In-vitro Comparative Dissolution Study of Different Brands (Glucomet, Met and Daomin) of Metformin Hydrochloride Tablets Available in Bangladesh

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

Submitted by

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Declaration by the Candidate

I, Md. Fazlay Rabbi, hereby declare that the dissertation entitled ***"In-vitro* comparative dissolution study of different brands (Glucomet, Met and Daomin) of Metformin Hydrochloride tablets available in Bangladesh"** submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, work carried out by me during the year 2016 of my research in the Department of Pharmacy, East West University, under the supervision and guidance of Tirtha Nandi, Lecturer, Department of Pharmacy, East West University. The thesis paper has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Certificate by the Supervisor

This is to certify that the thesis entitled **"*In-vitro* comparative dissolution study of different brands (Glucomet, Met and Daomin) of Metformin Hydrochloride tablets available in Bangladesh"** submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by Md. Fazlay Rabbi, ID: 2012-3-70-041, during the year 2016 of his research in the Department of Pharmacy, East West University, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Endorsement by the Chairperson

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Dedication

This research paper is dedicated to my beloved Parent and my Brother

Abstract

The aim of the present study was to evaluate and compare dissolution pattern of locally branded drug products of Metformin Hydrochloride available in Bangladesh with each other. Glucophage® is the patent drug of Metformin Hydrochloride. Branded drugs are expensive than locally marketed drug. Substitution of drugs is very essential for the people of under developing country. Three different brands of Metformin Hydrochloride tablets which are available in Bangladesh like Glucomet, Daomina and Met were collected from a reputed pharmacy store. Six tablets from each of the brands were used for the *in-vitro* dissolution study. Cumulative drug release was measured up to 50 minutes for all the brands. All the brands were compared with each other. Differential factor, f1 and similarity factor, f2 were determined. Few differences were observed during *in-vitro* drug release pattern of brand Glucomet, Met and Daomin with each other. Significant differences were found between Glucomet and Met and also between Glucomet and Daomin. The values of *f*1 found are respectively 27.51 and 28.52. And it is not acceptable. The values of *f*2 found are respectively 26.76 and 26.2. And it is also not acceptable. On the other hand, significant similarities were found between Met and Daomin. In conclusion, further investigations are needed to evaluate better dissolution study.

Keyword: Metformin HCl, Comparative dissolution, *In-vitro* drug dissolution study

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IP	Indian Pharmacopeia
ADRs	Adverse Drug Reactions
IR	Immediate Release
BCS	Biopharmaceutical Classification System
IVIVC	In Vivo-In Vitro Correlation
API	Active pharmaceutical Ingredient
FDA	Food and Drug Administration

Chapter1

INTRODUCTION

1.1 Overview

Metformin is a type of biguanide which is used for treating type 2 diabetes malitus. Metformin is an oral antihyperglycemic drug used in the management of type 2 diabetes. Chemical formula of Metformin hydrochloride is N, N-dimethylimidodicarbonimidic diamide hydrochloride. It is not chemically or pharmacologically related to any other classes of oral antihyperglycemic agents. Metformin hydrochloride is a white to off-white crystalline compound with a molecular weight of 165.63. Metformin hydrochloride is freely soluble in water and is practically insoluble in acetone, ether and chloroform. The pK, of metformin is 12.4. The pH of a 1% aqueous solution of metformin hydrochloride is 6.68. Metformin is type of bigunide. Most of the companies put metformin hydrochloride in the drug as it dissolves in water quickly. (Anand, 2011)

1.2 Diabetes

Diabetes mellitus commonly referred to as diabetes is a disease of the pancreas, an organ behind the stomach that produces the hormone insulin. Insulin helps the body use food for energy. When a person has diabetes, the human pancreas either cannot produce enough insulin, uses the insulin incorrectly, or both. Insulin works together with glucose in the bloodstream to help it enter the body's cells to be burned for energy. If the insulin isn't functioning properly, glucose cannot enter the cells. This causes glucose levels in the blood to rise, creating a condition of high blood sugar or diabetes, and leaving the cells without fuel. (Cleaveland clinic, 2017)

Types of diabetes

There are two common forms of diabetes: type 1 and type 2.

1. Type 1: Type 1 diabetes occurs because the insulin-producing cells of the pancreas (beta cells) are damaged. In type 1 diabetes, the pancreas makes little or no insulin, so glucose cannot get into the body's cells for use as energy. People with type 1 diabetes must utilize insulin injections to control their blood glucose. It is the most common form of diabetes in people under age 20-30, but it can occur at any age. Ten percent of people with diabetes are diagnosed with type 1.

2. Type 2: In type 2 diabetes, the pancreas does make some insulin. But it either doesn't produce enough insulin or the insulin does not work properly. Type 2 diabetes may sometimes be controlled with a combination of diet, weight management and exercise. However, treatment also may include oral glucose-lowering medications or insulin injections. Generally, type 2 diabetes is more common in people over age 40 who are overweight. Nine out of 10 people with diabetes have type 2 (Cleaveland clinic, 2017).

Symptoms of diabetes:

Common Symptoms:

1. Polydipsia
2. Polyuria
3. Fatigue
4. Dry mouth
5. Itchy skin
6. Blurred vision

Symptoms of type 1 diabetes:

1. Weight loss
2. Nausea
3. Vomiting

Symptoms of type 2 diabetes:

1. More susceptible to yeast infection.
2. Slow healing sores or cuts
3. Pain and numbness in feet

(WebMD, 2017)

1.3 Metformin

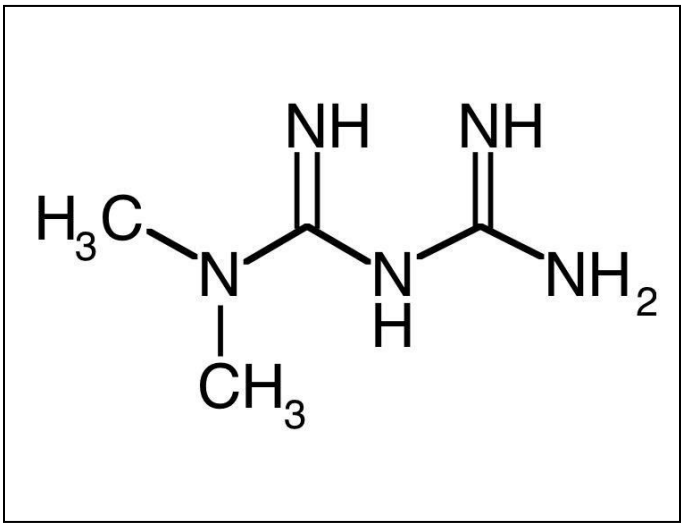

Fig: Structural formula of metformin. (Biohealthscience, 2017)

Metformin is the only currently available biguanide which is classed as an insulin sensitizer. It increases glucose uptake and utilization by target tissues, thereby decreasing insulin resistance. Like the sulfonylureas, metformin requires insulin for its action, but it differs from the sulfonylureas in that it does not promote insulin secretion. So, the amount of insulin which is secreted is same. This is a form of quantitative result. Hyperinsulinemia is not a problem. Thus, the risk of hypoglycemia is far less than that with sulfonylurea agents, and it may only occur if caloric intake is not adequate or exercise is not compensated for calorically. (Harvey et. al., 2008)

Two biguanide antidiabetics, phenformin and metformin were introduced in the 1950s. Because of higher risk of lactic acidosis, phenformin was withdrawn in many countries and has been banned in India since 2003. They differ markedly from sulfonylureas: cause little or no hypoglycemia in nondiabetic subjects, and even in diabetics episodes of hypoglycemia due to metformin are rare. They do not stimulate pancreatic β cells. Metformin is reported to improve lipid profile as well in type 2 diabetics (Tripathy K. D., 2011).

1.3.1 Mechanism of action of metformin

The main mechanism of action of metformin is reduction of hepatic glucose output, largely by inhibiting hepatic gluconeogenesis. Excess glucose produced by the liver is the major source of high blood glucose in Type 2 diabetic, accounting for the high blood glucose on waking in the morning. Metformin also slows intestinal absorption of sugars and improves peripheral glucose uptake and utilization. A very important property of this drug is its ability to modestly reduce hyperlipidemia. Low-density lipoprotein and very-low-density lipoprotein cholesterol concentrations fall, and high-density lipoprotein [HDL] cholesterol rises). These effects may not be apparent until 4 to 6 weeks of use. The patient often loses weight because of loss of appetite. The renowned ADA treatment algorithm recommends metformin which is used as the drug of choice for newly diagnosed Type 2 diabetics. Metformin may be used alone or in combination with one of the other agents, as well as with insulin. Hypoglycemia has occurred when metformin was taken in combination. If used with insulin, the dose of insulin may require adjustment, because metformin decreases the production of glucose by the liver. Pharmacokinetics and fate: Metformin is well absorbed orally, is not bound to serum proteins, and is not metabolized. Excretion is via the urine. (Harvey et. al., 2008)

1. The hypoglycemic actions of metformin are given below,
2. 1.Suppress hepatic gluconeogenesis and glucose output from liver: the major action.
3. Enhance insulin-mediated glucose disposal in muscle and fat. Though they do not alter translocation of GLUT4 (the major glucose transporter in skeletal muscle), they enhance GLUT1 transport from intracellular site to plasma membrane. The effect thus differs from that of insulin. 3. Retard intestinal absorption of glucose, other hexoses, amino acids and vit B12.
4. Interfere with mitochondrial respiratory chain—promote peripheral glucose utilization by enhancing anaerobic glycolysis. However, metformin binds less avidly to mitochondrial membrane. (Tripathy K. D., 2011)

1.3.2 Adverse effects of Metformin:

These are largely gastrointestinal. Metformin is contraindicated in diabetics with renal and/or hepatic disease, acute myocardial infarction, severe infection, or diabetic ketoacidosis. It should be used with caution in patients greater than 80 years of age or in those with a history of congestive heart failure or alcohol abuse. Diabetics being treated with heart-failure medications should not be given metformin because of an increased risk of lactic acidosis. Metformin should be temporarily discontinued in patients undergoing diagnosis requiring intravenous radiographic contrast agents. Rarely, potentially fatal lactic acidosis has occurred. Long-term use may interfere with vitamin B12 absorption. (Shah et. al., 2014)

1.3.3 Contraindications

Metformin hydrochloride tablets are contraindicated in patients with Renal disease or renal dysfunction or abnormal creatinine clearance which may also result from conditions such as cardiovascular collapse acute myocardial infarction, and septicemia. Congestive heart failure requiring pharmacologic treatment. Known hypersensitivity to metformin hydrochloride should also be considered. Cute or chronic metabolic acidosis, including diabetic ketoacidosis, with or without coma. Diabetic ketoacidosis should be treated with insulin. Metformin should be temporarily discontinued in patients undergoing radiologic studies involving intravascular administration of iodinated contrast materials, because use of such products may result in acute alteration of renal function. Lactic acidosis is a rare, but serious, metabolic complication that can occur due to metformin accumulation during treatment with metformin (Biohealthscience, 2017)

1.4 Pharmacokinetics

Absorption and Bioavailability

The absolute bioavailability of a metformin hydrochloride 500 mg tablet given under fasting conditions is approximately 50-60%. Studies using single oral doses of metformin tablets of 500 mg and 1500 mg, and 850 mg to 2550 mg, indicate that there is a lack of dose proportionality with increasing doses, which is due to decreased absorption rather than an alteration in elimination. Food decreases the extent of and slightly delays the absorption of metformin, as

shown by approximately a 40% lower mean peak concentration (C_{max}) and 25% lower area under the plasma concentration versus time curve (AUC), and a 35-minute prolongation of time to peak plasma concentration (T_{max}) following administration of a single 850 mg tablet of metformin with food, compared to the same tablet strength administered fasting (Tripathy K. D., 2011).

Distribution

Metformin is negligibly bound to plasma proteins in contrast to sulfonylureas which are more than 90% protein bound. Metformin partitions into erythrocytes, most likely as a function of time. At usual clinical doses and dosing schedules of metformin hydrochloride tablets, steady state plasma concentrations of metformin are reached within 24-48 hours. During controlled clinical trials, maximum metformin plasma levels did not exceed 5μg/mL, even at maximum doses (Cleaveland clinic, 2017).

Metabolism and Elimination

Intravenous single-dose studies in normal subjects demonstrate that metformin is excreted unchanged in the urine and does not undergo hepatic metabolism (no metabolites have been identified in humans) nor biliary excretion. Renal clearance (see Table 1) is approximately 3.5 times greater than creatinine. Clearance which indicates that tubular secretion is the major route of metformin elimination. Following oral administration, approximately 90% of the absorbed drug is eliminated via the renal route within the first 24 hours, with a plasma elimination half-life of approximately 6.2 hours. In blood, the elimination half-life is approximately 17.6 hours, suggesting that the erythrocyte mass may be a compartment of distribution (Tripathy K. D., 2011).

1.5 Special Populations

Patients with Type 2 Diabetes In the presence of normal renal function, there are no differences between single or multiple dose pharmacokinetics of metformin between patients with type 2 diabetes and normal subjects nor is there any accumulation of metformin in either group at usual clinical doses.

Renal Insufficiency In subjects with decreased renal function (based on measured creatinine clearance), the plasma and blood half-life of metformin is prolonged and the renal clearance is decreased in proportion to the decrease in creatinine clearance.

Hepatic Insufficiency

No pharmacokinetic studies have been conducted in subjects with hepatic insufficiency.

Geriatrics

Limited data from controlled pharmacokinetic studies of metformin in healthy elderly subjects suggest that total plasma clearance of metformin is decreased, the half-life is prolonged, and C_{max} is increased, compared to healthy young subjects. From these data, it appears that the change in metformin pharmacokinetics with aging is primarily accounted for by a change in renal function. Metformin treatment should not be initiated in patients more than 80 years of age unless measurement of creatinine clearance demonstrates that renal function is not reduced.

1.6 Precautions:

General Monitoring of renal function

Metformin is known to be substantially excreted by the kidney, and the risk of metformin accumulation and lactic acidosis increases with the degree of impairment of renal function. Thus, patients with serum creatinine levels above the upper limit of normal for their age should not receive metformin. In patients with advanced age, metformin should be carefully titrated to establish the minimum dose for adequate glycemic effect, because aging is associated with reduced renal function. In elderly patients, particularly those more than 80 years of age, renal function should be monitored regularly and, generally, metformin should not be titrated to the maximum dose. Before initiation of metformin therapy and at least annually thereafter, renal function should be assessed and verified as normal. In patients in whom development of renal dysfunction is anticipated, renal function should be assessed more frequently and metformin discontinued if evidence of renal impairment is present. Use of concomitant medications that may affect renal function or metformin disposition - Concomitant medications that may affect renal function or result in significant hemodynamic change or may interfere with the disposition

of metformin, such as cationic drugs that are eliminated by renal tubular secretion should be used with caution.

Radiologic studies

It involves the use of intravascular iodinated contrast materials E.g. Intravascular contrast studies with iodinated materials can lead to acute alteration of renal function and have been associated with lactic acidosis in patients receiving metformin. Therefore, in patients in whom any such study is planned, metformin should be discontinued at the time of or prior to the procedure, and withheld for 48 hours subsequent to the procedure and reinstituted only after renal function has been reevaluated and found to be normal.

Alcohol intake

Alcohol is known to potentiate the effect of metformin on lactate metabolism. Patients, therefore, should be warned against excessive alcohol intake, acute or chronic, while receiving metformin. Impaired hepatic function - Since impaired hepatic function has been associated with some cases of lactic acidosis, metformin should generally be avoided in patients with clinical or laboratory evidence of hepatic disease.

Hypoglycemia

Hypoglycemia does not occur in patients receiving metformin alone under usual circumstances of use, but could occur when caloric intake is deficient, when strenuous exercise is not compensated by caloric supplementation, or during concomitant use with other glucose-lowering agents (such as sulfonylureas) or ethanol. Elderly, debilitated or malnourished patients, and those with adrenal or pituitary insufficiency or alcohol intoxication are particularly susceptible to hypoglycemic effects. Hypoglycemia may be difficult to recognize in the elderly, and in people who are taking beta-adrenergic blocking drugs.

Loss of control of blood glucose

When a patient stabilized on any diabetic regimen is exposed to stress such as fever, trauma, infection, or surgery, a temporary loss of glycemic control may occur. At such times, it may be necessary to withhold metformin and temporarily administer insulin. Metformin may be reinstituted after the acute episode is resolved. The effectiveness of oral antidiabetic drugs in

lowering blood glucose to a targeted level decreases in many patients over a period of time. This phenomenon, which may be due to progression of the underlying disease or to diminished responsiveness to the drug, is known as secondary failure, to distinguish it from primary failure in which the drug is ineffective during initial therapy. Should secondary failure occur with metformin or sulfonylurea monotherapy, combined therapy with metformin and sulfonylurea may result in a response. Should secondary failure occur with combined metformin/sulfonylurea therapy, it may be necessary to consider therapeutic alternatives including initiation of insulin therapy.

1.7 Drug Interactions

Glyburide

In a single-dose interaction study in type 2 diabetes subjects, co-administration of metformin and glyburide did not result in any changes in either metformin pharmacokinetics or pharmacodynamics. Decreases in glyburide AUC and C max were observed, but were highly variable. The single-dose nature of this study and the lack of correlation between glyburide blood levels and pharmacodynamic effects, makes the clinical significance of this interaction is uncertain.

Furosemide

A single-dose, metformin-furosemide drug interaction study in healthy subjects demonstrated that pharmacokinetic parameters of both compounds were affected by co-administration. Furosemide increased the metformin plasma and blood C_{max} by 22% and blood AUC by 15%, without any significant change in metformin renal clearance. When administered with metformin, the C_{max} and AUC of furosemide were 31% and 12% smaller, respectively, than when administered alone, and the terminal half-life was decreased by 32%, without any significant change in furosemide renal clearance. No information is available about the interaction of metformin and furosemide when co-administered chronically.

Nifedipine

A single-dose, metformin-nifedipine drug interaction study in normal healthy volunteers demonstrated that co-administration of nifedipine increased plasma metformin C_{max} and AUC by 20% and 9%, respectively, and increased the amount excreted in the urine. T_{max}, and half-life were unaffected. Nifedipine appears to enhance the absorption of metformin. Metformin had minimal effects on nifedipine.

Other

Certain drugs tend to produce hyperglycemia and may lead to loss of glycemic control. These drugs include thiazide and other diuretics, corticosteroids, phenothiazines, thyroid products, estrogens, oral contraceptives, phenytoin, nicotinic acid, sympathomimetics, calcium channel blocking drugs, and isoniazid. When such drugs are administered to a patient receiving metformin, the patient should be closely observed to maintain adequate glycemic control. In healthy volunteers, the pharmacokinetics of metformin and propranolol and metformin and ibuprofen were not affected when co-administered in single-dose interaction studies. Metformin is negligibly bound to plasma proteins and is, therefore, less likely to interact with highly protein-bound drugs such as salicylates, sulfonamides, chloramphenicol, and probenecid, as compared to the sulfonylureas, which are extensively bound to serum proteins.

1.8 BCS Classification

1.8.1 The BCS

The BCS is a scientific framework for classifying a drug substance based on its aqueous solubility and intestinal permeability. It allows for the prediction of in vivo pharmacokinetics of oral immediate-release (IR) drug products by classifying drug compounds into four classes based on their solubility related to dose and intestinal permeability in combination with the dissolution properties of the dosage form. The interest in this classification system stems largely from its application in early drug development and then in .The Biopharmaceutical Classification System (BCS) is one of the experimental models that measures permeability and solubility under specific

conditions. The main purpose of the system was to aid in the regulation of post-approval changes, providing acceptance based on in vitro data when appropriate is available. Importantly, the system was designed around on oral drug delivery since the majority of drugs is and remains orally dosed. Waivers, permission to skip *in vivo* bioequivalence studies, are kept for drug products that meet certain requirements like solubility and permeability and that are also rapidly dissolving characters (Knott, 2016).

Table 1.4: The Bio pharmaceutics classification system

Class	Solubility	Permeability
I	High	High
II	Low	High
III	High	Low
IV	Low	Low

This classification is associated with a drug dissolution and absorption model, which identifies the key parameters controlling drug absorption as a set of dimensionless numbers. Ranitidine is in the Class III as it has high permeability and low solubility (Knott, 2016).

Class I

The drugs of this class exhibit high absorption number and high dissolution number. The rate-limiting step is drug dissolution, and if dissolution is very rapid, then the gastric-emptying rate becomes the rate-determining step. These compounds are well absorbed, and their absorption rate is usually higher than the excretion rate. Examples include metoprolol, diltiazem, verapamil, and propranolol.

Class II

The drugs of this class have a high absorption number but a low dissolution number. In vivo drug dissolution is then a rate-limiting step for absorption except at a very high dose number. The absorption for Class II drugs is usually slower than for Class I and occurs over a longer period of time. In vitro–in vivo correlation (IVIVC) is usually accepted for Class I and Class II drugs. The

bioavailability of these products is limited by their solvation rates. Hence, a correlation between the in vivo bioavailability and the in vitro solvation can be found (7, 9, and 10). Examples include glibenclamide, phenytoin, danazol, mefenamic acid, nifedinpine, ketoprofen, naproxen, carbamezapine, and ketoconazole (Knott, 2016).

Class III

Drug permeability is the rate-limiting step for drug absorption, but the drug is solvated very quickly. These drugs exhibit a high variation in the rate and extent of drug absorption. Since the dissolution is rapid, the variation is attributable to alteration of physiology and membrane permeability rather than the dosage form factors. Examples include cimetidine, ranitidine, acyclovir, neomycin B, atenolol, and captopril(Knott, 2016).

Class IV

The drugs of this class are problematic for effective oral administration. These compounds have poor bioavailability. They are usually not well absorbed through the intestinal mucosa, and a high variability is expected. Fortunately, extreme examples of Class IV compounds are the exception rather than the rule, and these are rarely developed and marketed. Nevertheless, several Class IV drugs do exist Examples include hydrochlorothiazide, taxol, and furosemide (Knott, 2016).

1.9 Dissolution

Dissolution is the primary quality control test to determine whether a drug product can release its active pharmaceutical ingredients in a timely manner.A dissolution test is a means of identifying and proving the availability of active drug materials in their delivered form. A dissolution test simulates the availability of active substance and allows the prediction of the time for complete release of the material from the dosage form. In the pharmaceutical industry, drug dissolution testing is routinely used to provide critical in vitro drug release information for both quality

control purposes, i.e., to assess batch-to-batch consistency of solid oral dosage forms such as tablets, and drug development, i.e., to predict in vivo drug release profiles (Knott, 2016).

1.9.1 Process of Dissolution

According to the rule *like dissolves like*, means that substances must have the same intermolecular forces to form solutions. After introducing a soluble solute is to solvent, the particles of solute interact with the particles of solvent. In the case of a solid or liquid solute, the interactions between the solute particles and the solvent particles are so strong that the individual solute particles separate from each other and, surrounded by solvent molecules, enter the solution. This process is known as solvation and is illustrated in Figure 1.1. When the solvent is water, then the salvation word is replaced by the word hydration.

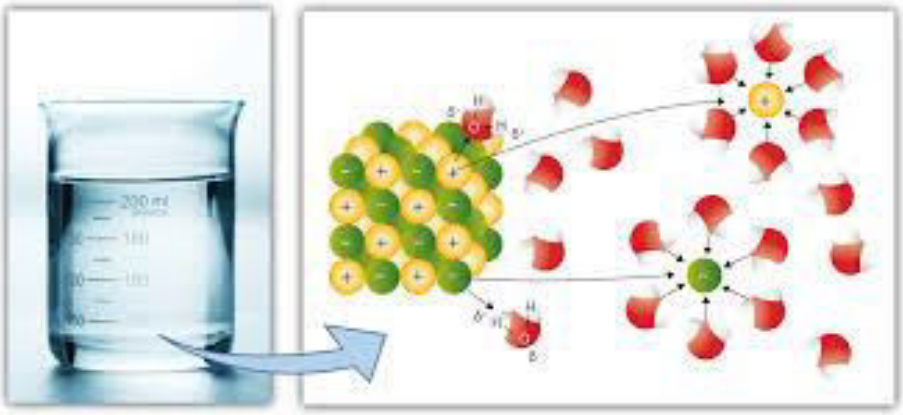

Figure 1.1: *Solvation* (Lapsurgery, 2014)

When a solute dissolves, the individual particles of solute become surrounded by solvent particles. Eventually the particle detaches from the remaining solute, surrounded by solvent molecules in solution (Lapsurgery, 2014).

In the case of molecular solutes like carbohydrates e.g. glucose, the particles are individual molecules. However, if the solute is ionic, the individual ions got separated from each other and become surrounded by solvent particles. That is, the ions of solute separate when the solute dissolves. This process is called dissociation. Soluble ionic compounds are often referred to as electrolytes. Many ionic compounds dissociate completely thus called strong electrolytes.

Sodium salts are example of strong electrolytes. Some compounds dissolve but get dissociated only in partial amount, and solutions of such solutes may conduct electricity only weakly. These solutes are called weak electrolytes. Acetic acid (CH3COOH) is counted as a very weak electrolyte (Lapsurgery, 2014).

1.9.2 Factors influence the dissolution of a substance

1. Temperature
2. Particular size of solute
3. Agitation
4. Solvent selection

Temperature

In most cases of dissolution of solute in a liquid depends on the absorption of heat. If the temperature is raised then the dissolution will be more rapid but in lower temperature the dissolution will be less. So, temperature has the significant influence on dissolution.

Particle Size

The dissolution rate depends on its particle size. In the case of small particle size, dissolution will be more but in the time of large particle size, dissolution will be less. The absorption depends upon the dissolution rate. So determination of dissolution rate of any solute is very important.

Agitation

Dissolution also depends on the concentration of the solvent. If the solvent is more concentrated dissolution will be less. If the solvent is less concentrated dissolution will be raised.

Solvent selection

Dissolution also depends on the type of the solvent. In water dissolution rate will be more than oily solvent (Yeomans, 2000).

1.10 Comparative dissolution

1.10.1 Basic concept of Comparative dissolution

Comparative dissolution testing is very important tool in drug development. Including serving as routine quality control tests, comparative dissolution tests is one of the best tools to support waivers for bioequivalence requirements, for approval of generic drug products. Accepting

product sameness under Scale-up and Post Approval (SUPAC)-related changes depends on the comparative dissolution test (Anand *et al.* 2011).

1.10.2 Specifications and Experimental Conditions

For immediate release products In United States the Centre for Drug Evaluation and Research (CDER) of the Food and Drug Administration (US FDA) pointed three categories of dissolution test specifications. These are single point specifications, two point specifications and dissolution profile comparison. Single and two-point specifications are sufficient to indentify drug products containing high solubility-high permeability substances. But the thing is, this is not suitable for characterization of low solubility products because such products have produced different dissolution profiles. Consequently, they may comply with the point estimates, thereby giving an erroneous impression of pharmaceutical equivalence in dissolution characteristics. It is recommended that dissolution profile comparison is for such products, as it is more precise and discriminative than point estimates others. At least three dissolution media is needed for comparative dissolution profile testing of drugs in order to study their stability and release describe in the different physiological conditions that they may be subjected to in vivo. The recommended dissolution media are 0.1 M HCl or buffer solution of pH 1.2 as well as buffer solutions of pH 4.5 and 6.8. Water can be used as an additional medium in the studies (Yuksel *et al*. 2000).

1.10.3 Methods for Comparison of Dissolution Profile Data

For *in vitro* dissolution profile there are three groups to taste the comparative dissolution profile:

i. Methods based on analysis of variance (ANOVA)

ii. Model-dependent methods

iii. Model-independent methods

ANOVA-based methods use in variety and multivariate approaches to measure the quantity in dissolution percentages. The cubic root law, which is a model depended method (Hixson and Crowell) mathematical model, the Weibull distribution model and the logistics (Rowlings) model for sigmoidal dissolution curves (Yuksel *et al*., 2000).

Moore and Flanner (1996) proposed a very simple model independent method to produce the fit factors to compare dissolution profile data of a pair of products under similar conditions. These fit factors directly compare the difference between percent drug dissolved per unit time for a test

and a reference product. These factors are denoted *f*1 (difference factor) and *f*2 (similarity factor) (Patel, 2009).

The difference factor (f1) is a measurement of the percent difference between two dissolution curves under comparison at each time point. It is a measure of the relative error between the two curves and is given by the formula:

$$f1 = \frac{\sum_{t=1}^{n}|Rt-Tt|}{\sum_{t=1}^{n} Rt} \; x \; 100$$

where, n is the number of testing time points; Rt is the average dissolution value of the reference product units at time t and Tt is the average dissolution value of the test product units at time t. Similarity of two dissolution curves is indicated by f1 values of 0 - 15% (Hasan *et al.,* 2007)
The similarity factor (*f*2) is a measurement of the similarity in the percent dissolution between two dissolution curves. It is inversely proportional to the average squared difference between the two profiles. It is a logarithmic reciprocal square root transformation of the sum of squared error and is given by the formula:

$$f2 = 50.log\left[1\Big/\sqrt{\left\{1+\frac{1}{n}\sum_{t=1}^{n}(Rt-Tt)^2\right\}}\; x\; 100\right]$$

where, n is the number of testing time points; Rt is the average dissolution value of the reference product units at time t and Tt is the average dissolution value of the test product units at time t. Similarity of two dissolution curves is indicated by f1 values of 0 - 15% (Hasan *et al.,* 2007)
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It is recommended for evaluation for similarity is availability of data for

six (6) or twelve (12) units of each product, availability of three or more dissolution time points, same conditions of testing for reference and test products and same dissolution time points for both profiles. As a further recommendation, it is suggested that only one measurement be considered after 85% dissolution of both products. (Ochekpe *et al.,* 2006).

The similarity factor has been adopted by the US FDA and the European Medicines Agency (EMEA) for dissolution profile comparison. When two dissolution profiles are identical, $f2$ = 100%. An average dissolution difference of 10% at all measured time points results in an $f2$ value of 50%. For this reason, the public standard for similarity of two dissolution profiles has been set at 50 - 100% (Shah, 2001).

1.11 Different brands of Metformin hydrochloride available in Bangladesh:

	Brand Name	**Company**
1	Bigmet	Renata Ltd
2	Comet	Square Pharmaceuticals Ltd.
3	D-Fo	Decent Pharmaceuticals Ltd.
4	Daomin	Acme Laboratories Ltd.
5	Dia-M	Medimet Pharmaceuticals Ltd.
6	Diabex	Gaco Pharmaceuticals Ltd.
7	Diafre	Mystic Pharmaceuticals Ltd.
8	Etform	Novartis Bangladesh Ltd.
9	Formin	Skylab Pharmaceuticals Ltd.
10	Formin	Zenith Pharmaceuticals Ltd.
11	G-Phase	Edruc Ltd.
12	Glucomet	Aristopharma Ltd.
13	Glunor	Eskayef Bangaladesh Ltd.

14	Gluphage XR	Silva pharmaceuticals Ltd.
15	Glymin	Healthcare Pharmaceuticals Ltd.
16	Hi-Met	Hudson Pharmaceuticals Ltd.
17	Info	Bristol Pharmaceuticals Ltd.
18	Informet	Beximco Pharmaceuticals Ltd.
19	Insimet	Ibn Sina Pharmaceuticals Ltd.
20	Kemin	Kemiko Pharmaceuticals Ltd.
21	Meforex	Jayson Pharmaceuticals Ltd.
22	Meforin	RAK Pharmaceuticals Ltd.
23	Met	Opsonin Pharmaceuticals Ltd.
24	Metarin	Popular Pharmaceuticals Ltd.
25	Metfar	White Horse Pharmaceuticals Ltd.
26	Metfast	Aexim Pharmaceuticals Ltd.
27	Metfen	Doctors Chemical Works Ltd.
28	Metfo	Pacific Pharmaceuticals Ltd.
29	Metform	ACI Ltd.
30	Metin	Supreme Pharmaceuticals Ltd.

(BDdrugs, 2017)

Chapter Two

LITERETURE REVIEW

2.0 Literature review:

Metformin hydrochloride is an orally administered anti-hyperglycemic agent, used in the management of non-insulin-dependent (type-2) diabetes mellitus. Unfortunately, a high percentage of patients suffering from type-2 diabetes are elderly people showing dysphagia. In this study, orally disintegrating tablets were prepared using direct compression and wet granulation method. First, the tablets of metformin were prepared using starch RX1500 and microcrystalline cellulose by direct compression. The tablets showed erosion behavior rather than disintegration. Then lactose was incorporated which created pores to cause burst release of drug. But these tablets did not give good mouth feel. Thus, Pearlitol SD 200 (spray dried mannitol) was used to prepare tablets by wet granulation (10% polyvinylpyrrolidone in Isopropyl alcohol as binder). The optimized batches of tablets (LMCT3 and MP13) not only exhibited desired mouth feel but also disintegration time, in vitro dispersion time, water absorption ratio, and in vitro drug release. All the batches contained 15% starch 1500 and 4% of croscarmellose sodium. The optimized batches prepared by direct compression and wet granulation showed 85% drug release at 4 min and 8min, respectively. The strong saline and slight bitter taste of the drug was masked using nonnutritive sweetener and flavor (Mohapatra, Parikh and Gohel, 2008).

This study was attempted to formulate a combination product of Glyburide and Metformin Hydrochloride Tablets USP 2.5mg/500mg and to evaluate their physico-chemical properties. Wet granulation method was adopted for preparation of tablet using different excipients namely Microcrystalline cellulose, Povidone K-30, Copovidone, Croscarmellose sodium and Sodium stearyl fumerate in six different formulations (F1-F-6). The granules for tabletting were evaluated for angle of repose, bulk density, tapped density, compressibility index and drug content etc. The tablets were subjected to thickness, hardness, friability, disintegration and *in vitro* release studies. The results of physical parameters of tablets showed that there were capping, hardness and friability problems in formulation F-1, F-2 and F-3. Granules of formula F-4, F-5 and F-6 showed

satisfactory flow properties, compressibility index and the physical parameters of tablets from these three formulations gave optimum result in comparison to innovator's brand. Disintegration time of these three formulations (7-8 min) was found similar with innovator's brand (6.30-7.30 min). Assay of formula F-6 of glyburide (97.97%) and Metformin Hydrochloride (100.2%) met the USP specification (90%-110%). It was also found that dissolution profile of Glyburide depends on particle size of Glyburide powder. When micronized and non micronized grade of Glyburide was used in a ratio of 3:1 (F-6) it gave similar dissolution profile as innovator's brand where the similarity factor (f2) was calculated as 59. On the other hand, dissolution profile of Metformin hydrochloride was found similar in all the three formulations (F-4, F-5, F-6) with reference to innovator having all f2 values above 50. Formulation F-6 possessed good stability in accelerated condition for 6 months study. By comparing the dissolution profiles with the innovator's drug glucovance® tablet, it was revealed that the formulation F-6 exhibit similar drug release profile for both Glyburide and Metformin Hydrochloride (Chowdhury, Nawreen and Rana, 2015).

The purpose of present investigation was to develop the dosage form containing metformin for both immediate and sustained release. The SR release tablets of metformin were not useful to control the fasting glucose levels whereas conventional metformin tablets cannot acts for prolonged time, But the tablets prepared by present method useful for control both fasting glucose levels and maintenance dose. Even though many combination therapies available in market as metformin for sustain release and other sulfonylureas for immediate release, The primary concern for considering metformin hydrochloride as monotherapy was its efficient activity, less cost and negligible cardiac risk factors. The immediate release dose was developed by direct compression method and sustained release beads were prepared by inotropic gelation method using sodium alginate and sodium CMC, CaCl2. The various batches of directly compressed tablets with different percentages of sustained release beads were prepared and evaluated for various physical properties and dissolution profile. Hardness (kg/cm2) of tablets was

decreased and percentage loss in friability is increased as concentration of beads in tablet increased. All the parameters are within range for tablets containing micro beads up to 35% thereafter loss in friability and Hardness are not within range (Movva, 2015).

The overall objective of the present work was to develop an oral sustained-release (SR) metformin tablet prepared by the direct compression method, using hydrophilic hydroxylpropylmethylcellulose (HPMC) and Guar gum polymer alone and in combination at different concentrations. Metformin hydrochloride (HCl), a biguanide, has a relatively short plasma half-life and low absolute bioavailability. All the batches were evaluated for thickness, weight variation, hardness and drug content uniformity and *in vitro* drug release. Mean dissolution time is used to characterize the drug release rate from a dosage form, and indicates the drug release-retarding efficiency of the polymer. The hydrophilic matrix of HPMC alone could not control the Metformin release effectively for 12 h whereas when combined with Guar gum, it could slow down the release of drug and, thus, can be successfully employed for formulating SR matrix tablets. Fitting the data to the Korsmeyer equation indicated that diffusion along with erosion could be the mechanism of drug release. Similarity factor $f2$ values suggest that the test and reference profiles are identical (Wadher, Umekar and Kakde, 2011).

In the present study hydrophilic gelling polymer based gastroretentive (floating) tablets of metformin hydrochloride were formulated and evaluated for increase bioavailability by increasing gastric residence time and sustained release of drug on the upper part of gastrointestinal tract thereby diminishing side effects and enhanced patient compliance. Metformin hydrochloride, an oral antidiabetic having narrow absorption window in the upper part of gastrointestinal tract, was formulated as floating matrix tablet using gas generating agent (potassium bicarbonate) and hydrophilic gelling polymer hydroxyl propyl methyl cellulose (hypromellose) by wet granulation technique. The formulation was optimized on the basis of in vitro drug release profile using 23 full factorial design with t50% and t80% as the kinetic parameters. The prepared formulations were evaluated for floating time and in vitro drug release characteristics using modified dissolution

method. All formulations possessed good floating properties with total floating time more than 12 hours. Formulations with high amount of hypromellose were found to float for longer duration and provide more sustained release of drug. The formulated drug delivery system was found to be independent of pH. Result showed the formulation F4 to closely match the extra design checkpoint (F9) formulation with a similarity factor value of 98.13. Matrix characterization included photomicrograph, scanning electron microscopy which showed definite entrapment of drug in the matrix. Release kinetics of formulations followed Higuchi model with anomalous non fickian diffusion. Hence it is evident from this study that gastroretentive tablets could be a promising delivery system for metformin hydrochloride with sustained drug release action and improved drug bioavailability (Flores et al., 2011).

An attempt was made to sustain the release of metformin HCl as well as to mask the bitter taste by complexation technique using strong cation-exchange resins, indion 244 and indion 264. The drug loading onto ion-exchange resin was optimized for mixing time, activation, effect of pH, mode of mixing, ratio of drug:resin and temperature. The resinate was evaluated for micromeritic properties, taste masking and characterized using XRPD and IR. Using resinate sustained release tablets were formulated using hydoxypropylmethylcellulose K100M.The tablets were evaluated for hardness, thickness, friability, drug content, weight variation and *in vitro* drug release.The release of metformin HCl from resinate controls the diffusion of drug molecules through the polymeric material into aqueous medium. Results showed that metformin HCl was successfully taste masked and formulated into a sustained dosage form as an alternative to the conventional tablet (Bhoyar and Biyani, 2010).

Metformin HCl is an oral Anti-diabetic drug belongs to the class of biguanide derivatives commonly used to treat type 2 diabetes mellitus. The study was conducted to assess the comparative in-vitro quality control parameters through the evaluation of mechanical strength, dissolution study in buffer solution, content and weight uniformity between the commercially available conventional and modified (sustained release) tablets of different

brand of Metformin in India. It can be concluded that standard quality control parameters always should be maintained not only for Metformin but also for all kinds of medicine for getting better drug products (Kumar, 2013).

A quality control assessment of five brands of metformin hydrochloride tablets marketed in Nigeria [Glucophage (R) (Merck, Quetta), Metformin BDC (Bangkok labs, Bangkok), Metformin (Medopharm, India), Glucophage (R) (Ilsan), Glucophage (Lipha)] was carried out in order to determine the brands that are interchangeable or switchable. The disintegration time, dissolution rate and absolute drug content were determined in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) without enzymes. The weight uniformity and hardness tests were also performed according to the official methods. A variation of the concept of dissolution efficiency (DE), known as predicted availability equivalent (PAE), was used to predict the likely in vivo bioavailability. Our results showed that all the five brands passed the uniformity of weight and disintegration tests. Dissolution efficiency was found to be higher in SGF than in SIF. In SGF, all the brands were bioequivalent. In SIF, all the brands, except Medopharm, were also bioequivalent. The study showed that four brands of metformin hydrochloride (Merck, BDC, Lipha and Ilsan) marketed in Nigeria are of acceptable standards and hence BDC, Lipha and Ilsan brands of glucophage are interchangeable with the innovator drug, glucophage (Merck, 2013).

A simple and sensitive spectrophotometric method has been developed and validated for the estimation of metformin hydrochloride in bulk and in tablet formulation. The primary amino group of metformin hydrochloride reacts with ninhydrin in alkaline medium to form a violet colour chromogen, which is determined spectrophotometrically at 570 nm. It obeyed Beer's law in the range of 8-18 μg/ml. Percentage recovery of the drug for the proposed method ranged from 97-100% indicating no interference of the tablet excipients. The proposed method was found to be accurate and precise for routine estimation of metformin hydrochloride in bulk and from tablet dosage forms (Sharma, Chaturvedi and Sahoo, 2008).

Metformin HCL, the only available biguanide, remains the first line drug therapy for patients with Type 2 diabetes mellitus acts by decreasing hepatic glucose output and peripheral insulin resistance. It has relatively short plasma half life, low absolute bioavailability. The overall objective of the present work was to develop an oral sustained release metformin tablet prepared by direct compression method, using hydrophilic hydroxyl propyl methylcellulose and Xanthan gum polymer as rate controlling factor. All the batches were evaluated for thickness, weight variation, hardness, and drug content uniformity and in vitro drug release. Mean dissolution time is used to characterize drug release rate from a dosage form and indicates the drug release retarding efficiency of polymer. Hydrophilic matrix of HPMC alone could not control the Metformin release effectively for 12 h whereas when combined with Xanthan gum could slow down the release of drug and can be successfully employed for formulating sustained-release matrix tablets (Wadher, Umekar and Kakde, 2011).

Chapter Three

MATERIALS AND METHODS

3.1 Introduction:

The study on comparative dissolution profiles of Metformin Hydrochloride was carried out by using dissolution method to see the release pattern of Metformin Hydrochloride with different time interval. The method was verified and the rotating condition of the dissolution machine is optimized before starting sample analysis.

Comparative dissolution testing is a tool in drug development and characterization. In addition, routine quality control tests and comparative dissolution tests have been used to support waivers for bioequivalence requirements, for approval of generic drug products and accepting product sameness under Scale-up and Post Approval (SUPAC) related changes (Ulrich, *et. al.* 2009).

3.2 Reagents, Chemicals and Solvents

All reagents used were of analytical reagent grade and distilled water was used for the preparation of all solutions. To observe the change in dissolution of Metformin Hydrochloride in dissolution media I used different brands of Metformin Hydrochloride tablet. I used active pharmaceutical ingredient (API) which was collect from Incepta Pharmaceuticals Ltd. As the dissolution media is water for dissolution of Metformin Hydrochloride, we used water as a solvent.

For preparing standard curve, I used API from Incepta Pharmaceuticals Ltd. Other tablets I used to see the release pattern with different time interval are Glucomet 500 mg from Aristopharma Ltd., Met 500 mg from Opsonin Pharma Limited and Daomin 500 mg from ACME Laboratories Ltd.

3.3 Methods for Comparison of Dissolution Profile Data

A simple model independent method proposed by Moore and Flanner (1996) uses fit factors to compare dissolution profile data of a pair of products under similar testing conditions. These fit factors directly compare the difference between percent drug dissolved per unit time for a test and reference product. These factors are denoted as f1 (difference factor) and f2 (similarity factor) (US FDA, 1997; Saranadasa and Krishnamoorthy 2005; Sath*, et. al.* 1996; Yuksel *et. al.* 2000).

3.4 Difference factor

The difference factor (f1) is a measurement of the percent difference between two dissolution curves under comparison at each time point.

It is a measure of the relative error between the two curves and is given by the formula:

$$f1 = \frac{\sum_{t=1}^{n}|Rt - Tt|}{\sum_{t=1}^{n} Rt}\ x\ 100$$

where, n is the number of testing time points; Rt is the average dissolution value of the reference product units at time t and Tt is the average dissolution value of the test product units at time t. Similarity of two dissolution curves is indicated by f1 values of 0 - 15% (US FDA, 1997; Hasan, *et. al.* 2007; Yuksel, *et. al.* 2000).

3.5 Similarity factor

The similarity factor (f2) is a measurement of the similarity in the percent dissolution between two dissolution curves. It is inversely proportional to the average squared difference between the two profiles. It is a logarithmic reciprocal square root transformation of the sum of squared error and is given by the formula:

$$f2 = 50.log\left[1\Big/\sqrt{\left\{1 + \frac{1}{n}\sum_{t=1}^{n}(Rt - Tt)^2\right\}}\ x\ 100\right]$$

Where, n is the number of testing time points; Rt is the average dissolution value of the reference product units at time t and it is the average dissolution value of the test product units at time t (US FDA, 1997; Hasan, *et. al.* 2007; Shah 2001; Yuksel, *et. al.* 2000). The terms for evaluation for similarity is the availability of data for six (6) or twelve (12) units of each product, availability of three or more dissolution time points, same conditions of testing for reference and test products and same dissolution time points for both profiles. As a further recommendation, it is suggested that only one measurement be considered after 85% dissolution of both products.

(US FDA, 1997; Hasan, *et. al.* 2007; Ochekpe, *et. al.* 2006).The similarity factor has been adopted by the US FDA and the European Medicines Agency (EMEA) for dissolution profile comparison. When two dissolution profiles are identical, f2 = 100%. An average dissolution

difference of 10% at all measured time point's results in an f2 value of 50%. For this reason, the public standard for similarity of two dissolution profiles has been set at 50 - 100% (EMEA 2010; USFDA 1997; Shah, 2001).

3.6 Dissolution Testing Methods for Metformin Hydrochloride

Table 3.1- Dissolution parameter

Dissolution media	Distilled water
RPM	50
Temperature	37ºC
Time	50 minutes
Wavelength	232nm

The release rate of Metformin Hydrochloride tablet was determined by using tablet dissolution tester USP XXII. The dissolution test was performed using 900ml water pH (7.4) at 37±0.5 degree C and 50 r.p.m. At every 10 mins interval sample of 5 ml were withdrawn from the dissolution medium and the amount was replace by 5 ml distilled water. The sample was filtered through a filter paper named Whatmaan Filter paper and diluted to a suitable concentration of distilled water. The absorbance of the solution was measured 232nm for drug Metformin Hydrochloride by using a Shimadzu UV-1201 UV/visible double beam spectrophotometer (Hach, Japan).Percentage of drug release was calculated using an equation obtained from standard curve. The dissolution was continued for 50 minutes to get simulated picture of drug release in the in vivo condition and drug dissolve at specified time periods was plotted as percent release versus time(hours) curve (Shah,*et al.*1998).

3.7 Preparation of Standard Curve:

To prepare the standard curve, at first different concentrations (5, 10, 15, 20 and 25) µg/ml of Metformin Hydrochloride was prepared. The concentration of the stock solution collected from Incepta Pharmaceuticals had been 0.5mg/ml or 500µg/ml. This solution was filtered in the volumetric flask. After that the solution was 10 times diluted and the concentrations of the solution become 50µg/ml. Then taken solution was 1 ml, 2 ml, 3 ml, 4 ml, 5 ml and added water was 9 ml, 8 ml, 7 ml, 6 ml, and 5 ml. Then spectrophotometer is turned on and 232nm wave length was set up. Then the spectrophotometer was adjusted for 0 and 100%.The solutions were placed on spectrophotometer to measure the absorbance. Then the absorbance was plotted against concentration. A straight line was found.

Table 3.2- Concentrations of Metformin Hydrochloride (Campanero, *et. al.*1998)

Serial no	Concentration(µg/ml)
1	5
2	10
3	15
4	20
5	25

3.8 Preparation for dissolution test:

3.8.1 Preparation of stock solution:

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

3.8.2 Method for dissolution test of Glucomet (Metformin Hydrochloride)

6L (6000ml) of stock solution (distilled water) was prepared. Each vessel of dissolution tester was filled with 900 ml of stock solution (distilled water) Time 1 hour; rpm 50 was set up in the dissolution machine. Then the machine was allowed to warm up until it reached at 37.5 degree C. Then one Glucomet tablet was placed in every vessel. After 10, 20, 30, 40 and 50 minutes 5 ml of solution was collected from each vessels and filtered, then from that 1 ml of solution was taken in another test tube and 9 ml distilled water was added to make it 10 ml. At last UV absorbance off the solutions were taken where the wave length was 232nm. (Lawrence, *et. al.,* 2002).

3.8.3 Method for dissolution test of Met (Metformin Hydrochloride)

6L (6000ml) of stock solution (distilled water) was prepared. Each vessel of dissolution tester was filled with 900 ml of stock solution (distilled water) Time 1 hour; rpm 50 was set up in the dissolution machine. Then the machine was allowed to warm up until it reached at 37.5 degree C. Then one Met tablet was placed in every vessel. After 10, 20, 30, 40 and 50 minutes 5 ml of solution was collected from each vessels and filtered, then from that 1 ml of solution was taken in another test tube and 9 ml distilled water was added to make it 10 ml. At last UV absorbance off the solutions were taken where the wave length was 232nm. (Lawrence, *et. al.,* 2002).

3.8.4 Method for dissolution test of Daomin (Metformin Hydrochloride)

6L (6000ml) of stock solution (distilled water) was prepared. Each vessel of dissolution tester was filled with 900 ml of stock solution (distilled water) Time 1 hour; rpm 50 was set up in the dissolution machine. Then the machine was allowed to warm up until it reached at 37.5 degree C. Then one Daomin tablet was placed in every vessel. After 10, 20, 30, 40 and 50 minutes 5 ml of solution was collected from each vessels and filtered, then from that 1 ml of solution was

taken in another test tube and 9 ml distilled water was added to make it 10 ml. At last UV absorbance off the solutions were taken where the wave length was 232nm. (Lawrence, *et. al.,* 2002).

3.9 Determination of physical parameters

3.9.1 Weight Variation Test

3.9.1.1 Procedure:

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

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

Table 3.3: Accepted percentage list for weight variation test of tablets

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

3.9.1.2 Equation:

Following equation was used to determine % weight variation of tablets

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

Where,

Initial Weight of Tablet, I (gm)

Average weight of Tablets, A (gm) (Dunnett, C. W., and R. Crisafio.1995)

3.9.2 Thickness test

3.9.2.1 Procedure

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

3.9.2.2 Calculation

Following formula was used to determine thickness of tablets.

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

3.9.3 Hardness test

3.9.3.1 Procedure

The slide scale of hardness tester was made zero. One tablet was placed vertically between the two jaws of the tester. Force was applied with a screw thread and spring until tablet fractured. Reading in Kg was taken from the sliding scale (Dunnett and Crisafio, 1995).

3.9.3.2 Materials

3.9.3.2.1 Sample Collection

To observe the change in dissolution pattern of Different brands of Metformin Hydrochloride tablets with each other, samples were collected from the local drug store in Dhaka.

Table 3.4: Samples used in the experiment including source

Brand Name	**Source**
Glucomet tablets	Aristopharma Ltd.
Met tablets	Opsonin Pharma Limited
Daomin tablets	ACME Laboratories Ltd.

3.9.3.2.2 Stock solution:

As Metformin Hydrochloride is soluble in water so distilled water was prepared in the laboratory of East West University and was used as stock solution for dissolution

3.9.3.2.3 Equipments:

Table 3.5: In the characterization of matrix tablets of Metformin Hydrochloride (Kuss, 1992)

No.	**Equipments**	**Source**	**Origin**
1	Dissolution tester USPXXII	RC-6B	CHINA
2	UV-Spectrometer	HANNA1201PC	JAPAN
3	pH meter	HANNA pH 210	PORTUGAL
4	Distill Water Plant	SMIC	CHINA
5	Safety Pipette Filler	Saffron	ENGLAND
6	Filter	Copley Instruments	ENGLAND
7	Electronic Balance	Precisa XB120A	SWITZERLAND
8	Friability tester	VEEGO(EF-2)	INDIA
9	Vernier Slide Calipers	TRICLYCLE RING	INDIA
10	Hardness tester	Monasnto manually operating hardness tester	CHINA

3.10 Instrumentation

3.10.1 Dissolution Test Apparatus

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

peristaltic pump with tygon tubing's, a microprocessor controlled sample collector and a sample tray capable of collecting 10 x 6 sets of samples. Polycarbonate dissolution vessels with a hemispherical bottom and a capacity of 1000 ml were used for the study. Bromide (E. Merck, Darmstadt, Germany) and a manually operated hydraulic pellet press (Perking Elmer GmbH, Uberlingen, Germany).

3.10.2 Ultra- Violet Spectrophotometer

The ultra-violet absorption spectrum for Metformin Hydrochloride working standard was recorded using a double beam T90+ UV/VIS spectrometer controlled via a computer using UVWIN spectrophotometer software version 5.2.0 (HACH UV-1201 PC, JAPAN) over a 10 mm path length using quartz cuvettes.

3.11 Samples and Chemical Reference Substances

Metformin Hydrochloride tablets from different manufacturers were used in the study. The samples were obtained from different private retail outlets within
Bangladesh (Kuss,1992).

3.12 Images of Instruments:

Some images of important instruments those were used in different testes during research work are given below-

Figure 3.1: Dissolution apparatus (Tresnainstrument, 2016)

Figure 3.2: UV-1800 Double Beam Spectrophotometer (Tresnainstrument, 2016)

Figure 3.3: Distill Water Apparatus (Tresnainstrument, 2016)

Figure 3.4: Electronic Balance (Tresnainstrument, 2016)

Figure 3.5: Hardness Tester (Tresnainstrument, 2016)

3.13 Dissolution Efficiency

The dissolution efficiency is not a parameter to compare dissolution pattern between two brands. It is just a parameter to indicate drug release. It is calculated by the following equation:

$$DE = \frac{\int_{t1}^{t2} y.dt}{y100 \times (t2 - t1)} \times 100$$

In the above equation, y is the percentage of drug release. The numerator of the equation indicates the area under within the time frame. The denominator indicates the rectangle of 100% drug release from 0 times throughout the time frame. The area under the curve is calculated by the help of Microsoft Excel software (Anderson et al. 1998; Parakh and Patil 2014).

3.14 Apparatus:

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

Table 3.6- Representing the apparatus (Kuss, 1992)

Serial no	**Apparatus**
1	Beakers
2	Test tubes
3	Volumetric flasks
4	Filter paper
5	Spatula
6	Mortar and pestle
7	Pipette pumper
8	Pipette (1 ml & 10 ml)

Chapter Four

RESULTS AND DISCUSSION

4.1 Physical properties

4.1.1 Weight Variation Test

Table 4.1: Average weight of tablets of different brands

Name of the Drug	**Weight of tablets (mg)**
Glucomet	598.00
Met	541.00
Daomin	573.00

The experiments were done with three different brands of Metformin Hydrochloride. After the test it was seen that variations of the weight of the tablets are not very significant. The weight of the Glucomet is 598.00 mg. The weight of Daomin is very close to Glucomet and Met.

4.1.2 Disintegration time:

Table 4.2: Disintegration test

Formulation	Sample I Time (Minutes)	Sample II Time (Minutes)	Sample III Time (Minutes)	Average Time (Minutes)
Glucomet	21.50	20.45	21.22	21.06
Met	22.46	23.32	24.57	23.35
Daomin	18.57	20.04	19.43	19.48

All three of the drugs are film coated. Disintegration time for film coated tablet is 30 mins. All three tablets are disintegrated within 30 mins without showing much disparity.

4.2 Standard curve of Metformin Hydrochloride.

For the calculation of drug release from the test brands, a standard curve was prepared within the concentration range of 0-10 µg/mL. The curve displayed sufficient linearity with a correlation coefficient(R^2)=0.9994 and provided an equation y=0.0741x+0.0058.

Table 4.3: Standard curve of Metformin Hydrochloride

Concentration (µg/ml)	Absorbance
0	0
2	0.142
4	0.279
6	0.439
8	0.594
10	0.734

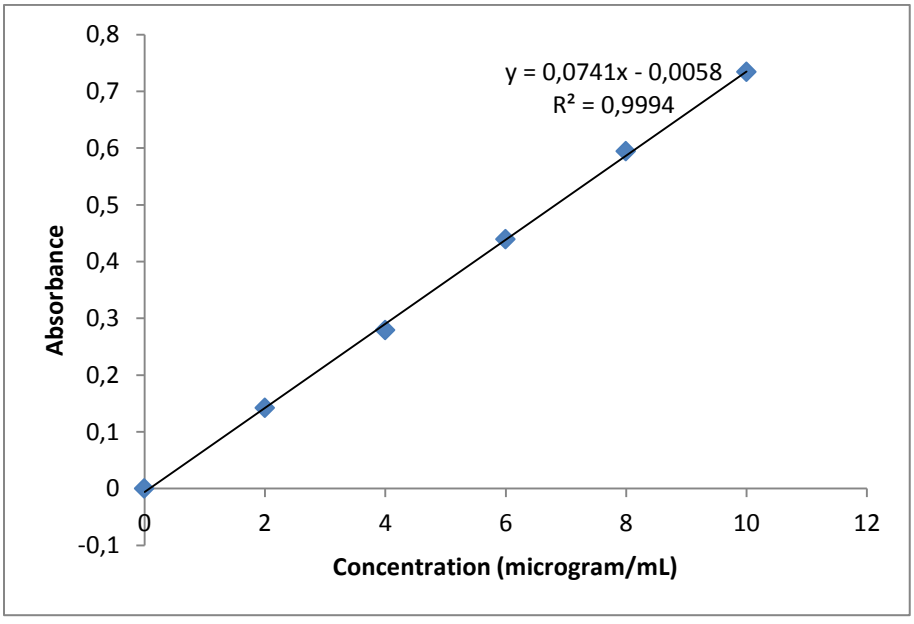

Figure 4.1: Standard curve Concentration Vs Absorbance (Metformin Hydrochloride)

By plotting the concentration against the absorbance of Metformin Hydrochloride we found a straight line. From the standard curve of Metformin Hydrochloride, we derived an equation y=0.0741x+0.0058 & R^2=0.9994 (Here, y= Absorbance and x=Concentration of drug).

4.3 Percent (%) release of Glucomet Tablet

Table 4.4: Percent (%) release of Glucomet Tablet

Time (Minutes)	**Drug Release (%)**
0.00	0.00
10.00	17.22
20.00	32.4
30.00	40.09
40.00	56.37
50.00	62.64

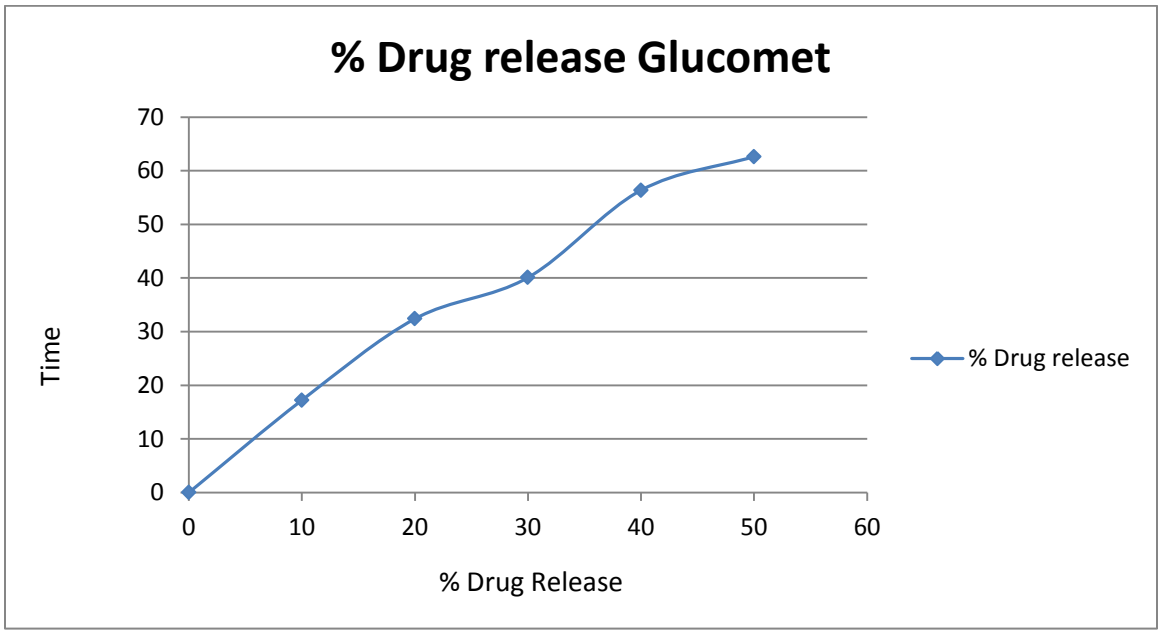

Figure 4.2: Time Vs Drug Release (%) Glucomet Tablet

Here the graph shows that the release of Glucomet (Metformin Hydrochloride) tablets are increased with time. We can see that the release pattern of the drug is increased with time. This graph does show the increasing of drug release according to the increasing of time. In 0.00 minutes the drug release was 0.00, In 10.00 minutes it was 17.22, In 20.00 minutes it was 32.4. In 30, 40, 50 minutes it was respectively 40.09, 56.37, 62.64.

4.4 Percent (%) release of Met tablet

Table 4.5: Percent (%) Release of Met tablet

Time (Minutes)	**Release (%)**
0.00	0.00
10.00	17.07
20.00	46.61
30.00	53.57
40.00	67.62
50.00	81.11

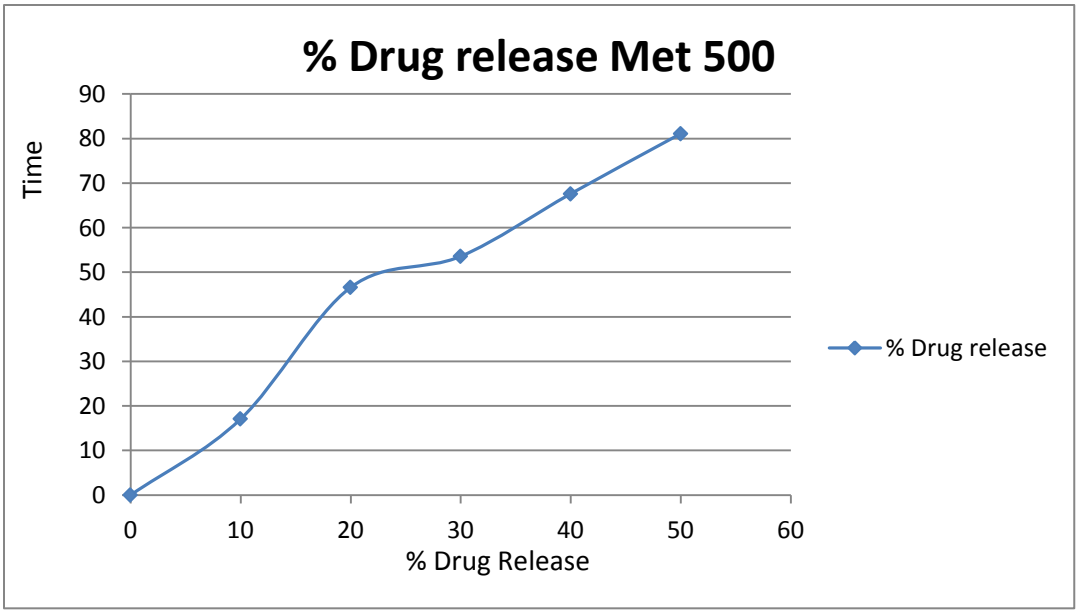

Figure 4.3: Time Vs Drug Release (%) of Met tablet

This graph represents that, the increase of drug release in accordance with the increasing of time. In 0.00 minutes the drug release was 0.00 , In 10.00 minutes it was 17.07 then In 20, 30 ,40 and 50 minutes it was respectively 46.61 ,53.57 , 67.62 , 81.11. Here X axis represents the time and Y axis is for Drug release.

4.5 Percent (%) release of Daomin tablet

Table 4.6: percent (%) release of Daomin tablet

Time (Minutes)	**Release** (%)
0.00	0.00
10.00	15.52
20.00	43.65
30.00	57.18
40.00	66.85
50.00	81.79

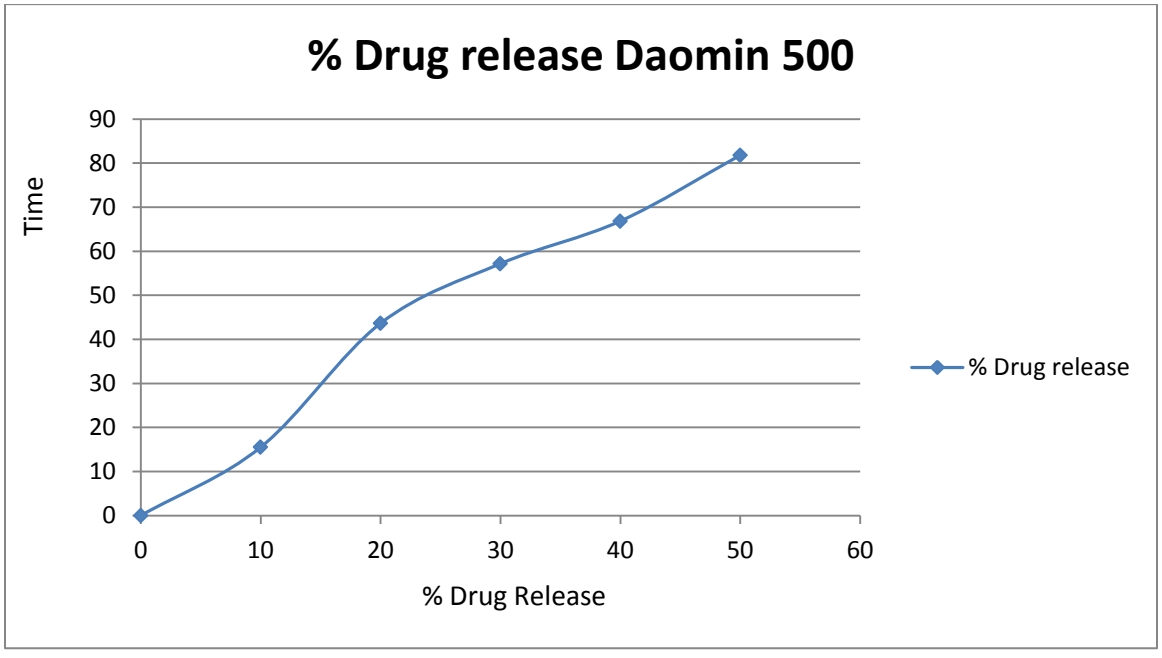

Figure 4.4: Time vs Drug Release (%) of Daomin tablet

This graph shows that the increasing of drug release according to the increasing of time. In 0.00 minutes the drug release was 0.00. In 10, 20, 30, 40, 50 minutes it was 15.52, 43.65, 57.18, 66.85, 81.79. Here X axis represents the time and Y axis is for Drug release.

4.6 Drug dissolution of different brands:

Table 4.7- Drug dissolution of different brands:

Time (Minutes)	**Glucomet(A) Release (%)**	**Met (B) Release (%)**	**Daomin (C) Release (%)**
0.00	0.00	0.00	0.00
10.00	17.22	17.07	15.52
20.00	32.44	46..61	43.65
30.00	40.09	53.57	57.18
40.00	56.37	67.62	66.85
50.00	62.64	81.11	81.79

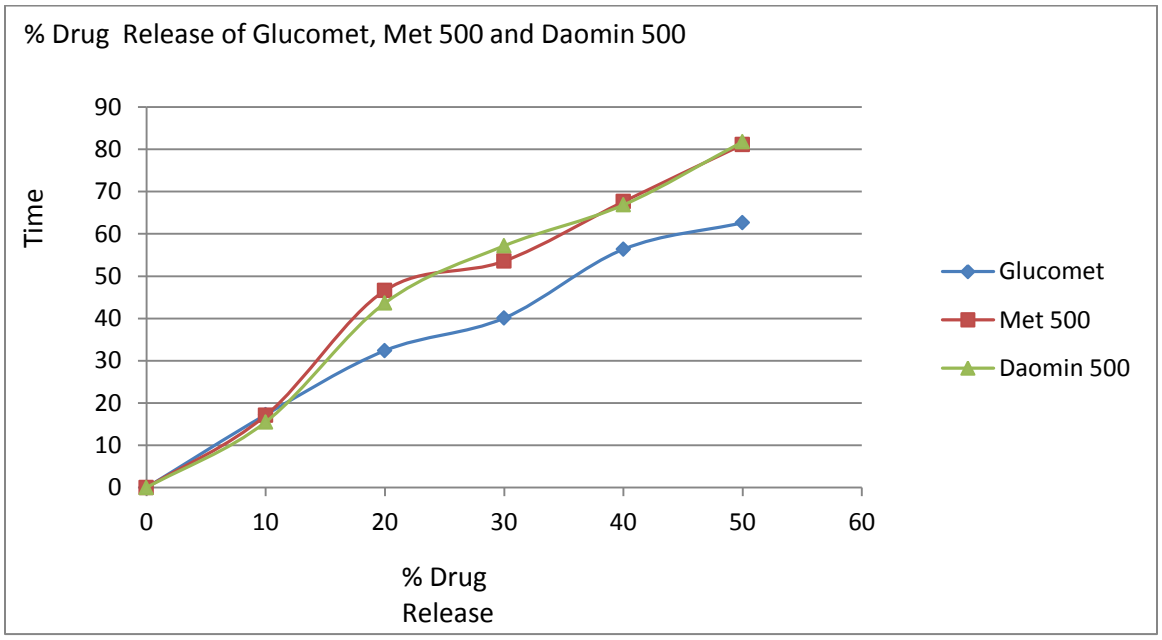

Figure 4.5: Drug dissolution profile of different brands

The graph shows that, the comparison of dissolution pattern of three different brands of drug with each other. The dissolution pattern of Daomin looks good in comparison with Met and Glucomet.

4.7 *f*1 Calculation for Glucomet (A) vs Met (B)

Difference Factor, *f*1 is the average difference between all the points of sampling between two brands e.g. Glucomet (A) and Met (B). Acceptable range of *f*1 is between 0-15. *f*1 value greater than 15 means significant difference between the excipients of two brands which is not acceptable (Lokhandwala et al. 2013; Parakh and Patil 2014; Patel, *et. al*. 2015; Qazi et al. 2013).

Table 4.8- *f*1 Calculation for Met (B) with respect to Glucomet (A)

Time (Minutes)	**Glucomet(A) Release %**	**Met (B) Release %**	**A–B**	**|A-B|**	***f* 1**
10	17.22	17.07	0.15	0.15	
20	32.40	46.61	-14.21	14.21	
30	40.19	53.57	-13.38	13.38	27.51
40	56.37	67.62	-11.25	11.25	
50	62.64	81.11	-18.47	18.47	
Total	208.82			57.46	

Acceptable range of *f*1 is between 0-15. *f*1 value greater than 15 means significant difference between two brands which is not acceptable. From the table 4.8 we see that the value of *f*1 is 27.51 which is not acceptable. It shows us that there are significant differences between these two brands of drugs in terms of excipients.

4.8 *f*1 Calculation for Glucomet (A) vs Daomin (C)

Table 4.9- *f*1 Calculation for Daomin (C) with respect to Glucomet (A)

Time (Minutes)	**Glucomet(A) Release %**	**Daomin (C) Release %**	**A-C**	**|A-C|**	***f* 1**
10	17.22	15.52	1.7	1.7	
20	32.40	43.65	-11.25	11.25	
30	40.19	57.18	-16.99	16.99	28.52
40	56.37	66.85	-10.48	10.48	
50	62.64	81.79	-19.15	19.15	
Total	208.82			59.57	

Acceptable range of *f*1 is between 0-15. *f*1 value greater than 15 means significant difference between two brands which is not accepted. From the table 4.9 we see that the values of *f*1 is 28.52 so it is not acceptable. It shows us that there are significant differences between these two brands of drugs in terms of excipients.

4.9 *f*1 Calculation for Met (B) vs Daomin (C)

Table 4.10- *f*1 Calculation for Daomin (C) with respect to Met (B)

Time (Minutes)	**Met (B) Release %**	**Daomin (C) Release %**	**B-C**	**|B-C|**	***f* 1**
10	17.07	15.52	1.55	1.55	
20	46.61	43.65	2.96	2.96	
30	53.57	57.18	-3.61	3.61	3.60
40	67.62	66.85	0.77	0.77	
50	81.11	81.79	-0.68	0.68	
Total	265.98			9.57	

Acceptable range of *f*1 is between 0-15. *f*1 value greater than 15 means significant difference between two brands which is not accepted. From the table 4.10 we see that the values of *f*1 is 3.60 so it is acceptable. It shows us that there are no significant differences between these two brands of drugs in terms of excipients.

4.10 *f*2 Calculation for Glucomet (A) vs Met (B)

Similarity Factor, *f*2 is calculated to determine significant similarity between two brands. The range of the *f*2 value is between 0 to 100. If the value remains between 50 to 100, it is acceptable (Lokhandwala et al. 2013; Parakh and Patil 2014; Patel et al. 2015; Qazi et al. 2013).

Table: 4.11- *f*2 Calculation for Glucomet (A) vs Met (B)

Time (Minutes)	**Glucomet(A) Release %**	**Met (B) Release %**	**A–B**	**|A-B|**	**$\|A-B\|^2$**	***f*2**
10	17.22	17.07	0.15	0.15	0.022	
20	32.40	46.61	-14.21	14.21	201.92	
30	40.19	53.57	-13.38	13.38	179.02	26.76
40	56.37	67.62	-11.25	11.25	126.56	
50	62.64	81.11	-18.47	18.47	341.12	
Total	208.82			57.46	848.66	

From the table 4.11 we see that the value of *f*2 is 26.76. The range of the *f*2 value is between 0 to 100. If the value remains between 50 to 100, it is acceptable. As the value of *f*2 is not within 50 to 100, it is not acceptable. It shows us that there are no significant similarities between these two brands of drugs in terms of excipients.

4.11 f2 Calculation for Glucomet (A) vs Daomin (C)

Table: 4.12 -*f*2 Calculation for Glucomet (A) vs Daomin (C)

Time (Minutes)	**Glucomet(A) Release %**	**Daomin(C) Release %**	**A-C**	**|A-C|**	**$\|A-C\|^2$**	***f*2**
10	17.22	15.52	1.7	1.7	2.89	
20	32.40	43.65	-11.25	11.25	126.56	
30	40.19	57.18	-16.99	16.99	288.66	
40	56.37	66.85	-10.48	10.48	109.48	26.2
50	62.64	81.79	-19.15	19.15	366.722	
Total	208.82			59.57	894.23	

From the table 4.12 we see that the value of *f*2 is 26.2. The range of the *f*2 value is between 0 to 100. If the value remains between 50 to 100, it is acceptable. As the value of *f*2 is not within 50 to 100, it is not acceptable. . It shows us that there are no significant similarities between these two brands of drugs in terms of excipients.

4.12 *f*2 Calculation for Met (B) vs Daomin (C)

Table:4.13- *f*2 Calculation for Met (B) vs Daomin (C)

Time (Minutes)	**Met (B) Release %**	**Daomin(C) Release %**	**B-C**	**|B-C|**	**$\|B-C\|^2$**	***f*2**
10	17.07	15.52	1.55	1.55	2.41	
20	46.61	43.65	2.96	2.96	8.76	
30	53.57	57.18	-3.61	3.61	13.03	64.52
40	67.62	66.85	0.77	0.77	.59	
50	81.11	81.79	-0.68	0.68	.47	
Total	265.98			9.57	25.26	

From the table 4.13 we see that the value of *f*2 is 64.52. The range of the *f*2 value is between 0 to 100. If the value remains between 50 to 100, it is acceptable. As the value of *f*2 is within 50 to 100, it is acceptable. . It shows us that there are significant similarities between these two brands of drugs in terms of excipients.

Chapter Five
General Discussion

5.1 General Discussion

In this study, comparisons of dissolution profiles of Metformin Hydrochloride oral formulations were made between three generic products. Comparison of the dissolution profiles was carried out by calculation of the similarity factor and difference factor. The criteria for similarity were taken as up to 15 an *f*2 value of 50 - 100 for both tablets and suspensions. The study was carried out at pH 7 and with the media as water and then it was calculated for the values of factors. It was ran for 50 minutes with the intervals of 10 minutes and found the results provided previous discussion. The influence of pH was ignored in this study.

The extreme variations in the API release profiles for Metformin tablets reflect differences in the quality of manufacturing. This could be due to differences in the source and quality of coating, formulation factors like the coating process, relative composition of the content of the polymers and other excipients.

According to the result, there are significant differences between Glucomet and Met, and Glucomet and Daomin. But no significant differencess are found between Met and Daomin as *f1* and *f2* value approved by FDA.

Generally, the similarity factor patterns observed in this study indicate that analyze and single point dissolution tests are not sufficient to prove efficacy or pharmaceutical equivalence of products tested. Lack of comparative dissolution data for pharmaceutical equivalence and then bioequivalence raises questions of product quality. These impacts on efficacy of the products raising further concerns about the effect of sub-therapeutic outcomes and repercussions of treatment failures especially for Biguanides.

Drug regulatory authorities are major to controlling the quality of products in circulation in any market. The Conference of Experts on the Rational Use of Drugs, held in Nairobi in 1985, and WHO's Revised Drug Strategy, adopted by the World Health Assembly in May 1986, identified effective functioning of national drug regulation and control systems as a vital means to assure

safety and quality of medicines (WHO 2007). The Pharmacy and Poisons Board (PPB) is the regulatory body responsible for approvals and granting of market authorization of drugs in Bangladesh. This includes determining the requirements and content of drug registration dossiers as per the Common Technical Document (CTD) guidelines, dossier review, quality control (QC) tests and good manufacturing practices (GMP) inspections. After market authorization, the PPB is responsible for conducting post-marketing surveillance through its pharmacovigilance programme with a view to ensure consistent good quality products in circulation. The pharmacovigilance (PV) programme must therefore be effective, sustained and targeted with clear regulatory actions on non-compliant products. The success of the PV programme also depends on sufficient manpower with the necessary education, training and experience to perform the PV functions. The PPB thus plays a key role in assuring the quality of drug products circulating in the Bangladesh market.

Chapter Six

Conclusion and Recommendation

6.1 Conclusion

In this study, comparisons of dissolution profiles of Metformin Hydrochloride oral formulations were made between three generic products. Comparison of the dissolution profiles was carried out by calculation of the similarity factor and difference factor. The criteria for difference factor *f1* were taken as up to 15 and similarity factor f2 value of 50 - 100 for both tablets and suspensions. Few differences were observed during *in-vitro* drug release pattern of brand Glucomet, Met and Daomin with each other. Significant differences were found between Glucomet and Met and also between Glucomet and Daomin. On the other hand, significant similarities were found between Met and Daomin. The extreme variations in the API release profiles for Metformin tablets reflect differences in the quality of manufacturing. This could be due to differences in the source and quality of coating, formulation factors like the coating process, relative composition of the content of the polymers and other excipients. The study shows that there are significant differences between the different brands of Metformin Hydrochloride available in Bangladeshi local market.

6.2 Recommendation

Results of assays and single-point dissolution tests should not be taken as proof of product quality, safety and efficacy. *In vitro* dissolution profile data for generic drug products should be included in routine QC and post-market surveillance tests in order to demonstrate comparative differences between locally marketed brands of a specific drug. In addition, stringent GMP inspections should be consistently conducted by the national drug regulatory authority, the PPB to ensure adherence to quality standards during the manufacture and storage of pharmaceutical products. As a further measure, post-market surveillance activities by the PPB should be regular and sustained as a tool for determining the consistency of good quality products in circulation. These measures are important steps in curbing sub-optimal therapeutic outcomes, treatment failures and microbial resistance incidences resulting from exposure to substandard therapeutic agents and will ensure patients get benefit from the generic drug products.

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