Drug - Drug Interaction study of Atorvastatin in combination by comparative in vitro dissolution

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A Dissertation submitted to the Department of Pharmacy, East West University, in Partial fulfillment of the Requirements for the Degree of Bachelor of Pharmacy

Dedicated

to

My Family and Friends

Declaration by the Candidate

I hereby declare that this dissertation, entitled "***Drug - Drug Interaction study of Atorvastatin in combination by comparative in vitro dissolution***" is an authentic and genuine research work carried out by me under the guidance of Mehreen Rahman, Lecturer, Department of Pharmacy, East West University, Dhaka.

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Certificate by the Invigilator

This is to certify that the dissertation, entitled *"**Drug - Drug Interaction study of Atorvastatin in combination by comparative in vitro dissolution***" is a bonafide research work done by MD. Samiul Islam , in partial fulfillment of the requirement for the Degree of Bachelor of Pharmacy.

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Endorsement by the Chairperson

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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

VLDL - Very Low Density Lipoprotein

IDL - Intermediate Density Lipoprotein

CYP3A4 - Cytochrome P450 3A4

THF - Tetrahydrofuran

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Last of all I would like to thank my parents and friends for their care and encouragement all the time.

Objective of the Study

The objective of the study is to -

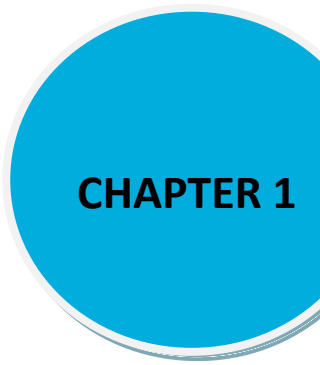
1. Compare the differences in dissolution behavior of Combination of different drugs with Atorvastatin to know the absorbance in different time intervals. It is a very important test and considered the rate limiting step in the sequence of steps leading to absorption of the drug into systemic circulation. 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.
2. Compare the difference in %dissolved of combination of drugs with Atorvastatin single dose.
3. If there is any synergistic or antagonistic activity if combination of different drugs are taken simultaneously with Atorvastatin.
4. Preparing a standard curve of the active ingredient to determine the % dissolved of the brands in different time intervals.

Atorvastatin is an INN drug (International Nonproprietary Name drug). This means the dissolution profile is not given in the BP (British Pharmacopoeia) or USP (United States pharmacopoeia). If there was 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. As it is a poorly soluble drug and the bioavailability is very less, 30% of drug dissolved within 30 minutes will be acceptable.

Abstract

The purpose of this research work was to determine if there is any drug drug interaction in case of combination drugs taken with Atorvastatin at same time by comparative in-vitro dissolution study. Single dose of Atorvastatin was the standard to compare with other drugs taken at the same time with Atorvastatin. The other drugs that was given with Atorvastatin at the same time, selected by sorting out prescriptions of different cardiovascular patients from different hospitals. Some prescriptions were selected where a patient has to administer Atorvastatin with Sedil® Nidocard® twice daily. So with the dissolution test we can determine the %dissolved of drug hence its bioavailability and to see if there was any interaction with other drugs to increase or decrease %dissolved and bioavailability. The dissolution rate was determined in phosphate buffer pH6.8 using paddles at a speed of 75 rpm. The dissolution assay was determined through UV analysis at maximum wavelength of 242nm respectively.

Keywords: Atorvastatin calcium, Dissolution test, Lipid lowering agent, Phosphate buffer ph6.8, Standard curve.



Introduction

1.1 Introduction to Arotvastatin

Atorvastatin, as a synthetic lipid-lowering agent, is an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme (HMG - CoA) reductase which catalyzes the conversion of HMG-Co A to mevalonate, a precursor of sterols , an early rate-limiting step in cholesterol biosynthesis. Atorvastatin is currently used as calcium salt for the treatment of hypercholesterolemia. Atorvastatin calcium ([R-(R*, R*)]-2-(4-fluorophenyl)-β ,γ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrol-1-heptanoic acid, hemi-alcium salt). Is a white to off-white crystalline powder that is insoluble in aqueous solution of pH 4 and below; it is very slightly soluble in water and slightly soluble at pH 7.4 phosphate buffers and acetonitrile, lightly soluble in ethanol and freely soluble in methanol. Atorvastatin calcium is highly susceptible to heat, moisture, a low pH environment and light. Again the amorphous form is many times unstable than its counterpart crystalline form. The intestinal permeability of atorvastatin is high at the physiologically intestinal pH (6 – 6.5). However, it is reported that the absolute bioavailability of atorvastatin is 12% after a 40mg oral dose. The empirical formula of atorvastatin calcium is (C33H34FN2O5)2Ca2+·3H2O with a molecular weight of 1209.42. Its structural formula is shown in the figure 1.1 (Ahjel, Lupuleasa, 2009)

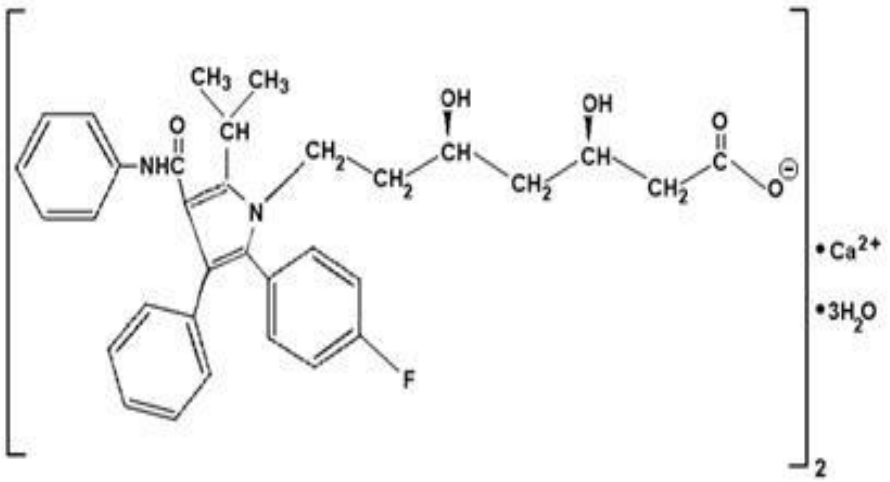


Figure 1.1: Chemical Structure of Atorvastatin Calcium

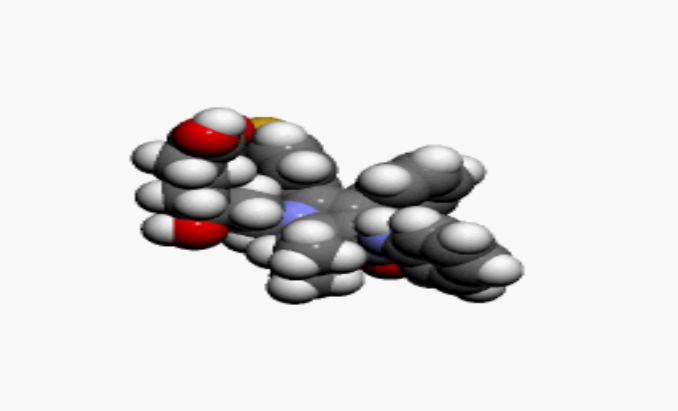


Figure 1.2: 3D Structure of Atorvastatin molecule

1.2 Synthesis of Atorvastatin calcium

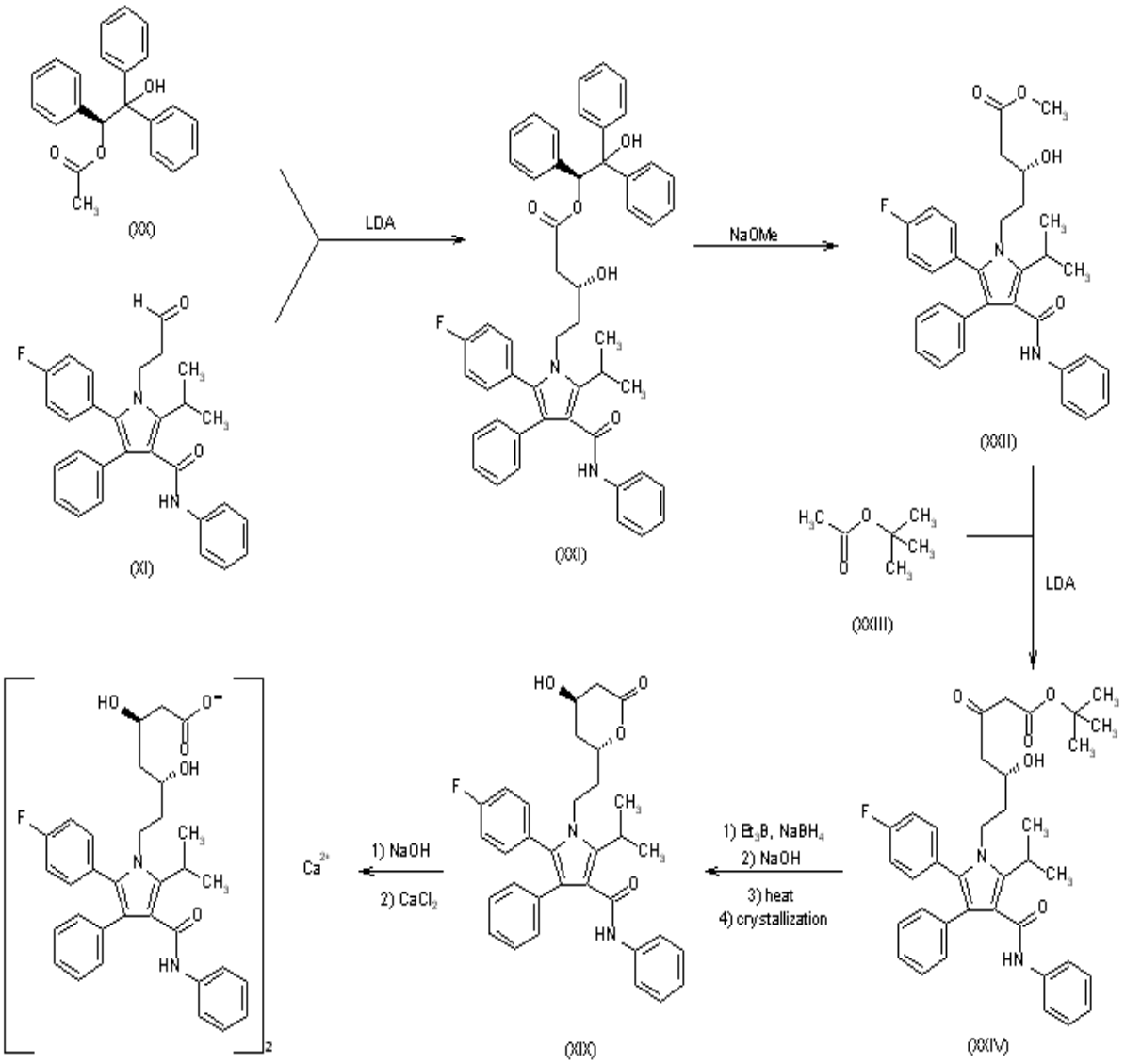


Figure 1.3: Synthesis of Atorvastatin Calcium

The condensation of the previously described aldehyde (XI) with (S)-(+)-2-acetoxy-1,1,2-triphenylethanol (XX) by means of lithium diisopropylamide (LDA) in THF gives 5-[2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(N-phenylcarbamoyl)pyrrol-1-yl]-3(R)-hydroxypentanoic acid 2-hydroxy-1(S),2,2-triphenylethyl ester (XXI), which is trans-esterified with sodium methoxide in methanol/THF yielding the expected methyl ester (XXII). The condensation of (XXII) with tert-butyl acetate (XXIII) by means of LDA in THF affords (R)-7-[2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(N-phenylcarbamoyl) pyrrol-1-yl]-5-hydroxy-3-oxoheptanoic acid tert-butyl ester (XXIV), which is reduced with triethylborane and NaBH4 in THF, hydrolyzed with NaOH, lactonized by heating in refluxing toluene and finally submitted to fractional crystallization in order to separate the two diastereomers of the obtained lactone, (R,R) and (R,S). The (R,R)-diastereomer (XIX), already obtained, is finally treated with NaOH and then with CaCl2.

1.3 Atorvastatin - Clinical Pharmacology

1.3.1 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. (Villa, Pratley, 2010)

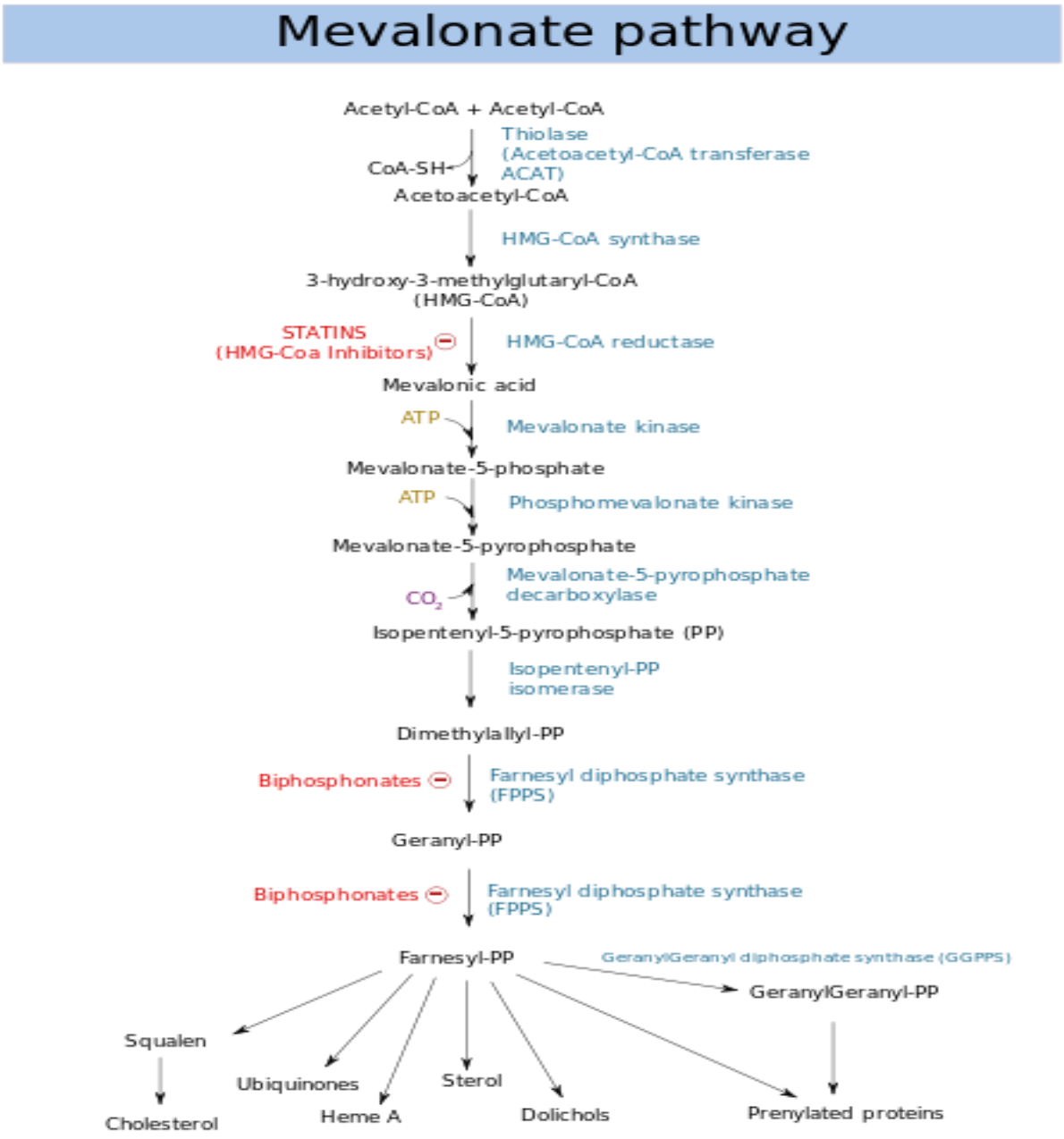


Figure 1.4: Flowchart of Mevalonate pathway

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, nonfamilial 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 increases HDL-C in patients with isolated hypertriglyceridemia. Atorvastatin reduces intermediate density lipoprotein cholesterol (IDL-C) in patients with dysbetalipoproteinemia.

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. (Villa, Pratley, 2010)

1.3.2 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

1.3.3 Pharmacokinetics

1.3.3.1 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 .

1.3.3.2 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. Based on observations in rats, Atorvastatin is likely to be secreted in human milk .

1.3.3.3 Metabolism: Atorvastatin is extensively metabolized to ortho- and parahydroxylated derivatives and various beta-oxidation products. *In vitro* inhibition of HMG-CoA reductase by ortho- and parahydroxylated metabolites is equivalent to that of Atorvastatin. Approximately 70% of circulating inhibitory activity for HMG-CoA reductase is attributed to active metabolites. *In vitro* studies suggest the importance of Atorvastatin metabolism by cytochrome P450 3A4, consistent with increased plasma concentrations of Atorvastatin in humans following co-administration with erythromycin, a known inhibitor of this isozyme . In animals, the ortho-hydroxy metabolite undergoes further glucuronidation.

1.3.3.4 Excretion: Atorvastatin and its metabolites are eliminated primarily in bile following hepatic and/or extra-hepatic metabolism; however, the drug does not appear to undergo enterohepatic recirculation. Mean plasma elimination half-life of Atorvastatin in humans is approximately 14 hours, but the half-life of inhibitory activity for HMG-CoA reductase is 20 to 30 hours due to the contribution of active metabolites. Less than 2% of a dose of Atorvastatin is recovered in urine following oral administration.

1.3.4 Adverse effects and contraindications

HMG-CoA inhibitors are contraindicated in pregnancy. Limited evidence from animal and human studies indicates that statins should not be taken during pregnancy

Liver dysfunction: Elevations of serum aminotransferase activity (up to three times normal) occur in some patients. This is often intermittent and usually not associated with other evidence of hepatic toxicity. In some patients, who may have underlying liver disease or a history of alcohol abuse, levels may exceed three times normal. This finding portends more

severe hepatic toxicity. A relatively common side effect of the statins (perhaps 1% of patients) is myositis, that is, inflammation of skeletal muscle accompanied by pain, weakness,and high levels of serum creatine kinase. Rhabdomyolysis,i.e., disintegration of muscle with urinary excretion of myoglobin and kidney damage, is serious side effect. The risk of muscle damage is said to increase with simultaneous use of the triglyceride-lowering fibrates.

1.3.5 Physical Parameters of Solid Dosage Forms (Tablet)

Tablets and capsules are the most preferred dosage forms of pharmaceutical scientists and clinicians because they can be accurately dosed and provide good patient compliance, they are easy for companies to manufacture, and they can be produced at a relatively low cost. This popularity of tablets coupled with an increased understanding of the physics of compression and of manufacturing process variables have matured the manufacture of tablets as a science in its own right. (Katzung, 2010)

1.3.6 Drug and food interactions

Interactions with clofibrate, fenofibrate, gemfibrozil, which are fibrates used in accessory therapy in many forms of hypercholesterolemia, usually in combination with statins, increase the risk of myopathy and rhabdomyolysis.

1.3.7 Drug and drug interactions

Co-administration of atorvastatin with one of CYP3A4 inhibitors such as itraconazole, telithromycin, and voriconazole, may increase serum concentrations of atorvastatin, which may lead to adverse reactions. This is less likely to happen with other CYP3A4 inhibitors such as diltiazem,erythromycin, fluconazole, ketoconazole, clarithromycin, cyclosporine, protease inhibitors, or verapamil, and only rarely with other CYP3A4 inhibitors, such as amiodarone and aprepitant. Often, bosentan, fosphenytoin, and phenytoin, which are CYP3A4 inducers, can decrease the plasma concentrations of atorvastatin.

Only rarely, barbiturates, carbamazepine, efavirenz, nevirapine, oxcarbazepine, rifampin, and rifamycin, which are also CYP3A4 inducers, can decrease the plasma concentrations of atorvastatin.

Oral contraceptives increased AUC values for norethindrone and ethinyl estradiol; these increases should be considered when selecting an oral contraceptive for a woman taking atorvastatin.

Antacids can rarely decrease the plasma concentrations of atorvastatin, but do not affect the LDL-C-lowering efficacy.

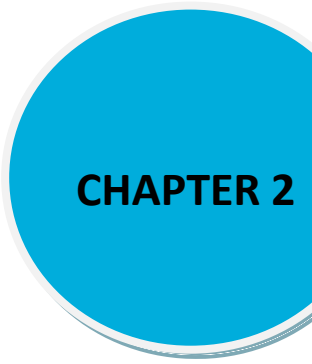
Niacin also is proved to increase the risk of myopathy or rhabdomyolysis.

Statins may also alter the concentrations of other drugs, such as warfarin or digoxin, leading to alterations in effect or a requirement for clinical monitoring.

Digoxin: When multiple doses of atorvastatin and digoxin were co-administered, steady state plasma digoxin concentrations increased by approximately 20%. Patients taking digoxin should be monitored appropriately.

Warfarin: Atorvastatin had no clinically significant effect on prothrombin time when administered to patients receiving chronic warfarin treatment.

Vitamin D supplementation lowers atorvastatin and active metabolite concentrations, yet synergistically reduces LDL and total cholesterol concentrations. Grapefruit juice components are known inhibitors of intestinal CYP3A4. Co-administration of grapefruit juice with atorvastatin may cause an increase in C_{max} and AUC, which can lead to adverse reactions or overdose toxicity. (Pfizer 2009)



Literature Review

2.1 **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 45min. Membrane permeability rate of selected brands were found to be proportional to the in vitro dissolution rate.(Islam, Oishi & Nimmi, 2011)

2.2 Formulation and characterization of atorvastatin calcium liquisolid compacts

The solubility and dissolution properties of any drug are vital determinants of its oral bioavailability. The dissolution rate of poorly soluble, highly permeable (BCS-II) drugs, such as atorvastatin calcium, can be improved by application of the liquisolid (LS) technique. Different liquisolid compacts were prepared using a mathematical model for calculating required quantities of powder and liquid ingredients to produce an acceptably flowable and compressible admixture. Avicel PH 102, Aerosil 200 and Explotab were employed as carrier, coating material and disintegrant, respectively. The liquisolid system showed acceptable micromeritic properties. The IR and DSC studies ruled out any significant interaction between the drug and excipients. This study shows that the liquisolid technique is a promising alternative for improvement of the dissolution and oral bioavailability of water insoluble drugs a confirmed by estimating the pharmacokinetic parameters in vivo in rabbits.(Gubbi, Jarag, 2010)

2.3 Stable and Bio-equivalent Formulation of HMG-CoA reductase inhibitor: Atorvastatin Calcium

The present study aims to develop stable and bioequivalent non-infringing generic formulation of highly bio-variable Atorvastatin calcium (ATV) using amorphous API. Atorvastatin undergoes two major degradation pathways: lactonization and oxidation; that affects its pharmaceutical efficacy and henceforth the useful shelf-life of the product. A randomized, two-treatment, four-period, two sequence, single dose, two-way replicate crossover bioequivalence study was carried out in 24 healthy human volunteers. Biostatistics of bioequivalence study clearly inveterate that optimization of the Tween® 80 (Polysorbate 80) concentration leads towards desired Cmax and AUC of test

formulation meeting the Bioequivalence criteria with respect to commercial reference formulation Lipitor®.(Mukharya et al., 2012)

2.4 Simultaneous Determination of Atorvastatin and Glimepiride by LC-MS/MS in Human Plasma and Its Application to a Pharmacokinetic Study

The aim of the proposed research work was to develop and validate a simple, selective, high sensitive and high-throughput assay for the simultaneous estimation of Atorvastatin and Glimepiride in human plasma using liquid chromatography tandem mass spectrometry (LC-MS/MS). Atorvastatin-Glimepiride combines a competitive inhibitor of HMG-CoA reductase and a sulfonylurea anti-diabetic drug. The purpose of this study was to develop single method for Atorvastatin and Glimepiride in plasma by liquid chromatography-tandem mass spectrometry (LC-MS/MS) that would result into a simultaneous estimation of Atorvastatin and Glimepiride avoiding acid-lactone inter conversions right from sample collections to analysis on the LC-MS/MS. Simultaneous estimation of Atorvastatin and Glimepiride is cost effective, reduces analysis cycle time, enables effective utilization of resources and reduces bleeding burden on human volunteers.(Hotha, Yarramu, Kandibedala, 2012)

2.5 Design, Development and Evaluation of immediate release drug combination

Therapeutic success if 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. For Atorvastatin only 2hour of dissolution study was performed and its release was found to be 97.6%. For Gliclazide dissolution study was performed upto complete 8hour and its release was found to be 89.314% and release rate was found to be nearly similar to marketed product.(Ugandhar, 2011)

2.6 Developing methods to compare tablet formulations 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. 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, Lacerda, Bonella, 2012)

2.7 Evaluation 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 (f 1), similarity factor (f 2), 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 (f 1<15 and f2>50) and not equivalent at pH 1.2 (f1>15 and f2<50). (Islam, Popy et al, 2012)

2.8 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 is 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.(Ahjel, Lupuleasa, 2009)

2.9 Design and Evaluation of Amlodipine Besilate and Atorvastatin Calcium Tablets

Amlodipine besilate is a calcium channel blockers used for the treating high blood pressure, certain types of angina and coronary heart failure. Atorvastatin calcium known as Statins is used for lowering blood cholesterol and in the treatment of primary hypercholesterolemia and dyslipidemia. The objective of the present investigation was to formulate and evaluate an oral administrable tablet containing Amlodipine besilate and Atorvastatin calcium by wet granulation method. The stability studies were carried out for the optimized batch for three months and it showed no significant changes in the physicochemical parameters and in vitro release pattern. The present study concludes that combined pill has the potential to improve the management of hypertensive patients with additional

cardiovascular risk factors, especially dyslipidemia by reducing pill burden and prescription costs.(Manikandan, Kannan et al, 2012)

2.10 Enhancement of dissolution rate of Atorvastatin calcium using solid dispersions by dropping method

The objective of the present investigation was to study the effect of polyethylene glycol 4000 (PEG 4000) and polyethylene glycol 6000 (PEG 6000) on in vitro dissolution of Atorvastatin Calcium (ATC) from solid dispersions. Initial studies were carried out using physical mixtures of the drug and carrier. Solid dispersions were prepared by the dropping method. The prepared solid dispersions showed marked increase in the saturation solubility and dissolution rate of Atorvastatin than that of pure drug. Finally solid dispersion of Atorvastatin: PEG 6000 prepared as 1:3 ratio by dropping method showed excellent physicochemical characteristics and was found to be described by dissolution release kinetics and was selected as the best formulation.(Narasaiah.V, Jimidi, Goli, Kanakam, 2011)

2.11 In vitro dissolution study of atorvastatin binary solid dispersion

The aim of the present study was to improve the solubility and dissolution rate of atorvastatin (ATV), a slight water-soluble drug, by solid dispersion (SD) technique using a hydrophilic carrier Poloxamer 188 (POL188). Physical mixing (PM) and solvent evaporation (SE) method were used to prepare ATV‑SD where different drug carrier ratios were used. In conclusion, binary SD prepared by both PM and SE technique using POL188 could be considered as a simple, efficient method to prepare ATV solid dispersions with significant improvement in the dissolution rate.(Islam, Tanwir, 2013)

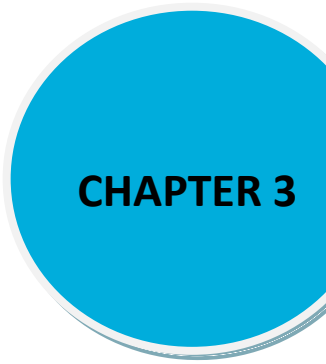
2.12 Enhancement of Atorvastatin Tablet Dissolution Using Acid Medium

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 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.(Hasson, 2010)

2.13 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. 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 namely ATNA1, ATNA2 and ATNA3. The layer of atorvastatin containing Cross-Povidone shown 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.(Mallikarjun et al., 2012)



Materials and Methods

3.1 Dissolution – Definition and Function

Dissolution is a process in which a solid substance solubilizes in a given solvent i. e. mass transfer from the solid surface to the liquid face. Rate of dissolution is the amount of drug substance that goes in solution per unit time under standardized conditions of liquid/ solid interface, temperature and solvent composition. (Banakar, 2009)

The principle function of the dissolution test may be summarized as follows:

1. Optimization of therapeutic effectiveness during product development and stability assessment.

2. Routine assessment of production quality to ensure uniformity between production lots.

3. Assessment of 'bioequivalence', that is to say, production of the same biological availability from discrete batches of products from one or different manufacturers.

4. Prediction of in-vivo availability, i.e. bioavailability (where applicable). (Banakar, 2009)

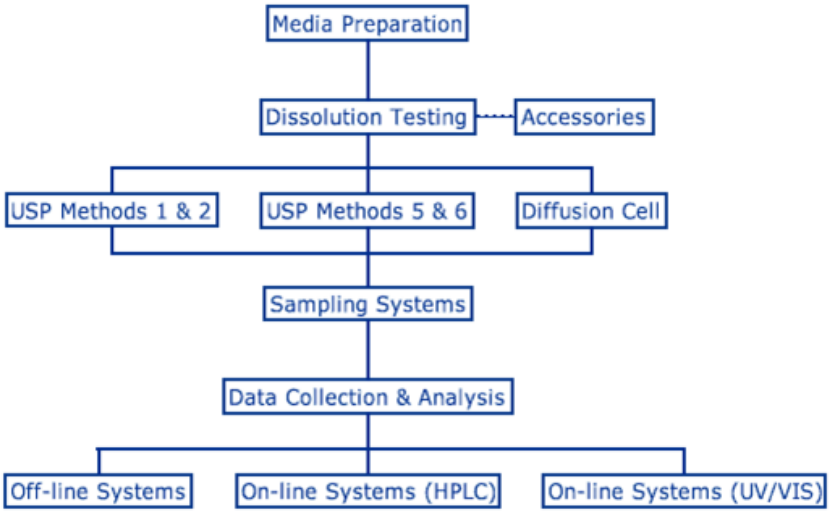


Figure 3.1: Stages in the Dissolution Testing Process

Although initially developed for oral dosage forms, the role of the dissolution test has now been extended to drug release studies on various other forms such as topical and transdermal systems and suppositories. (Banakar, 2009)

3.1.1 Steps involved in Dissolution

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. The process involved in dissolution of solid dosage forms is given below: (Banakar, 2009)

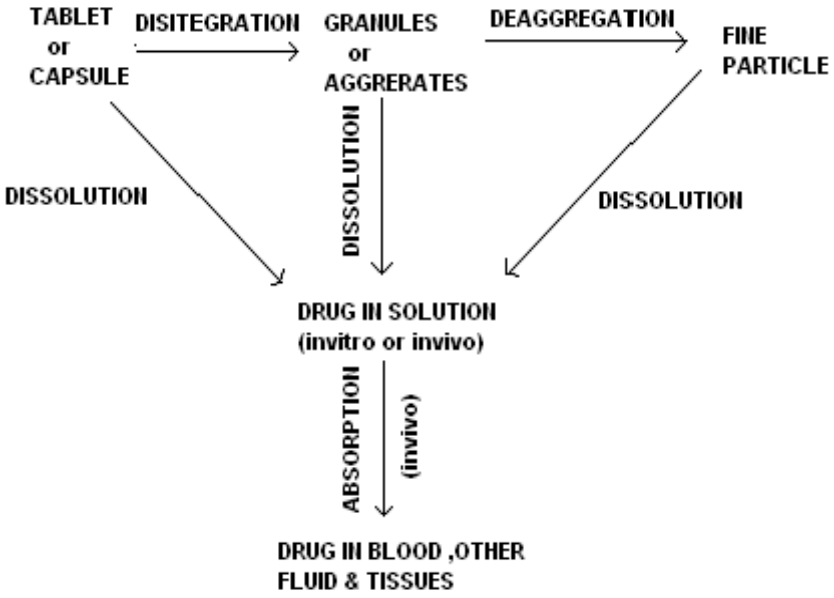


Figure 3.2: Schematic illustration of dissolution process of solid dosage forms

3.1.2 Importance of Dissolution Testing

The importance of dissolution testing is given below:

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. (Banakar, 2009)

3.1.3 Factors affecting Dissolution:

There are several factors which are involved in dissolution testing. They are:

1. Physicochemical Properties of Drug:
a. Drug Solubility
b. Salt Formation
c. Particle Size
d. Solid State Characteristics
e. Co-Precipitation
2. Drug Product Formulation Factors:
a. Diluents
b. Disintegrants
c. Binders And Granulating Agents
d. Lubricants
e. Surfactants
f. Water-Soluble Dyes
g. Coating Polymers
3. Processing Factors:
a. Method Of Granulation
b. Compression Force

c. Drug Excipient Interaction
d. Storage Conditions
4. Factors Relating Dissolution Apparatus:
a. Agitation
b. Stirring Element Alignment
c. Sampling Probe Position & Filter
5. Factors Relating Dissolution Test Parameters:
a. Temperature
b. Dissolution Medium
    - Effect of pH
    - Volume of dissolution medium and sink conditions
    - De-aeration of dissolution medium (Banakar, 2009)

3.1.4 Materials, Apparatus and Reagents used in Dissolution Studies

In this experiment, the following materials, reagents and apparatus were used:

1. 1000mL Beaker
2. 1000mL Measuring Cylinder
3. 100mL Beaker
4. 100mL Measuring Cylinder
5. Stirrer
6. Test Tubes
7. Test Tube Rack
8. Funnel
9. Filter Paper
10. Disodium hydrogen phosphate
11. Potassium dihydrogen phosphate
12. 0.1N HCL
13. Electrical Balance (Shimadzu, S/N: D30700184, Model: ATX224)
14. Labindia Dissolution Test Apparatus (Model: DS 8000)
15. Shimazdu UV Spectrophotometer (UV-1800)
16. Distilled Water
17. PH meter
18. Disposable Syringes

19. Thermometer
20. Mortar & Pestle

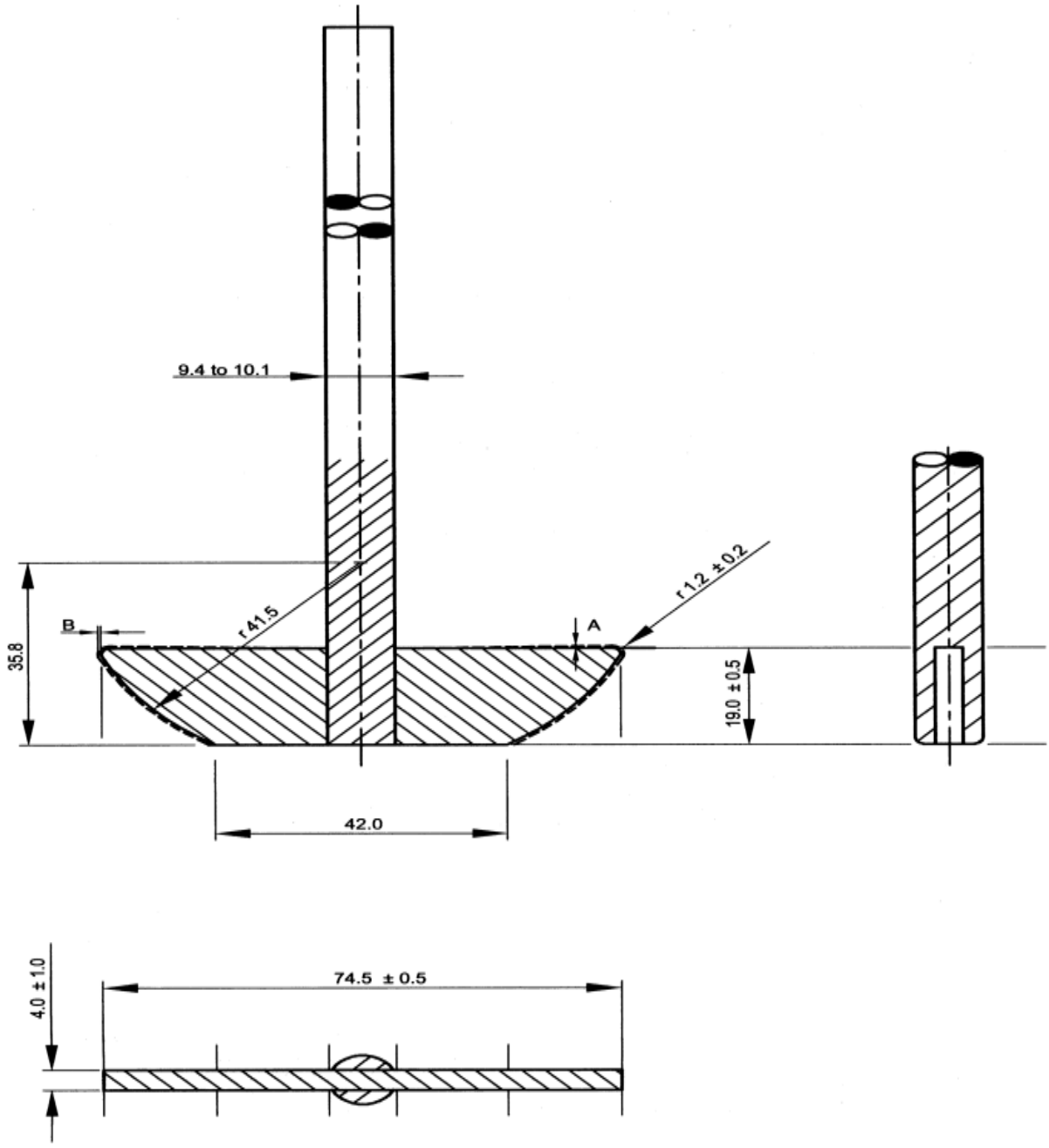


Figure 3.3: Apparatus 2, Paddle stirring element Dimensions in millimeters

Figure 3.4: Electrical Balance

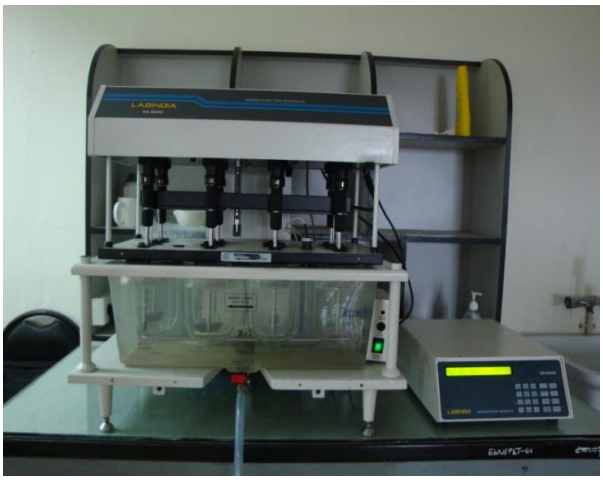


Figure 3.5: Dissolution Apparatus

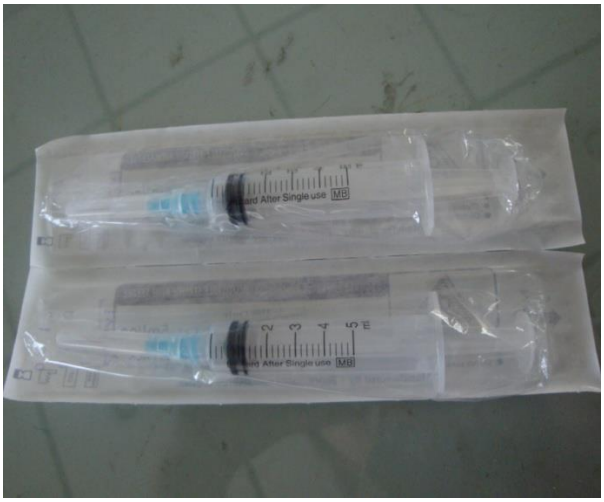


Figure 3.6: Disposable Syringes

Figure 3.7: Test Tubes

Figure 3.8: Filter Paper

Figure 3.9: Test Tubes(labeled)

3.1.5 Details of Dissolution Studies

Dissolution test apparatus: USP type II

Speed: 75 rpm

Stirrer: Paddle type II

Volume of medium: 900 mL

Volume withdrawn: 5 mL

Volume replaced: 5 mL

Medium used: Phosphate buffer, ph 6.8

Temperature: 37±0.5ºC

UV Wavelength: 242nm

$$\text{Dissolution \%} = \frac{A_S \times W_{st} \times 900 \times P}{A_{st} \times 100 \times \text{Dose of tablet}} \times 100\ \%$$

Where,

A_S = Absorbance of sample at 5 min, 10min, 15 min and 30 min

A_{st} = Absorbance of standard, 100% = 0.11

P = Potency of standard = 97 % or 0.97

Wst = weight of standard 0.11mg

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

3.1.6 Method for Dissolution Studies

1. At first, Phosphate buffer of ph 6.8 was prepared with Disodium hydrogen phosphate and Potassium dihydrogen phosphate.

2. 28.8g Disodium hydrogen phosphate was measured and made upto 1000ml with distilled water in a volumetric flask.
3. 11.45g Potassium dihydrogen phosphate was measured and made upto 100ml with distilled water in a volumetric flask.
4. Then 920ml of Disodium hydrogen phosphate and 80ml of Potassium dihydrogen phosphate was mixed and made 1000ml phosphate buffer. PH was adjusted to 6.8 with 0.1N HCL as needed. 900mL of this was used as dissolution medium.
5. The in vitro release of Atorvastatin 10mg tablet(single) and combination of Sedil, Nidocard with Atorvastatin was studied by running batches using Dissolution test apparatus – USP type II. Each batch contained 6 tablets when single Atorvastatin and 18 tablets when combination was studied.
6. The tablets were weighted using analytical balance keeping them on filter paper. Then tablets were placed in the vessels of the dissolution apparatus containing 900 mL of Phosphate buffer ph 6.8, using paddles at a speed of 75 rpm.
7. The temperature was maintained at 37°C during the 30 minutes of dissolution. The Temperature was measured using thermometer.
8. 5 mL sample from each vessel was withdrawn using 5 mL syringes at 5, 10, 15 and 30 minutes and kept in marked test tubes.
9. Fresh dissolution medium (5 mL) was added to the vessels after each sample was taken.
10. All the samples were filtered using funnel and filter paper.
11. Absorbance of the samples was measured using UV spectrophotometer at wavelength of 242 nm. Phosphate buffer ph 6.8 was used as blank.

3.2 Preparation of Standard Calibration Curve

Instrument calibration is an essential stage in most measurement procedures. It is a set of operations that establish the relationship between the output of the measurement system (e.g., the response of an instrument) and the accepted values of the calibration standards (e.g., the amount of analyte present). A large number of analytical methods require the calibration of an instrument. This typically involves the preparation of a set of standards containing a known amount of the analyte of interest, measuring the instrument response for each standard and establishing the relationship between the instrument response and analyte concentration.

This relationship is then used to transform measurements made on test samples into estimates of the amount of analyte present. (Barwick, 2003)

3.3 Preparation of the Standard Solution

A stock solution is prepared using an analytical balance. Five different percentage of standard solutions were prepared by pure Atorvastatin. These different percentage 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 adding small amount of methanol in each five standard samples to dissolve the Atorvastatin. And also added Phosphate buffer ph 6.8 upto 10 ml. No dilution was done. Then measure the absorbance of those solutions at the λmax 233nm.

3.4 Significance of the research study

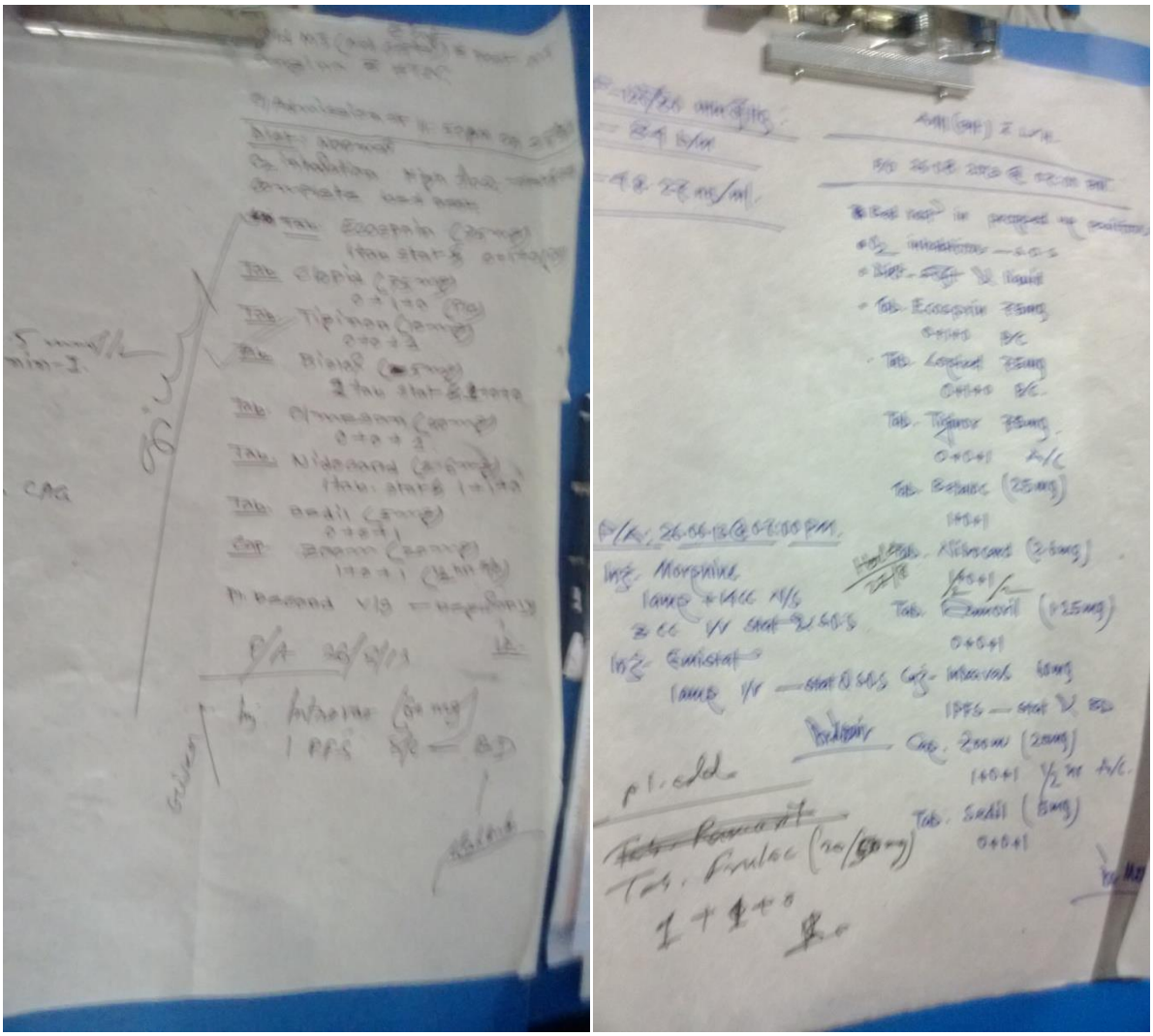
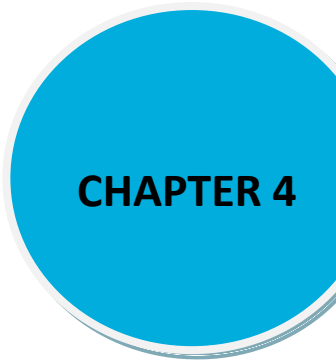


Figure 3.9: Prescriptions collected from different hospitals of cardiovascular patients



Results and Discussions

4.1. Results of Dissolution Studies

Table 4.1.: Absorbance of Lipicon-10® Atorvastatin 10mg at different time intervals

Brand	**Lipicon-10® Atorvastatin 10mg**			
Batch	**Batch 1**			
Time Interval (minutes)	Absorbance at 5 min	Absorbance at 10 min	Absorbance at 15 min	Absorbance at 30 min
Tablet 1	0.032	0.025	0.061	0.370
Tablet 2	0.021	0.025	0.076	0.490
Tablet 3	0.022	0.015	0.099	0.421
Tablet 4	0.028	0.037	0.044	0.454
Tablet 5	0.017	0.044	0.183	0.399
Tablet 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%

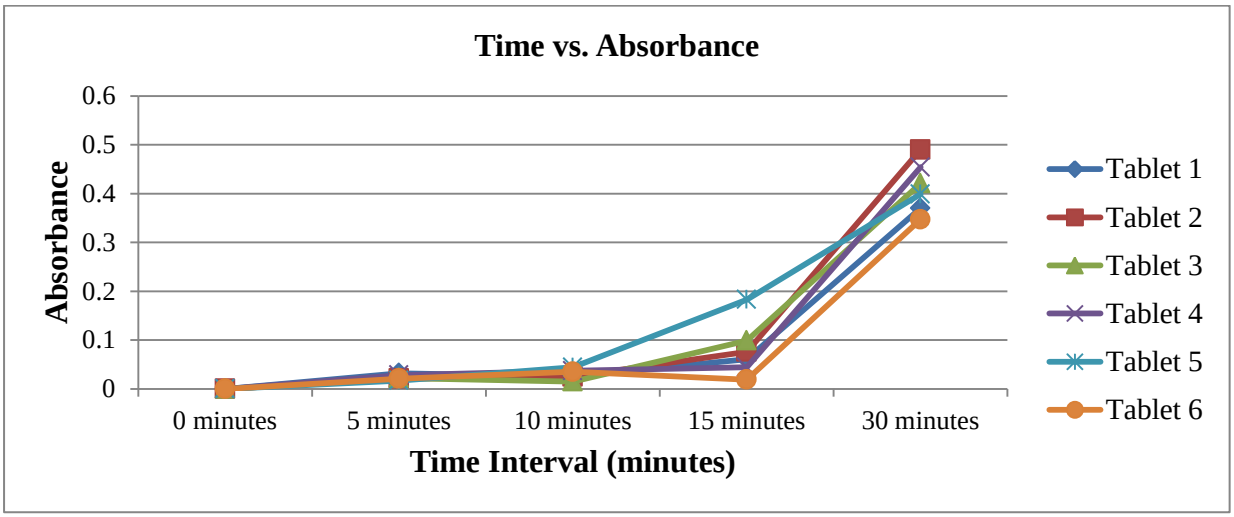


Figure 4.1: Time vs. Absorbance Curve for Lipicon-10® Atorvastatin

Table 4.2: Absorbance of Lipicon-10® Atorvastatin 10mg + Sedil® Diazepam BP 5mg + Nidocard® nitroglycerin usp 2.6mg at different time intervals

Brand	**Lipicon-10® Atorvastatin 10mg + Sedil® Diazepam BP 5mg + Nidocard® nitroglycerin usp 2.6mg**			
Batch	**Batch 1**			
Time Interval (minutes)	Absorbance at 5 min	Absorbance at 10 min	Absorbance at 15 min	Absorbance at 30 min
Combination 1	0.057	0.134	0.184	0.385
Combination 2	0.105	0.193	0.144	0.267
Combination 3	0.060	0.104	0.136	0.186
Combination 4	0.085	0.120	0.157	0.262
Combination 5	0.072	0.154	0.164	0.309
Combination 6	0.046	0.114	0.159	0.335
Average Absorbance	0.071	0.137	0.157	0.288
% Dissolved	6.2%	11.96%	13.71%	25.14%

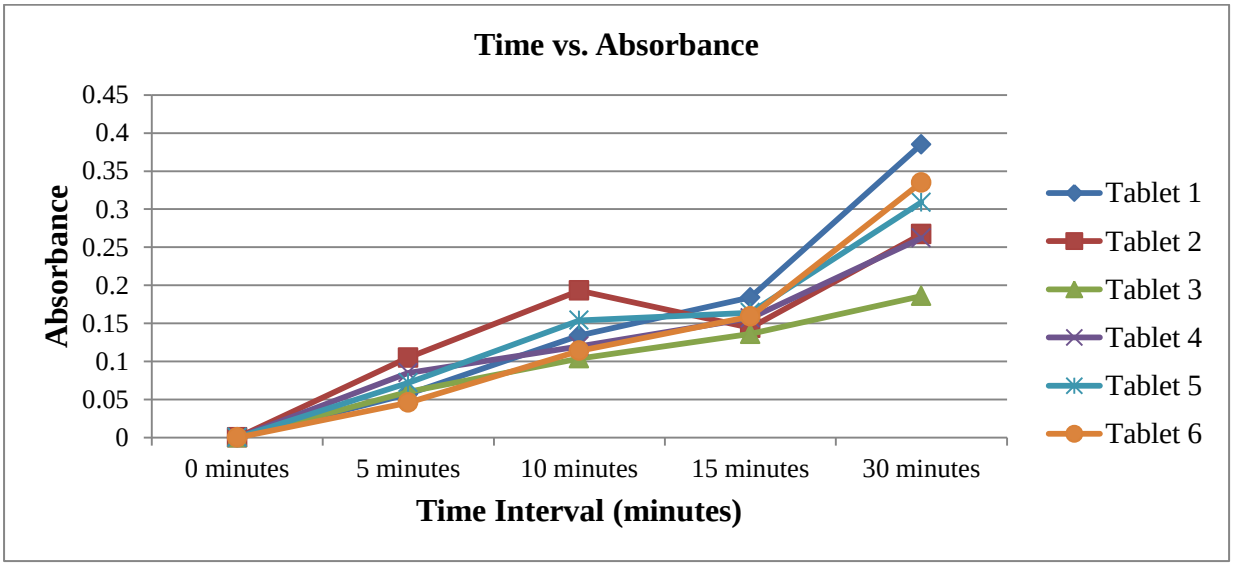


Figure 4.2: Time vs. Absorbance Curve for Lipicon-10® Atorvastatin 10mg + Sedil® Diazepam BP 5mg + Nidocard® nitroglycerin usp 2.6mg

4.2 Standard Solution

Table 4.3: Absorbance Test of different concentrations of Standard

Serial no.	Concentration (µg/mL)	Absorbance
1	80%	0.072
2	90%	0.087
3	100%	0.11
4	110%	0.136
5	120%	0.157

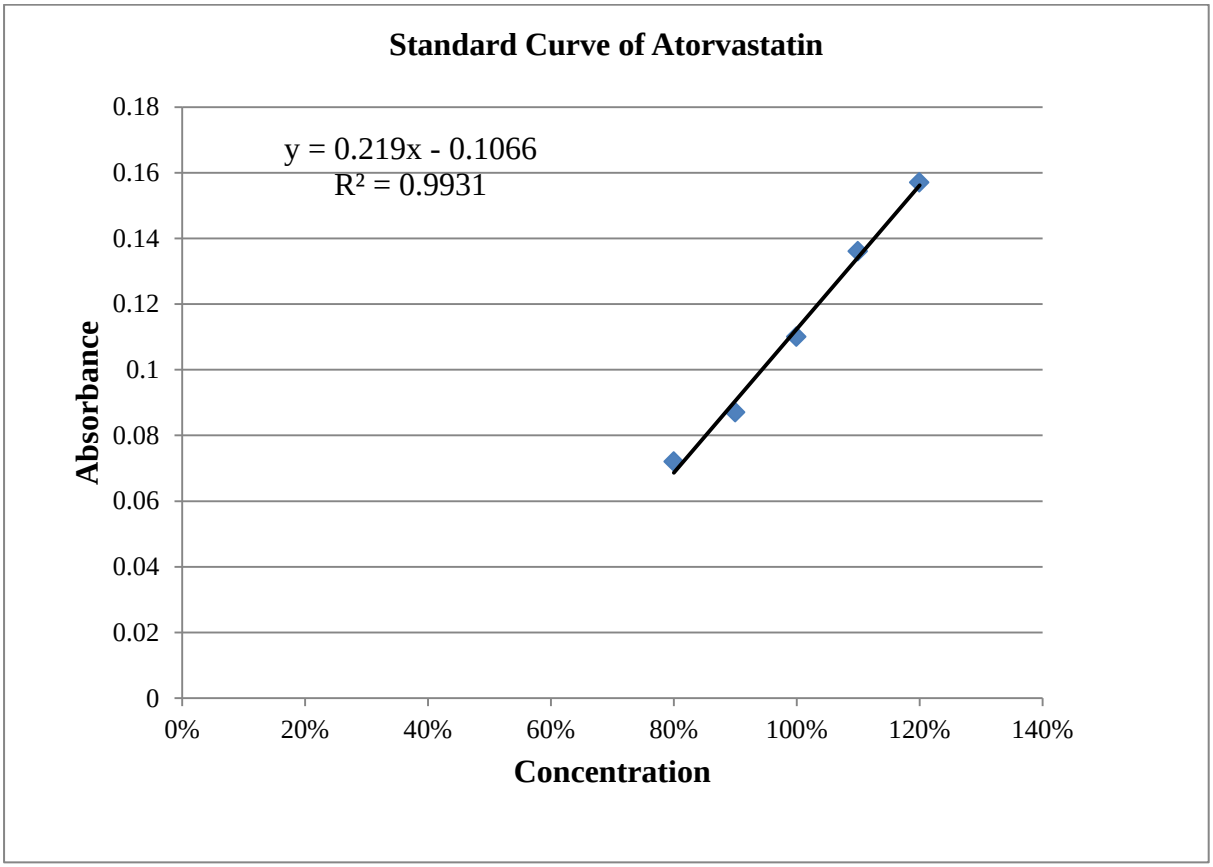


Figure 4.3: Standard Curve of Atorvastatin

$$\text{Dissolution \%} = \frac{A_S \times W_{st} \times 900 \times P}{A_{st} \times 100 \times \text{Dose of tablet}} \times 100\ \%$$

Where,

A_S = Absorbance of sample at 5 min, 10min, 15 min and 30 min

A_{st} = Absorbance of standard, 100% = 0.11

P = Potency of standard = 97 % or 0.97

Wst = weight of standard 0.11mg

4.3 Calculation of Atorvastatin

$$\text{Dissolution \% after 5 min} = \frac{0.024 \times 0.11 \times 900 \times 0.97}{0.11 \times 100 \times 10} \times 100\ \%$$

$$= 2.093\%$$

$$\text{Dissolution \% after 10 min} = \frac{0.0302 \times 0.11 \times 900 \times 0.97}{0.11 \times 100 \times 10} \times 100\ \%$$

$$= 2.64\%$$

$$\text{Dissolution \% after 15 min} = \frac{0.0803 \times 0.11 \times 900 \times 0.97}{0.11 \times 100 \times 10} \times 100\ \%$$

$$= 7.01\%$$

$$\text{Dissolution \% after 30 min} = \frac{0.414 \times 0.11 \times 900 \times 0.97}{0.11 \times 100 \times 10} \times 100\ \%$$

$$= 36.14\%$$

4.4 Calculation of Combination

$$\text{Dissolution \% after 5 min} = \frac{0.071 \times 0.11 \times 900 \times 0.97}{0.11 \times 100 \times 10} \times 100\ \%$$

$$= 6.2\%$$

$$\text{Dissolution \% after 10 min} = \frac{0.137 \times 0.11 \times 900 \times 0.97}{0.11 \times 100 \times 10} \times 100\ \%$$

$$= 11.96\%$$

$$\text{Dissolution \% after 15 min} = \frac{0.157 \times 0.11 \times 900 \times 0.97}{0.11 \times 100 \times 10} \times 100\ \%$$

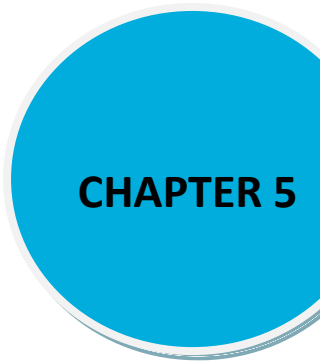
$$= 13.71\%$$

$$\text{Dissolution \% after 30 min} = \frac{0.288 \times 0.11 \times 900 \times 0.97}{0.11 \times 100 \times 10} \times 100\ \%$$

$$= 25.14\%$$

4.5 Discussion

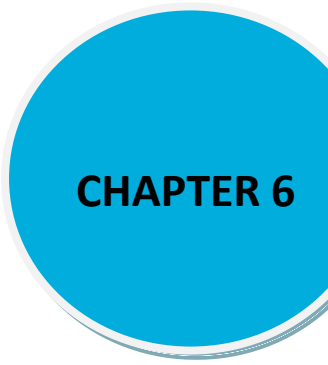
Atorvastatin calcium, a lipid-lowering drug, is much less bioavailable because of reduced solubility in acidic media. When Atorvastatin calcium was tested alone the %dissolved after 5, 10, 15, 30 minutes were 2.093%, 2.64%, 7.01% and 36.14% respectively. When Atorvastatin calcium was tested in combination with Diazepam and Nitroglycerin the % dissolved after 5, 10, 15, 30 minutes were 6.2%, 11.96%, 13.71%, 25.14% respectively. So we can see that after 30 minutes of study the %dissolved decreased from 36.14% to 25.14% therefore the effect of administering above mentioned drugs simultaneously in combination decreases the %dissolve of Atorvastatin thus lowering the bioavailability even more than usual. Though there may be some errors regarding the tests done due to various factors the results were comprehensive and should be taken into consideration.



Conclusion

Conclusion

In this study it was observed that taking other drugs such as Diazepam and Nitroglycerin in combination with Atorvastatin may decrease the %dissolved hence bioavailability of Atorvastatin compared to administering Atorvastatin alone. So there may be drug drug interaction between Atorvastatin, Diazepam and N*i*troglycerin in some way. Further studies must be done to see if there are any other effect when using Atorvastatin in combination. In my knowledge, not much work has been done regarding drug drug interaction with Atorvastatin in combination. During prescribing Atorvastatin with other drugs at the same time the interaction with other drugs should be taken under consideration.



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