In-Vitro Pharmaceutical Quality Control Testing: A Comparative study of Different Brands of Metformin 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. Rasel Shaikh, hereby declare that the dissertation entitled ***"In-Vitro Pharmaceutical Quality Control Testing: A Comparative study of Different Brands of Metformin 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 period 2017 of my research in the Department of Pharmacy, East West University, under the supervision and guidance of Nishat Nasrin, Assistant Professor, 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 Pharmaceutical Quality Control Testing: A Comparative study of Different Brands of Metformin 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. Rasel Shaikh, ID: 2014-1-70-077, during the period 2017 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

This is to certify that the thesis entitled ***"In-Vitro Pharmaceutical Quality Control Testing: A Comparative study of Different Brands of Metformin 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. Rasel Shaikh, ID: 2014-1-70-077, during the period 2017 of his research in the Department of Pharmacy, East West University.

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Prof. Chowdhury Faiz Hossain

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Dedication

This research paper is dedicated to my beloved Parents and my family members

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List of Abbreviation

AMPK	AMP-activated protein kinase
AMP	Adenosine Mono Phosphate
GLUT4	Glucose Transporter-4
ACC	Acetyl-CoA carboxylase
FDA	Food and Drug Administration
GFR	Glomerular Filtration Rate
LKB1	Liver Kinase B1
STK11	Serine/Threonine Kinase 11
BP	British Pharmacopoeia
USP	United States Pharmacopeia

Abstract

The aim of the present study was to evaluate and compare quality of locally branded drug products of Metformin Hydrochloride available in Bangladesh with each other. Three different brands of Metformin Hydrochloride tablets available in Bangladesh (Glunor, Daomin and Oramet) were collected from a reputed pharmacy store. The quality control parameters including weight variation, hardness, friability, disintegration test, dissolution test and potency test were performed to evaluate the tablets and to get a comparison between these marketed products. The specification about the drug was followed strictly as given in the USP and BP. For Daomin, Weight varition rage was from 1.9692 to -2.1569, Friability was 0.2496%, Average hardness was 4.19 kg/cm, Disintegration time was 7.5 miniutes, Dissolution(after 45min) was 88.68%, Potency was 93.8%. For Oramet, Weight variation range was from 1.5091 to -1.1644, Friability was 0.3746%, Average hardness was 4.40 kg/cm, Disintegration time was 6.3 minutes, Dissolution(after 45 min) was 85.97%, Potency was 101.2%. For Glunor, Weight variation range was from 1.3202 to -1.0557, Friability was 0.3300%, Average hardness was 4.18 kg/cm, Disintegration time was 9.8 minutes, Dissolution(after 45 min) was 92.22%, Potency was 92.2%. Various results were obtained from the test and compared with the specification. All the tablets met the specification and, hence, it can be concluded that the tablets of the local brands had the desired and optimum therapeutic efficacy.

Key words : Metformin, weight variation, hardness, friability, disintegration, dissolution, potency

Chapter one

Introduction

1.1 Overview

Type 2 diabetes mellitus results from impaired insulin secretion and reduced peripheral insulin sensitivity. Treatment options include diet, oral antihyperglycemic agents, and insulin. Metformin, an oral biguanide, ameliorates hyperglycemia by improving peripheral sensitivity to insulin, and reducing gastrointestinal glucose absorption and hepatic glucose production. Unlike sulfonylureas, it does not stimulate insulin secretion, aggravate hyperinsulinemia, or because hypoglycemia or weights gain (weight stabilizes or decreases). It also has beneficial effects on serum lipid profiles. In lean or overweight type 2 diabetic patients uncontrolled by diet, metformin monotherapy significantly improves glycemic control, compared with placebo, and to similar extents as sulfonylurea monotherapy. In secondary sulfonylurea failure, combination metformin-sulfonylurea treatment significantly improves glycemic control beyond that achieved with either agent along. Limited data suggest that metformin-insulin therapy may improve glycemic control, possibly reducing insulin requirements, in type 2 diabetic patients uncontrolled by insulin alone following secondary sulfonylurea failure. Gastrointestinal side effects are common, but usually tolerated. Lactic acidosis risk is minimal, provided that contraindications, particularly renal impairment, and prescribing guidelines are respected. Aside from elevated plasma metformin levels with cimetidine and synergistic hypoglycemia with sulfonylureas, few interactions occur. Thus, metformin is safe and effective both as monotherapy or in combination with other antihyperglycemic agents in type 2 diabetic patients requiring additional glycemic control and may be advantageous when weight control is desirable and/or hyperlipidemia exists (Davidson, 1997).

1.2 Biguanides

Biguanides (mainly Metformin) are widely prescribed antihyperglycemic agents that suppress hepatic glucose production, increase peripheral glucose uptake, and moderately reduce LDL cholesterol and triglyceride levels. Glucose control with the aid of biguanides appears to decrease the risk of diabetes-related complications, and is not associated with weight gain. This medication is reportedly associated with fewer hypoglycemic episodes than other lines of drugs. Biguanides activate AMPK, thus improving insulin signaling, whole-body energy balance, and the metabolism of glucose and fats. Activation of AMPK by biguanides inhibits

the expression of the hepatic gluconeogenic genes encoding PEPCK and G6Pase, thereby lowering glucose output. Increased AMPK activity in skeletal muscle by biguanides causes insulin-responsive, glucose transporter type 4, (GLUT4) positioning on the plasma membrane, leading to peripheral glucose uptake. Activation of AMPK by biguanides in hepatocytes and muscle cells reduces ACC activity and induces fatty acid oxidation. The most common adverse effect of biguanides is gastrointestinal distress, including diarrhea, cramps, nausea, vomiting, and increased flatulence. Long-term use of biguanides has been associated with decreased absorption of vitamin B12. The most serious and rare side effect of biguanides use is lactic acidosis, which in most cases appears to be related to comorbidities such as impaired liver or kidney function (Sofer, 2014).

1.3 Discovery of Metformin

The work of Dr Jean Sterne, a French clinician and his colleagues led to the discovery of metformin as an oral antidiabetic agent in the 1950s in Paris. The first synthesis of metformin (dimethyl biguanide) is attributed to Werner and Bell from Trinity College, Dublin, Ireland, in 1922, and was a basis for further experimental and clinical studies on the potential therapeutic application of biguanides, particularly metformin. The other two biguanide agents, phenformin and buformin, were soon withdrawn from widespread clinical use due to their toxicity, especially lactic acidosis. However, five decades were needed to promote metformin from a minor product to the 'gold standard' in the treatment of type 2 DM (Diabetes mellitus), with a wide safety profile (Andreja, 2010).

1.4 Development of Metformin

Guanidine found to lower blood glucose in animals in 1918, but too toxic. Alkyl-diguanides synthalin A and B were introduced into diabetes therapy in 1920s. Displayed efficacy comparable to insulin, but renal and hepatic damage resulted upon prolonged administration. Discontinued in the 1930s.The biguanides metformin (dimethylguanide), phenformin (phenylethylbiguanide) and buformin were introduced into clinical practice in 1950s as oral antihyperglycemics for the treatment of non-insulin dependent diabetes mellitus (NIDDM). Phenformin was initially regarded as the most potent biguanide and was used more extensively until its withdrawal in most countries by 1977 (Deruiter, 2003).

1.5 Chemical information of Metformine Hydrochloride

Metformin is a white, hygroscopic crystalline powder with a bitter taste. Chemically it is 1,1 dimethyl-biguanide hydrochloride with a mode of action and uses similar to other biguanides. This small molecule is soluble in water and 95% alcohol; on the other hand, it is practically insoluble in ether or chloroform. Its structure was generally represented in a wrong tautomeric form for several years, but that was corrected in 2005. When heated to decomposition, metformin emits toxic fumes of nitric oxides. It undergoes negligible hepatic metabolism and is excreted by the kidney with a half-life of approximately two hours.

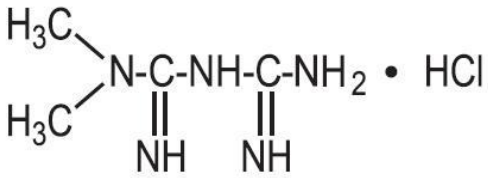

Fig 1.1: Structure of Metformin Hydrochloride (Patrick, 2013)

Several studies reported on crystallographic structure of metformin and its derivates – namely metformin hydrochloride, N, N-dimethylbiguanidium nitrate and metal complexes with metformin. These studies accentuate the importance of π-conjugation (multiple bond systems) and inter-molecular hydrogen bonding. Four primary end products of oxidation that result from the direct attack of hydroxyl radicals on metformin are a covalent dimer of metformin, hyproperoxide of metformin, methyl-biguanide and 2-amino-4-methylamino-1,3,5-triazine. Under similar conditions, the superoxide radicals are poor initiators of metformin oxidations, suggesting that metformin is not a powerful antioxidant (Patrick, 2013).

1.6 Mechanisms of action

Metformin's mechanisms of action differ from other classes of oral antihyperglycemic agents. Metformin decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization. These effects are mediated by the initial activation by metformin of AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin

signaling, whole body energy balance, and the metabolism of glucose and fats. Activation of AMPK is required for metformin's inhibitory effect on the production of glucose by liver cells. Increased peripheral utilization of glucose may be due to improved insulin binding to insulin receptors.

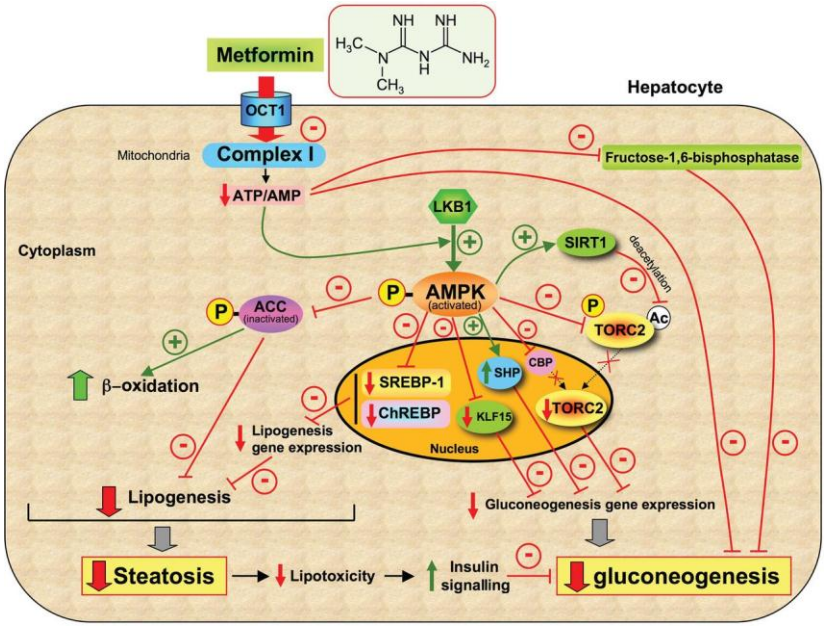

Fig 1.2: Mechanisms of action of Metformin (Alvarez, 2009)

Metformin administration also increases AMPK activity in skeletal muscle. AMPK is known to cause GLUT4 deployment to the plasma membrane, resulting in insulin-independent glucose uptake. The rare side effect, lactic acidosis, is thought to be caused by decreased liver uptake of serum lactate, one of the substrates of gluconeogenesis. In those with healthy renal function, the slight excess is simply cleared. However, those with severe renal impairment may accumulate clinically significant serum lactic acid levels. Other conditions that may precipitate lactic acidosis include severe hepatic disease and acute/decompensate heart failure (Musi, 2002).

1.7 Indication of metformin hydrochloride

1.7.1 Diabetes Mellitus

Metformin hydrochloride is used for the treatment of type 2 diabetes mellitus in adults, particularly in overweight patients, when dietary management and exercise alone does not result in adequate glycaemic control. Metformin may be used as initial treatment or in sulfonylurea failures either alone or in combination with a sulfonylurea and other oral agents. It is also used as an adjuvant therapy in insulin dependent diabetes especially if overweight (Akram, 2013).

1.7.2 Polycystic Ovary Syndrome

Metformin hydrchloride has been used in the management of metabolic and reproductive abnormalities associated with polycystic ovary syndrome (Akram, 2013).

1.8 Dose and method of administration

1.8.1 Monotherapy and combination with other oral antidiabetic agents in adults with normal renal function

Initially 500 mg should be taken once or twice a day and, if necessary, increased over a few weeks up to a maximum of 1 g three times per day. The dose should be titrated with gradual dose increments until the desired effect is obtained. 500 mg three times a day is often sufficient to obtain diabetic control. Control may be attained within a few days but occasionally requires up to two weeks. Once control has been obtained, the dosage should be reviewed and reduced to the lowest maintenance level consistent with good diabetic control. The maximum dose of 3g daily should only be used in patients with good renal function (ie creatinine clearance greater than 120ml/min). The action of Metformin is progressive and no final assessment of the patient's real response should be made before the 21st day of treatment; blood sugar estimations are recommended during the initial 15 days of stabilisation. Metformin will not produce a hypoglycaemic state when used alone; however, it increases insulin effectiveness (Ohkubo, 1998).

1.8.2 Combination with insulin or sulphonylureas in adult's patients

Metformin therapy with a sulphonylurea or insulin should be monitored by blood-sugar readings because combined therapy may cause hypoglycaemia. If it is decided to stabilise diabetic patients with metformin and insulin therapy, it is recommended that this is carried out in hospital because of the possibility of hypoglycaemia until the correct ratio of the two medicines is determined. (Ohkubo, 1998)

1.8.3 For renal Impairment

The risk of lactic acidosis is increased in patients with renal impairment. Metformin is contraindicated in patients with renal failure (creatinine clearance <15mL/min).Metformin may be used in patients with stable renal impairment (but see Warnings and Precautions).Where possible the dose should be titrated with gradual dose increments. The maximum daily dose for patients with creatinine clearance between 15-30mL/min is 500mg. The maximum daily dose for patients with creatinine clearance between 30-60mL/min is 1000 mg. The maximum daily dose for patients with creatinine clearance between 60-120mL/min is 2000mg. It is recommended that metformin concentrations are checked after steady state has been reached (after 48 hours) to ensure metformin concentrations remain below 5µg/mL (5mg/L).Renal function should be closely monitored (every 3-6 months).If the creatinine clearance drops below 15mL/min metformin must be discontinued (Ohkubo, 1998).

1.8.4 For elderly patients

The initial and maintenance dosing of metformin should be conservative in elderly patients, due to the potential for decreased renal function in this population. Any dosage adjustment should be based on a careful assessment of renal function. Generally, elderly patients should not be titrated to the maximum dose of metformin (Ohkubo, 1998).

1.8.5 Debilitated or malnourished patients

The dosing should be conservative and based on a careful assessment of renal function. (Akram, 2013)

1.8.6 For Children

Metformin is not recommended for use in children. (Akram, 2013)

1.8.7 Polycystic Ovary Syndrome

In polycystic ovary syndrome 1.5–2.25 g of metformin is given daily in divided doses generally.

(Akram, 2013)

1.8.8 Contraindications

Metformin is contraindicated in the following conditions: dehydration, diabetic coma, ketoacidosis, marked renal impairment, chronic liver disease, cardiac failure, recent myocardial infarction, alcoholism (both acute and chronic), conditions associated with hypoxaemia, states associated with lactic acidosis such as shock or pulmonary insufficiency in patients with a history of lactic acidosis and in the period around surgery. It is also contraindicated in case of hypersensitivity to metformin. It is contraindicated for paediatric use (Akinboboye, 2003).

1.9 Pharmacokinetics

1.9.1 Absorption and Bioavailability

The absolute bioavailability of a metformin hydrochloride 500 mg tablet given under fasting conditions is approximately 50% to 60%. Studies using single oral doses of metformin hydrochloride 500 to 1500 mg, and 850 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 plasma concentration (Cmax), a 25% lower area under the plasma concentration versus time curve (AUC), and a 35-minute prolongation of time to peak plasma concentration (Tmax) following administration of a single 850 mg tablet of metformin with food, compared to the same tablet strength administered fasting. The clinical relevance of these decreases is unknown. Following a single oral dose of metformin hydrochloride extended release tablet, Cmax is achieved with a median value of 7 hours and a range of 4 to 8 hours. Peak plasma levels are approximately 20% lower compared to the same

dose of metformin hydrochloride, however, the extent of absorption (as measured by AUC) is similar to metformin hydrochloride. At steady state, the AUC and Cmax are less than dose proportional for metformin hydrochloride extended release tablet within the range of 500 to 2000 mg administered once daily. Peak plasma levels are approximately 0.6, 1.1, 1.4, and 1.8 µg/mL for 500, 1000, 1500, and 2000 mg once-daily doses, respectively. The extent of metformin absorption (as measured by AUC) from metformin hydrochloride extended release tablet at a 2000 mg once-daily dose is similar to the same total daily dose administered as metformin hydrochloride tablets 1000 mg twice daily. After repeated administration of metformin hydrochloride extended release tablet, metformin did not accumulate in plasma. Within-subject variability in Cmax and AUC of metformin from metformin hydrochloride extended release tablet is comparable to that with metformin hydrochloride. Although the extent of metformin absorption (as measured by AUC) from the metformin hydrochloride extended release tablet increased by approximately 50% when given with food, there was no effect of food on Cmax and Tmax of metformin. Both high and low fat meals had the same effect on the pharmacokinetics of metformin hydrochloride (Day & Graham, 2007).

1.9.2 Distribution

The apparent volume of distribution (V/F) of metformin following single oral doses of metformin hydrochloride 850 mg averaged 654 ± 358 L. 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, steady state plasma concentrations of metformin are reached within 24 to 48 hours and are generally <1 µg/ml. During controlled clinical trials of metformin hydrochloride, maximum metformin plasma levels did not exceed 5 µg/mL, even at maximum doses (Day & Graham, 2007).

1.9.3 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) or biliary excretion. Renal clearance 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 (Day & Graham, 2007).

1.10 Pharmacodynamics

Metformin hydrochloride works mainly by suppressing excessive hepatic glucose production, through a reduction in gluconeogenesis. Other potential effects of metformin include an increase in glucose uptake, an increase in insulin signaling, a decrease in fatty acid and triglyceride synthesis, and an increase in fatty acid β-oxidation. Metformin may also increase glucose utilization in peripheral tissues, and possibly reduce food intake and intestinal glucose absorption. As metformin does not stimulate endogenous insulin secretion, it does not cause hypoglycemia or hyperinsulinemia, which are common side effects associated with other antidiabetic drugs.

The molecular mechanisms underlying metformin action appear to be complex and remain a topic of considerable debate. However, there is general agreement that the administration of metformin results in the phosphorylation and activation of AMP-activated protein kinase (AMPK) in the liver, which in turn may lead to diverse pharmacologic effects, including inhibition of glucose and lipid synthesis. Although the specific route of AMPK phosphorylation is not yet clear, the molecular components LKB1/STK11 and ATM have been shown to play a role in the phosphorylation of AMPK in the presence of metformin. However, ATM, LKB1, and AMPK are not the direct targets of metformin. A recent study using liver-specific AMPK-knockout mice has shown that inhibition of hepatic glucose production by metformin is preserved, suggesting that metformin may inhibit hepatic gluconeogenesis in an LKB1-independent and AMPK-independent manner. The findings from this study are yet to be replicated, and therefore, the role of AMP kinase in the inhibition of gluconeogenesis can still be considered. In a separate study in Oct-1-knockout mice, metformin both activated AMPK and reduced gluconeogenesis. A separate group has also concluded that metformin inhibits hepatic gluconeogenesis through AMPK-dependent regulation of SHP. Therefore, a reduction in

gluconeogenesis may occur both ways, in an AMPK-dependent and an AMPK-independent manner. Although the direct target is not fully elucidated, metformin specifically inhibits complex I of the mitochondrial respiratory chain, suggesting that this inhibition may activate AMPK by increasing the cellular AMP: ATP ratio. AMPK is a major cellular regulator of lipid and glucose metabolism. The activated AMPK phosphorylates and inactivates HMG-CoA reductase (encoded by gene HMGCR), MTOR (target of rapamycin); ACC-2 (encoded by gene ACACB); ACC (encoded by gene ACACA), glycerol-3-phosphate acyltransferase (encoded by gene GPAM); and carbohydrate response element-binding protein. The activation of AMPK by metformin also suppresses the expression of SREBP-1 (encoded by gene SREBF1), a key lipogenic transcription factors. Phosphorylated AMPK also activates SiRT1 and increases Pgc-1a (encoded by gene PPARGC1A) expression in the nucleus, leading to the downstream activation of mitochondrial biogenesis.

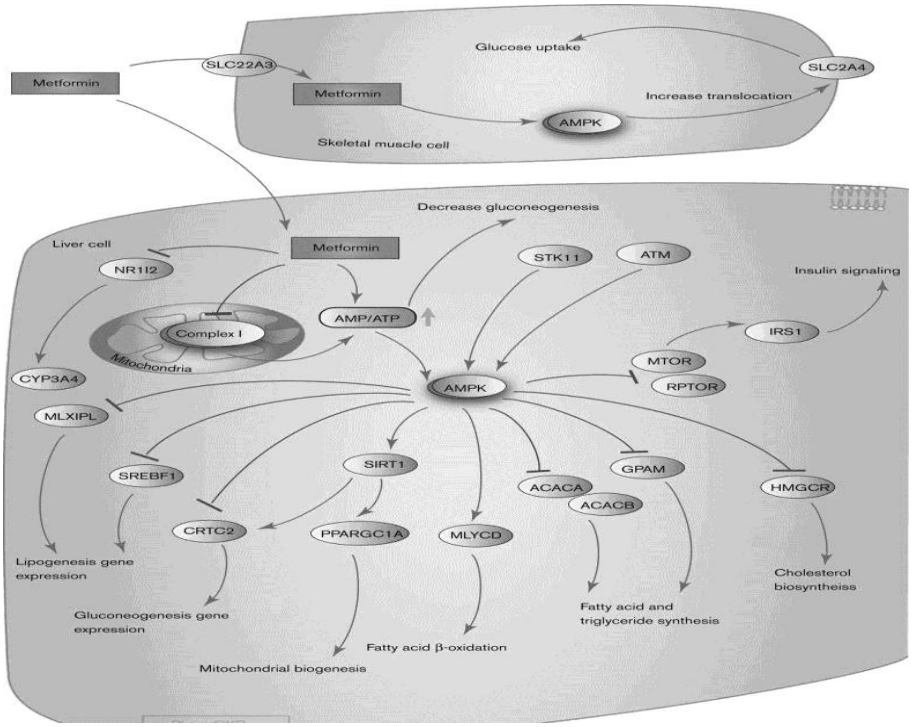

Fig 1.3: Pharmacodynamics of Metformin hydrochloride (Day & Graham, 2007)

Metformin disrupts the coactivation of PXR with SRC1, resulting in the downregulation of CYP3A4 gene expression. Finally, activation of AMPK results in an increase in glucose uptake in skeletal muscle by increasing the GLUT4 (encoded by gene SLC2A4) translocation activity.

The overall pharmacological effect of AMPK activation in the liver includes the stimulation of fatty acid oxidation with inhibition of cholesterol and triglyceride synthesis. Peripheral effects include the stimulation of fatty acid oxidation and glucose uptake in skeletal muscle as well as a systemic increase in insulin sensitivity. However, the role of metformin in insulin-mediated glucose uptake has been debated. Given the increased risk of cancer in T2DM (Type 2 Diabetes Mellitus) patients, metformin have also been evaluated for its tumor suppression ability and its potential to protect from cancer. Population studies have shown that metformin is associated with a significant reduction of neoplasia in multiple cancer types (cancer of the breast and prostate, in particular). Metformin may also inhibit the growth of cancer cells. The mechanisms underlying this protective effect are not well understood and may involve the activation of multiple pathways. The cell cycle arrest in metformin-treated breast cancer cells seems to involve the activation of AMPK and down-regulation of cyclin D1, and requires p27Kip1 or p21Cip1. Metformin was reported to suppress HER2 (ERBB2) oncoprotein overexpression through inhibition of the mTOR effector p70S6K1(RPS6KB1) in human breast carcinoma cells (Day & Graham, 2007).

1.11 Prescribing Limits

1.11.1 For pediatric Patients

For Children 10–16 years of age: Maximum 2 g daily as conventional tablets or oral solution. (Akram, 2013)

1.11.2 For Adults patient

Maximum 2.55 g daily as conventional tablets or oral solution, 2.5 g daily as extended-release tablets, or 2 g daily as certain other extended-release tablets. Switch to conventional tablets for further dosage titration if required dosage exceeds 2 g daily.Metformin Hydrochloride in Fixed Combination with Glyburide Maximum daily dose as second-line therapy is 2 g of metformin hydrochloride and 20 mg of glyburide.No experience with total daily dose exceeding 2 g of metformin hydrochloride and 10 mg of glipizide in clinical trials in patients receiving the fixed combination as initial therapy.Metformin Hydrochloride in Fixed Combination with Glipizide Maximum daily dose is 2 g of metformin hydrochloride and 20 mg of glipizide (Akram, 2013).

1.12 Prescribing limits for special populations

1.12.1 Renal Impairment

eGFR 30–45 mL/minute per 1.73 m^2: FDA recommends that metformin not be initiated; assess benefits and risks of continued treatment in patients already receiving metformin. eGFR <30 mL/minute per 1.73 m^2: Use of Metformin is contraindicated, discontinue in patients already receiving metformin. National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) states that the exact GFR cutoff for metformin use is controversial. NKF-KDOQI suggests reviewing use of metformin in GFR <45 mL/minute per 1.73 m^2. NKF-KDOQI and other clinicians suggest avoiding metformin therapy if GFR <30 mL/minute but suggest considering risk-benefit of such therapy if GFR stable (Akram, 2013).

1.12.2 Hepatic Impairment

Impaired hepatic function may significantly limit the ability to clear lactate, thus the product monograph recommendation to avoid metformin use in patients with hepatic failure. We should avoid the use of metformin in those with hepatic disease (Akram, 2013).

1.13 Special warnings and precautions for use

1.13.1 Lactic acidosis

Lactic acidosis is a rare but serious metabolic complication which can occur due to metformin accumulation during treatment. When it occurs, it is fatal in more than 25% of cases. Lactic acidosis is a medical emergency and must be treated in hospital immediately. The risk of lactic acidosis increases with the degree of renal dysfunction and the patient's age. Reported cases have occurred primarily in diabetic patients with acute conditions causing a significant decrease in renal function or tissue hypoxia. Hepatic dysfunction is also a risk as lactate clearance is reduced. Patients with long-term stable conditions should be carefully assessed prior to treatment for risk factors for lactic acidosis such as: poorly controlled diabetes, ketosis, prolonged fasting, excessive alcohol intake, hepatic insufficiency and conditions associated with hypoxia. Particular caution should be paid in situations where renal function may become impaired such as dehydration, when starting therapy with a diuretic or when starting therapy with a non-steroidal anti- inflammatory drug (NSAID). In these situations metformin should be temporarily discontinued. When metformin is implicated as the cause of lactic acidosis, metformin plasma

levels greater than 5µg/mL (5mg/L) is generally found. Diagnosis The risk of lactic acidosis must be considered in the event of non-specific signs such as malaise, myalgia, muscle cramps, respiratory distress, increasing somnolence and non-specific abdominal distress.Patients should be instructed to notify these signs to their physician immediately.As lactic acidosis progresses there may be associated hypothermia, hypotension and resistant bradyarrhythmias with more marked acidosis. This can be followed by acidotic dyspnea and coma. Lactic acidosis is characterised by acidosis (decreased blood pH), elevated lactate levels above 5mmol/L with increased lactate/pyruvate ratio and electrolyte disturbances with an increased anion gap. If there is any suspicion of metabolic/lactic acidosis metformin should be discontinued and the patient hospitalised immediately. Prompt haemodialysis is recommended to correct the acidosis and remove accumulated metformin (Giugliano, 1998).

1.13.2 Renal Impairment

Underlying renal disease, or deterioration in renal function, result in reduced clearance of metformin and drug accumulation and are therefore major risk factors in lactic acidosis. Creatinine clearance (this can be estimated from serum creatinine levels by using the Cockcroft-Gault formula) should be determined before initiating treatment and regularly thereafter:

• At least annually in patients with normal renal function

• At least twice a year in patients with impaired renal function and elderly patients

Decreased renal function in elderly subjects is frequent and asymptomatic. Metformin therapy should be temporarily stopped in the presence of any condition associated with hypoxaemia or dehydration, in patients suffering from serious infections or trauma (particularly if gastrointestinal disturbances are noted or acidosis is suspected) and in those undergoing surgery.

Prompt haemodialysis is recommended to correct the acidosis and remove accumulated metformin. Special caution should be exercised in situations where renal function may become impaired, for example when initiating antihypertensive therapy or diuretic therapy and when starting therapy with a (NSAID).Metformin is contraindicated in patients with creatinine clearance below 15ml/min (Akram, 2013).

1.13.3 Hepatic Impairment

Impaired hepatic function may significantly limit the ability to clear lactate. Metformin should be avoided in patients with severe hepatic insufficiency and used with caution in patients with milder disease (Giugliano, 1998).

1.13.4 Use in the elderly patients

The risk of lactic acidosis in association with metformin is increased in elderly patients on long-term therapy due to the physiological alteration of the renal function and the possible accumulation of metformin. Metformin may be used in the elderly when the issues raised under Contraindications and Warning and Precautions have been taken into consideration, the dosage is frequently reviewed and the renal function is closely monitored.
(Giugliano, 1998)

1.13.5 Heart Failure

Type 2 diabetic patients with heart failure are at an increased risk of hypo perfusion and possible renal insufficiency. Careful monitoring of renal function is recommended when metformin is used in patients with cardiac failure (Giugliano, 1998).

1.13.6 Surgery

Metformin must be discontinued 48 hours before elective surgery under general, spinal or peridural anaesthesia.Therapy may be restarted no earlier than 48 hours following surgery or resumption of oral nutrition and only if normal renal function has been established.
(Day & Graham, 2007)

1.13.7 Alcohol

Alcohol is known to potentiate the effect of metformin on lactate metabolism. Patients should therefore be warned against excessive alcohol intake, acute or chronic, while taking metformin.
(Day & Graham, 2007)

1.13.8 Other precautions

Periodic assessment of renal, hepatic and cardiovascular function is recommended during prolonged periods of treatment with metformin. Patients receiving continuous metformin therapy should have an annual estimation of vitamin B12 levels because of reports of decreased vitamin B12 absorption (Day & Graham, 2007).

1.13.9 Use in children

Metformin is not recommended for use in children, except those with insulin resistant diabetes who are being treated in hospital (Giugliano, 1998).

1.14 Interactions with other medicines

1.14.1 Pharmacokinetic interactions

a. Cimetidine: Reduced clearance of metformin has been reported during cimetidine therapy, so a dose reduction should be considered.
b. Anticoagulants: Metformin increases the elimination rate of vitamin K antagonists. Consequently, the prothrombin time should be closely monitored in patients in whom metformin and vitamin K antagonists are being coadministered. Cessation of metformin in patients receiving vitamin K antagonists can cause marked increases in the prothrombin time.
c. Nifedipine: A single dose, metformin/nifedipine drug interaction study in normal healthy volunteers demonstrated that coadministration of metformin and nifedipine increased plasma metformin Cmax and AUC by 20 and 9%, respectively, and increased the amount of metformin excreted in the urine. Tmax and half-life of metformin were unaffected. Nifedipine appears to enhance the absorption of metformin. Metformin had minimal effects on the pharmacokinetics of nifedipine.

(Wiholm, 1993)

1.14.2 Pharmacodynamic interactions

a. Sulfonylureas and repaglinide: During concomitant therapy with sulfonylureas and repaglinide, blood glucose should be monitored because combined therapy may cause hypoglycaemia.

b. Beta-blockers: Coadministration of metformin and beta-blockers may result in a potentiation of the hypoglycaemic action. In addition, some of the premonitory signs of hypoglycaemia, in particular tachycardia, may be masked. Monitoring of blood glucose should be undertaken during dosage adjustment of either agent.
c. ACE inhibitors: Coadministration of metformin and ACE inhibitors may result in a potentiation of the hypoglycaemic action. Monitoring of blood glucose should be undertaken during dosage adjustment of either agent.
d. Calcium channel blockers: Calcium channel blockers may affect glucose control in diabetic patients; regular monitoring of glycaemic control is recommended.
e. Thyroid products: Thyroid products tend to produce hyperglycaemia and may therefore lead to loss of control.
f. Corticosteroids: Corticosteroids tend to produce hyperglycaemia and may lead to loss of control.
g. Alcohol: Alcohol decreases blood glucose concentration by inhibiting hepatic glucose output, thus increasing the risk of hypoglycaemia and can also masks its warning symptoms. The CNS depressant effects of alcohol plus hypoglycaemia can make driving or the operation of dangerous machinery much more hazardous. Excessive consumption of alcohol while on metformin may result in elevation of blood lactate.
h. Thiazide diuretics: Thiazide therapy may impair glucose tolerance. Dosage adjustment of metformin may be required.

(Wiholm, 1993)

1.15 Undesirable effects

1.15.1 Gastrointestinal disorders

Mild gastrointestinal symptoms (such as diarrhoea, nausea, vomiting, and loss of appetite) are the most frequent reactions to metformin (> 1/10), especially during the initial treatment period. These symptoms are generally transient and resolve spontaneously during continued treatment.

Gastrointestinal side effects can possibly be avoided if Metformin is taken with meals and if the dose is increased slowly. Occasionally, a temporary dose reduction can be considered.

However occurrence of gastrointestinal symptoms, once a patient is stabilised on any dose of metformin, could be due to lactic acidosis or other serious disease (Chan, 1996).

1.15.2 Metabolism and nutrition disorders

Lactic acidosis is a very rare (< 1/10,000) but serious metabolic complication that can occur due to metformin accumulation during treatment. A decrease of vitamin B12 absorption with a decrease in serum levels has been observed in patients treated long-term with metformin. (Chan, 1996)

1.15.3 Skin and subcutaneous tissue disorders

Mild erythema, pruritus and urticaria have been reported in some hypersensitive individuals but the incidence is very rare < 1/10,000).
(Chan, 1996)

1.15.4 Hepatobiliary disorders

Isolated reports of liver function test abnormalities or hepatitis resolving upon metformin discontinuation (Chan, 1996).

1.16 Quality Control

Quality control is a process employed to ensure a certain level of quality in a product or service. It may include whatever actions a business deems necessary to provide for the control and verification of certain characteristics of a product or service. The basic goal of quality control is to ensure that the products, services, or processes provided meet specific requirements and are dependable, satisfactory, and fiscally sound.

Essentially, quality control involves the examination of a product, service, or process for certain minimum levels of quality. The goal of a quality control team is to identify products or services that do not meet a company's specified standards of quality. If a problem is identified, the job of a quality control team or professional may involve stopping production temporarily. Depending on the particular service or product, as well as the type of problem identified, production or implementation may not cease entirely (Riley, 2010).

1.17 Quality control parameters

1.17.1 Weight variation test

Weight variation of tablets is measured to ensure that a tablet contains the proper amount of the drug which indicates the criteria of the tablet formulation. If weight of the active ingredient is more than the accepted value, the patient may suffer from overmedication. On the other hand, if the determined weight is less than the accepted value, the patients will be experienced from under-medication. Weight variation test is done by electronic balance (Lachman, 2008).

1.17.2 Hardness Test

Tablet hardness is usually expressed as the load required crushing a tablet placed on its edge. Hardness is thus sometimes termed the tablet crushing strength. The suitability of a tablet in regard to mechanical stability during packaging and shipment can usually be predicted on the basis of hardness. Tablet hardness, in turn, influences tablet density and porosity. It may affect tablet friability and disintegration time. It usually affects the drug dissolution and release and it may affect bio-availability. Harness is measured by Monsanto harness tester (Lachman, 2008).

1.17.3 Friability Test

Friction and shock are the forces that most often cause the tablets to chip, chop or break. The friability test is closely related to tablet hardness and is designed to evaluate the ability of the tablet to withstand abrasion in packaging, handling and shipping. The value is expressed as a percentage. A maximum weight loss of not more than 1% of the weight of the tablets being tested during the friability test is considered generally acceptable and any broken or smashed tablets are not picked up. Normally, when capping occurs, friability values are not calculated. A thick tablet may have less tendency to cap whereas thin tablets of large diameter often show extensive capping, thus indicating that tablets with greater thickness have reduced internal stress (Nachaegari & Bansal, 2004).

1.17.4 Disintegration Test

The disintegration test is performed to find out the time it takes for a solid oral dosage form like a tablet or capsule to completely disintegrate. The time of disintegration is a measure of the quality. This is because, for example, if the disintegration time is too high; it means that the

tablet is too highly compressed or it may imply several other reasons. And also if the disintegration time is not uniform in a set of tablet being analyzed, it indicates batch inconsistency and lack of batch uniformity. Disintegration time may vary considering to its disintegrator used. Higher the disintegration time required lower the dissolution rate and followed to poor absorption. So disintegration is the crucial part of a drug for therapeutic action (Aulton, 2007).

1.17.5 Dissolution test

Dissolution is a test used by the Pharmaceutical industry to characterize the dissolution properties of the active drug, the active drug's release and the dissolution from a dosage formulation. Drugs administered orally in solid dosage forms, such as tablet or capsules, must dissolve in the contents of the gastrointestinal tract before drug absorption can occur. Often the rate of drug absorption is determined by the rate of drug dissolution from the dosage form. Therefore, if it is important to achieve high peak blood levels of a drug, it will usually be important to obtain rapid drug dissolution from the dosage form (Lachman, 2008).

1.17.6 Potency Test

Every tablet must contain the amount of drug substance intended with a little variation among the tablets within a batch. The uniformity of the tablet is necessary to obtain the desired biological effect also to avoid the undesirable side effect. So determining the content of the drug has become the principle purpose. Analyzing the potency in the tablets not only indicates the amount of drug substance in the dosage form but also prerequisite for the establishment of stability data (Lachman, 2008).

Significance of the study

Diabetes is a global public health problem. It is a chronic disease and is now growing as an epidemic in both developed and developing countries. As in many South Asian countries, diabetes is becoming a serious health concern in Bangladesh. Between 2000 and 2008, the proportion of people suffering from diabetes increased from 4% to 7%. Metformin Hydrochloride, which are used for the type-2 Diabetes Mellitus(DM), Which has great role to

control the blood sugar alone or combine with other drugs. According to the latest WHO data published in April 2011 Diabetes Mellitus Deaths in Bangladesh reached 19,598 or 2.05% of total deaths. The age adjusted Death Rate is 23.80 per 100,000 of population, ranks for Bangladesh #109 in the world. According to the IDF Diabetes Atlas, the diabetes cases in Bangladesh will have risen to 7.9 % by the year 2030 (Labu, 2013).

Asia is emerging as the epicenter of diabetes epidemic. Like all other develop and developing countries prevalence and incidence of type-2 DM is also increasing in Bangladesh. In 2010, the International Diabetes Federation (IDF) estimated that 5.7 million (6.1%) and 6.7 million (7.1%) of people living in Bangladesh is suffering from diabetes and impaired glucose tolerance (IGT) respectively. By 2030, that number of diabetic population is expected to rise to 11.1 million. Besides lifestyle intervention pharmacological interventions especially using metformin has also been shown to be effective in reducing the onset of diabetes in subjects with impaired glucose tolerance (IGT).Metformin is an antihyperglycemic agent which improves glucose tolerance in patients with type 2 diabetes, lowering both basal and postprandial plasma glucose. Its pharmacologic mechanisms of action are different from other classes of oral antihyperglycemic agents. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Unlike sulfonylureas, Metformin does not produce hypoglycemia in either patients with type 2 diabetes or normal subjects (Except in special circumstances), and does not cause hyperinsulinemia. With Metformin therapy, insulin secretion remains unchanged while fasting insulin levels and day-long plasma insulin response may actually decrease (Yousuf, 2006).

Poor quality of Metformin can cause serious trouble like health hazards and even mass death. If, available Metformin tablet contains high amount of drug it may cause hypoglycemia, it cannot give effective result.Again, if it contains less amount of drug in available Metformin preparation, it may cause hyperglycemia, which is life threating for diabetic patients. Poor quality Metformin preparation also causes other adverse effects. So the quality control study on Metformin is very essential. With quality control, inspection is intended to prevent faulty products reaching the customer.This approach means having specially trained inspectors, rather than every individual being responsible for his or her own work.Furthermore, it is thought that inspectors may be better placed to find widespread problems across an organization (Riley, 2012).

Objective of the study

a. To analyze different brands of Metformin in terms of physical parameters like weight variation, hardness, friability, disintegration etc.
b. To check the potency and dissolution profile of Metformin.
c. To assess and compare the rates of dissolution among three different brands of Metformin.

Chapter Two

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-F6). The granules for tableting 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, 2013).

- 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 (Aiache, 2014).

- 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 resonate 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 (Chavda, 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 (Anand, 2011).

- 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 (Yuksel, 2004).

- In this study, In-vitro dissolution profile of ten brands of Metformin hydrochloride sustained release matrix tablet was performed, which are commercially available in the pharmaceutical market in Bangladesh. All the brands except two brands (Code: MH-5 and MH-8) complied with the USP in vitro dissolution specification of 85% drug release at 10th hour in simulated intestinal medium. Drug release of 81.6 % and 80.3 % were showed by the brand code of MH-5 and MH-6 respectively within the specified time period which did not meet the terms of the USP guideline. To reveal the release kinetics of sustained release tablets of Metformin hydrochloride, release profiles were analyzed for zero order, first order and Higuchi equation and found that first order and Higuchi model showed high linearity with correlation coefficient (r2) value of 0.98 or more. In conclusion, our results indicated that all the brands of Metformin hydrochloride sustained release matrix tablets included in this study apart from MH-5, MH-8 showed high dissolution profile and hence good bioavailability (Akbar, 2011).

- The study was conducted to compare the quality of the metformin tablet formulations those are locally available in Trinidad & Tobago pharmaceutical market manufactured by various pharmaceutical companies with pharmacopoeia standards. The four popular brands (A, B, C, D) of metformin conventional tablet of 500 mg strength were chosen. The metformin tablets were obtained from government hospital pharmacies as well as from local private pharmacies. To compare the quality of tablet formulations of different brands various official parameters like friability, weight variation, disintegration time, dissolution and drug assay tests were performed as per the pharmacopoeia. The result of all these parameters of different brands were in the pharmacopoeial limits so it could be concluded that marketed pharmaceutical tablets of metformin of these brands are safe, effective and efficacious as well as satisfy quality control limits of pharmacopoeia (Gupta, 2016).

- The study was conducted to determine the biopharmaceutical and chemical equivalence of eight brands of Metformin tablets marketed in Nigeria using in vitro tests. The physicochemical equivalence of eight brands of Metformin hydrochloride tablets were assessed through the evaluation of both official and non-official standards such as uniformity of weight, friability, hardness, disintegration, Assay and dissolution rate. All the brands complied with the official specifications for uniformity of weight, disintegration and

dissolution tests. Brand B and C had the highest and lowest crushing strength respectively. However, for the friability test, one of the eight brands failed to meet the British pharmacopoeia specification for friability. Seven brands had values within the range specified for assay in the BP while Brand G failed the test. Only brand F, G and H met the BCS biowaiver criteria for very rapidly or rapidly dissolving tablets. Of all the eight brands evaluated in this study, only four brands could be regarded as being biopharmaceutically and chemically equivalent and therefore can be interchanged in the clinical practice (Akinleye, 2012).

- The study was conducted to develop formulation of metformin HCl 500 mg film coated tablets, compare in-vitro evaluation of self designed formulation with four different brands of metformin HCl 500 mg film coated tablets and to compare the physicochemical equivalency of the four brands. The tables were prepared by using wet granulation method. All the coated tablets passed weight variation test as the percentage of weight variation was within USP limits of ± 5% of the average weight. The chemical assay test of all the tablets showed that none had potency less than the required specifications of USP. The in vitro dissolution test results were found within the USP recommended limits for metformin HCl 500 mg film coated tablets. The comparative in-vitro study of FMet-HCl with four different brands showed that FMet-HCl has almost comparable characteristics with these brands. This study also proved the physicochemical equivalency of the four different brands. So if one brand is not available in the market then any of the other three brands can be taken in place of that unavailable brand (Nazir, 2013).

Chapter Three

Method & Materials

3.1 Sample Collection

Three brands were selected from three individual companies randomly. The names and brands of the selected companies are given below:

Table 3.1: Name and company of the selected brand of metformin.

Company	Brand
ACME Laboratories Ltd.	Daomin
Drug International Ltd.	Oramet
Eskayef Pharmaceuticals Ltd.	Glunor

3.2 Hardness test

Tablet hardness is defined as the force required for breaking a tablet in a diametric compression test.

Materials: Hardness Tester, Tablets.

Table 3.2: Name and specification of materials required in hardness test.

Materials	Specification
Hardness tester	Monsanto hardness tester

Method

The sliding scale of hardness tester has been set off to zero. The tablets have been placed vertically between the two jaws. Force has been applied with the screw thread and spring until the tablets has been fractured. A force of about 4-5 kg is considered to be the minimum for hardness according to The British Pharmacopoeia (Lachman, 2008).

Fig 3.1: Monsanto Hardness Tester

Limit of Acceptance: Oral tablets should have hardness minimum 4 kg. Where 1 kg = 9.81 Newton (Lachman, 2008).

3.3 Weight Variation test

Materials: Analytical balance, Tablets

Table 3.3: Name and specification of instrument required in weight variation test.

Materials	Specification
Electronic Balance	Shimadzu, Japan

Method

The experiment was started with 20 tablets and each tablet was weighted individually. The average weight of all the tablets was taken and considered as the standard weight of the individual tablet. All the tablet was weighed individually and observed whether the individual weight are within the range or not. The tablets meet the USP test if no more than two tablets are outside the percentage limit and if no tablet differs by more than two times the percentage limit (Gilbert, 1986).

Table 3.4: Limit of Weight variation test (Lachman, 2008)

Average weight	Percentage difference
130 mg or less	±10
More than 130 mg	±7.5
324 mg & above	±5

Fig 3.2: Electronic Balance (Shimadzu, Japan)

3.4 Friability test

It is another indicator of tablet strength. This device subjects a number of tablets to the combined effects of abrasion and shock by utilizing a plastic chamber that revolves at 25rpm, dropping the tablets a distance of six inches with each revolution.

Materials: Friability tester, electronic balance, tablets.

Table 3.5: Name and specification of materials required to friability test.

Materials	Specification
Friability Tester	Veego friability Tester; model:4310305
Electronic Balance	Shimadzu, Japan

Method

The experiment has been started by weighing 10 tablets which is considered as the initial reading. All the tablets have been placed in the drum of friability tester and rotate 100 times. Then the percentage loss has been calculated. According to BP the tablets should not lose more than 1% of their total weight (B.P. appendix: XVII, 2003).

Fig 3.3: VEEGO Friability Tester

Limit of acceptance: Conventional compressed tablets that lose less than 0.5% to 1% of their weight are generally considered acceptable (USP, 2007).

3.5 Disintegration test

A process through which the tablets are breakdown into small particles or granules prior to become solution is called disintegration.

Conditions:

Medium: 900ml distilled water

Time: 30 minutes.

Temperature: (37±2) °C

Table 3.6: Name and specification materials required to disintegration test

Materials	Specification
Disintegration tester	(BJ-2Vanguard pharmaceutical machinery, INC. USA) 1000 ml beaker, 6 tubes and disc, Demineralized water.

Method

The disintegration tester was assembled. An arbitrary figure appeared in the digital display. Then the time and temperature were set at prescribed in the specification. Then 600ml of the medium was placed in each 1000ml beaker. The temperature of the liquid was maintained at 35-39 0C. In each of the 3 tubes one tablet was placed. After placement of the tablet, 3 discs were placed above the table. The machine was then operated for the prescribed period. The entire tablet must disintegrate within the prescribed time (Lachman, 2008).

Fig 3.4: Vanguard Disintegration Tester

Table 3.7: Limit of Disintegration Time.

Type of tablet	Disintegration time
Uncoated tablet	15 minutes
Coated tablet	60 minutes or 1 hour

(Lachman, 2008).

3.6 Dissolution Test

Dissolution test is carried out to determine the amount of drug released during a specific period of time by using dissolution rate apparatus.

Conditions:

Medium: 900ml phosphate buffer, pH 6.8

Apparatus: Dissolution tester type 1 (basket)

Speed: 100rpm

Temperature: 37.5 C

Time: 45 minutes.

Analysis: UV-visible spectrophotometer.

λmax: 233nm

Materials: Dissolution tester, phosphate buffer, pH meter, 0.1N NaOH.

Table 3.8: Name and Specification Instrument Required to Dissolution Test.

Instrument	Specification
Dissolution Tester	Labinda DS- 8000

Method

On the dissolution test apparatus the water tank was filled and the temperature was set. Then 900 ml of the 0.68% w/v phosphate buffer was poured into one of the vessels at pH 6.8 and instruments were run till the set temperature was attained. One of the sample tablets was placed into the vessel and starts the run. Rotate the basket at 100 revolutions per minute. Run the test for 45 minutes. At the end of the time specified, 10ml of the sample was collected and filtered. Then 10ml of the filtered sample was diluted to 100 ml by adding distilled water and dilute 10ml of the resulting solution to 100ml with distilled water. Using the same procedure, as for the blank sample, use the distilled water. Finally the absorbance was measured at 233nm (BP, 2004).

Determination of the samples Dissolution of metformin can be measured by using the following equation,

$$\% \text{ Dissolution} = \frac{\text{Absorbance (a)}}{\text{A(1\%,1 cm)}} \times \frac{\text{Dilution factor} \times 900}{\text{Tablet weight (gm)}} \times 100$$

Fig 3.5: Labinda DS- 8000 Dissolution Tester

3.7 Potency Test

Every unit of tablet should contain the amount of drug substance equivalent to its label amount. For the evaluation of content, assay should be performed.

Method

20 tablets were weighed and crushed to make fine powder. Take a quantity of the powder containing 50 mg metformin in a 100 ml volumetric flask. Add 75 ml water in the flask and shaken for 15 minutes and sufficient water was added to make the volume up to 100 ml. It was mixed well and then filtered. The 5 ml of the filtrate was taken in a 50 ml volumetric flask. The volume was made up to 50 ml with water and mixed. The 5 ml of the resulting solution was taken in a 50 ml volumetric flask and was added to volume up to the mark with water and mixed.Then measure the absorbance at 232nm (BP, 2004).

Chapter Four

Result

4.1 Average weight of Tablets

Table 4.1: Average weight of Tablets

Brand name	Average weight (mg)
Daomin	564.68
Oramet	568.52
Glunor	778.62

4.2 Weight Variation test

4.2.1 Weight variation of Daomin

Table 4.2: Weight variation of Daomin

Tablet Number	Individual weight (mg)	Weight variation (%)	Highest variation	Lowest variation
1	564.7	0.0035		
2	554.2	-1.8559		
3	570.2	0.9775		
4	575.8	1.9692		
5	568.9	0.7470	1.9692	-2.1569
6	559.8	-0.8642		
7	563.9	-0.1381		
8	552.5	-2.1569		
9	562.7	-0.3506		
10	574.1	1.6682		

4.2.2 Weight variation of Oramet

Table 4.3: Weight variation of Oramet

Tablet Number	Individual weight (mg)	Weight variation (%)	Highest variation	Lowest variation
1	563.0	-0.9709		
2	577.1	1.5091		
3	568.6	0.0140		
4	564.6	-0.6895		
5	569.2	0.1196	1.5091	-1.1644
6	561.9	-1.1644		
7	569.7	0.2075		
8	568.1	-0.0738		
9	574.9	1.1222		
10	568.1	-0.0738		

4.2.3 Weight variation of Glunor

Table 4.4: Weight variation of Glunor

Tablet Number	Individual weight (mg)	Weight variation	Highest variation	Lowest variation
1	774.5	-0.5291		
2	771.8	-0.8759		
3	788.9	1.3202		
4	785.1	0.8322		
5	775.2	-0.4392	1.3202	-1.0557
6	775.8	-0.3621		
7	783.9	0.6781		
8	777.7	-0.1181		
9	782.9	0.5496		
10	770.4	-1.0557		

4.3 Friability test

Table 4.5: Friability test

Brand Name	Initial weight of 10 tablet (mg)	Final weight of 10 tablet (mg)	% Loss
Daomin	5646.8	5632.7	0.2496
Oramet	5685.2	5663.9	0.3746
Glunor	7786.2	7760.5	0.3300

4.4 Hardness test

4.4.1 Hardness test of Daomin

Table 4.6: Hardness test of Daomin

Tablet Number	Hardness (Kg/cm)	Average Hardness (Kg/cm)
1	4.5	
2	4.2	
3	4.1	
4	4.0	
5	4.2	4.19
6	4.3	
7	4.1	
8	4.0	
9	4.3	
10	4.2	

4.4.2 Hardness test of Oramet

Table 4.7 : Hardness test of Oramet

Tablet Number	Hardness (Kg/cm)	Average Hardness (Kg/cm)
1	4.6	
2	4.6	
3	4.3	
4	4.2	
5	4.4	4.40
6	4.3	
7	4.5	
8	4.4	
9	4.3	
10	4.4	

4.4.3 Hardness test of Glunor

Table 4.8 : Hardness test of Glunor

Tablet Number	Hardness (Kg/cm)	Average Hardness (Kg/cm)
1	4.4	
2	4.1	
3	4.0	
4	4.2	
5	4.2	4.18
6	4.3	
7	4.5	
8	4.0	
9	4.1	
10	4.0	

4.5 Disintegration Test

4.5.1 Disintegration test of Daomin

Table 4.9 : Disintegration test of Daomin

Tablet Number	Disintegration Time (minute)	Average Disintegration Time (minute)
1	7.2	
2	7.6	
3	7.9	7.5
4	7.3	
5	7.6	
6	7.4	

4.5.2 Disintegration test of Oramet

Table 4.10 : Disintegration test of Oramet

Tablet Number	Disintegration Time (minute)	Average Disintegration Time (minute)
1	6.2	
2	6.3	
3	6.5	6.3
4	6.3	
5	6.1	
6	6.4	

4.5.3 Disintegration test of Glunor

Table 4.11 : Disintegration test of Glunor

Tablet Number	Disintegration Time (minute)	Average Disintegration Time (minute)
1	9.8	
2	9.8	
3	9.7	9.8
4	10.2	
5	9.9	
6	9.5	

4.6 Dissoluliton Test

4.6.1 Dissolution test for Domin

Table 4.12 : Dissolution test for Domin

Sample No.	weight of sample (gm)	Absorbance of the sample	Dissolution (%)	Average Dissolution (%)
1	0.5647	0.233	92.15	
2	0.5702	0.234	91.65	
3	0.5758	0.215	83.39	88.68
4	0.5689	0.212	83.22	
5	0.5639	0.225	89.11	
6	0.5741	0.238	92.58	

4.6.2 Dissolution test for Oramet

Table 4.13 : Dissolution test for Oramet

Sample No.	weight of sample (gm)	Absorbance of the sample	Dissolution (%)	Average Dissolution (%)
1	0.5630	0.225	89.25	
2	0.5686	0.216	84.83	
3	0.5646	0.226	89.39	85.97
4	0.5692	0.210	82.39	
5	0.5619	0.214	85.05	
6	0.5681	0.216	84.91	

4.6.3 Dissolution test for Glunor

Table 4.14 : dissolution test for Glunor

Sample No	weight of sample (gm)	Absorbance of the sample	Dissolution (%)	Average Dissolution (%)
1	0.7745	0.329	94.87	
2	0.7718	0.319	92.30	
3	0.7752	0.310	89.31	92.22
4	0.7758	0.349	100.46	
5	0.7777	0.269	77.25	
6	0.7704	0.342	99.14	

4.7 Standard curve preparation

The standard was collected from Incepta Pharmaceuticals Ltd. and tried to make a standard curve. For different concentration of Metformin Hydrochloride different absorption were recorded. Five serial concentrations of the standards of cetirizine Hydrochloride were prepared for the purpose of creating a standard curve.

Table 4.15: Concentration & Absorption for Standard Curve of Metformin Hydrochloride

Concentration (µg/ml)	Absorbance (at 232 nm)
10	0.779
5.0	0.402
2.5	0.193
1.0	0.083
0.5	0.061

From this table we can see the absorption of pure Metformin Hydrochloride at different concentration. If we plot this data we can obtain a straight line curve. From that curve we can easily determine the % dissolve at different concentration.

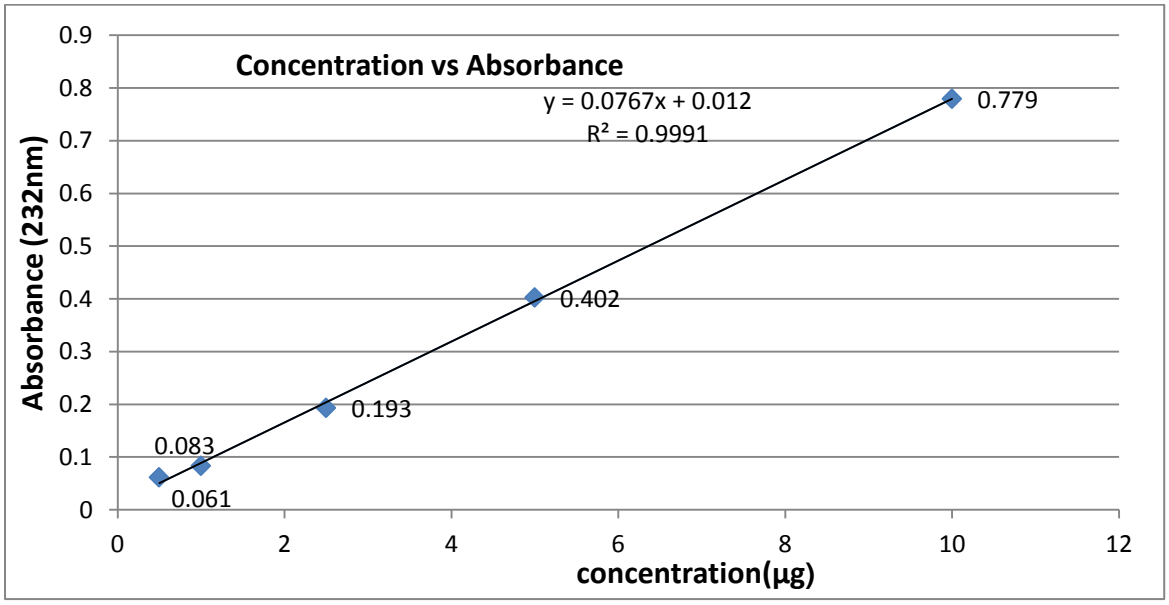

Fig 4.1: Standard curve of pure Metformin Hydrochloride

The figure indicates the standard curve of pure metformin Hydrchloride with five different concentrations. The curve shows a straight line which follows the Beer-lambert law.

4.8 Potency Test

Table 4.16: Potency Test of Three Brand

Brand Nmae	Absorbance	Potency (%)
Daomin	0.369	93.8
Oramet	0.397	101.2
Glunor	0.363	92.2

Chapter Five

Discussion

5.1 Weight Vriation

The weight variation ranged from 1.9692 to -2.1569 for Daomin. The weight variation ranged from 1.5091 to -1.1644 for Oramet. The weight variation ranged from 1.3202 to -1.0557 for Glunor.All the tablets of Daomin, Oramet, Glunor showed a percentage weight variation within the range of ±5% that meet the specification of USP (United States Pharmacopeia).

5.2 Friability

USP specifies that tablets of any batch must not lose 0.5% to 1% of their initial weight. The friability was 0.2496% for Daomin, 0.3746% for Oramet and 0.3300% for Glunor. All tablets of three brands (Daomin, Oramet and Glunor) had passed the friability test.

5.3 Hardness

The average hardness of the tablets is 4.19 kg/cm for Daomin and 4.40 kg/cm for Oramet and 4.18 kg/cm for Glunor. Daomin, Oramet and Glunor had hardness greater than 4kg/cm and therefore, met the USP specification and pass the quality control parameter.

5.4 Disintegration

The three brands have a disintegration time of 7.5 minutes for Daomin, 6.3 minutes for for Oramet and 9.8 minutes for Glunor and have met the specification of USP where uncoated tablets have a maximum disintegration time of 15 minutes.

5.5 Dissolution

After 45 minutes the dissolution parcentage of the three brands are 88.68% for Daomin, 85.97% for Oramet and 92.22% for Glunor.According to USP, the tolerance is not less than 70% of the labeled amount of Metformin hydrochloride is dissolved in 45 minutes.

5.6 Potency

Three brands have the potency 93.8% for Daomin, 101.2% for Oramet and 92.2% for Glunor. According to BP specification, % of potency for Metformin must be within 90-110%. Daomin, Oramet and Glunor had passed the potency specification.

Chapter Six

Conclusion

The quality control evaluations of three different brands of Metformin Hydrochloride tablets those are available in pharmaceutical market of Bangladesh were assessed by this study. This study showed that all three brands (Daomin, oramet, Glunor) of Metformin Hydrochloride tablets meet the pharmacopoeia specification of different parameters.The results of various quality control parameters for tablets like weight variation, friability, hardness, disintegration, dissolution, potency study all are in the pharmacopoeia limits. The efficacy of these tablets were well established which lead to diabetes mellitus patient will get expected therapeutic effects and minimum side effects. Everything was satisfactory and consistent with that for the cross-reference product. The quality of the products was acceptable. So this study revealed that collected samples of Metformin Hydrochloride 500mg tablet available in Dhaka, Bangladesh manufactured accordingly to cGMP as well as other standard monograph. Though this study has done with only one batch of each brand, further study can do with other batches to make sure the quality satisfaction of those brands.

Chapter Seven

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