In vitro dissolution study to determine the Drug-Drug interaction of Atorvastatin Calcium-Metformin HCL-Multivitamin Tablet

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

By

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DECLARATION BY THE CANDIDATE

I hereby declare that this thesis entitled **—In vitro dissolution study to determine the Drug-Drug interaction of Atorvastatin Calcium-Metformin HCL- Multivitamin Tablet** is a bonafide and genuine research work carried out by me, Farjana Islam Jui under the guidance of **Mahbubul Hoque Shihan**, Senior Lecturer, Department of Pharmacy, East West University, and Dhaka.

Date: January 17, 2014 Signature by the candidate

Place: Dhaka

CERTIFICATE

This is to certify that the Project- **"In vitro dissolution study to determine the Drug-Drug interaction of Atorvastatin-Metformin HCL-Multivitamin Tablet"** Submitted to the department of pharmacy, East West University in partial fulfillment of the requirements of the degree Bachelor of Pharmacy was carried out by Farjana Islam Jui (ID # 2010 – 1 – 70 – 038) and that all the sources of information and laboratory facilities availed of in this connection duly acknowledged. No Part of this thesis has been submitted for any other degree.

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CERTIFICATE

This is to certify that the Thesis on **—"In vitro dissolution study to determine the Drug-Drug interaction of Atorvastatin-Metformin HCL-Multivitamin Tablet"** is presented to the Department of Pharmacy, East West University is outcome of the investigations performed by **Farjana Islam Jui (ID # 2010 – 1 – 70 – 038)** under the supervision of **Mahbubul Hoque Shihan, Senior lecturer and co- supervision of Mehreen Rahman**, **Lecturer,** Department of Pharmacy, East West University. This is also certifying that no part of this project report has been or is being submitted elsewhere for the award of any degree or diploma.

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LIST OF ACRONYMS	
Acronyms	**Expansions**
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme
LDL	Low Density Lipoprotein
LDL-C	Low Density Lipoprotein-Cholesterol
U.S. FDA	United States Food & Drug Administration
HDL	High Density Lipoprotein
HDL-C	High Density Lipoprotein-Cholesterol
TG	Triglycerides
HMGRI	3-hydroxy-3-methylglutaryl-coenzyme reductase

ABSTRACT

The purpose of my research work was to determine the drug-drug interaction between Atorvastatin, Metformin HCL and Multivitamin by doing in vitro dissolution study. Because of drug-drug interaction dissolution rate can varies. To carry the study these drugs were collected from the market and drug-drug interaction study were determined.

Dissolution is a test used to characterize the dissolution properties of the active drug, the active drug's release and the dissolution from a dosage formulation. Drug is administered orally in solid dosage forms, such as tablet or capsules, must dissolve in the contents of the gastrointestinal tract before drug absorption can occur. Often the rate of drug absorption is determined by the rate of drug dissolution from the dosage form. Therefore, if it is important to achieve high peak blood levels of a drug, it will usually be important to obtain rapid drug dissolution from the dosage form. So, dissolution rate per minute is very important for better absorption and for better therapeutic activity.

To determine the drug-drug interaction and the differences in dissolution rate, Atorvastatin was given alone and it was given with Metformin and Multivitamin in a dissolution tester. This test was done on USPII Paddle apparatus, at 75rpm at 37degree Celsius. The dissolution medium was phosphate buffer at pH of 6.8 and it was analyzed by UV spectrometry at 242 nm. After dissolution test, the dissolution rate of Atorvastatin after 30 minute was 36.14% which is less than 50% and didn't pass the BP specified dissolution test. But in the combination the dissolution rate was only 5.58%.Though the dissolution rate of Atorvastatin was less than 50%, but it was far more than the rate of combination. In case of combination they gave antagonistic activity and that's why dissolution rate decreased.

Key words: Dissolution test, drug-drug interaction, Mechanism of action, standard solution and standard curve of Atorvastatin.

CHAPTER: 1

INTRODUCTION

1.1 Atorvastatin:

Atorvastatin calcium is a synthetic lipid-lowering agent. Atorvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate limiting step in cholesterol biosynthesis. Atorvastatin is used to lower cholesterol and triglyceride (fat-like substances) levels in the blood. Using this medicine may help prevent medical problems caused by such substances clogging the blood vessels. This medicine may also be used to prevent certain types of heart problems in adults with risk factors for heart problems. (FDA, 2013)

1.2 Chemistry of Atorvastatin:

Atorvastatin calcium is [R-(R*, R*)]-2-(4-fluorophenyl)-fluorophenyl)-ß, & dihydroxy-5 (1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate. The empirical formula of Atorvastatin calcium is (C33H34 FN2O5)2Ca•3H2O and its molecular weight is 1209.42. Its structural formula is:

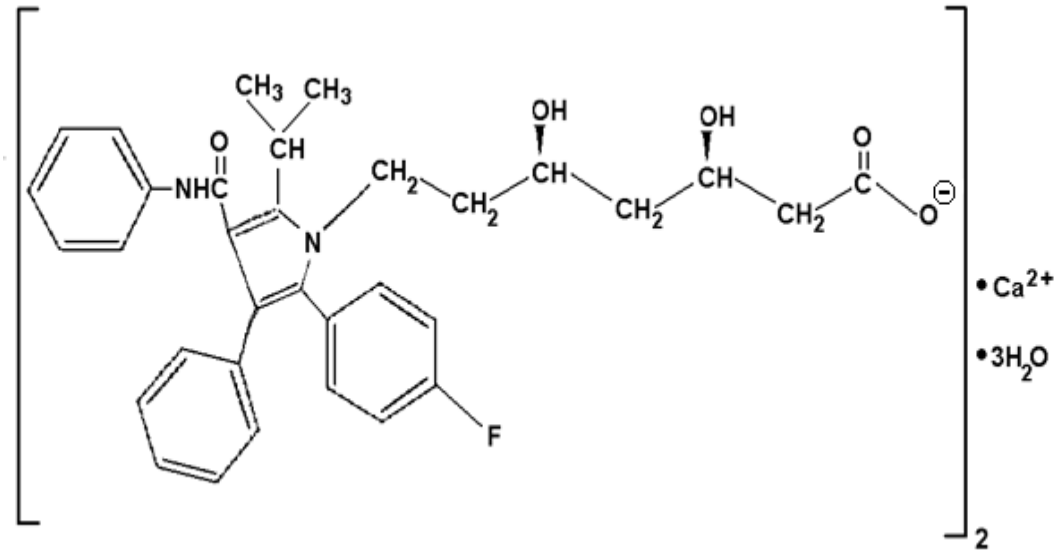

Fig 1: Chemical structure of Atorvastatin Calcium

Atorvastatin calcium is a white to off-white crystalline powder that is insoluble in aqueous solutions of pH 4 and below. Atorvastatin calcium is very slightly soluble in distilled water, pH 7.4 phosphate buffer, and acetonitrile, slightly soluble in ethanol, and freely soluble in methanol.

Atorvastatin tablets for oral administration contain 10, 20, 40 or 80 mg Atorvastatin and the following inactive ingredients: calcium carbonate, USP; candelilla wax, FCC; croscarmellose sodium, NF; hydroxypropyl cellulose, NF; lactose monohydrate, NF; magnesium stearate, NF; microcrystalline cellulose, NF; Opadry White YS-1-7040 (hypromellose, polyethylene glycol, talc, titanium dioxide); polysorbate 80, NF; simethicone emulsion. (Pfizer, 2009)

1.3 Discovery and development of Atorvastatin:

The search for potent and efficacious inhibitors of the enzyme HMG-CoA reductase (HMGRI) was the focus of considerable research in the 1980s. Building on the discovery of the fungal metabolite-derived inhibitors, mevastatin, lovastatin, prevastatin and simvastatin, a number of totally synthetic inhibitors were discovered and developed. This manuscript describes the discovery and development of one of those synthetic inhibitors, atorvastatin calcium, currently marketed in the United States as LIPITOR. This inhibitor was designed based in part on molecular modeling comparisons of the structures of the fungal metabolites and other synthetically derived inhibitors. In addition to development of the structure-activity relationships which led to atorvastatin calcium, another critical aspect of the development of this area was the parallel improvement in the chemistry required to prepare compounds of the increased synthetic complexity needed to potently inhibit this enzyme. Ultimately, the development of several chiral syntheses of enantiomerically pure atorvastatin calcium was accomplished through a collaborative effort between discovery and development. The impact of the progress of the required chemistry as well as external factors on internal decision-making with regards to the development of atorvastatin calcium will be discussed. (Lea, A., McTavish D. 2002)

1.4 Pharmacodynamics:

Atorvastatin as well as some of its metabolites are pharmacologically active in humans. The liver is the primary site of action and the principal site of cholesterol synthesis and LDL clearance. Drug dosage rather than systemic drug concentration correlates better with LDL-C reduction. Individualization of drug dosage should be based on therapeutic response. (Pfizer, 2009)

1.4.1 Mechanism of action:

Atorvastatin is a selective, competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme that converts 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate, a precursor of sterols, including cholesterol. Cholesterol and triglycerides circulate in the bloodstream as part of lipoprotein complexes. With ultracentrifugation, these complexes separate into HDL (high-density lipoprotein), IDL (intermediate-density lipoprotein), LDL (low-density lipoprotein), and VLDL (very-low-density lipoprotein) fractions. Triglycerides (TG) and cholesterol in the liver are incorporated into VLDL and released into the plasma for delivery to peripheral tissues.

LDL is formed from VLDL and is catabolized primarily through the high-affinity LDL receptor. Clinical and pathologic studies show that elevated plasma levels of total cholesterol (total-C), LDL-cholesterol (LDL-C), and apolipoprotein B (apo B) promote human atherosclerosis and are risk factors for developing cardiovascular disease, while increased levels of HDL-C are associated with a decreased cardiovascular risk. (Pfizer, 2009)

In animal models, Atorvastatin lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic LDL receptors on the cell-surface to enhance uptake and catabolism of LDL; Atorvastatin also reduces LDL production and the number of LDL particles. Atorvastatin reduces LDL-C in some patients with homozygous familial hypercholesterolemia (FH), a population that rarely responds to other lipid-lowering medication(s).A variety of clinical studies have demonstrated that elevated levels of total-C, LDL-C, and apo B (a membrane complex for LDL-C) promote human atherosclerosis. Similarly, decreased levels of HDL-C (and its transport complex, apo A) are associated with the development of atherosclerosis. Epidemiologic investigations have established that cardiovascular morbidity and mortality vary directly with the level of total-C and LDL-C, and inversely with the level of HDL-C. Atorvastatin reduces total-C, LDL-C, and apo B in patients with homozygous and heterozygous FH, non familial forms of hypercholesterolemia, and mixed dyslipidemia. Atorvastatin also reduces VLDL-C and TG and produces variable increases in HDL-C and apolipoprotein A-1. Atorvastatin reduces total-C, LDL-C, VLDL-C, apo B, TG, and non-HDL-C. And it increases HDL-C in patients with isolated

hypertriglyceridemia. Atorvastatin reduces intermediate density lipoprotein cholesterol (IDL-C) in patients with dysbetalipoproteinemia. (Pfizer, 2009)

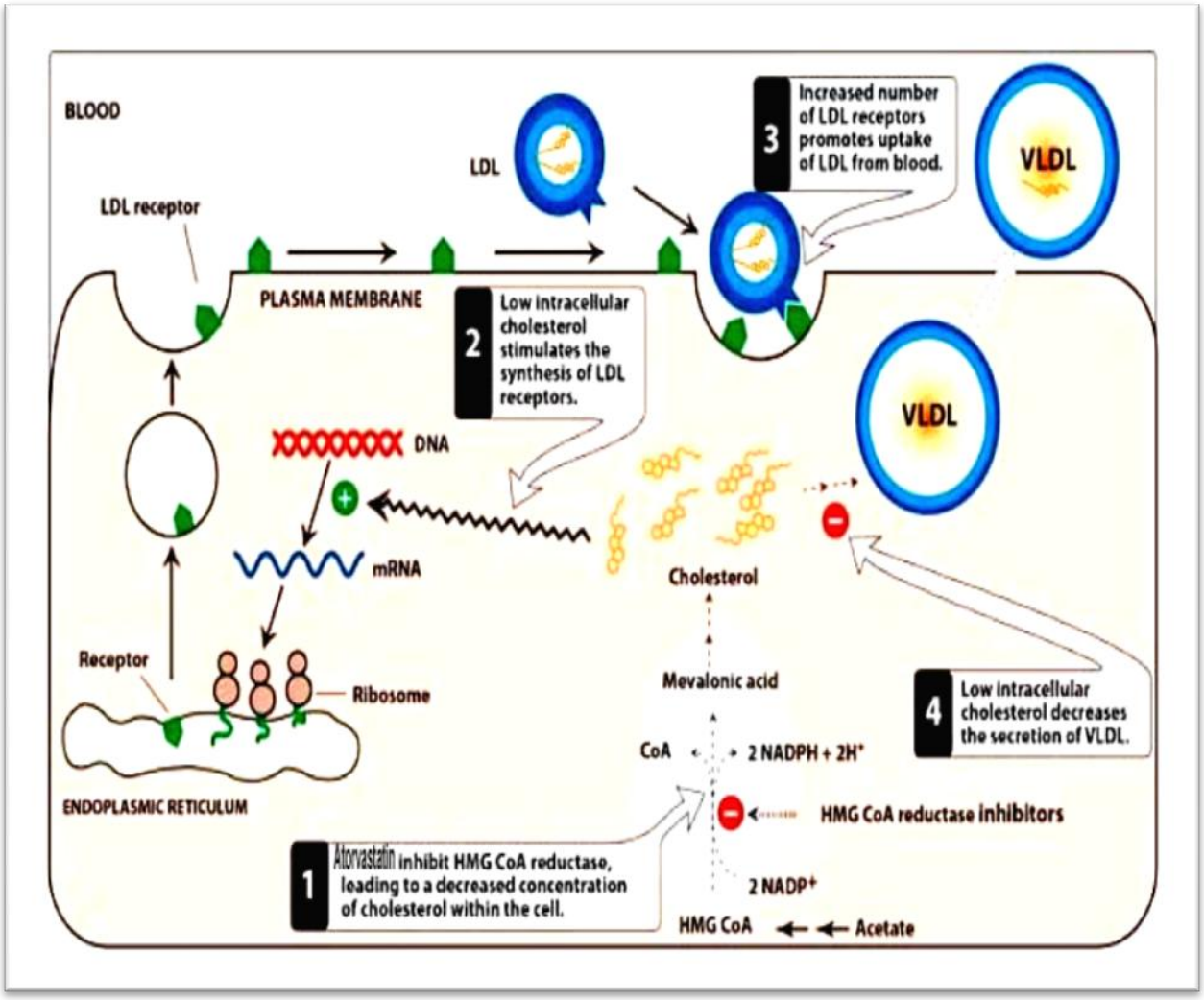

Fig 2: Mechanism of action of Atorvastatin

Like LDL, cholesterol-enriched triglyceride-rich lipoproteins, including VLDL, intermediate density lipoprotein (IDL), and remnants, can also promote atherosclerosis. Elevated plasma triglycerides are frequently found in a triad with low HDL-C levels and small LDL particles, as well as in association with non-lipid metabolic risk factors for coronary heart disease. As such, total plasma TG has not consistently been shown to be an independent risk

factor for CHD. Furthermore, the independent effect of raising HDL or lowering TG on the risk of coronary and cardiovascular morbidity and mortality has not been determined.

1.5 Pharmacokinetics:

1.5.1 Absorption:

Atorvastatin is rapidly absorbed after oral administration; maximum plasma concentrations occur within 1 to 2 hours. Extent of absorption increases in proportion to Atorvastatin dose. The absolute bioavailability of Atorvastatin (parent drug) is approximately 14% and the systemic availability of HMG-CoA reductase inhibitory activity is approximately 30%. The low systemic availability is attributed to presystemic clearance in gastrointestinal mucosa and/or hepatic first-pass metabolism. Although food decreases the rate and extent of drug absorption by approximately 25% and 9%, respectively, as assessed by Cmax and AUC, LDL-C reduction is similar whether Atorvastatin is given with or without food. Plasma atorvastatin concentrations are lower (approximately 30% for Cmax and AUC) following evening drug administration compared with morning. However, LDL-C reduction is the same regardless of the time of day of drug administration. (Pfizer, 2009)

1.5.2 Distribution:

Mean volume of distribution of Atorvastatin is approximately 381 liters. Atorvastatin is ≥98% bound to plasma proteins. A blood/plasma ratio of approximately 0.25 indicates poor drug penetration into red blood cells. (Pfizer, 2009)

1.5.3 Metabolism:

Atorvastatin is extensively metabolized to ortho- and parahydroxylated derivatives and various beta-oxidation products. The inhibition of HMG-CoA reductase by ortho-and parahydroxylated metabolites is equivalent to that of Atorvastatin. Approximately 70% of circulating inhibitory activity for HMG-CoA reductase is attributed to active metabolites. Atorvastatin metabolism is occurred by cytochrome P450 3A4, but when it is coadministered with erythromycin or a known inhibitor of this isozyme, then plasma concentrations of atorvastatin in humans increases. (Pfizer, 2009)

1.5.4 Excretion:

Atorvastatin is eliminated primarily in bile following hepatic and/or extrahepatic metabolism; however, the drug does not appear to undergo significant enterohepatic recirculation. Mean plasma elimination half-life of atorvastatin in humans is approximately 14 hours, but the half-life for inhibitory activity for HMG-CoA reductase is 20 to 30 hours due to the contribution of longer-lived active metabolites. Less than 2% of a dose of atorvastatin is recovered in urine following oral administration. (Pfizer, 2009)

1.6 Special Populations:

1.6.1 Geriatric:

Plasma concentrations of Atorvastatin are higher (approximately 40% for Cmax and 30% for AUC) in healthy elderly subjects (age ≥65 years) than in young adults. Clinical data suggest a greater degree of LDL-lowering at any dose of drug in the elderly patient population compared to younger adults. (Pfizer, 2009)

1.6.2 Pediatric:

Pharmacokinetic data in the pediatric population are not available.

1.6.3 Gender:

Plasma concentrations of Atorvastatin in women differ from those in men (approximately 20% higher for Cmax and 10% lower for AUC); however, there is no clinically significant difference in LDL-C reduction with Atorvastatin between men and women.

1.6.4 Renal Insufficiency:

Renal disease has no influence on the plasma concentrations or LDL-C reduction of Atorvastatin; thus, dose adjustment in patients with renal dysfunction is no necessary. (Pfizer, 2009)

1.6.5 Hemodialysis:

While studies have not been conducted in patients with end-stage renal disease, hemodialysis is not expected to significantly enhance clearance of Atorvastatin since the drug is extensively bound to plasma proteins.

1.6.6 Hepatic Insufficiency:

In patients with chronic alcoholic liver disease, plasma concentrations of Atorvastatin are markedly increased. Cmax and AUC are each 4-fold greater in patients with Childs-Pugh A disease. Cmax and AUC are approximately 16fold and 11-fold increased, respectively, in patients with Childs-Pugh B disease. (Pfizer, 2009)

1.7 Dose of Atorvastatin:

1.7.1 Pediatric patients:

If the suffer from Dyslipidemia, Heterozygous Familial Hypercholesteromia then the dose is oral. Initially 10 mg dose is daily for children of 10-17 years. Adjust dosage at intervals of ≥4 weeks until the desired effect on lipoprotein concentrations is observed or a daily dosage of20mg is reached. (Drugs.com, 2013)

1.7.2 Adult patients:

For the prevention of Cardiovascular events or management of Dyslipidemia or primary Hypercholesteromia (Heterozygous familial or Non familial) or Mixed Dyslipidemia then the dose is oral. Initially 10 or 20 mg is daily. Patients who require reductions of >45% in LDL-cholesterol concentration: May initiate therapy with 40 mg once daily.

Determine serum lipoprotein concentrations within 2–4 weeks after initiating and/or titrating therapy and adjust dosage accordingly. Usual maintenance dosage is 10-80mg once daily. In case of homozygous hyper cholesteromia dose is 10-80mg once daily. (Drugs.com, 2013)

1.8 Dosage form of Atorvastatin: Atorvastatin Calcium is found as a tablet, film coated tablet, oral formulation. (Drugs.com, 2013)

1.9 Indication and Usage:

1.9.1 Prevention of Cardiovascular Disease:

In adult patients without clinically evident coronary heart disease, but with multiple risk factors for coronary heart disease such as age, smoking, hypertension, low HDL-C, or a family history of early coronary heart disease, Atorvastatin is indicated to:

- Reduce the risk of myocardial infarction
- Reduce the risk of stroke
- Reduce the risk for revascularization procedures and angina.

In patients with type 2 diabetes, and without clinically evident coronary heart disease, but with multiple risk factors for coronary heart disease such as retinopathy, albuminuria, smoking, or hypertension, Atorvastatin is indicated to:

- Reduce the risk of myocardial infarction
- Reduce the risk of stroke.

In patients with clinically evident coronary heart disease, Atorvastatin is indicated to:

- Reduce the risk of non-fatal myocardial infarction
- Reduce the risk of fatal and non-fatal stroke.
- Reduce the risk for revascularization procedures
- Reduce the risk of hospitalization for CHF
- Reduce the risk of angina.

1.9.2 Hypercholesterolemia:

Atorvastatin is indicated:

a) As an adjunct to diet to reduce elevated total-C, LDL-C, apo B, and TG levels and to increase HDL-C in patients with primary hypercholesterolemia (heterozygous familial and nonfamilial) and mixed dyslipidemia *(Fredrickson* Types IIa and IIb);

b) As an adjunct to diet for the treatment of patients with elevated serum TG levels *(Fredrickson* Type IV);

c) For the treatment of patients with primary dysbetalipoproteinemia *(Fredrickson* Type III) who do not respond adequately to diet;

d) To reduce total-C and LDL-C in patients with homozygous familial hypercholesterolemia as an adjunct to other lipid-lowering treatments (e.g., LDL apheresis) or if such treatments are unavailable;

e) As an adjunct to diet to reduce total-C, LDL-C, and apo B levels in boys and postmenarchal girls, 10 to 17 years of age, with heterozygous familial hypercholesterolemia if after an adequate trial of diet therapy the following findings are present: a. LDL-C remains greater than or equal to

190 mg/dL or LDL-C remains greater than or equal to 160 mg/dL. (Pfizer, 2009)

1.9.3 Cardiovascular disease:

The cardiovascular benefits of Atorvastatin in primary prevention and secondary prevention, and acute coronary syndromes, across a wide age range and among patients with total cholesterol concentrations much lower than average.

1.10 Drug-Drug interactions:

1.10.1 Inhibitors of cytochrome P450 3A4:

Atorvastatin is metabolized by cytochrome P450 3A4. Concomitant administration of Atorvastatin with inhibitors of cytochrome P450 3A4 can lead to increases in plasma concentrations of Atorvastatin. The extent of interaction and potentiation of effects depends on the variability of effect on cytochrome P450 3A4. For example: Clarithromycin, Erythromycin, grapefruit juice etc. (Pfizer, 2009)

1.10.2 Combination of Protease Inhibitors:

Concomitant administration of Atorvastatin with ritonavir plus saquinavir resulted in a 3-fold increase in Atorvastatin AUC. Concomitant administration of Atorvastatin with lopinavir plus ritonavir resulted in a 5.9-fold increase in Atorvastatin AUC. (Pfizer, 2009)

1.10.3 Cyclosporine:

Atorvastatin and Atorvastatin-metabolites are substrates of the OATP1B1 transporter. Inhibitors of the OATP1B1 (e.g. cyclosporine) can increase the bioavailability of Atorvastatin. Concomitant administration of Atorvastatin and cyclosporine resulted in an 8.7-fold increase in Atorvastatin AUC. In cases where co-administration of Atorvastatin with cyclosporine is necessary, the dose of Atorvastatin should not exceed 10 mg. (Pfizer, 2009)

1.10.4 Inducers of cytochrome P450 3A4:

Concomitant administration of Atorvastatin with inducers of cytochrome P450 3A4 (e.g. Favirenz, Rifampin) can lead to variable reductions in plasma concentrations of Atorvastatin. Due to the dual interaction mechanism of Rifampin, simultaneous co-administration of Atorvastatin with Rifampin is recommended, as delayed administration of Atorvastatin after administration of Rifampin has been associated with a significant reduction in Atorvastatin plasma concentrations. (Pfizer, 2009)

1.11 Adverse effects:

Atorvastatin includes-

- Weakness
- Insomnia, dizziness
- Chest pain, Peripheral edema
- Rash
- Abdominal pain, Constipation, Diarrhea, Dyspepsia, Flatulence, Nausea
- Urinary tract infection
- Arthralgia, Myalgia, Back pain, Arthritis
- Sinusitis, Bronchitis, Pharyngitis, rhinitis
- Infection, Flu like syndrome, Allergic reaction (Pfizer, 2009)

1.12 Contraindications:

Atorvastatin is absolutely contraindicated in pregnancy; it is likely to cause harm to fetal development because of the importance of cholesterol and various products in the cholesterol biosynthesis pathway for fetal development, including steroid synthesis and cell membrane production. Nursing mothers are not recommended to take atorvastatin due to the possibility of adverse reactions in nursing infants, since experiments with rats indicate atorvastatin is likely to be secreted into human breast milk. Atorvastatin also contraindicate with active liver disease such as cholestasis, hepatic encephalopathy, hepatitis, and jaundice. Others are unexplained elevations in AST or ALT levels and breastfeeding. (Pfizer, 2009)

1.13 Precautions:

1.13.1General:

Before instituting therapy with Atorvastatin, an attempt should be made to control hypercholesterolemia with appropriate diet, exercise, and weight reduction in obese patients, and to treat other underlying medical problems.

1.13.2 Information for Patients:

Patients should be advised to report promptly unexplained muscle pain, tenderness, or weakness, particularly if accompanied by malaise fever. (Pfizer, 2009)

1.14 Quality control of Drug:

Quality control is an essential operation of the pharmaceutical industry. Drugs must be marketed as safe and therapeutically active formulations whose performance is consistent and predictable. New and better medicinal agents are being produced at an accelerated rate. At the same time more exacting and sophisticated analytical methods are being developed for their evaluation. Requirements governing the quality control of pharmaceuticals in accordance with the Canadian Food and Drugs Act are cited and discussed.(Drugs.com, 2013)

1.15 Importance of quality control study:

Quality must be built into a product and process design and it is influenced by the physical plant design, space, ventilation, cleanliness, and sanitation during routine production. The product and process design begins in research and development, and includes Preformulation and physical, chemical, therapeutic and toxicological considerations. It considers materials, in process and product control, including specifications and test for the active ingredients, the excipients and product itself, specific stability procedure for the product, freedom from microbial contamination and proper storage of the product provide functional protection of the product against such factors as moisture, oxygen, light, volatility, and drug package Quality must be built into a product and process design and it is influenced by the physical plant interaction (Drugs.com, 2013)

1.15.1 Quality control parameters for solid dosage form:

1. Hardness test
2. Weight variation test
3. Friability test
4. Disintegration test
5. Dissolution test

1.16 Dissolution study:

Dissolution is a test used by the Pharmaceutical industry to characterize the dissolution properties of the active drug, the active drug's release and the dissolution from a dosage formulation. Drugs administered orally in solid dosage forms, such as tablet or capsules, must dissolve in the contents of the gastrointestinal tract before drug absorption can occur. Often the rate of drug absorption is determined by the rate of drug dissolution from the dosage form. Therefore, if it is important to achieve high peak blood levels of a drug, it will usually be important to obtain rapid drug dissolution from the dosage form. (Drugs.com, 2013)

1.16.1 Importance of dissolution study:

Dissolution rate may be defined as amount of drug substance that goes in the solution per unit time under standard conditions of liquid/solid interface, temperature and solvent composition. It can be considered as a specific type of certain heterogeneous reaction in which a mass transfer results as a net effect between escape and deposition of solute molecules at a solid surface.

1. Results from in-vitro dissolution rate experiments can be used to explain the observed differences in in-vivo availability.
2. Dissolution testing provides the means to evaluate critical parameters such as adequate bioavailability and provides information necessary to formulator in development of more efficacious and therapeutically optimal dosage forms.
3. Most sensitive and reliable predictors of in-vivo availability.
4. Dissolution analysis of pharmaceutical dosage forms has emerged as single most important test that will ensure quality of product.
5. It can ensure bioavailability of product between batches that meet dissolution criteria.
6. Ensure batch-to-batch quality equivalence both in-vitro and in-vivo, but also to screen formulations during product development to arrive at optimally effective products.
7. Physicochemical properties of model can be understood needed to mimic in-vivo environment.
8. Such models can be used to screen potential drug and their associated formulations for dissolution and absorption characteristics.
9. Serve as quality control procedures, once the form of drug and its formulation have been finalized. If hydrophobic drug than the sodium lauryl sulphate can be added into simulated fluid to solublize the drug.(Lachman L., Liberman H.A, Kanig J.L. 2008)

The process involves in dissolution of solid dosage form:

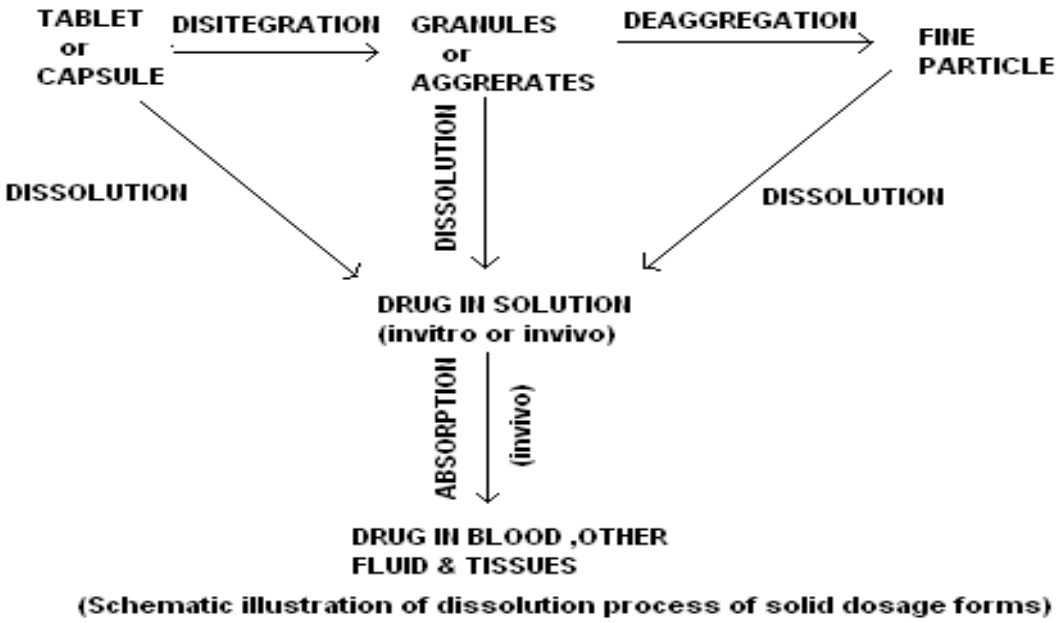

Fig 3: Dissolution process

1.16.2 Factors affecting dissolution rate:

1. Physicochemical Properties of Drug.
2. Drug Product Formulation Factors.
3. Processing Factors.
4. Factors Relating Dissolution Apparatus.
5. Factors Relating Dissolution Test Parameters (Lachman L., Liberman H.A, Kanig J.L. 2008)

1.17 INN (International Nonproprietary Names) drug:

International Nonproprietary Names (INN) facilitates the identification of pharmaceutical substances or active pharmaceutical ingredients. Each INN is a unique name that is globally recognized and is public property. A nonproprietary name is also known as a generic name. WHO has a constitutional mandate to "develop, establish and promote international standards with respect to biological, pharmaceutical and similar products". The World Health Organization collaborates closely with INN experts and national nomenclature committees to select a single name of worldwide acceptability for each active substance that is to be marketed as a pharmaceutical. To avoid confusion, this could jeopardize the safety of

patients pharmaceutical. To avoid confusion, which could jeopardize the safety of patients, trade-marks should neither be derived from INNs nor contain common stems used in INNs. (Umesh V. Banaker 2001)

The aim of the INN system has been to provide health professionals with a unique and universally available designated name to identify each pharmaceutical substance. The existence of an international nomenclature for pharmaceutical substances, in the form of INN, is important for the clear identification, safe prescription and dispensing of medicines to patients, and for communication and exchange of information among health professionals and scientists worldwide. (Umesh V. Banaker 2001)

INN has to be distinctive in sound and spelling, and should not be liable to confusion with other names in common use. To make INN universally available they are formally placed by WHO in the public domain, hence their designation as "nonproprietary". They can be used without any restriction whatsoever to identify pharmaceutical substances. (Umesh V. Banaker 2001)

1.18 Method Development:

If a USP method is available for the product, then the dissolution testing should be conducted using the USP method. If the USP method is not available, then the dissolution testing can be conducted using a method recommended by the FDA (FDA- recommended method). The FDA posts a list of its recommended dissolution method. If the recommended method is inadequate for the product then one needs to develop a dissolution method.

1.19 Aim & objective of the study:

The objective of the study is to -

- To compare the differences in dissolution rate, when Atorvastatin is given alone and when Atorvastatin is given with Metformin HCL, Multivitamin.
- To determine the Drug-Drug interaction, when Atorvastatin is given with Metformin HCL and Multivitamin.
- If Drug interaction occurs then is it increasing or decreasing the dissolution rate, this is determined.
- It is a very important test and considered the rate limiting step in the sequence of steps leading to absorption of the drug into systemic circulation. Absorption is the process of

transporting the drug substances from the gastrointestinal lumen into the systemic circulation. It is the first step before the distribution, metabolism and elimination (ADME) properties of drugs in the human body.

- An important feature of drug quality assurance includes the ability to confirm that the correct manufacturing procedures have been followed for a given batch, that the product performs effectively throughout its shelf life and that batch-to-batch reproducibility of the product meets regulatory requirements. The FDA guidance for industry indicates that for highly soluble drugs a single point dissolution test specification of 85% in 60 min or less is sufficient as a routine quality control test for batch-to-batch uniformity. Commercially, dissolution testing is used to confirm product consistency and to evaluate the quality of the product during its shelf life and to assess post approval changes.

1.20 Significance of the study:

Atorvastatin is an INN (International non proprietory drugs). This means the dissolution profile is not given in the BP (British Pharmacopoiea) or USP (United States pharmacopoeia). If there is a USP dissolution method available then dissolution testing data using USP method may be adequate for the submission. When there is no USP dissolution method for the product but there is a FDA recommended method, dissolution testing using the FDA recommended method may be adequate. If the percentage dissolved more than 85 then the brand is effective to give therapeutic activity. According to the British Pharmacopoiea it is said that if at least two tablets form a batch gives 50% dissolution, Dissolution test can be passed. From this study we can determine the dissolution rate of Atorvastatin, when it is given alone and when it is given as a combination of Atorvastatin Calcium-Metformin HCL-Multivitamin. From this study we also can determine, Drug –Drug interaction is occurring or not. If interaction happens does it have an effect of dissolution rate or not, it can also be determined.

Before doing the in vitro dissolution study, I collected some prescriptions from the nearest hospitals that these drugs are given at a same time or not. And I found out that they are given at a same time. The figure of some prescriptions is given below:-

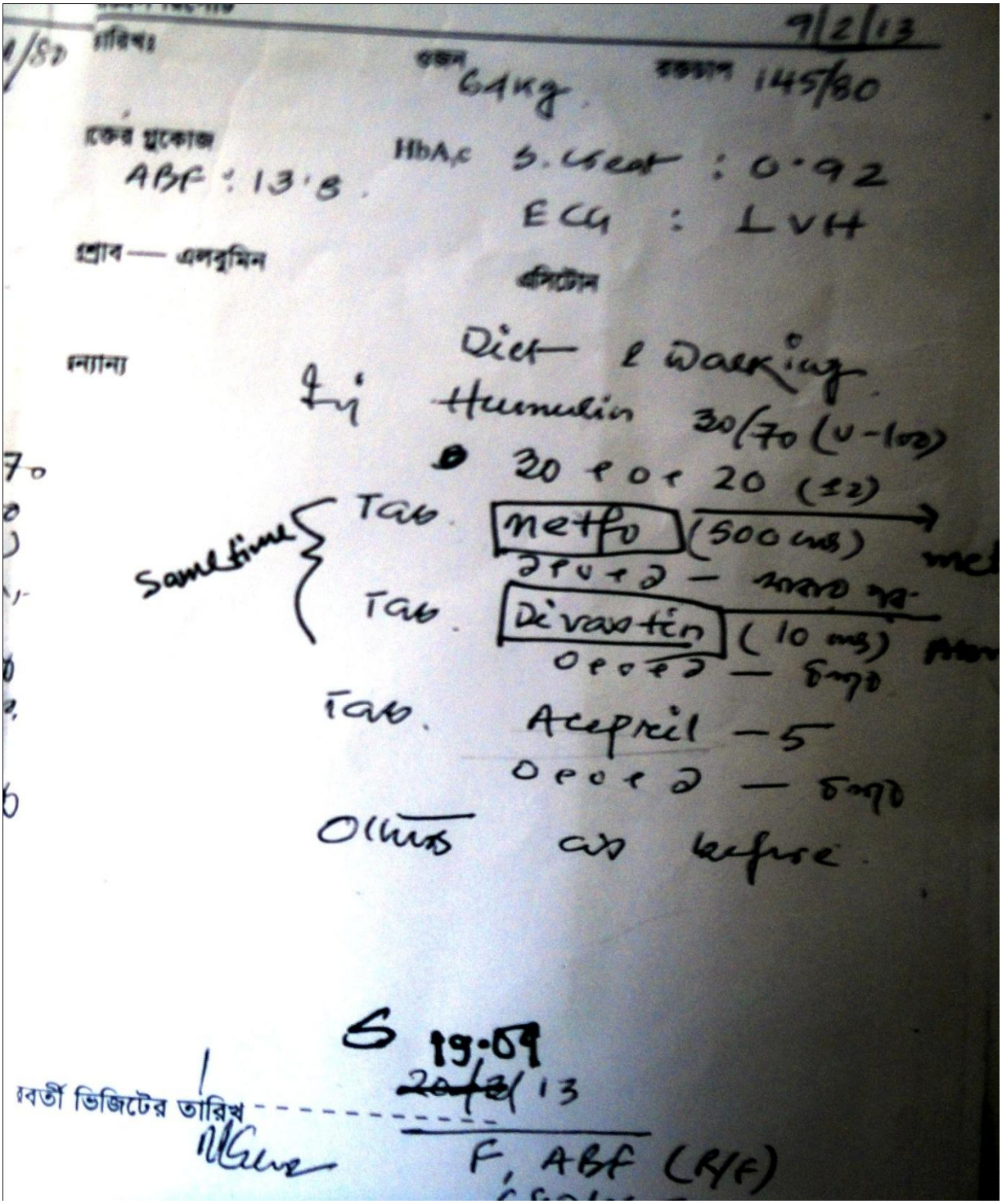

Fig 4: Prescriptions

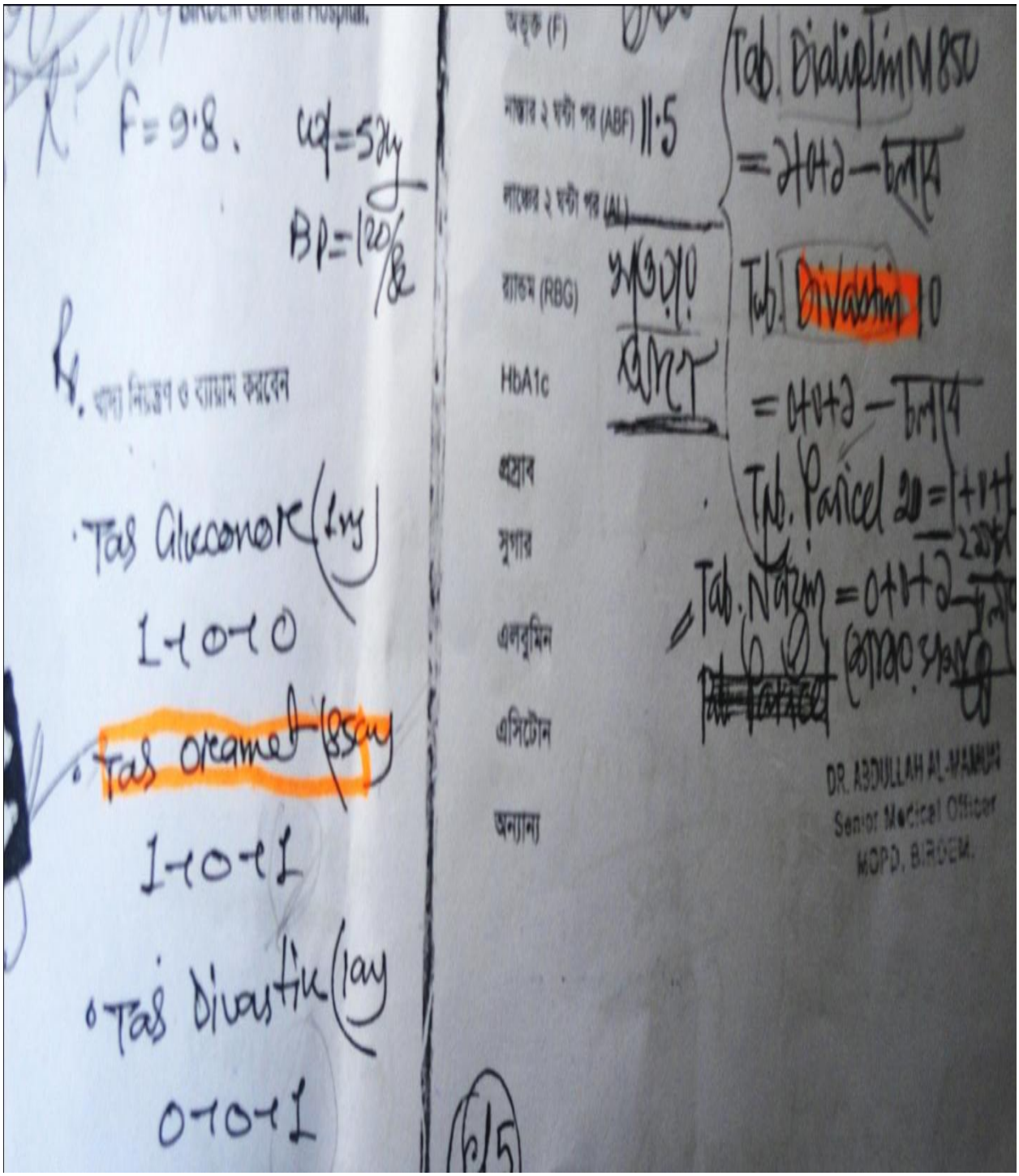

Fig 5: Prescriptions

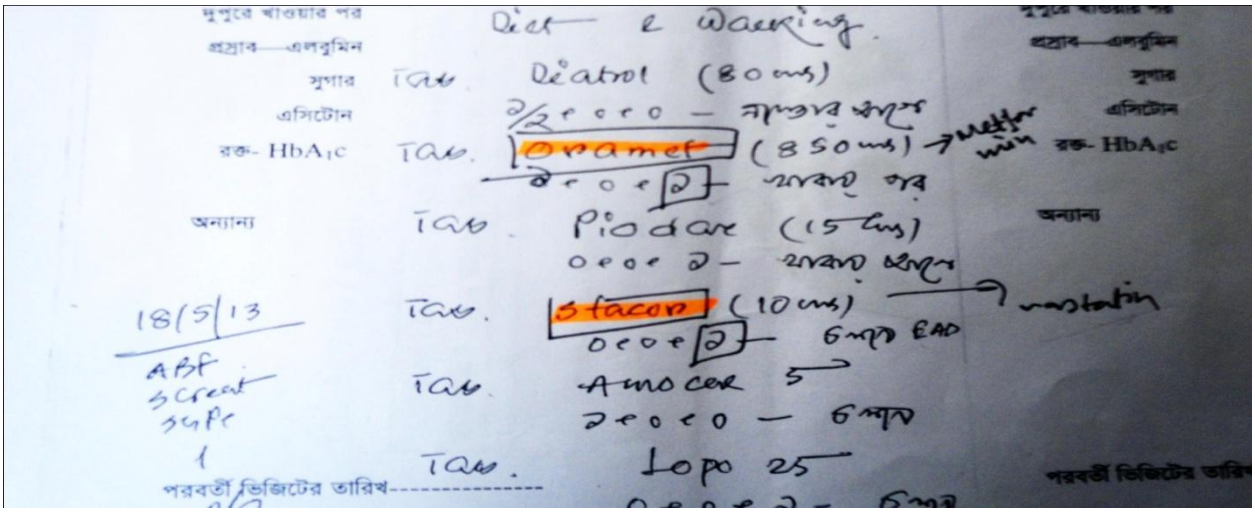

Fig 6: Prescriptions

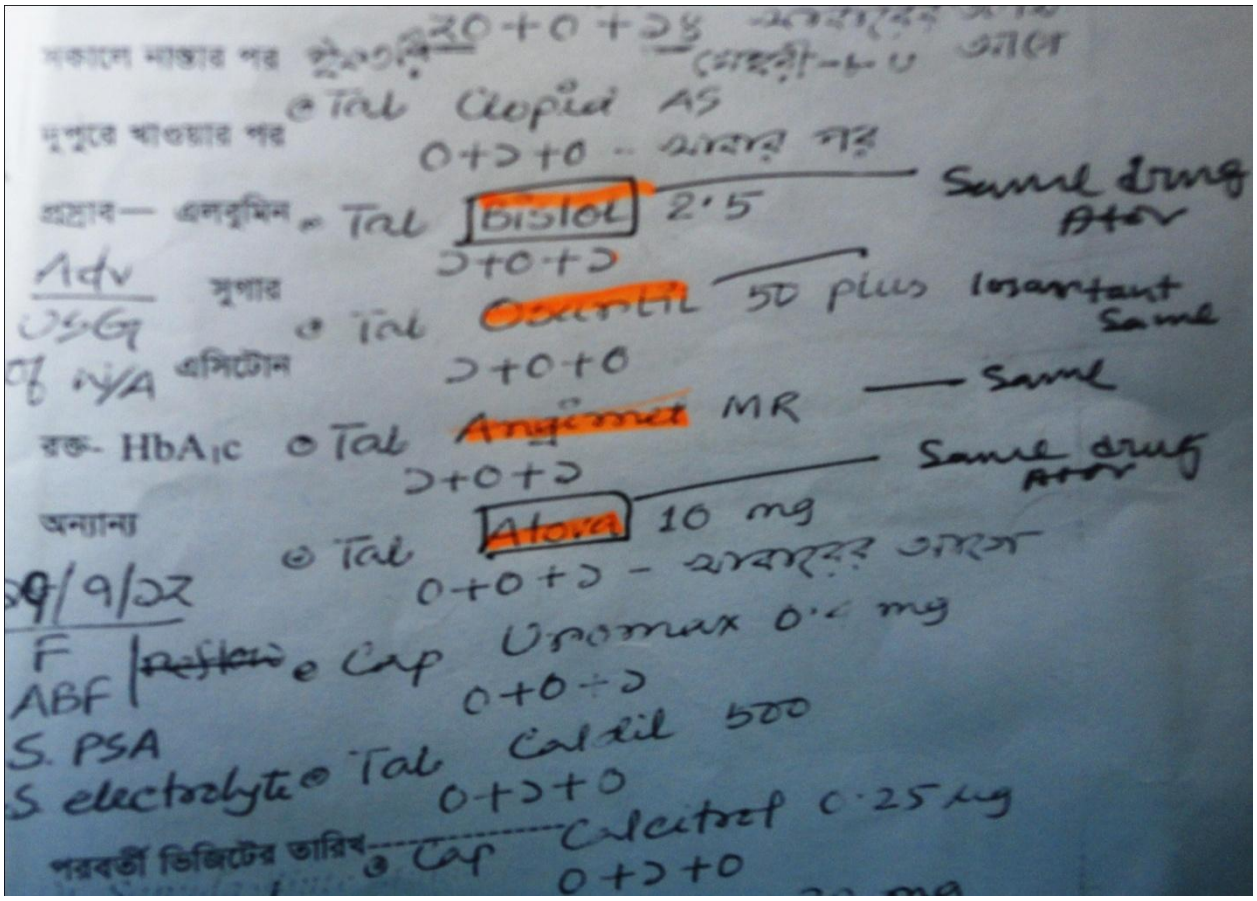

Fig 7: Prescriptions

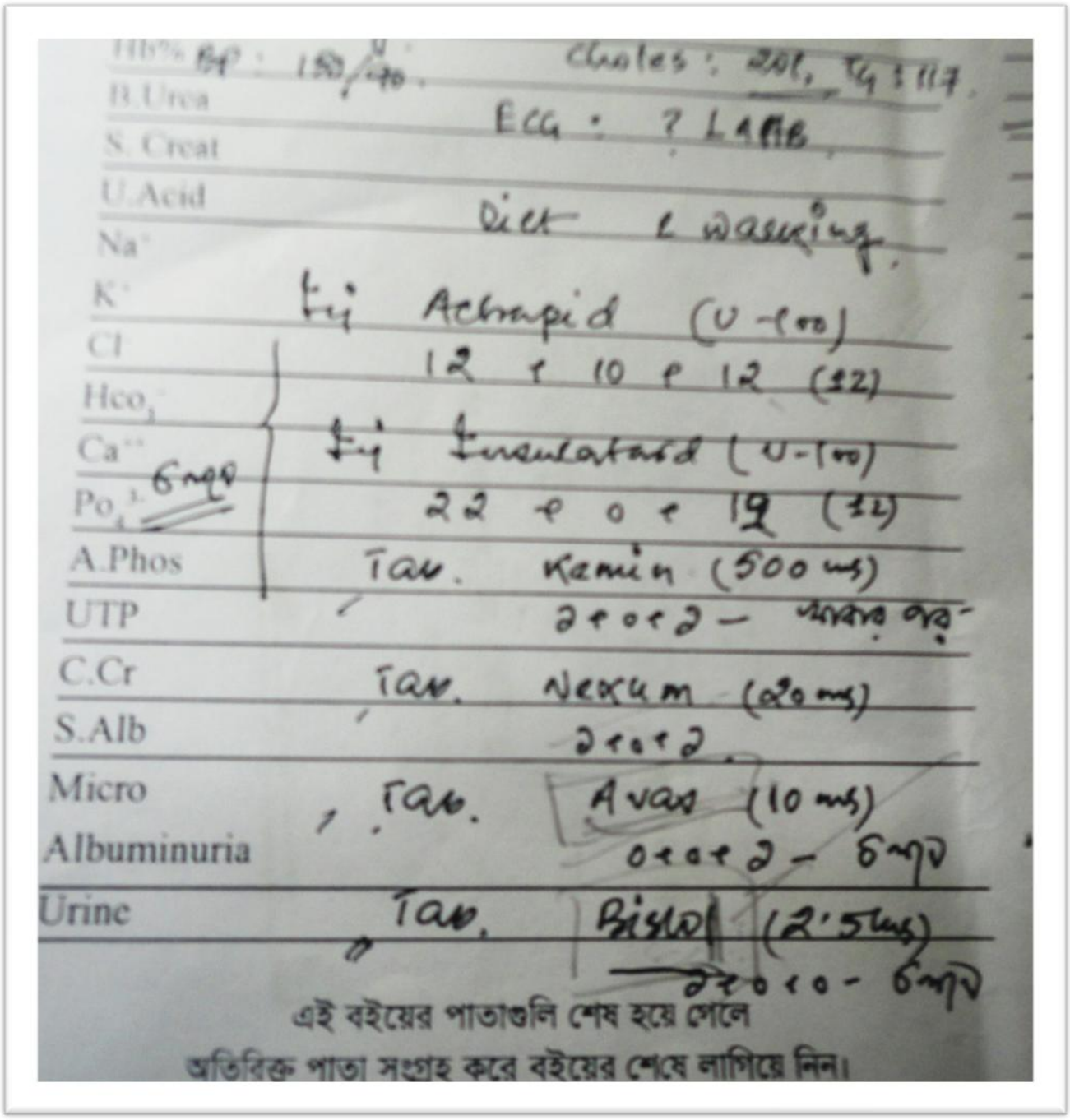

Fig 8: Prescriptions

CHAPTER: 2

LITERATURE REVIEW

2.1 Design, Development and Evaluation of Immediate Release Drug Combination:

Therapeutic success of any therapy depends on the patient's compliance toward the therapy. Tablets are the most popular dosage form because of its unique properties such as ease of administration, low cost and non-invasive therapy etc. The present study aims to develop and evaluate to provide polytherapy through a single tablet in which combination of Atorvastatin (antihyperlipidemic) and Gliclazide (antidiabetic) were used. Since Atorvastatin has t1/2 is near about 5 hours so its release was retarded. H.P.M.C. was used as a retardant material. Two grades of H.P.M.C. i.e. - H.P.M.C. 4000cps and H.P.M.C. 100cps were used. The tablets were prepared by wet granulation method and were compressed with punches of diameter 12.4mm. Granules property and tablets characteristics were evaluated following standard procedure. In vitro dissolution studies were conducted in USPXXIII apparatus at 50 rpm up to 8 hours. First two hour of dissolution study was carried out using medium 0.1N HCI and from 3rd to 8th hour the study was done using medium phosphate buffer of PH 6.8. For Atorvastatin only two hours of dissolution study was performed and its release was found to be 97.6%. For Gliclazide dissolution study was performed up to complete 8 hour and its release was found to be 89.314% and release rate was found to be nearly similar to marketed product. (Ugandhar, C. 2011)

2.2 Developing Methods to Compare Tablet Formulation of Atorvastatin:

Atorvastatin (ATV) is an antilipemic drug of great interest to the pharmaceutical industry. ATV does not appear in the monographs of Brazilian pharmacopoeia, and analytical methodologies for its determination have been validated. The chromatographic conditions used included: RP-18 column-octadecylsilane (250 x 4.6 mm, 5 mm), detection at 238 nm, mobile phase containing 0.1% phosphoric acid and acetonitrile (35:65% v/v), flow at 1.5 mL min-1, oven temperature at 30oC, and injection volume of 10 mL. ATV is classified as a class II product, according to the biopharmaceutical classification system. As such, a dissolution test was proposed to evaluate pharmaceutical formulations on the market today, under the following conditions: water as a dissolution medium, 1000 mL as a volume, paddle apparatus at a rotation speed of 50 rpm, 80% (Q) in 15 minutes with UV spectrophotometer readings at 238 nm. In the pattern condition proposed as the ideal dissolution test, which appropriately differentiates amongst formulations, the generic product was not considered pharmaceutically equivalent; however, in other less differential dissolution methods, which

also fall within appropriate legal parameters, this product could come to be regarded as generic.(*Oliveira, M., Lacerda, C., Bonella, A., 2012*)

2.3 Comparative in vitro Bioequivalence Analysis of Some Generic Tablets Of Atorvastatin, a BCS Class II Compound:

This study was aimed to assess the bioequivalence of ten generic Atorvastatin tablets from different manufacturers using in vitro dissolution and membrane permeability studies. Other general quality parameters of these tablets like weight variation, hardness, friability, disintegration time were also determined according to established protocols. The active ingredients were assayed by a validated HPLC method. All brands complied with the official specification for friability and disintegration time but two brands did not comply official specification for uniformity of weight. Assay of Atorvastatin tablets revealed that all samples contained over 98% (w/w) of labeled potency. The dissolution profiles showed inter brand and intra brand variability. Only four samples attained 70%dissolution within 45 min. Membrane permeability rate of selected brands were found to be proportional to the invitro dissolution rate. The test results were subjected to statistical analysis to compare the dissolution profile. A model independent approach of difference factor (f1), similarity factor (f2) and dissolution efficiency (%DE) were employed and the data indicated that only 4 brands may be used interchangeably.(Oishi, T., Nimmi, I., Islam, A., 2011)

2.4Evaluation of in vitro Equivalence for Tablets Containing the Poorly Water Soluble Compound Atorvastatin:

This paper describes the evaluation of the in vitro equivalence of tablets containing a poorly water-soluble compound, Atorvastatin, marketed in Bangladesh under biowaiver conditions. Drug release was compared with that of a reference product. The in vitro equivalence test was carried out in three different media (pH 1.2, pH 4.5, and pH 6.8). Test results were subjected to statistical analysis to compare the dissolution profiles. Model-independent approaches of difference factor ($f1$), similarity factor ($f2$), and dissolution efficiency (%DE) were employed. Dissolution profiles of test and reference (innovator) Atorvastatin are equivalent at pH 6.8 without statistical treatment. The test products are equivalent at pH 4.5 ($f1 < 15$ and $f2 > 50$) and not equivalent at pH 1.2 ($f1 > 15$ and $f2 < 50$). Other general quality parameters of these tablets (e.g., weight variation, crushing strength, friability, and disintegration time) were also determined according to established protocols,

and test results were within the limit. (Popy, F., Dewan, I., Most. Nazma Parvin, N., Islam, A., 2012)

2.5 Enhancement of Solubility and Dissolution rate Of Different Forms of Atorvastatin Calcium in Direct Compression Tablet Formulas:

The bioavailability of Atorvastatin is one of the key parameters for its therapeutic use and

dependent on the form of the Atorvastatin calcium to be used in the pharmaceutical formulation (amorphous, crystalline or a mixture of both). The patient should take a constant therapeutic daily dose, regardless to the pharmaceutical formulation of the Atorvastatin calcium. The major finding of this study was that the addition of buffering and/or alkalizing agent will dramatically increase both, the solubility and dissolution rate of Atorvastatin calcium regardless to the form (crystalline , amorphous or a mixture of both)used in the preparation of the direct compression formulas. The results also showed that it was possible to provide therapeutic equivalence of Atorvastatin calcium in the pharmaceutical formulation regardless to the form used in the preparation of the direct compression formulas since it was observed that addition of a buffering or alkalizing agent that can provide a pH equal to or greater than (pKa +1), i.e. (pH $\geq$ 6) can enhance both solubility and dissolution rate of Atorvastatin Calcium different forms. (Salam, W., Lupuleasa, D., 2009)

2.6 Enhancement of Atorvastatin Tablet Dissolution using Acid Medium:

In this study some generic commercial products of Atorvastatin tablets were evaluated by dissolution test in acid medium by comparing with that of parent drug Lipitor of Pfizer Company. Some of solubilizing agents were studied in formulation of Atorvastatin tablet including; surface active agent and PEG 6000 .The most effective factor was the use of PEG6000 in formulation of Atorvastatin tablet which improved the dissolution and the results of dissolution profile of formulated tablet in this work was bioequivalent to that of Lipitor .The quantitative analysis of this work was performed by using reversed phase liquid chromatography and a proper mixture of mobile phase which give a retention time for Atorvastatin about 6 minutes .(Kahtan, J., 2010)

2.7 Formulation and Evaluation of Bilayer tablets of Atorvastatin and Nicotinic acid in Different Media:

The aim of the present study is to formulate and evaluate the bilayer tablets using different media, to know the release studies in which media the release of the drug is in controlled manner. The bilayer tablets of Atorvastatin (AT) calcium and Nicotinic acid (NA) were prepared to give Atorvastatin as immediate release and controlled release of Nicotinic acid. Bi-Layer tablets consists of two layers i.e.immediate release layer containing of disintegrants like croscarmellose sodium and Cross-Povidone and controlled release layer containing HPMCK100M as retard layer. Combination of Atorvastatin and nicotinic acid is accepted to bring down cholesterol levels. Three different formulations were prepared by wet granulation method which consists of various disintegrants and polymer in different ratios namelyATNA1, ATNA2 and ATNA3.

The tablets were evaluated for various parameters like weight variation, thickness, hardness and Friability. The release studies were carried out using different media i.e. buffer PH 7.4, 6.8, 4.5 and 0.1NHCl using USP dissolution testing apparatus II (paddle type) for 12hrs.The controlled release layer of Nicotinic acid formulation containing HPMC K100 shows optimized drug in 0.1N HCl within 12 hours. The layer of Atorvastatin containing Cross-Povidone showed satisfactory release from dosage forms. Hence, from the above study we conclude that ATNA2 has shown good release in 0.1N HCl compared to other media with different ratios of polymers. (Shailesh Kumar, S., Rao, V. B., Kannappan, N., Dutta, A., 2009)

2.8 Formulation and stabilization of Atorvastatin Tablets:

The present study is planned to develop Atorvastatin calcium amorphous into immediate release tablets. Pre-formulation study and drug excipients compatibility study was done initially and the results obtained were directs the way and method of formulation. Preformulation and drug excipients compatibility study, prototype formulation carried out for the highest dose of Atorvastatin calcium (80 mg) and optimized to get the final formula. Atorvastatin calcium (amorphous) is highly susceptible to hydrolysis and oxidation. So wet granulation method was avoided. All the mentioned batches were done by dry granulation method by roller compaction. Granules were evaluated for tests such as loss on drying (LOD), bulk density, tapped density, compressibility index and Hauser’s ratio and sieve analysis before compression. Tablets were

tested for weight variation, thickness, hardness, friability and dissolution. In vitro dissolutions performed and Formulation 1 (F1) and Formulation 2 (F2) values were calculated. Dissolution profile ofF5 was matched perfectly with marketed (innovator) formulation and F2 value was found to be excellent. Also the impurity profile and stability result of F5 was found to be excellent. It can be concluded that the immediate release tablet was beneficial for delivering the drug which needs faster release to achieve the immediate action.(Wankhede, S. V., Krishnaprasad, M., Manjunath, S., Debnath, S., 2010)

2.9 Water Solubility Enhancement of Atorvastatin by Solid Dispersion Method:

Atorvastatin is currently used as Calcium salt for the treatment of hypercholesteromia.It. It is insoluble in aqueous solution of pH 4 and slightly soluble in water and pH7.4 phosphate buffer. In the present study an attempt was made to enhance the solubility and dissolution characteristics of Atorvastatin Calcium using solvent evaporation method. HPMC was used as polymer in different drug to polymer ratios. From the study it was found that HPMC at e drug to polymer ratio of 2:1 improves the water solubility of the drug by 2 folds when prepared as solid dispersions by solvent evaporation method. (Uddin, R., 2010)

2.10 Development of film coated Atorvastatin Calcium tablet using Opadry-OY:

The aim of this study was to develop and evaluate the stability of film coated Atorvastatin Calcium (AtC) tablets using Opadry-OY-B-28920. AtC uncoated tablets were developed and manufactured through the wet granulation process. Opadry-OY-B-28920 white aqueous coating dispersion was used as film coating material. The film coated tablets were completely disintegrated within 10 minutes in water media, it was also completely dissolved (more than 85% of the drug was released) within 30 minutes in pH 6.8 buffer solutions. The film coated tablets were studied under both long term and accelerated stability study and the results showed no significant variation in physical characteristics, color, hardness, no obvious defects or signs of peeling or chipping. These results reflect that the film coated system Opadry-OY-B-28920 can be successfully used in order to produce AtC film coated tablet that is protected from environmental conditions such as light and humidity. (Jodeh, S., 2013)

CHAPTER: 3

METHOD AND MATERIALS

3.1 Sample collection:

To determine the Drug-Drug interaction, three different oral drugs were collected and bought from the nearest drug store for in vitro dissolution study. The generic name of drug, brand name and companies names are given below:-

Table 1: Samples and Manufacturers name

Serial no.	Generic name (oral dosage form)	Brand name	Manufacturer Companies name
1.	Atorvastatin Calcium	Lipicon®10	Eskayef Bangladesh Ltd
2.	Metformin HCL	Oramet	Drug International
3.	MultiVitamin	Aristovit-M	Beximco Pharmaceutical Ltd

3.2 Equipments:

To determine the Drug –Drug interaction of Atorvastatin with Metformin HCL and MultiVitamin in vitro dissolution study was incorporated. The following equipments were used to run the dissolution test.

Table 2: List of equipments, source and their origin

Serial no	**Equipments name**	**Source**	**Origin**
1.	UV Spectroscopy	UV-1800 SHIMADZU	JAPAN
2.	Dissolution Tester	LABINDA-8000	INDIA
3.	Analytical Balance	PRECISA XB120A	SWITZERLAND
4.	pH Meter	HANNA pH-210	PORTUGAL
5.	Double rings filter paper	COPLY INSTRUMENTS	CHINA
6.	Distilled water plant	SMIC	CHINA

3.3 List of Apparatus / glassware used in Dissolution test:

Table 3: List Of glass wares

Serial no	Name of the apparatus
1.	Test tube
2.	Funnel
3.	Spatula
4.	Glass rod
5.	100 ml volumetric flask
6.	1000 ml volumetric flask
7.	100 and 1000 ml beaker
8.	100ml measuring cylinder
9.	1000ml measuring cylinder
10.	Disposable syringe

3.4 Powders used in phosphate buffer preparation:

Table 4: Name and amounts of powders

Name	Amount
Di sodium hydrogen phosphate anhydrous	24.4 gm
Potassium dihydrogen phosphate	11.45gm

3.5 Figures of Instruments:

Fig 9: Labinda DS-8000 Dissolution tester

Fig 10: UV-1800 Spectrometry

Fig 11: Analytical Balance

3.6 Methods:

Criteria of dissolution of tablets:

- Medium: Phosphate buffer, 900 ml, pH 6.8
- Apparatus: USP apparatus-II (Paddle)
- Speed: 75 RPM
- Time: 5, 10, 15, 30 minutes
- Analysis: UV visible spectrometer
- Lambda max: 242 nm
- Temperature: 37 degree Celsius (FDA, 2013)

3.6.1 Preparation of Phosphate buffer:

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

3.6.2 Procedure:

The release rate of Atorvastatin Calcium alone and in a combination with Metformin HCL and Multivitamin was determined by using Tablet Dissolution Tester USP apparatusII (Paddle). The dissolution tester was assembled. Water tank was filled by the water and operating parameters were settled. 900 mL of the distilled water was poured into 6 vessels and run the instrument till the set temperature was attained. 100 mL of the medium was remained for use as the blank. The tablets were placed into the baskets and start run. The dissolution test was performed using 900ml phosphate buffer (pH 6.8) at 37°C and 75 r.p.m. At every 5 min interval samples of 5ml were withdrawn from the dissolution medium and that amount was replaced with fresh medium to maintain the volume constant with disposable syringe. The samples were filtered through a Double rings filter paper. The absorbance of the solutions was measured at 242 nm for drugs by using a UV/Visible double beam spectrophotometer (Shimadzu, Japan). Percentage of drug release was calculated using an equation obtained from the standard curve. The dissolution study was continued for 30 min to get a simulated picture of the drug release in the in-vivo condition and drug dissolved at specified time periods was plotted as percent release versus time (hours) curve. This drug release profiles were fitted into several mathematical models to get an idea of the release mechanism of drugs from the tablets. By giving the three drugs as a combination Drug-Drug interaction was determined. And Atorvastatin has a dissolution effect or not because of other drugs, it was also determined. (BP, 2007)

3.6.3 Calculation:

$$\text{Dissolution \%} = \frac{\text{AS X Wst X 900 X P}}{\text{Ast X 100 x Dose of tablet}} \text{ x 100 \%}$$

Equation 1: Equation for the calculation of % dissolved of Atorvastatin

Where,

AS = Absorbance of sample at 5 minutes,10 minutes,15 minutes,30 minutes

Ast = Absorbance of standard

P = Potency of standard = 97 % or .97

Wst = weight of standard

3.6.4 Preparation of the Standard Solution:

A stock solution is prepared using an analytical balance. Five different percentages of standard solutions were prepared by pure Atorvastatin. These different percentages are 80%, 90%, 100%, 110% and 120% which contain 0.088 mg, 0.099 mg, 0.11 mg, 0.0121mg and 0.132 mg of pure Atorvastatin. After that, some small amount of methanol was added in each five standard samples to dissolve the Atorvastatin. And acetate buffer (pH 4.5) was also added up to 10 ml. No dilution was done. Then measure the absorbance of those solutions at the λmax233 nm.

CHAPTER: 4

RESULTS

4.1 In vitro comparative dissolution study:

Table 5: Absorbance test of only Atorvastatin

Absorbance test of Atorvastatin				
Tablet	Absorbance at 5 min	Absorbance at 10 min	Absorbance at 15 min	Absorbance at 30 min
1	0.032	0.025	0.061	0.37
2	0.021	0.025	0.076	0.49
3	0.022	0.015	0.099	0.421
4	0.028	.037	0.044	0.459
5	0.017	.044	0.183	0.399
6	0.021	0.035	0.019	0.347
Average Absorbance	0.024	0.0302	0.0803	0.414
% Dissolved	2.093%	2.64%	7.01%	36.14%

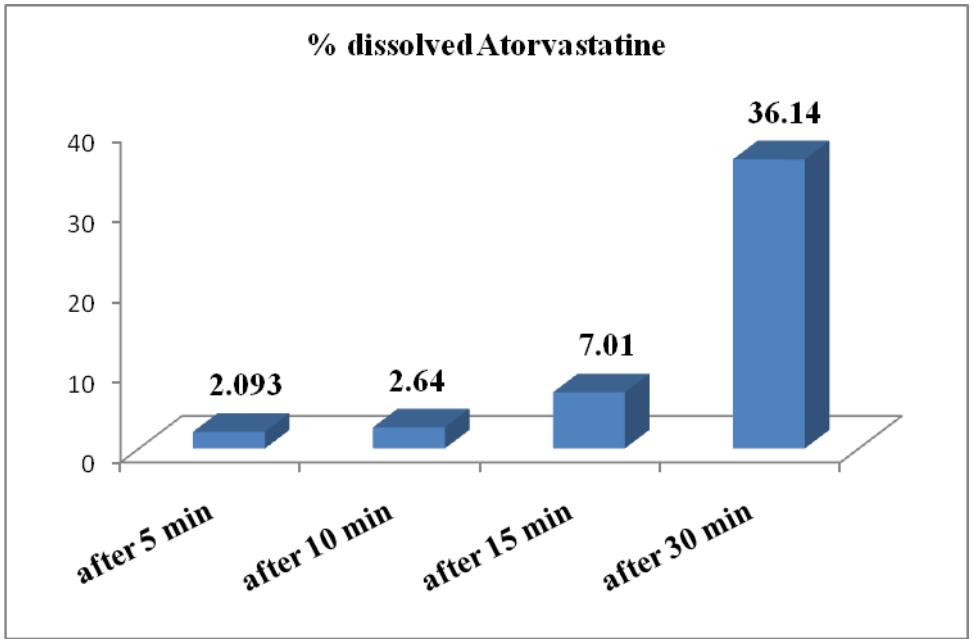

Fig 12: Bar Diagram of dissolution rate of Atorvastatin

Table 6: Absorbance test of Atorvastatin, Metformin and Multivitamin

Absorbance test of Atorvastatin, Metformin and Multivitamin				
Tablet	Absorbance at 5 min	Absorbance at 10 min	Absorbance at 15 min	Absorbance at 30 min
1	0.059	0.038	0.053	0.077
2	0.029	0.036	0.039	0.054
3	0.013	0.047	0.04	0.053
4	0.05	0.052	0.081	0.063
5	0.017	0.038	0.064	0.063
6	0.023	0.034	0.056	0.071
Average Absorbance	0.032	0.041	0.056	0.064
% Dissolved	2.79%	3.58%	4.89%	5.58%

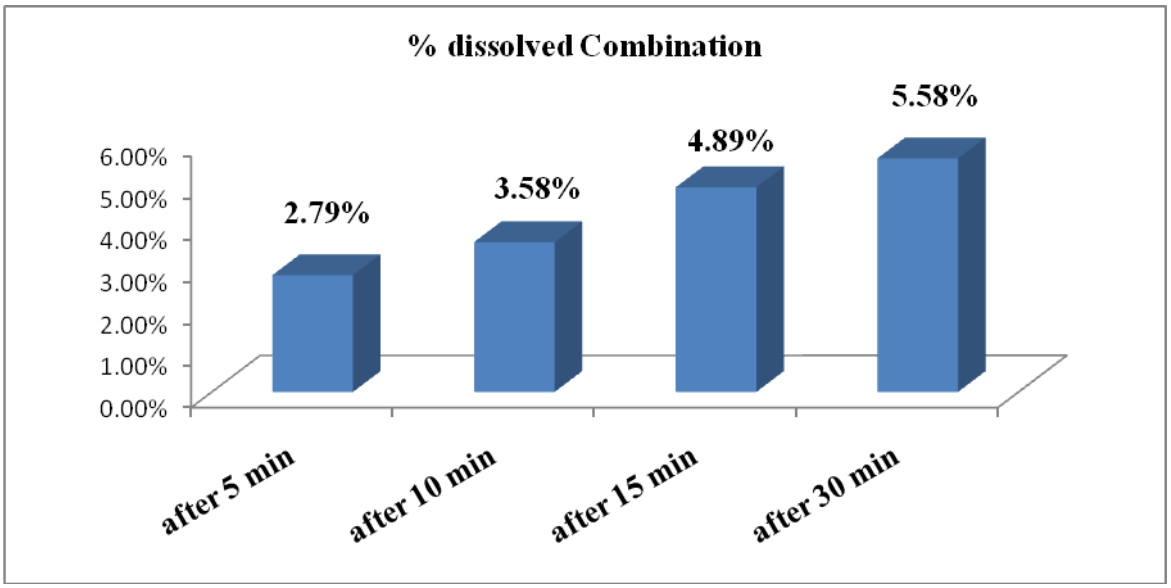

Fig 13: Bar Diagram of dissolution rate of combination

4.2 Calculation:

$$\text{Dissolution \%} = \frac{AS \times Wst \times 900 \times P}{Ast \times 100 \times \text{Dose of tablet}} \times 100\ \%$$

Where,

AS = Absorbance of sample at 5 mint, 10mint, 15 mint and 30 mint

Ast = Absorbance of the standard = .11

P = Potency of standard = 97 % or .97

Wst = weight of standard or .11mg

Dose of the tablet=10mg

4.2.1 Calculation of Atorvastatin:

$$\text{Dissolution \% after 5 min} = \frac{0.024 \times .11 \times 900 \times 0.97}{0 .11 \times 100 \times 10} \times 100\ \%$$

$$= 2.093\%$$

$$\text{Dissolution \% after 10 min} = \frac{0 .0302 \times .11 \times 900 \times 0.97}{0 .11 \times 100 \times 10} \times 100\ \%$$

$$= 2.64\%$$

$$\text{Dissolution \% after 15 min} = \frac{0.0803 \times .11 \times 900 \times 0.97}{0 .11 \times 100 \times 10} \times 100\ \%$$

$$= 7.01\%$$

$$\text{Dissolution \% after 30 min} = \frac{0 .414 \times .11 \times 900 \times 0.97}{0.087 \times 100 \times 10} \times 100\ \%$$

$$= 36.14\%$$

4.2.2 Calculation of Combination:

$$\text{Dissolution \% after 5 min} = \frac{0.032 \times .11 \times 900 \times 0.97}{0 .11 \times 100 \times 10} \times 100\ \%$$

$$= 2.79\%$$

$$\text{Dissolution \% after 10 min} = \frac{0.041 \times .11 \times 900 \times 0.97}{0.11 \times 100 \times 10} \times 100\ \%$$

$$= 4.89\%$$

$$\text{Dissolution \% after 15 min} = \frac{0.056 \times .11 \times 900 \times 0.97}{0 .11 \times 100 \times 10} \times 100\ \%$$

$$= 4.89\%$$

$$\text{Dissolution \% after 30 min} = \frac{0.064 \times .11 \times 900 \times 0.97}{0 .11 \times 100 \times 10} \times 100\ \%$$

$$= 5.59\%$$

4.3 Standard solution of Atorvastatin:

Table7: Absorbance Test of different concentrations of Standard

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

4.4 Standard curve of Atorvastatin:

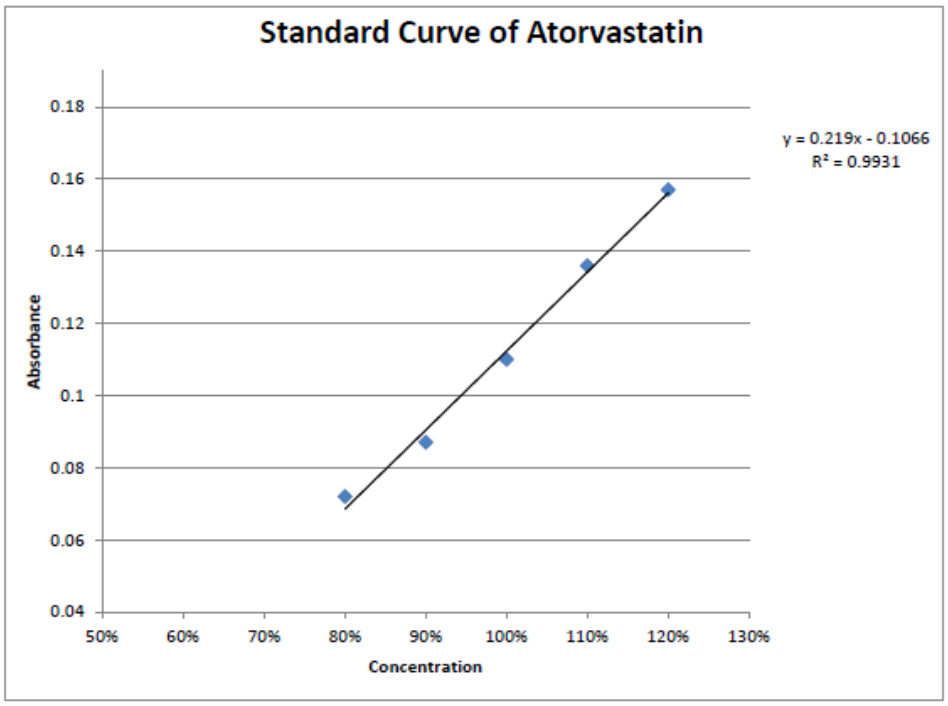

Figure 14: Standard Curve of Atorvastatin

CHAPTER: 5

DISCUSSION

According to the British Pharmacopoeia specifications, in order to pass the dissolution test at least two tablets from each batch must undergo 50% dissolution. But the dissolution rate of Atorvastatin and combination of Atorvastatin, Metformin and Multivitamin did not pass the specifications. If we see the dissolution percentage of Atorvastatin it was only 2.09% after five minutes. At ten minutes the dissolution rate didn't show any significant progress. After thirty minutes dissolution rate was only 36.14% which was unexpected.

Comparison between only Atorvastatin and combination was done in case of dissolution rate. Dissolution rate of Atorvastatin was more than in combination of Atorvastatin, Metformin and Multivitamin. After five minutes the dissolution rate was 2.79% and after 10 minute it was only 3.58%. And at the end after 30 minute it was only 5.58%. So, result says that drug- drug interaction occurred and they gave antagonistic activity and that's why dissolution rate decreased. So that means combination of Atorvastatin, Metformin and multivitamin give antagonistic activity.

CHAPTER: 6

CONCLUSION

Atorvastatin calcium is a synthetic is a lipid lowering drug. Now days it is being highly used for cardiovascular disease, hypercholesterolemia and many other diseases. Most of the patient suffers from diabetes and hypertension both. So physicians prescribe Metformin HCL and Atorvastatin at the same time. That's why this study was done and result showed antagonistic activity. Due to the limitation of the study we couldn't do any experiments to ensure the drug-drug interaction of Atorvastatin, Metformin and Multivitamin, and there also may be analytical error, manufacturing error .So further study needs to be conducted to determine the drug drug interaction.

CHAPTER: 7

REFERENCES

BP. (2007). British Pharmacpoeia (2010 ed., Vol. 4). London, United kingdom: The Stationery Office on behalf of the Medicines and Healthcare Products.

Drugs.com (2013), Atorvastatin Calcium, *Monograph of Atorvastatin*, available at http://www.drugs.com/monograph/atorvastatin-calcium.html

FDA, (2013) Atorvastatin Calcium Tablets, Description, FDA drug information, available at http://www.accessdata.fda.gov

Finkel, Richard; Clark, Michelle A.; Cubeddu, Luigi X (2008) , Lippincott's Illustrated Reviews: Pharmacology, 4th Edition 2008

Jodeh, S., (2013), Development of Film Coated Atrovastatin Calcium Tablet Using opadry-oy, International Journal of Drug Delivery Vol 4, 229-235; available at http://staff.najah.edu/sjodeh/published-research/development-film-coated-atrovastatin-calcium tablet-using-opadry-oy

Kahtan, J., (2010), Enhancement of Atorvastatin Tablet Dissolution Using Acid Medium, Iraqi J Pharm Sci, Vol.19(1) 2010; available at http://www.iasj.net/iasj?func=fulltext&aId=4395

Kumar, S., Rao, V. B., Kannappan, N., Dutta, A., (2009), Formulation and evaluation of atorvastatin calcium and nicotinic acid in a bilayer tablet, Journal of Pharmacy Research ISSN 0974-6943; available at http://www.journaldatabase.org/articles/formulation_evaluation_atorvastatin.html

Lachman L., Liberman H.A, Kanig J.L. 2008, The Theory and Practice of Industrial Pharmacy. 3rd edition, Bombay: Varghese publication. Page 300-804

Lea, A., McTavish D. (2002). The discovery and development of atorvastatin, a potent novel hypolipidemic agent. Available: www.ncbi.nlm.nih.gov/pubmed/12516521

Oishi, T., Nimmi, I., Islam, A., (2011), Comparative in vitro Bioequivalence Analysis of Some Generic Tablets of Atorvastatin, a BCS Class II Compound, Bangladesh Pharmaceutical Journal Vol. 14, No. 1, January 2011 ISSN 0301-4606: available at http://www.bps bd.org/journal/volume14/11.pdf

Oliveira, M., Lacerda, C., Bonella, A., (2012), Developing methods to compare tablet formulations of Atorvastatin, Brazilian Journal of Pharmaceutical Sciences, Vol 48,No 4

Pfizer. (June 2009). Lipitor: Prescribing Information. Website: http://www.meds-help.com/atorvastatin-systemic/description.htm

Popy, F., Dewan, I., Most. Nazma Parvin, N., Islam, A., (2012), Evalution of in-vitro equivalence for tablets containing the poorly water soluble compound Atorvastatin, dissolution technologies, november 2012; available at http://www.dissolutiontech.com/DTresour/201211Articles/DT201211_A04.pdf

Salam, W., Lupuleasa, D., (2009), Enhancement of Solubility and Dissolution rate of different forms of Atorvastatin Calcium in direct compression tablet formulas, FARMACIA, 2009, Vol. 57, 3; available at http://www.revistafarmacia.ro/20093/issue32009art04.pdf

Uddin, R., (2010), Water Solubility Enhancement of Atorvastatin by Solid Dispersion Method, S.J. Pharm, Sci. 3 (2): 43-46 : available at https://www.academia.edu

Ugandhar, C. (2011),Design, developmentand evaluationof immediaterelease drugcombination, Asian Journal of Pharmaceutical and Clinical Research ISSN –0974-2441,vol4

Umesh V. Banaker (2001), Pharmaceutical dissolution testing page :345-425

Wankhede, S. V., Krishnaprasad, M., Manjunath, S., Debnath, S., (2010), Formulation and stabilization of Atorvastatin tablets, Journal of Chemical and Pharmaceutical Research ISSN No: 0975-7384; available at www.jocpr.com