IMPACT OF CALCIUM AND VITAMIN-D SUPPLEMENT ON THE DISSOLUTION PROFILE OF ROSUVA™ (ROSUVASTATIN)

A dissertation submitted to the Department of Pharmacy, East West University, Bangladesh for the partial fulfillment of the Degree of Bachelor of Pharmacy

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Declaration

The research work entitled “Impact of Calcium and Vitamin D supplement on the dissolution profile of Rosuva^{TM} (Rosuvastatin)” is submitted as dissertation for the partial fulfillment of the Bachelor Degree of Pharmacy, under the supervision and guidance of Md. Anisur Rahman, Assistant Professor, Department of Pharmacy, East West University, Dhaka.

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Certification by the Supervisor

The under signed certify that the research work which is presented here was completely done by the author as well as to the style and contents. This thesis is therefore suitable for submission. No part or whole of this work was submitted before other degree. We further certify that the source of information has been availed of this connection is duly acknowledged.

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Certification by the Chairperson

This is to certify that the thesis entitled "Impact of Calcium and Vitamin D supplement on the dissolution profile of Rosuva^{TM} (Rosuvastatin)" submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree of Bachelor of Pharmacy is a record of original and genuine research work carried out by Jannatul Ferdous Nova, ID: 2013-1-70-034 in 2016.

……..

Dr. Shamsun Nahar Khan
Associate Professor and Chairperson
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Dedicated To

My Parents

&

Honorable teachers

Acknowledgement

All praises to the almighty 'Allah' for the strengths and blessings given to me in completing this research.

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Table of contents

List of Table

List of Figure

Abstract

The objective of the research work was to investigate the impact of Calcium and Vitamin D supplement on the dissolution profile of Rosuva^{TM} 10 mg (Rosuvastatin Calcium) tablet. The dissolution test was performed by using distilled water (as dissolution medium) with USP dissolution apparatus II. The amounts of drugs released were measured using UV spectroscopy. A standard curve equation of Rosuvastatin was established for the calculation of percent dissolved amount of drug. The dissolution of individual Rosuvastatin (Rosuva^{TM} 10 mg) 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 Rosuvastatin (Rosuva^{TM} 10 mg) tablets and combination with the Calcium and Vitamin D supplement drugs were subjected to determine the dissolution profile. After an hour the average percent dissolved amount of individual Rosuva and Rosuva with Calcium and Vitamin D supplement were respectively 71.13% and 50.25%. From the result it was assumed that Calcium and Vitamin D supplement has extreme effect on the dissolution of Rosuva. The dissolution rate of Rosuva was decreased by the effect of Calcium and Vitamin D supplement may be due to the common-ion effect. As Calcium and Vitamin D supplement has the extreme effect on the dissolution profile of Rosuvastatin Calcium, they should not be used concomitantly.

Key words: UV spectroscopy, USP dissolution apparatus II, Hardness, Thickness, Weight variation, Dissolution impact, Common-ion effect

Chapter One

Introduction

1.1 Objective

Concomitant use of more than one drug is very prevailing practice among the patients. If two or more drugs are being taken, there is chance that there will be an interaction among the taken drugs. This type of interaction may increase or decrease the effectiveness of the drugs or the side effects of the drugs. The possibility of drug interactions increases as the number of the drugs being taken. Hence, people who administer several drugs at a time are at the greater risk for interactions. The main aspiration of this research project was to determine the impact of calcium and vitamin D supplement on dissolution of Rosuvastatin. During this experiment, we calculated the percent release of a drug product individually and also in combination with calcium and vitamin D supplement drug and then determine the impact on dissolution of Rosuvastatin. In this research work, Rosuvastatin was selected as a main drug product and calcium and vitamin D supplement drug was selected as co-administered product.

Rosuva^{TM} (10mg) prepared by Square Pharmaceuticals Limited was the subject of my research project. Briefly, my project was to determine the impact of calcium and vitamin D supplement drug on the dissolution profile of Rosuvastatin.

To reach the result, we had performed in vitro test such as; dissolution test of individual Rosuvastatin (Rosuva ^{TM} 10 mg) and dissolution test of Rosuvastain with calcium and vitamin D supplement (Calvimax-D 500mg) tablet.

1.2 Cholesterol

Cholesterol is a complex monohydric secondary alcohol which is a very important member of the steroid class. It may exist in all cells- both in cytoplasm and cell membrane. It is available in free and ester form. Normal blood cholesterol deviates between 150-200 mgm per 100 ml which is equally dispersed between plasma and corpuscles. But in the corpuscles, cholesterol mainly present in the free form while in the plasma it remains as ester. (Chatterjee, 2003, p 566)

1.2.1 Function of Cholesterol

1. **Essential component of all cells:** It is a component of the ‘element constant’ of the cells. Its constant amount in the cell revealed that it is concerned with the functions of the cell.
2. **Controls cell permeability**: since it is a constant component of cell membrane it helps to control in permeability of the tissue cells.

3. **Defensive action:** During severe infections blood cholesterol level falls and it will rise again after recovery.
4. **Transportation of fat:** Cholesterol esters are responsible for transportation of large part of fat.
5. **Formation of cholic acid (bile salts):** Cholic acid is a component of bile salt and it is synthesized from cholesterol.
6. **Others:** Cholesterol is responsible for preventing hemolysis, antilipotropic action, formation of steroid hormones etc. (Chatterjee,2003, p 568)

There are two main types of cholesterol.

1. **Low- density lipoproteins (LDL):** It is referred to as "bad" cholesterol. High levels of this can deposit in arteries which causes heart diseases.
2. **High- density lipoproteins (HDL):** It is called "good" cholesterol. This good cholesterol transports cholesterol from other parts of body to the liver where it is processed to be excreted.

It is essential to maintain a healthy level of both types of cholesterol.

1.2.2 Healthy level of cholesterol

Accepted level of LDL cholesterol should be less than 160mg/dl and HDL cholesterol should be atleast 35 mg/dl. (Barcley, 2016)

1.3 Statin

If LDL cholesterol levels are high, deposition of this in arteries make the blood vessels hardened and narrow, gradually resulting in blocked arteries (atherosclerosis) and reducing the flow of oxygen rich blood to the heart. This can result in heart diseases. Hence it is essential to maintain a healthy level of cholesterol for avoiding heart diseases. The main concern of cholesterol treatment is to reduce the LDL (bad cholesterol) levels. Statins are the most prominent choices of doctors when diet and exercise are not enough to low the level of LDL. Statin can reduce the

formation of plaques in the arteries by preventing the synthesis of cholesterol. (Medlineplus, n.d.)

1.3.1 General Mechanism of Statins

Statins imitate the natural substrate molecule, 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) by competitively inhibiting HMG-CoA reductase enzyme which is the first and key-limiting enzyme of the cholesterol biosynthetic pathway. HMG-CoA reductase enzyme catalyzes the reduction of HMG-CoA to mevalonate which is the precursor of the synthesis of cholesterol and steroids and isoprenoids. Since statins competitively inhibit HMG-CoA reductase enzyme in the liver, this competition slows the rate of mevalonate production and consequently reduces the endogenous synthesis of cholesterol. Furthermore, liver cells aware about the reduced levels of liver cholesterol synthesis with statin use and try to minimize the effect by synthesizing more LDL receptors on the cell surface of liver, which increases catabolism of plasma LDL, resulting the lowering of serum apolipoprotein B levels (the protein component of LDL) and reduced plasma cholesterol level. By reducing total plasma cholesterol level and LDL cholesterol level, the formation of atherosclerotic plaques is decreased. Additionally, statins are found to have other beneficial effects including stabilization of atherosclerotic plaques, improvement of endothelial function and also prevention of thrombus formation. (Goodman and Gilman, 2011)

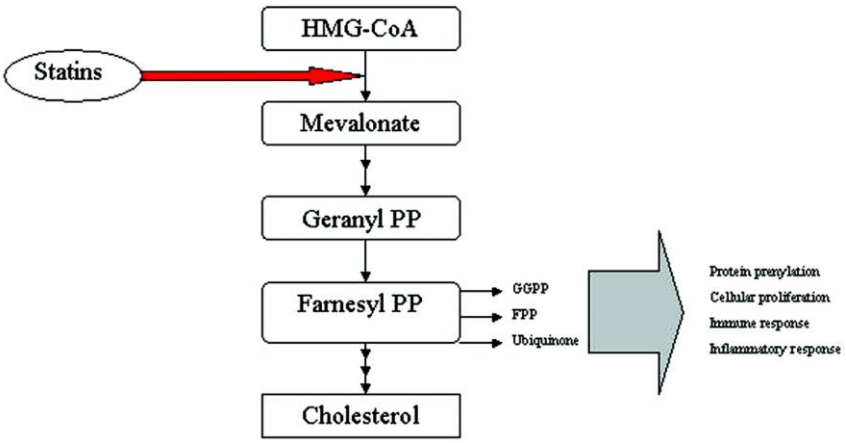

Figure 1.1: Mechanism action of Statin (Belay et al., 2007)

1.3.2 Types of statins

There are two types of statin based on their sources.

1. **Type 1 statins:** These are drevied from natural sources or modifications of natural molecules; e.g. lovastatin, pravastatin and simvastatin.
2. **Type 2 statins**: These are made in the laboratory after understanding the structures of various natural statins or synthetically derived. Synthetic statins are fluvastatin, atorvastatin, Rosuva® (10mg)statin, cerivastatin. (Atrainceu.com, 2012)

1.3.3 Indication of statins

1. Statins are indicated to use in the reduction of the risk of myocardial infarction, stroke and angina and slow the progression of coronary atherosclerosis in patients with heart disease.

2. These drugs are also indicated to lower the risk of myocardial infarction, stroke and angina in patients without heart disease but who are in risk of heart disease by displaying risk factors such as smoking, hypertension, diabetes or a family history of early coronary heart disease.

3. Satins are also declared to lower cholesterol levels in patients with primary hypercholesterolemia.

4. Statins are used for the treatment of adolescents and adults with heterozygous familial hypercholesterolemia.

However, statin therapy is normally administered if the patient's LDL level exceeds a certain threshold value. In patients with coronary heart disease, statin therapy is prescribed if LDL levels are over 130mg/dl. In patients without heart disease but displaying various risk factors, treatment begins when the LDL level is within 130-160 mg/dl. In patients without risk factors for heart disease, therapy cannot be taken until LDL level reach 160-190 mg/dl. (Acls.com, 2014)

1.3.4 Side effects of statin

Statins are prescribed for people with high cholesterol to reduce their total cholesterol level and lower their risk of a heart attack or stroke. Most people who use statin drugs tolerate them very well but some can't. They experience moderate to severe side effects.

Statin side effects are-

1. **Muscle pain and damage:** One of the most common side effects of patient taking statins is muscle pain. This pain can be felt as a soreness, tiredness or weakness in muscles. The pain can be severe or mild discomfort. In addition, most controlled studies of statins declare that patient taking statins experience muscle pain at the same rate as people taking placebo but up to 29% of patient who start taking statins develop muscle pain and many discontinue statins because of this side effects. Many patients avoid these side effects by switching to a different variety of statin.

   Hardly ever, statins can cause rhadomyolysis; life threatening muscle damage which is responsible for severe muscle pain, liver damage, kidney failure and death. This rare side effect can occur when statins in combination with certain drugs are taken or a high dose of statins are taken.
2. **Damage in liver:** Infrequently, statin therapy can cause an increase in the level of enzymes which is responsible for liver inflammation.
3. **Increased blood sugar or type- 2 diabetes:** Statin therapy may increase the blood glucose level which may cause to develop type-2 diabetes. The risk is small but FDA has issued a warning on statin labels in the case of blood glucose levels and diabetes.
4. **Neurological side effects:** Some patients may experience memory loss or confusion while taking statin therapy. The FDA has issued a warning on statin labels that some people have experienced confusion or memory in statin therapy. (Mayoclinic, 2016)

1.3.5 Who may experience statin side-effects?

Some patients may experience at a greater risk than are others. Risk factors are:

- Taking combination therapy with other drugs which lower cholesterol level
- Female

- Possessing smaller body frame
- Age 65 or older
- Kidney or liver disease patient
- Too much alcohol drinking (Mayoclinic, 2016)

1.4 Calcium

1.4.1 General information of Calcium

Calcium is a mineral which is needed for the formation of bone and teeth. It also performs other bodily firm work such as muscle contraction. Lack of calcium causes rickets which are mainly occurred in early childhood and also cause osteoporosis which is occurred in later life. An adult needs 700 mg calcium per day. To avoid deficiency, health care provider suggests taking calcium supplement. (WebMd.com, 2016)

1.4.2 Sources of calcium

Sources of calcium include-

- Dairy products include milk, cheese etc.
- Nuts
- Fish
- Soya drinks with calcium
- Green leafy vegetables
- bread
- Soya beans (NHS choices, 2015)

1.4.3 Function of calcium

The bones and teeth are composed of calcium. Calcium is stored in the bone and at the time of functions, calcium is released. However, calcium is also available in the blood, muscles and other tissues. The concentration of calcium is decreasing with the increased age. In women, the absorption of calcium is decreased due to the reduction of estrogen levels. As a result, bones become soft which leads to the breakdown of bones. But bones are always breaking down and rebuilding in nature. In this case, calcium is essential for the reformation of the bones. So,

calcium supplement are needed for the growth of the bones and makes them strong. (WebMd.com, 2016)

1.4.4 Indication

Calcium is used for the prevention of bone related problems which include-

- Osteoporosis
- Rickets
- Osteomalacia
- Premenstrual syndrome
- Pre-eclampsia
- Leg pain during pregnancy

Calcium is also used for the reduction of risk of rectal and colon cancers. It is also used to reduce complication after bypass surgery. (WebMd.com, 2016)

1.4.5 Overdoses

If calcium is taking over 1500 mg per day, then it will lead to stomach pain and diarrhea.

(NHS choices, 2015)

1.5 Vitamin D

1.5.1 Description

The fat soluble vitamin D is available in few foods and it can also be available as a supplement.

When ultra-violet ray strikes the skin, it leads the synthesis of vitamin D. The vitamin D we obtain from food, sun exposure and as a supplement are biologically inert.For their activation, they have to undergo hydroxylation in the body by two ways.

- Vitamin D converts to 25-hydroxyvitamin D (calcidiol) in the liver
- Vitamin D converts to 1, 25- dihydroxyvitamin (calcitriol) in the kidney

Vitanin D improves the absorption of calcium in the gut and prevent hypolcemic tetany by maintaining the concentratiuon of serum calcium and phosphate. It is important for the growth of bone otherwise the bone will be thin, brittle. Co-administration of calcium with vitamin D, can help to prevent osteoporosis. Vitamin D can also perform neuromuscular and immune function, reduction of inflammation and modulation of cell growth. (NHS choices, 2015)

1.5.2 Sources of vitamin D

The following are the sources of vitamin D which include-

- Cheese
- Beef liver
- Egg yolk
- Fish liver oil
- Flesh of fish such as tuna, salmon etc. (NHS choices, 2015)

1.5.3 Risky groups

People who are at the risk of vitamin D deficiency include-

- **Breastfed infants**

Vitamin D passes into breast milk. Human milk only cannot provide the full needs of vitamin D because it is related to the amount of vitamin D that a mother contains.

The amount is generally less than 25 IU/ L to 78 IU/L. So, mother should be given vitamin D supplement with 400 IU per day to meet the demand of an infant. Otherwise, there is a chance of rickets among the infants.

- **Older adults**

Older adults are developing vitamin D insufficiency due to age. Most of the time, they remain in the home so their skin can not to be exposed to the sunlight. Sunlight helps to synthesize vitamin

D. Moreover, they do not take vitamin supplement and cannot meet up their demand for vitamin D.

- **People with limited sun exposure**

Basically women who remain in the home for religious reasons and people with occupations have limited exposure to sun light. People who use sunscreen may reduce vitamin D synthesis is unknown. All of these cause vitamin D deficiency. So, they should be provided the supplement according to their needs.

- **People with dark skin**

The dark skin results from increased amount of melanin pigment in the epidermal layer. The increased amount of melanin pigment reduces the production of vitamin D from sun light. Evidence shows that the level of vitamin D is greater in the white than in the black.

- **People with inflammatory bowel disease and other conditions causing fat malabsorption**

Liver disease, cystic fibrosis, celiac disease and crohn's disease etc. associated with fat malabsorption which require low intake of vitamin D which causes vitamin D deficiency.

(NHS choices, 2015)

1.5.4 Vitamin D deficiency

Vitamin D deficiency result from lower intake of vitamin D, improper absorption, limited exposure to sunlight, various diseases which lower the intake or sometimes kidney cannot convert vitamin D's to its activate form. That means vitamin deficiency can occur when demand is greater than the requirement.

Rickets and osteomalacia are the diseases caused by vitamin D deficiency. Rickets are the disease occurred in children who cause soft bone instead of hard bone and skeletal deformation.

Rickets occur due to prolong breastfeeding by mothers who have lack of vitamin D. Sometimes, baby day care programs (exposure to the sun) also lead to the initiation of rickets.

Osteomalacia occurs in adult which leads to the formation of weak bones. Symptoms of osteomalacia include muscle weakness and bone pain which is not detected in the primary stages. (National Institute of Health, 2016)

1.5.5 Dietary supplements

Vitamin D is found in two forms in few foods and supplements. The two forms are vitamin $D_2$and vitamin D_3. The differences between this two vitamin is found in their side chain structure.

- Vitamin D_2 is also known as ergocalciferol which is made by the irradiation of ergosterol in yeast.

- Vitamin $D_3$is also known as choecalciferol which is made by the irradiation of 7-dehydrocholesterol from lanolin and the chemical conversion of cholesterol.

This two forms are used for the recovery of rickets, osteomalacia. However, these 2 forms are equivalent at nutritional doses but vitamin D_2 is less potent than vitamin D_3 at high doses. (National Institute of Health, 2016)

1.5.6 Drug-Drug interaction

Vitamin D can also interact with other drugs. So, patient should inform the physician that he or she is already taking vitamin D. Few example of vitamin D interaction with other drug are given below-

- **Prednisone:** It is used to reduce inflammation. Together with vitamin D, prednisone reduces calcium absorption and affect vitamin metabolism. As a result, it causes bone loss and leads to the development of osteoporosis.

- **Orlistat:** It is used to reduce weight. Together with vitamin D, it reduces the absorption of vitamin D.

- **Cholestyramine:** It is used to reduce cholesterol. Together with vitamin D, it reduces the absorption of vitamin D.

- **Phenobarbital:** It is used to prevent the epileptic seizures**.** Together with vitamin D, it reduces the calcium absorption and affects the metabolism of vitamin D. (National Institute of Health, 2016)

1.5.7 Overdoses

Overdoses of vitamin D can cause toxicity and show non-specific symptoms which include

- Anorexia
- Weight loss
- Polyuria
- Heart arrhythmias
- Tissue calcification

However,the use of both calcium and vitamin D supplement, increases the risk of kidney stones in case of postmenopausal women. (National Institute of Health, 2016)

1.5.8 Healthful Diets

For the maintenance of vitamin D level from diets, the following should be followed-

- Takes variety of vegetables, fruits, fat free milk, milk products, cheese etc.

- Takes variety of protein foods such as meats, eggs and sea foods etc.

- Takes sugar, sodium etc. (National Institute of Health, 2016)

1.6 Rosuvastatin

1.6.1 Rosuvastatin general information

Rosuvastatin is a member of the statin drug class which is first marketed as Crestor. Rosuvastatin is used in combination with exercise, diet and weight-loss for the treatment of high cholesterol and to stave off cardiovascular disease. Shionogi was developed this drug.

Active ingredient of Rosuvastatin film-coated tablet is 7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl (methyl-sulfonyl) amino] pyrimidin-5-yl] (3R,5S)-3,5-dihydroxyhept-6-enoic acid.

(Patil, Shinde & Chaudhuri, 2012)

Rosuvastatin has the given structure:

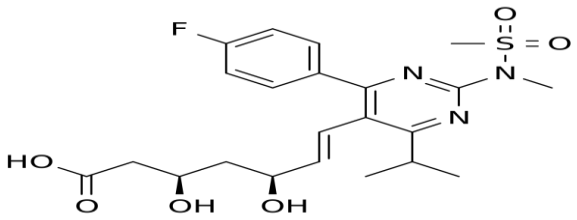

Figure 1.2: Rosuvastatin structure (Ahmad et al, 2012)

The molecular formula of Rosuvastatin is $C_{22}H_{28}FN_3O_6S$; having molecular weight of 481.539 g/mol. It is sparingly soluble in water and methanol and it is also soluble in ethanol at some extent. It seems as amorphous powder in white color. (Ahmad et al, 2012)

There are four available doses of Rosuvastatin tablets; such as

- ✓ 5 mg film-coated tablet
- ✓ 10 mg film-coated tablet
- ✓ 20 mg film-coated tablet
- ✓ 40 mg film-coated tablet

Each Rosuvastatin tablet contains inactive ingredients which are known as excipients like cellulose, lactose monohydrate, silica, crospovidone, magnesium oxide, magnesium stearate, iron oxide red (E172) for making tablet core and in terms of making film-coating, lactose monohydrate, hypromellose, titanium dioxide (E171), triacetin, Sunset Yellow FCF (E110), allura red(E129), indigo carmine(E132) are used. (Medicines.ie, 2015)

Figure 1.3: Rosuva™ Tablet (SquarePharma, 2016)

1.6.2 Pharmacology of Rosuvastatin

Rosuvastatin acts as a selective and competitive inhibitor of HMG-CoA reductase enzyme which is responsible for the convertion of 3-hydroxy-3-methylglutaryl coenzyme A to mevalonate, a precursor of cholesterol. Rosuvastatin produces its lipidmodifying effects in two ways in vivo studies in animals and in vitro studies in cultured animal and human cells. In first way, it

produces greater amount of hepatic LDL receptors on the cell surface to enhance and uptake of LDL. Secondly, Rosuvastatin inhibits hepatic synthesis of VLDL which consequently reduces the total number of VLDL and LDL particles. (SquarePharma, 2016)

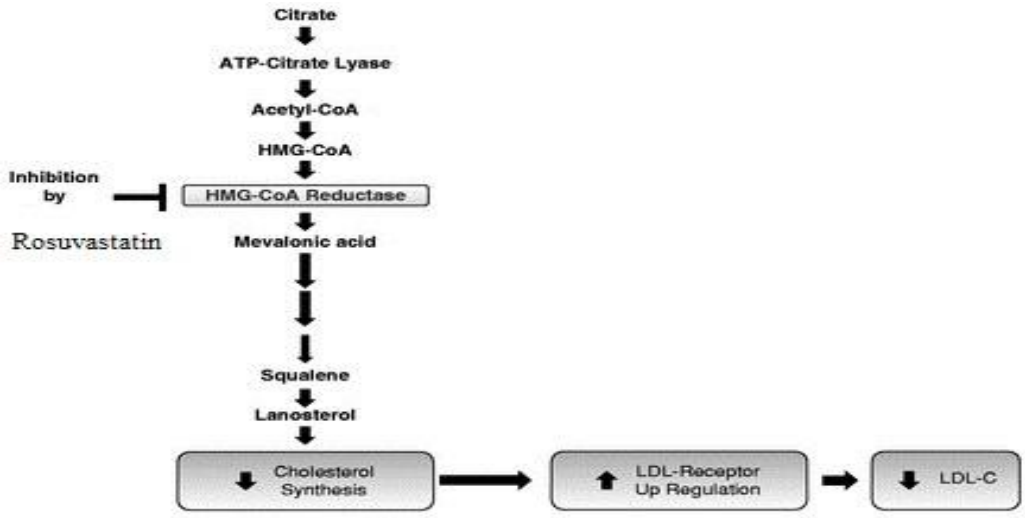

Figure 1.4: Pharmacology of Rosuvastatin (Belay et al., 2007)

1.6.3 Therapeutic indications of Rosuvastatin

1. **Heterozygus hypercholesterolemia:** Heterozygus hypercholesterolemia is a common genetic disorder marked by elevated cholesterol levels specifically very high levels of low-density lipoprotein in the blood due to the mutation of the LDL receptor protein gene which is normally responsible for the removal of LDL from blood. People who have one abnormal copy of LDL receptor gene are known as heterozygous. This common genetic disorder can be treated by Rosuvastatin. (Rader et.al, 2003)
2. **Homozygous hypercholesterolemia:** It is also a rare genetic disorder where cholesterol levels are elevated due to the presence of two abnormal copy of LDL receptor gene. Treatment of this disorder is not easy. High dose of Rosuvastatin is prescribed for this genetic disorder. (Rader et.al, 2003)
3. **Mixed Dyslipidemia :** Mixed dyslipidemia is specified as elevations in LDL cholesterol and Triglycerides levels concurring with low levels of HDL cholesterol. This disorder

happens due to hepatic overproduction of very low density lipoprotein (VLDL) particles leading to increases in both LDL cholesterol and triglycerides level. Mixed dyslipidemia can also maintained by taking Rosuvastatin tablets. (Rader et.al, 2003)

4. **Hypertriglyceridemia:** It is a condition in which triglyceride levels are elevated than normal level causing by uncontrolled diabetes mellitus, obesity and sedentary habits. This disorder is usually asymptomatic until triglycerides are greater than 1000-2000 mg/dl. For the treatment of adult patients with hyperglyceridemia, Rosuvastatin tablets are prescribed as addition therapy with diet. (Rader et.al, 2003)
5. **Primary prevention of cardiovascular disorder:** Rosuvastatin is used to-
    - Reduce the risk of stroke
    - Detract the risk of myocardial infarction
    - Reduce the risk of arterial revascularization procedures
    - Retard the progression of atherosclerosis (ACI, n.d.)

1.6.4 Pharmacodynamics of Rosuvastatin

The production of Rosuvastatin is done by synthetically to lower the level of total cholesterol, LDL cholesterol, apolipoprotein B, triglyceride and to increase the level of HDL cholesterol in blood. The increased level of LDL and triglycerides increases the incident of atherosclerosis, coronary artery disease and other cardiovascular disease. In case of lowering the LDL level and triglyceride in blood, Rosuvastatin is the good choice of reducing LDL level and increasing the amount of good cholesterol level which is known as HDL also. (Drugs.com, 2016)

1.6.5 Pharmacokinetics of Rosuvastatin

Absorption: After administration of tablet orally, peak plasma concentrations of Rosuvastatin are reached 3-5 hours. Both peak concentration (Cmax) and area under the plasma concentration-time curve (AUC) increase proportionally to Rosuvastatin dose. Approximately 20% is the absolute bioavailability of Rosuvastatin. If Rosuvastatin is administered with food, the rate of drug absorption is decreased by 20 % as assessed by Cmax, but there is no effect on the extent of absorption as assessed by AUC. (SquarePharma, 2016)

Distribution: At steady state of Rosuvastatin, mean volume of distribution is approximately 134 liters. 88% Rosuvastatin is bound to plasma proteins, mainly albumin. (SquarePharma, 2016)

Metabolism: Rosuvastatin is not widely metabolized. Approximately 10% of a radiolabeled dose is retrieved as metabolite. N-desmethylRosuvastatin is the major metabolite that is formed by cytochrome P450 2C9. In vitro studies have explained that N-desmethylRosuvastatin has approximately one-sixth to one-half the HMG-CoA reductase inhibitory activity of Rosuvastatin or 90% Rosuvastatin has the greater inhibitory activity against HMG-CoA reductase. (SquarePharma, 2016)

Elimination: After oral administration, Rosuvastatin and its metabolites are mainly excreted in the feces (90%). 19 hours is the elimination half life (t1/2) of Rosuvastatin. (SquarePharma, 2016)

1.6.6 Dosage and administration

The dose range for Rosuvastatin is 5 to 40 mg orally once daily. 5-10 mg is the starting dose. Rosuvastatin can be administered as a single dose at any time of the day, with or without food. When initiating Rosuvastatin therapy or switching from another HMG-CoA reductase inhibitor therapy, the appropriate Rosuvastatin starting dose first be utilized and only then titrated according to the patients' response and individualized goals of therapy. After initiation or upon titration of Rosuvastatin, lipid level should be analyzed within 2 to 4 weeks and the dosage being adjusted gradually. (ACI, n.d.)

Heterozygus hypercholesterolemia and mixed dyslipidemia: 10 mg once daily is the usual recommended starting dose of Rosuvastatin. For the patients requiring less aggressive LDL cholesterol reductions may initiate their therapy with 5 mg once daily. 20 mg starting dose may be considered for patients with marked hypercholesterolemia (LDL is greater than 190 mg/dl) and aggressive lipid targets. Those patients who have no achieved goal LDL-C at 20 mg can start taking the 40 mg dose.

***Homozygous hypercholesterolemia*:** In patients with homozygous hypercholesterolemia, 20 mg is the recommended starting dose of Rosuvastatin. 40 mg is the maximum recommended starting daily dose.

Dosage in patients with insufficiency of renal: For patients with mild to moderate renal insufficiency, dosage range modification is not necessary. (SquarePharma, 2016)

1.6.7 Cotraindiaction of Rosuvastatin:

Rosuvastatin is contraindicated –

- In patients who are hypersensitive to Rosuvastatin or to any other excipients.
- In patients with active hepatic disease including persistant elevations of serum transminases and any serum transminase elevation exceeding 3X the upper limit of normal (ULN).
- In patients with severe renal impairment in which creatinine clearance is less than 30 ml/min.
- In myopathy patients
- In those patient who are receiving concomitant ciclosporin
- During pregnancy and lactation

Patients with pre-disposing factors for myopathy/ rhabdomyolysis are contraindicated to 40 mg dose. Some following factors are included:

- Moderate renal imapairment (clearance of creatinine less than 60 ml/min)
- Hypothyroidism
- Family history of hereditary muscular disorders
- Previous history of muscular toxicity with another HMG-CoA reductase inhibitor or fibrate
- Alcohol consumption
- Asian patients
- Concomitant use of fibrates (Medicines.ie, 2015)

1.6.8 Side effects

Rosuvastatin is mainly well tolerated drug. But this drug may have some side effects. Such as;

- Constipation
- Stomach pain
- Dizziness
- Difficulty in falling asleep or staying asleep
- Depression

- Joint pain
- Headache
- Memory loss
- Confusion

Some side effects are more serious and they are:

- Muscle pain
- Lack of energy
- Fever
- Chest pain
- Yellowing of the skin or eyes
- Dark colored urine
- Pain in the upper right part of the abdomen
- Nausea
- Fatigue
- Hives
- Rash
- Anorexia
- Flu like symptoms
- Itching
- Difficulty in breathing or swallowing
- Hoarseness
- Swelling of the face, throat, tongue, lips, eyes, hands, feet, ankles or lower legs

(Medlineplus, 2016)

1.6.9 Special precautions which should be taken for oral administration

Renal effects: Patients who take higher doses of Rosuvastatin has been observed developing proteinuria which is detected by dipstic testing method. In acute or progressive renal disease, proteinuria is not predictive. This problem has been observed in the use of 40 mg dose of

Rosuvastatin. An assessment of renal function should be done during routine follow-up patients treated with 40 mg dose. (Medicines.ie, 2015)

Skeletal muscle effects: In the higher doses Rosuvastatin therapy, myalgia, myopathy and rareky rhabdomyolysis have been reported. With the use of ezetimibe in combination with HMG-CoA reductase inhibitor, rhabdomyolysis has been informed which is happened very rarely. At the 40 mg dose of Rosuvastatin with other HMG-Co A reductase inhibitors, rhabdomyolysis has been reported. In patients receiving fusidic acid and statins in combination, rhabdomyolysis has been observed. In accordance, Rosuvastatin must not be co-administered with systemic formulations of fusidic acid. (Medicines.ie, 2015)

Liver effects: Patients who consume excessive quantities of alcohol or having a history of liver disease should use Rosuvastatin with caution. If the level of serum transminases is greater than 3 times the upper limit of normal, Rosuvastatin should be discontinued or the dose should be reduced. (Medicines.ie, 2015)

Race: Increased exposure of pharmacokinetic studies has been shown in Asian objects than Caucasians. 40 mg dose of Rosuvastatin should be taken with caution in these patients. (Medicines.ie, 2015)

Protease inhibitors: In HIV patients who receive protease inhibitor with Rosuvastatin, plasma concentration of Rosuvastatin is increased due to the concomitant use with protease inhibitor. Accordingly, the concomitant use of Rosuvastatin with protease inhibitor is not recommended. (Medicines.ie, 2015)

Interstitial lung disease: In long term Rosuvastatin therapy, interstitial lung disease has been developed rarely. This disease can be identified by dyspnea, non-productive cough and deterioration in general health. When it is suspected that a patient has developed this disease, statin therapy should be discontinued. (Medicines.ie, 2015)

Diabetes mellitus: Some studies suggests that Rosuvastatin as a class of raise blood glucose and in some patients, at high risk of future diabetes, may experience a level of hyperglycemia where formal diabetes care is appropriate. Diabetic patients at risk should be monitored both clinically and biochemically according to national guidelines. In the JUPITER study, 2.8% in Rosuvastatin

and 2.3% in placebo were being the frequency of diabetes mellitus in patients with fasting glucose 5.6 to 6.9 mmol/L. (Medicines.ie, 2015)

Paediatric population: In a clinical trial of children giving Rosuvastatin for 52 weeks, muscle symptoms were observed more often compared to adults. (Medicines.ie, 2015)

Excipients: Film coated tablet of Rosuvastatin contain lactose monohydrate as excipients. Patients who have rare hereditary problems of galactose intolerance should not take this medicine. This tablet also contains Allura red and Sunset yellow, which may cause allergic reactions. (Medicines.ie, 2015)

1.6.10 Use in pregnancy and lactation

In women of childbearing age should have been informed of the potential hazard during Rosuvastatin administration. During pregnancy, Rosuvastatin therapy should be discontinued immediately and the patient apprises of the potential hazard to the fetus. It is not determined that Rosuvastatin is excreted in human milk. (SquarePharma, 2016)

1.6.11 Use in Pediatric patient

In pediatric patients, the safety and effectiveness of Rosuvastatin have not been established. (SquarePharma, 2016)

1.6.12 Drug interactions:

Cytochrome P450 enzymes: Rosuvastatin is neither an inhibitor nor an inducer of cytochrome P450 enzymes which has been observed from in vitro and in vivo studies. Accordingly, drug interaction resulting from cytochrome P450- mediated metabolism is not expected and clearance of Rosuvastatin is not dependent on metabolism by this enzyme. (SquarePharma, 2016)

Ketoconazole: There is no change in plasma concentration of Rosuvastatin when it is co-administered with ketoconazole. (SquarePharma, 2016)

Antacid: The decreased plasma concentration of Rosuvastatin has been observed when it is combined with an antacid suspension containing aluminium and magnesium hydroxide. This

effect can be minimized when the antacid is dosed 2 hours after Rosuvastatin. (Medicines.ie, 2015)

Erythromycin: Combined use of Rosuvastatin and erythromycin resulted in a 20% decrease in $AUC_{(0-t)}$ and a 30 % decrease in C_{max} of Rosuvastatin. This interaction may be caused by the increase in gut motility due to the use erythromycin. (Medicines.ie, 2015)

Ezetimibe: Combined use of 10 mg of Rosuvastatin and 10 mg ezetimibe resulted in a 1.2 fold increase in AUC or Rosuvastatin. A pharmacodynamic interaction, in case of adverse effects, between Rosuvastatin and ezetimibe cannot be eliminated. (Medicines.ie, 2015)

Itraconazole: Increase in AUC of Rosuvastatin has been resulted when it is combinely used with itraconazole. (SquarePharma, 2016)

Fluconazole: 14% increase in AUC of Rosuvastatin has been reported in the co-administration of fluconazole with Rosuvastatin. (SquarePharma, 2016)

Warfarin: Concomitant use of warfarin (20 mg) with Rosuvastatin (40 mg) did not change warfarin plasma concentration but increased the International Normalized Ratio(INR). (SquarePharma, 2016)

Digoxin: Concomitant use of digoxin (0.5 mg) with Rosuvastatin (40 mg) resulted in no change to digoxin plasma concentration. (SquarePharma, 2016)

Fenofibrate: No significant changes in plasma concentrations of Rosuvastatin or fenofibrate results in co-administration of fenofibrate with Rosuvastatin. (SquarePharma, 2016)

Gemfibrozil: Concomitant use of gemfibrozil (600 mg twice daily for 7 days) with Rosuvastatin (80 mg) resulted in a 90% and 120 % increase for AUC and Cmax of Rosuvastatin. (SquarePharma, 2016)

Oral contraceptives: Concomitant use of oral contraceptives (ethinyl estradiol and norgestrel) with Rosuvastatin resulted in an increase in plasma concentrations of ethinyl estradiol and norgestrel by 26% and 34% respectively. (SquarePharma, 2016)

1.6.13 Storage condition

Rosuvastatin tablet should be stored in a cool and dry place and should be protected from light and moisture. (SquarePharma, 2016)

1.7 Information of Calvimax-D (500mg)

1.7.1 Therapeutic group

Vitamins and minerals (Incepta Pharma, 2016)

1.7.2 Description

Calcium is an important element which plays a vital role in the formation of bone. Calcium is used to prevent osteoporosis and other related fractures which are caused by imbalances in the level of calcium in the bone and that is established by clinical evidence. Vitamin D is also a vital element and it helps in calcium absorption which is needed for strong bones. Both Calcium and Vitamin D are important for bone growth because they have synergistic effect on the growth of bones. In the treatment of osteoporosis and other related fractures, both Calcium and Vitamin D are used. (Incepta Pharma, 2016)

Figure1.5 : Calvimax-D(Incepta Pharma, 2016)

1.7.3 Composition

Each tablet contains calcium BP 500 mg and Vitamin D3 BP 200 IU. (Incepta Pharma, 2016)

1.7.4 Indication

Calvimax is used for the following cases-

- Osteoporosis
- Osteomalacia
- Rickets
- Hypoparathyroidism
- Tetany
- During pregnancy and lactation
- Kidney disease
- Used with antiseizure medication (Incepta Pharma, 2016)

1.7.5 Dosage and Administration

- Age above 12 years, the tablet should take twice a day. One in the morning and other in the evening.
- Age below 12 years, calvimax is not recommended. (Incepta Pharma, 2016)

1.7.6 Side effects

The side effects caused by calcium supplement include-

- constipation
- diarrhoea
- flatulence
- nausea
- gastric pain

The side effects caused by Vitamin D supplement include-

- skin rashes
- hypercalciuria

- hypercalcaemia (in case of long term treatment) (Incepta Pharma, 2016)

1.7.7 Precautions

- Patients should be supervised carefully having mild to moderate renal failure.
- Plasma calcium level and urinary calcium excretion should be checked in patients having mild to moderate renal failure.
- Should be measured urinary calcium excretion in patient having history of renal stone.
- Serum and urinary calcium level should be monitored in case of long term treatment.

(Incepta Pharma, 2016)

1.7.8 Contraindications:

- Hypercalcaemia
- Primary hyperparathyroidism
- Vitamin D over dosage
- Severe renal damage
- Renal stones
- Severe hypercalciuria
- Hypersensitivity to any ingredients of the tablet (Incepta Pharma, 2016)

1.7.9 Use in pregnancy and lactation

Women should be followed the direction of physician during pregnancy and lactation. During that time, the demand for calcium and Vitamin D are increased. So, to meet up the demand, calcium and Vitamin supplements are used with following condition-

- If calcium and iron supplement are taken by the patients, they should be taken that supplements at different times.
- Over doses of calcium have teratogenic effects in pregnant patient because vitamin D and its metabolites pass into the breast milk. So, the dosing should be perfect.

(Incepta Pharma, 2016)

1.7.10 Overdose

Overdoses of calvimax causes hypercalcemia followed by Vitamin D toxicity. To avoid toxicity, stop taking of calcium and vitamin D supplement. (Incepta Pharma, 2016)

1.8 Dissolution

1.8.1 Dissolution General Information

Dissolution is referred to as the transfer of molecules of ions from solute state in a solution. In simpler way, dissolution is the process by which a substance turns into solution in a solvent. In case of solids dissolution is the process by which break down of the crystal lattice into individual ions atoms or molecules happens. Thermodynamic energies such as the heat of solution and entropy of solution control the result of dissolution. For having the net dissolution, the free energy must be negative. (Sirius-analytical, 2016)

1.8.2 Rate of dissolution

The rate of total dissolution indicates the speed of the total process. Rate of dissolution depends on the chemical natures of the solvent and solute, such as; temperature, the degree of unsaturation, the interfacial surface area and the presence of inhibitors like substances which are absorbed on the surface.

Noyes-Whitney equation or the Nernst and Brunner equation can express the rate of dissolution. The equation is:

$dm/dt = AX \{ D/d\}X(C_s-C_b)$;

where, m= mass of solute material

t= time

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

D= diffusion coefficient

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

C_s= mass concentration of the substance on the surface

C_b= mass concentration of the substance in the bulk of the solvent

In case of dissolution limited by diffusion, C_s 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 denoted by kg/m^2S and termed as 'intrinsic dissolution rate'. (Sirius-analytical, 2016)

1.8.3 Dissolution process

Based on the rule, ' like dissolves like'; means that substances must have the same intermolecular forces to form solutions. The particles of solute interact with the particles of solvent after the introduction of a soluble solute into the solvent. In solid or liquid solute, the interactions between the solute particles and the solvent particles are so strong that the individual solute particles separate from each other and surrounded by solvent molecules, traverse to the solution. This is process is referred to as salvation and is illustrated in the following figure:

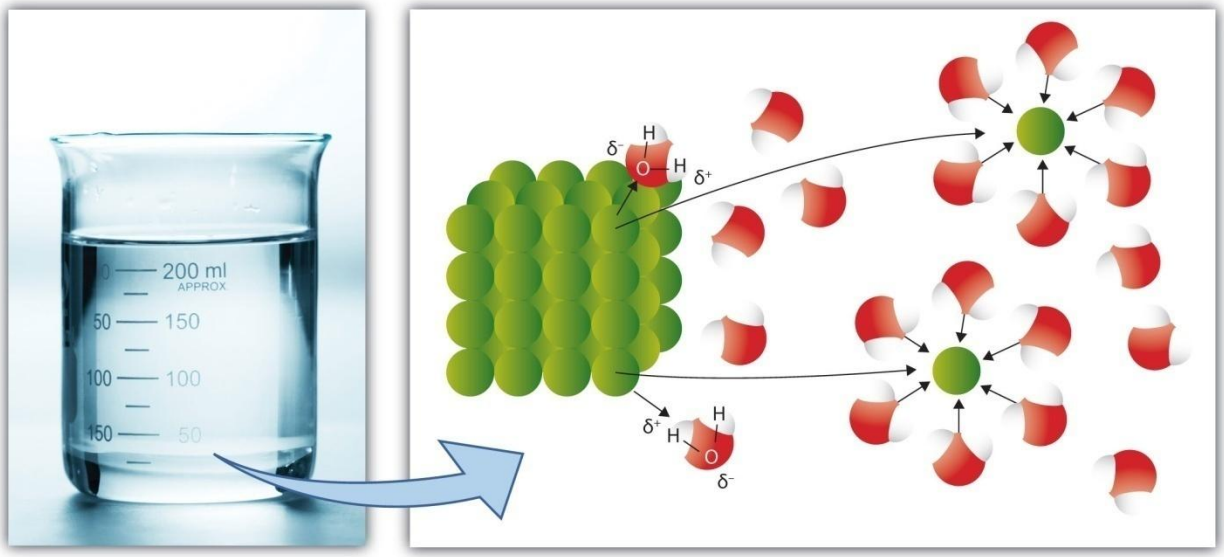

Figure1.6: Solvation (Lapsurgery, 2014)

In the case of water as solvent, the salvation word is replaced by the word hydration. After dissolving a solute, the individual particles of solute become surrounded by solvent particles. Eventually the particle separates from the remaining solute, surrounded by solvent molecules in solution. Moreover, if the solute is ionic, the individual ions get separated from each other and become surrounded by solvent particles. So the ions of solute separate when the solute dissolves. This process is known as dissociation. Soluble ionic compounds are often known as electrolytes. Many ionic compounds dissociate completely and they are called strong electrolytes. Sodium salts are example of strong electrolytes. Weak electrolytes are those which are dissolved but get dissociated in partial amount and solutions of such solutes may conduct electricity weakly such as; acetic acid. (Lapsurgery, 2014)

1.8.4 Factors which influence the dissolution of a substance

- Temperature
- Particle size of solute
- Agitation
- Solvent selection

Temperature

In most cases of dissolution of solute in a liquid is based on the absorption of heat. The dissolution will be more rapid, if the temperature is elevated but in lower temperature the dissolution will be less. So, temperature has the important influence on dissolution.

Particle size

Particle size also influences the dissolution rate. The more size of particles, the less the rate of dissolution. The absorption depends on the dissolution rate. So the rectification of dissolution rate of any solute is very important.

Agitation

Concentration of the solvent also determines the dissolution. The more concentrated the solvent, the less the rate of dissolution.

Solvent selection

Dissolution rate also depends upon the type of solvent. In oily solvent dissolution rate is slower than in water. (Yuksel et al. 2000)

1.9 Comparative dissolution

1.9.1 Basic concept of comparative dissolution

Comparative dissolution testing is very significant tool in drug development including serving as routine quality control tests. Comparative dissolution tests are one of the most important tools to support waivers for bioequivalence requirements, for approval of generic drug products. (Anand et al. 2011)

1.9.2 Specifications and experimental conditions

The Centre for Drug Evaluation and Research (CDER) of the Food and Drug Administration (USFDA) declared three categories of dissolution test specifications for immediate release products. Such as;

- Single point specifications
- Two point specifications
- Dissolution profile comparison

Single and two point specifications are important to identify drug products which contain high solubility-high permeability substances. But the matter is, this is not applicable for characterization of low solubility products because such products have produced different profiles of dissolution. In addition, they may comply with the point estimates, thereby giving an erroneous impression of pharmaceutical equivalence in dissolution characteristics. Since dissolution profile comparison is more precise and discriminative than point estimates others, it is recommended that dissolution profile comparison is for such products.

For comparative dissolution profile testing of drugs in order to study their stability and release described in the different physiological conditions, at least three dissolution media is needed.

The recommended dissolution media are 0.1M HCL or pH 1.2 buffer solution as like as buffer solutions of pH 4.5 and 6.8. Water can be implemented as an additional medium in the studies.

(Yuksel et al. 2000)

1.9.3 Methods for comparison of dissolution profile data

In case of in vitro dissolution profile, there are three groups to test the comparative dissolution profile:

- Methods based on analysis of variance (ANOVA)
- Model-dependent methods
- Model-independent methods

ANOVA – based methods implement in variety and multivariate approaches to measure the quantity in dissolution percentages. The cubic root law is a model depended method. (Yuksel et al. 2000)

Moore and Flanner (1996) declared a very simple model independent method to produce the fit factors to compare dissolution profile data of a pair of products under similar conditions. These fit factors directly analogize the difference between percent drug dissolved per unit time for a test and a reference product. These factors are described as *f*1 (difference factor) and *f*2 (similarity factor). (Patel, 2009)

Measurement of the percent difference between two dissolution curves under comparison at each point is denoted as the difference factor (f1). It is a measurement of the relative error between the two curves. Similarity of two dissolution curves is denoted by f1 values of 0-15%. (Patel, 2009)

Measurement of the similarity in the percent dissolution between two dissolution curves is known as the similarity factor (f2). The similarity factor is inversely proportional to the average

squared difference between the two profiles. It is expressed as a logarithmic reciprocal square root transformation of the sum of squared error. (Shah and Amidon, 2014)

Chapter two
Literature Review

Different types of research projects about Statins had done by researchers before I did. Among those research works some of are mentioned below:

In the year 1998, one research study was done to determine whether 12 months of simvastatin therapy, an HMG-Co reductase inhibitor would dissolve gallstones. In this research, 27 subjects were selected and they all had a fasting oral cholecystogram, ultrasound examination and fasting serum lipids. According to this study, researchers decided that 12 months of therapy with simvastatin was effective in lowering serum lipids but not effective in dissolving gallstones. (Chapman et al., 1998)

At year 1998, a study was done to determine if a combination therapy with simvastatin and Urodeoxycholic Acid was more effective for cholesterol sallstone dissolution than was Urodeoxycholic Acid monotherapy. In this study fifty patients with radiolucent gallstones in a gallbladder opacifying at drip infusion cholecytography 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 et al., 1998)

In the past year of 2000, 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. (Wang and Asgharmejad, 2000)

In the year of 2006, one research was studied 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, 2006)

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)

At 2007, one study was done to improve the solubility and dissolution rate of a poorly water-soluble drug, Lovastatin. In this study, solid dispersion technique as method was used. The result of this study was if tablet contains PEG and PVP, its solubility will be increased. (Patel and Patel, 2007)

At 2008, simvastatin was studied in a research paper to enhance the solubility and dissolution by using hydrophilic, low viscosity grade polymer hydroxypropyl methylcellulose. In this thesis, co solvent evaporation and spray drying method were used. Result from the research showed that the conversion of crystalline form of simvastatin into amorphous. (Pandya et al., 2008)

At 2009, one study evaluated a new method to prepare nanoparticles of a poorly water-soluble drug, simvastatin by evaporation of all solvents from spontaneously formed oil-in-water microemulsion. Freeze-drying was applied in this research. Result of this experiment showed that simvastatin nanoparticles were amorphous. (Margulis-Goshen and Magdassi, 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 dissolion rate of that drug. (Rao et al., 2010)

At 2011, one 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. (Sahoo, 2011)

In the year 2011, an investigation was done to increase the solubility and dissolution rate of simvastatin by the preparation of nanosuspensions with Pluronic F127 and zirconium oxide beads. In this study wet-milling technique was applied at the laboratory scale. The result of this investigation showed that the preparation of simvastatin loaded nanosuspensions significantly improved the in vitro dissolution rate, thusly enhancing the fast onset of therapeutic drug effect. (Pandya,Patel and Patel, 2011)

In the year 2011, 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 et al., 2011)

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. (Jiang et al., 2012)

At 2012, a study was done to enhance the dissolution profile, absorption efficiency and bioavailability of lovastatin. In this project, solid dispersions, superdisintegrants and sublimation were used as techniques. The result of this experiment suggested that superdisintegrants method was preferred. (Neduri, Bontha and Vemula, 2013)

In the year 2012, some researchers wrote a paper about the pharmacology of Rosuva® (10mg)statin and its efficacy and safety and also the major clinical trials with prevention. (Luvai 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. (Jahan et al., 2013)

At 2013, one 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)

At 2013, a study was done to demonstrate the enhancement of release pattern, drug release rates of poorly water soluble drug Rosuvastatin Calcium. In this research, Liquisolid compact was used as technique. According to this study, researchers concluded that liquisolid compacts was a promising strategy in improving the dissolution of poorly water-soluble drugs and formulating immediate release solid dosage forms. (Kapure, Pande and Deshmukh, 2013)

At 2013, one study was accomplished to improve the dissolution and permeability of Rosuvastatin Calcium by formulating it as a niosomal dosage form. In this research, non-ionic surfactants, cholesterol nad lecithin in different ratios were used by film hydration method. The result f 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. (Salih, Samein and Ali, 2013)

At 2014, one work had done 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. (Jahan et al., 2014)

In the year of 2014, a research project was studied to increase the solubility of Rosuvastatin as it is poorly water soluble drug. In this research, liquisolid systems were used for the determination of flow property and drug-excipients interactions by Infrared spectra (IR) analysis, differential scanning calorimetry (DSC) and X-ray powder diffraction (XRPD). This study showed that liquisolid technique was a promising technique for the improvement of the dissolution rate of water insoluble drug. (Kamble, Shaikh and Chaudhari, 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. (Panghal *et al.*, 2014)

In the year 2014, researchers did a research to enhance the dissolution of the poorly soluble drugs, Lovastatin. Liquisolid compacts technique was used in this research. The results reflected that liquisolid compacts demonstrated significantly higher drug release rates than direct compressed tablets. (Chapman et al., 2014)

At year 2014, the purpose of a research work was 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 thusly dissolution rate decreased. (Jui, 2014)

At 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)

At 2015, a research work had done to prepare microparticles to enhance solubility of Rosuva® (10mg)statin calcium. This study was evaluated by Fourier Transform Infrared spectroscopy (FTIR), thermal analysis, dissolution studies, powder X-ray diffraction (PXRD) and scanning electron microscopy (SEM). This study showed that In PXRD, drug nature was changed from crystalline to amorphous and FTIR and thermal analysis assured that the complex was formed between drug and beta-cyclodextrin and SEM images showed that microparticles had small size loaded with drug. (Sarfarz *et al.* 2015)

At 2015, one work was aimed to develop an immediate release tablet formulation of Fenofibrate and Rosuvastatin in combination for the well treatment of hypercholesterolemia and prevention of candiovascular diseases. Hot-Melt technology was used to develop this study. The result of this study was the release rate and dissolution profile of fenofibrate and Rosuvastatin was improved. (Eamen *et al.*,2015)

In this present year 2016, Pitavastatin was studied to enhance the solubility, dissolution rate, and bioavailability. In this project, Liquisolid technology and solid dispersions methods were used. By this study, it was ensured that the solubility, dissolution rate, bioavailability of water insoluble drug pitavastatin was being enhanced. (Messa and Ampati, 2016)

In this present year 2016, one study had done to explore novel carrier VBP-1 (organosulphur compound) for formulating a solid dispersion. In this research, co-grinding technique was used. In result, they concluded that novel carrier VBP-1 was successfully employed to enhance the dissolution of atorvastatin calcium. (Prabhu and Patravale, 2015)

At 2016, one study was done to develop a novel method for the fabrication of amorphous nano-solid dispersions of atorvastatin calcium, ezetimibe and atorvastatin and ezetimibe combination as poorly water soluble drugs. Here, electrospraying method was applied and it was proved to be an effiecient method for the fabrication of amorphous nano-solid dispersions of atorvastatin calcium, ezetimibe and atorvastatin and ezetimibe combination as poorly water soluble drugs. (Jahangiri et al.,2016)

Chapter Three

Materials and Method

3.1 Sample collection

To observe the change in dissolution of Rosuvastatin with the presence of two supplements- 12 tablets of Rosuva^{TM} (10 mg) and 6 tablets of Calvimax-D (500mg) that were collected from local drugstore in Dhaka as sample.

Table 3.1: Samples used in the experiment and their sources

Sample name	**Source (Suppliers' name)**
Rosuva^{TM} (10mg)	Square Pharmaceuticals Limited
Calvimax-D (500mg)	Incepta Pharmaceuticals Limited

3.2 Reagent(s)

Distill water which was prepared in the laboratory of East West University.

3.3 Instruments

Table 3.2: Instruments used in the experiment

Serial no.	**Equipment**	**Source (supplier name)**	**Origin**
1.	UV-Spectrophotometer	Shimadzu UV-1800	Japan
2.	Dissolution Tester	SMIC	China
3.	Distill water plant	SMIC	China
4.	Electronic Balance	Precisa XB120A	Switzerland
5.	Friability tester	Veegoindia	India
6.	Vernier Caliper	China supplier	Shanghai, China

7.	Hardness tester	Manually operated hardness tester	India

3.4 Apparatus

Table 3.3: Apparatus used throughout the experiment

Serial no.	Apparatus
1	Beaker
2	Test tube
3	Filter paper
4	Glass rod
5	Morter and pestle
6	spatula
7	funnel
8	Pipette (1 ml, 5 ml, 10 ml)
9	Pipette pumper
10	Volumetric flask (25 ml, 50 ml, 100 ml, 1000ml)

Table 3.4: *In vitro* dissolution study

Dissolution medium	Distilled water
RPM	50
Time	60 minutes

3.5 Procedure

The release rate of Rosuvastatin tablet was determined by using tablet dissolution tester USPXXII. By using 900 ml water at pH 7.4 and at 37 degree Celsius and 50 RPM, the dissolution test was performed. At first 10 minutes and with the interval of 10 minutes sample of 10 ml was collected from the dissolution medium and the amount was replaced by 10 ml distill water. The sample was filtered through a Whatman filter paper. The absorbance of the solution was measured 241nm for Rosuvastatin drug by using a Shimadzu UV-1202 UV/ Visible double beam spectrophotometer. Percentage of drug release was calculated by using an equation which is obtained from standard curve. The dissolution was continued for 60 minutes to get stimulated picture of drug release in vivo condition and drug dissolve at specific time period was plotted as percent release versus time curve. (Shah et al. 1998)

3.6 Preparation of dissolution medium for standard curve

Since Rosuvastatin is a water soluble drug, 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.

3.7 Preparation of Standard Curve

In order to prepare standard curve, at first different concentrations of Rosuvastatin (0.001 mg/ml, 0.002 mg/ml, 0.003 mg/ml etc) were prepared. The following steps were followed to prepare these concentrations:

- Three Rosuva[TM] (10mg) tablet were crushed in mortar and pestle.
- Equivalent weight of 10 mg of tablet was callipered and then it was dissolved in 100 ml of distilled water. According to this procedure the concentration of the stock solution became .01mg/ml.
- Then the solution in the volumetric flask was filtered and it was become the stock solution for the preparation of 0.001 mg/ml concentration.

3.8 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 aginse 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 3.5: Prepared Concentration of Rosuvastatin

Serial no.	Concentration of Rosuvastatin (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

- Afterwards preparing the desired concentrations, the spectrophotometer was turned on and 241nm wavelength was set up as 241 nm was to be determined for showing the accurate result.
- The spectrophotometer was adjusted for 0 and 100% transmittance (T).
- The absorbances of the prepared solutions were measured later on.
- Then the absorbences were plotted against concentrations and a straight line was observed.

3.9 Preparation for dissolution test

3.9.1 Preparation of stock solution

Distilled water was made in the laboratory and was used as stock solution for dissolution test. 6 liters of distilled water was prepared for each batch.

3.9.2 Method for dissolution test of Rosuva™ (10mg)

6 liters or 600ml of stock solution (distilled water) was prepared. Each vessel of dissolution tester was poured to 900 ml of distilled water. 1 hour time and 50 RPM were set up in the dissolution machine. After that, the dissolution machine was warmed up until it reached at 37.5 degree Celsius. Then one Rosuva® (10mg) 10 mg tablet was placed in every vessel. After 10, 20, 30, 40, 50 and 60 minutes, 10 ml of solution was withdrawn from each vessels and filtered, then UV absorbance of the solution was taken at the wavelength of 241nm.

3.9.3 Method for dissolution test of Rosuva™ (10mg) and Calvimax-D (500mg)

6 liters or 600ml of stock solution (distilled water) was prepared. Each vessel of dissolution tester was poured to 900 ml of distilled water. 1 hour time and 50 RPM were set up in the dissolution machine. After that, the dissolution machine was warmed up until it reached at 37.5 degree Celsius. Then one Rosuva® (10mg) 10 mg tablet and one Calvimax-D (500mg) 500 mg tablets were placed in every vessel. After 10, 20, 30, 40, 50 and 60 minutes, 10 ml of solution was withdrawn from each vessels and filtered, then UV absorbance of the solution was taken at the wavelength of 241nm.

3.10 Determination of physical parameters

3.10.1 Weight Variation Test

3.10.1.1 Procedure

10 tablets were taken and then weighed. The average was calculated and it was considered as the standard weight of an individual tablet. All tablets were weighed individually and observed whether the individual tablets are in between the range or not. Noted that the variation from the average weight in the weights not more than two tablets must not differ more than the percentage listed below:

Table 3.6: Accepted percentage list of weight variation test of tablets

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

3.10.1.2 Equation

The given equation was used to determine % weight variation of tablets:

% weight variation = (A-I/A) * 100

Where, I= initial weight of tablet

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

3.10.2 Thickness test

3.10.2.1 Procedure

At first the tablet was placed in between the two jaws of vernier caliper. Then the main scale reading was observed and taken and at next vernier scale reading was taken also. Then the two readings were added together and then multiply with the vernier constant 0.1cm. (Dunnet and Crisafio, 1995)

3.10.2.2 Calculation

Following equation was used to determine the thickness of tablets.

Thickness of the tablet= Reading of cm scale + Reading of vernier scale × Vernier constant (0.01) + Vernier error (Dunnet and Crisafio, 1995)

3.10.3 Hardness test

3.10.3.1 Procedure

The slide scale of hardness tester was become zero. One tablet was placed vertically in between the two jaws of the tester. Then force was applied with a screw thread and spring until tablet fractured. From the sliding scale, reading in Kg was taken. (Dunnet and Crisafio, 1995)

3.11 Instrumentation

3.11.1 Dissolution test apparatus

A dissolution tester USPXXII (source RC-6B, made in china) was implemented for dissolution experiments. It is incorporated with a clear acrylic water bath, a stirrer hood with paddle shafts, an automatic sampling unit and a control unit supported by microcontroller software with a non-volatile memory for 15 methods. An immersion circular with an in-built thermostat was being incorporated with water bath for temperature control. An external temperature sensor, a water level sensor and a lid were used to support for eight dissolution bowls. The stirrer hood was incorporated with 8 paddle shafts fitted with USP apparatus 2 and a tablet dispenser with 8 conical shaped dissolution bowl lids. 10 in-line filters, a bi-directional 12- channel peristaltic pump with tygon tubings, microprocessor controlled sample collector and a sample tray capsule of collecting 10X6 sets of samples were consisted the automatic sampling unit. In this study, polycarbonate dissolution vessels with a hemispherical bottom and a capacity of 1000 ml were used.

3.11.2 Ultra-Violet Spectrophotometer

The ultra-violet absorption spectrum for Rosuvastatin working standard was recorded using a double beam T90 + UV/VIS spectrometer controlled via a computer using UVWIN spectrophotometer software version 5.2.0. over a 10 mm path length using quartz cuvettes.

3.11.3 Images of Instruments:

Some images of instruments which are used in this experiment are given below:

Figure 3.1: Distilled water apparatus (Tresnainstrumen, 2016)

Figure 3.2: Dissolution tester (Tradeindia, 2016)

Figure 3.3: Hardness tester (Tradeindia, 2016)

Figure 3.4: Vernier Caliper (Tradeindia, 2016)

Figure 3.5: Electronic Measuring Balance (Tradeindia, 2016)

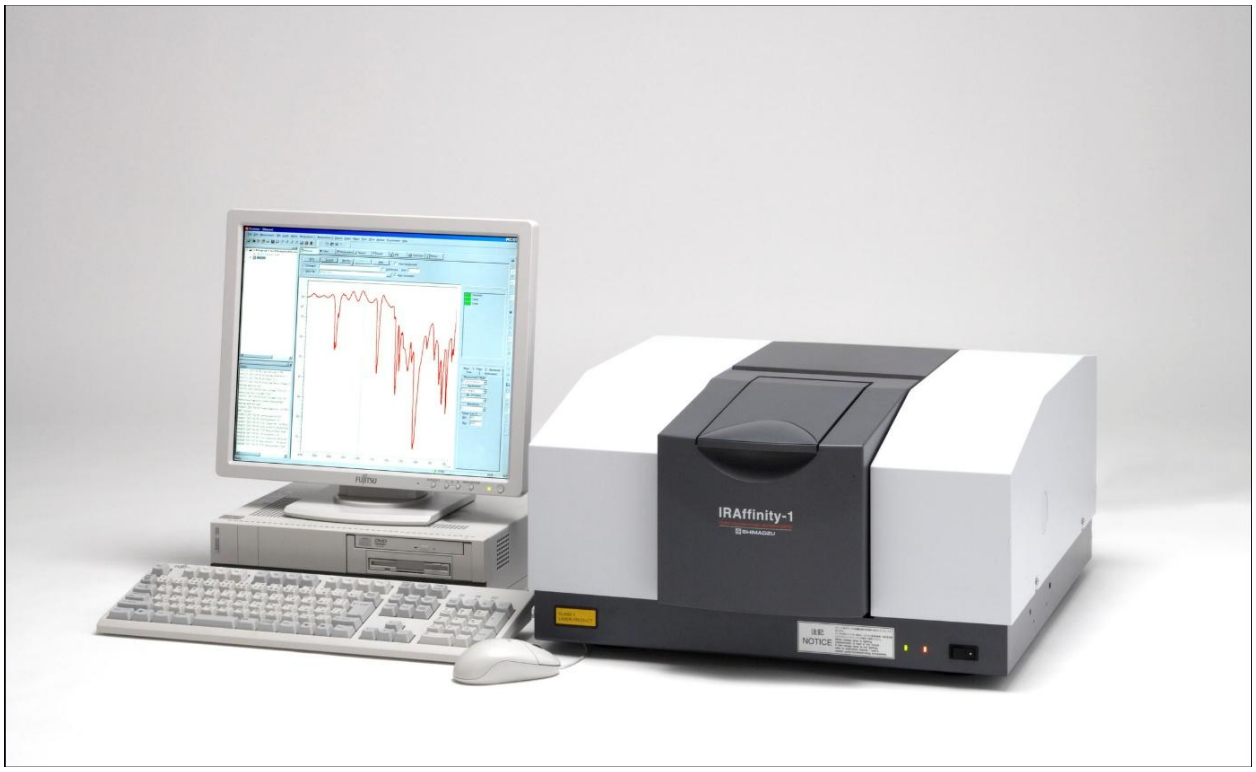

Figure 3.6: UV-Visible Spectrscopy (Tradeindia, 2016)

Chapter Four

Results

4.1 General Information

The Rosuvastatin samples were subjected to assay and dissolution profile analysis under the optimum situations. The objective of the assay was to assess the samples for compliance with pharmacopeias limits for content.

4.2 Physical parameters

Table 4.1: Results from weight variation test:

Tablet No.	Initial Weight	Average weight	% Weight variation
	I (mg)	A(mg)	(A-I)/I*100
1.	143		0.699
2.	145		-0.694
3.	143		0.699
4.	144		0
5.	143	144	0.699
6.	145		-0.694
7.	143		0.699
8.	144		0
9.	143		0.699
10.	143		0.699

Table 4.2: Results from hardness test:

Tablet No.	Hardness (kg)	Average
1	3.3	
2	3.2	
3	3.1	
4	3.5	
5	3.3	3.3
6	3.2	
7	3.2	
8	3.4	
9	3.3	
10	3.4	

Table 4.3: Results from thickness test

Tablet No.	Main scale reading (M)	Vernier scale reading (V)	Vernier constant (V_c)	Vernier error	Thickness of tablet
	cm	cm			M+(V× Vc) cm
1	0.3	1	0.1	0	0.4
2	0.3	1	0.1	0	0.4
3	0.3	1	0.1	0	0.4
4	0.3	1	0.1	0	0.4
5	0.3	1	0.1	0	0.4
6	0.3	1	0.1	0	0.4
7	0.3	1	0.1	0	0.4
8	0.3	1	0.1	0	0.4
9	0.3	1	0.1	0	0.4
10	0.3	1	0.1	0	0.4

4.3 Standard Curve Preparation

Table 4.4: Concentration and absorbance for Standard curve of Rosuvastatin

Serial No.	Concentration (µg/ml)	Absorbance at 241nm
1	0	0
2	0.001	0.031
3	0.002	0.055
4	0.003	0.086
5	0.004	0.108
6	0.005	0.134
7	0.006	0.156
8	0.007	0.184
9	0.008	0.207
10	0.009	0.237

By plotting the concentration against the absorbance of Rosuvastatin, we have found a straight line. From the standard curve of Rosuvastatin, we derived an equation $y = 25.73x + 0.004$ and $R^2 = 0.998$. Here, y= Absorbance

x= Concentration of drug

R^2= Co-efficient of determination

By using this equation we can get the concentration from different samples absorbance of Rosuvastatin.

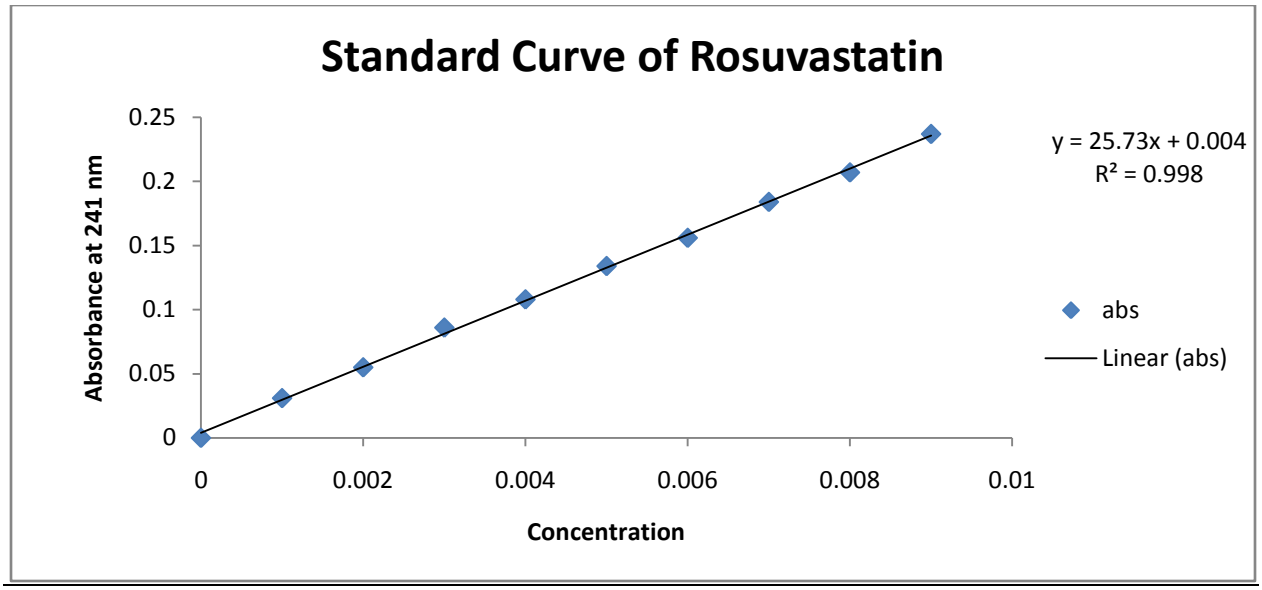

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Figure 4.1: Standard curve of Rosuvastatin

By observing this graph, we can see that the drug release is increasing with the increasing of time which makes the graph accurate. This graph is named as the standard cure for the following drugs. Here X axis reflects the concentration and Y axis is for absorbance.

4.4. Results of the dissolution test of individual Rosuva™(10mg), Rosuva™ (10mg) with Calcium and Vitamin D supplement and the impact of supplements on the dissolution of Rosuva™ (10mg) after 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minues and 60 minutes.

4.4.1 Dissolution test of Rosuva™ (10mg) without any supplement

Table 4.5: UV- absorbance of Rosuva™ (10mg) without any supplement

Sample no.	Absorbance at 241 nm					
	10 minutes	20 minutes	30 minutes	40 minutes	50 minutes	60 minutes
1	0.153	0.169	0.175	0.183	0.195	0.2
2	0.152	0.167	0.174	0.182	0.193	0.211
3	0.157	0.168	0.178	0.184	0.192	0.209
4	0.158	0.166	0.177	0.187	0.198	0.202
5	0.156	0.163	0.178	0.187	0.196	0.21
6	0.151	0.166	0.176	0.189	0.191	0.212

4.4.2 Calculation

The dissolved amount of Rosuva™ (10 mg) is calculated by the following equation that is obtained from the standard curve:

$y = 25.73x + 0.004$

Here, y= absorbance and x= concentration

and the dilution factor is 900.

When the absorbance was 0.153, then the following equation can be written as-

$0.153 = 25.73x + 0.004$

Or, $25.73x = 0.153 - 0.004$

Or, $25.73x = 0.149$

Or, $x = 0.149/25.73$

$x = 0.006$

So, the dissolved amount of Rosuva™ (10 mg) was= $0.006 \times 900 = 5.4$ mg. By putting the other absorbance values in the same equation, different dissolved amount of Rosuva™ (10mg) was calculated.

Table 4.6: Determination of dissolved amount of Rosuva™ (10mg) without supplement

Time	Sample no.	Absorbance at 241 nm	Drug Release(mg)	% Drug Release
10 minutes	1	0.153	5.212	52.118
	2	0.152	5.177	51.768
	3	0.157	5.352	53.517
	4	0.158	5.387	53.867
	5	0.156	5.317	53.168
	6	0.151	5.142	51.419
Time	**Sample no.**	**Absorbance**	**Drug Release**	**% Drug Release**
20 minutes	1	0.169	5.771	57.715
	2	0.167	5.702	57.015
	3	0.168	5.736	57.365
	4	0.166	5.667	56.665
	5	0.163	5.562	55.616
	6			
Time	**Sample no.**	**Absorbance**	**Drug Release**	**% Drug Release**
30 minutes	1	0.175	5.981	59.813
	2	0.174	5.946	59.464
	3	0.178	6.086	60.863
	4	0.177	6.051	60.513
	5	0.178	6.086	60.863
	6	0.176	6.016	60.163
Time	**Sample no.**	**Absorbance**	**Drug Release**	**% Drug Release**
40 minutes	1	0.183	6.261	62.612
	2	0.182	6.226	62.262
	3	0.184	6.296	62.962
	4	0.187	6.401	64.011
	5	0.187	6.401	64.011

	6	0.189	6.471	64.710
Time	**Sample no.**	**Absorbance**	**Drug Release**	**% Drug Release**
50 minutes	1	0.195	6.681	66.809
	2	0.193	6.611	66.110
	3	0.192	6.576	65.760
	4	0.198	6.786	67.859
	5	0.196	6.716	67.159
	6	0.191	6.541	65.410
Time	**Sample no.**	**Absorbance**	**Drug Release**	**% Drug Release**
60 minutes	1	0.2	6.856	68.558
	2	0.211	7.241	72.406
	3	0.209	7.171	71.706
	4	0.202	6.926	69.258
	5	0.21	7.206	72.056
	6	0.212	7.276	72.756

4.4.3 Dissolution test of Rosuva™ (10mg) with Calvimax-D (500mg) supplement

Table 4.7: UV- absorbance of Rosuva™ (10mg) with Calvimax-D (500mg) (150mg) supplement

Sample no.	**Absorbance at 241 nm**					
	10 minutes	20 minutes	30 minutes	40 minutes	50 minutes	60 minutes
1	0.119	0.129	0.135	0.139	0.145	0.149
2	0.118	0.125	0.133	0.138	0.144	0.148
3	0.119	0.129	0.135	0.139	0.145	0.149
4	0.117	0.124	0.131	0.136	0.142	0.146
5	0.12	0.129	0.135	0.139	0.145	0.149
6	0.114	0.123	0.129	0.133	0.141	0.145

Table 4.8: Determination of dissolved amount of Rosuva™ (10mg) with Calvimax-D (500mg) supplement

Time	Sample no.	Absorbance at 241 nm	Drug Release(mg)	% Drug Release
10 minutes	1	0.119	4.02	40.23
	2	0.118	3.99	39.88
	3	0.119	4.02	40.23
	4	0.117	3.95	39.53
	5	0.12	4.06	40.58
	6	0.114	3.85	38.48
Time	**Sample no.**	**Absorbance**	**Drug Release(mg)**	**% Drug Release**
20 minutes	1	0.129	4.37	43.72
	2	0.125	4.23	42.32
	3	0.129	4.37	43.72
	4	0.124	4.20	41.97
	5	0.129	4.37	43.72
	6	0.123	4.16	41.62
Time	**Sample no.**	**Absorbance**	**Drug Release(mg)**	**% Drug Release**
30 minutes	1	0.135	4.58	45.82
	2	0.133	4.51	45.12
	3	0.135	4.58	45.82
	4	0.131	4.44	44.42
	5	0.135	4.58	45.82
	6	0.129	4.37	43.72
Time	**Sample no.**	**Absorbance**	**Drug Release(mg)**	**% Drug Release**
40 minutes	1	0.139	4.72	47.22
	2	0.138	4.69	46.87
	3	0.139	4.72	47.22
	4	0.136	4.62	46.17

	5	0.139	4.72	47.22
	6	0.133	4.51	45.12
Time	**Sample no.**	**Absorbance**	**Drug Release(mg)**	**% Drug Release**
50 minutes	1	0.145	4.93	49.32
	2	0.144	4.90	48.97
	3	0.145	4.93	49.32
	4	0.142	4.83	48.27
	5	0.145	4.93	49.32
	6	0.141	4.79	47.92
Time	**Sample no.**	**Absorbance**	**Drug Release(mg)**	**% Drug Release**
60 minutes	1	0.149	5.07	50.72
	2	0.148	5.04	50.37
	3	0.149	5.07	50.72
	4	0.146	4.97	49.67
	5	0.149	5.07	50.72
	6	0.145	4.93	49.32

4.4.4 Impact of Calvimax-D (500mg) on the dissolution of Rosuva™ (10mg) after 10 minutes.

Table 4.9: Percentage calculation for dissolved amount of Rosuva™ (10mg) (Rasuvastatin), Rosuva™ 10mg (Rasuvastatin) with Calvimax-D (500mg) (Calcium and Vitamin D supplement) and the impact of Calvimax-D (500mg) on the dissolution of Rosuva™ (10mg) (Rasuvastatin) after 10 minutes

Rosuva™ (10mg) without any supplement				Rosuva™ (10mg) with Calvimax-D (500 mg)				
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 (%)
5.21		52.12		4.02		40.23		
5.18		51.77		3.99		39.88		
5.35	5.27	53.52	52.65	4.02	3.98	40.23	39.82	-24.37
5.39		53.87		3.95		39.53		
5.32		53.17		4.06		40.58		
5.14		51.42		3.85		38.48		

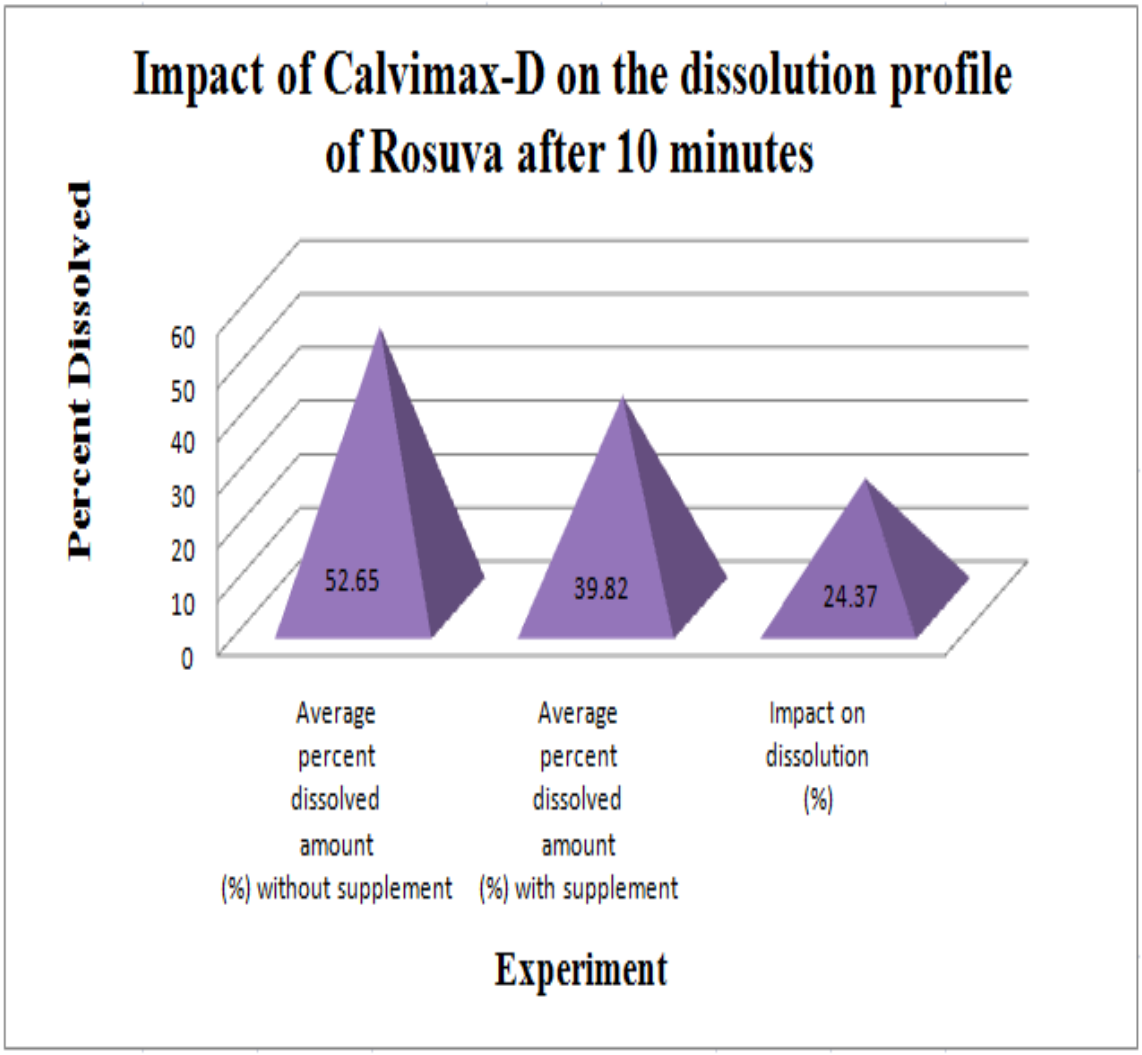

Figure 4.2: Graph showing the impact of Calvimax-D on the dissolution profile of Rosuva after 10 minutes

Table 4.10: Percentage calculation for dissolved amount of Rosuva™ (10mg) (Rasuvastatin), Rosuva™ (10mg) (Rasuvastatin) with Calvimax-D (500mg) (Calcium and Vitamin D supplement) and the impact of Calvimax-D (500mg) on the dissolution of Rosuva™ (10mg) (Rasuvastatin) after 20 minutes

Rosuva™ (10mg) without any supplement				**Rosuva™ (10mg) with Calvimax D (500 mg)**				
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 (%)**
5.77		57.71		4.37		43.72		
5.70		57.02		4.23		42.32		
5.74	5.69	57.36	56.84	4.37	4.28	43.72	42.83	-24.65
5.67		56.67		4.20		41.97		
5.56		55.62		4.37		43.72		
5.67		56.67		4.16		41.62		

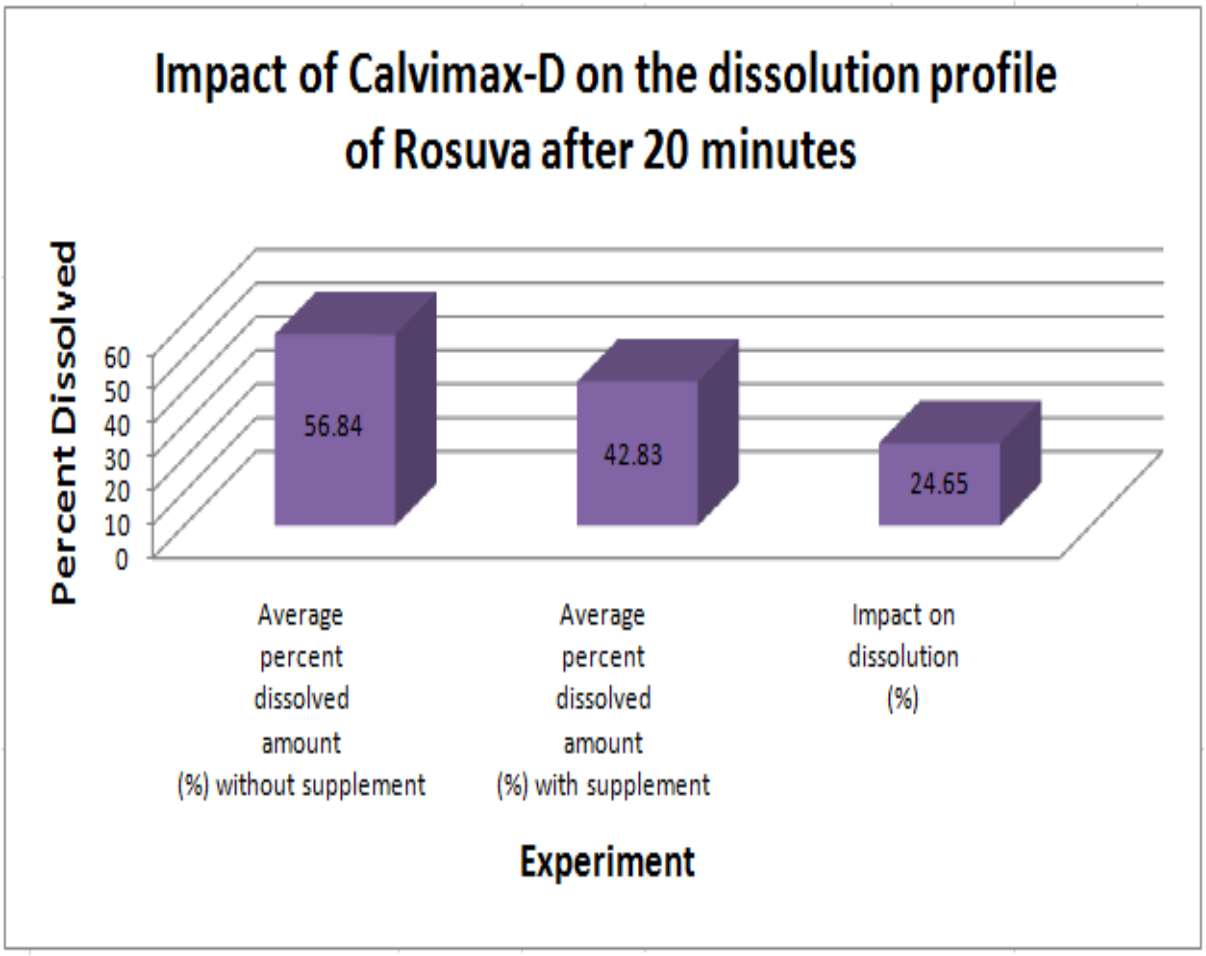

Figure 4.3: Graph showing the impact of Calvimax-D on the dissolution profile of Rosuva after 20 minutes

Table 4.11: Percentage calculation for dissolved amount of Rosuva™ (Rasuvastatin) (10mg), Rosuva™ (10mg) (Rasuvastatin) with Calvimax-D (500mg) (Calcium and Vitamin D supplement) and the impact of Calvimax-D (500mg) on the dissolution of Rosuva™ (10mg) (Rasuvastatin) after 30 minutes

Rosuva™ (10mg) without any supplement				Rosuva™ (10mg) with Calvimax D (500 mg)				
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 (%)**
5.98		59.81		4.58		45.82		
5.95		59.46		4.51		45.12		
6.09	6.03	60.86	60.28	4.58	4.51	45.82	45.10	-25.18
6.05		60.51		4.44		44.42		
6.09		60.86		4.58		45.82		
6.02		60.16		4.37		43.72		

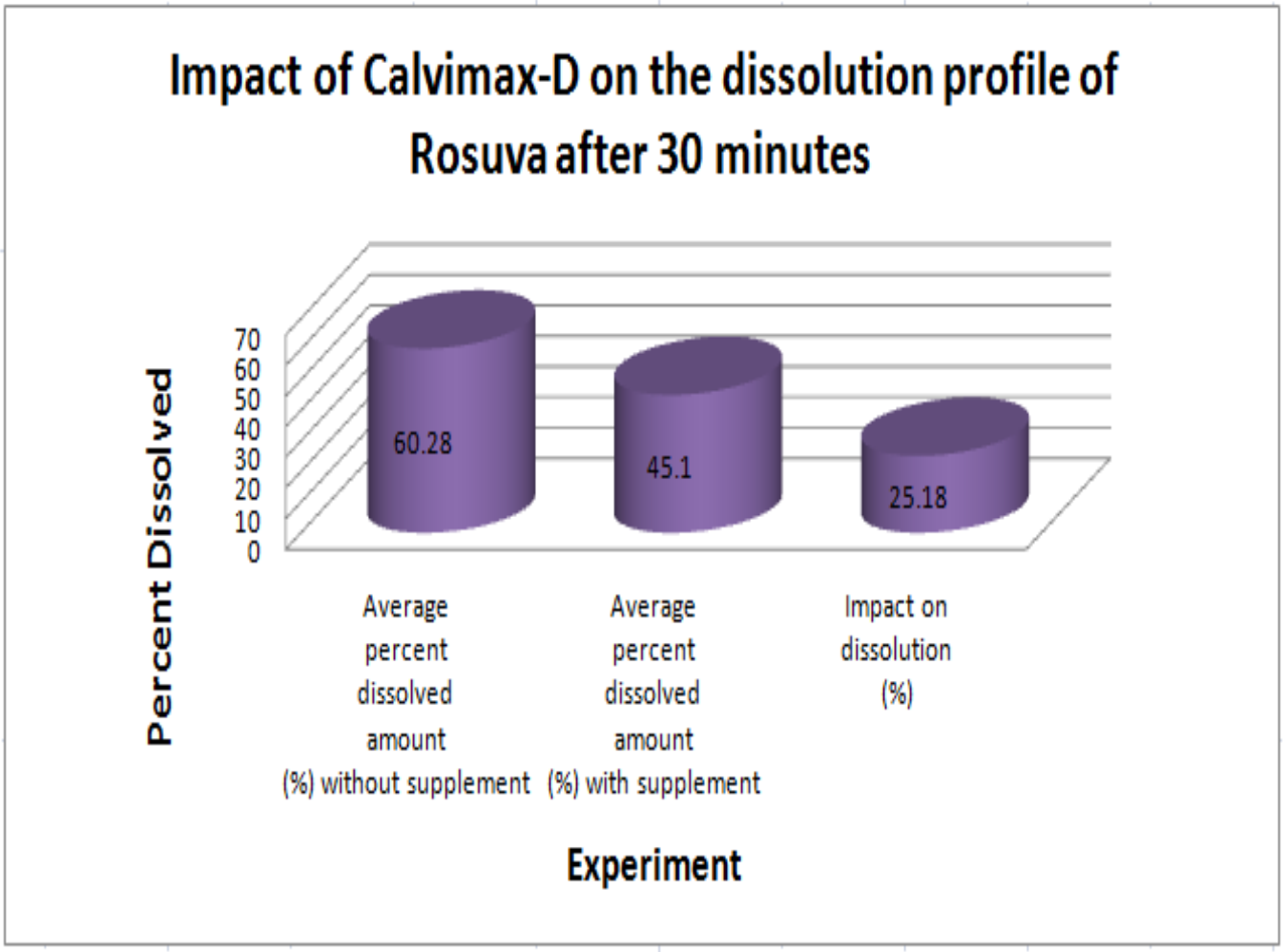

Figure 4.4: Graph showing the impact of Calvimax-D on the dissolution profile of Rosuva after 30 minutes

Table 4.12: Percentage calculation for dissolved amount of Rosuva™ (10mg) (Rasuvastatin), Rosuva™ (10mg) (Rasuvastatin) with Calvimax-D (500mg)(Calcium and Vitamin D supplement) and the impact of Calvimax-D (500mg) on the dissolution of Rosuva™ (10mg) (Rasuvastatin) after 40 minutes

Rosuva™ (10mg) without any supplement				Rosuva™ (10mg) with Calvimax D (500 mg)				
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 (%)**
6.26		62.61		4.72		47.22		
6.23		62.26		4.69		46.87		
6.30	6.34	62.96	63.43	4.72	4.66	47.22	46.63	-26.48
6.40		64.01		4.62		46.17		
6.40		64.01		4.72		47.22		
6.47		64.71		4.51		45.12		

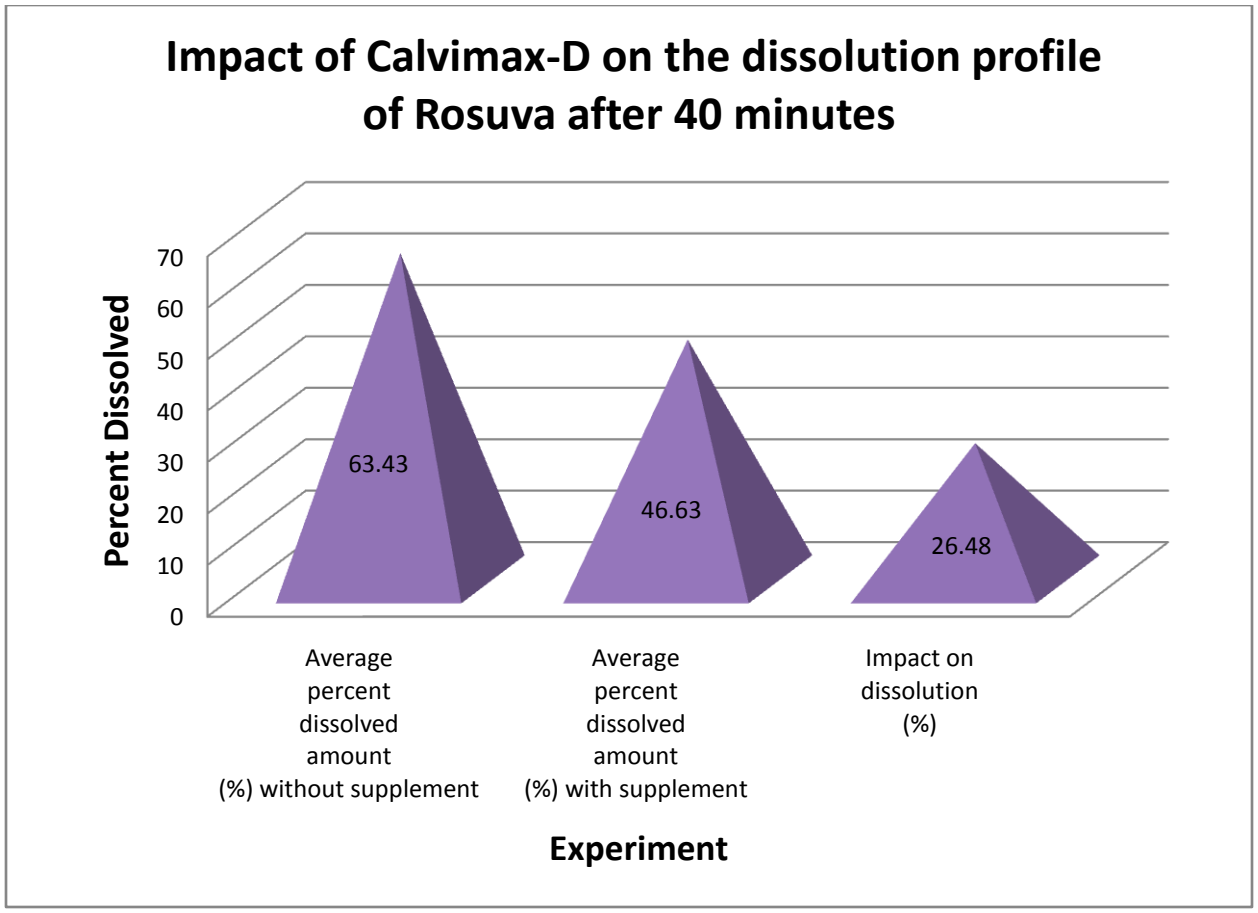

Figure 4.5: Graph showing the impact of Calvimax-D on the dissolution profile of Rosuva after 40 minutes

Table 4.13: Percentage calculation for dissolved amount of Rosuva™ (10mg) (Rasuvastatin), Rosuva™ (10mg) (Rasuvastatin) with Calvimax-D (500mg) (Calcium and Vitamin D supplement) and the impact of Calvimax-D (500mg) on the dissolution of Rosuva™ (10mg)(Rasuvastatin) after 50 minutes

Rosuva™ (10mg) without any supplement				Rosuva™ (10mg) with Calvimax-D(500 mg)				
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 (%)**
6.68		66.81		4.93		49.32		
6.61		66.11		4.90		48.97		
6.58	6.65	65.76	66.52	4.93	4.89	49.32	48.85	-26.56
6.79		67.86		4.83		48.27		
6.72		67.16		4.93		49.32		
6.54		65.41		4.79		47.92		

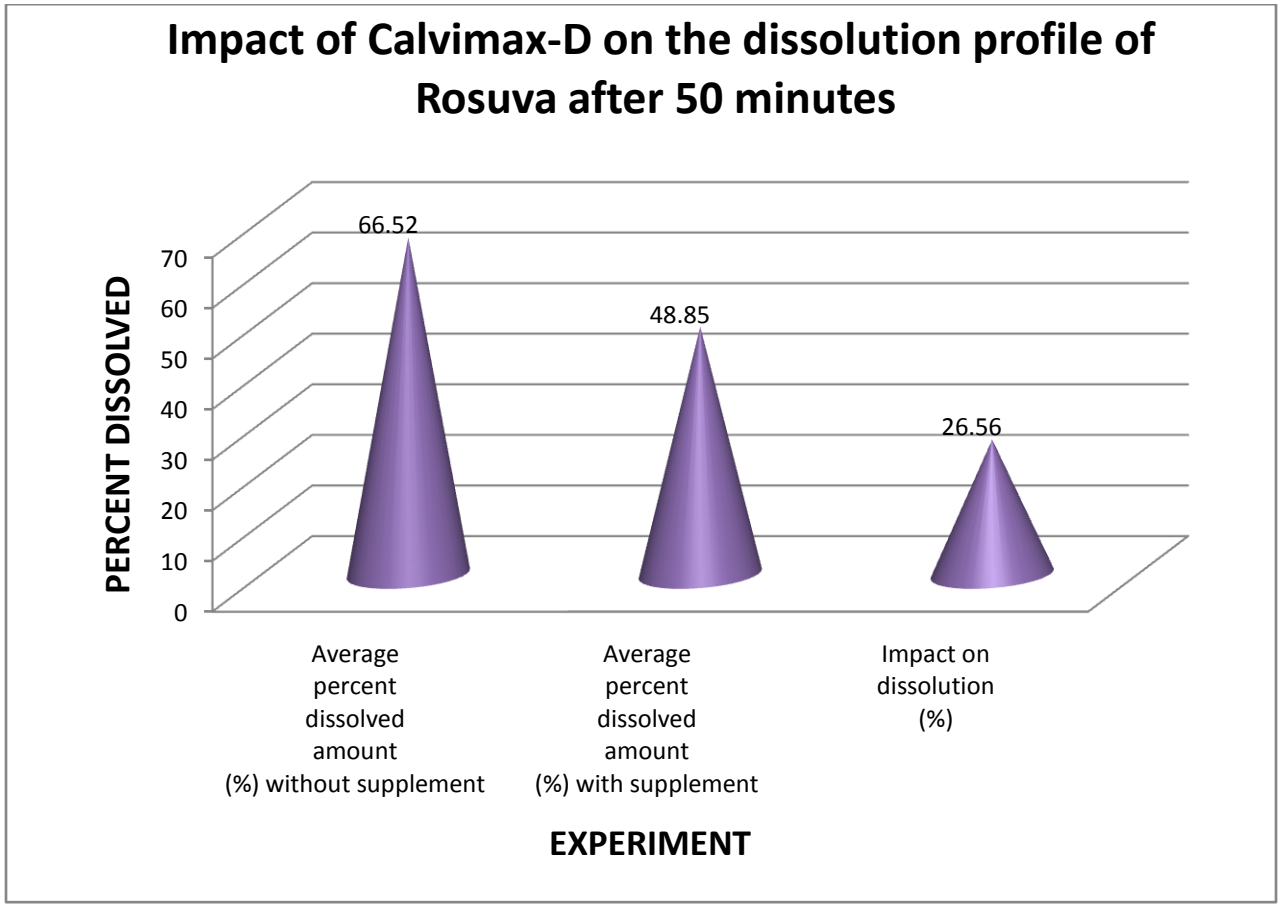

Figure 4.6: Graph showing the impact of Calvimax-D on the dissolution profile of Rosuva after 50 minutes

Table 4.14: Percentage calculation for dissolved amount of Rosuva™ (10mg) (Rasuvastatin), Rosuva™ (10mg) (Rasuvastatin) with Calvimax-D (500mg)(Calcium and Vitamin D supplement) and the impact of Calvimax-D (500mg) on the dissolution of Rosuva™ (10mg) (Rasuvastatin) after 60 minutes

Rosuva™ (10mg)without any supplement				Rosuva™ 10mg) with Calvimax D(500 mg)				
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 (%)**
6.86		68.56		5.07		50.72		
7.24		72.41		5.04		50.37		
7.17	7.12	71.71	71.13	5.07	5.03	50.72	50.25	-29.35
6.93		69.26		4.97		49.67		
7.21		72.06		5.07		50.72		
7.28		72.76		4.93		49.32		

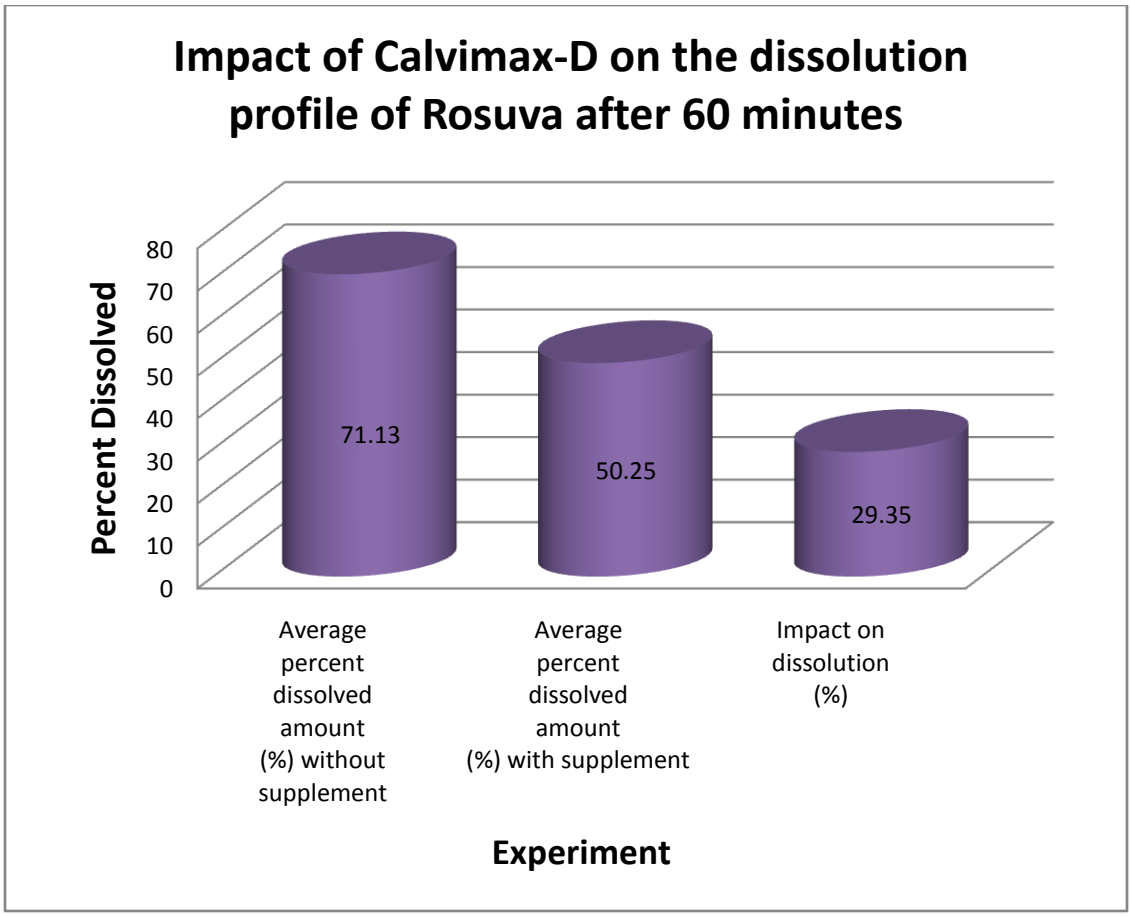

Figure 4.7: Graph showing the impact of Calvimax-D on the dissolution profile of Rosuva after 60 minutes

Table 4.15: Comparison among the impact (%) of Calvimax-D (500mg) on Rosuva™ (10 mg) in 10, 20, 30, 40, 50, 60 minutes

	10 minutes	20 minutes	30 minutes	40 minutes	50 minutes	60 minutes
Impact on dissolution (%)	24.37	24.65	25.18	26.48	26.56	29.35

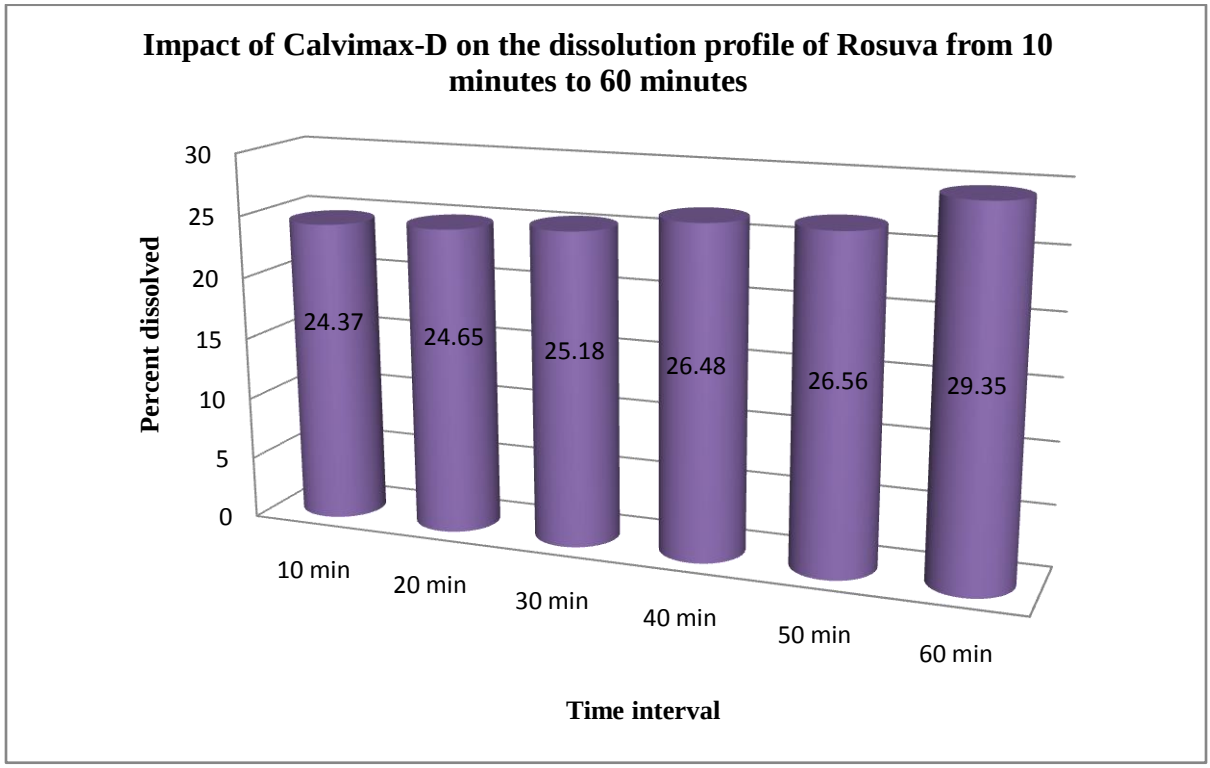

Figure 4.8: Graph showing the impact of Calvimax-D on the dissolution profile of Rosuva 10 minutes to 60 minutes

Chapter Five

Discussion

5. Discussion

The weight variation of Rosuva^{TM} (10mg) denoted that the solid dosage forms were maintained uniformity. According to USP, tablets of specific weight range have a particular limitation of weight variation and Rosuva^{TM} (10mg) meets those specifications. Weight variation test indicates that a tablet has its appropriate size, the content of the formulation is uniform and ultimately it reflects good manufacturing practices (GMP). (Nasrin et al., 2011)

The variation result for tablet thickness is also connected with tablet hardness. If the thickness of tablet changes significantly then the tablet hardness comparison will be incorrect. (Pitt and Heasley, 2013). For the determination of the thickness of ten tablets of Rosuva^{TM} (10mg), vernier caliper was used. The results from the calculation 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). Since 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 result in tablet hardness. (Chowhan and Palagyi, 1978)

The result of the dissolution tests given for Rosuva^{TM} (10mg) was increasing with time. In 10 minutes the average percent release of Rosuva^{TM} (10mg) was 52.65% whereas in 60 minutes, the average percent release was 71.13 %. Thusly we can conclude that the more time is passed, the more release of drug occurs.

When Rosuva^{TM} (10mg) was examined with Calvimax-D (500mg) (Calcium carbonate and vitamin D), then the dissolution profile of Rosuva^{TM} (10mg) would be altered. The drug release rate was decreased with time. At 10 minutes, the average percent of drug release was 39.82 % which create an impact on dissolution of Rosuva^{TM} (10mg) 24.37%. After 60 minutes, the average percent of drug release was 50.25% and the impact on dissolution of Rosuva^{TM} (10mg) was 29.35%. So it is clear that the dissolution rate or the drug release of Rosuva^{TM} (10mg) is decrease with increasing time when it is experimented with calcium and vitamin D supplement and the impact of calcium and vitamin D supplement on dissolution of Rosuva^{TM} (10mg) is increased with time.

The incident of decrease of drug release happens 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)

In this experiment, Rosuva^{TM} (10mg) which contains Rosuvastatin Calcium and Calvimax-D (500mg) containing Calcium carbonate and vitamin D were subjected. Here Rosuvastatin is poorly soluble drug and for increasing the solubility calcium salt is used. When we combined these two drugs, calcium ion became the common ion of the solution. According to Le Chatelier's principle, the precipitation of calcium ion occurred and solubility and the dissolution rate of Rosuva^{TM} (10mg) decreased by the effort of the common ion present in aqueous medium. Since the dissolution rate of Rosuva^{TM} (10mg) is altered, Calvimax-D (500mg) may interfere with the absorption of Rosuvastatin and reduce its effectiveness. (Drugs.com, 2016)

In conclusion, it can be said that Rosuva^{TM} (10mg) and Calvimax-D (500mg) should not be used concomitantly. There should be at least 2 hours time interval for administering these two drugs. (Drugs.com, 2016)

Chapter Six
Conclusion

Conclusion:

Rosuvastatin is an anti-hyperlipidemic drug which is not included in any of the standard pharmacopoeias such as United States Pharmacopoeia and British Pharmacopoeia. Hence, this drug is inserted in the list of International Non-proprietary Names (INN) and thus marketed as such. Rosuvastatin is also a drug belonging to a group that comes with many type of Drug-Drug interaction as described in the introduction part. Thusly it is very essential that it is manufactured according to Good Manufacturing Practice (GMP). In this project, a Bangladeshi brand of Rosuvastatin tablet titled Rosuva^{TM} (10mg) manufatured by Square Pharmaceutical Limited was subjected to dissolution studies both alone and also in combination with a Bangladeshi Brand of calcium and vitamin D supplement branded Calvimax-D (500mg) manufactured by Incepta Pharmaceutical Limited. However, the investigation report of the study showed the extreme impact of Calvimax-D (500mg) on the dissolution profile of Rosuva^{TM} (10mg) and result showed that these two drugs should not be used concomitantly. Further studies are required on live subjects both animals and humans in order to find out the impact of such co-administration. The results from this experiment can be extrapolated to the wider Bangladeshi laboratory.

Chapter Seven

References

References

ACI limited (n.d.) *Rosetor* [Online] Available from: https://www.aci-bd.com/Brand/Rosetor.pdf [Accessed: 3rd December 2016]

ACLS Certification Institute (n.d) *Statins (HMG-CoA Reductase Inhibitors)* [Online] Available from: https://acls.com/free-resources/medication-medication-delivery/statins-hmg-coa-reductase-inhibitors (Accessed: 26 November, 2016)

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.p.23

Anand,O., Yu, L., Conner, D. and Davit, B. (2011) *Dissolution testing for Generic Drugs: An FDA perspective.* The American Association of Pharmaceutical Scientists Journal, 13(3),pp.328-335.

Atrainceu.com.(2016) *ZZZ_76_Statins:Controlling Cholesterol: Module6.* [Online] Available from: http://www.atrainceu.com/course-module/15552782-zzz_76_statins-controlling-cholerol-module-6 (Accessed: 26 November, 2016)

Barclay. R (2016) *Facts About LDL: The Bad kind of Cholesterol* [Online] Available from: http://www.healthline.com/health/ldl-cholesterol#Overview1 (Accessed: 26 November, 2016)

Belay, B., Belamarich, F. P. et al. (2007) The Use of Statins in Pediatrics: Knowledse Base, Limitations and Future Directions. *Pediactrics.* [Online] vol. 119 (2) Available from: http://pediatrics.aappublications.org/content/119/2/370 [Accessed: 3rd December 2016]

Bolourchian, N., Mahboobian, M. and Dadashzadeh, S. (2013). The Effect of PEG Molecular Weights on Dissolution Behavior of Simvastatin in Solid Dispersions. *Iranian Journal for Pharmaceutical Research*,12(4),p.1

Chapman, B.A., Burt, M.J., Chisholm, R.J., Allan, R.B., Yeo, K.H.J. and Ross, A.G., (1998). 'Dissolution of gallstones with simvastatin, an HMG CoA reductase inhibitor.' *Digestive diseases and sciences*, *43*(2), pp.349-353.

Chowhan,Z. and Palagyi,L.(1978) *Hardness Increase Induced By Partial Moisture Loss In Compressed Tablets And Its Effect On In Vitro Dissolution*. Journal of Pharmaceutical Sciences,67(10),1385-1389

Drugs.com (2016) *Drug interactions between calcium/vitamin d and Crestor* [Online] Available from: https://www.drugs.com/drug-interactions/calcium-vitamin-d-with-crestor-2917-0-2031-1316.html [Accessed: 3rd December 2016]

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*.3(1),pp.221-222

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

Jahan, R., Islam, M., Tanwir, A. and Chowdhury, J. (2013). In vitro dissolution study of atorvastatin binary solid dispersion. *Journal of Advanced Pharmaceutical Technology & Research*, 4(1), p.1

Jahangiri,A., Jalali, M., Javadjadeh, Y., Hamishekhar, H., Adibkia, K. (2016) ' Physicochemical characterization of atorvastatin calcium/ezetimibe amorphous nano-solid dispersions prepared by electrospraying method.'A*rtifical cells, Nanomedicine and Biotechnology,International Journal*.4(1),pp.22-25

Jiang, T., Han, N., Zhao, B., Xie, Y. and Wang, S., 2012. Enhanced dissolution rate and oral bioavailability of simvastatin nanocrystal prepared by sonoprecipitation. *Drug development and industrial pharmacy*, *38*(10), pp.1230-1239.

Jui, F.I., 2014. *In vitro dissolution study to determine the Drug-Drug interaction of Atorvastatin Calcium-Metformin HCLMultivitamin Tablet*(Doctoral dissertation, East West University).

Jun, S.W., Kim, M.S., Kim, J.S., Park, H.J., Lee, S., Woo, J.S. and Hwang, S.J. (2007) 'Preparation and characterization of simvastatin/hydroxypropyl-β-cyclodextrin inclusion

complex using supercritical antisolvent (SAS) process.' *European Journal of Pharmaceutics and Biopharmaceutics*, *66*(3), pp.413-421.

Kapure, V., Pande, V. and Deshmukh, P. (2013). Dissolution Enhancement of Rosuvastatin Calcium by Liquisolid Compact Technique. *Journal of Pharmaceutics*, 2013.5(2).p.1

Kamble, P., Shaikh, K. and Chaudhari, P. (2014). Application of Liquisolid Technology for Enhancing Solubility and Dissolution of Rosuvastatin. *Advanced Pharmaceutical Bulletin*, 4(2), p.1

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

Luvai, A., Mbagaya, W., Hall, A. and Barth, J. (2012). Rosuvastatin: A Review of the Pharmacology and Clinical Effectiveness in Cardiovascular Disease. *Clinical Medicine Insights: Cardiology*, 6, p.1

Mayo Clinic (2016) *Statin side effects: Weigh the benefits and risks* [Online] Available from: http://www.mayoclinic.org/diseases-conditions/high-blood-cholesterol/in-depth/statin-side-effects/art-20046013 (Accessed: 26 November, 2016)

Medicines.ie (2015) *Rosuvastatin Mylan 5 mg, 10 mg, 20 mg & 40 mg Film-coated Tablets* [Online] Available from: http://www.medicines.ie/medicine/15445/SPC/Rosuvastatin+Mylan+5mg+10mg+20mg+%26+40mg+Film-coated+Tablets/ (Accessed: 26 November, 2016)

MedlinePlus (2016) *Drugs, Herbs and Supplements: Rosuvastatin* [Online] Available from: https://medlineplus.gov/druginfo/meds/a603033.html [Accessed: 3rd December 2016]

Medline Plus (n.d) *Cholesterol* [Online] Available from: https://vsearch.nlm.nih.gov/vivisimo/cgi-bin/query-meta?v%3Aproject=medlineplus&v%3Asources=medlineplus-bundle&query=cholesterol&_ga=1.154980472.1590717793.1479560807 (Accessed: 26 November, 2016)

Margulis-Goshen, K. and Magdassi, S. (2009) 'Formation of simvastatin nanoparticles from microemulsion.' *Nanomedicine: Nanotechnology, Biology and Medicine*, *5*(3), pp.274-281

Messa, R. and Ampati, S., 2016. Comparative Studies for Enhancement of the Dissolution Profile of Pitavastatin.6(4).pp.123-124

Nasrin,N., Asaduzzaman,M., Mawla, R., Rizwan, F. and Alam, A.(2011) 'A comparative study of physical parameters of selected ketorolac tromethamine tablets available in the pharma market of Bangladesh.' *Journal of Pharmaceutical Science*,01(08),101-103

Neduri, K., Bontha, V. and Vemula, S. (2013). DIFFERENT TECHNIQUES TO ENHANCE THE DISSOLUTION RATE OF LOVASTATIN: FORMULATION AND EVALUATION. *Asian Journal of Pharmaceutical and Clinical Research*, 6(1), p.1

NHS Choices (2015) *Vitamins and minerals-Calcium* [Online] Available from: http://www.nhs.uk/Conditions/vitamins-minerals/Pages/Calcium.aspx (Accessed: 28 November 2016)

OMICS international (2014). *Common Ion Effect.* [Online] Available from: http://research.omicsgroup.org/index.php/Common-ion_effect (Accessed 2 Dec. 2016)

Pandya, P., Gattani, S., Jain, P., Khirwal, L. 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.' *AAPS pharmscitech*, *9*(4), pp.1247-1252

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.

Panghal, D., Nagpal, M., Thakur, G. and Arora, S. (2014). Dissolution Improvement of Atorvastatin Calcium using Modified Locust Bean Gum by the Solid Dispersion Technique. *Scientica Pharmaceutica*, 82(1), p.23

Patel, R. and Patel, M. (2008) 'Preparation, characterization, and dissolution behavior of a solid dispersion of simvastatin with polyethylene glycol 4000 and polyvinylpyrrolidone K30.' *Journal of Dispersion Science and Technology*, *29*(2), pp.193-204.

Patel, R.P. and Patel, M.M. (2007) ' Physicochemical characterization and dissolution study of solid dispersions of lovastatin with polyethylene glycol 4000 and polyvinylpyrrolidone K30.' *Pharmaceutical development and technology*, *12*(1), pp.21-33.

Patel, D. and Sawant, K. (2009) 'Self Micro-Emulsion Drug Delivery System: Formulation Development and Biopharmaceutical Evaluation of Lipophilic drugs.' *Current Drug Delivery, 6(4),*pp.419-424

Pitt,K.and Heasley,M.(2013) 'Determination of the tensile strength of elongated tablets.' *Powder Technology,*238,169-175.

Prabhu, P. and Patravale, V., 2015. Dissolution enhancement of atorvastatin calcium by co-grinding technique. *Drug delivery and translational research*, pp.1-12.

Rao, M., Mandage, Y., Thanki, K. and Bhise, S. (2010) 'Dissolution improvement of simvastatin by surface solid dispersion technology.' *Dissolution technologies*, *61*, pp.27-34.

Rader. DJ., Cohen. J., Hobbs .,HH. (2003). "Monogenic hypercholesterolemia: new insights in pathogenesis and treatment". *J. Clin. Invest. 111(12).*pp. *1795–803.*

Salih, O., Samein, L. And Ali, W. (2013). FORMULATION AND IN VITRO EVALUATION OF ROSUVASTATIN CALCIUM NIOSOMES. *International Journal of Pharmacy and Pharmaceutical Sciences,*5(4), p.1

Sahoo, H.B.(2011) 'Formulation Of Dosage Form Of Rosuvastatin Calcium And Development Of Validated Rp-Hplc Method For Its Estimation.'6(4).pp231-234

Sarfraz, R.M., Ahmad, M., Mahmood, A., Minhas, M.U. And Yaqoob, A., 2015. Development and Evaluation of Rosuvastatin Calcium Based Microparticles for Solubility Enhancement: An In Vitro Study. *Advances in Polymer Technology*.13(5).p.31

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

Shaikh, K., Patwekar, S., Payghan, S. and D Souza, J. (2011). Dissolution and Stability Enhancement of Poorly Water Soluble Drug – Lovastatin by Preparing Solid Dispersions. *Asian Journal of Biomedical and Pharmaceutical Sciences*,1(4), p.2

Sirius-analytical. (2016) *Dissolution Definitions*. [Online] Available from: http://www.sirius-analytical.com/science/dissolution/dissolution-definitions (Accessed: 28 November 2016)

Square Pharmaceuticals Ltd.,(2016) *Rosuva10mg* [Online] Available from: http://www.squarepharma.com.bd/downloads/Rosuva.pdf (Accessed: 26 November, 2016)

Sweeney.M.T (2016) *Hypertriglyceridemia* [Online] Available from: http://emedicine.medscape.com/article/126568-overview (Accessed: 26 November, 2016)

Tazuma, S., Kajiyama, G., Mizuno, T., Yamashita, G., Miura, H., Kajihara, T., Hattori, Y., Miyake, H., Nishioka, T., Hyogo, H. and Sunami, Y.(1998) 'A combination therapy with simvastatin and ursodeoxycholic acid is more effective for cholesterol gallstone dissolution than is ursodeoxycholic acid monotherapy.' *Journal of clinical gastroenterology*, *26*(4), pp.287-291

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)

Tresna instrument. (2016). *Vernier Calipers*. [Online] Available from: http://www.tresnainstrument.com/vernier_calipers.html (Accessed: 28 November 2016)

Rodde, M.S., Divase, G.T., Devkar, T.B. and Tekade, A.R., 2014. Solubility and Bioavailability Enhancement of Poorly Aqueous Soluble Atorvastatin: In Vitro, Ex Vivo, and In Vivo Studies. *BioMed research international*, *2014*,23(4).p.23

Wang, L. and Asgharnejad, M. (2000) 'Second-derivative UV spectrometric determination of simvastatin in its tablet dosage form.' *Journal of pharmaceutical and biomedical analysis*, *21*(6), pp.1243-1248.

WebMd.com (2016) *Calcium overview information* [Online] Available from: http://www.webmd.com/vitamins-supplements/ingredientmono-781-calcium.aspx?activeingredientid=781(Accessed: 28 November 2016)

Yuksel,L., Carlin,A., Amidon,G. and Hussain,A. (2004) 'Feasibility Studies Of Utilizing Disk Intrinsic Dissolution Rate To Classify Drugs'. *International Journal of Pharmaceutics*,270(1-2),pp.221-227