IN VITRO DISSOLUION STUDY TO DETERMINE DRUG – DRUG INTERACTION OF ATORVASTATIN CALCIUM

A thesis report submitted to the Department of Pharmacy, East West University, Aftabnagar, Dhaka, Bangladesh, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

By

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DECLARATION BY THE THESIS CANDIDATE

I, Md. Nazmul Islam (ID # 2009 – 3 – 70 – 038) , hereby declare that the thesis entitled "**COMBINED DISSOLUTION STUDY OF ATORVASTATIN TABLETS WITH PANTOPRAZOLE & CALCIUM CARBONATE TABLETS**", submitted by me to the Department of Pharmacy, East West University, Aftabnagar, Dhaka, Bangladesh in the partial fulfillment of the requirement for the award of the Degree of Bachelor of Pharmacy (Honors) is a bonafide record of original Thesis work carried out by me during 2013 under the supervision and guidance of Mrs. Mehreen Rahman, Lecturer, Department of Pharmacy, East West University, Aftabnagar, Dhaka, Bangladesh and it has not formed the basis for the award of any other Degree / Diploma / Fellowship or other similar title to any candidate of any University.

Place: Dhaka Md. Nazmul Islam

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Date:_____

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CERTIFICATE

This is to certify that the Thesis entitled "**COMBINED DISSOLUTION STUDY OF ATORVASTATIN TABLETS WITH PANTOPRAZOLE & CALCIUM CARBONATE TABLETS**" is submitted to the department of pharmacy, East West University in partial fulfillment of the requirements of the degree of Bachelor of Pharmacy which was carried out by Md. Nazmul Islam (ID # 2009 – 3 – 70 – 038) under our guidance and supervision and that no part of the thesis has been submitted for any other degree. We further certify that all the sources of information and laboratory facilities availed of in this connection is duly acknowledged.

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I, **Md. Nazmul Islam** (ID # 2009 – 3 – 70 – 038), a student of the Department of Pharmacy in East West University at Aftabnagar in Dhaka cordially give my thanks to the Almighty Allah for successfully completing my project.

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LIST OF ABBREVIATIONS

Abbreviations	Expansions
ACE	Angiotensin Converting Enzyme
Apo – AI	Apolipoprotein AI
Apo – AII	Apolipoprotein AII
Apo – B	Apolipoprotein B
ATV	Atorvastatin
CAM	Contemporary and Alternative Medicine
CYP	Cytochrome P450 Enzyme
GFJ	Grape Fruit Juice
HCV	Hepatitis C Virus
HDL – C	High Density Lipoprotein Cholesterol
HMG – CoA	Hydroxy Methyl Gluteryl Co – Enzyme A
HMGRI	Hydroxy Methyl Gluteryl Reductase Inhibitors
IHD	Ischemic Heart Disease
LDL – C	Low Density Lipoprotein Cholesterol
MI	Myocardial Infarction
NAFLD	Non – Alcoholic Fatty Liver Disease
NCEP	National Cholesterol Education Program
OR	Odds Ratios
PAD	Peripheral Arterial Disease
PVD	Peripheral Vascular Disease
TCM	Traditional Chinese Medicine
TRL – C	Triglyceride – rich Lipoprotein Cholesterol
VLDL – C	Very Low Density Lipoprotein Cholesterol
WHO	World Health Organization
INN	International Non – proprietary Names
USP	United States Pharmacopoeia

ABSTRACT

Background

Atorvastatin calcium is a selective and competitive inhibitor of HMG – CoA reductase which is responsible for converting HMG – CoA to mevalonate, a precursor of cholesterol synthesis. Thus Atorvastatin is extensively used to lower cholesterol and triglycerides in patients with hypercholesterolemia, mixed dyslipidemia.

Objective

Drug – Drug and Drug – Food Interaction of Atorvastatin is well established. Thus, the objective of the present research was to study and compare the dissolution profile of Atorvastatin Calcium alone and with Pantoprazole and Calcium Carbonate.

Methods

For the present study, Dissolution Apparatus II, USP XIII was used to conduct the experiment. Six tablet of atorvastatin alone was subjected to dissolution study followed by another six tablets of atorvastatin with six of Pantoprazole and Calcium carbonate each. Same media of phosphate buffer of pH 6.8 was used in conduction of both experiments. Then in both cases, 5 ml samples were withdrawn at time intervals of 5, 10, 15 and 30 minutes. The samples were then subjected to UV-Vis Spectroscopy and the UV absorbances were recorded.

Findings

From the UV absorbances percentage dissolutions were calculated by the designated formulas. And the data of Atorvastatin alone and in combination were compared in order

to assess the changes in the dissolution profile. The changes in percentage dissolution in both cases were considerably different indicating the effect of Pantoprazole & Calcium carbonate on the dissolution profile of Atorvastatin.

Conclusion

Bioavailability of atorvastatin is 12%. Initial Increases in dissolution profile of atorvastatin with Pantoprazole and calcium carbonate followed by a substantial decrease can lead to variable dissolution & bioavailability of atorvastatin in presence of other drugs while, also increases the risk of side – effects and toxicity of atorvastatin. Thus, further studies are needed to confirm whether, such effect on dissolution profile is beneficial or harmful.

Keywords

Lipid-lowering Drugs, Statins, Atorvastatin, Hyperlipidemia, Atherosclerosis, Cardiovascular Disease, Cardiovascular Drugs, Drug Interaction, Dissolution Test, UV – Vis Spectroscopy

CHAPTER 1

INTRODUCTION

CHAPTER 1: INTRODUCTION

1.1 Modern Aspects of Therapeutics

Use of natural substances as therapeutic agents in modern medicine has sharply declined from the early decades of last century, but search for bioactive compounds from nature such as plants, animals, microorganisms continues to play an important role in development of new medicinal agents. With the advent of modern techniques, instrumentation and automation in isolation and structural characterization, enormous repository of natural compounds can be obtained very easily. Thus, researches are being conducted in order to make significant contributions in the domain of discovery and development of new medicinal products, (Dev, 2010).

1.2 Cardiovascular Diseases

Cardiovascular disease or Heart disease is a class of diseases that involve the heart, the blood vessels. It refers to any disease that affects the cardiovascular system, principally cardiac disease, vascular diseases of the brain and kidney, and peripheral arterial disease. The causes of cardiovascular disease are diverse but atherosclerosis and/or hypertension are the most common, (Kelly, 2010; Dantas, Jimenez-Altayo, & Vila, 2012).

Cardiovascular disease is the leading cause of deaths worldwide especially in low – and middle – income countries. Although cardiovascular disease usually affects older adults, the antecedents of cardiovascular disease, notably atherosclerosis, begin in early life, making primary prevention efforts necessary from childhood. There is therefore increased emphasis on preventing atherosclerosis by modifying risk factors, such as

healthy eating, exercise, and avoidance of smoking tobacco, (McGill, McMahan, & Gidding, 2008; Mendis, Puska, & Norrving, 2011; Finegold, Asaria, & Francis, 2012).

1.2.1 Different Cardiovascular Diseases

1.2.1.1 Coronary Artery Disease

Coronary Artery Disease (CAD) also known as **atherosclerotic heart disease**, **coronary heart disease** or **ischemic heart disease** (IHD) is the most common type of heart disease and cause of heart attacks. The disease is caused by plaque building up along the inner walls of the arteries of the heart, which narrows the arteries and reduces blood flow to the heart, (Bhatia, 2010). IHD presents itself as chest pain or other symptoms at rest, or rapidly worsening angina. The risk of artery narrowing increases with age, smoking, high blood cholesterol, diabetes, high blood pressure, Prinzmetal's angina, (Finegold, Asaria, & Francis, 2012).

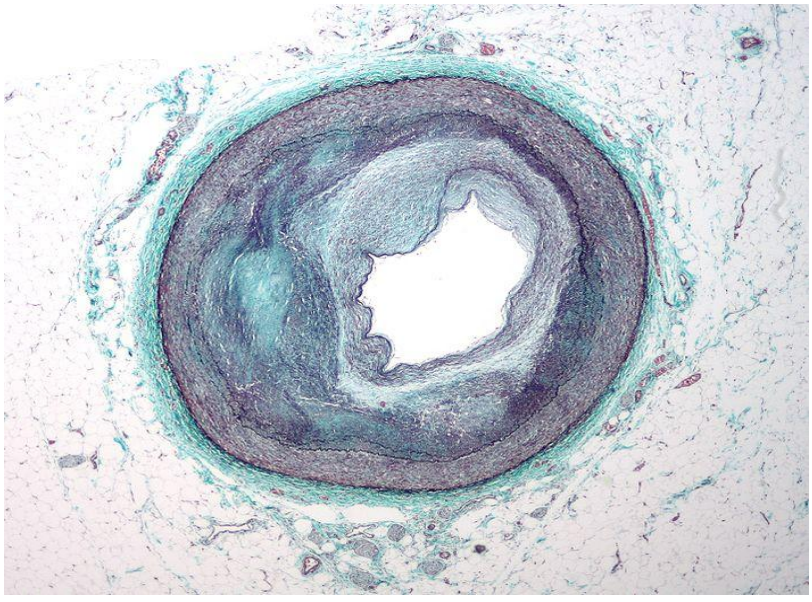

Figure : Micrograph of a coronary artery with the most common form of coronary artery disease (atherosclerosis) and marked luminal narrowing

1.2.1.2 Hypertensive Heart Disease

Hypertensive heart disease includes a number of complications of systemic arterial hypertension or high blood pressure that affect the heart. The 10th revision of the International Classification of Diseases categorized heart failure and other cardiac complications of hypertension one of the most common cause of death, (Alegría-Ezquerra, González-Juanatey, & González-Maqueda, 2006).

The signs and symptoms of hypertensive heart disease are Fatigue, Irregular pulse or palpitations, Swelling of feet and ankles, Weight gain, Nausea, Shortness of breath, Difficulty sleeping flat in bed (orthopnea), Bloating and abdominal pain, Greater need to urinate at night, Altered mentation (in severe cases), and enlarged heart (cardiomegaly), (Alegría-Ezquerra, González-Juanatey, & González-Maqueda, 2006).

Stages of Elevated BP and Hypertension According to The Seventh Report of the Joint National Committee (JNC7) on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, (Chobanian, et al., 2003) are as followed.

Table : Different Stages of Hypertension

Category	Systolic BP (mm Hg)	Diastolic BP (mm Hg)
Optimal	< 120	< 80
Prehypertension	120-139	80-89
Stage I	140-159	90-99
Stage II	>160	>100

1.2.1.3 Heart Failure

Heart failure (HF), Congestive Heart Failure (CHF) and Congestive Cardiac Failure (CCF) are synonymous terms. It occurs when the heart is unable to provide sufficient pump action to maintain blood flow to meet the sufficient requirements of the body. Sign and symptoms include shortness of breath, leg swelling, and exercise intolerance, (Dorland, 2011).

Common causes of heart failure include myocardial infarction and other forms of ischemic heart disease, hypertension, valvular heart disease, and cardiomyopathy. The term heart failure is sometimes incorrectly used for other cardiac – related illnesses, such as myocardial infarction (heart attack) or cardiac arrest, which can cause heart failure but are not equivalent to heart failure, (Dickstein, et al., 2008).

1.2.1.4 Peripheral Vascular Disease

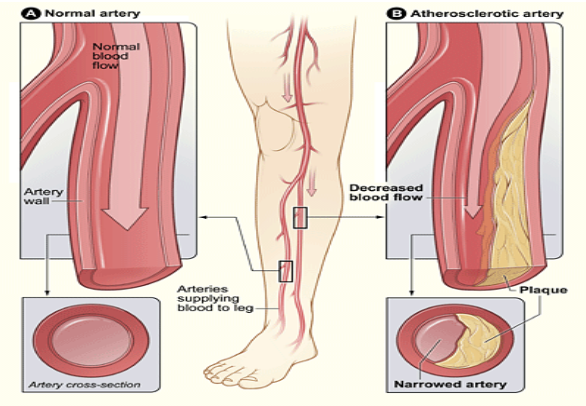

Figure : The illustration shows how PAD affects the arteries in the legs. Figure a show a normal artery with normal blood flow. Figure B shows an artery with plaque buildup that's partially blocking

Peripheral Vascular Disease (PVD) or Peripheral Artery Disease (PAD) refers to the obstruction of large arteries of the periphery of the body. It can result from

atherosclerosis, inflammatory processes leading to stenosis, embolism or thrombus formation. It causes either acute or chronic ischemia (lack of blood supply). Often it is a term used to refer to atherosclerotic blockages found in the lower extremity, (Porter & Kaplan, 2011).

1.2.1.5 Atherosclerosis

Atherosclerosis or Arteriosclerotic Vascular Disease is a specific form of arteriosclerosis in which an artery wall thickens as a result of the accumulation of calcium and fatty materials such as cholesterol and triglyceride. It is a syndrome affecting arterial blood vessels, a chronic inflammatory response in the walls of arteries, caused largely by the accumulation of macrophages and white blood cells and promoted by low-density lipoproteins without adequate removal of fats and cholesterol from the macrophages by functional high-density lipoproteins (HDL). It is commonly referred to as a hardening of the arteries. It is caused by the formation of multiple plaques within the arteries, (Finn, Nakano, Narula, Kolodgie, & Virmani, 2010).

It is a chronic disease that remains asymptomatic for decades. Atherosclerotic lesions or atherosclerotic plaques are separated into two broad categories: Stable and unstable. Stable atherosclerotic plaques are rich in extracellular matrix and smooth muscle cells while, unstable plaques are rich in macrophages and foam cells and the extracellular matrix separating the lesion from the arterial lumen (also known as the fibrous cap) is usually weak and prone to rupture. Ruptures of the fibrous cap expose thrombogenic material, such as collagen to the circulation and eventually induce thrombus formation in the lumen. Upon formation, intraluminal thrombi can occlude

arteries outright, but more often they detach, move into the circulation and eventually occluding smaller downstream branches causing thromboembolism, (Didangelos, Simper, C, & Mayr, 2009).

1.3 Hyperlipidemia

Hypercholesterolemia is an established major cardiovascular risk factor, and the beneficial effect of lipid modifiers, primarily statins, on global vascular risk education is well recognized, and presumed to be largely derived from low density lipoprotein (LDL) cholesterol reduction, (Brugts, Yetgin, & Hoeks, 2009; Lee, Saver, Towfighi, Chow, & Ovbiagele, 2011).

Atherogenic dyslipidemia is a common form of dyslipidemia characterized by three lipid abnormalities: elevated serum triglycerides, small LDL particles, and reduced serum high-density lipoprotein (HDL) cholesterol. High triglyceride and low HDL cholesterol levels have been shown to singly and collectively boost the risk of cardiovascular events independent of conventional risk factors in large cohort studies, (Bansal, Buring, Rifai, Mora, Sacks, & Ridker, 2007; Barter, Gotto, & LaRosa, 2007).

1.4 Cardiovascular Agents

1.4.1 Drugs Acting on Renin – Angiotensin – Aldosterone System

1.4.1.1 ACE Inhibitors

ACE Inhibitors are inhibitors of Angiotensin Converting Enzyme, a membrane bound zinc containing enzyme found on the endothelial cells of vast vascular endothelium of the lungs. The first commercially marketed ACE inhibitor, Captopril prevents the binding interaction of Angiotensin I with ACE. Affinity of Captopril

towards ACE is 30000 times greater than Angiotensin I, (Cutler, 2011, p. 608; Rang, Dale, Ritter, Flower, & Henderson, 2012, p. 275).

Other drugs of this group are Lisinopril, Enalapril, Ramipril, Fosinopril, Quinapril, Trandolapril etc, (Cutler, 2011, pp. 609-612). Clinical uses of these drugs are Hypertension, Cardiac Failure, Cardiac following Myocardial infarction, Ischemic Heart Disease, Diabetic Nephrotrophy and Progressive Renal Insufficiency, (Rang, Dale, Ritter, Flower, & Henderson, 2012).

1.4.1.2 Angiotensin II Receptor Blockers

Losartan, Candesartan, Valsartan, Irbesarten are Angiotensin II Receptor Blockers (ARB). ARBs are prominently used to control hypertension in young patients, diabetic patients and in patients with left ventricular hypertrophy. These are also used in Heart Failure and in Diabetic Nephropathy, (Rang, Dale, Ritter, Flower, & Henderson, 2012).

1.4.1.3 Renin Inhibitors

Renin inhibitors (current commercially available drug is Aliskiren) are unique in their effects on the RAAS. Both ACE inhibition and angiotensin receptor blockade lead to a reactive rise in plasma renin activity (PRA) and thus to an increase in the Angiotensin peptides, both angiotensin I and angiotensin II in the case of angiotensin receptor blockers (ARBs) and angiotensin I with ACE inhibitors. Renin inhibitors, operating at the first and rate-limiting step of the cascade, render the entire pathway quiescent. Because renin is specific for the substrate angiotensinogen, renin inhibitors do not cause stimulation of bradykinin or prostaglandins, (Fisher & Meagher, 2011).

1.4.1.4 Aldosterone Antagonists

Spironolactone is a nonselective aldosterone receptor antagonist that is metabolized extensively in the liver to its active metabolites. Eplerenone is a selective aldosterone receptor antagonist derived from spironolactone but with limited affinity for the progesterone and androgen receptors and therefore lacks sex-related adverse side effects. Clinically, these drugs are used in Hypertension, Congestive Heart Failure and Hyperaldosteronism associated chronic kidney disease, (Maron & Leopold, 2010).

1.4.2 Vasodilators

1.4.2.1 Nitrovasodilators

Nitrovasodilators are a group of drugs with heterogeneous chemical structures such as glyceryl trinitrate, isosorbide dinitrate, isosorbide nitrate, pentaerythritol tetranitrate. These are used for the acute and preventive treatment of myocardial ischemia. In addition inorganic compounds (such as sodium nitroprusside) are also clinically used nitrovasodilators, (Harrison & Bates, 1993).

1.4.2.2 Calcium Channel Blockers

Calcium channel blockers, which dilate arteries by reducing calcium flux into cells, effectively lower blood pressure, especially in combination with other drugs, and some formulations of agents of this class are approved for treating angina or cardiac dysrhythmias. Calcium channel blockers reduce blood pressure across all patient groups, regardless of sex, race ⁄ ethnicity, age, and dietary sodium intake. Calcium channel blockers (CCBs) inhibit the flow of extracellular calcium through ion-specific channels that span the cell wall. Although several types of such channels have been identified,

currently available CCBs inhibit the L-type channels in humans. When inward calcium flux is inhibited, vascular smooth muscle cells relax, resulting in vasodilation and a lowering of blood pressure (BP), (Elliott & Ram, 2011).

1.4.3 Adrenergic Drugs

1.4.3.1 β – Adrenergic Receptor Blockers

β – Blockers are currently recommended as 1st line drug therapy for hypertension in presence of associated diseases such as Myocardial Infarction. Propranolol nonselectively acts at both β_1 and β_2 adrenergic receptors. Selective β_1 receptor blockers, such as metoprolol and atenolol, are commonly prescribed β – blockers, and used cautiously to hypertensive patients who also have asthma. The nonselective β – blockers, such as propranolol and nadolol, are contraindicated due to their blockade of β_2 – mediated bronchodilation. β – Blockers are more effective for treating hypertension in white and young patients, (Clark, Finkel, Rey, Whalen, & Harvey, 2012).

1.4.3.2 α_1 – Adrenergic Receptor Antagonist

Prazosin, doxazosin, and terazosin produce a competitive block of α_1 – adrenoceptors. They decrease peripheral vascular resistance and lower arterial blood pressure by causing relaxation of both arterial and venous smooth muscle. In some individuals, α_1 – Blockers may be used to treat mild to moderate hypertension, (Clark, Finkel, Rey, Whalen, & Harvey, 2012, p. 238).

1.4.3.3 α & β – Adrenergic Receptor Antagonists

Labetalol and Carvedilol block α_1, β_1, and β_2 adrenergic receptors. Carvedilol, although an effective antihypertensive, is mainly used in the treatment of heart failure.

Carvedilol, as well as metoprolol, a selective β_1 antagonist, have been shown to reduce morbidity and mortality associated with heart failure, (Clark, Finkel, Rey, Whalen, & Harvey, 2012, p. 238).

1.4.3.4 Centrally Acting α_2 – Adrenergic Agonists

Clonidine, a centrally acting α_2 – adrenergic agonist used for the treatment of hypertension that has not responded adequately to treatment with two or more drugs. α_2 – methyldopa, another, centrally acting α_2 – agonist is converted to methylnorepinephrine to diminish adrenergic outflow from the CNS. This decreases peripheral resistance and blood pressure. It used for treating hypertensive patients with renal insufficiency. It has been used in hypertensive pregnant patients, (Clark, Finkel, Rey, Whalen, & Harvey, 2012, pp. 238-239).

1.4.4 Antihyperlipidemic Drugs

1.4.4.1 HMG – CoA Reductase Inhibitors

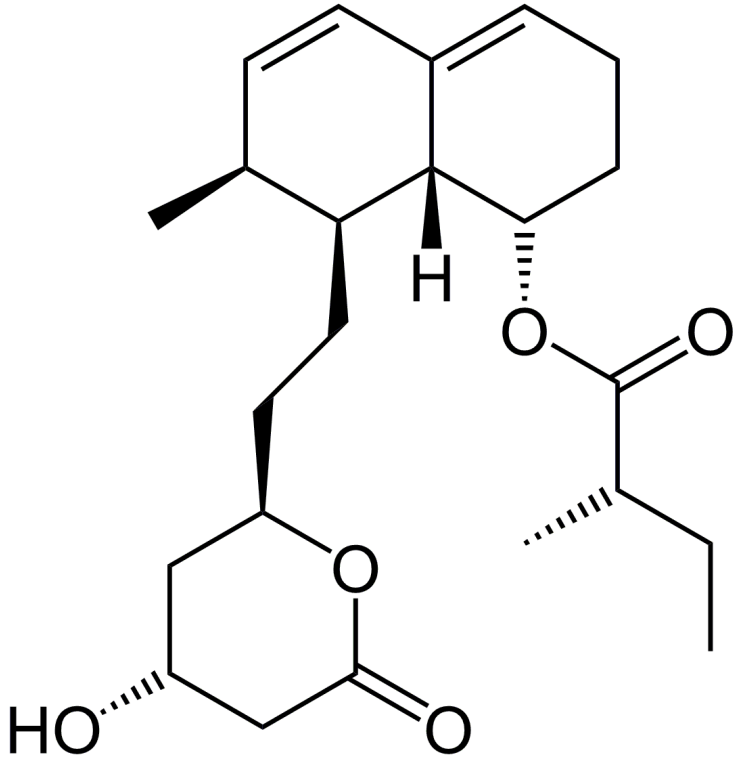

Figure : Chemical Structure of Mevastatin, the prototypic Statin, first isolated from a mold Penicillium citrinum

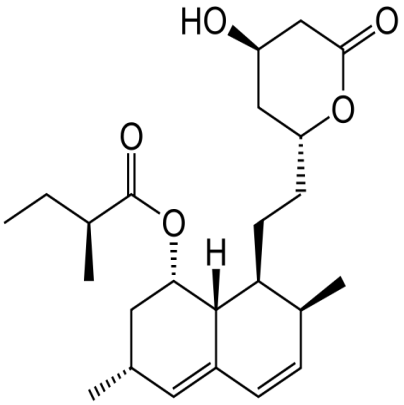

Figure : Chemical Structure of Lovastatin, the first commercially marketed Statin, naturally found in Oyster Mushrooms and first isolated from Aspergillus terreus

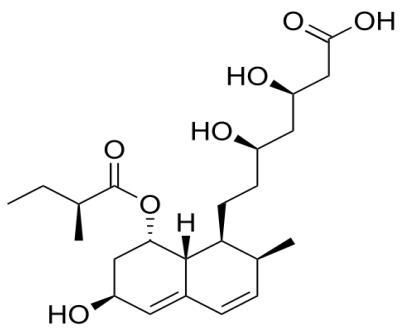

Figure : Chemical Structure of Pravastatin, a derivative of Lovastatin

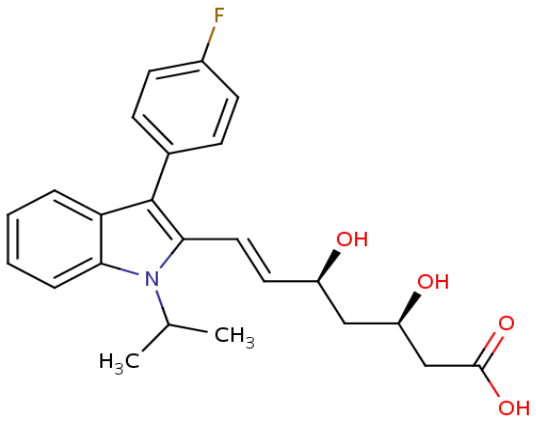

Figure : Chemical Structure of Fluvastatin, another derivative of Lovastatin

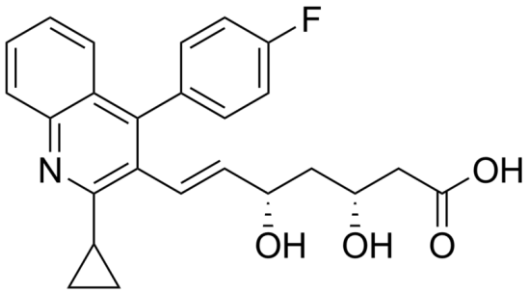

Figure : Chemical Structure of Pitavastatin

Statins were first isolated from a mold, *Penicillium citrinum*, and identified as inhibitors of cholesterol biosynthesis in 1976 by Akira Endo. Brown and Goldstein established that statins act by inhibiting HMG – CoA reductase. The isolated compound

was **Mevastatin**, which was also first statin used for clinical trial on humans and demonstrated the therapeutic potential of this class of drugs. The first statin approved for use in humans was **Lovastatin**, which was isolated from the fungus *Aspergillus terreus*. **Pravastatin** and **Simvastatin** are chemically modified derivatives of **Lovastatin**. **Atorvastatin**, **Fluvastatin**, **Rosuvastatin**, and **Pitavastatin** are structurally distinct synthetic compounds, (Goodman & Gilman, 2010).

Statins are a class of powerful LDL – lowering drugs that are widely used in clinical practice. These agents reduce the risk of essentially every clinical manifestation of the atherosclerotic process. They are easy to administer, with good patient acceptance. And, there are very few drug to drug interactions. According to the United States NCEP, Adult Treatment Panel III report Patients with CHD, other forms of atherosclerotic disease, diabetes mellitus, multiple risk factors imparting high risk, and severe hypercholesterolemia are ideal candidates for these drugs, (Pasternak, Smith, Bairey-Merz, Grundy, Cleeman, & Lenfant, 2002).

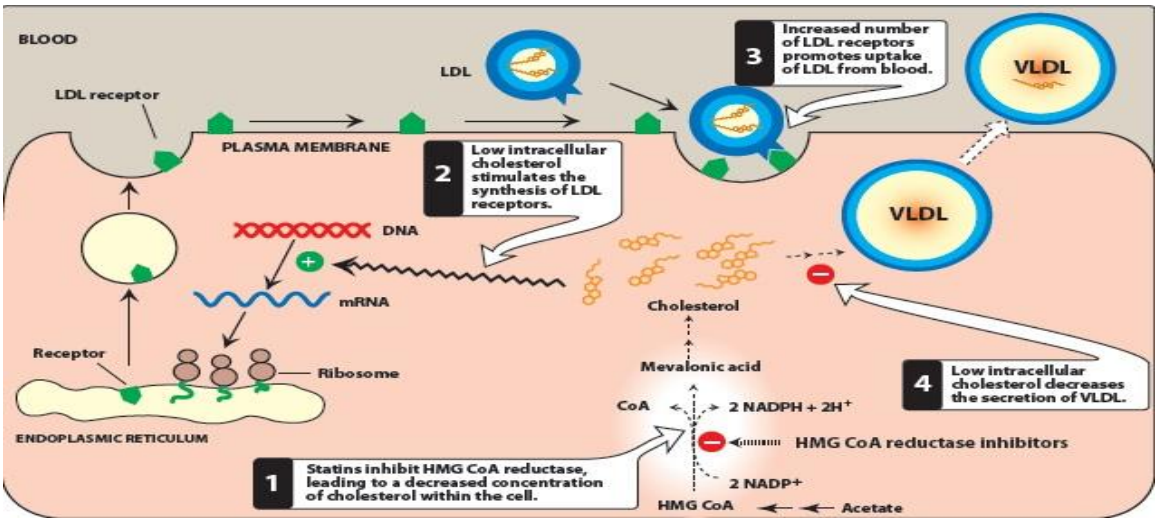

Figure : Mechanism of Action of HMG-CoA Reductase Inhibitors

Statins are analogs of HMG, the precursor of cholesterol. Lovastatin and simvastatin are prodrugs that are hydrolyzed to their active forms. However, Pravastatin and Fluvastatin are active. Due to high affinity, all drugs compete effectively to inhibit HMG – CoA reductase to limit cholesterol synthesis. By inhibiting rate – limiting cholesterol synthesis, they deplete the intracellular supply of cholesterol. Depletion of intracellular cholesterol causes the cell to increase the number of specific cell-surface LDL receptors that can bind and internalize circulating LDLs. Thus, the end result is a reduction in plasma cholesterol, both by lowered cholesterol synthesis and by increased catabolism of LDL, (Clark, Finkel, Rey, Whalen, & Harvey, 2012, pp. 269-270).

1.4.4.2 Fibrates

Fibrates such as Fenofibrate and gemfibrozil are derivatives of fibiric acid. They reduce elevated plasma triglycerides and cholesterol. The effectiveness depends on the patient's pretreatment lipoprotein status and the potency of the fibrate administered. There are 5 major mechanisms of Fibrates that have been identified. These are **1**) **Induction of lipoprotein lipolysis**: Fibrates increases the intrinsic lipoprotein lipase (LPL) activity or accessibility of TRLs for lipolysis by LPL is increased leading to a reduction of TRL content, **2**) **Induction of Hepatic Fatty Acid uptake and Reduction of Hepatic Triglyceride Synthesis**: Inhibition of hormone – sensitive lipase by Fibrates increases fatty acid uptake by fatty acid transporter proteins and converts it into acyl – CoA by acyl – CoA synthetase in the liver. This decreases the availability of Fatty Acids required for triglyceride synthesis, **3**) **Increased Removal of LDL Particles**: Fibrate treatment increases the formation of LDL with a higher affinity for the LDL receptor, which are thus catabolized more rapidly, **4**) **Reduction in Neutral Lipid** (cholesteryl

ester and triglyceride) exchange between VLDL and HDL results from decreased plasma levels of TRL, 5) **Increase in HDL Production and Stimulation of Reverse Cholesterol Transport**: Fibrates increase the production of apo – lipoprotein A – I and apo – lipoprotein A – II in liver which may contribute to the increase of plasma HDL concentrations and a more efficient reverse cholesterol transport, (Staels, Dallongeville, Auwerx, Schoonjans, Leitersdorf, & Fruchart, 2008).

1.4.4.3 Niacin

Niacin or Vitamin B_3 is a naturally occurring human nutrient found in abundance in meat, yeast, vegetables and seeds. Originally isolated in the late 19^{th} century, it was not discovered until the 1930's that deficiencies of this essential nutrient lead to pellagra in man. Subsequently, it has been found to be useful in the treatment of hypercholesterolemia. It is the precursors to the co – enzyme nicotinamide adenine dinucleotide (NAD). It decreases all atherogenic apolipoprotein B (ApoB) by inhibiting diacylglycerol acyltransferase 2 (DGAT2) in the liver. This leads to a decrease in production of triglycerides and ApoB. It also increases all antiatherogenic apolipoprotein AI by inhibiting the HDL apolipoprotein AI catabolism pathway. This leads to an increased production of HDL. Conventional nicotinic acid therapy has notable limitations that include flushing, most often seen with IR formulations, and hepatotoxicity, (Eswaran, Alvey, Fayek, Shah, & Chan, 2013).

1.4.4.4 Cholesterol Absorption Inhibitors

Interruption of absorption of cholesterol from the intestine by pharmacological means reduces the plasma cholesterol concentration, since it is influenced by the level of

intestinal cholesterol absorption. This reduces the concentration of plasma LDL cholesterol which is the latter a risk factor that is tightly linked to the development of atherosclerosis. For example, administration of ezetimibe the only commercially available cholesterol absorption inhibitor reduces LDL cholesterol concentrations by 20%, (Temel, et al., 2013).

Ezetimibe has a mechanism of action that differs from other classes of cholesterol reducing compounds. The molecular target of ezetimibe is the sterol transporter protein, Niemann – Pick C1 – Like 1 (NPC1L1) at the jejunal enterocyte brush border and Ezetimibe then localizes there to inhibit the absorption of cholesterol from the gastrointestinal tract. Ezetimibe therefore inhibits the absorption of cholesterol, leading to a decrease in the delivery of intestinal cholesterol to the liver. This causes a reduction of hepatic cholesterol stores and an increase in clearance of cholesterol from the blood, (Inazawa, et al., 2013).

1.4.4.5 Bile Acid Sequestrants

Cholestyramine, Colestipol and Colesevelam are bile acid sequestrants. These molecules are positively charged non – digestible resins that bind to bile acids in the intestine to form an insoluble complex, which is excreted in the feces. They are used mainly for the treatment of primary hypercholesterolemia and hypercholesterolemia associated with mild hypertriglyceridemia, in patients not responding to dietary treatment as well as a second line-treatment for pruritus associated with cholestatic disease, in patients with incomplete biliary obstruction, (Scaldaferri, Pizzoferrato, Ponziani, Gasbarrini, & Gasbarrini, 2013).

1.4.4.6 ω_3 – Polyunsaturated Fatty Acids (ω_3 – PUFA)

The hypotriglyceridemic effect is the best defined metabolic action of ω_3 PUFAs, with a mechanism likely to be related to activation of peroxisome proliferator – activated receptors. Potential beneficial effects of ω_3 PUFAs are cardiovascular effects (reduction of susceptibility to ventricular arrhythmia, antithrombogenic and antioxidant effect;, retardation of the atherosclerotic plaque growth by reduced expression of adhesion molecules and platelet – derived growth factor and promotion of endothelial relaxation by induction of nitric-oxide production, and mild hypotensive effect, (Siscovick, Raghunathan, & King, 1995; Calabresi, Villa, & Canavesi, 2004).

1.5 Dissolution Test

Dissolution test is the method of determining the amount of release of active ingredient released from a solid oral dosage form, such as a tablet or a capsule by using a specific volume of dissolution medium within a predetermined length of time. All parts of the apparatus that comes in contact with the sample or the dissolution medium must be chemically inert, non – reactive and non – interfering. The assembly of the apparatus is constructed in order to minimize any vibration generated. The Paddle Apparatus consists of a cylindrical glass – vessel with a hemispherical bottom with a capacity of 1000 ml. The vessel is covered with a cover to prevent evaporation of the medium. The cover has holes, one in the centre to accommodate the shaft of the stirrer and others for the thermometer and for devices for withdrawal of liquid. The stirrer consists of a vertical shaft with a blade at the lower end. The blade is constructed around the shaft so that it is flush with the bottom of the shaft. When placed inside the vessel, the shaft's axis is within 2 mm of the axis of the vessel and the bottom of the blade is 25 ± 2mm from the

inner bottom of the vessel. The upper part of the shaft is connected to a motor provided with a speed regulator so that smooth rotation of the stirrer can be maintained without any significant wobble. The apparatus is placed in water – bath that maintains the dissolution medium in the vessel at 37 ± 0.5 °C, (WHO, 2011).

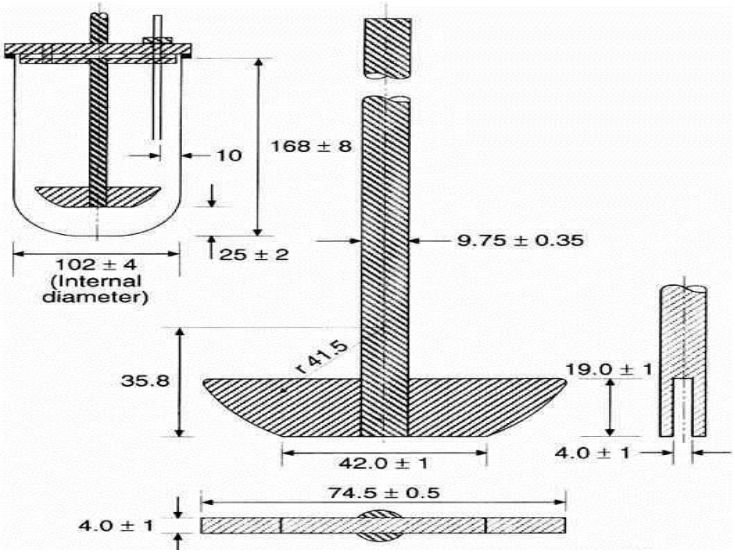

Figure : Dissolution Test Apparatus

CHAPTER 2

DRUG INFORMATION

CHAPTER 2: DRUG INFORMATION

2.1 Atorvastatin

LIPITOR® developed by Pfizer is the commercial brand name of **Atorvastatin**. It was the best selling drug in the World from 2002 to 2009, generating gross revenue of approximately 9.3 billion dollars. Atorvastatin (ATV) calcium is used to reduce the levels of lipoproteins rich in cholesterol and reduce the risk of coronary artery disease. This is due to this drug's inhibitory action on the hydroxy – methyl – glutaryl – CoA reductase (HMG – CoA reductase) enzyme, which is important in cholesterol biosynthesis, (Oliveira, Yoshida, Belinelo, & Valo, 2013).

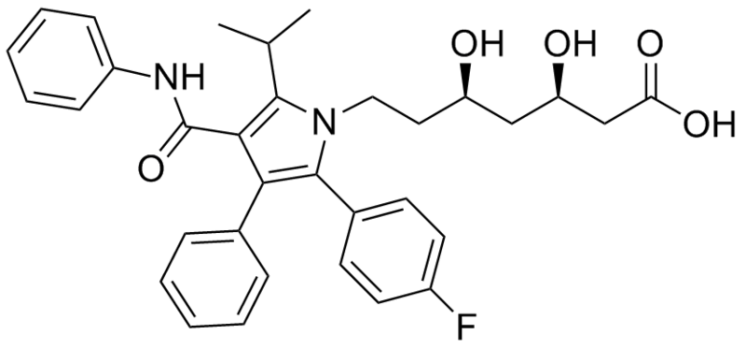

Figure : Two Dimensional Structure of Atorvastatin

2.2 Discovery & Development of Atorvastatin

The HMG-CoA reductase Inhibitors (HMGRI) **Mevastatin** and **Lovastatin** the fungal metabolites and their derivatives **Pravastatin** and **Simvastatin** were discovered and developed respectively in 1980s. **Atorvastatin calcium** currently marketed in the United States as **LIPITOR®** was designed based on molecular modeling comparisons mentioned drugs. In addition, the advancements in parallel synthesis fast – forwarded the

development of the structure – activity relationships which led to atorvastatin calcium in a very short time. Ultimately, several pure enantiomers of **Atorvastatin Calcium** were developed through chiral synthesis, (Lea & McTavish, 2002).

2.3 Chemistry of Atorvastatin

Molecular formula and molecular weight atorvastatin are $\mathbf{C_{66}H_{68}CaF_2N_4O_{10}}$ $\mathbf{.3H_2O}$ and 1,209.4 g/mol respectively. It is a white crystalline powder with a partition coefficient of 6.36 and a dissociation constant of 4.46. The drug is insoluble in aqueous solutions with pH ≤ 4.0, very slightly soluble in water plus phosphate buffer (pH 7.4) and in acetonitrile, slightly soluble in ethanol and very soluble in methanol, (Oliveira, Yoshida, Belinelo, & Valo, 2013).

2.4 Pharmacokinetic Profile of Atorvastatin

2.4.1 Absorption Profile of Atorvastatin

Atorvastatin calcium exhibits high permeability through biological membranes, but its absorption after oral administration is limited by its low dissolution rate due to its very low aqueous solubility. For this, atorvastatin is given in its acid form which is highly soluble and permeable, and in this form it is completely absorbed after oral administration. Upon oral administration, it achieves its maximum plasma concentrations occur within 1 to 2 hours. Extent of absorption increases in proportion to atorvastatin dose. The absolute bioavailability of atorvastatin (parent drug) is approximately 14% and its bioavailability is approximately 30%. This low bioavailability is due to first – pass metabolism in gastrointestinal mucosa and / or Liver, (Lennernäs, 2003; Gubbi & Jarag, 2010).

2.4.2 Distribution of Atorvastatin

Mean volume of distribution of atorvastatin is approximately 381 liters. Atorvastatin is about 98% bound to plasma proteins, (Lennernäs, 2003).

2.4.3 Metabolism of Atorvastatin

Atorvastatin, as well as other statins such as Simvastatin, and Lovastatin, are predominantly metabolized by CYP3A4. Atorvastatin is extensively metabolized to ortho – and para – hydroxylated derivatives and various beta – oxidation products. In vitro inhibition of HMG-CoA reductase by ortho- and parahydroxylated metabolites is equivalent to that of atorvastatin. Approximately 70% of circulating inhibitory activity for HMG-CoA reductase is attributed to active metabolites, (Park, et al., 2012).

2.4.4 Excretion Profile of Atorvastatin

Atorvastatin and its metabolites are eliminated primarily in bile following hepatic and / or extra – hepatic metabolism. However, the drug undergoes three enterohepatic recirculations. Mean plasma elimination half – life of atorvastatin in humans is approximately 14 hours, but the half – life of inhibitory activity for HMG – CoA reductase is 20 to 30 hours due to the contribution of active metabolites. Less than 2% of a dose of atorvastatin is recovered in urine following oral administration, (Park, et al., 2012; Goodman & Gilman, 2010).

2.5 Therapeutic Uses of Atorvastatin

Atorvastatin is primarily used for treating dyslipidemia and to prevention of cardiovascular diseases. It is recommended to be used only after other measures such as diet, exercise, and weight reduction have not improved cholesterol levels, (McCrindle,

Ose, & Marais, 2003). The drug is used also to treat Hypercholesterolemia and mixed dyslipidemia to reduce total cholesterol, LDL – C, apo – B, triglycerides levels, as well as to increase HDL levels, (Nissen, et al., 2006).

Atorvastatin is also for secondary prevention in people with CHD and multiple risk factors for MI, Angina pectoris. It is best for preventing MI and stroke prophylaxis in Type II Diabetic Patients. Atorvastin can be used in combination with bile acid sequestrants, (Ozaki, et al., 2006; Neil, et al., 2006).

2.6 Adverse Effects of Atorvastatin

Hepatotoxicity, Liver Failure, Myopathy and Rhabdomyolysis are some the serious side -effects of Atorvastatin. The Others are weakness, Insomnia and dizziness, Chest pain, peripheral edema, Rash, Abdominal pain, Constipation, Diarrhea, Dyspepsia, Flatulence, Nausea, Urinary tract infection, Arthralgia, myalgia, back pain, Arthritis, Sinusitis, pharyngitis, bronchitis, rhinitis, (Goodman & Gilman, 2010; Park, et al., 2012).

2.7 Contra – indications of Atorvastatin

Atorvastatin is contra – indicated in patients with liver dysfunction and active liver diseases such as Cholestasis, Hepatic Encephalopathy, Hepatitis and Jaundice and in liver conditions involving unexplained level of Aspartate transaminase, Alanine transaminase. The drug must be avoided during pregnancy and in breast – feeding period, because cholesterol and its various products are important for fetal development, (Williams & Feely, 2002).

Atorvastatin must be discontinued immediately when diagnosed with rhabdomyolysis, since it can lead to acute renal failure due to myoglobinuria, (Hermann, et al., 2012).

2.8 Drug Interactions of Atorvastatin

2.8.1 Drug – Drug Interactions

Co – administration of Atorvastatin with Fibrates (e.g. clofibrate, fenofibrate, gemfibrozil) in hypercholesterolemia increases the risk of myopathy and rhabdomyolysis. This because, the fibrates inhibits 1) uptake of the active hydroxy acid forms of statins into hepatocytes by and 2) interferes with the transformation of most statins by glucuronidases. This nearly doubles the plasma concentration of the statin hydroxy acids, (Steiner, 2007; Goodman & Gilman, 2010).

Co – administration of atorvastatin with any of the CYP3A4 inhibitors such as itraconazole, telithromycin, and voriconazole, may increase serum concentrations of atorvastatin, leading to adverse reactions. Chances are less with other CYP3A4 inhibitors such as diltiazem, erythromycin, fluconazole, ketoconazole, clarithromycin, cyclosporine, (Neuvonen, Niemi, & Backman, 2006).

However, CYP3A4 inducers such as bosentan, fosphenytoin, and phenytoin, barbiturates, carbamazepine, efavirenz, nevirapine, rifampin, and rifamycin can decrease the plasma concentrations of atorvastatin, leading to therapeutic failure. Oral contraceptives increased AUC values for norethindrone and ethinyl estradiol; these increases should be considered when selecting an oral contraceptive for a woman taking atorvastatin, (Backman, Luurila, Neuvonen, & Neuvonen, 2005).

Vitamin D supplementation lowers atorvastatin and active metabolite concentrations, yet synergistically reduces LDL and total cholesterol concentrations, (Schwartz, 2009).

2.8.1.1 Drug – Drug Interaction with Amidarone

Amidarone is a CYP3A4 inhibitor, the enzyme responsible for the metabolism of Atorvastatin. Co – administration of atorvastatin with Amidarone decreases its metabolism, thus increasing the risk of myopathy caused by atorvastatin. When such cases cannot be avoided, dose of atorvastatin should be limited, (Williams & Feely, 2002; Wyeth, 2011).

2.8.1.2 Drug – Drug Interaction with Azole Antifungals

Itraconazole, Ketoconazole, Posaconazole and voriconazole are strong inhibitors of CYP3A4 enzyme. Co – administration of these drugs with atorvastatin reduces its metabolism resulting in increased risk of myopathy and rhabdomyolysis. In such cases atorvastatin must be stopped during itraconazole treatment, while dose of atorvastatin should be reduced is used with ketoconazole, posaconazole and voriconazole, (PL, 2009; NIH, 2007; Pfizer, 2011). Although Fluconazole is moderate inhibitor of CYP3A4, it advised that dose of atorvastatin should be reduced to 50% during co – administration with fluconazole, (Shaukat, Benkli, Vladutiu, Slack, Wetzer, & Baer, 2003).

2.8.1.3 Drug – Drug Interaction with Bile – acid Sequestrants

Bile – acid Sequestrants such as Cholestyramine and Colestipol decrease the bioavailability of atorvastatin due to the drug – drug complexation between them in the

intestine. It is recommended that there should be at least 2 hrs difference between atorvastatin administration and Bile – acid sequestrant administration, (Pfizer, 2011).

2.8.1.4 Drug – Drug Interaction with Calcium Channel Blockers

Calcium Channel Blockers such as amlodipine, diltiazem and verapamil are CYP3A4 inhibitors and thus their co – administration with atorvastatin increases the risk of myopathy and rhabdomyolysis. In such cases, atorvastatin doses should be limited or therapy should be done with calcium channel blockers that do not inhibit atorvastatin metabolism, (Neuvonen, Niemi, & Backman, 2006).

2.8.1.5 Drug – Drug Interaction with Colchicine

Colchicine is a strong p – glycoprotein inhibitor, thus it increases the plasma level of free atorvastatin. Thus, it can increase the risk of myopathy cause by atorvastatin, (Bristol-Myers, 2012).

2.8.1.6 Drug – Drug Interaction with Cyclosporine

Cyclosporine in an inhibitor of both CYP3A4 enzyme (major metabolic enzyme for Atorvastatin) and P – glycoprotein (the plasma binding protein for atorvastatin), thus it increases the plasma level of free atorvastatin as well as decreases the level of metabolism of it significantly. This increases the risk of myopathy. And, since cyclosporine itself causes myopathy, co – administration of both drugs is highly contra – indicated, (Williams & Feely, 2002; FDA, FDA Safety communication: important safety label changes to cholesterol-lowering statin drugs, 2012).

2.8.1.7 Drug – Drug Interaction with Danazol

Danazol is a strong inhibitor of CYP3A4 enzyme. Thus, co – administration of both danzol and atorvastatin decreases its rate of metabolism leading to increased risk of myopathy and rhabdomyolysis, (Williams & Feely, 2002).

2.8.1.8 Drug – Drug Interaction with Digoxin

Steady sate plasma concentration of digoxin increases by 20% when co – administered with atorvastatin, because 98% of atorvastatin in plasma remains bound to p – glycoprotein resulting in increased level of digoxin. In such cases, digoxin effect and it serum level should be monitored at initiation of therapy and after dose adjustment of both of the drugs, (Boyd, Stern, & Stewart, 2000).

2.8.1.9 Drug – Drug Interaction with Macrolide Antibiotics

Macrolide Antibiotics such as Erythromycin and Clarithromycin are inhibitors of CYP3A4 enzyme, the major metabolic enzyme for atorvastatin. This increases the risk of myopathy and rhabdomyolysis cased by atorvastatin upon co – administration. In such cases, dose of atorvastatin should not exceed 20 mg with clarithromycin and erythromycin. And, it is best to change the macrolide into azithromycin to avoid the interaction due to low binding, (Williams & Feely, 2002; Pfizer, 2011; Westphal, 2000).

2.8.1.10 Drug – Drug Interaction with Protease Inhibitor

Protease inhibitors such as atazanavir, boceprevir, darunavir, fosamprenavir, indinavir, ritonavir, nelfinavir, saquinavir, telaprevir, tipranavir are very strong inhibitors of CYP3A4 enzyme the major metabolic enzyme for atorvastatin. Thus upon co –

administration, they significantly reduce the rate of atorvastatin metabolism and increases the risk of myopathy and rhabdomyolysis caused by atorvastatin, (PL, 2009).

Atorvastatin therapy must be stopped in case of ritonavir, telaprevir and tipranavir, (FDA, FDA drug safety communication: interactions between HIV and Hepatitis C drug and cholesterol-lowering statin drugs can increase the risk of muscle injury, 2012). In case of co – administration with azatanavir and indinavir, atorvastatin dose must not exceed 10 mg, while for saquinavir, darunavir, fosamprenavir and boceprevir, the dose must not exceed 20 mg, but for nelfinavir, dose of atorvastatin can be way up to 40 mg, (Pfizer, 2011).

2.8.1.11 Drug – Drug Interaction with Telithromycin

Telithromycin is a strong CYP3A4 inhibitor and co – administration with atorvastatin increases the risk of myopathy and rhabdomyolysis caused by atorvastatin, (PL, 2009).

Atorvastatin should be stopped during telithromycin therapy. In case of long – term telithromycin therapy pravastatin, rosuvastatin or Fluvastatin can be used instead of atorvastatin, (FDA, FDA Safety communication: important safety label changes to cholesterol-lowering statin drugs, 2012).

2.8.1.12 Drug – Drug Interaction with Rifampin

Rifampin is a cytochrome p450 inducer. Simultaneous administration of rifampin and atorvastatin increases the AUC of atorvastatin by 196%. If rifampin is administered

first, then plasma level of atorvastatin drops by 80% leading to therapeutic failure, (Pfizer, 2011).

2.8.2 Drug – Food Interactions

Co – administration of grapefruit juice has the potential to increase plasma concentrations of HMG – CoA reductase inhibitors including atorvastatin. The equivalent of 1.2 litres per day resulted in a 2.5 fold increase in AUC of atorvastatin. Consumption of excessive grapefruit juice with atorvastatin is not recommended, (Pfizer, 2011).

CHAPTER 3

LITERATURE REVIEW

CHAPTER 3: LITERATURE REVIEW

3.1 Study of the Pharmacokinetics of Atorvastatin and its metabolites in Hypercholesterolaemic Haemodialysis Patients

The objective is to study the Pharmacokinetics of atorvastatin and its metabolites after single and multiple dosing in hypercholesterolaemic haemodialysis patients. For this, hypercholesterolaemic haemodialysis patients received 40 mg or 80 mg atorvastatin once daily, first as a single dose and then continuously for 2 weeks. Plasma levels of atorvastatin and its active and inactive metabolites were measured and pharmacokinetic parameters were compared between single and multiple dosing, and between the different doses. The pharmacokinetic parameters of the parent drug atorvastatin acid were not significantly different after single and 2 week multiple dosing. Dose proportionality and absence of accumulation was also observed for the major active metabolite ortho – hydroxy – atorvastatin and the inactive metabolites atorvastatin lactone and ortho – hydroxy – atorvastatin lactone, but the levels of the active metabolite were relatively lower. Haemodialysis did not cause enhanced clearance of atorvastatin or its metabolites, the drug was well tolerated and there were no serious adverse events, (Lins, et al., 2003).

3.2 Long – term Study of Atorvastatin & Placebo in Serum Cholesterol Reduction

The objective of the study was to find out whether or not reduction in blood plasma cholesterol produces any benefit in the prevention of CHD in patients with hypertension and hyperdyslipidemia in long term administration of Atorvastatin. For this, 20610 hypertensive patients aged between 40 to 70 years with at least three other cardiovascular risk factors already receiving two of the antihypertensive were divided

into 2 groups. 10305 patients with non – fasting total cholesterol concentrations 6·5 mmol / L or less were randomly received additional atorvastatin 10 mg or placebo for 5 years. These patients formed the lipid-lowering arm of the study. Treatment was stopped after a median follow-up of 3·3 years. By that time, 100 primary events had occurred in the atorvastatin group compared with 154 events in the placebo group. This benefit emerged in the first year of follow-up. The total cardiovascular events were 389 vs 486. There were 185 deaths in the atorvastatin group and 212 in the placebo group. And, Atorvastatin lowered total serum cholesterol by about 1·3 mmol/L compared with placebo at 12 months, and by 1·1 mmol/L after 3 years of follow-up, (Sever, et al., 2003).

3.3 Pharmacokinetic Co – administration experiments with Atorvastatin and Rifampicin

Pharmacokinetic co – administration experiments with atorvastatin and rifampicin in rats were performed to investigate the potential involvement of hepatic uptake transporters during hepatic drug elimination. Pharmacokinetic parameters were compared between Atorvastatin control and Rifampicin – treatment groups following oral (10 mg / kg) and IV (2 mg / kg) administration to rats in the absence (Control group) and presence (Treatment group) of a single intravenous dose of Rifampicin (20 mg / kg). Rifampicin markedly increased the plasma concentrations of atorvastatin and its metabolites upon oral administration. The AUC Atorvastatin also increased significantly after IV dosing of Atorvastatin with Rifampicin, but the extent was much less than that oral Atorvastatin dosing. Significant increases in plasma levels were observed for both metabolites as well, (Lau, Okochi, Huang, & Benet, 2006).

3.4 Study on Once – daily Atorvastatin Patient Adherence

The objective of this study was to estimate the effect of a pharmaceutical care program on the adherence of once-daily Atorvastatin treatment in patients with elevated cholesterol levels in Belgium. 392 Hyperdyslipidemic patients were subjected to Atorvastatin for at least 3 months. 'Adherence' was defined as the proportion of days during which the electronic device record showed that the patient had taken the daily dose. Around 6.5% patients were adhered to the Once – daily treatment regimen for all of the 3 months, (Vrijens, Belmans, Matthys, De Klerk, & Lesaf, 2006).

3.5 Study of the Effect of Atorvastatin in Patients with NAFLD

The goal of the study is to evaluate the effectiveness and safety of atorvastatin in dyslipidemic, non – alcoholic fatty liver patients. For this 25 hyperdyslipidemic patients with NAFLD aged between 37 to 47 years were enrolled where 22 (14 men and 8 women) of them completed the study receiving 10 – 80 mg dose of Atorvastatin daily according to serum cholesterol level. The target of the study was to normalize liver transaminases and / or improve liver. After 6 months of treatment, eight patients (36.3%) presented normal transaminase levels. The remaining patients continued treatment for 12 months when 20% of patients presented with normal transaminase levels. Mean cholesterol levels were 268.5±44.2 and 186.8±14.4 mg/dL before and after treatment, respectively. No side effects were reported. Serum aminotransferase and lipid levels were reduced significantly in all patients with atorvastatin treatment. Therapy with atorvastatin in NAFLD patients with hyperlipidemia was found to be both effective and safe, (Gomez-Dominguez, Gisbert, Moreno-Monteagudo, Garcia-Buey, & Moreno-Otero, 2006).

3.6 Study of Anti – viral Activity of Atorvastatin against HCV

Cholesterol biosynthesis is a vital part of HCV RNA replication. *In vitro* studies have showed that several HMG – CoA reductase inhibitors can decrease HCV RNA replication. Therefore, a clinical trial was designed to evaluate the effect of atorvastatin on HCV RNA levels. In this prospective clinical trial, 10 HCV – infected patients who required treatment for high cholesterol were given 20 mg atorvastatin per day. Although serum cholesterol and LDL predictably decreased significantly, there was no statistically significant change in HCV RNA levels. It is unclear whether the addition of an HMG-CoA reductase inhibitor to interferon or a more potent inhibitor of cholesterol biosynthesis may be required to inhibit HCV RNA replication *in vivo*. In conclusion, atorvastatin does not inhibit HCV RNA replication *in vivo* at conventional doses, (O'Leary, Chan, McMahon, & Chung, 2007).

3.7 Study of Co – administration of Atorvastatin with CYP3A4 Inhibitors

The objective of the study was to detect co – prescriptions of CYP3A4 inhibitors such as diltiazem, verapamil, clarithromycin, erythromycin, fluconazole, itraconazole and ketoconazole with simvastatin or atorvastatin in community pharmacies and to assess the risk – preventive actions taken by the prescribing physicians who were alerted about the co – prescription by the pharmacist. The study was performed during four separate 6 weeks and involved 110 Norwegian community pharmacists (25 – 30 in each period). As co – prescription of the selected CYP3A4 inhibitors with either simvastatin or atorvastatin was detected, pharmacist alerted the prescribing physician about the co – prescription as well as information on possible strategies to minimize the risk. In total, 245 co – prescriptions of CYP3A4 inhibitors with simvastatin (134 events) or atorvastatin

(111) were detected. Diltiazem (86 events), verapamil (72), erythromycin (48) and clarithromycin (29) were the most commonly coprescribed CYP3A4 inhibitors. Physicians were informed in 68.6% of cases. And, the prescription was subsequently changed in 59.5%. Another 50 physicians consulted with the patient and monitor potential adverse effects in 29.8% cases. Nine out of ten physicians changed prescriptions or monitored potential adverse effects when informed by community pharmacists about the risk associated with co – prescription of CYP3A4 inhibitors with simvastatin or atorvastatin. This suggests that an important risk factor for myotoxicity due to these statins could be minimized through interdisciplinary co – operation, (Molden, Skovlund, & Braathen, 2008).

3.8 Study of Co – administration of Dabigatran etexilate & Atorvastatin

The objective is to evaluate the potential impact of atorvastatin co – administration on the pharmacokinetics, pharmacodynamics, and safety of dabigatran etexilate, an oral direct thrombin inhibitor used for the prophylaxis of thromboembolism. For this, 22 healthy male and female volunteers were recruited. They received dabigatran etexilate 150 mg twice daily on days 1 to 3 and once daily on day 4 and received atorvastatin 80 mg once daily on days 1 to 4, or both treatments together on days 1–4. In Co – administration of Atorvastatin and dabigatran etexilate, 1) AUC of dabigatran etexilate was reduced by 18%; plasma concentration of atorvastatin was increased by 18% and 15% and 30% for its 2’ – hydroxy and 4’ – hydroxy metabolite. 8 subjects in the dabigatran treatment group, 6 subjects in the atorvastatin treatment group and 6 subjects during combination treatment reported reversible adverse events such as dizziness, headache and fatigue. Results of this study showed that atorvastatin had no influence on

the pharmacokinetic/ pharmacodynamic profile of dabigatran, and vice versa, (Stangier, Rathgen, Stahle, Reseski, Kornicke, & Roth, 2009).

3.9 Study of the Effect of Grapefruit Juice on Pharmacokinetic Profile of Atorvastatin & Pitavastatin

The aim of the study was to compare the effects of grapefruit juice (GFJ) on the pharmacokinetics of Atorvastatin and Pitavastatin in a randomized, 4 phase crossover study. For this, 8 healthy subjects consumed either GFJ or water twice – a – day for 4 days in each trial. On each final day, a single dose of 4 mg Pitavastatin or 20 mg atorvastatin was administered. GFJ increased the mean AUC of atorvastatin acid by 83% and that of pitavastatin acid by 13%. Pitavastatin, unlike atorvastatin, appears to be scarcely affected by the CYP3A4 – mediated metabolism, (Ando, et al., 2010).

3.10 Dissolution Study of Different Brands of Atorvastatin

The aim of the study was to evaluation the in vitro equivalence under biowaiver conditions of atorvastatin tablets marketed in Bangladesh. Drug release was compared with that of a reference product. The in vitro equivalence test was carried out in three different media, hydrochloric acid solution (pH 1.2), Acetate buffer solution (pH 4.5), and Phosphate buffer solution (pH 6.8). Test results were subjected to statistical analysis to compare the dissolution profiles. Model – independent approaches of difference factor (f_1), similarity factor (f_2), and dissolution efficiency (%DE) were employed. Dissolution profiles of test and reference atorvastatin were equivalent at pH 6.8 without statistical treatment. The test products were equivalent at pH 4.5 and not equivalent at pH 1.2, (Akter, Dewan, Parvin, & Islam, 2012).

3.11 Study to enhance the solubility and dissolution rate of Atorvastatin calcium by Co – solvent evaporation technique

The aim of the study was to enhance the solubility and dissolution rate of Atorvastatin calcium by co – solvent evaporation technique. For this, HPMC E5 LV and MCC were used to prepare solid dispersions (SD) of Atorvastatin calcium by rotary evaporation method in 1:1 and 1:2 ratios and by physical mixture in the ratio of 1:2. Solid dispersions obtained by both methods were characterized by solubility studies, scanning electron microscopy which inferred that the pure drug was crystalline while the drug in SD was in amorphous form. Drug release from Solid Dispersion prepared by solvent evaporation method was increases with increasing concentration of the polymer. Tablets prepared by the Solid Dispersion of atorvastatin calcium and HPMC in 1:2 ratio showed maximum percentage drug release (98.3%). Optimized formulation compared with marketed product was found bioequivalent, (Geethalakshmi, Divya, & Mahalingan, 2013).

3.12 Identification of Atorvastatin metabolites in rat hepatocytes by using UPHPLC

The purpose of the study was identification of Atorvastatin metabolites in rat hepatocytes their comparison with electrochemically generated oxidation products. Atorvastatin was incubated with rat hepatocytes for 24 h. Electrochemical oxidation of AT was performed by use of a three – electrode offline system with a glassy carbon working electrode. Atorvastatin undergoes oxidation by a single irreversible process at approximately +1.0 V vs. Ag / AgCl electrode. The results obtained revealed a simple and relatively fast way of determining the type of oxidation and its position.

Unfortunately, different products were obtained by electrochemical oxidation and biotransformation of Atorvastatin. High-mass-accuracy measurements combined with different UHPLC–MS–MS scans enable rapid identification of drug – related compounds. β – Oxidation, aromatic hydroxylation, sulfation, lactone and glycol products were observed in rat biotransformation samples. In contrast, a variety of oxidation reactions on the conjugated skeleton of isopropyl substituent of Atorvastatin were identified as products of electrolysis, (Jirásko, Mikysek, Chagovets, Vokřál, & Holčapek, 2013).

3.13 Study of the Role of Atorvastatin on Progenitor Cell Mobilization

The aim is to study the role of Atorvastatin on progenitor cell mobilization with a focus on bone metabolism. For this, $FGF2^{-/-}$ and wild type mice were treated with atorvastatin or placebo. In contrast to wild type, the number of positive progenitor cells in peripheral blood (PB) in atorvastatin treated $FGF2^{-/-}$ mice did not increase, and was accompanied by a defective reendothelialization after injury of the common carotid artery. In wild type, Atorvastatin treatment was associated with increased levels of Receptor Activator of NF-κB ligand (RANKL) in bone marrow supernatant. To measure the release of positive progenitor cells from the bone marrow, in situ perfusion experiments on isolated hind limbs was performed. Atorvastatin treatment increases RANKL levels with no measurable effect on bone metabolism and mobilization of progenitor cells from Bone Marrow to Peripheral Blood, (Steinmetz, Pelster, Lucanus, Arnal, Nickenig, & Werner, 2013).

Table : List of Literature Review

Article Title	**Author(s)**	**Source**	**Year**
Pharmacokinetics of atorvastatin and its metabolites after single and multiple dosing in hypercholesterolaemic haemodialysis patients	Lins, R. L., Matthys, K. E., Verpooten, G. A., Peeters, P. C., Dratwa, M., Stolear, J.-C., et al.	*Nephrology Dialysis Transplant*	2003
Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial—Lipid Lowering Arm (ASCOT-LLA): a multicentre randomise.	Sever, P. S., Dahlof, B., Poulter, N. R., Wedel, H., Beevers, G., Caulfield, M., et al.	*The Lancet*	2003
Pharmacokinetics of Atorvastatin and Its Hydroxy Metabolites in Rats and the Effects of Concomitant Rifampicin Single Doses: Relevance of First-Pass Effect from Hepatic Uptake Transporters, And Intestinal and Hepatic Metabolism.	Lau, Y. Y., Okochi, H., Huang, Y., & Benet, L. Z	*Drug Metabolism and Disposition*	2006
Effect of intervention through a pharmaceutical care program on patient adherence.	Vrijens, B., Belmans, A., Matthys, K., De Klerk, E., & Lesaf, E.	*Pharmacoepidemiological Drug Safety*	2006
A pilot study of atorvastatin treatment in dyslipemid, non-alcoholic fatty liver patients.	Gomez-Dominguez, E., Gisbert, J. P., Moreno-Monteagudo, J. A., Garcia-Buey, L., & Moreno-Otero, R.	*Alimentary Pharmacology & Therapeutics*	2006
Atorvastatin Does Not Exhibit Antiviral Activity Against HCV at Conventional Doses: A Pilot Clinical Trial.	O'Leary, J. G., Chan, J. L., McMahon, C. M., & Chung, R. T.	*Hepatology*	2007

Table : List of Literature Review (Contd.)

Article Title	Author(s)	Source	Year
Risk Management of Simvastatin or Atorvastatin Interactions with CYP3A4 Inhibitors	Molden, E., Skovlund, E., & Braathen, P.	*Drug Safety*	2008
Coadministration of Dabigatran Etexilate and Atorvastatin	Stangier, J., Rathgen, K., Stahle, H., Reseski, K., Kornicke, T., & Roth, W.	*Americal Journal of Cardiovascular Drugs*	2009
Effects of grapefruit juice on the pharmacokinetics of pitavastatin and atorvastatin	Ando, H., Tsuruoka, S., Yanagihara, H., Sugimoto, K.-i., Miyata, M., Yamazoe, Y., et al.	*British Journal of Clinical Pharmacology*	2010
Evaluation of In Vitro Equivalence for Tablets Containing the Poorly Water- Soluble Compound Atorvastatin	Akter, F. P., Dewan, I., Parvin, M. N., & Islam, S. M.	*Dissolution Technologies*	2012
Enhhancement of Solubility and Dissolution Rate of Atorvastatin Calcium by by Co-solvent Evaporation.	Geethalakshmi, A., Divya, V., & Mahalingan, K.	*World Journal of Pharmacy and Pharmaceutical Sciences*	2013
Structural characterization of electrochemically and in vitro biologically generated oxidation products of atorvastatin using UHPLC/MS/MS.	Jirásko, R., Mikysek, T., Chagovets, V., Vokřál, I., & Holčapek, M.	*Analogy of Bioanalytical Chemistry*	2013
Atorvastatin-induced increase in progenitor cell levels is rather caused by enhanced receptor activator of NF-kappaB ligand (RANKL) cell proliferation than by bone marrow mobilization.	Steinmetz, M., Pelster, B., Lucanus, E., Arnal, J. F., Nickenig, G., & Werner, N.	*Journal of Molecular and Cellular Cardiology*	2013

CHAPTER 4

MATERIALS & METHODS

CHAPTER 4: MATERIALS & METHODS

4.1 Significance of the Study

The objective of the study was to discover the effect on the dissolution profile of atorvastatin in presence of Pantoprazole and Calcium carbonate in *in vitro* dissolution medium. Since, drug – drug interaction profile of atorvastatin is well established, several prescriptions prescribed by the physicians were surveyed & photographed to find out the drugs co – administered with atorvastatin. It was discovered that atorvastatin was frequently co – administered with Pantoprazole and Calcium carbonate. Thus, it was determined to study the dissolution profile of the atorvastatin in presence of Pantoprazole and calcium carbonate. A photograph of one of the prescriptions is given below.

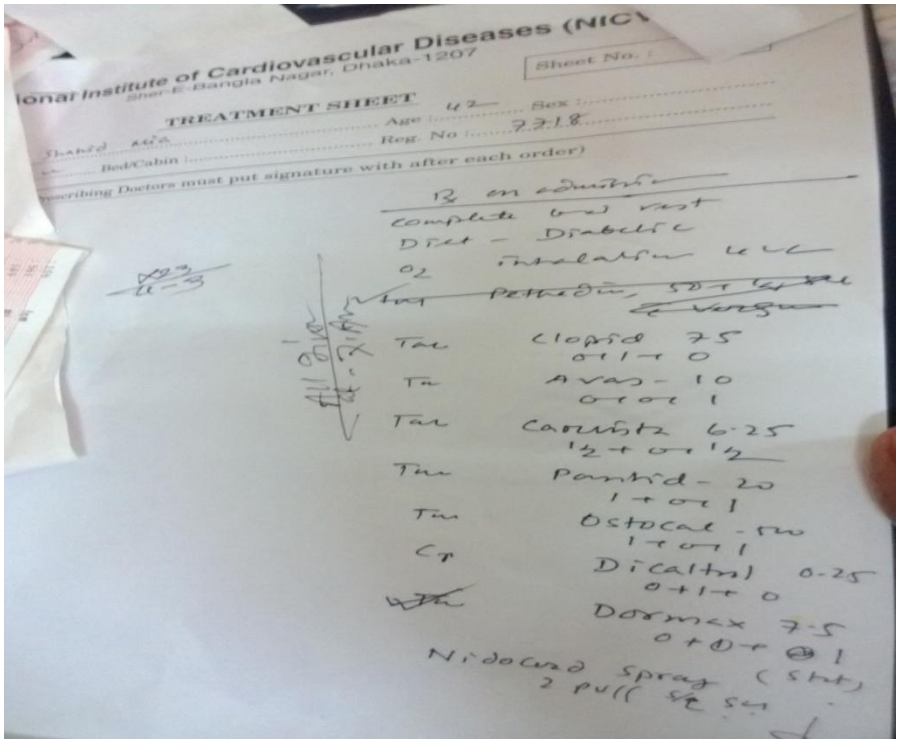

Figure : Prescription Showing Co – treatment of Atorvastatin with Pantoprazole and Calcium carbonate

4.2 Method of Dissolution Test

The release rate of Atorvastatin Calcium Tablets with Omeprazole and Calcium Tablets was determined by using Tablet Dissolution Tester USP XXIII. The dissolution test was performed by using 900 ml phosphate buffer (pH 6.8) at 37°C ± 0.5°C and 100 rpm. At every 5 min interval samples of 6 ml were withdrawn from the dissolution medium and that amount was replaced with fresh medium to maintain the volume constant. The samples were filtered through a Whatman No. 1 filter paper and diluted to a suitable concentration with distilled water. The absorbance of the solutions was measured at 242 nm for drug Atorvastatin Calcium by using a Shimadzu UV – 1201 UV / Visible double beam spectrophotometer (Shimadzu, Japan). Percentage of drug release was calculated using an equation obtained from the standard curve. The dissolution study was continued for 30 min to get a simulated picture of the drug release in the *in vivo* condition and drug dissolved at specified time periods was plotted as percent release versus time (hrs,) curve. The % Dissolution of Atorvastatin alone and Atorvastatin with Omeprazole & Calcium Tablets was calculated by using the equation below –

$$\textbf{\% Dissolution} = \frac{\mathbf{a}}{\mathbf{A}} \times \frac{\mathbf{P \times W}}{\mathbf{D}} \times \frac{\mathbf{900}}{\mathbf{100}} \times \mathbf{100\ \%} = \frac{\mathbf{a}}{\mathbf{0.11}} \times \frac{\mathbf{0.97 \times 0.11}}{\mathbf{10}} \times \mathbf{9 \times 100\ \%}$$

Here,

P = Potency = 97 % = 0.97

A = Specific Absorbance of Atorvastatin Standard = 0.11

a = UV Absorbance of Atorvastatin Samples taken at different time intervals

W = Weight of Atorvastatin Standard = 0.11 mg

D = Dose of Atorvastatin Tablets = 10 mg

4.2.1 Specifications

Table : Specifications for Dissolution Test for Atorvastatin

Parameters	**Values**
Dissolution medium	900 ml Phosphate buffer (pH 6.8)
Apparatus	Apparatus 2 (paddle Apparatus)
RPM	100
Time	30 min
Sampling time	5, 10, 15, 30 min
λ_{max}	242 nm

4.2.2 Preparation of Phosphate Buffer

To prepare phosphate buffer, at first 28.4 gm disodium hydrogen phosphate and 11.45 mg potassium dihydrogen phosphate was weighed. Then sodium salt was passed to the 1000 ml beaker and potassium salt was passed to the 100 ml beaker for stirring. Then sodium salt was passed to the 1000 ml volumetric flask and potassium salt was passed to the 100 ml volumetric flask for better dissolution. Then 920 ml sodium salt and 80 ml potassium salt was passed to the 1000 ml measuring cylinder. Then pH of the phosphate buffer was adjusted to 6.8 by HCL. By this way phosphate buffer was made.

4.3 Materials Required for Dissolution Test

Table : Materials for Dissolution Test

Materials	**Generic Name**	**Company Name**	**Batch no.**	**Quantity**
Lipicon – 10 Tablet	Atorvastatin, INN	Eskayef	2022	12
Pantid – 20 Tablets	Pantoprazole, USP	Opsonin	Tai210	6
Ostocal	Calcium carbonate, USP	Eskayef	2037	6

Table : Apparatus Used for Dissolution Test

Apparatus	Company	Origin	Quantity
Tablet Dissolution Tester Apparatus II, USP XXIII	Vanguard® Pharmaceuticals	England	1
Double Beam UV – VIS Spectrophotometer	Shimadzu	Japan	1
pH Meter	Hanna pH210	Portugal	1
Stop Watch	Casio	Japan	1
1000 ml Beaker	*	*	1
100 ml Beaker	*	*	1
1000 ml Volumetric Flask	*	*	1
100 ml Volumetric Flask	*	*	1
1000 ml Measuring Cylinder	*	*	1

Figure : USP XIII Tablet Dissolution Apparatus II

Figure : Double Beam UV-Vis Spectrophotometer

Table : Reagents Used for Dissolution Test

Materials	Quantity
Disodium hydrogen phosphate	28.4 gm
Potassium Dihydrogen Phosphate	11.45 mg
Hydrochloric Acid	q.s
Disodium Hydrogen phosphate Solution	920 ml
Potassium Dihydrogen Phosphate Solution	80 ml
Phosphate Buffer (pH 6.8)	900 ml
Distilled Water	1800 ml

CHAPTER 5

RESULT

CHAPTER 5: RESULT

5.1 Dissolution Test of Atorvastatin

Table : Absorbance Test of Atorvastatin (Lipicon – 10)

Tablet #	Absorbances of sample at Different Time Interval (a)			
	5 min	10 min	15 min	30 min
1	0.032	0.025	0.610	0.370
2	0.021	0.025	0.076	0.490
3	0.022	0.015	0.099	0.421
4	0.028	0.037	0.044	0.454
5	0.017	0.044	0.183	0.399
6	0.210	0.035	0.019	0.347
Average	0.055	0.030	0.172	0.414
% Dissolution = (a / 0.11) × {(0.97 × 0.11) / 10} × 9 × 100%	4.802%	2.634%	15.001%	36.099%

5.2 Dissolution Test of Atorvastatin with Pantoprazole & $CaCO_3$ Tablets

Table : Absorbance Test of Atorvastatin (Lipicon – 10) with Pantoprazole (Pantid – 20) & Calcium Carbonate (Ostocal) Tablets

Tablet #	Absorbances of sample at Different Time Interval (a)			
	5 min	10 min	15 min	30 min
1	0.081	0.288	0.194	0.194
2	0.047	0.109	0.329	0.322
3	0.175	0.572	0.782	0.743
4	0.328	0.100	0.666	0.705
5	0.294	0.258	0.115	0.376
6	0.318	0.315	0.268	0.120
Average	0.207	0.274	0.392	0.410
% Dissolution = (a / 0.11) × {(0.97 × 0.11) / 10} × 9 × 100%	18.086%	23.891%	34.251%	35.793%

5.3 Comparison between % Dissolution of Atorvastatin & Atorvastatin with Pantoprazole & Calcium Carbonate Tablets

Table : Comparison between percent Dissolution of Atorvastatin & Atorvastatin with Pantoprazole & Calcium Carbonate Tablets

Time Intervals (min)	% Dissolution of Atorvastatin	% Dissolution of Atorvastatin with Pantoprazole & $CaCO_3$	Change in % Dissolution
At 5 min	4.802%	18.086%	13.284%
At 10 min	2.634%	23.891%	21.258%
At 15 min	15.001%	34.251%	19.250%
At 30 min	36.099%	35.793%	-0.306%
Equation of Regression Line	y = 0.0137x - 0.0595	y = 0.0068x + 0.1787	*
Value of R^2	0.9395	0.7439	*

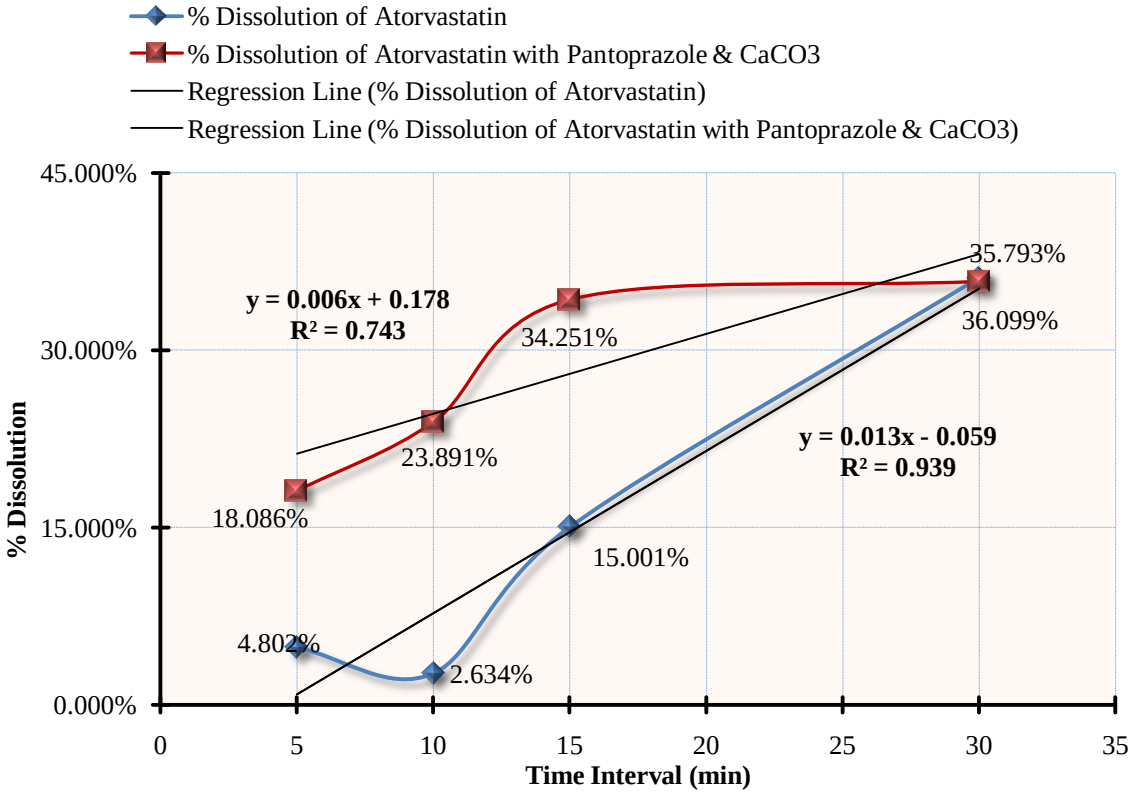

Figure : Comparison between percent Dissolution of Atorvastatin & Atorvastatin with Pantoprazole & Calcium Carbonate Tablets

5.4 UV Absorbances of Different Concentrations of Atorvastatin Standard

Table : UV Absorbances of Different Concentrations of Atorvastatin Standard

Concentration	UV Absorbance
80%	0.072
90%	0.087
100%	0.11
110%	0.136
120%	0.157

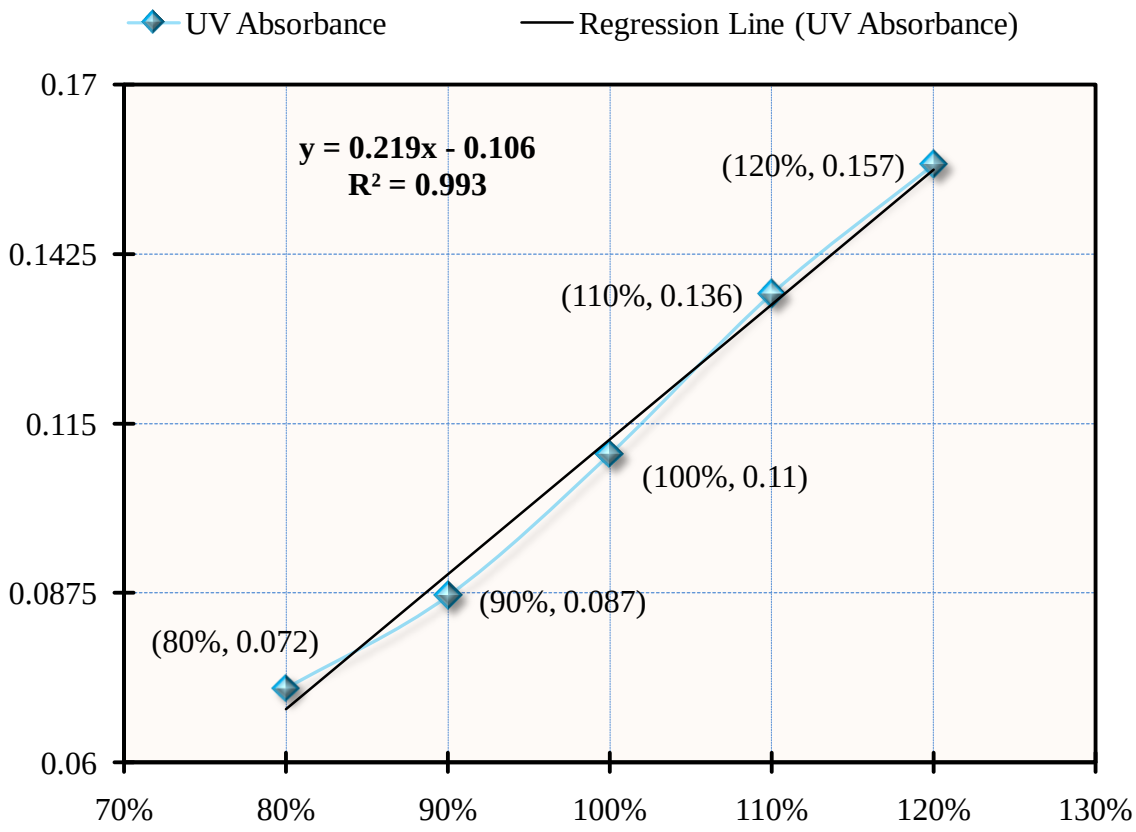

Figure : Line Diagram & Regression Analysis of the UV Absorbances Different Concentrations of Atorvastatin Standard

CHAPTER 6

DISCUSSIONS

CHAPTER 6: DISCUSSIONS

6.1 Dissolution Test of Atorvastatin Tablets

According to British Pharmacopoeia specifications, in order to pass the dissolution test at least two tablets from a batch must undergo 50% dissolution. In the present study, Atorvastatin tablets of the brand Lipicon®10 from Eskayef were subjected to the dissolution test studies. And it was found that none of the 6 tablets from any batches had average % dissolution above 50 at any time interval. So, the batch did not passed the dissolution test. The highest % dissolution observed was 36.099 % at 30 min interval. (See table 8)

6.2 Dissolution Test of Atorvastatin with Pantoprazole and $CaCO_3$ Tablets

According to British Pharmacopoeia specifications, in order to pass the dissolution test at least two tablets from a batch must undergo 50% dissolution. In the present study, Atorvastatin tablets of the brand Lipicon®10 from Eskayef Pharmaceutical, with Pantoprazole Tablets of the brand Pantid® 20 from Opsonin Pharmaceutical and Calcium carbonate tablets of the brand Ostocal® from Eskayef Pharmaceutical were subjected to the dissolution test studies. And it was found that none of the 6 atorvastatin tablets from the batches had % dissolution above 50 at any time interval. So, the batch did not passed the dissolution test. The highest % dissolution observed was 35.793 % at 30 min interval. (See table 9)

6.3 Comparison between the % Dissolution of Atorvastatin & Atorvastatin with Pantoprazole and Calcium Carbonate Tablets

Although none of the tablets under any conditions passed the dissolution test studies, considerable increase can be observed in case of combined dissolution study of Atorvastatin with Pantoprazole and $CaCO_3$ tablets compared to that of Atorvastatin alone (see table 10 & figure 15). In this case, at 5, 10, 15 & 30 minutes interval the % dissolution of Atorvastatin and Atorvastatin with Pantoprazole & Calcium Carbonate Tablet were 4.802% & 18.086%, 2.634% & 23.891%, 15.001% & 34.251% and 36.099% & 35.793% respectively.

% dissolution of Atorvastatin at 5, 10, 15 & 30 min differs by 13.284%, 21.258%, 19.250% and –0.306%. For dissolution study of Atorvastatin tablet only, % dissolution increases by 21.258%% from 5 minute to 10 minute time interval while, it decreases to –0.306% at 30 minute time interval in case of combined dissolution study of Atorvastatin with Pantoprazole & Calcium carbonate tablet. (See figure 13 & 15)

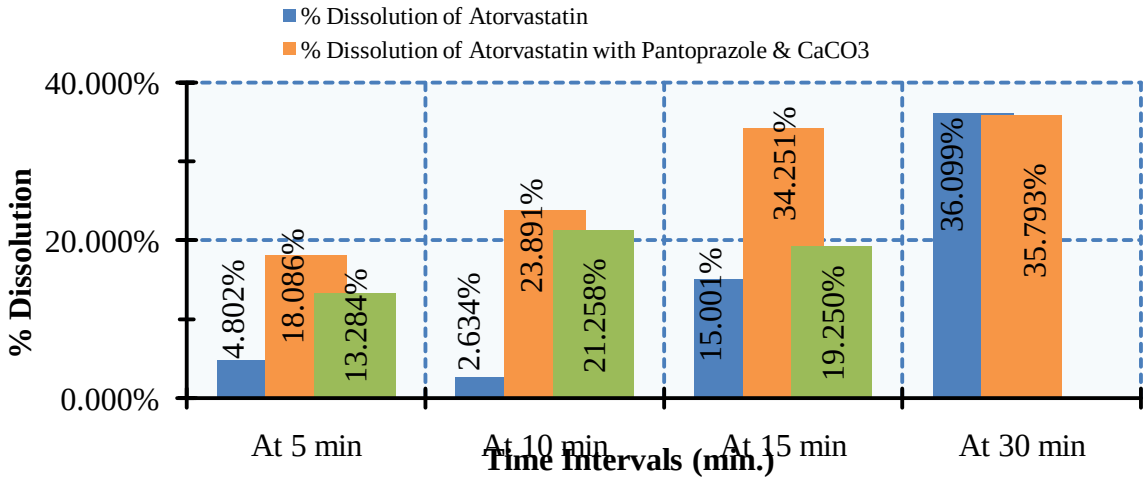

Figure : A Horizontal Bar Diagram showing the Change in % Dissolution of Atorvastatin

These results indicated that dissolution profile of atorvastatin tablets increases noticeably upon co – administration with Pantoprazole and calcium carbonate tablets. However, this requires more studies since; the value of R^2 is 0.7397 in case of the regression analysis of the data obtained from % dissolution studies of Atorvastatin with Pantoprazole & Calcium carbonate tablets. (See figure 16).

CHAPTER 7

CONCLUSION

CHAPTER 7: CONCLUSION

Atorvastatin is an anti – hyperlipidemic drug. And, it is a drug that is not included in any of the standard pharmacopoeias such as United States Pharmacopoeia and British Pharmacopoeia. Hence, it is included in the list of International Non – proprietary Names (INN) and thus marketed as such. It is also a drug belonging to a group that comes with many type of Drug – Drug and Drug – Food Interaction, as discussed in the section of this reports and citations. So, it is very essential that it is manufactured by strictly following Good Manufacturing Practice (GMP). In this study, a Bangladeshi brand of Atorvastatin tablet titled Lipicon® 10 manufactured by Eskayef Pharmaceutical Company was subjected to Dissolution Studies both alone and also in combination with a Bangladeshi Brand of Pantoprazole tablet titled Pantid® 20 and Calcium carbonate tablet branded Ostocal® manufactured by Opsonin and Eskayef Pharmaceutical Companies respectively. Unfortunately, in none of the case the tablets passed the Dissolution Tests. However, the studies have provided substantial knowledge on the Drug – Drug Interaction with Pantoprazole and Calcium Carbonate, since dissolution profile of Atorvastatin greatly increases in such combination. The result of the study signifies that co – administration of Pantoprazole & Calcium carbonate with Atorvastatin can lead to increased blood plasma concentration due to high dissolution profile in their presence. Thus, further studies are required on live subjects both animals and on humans in order to find out the impact of such co – administration.

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