ORAL BIOAVAILABILITY & BIOEQUIVALENCE STUDY OF FENOFIBRIC ACID

Anchal Sharma¹, Dr. Rajesh Asija², Dr. Radheshyam Kumawat³, Mrs. Richa Agarwal⁴

Maharishi Arvind Institute of Pharmacy, India

ABSTRACT

Fenofibric acid is generally used to treat Hyperlipidemia, Mixed Dyslipidemia, Hypertriglyceridemia and Hypercholesterolemia in adults who don’t respond to non-pharmacological treatment. The study is conducted to evaluate the bioequivalence of Fenofibric Acid 135 mg Delayed Release Capsules in 6 normal, healthy, adult, male subjects under fasting conditions.

Keyword: Hyperlipidemia, Pharmacokinetics, bioequivalence, Adverse Drug Reaction

1. INTRODUCTION

Bioavailability is used to describe the fraction of an administered dose of medication that reaches the systemic circulation, one of the principal properties of the drugs. By definition, when the drug is administered intravenously, its bioavailability is 100%. However when a medication is administered via other routes (such as by mouth), its bioavailability decreases (due to incomplete absorption and first-pass metabolism). Bioavailability is one of the essential tools in pharmacokinetics, as bioavailability must be considered when calculating dosages for non-intravenous routes of administration.

Bioavailability and bioequivalence of drug products, and drug product selection have emerged as critical issues in pharmacy and medicine during the last three decades. Concern about lowering health care costs is resulted in a tremendous increase in the use of generic drug products; currently about one half of all prescriptions written are for drugs that can be substituted with a generic product.

Inherent in the currently accepted guidelines for product substitution is the assumption that a generic drug considered to be bioequivalent to a brand-name drug would elicit the same clinical effect. Numerous papers in the literature indicate that there is concern that the current standards for approval of generic drugs may not always ensure therapeutic equivalence. The availability of different formulations of the same drug substance given at the same strength and in the same dosage form poses a special challenge to health care professionals.

If the size of the dose to be administered is same, then bioavailability of a drug from its dosage form depends upon three major factors, Pharmaceutical factors related to physiochemical properties of the drug and characteristics of dosage form.

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2. HEART

Heart which is a hallow organ located in the centre of the chest forms a part of the circulatory system that is also inclusive of blood vessels and lungs. A strong muscle known as myocardium makes up the walls of the heart (7). In an average adult, about 4.7 liters of blood is pumped by the heart every minute and distributed throughout the body by rhythmic contractions. Providing a continuous circulation of blood throughout the body, a healthy human weighs approximately 250-350 grams (8).

Located in the thoracic cavity in between the lungs, heart is cone shaped and has a broad base at the top. The large blood vessels enter and exit from the top. The tip of the heart lies close to the sternum. The position of the heart is such that it is anterior to the vertebral column and posterior to the sternum. Enclosed in a protective sac called pericardium, the heart is covered by a lubricating pericardial fluid (9). This helps in the sliding of the two walls over one another during the movements of the heart. Three layers make up the outer wall of the heart. These are Epicardium, myocardium and endocardium. Epicardium is the outermost
layer of the heart. During the contraction of the ventricles, the movement of the wave of depolarization occurs from endocardial to epicardial surface. Myocardium is made up of involuntary striated muscles. The innermost layer that lines the chambers of the heart is the endocardium. This performs the function of proving protection to the valves and heart. The heart consists of four main chambers. The two upper chambers are called as the left and the right atrium while left and right ventricles are the lower chambers. The right side of the heart is separated from the left side by a dividing wall of muscle known as the septum (10). Ventricular septum is the part of muscle that separates the ventricles. The two atria are separated by the atrial septum. With each heart beat the same amount of blood is pumped into the lungs by the right ventricle as is pumped out into the body by the left ventricle.

The atria are the receiving chambers of the heart. They receive blood and arte connected to the veins that bring blood to the heart. The ventricles are larger and stronger than atria. These are the pumping chambers and pump the blood out of the heart. Blood is brought to the right atrium by the cranial vena cava, coronary sinus and the caudal vena cava (11). The right atrium has sino-atrial node. Opening of the right atrium is guarded by the tricuspid valve. Oxygenated blood is carried to the left atrium by the pulmonary veins. The passage of blood from the left atrium to the left ventricle is guided by the bicuspid or the left atrioventricular valve.

The location of the left atrium is under tracheal bifurcation. Enlargement of this area of the heart leads to the difficulty in breathing. Blood passes from the left ventricle to the ascending aorta through the aortic semi lunar valve. The ascending aorta branches it into the coronary arteries.

2.1. Physiology of the heart

At any given time, there are two states in which the chambers of the heart may be found. They are the following:

- Systole – This is the state in which contraction of the cardio muscle tissue takes place in order to the blood out of the chamber (12).
- Diastole – In this state, relaxation of the cardiac muscle cells takes place so that the chambers are allowed to be filled with the blood. During ventricular systole the blood pressure increases in the major arteries while it decreases during the ventricular systole.

2.2. The cardiac cycle

A cardiac cycle includes all those events that take place during a single heart beat. This consists of three phases. They are the following:

- Atrial systole- This is characterized by the contraction of the atria to push the blood into the ventricles. During this phase, the ventricles remain in the diastole phase.
- Ventricular systole- during ventricular systole, contraction of the ventricles occurs for pushing the blood into the aorta and pulmonary trunk (13). Due to the pressure of the ventricles the semi lunar valves are forced to open whereas the AV valve closes.
- Relaxation phase- This is the phase in which all the four chambers of the heart are in diastole. During this, the blood pours form the veins into the heart. The ventricles get filled with blood to about 75 per cent of their capacity (7). Once the atria enter the systole, the ventricles get completely filled.

The circulation of blood in the blood vessels in heart muscle is known as coronary circulation. Oxygen rich blood is delivered to the myocardium with the help of coronary arteries (29). Right coronary artery supplies blood to the right atrium, right ventricle, bottom portion of the left ventricle and septum. The left coronary artery divides into two branches known as circumflex artery and left anterior descending artery.

The blood vessels that deliver oxygen rich blood from the heart to the tissues of the body are known as arteries. Each artery is in the form of a muscular tube that is lined by smooth muscle (28). Aorta is the largest artery which is the main pipeline connected to the left ventricle of the
heart. The aorta extends throughout the body in the form of a network of branched smaller arteries. The arteries further branch into arterioles and capillaries.

3. DISEASES OF HEART

Being a complex organ, the heart is prone to several diseases. The diseases of the heart involve the heart and the blood vessels including arteries, capillaries and veins. Heart diseases can be divided into five main types. These are:

3.1 Rheumatic heart disease-

These are caused by the damage caused to the heart, particularly its valves, due to one or more attack of the rheumatic fever (14). Rheumatic fever that occurs in the childhood affects the heart and may cause the valves to get scarred. This affects their normal opening and closing. The muscles of the heart weaken and the sac enclosing the heart gets damaged. Rheumatic fever is an inflammatory disease that affects the connective tissues of the body especially those of the heart. As a result of this, mitral valve is most commonly affected. Fibrosis of the heart valves that is caused by the rheumatic fever results in crippling valvular heart disease, heart failure and death. Currently, the global burden of the rheumatic heart disease falls on children and young adults who reside in low income countries. This category of heart diseases causes 233, 000 deaths every year (30).

3.2 Hypertensive heart disease-

Hypertensive heart disease is inclusive of a number of complications of systemic arterial hypertension. These heart diseases occur as a result of the high blood pressure which may of unknown origin known as primary hypertension. Secondary hypertension may be caused due to certain specific diseases or infections such as tumor in the adrenal glands, disease of the kidneys and their blood vessels (15). Due to high pressure, the heart and the blood vessels may get overburdened which may cause disease. As per the World Heart Federation, total number of deaths due to hypertensive heart disease in the world is 1, 153,000. Blood is transported to the muscles of the heart with the help of coronary arteries. Due to high blood pressure, the blood vessels become narrow. As such, the blood flow to the heart can slow or stop. This condition, known as the coronary heart disease can make it difficult for the heart to function normally. There are various types of hypertensive heart diseases. These are the following:

- Aneurism
- Atherosclerosis
- High blood pressure (hypertension)
- Peripheral Heart Disease
- Ischemic heart disease
- Cerebrovascular Disease
- Inflammatory Disease

Hyperlipidemia

The scientific term for fats in blood is lipids. At proper levels, important functions are performed by lipids in the human body. But if they are present in excess, several problems can be caused. The term hyperlipidemia refers to high lipid levels in the body (27). It is a heterogeneous group of disorders which is characterized by increased levels of lipid in the blood stream. These lipids are cholesterol, phospholipids, cholesterol esters and triglycerides. Hyperlipidemia is characterized by increased risk of atherosclerosis and other serious conditions. Being of common occurrence in the general population, lipids and abnormalities of lipoprotein are modifiable risk factors for cardiovascular diseases (26). This is because they influence atherosclerosis. Depending upon the type of lipids elevated, hyperlipidemia is classified into the following categories.

- Hyperlipoproteinemia
- Hypercholesterolemia or elevated levels of cholesterol
- Elevated very low density lipoprotein (VLDL)
- Low density lipoprotein (LDL) levels
- Hypertriglyceremia or elevated levels of triglycerides

Being of typical asymptomatic nature, hyperlipidemia is generally detected during routine screening. Hyperlipidemia is of two types. They are the following:

- Primary hyperlipidemia
- Secondary hyperlipidemia

Causes:

There are various factors that result into hyperlipidemia. These are delayed or defective clearance, overproduction of VLDL by the liver.
This is subsequently transformed into LDL. Hepatic and non hepatic LDL receptors are involved in familial hypercholesterolemia. The production of VLDL by the liver is increased due to excess intake of saturated fats. This occurs via a molecular mechanism which involves protein activators (3). The main sources of saturated fats are animal products such as meat, whole milk dairy products such as milk, cream and cheese, and butter and tropical oils such as palm, coconut and palm kernel. Among the lifestyle contributors are included obesity, lack of physical activity and smoking. The chances of developing hyperlipidemia are more in males of more than 45 years of age and females of more than 55 years of age.

**Risk Factors**

There are number of preventable risk factors that increase a person’s risk of developing hyperlipidemia. They are the following:

- Poor diet
- Diabetes mellitus
- Chronic renal failure – This has association with hypertriglyceridemia (1).
- Nephritic syndrome
- Obesity
- Physical inactivity
- Smoking

**Symptoms of Hyperlipidemia**

There are no noticeable symptoms of hyperlipidemia. It is generally detected during the routine examination. Detection of the disorder also occurs during evaluation for atherosclerotic cardiovascular disease. However, there may be deposits of cholesterol under the skin known as xanthomas (4). These are especially found around the eyes or along Achilles tendons in those individuals that suffer from familial type of hyperlipidemia.

**Types of Lipids**

Lipids are a group of molecules that occur naturally. These are fats, sterols, waxes, monoglycerides, diglycerides, triglycerides, phospholipids and fat-soluble vitamins (16). These perfume the functions of signaling, storing energy and acting as structural components of cell membranes. Lipids are broadly classified into the following categories mentioned below.

**Storage lipids**

**Storage lipids are of three types:**

**Fatty acids**: Being the defining components of lipids, fatty acids are largely responsible for distinctive p-physical and metabolic properties. They also find their importance in non-esterified form. During fasting, these are released in the body by the Triacylglycerols. This occurs to provide a source of energy to the body. Linoleic and linolenic acids are essential fatty acids (1). These are not produced in the human body. Instead they are derived from the consumption of plants. These fatty acids in diet are in short and medium chain length. These are not usually esterified. When they are in the body, they rapidly get oxidized in the tissues and act as source of fuel. Esterification of the longer fatty acids converts them to Triacylglycerols or structural lipids in the tissues.

**Triacylglycerols**: the long chain fatty acids are stored in the form of Triacylglycerols to be used for energy and structure formation of cells. They consist of glycerol and 3 fatty acids. These yield a triester. Blood tests help in the detection of triglycerides (17). Being an important constituent of the phospholipids polyunsaturated fatty acids are responsible for forming the membrane of the cells. These are found in most of the natural fats and oils.

**Tri-, Di- and Monoacylglycerols**: Biosynthesis of Triacylglycerols yields 1, 2-Diacylglycerols. These perform the function of second messengers in many processes of the cells. Digestion of Triacylglycerols in the intestines produces monoacylglycerols.

**Sterols**: an omnipresent component of all the animal tissues is cholesterol (18). Most of the cholesterol is present in the membranes. It generally occurs in the free form. After esterification it gets converted into long chain fatty acids. These include the plasma lipoproteins cholesterol act as precursors of bile acids, steroidal hormones and vitamin D.

**Structural lipids**

Transportation of materials is controlled by the cellular membranes. This includes providing signal to the molecules. These cell membranes are formed of a water loving component and a water fearing component. These are of the following types:
• **Phospholipids** - These are of two types, glycerophospholipids and sphingolipids. The molecules that are composed of glycerol substituted with two fatty acid esters are known as phosphatides. These are composed of three alcohols known as ethanolamine, serine and choline.

• **Sphingolipids** - Are composed of long chain sphingoid base (19). The most abundantly present sphingolipids in animal tissues is sphingomyelin. This serves as the most important building block of membranes.

• **Saccharolipids** - These are the molecules that contain fatty acids linked directly to sugar backbone. Saccharolipids form a constituent of the bilayer.

**Other lipids**

Among other lipids are included the proteolipids and lipoproteins as well a polyketides.

**Proteolipids and Lipoproteins** - These are proteins that form covalent bonds with fatty acids such as cholesterol. They include HDL (high density lipoprotein), LDL (low density lipoprotein) and VLDL (Very low density lipoprotein). The above characterization is done on the basis of molecular size.

**Polyketides** - They are responsible for the formation of a large number of metabolites and natural products from animal, bacterial and plant sources (17). Antibiotics such as erythromycins, tetracycline and anticancer agents such as epothilones come in the category of Polyketides.

**Role of lipids in Hyperlipidemia**

Hyperlipidemia is a metabolic disorder that is characterized by increase of the concentration of fat in the blood serum. It is general term that depicts elevated levels of any or all then lipids in the plasma.

**Role of cholesterol**

This is a lipid or fat chemical which is manufactured in the liver form the intake of fatty foods. A certain amount of cholesterol that is present in the blood stream is required to keep the body healthy. This cholesterol is carried in the blood by molecules known as lipoproteins. The levels of cholesterol are measured as milligrams of cholesterol per deciliter of blood. As per the guidelines of NCEP, the desirable cholesterol concentrations are those below 200 mg/dL. A reading of 200 to 239 mg/dL is considered as borderline high concentration. Cholesterol concentration of greater than 240 mg/dL is indicative of hypercholesterolemia (25). As the levels of total cholesterol decrease, the risk of developing cardiovascular diseases also falls.

Being a fat like substance circulating in the blood, about 25 per cent of it comes from food whole 75 per cent is manufactured in the body. It is vital for life as it is a component of cell membranes. It is also involved in proper functioning of cells. Cholesterol also finds its use in the formulation of hormones and vitamins. However, if present in excess amount, it may become a major health risk as it may result in serious diseases and heart attacks (5). Total cholesterol level is not of much importance as it may be possible that a person having a total cholesterol level of less than 200 mg/dL has unhealthy levels of High density lipoprotein or low density lipoprotein. In general, there are two main types of cholesterol. These are the following:

**LDL cholesterol** - Low density lipoprotein or LDL cholesterol is also known as bad cholesterol. A LDL cholesterol level of below 70 mg/Dl is considered to be ideal for those people who are at high risk of heart disease. While a level of 100-129 mg/dL is considered to be near ideal. However, a LDL concentration of 130-159 mg/dL is regarded as borderline high (6). LDL levels of 190 mg/dL and above are considered to be very high. If a person has Low density lipoprotein cholesterol above 130 mg/dL, he is considered to be suffering from hyperlipidemia.

**HDL cholesterol** - High density lipoprotein is also known as good cholesterol. These are scavengers of cholesterol. They perform the function of picking up extra cholesterol from the blood. This extra cholesterol is taken back to the liver where it is broken down. With higher level of HDL in blood the level of bad cholesterol is lowered. A HDL level of 60 mg/dL is considered to be desirable for an individual. Each bit of HDL cholesterol is a microscopic blob (2). This is inclusive of a rim of lipoprotein surrounding a cholesterol centre. The particle of HDL cholesterol is dense as compared to other types of cholesterol particles. It is for this reason that it is called as highly density lipoprotein. The LDL is reduced, reused and recycled by high density lipoprotein cholesterol. This is done by transporting LDL to the liver for further processing. In this manner, it
performs the functions of a maintenance crew for the inner walls of the blood vessels.

Cholesterol being an essential component of the cell membranes is needed for carrying out many functions of the body one of them being production of hormones. Cholesterol is produced on digestion of the foods containing oil and fat. Its production also takes in the liver. Cholesterol is found in blood in the form of two types of particles. The cholesterol in low density proteins (LDL) contributes to the diseases of the heart by sticking to the walls of the arteries that supply the heart and narrowing them. High density lipoprotein (HDL) cholesterol performs the function of checking the levels of LDL (3). When the levels of LDL cholesterol in blood become higher than the normal, it leads to hypercholesterolemia. Familial hypercholesterolemia is an inherited condition. The delivery of cholesterol to the cells occurs via the bloodstream. Normally, the tiny particles of LDL cholesterol get attached to the receptor sites on the targeted cells. These are the absorbed by the cells. The production of these receptors is controlled by LDLR gene on chromosome 19. When mutation of this gene occurs, it results into a change in the way of development of the receptors either in their number or in their structure. As a result of this, Low density cholesterol could be absorbed by the cells. It keeps on circulating in the blood. As such, its level is increased that leads to hypercholesterolemia which is a type of primary Hyperlipidemia.

Role of Triglyceride

These are the main form of fats that are stored in the body. Triglycerides are formed as an end product after breaking and digestion of bulky fats ingested through food. Triglycerides are formed in the shape of bundles and are transported through blood by the lipoproteins. These are released by the hormones for energy between the meals. Normal level of triglycerides is less than 150 mg/dL. A triglyceride level of 150 to 199 mg/dL is borderline high level. If the level of this lipid is more than 200 mg/dL, it is regarded as high (1). As per American Heart Association, optimal level of triglyceride is 100 mg/dL. Hence, triglycerides are wasted calories with the help of which, energy is provided to the body.

Treatment of Hyperlipidemia

The treatment for hyperlipidemia is dietary and lifestyle modification, followed by drug therapy, as if required. Hyperlipidemia should not be considered refractory to dietary treatment if the therapeutic regimen included animal products or more than minimal amounts of vegetable oils. Such diets do not lower LDL cholesterol concentrations as effectively as high-fiber; low-fat diets that exclude animal products. Regular exercise can improve lipid concentrations. Low to moderate amounts of physical activity such as walking lower triglyceride concentrations by an average of 10 mg/dL, while raising HDL by 5 mg/dL (these numbers are means drawn from large groups).

Bile acid sequestrants (eg, cholestyramine, colestipol) are second-line agents for the treatment of elevated LDL cholesterol. These medications can produce gastrointestinal distress, constipation, and impaired absorption of other drugs.

Fibrates (eg, gemfibrozil, fenofibrate) are used as first-line treatment for elevated triglyceride concentrations and may be prescribed in combination with the above drug classes. Gallstones, dyspepsia, and myopathy may occur. Myopathy risk may be particularly high when fibrates are combined with statins.

Nicotinic acid (niacin) is a second-line therapy for all lipid disorders. Niacin is often combined with statins, but is also effective as a single agent. Its use is often limited by skin itching or burning. Other side effects include GI distress, hepatotoxicity, hyperglycemia, and gout.

Ezetimibe and colesevelam decrease GI cholesterol absorption, and have emerged as a favored second-line therapy due to their effectiveness, safety, and lack of side effects. They lower LDL and often raise HDL, and are particularly effective when combined with statins (often achieving lipid targets at lower statin doses). Ezetimibe has emerged as the more effective drug (7, 8).

Drug Profile

Fenofibric Acid
**Mechanism of Action**

The Fenofibric acid has a lipid modifying effect which has been revealed in clinical studies in-vivo in transgenic mice and in-vitro in human hepatocyte cultures by the PPARα (peroxisome proliferator activated receptor α) activation. It activates lipoprotein lipase and decreases the production of Apo CIII (an inhibitor of lipoprotein lipase activity) to increase lipolysis and elimination of triglyceride-rich particles from plasma.

The reduction in triglyceride results in change in LDL composition from small dense particles to large buoyant particles. The cholesterol receptor has greater affinity for larger particles & thus catabolized them rapidly. While the activation of PPARα, increases the synthesis of HDL-C and Apo AI & AIL.

**Side Effects**

In rare incidences, Fenofibric acid causes breakdown of skeletal muscle tissue which may lead to kidney failure. The side effects of Fenofibric acid can be classified in to three categories:-

<table>
<thead>
<tr>
<th>Allergic Reactions</th>
<th>Mild Side Effects</th>
<th>Serious Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hives</td>
<td>Headache, Dizziness</td>
<td>Severe stomach pain</td>
</tr>
<tr>
<td>Difficulty in breathing</td>
<td>Back pain</td>
<td>Nausea, Vomiting</td>
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<tr>
<td>Swelling on face,</td>
<td>Joint pain</td>
<td>Fever, Jaundice</td>
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<tr>
<td>lips tongue or throat</td>
<td>Diarrhea, Upset stomach</td>
<td>Pain, swelling &amp; redness in legs</td>
</tr>
<tr>
<td></td>
<td>Sneezing, Sore Throat</td>
<td>Chest Pain, Wheezing &amp; Rapid fast breathing</td>
</tr>
</tbody>
</table>

**Interactions**

The drug Fenofibric acid (Choline) has oral interactions with blood thinners such as “warfarin”.

It may also interact with the drugs which are harmful to kidneys such as NSAID’s, Ibuprofen, Cyclosporine (31).

**Drug Brief Detail**

**Reference Product**

Trilipix is an oral delayed release capsule of Fenofibric acid. It is a lipid regulating agent and each capsule contains choline fenofibrate equivalent to 45 mg to 135 mg of Fenofibric acid.

**Composition:**

Each delayed release capsule contains choline fenofibrate as active ingredient and following inactive ingredient: Hypromellose, povidone, water, hydroxypropyl cellulose, colloidal silicon dioxide, sodium stearyl fumarate, methacrylic acid copolymer, talc, triethyl citrate, gelatin, titanium dioxide, and yellow iron oxide. Additionally, 135mg capsule shell contains FD&C Blue #2.

**Pharmacokinetic Properties**

Absorption: Fenofibric Acid is absorbed well in gastrointestinal tract. Its absolute bioavailability is around 81%. After 3hrs-5hrs of single dose administration of Trilipix capsule under fasting conditions peak plasma concentration is achieved.

Distribution: Fenofibric Acid shows 99% of serum protein binding in both normal and dyslipidemic subjects. The steady state of Fenofibric acid level reached within 8 days of multiple dosing.

Metabolism: Fenofibric acid is conjugated with glucuronic acid and then excreted in urine. While a small amount is reduced at the carbonyl moiety to a benzhydryl metabolite which in turn, conjugated with glucuronic acid and excreted in urine.

Excretion: The half life of Fenofibric acid is 20 hours after single dose administration in a day. It is excreted in the urine in the form of fenofibric acid and fenofibric acid glucuronide (31, 32).

**Test Product**

The active ingredient content of test product is Fenofibrate. The type of formulation is oral delayed release capsule. The strength of capsule is 135 mg and it is manufactured by the Sponsor.
4. AIM & OBJECTIVE

**Aim –**

The aim of the study is to determine the oral bioequivalence of Fenofibric Acid 135 mg Delayed Release Capsules manufactured by Sponsor with Trilipix™ 135 mg (Fenofibric Acid) Delayed Release Capsules manufactured by Abbott Laboratories in 6 normal, healthy, adult, male subjects under fasting conditions.

**Objective –**

The Sponsor is intended to determine the bioequivalence of Fenofibric Acid 135 mg Delayed Release Capsules. For this reason a single dose of Fenofibric Acid 135 mg Delayed Release Capsules manufactured by Sponsor will be compared with Trilipix™ 135 mg (Fenofibric Acid) Delayed Release Capsules in 6 normal, healthy, adult, male subjects under fasting conditions.

5. METHODOLOGY

**Study Design**

The study is designed as an open-label, randomized, two treatment, two sequence, two period, single dose, two way crossover oral bioequivalence study in 6 normal, healthy, adult, male subjects under fasting conditions.

**Study Plan and Procedure**

**Study Population**

A sufficient number of normal, healthy, adult, male subjects will be screened and enrolled in to the study in order to ensure that 06 subjects will be dosed at the beginning of the study.

**Subject Screening**

All subjects will undergo a screening procedure comprising clinical examination, recording of electrocardiogram, radiological investigation (Chest X-Ray, if not done in the past 6 months or if clinically indicated) and laboratory investigations of blood as well as urine less than 21 days prior to first dosing.

**Inclusion Criteria**

- Male subjects aged from 18 to 45 years (inclusive of both).
- Subjects within the BMI range from 18.5 to 24.9 kg/m2 (inclusive of both) and the body weight is ≥ 45 kgs.
- Subjects with normal vital signs (Blood pressure, Pulse rate, Respiratory rate and Body temperature).
- Subjects with normal medical and surgical history as determined by the physician or principal investigator prior to start of the study.
- Subjects with normal functioning of Cardiovascular, Respiratory, Gastrointestinal, Nervous System, Musculoskeletal, Vascular, Genitourinary, Endocrine/Metabolic systems.
- Subjects with normal lymph nodes and other systems like head, neck, eyes, ears, nose, throat and skin.
- Subjects with normal laboratory values of investigations as mentioned in the table below.

<table>
<thead>
<tr>
<th>Blood Tests</th>
<th>Biochemical Tests</th>
<th>Urine Analysis</th>
<th>Serological Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematology</td>
<td>Serum Creatinine</td>
<td>Colour</td>
<td>HIV (1 &amp; 2 antibodies)</td>
</tr>
<tr>
<td>RBC</td>
<td>Blood Urea</td>
<td>Appearance</td>
<td>HCV antibodies</td>
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<td>WBC</td>
<td>Total Cholesterol</td>
<td>pH</td>
<td>VDRL</td>
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<td>Differential WBC Count</td>
<td>Triglyceride</td>
<td>Chemical Examination</td>
<td>Hepatitis B surface antigen</td>
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<td>Fasting Blood Glucose</td>
<td>Microscopic Examination</td>
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<td>Platelet Count</td>
<td>Total Bilirubin</td>
<td>Pus Cells</td>
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<tr>
<td>ESR</td>
<td>Total Protein</td>
<td>RBC</td>
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<tr>
<td>Blood Group</td>
<td>Serum Albumin</td>
<td>Crystals</td>
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- Subjects having normal 12-lead electrocardiogram (ECG).
- Subjects having normal chest X-Ray (P/A view).
- Subjects able to communicate effectively.
- Subjects willing to provide consent and adhere to the protocol requirements.

**Exclusion Criteria**

- Contraindications or hypersensitivity to Fenofibrate or related group of drugs.
- History or presence of any disease in the recent past (hepatic, gastrointestinal, metabolism endocrinological, any type of phrophiria, neurological etc.) according to the opinion of the physician.
- History or presence of significant alcoholism or drug abuse in the past one year.
- History or presence of significant smoking (more than 09 cigarettes or bidis/day) or consumption of tobacco products (pan, gutkha) and refusal to restrain from smoking for 48.00 hours before first period check-in until last sample collection.
- History or presence of significant asthma, urticaria or other allergic reactions.
- Difficulty in donating blood or difficulty in accessibility of veins.
- Difficulty in swallowing capsules.
- Systolic blood pressure less than 110 mm Hg or more than 139 mm Hg.
- Diastolic blood pressure less than 70 mm Hg or more than 89 mm Hg.
- Pulse rate less than 60/minute or more than 100/minute.
- Use of any prescribed medication during last two weeks or OTC medicines or medicinal products during the last one week preceding the first period check-in until completion of the study.
- Use of any pharmacological agents (Eg: Cyclosporine, Anticoagulants, Cytochrome P-450 Enzymes) known to significantly induce or inhibit drug metabolizing enzymes within 14 days of start of the study.
- Refusal to abstain from grapefruit and orange products from 7 days prior to the check-in until the last sampling.
- Major illness during 3 months before screening.
- Subjects who have been on an abnormal diet for whatever reason e.g. because of fasting due to religious reasons during the four weeks before screening.
- Participation in a drug research study within past 3 months.
- Donation of blood in the past 3 months before screening.
- Refusal to abstain from water for at least 01.00 hour prior to dosing and for at least 02.00 hour post dose in each period.
- Refusal to abstain from food for at least 10.00 hours prior dosing and for at least 04.00 hours post dose.
- Refusal to abstain from alcohol or methylxanthine-containing beverages or foods (coffee, tea, carbonated drinks, chocolate) from 48.00 hours prior to first period check-in until last sample collection.
- Presence of disease markers Hepatitis B virus, Hepatitis C virus and VDRL.
- HIV positive.
- Found positive in the alcohol breath test done at the time of check-in.
- Found positive in the urine drugs of abuse done at the time of check-in.
- Evidence of an uncooperative attitude.

**Criteria for discontinuation/withdrawal of subjects**

- Subject wishes to withdraw on his own accord during the study.
The Principal investigator may withdraw a subject from the study for any of the following:
- Emesis occurred at any time during the labeled dosing interval.
- The subject requires concomitant medication, which could interfere with the pharmacokinetic property of the study medication.
- The subject suffers from significant intercurrent illness or undergoes any surgery during the course of the study.
- Subject with significant adverse event.
- Investigator felt that it is not reasonable to continue the study for the best of subject’s health.
- Subject is non-cooperative and undisciplined or violating any restrictions mentioned in the protocol.

Any subject who discontinues the study after his check-in will be declared as a drop-out. For each subject being dropout/withdrawn from the study prior to regular termination of the individual study period, due to any reason, the reason for dropout/withdrawal will be documented in the respective form, the pagination number of the same will be mentioned in case report form and safety assessment will be done.

Treatment to subject

Housing

Subjects will be admitted and housed in the clinical facility from not less than 10.50 hours before dosing and will be checked out 24.00 hours after dosing in each period, if the subjects do not suffer from any adverse event. In case of adverse event the subject will be housed in the facility at discretion of the physician.

Dose Regimen & Administration

All subjects will be in a fasting state for at least 10.00 hours before scheduled time of dosing. The subjects will be administered as per the randomization schedule, either single dose of Test (T): Fenofibric acid 135 mg Delayed Release Capsule or Reference (R): TrilipixTM 135mg (Fenofibric acid delayed release) Capsule with 240±5 mL of water in each period under the supervision of Principal Investigator.

Subjects will be instructed not to chew or crush the capsule but to consume as a whole. Administration of investigational products will be done while the subjects are in standing posture and they will be instructed to remain in upright posture (sitting) for the first two hours after dosing in each period except when clinically indicated to change the posture or in case of any natural exigency (31, 32, 33).

Sampling Schedule

Twenty-two (22) blood samples will be collected from each subject during each period. The venous blood samples (05ml each) will be withdrawn at pre-dose [(00.00) with in 02.00 hours prior to dosing] and at 0.25, 0.50, 0.75, 01.00, 01.25, 01.50, 01.75, 02.00, 02.25, 02.50, 03.00, 03.50, 04.00, 04.50, 05.00, 6.00, 8.00, 10.00, 12.00, 16.00, and 24.00 hours post dose.

Sampling Procedure

Intravenous Cannula Insertion and Removal Time

Intravenous cannula will be inserted before pre-dose sample and will remain till last sample collection.

Blood samples will be collected at bed side through an indwelling cannula placed in a forearm vein using disposable syringe.

Any difficulty in blood withdrawal due to cannula blockade, swelling or pain at the insertion site, then cannula will be removed and remaining blood samples will be collected through fresh vein puncture or by recannulation.

Blood Samples Collection

Five (05) mL of blood sample will be collected at each sampling time point except pre-dose (for pre-dose 10 mL) and transferred to pre-labeled vacutainers containing K2EDTA as an anticoagulant.

Before every blood sample collection (except pre-dose) 0.5 mL of blood will be discarded through I.V. cannula. After every blood sample collection 0.5 mL of heparinized saline (1 mL of 5 IU/mL of heparin in normal saline solution as an Anticoagulant will be injected in to the I.V. cannula to maintain the cannula patent.

The pre-dose (00.00 hour) blood sample will be collected within 02.00 hours prior to dosing and the post-dose in-house samples will be collected within ± 2 minutes from the scheduled sampling time. The mid-point time of collection of each
blood sample (to the nearest minute) will be recorded on the appropriate CRF. The deviations greater than mentioned in this protocol from the scheduled sampling time will be reported as protocol deviations.

**Sample Handling and Processing**

After collection of blood samples from all the subjects at each time point, samples will be centrifuged at 4000 RPM for 10 minutes at 5°C. All plasma samples will be transferred into pre-labeled polypropylene tubes. The plasma samples will be stored at -25±5°C for maximum period of 12.00 hours. The plasma samples will be transferred to bioanalytical department in an appropriate container containing dry ice and stored at -75±10°C until subjected for analysis.

**Total Blood Withdrawn**

| Cmax | Maximum measured plasma concentration. |
| AUC0-t | Area under the plasma concentration-time curve from zero hours to last quantifiable concentration to be calculated using the linear trapezoid rule. |
| AUC0-∞ | Area under the curve from zero hours to infinity time (AUC0-t plus additional area extrapolated to infinity, calculated using the formula AUC0-∞ = C0/Kel, where C0 is the last measurable drug concentration and Kel is the elimination rate constant). |
| Tmax | Time of the maximum measured plasma concentration. If the maximum value occurs at more than one point, Tmax is defined as the first time point with this value. |
| Kel | First order rate constant associated with the terminal portion of the curve. (Calculated using the formula Kel = -2.303* b where b is the slope). |
| t1/2 | Elimination half-Life (Calculated using the formula t1/2 = ln 2/ Kel). |

**Statistical Analysis**

Statistical analyses will be performed on the pharmacokinetic parameters using the SAS Statistical Software Version 9.1.3 or higher, SAS Institute, Inc., Cary, USA. Data from the subjects who vomit during the course of the study will not be considered for statistical analysis if vomiting occurs at any time during the labeled dosing interval. If a pharmacokinetic parameter cannot be determined for one period, the corresponding subject will be excluded for that particular statistical comparison.

**Summary Statistics**

Mean, minimum, maximum, standard deviation, standard error, median, CV% geometric mean and coefficient of variation will be calculated for plasma concentration of Fenofibric acid at respective time points as well as for the pharmacokinetic parameters Cmax, AUC0-t and AUC0-inf. In addition, following statistical information will be provided for Cmax, AUC0-t and AUC0-inf:

- Geometric Mean,
✓ Ratios of means
✓ 90% Confidence Interval

Analysis of Variance

The Ln-transformed pharmacokinetic parameters Cmax, AUC0-t and AUC0-inf for Fenofibric acid will be statistically analyzed using SAS Statistical Software Version 9.1.3 or higher, SAS Institute. Inc., Cary, USA. The factors included in this model will be the treatment received, the period at which it is given along with the sequence in which each treatment being received and the subject effect (nested within the sequence). The sequence effect will be tested using the subject nested within sequence mean square from the ANOVA as the error term. Each analysis of variance will include calculation of least square mean (LSM).

Two one sided t-test

Two one-sided t tests at 5% level of significance were used to compare the average values of pharmacokinetic parameters determined after administration of test and reference products. Consistent with the two one-sided t tests for bioequivalence, 90% confidence interval for the difference between treatments, least-square means were calculated for Ln-transformed pharmacokinetic parameters Cmax, AUC0-t and AUC0-inf.

Confidence Interval

90% confidence intervals for the difference between treatments, least-square means will be calculated for Ln-transformed Cmax, AUC0-t and AUC0-inf. The confidence intervals are expressed as a percentage relative to the LSM of the reference treatments.

Ratio Analysis

Ratio analysis will be reported for Ln-transformed Cmax, AUC0-t and AUC0-inf parameters. The least square mean values will be reported for Ln-transformed data.

Bioequivalence Criteria

Based on the statistical results of the 90% confidence intervals for the difference of means of Ln-transformed Cmax, AUC0-t and AUC0-inf conclusions will be drawn whether the test product is bioequivalent to the reference product under fasting conditions. The acceptance range for bioequivalence is ≥ 80.00% and ≤ 125.00% for the 90% confidence intervals for the difference of means of Ln-transformed Cmax, AUC0-t and AUC0-inf for Fenofibric acid.

Power Test

The power (i.e. probability of detecting a 20% differences relative to the reference treatment LSM at the 5% significance levels using a t-test under null hypothesis of zero differences) will be calculated for Ln transformed Cmax, AUC0-t and AUC0-inf.

Ethical Consideration

Institutional Ethics Committee

This protocol and corresponding information and consent document (containing information about the study and used to obtain consent from the subjects) will be reviewed by the Institutional Ethics Committee (IEC). Subjects will be enrolled into the study only after the IEC approves the protocol and the information and consent document as submitted or with modification(s).

Information and Consent Document

The information and consent document which is study document containing information about the study (in English, Hindi and/or Telugu or in a language understandable by the subject) will be distributed to the subjects before initiation of study. The Principal Investigator or designated study personnel will inform the subjects through an oral presentation regarding the purpose, procedures to be carried out, investigational products, potential hazards and rights of the study subjects stated in the information and consent document. The subjects will be required to understand and sign the information and consent document prior to check-in for the study in the first period and one photocopy of the signed information and consent document will be given to each subject while the original will be filed in the trial master file.

Compensation for Inconvenience during the Study

The subjects will be given an adequate (IEC approved) compensation on account of the inconvenience caused due to participation in the study. In case of dropout/withdrawal of a subject before completion of the study, the subjects will be given a pro-rated compensation depending upon the extent of participation. Controversy
pertaining to this compensation will be forwarded to the IEC and the decision of the IEC will be final as well as binding on both the subjects and CRBio.

Plan of Work
I. Literature survey on –
II. Anti-lipidemic drugs
III. New group of anti-lipidemic drugs
IV. Pharmacology of Fenofibric Acid

Pharmacological activity of Trilipix 135mg.

Clinical Work
1. Review of ICF
2. Review of Protocol
3. Approval of ICF for Protocol
4. Approval of Protocol form general ethics committee
5. Selection of Patients:
   I. Inclusion Criteria
   II. Exclusion Criteria
   1. Preparation of CRF
   2. Maintaining contact list
   3. Maintaining phone contacts
   4. Updating status databases
   5. Preparation of documents

Follow-up study

Follow-up-study data will be perform

- Side effects will be noted in the CRF
- Specify any other side effect in the CRF will be observe in the study period Statistical Analysis will be perform

REFERENCES

Journals
[14] Cleland, FGJ, Findlay, I, Jafri, S, et.al., The Warfarin/Aspirin study in heart failure (WASH): a randomized trial comparing


