In-vitro Comparative Dissolution Study of Three Different Brands (Comet, Informate, Bigmet) of Metformin Hydrochloride Tablets Available in Bangladesh

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

Bachelor of Pharmacy.

Submitted by

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Declaration by the Research Candidate

Nusrat Jahan, ID: 2013-1-70-065, hereby declare that the dissertation entitled "In-vitro comparative dissolution study of different brands of Metformin Hydrochloride tablets available in Bangladesh" which is submitted by me to the Department of Pharmacy, under the supervision and guidance of Tirtha Nandi, Lecturer, Department of Pharmacy, East West University, Dhaka.

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Certificate by the Supervisor

This is to certify that the thesis entitled “In-vitro comparative dissolution study of different brands 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, is a original record and genuine research work carried out by Nusrat Jahan, ID: 2013-1-70-065 in 2017 of his research in the Department of Pharmacy, East West University, under my supervision and guidance.

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Certificate by the Chairperson

This is to certify that the thesis entitled “In-vitro comparative dissolution study of different brands 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 is a record of original and genuine research work carried out by Nusrat Jahan, ID: 2013-1-70-065 in 2017.

……..

DR. SHAMSUN NAHAR KHAN

Associate Professor and Chairperson

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Dedication

This research paper is dedicated to

my beloved parents, who are my biggest inspirations.

Abstract

The aim of the study was to evaluate and compare dissolution pattern of locally branded drug products of Metformin Hydrochloride in Bangladesh with each other. Glucophase® is the patent drug of Metformin Hydrochloride. Branded drugs are expensive than locally marketed drug. Substitution of dugs is very essential for the people of under developed country. Three different brands of Metformin Hydrochloride tablets in Bangladesh like Comet, Informate, Bigmet were collected from a reputed pharmacy store. Six tablets of each of the brands were used for the *In-Vitro* dissolution study. Cumulative drug release were measured up to 50 minutes for all the brands. All the brads 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 Comet, Informate and Bigmet with each other. f1 value of Informate with respect of comet was 6.18% and f2 value was 43.5. f1 value of Bigmet with respect of Informate was 10.6% and f2 value was 42.1. f1 value of Comet with respect of Bigmet was 7.70% and f2 value was 41.5.

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

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Chapter 1

Introduction

1.1 Objective:

World Health Organization has estimated that about 80% of people suffering from diabetes live in low- and middle-income countries. Like all other developed and developing countries prevalence and incidence of type-2 Diabetes Mellitus is also increasing in Bangladesh.The aim of the research that to find out good quality of marketed metformin Hydrochloride which are used for the type-2 diabetes Mellitus. Which has great role to control the blood sugar alone or combine with other drugs.(Labu,Debnath,2013)

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 (5). In 1922 The very first synthesis of metformin (dimethyl biguanide) is attributed to Werner and Bell from Trinity College which is situated in Dublin, Ireland 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 because of their toxicity, especially lactic acidosis. However 50 years were needed to promote metformin from a minor product to the 'gold standard' in the treatment of type 2 DM, with a wide safety profile.(Marić, A., 2010)

Metformin is considered as the first-line oral hypoglycemic agent in the treatment of type 2 diabetes mellitus. Metformin is the drug of choice in obese patients. Metformin activates adenosine monophosphate activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, total body energy balance and metabolism of glucose and fats. Activation of AMPK is required for metformin's inhibitory effect on the production of glucose by liver cells.(Bennett, Wendy L,2011)

Among several antidiabetic drugs; metformin is an ideal antihyperglycemic but it is not hypoglycemic drug because it does not affect insulin release from the pancreas and does not cause hypoglycemia, even in large doses. Metformin drcreases glucose levels primarily by decreasing hepatic glucose production and by increasing insulin action in muscle and fat. The mechanism by which metformin reduces hepatic glucose production is controversial but thereis an effect on reducing gluconeogenesis.Metformin is manufactured by several pharmaceutical companies of Bangladesh in different brand names it is very much important to know that the compatibility of

the drug and its excipients in formulation may impair the efficacy of the drugs. (Islam, M. A., 2014)The existence of poor quality drugs in many third world countries has been reported previously. Bangladesh is one of the medium earning countries so it is very important to have an observation of the regular drugs used by the mass population (Birhanu et al., 2013).

Under the research protocol local brands of Bangladesh is compared with the standard of metformin (Active Pharmaceutical Ingredient).Drugs are Informet (Beximco Pharmaceuticals Ltd), Daomin (ACME Laboratories Ltd.), Met (Opsonin Pharma Limited).

1.2 Medications for type 2 Diabetes Mellitus:

These are the drugs normally used

- Alpha-glucosidase inhibitors
- Biguanides
- Dopamine agonist
- DPP-4 inhibitors
- Glucagon-like peptides (incretin mimetics)
- Meglitinides
- Sodium glucose transporter (SGLT) 2 inhibitors
- Sulfonylureas
- Thiazolidinediones. (Healthline,2016)

Biguanides:

Metformin was first discovered as a product in the synthesis of N,N dimethylguanidine by Emil Werner and James Bellandin 1922. Later in 1929, it was found that it reduce blood sugar level.Metformin was described in 1957 and it became available in the British National Formulary in the year of 1958.(Wai, Kin,2008)

1.2.1 Biguanides general informations:

Biguanides are insulin sensitizers.

Biguanides lower blood sugar by the following ways:

- By decreasing the amount of sugar produced by the liver.
- By increasing the amount of sugar absorbed by muscle cells and fat.
- By decreasing the body's need for insulin.

Metformin does not act on the pancreas to produce more insulin. It should not cause low blood sugar (hypoglycemia) or weight gain. HealthLink, 2016)

1.2.2 Mechanism of Action:

The principle activity of metformin is to reduce the glucose level of blood. 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), which is a liver enzyme that plays a vital role in insulin signaling,the 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. Metformin intake 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/decompensated heart failure. (DrugBank,2016)

1.3 Side effects:

The more common side effects that occur with metformin are the following:

Stomach problems, Diarrhea, Nausea, Stomach pain, Heartburn, Gas

If these effects are mild,then they may go away within a few days or a couple of weeks.

1.3.1 Serious side effects:

Lactic acidosis: Symptoms include:

Tiredness, Weakness, Stomach pains, nausea, or vomiting, Dizziness or lightheadedness, Slow or irregular heart rate, unusual muscle pain, trouble breathing, unusual sleepiness

Using the following drugs with metformin may increase your risk of lactic acidosis.

drugs include:

Acetazolamide, Brinzolamide, Dorzolamide, Methazolamide, Topiramate, Phenytoin

Low blood sugar. Symptoms include:

Headache, Weakness, Confusion, Shaking or feeling jittery, Drowsiness, Dizziness, Irritability, Sweating, Hunger, Fast heart rate

1.4 Metformin VS Sulfonylureas:

Metformin was the most widely prescribed drug, followed by second-generation sulfonylureas. As we know that many patients took more than one diabetes drug.

Among the major findings:

Compared with metformin-

- Single-drug treatments with first- or second-generation sulfonylureas was associated with (24 to 61)% increased risk of death from all causes.
- Second-generation sulfonylurea use- was associated with (18 to 30)% increased risk for heart failure.
- Treatment with Actos was associated with (31 to 39)% decreased risk for death.

Metformin as a First-Line Treatment

First-generation sulfonylureas include the drugs, acetohexamide (Dymelor), chlorpropamide (Diabinese), tolbutamide, and tolazamide (Tolinase).

Second-generation versions include glipizide (Glucotrol), gliclazide, glimepiride (Amaryl), and glyburide (Diabeta, Micronase, Glycron, Glynase). (WebMD, 2009)

1.5 Metformin general information:

Metformin Hydrochloride (HCl) Tablets, USP is an oral antihyperglycemic drug used in the management of type 2 diabetes. Metformin HCl, USP (N,N-dimethylimidodicarbonimidic diamide hydrochloride). Metformin HCl, USP is a white to off-white crystalline compound with a molecular formula of C4H11N5 • HCl and a molecular weight of 165.62. Metformin HCl, USP is freely soluble in water and is practically insoluble in acetone, ether, and chloroform. The pKa of Metformin is 12.4. The pH of a 1% aqueous solution of Metformin HCl, USP is 6.68.

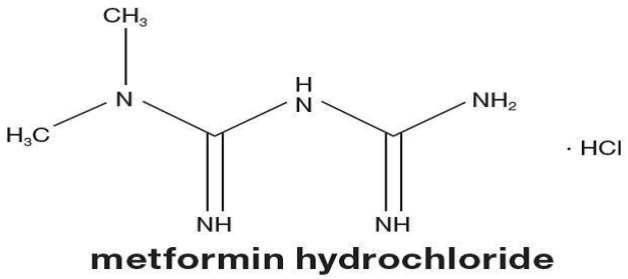

Figure 1.1: Metformin Hydrochloride. (Drugs.com,2016)

Metformin HCL Tablets, USP, contains 500 mg, 850 mg, or 1000 mg of Metformin HCl, USP. Each tablet contains the excipients povidone, microcrystalline cellulose, croscarmellose sodium and magnesium stearate.The coating for the 500 mg, 850 mg and 1000 mg tablets contain titanium dioxide, talc, polyethylene glycol, polyvinyl alcohol, gum acacia, maltodextrin, propylene glycol and natural flavors. (Drugs.com, 2016)

Storage:

Store at 20° to 25° C (68° to 77° F); excursions permitted to 15° to 30° C (59° to 86° F).

1.6 Dosage Indication and Administration:

1. Metformin HCl Tablets, USP usually have a starting dose of 500 mg twice a day or 850 mg once a day, which is given with meals. Dosage increases are made in increments of 500 mg weekly or 850 mg every 2 weeks, up to a total of 2000 mg per day can be given in divided doses. Patients can also be titrated from 500 mg twice a day to 850 mg twice a day after 2 weeks of time.

2. The patientsrequiring additional glycemic control, Metformin HCl, USP may be given to a maximum daily dose of 2550 mg per day. Doses above 2000 mg may be better tolerated and can be given three times a day with meals.

Pregnancy-

Metformin HCl, USP is not recommended during pregnancy.

Elderly-

The initial and maintenance dosing of Metformin HCl, USP should be conservative in patients with advanced age, 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, debilitated, and malnourished patients should not be titrated to the maximum dose of Metformin HCl, USP.

Monitoring of renal function is necessary to aid in prevention of lactic acidosis, particularly in the elderly.

Metformin HCl, USP is not recommended in patients below the age of 10 years.(Druglib,2015)

Laboratory Tests

All diabetic therapies responses should be monitored by periodic measurements of fasting blood glucose and glycosylated hemoglobin levels, with a goal of decreasing these levels toward the normal range. During initial dose titration, fasting glucose can be used to determine the therapeutic response. Thereafter, both glucose and glycosylated hemoglobin should be monitored.

Measurements of glycosylated hemoglobin may be especially useful for evaluating long-term control.

Initial and periodic monitoring of hematologic parameters (e.g., hemoglobin/hematocrit and red blood cell indices) and renal function (serum creatinine) should be performed, at least on an annual basis. While megaloblastic anemia has rarely been seen with Metformin HCL therapy, if this is suspected, Vitamin B12 deficiency should be excluded.

1.7 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.
- In patients in whom development of renal dysfunction is anticipated, renal function should be assessed more frequently and Metformin HCL discontinued if evidence of renal impairment is present.
- Use of concomitant medications that may affect renal function or Metformin disposition—such as cationic drugs that are eliminated by renal tubular secretion should be used with caution.

Hypoxic states-

Cardiovascular collapse (shock) from whatever cause, acute congestive heart failure, acute myocardial infarction and other conditions characterized by hypoxemia have been associated with lactic acidosis and may also cause prerenal azotemia. When such events occur in patients on Metformin HCL therapy, the drug should be promptly discontinued.

Surgical procedures-

Metformin HCL therapy should be temporarily suspended for any surgical procedure and should not be restarted until the patient's oral intake has resumed and renal function has been evaluated as 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 HCL.

Impaired hepatic function-

Since impaired hepatic function has been associated with some cases of lactic acidosis, Metformin HCL should generally be avoided in patients with clinical or laboratory evidence of hepatic disease.

Hypoglycemia-

Hypoglycemia does not occur in patients receiving Metformin HCL 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 and insulin) or ethanol.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 HCL and temporarily administer insulin. Metformin HCL may be reinstituted after the acute episode is resolved.

1.8 Drug Interactions:

Glyburide-

Co-administration of Metformin and glyburide did not result in any changes in either Metformin pharmacokinetics or pharmacodynamics. Decreases in glyburide AUC and Cmax 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 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 Cmax by 22% and blood AUC by 15%, without any significant change in Metformin renal clearance. When administered with Metformin, the Cmax 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 Cmax and AUC by 20% and 9%, respectively, and increased the amount excreted in the urine. Tmax and half-life were unaffected. Nifedipine appears to enhance the absorption of Metformin. Metformin had minimal effects on nifedipine.

Cationic drugs-

Cationic drugs (e.g., amiloride, digoxin, morphine, procainamide, quinidine, quinine, ranitidine, triamterene, trimethoprim, or vancomycin) that are eliminated by renal tubular secretion theoretically have the potential for interaction with Metformin by competing for common renal tubular transport systems. Such interaction between Metformin and oral cimetidine has been observed in normal healthy volunteers in both single- and multiple-dose, Metformin-cimetidine drug interaction studies, with a 60% increase in peak Metformin plasma and whole blood concentrations and a 40% increase in plasma and whole blood Metformin AUC. There was no change in elimination half-life in the single-dose study. Metformin had no effect on cimetidine pharmacokinetics. Although such interactions remain theoretical (except for cimetidine), careful patient monitoring and dose adjustment of Metformin HCL and/or the interfering drug is

recommended in patients who are taking cationic medications that are excreted via the proximal renal tubular secretory system.

Other-

Certain drugs tend to produce hyperglycemia and may lead to loss of glycemic control. These drugs include the thiazides 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 HCL, the patient should be closely observed for loss of blood glucose control. When such drugs are withdrawn from a patient receiving Metformin HCL, the patient should be observed closely for hypoglycemia.

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.(Drugs.com,2016)

1.8.1 Metformin May Interact with Other Medications:

Oral Tablet:

Metformin can interact with other drugs, herbs or vitamins that are a person might be taking.

-Diabetes drugs

Using these following drugs with metformin may cause low blood sugar levels:

Drugs include:

Insulin, Medications that release insulin, Heart or blood pressure drugsmay reduce the effectiveness of metformin, Diuretics, Calcium channel blockers, such as Nifedipine, Heart rhythm problem drugs.

Metformin may increase the levels of these medications in patient body. This raises the risk of side effects.

Drugs include:

Digoxin, Procainamide, Quinidine, Cholesterol drugs, Trimethoprim, Vancomycin, Cimetidine, Ranitidine, Phenothiazines, Morphine, Malaria drugs, Quinine, Hormone drugs.

These following drugs may make metformin less effective in lowering the blood sugar level.

Drugs include

Antibiotics, Stomach problems drugs, Pain medications, Nicotinic acid, Glaucoma drugs, Corticosteroids (inhaled and oral), Levothyroxine, Liothyronine, Liotrix, Estrogens, Conjugated estrogens, Estradiol, Tuberculosis drugs, Isoniazid, Thyroid drugs, dessicated thyroid

Alcohol interaction:

The use of drinks that contain alcohol can increase the risk of lactic acidosis of a patient taking metformin. Alcohol may also raise or lower your blood sugar levels. (Healthline,2016)

Acetazolamide: The risk or severity of adverse effects can be increased when Acetazolamide is combined with Metformin.

Acetylsalicylic acid: Acetylsalicylic acid may increase the hypoglycemic activities of Metformin.

Aminosalicylic Acid: Aminosalicylic Acid may increase the hypoglycemic activities of Metformin.

Aripiprazole: The therapeutic efficacy of Metformin can be decreased when used in combination with Aripiprazole.

Arsenic trioxide: The therapeutic efficacy of Metformin can be decreased when used in combination with Arsenic trioxide.

Articaine: The therapeutic efficacy of Metformin can be decreased when used in combination with Articaine.

Asenapine: The therapeutic efficacy of Metformin can be decreased when used in combination with Asenapine.

Atazanavir: The therapeutic efficacy of Metformin can be decreased when used in combination with Atazanavir.

Balsalazide: Balsalazide may increase the hypoglycemic activities of Metformin.

Bendroflumethiazide: The therapeutic efficacy of Metformin can be decreased when used in combination with Bendroflumethiazide.

Benmoxin: Benmoxin may increase the hypoglycemic activities of Metformin.

Betamethasone: The therapeutic efficacy of Metformin can be decreased when used in combination with Betamethasone.

Food Interactions:

- Avoid alcohol.
- Take with food to reduce gastric irritation.

Half life: 6.2 hours. Duration of action is 8-12 hours.

Clearance: 718-1552 mL/minute following single oral dose of 0.5-1.5 g. Metformin is removed by hemodialysis at a rate of approximately 170 ml/min under good hemodynamic conditions.

Toxicity:

Acute oral toxicity (LD50): 350 mg/kg [Rabbit]. It would be expected that adverse reactions of a more intense character including epigastric discomfort, nausea, and vomiting followed by diarrhea, drowsiness, weakness, dizziness, malaise and headache might be seen.

Affected organisms: Humans and other mammals.(DrugBank,2016)

1.9 Teratogenic Effects:

Recent information strongly suggests that abnormal blood glucose levels during pregnancy are associated with a higher incidence of congenital abnormalities. Most experts recommend that insulin be used during pregnancy to maintain blood glucose levels as close to normal as possible. Because animal reproduction studies are not always predictive of human response, Metformin HCL should not be used during pregnancy unless clearly needed.

1.10 Overdose:

Overdose of metformin HCl has occurred, including ingestion of amounts greater than 50 grams. Hypoglycemia was reported in approximately 10% of cases, but no causal association with metformin HCl has been established. Lactic acidosis has been reported in approximately 32% of metformin overdose cases (see WARNINGS). Metformin is dialyzable with a clearance of up to 170 mL/min under good hemodynamic conditions. Therefore, hemodialysis may be useful for removal of accumulated drug from patients in whom metformin overdosage is suspected.(Drugs.com,2016)

1.11 Contraindications:

Metformin HCl, USP is contraindicated in patients with:

- Renal disease or renal dysfunction (e.g., as suggested by serum creatinine levels ≥1.5 mg/dL [males], ≥1.4 mg/dL [females] or abnormal creatinine clearance) which may also result from conditions such as cardiovascular collapse (shock), acute myocardial infarction, and septicemia.
- Known hypersensitivity to metformin HCl, USP.
- Acute or chronic metabolic acidosis, including diabetic ketoacidosis, with or without coma. Diabetic ketoacidosis should be treated with insulin.

Metformin HCl, USP 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.(DrugLib,2015)

1.12 Pharmacokinetics of Metformin:

1.12.1 Absorption and Bioavailability

The absolute bioavailability of a Metformin HCL 500 mg tablet given under fasting conditions is approximately 50 to 60%. Studies using single oral doses of Metformin HCL 500 mg to 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 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.

1.12.2 Distribution:

The apparent volume of distribution (V/F) of Metformin following single oral doses of Metformin HCL 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 HCL, steady-state plasma concentrations of Metformin are reached within 24 to 48 hours and are generally <1 mcg/mL. During controlled clinical trials of Metformin HCL, maximum Metformin plasma levels did not exceed 5 mcg/mL, even at maximum doses.

1.12.3 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) 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.(Drugs.com,2016)

1.12.4 Special population:

Pediatric:

The safety and effectiveness of Metformin HCL for the treatment of type 2 diabetes have been established in pediatric patients ages 10 to 16 years. Use of Metformin HCL in this age group is supported by evidence from adequate and well-controlled studies of Metformin HCL in adults with

additional data from a controlled clinical study in pediatric patients ages 10-16 years with type 2 diabetes, which demonstrated a similar response in glycemic control to that seen in adults. In this study, adverse effects were similar to those described in adults. A maximum daily dose of 2000 mg is recommended.

Gender:

Metformin pharmacokinetic parameters did not differ significantly between normal subjects and patients with type 2 diabetes when analyzed according to gender (males = 19, females = 16). Similarly, in controlled clinical studies in patients with type 2 diabetes, the antihyperglycemic effect of Metformin HCL was comparable in males and females.

Race:

No studies of Metformin pharmacokinetic parameters according to race have been performed. In controlled clinical studies of Metformin HCL in patients with type 2 diabetes, the antihyperglycemic effect was comparable in whites (n=249), blacks (n=51), and Hispanics (n=24).

Geriatric:

Metformin is known to be substantially excreted by the kidney and because the risk of serious adverse reactions to the drug is greater in patients with impaired renal function, Metformin HCL should only be used in patients with normal renal function. Because aging is associated with reduced renal function, Metformin HCL should be used with caution as age increases. Care should be taken in dose selection and should be based on careful and regular monitoring of renal function. Generally, elderly patients should not be titrated to the maximum dose of Metformin HCL.(Drugs.com,2016)

1.13 Scanning of metformin hydrochloride by UV-visible spectrophotometery:

The drug was accurately weighed on electronic balance and was solubilized in distilled water. The drug solution was diluted up to the appropriate dilution in different concentrations. The absorbance was deter-mined spectrophotometricall and also scanned for the λmax, characteristics feature of the drug. The observed λmax was compared with reference value for the drug.

1.14 Calibration curve for metformin hydrochloride:

The standard solution of metformin hydrochloride was prepared in different concentrations. The absorbance of different samples was determined spectrophtometrically on scanned λmax and calculated by using linear regression equation.(Sharma,V.K., 2010)

1.15 BCS and Dosage Form Trends:

The simplest and easiest way of drug administration is oral route. The formulation of poorly soluble compounds for oral delivery at present is one of the most frequent and greatest challenges to formulation scientists in the pharmaceutical industry. It is frequently reported that 40% of New Drug Molecule in pharmaceutical industry are poorly water soluble. especially those belonging to the Biopharmaceutics Classification System1 (BCS) Class IV, dissolve slowly, poorly and irregularly, and hence poses serious drug delivery challenges like incomplete release from the dosage form, poor bioavailability leads to decreased release rate & poor bioavailability. So large dose is required for therapeutic activity but that may leads to

toxicity of the drug. Solubility is one of the important parameter to achieve desired concentration of drug insystemic circulation for pharmacological response. (BIYANI,2014)

1.15.1 Purpose of the BCS Guidance:

1.Expands the regulatory application of the BCS and recommends methods for classifying drugs.

2.Explains when a waiver for in vivo bioavailability and bioequivalence studies may be requested based on the approach of BCS.

Classes of Biopharmaceutical Classification System

Class I -

High Permeability, High Solubility: Those compounds are well absorbed and their absorption rate is usually higher than excretion. 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. They dissolve rapidly when presented in immediate release form, and are also transported across the gut wall.

Class II –

High Permeability, Low Solubility: These drugs 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. These drug exhibited variable bioavailability and need the enhancement in dissolution for increasing the bioavailability. These compounds are suitable for design the SR and CR formulations. In vitro-in vivo correlation (IVIVC) is usually expected for class II drugs.

Class III -

Low Permeability, High Solubility:The absorption is limited by the permeation rate but the drug is solvated very fast. Drug permeability is the rate-limiting step for drug absorption, but the drug is solvated very quickly.

Class IV -

Low Permeability, Low Solubility: Those compounds have a poor bioavailability. Usually they are not well absorbed over the intestinal mucosa and a high variability is expected.

Figure 1

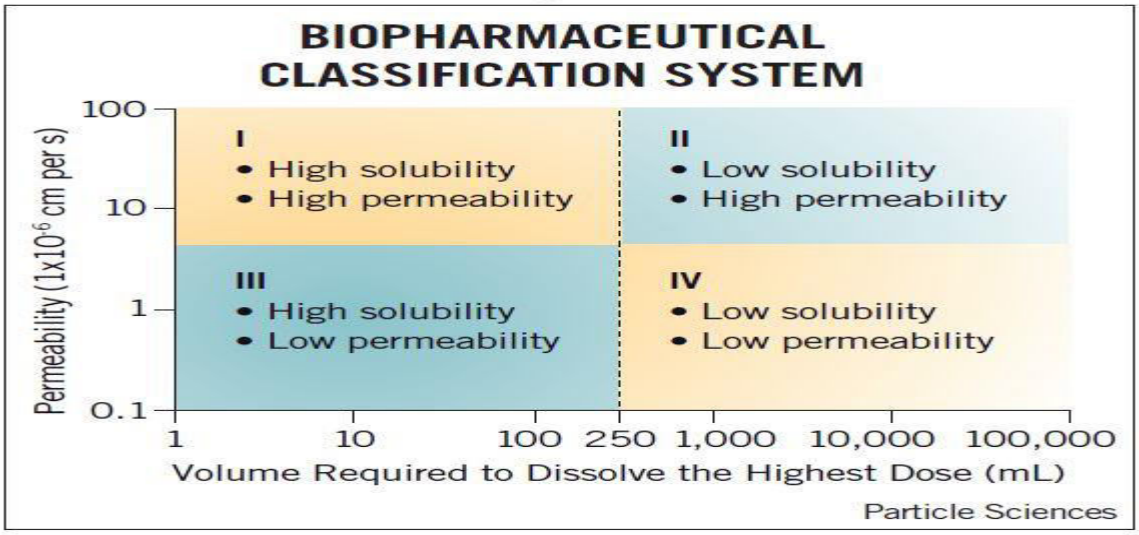

Figure 1.2: Biopharmaceutical Classification System (Particlesciences, 2011)

Extension to BCS (BCS Containing Six Classes): Bergstrom devised a modified Biopharmaceutical Classification System, in which they categorized the drugs into six classes based on the solubility and permeability. The solubility was classified as "high" or "low" and the permeability was allotted as "low", "intermediate," or "high". This new classification was developed based on the calculated surface area descriptors on the one hand and solubility and permeability on the other. Surface areas related to the non-polar part of the molecule resulted in good predictions of permeability. It was tentatively concluded that these models would be useful for early indication with regard to the absorption profiles of the compound during the early stages of drug discovery so that the necessary modifications can be made to optimize the pharmacokinetic parameters.

According to the current Biopharmaceutics Classification System (BCS), Metformin hydrochloride should be assigned to Class III.

1.15.2 Parameters of BCS

The drugs are classified in BCS on the basis of following parameters:

1. Solubility

2. Permeability

3. Dissolution.

The class boundaries for the parameters are:

1. Solubility class boundaries.

2. Permeability class boundaries.

3. Dissolution class boundaries.(Siya, Kunde, 2015)

1.16 Formulation Approach:

A pre-defined system in which decisions can be made based on data is necessary for efficient drug development. Inputs into such a system in addition to BCS class, a detailed solubility profile, polymorph status, desired dosage form, target dose and dosing regimen, drug stability, excipient compatibility and knowledge of transporter and metabolic pathways. Non-technical factors like as

a practical matter, need to be considered are such things as cost, intellectual property and distribution chain limitations. Integration of these into a methodical systematic approach will maximize the chances of a successful outcome. As R&D dollars become ever more scarce, it becomes increasingly evident that early consideration of as many factors as possible is the most efficient way to proceed. This is true independent of theroute of administration. This leads to the strategy of getting to FIH as quickly as possible with a formulation strategy that accounts for both physicochemical properties and physiologic influences. First, it is critical to characterize the compound of yours. Understanding the basic behavior of a given compound in various solvents and across a range of pH is fundamental to design a dosage form. For instance, a compound soluble only at lower pHs will require a different formulation than one freely soluble at, for example, pH 7. Very importantly, this is true whether one is administering the drug, for example, IV or orally. It is important that the drug developer or the CRO be equipped with a range of technologies to address the various patterns that emerge. There is a very few thing the world that wastes more time and money than trying to fit a drug to a specific preordained delivery technology. Armed with the proper set of tools one can rapidly narrow down the potential approaches. For the most part, all drug delivery strategies are trying to control drug exposure. Most often, one is trying to maximize it over time and/or concentration but frequently goals also include extended release and/or site specific delivery.

Table 1

BCS Class	Solubility	Permeability	Oral Dosage Form Approach	Chances of Non-oral Dosage Form being Required
1	High	High	Simple solid oral dosage form	
2	Low	High	• Techniques to increase surface area like particle size reduction, solid solution, solid dispersion • Solutions using solvents and/ or surfactants	
3	High	Low	Incorporate permeability enhancers, maximize local lumenal concentration	
4	Low	Low	Combine 2 and 3	

Figure 1.3: BCS Classification (Particlesciences, 2011)

In addition to the delivery goals, other functions are often required such as API stabilization or taste masking as two examples. In short, no one formulation approach will ever satisfy all or even a substantial portion of drug delivery demands.If formulation conditions dictate that a non-oral dosage form be used, similar charts exist for virtually all routes of administration. Each route of administration will must have different options but they are all ruled by the interplay of the drug's physicochemical properties and the local and systemic physiology they encounter. Independent of the final dosage form, ideal drug development involves an iterative process of setting goals, performing formulation work and developmental stage appropriate testing. Early on, for example, after physicochemical evaluations are complete, screening BCS testing and early polymorph screens might be performed. After thorough preformulation including solubility and stability testing, early formulations might again be screened for their impact on dissolution or bioavailability. This approach is repeated such that at each inflection point data is gathered to support the development plan. In this way, FIH is achieved most efficiently and in such a way as to insure clinically relevant data is obtained (Particlesciences, 2011).

1.17 Dissolution:

1.17.1 Dissolution General information:

The transfer of molecules of loins form solute state in a solution is known as dissolution.It is the process of dissolving solid part (solute) in the solvent (liquid). So, we can say that Dissolution is the process by which a substance turns in to solution in a solvent. For solids,dissolution is explained as the breakdown of the crystal lattice into individual ions, atoms ormolecules. Dissolution is a total kinetic process. The result of dissolution is controlled by thethermodynamic energies involved in the process, such as the heat of solution and entropy ofsolution, but the dissolution itself is not. Overall the free energy must be negative for netdissolution to occur. In turn, those energies are controlled by the way in which different chemicalbond types interact with those in the solvent (Sirius-analytical, 2016).

1.17.2 Rate of Dissolution:

The rate of dissolution determines the speed of the total process. It depends on the chemical natures of the solvent and solute these are the temperature, the degree of unsaturation, the interfacial

surface area, and the presence of "inhibitors" Like, substances adsorbed on the surface. The rate can be often expressed by the Noyes-Whitney Equation or the Nernst and Brunner equation of the form

dm/dt = AX{D/d}X(Cs-Cb)

Where:

m, mass of solute material

D is diffusion coefficient

d is thickness of the boundary layer of the solvent at the surface of the dissolving substance

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

Cs is mass concentration of the substance on the surface

Cb is mass concentration of the substance in the bulk of the solvent.

t is time

For dissolution limited by diffusion, Cs is equal to the solubility of the solute. When the dissolution rate of a pure substance is normalized to the surface area of the solid, then it is expressed in kg/m2S and termed as "intrinsic dissolution rate", which is defined by the United States Pharmacopeia (Lentle and Janssen, 2011).

1.17.3 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.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).

1.17.4 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 thetemperature is raised then the dissolution will be more rapid but in lower temperature thedissolution 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 willbe more but in the time of large particle size, dissolution will be less. The absorption dependsupon 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 concentrateddissolution 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 thanoily solvent (Yeomans, 2000).

1.18 Comparative dissolution:

1.18.1 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).

1.18.2 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 etal. 2000).

Methods for Comparison of Dissolution Profile Data:

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

- Methods based on analysis of variance (ANOVA).
- Model-dependent methods.
- 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 f1 (difference factor) and f2 (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:

$$f_1 = \frac{\sum_{t=1}^{n}(\mathrm{IRt} - \mathrm{TtI})}{\sum_{t=1}^{n}\mathrm{Rt}} X100$$

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 (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:

$$f_2 = 50\log\{1 + (1/n)\sum_{t=1}^{n}(Rt - Tt)^{-0.5}\}X100$$

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 (Yuksel et al., 2000).

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).

Chapter 2
Literature Review

The main purpose of type 2 diabetes mellitus treatment is to achieve and maintain good glycemic control, and to minimize the mortality and risk of microvascular and macro-vascular complications. Most recent algorithms for medical management of type 2 diabetes mellitus has recommend a combination of lifestyle intervention and metformin as initial therapy. Various studies suggest positive antihyperglycemic and metabolic effects of metformin, which have a wide safety profile. There is an increasing evidence for the potential efficacy of this drug in other diseases such as polycystic ovary syndrome, nonalcoholic steatohepatitis, HIV lipodystrophy, and neoplasms. (Marić, A., 2010)

Metformin hydrochloride is a drug of biguanide category in the treatment and management of diabetes mellitus. The drug sample was scanned for λmax by spectrophotometer and also by HPLC. The FTIR spectrum of the sample drug was compared with reference for genuinety. The thermal stability of the drug was studied at the temperature of 30, 40, 50 and 70 ºC in aqueous medium as it is freely soluble in water. The data obtained was treated by Arrhenius, zero order and first order kinetics. Metformin followed zero order kinetics in thermal degradation and covered 208 h to be decomposed up to 10 %. (Sharma, V.K., Nautiyal, V., Goel, K.K. and Sharma, A., 2010)

Metformin was subjected to different stress conditions as per (ICH) International Conference on Harmonization guidelines. A UV spectroscopic method was developed for analysis of the drug in the presence of the degradation products. As solvents Methanol and distilled water were used. The amount of degraded drug was calculated by taking absorbance at 237 nm. According to the assay limit of USP specified that the content should not be less than 95% and not more than 105% of labelled amount. Brand A, C and E degraded after heating. Brand A,B,C, and D degraded by UV light exposure .On basic pH brand A, and C showed degradation after the addition of 0.1 N NaOH while other brands does not degraded as base has no impact on metformin concentration and the original pH of metformin HCL was 6.68 before addition of acid and base. On addition of 0.1 N HCL all brands showed heavy degradation. After 15 days the time affects and degrades the metformin concentration of all brands. The method was found to be simple and less time consuming and cost effective.(Naveed, S., Shafiq, A., Khan, M., Jamal, M., Zafar, H., Hashim, H. and Urooj, L., 2014)

Delayed-release metformin (Met DR) is formulated in the aim to deliver the drug to the lower bowel to leverage the gut-based mechanisms of metformin action with lower plasma exposure. Met DR was assessed in two studies. Study 1 was compared the bioavailability of single daily doses of Met DR to currently available immediate release metformin (Met IR) and extended-release metformin (Met XR) in otherwise healthy volunteers. Study 2 was assessed glycemic control in subjects with type 2 diabetes (T2DM) over 12 weeks. (Buse, J.B., DeFronzo, R.A., Rosenstock, J., Kim, T., Burns, C., Skare, S., Baron, A. and Fineman, M., 2016)

Metformin is nowadays the most widely prescribed oral hypoglycemic agent. The main mechanisms of action includes reduction of appetite and by decreasing intestinal carbohydrate absorption, inhibition of hepatic gluconeogenesis, and increased glucose uptake by peripheral tissues. This agent may also be safely and efficaciously combined with all other oral hypoglycemic agents, enabling a useful additive effect. Additionally, it may be prescribed in combination with insulin The efficacy of metformin is accompanied by excellent safety: caution is only needed to avoid the drug in patients with obvious contraindications (mainly chronic renal failure, congestive heart failure, chronic obstructive pulmonary disease, liver disease. Generally, metformin is an excellent choice both in the specialized setting and in primary health care. (Papanas, N. and Maltezos, E., 2009)

The study was carried out on the ten brands of Metformin hydrochloride matrix tablets using dissolution test-1 method apparatus -2 (paddle) at 100 rpm in 1000 ml simulated intestinal medium (pH 6.8 ± 0.1) for 10 hour time period according to the guideline of United States Pharmacopeia (USP). In this study, 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 79.7 % 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, Mohammad A.; Mawla, Mahbub; Khan, Mohammad A.; Hye, Tanvirul; Asaduzzaman, Muhammad; Muhit, Md. A.; Raihan, Sheikh Z, 2011).

The intestinal absorption of oral-anti diabetic drugs in the treatment of type-II diabetes mellitus is altered when they are administered with synthetic drugs, food supplements and others. Diabetic health care consumers needs sweetening agents to take drugs, foods and eatables. A randomized cross over study in two phases and a washout period of 4 weeks was carried out to evaluate the bioavailability of anti diabetic drug Metformin hydrochloride. In the present study 10 healthy human volunteers received stevias (1g) for 5 days. After overnight fasting on 6th day a single dose of Metformin hydrochloride (500mg) was given. The blood samples following the intake were taken at different time intervals of 1, 2, 3, 4, 5, 7, 9 and 12 hours. The plasma samples (100µl) were injected into HPLC system after separation. The mobile phase comprised of Methanol: acetonitrile: mixed phosphate buffer (pH 2.6) at a ratio of (40:12:48). Analyses were run using cyano column (7.5mm x 4.6mm i.d, 5µm) at a flow rate of 1.2 ml.min-1 with diode array detector operating at a detection wave length of 234 nm in HPLC and the pharmacokinetic parameters were calculated by using the software Kinetica (Version 4.4.1Innaphase, USA). This study reveals that there is no significant change in the plasma concentration of Metformin hydrochloride when it was concomitantly administered with Stevias. (Gopi, G., Manikandan, M., Roja, D.N., Thirumurugu, S., Kannan, K., Arumainayagam, D.C. and Manavalan, R.,2012)

Pharmacokinetics of metformin extended release (XR) formulation were studied under fasting and fed conditions and compared to those of immediate release (IR) under fasting conditions in humans. 78 healthy human volunteers participated in 3 independent studies (26 subjects per study) were given either 1000 mg oral dose metformin IR or 750 mg metformin XR. Plasma samples were obtained up to 24 hours after dosing. Pharmacokinetic parameters in plasma were calculated by non-compartmental analysis using Kinetica program. Results have shown increased XR bioavailability and delayed time to reach the maximum concentration (Cmax) in the fed state as compared to fasted state, with no significant difference in Cmax and half life values. On the other hand, the IR formulation showed significant differences in all parameters as compared to XR formulation, yet the half life was similar. In conclusion, XR formulation was shown similar to IR

formulation with less possible side effects. (Idkaidek, N., Arafat, T., Melhim, M., Alawneh, J. and Hakooz, N., 2012)

Metformin lowers blood glucose levels by inhibiting hepatic glucose production (HGP), an effect originally postulated to be due to a hepatic AMP-activated protein kinase (AMPK)-dependent mechanism. Studies have questioned the contribution of hepatic AMPK to the effects of metformin on lowering hyperglycemia and a gut–brain–liver axis that mediates intestinal nutrient and hormone induced lowering of HGP has been identified. Thus, it is possible that metformin affects HGP through this inter-organ crosstalk. Here we show that intra-duodenal infusion of metformin for 50 min activated duodenal mucosal Ampk and lowered HGP in a rat 3 d high fat diet (HFD)-induced model of insulin resistance. Inhibition of duodenal Ampk negated the HGP-lowering effect of intra-duodenal metformin, and both duodenal glucagon-like peptide-1 receptor (Glp-1r)–protein kinase A (Pka) signaling and a neuronal-mediated gut–brain–liver pathway were required for metformin to lower HGP. Pre -absorptive metformin also lowered HGP in rat models of 28 d HFD–induced obesity and insulin resistance and nicotinamide (NA) streptozotocin (STZ)–HFD-induced type 2 diabetes. In an unclamped setting, inhibition of duodenal Ampk reduced the glucose-lowering effects of a bolus metformin treatment in rat models of diabetes. These findings show that, in rat models of both obesity and diabetes, metformin activates a previously unappreciated duodenal Ampk–dependent pathway to lower HGP and plasma glucose levels. (Duca, F.A., Côté, C.D., Rasmussen, B.A., Zadeh-Tahmasebi, M., Rutter, G.A., Filippi, B.M. and Lam, T.K., 2015)

One aspect of the effects of metformin on glucagon-like peptide (GLP)-1 might be associated with the mechanism by which the cross talk between insulin and Wnt signaling enhances GLP1 secretion, due to the action of metformin as an insulin sensitizer. However, this remains completely unknown. In this study, it has investigated the mechanisms of the action of metformin on cross talk between insulin and Wnt signaling. GLP1 enhancement by meformin was determined in human NCI-H716 intestinal L-cells and hyperglycemic db/db mice treated with metformin (0.25 and 0.5 mM and/or 12.5 mg/kg body weight) for 24 h and 2 months. Metformin increased GLP1 secretion in L-cells and db/db mice. Metformin stimulated the nuclear translocation of β-catenin and TOPflash reporter activity, and gene depletion of β-catenin or enhancement of mutation of

transcription factor 7-like 2 binding site offset GLP1. In addition, insulin receptor substrate 2 gene depletion blocked metformin-enhanced β-catenin translocation. These effects were preceded by an increase in glucose utilization and calcium influx, the activation of calcium-dependent protein kinase, and, in turn, the activation of insulin signaling, and the inhibition of glycogen synthase kinase 3β, a potent inhibitor of β-catenin. High blood glucose levels were controlled via GLP1 receptor-dependent insulinotropic pathways in db/db mice, which were evidenced by the increase in GLP1 and insulin levels at 30 min after oral glucose loading and pancreatic insulinotropic gene expression. The findings indicate that the cooperation between Wnt and its upstream insulin signaling pathways might be a novel and important mechanism underlying the effects of metformin on GLP1 production. (Kim, M.H., Jee, J.H., Park, S., Lee, M.S., Kim, K.W. and Lee, M.K., 2014).

Metformin is a first-line antidiabetic agent taken by 150 million people across the world every year, yet its mechanism remains only partially understood and controversial. It was proposed that suppression of glucose production in hepatocytes by metformin is AMPK-independent; however, unachievably high concentrations of metformin were employed in these studies. In the current study, we find that metformin, via an AMP-activated protein kinase (AMPK)-dependent mechanism, suppresses glucose production and gluconeogenic gene expression in primary hepatocytes at concentrations found in the portal vein of animals (60–80 μM). Metformin also inhibits gluconeogenic gene expression in the liver of mice administered orally with metformin. Furthermore, the cAMP-PKA pathway negatively regulates AMPK activity through phosphorylation at Ser-485/497 on the α subunit, which in turn reduces net phosphorylation at Thr-172. Because diabetic patients often have hyperglucagonemia, AMPKα phosphorylation at Ser-485/497 is a therapeutic target to improve metformin efficacy. (Cao, J., Meng, S., Chang, E., Beckwith-Fickas, K., Xiong, L., Cole, R.N., Radovick, S., Wondisford, F.E. and He, L., 2014).

Metformin, a biguanide derivate, has pleiotropic effects beyond glucose reduction, including improvement of lipid profiles and lowering microvascular and macrovascular complications associated with type 2 diabetes mellitus (T2DM). These effects have been ascribed to adenosine monophosphate-activated protein kinase (AMPK) activation in the liver and skeletal muscle. However, metformin effects are not attenuated when AMPK is knocked out and intravenous metformin is less effective than oral medication, raising the possibility of important gut

pharmacology. It has been hypothesized that the pharmacology of metformin includes alteration of bile acid recirculation and gut microbiota resulting in enhanced enteroendocrine hormone secretion. In this study we evaluated T2DM subjects on and off metformin monotherapy to characterize the gut-based mechanisms of metformin. Subjects were studied at 4 time points: (i) at baseline on metformin, (ii) 7 days after stopping metformin, (iii) when fasting blood glucose (FBG) had risen by 25% after stopping metformin, and (iv) when FBG returned to baseline levels after restarting the metformin. At these time points we profiled glucose, insulin, gut hormones (glucagon-like peptide-1 (GLP-1), peptide tyrosine-tyrosine (PYY) and glucose-dependent insulinotropic peptide (GIP) and bile acids in blood, as well as duodenal and faecal bile acids and gut microbiota. We found that metformin withdrawal was associated with a reduction of active and total GLP-1 and elevation of serum bile acids, especially cholic acid and its conjugates. These effects reversed when metformin was restarted. Effects on circulating PYY were more modest, while GIP changes were negligible. Microbiota abundance of the phylum Firmicutes was positively correlated with changes in cholic acid and conjugates, while Bacteroidetes abundance was negatively correlated. Firmicutes and Bacteroidetes representation were also correlated with levels of serum PYY. Our study suggests that metformin has complex effects due to gut-based pharmacology which might provide insights into novel therapeutic approaches to treat T2DM and associated metabolic diseases. (Napolitano, A., Miller, S., Nicholls, A.W., Baker, D., Van Horn, S., Thomas, E., Rajpal, D., Spivak, A., Brown, J.R. and Nunez, D.J., 2014).

The antidiabetic drug metformin is often associated with a small reduction in total circulating cholesterol, but the mechanism responsible is unknown. As bile salts contribute significantly to cholesterol homeostasis, this study has investigated the effect of metformin on the absorption of bile salts by the jejunum and ileum, and their transfer into bile. The method used was, Sodium-[1-14C]-glycocholate was administered into the jejunum or ileum of anaesthetized rats with and without metformin (250 mg/kg). Appearance of 14C-glycocholate in plasma and bile was followed for 150 min. Result was Absorption of 14C-glycocholate from the ileum, which is a high-capacity active process, was 10-fold greater than absorption from the jejunum, which is mainly a passive process. Metformin increased threefold the absorption of 14C-glycocholate from the jejunum. Metformin similarly increased the appearance of jejunal 14C-glycocholate in plasma and bile. In contrast to the jejunum, absorption of 14C-glycocholate from the ileum was suppressed by more

than half with metformin. This was associated with corresponding reductions of 14C-glycocholate in plasma and bile. Thus, metformin induced a large suppression of active bile salt absorption from the ileum compared with a small increase in passive absorption from the jejunum. This suggests that the ileal effect of metformin to reduce overall bile salt absorption could contribute to the modest cholesterol-lowering effect of this drug. (Carter, D., Howlett, H.C.S., Wiernsperger, N.F. and Bailey, C.J., 2003)

Metformin-induced changes in the gut microbiota have been reported; however, the relationship between metformin treatment and the gut microbiota remains unclear. In this study, the composition of the gut microbiota was investigated using a mouse model of high-fat-diet (HFD)-induced obesity with and without metformin treatment. As expected, metformin treatment improved markers of metabolic disorders, including serum glucose levels, body weight, and total cholesterol levels. Moreover, Akkermansia muciniphila (12.44% ± 5.26%) and Clostridium cocleatum (0.10% ± 0.09%) abundances increased significantly after metformin treatment of mice on the HFD. The relative abundance of A. muciniphila in the fecal microbiota was also found to increase in brain heart infusion (BHI) medium supplemented with metformin in vitro. In addition to the changes in the microbiota associated with metformin treatment, when other influences were controlled for, a total of 18 KEGG metabolic pathways (including those for sphingolipid and fatty acid metabolism) were significantly upregulated in the gut microbiota during metformin treatment of mice on an HFD. Our results demonstrate that the gut microbiota and their metabolic pathways are influenced by metformin treatment. (Lee, H. and Ko, G., 2014)

DPP-4 inhibitors prevent degradation of incretin hormones (GLP-1 and GIP) while metformin may increase GLP-1 levels. We examined, in a 4-period cross-over trial, the influence of metformin (2000 mg/d), sitagliptin (100 mg/d), or their combination, on GLP-1 responses and on the incretin effect in 20 patients with type 2 diabetes comparing an oral glucose challenge (75 g, day 5) and an "isoglycemic" intravenous glucose infusion (day 6). Fasting total GLP-1 was significantly increased by metformin and not changed by sitagliptin. After oral glucose, metformin increased and sitagliptin significantly decreased (by 53 %) total GLP-1. Fasting and post-load intact GLP-1 increased with sitagliptin, but not with metformin. After oral glucose, only sitagliptin, but not metformin significantly augmented insulin secretion, both in monotherapy and as add-on to

metformin. The incretin effect was not changed numerically with any of the treatments. sitagliptin increased intact GLP-1 and GIP through DPP-4 inhibition, but reduced total GLP-1 and GIP (feedback inhibition) without affecting the numerical contribution of the incretin effect. Insulin secretion with sitagliptin treatment was similarly stimulated with both oral and "isoglycemic" intravenous glucose. This points to an important contribution of small changes in incretin concentrations within the basal range, or to additional insulinotropic agents besides GLP mediating antidiabetic effects of DPP-4 inhibition. (Vardarli, I., Arndt, E., Deacon, C.F., Holst, J.J. and Nauck, M.A., 2013)

The main effect of metformin is to suppress glucose production in the liver. There is no reliable biomarker to assess the effectiveness of metformin administration. Our previous studies have shown that phosphorylation of CBP at S436 is important for the regulation of hepatic glucose production by metformin. In current study, we found that CBP could be phosphorylated in white blood cells (WBCs), and CBP phosphorylation in the liver and in WBCs of mice had a similar pattern of change during a fasting time course experiment. These data suggests that CBP phosphorylation in WBCs may be used as a biomarker of metformin action in the liver. (He, L., Meng, S., Germain-Lee, E.L., Radovick, S. and Wondisford, F.E., 2014).

To assess the effect of metformin on insulin sensitivity, glucose tolerance and components of the metabolic syndrome in patients with impaired glucose tolerance (IGT). Forty first-degree relatives of patients with Type 2 diabetes fulfilling WHO criteria for IGT and participating in the Botnia study in Finland were randomized to treatment with either metformin 500 mg or placebo for 6 months. An oral glucose tolerance test (OGTT) and a euglycaemic hyperinsulinaemic clamp in combination with indirect calorimetry was performed at 0 and 6 months. The patients were followed after stopping treatment for another 6 months in an open trial and a repeat OGTT was performed at 12 months. Metformin treatment resulted in a 20% improvement in insulin-stimulated glucose metabolism (from 28.7 ± 13 to 34.4 ± 10.7 μmol/kg fat-free mass (FFM)/min) compared with placebo (P = 0.01), which was primarily due to an increase in glucose oxidation (from 16.6 ± 3.6 to 19.1 ± 4.4 μmol/kg FFM; P = 0.03) These changes were associated with a minimal improvement in glucose tolerance, which was maintained after 12 months. Metformin improves insulin sensitivity in subjects with IGT primarily by reversal of the glucose fatty acid

cycle. Obviously large multicentre studies are needed to establish whether these effects are sufficient to prevent progression to manifest Type 2 diabetes and associated cardiovascular morbidity and mortality. (Lehtovirta, M., Forsen, B., Gullström, M., Häggblom, M., Eriksson, J.G., Taskinen, M.R. and Groop, L., 2001)

Investigation shows whether the addition of metformin to the treatment of overweight and obese individuals further reduces the incidence of type2 diabetes mellitus (T2DM), prediabetes and metabolic syndrome (MetS) and improves cardiovascular disease (CVD) risk factors (RFs). Design and methods: We studied 366 adults (mean age 53.0±0.5 SE years, and mean BMI 32.3±0.2 SE Kg/m2) without CVD. All subjects received lifestyle recommendations and drug management of CVD-RFs, whilst 95 of them were additionally given metformin. The follow-up period lasted 12 months. Results: At the end of the study the frequency of T2DM in the metformin and non-metformin group was 1.1 and 8.1%, respectively (risk difference=−7% with 95% CI from −12.7% to −1.4%, p=0.012). Participants with prediabetes displayed a greater reduction in the incidence of T2DM after taking metformin compared to those who had not received this drug (risk difference=−18.5% with 95%CI from −33.1% to −3.9%, p=0.010). Metformin had a similar beneficial impact on subjects with Mets (risk difference=−12.9% with 95% from −25% to −0.7%, p=0.040) and this was attributed to the greater increase in HDL-C (p=0.046) and decrease in fasting plasma glucose levels (p=0.024). Metformin also achieved a greater reduction in total cholesterol and LDL-C levels (metformin vs. non-metformin treated subjects: −31.9 vs. −17.3 mg/dl, p=0.001, and −26.2 vs. −15.9 mg/dl, p=0.006, respectively). It can be said that, Metformin reduces the occurrence of T2DM in overweight and obese non-diabetic adults and decreases the rate of MetS by improving the CVD risk factor profile. (Andreadis, E.A., Katsanou, P.M., Georgiopoulos, D.X., Tsourous, G.I., Yfanti, G.K., Gouveri, E.T. and Diamantopoulos, E.J., 2009).

To determine the proportion of the American population who would merit metformin treatment, according to recent American Diabetes Association (ADA) consensus panel recommendations to prevent or delay the development of diabetes. Research design and methods, Risk factors were evaluated in 1,581 Screening for Impaired Glucose Tolerance (SIGT), 2,014 Third National Health and Nutrition Examination Survey (NHANES III), and 1,111 National Health and Nutrition Examination Survey 2005–2006 (NHANES 2005–2006) subjects, who were non-Hispanic white

and black, without known diabetes. Criteria for consideration of metformin included the presence of both impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), with ≥1 additional diabetes risk factor: age <60 years, BMI ≥35 kg/m2, family history of diabetes, elevated triglycerides, reduced HDL cholesterol, hypertension, or A1C >6.0%. RESULTS Isolated IFG, isolated IGT, and IFG and IGT were found in 18.0, 7.2, and 8.2% of SIGT; 22.3, 6.4, and 9.4% of NHANES III; and 21.8, 5.0, and 9.0% of NHANES 2005–2006 subjects, respectively. In SIGT, NHANES III, and NHANES 2005–2006, criteria for metformin consideration were met in 99, 96, and 96% of those with IFG and IGT; 31, 29, and 28% of all those with IFG; and 53, 57, and 62% of all those with IGT (8.1, 9.1, and 8.7% of all subjects), respectively. More than 96% of individuals with both IFG and IGT are likely to meet ADA consensus criteria for consideration of metformin. Because >28% of all those with IFG met the criteria, providers should perform oral glucose tolerance tests to find concomitant IGT in all patients with IFG. To the extent that our findings are representative of the U.S. population, ∼1 in 12 adults has a combination of pre-diabetes and risk factors that may justify consideration of metformin treatment for diabetes prevention. (Rhee, M.K., Herrick, K., Ziemer, D.C., Vaccarino, V., Weintraub, W.S., Narayan, K.V., Kolm, P., Twombly, J.G. and Phillips, L.S., 2010)

In the Diabetes Prevention and treatment Program, treatment of subjects with impaired glucose tolerance with metformin >3.2 years reduced the risk of developing type 2 diabetes by 30% compared with placebo. This study describes the mechanisms of this effect. In proportional hazards regression models with 2,155 subjects, changes in weight, the insulinogenic index (IGR), fasting insulin, and proinsulin were predictive of diabetes, though to different degrees within each group. The mean change in weight, fasting insulin, and proinsulin, but not IGR, differed between groups during the study. The 1.7-kg weight loss with metformin versus a 0.3-kg gain with placebo alone explained 64% of the beneficial metformin effect on diabetes risk. Adjustment for weight, fasting insulin, proinsulin, and other metabolic factors combined explained 81% of the beneficial metformin effect, but it remained nominally significant (P = 0.034). After the addition of changes in fasting glucose, 99% of the group effect was explained and is no longer significant. Treatment of high-risk subjects with metformin results in modest weight loss and favorable changes in insulin sensitivity and proinsulin, which contribute to a reduction in the risk of diabetes apart from the

associated reductions in fasting glucose. (Lachin, J.M., Christophi, C.A., Edelstein, S.L., Ehrmann, D.A., Hamman, R.F., Kahn, S.E., Knowler, W.C. and Nathan, D.M., 2007).

The goal of this study was to determine the effects of genetic variation in the organic cation transporter 1, OCT1, on the pharmacokinetics of the antidiabetic drug, metformin. Twenty healthy volunteers with known OCT1 genotype agreed to participate in the study. Each subject received two oral doses of metformin followed by collection of blood and urine samples. OCT1 genotypes had a significant ($P<0.05$) effect on metformin pharmacokinetics, with a higher area under the plasma concentration–time curve (AUC), higher maximal plasma concentration (Cmax), and lower oral volume of distribution (V/F) in the individuals carrying a reduced function OCT1 allele (R61C, G401S, 420del, or G465R). The effect of OCT1 on metformin pharmacokinetics in mice was less than in humans possibly reflecting species differences in hepatic expression level of the transporter. Our studies suggest that OCT1 genotype is a determinant of metformin pharmacokinetics. (Shu, Y., Brown, C., Castro, R.A., Shi, R.J., Lin, E.T., Owen, R.P., Sheardown, S.A., Yue, L., Burchard, E.G., Brett, C.M. and Giacomini, K.M., 2008)

Interindividual variation in response to metformin, first-line therapy for type 2 diabetes, is substantial. Given that transporters are determinants of metformin pharmacokinetics, we examined the effects of promoter variants in both multidrug and toxin extrusion protein 1 (MATE1) (g.–66T→C, rs2252281) and MATE2 (g.–130G→A, rs12943590) on variation in metformin disposition and response. The pharmacokinetics and glucose-lowering effects of metformin were assessed in healthy volunteers ($n = 57$) receiving metformin. The renal and secretory clearances of metformin were higher (22% and 26%, respectively) in carriers of variant MATE2 who were also MATE1 reference ($P < 0.05$). Both MATE genotypes were associated with altered post-metformin glucose tolerance, with variant carriers of MATE1 and MATE2 having an enhanced ($P < 0.01$) and reduced ($P < 0.05$) response, respectively. Consistent with these results, patients with diabetes ($n = 145$) carrying the MATE1 variant showed enhanced metformin response. These findings suggest that promoter variants of MATE1 and MATE2 are important determinants of metformin disposition and response in healthy volunteers and diabetic patients. (Stocker, S.L., Morrissey, K.M., Yee, S.W., Castro, R.A., Xu, L., Dahlin, A., Ramirez, A.H., Roden, D.M., Wilke, R.A., McCarty, C.A. and Davis, R.L., 2013).

Chemically, it is a hydrophilic base which exists at physiological pH as the cationic species (>99.9%). Consequently, its passive diffusion through cell membranes should be very limited. The mean ± SD fractional oral bioavailability (F) of metformin is 55 ± 16%. It is absorbed predominately from the small intestine. Metformin is excreted unchanged in urine. The elimination half-life (t1/2) of metformin during multiple dosages in patients with good renal function is approximately 5 hours. From published data on the pharmacokinetics of metformin, the population mean of its clearances were calculated. The population mean renal clearance (CLR) and apparent total clearance after oral administration (CL/F) of metformin were estimated to be 510 ± 130 mL/min and 1140 ± 330 mL/min, respectively, in healthy subjects and diabetic patients with good renal function. Over a range of renal function, the population mean values of CLR and CL/F of metformin are 4.3 ± 1.5 and 10.7 ± 3.5 times as great, respectively, as the clearance of creatinine (CLCR). AS the CLR and CL/F decrease approximately in proportion to CLCR, the dosage of metformin should be reduced in patients with renal impairment in proportion to the reduced CLCR. The oral absorption, hepatic uptake and renal excretion of metformin are mediated very largely by organic cation transporters (OCTs). An intron variant of OCT1 (single nucleotide polymorphism [SNP] rs622342) has been associated with a decreased effect on blood glucose in heterozygotes and a lack of effect of metformin on plasma glucose in homozygotes. An intron variant of multidrug and toxin extrusion transporter [MATE1] (G>A, SNP rs2289669) has also been associated with a small increase in antihyperglycaemic effect of metformin. Overall, the effect of structural variants of OCTs and other cation transporters on the pharmacokinetics of metformin appears small and the subsequent effects on clinical response are also limited. However, intersubject differences in the levels of expression of OCT1 and OCT3 in the liver are very large and may contribute more to the variations in the hepatic uptake and clinical effect of metformin. Lactic acidosis is the feared adverse effect of the biguanide drugs but its incidence is very low in patients treated with metformin. We suggest that the mean plasma concentrations of metformin over a dosage interval be maintained below 2.5 mg/L in order to minimize the development of this adverse effect. (Graham, G.G., Punt, J., Arora, M., Day, R.O., Doogue, M.P., Duong, J., Furlong, T.J., Greenfield, J.R., Greenup, L.C., Kirkpatrick, C.M. and Ray, J.E., 2011)

The research group examined the pharmacokinetic and pharmacodynamic rationales to develop controlled release (CR) formulations of metformin. Unrestrained diabetic rats received the drug as

intravenous bolus (i.v.), oral solution (p.o.), intra-duodenal bolus, 4-h infusion, or intra-colonic bolus. In addition, we developed two CR-gastroretentive dosage forms (CR-GRDF) that released the drug over 3 or 6 h (in vitro), and retained in the rats' stomach for 8–10 h. Metformin exhibited flip-flop PK. The colonic absorption was low but sustained and was associated with highly variable glucose-lowering effects, thus providing a PK rationale to develop CR-GRDF. In addition, the glucose-lowering effect was greater following p.o. vs. i.v. administration, despite equivalent AUC, indicating a first pass PD effect, thus, adding a PD rationale to develop metformin CR-GRDF. When administered to the diabetic rats, CR-GRDFs produced bioavailability and extent of glucose-lowering effects that were similar to those of the duodenal infusion and p.o. metformin administration. These findings are attributed to the adsorption of metformin to the intestine that yields slow and prolonged absorption even following p.o. administration of drug solution. The data indicates that unless the CR formulation could significantly extend the absorption period, it is not likely to improve glucose-lowering efficacy. (Stepensky, D., Friedman, M., Srour, W., Raz, I. and Hoffman, A., 2001)

Genetic polymorphisms of organic cation transporter 2 (OCT2) have been recently described, but their genotype–phenotype relationship in humans is unknown. They performed this study to (i) characterize genetic variations of the OCT2 gene in the Chinese population and (ii) investigate the potential functional significance of OCT2 polymorphisms using metformin (an OCT2 substrate) alone or in the presence of its transport inhibitor (cimetidine). Direct sequencing of all OCT2 exons and the surrounding introns was performed using genomic DNA from 112 healthy Chinese participants. To evaluate the potential functional change of a common 808G>T variant (Ala270Ser) identified in this population, 15 healthy participants with different 808G>T mutation status were recruited in a pharmacokinetic study of metformin with or without cimetidine. A total of 14 genetic variants were identified and 13 had frequency more than 1%. The renal tubular clearance (CLt) of metformin averaged 8.78±1.75, 7.68±0.672, and 6.32±0.954 ml/min/kg for participants with GG (n=6), GT (n=5), and TT (n=4) genotypes, respectively (P=0.037, one-way analysis of variance). In the presence of cimetidine, metformin CLt was decreased in all participants, but the decrease was significantly lower in TT than GG group (18.7 vs. 48.2%, P=0.029). Our study results demonstrated for the first time the existence of genetic polymorphisms of OCT2 in the Chinese population, and further showed that the 808G>T polymorphism is

associated with a reduced metformin renal or tubular clearance. Moreover, the inhibition of metformin renal tubular secretion by cimetidine also appeared to be dependent on this mutation. (Wang, Z.J., Yin, O.Q., Tomlinson, B. and Chow, M.S., 2008)

Organic cation transporters (OCTs) are responsible for the hepatic and renal transport of metformin. In this study we analyzed variants of OCT1 and OCT2 genes in 33 patients (24 responders and nine non-responders) based on the hypothesis that polymorphisms in both genes contribute to large inter-patient variability in the clinical efficacy of metformin. The sequences of the 5′-flanking and coding regions of the two genes of interest were screened by single-strand conformation polymorphism (SSCP) analysis. To compare the causative factors between responders and non-responders, we performed stepwise discriminant functional analysis. Age, body mass index (BMI) and treatment with lipid-lowering agents were demonstrated as positive predictors, and two mutations in the OCT1 gene, −43T > G in intron 1 and 408Met > Val (1222A > G) in exon 7, were negative and positive predictors, respectively, for the efficacy of metformin; the predictive accuracy was 55.5% ($P < 0.05$). Subsequent study indicated that OCT1 mRNA levels tended to be lower in human livers with the 408Met (1222A) variant, though the differences did not reach the level of significance. In this study it is suggested that OCT1 and OCT2 gene polymorphisms have little contribution to the clinical efficacy of metformin. (Shikata, E., Yamamoto, R., Takane, H., Shigemasa, C., Ikeda, T., Otsubo, K. and Ieiri, I., 2007)

Metformin is a widely used oral antihyperglycemic drug for the treatment of type II diabetes mellitus. The intestinal absorption of metformin is dose-dependent and involves an active, saturable uptake process. Metformin has been shown to be transported by the human organic cation transporters 1 and 2 (hOCT1-2). We recently cloned and characterized a novel proton-activated organic cation transporter, plasma membrane monoamine transporter (PMAT). We previously showed that PMAT transports many classic organic cations (e.g., monoamine neurotransmitters, 1-methyl-4-phenylpyridinium) in a pH-dependent manner and its mRNA is expressed in multiple human tissues. The goal of this study is to investigate whether metformin is a substrate of PMAT and whether PMAT plays a role in the intestinal uptake of metformin. Using Madin-Darby canine kidney cells stably expressing human PMAT, we showed that metformin is avidly transported by PMAT, with an apparent affinity (Km = 1.32 mM) comparable to those reported for hOCT1-2.

Interestingly, the concentration-velocity profile of PMAT-mediated metformin uptake is sigmoidal, with a Hill coefficient of 2.64. PMAT-mediated metformin transport is greatly stimulated by acidic pH, with the uptake rate being ~4-fold higher at pH 6.6 than at pH 7.4. Using a polyclonal antibody against PMAT, we showed that the PMAT protein (58 kDa) was expressed in human small intestine and concentrated on the tips of the mucosal epithelial layer. Taken together, our results suggest that PMAT transports metformin, is expressed in human intestine, and may play a role in the intestinal absorption of metformin and possibly other cationic drugs. (Zhou, M., Xia, L. and Wang, J., 2007)

The objective of the present study was to develop a hydrodynamically balanced system of metformin as a single unit floating capsule. Various grades of low-density polymers were used for the formulation of this system. They were prepared by physical blending of metformin and the polymers in varying ratios. The formulation was optimized on the basis of in vitro buoyancy and in vitro release in simulated fed state gastric fluid (citrate phosphate buffer pH 3.0). Effect of various release modifiers was studied to ensure the delivery of drug from the HBS capsules over a prolonged period. Capsules prepared with HPMC K4M and ethyl cellulose gave the best in vitro percentage release and were taken as the optimized formulation. By fitting the data into zero order, first order and Higuchi model it was concluded that the release followed zero order release, as the correlation coefficient (R2 value) was higher for zero order release. It was concluded from R2 values for Higuchi model that drug release followed fickian diffusion mechanism. In vivo studies were carried out in rabbits to assess the buoyancy, as well as the pharmacokinetic parameters of the formulation using gamma scintigraphy. The formulation remained buoyant during 5 h of study in rabbits. The comparative pharmacokinetic study was performed by administration of the optimized HBS capsules and immediate release capsules, both with radiolabeled metformin, using gamma counter. There was an increase in AUC in optimized HBS capsules of metformin when compared with immediate release formulation. (Ali, J., Arora, S., Ahuja, A., Babbar, A.K., Sharma, R.K., Khar, R.K. and Baboota, S., 2007)

Metformin hydrochloride is an orally administered antihyperglycemic agent, used in the management of non-insulin- dependant (type-2) diabetes mellitus. Difficulty in swallowing (dysphagia) is common among all age groups, especially in elderly and pediatrics. 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 8 min, respectively. The strong saline and slight bitter taste of the drug was masked using nonnutritive sweetener and flavor. (Mohapatra, A., Parikh, R.K. and Gohel, M.C., 2014)

The Biopharmaceutics Classification System (BCS) represents the framework for predicting the intestinal drug absorption based on its solubility and intestinal permeability. Research has lead to the use of in vitro tests to waive additional in vivo bioequivalence studies for some pharmaceutical products (eg. biowaiver). The current regulations permit waivers for BCS Class I (highly soluble/highly permeable) drug substances, which represent up to 25 % of the drugs. Efforts in both the science and regulatory bodies are being made to extend biowaivers to certain Class II and III products, which would represent more than 50 % of all drugs coming to the market. The aim of this study was to investigate the influence of experimental conditions on metformin hydrochloride (CAS 1115-70-4) release from two immediate-release tablet formulations with proven bioequivalence and justify the biowaiver request for dissolution profile similarity in three pH media. Obtained results indicate that differences in drug dissolution observed in vitro were not reflected in vivo. Such data support the existing idea that BCS Class III drugs are eligible biowaiver candidates, even if a very rapid dissolution criterion is not fulfilled. (Homšek, I., Parojčić, J., Dačević, M., Petrović, L. and Jovanović, D., 2010)

Chapter 3
Experimental and Methodology

3.1 Experimental and Methodology

3.1.1 Specifications and Experimental Conditions:

There are three categories of dissolution test specifications for immediate release products as described by The Centre for Drug Evaluation and Research (CDER) at the United States Food and Drug Administration (US FDA. These are single point specifications, two point specifications and dissolution profile comparison. Single and two-point specifications are sufficient to characterize drug products containing high solubility-high permeability substances. However, this is not suitable for characterization of low solubility products because such products have inherent different dissolution profiles. Consequently, they may comply with the point estimates, thereby giving an erroneous impression of pharmaceutical equivalence in dissolution characteristics. Dissolution profile comparison is recommended for such products, as it is more precise and discriminative than point estimates.Comparative dissolution profile testing of drugs is carried out in at least three dissolution media in order to study their stability and release characteristics in the different physiological conditions that they may be subjected to in vivo. The recommended dissolution media 900ml distill water (Ahmed et al., 1993)

3.2 Methods for Comparison of Dissolution Profile Data:

The methods for the comparison of in vitro dissolution profiles can be classified into three groups:

i. Methods based on analysis of variance (ANOVA)

ii. Model-dependent methods.

iii. Model-independent methods.

ANOVA-based methods use univariate and multivariate approaches to quantify differences in dissolution percentages at each time point and among different products. Model-dependent methods include the cubic root law (Hixson and Crowell) mathematical model, the Weibull distribution model and the logistics (Rowlings) model for sigmoidal dissolution curves. ((Yuksel et al., 2000).

3.2.1 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 a reference product. These factors are denoted f1 (difference factor) and f2 (similarity factor) (Yuksel et al,. 2000).

Comparison of the dissolution profiles of ranitidine can be satisfactorily carried out using the model independent approaches. 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

$$f_1 = \frac{\sum_{t=1}^{n}(\mathrm{IRt}-\mathrm{TtI})}{\sum_{t=1}^{n}\mathrm{Rt}}\ X100$$

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% .(Yukselet al. 2000).

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:

$$f_2 = 50\log\{1 + (1/n)\sum_{t=1}^{n}(Rt - Tt)^{-0.5}\}X100$$

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.(Yuksel et al., 2000).

The proviso 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.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% (Ochekpe et al., 2006).

3.3 Comparative Dissolution Studies and Generic Prescribing

The in vitro dissolution test is important in characterization of drug product performance.

It is useful for quality control and in the prediction of in vivo performance of pharmaceutical

products. Comparative in vitro dissolution testing of generic drugs versus innovator products serves as a tool to determine pharmaceutical equivalence of the two products. Two products are considered pharmaceutically equivalent if they contain the same amounts of API in the same dosage forms that meet the same or comparable standards. Determination of pharmaceutical equivalence serves as a surrogate for in vivo bioequivalence tests that are expensive and not readily undertaken by generic drug manufacturers. The in vitro dissolution test is therefore a useful surrogate for assessment of bioequivalence. It plays an important role in comparison of therapeutic performances of pharmaceutical products containing the same API and has for this reason gained importance since the inception of generic equivalents of innovator drugs as a cost-cutting measure in healthcare (Yuksel et al., 2000).

Establishment of bioequivalence is essential to interchangeability of drug products. Whereas pharmaceutical equivalence does not necessarily imply bioequivalence, it is an important determinant in establishing interchangeability. Theoretically, any generic drug 15 that is bioequivalent to its innovator counterpart may be interchanged with it. It is expected that the generic formulations have an equivalent clinical effect and safety profile to the innovator formulation. In settings where bioequivalence studies are not viable, comparative dissolution testing can be used to determine which products can be used interchangeably (Ruiz et al.,2012).

3.4 Dissolution Testing Sample, Reagents and Instruments

Table 3.1: Sample of Metformin used in the experiment

Sample name	Manufacturer	Source
Comet	Square Pharmaceuticals Ltd.	Lazz Pharma
Informate	Beximco Pharmaceuticals Ltd.	United Pharmacy
Bigmet	Renata Ltd.	Foraizy Pharmacy

Table 3.2: Reagents used in the experiment

Reagent name	Source (Supplier name)
Distilled water	Laboratory (East West University)
Metformin API	Insecpta Pharmaceuticals

Table 3.3: Instruments used in the experiment

Serial no.	Equipments	Source(supplier name)	Origin
1.	UV-Spectrophotometer	Shimadzu UV-1800	Japan
2.	Dissolution tester	SMIC	China
3.	Distill water plant	SMIC	China
4.	Electronic balance	PrecisaXB120A	Switzerland

Table 3.4: Apparatus used throughout the experiments:

Serial no.	**Apparatus**
1.	Beaker
2.	Test tube
3.	Filter paper
4.	Glass rod
5.	Morter and pastle
6.	Spatula
7.	Volumetric flask(25ml,50ml,100ml,1000ml)
8.	Pipette pumper
9.	Funnel
10.	Pipette(1ml,5ml,10ml)

Table 3.5: In Vitro dissolution study:

Dissolution medium	Distilled water
RPM	50
Time	50 minutes

3.5 Preparation of Standard Curve:

To prepare standard curve, at first different concentrations (2, 4, 6, 8 and 10) µg/ml ofMetformin was prepared. For the preparation of different concentrations of Metformin.To prepare the solutions used for standard curve at first, 100 mg Metformin Hydrochloride was dissolved in 250 ml distilled water The concentration of the solution was then 400 µg/ml, this was mother solution 1(MS1).Then the solution was filtered in a volumetric flask .From the mother solution 1, 5ml was taken and made diluted into 100 ml solution, concentration was 20µg/ml(mother solution 2, MS2). Wavelength used – 232nm.

Table 3.6: Preparation of standard curve

MS2	Distilled water	Concentration	Absorbance
1ml	9ml	2µg/ml	0.142
2ml	8ml	4µg/ml	0.279
3ml	7ml	6µg/ml	0.439
4ml	6ml	8µg/ml	0.594
5ml	5ml	10µg/ml	0.734

3.6 Preparation for dissolution test:

3.6.1 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.

3.6.2 Method for dissolution test of Comet

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 ºC. Then 1 comet 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.

3.6.3 Method for dissolution test of Informate:

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 1 Informate 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.

3.6.4 Method for dissolution test of Bigmet

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 1 Bigmet tablet was placed in every vessel. After10, 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.

3.7 Instrumentation:

Dissolution Test Apparatus:

A Dissolution tester USPXXII (source RC-6B, made in China) was used for dissolution experiments. It incorporated a clear acrylic water bath, a stirrer hood with paddle shafts, an

automatic sampling unit and a control unit supported by microcontroller software with a nonvolatile memory for 15 methods. The water bath incorporated an immersion circulator with an in-built thermostat for temperature control, an external temperature sensor, a water level sensor and a lid with support for eight dissolution bowls. The stirrer hood was equipped with 8 paddle shafts fitted with USP apparatus 2 and a tablet dispenser with 8 conical shaped dissolution bowl lids. The automatic sampling unit consisted of 10in-line filters, a bi-directional 12- channel peristaltic pump with tygontubings, a microprocessor controlled sample collector and a sample tray capable of collecting 10 x 6 sets of samples. Polycarbonate dissolution vessels with a hemispherical bottom and a capacity of 1000 ml were used for the study.

3.8 Images of Instruments

Some images of important instruments those were used in different testes during research work

Figure 3.1:Distilled Water apparatus (Tresnainstrument,2006)

Figure 3.2 Dissolution apparatus (Tradeindia,2016).

Figure 3.3: UV-1800 Double Beam Spectrophotometer (Tradeindia, 2016)

Figure 3.4: Electronic Balance (Tradeindia, 2016)

Chapter 4
Result and Discussion

4.1 General Information:

The Metformin samples were subjected to assay and dissolution profile analysis under the optimum conditions. The purpose of assay was to assess the samples for compliance with pharmacopoeias limits for content.

Metformin HCL Tablets, USP, contains 500 mg, 850 mg, or 1000 mg of Metformin HCl, USP. Each tablet contains the inactive ingredients povidone, microcrystalline cellulose, croscarmellose sodium and magnesium stearate. In addition, the coating for the 500 mg, 850 mg and 1000 mg tablets contain polyethylene glycol, polyvinyl alcohol, titanium dioxide, talc, gum acacia, maltodextrin, propylene glycol and natural flavors.

4.2 Physical Parameters:

Table 4.1 Weight

Name of the drug	Weight(mg)
Bigmet	687 mg
Informate	700 mg
Comet	712 mg

4.3 Preparation of Standard Curve:

To prepare standard curve, at first different concentrations (2, 4, 6, 8 and 10) µg/ml of Metformin was prepared. For the preparation of different concentrations of Metformin. To prepare the solutions used for standard curve at first, 100 mg Metformin Hydrochloride was dissolved in 250 ml distilled water The concentration of the solution was then 400 µg/ml, this was mother solution 1(MS1). Then the solution was filtered in a volumetric flask .From the mother solution 1, 5ml was taken and made diluted into 100 ml solution, concentration was 20µg/ml (mother solution 2, MS2). Wavelength used – 232nm.

Table 4.2: Standard Curve of Metformin:

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

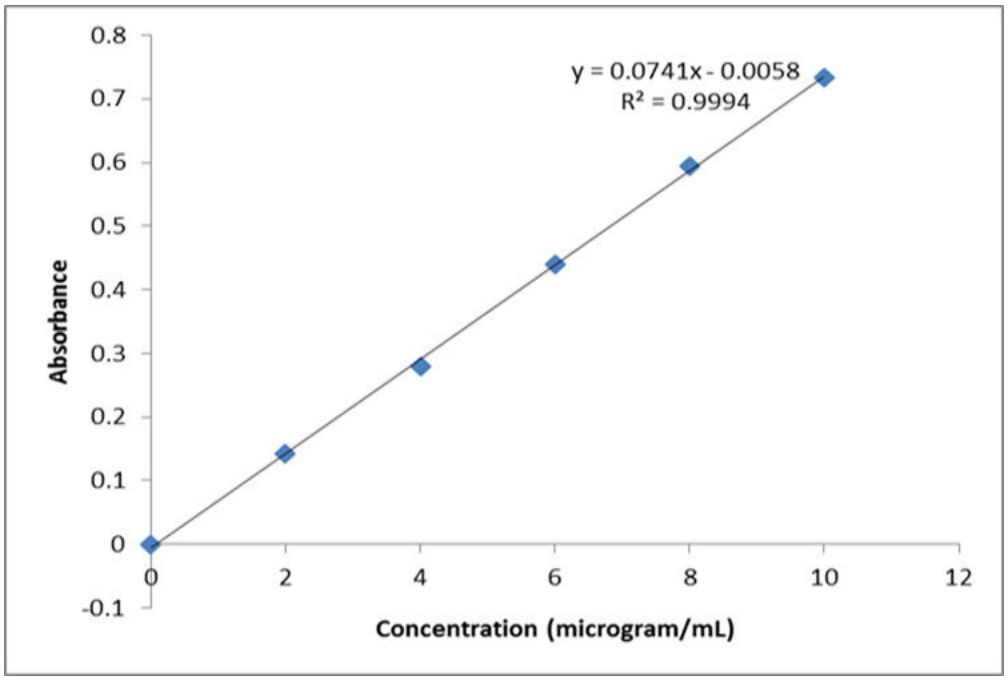

Figure 4.1: Concentration VS Drug release of Metformin

Metformin HCl API at concentration 2µg/ml gives absorbance 0.142. At concentration 4µg/ml it shows absorbance 0.279, at concentration 6µg/ml shows 0.439, at concentration 8µg/ml shows 0.594,at concentration 10µg/ml shows 0.734.

Table 4.3: Drug Release of Comet 500mg tablets:

Time (Minute)	Drug release(%)
0	0
10	21.39
20	32.89
30	44.1
40	53.09
50	65.02

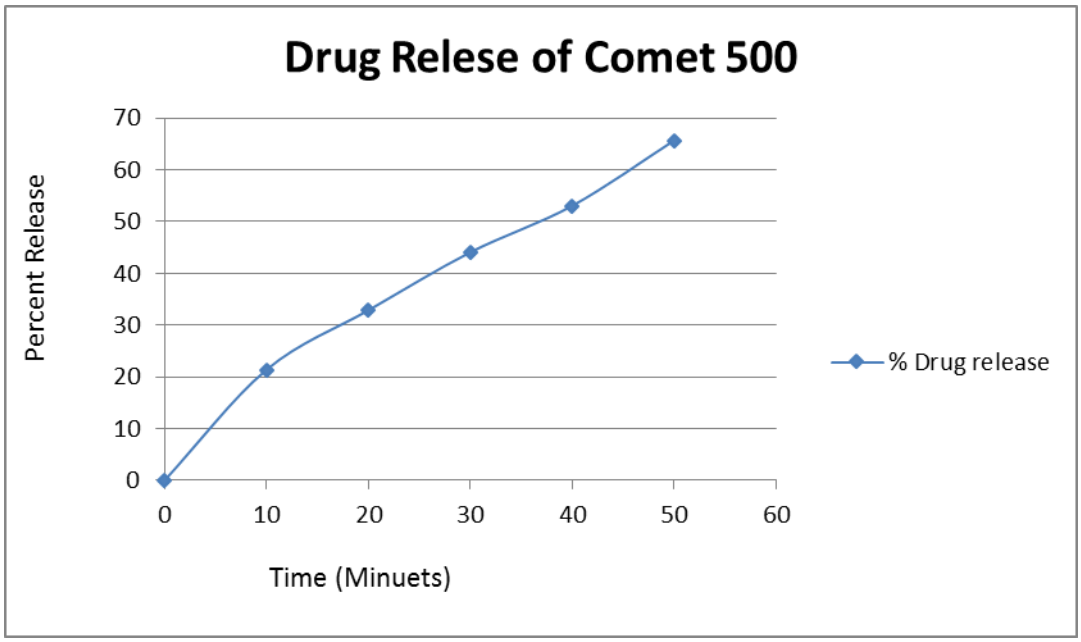

Figure 4.2: Time VS Drug release (%) of Comet 500 mg

This graph does mean the increasing of drug release in according to the counting of time. In 0.00the drug release was 0.00 and then 10.00 minutes has 21.39 then 20.00 minutes was 32.89, 30.00 minutes has 44.1, 40.00 minutes has 53.09, 50.00 has65.02. Here X axis represents the time and Y axis is for Drug release.

Table 4.4Drug release of Informate 500mg:

Time (minute)	Drug release(%)
0	0
10	32.04
20	38.88
30	47.26
40	60.78
50	72.56

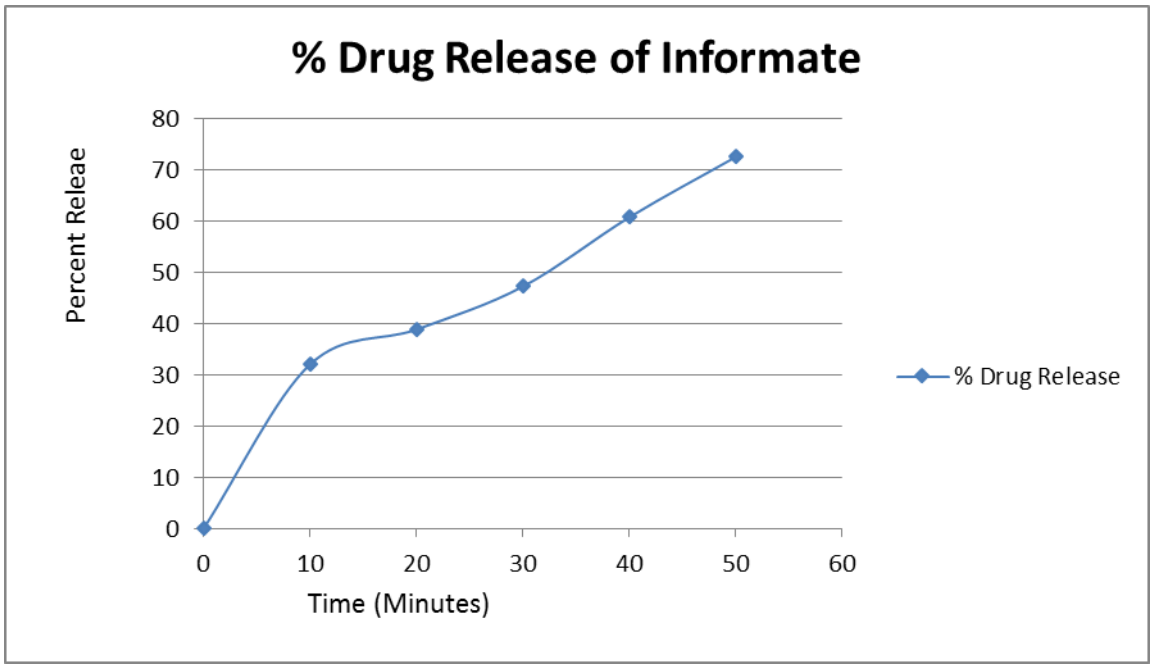

Figure 4.3: Time Vs Drug release (%) of Informate 500 mg

This graph does mean the increasing of drug release in according to the counting of time. In 0.00the drug release was 0.00 and then 10.00 minutes has 32.04 then 20.00 minutes was 38.88, 30.00 minutes has 47.26, 40.00 minutes has 60.78, 50.00 has72.56. Here X axis represents the time and Y axis is for Drug release.

Table 4.5 Drug release of Bigmet 500mg):

Time (minute)	Drug release (%)
0	0
10	15.44
20	40.98
30	50.29
40	57.42
50	71.02

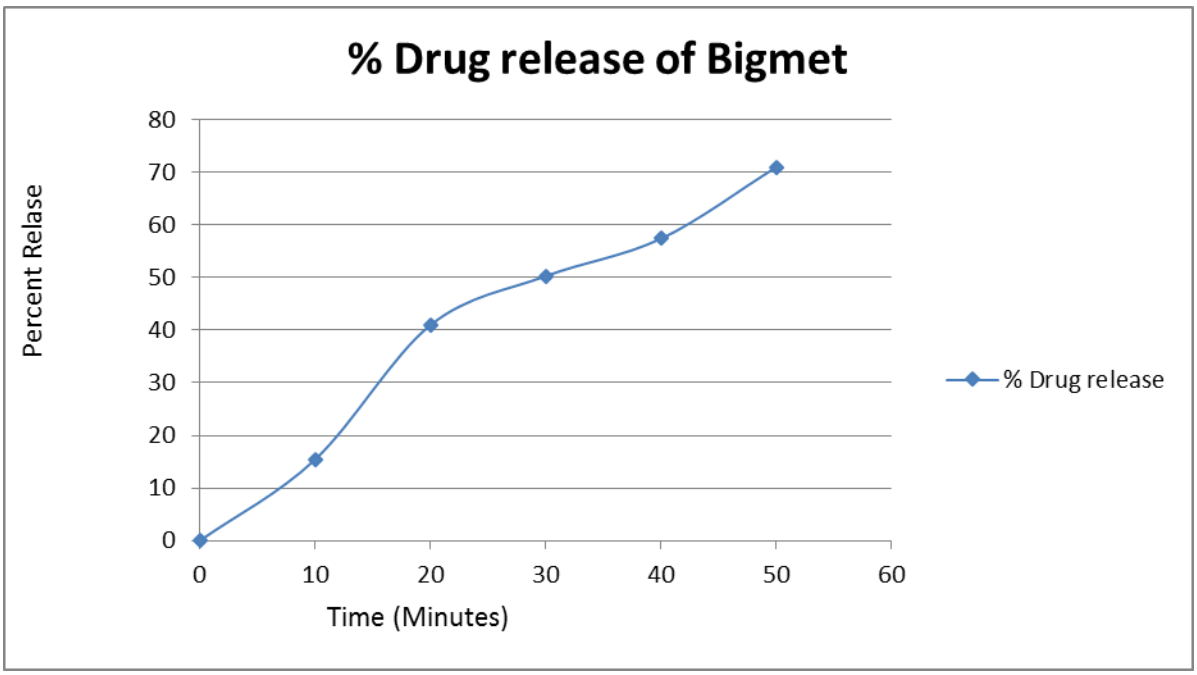

Figure 4.4: Time Vs Drug release (%) of Bigmet 500mg

This graph does mean the increasing of drug release in according to the counting of time. In 0.00the drug release was 0.00 and then 10.00 minutes has 15.44 then 20.00 minutes was 40.98, 30.00 minutes has 50.29, 40.00 minutes has 57.42, 50.00 has71.02. Here X axis represents the time and Y axis is for Drug release.

Table 4.6: Compiled result of Comet, Informate and Bigmet:

Time (Minutes)	Comet Drug (A) Release %	Informate Drug (B) Release %	Bigmet Drug (C) Release %
0	0	0	0
10	21.39	32.04	15.44
20	32.89	38.88	40.98
30	44.1	47.26	50.29
40	53.09	60.78	57.42
50	65.02	72.56	71.02

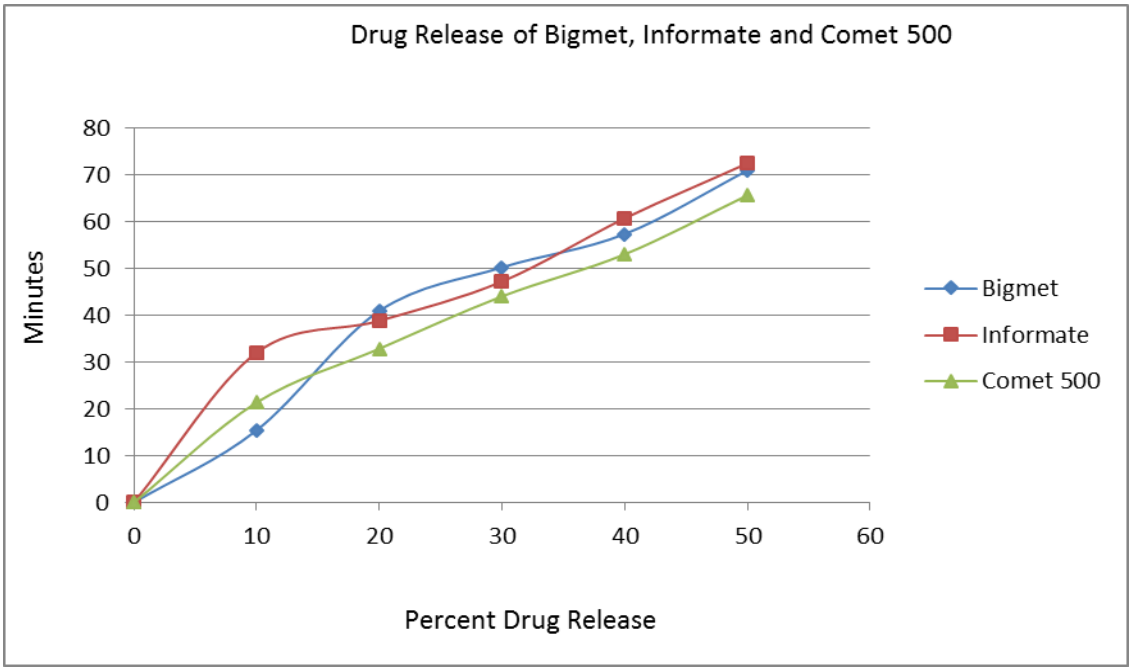

Figure 4.5:TimeVs Drug release (%) (Bigmet, Informate, Comet 500 mg)

This graph does mean the increasing of drug release in according to the counting of time. Here drug release is given in the serial of Comet, Informate, Bigmet. In 0.00 all drug release was 0.00 and then 10 minutes has21.39,32.04 and 15.44 then 20.00 minutes was 32.89,38.88 and 40.98 , 30.00 minutes has 44.1, 47.26 and 50.29, 40.00 minutes has 53.09, 60.78 and 57.42, 50.00 minutes has 65.02, 72.56 and 71.02. Here X axis represents the time and Y axis is for Drug release.

4.4 Calculation

4.4.1: *f1* (Difference factor) calculation

Among several methods investigated for dissolution profile comparison, *f2* is the simplest. Moore and Flanner proposed a model independent mathematical approach to compare the dissolution profileusingtwo factors, *f1* and *f2*. *f1* or difference factor should be between 0 to 15, more than this will not be accepted. *f1* measures the difference between two profiles at different time points.

Table 4.7:*f1* calculation for Informatewith Respect of Comet:

Time (Minutes)	Comet(R)	Informate(T)	R-T	|R-T|	*f1*
10	21.39	32.04	-10.65	10.65	
20	32.89	38.88	-5.99	5.99	
30	44.1	47.26	-3.16	3.16	6.18 %
40	53.09	60.78	-7.69	7.69	
50	65.02	72.56	7.54	7.54	
Total	216.50			35.03	

Here the both value of *f*1 within the range means it is below the 15. The brands can be accepted as well manufactured.

Table 4.8:*f1* calculation for BigmetWith Respect of Informate:

Time (Minutes)	Informate(R)	Bigmet(T)	R-T	|R-T|	*f1*
10	32.04	15.44	16.6	16.6	
20	38.88	40.98	-2.1	2.1	
30	47.26	50.29	-3.03	3.03	10.6%
40	60.78	57.42	3.36	3.36	
50	72.56	71.02	1.54	1.54	
Total	251.52			26.63	

The values of *f1* are within the range means it is below the 15. So the brand can be accepted as well manufactured.

Table 4.9:*f1* calculation for Comet with Respect of Bigmet:

Time (Minutes)	Bigmet (R)	Comet (T)	R-T	|R-T|	*f1*
10	15.44	21.39	-5.95	5.95	
20	40.98	32.89	8.09	8.09	
30	50.29	44.1	6.19	6.19	7.70%
40	57.42	53.09	4.33	4.33	
50	71.02	65.02	6	6	
Total	235.15			30.56	

The values of f1 are within the range means it is below the 15. So the brand can be accepted as well manufactured.

Here the both values of f1 are within the range means it is below the 15. So the brands can be accepted as well manufactured.

4.4.2: *f2* (Similarity factor) calculation:

It is suggested that only one measurement be considered after 85% dissolution of both products.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%

f2 measures the degree of closeness between two profiles. Since f2 tells how close the two dissolution profiles, it is widely used in pharmaceutical industry to measure the profile similarity between generic and innovator products. *f2* value should be between50-100.

Table 4.10:*f2* calculation for Informate with Respect of Comet:

Time (Minutes)	**Comet(R)**	**Informate(T)**	**R-T**	**|R-T|**	**|R-T|²**	***f2***
10	21.39	32.04	-10.65	10.65	113.42	
20	32.89	38.88	5.99	5.99	35.88	
30	44.1	47.26	-3.16	3.16	10	43.5
40	53.09	60.78	-7.69	7.69	59.14	
50	65.02	72.56	-7.54	7.54	56.85	
Total	216.50			35.03	275.29	

f2 measures the degree of closeness between two profiles. Since *f2* tells how close the two dissolution profiles, it is widely used in pharmaceutical industry to measure the profile similarity between generic and innovator products.

f2 value should be between50-100.

Table 4.11: *f2* calculation for BigmetWithRespect of Informate:

Time (Minutes)	Informate(R)	Bigmet(T)	R-T	|R-T|	|R-T|²	*f2*
10	32.04	15.44	16.6	16.6	275.56	
20	38.88	40.98	-2.1	2.1	4.41	
30	47.26	50.29	-3.03	3.03	9.18	42.1
40	60.78	57.42	3.36	3.36	11.29	
50	72.56	71.02	1.54	1.54	2.37	
Total	251.52			26.63	302.18	

Table 4.12: *f2* calculation for Comet With Respect of Bigmet:

Time (Minutes)	Bigmet (R)	Comet (T)	R-T	|R-T|	|R-T|²	*f2*
10	15.44	21.39	5.6	5.60	31.36	
20	40.98	32.89	8.09	8.09	65.5	
30	50.29	44.1	6.19	6.19	38.31	41.5
40	57.42	53.09	4.33	4.33	18.75	
50	71.02	65.02	6	6.0	36	
Total	235.15			30.21	190.0	

In this case though Comet – 41.5,Informate - 43.5and Bigmet – 41.5, have the value of below 50 that means it is not in the range of 50-100 and made the brands has some manufacturing problem ,so those can't be accepted. This problem can be due to manufacturing problems or can have instrumental problems too.

In this study, comparisons of dissolution profiles of Metformin HCL 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 f2 value of 50 - 100 for both tablets and suspensions. The study was carried out at pH 7 normal range and with the media 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 variations in the API release profiles for Metformin tablet 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.

Generally, the similarity factor patterns observed in this study indicate that assay and single point dissolution tests are not sufficient to prove efficacy or pharmaceutical equivalence of the products tested. Lack of comparative dissolution data for pharmaceutical equivalence and subsequently, 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 Biguanide drugs.

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 pharmacovigilanceprogramme with a view to ensuring 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 5
Conclusion

Conclusion:

In the study, significant differences were observed in the dissolution profiles of the Metformin products tested. While all products complied with assay specifications, generic products tested did not comply with the specifications for similarity factor f2 in relation to the innovator product. The results obtained from this study can be extrapolated to the wider Bangladesh market. The city harbours many pharmaceutical manufacturing industries and acts as a centre of distribution for imported drugs. In addition, the sub-counties in Dhaka focus the economic capacities of the Bangladesh population, which in turn affects stocking patterns for the drug products. A significant percentage of generic products in the market may not be pharmaceutically equivalent to others. As such, results of clinical studies conducted on the innovator product may not necessarily be applicable to generic products. Consequently, the generic products in the Bangladesh market may not be interchangeable with the innovator product and their efficacy may also not be comparable to that of innovator drugs.

Recommendation:

Results of assays and single-point dissolution tests should not be taken as proof of pharmaceutical equivalence, 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 consistent pharmaceutical equivalence to the innovator products. 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 well as 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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