EAST WEST UNIVERSITY

IMPACT OF CALCIUM AND VITAMIN-D SUPPLEMENT ON THE DISSOLUTION PROFILE OF ROCOVUS®

A dissertation submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirement for the degree of bachelor of pharmacy.

Submitted by

Sabera Rahman

ID: 2013-1-70-035

Department of Pharmacy

East West University

Submitted to

Md. Anisur Rahman

Assistant Professor

Department of Pharmacy

East West University

Declaration by the Research Candidate

I, **Sabera Rahman**, hereby declare that the dissertation, entitled "Impact of Calvimax-D on the Dissolution Profile of Rocovus®" submitted by me to the Department of Pharmacy, east West University and in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, under the supervision and guidance of **Md. Anisur Rahman**, Assistant Professor, department of Pharmacy, east West University, Dhaka.

.....

Sabera Rahman
ID: 2013-1-70-035
Department of Pharmacy
East West University

Certificate by the Supervisor

This is to certify that the thesis entitled "Impact of Calvimax-D on the Dissolution Profile of Rocovus®", submitted to the Department of Pharmacy, East west University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, is a original record and genuine research work carried out by **Sabera Rahman, ID: 2013-1-70-035** in 2016 of her research project in the Department of Pharmacy, East West University, under my supervision and guidance.

.....

Md. Anisur Rahman
Assistant Professor
Department of Pharmacy
East West University

Certified by the Chairperson

This is to certify that the thesis entitled "Impact of Calvimax-D on the Dissolution Profile of Rocovus®", submitted to the Department of Pharmacy, East west University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, is a original record and genuine research work carried out by **Sabera Rahman, ID: 2013-1-70-035** in 2016.

.....

DR. SHAMSUN NAHAR KHAN
Associate Professor and Chairperson
Department of Pharmacy
East West University

Acknowledgement

At first, I would like to thank Almighty Allah for enabling me to successfully completing my research work soundly and orderly.

I would like to express my deepest gratitude to my supervisor, **Md. Anisur Rahman**, Assistant Professor, Department of Pharmacy, East West University, for his cordial support throughout this research project. His generous advice, constant supervision, intense support and enthusiastic encouragements during the research work helped me to shape this research in a better way. His motivation, support, patience and advices have made it possible for me to complete this research.

I put forward my sincere regards and profound gratitude to Chairperson, **Dr. Shamsun Nahar Khan**, Associate Professor, Department of Pharmacy, East West University, for her inspiration in my study.

I express my sincere thankfulness to my parents and my sister for their support and guidance all through my life, including that for my research project.

I want to give special thanks to my dear friends **Jannatul Ferdous Nova**, **Sayma Afroz** and **Tasnim Kabir Taki** for their support and guidance throughout this research project.

I would also like to thank **Omer Fayshal Pavel** for his guidance and help throughout the research project.

I would like to thank our lab instructor **Sujit Kumer** for his support in this project and also all other lab instructors, **Ruchira Chakraborty**, **Shipra Sarkar** and **Razia Sultana** for their help and support throughout this research project. Again the help from the stuffs of Pharmacy Department cannot be neglected. My sincere thanks goes to each and every person who has contributed to complete this research work successfully.

Dedication

This research work is dedicated to my beloved parents, who are my biggest inspiration.

Table of Content

List of Tables

List of Figures

Abstract

The aim of this study is to find the impact of calcium-vitamin D supplement on the dissolution profile of Rocovus® (rosuvastatin calcium). Rosuvastatin is a cholesterol lowering drug and is used in hyperlipidemia and dyslipidemia. On the other hand, calcium-vitamin D supplements are used in patients who lack in calcium and vitamin D. Dissolution test of these two agents are performed to observe if there is any change in their release pattern when they are combined than they are dissolved individually. The test is performed under optimum conditions. The dissolution test was performed by using distilled water (as dissolution medium) with USP dissolution apparatus II. The dissolution tester was run in 37°C temperature at 50 rpm for 60 minutes. The absorbance of the samples were measured by UV-spectrophotometer at 241 nm. A standard curve equation of Rosuvastatin was established for the calculation of percent dissolved amount of drug. The dissolution of individual Rocovus® tablets and also combination with the Calcium and Vitamin D supplement drugs were determined after 10, 20, 30, 40, 50, 60 minutes. Six samples of each individual Rocovus® tablets and combination with the Calcium and Vitamin D supplement drugs were subjected to determine the dissolution profile. It was observed that at 10 minutes the average percent of drug release was 40.74% that create an impact of 24.36%. After 60 minutes the average percent of drug release was 50.03% and the impact of dissolution altered was 29.90%. So, it was clear that with increasing time the dissolution or the drug release of Rocovus® is decreased when it is combined with calcium and vitamin D supplement. The reason for such decrease in drug release is due to common ion effect. Finally, it can be concluded that rosuvastatin should not be co-administered with calcium-vitamin D supplement.

Chapter One

Introduction

Due to the convenience and ease of drug delivery the oral route of drug administration is mostly preferred. Swallowing drug is also familiar and comfortable to patients if they are given the dosage form in oral route. There are a number of reasons for which mode of drug delivery can be inefficient and problematic. When a drug is administered orally then a common problem that hampers the bioavailability of drug is its poor drug absorption. (Ramu et al., 2013)

A drug needs to undergo dissolution before going to the gastrointestinal site for absorption. If the drug is not dissolved in the gastrointestinal fluid then it may lead to poor drug bioavailability. Again if the drug is co-administered with other drug then also the absorption may be hampered (Swati et al., 2013).

This research project was done to observe the impact of calcium and vitamin D supplement when they are co-administered with rosuvastatin drug. Rosuvastatin is a lipid lowering drug that is prescribed in several disease conditions and to reduce the risk of cardiovascular diseases. Calcium and vitamin D supplement are also prescribed to prevent osteoporosis and for many other conditions.

In this research project Rocovus® (Rosuvastatin) from Incepta Pharmaceuticals and Calvimax-D (Calcium and Vitamin D) from Incepta pharmaceuticals were taken. The strength of Rocovus® was 10 mg and the strength of Calvimax-D was 500 mg. The impact of drug dissolution as both individual and in combination with supplement was observed for 60 minutes. Samples were collected as an interval of 10 minutes. Then the absorbance of the sample was measured in the UV spectrophotometer at a wavelength of 241 nm and the percent of drug release was finally calculated.

1.2 Cholesterol in Human Body:

Cholesterol is a fat-like and waxy substance that is found in all cells of the body. As cholesterol is water insoluble so they travel in the bloodstream by lipoproteins (Gill, 2016). These lipoproteins are macromolecules and consist of free and esterified cholesterol, phospholipid and triglyceride (Goodman, Gilman and Brunton, 2008).

1.2.1 Type of Lipoprotein:

Two types of lipoproteins carry cholesterol and they are:

- **Low-density lipoprotein (LDL):** The major constituent of LDL is cholesterol ester. The liver removes ~75% of all LDL from plasma by expressing a large complement of LDL receptors. Hepatic LDL receptor expression can be enhanced by diet such as reducing saturated fat and cholesterol intake or by pharmacological treatment (lipid lowering drugs).
- **High-density lipoprotein (HDL):** In HDL the major constituents are phospholipids and cholesterol esters. HDL plays a role in reverse cholesterol transport. HDL acquires excess cholesterol from cell and then they are transported to liver for excretion. Thus increased level of HDL reduces the risk of coronary heart disease (CHD). (Goodman, Gilman and Brunton, 2008)

1.2.2 Functions of Cholesterol:

- Cholesterols play major role in cell structure by maintaining the shape of cell while making them flexible.
- Corticosteroids are the hormones that protect the body from disease and stress and these corticosteroids are produced from cholesterol. Again cholesterol is also a precursor of important sex hormones for example estrogen, testosterone, progesterone etc.
- Cholesterols not only strengthen the intestinal wall of the digestive system but also aids in the synthesis of bile salts.
- Vitamin D is produced by cholesterol when ultraviolet ray of sun reaches human skin.

- Healthy nervous system is produced by cholesterol as they serve as insulator around the nerves. This function is essential for brain as it prevents the nervous system from diseases.
- An important neurotransmitter of brain is serotonin that requires cholesterol for functioning properly. (Walling, 2009)

1.2.3 Cholesterol Reading:

Table 1.1: Cholesterol readings that may increase the risk for various diseases:

	Amount (mg/dl)	**Comment**
Total Cholesterol	<200	Desirable
	200-239	Borderline high
	≥240	High
High-density lipoprotein (HDL) cholesterol	<40	Low
	>60	High
Low-density lipoprotein (LDL) cholesterol	<70	Optimal for very high risk for patients with coronary heart disease (CHD).
	<100	Optimal
	100-129	Near optimal
	130-159	Borderline high
	160-189	High
	≥190	Very high

(Goodman, Gilman and Brunton, 2008)

1.2.4 Factors Increasing the Risk for High Cholesterol:

- **Diet:** Saturated and trans fat increases the cholesterol level in body. These fats are found in many food items such as red meat, dairy products with full-fat, commercially baked snacks etc.
- **Obesity:** If the body mass index (BMI) of an individual is increased to 30 or greater then there is a risk of high level of cholesterol.
- **Lack of physical activity:** Exercise or physical activity helps to increase the level of HDL and reduces the level of LDL by increasing the particle size.
- **Smoking:** Smoking lowers the level of high-density lipoproteins. Due to cigarette smoking the blood vessels are damaged and fat accumulation can occur in the damaged walls of vessels.
- **Diabetes:** Diabetic patients were observed to have a high level of LDL and a low level of HDL. Again, high bold glucose level can damage the artery lines. (Mayo Clinic, 2016)
- **Age and Gender:** Cholesterol level rises with increasing age of both men and women. Again, after menopause the LDL level rises in women.
- **Heredity:** High level of cholesterol can be hereditary as the genes determine the amount of cholesterol to be synthesized for body. (NIH, 2005)

1.2.5 Conditions Associated with Hyperlipidemia and Dyslipidemia:

Atherosclerosis is caused by hyperlipidemia and dyslipidemia and there are several conditions that are associated with atherosclerosis and it includes:

- **Coronary Heart Disease:** Deposition of cholesterol in the artery walls may lead to plaque formation and that will narrow the arteries. Blood flow is reduced resulting in coronary heart disease.
- **Stroke:** If excess cholesterol is stored in the blood vessel of brain then the blood supply to brain will be reduced. As a result stroke occurs due to lack of oxygen.

- **Peripheral Vascular Disease:** Lack of blood supply is observed when major arteries are blocked by high level of cholesterol. This situation causes muscle cramp, weakness and numbness.

Again there can be some other critical conditions that are associated with high level of cholesterol such as hypertension, kidney disease, diabetes etc. (Goodman, Gilman and Brunton, 2008, NHS choice, 2015)

1.2.6 Management of High Cholesterol Level:

A healthy lifestyle is essential for maintaining a balanced level of cholesterol. A healthy diet and enough physical activity are very important for a healthy life. Again, quitting smoking may also lessen the risk of high level of cholesterol.

If the cholesterol level is high and an individual is not able to control it then he should consult a doctor for proper medication. (Mayo Clinic, 2015)

1.3 Statin:

Statins are a class of drugs that are used to lower the level of cholesterol in the blood. These drugs reduce the production of cholesterol in the liver by blocking an enzyme, hydroxy-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), which is responsible for the production of cholesterol. So, statins are called HMG-CoA reductase inhibitors. (Crosta, 2016)

Statins are used for stable coronary artery disease (CAD). It also stabilizes existing atherosclerotic plaque and prevents formation of new atherosclerotic lesions. Again statins help to decrease vascular inflammation, reduce platelet aggregation and deposition of thrombus and increase nitric oxide production in endothelium tissue. A recent study suggests that statins may also promote neovascularization of ischemic tissue in animals with normal cholesterol level. (Vasa et al., 2001)

1.3.1 Types of Statins:

- Atorvastatin
- Cerivastatin
- Fluvastatin
- Lovastatin
- Mevastatin
- Pitavastatin
- Pravastatin
- Rosuvastatin
- Simvastatin (Crosta, 2016)

Atorvastatin, fluvastatin and rosuvastatin are synthetic compounds that are structurally different. Chemical modification of lovastatin results in pravastatin and simvastatin. Higher doses of the statins that are more potent for example atorvastatin, simvastatin or rosuvastatin can limit cholesterol biosynthesis as well as reduce the level of triglyceride. (Goodman, Gilman and Brunton, 2008)

1.3.2 Actions of Statin:

Statins are not only lipid lowering drugs but they also exert many other actions in the body:

- Atherosclerotic plaque is stabilized
- Exert antithrombotic action by reducing platelet aggreability
- Fibrinolysis is enhanced
- Endothelial function is improved
- Vascular inflammation is reduced
- Neovascularization of ischemic tissue is increased
- Gives protection against sepsis. (Rang et al., 2012)

1.3.4 Absorption, Metabolism and Excretion of Statin:

Intestinal absorption of statins after oral administration varies between 30%-85%. Except simvastatin and lovastatin the other statins are administered as active b-hydroxy acids. Lovastatin and simvastatin are administered as inactive lactones and they are metabolized in the liver to produce b-hydroxy acids. All statins undergo extensive first-pass hepatic metabolism but the mechanism may be varied. Atorvastatin, pravastatin and rosuvastatin is up taken by the organic anion transporter 2 (OATP2). Lovastatin and simvastatin in their lipophilic lactones forms enter the liver by simple diffusion.

The systemic bioavailability of the administered doses of statins varies between 5%-30% due to their extensive first-pass metabolism. The enzyme responsible for the metabolism of atorvastatin, lovastatin and simvastatin is CYP3A4. Fluvastatin is mainly metabolized by CYP2C9 but CYP3A4 and CYP2C8 also play role in its metabolism. Small amounts of statins along with their metabolites are found in the circulation under steady-state conditions. Statins and their metabolites are highly protein bound (>95%). Pravastatin is an exception and the parent drug with its metabolite is 50% plasma protein bound. The peak plasma concentration for statin is achieved in 1-4 hours after oral administration. Atorvastatin and rosuvastatin have half-lives of ~20 hours that shows their better efficacy while the others statins have half-lives of 1-4 hours.

Statin metabolism occurs in the liver and >70% of statin metabolites are eliminated in the feces. (Goodman, Gilman and Brunton, 2008)

1.3.5 Adverse Effect and Drug Interaction of Statins:

There is minor or no side effects associated with statins. Among the minor side effects headache, abdominal pain, diarrhea, bloating, rash are common. (Crosta, 2016)

Hepatotoxicity:

Statins rarely causes serious hepatotoxicity (~one case of hepatotoxicity per million people who used them for years).

Myopathy:

The incidence of myopathy associated with statin is very low (~0.01%) but it is a significant adverse effect. The risk of rhabdomyolysis (severe muscle damage) may increase with increased plasma statin concentration.

Statin interactions can commonly occur with fibrates. Among them gemfibrate (38%) is the most common agent that induce myopathy. It interferes with the metabolism of statins by cytochrome enzymes and glucoronidases and inhibits hepatocyte to uptake statin as the active hydroxy acid form. The plasma concentration of rosuvastatin can become double if the drug is co-administered with gemfibrozil. On the other hand fenofibrate when combined with statin do not increase the risk of myopathy as there is no interference of glucuronidation process. Other drugs that may interact with statin are azoles antifungal (1%), cyclosporine (4%), digoxin (5%), macrolide antibiotics (3%) and warfarin (4%). The risk of myopathy may be rarely increased by the interaction of statin with amiodarone, HIV protease inhibitors, nefazodone and niacin. (Goodman, Gilman and Brunton, 2008)

1.3.6 Statin in Pregnancy:

The safety of statin use in pregnancy is not established so statins should be avoided during this period.

1.3.7 Statin Use by Children:

Children with heterozygous familial hypercholesterolemia are prescribed with some statins. Among them atorvastatin, lovastatin and simvastatin are approved for children age 11or older and for children age 8 or older pravastatin is approved.

1.3.8 Statin in Combination with Other Lipid-lowering Drugs:

Cholestyramine and colestipol if combined with statin then they may exert 20%-30% greater effect then statin alone.

This greater effect can also be observed with niacin combination but there is a chance of myopathy if the dose of statin is increased to 25% of maximal dose.

Patients with hypertriglyceridemia and high LDL-C level can be benefited if statins are combined with fibrates such as fenofibrate, gemfibrozil and clofibrate. If the dose of statin does not exceed the maximum dose of 25% when combined with fibrates then the chance of myopathy can be decreased.

LDL-C level can be reduced up to 70% if triple therapy is given with statin, niacin and resin.

LDL-C levels can also be reduced up to 60% when a fixed combination of simvastatin (10, 20, 40 or 80 mg) and ezetimibe (10 mg) are given for 24 weeks. (Goodman, Gilman and Brunton, 2008)

1.3.9 Dose and Administration of Statin:

- Lovastatin (MEVACOR) 20 mg is recommended to be taken at evening than at bedtime because it is slightly more effective. The dose of lovastatin can be increased to a maximum of 80 mg/day but it is more effective if the dose is given as 40 mg twice daily. Again, lovastatin (20 mg) can be combined with niacin (500 mg, 750 mg or 1000 mg) for better activity.
- Simvastatin (ZOCOR) is approved as 20 mg starting dose but if the LDL-C level need to be reduced to 45% then only it can be prescribed as 40 mg as a starting dose. The maximum dose for simvastatin is 80 mg/day. If the drug is co-prescribed with fibrates, niacin or cyclosporine then more than 20 mg of simvastatin should not be prescribed.
- Pravastatin (PRAVACHOL) is approved as 20 mg or 40 mg as a starting dose but the dose should not exceed 80 mg/day. If the drug is co-prescribed with resins then there should be a dose interval of 2 to 3 hours.

- Fluvastatin (LESCOL) therapy is started with 20 mg to 40 mg with a maximum dose of 80 mg per day. A time interval of several hours is maintained if the drug is prescribed with bile-acid sequestrant.

Atorvastatin (LIPITOR) has a long half-life and due to this reason can be prescribed at any time of the day. 10 mg starting dose is preferential but the daily dose should not exceed more than 80 mg. Atorvastatin is also found to be combined with amlodipine (calcium channel blocker) for patients with hypertension and hypercholesterolemia. (Goodman, Gilman and Brunton, 2008)

1.4 Rosuvastatin:

1.4.1General Description of Rosuvastatin

1.4.1.1 Physical Properties:

- Color: White
- State: An amorphous powder
- Melting point: 122° C
- Solubility: In water and methanol it is moderately soluble and to some extent it is soluble in ethanol. (Ahmad et al., 2012)

1.4.1.2 Chemical Properties:

The molecular formula for rosuvastatin is $(C_{22}H_{27}FN_3O_6S)_2Ca$.

Molecular weight of rosuvastatin is 1001.14.

The chemical name of rosuvastatin is bis{(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino]pyrimidine-5-yl] (3R,5S)-3,5-dihydroxyhept-6-enoic acid} calcium salt. (Ahmad et al., 2012)

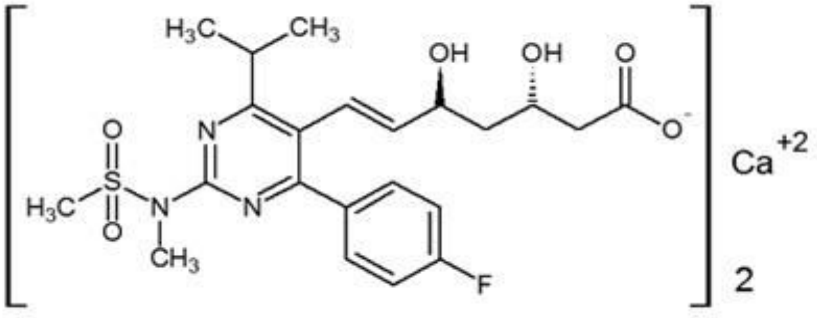

Figure 1.1: Chemical structure of rosuvastatin (Ashfaq et al., 2013)

The pharmacophore of rosuvastatin is the heptenoic acid that is present in its molecular structure. The other portion of rosuvastatin also differs from other statin drugs. The hydrophilic nature of rosuvastatin is due to the presence of a polar hydroxy group and methane sulphonamide group. (Ahmad et al., 2012)

1.4.2 Synthesis of Rosuvastatin:

The synthetic pathway of rosuvastatin includes several steps where cyclocondensation, dehydrogenation and modified Wittig reactions take place.

There is an aldehyde functionalized pyrimidine intermediate in the synthetic pathway that allows the introduction of a modified Wittig reaction that lock the E-alkene stereochemistry of the rosuvastatin intermediate (R8). As a result the protecting groups provide a high purity of (3R, 5S) rosuvastatin enantiomeric methyl ester (R9 and R10). The methyl ester intermediate (R10) undergoes a two step saponification process and then it forms sodium salt of rosuvastatin base (R11). Finally it is converted to calcium salt form of rosuvastatin in the final step of reaction scheme (R12). (Fairchild and Jones, 2015)

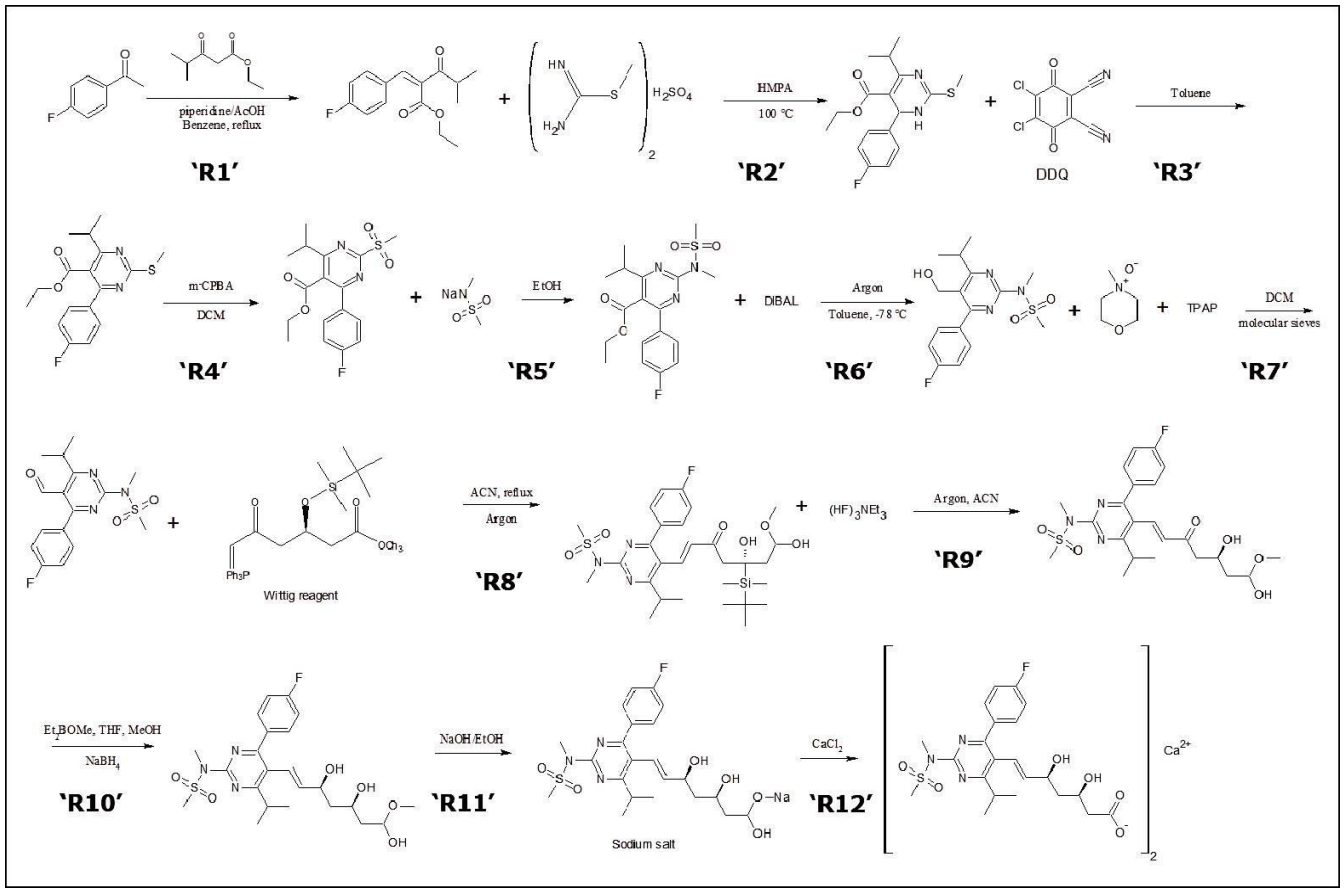

Figure 1.2: Synthetic pathway of rosuvastatin (Fairchild and Jones, 2015)

1.4.3 Mechanism of Action:

There are two possible mechanisms by which rosuvastatin can exert its effect.

a. Inhibition of HMG CoA reductase:

Rosuvastatin is an analogue of hydroxy-methylglutaryl (HMG) which is the cholesterol precursor. HMG CoA reductase catalyzes the conversion of HMG CoA to mevalonic acid in the cholesterol synthesis pathway. This drug has a strong affinity for the hydroxy-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) enzyme and effectively inhibits this rate-limiting step of cholesterol biosynthesis. As a result, the intracellular cholesterol is depleted and the LDL cholesterol level is lowered.

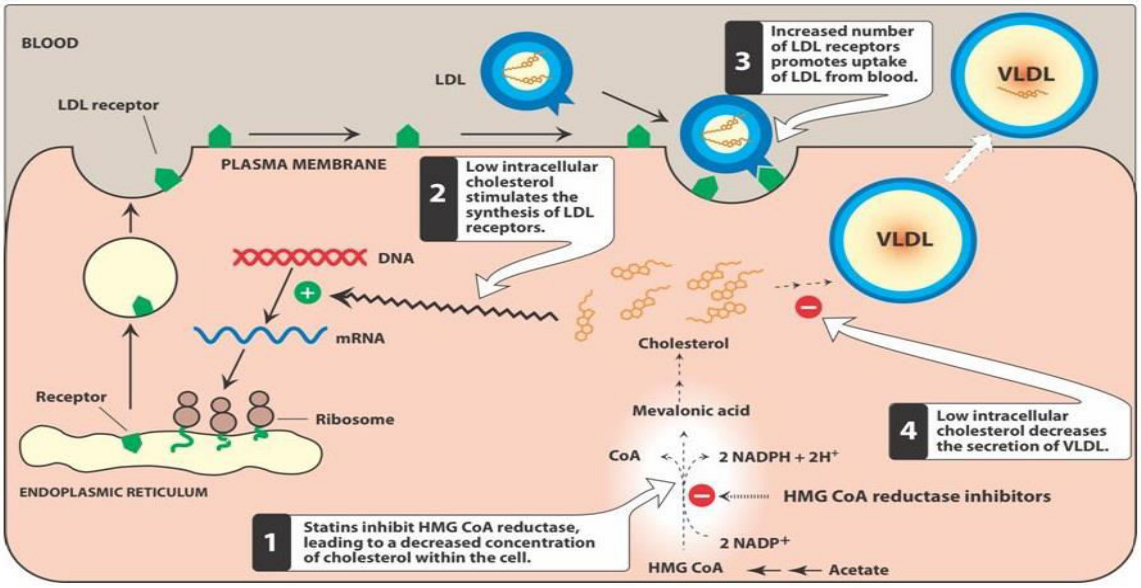

Figure 1.3: Mechanism of action of rosuvastatin (Clark et al., 2012)

b. Increase in LDL receptor:

The number of specific cell-surface LDL receptor is increased when intracellular cholesterol level is depleted. They can then bind with the circulating LDL and thus the plasma cholesterol level is reduced.

So rosuvastatin works both by limiting cholesterol synthesis and by increasing LDL catabolism. (Clark et al., 2012)

1.4.4 Indication of Rosuvastatin:

- Hyperlipidemia
- Hypertriglyceridemia
- Homozygous familial hypercholesterolemia
- Primary dysbetalipoproteinemia
- Primary prevention of cardiovascular disease
- Slow down the progression of atherosclerosis (Ahmad et al., 2012)

1.4.5 Dosage Form and Strength:

- Rosuvastatin is available as film coated tablet with the strength of 5 mg, 10 mg, 30 mg and 40mg. (Ahmad et al., 2012)

1.4.6 Therapeutic Dose of Rosuvastatin:

The dose regimen of rosuvastatin varies between 5 mg to 40 mg. the starting dose of rosuvastatin is 5-10 mg daily and it can be increased if the dose need to be adjusted.

The dose of rosuvastatin should not be increased to more than 10 mg if it is combined with gemfibrozil (fibrates). A clinical study showed that rosuvastatin can cause renal failure, proteinuria and hematuria if the dose is increased to 80 mg (FDA does not approve) (Goodman, Gilman and Brunton, 2008).

Table 1.2: Dose of rosuvastatin for specific population:

Specific Population	**Dose**
Hypercholesterolemia (heterozygous familial) in pediatric patients of 10 to 17 years of age	5-20 mg/day is the usual dose Maximum dose that is recommended for use is 20 mg/day.
Hypercholesterolemia (homozygous familial)	20 mg/day should be the starting dose
With cyclosporine therapy	Maximum dose should be 5 mg/day
With Liponavir/Ritonavir or Atazanavir/ Ritonavir therapy	10 mg/day should be the maximum dose
With mild to moderate renal impairment	Dosage adjustment is not required
With severe renal impairment	Starting dose of 5 mg/day and maximum dose should be 10 mg/day

(ACI limited)

1.4.7 Rosuvastatin in Pregnancy and Nursing Period:

Rosuvastatin is not prescribed during pregnancy as there is a risk of harm to the fetus. However, there is no evidence that suggest that the drug can be released in the breast milk of nursing mother. (Ogbru, 2016)

1.4.8 Pharmacokinetic Properties of Rosuvastatin:

1.4.8.1 Absorption:

After the oral administration of rosuvastatin the peak plasma concentration reached between 3 to 5 hours. The C_{max} and AUC increased in approximate proportion with the dose of rosuvastatin. The absolute bioavailability for rosuvastatin was observed to be approximately 20%.

Food do not affect the AUC of rosuvastatin and the drug can be taken any time of day as there is no optimum time of dosing for rosuvastatin. ((RxList, 2016; Schachter, 2004)

1.4.8.2 Distribution:

The mean volume of distribution of rosuvastatin at steady state is approximately 134 liters. This drug mostly bound with albumin and the plasma protein binding is 88%. This binding is independent of plasma concentration and is also irreversible. (RxList, 2016)

1.4.8.3 Metabolism:

Rosuvastatin does not undergo extensive metabolism and as a metabolite approximately 10% of a radiolabeled dose is recovered. Cytochrome P450 enzyme metabolizes rosuvastatin and forms N-desmethyl rosuvastatin. (RxList, 2016)

1.4.8.4 Excretion:

After oral administration, rosuvastatin and its metabolites are excreted by feces (90%). Rosuvastatin has an elimination half life of approximately 19 hours.

If the drug is administered intravenously then the body clearances via the renal route and hepatic route are 28% and 72% respectively. (RxList, 2016)

1.4.9 Side Effects Associated with Rosuvastatin:

Common side effects include:

- Headache
- Nausea
- Vomiting
- Diarrhea
- Muscle pain

Some other important side effects may include:

- Forgetfulness
- Confusion
- Post-marketing reports of memory loss
- Amnesia

These symptoms may arise after starting the medication or by using medication for years and the symptoms may be resolved within 3 weeks after stopping the medication.

Severe side effects of rosuvastatin are rare but it may include:

- **Liver failure:** Liver failure due to use of rosuvastatin is very rare. But if the drug is used for chronic period of time liver tests should be performed for patient's safety.
- **Rhabdomyolysis (breakdown of muscle):** The process where severe injury of muscle occurs with the release of muscle protein in blood is called rhabdomyolysis. In this condition severe muscle pain occurs and kidney failure may also be caused by myoglobin.

Renal impairment: The amount of protein excreted by the kidney may be reversibly increased by use of rosuvastatin and it may cause kidney failure also. If the dose is more than 40 mg then only this situation may arise. (Ogbru, 2016)

1.4.10 Drug Interactions of Rosuvastatin:

Table 1.4: Drug interactions of rosuvastatin

Name of drug that interact with rosuvastatin	Effect of interaction
Erythromycin	Interaction between erythromycin and rosuvastatin may increase gastrointestinal motility and then the AUC of rosuvastatin can decrease by 20% and C_{max} decrease by 30%
Lopinavir/Ritonavir	When rosuvastatin is administered with liponavir/ritonavir then the AUC of rosuvastatin increases to 2.1 times and C_{max} increases to 4.7 times.
Antifungal agents	Studies suggest that the AUC and Cmax of rosuvastatin is not altered when they are co-administered with antifungal agents such as fluconazole and ketoconazole
Rifampicin	No significant change in the pharmacokinetic properties of rosuvastatin was observed when they were co-administered with rifampicin
Silymarin	Silymarin is a hepatoprotective agent that do not alter rosuvastatin's pharmacokinetic properties when co-administered
Cyclosporin	Combination of rosuvastatin with cyclosporine increases the AUC of rosuvastatin by 7 times
Vitamin K antagonist	If vitamin K antagonist (warfarin) is administered with rosuvastatin then the plasma level of patients should be monitored for safety issues
Fibrates	Increased risk of myopathy results when combination of rosuvastatin

	and fibrates (gemfibrozil, fenofibrate) are administered
Antacid	Plasma concentration of rosuvastatin can be reduced to 50% if they are co-prescribed with antacids
Hormone replacement therapy/ oral contraceptive	Co-administration of rosuvastatin and oral contraceptive can increase the plasma concentration of rosuvastatin.

(Ahmad et al., 2012; Chemicalbook, 2016)

1.4.11 Special Warnings and Precautions:

Hypercholesterolemia should be first managed with proper diet, physical activity, weight loss and treatment of any related health problems before starting the drug therapy of rosuvastatin.

- **Renal Effect:** Patients with severe renal impairment has shown 3 times greater plasma concentration when prescribed with rosuvastatin 20 mg/day. (Healthcare pharmaceuticals). Again if dose is increased to 40 mg/day then it may cause proteinuria. So the renal function of patients should be monitored if higher doses of rosuvastatin are used.
- **Skeletal Muscle Effect:** Dose greater than 20 mg/day has shown some serious side effects in patients such as myalgia, myopathy and very rarely rhabdomyolysis. So before prescribing higher doses the risk should be taken into consideration.

Before Treatment

Patients with pre-disposing factors for skeletal problems should be given rosuvastatin by considering the following factors:

a. Hypothyroidism
b. Renal impairment
c. Muscular disorder
d. Age >70 years
e. Alcohol abuse
f. Co-administration of fibrates and also if there is any previous report of fibrate toxicity.

Whilst on Treatment

Patients with any muscle problem during treatment should report physician. Therapy may be discontinued if severe problem arises.

If severe problem arises then therapy should be discontinued.

- **Liver effect:** patients who have a history of liver disease or consume alcohol frequently should be given the drug cautiously. If hypothyroidism or nephritic syndrome causes secondary hypercholesterolemia then it should be treated prior to rosuvastatin therapy.

- **Race:** Asian subjects have shown more exposure in the pharmacokinetic studies than the Caucasians.

- **Protease Inhibitors:** In certain cases the concomitant use of rosuvastatin with protease inhibitors are not preferred until the dose of rosuvastatin is adjusted.

- **Interstitial Lung Disease:** long term therapy of rosuvastatin may develop some interstitial lung diseases and in such cases therapy should be discontinued.

- **Diabetes Mellitus:** Rosuvastatin may increase the blood glucose level and some patients may experience hyperglycemia. Thus the blood glucose level of such patients should be monitored. (AstraZeneca, 2016)

1.4.12 Contraindications:

Rosuvastatin is contraindicated in patients with the following conditions:

- Patients who have hypersensitivity with rosuvastatin
- Patients with active liver disease where serum transaminase is increased more than three times than the normal level
- In patients with severe renal impairment
- Patients with myopathy and also to those who have risk to develop myopathy or rhabdomyolysis
- In patients who are prescribed with concomitant cyclosporine. (AstraZeneca, 2016)

1.4.13 Special Population:

- **Age and Gender:** the pharmacokinetic of rosuvastatin is not affected by age or gender of patient.
- **Race:** Asian subjects for example Japanese, Chinese, Koreans have shown an elevation of approximately 2-fold of AUC and C_{max} than the Caucasians when the pharmacokinetic profile of rosuvastatin were observed. In case of Asian-Indians the elevation of AUC and C_{max} was 1.3 fold.
- **Renal Insufficiency:** Patients with mild to moderate renal insufficiency have shown no influence in plasma concentration of rosuvastatin but in severe impairment the concentration may increase to 3-fold than the normal level.
- **Hepatic Insufficiency:** Mild to moderate hepatic insufficiency has shown no influence in plasma concentration of rosuvastatin but in severe condition the concentration may increase for some people.
- **Pediatric Population:** A pharmacokinetic study on pediatric patients with hypercholesterolemia suggested that the rosuvastatin exposure may decrease when used for a 2 year period. (AstraZeneca, 2016)

1.4.14 Toxicity:

Central Nervous System Toxicity:

Statins other than rosuvastatin have shown CNS vascular lesions, characterized by edema and perivascular hemorrhages. Animal study suggests that dose dependent optic nerve degeneration can occur with a drug that is chemically similar to rosuvastatin. Rosuvastatin so far observed is well tolerated in human beings. (RxList, 2016)

Juvenile Toxicology Study:

No effect of toxicity was observed with an elevated dose of rosuvastatin when animal study was performed. (RxList, 2016)

1.4.15 Proper Storage of Rosuvastatin:

The drug can be stored at room temperature 20° C to 25° C. the storage should be done away from heat, light and moisture. (Drugs.com, 2016)

1.5 Calcium and Vitamin D Combination:

1.5.1 Calcium:

Calcium is a mineral which is essential for life. It helps to build bones and keep them healthy as well as it helps in blood clotting, muscle contraction and sending messages to nerves. Approximately 99% of calcium found in human body is from bones and teeth. Human body cannot produce calcium. (NOF, 2016)

1.5.1.1 Requirement of Calcium for Body:

The amount of calcium that a person needs everyday depends on age and gender of that person.

Table 1.5: Requirement of calcium for body

Women	
≤50 years	1000 mg daily
≥51 years	1200 mg daily
Men	
≤70 years	1000 mg daily
≥71 years	1200 mg daily

(NOF, 2016)

1.5.1.2 Sources of Calcium:

The major source of calcium is food that is rich in calcium. Dairy products including low-fat milk, yogurt, and cheese are rich in calcium. Calcium is also present in small amount in some

green vegetables. If food is not sufficient to fulfill the calcium demand of body then calcium supplement can also be taken. (NOF, 2016)

1.5.2 Vitamin D:

Vitamin D is necessary for protecting bones and again the bones require Vitamin D to absorb the calcium. If there is a deficiency of calcium then it may lead to lower the bone density and make the bones more likely to break or fracture. (NOF, 2016)

1.5.2.1 Requirement of Vitamin D for Body:

Table 1.6: Requirement of Vitamin D for body:

Men and Women	
Under age 50	400-800 IU daily
Age 50 and older	800-1000 IU daily

IU: International Unit (NOF, 2016)

1.5.2.2 Sources of Vitamin D:

Vitamin D can be obtained from:

Sunlight: Body makes vitamin D with the help of sunlight (UVB ray) and can store it for further use.

Food: Vitamin D is naturally available in fatty fishes such as wild-caught mackerel, tuna and salmon.

Supplement: If the amount of vitamin D obtained from food and sunlight is not sufficient then supplement is necessary. (NOF, 2016)

1.5.3 Complications Associated with Calcium and Vitamin D Deficiency:

Bone is a living tissue that has the ability to constantly break down and builds back up. The bone mass in adult is acquired by the age of 20. For the formation of this bone mass calcium is essential. If the bone mass drop due to deterioration of bone tissue then a clinical condition arises this is termed as 'Osteoporosis'. Osteoporosis can cause bones to be susceptible to fractures and the risk for breakdown of bone also increases.

Research has shown that women who have reduced net calcium absorption, lower rate of bone formation and higher rate of urinary calcium excretion may face some complications during menstruation. Reduced calcium retention and lower bone mass can also induce amenorrhea. (Harbolic, 2015)

Common effect of vitamin D deficiency includes the following:

- Thin or brittle bones that increase the risk of osteoporosis
- Bone softening can lead to 'Rickets' in children
- Can create insulin resistance
- Immune system functioning can be impaired also.

Research suggests that deficiency of vitamin D can play a role in depressive disorder. Another study suggest that breast cancer growth in animal model has become faster when there was a deficiency of vitamin D. (Nall, 2016)

1.5.4 Indication of Calcium and Vitamin D Combination:

- Calcium deficiency in blood
- Vitamin D deficiency in blood
- Osteoporosis
- Bone weakness (Rickets)
- Post-menopausal osteoporosis prevention
- Hypoparathyroidism (WebMD, 2016)

1.5.5 Side Effects of Calcium-Vitamin D:

Common side effects include:

- Constipation
- Diarrhea
- Headache
- Loss of appetite
- Nausea, vomiting

Some other side effects that are usually uncommon but can occur include:

- Dry mouth
- High blood pressure
- Confusion
- Allergic reaction like rash, itching etc
- Irregular heartbeat
- Increased urination
- Increased hunger or thirst
- Metallic taste
- Weight loss (Healthline, 2009)

1.5.6 Drug Interactions with Calcium-Vitamin D:

- Calcium and vitamin D can interact with the following drugs:
- Ammonium chloride
- Methenamine
- Antibiotics like ciprofloxacin, tetracycline, gatifloxacin
- Captopril
- Delavirdine
- Diuretics
- Iron supplements

- Antifungal agents like ketoconazole and itraconazole
- Thyroid medicine (Healthline, 2009)

1.6 Dissolution

1.6.1 General Information:

Dissolution is a process where transfer of ions from molecules of solute state in solution state takes place. It is the process of dissolving solid part (solute) in the solvent (liquid). Dissolution can be simply defined as the process by which a substance turns into solution in a solvent. For solid, dissolution is explained as the breakdown of the crystal lattice into individual ions, atoms or molecules. It is a total kinetic process. Thermodynamic energies that are involved in the dissolution process can be changed for example heat of solution and entropy of solution to control the result of dissolution. Overall free energy must be negative for net dissolution to occur. In turn, those energies are controlled by the way in which different chemical bond types interact with those in the solvent. (Sirius-analytical, 2016).

1.6.2 Rate of Dissolution:

The rate of dissolution determines the speed of the total process. It depends on the chemical natures of the solvent and solute which are temperature, degree of unsaturation, the interfacial surface area and the presence of 'inhibitors' for example substances adsorbed on the surface.

The rate can be often expressed by the *Noyes-Whitney* equation or the Nernst and Brunner equation of the form

dm/dt = AX{D/d}X(Cs-Cb)

Where: m= mass of solute

t= time

A= surface area of the interface between the dissolving substance and the solvent

D= diffusion co-efficient

d= thickness of the boundary layer of the solvent at the surface of the dissolving substance

Cs= mass concentration of the substance on the surface

Cb= mass concentration of the substance in the bulk of the solvent.

For dissolution limited by diffusion, Cs is equal to the solubility of the solute. When the dissolution rate of a pure substance is normalized to the surface area of the solid, then it is expressed in kg/ms^2 and is termed as 'intrinsic dissolution rate', which is defined by the United States Pharmacopeia (Lentle and Janssen, 2011)

1.6.3 Dissolution Process:

Dissolution requires that substances must have the same intermolecular force to go to the solution. When a solute is introduced in a solvent then the particles of both solute and solvent interact with each other. The interaction between solid or liquid solute and solvent is so strong that solute particles separate from each other and solvent molecules surround them. The process of entering into solution is termed as salvation. If water is used as the solvent then it is called hydration (Lapsurgery, 2014).

Certain molecules are itself individual molecules for example carbohydrates (e.g. glucose). But ionic solutes get separated from each other and the individual ions are then surrounded by solvent particles. When the solute dissolves then the ions of solute are separated and this process is called dissociation and the soluble ionic compounds are referred to as electrolytes. (Lapsurgery, 2014)

1.6.4 Factors Influencing Dissolution:

- **Temperature:** Temperature has significant influence on dissolution. If the temperature is increased then dissolution will be rapid. Dissolution of solute in a liquid depends on the absorption of heat.

- **Particle size of solute:** Particle size of solute greatly influences the dissolution rate. If the particle size is small then faster dissolution is observed and for larger particle size dissolution rate is slower.
- **Agitation:** Dissolution also depends on solvent concentration. If the solvent is highly concentrated then less dissolution will be observed. Similarly, dissolution is faster for less concentrated solvent.
- **Solvent selection:** Type of solvent is also important for better dissolution. Dissolution rate in water is more than dissolution rate in oily solvent (Yeomans, 2000)

1.7 Instrumentation

1.7.1 Dissolution Test Apparatus:

Dissolution tester incorporates a clear acrylic water bath, paddle or basket and vessels that contain a fixed volume of fluid. In this experiment USPXXII (source RC-6B, made in China) dissolution tester was used. The tester has a stirrer hood with paddle shaft. It also has a control unit with microcontroller software. For controlling the temperature of the water bath there was an in-built thermostat, sensor for external temperature and water level sensor.

Figure 1.4: Dissolution Tester (Tradeindia, 2016)

This dissolution tester has a capacity of eight vessels and also stirrer hood that was equipped with eight paddle shafts fitted with USP apparatus 2. There was also a tablet dispenser which has

dissolution bowl lids of conical shape. The vessels are made of glass that can contain 1000 ml of solvent. (Crist, 2007)

1.7.2 Ultraviolet Spectrophotometer

In a UV spectrophotometer the radiation of specific wavelength is passed through the sample of interest. A detector is placed that detects the light that absorbed at a specific wavelength and measures the extent of absorption. (Soderberg, 2016)

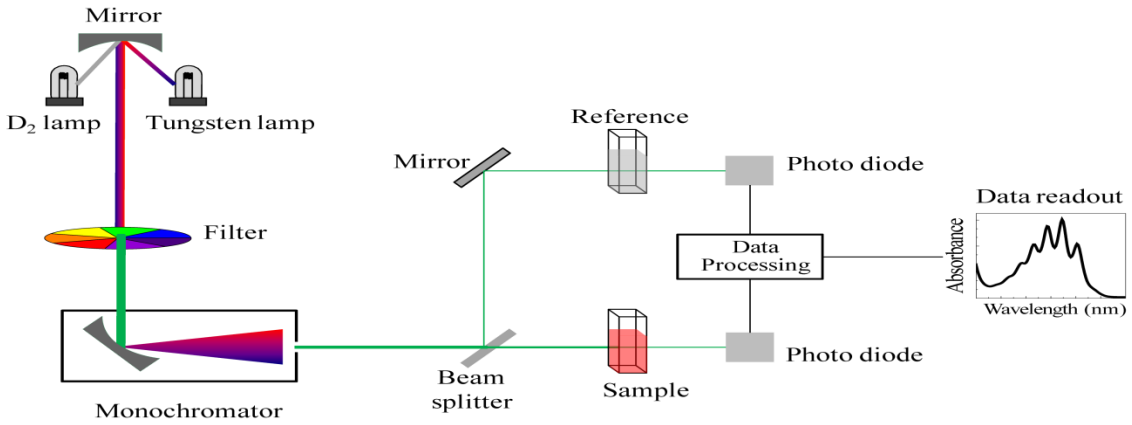

Figure 1.5: Schematic diagram for UV spectroscopy (Soderberg, 2016)

In this experiment the ultraviolet absorption of drug samples were measured by using a double beam T90+ UV/VIS spectrometer that is controlled by a computer having a specific software named 'UVWIN spectrophotometer', version 5.2.0 over a 10 mm path length. Quartz cuvettes were used as the sample holder.

Chapter Two

Literature Review

A literature review was done to evaluate the previous works that were done on the dissolution profile of statins. It was observed that the studies done on statins were not similar to this research project. But those studies helped to find the information's that helped in the research work and also helped to compare this research work with other research projects. A brief of those studies is given below:

A prospective study was performed to evaluate the role of HMG-CoA reductase inhibitors as gallstone-dissolving agents. Fifty patients with radiolucent gallstones in a gallbladder opacifying at drip infusion cholecystography were treated with either 10 mg/day simvastatin plus 600 mg/day ursodeoxycholic acid or 600 mg/day ursodeoxycholic acid alone for 12 months. The result of this study showed that combination therapy with simvastatin and ursodeoxycholic acid was more effective for cholesterol gallstones dissolution than ursodeoxycholic acid monotherapy. (Tazuma, 1998)

A research was accomplished to develop a second derivative UV spectroscopic method for the determination of simvatatin in the tablet dosage form. This method was done by using UV spectroscopy. According to this study, the result reflected that a second derivative UV spectroscopy method was an excellent alternative to HPLC method for the dissolution and release testing of Simvastatin. (Asgharnejad and Wang, 2000)

A study was performed with simvastatin (SV), and its inclusion complex with hydroxypropyl β-cyclodextrin (HP-β-CD) by using supercritical antisolvent (SAS) to improve the aqueous solubility and the dissolution rate of drug. Inclusion complexation in aqueous solution and solid state was evaluated by the phase solubility diagram, differential scanning calorimetry (DSC), powder X-ray diffractometry (PXRD), Fourier-transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM). Results showed that aqueous solubility and dissolution studies showed that the dissolution rates were remarkably increased in inclusion complex, compared with the physical mixture and drug alone. (Seoung et al, 2006)

In the year of 2006, a study was performed where a method was established to determine the dissolution rate of atorvastatin calcium soft capsules. The phosphate buffer solution was used as dissolution medium and rotary basket method was also used. HPLC was used to determine the dissolution content. Finally it was found that the atorvastatin calcium soft capsules were good as

they showed dissolution content more than 90 percent. (Zhang-wen, Xuan-bo and Xue-jian, 2006)

A study was done to improve the solubility and dissolution rate of a poorly water-soluble drug that is lovastin. The technique used was solid dispersion technique. These solid dispersions were prepared by polyethylene glycol 4000 (PEG 4000) and polyvinylpyrrolidone K30 (PVP K 30) with different ratios. The new formulations were screened with, calorimetry, X-ray power diffraction and FT-IR spectroscopy. Finally it was found that the lovastin tablets that were prepared with PEG and PVP showed better solubility and dissolution rate than the lovastin tablets alone. (Patel and Patel, 2007)

A study described the evaluation of atorvastatin and amlodipine combination by reverse phase HPLC. Combination of atorvastatin and amlodipine along with only atorvastatin and only amlodipine were subjected to thermal, photolytic, hydrolytic and oxidative stress. In-vitro dissolution test were also performed. The average percentage of drug release was found to be more than 70% within 30 minutes for both drugs. This method can also be used to determine the in-vitro dissolution rate of combination drug products. (Chaudhari, Patel and Shah, 2007)

At 2007, one research project was accomplished to prepare simvastatin and its inclusion complex with hydroxypropyl beta-cyclodextrin by using supercritical antisolvent process (SAS) to investigate the improvement of the aqueous solubility and the dissolution rate of drug. In result, it was declared that SAS process could be a useful methos for the preparation of the inclusion complex of drug with hydroxypropyl beta-cyclodextrin and its solubility and dissolution rate were increased significantly.(Jun et al., 2007)

In the same year of 2008, another research was conducted to improve the solubility and dissolution rate of a poorly water-soluble drug, Simvastatin by solid dispersion technique. By this study, it was determined that tablets containing solid dispersion prepared with PEG and PVP showed significant improvement in the release profile of Simvastatin as compared to tablet containing Simvastatin without PEG or PVP. (Patel and Patel, 2008)

In drug development enhancement of drug solubility and dissolution rate is a challenging task. A hydrophilic and low viscosity polymer hydroxyproply methyl cellulose (HPMC K_3LV) was used to increase the solubility of simvastatin. For solvent evaporation two methods were used that are

spray drying and rotaevaporation. The crystalline form of simvastatin was converted to amorphous form. When the co-solvent evaporated mixtures were compared with simvastatin then there was an increasing rate of dissolution. These co-solvent evaporated mixtures showed better therapeutic effect because of its better solubility and dissolution rate. (Pandya et al., 2008)

The objective of a study was to increase the solubility, dissolution rate and stability of atorvastatin calcium (ATN) through inclusion complexation with β-cyclodextrin (β-CD). Solid complexex were prepared by physical mixing, kneading, co-evaporation and freeze-drying and for screening purpose calorimetry, fourier transform infrared spectroscopy, and powder X-ray diffactometry were used. The solubility and dissolution rate of atorvastatin complex with β-CD were increased than atorvastatin alone when in-vitro studies were performed. Again, the freeze dried products also showed higher solubility and dissolution rate in combination of atorvastatin and β-CD. (Palem, Patel and Pokharkar, 2009)

In the year of 2010, a study was done which objective was to formulate surface solid dispersions of simvatatin to improve the aqueous solubility and dissolution rate to facilitate faster onset of action. To accomplish this study, co-evaporation method was used. The result showed that solid dispersion increased dissolution rate of that drug. (Rao et al., 2010)

The dissolution rate of simvastatin was investigated in a study to identify if the nanosuspension preparation of simvastatin has a better dissolution rate. The nanosuspension of simvastatin was prepared with Pluronic F127 and zirconium oxide (ZnO_2) beads by using a wet-milling technique. The researchers found that the therapeutic drug effect can be faster by maintaining nano-sized particles of simvastatin as the in vitro dissolution rate increases with nanosuspensions. (Pandya et al., 2011)

A research was done to establish a new, simple, precise, rapid and accurate formulation method for Rosuvastatin Calcium as active ingredients. In this research, High Performance Liquid Chromatography was used as method. By this method, accurate formulation of Rosuvastatin Calcium was developed. (Chakraborty, Mishra and Sahoo, 2011)

The intent of the study was to enhance the dissolution profile, absorption efficiency and bioavailability of water insoluble drugs like Lovastatin by using various techniques. This study includes methods like solid dispersions, superdisintegrants and sublimation with respective to

conventional drug. Solid dispersions of lovastatin showed enhancement in its dissolution efficiency when compared to conventional preparation. The results of this research suggest that there was satisfactory dissolution enhancement from all three methods and could potentially lead to improvement in bioavailability of oral lovastatin products, due to its simplicity, low cost and industrial feasibility super disintegrant method was preferred. (Karthik et al, 2012)

In the year of 2012, one article was written which objective was to prepare simvastatin nanocrystals to enhance its dissolution rate and bioavailability by using sonoprecipitation method. The result of this study showed that this method could produce small and uniform simvastatin nanocrystals with an improved saturation solubility, dissolution rate and oral bioavailability. (Wang et al., 2012)

Lovastatin was studied to enhance the surface area, the dissolution rate and the bioavailability. For this study, solvent evaporation method was used. The result of this study showed that the peaks of Lovastatin and polymers were distinguishable and there was no chemical interaction between drug and polymer. (Shaikh, 2012)

Atorvastatin calcium (ATC) were developed to multiple-unit floating microcapsules to improve its solubility and dissolution rate. Emulsion-solvent evaporation technique was used to prepare the floating microcapsules. As a dissolution enhancer dioctyl sodium sulphosuccinate (DSS) was used. The microcapsules were assessed for shape, size, drug entrapment efficiency, stability and *in-vitro* drug dissolution rate and were subjected to SEM, DSC and PXRD studies. The floating microspheres containing DSS had significantly higher drug dissolution rates than those without DSS. The present study indicates that the use of multi-unit floating microcapsules for delivery of ATC can improve its bioavailability. (Dehghan and Khan, 2012)

In this research, The solvent evaporation method was found to be a promising method for formulating uniform and stable lovastatin solid dispersions with enhanced surface area and dissolution rate. The bioavailability also increased due to increased wettability of the solid dispersions. For this, Lovastatin solid dispersions were prepared by solvent evaporation method. The prepared solid dispersions were characterized by Fourier transform infrared (FT-IR) spectroscopy and evaluated for various parameters like drug content, solubility and dissolution studies and different physical properties. The data showed that solubility raised in all cases.

Dissolution data of all solid dispersions also indicated increase in dissolution as compared to pure drug and increase was due to wetting phenomenon of superdisintegrants used for preparation of solid dispersions. (Khayyam et al., 2012)

In the year 2013, a research was done for the improvement of the solubility and dissolution rate of atorvastatin by 'Solid Dispersion' technique and solvent evaporation method. By using this method, it was found that hydrophilic carrier Poloxamer 188 was found to be played a vital role in the improvement of the dissolution property. (Tanwir et al., 2013)

A study was done which objective was to investigate the effect of polyethylene glycol (PEG) molecular weights as solid dispersion carriers on the dissolution behavior of simvastatin. Here solvent method was used to determine this project. From this preoject result confirmed the influence of PEG molecular weight on drug dissolution rate from solid dispersion systems. (Bolourchian, Mahboobian and Dadashzadeh, 2013)

In a research project, the technique for changing fluid medicines of water insoluble medications in non-volatile fluid vehicles over into acceptably flowing and compressible powder form was used. For precompression parameters, defined frameworks were evaluated like flow properties of liquisolid framework, Fourior change infra red spectra (FTIR) examination, X-beam powder diffraction (XRPD), differential filtering calorimetry (DSC), and post pressure parameters like substance consistency, weight variety, hardness and friability, wetting time, in vitro disintegration concentrates on, impact of disintegration volume on medication discharge rate, and estimation of portion of molecularly scattered medication in fluid medicine. As liquisolid compacts exhibited fundamentally higher medication discharge rates, they prompt to conclusion through their research that it could be a promising procedure in enhancing the disintegration of poor water dissolvable medications and figuring quick release strong dose forms.(Deshmukh, Kapure and Pande, 2013)

A study found that Niosomes were promising dosage form for enhance dissolution and permeability of slightly soluble drugs prepared by film hydration method. For this research, non-ionic surfactants (Span 20,Span 60,span 80), cholesterol and lecithin in different ratios were used by film hydration method and evaluated the formulas by HPLC, particle size, morphology, in-vitro drug release and ex-vivo permeation study. The result of this study showed that niosomes

were the excellent dosage form for the enhancement of dissolution and permeability of poorly soluble drugs prepared by film hydration method. (Omar S., 2013)

A study was done to prove that liquisolid technique is a promising alternative for improvement of the dissolution rate of water insoluble drug. The IR and DSC studies demonstrated that there is no significant interaction between the drug and excipients. Liquisolid compacts demonstrated significantly higher drug release rates than those of conventional and marketed tablet due to increasing wetting properties and surface area of the drug. (Chaudhari, Kamble, and Shaikh, 2014)

A work had done in 2014 to prepare a self emulsifying drug delivery system(SEDDS) of rosuvastatin calcium with the least amount of surfactant which could enhance its solubility and oral bioavailability. For this research, spectrophotometric method was implemented. From this study, it was concluded that solid SEDDS could be a promising approach to enhance the solubility and drug release of rosuvastatin calcium. (Nagda, Nagda and Rokad, 2014)

This research showed that liquisolid compacts demonstrated significantly higher drug release rates than those of conventionally made directly compressed tablets. As the poor dissolution rate of water-insoluble drugs is still a major problem confronting the pharmaceutical industry. In this study, the dissolution behavior of lovastatin from liquisolid compacts was investigated in simulated gastric fluid (SGF, p^H1.2). To this end, several liquisolid tablets formulations containing various ratios of drug: Propylene glycol were prepared. The ratio of starch and microcrystalline cellulose (carrier) to silica (coating powder material) was kept constant in all formulations. (Shyam et al., 2014)

A research was done with the purpose to determine the drug-drug interaction between Atorvastatin, Metformin HCL and Multivitamin by doing in vitro dissolution study. In this study, result had shown that combination gave antagonistic activity and so the dissolution rate decreased. (Islam, 2014)

In the year of 2014, a research was aimed at the improvement of the dissolution rate of Atorvastatin calcium by the solid dispersion technique using modified locust bean gum. According to this study, it is concluded that modified locust bean gum could be a promising

carrier for the solubility enhancement of poorly water-soluble drugs, rosuvastatin calcium. (Nagpal et al., 2014)

A research work was done which objective was to improve the solubility of the poorly water soluble drug Atorvastatin. In this project, Solid dispersion technique was used with Neem Gum as a hydrophilic carrier. From this study, it was concluded that hydrophilic NG was essential in enhancing the solubility, dissolution rate and bioavailability of atorvastatin. (Rodde et al., 2014)

The work was done to develop an immediate release tablet formulation with fenofibrate and rosuvastatin and also to improve their dissolution profile. In this project 'Hot-Melt' technology was used to improve the dissolution profile. The formulated tablets were examined for hardness test, weight uniformity, friability test and dissolution test. The result showed that all tablets met the requirements and the release of dissolution profile was also increased in the combination form of fenofibrate and rosuvastatin. (Sarkar et al., 2015)

The main objective of this study was to enhance the solubility, dissolution rate, bioavailability of water insoluble drug pitavastatin by liquisolid technology and solid dispersions. The liquisolid compacts were prepared by different ratios of polyethylene glycol 400, micro crystalline cellulose used as carrier material and colloidal silicon dioxide as coating material. In-vitro dissolution profiles of liquisolid formulation, solid dispersions were studied and compared with that of pure pitavastatin tablet formulation in 0.1N HCL. It was found that liquisolid formulation tablets showed higher dissolution rate. (Rajendra et al,2016)

Chapter Three

Materials and Method

3.1 Sample Collection:

To observe the impact of calcium with vitamin D in the dissolution profile of Rosuvastain calcium samples of 50 tablets of Rocovas® (10 mg) and 10 tablets of Calvimax-D (500 mg) were collected from local drug store in Dhaka.

Table 3.1: Samples used in the experiment with their sources

Name of Sample	Source (Supplier)
Rocovus® tablets	Incepta Pharmaceuticals Ltd.
Calvimax-D tablets	Incepta Pharmaceuticals Ltd.

Figure 3.1: Rocovus® 10 mg (Rosuvastatin) and Calvimax-D (Incepta Pharmaceuticals, 2016)

Table 3.2: Reagent used in the experiment

Reagent Name	Source (Supplier)
Distilled water	East West University Laboratory

Table 3.3: Instruments used in the experiment

Serial no.	Equipments	Source (Name of supplier)	Origin
1	Dissolution Tester	SMIC	China
2	UV Spectrophotometer	Shimadzu UV-1800	Japan
3	Distill water plant	SMIC	China
4	Electronic balance	PrecisaXB120A	Switzerland
5	Vernier caliper	China supplier Shanghai	China
6	Friability tester	Veegoindia	India
7	Hardness tester	Manually operated hardness tester	India

Table 3.4: Apparatus that were used throughout the experiments

Serial Number	Apparatus
1	Beaker
2	Test tube
3	Filter paper
4	Mortar and pestle
5	Funnel
6	Volumetric flask (50 ml, 100 ml)
7	Pipette pumper
8	Pipette (10 ml, 1 ml)
9	Spatula
10	Syringe (10 ml)

Table 3.5: *In vitro* dissolution study

Dissolution medium	Distilled water
RPM	50
Time	60 minutes

3.2 Important Images

Some important images of instruments that were used during different steps of experiments:

Figure 3.2.1: Dissolution tester (Electrolab)

Figure 3.2.2: UV Spectrophotometer (Biocompare)

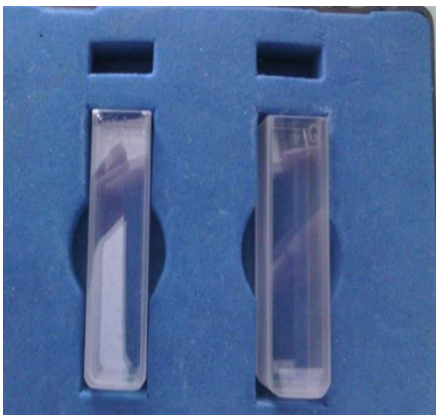

Figure 3.2.3: Quartz cuvette

Figure 3.2.4: Water distillation machine (Bpress, 2014)

Figure 3.2.5: Vernier capilar (KBC tool and machinery)

Figure 3.2.6: Electric analytical balance (Contech Instrument Ltd)

Figure 3.2.7: Hardness tester (Natoli)

3.3 Method:

3.3.1 Preparation of Dissolution Medium for Standard Curve:

Rosuvastatin is a water soluble drug. So, for dissolution and standard curve of rosuvastatin distilled water was used. The distilled water propagating apparatus of East West University was used to prepare 500 ml of distilled water that was used for standard curve preparation.

3.3.2 Preparation of Standard Curve:

For obtaining standard curve at first different concentrations of rosuvastatin calcium (0.001 mg/ml, 0.002 mg/ml, 0.003 mg/ml etc) were prepared. The following steps were followed:

- Rocovus® tablet was crushed in mortar and pestle.
- Equivalent weight of 10 mg of tablet was measured and then it was dissolved in 100 ml of distilled water. By this procedure the concentration of the stock solution became .01mg/ml.
- Then the solution in the volumetric flask was filtered.

3.3.2.1 Calculations:

For the preparation of 0.001 mg/ml,

V_1= 0.001 mg/ml

$S_{1=}$ 15 ml

S_2= .01 mg/ml

V_2= ?

We know that, $V_1 S_1 = V_2 S_2$

Or, $V_{2=} V_1 S_1 / S_2$

$V_{2=}$ [(0.001×15)/.01] ml

V_2= 1.5 ml

This 1.5 ml of stock solution was added with 13.5 ml of distilled water to obtain 15 ml of solution.

The same calculation was followed for the preparation of 0.002 mg/ml, 0.003 mg/ml, 0.004 mg/ml, 0.005 mg/ml, 0.006 mg/ml, 0.007 mg/ml, 0.008 mg/ml and 0.009 mg/ml.

For,

- 0.002 mg/ml, 3 ml of stock solution was added with 12 ml of distilled water.
- 0.003 mg/ml, 4.5 ml of stock solution was added with 10 ml of distilled water.
- 0.004 mg/ml, 6 ml of stock solution was added with 9 ml of distilled water.
- 0.005 mg/ml, 7.5 ml of stock solution was added with 7.5 ml of distilled water.
- 0.006 mg/ml, 9 ml of stock solution was added with 6 ml of distilled water.
- 0.007 mg/ml, 10.5 ml of stock solution was added with 4.5 ml of distilled water.
- 0.008 mg/ml, 12 ml of stock solution was added with 3 ml of distilled water.
- 0.009 mg/ml, 13.5 ml of stock solution was added with 1.5 ml of distilled water.

Table 3.6: Concentration of Rosuvastatin Prepared

Serial no.	Concentration of Rosuvastatin calcium (mg/ml)
1	0.001
2	0.002
3	0.003
4	0.004
5	0.005
6	0.006
7	0.007
8	0.008
9	0.009

- After preparing the desired concentrations, the spectrophotometer was turned on and 241nm wavelength was set up.
- The spectrophotometer was adjusted for 0 and 100% T (transmittance).
- The absorbance of the prepared solutions were measured
- Then the absorbencies were plotted against concentrations and a straight line was found.

3.3 Preparation for Dissolution Test

3.3.1 Preparation of Dissolution Medium

For each batch 6 liters of distilled water was prepared in the laboratory and was used as the dissolution medium for dissolution test.

3.3.2 Method for Dissolution Test of Rocovus® (Rosuvastatin)

1. 6 L (6000 ml) of distilled water was prepared.
2. Each vessel of the dissolution tester was filled with 900 ml of distilled water.

3. The dissolution tester was set at a temperature of 37°C and 50 rpm (rotation per minute) for 1 hour.
4. The machine was allowed to reach the desired temperature before operation.
5. When temperature was reached then each vessel were provided with one tablet of Rocovus®.
6. Samples were collected from each vessel after 10, 20, 30, 40, 50 and 60 minutes.
7. The solutions were filtered and in the UV spectrophotometer, the absorbance of the samples was measured at a wavelength of 241nm.

3.3.3 Method for Dissolution Test of Rocovus® (Rosuvastatin) and Calvimax-D (Calcium and vitamin D supplement)

1. 6 L (6000 ml) of distilled water was prepared.
2. Each vessel of the dissolution tester was filled with 900 ml of distilled water.
3. The dissolution tester was set at a temperature of 37°C and 50 rpm (rotation per minute) for 1 hour.
4. The machine was allowed to reach the desired temperature before operation.
5. When temperature was reached then each vessel were provided with one tablet of Rocovus® and one tablet of calvimax-D.
6. Samples were collected from each vessel after 10, 20, 30, 40, 50 and 60 minutes.
7. The solutions were filtered and in the UV spectrophotometer, the absorbance of the samples was measured at a wavelength of 241nm.

3.4 Impact of Calvimax-D on the Dissolution Profile of Rocovus®

The impact calcium-vitamin D that may alter the dissolution of rosuvastatin is calculated by the following equation:

Impact on dissolution=(Average percent of drug release in combination-average percent of drug release in rosuvastatin)/ average percent of drug release in rosuvastatin×100

3.5 Determination of Physical Parameters

3.5.1 Weight Variation Test

3.5.1.1 Test Procedure

- The weight of 10 tablets was taken.
- The average weight was taken and it was considered as the standard weight of an individual.
- Individually all tablets were weighted and then they were compared to determine if they are within the range or not.

Noted that among the weights the variation of the average weight not more than two tablets must not differ more than the percentage listed below:

Table 3.7: Accepted percentage list for weight variation test of tablets

Weight of tablets	Percentage difference
130 mg or less	±10%
More than 130 mg to 324 mg	±7.5%
More than 324 mg	±5%

3.5.1.2 Equation:

To determine the % weight variation of tablets the following equation was used:

$$\% \text{ Weight variation} = (A-I/I) \times 100$$

Here, I= initial weight of tablet (g)

A= average weight of tablet (g) (Dunnet and Crisafio, 1995)

3.5.2 Thickness Test:

3.5.2.1 Procedure:

First Vernier error is calculated, if present.

Then the tablet was placed between two jaws of the Vernier caliper.

Then the main scale reading and Vernier scale reading were taken.

The two readings were added together for multiplying with the Vernier constant 0.1 Cm.

3.5.2.2 Calculation:

To determine the thickness of tablets the following formula is used:

Thickness of tablet= Reading of Cm scale+ reading of Vernier scale× Vernier constant (0.01)+ Vernier error

3.5.3 Hardness Test

3.5.3.1 Procedure:

- The slide scale of the hardness tester was made zero.
- Between the two jaws of the tester, one tablet was placed vertically.
- Forced was applied with a screw thread and spring until tablet fractured.
- Reading in Kg was taken from the sliding scale.

Chapter Four

Results

4.1 Result

4.1.1 General Information:

The Rosuvastatin samples were subjected to assay and dissolution profile analysis under the optimum conditions. The purpose of assay was to determine the impact of dissolution profile of rosuvastatin when given in combination with other agents.

4.2 Physical Parameter Test

4.2.1 Weight Variation Test

Table 4.1: Results of weight variation test for Rocovus®

Tablet no.	Initial weight of Tablet (g), I	Average weight of Tablet (g), A	% Weight variation, (A-I)/I×100
1	0.145	0.145	0
2	0.144		0.69
3	0.145		0
4	0.146		-0.68
5	0.144		0.69
6	0.145		0
7	0.145		0
8	0.145		0
9	0.145		0
10	0.145		0

4.2.2 Thickness Test

Table 4.2: Result of thickness test of Rocovus®

Tablet no.	Main scale reading, M (cm)	Vernier scale reading, V (cm)	Vernier constant, Vc	Vernier error	Thickness of the tablet, M+(V× Vc) (cm)
1	0.3	2	0.1	0	0.5
2	0.3	2			0.5
3	0.3	2			0.5
4	0.3	2			0.5
5	0.3	2			0.5
6	0.3	2			0.5
7	0.3	2			0.5
8	0.3	2			0.5
9	0.3	2			0.5
10	0.3	2			0.5

4.2.3 Hardness Test

Table 4.3: Result of hardness test for Rocovus®

Tablet no.	Hardness (kg)	Average (kg)
1	1	1.45
2	1.5	
3	2	
4	2	
5	1	

6	1.5	
7	1	
8	1.5	
9	1.5	
10	1.5	

4.3 Standard Assay

4.3.1 Standard Curve:

10 mg of Rosuvastatin calcium (Rocovus®) was taken for preparing standard curve and the concentrations were raised gradually from 0.001 to 0.009 and the result of absorbencies are listed below:

Table 4.4: Standard curve value

Serial no.	Concentration (mg/ml)	Absorbance at 241nm
1	0.001	0.049
2	0.002	0.083
3	0.003	0.124
4	0.004	0.158
5	0.005	0.231
6	0.006	0.240
7	0.007	0.278
8	0.008	0.317
9	0.009	0.362

By plotting the concentration against the absorbance of Rocovus® (Rosuvastatin), a straight line was found.

From the standard curve of Rocovus®, an equation was derived and that is y = 39.91x + 0.003 and R^2 = 0.993. Here, y= absorbance and x= concentration of drug.

This equation is used for further calculations of Rocovus®.

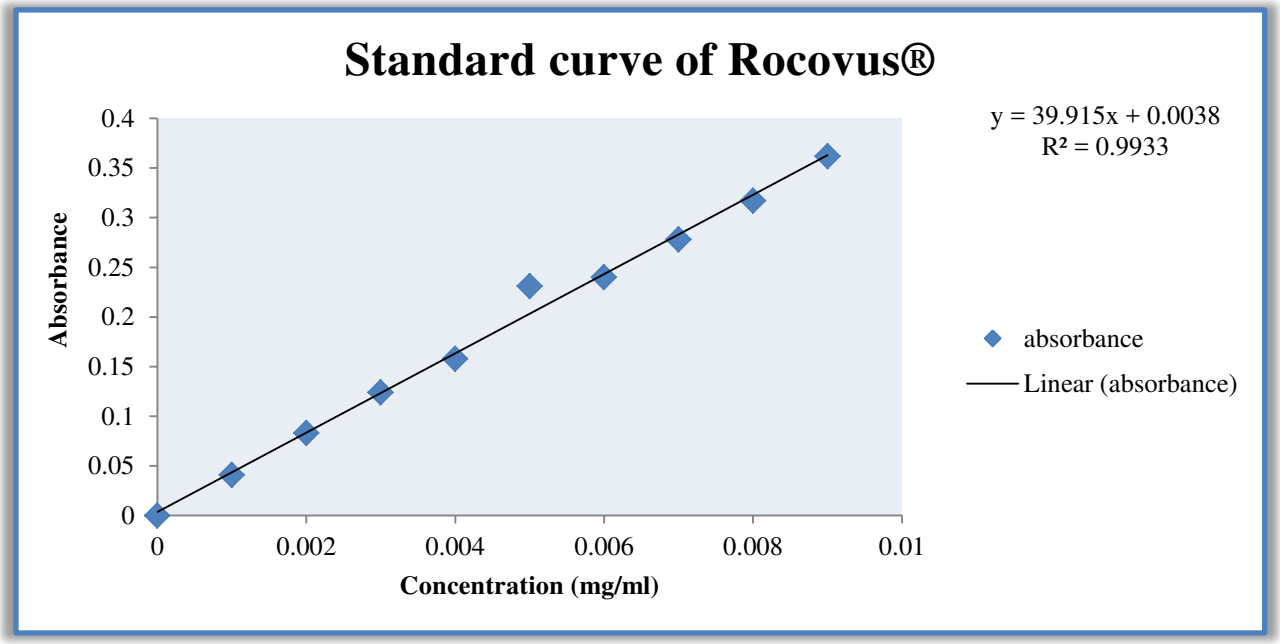

Figure 4.1: Standard curve of Rocovus® (Rosuvastatin calcium)

Here the release of drug is increasing with the increasing time. This graph is considered as the standard curve for the following drugs. Here, X axis represents the concentration in mg/ml and Y axis is for drug absorbance at 241 nm.

4.4 Results of the Dissolution Test

Result of the dissolution test of individual Rocovus® and Rocovus® with Calvimax-D® is calculated at a specific time interval under optimum condition and the following results were obtained:

4.4.1 Dissolution Test of Rocovus® without any Supplement

Table 4.5: UV absorbance of only Rocovus® (Rosuvastatin calcium) 10 mg

Sample no.	Absorbance					
	10 minutes	20 minutes	30 minutes	40 minutes	50 minutes	60 minutes
1	0.245	0.253	0.272	0.284	0.301	0.317
2	0.244	0.259	0.271	0.287	0.303	0.321
3	0.239	0.254	0.274	0.285	0.298	0.314
4	0.241	0.262	0.274	0.290	0.307	0.324
5	0.242	0.258	0.273	0.288	0.302	0.322
6	0.240	0.252	0.275	0.286	0.295	0.319

4.4.1.1 Calculation:

The dissolved amount of Rocovus® (Rosuvastatin calcium) is calculated by the following equation that is obtained from the standard curve:

$y = 39.91x + 0.003$

Where, y= absorbance and x= concentration

Here the dilution factor is 900.

When the absorbance was 0.245, then the following equation can be written as-

$0.245 = 39.91x + 0.003$

Or, $39.91x = 0.245 - 0.003$

Or, $39.91x = 0.242$

Or, $x = 0.242/39.91$

$x = 0.006$

So, dissolved amount of Rocovus® (Rosuvastatin calcium) was= $0.006 \times 900 = 5.46$

By putting the other absorbance values in the same equation different dissolved amount of Rocovus® was calculated.

Table 4.6: Determination of dissolved amount of Rocovus® without supplement

Time	Sample no.	Absorbance	Drug Release (mg)	% Drug Release
10 minutes	1	0.245	5.46	54.57
	2	0.244	5.43	54.35
	3	0.239	5.32	53.22
	4	0.241	5.37	53.67
	5	0.242	5.39	53.90
	6	0.240	5.34	53.45
20 minutes	1	0.253	5.64	56.38
	2	0.259	5.77	57.73
	3	0.254	5.66	56.60
	4	0.262	5.84	58.41
	5	0.258	5.75	57.50
	6	0.252	5.62	56.15
30 minutes	1	0.272	6.07	60.66
	2	0.271	6.04	60.44
	3	0.274	6.11	61.11
	4	0.274	6.11	61.11
	5	0.273	6.09	60.89
	6	0.275	6.13	61.34

Time	Sample no.	Absorbance	Drug Release (mg)	% Drug Release
40 minutes	1	0.284	6.34	63.37
	2	0.287	6.40	64.04
	3	0.285	6.36	63.59
	4	0.290	6.47	64.72
	5	0.288	6.43	64.27
	6	0.286	6.38	63.82
50 minutes	1	0.301	6.72	67.20
	2	0.303	6.77	67.65
	3	0.298	6.65	66.52
	4	0.307	6.86	68.55
	5	0.302	6.74	67.43
	6	0.295	6.58	65.85
60 minutes	1	0.317	7.08	70.81
	2	0.321	7.17	71.71
	3	0.314	7.01	70.13
	4	0.324	7.24	72.39
	5	0.322	7.19	71.94
	6	0.319	7.13	71.26

4.4.2 Dissolution test of Rocovus® (Rosuvastatin) and Calvimax-D (Calcium-vitamin D)

Table 4.7: UV absorbance of Rocovus® (Rosuvastatin) with Calvimax-D (Calcium-vitamin D)

Sample no.	Absorbance					
	10 minutes	20 minutes	30 minutes	40 minutes	50 minutes	60 minutes
1	0.192	0.199	0.211	0.22	0.231	0.234
2	0.179	0.191	0.205	0.215	0.22	0.223
3	0.189	0.197	0.206	0.21	0.223	0.227
4	0.181	0.195	0.203	0.207	0.218	0.221
5	0.183	0.196	0.202	0.209	0.219	0.224
6	0.178	0.189	0.198	0.206	0.215	0.22

4.4.2.1 Calculation:

Calculation for dissolved amount of (mg) of Rocovus® (Rosuvastatin) with Calvimax-D (Calcium-vitamin D):

$y = 39.91x + 0.003$

Where, y= absorbance and x= concentration

Here the dilution factor is 900.

When the absorbance was 0.192, then the following equation can be written as-

$0.192 = 39.91x + 0.003$

Or, $39.91x = 0.192 - 0.003$

Or, $39.91x = 0.189$

Or, $x = 0.189/39.91$

$x = 0.005$

So, dissolved amount of Rocovus® with Calvimax-D was= $0.005 \times 900 = 4.28$

By putting the other absorbance values in the same equation different dissolved amount of Rocovus® with Calvimax-D was calculated.

Table 4.8: Determination of dissolved amount of Rocovus® (10 mg) with Calvimax-D (500 mg)

Time	Sample no.	Absorbance	Drug Release (mg)	% Drug Release
10 minutes	1	0.192	4.26	42.62
	2	0.179	3.97	39.69
	3	0.189	4.19	41.94
	4	0.181	4.01	40.14
	5	0.183	4.06	40.59
	6	0.178	3.95	39.46
20 minutes	1	0.199	4.42	44.20
	2	0.191	4.24	42.40
	3	0.197	4.37	43.75
	4	0.195	4.33	43.30
	5	0.196	4.35	43.52
	6	0.189	4.19	41.94
30 minutes	1	0.211	4.69	46.91
	2	0.205	4.56	45.55
	3	0.206	4.58	45.78
	4	0.203	4.51	45.10
	5	0.202	4.49	44.88
	6	0.198	4.40	43.97

Time	Sample no.	Absorbance	Drug Release (mg)	% Drug Release
40 minutes	1	0.22	4.89	48.94
	2	0.215	4.78	47.81
	3	0.210	4.67	46.68
	4	0.207	4.60	46.00
	5	0.209	4.65	46.45
	6	0.206	4.58	45.78
50 minutes	1	0.231	5.14	51.42
	2	0.220	4.89	48.94
	3	0.223	4.96	49.61
	4	0.218	4.85	48.48
	5	0.219	4.87	48.71
	6	0.215	4.78	47.81
60 minutes	1	0.234	5.21	52.09
	2	0.223	4.96	49.61
	3	0.227	5.05	50.51
	4	0.221	4.92	49.16
	5	0.224	4.98	49.84
	6	0.220	4.89	48.94

4.5 Impact of Calcium and Vitamin-D on the Dissolution Profile of Rocovus®

Table 4.9: Average percentage calculation for dissolved amount of Rocovus®, Rocovus® with Calvimax-D (Calcium-vitamin D) and the impact of calcium and vitamin-D on the dissolution of Rocovus® after 10 minutes

Rocovus® without supplement				**Rocovus® with Calvimax-D**				**Result**
Dissolved amount (mg)	Average dissolved amount (mg)	Percent dissolved amount (%)	Average percent dissolved amount (%)	Dissolved amount (mg)	Average dissolved amount (mg)	Percent dissolved amount (%)	Average percent dissolved amount (%)	Impact on dissolution (%)
5.46		54.57		4.26		42.62		
5.43		54.35		3.97		39.69		
5.32	5.39	53.22	53.86	4.19	4.07	41.94	40.74	-24.36
5.37		53.67		4.01		40.14		
5.39		53.90		4.06		40.59		
5.34		53.45		3.95		39.46		

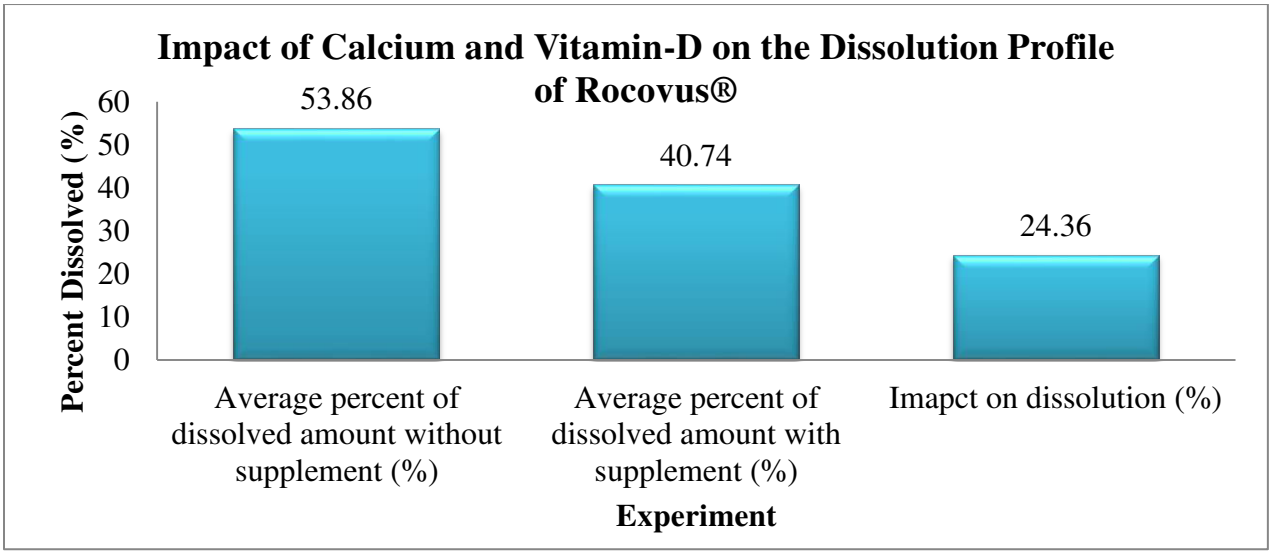

Figure 4.2: Impact of drug dissolution after 10 minutes

Table 4.10: Average percentage calculation for dissolved amount of Rocovus®, Rocovus® with Calvimax-D (Calcium-vitamin D) and the impact of calcium and vitamin-D on the dissolution of Rocovus® after 20 minutes

Rocovus® without supplement				**Rocovus® with Calvimax-D**				**Result**
Dissolved amount (mg)	Average dissolved amount (mg)	Percent dissolved amount (%)	Average percent dissolved amount (%)	Dissolved amount (mg)	Average dissolved amount (mg)	Percent dissolved amount (%)	Average percent dissolved amount (%)	Impact on dissolution (%)
5.64		56.38		4.42		44.20		
5.77		57.73		4.24		42.40		
5.66	5.71	56.60	57.13	4.37	4.32	43.75	43.19	-24.40
5.84		58.41		4.33		43.30		
5.75		57.50		4.35		43.52		
5.62		56.15		4.19		41.94		

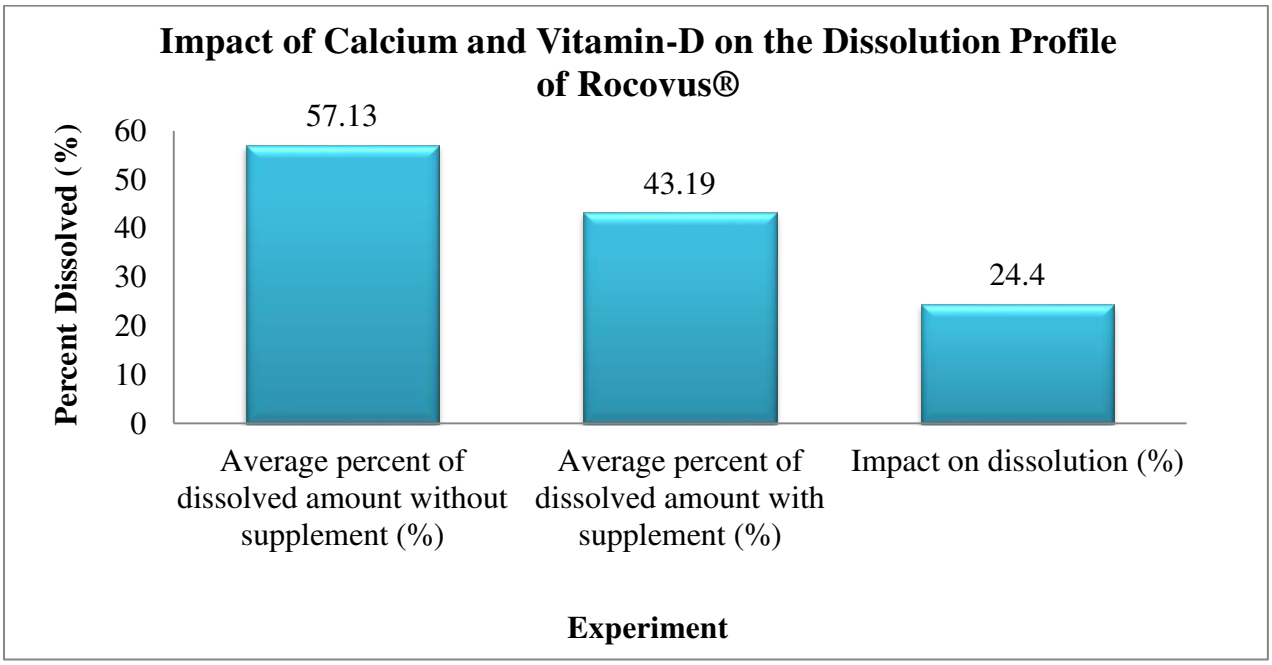

Figure 4.3: Impact of drug dissolution after 20 minutes

Table 4.11: Average percentage calculation for dissolved amount of Rocovus®, Rocovus® with Calvimax-D (Calcium-vitamin D) and the impact of calcium and vitamin-D on the dissolution of Rocovus® after 30 minutes

Rocovus® without supplement				**Rocovus® with Calvimax-D**				**Result**
Dissolved amount (mg)	Average dissolved amount (mg)	Percent dissolved amount (%)	Average percent dissolved amount (%)	Dissolved amount (mg)	Average dissolved amount (mg)	Percent dissolved amount (%)	Average percent dissolved amount (%)	Impact on dissolution (%)
6.07		60.66		4.69		46.91		
6.04		60.44		4.56		45.55		
6.11	6.09	61.11	60.93	4.58	4.54	45.78	45.37	-25.54
6.11		61.11		4.51		45.10		
6.09		60.89		4.49		44.88		
6.13		61.34		4.40		43.97		

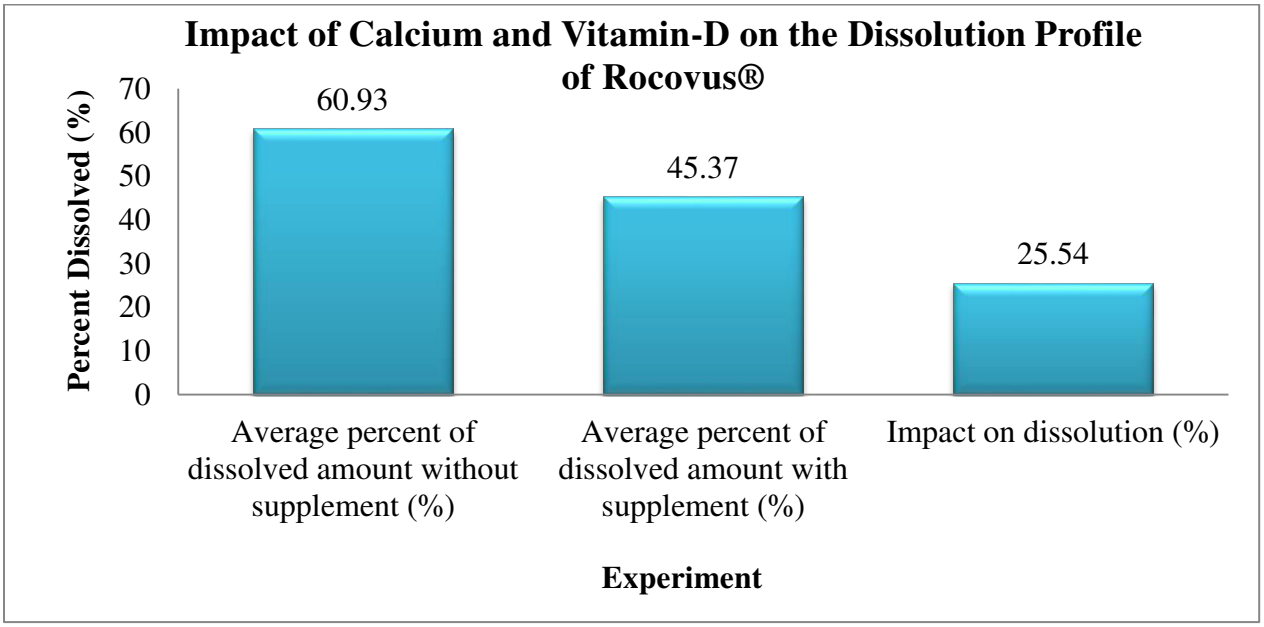

Figure 4.4: Impact of dissolution after 30 minutes

Table 4.12: Average percentage calculation for dissolved amount of Rocovus®, Rocovus® with Calvimax-D (Calcium-vitamin D) and the impact of calcium and vitamin-D on the dissolution of Rocovus® after 40 minutes

Rocovus® without supplement				**Rocovus® with Calvimax-D**				**Result**
Dissolved amount (mg)	Average dissolved amount (mg)	Percent dissolved amount (%)	Average percent dissolved amount (%)	Dissolved amount (mg)	Average dissolved amount (mg)	Percent dissolved amount (%)	Average percent dissolved amount (%)	Impact on dissolution (%)
6.34		63.37		4.89		48.94		
6.40		64.04		4.78		47.81		
6.36	6.40	63.59	63.97	4.67	4.70	46.68	46.94	-26.62
6.47		64.72		4.60		46.00		
6.43		64.27		4.65		46.45		
6.38		63.82		4.58		45.78		

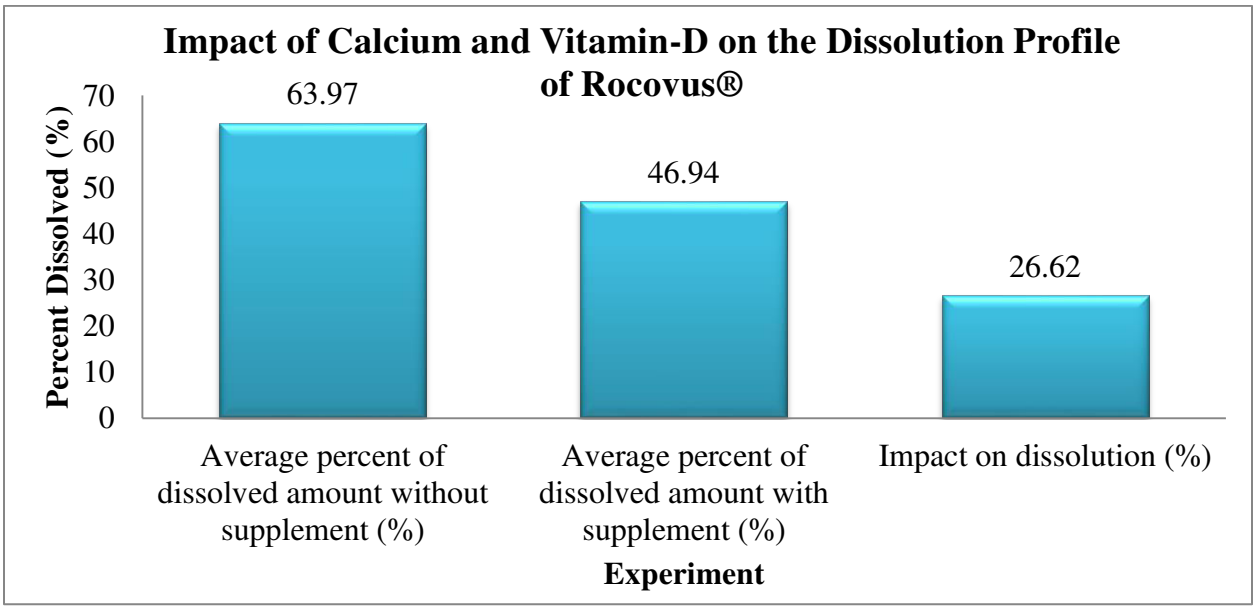

Figure 4.5: Impact of dissolution after 40 minutes

Table 4.13: Average percentage calculation for dissolved amount of Rocovus®, Rocovus® with Calvimax-D (Calcium-vitamin D) and the impact of calcium and vitamin-D on the dissolution of Rocovus® after 50 minutes

Rocovus® without supplement				**Rocovus® with Calvimax-D**				**Result**
Dissolved amount (mg)	Average dissolved amount (mg)	Percent dissolved amount (%)	Average percent dissolved amount (%)	Dissolved amount (mg)	Average dissolved amount (mg)	Percent dissolved amount (%)	Average percent dissolved amount (%)	Impact on dissolution (%)
6.72		67.20		5.14		51.42		
6.77		67.65		4.89		48.94		
6.65	6.72	66.52	67.2	4.96	4.92	49.61	49.16	-26.85
6.86		68.55		4.85		48.48		
6.74		67.43		4.87		48.71		
6.58		65.85		4.78		47.81		

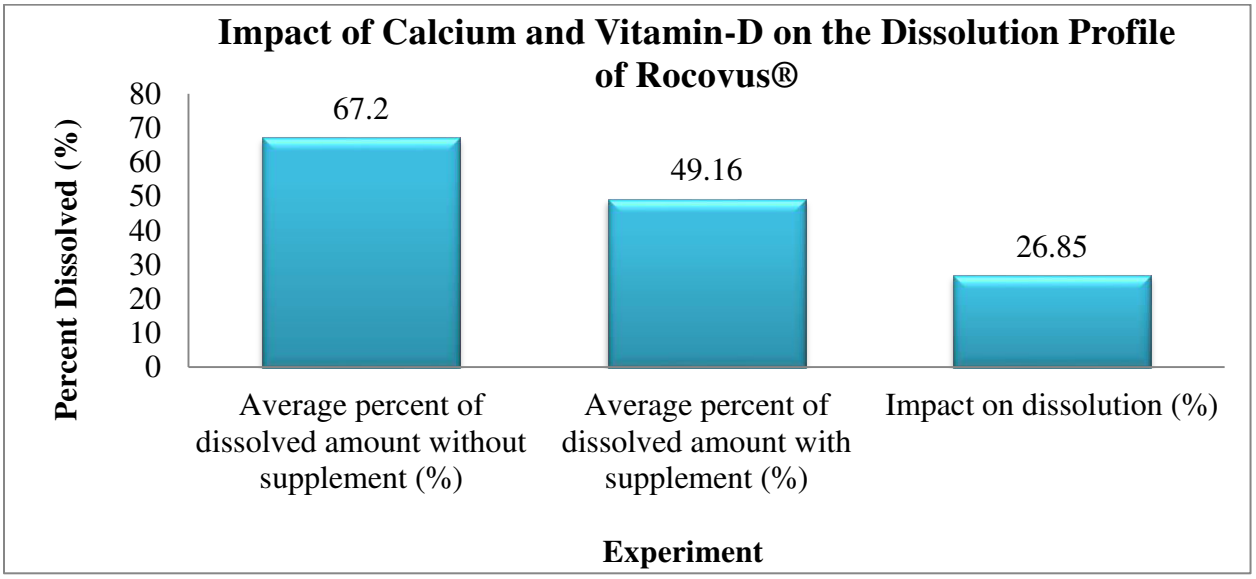

Figure 4.6: Impact of dissolution after 50 minutes

Table 4.14: Average percentage calculation for dissolved amount of Rocovus®, Rocovus® with Calvimax-D (Calcium-vitamin D) and the impact of calcium and vitamin-D on the dissolution of Rocovus® after 60 minutes

Rocovus® without supplement				**Rocovus® with Calvimax-D**				**Result**
Dissolved amount (mg)	Average dissolved amount (mg)	Percent dissolved amount (%)	Average percent dissolved amount (%)	Dissolved amount (mg)	Average dissolved amount (mg)	Percent dissolved amount (%)	Average percent dissolved amount (%)	Impact on dissolution (%)
7.08		70.81		5.21		52.09		
7.17		71.71		4.96		49.61		
7.01	7.14	70.13	71.37	5.05	5.00	50.51	50.03	-29.90
7.24		72.39		4.92		49.16		
7.19		71.94		4.98		49.84		
7.13		71.26		4.89		48.94		

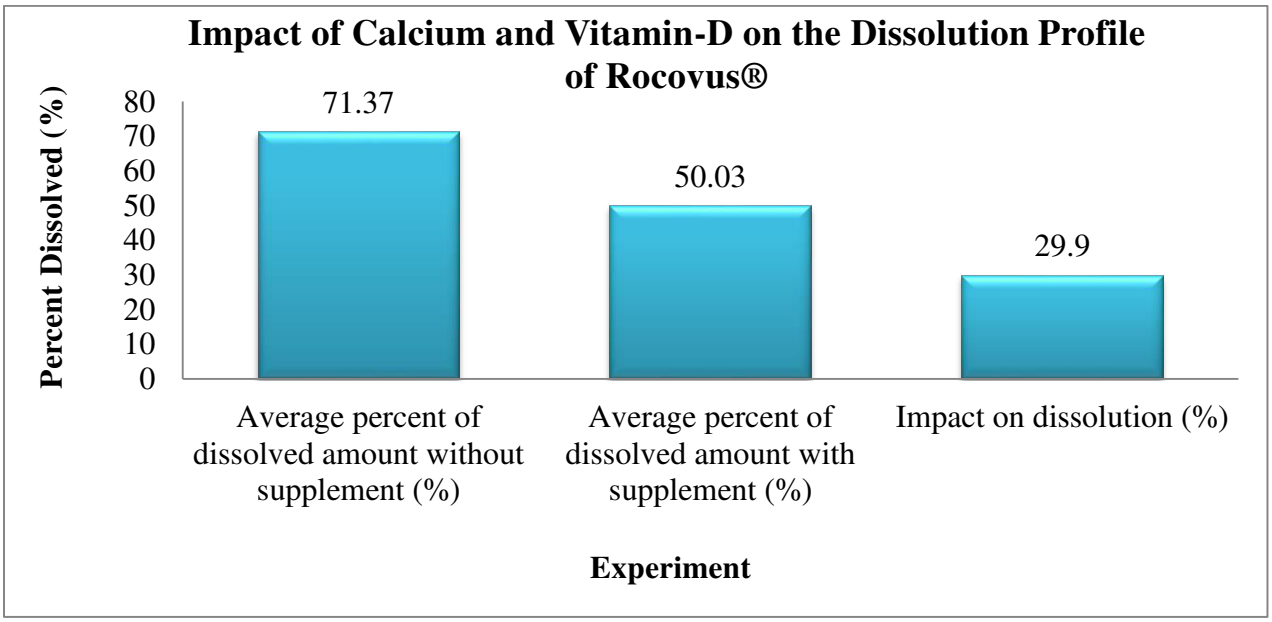

Figure 4.7: Impact of dissolution after 60 minutes

Chapter Five
Discussion

5.1 Discussion

The weight variation of Rocovus® indicated that the solid dosage forms were uniform. According to USP, tablets of specific weight range have a particular limitation of weight variation and Rocovus® meets those specifications. Weight variation test indicates that a tablet is of appropriate size, the content of the formulation is uniform and ultimately it indicates good manufacturing practices (GMP) (Nasrin et al., 2011).

The variation result for tablet thickness is also related with tablet hardness. If thickness of tablet varies significantly then the tablet hardness comparison will be incorrect (Pitt and Heasley, 2013). Vernier caliper was used to determine the thickness of ten tablets of Rocovus® and the results were same for all the tablets.

If tablet is too hard then it may not be able to disintegrate in the required time period to meet the dissolution specification (Nasrin et al., 2011). As the dissolution of drug product depends on the tablet hardness, so it is important to determine the hardness of tablet. Lack of moisture can increase compression load and results in tablet hardness (Chowhan and Palagyi, 1978).

The result of the dissolution tests showed for Rocovus® were increasing with time. In 10 minutes the average percent release of Rocovus® was 53.86% whereas in 60 minutes the percent release was 71.37%. So it was observed that with increasing time the release of drug is also increasing gradually.

Rocovus® when examined with Calvimax-D (calcium-vitamin D) then the dissolution of Rocovus® was altered. The drug release rate was decreased. At 10 minutes the average percent of drug release was 40.74% that create an impact of 24.36%.

After 60 minutes the average percent of drug release was 50.03% and the impact of dissolution altered was 29.90%. So, it was clear that with increasing time the dissolution or the drug release of Rocovus® is decreased when it is combined with calcium and vitamin D supplement.

This decrease of drug release is due to the common ion effect. When a soluble or weakly soluble compound is combined with its ionic part then the solubility decreases. According to Le Chatelier's principle if the concentration of any ion is increased in the solution then the

equilibrium of the solution is shifted to the left to make a balance between free ion and bound ion. As a result the solubility of the salt is reduced. (OMICS International, 2014)

Finally, it can be said that Rocovus® and Calvimax-D should not be co-administered. There should be a minimum time interval for administering these two drugs.

Chapter Six

Conclusion

6.1 Conclusion:

From the experiment it was observed that the dissolution profile of Rocovus® decreases due to the presence of Calvimax-D. Both Rocovus® and Calvimax-D are important for various health conditions. Rosuvastatin is a drug that is used in hyperlipidemia and dyslipidemia and also it lowers the risk for heart diseases. Calcium and vitamin-D is also important for patients who lack in those elements in body. These supplements also serve many important purposes. But it is to be noted that in Bangladesh the calcium-vitamin D supplements are often used without any major health issues. Any drug or supplement can be harmful if proper use is not ensured. As Rocovus® (rosuvastatin) is used in many serious health problems so it is advisable that the drug should not be co-prescribed with Calvimax-D (calcium-vitamin D). If there is a need to prescribe Rocovus® with Calvimax-D then there should be a minimum time interval between administration of these two agents.

Chapter Seven

Reference

References:

Abe, M.D., Adachi, M.D., Gunji, M.D., Hattori, Y., Hidenori, M.D., Hideyuki, M.D., Hiroaki, M.D., Hiroyuki, M.D., Hisao, M.D., Hyogo, S.Y., Kajiyama, G.M., Kajihara, T., Mizurio, T., Miyaki, N., Ochi, M., Ohguri, S., Omata, F., Shibata, K., Shigehiro, M.D., Sugata, F., Tazuma, S., Tazuma, S., Tomoji, M.D., Tsuyoshi, M.D., Ueno, F., Yamashita, M., and Yasumiba, S. (1998) Combination Therapy with Simvastatin and Ursodeoxycholic Acid is More Effective for Cholesterol Gallstone Dissolution than is Ursodeoxycholic Acid Monotherapy. *Journal of Clinical Gastroenterology*, 26, pp. 287-291.

ACI Limited. Rosetor® (Rosuvastatin) [Online] Available from: https://www.aci-bd.com/Brand/Rosetor.pdf (Accessed: 20 November 2016)

Adler, K., Aicher, A., Dimmeler, S., Fichtlscherer, S., Martin, H., Vasa, M. and Zeiher, A. (2001) Increase in Circulating Endothelial Progenitor cells by Statin Therapy in Patients with Stable Coronary Artery Disease. *Clinical Investigation and Reports,* 103.

Ahmad, M., Sohail, M. and Usman, M. (2012) Pharmacokinetic Interactions of Rosuvastatin: A *Review. Journal of Pharmacy and Alternative Medicine,* 2.

Ahmad, H., Ashfaq, M., Mustafa, G. and Khan, I. (2013) LC Determination of Rosuvastatin and Ezetimibe in Human Plasma. *Journal of the Chilean Chemical Society,* 58.

Ampati, S. and Messa, R. (2016) Comparative Studies for Enhancement of the Dissolution Profile of Pivastatin. *International Journal of Medicine and Nanotechnology*, 3 (1), pp. 311-315.

Arora, S., Nagapl, M., Panghal, D. and Thakur, G.S. (2014) Dissolution Improvement of Atorvastain calcium using Modified Locust Bean Gum by the Solid Dispersion Technique. *Scientia Pharmaceutica*, 82 (1) pp. 177-191.

Asghamejad, M. and Wang, L. (2000) Second-derivative UV Spectrometric Determination of Simvastatin in Its Tablet Dosage Form *Journal of Pharmaceutical and Biomedical Analysis*, 21 pp. 1243-1248.

AstraZeneca (2016) Crestor 5mg, 10mg, 20mg and 40mg Film-coated Tablets [Online] Available from: https://www.medicines.org.uk/emc/medicine/11976 (Accessed: 19 November 2016)

Bhise, S., Mandage, y., Rao, M. and Thanki, K. (2010) Dissolution Improvement of Simvastatin by Surface Solid Dispersion Technology *Dissolution Technologies*.

Biocompare (2016) Double Beam UV-Vis Spectrophotometer [Online] Available from: http://www.biocompare.com/Lab-Equipment/22959-Double-Beam-UV-Vis-Spectrophotometers/ (Accessed: 25 November 2016)

Bolourchian, N., Dadashzadeh, S. and Mahboobian, M.M. (2013) The Effect of PEG Molecular Weights on Dissolution Behavior of Simvastatin in Solid Dispersions *Iranian Journal on Pharmaceutical Research*, 12, pp. 11-20.

Bontha, V.K., Neduri, E.K. and Vemula, S.K. (2012) Different Techniques to Enhance the Dissolution Rate of Lovastin: Formulation and Evaluation *Asian Journal of Pharmaceutical and Clinical Research,* 6, pp. 56-60.

Chaudhari, B.G., Patel, N.M. and Shah, P.B. (2007) Stability Indicating RP-HPLC Method for Simultaneous Determination of Atorvastatin and Amlodipine from Their Combination Drug Products. *Chemical and Pharmaceutical Bulletin*, 55, pp. 241-246.

Chaudhari, P.D., Kamble, P.R. and Shaikh, K.S. (2014) Application of liquisolid technology for enhancing solubility and dissolution of rosuvastatin. *Advanced Pharmaceutical Bulletin*, 4 (2) pp.197-204.

ChemicalBook (2016) Rosuvastatin Calcium [Online] Available from: http://www.chemicalbook.com/chemicalproductproperty_en_cb9482678.htm (Accessed: 19 November 2016)

Crosta, P. (2016) *Statins: Uses, Side Effects, and Risks* [Online] Available from: http://www.medicalnewstoday.com/articles/8274.php (Accessed: 18 November 2016)

Chakrabortya, A.K., Mishrab, S.R. and Sahoob, H.B. (2011) Formulation of Dosage Form of rosuvastatin calcium and Development of Validated RP-HPLC Method for Its Estimation *International Journal of Analytical and Bioanalytical Chemistry*, 1 (3), pp. 89-101.

Chowdhury, J.A., Islam, M.S., Jahan, R. and Tanwir, A. (2013) In-vitro Dissolution Study of Atorvastatin Binary Solid Dispersion *Journal of Advanced Pharmaceutical Research and Technology*, 4 (1), pp. 18-24.

Clark, M.A., Finkel, R., Rey, J.A. and Whalen, K. (2012) *Lippincott's Illustrated Reviews Pharmacology* Republic of China: Wolters Kluwer Health.

Dehgan, M.H. and Khan, F.N. (2012) Enhanced Bioavalability and Dissolution of Atorvastatin calcium from Floating Microcapsules using Minimum Additives *Scientia Pharmaceutica*, 80, pp. 215-228.

Deshmukh, P.K., Kanpure, V.J. and Pande, V.V. 2013. Dissolution enhancement of rosuvastatin calcium by liquisolid compact technique. *Journal of pharmaceutics*, *2013*.

Devkar, T.B., Divase, G.T., Rodde, M.S. and Tekade, A.R. (2014) Solubility and Bioavailability Enhancement of Poorly Aqueous Soluble Atorvastatin: In-vitro, Ex-vitro and In-vivo Studies. *BioMed Research International*.

Drugs.com (2016) Rosuvastatin [Online] Available from: https://www.drugs.com/cdi/rosuvastatin.html (Accessed: 16 November 2016)

D'Souza, J., Patwekar, S., Payghan, S. and Shaikh, K. (2011) Dissolution and Stability Enhancement of Poorly Water Soluble Drug-Lovastin by Preparing Solid Dispersions *Asian Journal of Biomedical and Pharmaceutical Sciences,* 1(4), pp. 24-31

Eamen, S., Hossen, M.B., Mucktady, M.G. and Sarkar, P. (2015) Formulation Development and Evaluation of Fenofibrate and Rosuvastatin in Combination tablets and Release Rate Improvement. *International Journal of Pharmaceutical Sciences and Research,* 6 (9), pp. 4075-4079

Electrolab (2016) Dissolution Tester [Online] Available from: http://www.electrolabindia.com/ProductDetails.aspx?SEOType=BSuwhmB9Ll4%3D&PID=a3NgbKlWisU%3D&CID=7x0VaNKZAfw%3D&CType=ZgDIC937E8s%3D (Accessed: 19 November 2016)

Fairchild, J. and Jones, M. (2015) Reaction Monitoring of a Rosuvastatin Synthesis Featuring Enantiopurity Determination. *Waters: the Science of What's Possible.* [Online] Available from: http://www.waters.com/webassets/cms/library/docs/720005410en.pdf (Accessed: 19 November 2016)

Goodman, L., Gilman, A. and Brunton, L. (2008). *Goodman & Gilman's manual of pharmacology and therapeutics*. New York: McGraw-Hill Medical.

Gattani, S., Jain, P., Khirwal, L., Pandya, P., and Surana, S. (2008) Co-solvent Evaporation Method for Enhancement of Solubility and Dissolution Rate of Poorly Aqueous Soluble Drug Simvastatin: In-vitro-In-vivo Evaluation *APPS PharmSciTech*, 9, pp. 1247-1252.

Han, N., Jiang, T., Wang, S., Xie, Y. and Zhao, B. (2012) Enhanced Dissolution Rate and Oral Bioavailability of Simvastatin Nanocrystal Prepared by Sonoprecipitation *Drug Development and Industrial Pharmacy,* 38, pp. 1230-1239.

Harbolic, B. K. (2015) Vitamin and Calcium Supplement *Medicine.Net.com* [Online] Available from: http://www.medicinenet.com/vitamins_and_calcium_supplements/page2.htm (Accessed: 24 November 2016)

Healthcare Pharmaceuticals. Rovast® (Rosuvastatin INN) [Online] Available from: http://www.hplbd.com/downloads/Rovast-Ins.pdf (Accessed: 21 November 2016)

Healthline (2009) *Calcium-Vitamin D, Oral Tablet* [Online] Available from: http://www.healthline.com/drugs/calcium-vitamin-d/oral-tablet#Highlights1 (Accessed: 21 November 2016)

Hwang, S., Jun, S.W., Kim, M.S., Kim, J.S., Lee, S. and Woo, J.S. (2007) Preparation and Characterization of Simvastatin/Hydroxypropyl-beta-cyclodextrin Inclusion Complex using Supercritical Antisolvent (SAS) Process *European Journal of Pharmaceutics and Biopharmaceutics,* 66, pp. 413-421.

Incepta Pharmaceuticals Limited (2016) Rocovas (Rosuvastatin) [Online] Available from: http://www.inceptapharma.com/view-product.php?pid=371 (Accessed: 28 November 2016)

Incepta Pharmaceuticals Limited (2016) Calvimax-D (Calcium-vitamin D) Available from: http://www.inceptapharma.com/view-product.php?pid=178 (Accessed: 26 November 2016)

Islam, F. (2014) In-vitro Dissolution Study to Determine the Drug-Drug Interaction of Atorvastatin Calcium-Metformin HCl Multivitamin Tablet. *EWU Institutional Repository* [Online] Available from: http://dspace.ewubd.edu/handle/123456789/679 (Accessed: 23 November 2016)

Lapsurgery.com (2016) *Gerd,Acid Reflux, Heartburn, Hiatal Hernia.* [Online] Available from: http://www.lapsurgery.com/gerd.html (Accessed: 28 November 2016)

Madhavi, K., Krishna, R., Krishna, V., Kumar, S. and Shyam, J. (2014) Enhancement of Solubility and Dissolution Properties of Lovastatin by Liquisolid Technique. *International Journal of Research in Pharmacy and Chemistry*, 4 (3), pp. 713-722.

Nagda, C., Nagda, D. and Rokad, V. (2014) Design and Evaluation of Solid Self-emulsifying Drug Delivery System of Rosuvastatin Calcium. *Journal of Young Pharmacists*, 6, pp. 37-45.

Nall, R. (2016) The Effect of Vitamin D Deficiency *Healthline* [Online] Available from: http://www.healthline.com/health/vitamin-d-deficiency#Overview1 (Accessed: 24 November 2016)

Natoli (2015) Hand-held Hardness tablet Tester [Online] Available from: https://natoli.com/product/hand-held-hardness-tablet-tester-2/ (Accessed: 17 November 2016)

NOF (2016) Calcium/Vitamin D *National Osteoporosis Foundation* [Online] Available from: https://www.nof.org/patients/treatment/calciumvitamin-d/ (Accessed: 17 November 2016)

Ogbru, O. (2016) *MedicineNet.com* [Online] Available from: http://www.medicinenet.com/rosuvastatin/article.htm (Accessed: 15 November 2016)

OMICS International (2014) Common Ion Effect [Online] Available from: http://research.omicsgroup.org/index.php/Common-ion effect (Accessed: 16 November 2016)

Palem, C.R., Patel, S. and Pokharkar, V.P. (2009) Solubility and Stability Enhancement of Atorvastatin by Cyclodextrin Complexation. *PDA Journal of Pharmaceutical Science and Technology*, 63(3), pp.217-225.

Pandya, V.M., Patel, J.K. and Patel, D.J., 2011. Formulation and optimization of nanosuspensions for enhancing simvastatin dissolution using central composite design. *Dissolution Technologies*, *18*(3), pp.40-45.

Patel, R. P., and Patel, M. M. (2007) Physicochemical Characterization and Dissolution Study of Solid Dispersions of Lovastin with Polyethylene Glycol 4000 and Polyvinylpyrrolidone K30 *PubMD,* 12 (1), pp. 21-33.

Rang, H.P., Dale, M.M., Ritter, J.M., Flower, R.J. and Henderson, G. (2012) Rang and Dale's pharmacology. 7th ed. Edinburgh: Elsevier Churchill Livingstone.

RxList (2016) Crestor [Online] Available from: http://www.rxlist.com/crestor-drug/clinical-pharmacology.htm (Accessed: 20 November 2016)

Schachter, M. (2004) Chemical, Pharmacokinetic and Pharmacology Properties of Statin. *Fundamental and Clinical Pharmacology,* 19, p.117-125.

Sirius-analytical (2016) Dissolution Definitions. [Online] Available from: http://www.siriusanalytical.com/science/dissolution/dissolution-definitions (Accessed: 20 November 2016)

Shah,V. and Amidon, G. (2014) A Theoretical Basis For A Biopharmaceutic Drug Classification:The Correlation Of In Vitro Drug Product Dissolution And In Vivo Bioavailability. *The American Association of Pharmaceutical Scientists Journal*,16(5), pp.894-898

Soderberg, T. (2016) UV and Visible Spectroscopy. *Chemistry Libretexts* [Online] Available from:http://chem.libretexts.org/Textbook_Maps/Organic_Chemistry_Textbook_Maps/Map%3A_Organic_Chemistry_With_a_Biological_Emphasis_(Soderberg)/Chapter_04%3A_Structure_Determination_I/4.3%3A_Ultraviolet_and_visible_spectroscopy (Accessed: 22 November 2016)

Stuart Equipment (2014) Water Distillation Machine 41/h|D4000. [Online] Available from: http://www.bpress.cn/im/tag/Stuart-Equipment/ (Accessed: 19 November 2016)

Tradeindia.(2016) *Tablet Dissolution Test Apparatus In Mumbai, Maharashtra,India-VEEGO instruments*. [Online] Available from: http://www.tradeindia.com/fp194365/Tablet-Dissolution-Test-Apparatus.html (Accessed: 28 November 2016)

Walling, E. (2009) Dispel the Myths: The True function of Cholesterol in the Body. *Natural News* [Online] Available from: http://www.naturalnews.com/025849_cholesterol_body_health.html (Accessed: 25 November 2016)

Web.MD (2016) *Calcium 500 with Vitamin D* [Online] Available from: http://www.webmd.com/drugs/2/drug-59789/calcium-500-with-vitamin-d-oral/details# (Accessed: 22 November 2016)

Yeomans, N., Tulassay, Z., Juhasz, L., Rácz, I., Howard, J., van Rensburg, C., Swannell, A. and Hawkey, C. (2000). A Comparison of Omeprazole with Ranitidine for Ulcers Associated with Nonsteroidal Antiinflammatory Drugs. *New England Journal of Medicine*, 338(11), pp.719-726.

Zhang-Wen, S.Y., Xuan-bo, H. and Xue-jian, M. (2006) Detrmination on Dissolution of Atorvastatin Calcium Soft Capsule. *Cnki.com* [Online] Available from: http://en.cnki.com.cn/Article_en/CJFDTOTAL-SDYG200612016.htm (Accessed: 17 November 2016)