Impact of Calcium and Vitamin D supplement on the Dissolution profile of Rosutin® (Rosuvastatin)

A Dissertation submitted to the East West University, Bangladesh

For the partial fulfillment of the Degree of

Bachelor of Pharmacy

Submitted by

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DECLARATION

The research work entitled “Impact of Calcium and Vitamin D supplement on the dissolution profile of Rosutin® (Rosuvastatin) is submitted as a 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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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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Dr. Shamsun Nahar Khan

Associate Professor & Chairperson

Department of Pharmacy

East West University, Bangladesh

Dedicated to

My Parents

&

Honourable Teachers

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Abstract

The objective of the research work was to investigate the impact of Calcium and Vitamin D supplement on the dissolution profile of Rosutin® 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 (Rosutin® 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 (Rosutin® 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 Rosutin® and Rosutin® with Calcium and Vitamin D supplement were respectively 76.235 % and 61.416 %. From the result it was assumed that Calcium and Vitamin D supplement has extreme effect on the dissolution of Rosutin®. The dissolution rate of Rosutin® 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.Cardiovascular disease:

The term cardiovascular disease refers to the conditions affecting the heart or blood vessels. It is mainly related to building up of fatty deposits in the wall of arteries which is called atherosclerosis and also increases the risk of blood clots. Most of the time, damages the arteries. (NHS, 2016)

1.1.1. Types of cardiovascular disease:

- Coronary heart disease
- Strokes
- Peripheral arterial disease
- Aortic disease

The development of cardiovascular diseases in the human is mainly associated with the imbalance of cholesterol in the body.

1.1. Cholesterol:

Cholesterol is a fatty substance made by liver, also known as a lipid and plays an important role in the body. Protein carries the cholesterol in the body. The combination of cholesterol and protein are called lipoprotein. There are two main types of lipoprotein which include:

- High density lipoprotein which remove cholesterol from the cells and return back to the liver where it is broken down or excrete from the body as a waste product. So, it is called good cholesterol. An ideal level of HDL is above 1mmol/L. A lower level of HDL can increase the risk of heart disease.
- Low density lipoprotein carries cholesterol to the cells. If the level is high, it forms fatty deposits in the wall of arteries and leading to various cardiovascular disease such as Coronary heart disease, Strokes, Peripheral arterial disease, Aortic disease etc. So, it is called bad cholesterol. (NHS, 2016)

1.2.1. Causes of cholesterol:

- Unhealthy died
- Smoking
- Diabetes
- High blood pressure (NHS, 2016)

1.2.2. Level of cholesterol:

- The total level of cholesterol should be 5 mmol/ L or less for healthy
- 4 mmol/L or less for those who are at risk (NHS, 2016)

1.3. Information of Statin:

1.3.1. Description:

Statins are a medicine that lower production of low density lipoprotein cholesterol in the liver that is known as bad cholesterol. Statin blocks the hydroxy-methylglutaryl CoA reductase enzyme in the liver so statins are called HNG CoA reductase inhibitor.

Cholesterol is responsible for the development of atherosclerosis by forming plaques within arteries. This plaque blocks the arteries that supplies the blood to the tissues. This reduction of blood flow to the heart, leads to the initiation of angina or heart attack and if reduction of blood flow causes in the brain, the ultimate result is stroke. Intermittent claudication is the result of reduction of blood flow in the legs.

Atherosclerosis is a complex process. Scientist have discovered that the inflammation in the wall of the arteries can also lead to the development of atherosclerosis.

By reducing the production of cholesterol that are responsible for the development of atherosclerosis, statin can slow the formation of plaque or reduce the plaque that are present in the arteries. (Medicine Net.com, 2016)

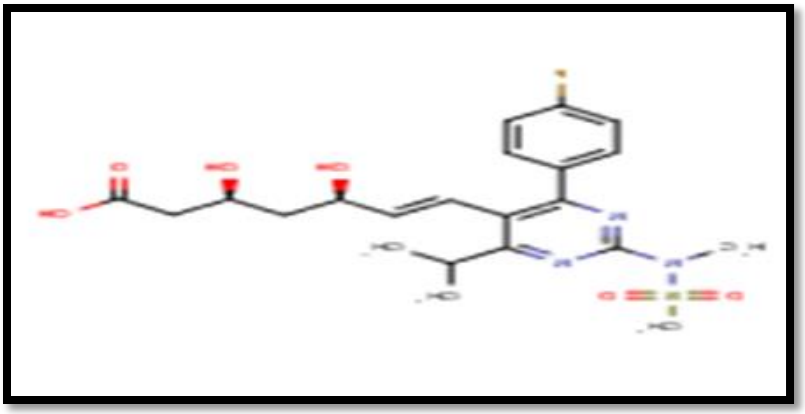

Figure 1.1: General structure of statin (nature, 2016)

1.3.2. Types of statin: The following types of statin are available-

- Atorvastatin
- Fluvastatin
- Pravastatin
- Rosuvastatin
- Simvastatin (NHS, 2016)

1.3.3. Alternative to statins:

Statins are used to reduce the risk of cardiovascular diseases. There are some alternative that are also used to reduce the risk of cardiovascular diseases which include:

- By eating balance diet
- By controlling weight
- By exercising regularly
- By stopping smoking
- By reducing the amount of alcohol, taking by individual (NHS, 2016)

1.3.4. Indications: Statin are used for the following cardiovascular diseases-

- Angina
- Heart attack
- Stroke

- Atherosclerosis (Medicine Net.com, 2016)

1.3.5. Side effects:

The side effects statin are:

- Headache
- Nausea
- Vomiting
- Constipation
- Rash
- Weakness
- Muscle pain
- Liver failure
- Kidney failure (Medicine Net.com, 2016)

1.3.6. Caution: One have to stop taking statin when

- One have achieve muscle pain and weakness
- During pregnancy
- Nursing mother
- Using medications such as antibiotic or antifungal drug
- Increasing liver enzyme
- Worsening the lung functions (Health, 2016)

1.3.7. Drug-Drug interaction:

Statin shows interaction with other medication as well as grapefruit juice.

- Niacin causes liver failure. When it is administered with statin, statin increase the level of liver failure.
- Fibric acid shows the same result as niacin when it is administered with statin.
- Statin reduces the absorption of cholestyramin when it is used with statin.
- Colestid also shows the same result as cholestyramin when it is administered with statin.
- The effects of warfarin is increased when it is administered with statin.

- When red yeast rice is used with statins that can lead to serious side effects such as muscle breakdown (myopathy). (Medicine Net.com, 2016)

1.3.8. Precaution:

- Statin tablet should be taken once a day
- Treatment with statin should be continued for life time otherwise cholesterol level will increase again
- If one forget to take medicine, he or she need not take extra dose for adjustment
- If one forget to take medicine, he or she need not take extra dose for adjustment

(NHS, 2016)

1.4.Information of vitamin D:

1.4.1.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.

(NIH, 2016)

Figure 1.2: vitamin D supplement (On Health, 2016)

1.4.2.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.

(NIH, 2016)

1.4.3. Risky groups: People who are at the risk of vitamin D deficiency include-

- **Breastfed infants**

Vitamin D passes into breast milk. Human milk only can not 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 can not 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 these causes vitamin D deficiency. So, they should be provided the supplement according to their needs.

- **People with dark skin**

The dark skin result 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 disease etc. associated with fat malabsorption which require low intake of vitamin D which causes vitamin D deficiency.

(NIH, 2016)

1.4.4. Recommendation for vitamin D: The amount of vitamin D which is needed for humans are given below-

Age	Male	Female	Pregnancy	Lactation
0-12 months	400 IU	400 IU		
1-13 years	600 IU	600 IU		
14-18 years	600 IU	600 IU	600 IU	600 IU
19-50 years	600 IU	600 IU	600 IU	600 IU
51-70 years	600 IU	600 IU		
Above 70 years	800 IU	800 IU		

(NIH, 2016)

1.4.5. 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 can not 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 which causes 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 are not detected in the primary stages.

(NIH, 2016)

1.4.6 .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 vitamins 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, this 2 forms are equivalent at nutritional doses but vitamin D_2 is less potent than vitamin D_3 at high doses.

(NIH, 2016)

1.4.7. 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 reduce 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.

(NIH,2016)

1.4.8. 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.

(NIH, 2016)

1.4.9. 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.

(NIH, 2016)

1.5. Information of Calcium:

1.5.1. Description:

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 suggest to take calcium supplement. (WebMed, 2016)

Figure 1.3: calcium supplement (USA TODAY, 2016)

1.5.2. 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, 2016)

1.5.3. 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.

(WebMed, 2016)

1.5.4. 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. (WebMed, 2016)

1.5.5 .Overdoses:

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

(NHS, 2016)

1.6. Information of Rosuvastatin (Rosutin®):

Rosuvastatin belongs to a drug class of statins which is called HMG-CoA reductase inhibitor. The chemical formula of rosuvastatin is $C_{22}H_{28}FN_3O_6S$ and the IUPAC name of rosuvastatin is (3R, 5S, 6E)-7-[4-(4-fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2-yl) pyrimidin-5yl]-3, 5-dihydroxyhept-6-enoic acid. The molecular structure of rosuvastatin is given below: (Drug bank, 2016)

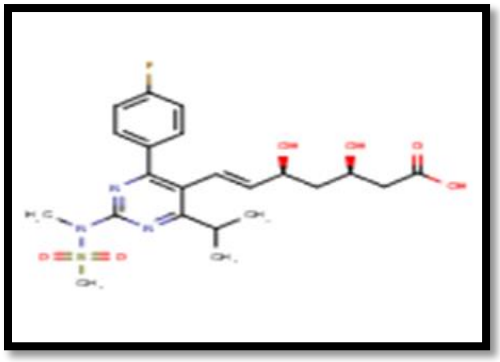

Figure 1.4: Molecular Structure of Rosuvastatin

(Drug bank, 2016)

1.6.1. Description:

It is a lipid lowering drug, works by slowing production of cholesterol in the body which is responsible for the blocking of blood flow to heart, brain and other parts of the body. It is used together with diet, exercise to minimize the chance of diseases like atheroclerosis, several heart problems (heart attack, stroke) etc.

(PubMed health, 2016)

1.6.2. Composition:

10 mg tablet: Each tablet contains 10 mg rosuvastatin as rosuvastatin calcium.

(Drug bank, 2016)

1.6.3. Mechanism of Action:

Rosuvastatin is a HMG CoA reductase inhibitor which competitively inhibit HMG CoA reductase in the synthesis of cholesterol and that step is known as rate limiting step. As a result cholesterol synthesis is reduced and act as a lipid lowering agent.

(MEDICO TIPS, 2016)

1.6.4. Pharmacodynamics:

Rosuvastatin is synthetically produced for the purposes of lowering the level of total cholesterol, low density lipoprotein cholesterol, apolipoprotein B, triglyceride and at the same time increasing the high density lipoprotein cholesterol which is known as good cholesterol. The imbalances in the level of cholesterol and triglyceride increase the risk of atherosclerosis, coronary artery disease and other cardiovascular disease. In this case, rosuvastatin is used to reduce the risk of cardiovascular diseases by decreasing low density lipoprotein cholesterol and triglyceride and at the same time increasing the good cholesterol (HDL).

(Drug bank, 2016)

1.6.5. Pharmacokinetics

Absorption:

In clinical trial, it is found that the drug reach the peak plasma concentration by 3 to 5 hours after orally administered to a man. The increase in dose leads to the increase in both Cmax and AUC.

The rosuvastatin is approximately 20% orally bioavailable.

Distribution:

The rosuvastatin has a volume of distribution of 134 liters and it is bound to plasma protein which is 88%. The binding is not dependent on plasma concentration.

Metabolism:

The metabolism of rosuvastatin is not extensive. As a metabolite 10% of dose is recovered which is metabolized by the enzyme cytochrome P450 or cytochrome 2C9.

Excretion:

90% of rosuvastatin and its metabolite are excreted in the feces and its elimination half-life is 19 hours. (RxList, 2016)

1.6.6. Route of administration: Oral (Drug bank, 2016)

1.6.7. Therapeutic indication:

Rosuvastatin is used for the following purposes-

- to reduce the blood cholesterol level and triglycerides
- to increase high-density lipoprotein cholesterol levels which is known as good cholesterol
- to reduce the risk of heart attacks, stroke, and other multiple risk factors for heart disease

(Medicine Net.com, 2016)

1.6.8. Side effects: The general side effects of rosuvastatin include:

- headache
- muscle pain
- nausea
- vomiting
- diarrhea

- forgetfulness
- amnesia
- confusion
- memory impairment
- constipation
- stomach pain
- dizziness
- difficulty falling asleep or staying asleep
- depression

Some serious side effects include:

- liver failure
- muscle breakdown
- kidney failure

(Drugs.com)

1.6.9. Contraindication:

- having allergy to any ingredient that the rosuvastatin contains
- having liver failure or disease
- during pregnancy
- nursing mothers (Drugs.com)

1.6.10. Toxicity:

Generally, rosuvastatin is well-tolerated and shows various side effects which include muscle pain, liver failure, kidney failure, dizziness etc. Over doses may show toxicity. To avoid toxicity, the doses should be minimized. (Drug bank, 2016)

1.6.11. Administration and Doses: According to FDA

Adult Indications and Dosage:

- **For General Dosing Information**
    - Take 5 to 40 mg orally once daily.

- **For Homozygous Familial Hypercholesterolemia**
    - Take 20 mg once daily

- **Prophylaxis for Cardiovascular Event in Percutaneous Coronary Intervention (PCI)**
    - Take 40 mg/day

- **Prophylaxis for Venous Thromboembolism**
    - Take 20 mg/day

- **Metabolic Syndrome**
    - Take 10 mg/day

Pediatric Indications and Dosage:

- Heterozygous Familial Hypercholesterolemia in Pediatric Patients (10 to 17 years of age)
    - Take 5-20 mg/day; the maximum recommended dose is 20 mg/day

(WikiDoc, 2016)

1.6.12. Precautions:

One have to follow the followings before taking rosuvastatin-

- What medicines we have taking or planing to take (vitamins, nutritional supplements etc.) that should be informed the doctor by us. Otherwise, there is chance of drug-drug interaction. For example, Abiraterone decreases the metabolism of Rosuvastatin

- We should be taken aluminum and magnesium hydroxide at least 2 hours before taking rosuvastatin
- We should inform the doctor if we having any hypersensitivity to the ingredient of rosuvastatin or other medication
- We should Inform the doctor if we are able to take rosuvastatin

- We should inform the doctor that we are allowed to take rosuvastatin or not when we taking alcohol because it can increases the chance of side effect
  (Medicine Plus, 2016)

1.6.13. Drug interaction:

Drug	Interaction
Abiraterone	Abiraterone decreases the metabolism of Rosuvastatin.
Acenocoumarol	The anticoagulant properties of Acenocoumarol are increased by Rosuvastatin.
Acipimox	The myopathicrhabdomyolysis properties of Rosuvastatin are increased by Acipimox.
Alogliptin	Alogliptin increases the serum concentration of Rosuvastatin.
Alpha-1-proteinase inhibitor	Alpha-1-proteinase inhibitor increases the serum concentration of Rosuvastatin.
Aluminum hydroxide	Aluminum hydroxide decreases the serum concentration of Rosuvastatin.

Aluminum phosphate	Aluminum phosphate decreases the serum concentration of Rosuvastatin.
Gemfibrozil	Rosuvastatin exposure are increased by Gemfibrozil.
Lopinovir	Rosuvastatin exposure are increased by Lopinovir.
Cyclosporin A	Rosuvastatin exposure are increased by Cyclosporin A
Ritonavir	Rosuvastatin exposure are increased by Ritonavir.
Simeprevir	Simeprevir increases the plasma concentration of Rosuvastatin.

(Drug bank, 2016)

1.6.14 .Storage Conditions:

Tablet

- Should be stored at room temperature (15°C-30°C).
- Should be protected from moisture and heat.

(DRUG, 2016)

Figure 1.5: Rosuvastatin Tablet

(BEXIMCO PHARMA, 2016)

1.7. Information of calvimax-D (Calcium Carbonate & Vitamin D):

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)

Figure1.6: Calvimax-D

(Incepta Pharma, 2016)

1.7.3. Composition:

Calvimax-D tablet: 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 medications

(Incepta Pharma, 2016)

1.7.5. Dosage and Administration:

- Age above 12 years, take the tablet 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 cause by calcium supplement include-

- constipation
- diarrhoea
- flatulence
- nausea
- gastric pain

The side effects cause 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 hypercalcaemea 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 refers to the transfer of molecules of ions from solute state in a solution. In other words, the process in which solutes are dissolved in the solvent is called dissolution. In the term of solids, dissolution is described by the breakdown of the crystal lattice into ions, atoms or molecules. Dissolution is a total kinetics process and the result of dissolution is controlled by the thermodynamic energies involved in the process. Overall the free energy must be negative for net dissolution to occur. In turn, those energies are controlled by the way in which different chemical bond types interact with those in the solvent.

(Sirius-analytical, 2016)

1.8.2. Rate of Dissolution

The speed of the total process is expressed by the rate of dissolution. It depends on the chemical natures of the solvent and solute (the temperature, the degree of unsaturation, the interfacial surface area, and the presence of "inhibitors" Like, substances adsorbed on the surface). The rate can be often stated by the Noyes-Whitney Equation or the Nernst and Brunner equation. The equation is dm / dt = AX{D/d}X(Cs-Cb).

Where:

m = mass of solute t is time

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

D = diffusion coefficient d is thickness of the boundary layer of the solvent at the surface of the dissolving substance

Cs = mass concentration of the substance on the surface

Cb = mass concentration of the substance in the bulk of the solvent. For dissolution limited by diffusion

Cs =equal to the solubility of the solute.

When the dissolution rate of a pure substance is normalized to the surface area of the solid, then it is expressed in kg/m2S and termed as "intrinsic dissolution rate", which is defined by the United States Pharmacopeia.

(Lentle and Janssen, 2011)

Chapter: Two

Literature Review

Statins belong to group of poorly water soluble drugs. Many research works were done on statin group of drugs for improving the solubility, dissolution, and bioavailability. Some work done on developing uniform dosage form by using various methods. Some work done on comparison dissolution studies. Among the works, maximum works were done on dissolution profile of statin group of drugs. Some example of these works are given below:

UV spectroscopy was used for the development of simvastatin solid dosage form. This study showed comparison between UV spectroscopy and HPLC method. The UV method is very reliable, easy and less time consuming compare to HPLC method. Thus, for the dissolution and release testing of simvastatin in the solid dosage form, this method was found authorized to a substitute of HPLC method.

(Wang and Asgharnejad, 2000)

For improving the dissolution rate as well as bioavailability, Solid dispersions in water soluble carriers is a very noble method. The aim of this study was to apply solid dispersion technique prepared by polyethylene glycol 4000 and polyvinylpyrrolidone for the enhancement of the solubility and dissolution rate of the Lovastatin, a poorly soluble drug. The method showed good result in the drug release of Lovastatin.

(Patel and Patel, 2007)

In this study, Supercritical antisolvent process was used for the preparation of Simvastatin and its complex with hydroxypropyl β-cyclodextrin. This process was used for the enhancement of the solubility, dissolution rate as well as bioavailabity of Simvastatin and its complex with hydroxypropyl β-cyclodextrin. Result showed that Supercritical antisolvent process could be a promising method for the enhancement of the solubility, dissolution rate as well as bioavailability of the drug because of the formation of complex between Simvastatin and hydroxypropyl β-cyclodextrin.

(Jun et al., 2007)

For improving the solubility as well as dissolution rate, Solid dispersions in water soluble carriers is a very noble method. The aim of this study was to apply solid dispersion technique prepared by polyethylene glycol 4000 and polyvinylpyrrolidone for the enhancement of the solubility and dissolution rate of the Simvastatin, a poorly soluble drug. The method showed good result in the drug release of Simvastatin.

(Patel and patel ,2008)

This study was done for the improvement of the solubility of poorly water soluble drug, Simvastatin by Co-solvent evaporation method using hydroxypropyl methylcellulose (HPMC K_3LV). The result showed that the developed formulation gave better enhancement in the solubility as well as dissolution rate compared to Simvastatin. Thus, Co-solvent evaporation method was proven promising method for the Improvement of dissolution rate of Simvastatin.

(Pandya et al.,2008)

This study was done to prepare nanoparticles of Simvastatin which is a poorly water soluble drug, a method was applied by evaporation of all the solvents from O/W microemulsions. In this method oil phase was converted into nanoparticles by the process of freeze drying. The nanoparticles of Simvastatin showed the improvement dissolution profile compared with the marketed tablets. So, that method was really successful to fulfill the expectation.

(Margulis-Goshen and Magdassi, 2009)

The aim of the study was to improve the dissolution rate as well as bioavailability of Lovastatin by using solid dispersion made by solvent evaporation method. That method was proven auspicious for preparing uniform dosage form with improved surface area, dissolution rate as well as improved bioavailability.

(Sheikh,2011)

The aim of the investigation was to use exploiting sonoprecipitation to prepare simvastatin nanocrystals for the improvement of dissolution as well as bioavailability. The result showed an improved solubility, dissolution rate and oral bioavailability due to the production of small and uniform nanocrystals of simvastatin. (Jiang et al., 2012)

A research was done for the purpose of improving the solubility and dissolution rate of Atorvastatin, a drug belong to the group of statin by Solid Dispersion (SD) technique which was made by physical mixing and solvent evaporation method. Both method used a hydrophilic carrier Poloxamer 188 which was found to be played a vital role in the improvement of the dissolution property.

(Jahan et al., 2013)

An investigation was done for the improvement of the dissolution of Rosuvastatin Calcium by using a technique called liquisolid compaction As liquisolid compacts established a significantly higher rate of drug release, this technique could be an auspicious strategy in enhancing the dissolution of poor water soluble drugs and formulating immediate release solid dosage forms.

(Deshmukh,2013)

Rosuvastatin is a lipid lowering drug act by inhibiting the HMG-CoA reductase enzyme. A formulation was established for the disintegrating tablets of Rosuvastatin by using superdisintegrant addition method followed by direct compression technique. Among the formulation, the formulated one which containing crosspovidone and sodium starch glycolate showed better result than the others.

(Rohini,2013)

Rosuvastatin Calcium has a problem of low bioavailability for its poor solubility and permeability of slightly soluble drug. In this study, non-ionic surfactants, cholesterol and lecithin in different ratios by film hydration was used for the formulation of expected dosage form.Niosoms were proved promising dosage form made by film hydration method for enhance dissolution and permeability of slightly soluble drugs.

(SALIH et al ,2013)

For the improvement of the dissolution profile, absorption efficacy and bioavailability of Lovastatin, a study was done by using different technique which include solid dispersion, superdisintegrants and sublimation method. The result showed, the dissolution efficacy was high in Lovastatin prepared by superdisintegrants method compared to other. But all three showed good result in dissolution profile. Due to the simplicity, low cost and industrial feasibility,

superdisintegrants method was selected as promising method over solid dispersion and sublimation method.

(Neduri,Bontha and Vermula, 2013)

A study was done to examine the dissolution behavior of simvastatin by using various polyethylene glycol molecular weights which include 6000, 12000 and 20000 molecular weight as a carriers of solid dispersion system. Among the polyethylene glycols, the PEG 12000 exhibited 3 fold increase in the rate of dissolution. From the result, it was understood that the molecular weight of polyethylene glycol from solid dispersion systems had an effect on the rate of dissolution of Simvastatin.

(Bolourchian, Mahboobian, and Dadashzadeh, 2013)

For the enhancement of solubility and oral bioavailability, this research was done to establish a self-emulsifying drug delivery system using surfactant.The developed formulation exhibit negative zeta potential, optimal particle size as well as outstanding self-emulsifying ability together with a maximum solubilizing capability. The liquid self-emulsifying drug delivery system was used for formation of the self-emulsifying drug delivery system formulation which disclosed maximum rate of drug release in in vitro test compared to other prepared and marketed formulation. So, it could be a promising method to improve the solubility and drug release of Rosuvastatin.

(Rokad, Nagda and Nagda,2014)

Lovastatin belongs to a statin group which is a poorly water soluble drug. For the improvement of the solubility and dissolution rate of the low soluble drug, various techniques were used. A study was done for the improvement of the solubility and dissolution rate of Lovastatin by using a technique called liquisolid compacts. In this technique, significantly high release of drug was observed than those formulations prepared by direct compression.

(Shyam et al.,2014)

For the improvement of the dissolution rate of Atorvastatin Calcium, Solid Dispersion technique was used which was made by modified solvent evaporation method by using modified locust bean gum. In this study, other mixtures were used which were made by physical mixing, co-grinding and kneading method. Modified locust bean gum showed outstanding result in the improvement of the dissolution rate of Atorvastatin Calcium. Besides, Co-grinding mixture showed good result in the improvement of the dissolution rate of Atorvastatin Calcium. So, it can be used as an alternative in stead of solid dispersion.

(Panghal et al.,2014)

A dissolution study was done to show the drug-drug interaction between Atorvastatin, Metformin HCL and Multivitamin. In the result, the combination showed antagonist activity compared to individual. That's why the rate of dissolution was decreased.

(Jui, 2014)

For the improvement of the solubility of Atorvastatin, a technique is used which is known as solid dispersion technique with Neem Gum. The result showed an increase in the solubility of the Atorvastatin with an increase in the concentration of Neem gum. But this varies from method to method. Beside, an in vivo study was also done. By analysis all the studies that was performed, indicating the successful use of Neem Gum in enhancing the dissolution properties of Atorvastatin.

(Rodde, M.S. et al., 2014)

A research was done which showed that Rosuvastatin is a poorly water soluble drug and the rate of its oral absorption is controlled by the rate of dissolution. For the improvement of the solubility, liquisolid system is a good method because it shows acceptable flow properties. So, this technique could be an auspicious strategy for the improvement of water insoluble drug.

(Kamble,Shaik and Chaudhari,2014)

Atorvastatin Calcium shows poor solubility because of its poor dissolution property. For the purpose of enhancing the solubility of Atorvastatin Calcium and developing an oral dosage form, we used Co-grinding technique (for increasing solubility) using VBP-1(organosulphur

compound). VBP-1 proved the ability for increasing the dissolution of Atorvastatin Calcium followed by Co-grinding technique.

(Prabhu and Patravale,2015)

A study was done to establish an immediate release tablet formulation of Fenofibrate and Rosuvastatin in combination for the treatment of cardiovascular diseases. To enhance the dissolution property of Fenofibrate, the particular target was set up. Hot-Melt Technology was used for the enhancement of the dissolution profile of combined formulation. The result showed that the release rate of Fenofibrate was enhanced as well as enhanced dissolution profile both for Fenofibrate and Rosuvastatin.

(Sarkar etal.,2015)

For increasing a dissolution rate and bioavailability of poorly soluble drug like Rosuvastatin, liquisolid systems were used. The objective of this research work was to estimate differences among the liquisolid systems those containing Kollidon® CL-F, Vivasol® and Explotab® as superdisintegrants. In the result, liquisolid systems containing Kollidon® CL-F and Vivasol® showed the maximum release of drug compared to the other liquisolid systems. So, the liquisolid systems containing Kollidon® CL-F and Vivasol® were proven as a promising methods.

(Vraníková ,Gajdziok, and Doležel, 2015)

Atorvastatin is a poorly water soluble drug. A study was done to increase the solubility and dissolution of Atorvastatin with Cyclodextrins. Different techniques were followed for the binary system of Atorvastatin with Cyclodextrins. Both in vivo and in vitro performance were done for the determination of solid complex's effectiveness on bioavailability. In binary system, the dissolution rate was increased, compared with the physical mixture and pure drug. Again, the binary systems with HPβCD followed by freeze drying method exhibited better effect on solubility and dissolution of Atorvastatin.

(Palanisamy, James and Khanam, 2016)

For the fabrication of amorphous nano-solid dispersions (N-SDs) of atorvastatin calcium (ATV), ezetimibe (EZT), and ATV/EZT combination as poorly water-soluble drugs, electrospraying was

applied as an innovative method. This method means electrospraying method was proved as a novel method for the fabrication of amorphous nano-solid dispersions (N-SDs) of poorly water-soluble drugs.

(Jahangiri et al.,2016)

For the enhancement of the solubility of Rosuvastatin Calcium, polymers are played a major role in this case which include chitosan, polyvinyl pyrolydone, polyvinyl alcohol, β- cyclodextrin etc. Among all the polymers, β-cyclodextrin is the most efficient polymer to work as a carrier for these drugs to enhance solubility. The formulation prepared by solid dispersion of drug and β-cyclodextrin had achieved maximum release of drug 97 % within 45 minutes that was found from the result.

(Sarfraz et al.,2016)

A research was done to improve the solubility, dissolution rate, bioavailability of water insoluble drug like pitavastatin followed by liquisolid technology and solid dispersions. In the study, higher drug release was observed from liquisolid formulation tablets prepared with microcrystalline cellulose and that drug release was higher than pure drug. So, this technique was proven efficient.

(Messa and Ampati,2016)

By the preparation of nanosuspensions with Pluronic F127 and zirconium oxide (ZrO2) beads using a wet-milling technique at the laboratory scale, a study was done for the improvement of solubility and dissolution rate of Simvastatin. The used of nanosuspensions with Pluronic F127 and zirconium oxide (ZrO2) at high concentration, increased the dissolution rate of Simvastatin because of nano sized particles. So, this method could be a promising method for improving dissolution profile of Simvastatin.

(Vraníková, Gajdziok and Doležel, 2016)

Surface Solid Dispersion of simvastatin was done using Co-evaporation method to enhance the solubility and dissolution rate of Simvastatin. The result showed a decreased in crystallinity of pure drug which was remained in solid dispersions which caused an increase in the dissolution rate of Simvastatin. Finally this method was proven an efficient method.

(Vraníková, Gajdziok and Doležel, 2016)

For the enhancement of the solubility and dissolution rate of Simvastatin, a study was done. Two methods were used for the following purpose which include solid dispersion by fusion method and complexes with HP-β-cyclodextrin by kneading method. In the dissolution profile, the solid dispersion which were made in the ratio of 1:1 by the HP-β-cyclodextrinexihibited highest dissolution rate of Simvastatin comparing to others.

(Sarfraz et al.,2016)

For the enhancement of the solubility of Rosuvastatin Calcium, polymers are played a major role in this case which include chitosan, polyvinyl pyrolydone, polyvinyl alcohol, β- cyclodextrin etc. Among all the polymers, β-cyclodextrin is the most efficient polymer to work as a carrier for these drugs to enhance solubility. The formulation prepared by solid dispersion of drug and β-cyclodextrin had achieved maximum release of drug 97 % within 45 minutes that was found from the result.

(Tazuma et al.,2016)

Chapter: Three

Materials & Methods

3.1. Materials

3.1.1. Sample collection: To observe the change in dissolution of Rosutin® with the present of calcium and vitamin D supplement. 6 tablets of Rosutin® and 6 tablets of Calvimax-D (500 mg) were collected from local drug store in Dhaka as a sample.

Table 3.1: Samples used in the experiment and their sources

Sample Name	Sources (Supplier)
Rosutin® tablets (10 mg)	Beximco pharmaceutical Limited
Calvimax-D tablets (500 mg)	Incepta Pharmaceutical Limited

3.1.2. Reagent(s):

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

3.1.3. Equipment & Instruments:

Table 3.2: List of Equipment's used in the experiment

Serial No.	Equipment	Source (Supplier name)	Origin
1	UV-spectrophotometer	Shimadazu UV1800	Japan
2	Electronic balance	Precise XB120A	Switzerland
3	Distill water plant	SMIC	China
4	Dissolution tester	SMIC	China
5	Vernier caliper	China supplier	Shanghai, china
6	Hardness tester	Manually operated hardness tester	India

3.1.4. Apparatus:

Some apparatus are listed in the following table those were used throughout the experiments.

Table 3.3: List of Apparatus

Serial No.	Apparatus
1	Beaker
2	Test tubes
3	Volumetric flasks
4	Filter papers
5	Mortar & pestles
6	Spatula
7	Glass Rod
8	Syringe (10ml)
9	Pipette pumper
10	Pipette (1ml, 2ml, 10ml)
11	Glass and plastic funnel

Images of some important instruments those were used in the different tests during research work.

Images of some important instruments those were used in the different tests during research work.

Figure 3.1*:* Dissolution Apparatus

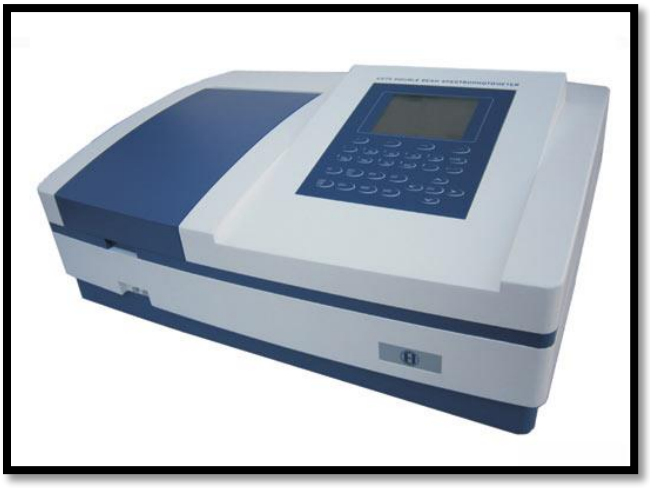

Figure 3.2: UV-1800 Double Beam Spectrophotometer

Figure 3.3: Distill Water Propagating apparatus

Figure 3.4: Electronic Balance

Figure 3.5: Vernier caliper

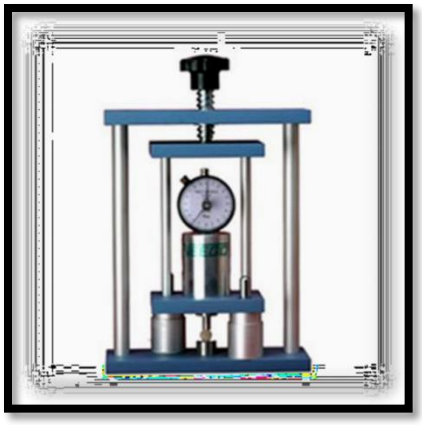

Figure 3.6: Hardness tester

3.2. Methods

3.2.1.1. Preparation of dissolution medium for Standard Curve

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

3.2.1.2. Preparation of Standard Curve:

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

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

Calculations:

For the preparation of 0.001 mg/ml,

V_1= 0.001 mg/ml

$S_{1=}$ 15 ml

S_2= .01 mg/ml

V_2=?

We know that, $V_1 S_1 = V_2 S_2$

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

$V_{2=}$ [(0.001×15)/.01] ml

V_2= 1.5 ml

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

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

For,

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

Table 3.4: Concentration and Absorbance of Rosuvastatin Prepared

Serial No.	Concentration((mg/ml)	Absorbance at 241 nm
1	**0.001**	**0.039**
2	**0.002**	**0.089**
3	**0.003**	**0.125**
4	**0.004**	**0.148**
5	**0.005**	**0.188**
6	**0.006**	**0.223**
7	**0.007**	**0.266**
8	**0.008**	**0.303**
9	**0.009**	**0.361**

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

3.2.2. Preparation of dissolution medium

3.2.2.1. Preparation of distill water

Distilled water was prepared in the laboratory and was used as dissolution medium for dissolution test.

3.2.2.2. Method for dissolution test of Rosutin®(Rosuvastatin)

- 5L (5000 ml) of distilled water (dissolution medium) was prepared.
- Each vessel of dissolution tester was filled with 900 ml of distilled water.
- Time 1 hour, rpm 50 was set up in the dissolution machine. Then the machine was allowed to warm up until it reached at 37.5 degree C.

- Then 1Rosutin®tablet was placed in every vessel. After 10, 20, 30, 40, 50 and 60 minutes 10 ml of solution was collected from each vessels and filtered.
- At last UV absorbance off the solutions were taken where the wave length was 241nm. 5L (5000 ml) of distilled water was prepared.

3.2.2.3. Method for dissolution test of Rosutin®(Rosuvastatin) with Calvimax-D (Calcium and vitamin D supplement)

- Each vessel of dissolution tester was filled with 900 ml of distilled water.
- Time 1 hour, rpm 50 was set up in the dissolution machine.
- Then the machine was allowed to warm up until it reached at 37.5 degree C.
- Then 1 Rosutin®tablet and 1 Calvimax-D were placed in every vessel.
- After 10, 20, 30, 40, 50 and 60 minutes 10 ml of solution was collected from each vessels and filtered.
- At last UV absorbance off the solutions were taken where the wave length was 241 nm.

3.2.3. Determination of physical parameters

3.2.3.1Weight Variation Test

Procedure

- 10 tablets were taken and weighed.
- The average was taken and it was considered as the standard weight of an individual tablet.
- All tablets were weighed individually and observed whether the individual tablets are within the range or not.

N.B: The variation from the average weight in the weights not more than two tablets must not differ more than the percentage listed below:

Table 3.5: Accepted percentage list for weight variation test of tablets

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

Equation:

Following equation was used to determine % weight variation of tablets

% Weight Variation = (A-I/I) × 100

Where,

Initial Weight of Tablet, I (gm)

Average weight of Tablets, A (gm)

3.2.3.2. Thickness test

Procedure

- First the tablet was placed between the two jaws of the Vernier caliper.
- Then the main scale reading was taken.
- Next Vernier scale reading was taken also.
- The two readings were added together for multiplying with the Vernier constant 0.1Cm.

Calculation

Following formula was used to determine thickness of tablets.

Thickness of the tablet = Reading of Cm scale + Reading of Vernier scale ×

Vernier constant (0.01) + Vernier error

3.2.3.3. Hardness test

Procedure

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

3.3. Instrumentation

3.3.1. Dissolution Test Apparatus

A Dissolution tester USPXXII (source RC-6B, made in China) was used for dissolution experiments. It incorporated 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 nonvolatile memory for 15 methods. The water bath incorporated an immersion circulator with an in-built thermostat for temperature control, an external temperature sensor, a water level sensor and a lid with support for eight dissolution bowls. The stirrer hood was equipped with 8 paddle shafts fitted with USP apparatus 2 and a tablet dispenser with 8 conical shaped dissolution bowl lids. The automatic sampling unit consisted of 10in-line filters, a bi-directional 12- channel peristaltic pump with tygontubings, a microprocessor controlled sample collector and a sample

tray capable of collecting 10 x 6 sets of samples. Polycarbonate dissolution vessels with a hemispherical bottom and a capacity of 1000 ml were used for the study.

3.3.2. Ultra- Violet Spectrophotometer

The ultra-violet absorption spectrum for rosutin® 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.

3.3.3. Samples and Chemical Reference Substance

Rosutin® tablets from different manufacturers were used in the study. The samples were obtained from different private retail outlets within Bangladesh.

Chapter: Four

Result & Discussion

4.1. General Information

The Rosuvastatin samples were subjected to assay and dissolution profile analysis under the optimum conditions. The purpose of assay was to determine the impact of dissolution profile of rosuvastatin when given in combination with Calcium and vitamin D supplement (Calvimax-D)

4.2. Standard curve preparation

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

Table 4.1: Concentrations and absorbance of Rosutin®

Serial No.	Concentration((mg/ml)	Absorbance
1	0.001	0.039
2	0.002	0.089
3	0.003	0.125
4	0.004	0.148
5	0.005	0.188
6	0.006	0.223
7	0.007	0.266
8	0.008	0.303
9	0.009	0.361

By plotting the concentration against the absorbance of Rosutin we found a straight line. From the standard curve Rosutin, we derived an equation y=38.279x+0.0019 & R^2=0.9951 (Here, y= Absorbance and x=Concentration of drug). We use this equation to get the concentration from different samples absorbance of Rosutin.

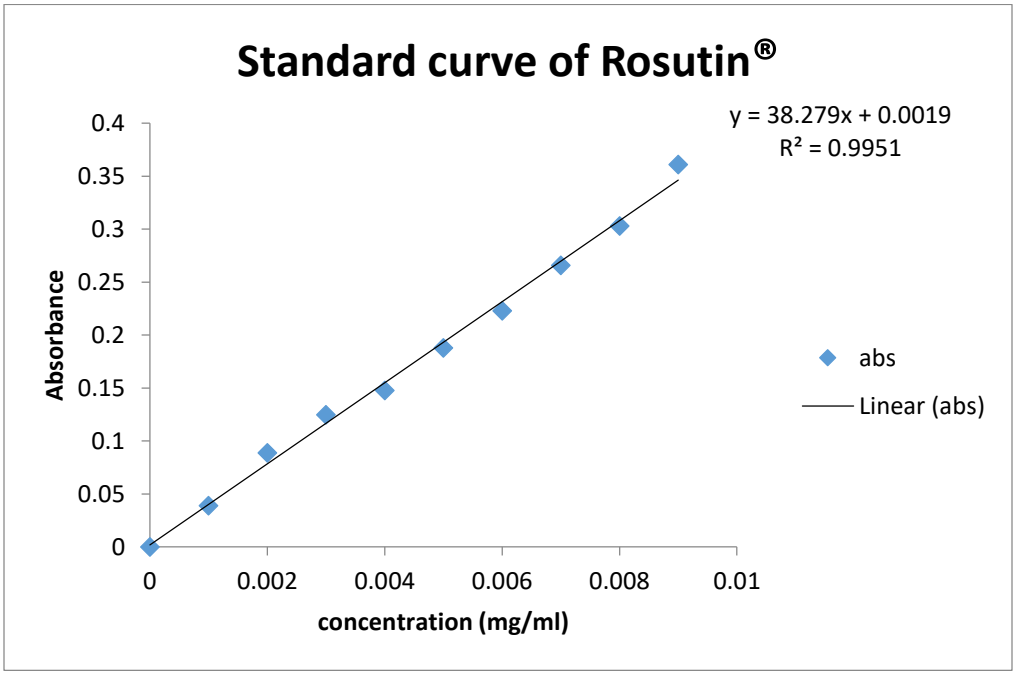

Figure 4.1: Graph showing straight line for absorbance with respect to concentration for Rosutin

4.3. Results of the dissolution test of individual Rosutin with calcium and vitamin D supplement drugs and the impact of supplements on the dissolution of Rosutin® after 10minute, 20minute, 30 minute, 40 minute, 50 minute and 60 minute.

Dissolution test of Rosutin without any supplement

Table 4.2: UV absorbance of only Rosutin® (Rosuvastatin) 10 mg tablet

Absorbance at 241nm						
Serial No.	After 10 minutes	After 20 minutes	After 30 minutes	After 40 minutes	After 50 minutes	After 60 minutes
1	0.217	0.238	0.258	0.288	0.301	0.324
2	0.218	0.235	0.255	0.285	0.308	0.326
3	0.219	0.236	0.257	0.287	0.304	0.322
4	0.217	0.238	0.255	0.285	0.301	0.325
5	0.218	0.234	0.258	0.288	0.305	0.328
6	0.219	0.237	0.256	0.286	0.306	0.326

4.4. Calculation of Drug release for Rosutin® (Rosuvastatin)

From the standard curve an equation was found which was, y = 38.279x+0.0019

Here, y = Absorbance, x=concentration=?

Dilution factor=900

When the absorbance was 0.217, the following equation can be written as-

0.217= 38.279x + 0.0019

38.279x = 0.217-0.0019

38.279x= 0.2151

X= 0.2151/38.279 =0.005078

So, Drug release of Rosutin® (Rosuvastatin) was = 0.00561*900 =5.080 mg

By putting the other absorbance values in the same equation different Drug release of Rosutin® (Rosuvastatin) was calculated.

4.5. Calculation of % Drug release for Rosutin® (Rosuvastatin)

% Drug release= Drug release *100/10

For Drug release 5.080 mg

% Drug release= 5.080*100/10

= 50.80 %

By putting the other absorbance values in the same equation different % Drug release of Rosutin® (Rosuvastatin) was calculated.

Table 4.3: Determination of Drug release and % Drug release of Rosutin® (Rosuvastatin) for 10 minutes without any supplement

After 10 minutes			
Sample no.	**Absorbance**	**Drug Release (mg)**	**% Drug Release**
1	0.217	5.08	50.80
2	0.218	5.10	51.03
3	0.219	5.13	51.27
4	0.217	5.08	50.80
5	0.218	5.10	51.03
6	0.219	5.13	51.27

Table 4.4: Determination of Drug release and % Drug release of Rosutin® (Rosuvastatin) for 20 minutes without any supplement

After 20 minutes			
Sample no.	**Absorbance**	**Drug Release (mg)**	**% Drug Release**
1	0.238	5.57	55.74
2	0.235	5.50	55.03
3	0.236	5.53	55.27
4	0.238	5.57	55.74
5	0.234	5.48	54.79
6	0.237	5.55	55.50

Table 4.5: Determination of Drug release and % Drug release of Rosutin® (Rosuvastatin) for 30 minutes without any supplement

After 30 minutes			
Sample no.	**Absorbance**	**Drug Release (mg)**	**% Drug Release**
1	0.258	6.04	60.44
2	0.255	5.97	59.73
3	0.257	6.02	60.20
4	0.255	5.97	59.73
5	0.258	6.04	60.44
6	0.256	6.00	59.97

Table 4.6: Determination of Drug release and % Drug release of Rosutin® (Rosuvastatin) for 40 minutes without any supplement

After 40 minutes			
Sample no.	**Absorbance**	**Drug Release (mg)**	**% Drug Release**
1	0.288	6.75	67.49
2	0.285	6.68	66.79
3	0.287	6.73	67.26
4	0.285	6.68	66.79
5	0.288	6.75	67.49
6	0.286	6.70	67.02

Table 4.7: Determination of Drug release and % Drug release of Rosutin® (Rosuvastatin) for 50 minutes without any supplement

After 50 minutes			
Sample no.	**Absorbance**	**Drug Release(mg)**	**% Drug Release**
1	0.301	7.06	70.55
2	0.308	7.22	72.20
3	0.304	7.13	71.26
4	0.301	7.06	70.55
5	0.305	7.15	71.49
6	0.306	7.17	71.73

Table 4.8: Determination of Drug release and % Drug release of Rosutin® (Rosuvastatin) for 60 minutes without any supplement

After 60 minutes			
Sample no.	**Absorbance**	**Drug Release(mg)**	**% Drug Release**
1	0.324	7.60	75.96
2	0.326	7.64	76.43
3	0.322	7.55	75.49
4	0.325	7.62	76.20
5	0.328	7.69	76.90
6	0.326	7.64	76.43

4.6. Dissolution test of Rosutin® with Calcium and vitamin D supplement

Table 4.9: UV absorbance of Rosutin® (Rosuvastatin) 10 mg tablets with Calcium and vitamin D supplement

Absorbance at 241 nm						
Serial No.	After 10 minutes	After 20 minutes	After 30 minutes	After 40 minutes	After 50 minutes	After 60 minutes
1	0.181	0.196	0.209	0.232	0.247	0.261
2	0.182	0.199	0.209	0.234	0.249	0.264
3	0.182	0.193	0.213	0.235	0.247	0.262
4	0.184	0.194	0.214	0.232	0.25	0.261
5	0.186	0.196	0.210	0.236	0.245	0.259
6	0.185	0.192	0.211	0.234	0.247	0.266

4.7. Calculation of Drug release for Rosutin® (Rosuvastatin) and Calcium and vitamin D supplement

From the standard curve an equation was found which was, $y = 38.279x+0.0019$

Here, y = Absorbance, x=concentration=?

Dilution factor=900

When the absorbance was 0.181, the following equation can be written as-

$0.181= 38.279x + 0.0019$

38.279x =0.181-0.0019

38.279x=0.1791

X= .00467

So, Drug release of Rosutin® (Rosuvastatin) and Calcium and vitamin D supplement were =.00467*900 = 4.187 mg

By putting the other absorbance values in the same equation different Drug release of Rosutin® (Rosuvastatin) and Calcium and vitamin D supplement were calculated.

4.8. Calculation of % Drug release for Rosutin® (Rosuvastatin) and Calcium and vitamin D supplement

% Drug release= Drug release*100/10

For Drug release 4.187 mg,

% Drug release= 4.187*100/10

= 41.87%

By putting the other absorbance values in the same equation different % Drug release of Rosutin® (Rosuvastatin) and Calcium and vitamin D supplement were calculated.

Table 4.10: Determination of Drug release and % Drug release of Rosutin® (Rosuvastatin) for 10 minutes with Calcium and vitamin D supplement

After 10 minutes			
Sample no.	**Absorbance**	**Drug Release (mg)**	**% Drug Release**
1	0.181	4.19	41.87
2	0.182	4.23	42.34
3	0.184	4.23	42.34
4	0.184	4.28	42.81
5	0.186	4.33	43.28
6	0.185	4.35	43.51

Table 4.11: Determination of Drug release and % Drug release of Rosutin® (Rosuvastatin) for 20 minutes with Calcium and vitamin D supplement

After 20 minutes			
Sample no.	**Absorbance**	**Drug Release(mg)**	**% Drug Release**
1	0.196	4.59	45.86
2	0.199	4.66	46.56
3	0.193	4.52	45.15
4	0.194	4.54	45.39
5	0.196	4.59	45.86
6	0.192	4.49	44.92

Table 4.12: Determination of Drug release and % Drug release of Rosutin® (Rosuvastatin) for 30 minutes with Calcium and vitamin D supplement

After 30 minutes			
Sample no.	**Absorbance**	**Drug Release (mg)**	**% Drug Release**
1	0.209	4.86	48.60
2	0.209	4.86	48.60
3	0.213	4.96	49.60
4	0.214	5.98	49.80
5	0.210	4.89	48.90
6	0.211	4.91	49.10

Table 4.13: Determination of Drug release and % Drug release of Rosutin® (Rosuvastatin) for 40 minutes with Calcium and vitamin D supplement

After 40 minutes			
Sample no.	**Absorbance**	**Drug Release(mg)**	**% Drug Release**
1	0.232	5.43	54.32
2	0.234	5.48	54.79
3	0.235	5.50	55.03
4	0.232	5.43	54.32
5	0.236	5.53	55.27
6	0.234	5.48	54.79

Table 4.14: Determination of Drug release and % Drug release of Rosutin® (Rosuvastatin) for 50 minutes with Calcium and vitamin D supplement

After 50 minutes			
Sample no.	**Absorbance**	**Drug Release (mg)**	**% Drug Release**
1	0.247	5.79	57.85
2	0.249	5.83	58.32
3	0.247	5.79	57.85
4	0.25	5.86	58.56
5	0.245	5.74	57.38
6	0.247	5.79	57.85

Table 4.15: Determination of Drug release and % Drug release of Rosutin (Rosuvastatin) for 60 minutes with Calcium and vitamin D supplement

After 60 minutes			
Sample no.	**Absorbance**	**Drug Release (mg)**	**% Drug Release**
1	0.261	6.11	61.14
2	0.264	6.19	61.85
3	0.262	6.14	61.38
4	0.261	6.11	61.14
5	0.259	6.07	60.67
6	0.266	6.23	62.32

4.9. Impact of Calcium and vitamin D supplement on the Dissolution Profile of Rosutin®

Table 4.16: Showing % Average drug release of Rosutin® without Supplement and % Average drug release of Rosutin® with Calcium and vitamin D Supplement and impact of Calcium and vitamin D supplement on the dissolution profile after 10 minutes

After 10 minutes								**Result**
Rosutin® Without Supplement				**Rosutin® With Calcium and vitamin D Supplement**				**Impact on dissolution**
Drug release (mg)	Average drug release (mg)	% Drug release	% Average drug release	Drug release (mg)	Average drug release (mg)	% Drug release	% Average drug release	
5.08		50.80		4.19		41.87		
5.10		51.03		4.23		42.34		
5.13	5.103	51.27	51.033	4.23	4.268	42.34	42.691	-16.346%
5.08		50.80		4.28		42.81		
5.10		51.03		4.33		43.28		
5.13		51.27		4.35		43.51		

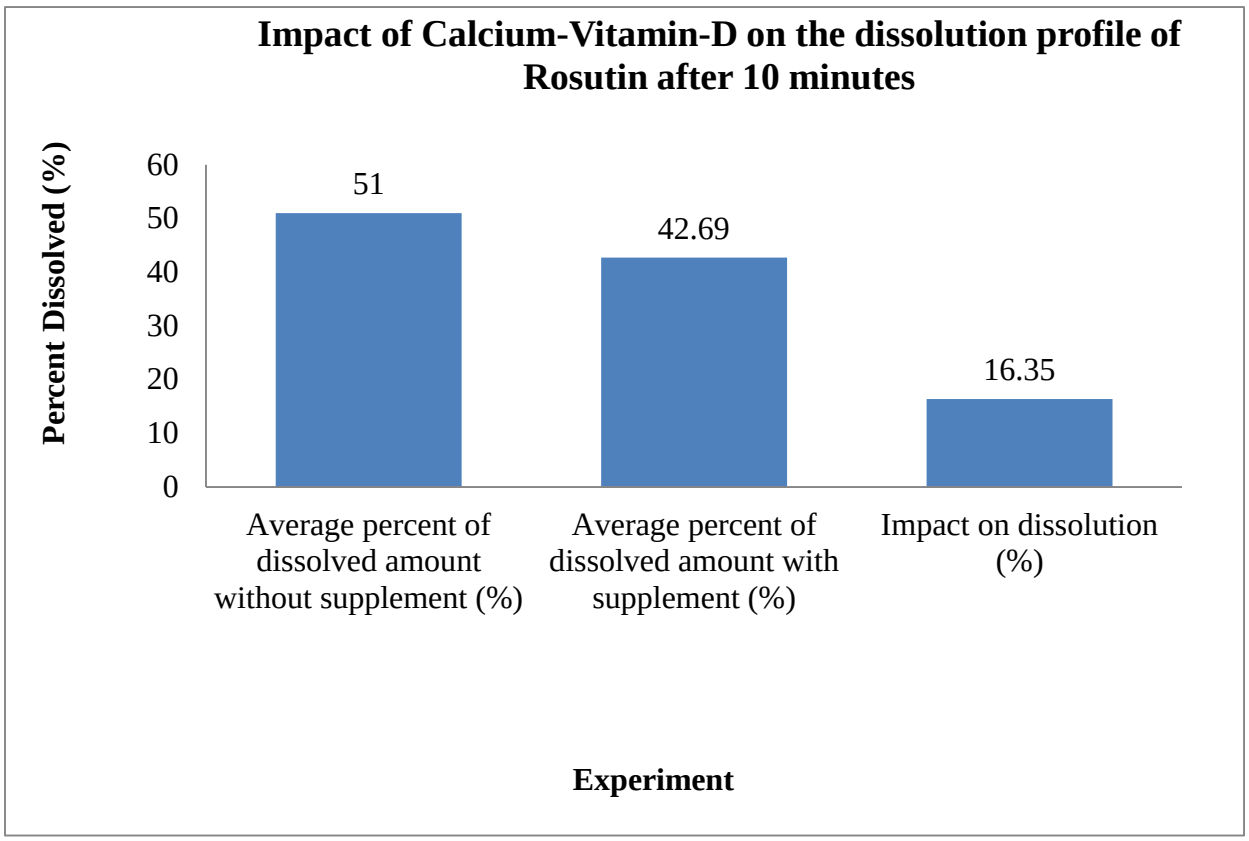

Figure 4.2: Impact of dissolution after 10 minutes

Table 4.17: Showing % Average drug release of Rosutin® without Supplement and % Average drug release of Rosutin® with Calcium and vitamin D Supplement and impact of Calcium and vitamin D supplement on the dissolution profile after 20 minutes

After 20 minutes								**Result**
Rosutin® Without Supplement				**Rosutin® With Calcium and vitamin D Supplement**				**Impact on dissolution**
Drug release (mg)	Average drug release (mg)	% Drug release	% Average drug release	Drug release (mg)	Average drug release (mg)	% Drug release	% Average drug release	
5.57		55.74		4.59		45.86		
5.50		55.03		4.66		46.56		
5.53	5.533	55.27	55.345	4.52	4.565	45.15	45.623	-17.566%
5.57		55.74		4.54		45.39		
5.48		54.79		4.59		45.86		
5.55		55.50		4.49		44.92		

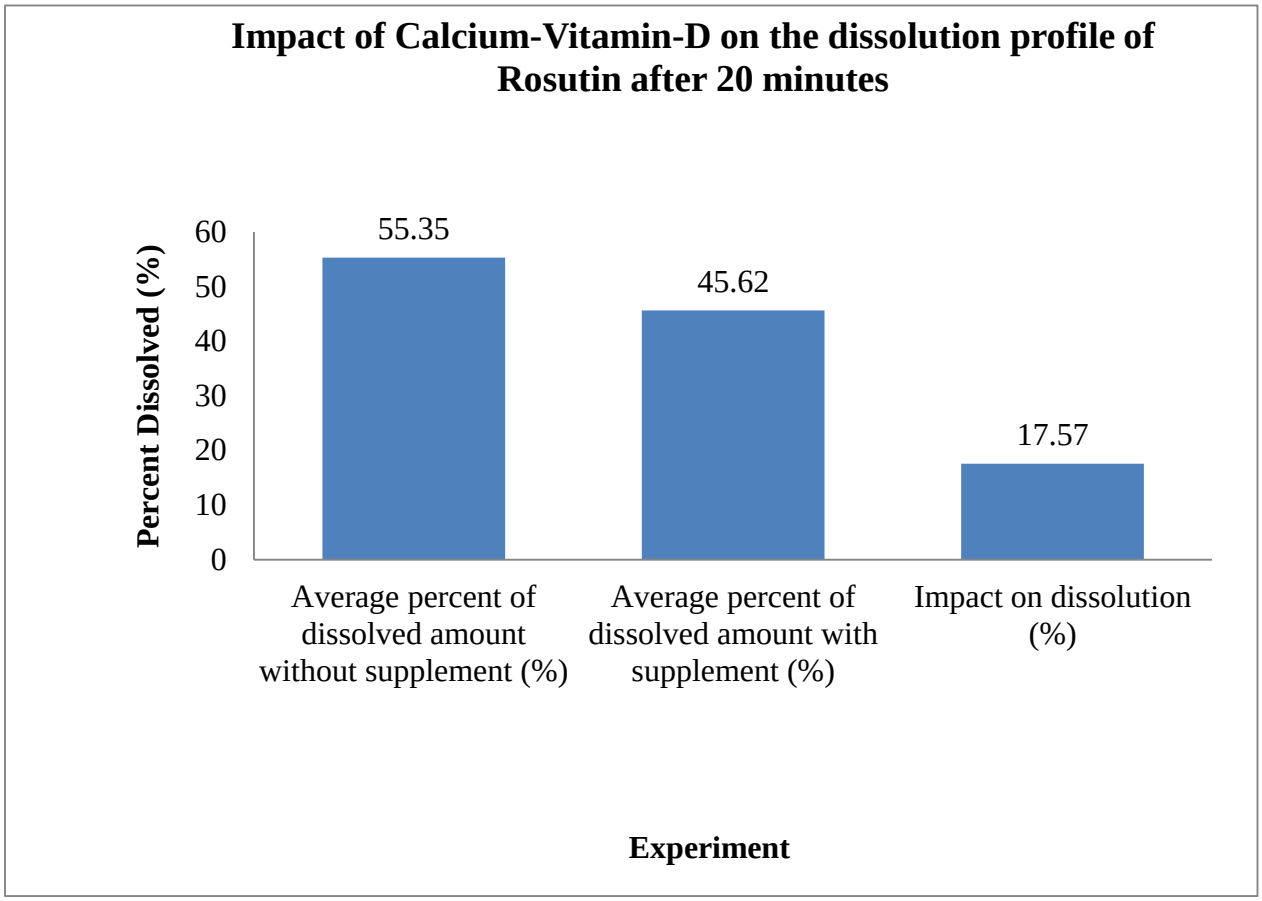

Figure 4.3: Impact of dissolution after 20 minutes

Table 4.18: Showing % Average drug release of Rosutin® without Supplement and % Average drug release of Rosutin® with Calcium and vitamin D Supplement and impact of Calcium and vitamin D supplement on the dissolution profile after 30 minutes

After 30 minutes								**Result**
Rosutin® Without Supplement				**Rosutin® With Calcium and vitamin D Supplement**				**Impact on dissolution**
Drug release (mg)	Average drug release (mg)	% Drug release	% Average drug release	Drug release (mg)	Average drug release (mg)	% Drug release	% Average drug release	
6.04		60.44		4.86		48.60		
5.97		59.73		4.86		48.60		
6.02	6.006	60.20	60.085	4.96	4.911	49.60	49.112	-18.282%
5.97		59.73		4.98		49.80		
6.04		60.44		4.89		48.90		
6.00		59.97		4.91		49.10		

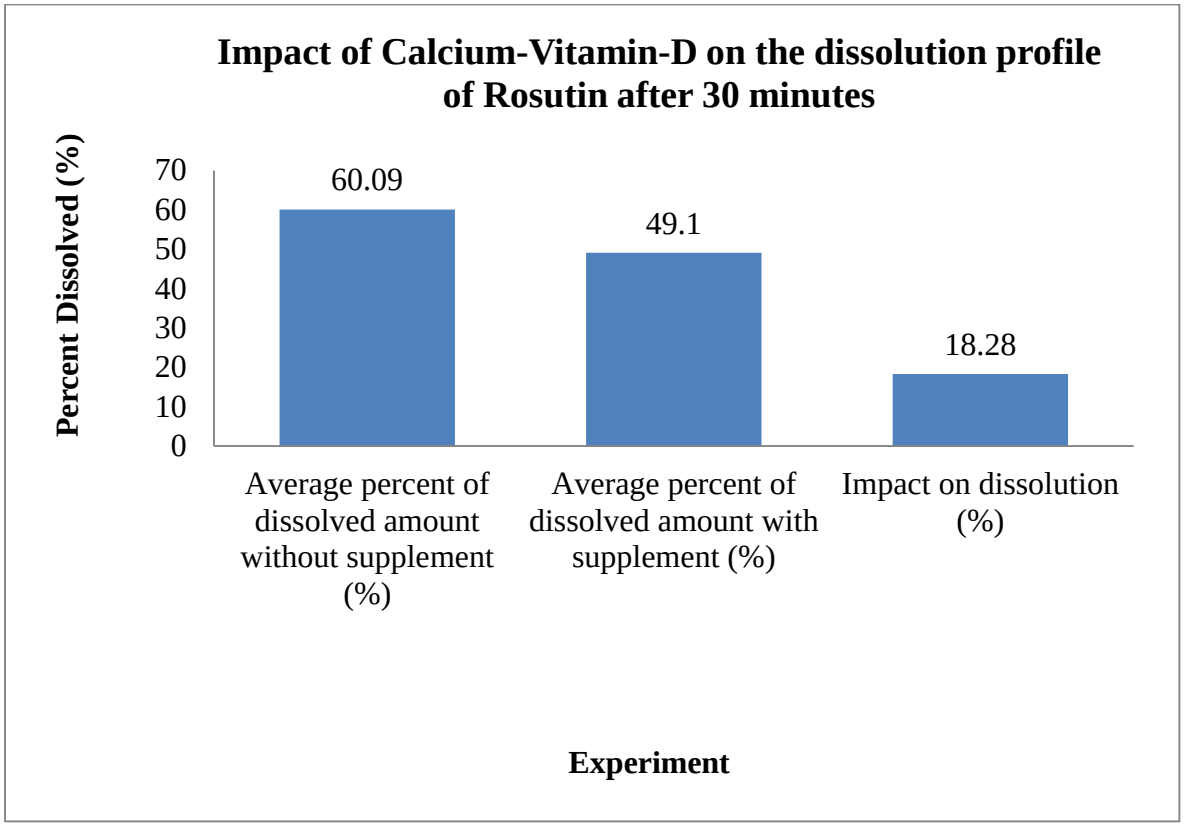

Figure 4.4: Impact of dissolution after 30 minutes

Table 4.19: Showing % Average drug release of Rosutin® without Supplement and % Average drug release of Rosutin® with Calcium and vitamin D Supplement and impact of Calcium and vitamin D supplement on the dissolution profile after 40 minutes

After 40 minutes								**Result**
Rosutin® Without Supplement				**Rosutin® With Calcium and vitamin D Supplement**				**Impact on dissolution**
Drug release (mg)	Average drug release (mg)	% Drug release	% Average drug release	Drug release (mg)	Average drug release (mg)	% Drug release	% Average drug release	
6.75		67.49		5.43		54.32		
6.68		66.79		5.48		54.79		
6.73	6.715	67.26	67.14	5.50	5.475	55.03	54.753	-18.44%
6.68		66.79		5.43		54.32		
6.75		67.49		5.53		55.27		
6.70		67.02		5.48		54.79		

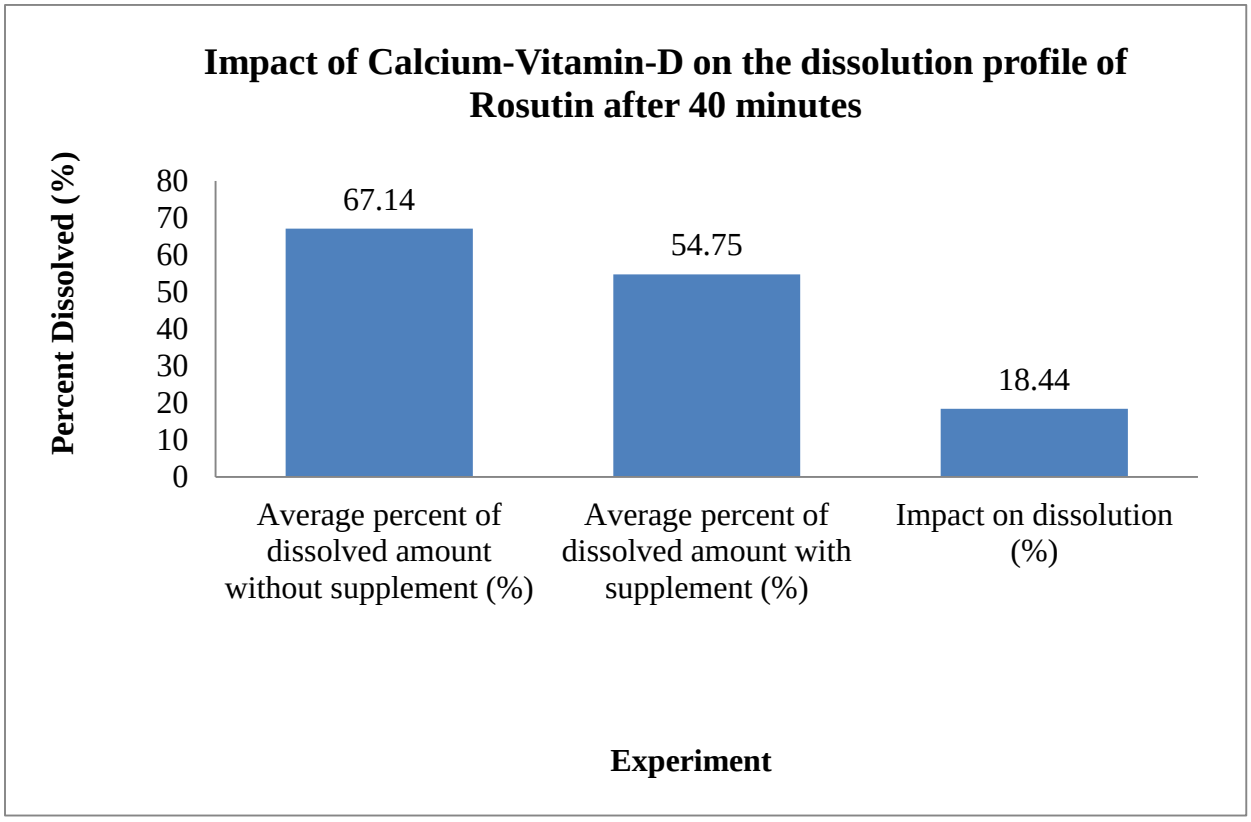

Figure 4.5: Impact of dissolution after 40 minutes

Table 4.20: Showing % Average drug release of Rosutin® without Supplement and % Average drug release of Rosutin® with Calcium and vitamin D Supplement and impact of Calcium and vitamin D supplement on the dissolution profile after 50 minutes

After 50 minutes								**Result**
Rosutin® Without Supplement				**Rosutin® With Calcium and vitamin D Supplement**				**Impact on dissolution**
Drug release (mg)	Average drug release (mg)	% Drug release	% Average drug release	Drug release (mg)	Average drug release (mg)	% Drug release	% Average drug release	
7.06		70.55		5.79		57.85		
7.22		72.20		5.83		58.32		
7.13	7.131	71.26	71.296	5.79	5.80	57.85	57.968	-18.69%
7.06		70.55		5.86		58.56		
7.15		71.49		5.74		57.38		
7.17		71.73		5.79		57.85		

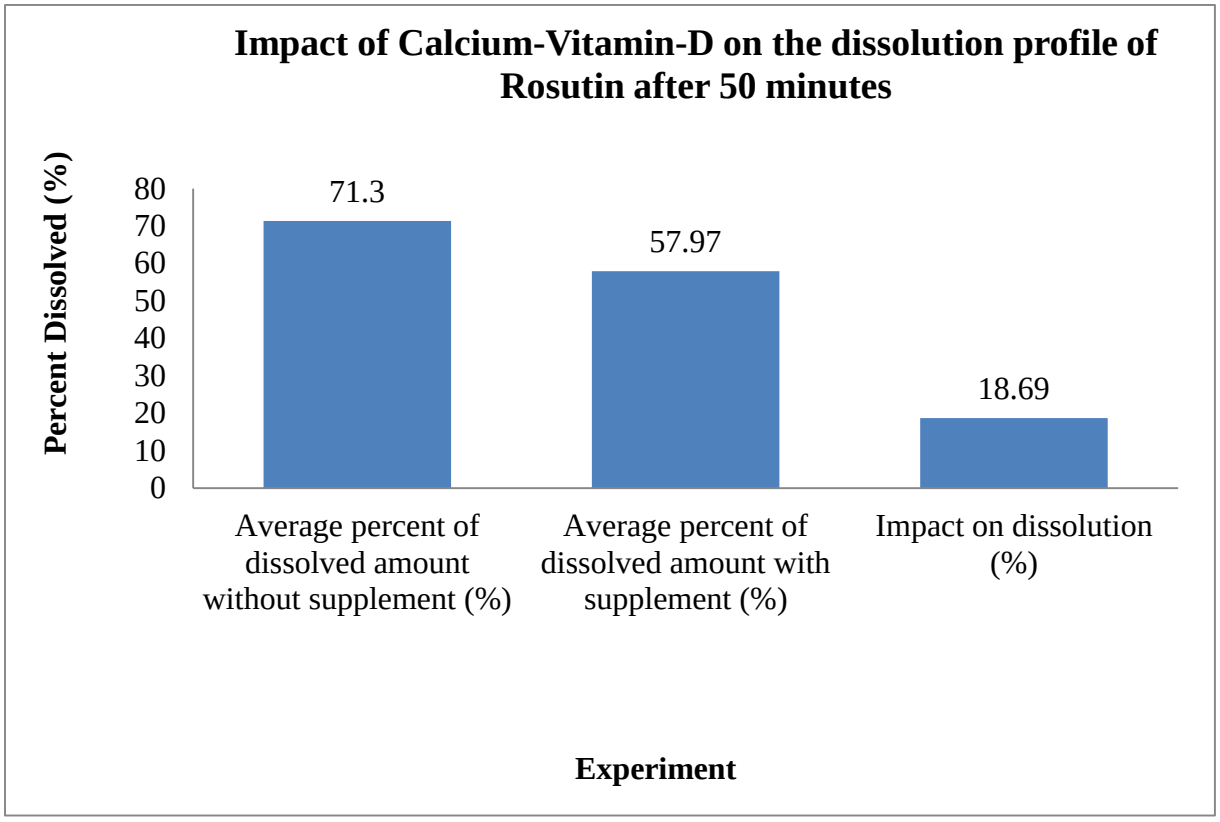

Figure 4.6: Impact of dissolution after 50 minutes

Table 4.21: Showing % Average drug release of Rosutin® without Supplement and % Average drug release of Rosutin® with Calcium and vitamin D Supplement and impact of Calcium and vitamin D supplement on the dissolution profile after 60 minutes

After 60 minutes								**Result**
Rosutin® Without Supplement				**Rosutin® With Calcium and vitamin D Supplement**				**Impact on dissolution**
Drug release (mg)	Average drug release (mg)	% Drug release	% Average drug release	Drug release (mg)	Average drug release (mg)	% Drug release	% Average drug release	
7.60		75.96		6.11		61.14		
7.64		76.43		6.19		61.85		
7.55	7.623	75.49	76.235	6.14	6.141	61.38	61.416	-19.438%
7.62		76.20		6.11		61.14		
7.69		76.90		6.07		60.67		
7.64		76.43		6.23		62.32		

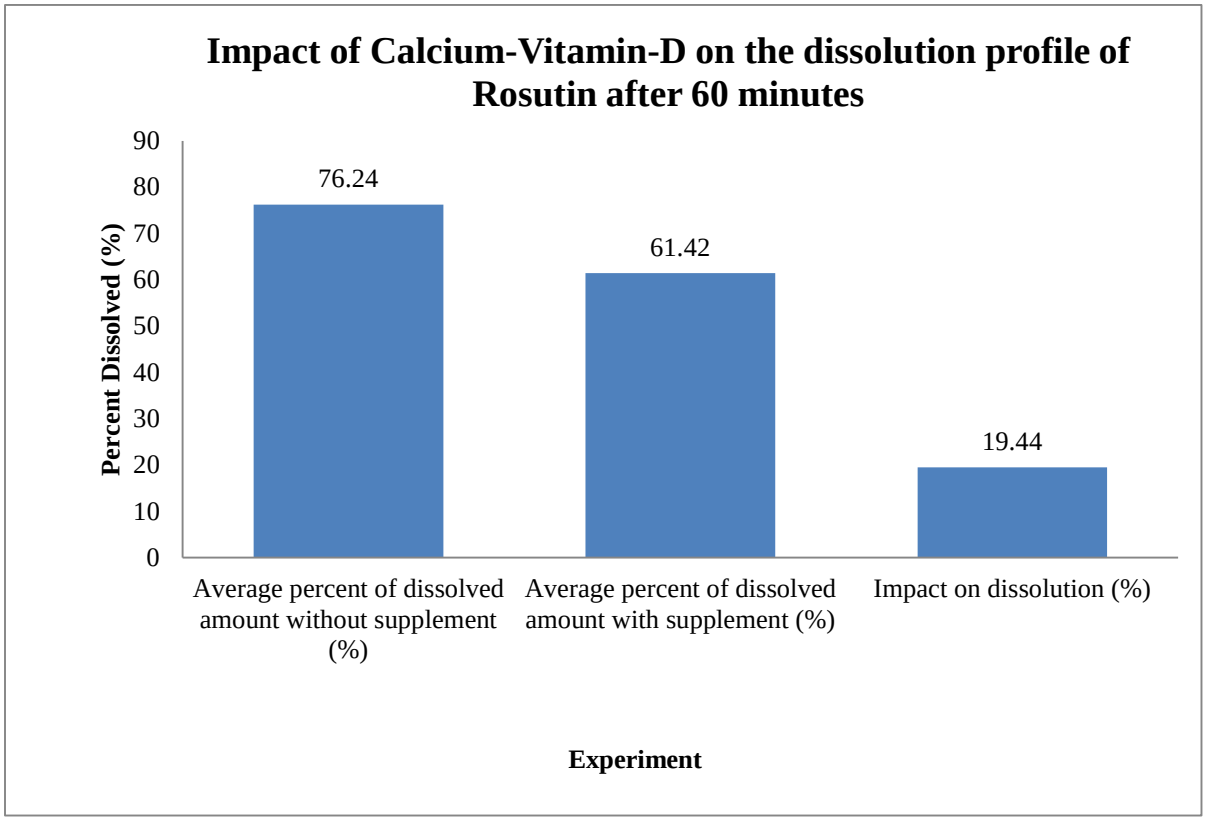

Figure 4.7: Impact of dissolution after 60 minutes

4.10. Result from weight variation test:

Table 4.22: Weight variation of Rosutin® (Rosuvastatin)

Tablet Number	Initial Weight I (gm)	Average weight A (gm)	% Weight Variation (A-I)/I*100
1	0.166		-0.602
2	0.168		-1.82
3	0.166		-0.61
4	0.162		1.82
5	0.163	0.165	1.21
6	0.165		0
7	0.161		2.42
8	0.167		1.82
9	0.163		1.21
10	0.164		0.61

4.11. Results from Hardness test:

Table 4.23: Hardness test of Rosutin® (Rosuvastatin)

Tablet Number	Hardness(kg)	Average (kg)
1	1.4	
2	1.4	
3	1.6	
4	2.4	
5	2.5	2.04
6	2.2	
7	1.9	
8	2.5	
9	2.5	
10	2	

4.12. Results from Thickness test

Table 4.24: Thickness test of Rosutin® (Rosuvastatin)

Tablet Number	**Main scale Reading (cm), M**	**Vernier Scale Reading (cm),V**	**Vernier constant, Vc**	**Vernier Error, VE**	**Thickness of the tablet M+(V×Vc) (cm)**
1	0.3	2	0.1		0.5
2	0.3	2	0.1		0.5
3	0.3	2	0.1		0.5
4	0.3	2	0.1		0.5
5	0.3	2	0.1	0	0.5
6	0.3	2	0.1		0.5
7	0.3	2	0.1		0.5
8	0.3	2	0.1		0.5
9	0.3	2	0.1		0.5
10	0.3	2	0,1		0.5

Discussion

Weight variation of sample tablets (Rosutin®) indicated the uniformity of the solid dosage forms. USP provides an accepted percentage for weight variation test and our products were within that range. The hardness of the tablets are slightly increased with the increase in weight content without much variation in content uniformity of weight. Weight variation test indicates the good manufacturing practice (GMP), appropriate size of the tablets and the content uniformity of the formulation. (Nasrin et al., 2011)

Hardness determination was important because the dissolution of a drug product depends on its hardness. The hardness increase caused by higher compression loads in the absence of a moisture-induced effect, which is responsible for decrease in the in vitro dissolution as the hardness was increased. (Chowhan and Palagyi, 1978)

If the tablet is too hard, it may not disintegrate in the required period of time to meet the dissolution specifications. (Nasrin et al., 2011)

The thickness of all tablets (Rosutin®) were determined by vernier calipers and all values were same that indicates there is vernier error. Thickness determination was important because it relates with tablet hardness. If the thickness of a tablet is materially changed, then all tablet hardness comparisons will become incorrect. (Pitt and Heasley, 2013)

From the result of dissolution tests of Rosutin®, it was observed that the percent release of drug for Rosutin® was increasing with time. After 10 minutes, 20 minutes,30 minutes, 40 minutes,50 minutes and 60 minutes, the average percent release of drug were 51.03%, 55.34% , 60.08%, 67.14% ,71.29% and 76.235% respectively. So, it was observed that with increasing time the release of drug is also increasing gradually.

When Rosutin® was tested with Calvimax- D (calcium-vitamin D supplement), then the dissolution was changed. After 10 minutes, 20 minutes,30 minutes, 40 minutes,50 minutes and 60 minutes, the average percent release of drug were 42.69%, 45.62%, 49.11%, 54.75%, 57.16% and

61.41% respectively. So, it was clear that with increasing time the dissolution or the drug release of Rosutin® is decreased when it is combined with calcium and vitamin D supplement.

This decrease of drug release is due to the common ion effect. A compound of low solubility from two ions in a saturated solution, when addition of either of these two ions will decrease the soilubility of the compound.

Rosutin® (Rosuvastatin Ca) was applied on the dissolution media, it become saturated. Since this solution is at equilibrium, the drug was dissolved and precipitated all the at rates which just balance each other. This remain constant through enternity unless we do something.

When Calcium and vitamin D was applied with Rosutin® (RosuvastatinCa), common ion effect was found. According to the Le Chatelier's principle, increasing the Ca concentration, the equilibrium was shifted to the left. That mean's addition of Calcium and vitamin D decreased the solubility. The decreased of solubility also decreased the dissolution rate.

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

(Shin et al., 2013)

Chapter: Five

Conclusion

Conclusion

The investigation report of the study showed the impact of Calcium and vitamin D supplement on the dissolution profile of Rosutin® (Rosuvastatin). In the research, we observed that the individual release of Rosutin® was increased gradually from 10 minutes to 60 minutes. But when calcium and vitamin D supplement was combined with Rosutin®, it was shown that the release of Rosutin® was decreased. That was happened because of common ion effect which decreased the solubility of the drug.So, Rosutin® (Rosuvastatin) can not be co-administered with Calcium and vitamin D supplement.

Chapter: Six

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