

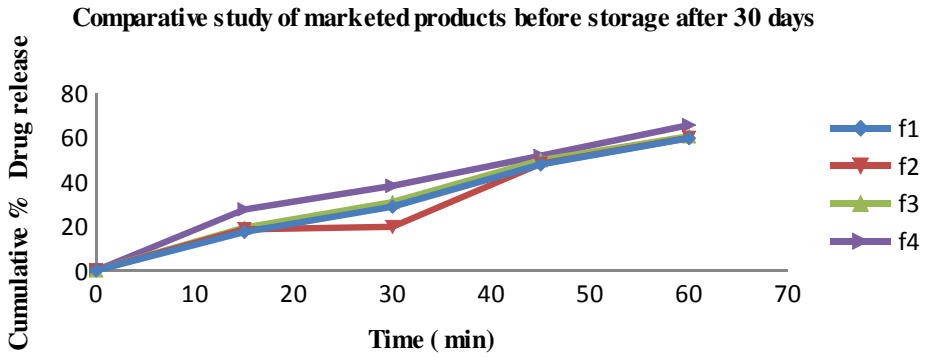

Fig-12

Table.58 In vitro drug release profile for formulation F1 after 45 days at 40^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.044	3.21	28.89	19.26 ±0.547
30	0.067	4.81	43.30	88.87 ±0.321
45	0.11	7.911	71.20	47.42 ±0.145
60	0.138	9.89	89.01	59.34 ± 0.732
				**P<0.01

Table.59 In vitro drug release profile for formulation F2 after 45 days at 40^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.042	3.06	77.54	18.36 ± 0.257
30	0.063	4.57	41.14	27.43 ± 0.236
45	0.107	7.71	69.45	46.30 ± 0.017
60	0.137	9.85	88.65	59.11 ± 0.997
				**P<0.01

Table.60 In vitro drug release profile for formulation F3 after 45 days at 40^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.045	3.25	29.23	19.52 ± 0.021
30	0.071	5.14	46.35	30.89 ± 0.063
45	0.074	5.30	74.76	49.82 ± 0.034
60	0.14	10.02	90.18	60.12 ± 0.134
				**P<0.01

Table.61 In vitro drug release profile for formulation F4 after 45 days at 40^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.059	4.28	38.55	25.70 ± 0.048
30	0.084	6.03	54.27	36.18 ± 0.011
45	0.113	8.11	72.99	48.66 ± 0.031
60	0.149	10.69	96.21	64.14±0.048
				**P<0.002

The percentage degradation of rifampicin was found to be 64% and drug release is reduced more due to degradation of rifampicin is more after 45 days.

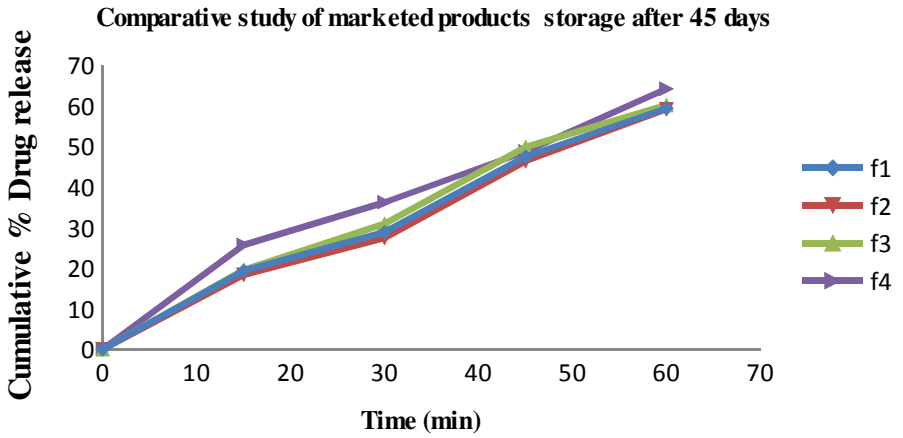

Fig-13

The percentage degradation of rifampicin was found to be 64% and drug release is reduced more due to degradation of rifampicin is more after 45 days.

Table.62 In vitro drug release profile for formulation F1 after 60 days at 40^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.022	1.63	14.73	9.82 ± 0.237
30	0.046	3.32	29.91	19.94 ± 0.215
45	0.064	4.60	41.43	27.62 ± 0.228
60	0.085	6.13	55.2	36.89 ± 0.179
				**P<0.01

Table.63 In vitro drug release profile for formulation F2 after 60 days at 40^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.024	1.77	16.0	10.67 ± 0.156
30	0.048	3.45	31.09	20.73 ± 0.024
45	0.068	4.91	44.20	29.47 ± 0.023
60	0.094	6.73	60.06	40.46 ± 0.051
				**P<0.01

Table.64 In vitro drug release profile for formulation F3 after 60 days at 40^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.042	3.0	27.06	18.04 ± 0.019
30	0.057	4.10	36.94	24.73 ± 0.026
45	0.076	5.46	49.21	32.81 ± 0.061
60	0.131	9.4	84.6	56.42 ± 0.110
				**P<0.01

Table.65 In vitro drug release profile for formulation F4 after 60 days at 40^0C

Time(min)	Absorbance (nm)	Concentration (µg/ml)	Amount Of Drug Release(mg)	Cumulative %Drug Release
15	0.028	2.07	18.69	12.46 ± 0.271
30	0.057	4.09	36.88	24.59 ± 0.233
45	0.074	5.29	47.69	31.75 ± 0.030
60	0.10	7.43	66.9	44.69 ± 0.165
				**P<0.01

When compared to 15,30,45 and 60 days drug release at 60 days was found to be less (44%) due to more amount of rifampicin get degraded due to gain in moisture by ethambutol .

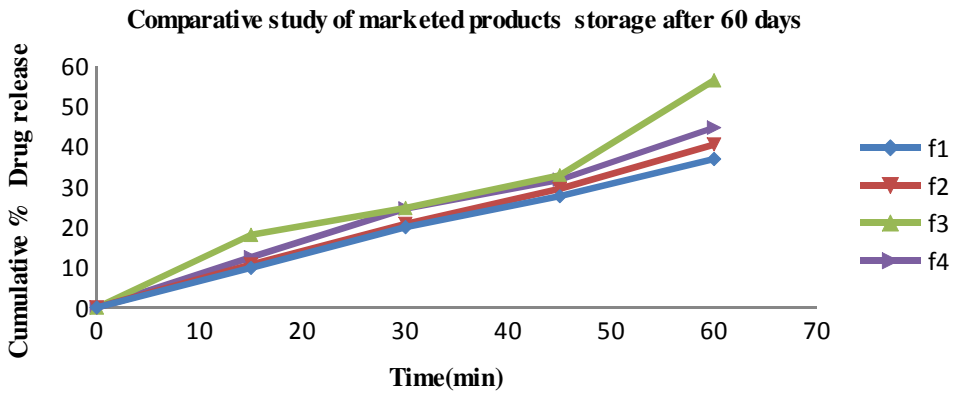

Fig-14

Table. 66 Comparative degradation of F1, F2, F3 and F4 formulations after 15, 30, 45 and 60 days at 4^0C

Formulation code	Degradation after 15 days (%)	Degradation after 30 days (%)	Degradation after 45 days (%)	Degradation after 60 days (%)
F1	1.9 ± 0.165	2.6 ± 0.138	3.20 ± 0.283	4.23 ± 0.237
F2	1.96 ± 0.239	2.70 ± 0.319	3.73 ± 0.127	4.57 ± 0.521
F3	1.0 ± 0.168	2.12 ± 0.148	2.96 ± 0.693	4.16 ± 0.025
F4	1.69 ± 0.265	2.0 ± 0.293	2.44 ± 0.181	3.22 ± 0.179
				**P<0.01

Fig-15

Fig-15

Table.67 Comparative degradation of F1, F2, F3 and F4 formulations after 15, 30, 45 and 60 days at 30^0

Formulation code	Degradation after 15 days (%)	Degradation after 30 days (%)	Degradation after 45 days (%)	Degradation after 60 days (%)
F1	2.59 ± 0.261	5.27 ± 0.273	5.55 ± 0.069	6.68 ± 0.133
F2	2.70 ± 0.163	5.41 ± 0.076	6.53 ± 0.257	7.93 ± 0.192
F3	2.6 ± 0.242	4.62 ± 0.275	6.38 ± 0.174	7.40 ± 0.181
F4	2.27 ± 0.816	4.01 ± 0.525	4.97 ± 0.195	6.10 ± 0.056
				**P<0.01

Fig-16

Table.68 Comparative degradation of F1, F2, F3 and F4 formulations after 15, 30, 45 and 60 days at 40^0C

Formulation code	Degradation after 15 days (%)	Degradation after 30 days (%)	Degradation after 45 days (%)	Degradation after 60 days (%)
F1	11.6 ± 0.024	20 ± 0.154	39 ± 0.184	37.2 ± 0.179
F2	10.8 ± 0.312	16 ± 0.142	32 ± 0.199	38 ± 0.352
F3	8.8 ± 0.242	15 ± 0.359	33.85 ± 0.274	39 ± 0.251
F4	7.4 ± 0.139	13.3 ± 0.241	29 ± 0.349	34.2 ± 0.187
				**P<0.01

DISCUSSION

RESULTS AND DISCUSSION

By comparing all four F1, F2, F3 and F4 formulations stored at different temperatures like 4^0C, 30^0C and 40^0C with 75%RH. Formulations when stored at 4^0C were found to having the hardness with in prescribed limits. Among the four formulations, formulation F4 is found to be within the range of 4.2 to 4.6 and hence F4 formulation may be considered as the best formulation.

The percentage weight variation of all formulation was found to be within the pharmacoepial limits. When compared to different temperatures for formulations stored at 4^0C there was very less fall in drug content i.e. 91.2% which shows that the degradation of drug is very low at 4^0C when compared to other temperatures

In- vitro stability studies:

Among all the four packaged formulations degradation of rifampicin was found to be more in blister packages when compared to strip packages because blister packaged tablets showed discoloration and bleeding inside package due to allowing the moisture to ingress and resulted in higher degradation which was further supported by the previous study.[11] The another reason for degradation of rifampicin within formulation is due to the formation hydrazine (HYD) which is the main degradation product whenever rifampicin and isoniazid are combined together in FDC formulation and the another reason for rapid degradation is due to hygroscopicty of ethambutol that is present in the four FDC.

By observing the above reasons among all FDC formulations which is packed in strip package is found to be the best formulation since less amount of rifampicin was degraded when compared to other formulation and there was no influence of light on tablets packed in strips. Hence the strip products undergo less degradation when compared to blister packages.

SUMMARY & CONCLUSION

- A salient finding of this study is fixed dose combinations containing rifampicin, isoniazid, pyrazinamide and ethambutol stored in freezer (4^0C) showed less amount of degradation of rifampicin when compared to other temperatures like 30^0C and 40^0C with 75%RH.
- This study showed that strip package undergo less degradation when compared to blister package because strip packages show low moisture gain while blister package show high moisture gain.
- Therefore manufacturers should use packaging materials that provide strong barriers.

REFERENCES

REFERENCES

1. Frieden TR, Sterling TR, Munsiff SS, Watt CJ, Dye C: Tuberculosis *The Lancet* 2003; 362(9387):887-899

2. Blomberg B, Spinaci S, Fourie B, Laing R. The rationale for recommending fixed-dose combination tablets for treatment of tuberculosis. Bull WHO 2001; 79: 61-68.

3. Bhutani, H., Mariappan, T.T., Singh, S., 2004. A study on the physical and chemical stability of anti-tuberculosis fixed-dose combination (FDC) products under accelerated climatic conditions. Int. J. Tuber. Lung Dis. 8, 1073-1080

4. Mitchison DA: Mechanism of drug action in short-course chemotherapy**.** *Bulletin International Union against Tuberculosis* 1985, **65:**30-7.
5. Bhutani H, Singh S,Chakraborti A K, Jindal K C. Mechanistic explanation to the catalysis by Pyrazinamide and Ethambutol of reaction between Rifampicin and Isoniazid in anti-TB FDCs. *J Pharm Biomed Anal* 2005; 39: 892-899
6. Shishoo C. J., Shah S.A., Rathod I.S., and Savale S.S., 2001. Impaired bioavailability of rifampicin from fixed dose combination (FDC) formulations with Isoniazid. Indian J. Pharm. Sci. 63 (6), 443-449.
7. Shishoo, C.J., Shah, S.A., Rathod, I.S., Savale, S.S., Kotecha, J.S., Shah, P.B., 1999. Stability of rifampicin in dissolution medium in presence of isoniazid. Int. J. Pharm. 190, 109-23.
8. Singh, S., Mariappan, T.T., Sankar, R., Sarda, N., Singh, B., 2001. A critical review of the probable reasons for the poor/variable bioavailability of rifampicin from anti-tubercular fixed-dose combination (FDC) products, and the likely solutions to the problem. Int. J. Pharm. 228, 5–17.
9. WHO Expert Committee on Specifications for Pharmaceutical Preparations (1999: Geneva, Switzerland) WHO Expert Committee on Specifications for Pharmaceutical Preparations: thirty-sixth report.

10. Amidon E. G and Middleton R.K.1988. Accelerated physical stability testingand long term predictions of changes in crushing strength of tablets storedin blister packages Int J.pharm., 45: 79-89
11. Singh, S., Mohan, B., 2003. A pilot stability study on anti-tuberculosis four drug fixed dose combination products. Int. J. Tuber. Lung Dis. 7, 298-303
12. www.Tuberculosis.com
13. WHO/HTM/TB/2008.401
14. Panchagnula R, Agrawal S, Kaul CL. Fixed-dose combinations in the treatment tuberculosis. *Ind J Pharm Sci*, 2001; 63:1-9.
15. World Health Organization – Geneva 2003: Treatment of TB: Guidelines for National Programmes.
16. World Health Organization. Communicable Diseases Cluster: Fixed-dose combination tablets for the treatment of tuberculosis. 1999.
17. Heifets LB, Lindholm-Levy P: Comparison of bactericidal activities of streptomycin, amikacin, kanamycin, and capreomycin against *Mycobacterium avium* and *M tuberculosis*
18. Rieder HL. Epidemiologic basis of tuberculosis control. Paris: International Union against Tuberculosis and Lung Disease, 1999; pp. 1-162.
19. John Dewey Stability & Human bioavailability of novel rifampicin and isoniazid FDC.
20. Singh, S., Mariappan, T.T., Sharda, N., Kumar, S., Chakraborti, A.K., 2000. The reason for an increase in decomposition of Rifampicin in the presence of Isoniazid under acid conditions.Pharm. Pharmacol. Commun. 6, 405- 410.
21. Savale, S.S., 2003. Dissolution study and its correlation with bioavailability of the drugs.Ph.D. Thesis, Gujarat University, Ahmedabad
22. Satish Balkrishna Bhise, Sevukarajan Mookkan Formulation and Evaluation of Novel FDCs of Antitubercular Drugs ISSN: 0974-6943.
23. ICH, 2003. Stability testing of the new drug substances and products, Q1A (R2). Geneva, Switzerland.
24. ICH Guidline on Stability Testing of New Drug Substances And Products.Recommended for Adoption under Step 4 of the ICH Process on 8 November 2000 by the ICH Steering Committee.
25. .Waterman C.K. and Adami C.R. 2005 Accelerated aging: prediction of chemical stability of pharmaceuticals Int.J. Pharm., 293: 101-125.
26. Airaksinen, S.T.T., 2005. Role of excipients in moisture sorption and physical stability of solid pharmaceutical formulations.

27. Hu Y, Coates AR, Mitchison DA. Sterilising activites of fluroquinolones against rifamintolerant populations of mycobacterium tuberculosis.Antimicrob Agents Chemotheraphy 2003;47:653.
28. Brogden RN, Fitton A. Rifabutin. A review of its antimicrobial activity, pharmacokinetic properties and therapeutic efficacy. Drugs 1994; 47: 983-1009.
29. Eustice DC, Feldman PA, Zajac I, Slee AM. Mechanism of action of DuP 721: inhibition of an early event during initiation of protein synthesis. Antimicrob Agents Chemother 1988; 32: 1218-22.
30. Ashtekar DR, Costa-Perira R, Nagrajan K, Vishvanathan N, Bhatt AD, Rittel W. In vitro and in vivo activities of the nitroimidazole CGI 17341 against *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 1993; 37: 183-6.
31. Singh, S., Mohan, B., 2003. A pilot stability study on anti-tuberculosis four drug fixed dose combination products. Int. J. Tuber. Lung Dis. 7, 298-303.
32. Shrutidevi Agrawal, Ramesh Panchagnula 2004 Dissolution test as a surrogate for quality evaluation ofrifampicin containing fixed dose combination formulations International Journal of Pharmaceutics 287 (2004) 97–112.
33. Mukesh C. Gohel and Krishnakant G. Sarvaiya 2007 A Novel Solid Dosage Form of Rifampicin and Isoniazid With Improved Functionality
34. Singh, S., Bhutani, H., Mariappan, T.T., 2006. Quality problems of anti-tuberculosis fixed- dose combinations (FDCs): A way forward. Indian J. Tuberc. 53, 201-205.
35. Indian Pharmacopeia 2010 volume III page 2054-2055.
36. Walter Wehrli.Rifampin: Mechanism of Action and Resistance.Rev Infect Dis 1983; 5:407-11.
37. Van Scoy RE, Wilkowske CJ. Antituberculosis agents. Mayo Clin Proc 1987; 62:1129-36.
38. R.S. Satoskar, S.D. Bhandarkar, Nirmala N. Rege. The pharmacology and pharmacotherapeutics. 20th edition; page 737.
39. Meyer H, Mally J. Über Hydrazinderivate der Pyridincarbonsäuren. Monatsheftefür Chemie und verwandte Teile anderer Wissenschaften 1912; 23: 393-414.
40. Indian Pharmacopeia 2010 volume II page 1515-1517.
41. Winder FG, Collins PB. Inhibition by isoniazid of synthesis of mycolic acids in *Mycobacterium tuberculosis*. J Gen Microbiol 1970; 63: 41-8.

42. Baciewicz AM & Self TH: Isoniazid interactions. South Med J 1985; 78:714-718.
43. Siskind MS, Thienemann D, Kirlin L. Isoniazid-induced neurotoxicity in chronic dialysis patients: report of three cases and a review of the literature. Nephron 1993; 64: 303-6.
44. Jimenez-Lucho VE, Del Busto R, Odel J. Isoniazid and ethambutol as a cause of optic neuropathy. Eur J Respir Dis 1987; 71: 42-5.
45. Indian Pharmacopeia 2010 volume III page 2004-2005.
46. Zhang Y, Scorpio A, Nikaido H, Sun Z. Role of acid pH and deficient efflux of pyrazinoic acid in unique susceptibility of *Mycobacterium tuberculosis* to pyrazinamide.J Bacteriol 1999; 181: 2044-9.
47. Leonin TA, Julian EV, Baluis CR. A review of the hepatotoxic effects of anti-TB drugs at the Veterans Memorial Medical Center. Chest 1979; 11: 140-8.
48. Indian Pharmacopeia 2010 volume II page 1299-1301.
49. Zhang Y, Telenti A. Genetics of drug resistance in *Mycobacterium tuberculosis*. *In:* Hatfull GF, Jacobs WR, Jr., Eds. Molecular genetics of mycobacteria. Washington, DC: ASM Press, 2000; 235-254.
50. Peets EA, Sweeney WM, Place VA, Buyske DA. The absorption, excretion, and metabolic fate of ethambutol in man. Am Rev Respir Dis 1965; 91: 51-8.
51. Campbell IA, Ormerod LP. Ethambutol and the eye. (Correspondence). Lancet 1988; 2: 1134.
52. Indian Pharmacopeia 2010 volume I page 560.
53. Benetton SA, Kedor-Hackmann ER, Santoro MI, Borges VM. Visible spectrophotometric and first derivative UV spectrophotometric determination of rifampicin and isoniazid in pharmaceutical preparation Talanta 1998;47:639-43
54. Mariappan TT, Saranjit. Gastrointestinal permeability studies using combinations of rifampicin and nucleoside analogue reverse transcriptase inhibitors in rats Ind J. Pharmacol 2007;39,248-90.