

Evaluation and Comparison of Release Kinetics of an Anti Diabetic Formulation

A project submitted

by

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Inspiring Excellence

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Certification Statement

This is to certify that this project titled „Evaluation and Comparison of Release Kinetics of an Anti Diabetic Formulation“ submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Dr. Eva Rahman Kabir, Associate Professor, Department of Pharmacy, BRAC University and that appropriate credit is given where I have used the language, ideas or writings of another.

Signed,

Sumaiya 20.08.2015

Countersigned by the supervisor

EKall 20/08/15

Dedicated to my respectable parents and those who love me a lot

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Abstract

Diabetes, a persistent endocrine disorder causing high rate of mortality, has been treated with adequate methods for the last hundred years after the discovery of insulin by Banting and Best though its clinical features were first described by the Egyptians 3000 years ago. The effective treatment strategies started to develop when the first oral hypoglycemic drugs, tolbutamide and carbutamide, came into the market. Presently, the establishment of new methods and strategies of combination therapy is considered to be more convenient over monotherapy. The main objective of this current study was to estimate a DPP-4 inhibitor named sitagliptin either alone or in a combination formulation with metformin. The quality of both available single and combination tablets of local companies with the innovator brand were also compared.

The method followed for the determination of sitagliptin employed a RP-HPLC procedure with PDA detector consisting of Luna 5 μ C18column (250 mm x 4.60 mm) inserting an injection volume of 10 μ L and eluted by the mobile phase of 0.02 M potassium dihydrogen phosphate (pH4) and acetonitrile in a ratio of 60:40 respectively, which is pumped at a flow rate of 1.0 mL/min. The method carried out the detection at a wavelength of 252 nm for the binary mixture and of 210 nm for the single sitagliptin; and had the elution time of 3.45 and 2.28 minutes for sitagliptin and metformin respectively. All the values for system suitability parameters were also feasible with this method.

The release kinetics profile of sitagliptin was very precise and accurate and can be concluded that this method is suitable for any tablet containing sitagliptin in the routine analysis of quality control laboratory.

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List of Acronyms

AGIs= Alpha Glucosidase Inhibitors

API= Active Pharmaceutical Ingredient

DM= Diabetes Mellitus

DPP-4=Di-Peptidyl Peptidase-4

FDC= Fixed Dose Combination Therapy

GLP-1= Glucagon Like Peptide-1

GQCLP= Good Quality Control Laboratory Practice

HLA= Human Leucocyte Antigen

HPLC = High Performance Liquid Chromatography

IDDM= Insulin Dependent Diabetes Mellitus

IGT = Impaired Glucose Tolerance

K_{ATP}= Adenosine Tri Phosphate -Sensitive Potassium

Metformin HCl= Metformin Hydrochloride

NIDDM= Non-Insulin Dependent Diabetes Mellitus

NPH= Neutral Protamine Hagedorn

NSAIDs= Non-Steroidal Anti Inflammatory Drugs

NYHA= New York Heart Association

OGTT= Oral Glucose Tolerance Test

PDA= Photo Diode Array

PPAR γ = Peroxisome Proliferator Activated Receptor γ

PrevAGT= Previous Abnormality of Glucose Tolerance

PotAGT= Potential Abnormality of Glucose Tolerance

Rpm= Rotation per minute

RP-HPLC= Reverse Phase High Performance Liquid Chromatography

RSD = Relative Standard Deviation

SURs= Sulfonylurea Receptors

T1DM= Type 1 Diabetes Mellitus

T2DM= Type 2 Diabetes Mellitus

USP= United State Pharmacopeia

UV= Ultra Violet

Chapter 1

Introduction

Chapter 1

Introduction

The term diabetes refers to a group of chronic metabolic disorders resulting from multiple etiologies which is characterized by chronic hyperglycemia, glycosuria, polyuria with interruption of carbohydrate metabolism mainly that initiates from different degree of decreased insulin secretion, insulin insensitivity, or both (WHO, 2015).

A number of synchronizing system and pathways work jointly in order to maintain a healthy physiological state in human body. Homeostasis lies at the core of these processes. An anomaly of this homeostasis promotes to the initiation of an injury or a pathological state in various organs. Diabetes is such a type of metabolic disease which causes a number of major and some minor complications (Kaul et al., 2012). These complications include the risk of cardiovascular diseases (International Diabetes Federation Annual Report, 2013), infectious diseases, ophthalmic diseases (Chait and Bornfeldt, 2008; Libby, 2002; American diabetes association, 2004; Global data on visual impairments, 2012), kidney damage (Global status report on noncommunicable diseases, 2011) etc. As a result the risk of mortality among the people with diabetes is two times higher than the non-diabetic people (Roglic et al., 2005). In addition to that, people with diabetes may develop other complications which include complications of pregnancy, fatty liver disease, periodontal disease, hearing loss and depression, etc. (National Diabetes Statistics Report, 2014; American diabetes association, 2004).

1.1. Data of Increasing Number of Diabetic Patients

Diabetes has revealed as a global epidemic with the emergence of industrialization worldwide and thus the burden of diabetes has become tremendous and elevating at a hazardous rate. The standard and the deviation in methods of data collection in different parts of the world are the two major reasons which make the process difficult to reach an accurate measure of prevalence. However, surveys give us evidence that in 1985, 30 million people had diabetes, while the number elevated to 150 million in 2000 that is shown in Figure 1. It is confirmed that in 2010, 285 million adult people which was almost 6.6% of the global population were found to be diabetic and the estimated number of diabetic patients by 2030 will be 435 million that is approximately 7.8% of the adult population (Reichal and Rao, 2014; IDF Diabetes Atlas, Fourth edition, 2009).

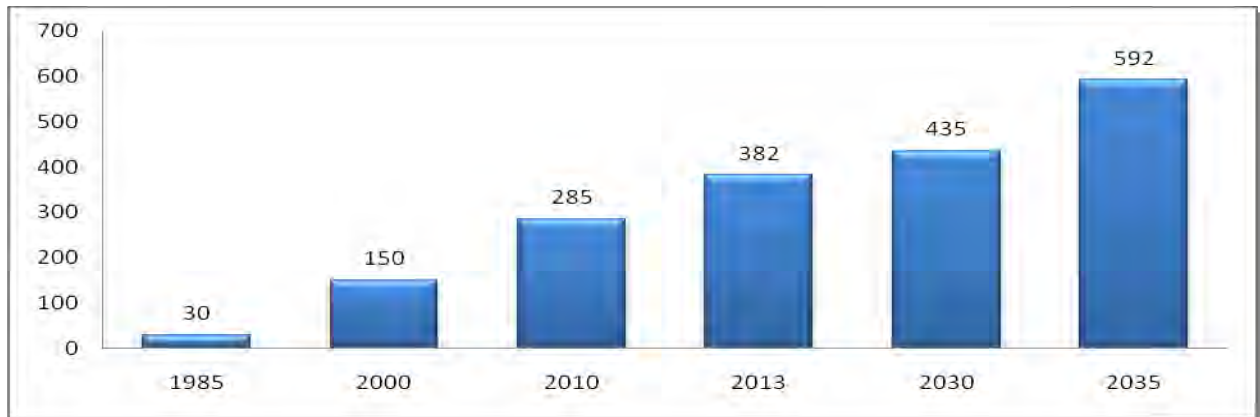


Figure 1: Number of Diabetic Patients in Million According to the Year (Reichaland Rao, 2014; IDF Diabetes Atlas, Sixth edition, 2013)

By 2025 the most affected countries will be India, China and the USA. Moreover, even today a large number of diabetic patients remain undiagnosed (WHO, 2015; IDF Diabetes Atlas, Sixth edition, 2013).

An estimated 1.5 million deaths were directly caused by diabetes in 2012 and more than 80% of these deaths occur in low and middle income countries (WHO, 2015). According to WHO diabetes will be the seventh leading cause of death in 2030 (Mathers and Loncar, 2006).

Consequently, diabetes has a major impact on economy of the country. It has costed the world economy near \$376 billion in 2010 which was 11.6% of total world healthcare expenditure (Reichal and Rao, 2014).

Table 1 shows the regional overview recorded by the WHO in 2015, which slightly contradicts the predicted number of diabetic patients given by IDF.

Table 1: Regional Overview with the Estimated Number of Diabetic Patients (WHO, 2015)

Region	Year 2000 (number of diabetic patients)	Year 2030 (estimated number)
South East Asia Region	46,903,000	119,541,000
Western Pacific Region	35,771,000	71,050,100
European Region	33,332,000	47,973,000
American Region	33,016,000	66,812,000
Eastern Mediterranean Region	15,188,000	42,600,000
African Region	7,020,000	18,234,000
World	171,000,000	366,000,000

1.2. Classification of Diabetes and Other Categories of Glucose Intolerance

A classification of diabetes and other categories of glucose intolerance are shown in Table 2:

Table 2: Classification of Diabetes and Other Categories of Glucose Intolerance (National diabetes data group, 1979; American diabetes association, 2004; National Diabetes Statistics Report, 2014)

Class	Sub class	Former Terminology	
Diabetes Mellitus (DM)	Insulin-dependent diabetes mellitus (IDDM or Type I DM)	Juvenile diabetes	
		Juvenile onset diabetes	
		Juvenile onset- type diabetes Ketosis-prone diabetes	
		Brittle diabetes	
		Adult-onset diabetes	
	Noninsulin dependent diabetes mellitus (NIDDM or Type II DM)	• Non obese NIDDM	Maturity-onset diabetes
		• Obese NIDDM	Maturity-onset-type diabetes
			Ketosis resistant diabetes
			Stable diabetes
		Diabetes mellitus associated with certain conditions and syndromes:	Secondary diabetes
Impaired Glucose Tolerance (IGT)	Non obese IGT	Asymptomatic diabetes	
		Chemical diabetes	
		Subclinical diabetes	
		Borderline diabetes	
		Latent diabetes	
	Obese IGT		
IGT associated with certain conditions and syndromes which may be-	• Pancreatic disease		
	• Hormonal		
	• Drug or chemical induced		
	• Insulin receptor abnormalities		
	• Certain genetic syndromes		

Table 2 (Continued)

Gestational Diabetes	
Diabetes insipidus	
Previous Abnormality of Glucose Tolerance (PrevAGT)	Latent diabetes Prediabetes
Potential Abnormality of Glucose Tolerance (PotAGT)	Prediabetes Potential diabetes

1.3. Diabetes Mellitus: Ancient History and Discovery of Insulin

The term diabetes mellitus has been revealed with a long history in which it was considered to be a disease of the kidneys. Beginning in antiquity, it was first recorded as abnormal polyuria along with weight loss in the Egyptian Papyrus Ebers as early as 1500 BC. In first or second century BC Demetrius of Apameia first introduced the term diabetes that was based from Ionic and Latin terms meaning that to pass through siphon. It was the Greek physician Aertaeus of Cappadocia (AD 30-90) who defined this term that was considered as the first accurate clinical description of diabetes. Diabetes was recognized as a disease of kidneys by another Greek physician Claudius Galen (AD 129-200). Later, Avicenna (AD 960-1037), an Arab physician, explained accurately the clinical features with some complications of diabetes i.e. peripheral neuropathy, gangrene and erectile dysfunction. Both Avicenna and Paracelsus (AD 1493-1541) emphasized on the idea of sweet taste of urine which was also mentioned in the Hindu medical textbooks from the fifth century. Indian texts referred it as illness of excessive urine, coupled with thirst and emaciation that affected rich people who consumed large quantities of carbohydrate. Urine was described here as *ksaudra* (sweet) or *madhu meha* (honey urine). Afterwards, another Arab physician, Abdel Latif el Baghdadi wrote the first extant treatise dedicated to diabetes in 1225. After the discovery of the circulation in 1628 by William Harvey, Thomas Willis, a physician at Guy's Hospital in London, located the origin of sweetness in blood prior to urine in 1674. Therefore, Willis's simple observation gave the disease new name as diabetes mellitus where the Latin word mellitus referring to honey or sweetness. On the basis of the presence of sugar like substances

the disease was characterized as diabetes insipidus, tasteless urine and diabetes vera, sweet urine by Frank after four years. The presence of excess sugar in blood was substantiated by both Robert Wyatt and Matthew Dobson (a Liverpool physician) more than a century later in 1774 and 1776 respectively. Dobson believed this as a system disorder. In 1809 John Rollo, a French physician established the link between the consumed food and the amount of sugar in the urine by his pioneering work. He boosted the idea of a diet i.e. low in carbohydrates and high in fat and protein as a treatment which was undoubtedly a milestone until the discovery of insulin. In 1815 a French chemist Michel Eugene Chevreul identified the sugar as glucose and then in 1857 Claude Bernard (France) showed the glycogenic properties of the liver which was the initiating activity towards the discovery of the role of the pancreas as the source of insulin. Afterwards, diabetes had been linked to pancreas by both Richard Bright and Von Recklinhausen in 1831 and 1864 respectively which was previously observed by Thomas Cawley in 1788. Cawley's initial simple observation was later confirmed by Oscar Minkowski and Joseph Mering in 1889 when they showed that pancreatectomized dogs developed diabetes. Therefore, they were the real explorers of the role of pancreas in pathogenesis of diabetes. Meanwhile, Paul Langerhans in 1869 had given the explanation of unique morphologic features of the pancreatic islands. It was a great revolution when Edward Sharpey-Schafert recommended in 1916 that the islets of Langerhans produced a glucose regulating hormone which he termed insulin. At last Frederick Banting and Charles Best of Toronto, Canada, isolated this hypothetical hormone in the summer of 1922 that was an extraordinary innovation in the history of medicine. Today the endocrine nature of diabetes is clearly established which interprets diabetes is not a disease of the kidneys rather it is a cause of kidney disease(Eknoyan and Nagy, 2005; Kirchhof et al., 2008; Ahmed, 2002).

1.3.1. Diabetes Mellitus

Diabetes mellitus, a foremost factor of morbidity and premature mortality, is the disorder of different degrees of peripheral insulin resistance and decreased insulin secretion because of damaged β cell (Kaul et al., 2012).

Some characteristic symptoms have been observed in the patients with diabetes mellitus which are discussed previously i.e. hyperglycemia, glycosuria, polyuria, polydypsia with polyphagia, fatigue, frequent episodes of thrush in penis or vagina and slow wound healing (NHS, 2014). The most severe forms are ketoacidosis or a non-ketotic hyperosmolar state which may develop and

lead to stupor, coma and, ultimately death. The effects also include long-term damage, dysfunction and failure of various organs.

The pathogenic processes that are incorporated in estimation of diabetes range from the abnormalities in insulin action and autoimmune destruction of pancreatic β cells with impaired insulin secretion. As a consequence abnormal metabolism of carbohydrate, fat, and protein occurs. Insufficient insulin action may be the consequence of inadequate insulin secretion and/or decreased tissue responses to insulin. Patients may suffer from either one or both of the consequences but it is often unrevealed that which abnormality is the primary cause of the hyperglycemia, if either alone is considered (WHO, 2015; American diabetes association, 2004). Pancreatic β cells release insulin which stimulates the other cell of body to absorb glucose during high blood glucose level. Subsequently, the glucose is converted to glycogen, and to some extent triglycerides and protein. During decreased glucose level, α cell of pancreas secretes glucagon which further stimulates the liver to release glucose by the breakdown of stored glycogen. Insufficient insulin secretion and action result dysfunction in this homeostasis process (Kaul et al., 2012).

1.3.2. Classification of Diabetes Mellitus

The vast majority of cases of diabetes mellitus have been fallen into two broad etiopathogenic categories because of the dissimilarity in the mechanisms for developing the disease (Kaul et al., 2012; National diabetes data group, 1979; American diabetes association, 2004; National Diabetes Statistics Report, 2014).

➤ Type 1 Diabetes Mellitus

T1DM, previously encompassed as insulin dependent diabetes or juvenile onset diabetes, is an autoimmune disorder which results an absolute deficiency of insulin secretion due to the impairment of the β cells by activated $CD4^+$ and $CD8^+$ T cells and macrophages attacking the pancreatic islets that accounts for only 5–10% of those with diabetes (Noble et al., 2011; Phillips et al., 2009; American diabetes association, 2004; IDF Diabetes Atlas, Sixth edition, 2013; Diabetes Update, 2015). The onset of this type of diabetes usually occurs in childhood as well as early adulthood (IDF Diabetes Atlas, Sixth edition, 2013). Since this is an autoimmune disease, patients suffering from this also may develop other types of autoimmune disorder like Addison's

disease, Graves' disease, Hashimoto's thyroiditis, vitiligo, celiac spure, myasthenia gravis, pernicious anemia and autoimmune hepatitis (American diabetes association, 2004).

Idiopathic diabetes is another type of T1DM which does not incorporated with autoimmunity. Though the etiology behind it is unknown and only a small number of patients may develop it, this may lead to permanent insulinopenia and ketoacidosis (Kaul et al., 2012; American diabetes association, 2004).

Fulminant Type 1 Diabetes Mellitus is another subtype of T1DM which has been recently discovered, causes extremely rapid and almost complete destruction of β cells (Hanafusa and Imagawa, 2007).

Both genetic and environmental factors are known to contribute to the susceptibility to this type of diabetes (Kaul et al., 2012; IDF Diabetes Atlas, Sixth edition, 2013).

○ **Genetic Factors**

Genetic studies have shown that the most effective genes contributing to T1DM susceptibility are located in the HLA (human leucocyte antigen) locus on chromosome 6 (Kaul et al., 2012; Noble et al., 2011; Knip et al., 2003). Locating on the cell surface the HLA proteins help the immune system to distinguish body's normal cells from foreign infectious and non-infectious agents. However, in T1DM, an abnormality in the HLA proteins leads to an autoimmune reaction against the β cells (Kaul et al., 2012) where HLA also linked to DQA and DQB genes, and is influenced by the DRB genes (American diabetes association, 2004).

○ **Environmental Factors**

The following environmental factors may be responsible for type 1 diabetes mellitus (Knip et al., 2003).

1. Dietary factors

Cow's milk proteins

- Casein
- Bovine serum albumin
- Beta-lacto globulin
- Bovine insulin

Gluten and other plant proteins

Fats

Nitrate and nitrite

Coffee, tea

Deficiency of zinc

Vitamin D deficiency

Frequent intake of solid foods rich in carbohydrate and protein

2. Viral infections

Mumps

Rubella

Cytomegalovirus

Epstein-Barr virus

Enterovirus

Retroviruses

Rotavirus

3. Toxins

Alloxan

Streptozotocin

N-nitroso compounds

Bafilomycin A1

4. Growth

Infant growth

Childhood growth

5. Standard of hygiene and vaccinations

6. Psychosocial factors

7. Latitude and temperature

8. Antenatal and perinatal risk factors

➤ **Type 2 Diabetes Mellitus**

This type of diabetes mellitus is also known as non-insulin dependent diabetes or adult-onset diabetes occurs due to the combination of resistance to insulin action and an inadequate compensatory insulin secretory response. Though the specific etiologies are unknown, this form of diabetes accounts for 90–95% of diabetic patients. However, it is confirmed that autoimmune

destruction of β -cells does not occur. Today it is increasingly observed in children and adolescents too. There are several important risk factors which may promote the disease i.e.

- Obesity
- Poor diet
- Dyslipidemia
- Hypertension
- Physical inactivity
- Advancing age
- Ethnicity
- High blood glucose during pregnancy affecting the unborn child
- Family history of diabetes

Strong genetic predisposition may be involved with it but the genetics of this form of diabetes are complex and not clearly defined (American diabetes association, 2004; IDF Diabetes Atlas, Sixth edition, 2013).

T2DM is divided into two subgroups such as obese and non-obese. Modification in cell receptor develops endogenous insulin resistance which is associated with distribution of abdominal fat and this type of patients fall under the first group. On the other hand, insulin resistance at the post receptor levels causes non obese T2DM (Kaul et al., 2012; National diabetes data group, 1979).

1.3.3. History of Diagnosis

Although the signs and symptoms of diabetes have been recorded since the beginnings of civilization, it was the nineteenth century when the diagnostic tests to define this disease have been started to develop. Theophilus Protospatharios (AD 630) was the first who applied heat to urine as a diagnostic test for diabetes. Thereafter, the first clinical test for glycosuria was developed in 1841 by Karl Trommer, which was a qualitative test involving the reaction of urine sample with a strong acid for acid hydrolysis of disaccharides into monosaccharides which ultimately yielded glucose. Based on this work Hermann Von Fehling developed a quantitative test to measure sugar content. A quantitative relationship between the degree of hyperglycemia and glycosuria based on Fehling's test was inaugurated by Frederick Pavy (1829-1911). Stanley Benedict, in 1907, developed a milder test for glycosuria using a copper reagent with a carbonate base.

A pioneering method was explored by Ivar Bang in 1913 to test blood glucose levels. The first “stick” or “strip” test (Clinitest) was introduced in 1941 by the Ames Company which was still based on the old methodology of Trommer’s test involving copper sulfate reduction. Afterwards, the Ames Company produced the more accurate Clinistix on the basis of enzymatic reaction of glucose oxidase. In 1979, the diagnostic criteria of diabetes have been developed by the National Diabetes Data Group and the World Health Organization which incorporated measuring glucose tolerance using an oral glucose tolerance test (OGTT). American Diabetes Association updated these guidelines in 1997, and these were then revised in 2003. In order to diagnose diabetes the new guidelines must require meeting one of three criteria(Kirchhof et al., 2008).

1.3.4. Diagnostic Criteria

Sustained elevation of blood glucose defines the diabetic state clinically. It is very common to find the glucose concentration exceeding the normal upper limit. Today the most preferred method for the diagnosis of diabetes suggests measuring the blood glucose levels at different situation which is given below:

1. Random plasma glucose is equal or higher than 200 mg/dL (11.1 mmol/L)
2. Fasting plasma glucose is equal or higher than 126 mg/dL (7.0 mmol/L)
3. Oral glucose tolerance test (measure of plasma glucose levels 2 hr after glucose is given orally is greater than 200 mg/dL (11.1 mmol/L)

According to WHO the test should be performed by administering glucose load which contains the equivalent of 75 g anhydrous glucose dissolved in water.

These criteria should be confirmed by repeat testing on different day in absence of unambiguous hyperglycemia. For routine clinical purpose the third measure is not recommended(Mehta and Wolfsdorf, 2010; American diabetes association, 2004; Report of a WHO/IDF Consultation, 2006; Kirchhof et al., 2008).

1.4 Treatment of Diabetes Mellitus

All types of diabetes should be treated under a close collaboration and effective management of diabetes requires a partnership between the diabetic patients and health professionals. The goal is to keep the blood glucose levels as near to normal as possible avoiding hypoglycemia, since there is no cure of diabetes (Kaul et al., 2012; NHS, 2014). Today the treatment strategies for

diabetes have been evaluated tremendously. However, the treatment regimens used has also significantly increased the risk of severe hypoglycemia (Mehta and Wolfsdorf, 2010)

1.4.1. Treatment of Type 1 Diabetes Mellitus

Avoiding its large fluctuation sustaining normal blood glucose level as near normal as possible is the greatest challenge in treating T1DM in order to prevent the development of microvascular and arterial complications. Insulin is the only treatments of T1DM which can be administered in injectable or inhaled forms (Press Release, 2015). Diabetes associated complications are prevented by using self-monitoring devices for blood glucose that also helps to adjust insulin dosage. It has been observed that in the early stage of β cell impairment small number of patients may able to secrete some insulin. Hence, insulin secretagogues and drugs are beneficial in this case (Kaul et al., 2012).

1.4.2. Treatment of Type 2 Diabetes Mellitus

First line defense against T2DM are change in lifestyle, diet, and weight control. Nevertheless, patients who do not respond to these, oral anti-diabetic medicines are used in the treatment which includes Sulfonylureas, Thiazolidinediones derivatives, Biguanides, Meglitinide analogue and DPP- 4 inhibitors (Kaul et al., 2012; Reichal and Rao, 2014; NHS, 2014).

1.5 Classification of Hypoglycemic Agents

1.5.1. Parenteral Hypoglycemic Agents

➤ Drug Class: Insulin

The hormone insulin is endogenously released from β cells of the pancreas which is found to be deficient in all type 1 diabetic patients as a lifelong treatment. It is also administered in type 2 diabetic patients as either adjunct therapy to oral antidiabetic agents or as monotherapy with the progress of disease. Insulin molecule has been substituted and modified extensively which leads to multiple types of insulin. Based on their pharmacodynamic and pharmacokinetic properties such as onset, peak, and duration of action these are categorized as long-acting, intermediate acting, rapid-acting, and short-acting (Ruchalski, n.d., para. 3).

Mechanism of Action

The binding of insulin to α subunits of the insulin receptor results in the activation of the enzyme tyrosine kinase in β subunit in order to assist auto phosphorylation of it. Insulin signals the Liver

to convert glucose into glycogen. Insulin also triggers the glucose to adipose and skeletal muscle cells via glucose transporter (Kaul et al., 2012).

The following therapeutic usage, adverse drug reactions, drug interactions and contraindications have been found (Ruchalski, n.d., para. 3):

Therapeutic Usage

- Type 1 diabetes mellitus
- Type 2 diabetes mellitus
- Hyperkalemia
- Diabetic coma

Adverse Drug Reactions

- Hypoglycemia (anxiety, blurred vision, palpitations, shakiness, slurred speech, sweating)
- Weight gain

Major Drug Interactions

Drugs affecting Insulin (Decreased Hypoglycemic Effect)

- Acetazolamide
- Diuretics
- Oral contraceptives
- Albuterol
- Epinephrine
- Phenothiazines
- Asparaginase
- Estrogens
- Terbutaline
- Corticosteroids
- HIV antivirals
- Thyroid Hormones
- Diltiazem

Drugs affecting Insulin (Increased Hypoglycemic Effect)

- Alcohol
- Fluoxetine
- Anabolic Steroids

- β -Blockers
- Sulfonamides
- Clonidine

Contraindications

- Severe hypoglycemia
- Allergy or Sensitivity to any ingredient of the product

Members of the Drug Class

Members of this drug class are as follows (Ruchalski, n.d., para. 3):

- Rapid acting: Insulin glulisine, Insulin lispro, Insulin aspart
- Intermediate acting: Insulin NPH
- Short acting: Insulin regular
- Long acting: Insulin glargine, Insulin detemir
- Others: 70% NPH and 30% Regular Insulin mixture, 50% NPH and 50% Regular Insulin mixture, 75% Intermediate-Acting Lispro Suspension and 25% Rapid-Acting Lispro Solution, 70% Intermediate-Acting Aspart Suspension and 30% Rapid-Acting Aspart Solution

A comparison of insulin products is shown in Table 3.

Table 3: Comparison of Insulin Products(Ruchalski, n.d., para. 3)

Product	Onset (hours)	Peak (hours)	Duration (hours)
Long-Acting			
Insulin glargine	4	N/A	12-24
Insulin detemir	4	N/A	24
Intermediate-Acting			
Insulin NPH	2-4	6-10	10-16
Short-Acting			
Insulin regular	0.5-1	2-3	3-6
Rapid-Acting			
Insulin glulisine	0.25	1-2	3-5
Insulin lispro	0.25	1	3-4
Insulin aspart	0.25	0.5-1.5	3-4

1.5.2. Oral Hypoglycemic Agents

➤ History

Some sulphonamides with antibacterial activity could not be used clinically because of their convulsive side effect which brought hypoglycemia. This was firstly detected in 1930. Later, in 1942 when a Professor of Pharmacology at Montepelier, South of France named M. J. Janbon was working for a cure for typhoid fever this issue was reactivated. By 1946, Loubatieres experimentally identified the sulphonamide group as a responsible factor for the hypoglycemic action. Frank and Fuchs in Berlin, Germany rediscovered sulphonamides after 10 years later which resulted in the development of the first two compounds (tolbutamide and carbutamide). It also initiated the discovery of tolazamide and chloropromide in the next decade(Ahmed, 2002).

➤ Drug Class: Sulfonylurea-Insulinotropic Drugs

The sulfonylureas are used in the treatment of patients with type 2 diabetes mellitus as adjuncts to diet and exercise. Sulfonylureas are commonly used in combination with other oral antidiabetic agents sometimes in the same formulation to treat those patients who do not reach glycemic goals(Ruchalski, n.d., para. 4).

Mechanism of Action

Sulfonylureas lowers blood glucose by stimulating insulin release from β cells of the pancreatic islets. Their target is the ATP-sensitive potassium (K_{ATP}) channel which inhibition by sulfonylureas results depolarization of the β -cell membrane that in turn triggers the opening of voltage-gated Ca^{2+} channels to generate Ca^{2+} influx and a rise in intracellular Ca^{2+} . As a consequence of which the exocytosis of insulin-containing secretory granules are stimulated (Proks et al., 2002).

The following therapeutic usage, adverse drug reactions, drug interactions and contraindications have been found (Ruchalski, n.d., para. 4):

Therapeutic Usage

- Type 2 diabetes mellitus

Adverse Drug Reactions

- Hypoglycemia
- Gastro Intestinal distress
- Dizziness

Major Drug Interactions

Drug Affecting Sulfonylureas

- Enhanced hypoglycemic effects
 - Anticoagulants
 - Azole antifungals
 - Gemfibrozil
- Decreased hypoglycemic effects
 - β-Blockers

Sulfonylurea Affecting Other Drugs

- Digoxin: Increased levels

Contraindications

- Diabetes complicated by ketoacidosis, with or without coma
- Diabetes complicated by pregnancy
- Type 1 diabetes mellitus

Members of the Drug Class

Members of this drug class are given below (Ruchalski, n.d., para. 4):

- First generation: Acetohexamide, Tolbutamide, Chlorpropamide, Tolazamide
- Second generation: Glibenclamide, Gliclazide, Glimepiride, Glipizide, Glyburide

➤ **Drug Class: Meglitinide Analogue-Insulinotropic Drugs**

The meglitinide analogues are the class of oral antidiabetic agents which stimulate insulin secretion from the pancreatic β cell. Their properties assume that they have the ability to produce a rapid, short-lived insulin output. Meglitinides may be used as monotherapy as an adjunct to diet and exercise or in combination with other oral antidiabetic agents and/or insulin to reach glycemic goals in patients who do not receive it on those therapies (Black et al., 2009).

Mechanism of Action

Meglitinides bind competitively to sulfonylurea receptors (SURs) to inhibit K_{ATP} channels and to stimulate insulin secretion. The insulinotropic action of meglitinide is also mediated via adenosine triphosphate (ATP) dependent potassium channels like the sulfonylureas. It stimulates insulin secretion by blocking ATP- dependent potassium channels (K_{ATP}) of the pancreatic β cell which results in membrane depolarization and calcium influx through voltage-gated calcium

channels. Finally, these activities lead to an increase in intracellular calcium and following exocytosis of insulin-containing granules (Mendoza et al., 2013).

Therapeutic Usage

- Type 2 diabetes mellitus

Adverse Drug Reactions

Adverse drug reactions which have been found most commonly are given below (Black et al., 2009):

- Weight gain
- Diarrhea
- Hypoglycemia

Major Drug Interactions

Major drugs which interact with meglitinides are as follows (DeRuiter, 2003):

- Increased Action
 - NSAIDs
 - Azole antifungal (ketoconazole, miconazole)
 - Antibiotics including erythromycin
 - Highly protein bound drugs- clyates, sulfonamides, chloramphenicol, coumarins, probenecid
- Decreased Action
 - Troglitazone
 - Rifampin
 - Barbiturates
 - Carbamazepine

Contraindications

The most common contraindications are as follows (Wang et al., 2015; Culy and Jarvis, 2001; Tornio et al., 2014):

- Clopidogrel (a medicine used to prevent blood clot)
- Type 1 diabetes mellitus
- Diabetic keto acidosis

Members of the Drug Class

Members of this drug class are given below(Black et al., 2009):

- Repaglinide
- Nateglinide
- **Drug Class: Di-Peptidyl Peptidase-4 (DPP-4) Inhibitor-Insulinotropic Drugs**

Di-Peptidyl Peptidase-4 Inhibitor is the newer member in the family of oral anti diabetic drugs which also stimulates insulin secretion from the pancreatic β cell.

Mechanism of Action

Di-Peptidyl Peptidase-4 (DPP-4) Inhibitor inhibits the breakdown of active Glucagon-like peptide-1 (GLP-1) to inactive GLP-1 by inhibiting the enzyme DPP-4. In response to food intake active GLP-1 is released from α cell of the pancreas. GLP-1 maintains blood glucose by enhancing the secretion of insulin from the pancreas in a glucose-dependent manner. This class of drugs is now being used as monotherapy as an adjunct to diet and exercise or in combination with other oral antidiabetic agents and/or insulin to reach glycemic goals in patients who do not receive it in the present therapy (Ruchalski, n.d., para. 2).

The following therapeutic usage, adverse drug reactions and drug interactions have been found (Ruchalski, n.d., para. 2):

Therapeutic Usage

- Type 2 diabetes mellitus

Adverse Drug Reactions

- Nasopharyngitis
- Nausea
- Vomiting
- Diarrhea
- Hypoglycemia
- Weight loss

Major Drug Interactions

DPP-4 Inhibitors Affecting Other Drugs

- Digoxin: Increased levels

Members of the Drug Class

Members of this drug class are as follows (Ruchalski, n.d., para. 2):

- Sitagliptin (first member in this class)
- Saxagliptin

➤ **Drug Class: Biguanides- Insulin Sensitizing Agents**

The biguanides are the drugs of first choice for a newly diagnosed patient with type 2 diabetes which are used as an adjunct to diet and exercise. They are frequently used in the combination with other oral antidiabetic agents and/or insulin in patients who do not reach glycemic goals on the current therapies.

Mechanism of Action

Biguanides lower both basal and postprandial plasma glucose. Hepatic glucose production and intestinal absorption of glucose production are decreased by them. In fact peripheral glucose uptake and utilization are also improved(Ruchalski, n.d., para. 1).

The following therapeutic usage, adverse drug reactions, drug interactions and contraindications have been found (Ruchalski, n.d., para. 1):

Therapeutic Usage

- Type 2 diabetes mellitus
- Antipsychotic-induced weight gain

Adverse Drug Reactions

- Diarrhea
- Vomiting
- Dyspepsia
- Flatulence
- Metallic taste
- Weight loss

Major Drug Interactions

Drugs Affecting Metformin

- Alcohol
- Iodinated contrast media

Contraindications

- Renal disease
- Heart failure requiring pharmacologic therapy
- Acute or chronic metabolic acidosis
- Active liver disease

Members of the Drug Class

Members of this drug class are given below (Ruchalski, n.d., para. 1):

- Metformin
- Fenformin
- Buformin
- **Drug Class: Thiazolidinediones- Insulin Sensitizing Agents**

Like biguanides thiazolidinediones also decrease insulin resistance by enhancing insulin-receptor sensitivity and are used as adjuncts to diet and exercise in patients with type2 diabetes mellitus. They are frequently used in combination with other oral antidiabetic agents and/or insulin in patients who do not reach glycemic goals on the current therapies.

Mechanism of Action

Insulin sensitivity is increased because of their agonistic activity to peroxisome proliferator-activated receptor γ (PPAR γ) in turn of which insulin resistance is decreased in adipose tissue, skeletal muscle, and the liver(Ruchalski, n.d., para. 5).

The following therapeutic usage, adverse drug reactions, drug interactions and contraindications have been found (Ruchalski, n.d., para. 5):

Therapeutic Usage

- Type 2 diabetes mellitus.

Adverse Drug Reactions

- Weight gain
- Edema
- Hypoglycemia (when used with insulin or other oral antidiabetic drugs)
- Increased risk of myocardial infraction

Major Drug Interactions

Drug Affecting Thiazolidinediones

- Gemfibrozil: Increased levels
- Rifampin: Decreased levels

Thiazolidinedione Affecting Other Drugs

- Oral contraceptives: Decreased efficacy

Contraindications

- Patients with NYHA (New York Heart Association) class III and IV heart failure

- Concurrent insulin or nitrate use with rosiglitazone
- Patients with hepatic dysfunction

Members of the Drug Class

Members of this drug class are as follows (Ruchalski, n.d., para. 5):

- Pioglitazone
- Rosiglitazone
- **Drug Class: α -Glucosidase Inhibitors- Insulin Sensitizing Agents**

Alpha glucosidase inhibitors (AGIs), enzyme inhibitors, are the unique class of anti-diabetic drugs which delay carbohydrate absorption in the gastrointestinal tract. By this way, they control postprandial hyperglycaemia.

Mechanism of Action

In order to digestion the carbohydrates need to be broken down to monosaccharides most of which are present as oligo or poly saccharides. Alpha amylase breaks down the starch whereas AGIs inhibit both alpha amylase and the other alpha-glucosidases preventing the absorption of starch and other carbohydrates from the brush border of the intestine.

Therapeutic Usage

- Type 2 diabetes mellitus

Adverse Drug Reactions

Undigested disaccharides which remain in the intestinal lumen may cause flatulence, diarrhea and abdominal pain (Kalra, 2014).

Major drugs which may interact commonly with AGIs and the contraindications of AGIs are mentioned below (Bayer HealthCare Pharmaceuticals Inc, 2011):

Major Drug Interactions

- Thiazides and other diuretics
- Corticosteroids
- Phenothiazines
- Thyroid products
- Estrogens
- Oral contraceptives
- Phenytoin
- Nicotinic acid

- Sympathomimetics
- Calcium channel-blockers
- Isoniazid
- Sulfonylureas
- Insulin
- Digoxin

Contraindications

- Inflammatory bowel disease
- Disorder of digestion
- Colonic ulceration
- Partial intestinal obstruction
- Cirrhosis of the liver

Members of the Drug Class

Members of this drug class are as follows (Kalra, 2014):

- Acarbose
- Voglibose
- Miglitol

1.6. Management of Diabetes Mellitus and Fixed Dose Combination (FDC) Therapy

Depending upon the condition of the patient, some factors need to be taken under consideration for the treatment of diabetes mellitus such as degree of hyperglycemia and properties of anti-hyperglycemic drugs.

Management of diabetes mellitus can be classified into mainly two categories i.e.

- a) Mono-therapy medicines
- b) Combination medicines

1.6.1. Need for Combination Therapy

Fixed dose combination therapy (FDC) is referred to as a combination of two or more active ingredients in a fixed ratio of doses. According to the suggestion of International Diabetic Federation and American Diabetic Federation if the monotherapy fails along with lifestyle modification the patient should follow by combination therapy. It has been proved that

combination therapy helps to achieve and maintain the desired therapeutic targets (Reichal and Rao, 2014).

The advantages of Fixed Dose Combination are as follows (Reichal and Rao, 2014)-

- Ease of administration
- Convenience
- Synergistic effect
- Complementary mechanism of action
- Low dose with fewer side effects
- Economical
- Reduce the pill burden
- Improve adherence to treatment
- Improve tight glycemic control
- Decrease adverse drug reactions
- Delay the need for insulin therapy

Various works have been done previously with the combination of diabetic drugs in bilayer tablets which are shown in the Table 4.

Currently Fixed Dose Combination (FDC) of diabetic drugs is fabricated for various reasons which are discussed in the Table 5.

Table 4: Combination of Diabetic Drugs in Bilayer Tablets (Reichal and Rao, 2014)

Drugs	Rationale
1. Glibenclamide +Metformin HCl	Frequency of administration is reduced Patient compliance is improved
2. Pioglitazone+ Metformin HCl	Patient compliance is improved
3. Metformin HCl +Gliclazide	Prolong the release up to 12hrs Patient compliance is improved
4. Glimepiride + Metformin HCl	Improve oral therapeutic efficacy with optimal control of plasma drug level
5. Pioglitazone + Gliclazide	Provide synergistic action
6. Glipizide +Metformin HCl	Provide synergistic action

Table 5: List of Current Fixed Dose Combinations (FDCS)(Reichal and Rao, 2014)

Combination of Drugs	Mechanism of Action	Rationale
Metformin+ Sulfonyl Urea	Metformin suppresses hepatic gluconeogenesis to reduce fasting glycemia and the peripheral glucose uptake. Sulfonyl ureas increase insulin release from β cells residual function is present	Better than monotherapy Synergistic effect is obtained
Metformin+ Pioglitazone	Pioglitazone increases insulin sensitivity in adipose tissue and inhibit β cell loss	Synergistic effect is obtained
Metformin+ DPP-4 inhibitors	Inhibits the breakdown of GLP-1 by DPP-4 therefore increases GLP-1 levels, resulting in increased glucose-dependent insulin release and decreased level of circulating glucagon and hepatic glucose production	Safety and tolerability (mainly used for early combination therapy)
Metformin+ α Glucosidase Inhibitors	Metformin acts on gluconeogenesis and Acarbose, voglibose reduce intestinal glucose absorption to control post prandial glycemia	Synergistic effect is obtained

Chapter 2

Methodology

Chapter 2

Methodology

The research methodology of this project has been followed based on an extensive literature review of the release kinetics and quality assurance of sitagliptin and metformin.

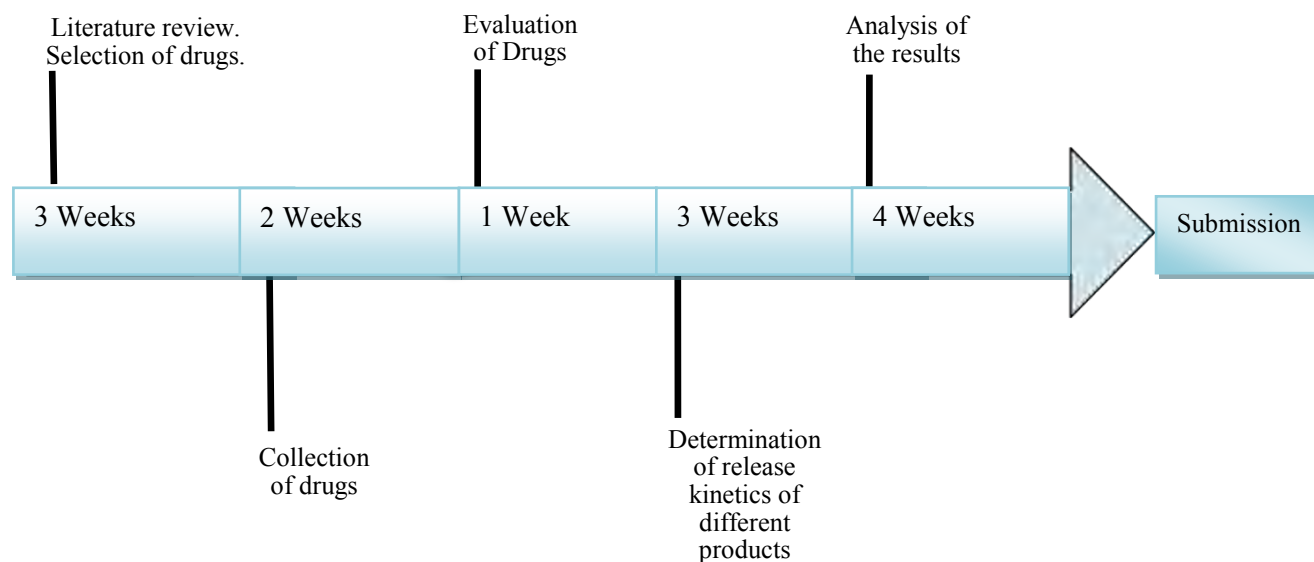
A method should be developed with a goal to rapidly test preclinical samples, formulation prototypes, and commercial samples (Breux et al., 2003). The Good Quality Control Laboratory Practice (GQCLP) requires test methods to assess the compliance of pharmaceutical product with established specification and to meet proper standard of accuracy and reliability. The validated method will give consistent and reliable results which are mainly concerned with source of errors and their estimation in the experiment. If the estimated errors are within the acceptable limit, then the method is said to be validated and qualified for its intended use.

For good quality control laboratory practice, numerous methods need to be developed to ascertain the identity, claimed potency, strength, quality and purity of different drug substance and drug product. These physicochemical properties of any drug substance or others are checked through different test methods such as assay test or content uniformity test, dissolution test, and disintegration test etc. These test methods vary from one API to another. Therefore, before manufacturing or launching any new product to the market, different test methods specific to the product need to be fixed initially so that the physicochemical properties of that drug product could be checked whenever needed to ensure the product safety and efficacy throughout its shelf life including storage, distribution and use (Patil et al., 2001).

For this purpose, pure sample of sitagliptin and metformin, available market tablets of sitagliptin and the combination formulation of sitagliptin and metformin were collected in the initial phase of the study. A system of documentation relating to the study was prepared and maintained from the very beginning of the study. The name of marketed products and chemicals used as reagents along with the apparatus used for the studies are given in the appendix 1.

Several generic products containing sitagliptin alone and in combination with metformin have been registered in the world as well as in Bangladesh by several pharmaceutical companies and are available in the market. From a quality control point of view, to perform a comparative analytical evaluation between trademark and generic formulations containing sitagliptin several physicochemical parameters was performed to assure the quality of the generics.

Following is a timeline showing the details of the study:



2.1. Literature Review

The study commenced with an extensive review of literature. The papers related to the present study were selected and information was reviewed. Several HPLC methods have been reviewed for the determination of sitagliptin when used alone and in combination with metformin (Ramzia et al., 2011; Nashwahgadallah, 2014; Rezk et al., 2013; Lathareddy and Rao, 2013; Vani et al., 2014; Karimulla et al., 2013; Juvvigunta et al., 2013).

Similarly, a survey of the analytical literature for HPLC, UV spectrophotometric determination of sitagliptin when used alone and in combination with metformin (Bhende et al., 2012; Raja and Rao, 2012; Jeyabalan and Nyola, 2012; Loni et al., 2012; Sankar et al., 2013), in pharmaceutical preparations has also been described.

2.2. Selection of drugs

Based on extensive literature review sitagliptin and metformin (appendix 2) were selected for the study.

2.3. Sample Collection

Five commercially available samples from different companies were collected among which Glipita[®] 50 a gift sample was received from Beximco Pharmaceuticals Ltd., Bangladesh, while others were purchased. Each film coated tablet contains 64.25 mg of sitagliptin Phosphate

Monohydrate that is equivalent to 50 mg of sitagliptin. The reference product for the present study was Januvia[®], the innovator brand manufactured by Merck Sharp & Dohme (MSD), Italy. Similarly for combination preparation, five commercially available samples from different brand containing 64.25 mg of sitagliptin Phosphate Monohydrate that is equivalent to 50 mg of sitagliptin and 500 mg of metformin Hydrochloride in each film coated tablet were collected. Glipita[®]-M 50/500, a gift sample was received from Beximco Pharmaceuticals Ltd., Bangladesh, while others were purchased. For the present study, Janumet[®] was purchased that was the innovator brand, Merck Sharp & Dohme (MSD), The Netherlands to use as reference product. Pure sample of powdered sitagliptin and metformin was received from Beximco Pharmaceuticals Ltd., Bangladesh as a gift having a potency of 96.4% and 99.5% respectively. All the products tested were stored within specified conditions and were within their expiry date.

2.4. Tablet Evaluation

The quantitative evaluation of a tablet's physicochemical parameters is essential in the design of the dosage form and to monitor its quality. Since chemical breakdown or interactions between the tablet components may occur during its manufacturing, storage and use which ultimately alter its physical properties as well as bioavailability, evaluation is highly important. Regarding the quality of tablets there are several standards which have been set in the various pharmacopoeias.

The following physicochemical parameters were checked:

1. Average weight-weight variation
2. Friability
3. Hardness
4. Disintegration

2.4.1. Average Weight-Weight Variation

Factors which affect tablet weight are mainly tooling of the compression machine, head pressure, machine speed and flow properties of the powder. Common sources of weight variation are inconsistent powder or granulate density and particle size distribution during compression. Uniformity of weight is important to ensure the consistency of dosage units during compression.



Figure 2:Electronic Balance

Procedure

- Twenty tablets were taken and weighed individually using the electronic balance shown in Figure 2.
- The average weight was then calculated and it was compared to the individual tablet weight.

The tablet pass the USP test if no more than 2 tablets are outside the percentage limit and if no tablet differs by more than 2 times the percentage limit. The USP standard of percent difference according to average weight is shown in Table 6.

Table 6: Pharmacopeial Standard (USP29-NF24)

Average weight	Percent difference
USP	
130mg or less	±10%
More than 130mg through 324mg	±7.5%
More than 324mg	±5%

2.4.2. Friability

It is another measure of a tablet's strength. Tablets may tend to powder and fragment during handling which cause lack of elegance and consumer acceptance. Friability also interferes in uniformity of weight as well as content uniformity.

Friability test subjects a number of tablets to the combined effects of abrasion and shock by utilizing a plastic chamber that revolves at 25 rpm, dropping the tablets with each revolution.



Figure 3: Friability Tester

Procedure

- Ten pre-weighed tablets were placed in the friability tester which is shown in Figure 3. This was operated for 100 revolutions at 25 rpm.
- The tablets were reweighed.

The equation by which the amount of friability can be calculated is mentioned below(Nasrin, 2011):

$$\% \text{ friability} = \frac{W_0 - W_f}{W_0} \times 100\%$$

W_0 = initial weight

W_f = final weight

2.4.3. Hardness

Tablets require a certain amount of strength or hardness to withstand mechanical shocks of handling in manufacture, packaging and shipping as well as to withstand reasonable abuse when

in the hands of the consumer. The hardness of tablets is related to both disintegration and dissolution or, in other words, bioavailability.

Hardness of tablet is the force required to break a tablet along its diameter by applying compression loading.

Hardness variation depends on:

- Compression force
- Concentration and type of binding agent

If it is too soft, it may not withstand the necessary multiple shocks occurring during handling, shipping, and dispensing. If the tablet initially is too hard, it may not disintegrate in the requisite period of time.



Figure 4: Hardness Tester

Procedure

- A tablet was placed between two anvils, force was applied to the anvils, and the crushing strength that just caused the tablet to break was recorded (in kg) using the hardness tester shown in Figure 4.
- In this way, hardness of 10 tablets was recorded.

2.4.4. Disintegration Test

This test determines whether tablets disintegrate within the prescribed time when placed in a liquid medium under the experimental conditions or not.

The USP device to test disintegration uses 6 glass tubes that are 3 inch long; open at the top and 10 mesh screens at the bottom end. The disintegration tester which was used is shown in Figure 5.



Figure 5: Disintegration Tester

Procedure

- One tablet was placed in each tube and the basket rack was positioned in a 1L beaker of water which contained distilled water at $37 \pm 0.5^\circ\text{C}$.
- The basket rack was kept such a way that during upward movement the tablet remained 2.5 cm below the surface of liquid and during downward movement not closer than 2.5 cm from the bottom of the beaker.
- At a frequency of 28 cycles per minute the basket containing the tablets was moved up and down through a distance of 5 cm.
- A perforated plastic disc was placed on each tablet in order to prevent floating of the tablets.

The time when the tablets disintegrated and all particles passed through the 10 mesh screen was recorded.

- Disintegration time for uncoated tablet: 5-30 minutes
- Disintegration time for coated tablet: 1-2 hours

2.5. Determination of Release Kinetics

In vitro dissolution measures the portion (%) of the API that has been released from the dosage form and has dissolved in the dissolution medium during controlled testing conditions within a defined period. It gives the prediction on bioavailability and thus it is one of the most important parameters for pharmaceutical dosage form.

Standard dissolution method for sitagliptin alone and in combination with metformin is shown in Table 7 according to U.S. Food and Drug Administration.

Table 7: Standard Dissolution Method for Sitagliptin Alone and in Combination with Metformin (FDA-Recommended Dissolution Methods, 2015.)

Drug Name	Dosage Form	USP Apparatus	Speed (RPMs)	Medium	Volume (mL)
Sitagliptin Phosphate	Tablet	I (Basket)	100	Water	900
Metformin	Tablet	II (Paddle)	75	0.025 M NaCl	900
HCl+Sitagliptin Phosphate					

USP Dissolution Apparatus

There are two types of apparatus for dissolution. The assembly of dissolution apparatus consists of the following:

A vessel made of glass or other inert, transparent material that does not interfere with the dosage form, a motor, a drive shaft, and a cylindrical basket in case of USP apparatus I and instead of basket there is paddle in USP apparatus II. The paddle is formed from a blade and a shaft which is used as the stirring element. Temperature inside the vessel is maintained at 37 ± 0.5 °C during the test by immersing the vessel partially in a suitable water-bath of any convenient size or heated by a suitable device such as a heating jacket. It keeps the dissolution medium in a constant and smooth motion. No other part of the assembly does not cause significant motion, agitation or vibration beyond which because of the smoothly rotating stirring element. The vessel having a capacity of 1 liter is cylindrical with a hemispherical bottom. At the top its sides are flanged. A fitted cover can be used to retard evaporation which spares adequate openings to allow ready insertion of the thermometer and withdrawal of samples. The shaft is positioned in

order to fix its axis that is not more than 2 mm at any point from the vertical axis of the vessel. This ensures the rotation smooth without significant wobble that could affect the results.



Figure 6: Dissolution Tester

Preparation of Sodium Chloride Solution: 8.775 g of sodium chloride was weighed and transferred into a media bucket with 6 liter of distilled water.

Procedure

During the dissolution test of sitagliptin in combination with metformin the stated volume of the dissolution medium ($\pm 1\%$) was placed in the vessel of the specified apparatus that is shown in Figure 6. The apparatus was then assembled and the dissolution medium was equilibrated to 37 ± 0.5 °C. Six tablets were taken and one tablet was placed in each of the six vessels. Subsequently, the apparatus was operated at the specified rate. After 30 minutes from the starting time 10 mL of sample was collected from a point midway between the stirrer and the glass vessel.

Since sitagliptin required USP apparatus I which is of basket type, tablets were placed here into the baskets instead of vessels. The other procedures were as same as the process of the dissolution of sitagliptin in combination with metformin.

2.5.1. Choice of Method

For the estimation of sitagliptin and metformin, several methods were reviewed from different papers and the preferable methodology among them was eventually adopted and modified after undertaking several trial and error steps.

- I. According to (Bhende et al., 2012) the elution of sitagliptin and metformin was carried out utilizing XTerraC8 column (4.6x100 mm, 3 μ m) with a mobile phase combination of phosphate buffer solution (pH 9), acetonitrile and methanol in the ratio 35:45:20 respectively at flow rate of 0.6mL/min. The detection was carried out at a wavelength of 260 nm.
- II. In another literature the estimation of sitagliptin and metformin was carried out on an XTerraC8 column (4.6x100 mm, 5 μ m) using a mobile phase of phosphate buffer solution (pH 8) in combination with acetonitrile and methanol in the ratio 45:35:20 respectively which was pumped at a flow rate of 1.0 mL/min with the injection volume of 20 μ L at ambient temperature. The detection was carried out at a wavelength of 254 nm in accordance with (Raja and Rao, 2012).
- III. According to (Jeyabalan and Nyola, 2012) Phenomenex C18 column(4.6x250 mm, 5 μ m) in isocratic mode was used to estimate sitagliptin and metformin by a mobile phase of 0.02 M potassium dihydrogen phosphate (pH 4.3) with acetonitrile in a ratio 55:45 respectively. The flow rate was 1.0 mL/min with the injection volume of 20 μ L and the final analytical determination was done using UV detector at wavelength of 252 nm.
- IV. A literature from (Loni et al., 2012) Hi-Q Sil C18 column (4.6x250 mm, 5 μ m) was used with a mobile phase containing phosphate buffer solution (pH 4), acetonitrile and methanol in a ratio 50:20:30 respectively and a flow rate of 1.2 mL/min with 20 μ L injection volume. The analytes were detected through UV detector at wavelength of 258 nm.
- V. According to (Sankar et al., 2013) the determination of sitagliptin and metformin was carried out on a Phenomenex Luna C18 column (4.6x250 mm, 5 μ m) in isocratic mode. An injection volume of 20 μ L was injected and eluted with mobile phase 0.02 M potassium dihydrogen phosphate (pH 4) and acetonitrile in a ratio 60:40 respectively pumped at a flow rate of 1.0 mL/min. The detection was carried out at a wavelength of 252 nm.

VI. Finally, one method was done as a trial and error method using Luna 5 μ C18column (250 mm x 4.60 mm) in isocratic mode through a mobile phase consisting of dipotassium hydrogen phosphate (pH 7.2) and acetonitrile in a ratio 70:30 respectively. An injection volume of 10 μ L was injected with a flow rate of 1.0 mL/min. The analytes were detected through UV detector at wavelength of 210 nm.

This method was adopted from a company (primary source).

All the tests mentioned above were carried out and method according to Sankar et al., 2013 was found to give the most optimum response with least interference.

2.5.2. Choice of Mobile Phase

At the initial point of the study, for the selection of mobile phase, the various compositions of mobile phase verification were carried out for the gradient elution of sitagliptin and metformin are mentioned as follows:

Mobile Phase 1	: pH 4 0.02 M Potassium dihydrogen phosphate: acetonitrile (60:40)
Mobile Phase 2	: pH 4 Phosphate buffer solution: acetonitrile: methanol (50:20:30)
Mobile Phase 3	: pH 4.3 0.02 M Potassium dihydrogen phosphate: acetonitrile (55:45)
Mobile Phase 4	: pH 7.2 Dipotassium hydrogen phosphate: acetonitrile (70:30)
Mobile Phase 5	: pH 8 Phosphate buffer solution: acetonitrile: methanol (45:35:20)
Mobile Phase 6	: pH 9 Phosphate buffer solution: acetonitrile: methanol (35:45:20)

Initially mobile phase containing dipotassium hydrogen phosphate (pH 7.2) and acetonitrile (70:30) had been selected but it had a run time of 20 minutes which was undoubtedly very much time consuming as well as expensive. Similarly we had observed the trial at higher pH using Mobile Phase 5 and Mobile Phase 6. After that we came to a very interesting conclusion that was pH had a major effect on the elution of these analytes specially sitagliptin. This was one of the reasons why these particular mobile phases with high pH system were discarded.

On the other hand among the mobile phase of lower pH, Mobile Phase 1 was chosen because of its least interference on the system and sharp as well as completely resolved peak.

The chromatographic condition is given in Table 8.

Table 8: Chromatographic Condition

Chromatographic Mode	Chromatographic condition
Mobile Phase	0.02 M Potassium dihydrogen phosphate pH4: acetonitrile (60:40% v/v)
Stationary phase	Luna 5 μ C18column (250 mm x 4.60 mm)
Flow Rate	1.0 mL/min
Injection Volume	10 μ L
Diluent	Water
Temperature	25°C
Elution	Isocratic
Detection for sitagliptin alone	UV, absorbance at 252 nm
Detection for sitagliptin in combination with metformin	UV, absorbance at 210 nm
Run Time	4 minutes
Retention time	Metformin: Approximately 2.4 minutes Sitagliptin: Approximately 3.4 minutes



Figure 7: HPLC Machine



Figure 8: pH Meter

2.5.3. Instrumentation (Mubtasim et al., 2015)

The HPLC system was composed of following parts (Figure 7):

- A high pressure binary gradient pump which is Shimadzu LC-20AT
- Auto sampler named SIL-20AHT
- CTO-10ASvp column temperature oven
- PDA detector named SPD-M20A
- CBM-20 Alite system controller
- Lab solution LC workstation multi PDA software

2.5.4. Preparation

Buffer Solution: Accurately 2.72 g of potassium dihydrogen phosphate was weighed and transferred into a volumetric flask containing distilled water. pH 4 was adjusted by adding diluted orthophosphoric acid using a pH meter shown in Figure 8. Final volume was adjusted up to 1 liter by the addition of distilled water.

Mobile Phase: A mixture of buffer solution and acetonitrile (HPLC Grade) was prepared at a ratio of 60:40 and it was then filtered through 0.22 μm Restek membrane filter.

Standard Solution for Sitagliptin in Combination with Metformin: 12.5 mg of sitagliptin and 125 mg of metformin were weighed and then transferred into 250 ml volumetric flask. The

mixture was dissolved with the dissolution media i.e. 0.025 M NaCl solution and the final volume was adjusted to 250 ml with the same solvent.

Standard Solution for Sitagliptin Alone: 12.5 mg of sitagliptin was weighed which was then transferred into 250 ml volumetric flask. It was dissolved with the dissolution media i.e. distilled water and the final volume was adjusted to 250 ml using the same solvent.

Sample Solution: The 10 mL of sample that was withdrawn from a point midway between the stirrer and the glass vessel after 30 minutes from the starting time of dissolution was filtered through 0.22 μm Restek syringe filter.

Procedure:

The vials containing standard and sample were placed into the tray of auto sampler of Shimadzu HPLC and they were injected under the following chromatographic conditions. The concentrations of standard and sample are as below:

- Concentration of metformin in Standard and Sample: 0.5mg/mL
- Concentration of sitagliptin in Standard and Sample: 0.05 mg/mL

Chromatographic Conditions

<u>Apparatus</u>	: Shimadzu HPLC-prominence integrated with PDA detector
<u>Column</u>	: Luna 5 μ C18column (250 mm x 4.60 mm)
<u>Mobile Phase</u>	: 0.02 M Potassium dihydrogen phosphate pH-4: acetonitrile (60:40% v/v)
<u>Flow Rate</u>	: 1.0 mL/min
<u>Diluent</u>	: Water
<u>Injection Volume</u>	: 10 μL
<u>Temperature</u>	: 25°C
<u>Detection</u>	: UV, absorbance at 252 nm for sitagliptin alone and at 210 nm for sitagliptin in combination with metformin
<u>Run Time</u>	: 4 Minutes
<u>Elution</u>	: Isocratic

Chapter 3

Data Analysis

Chapter 3

Data Analysis

All the data obtained were then carefully analyzed and the results interpreted as described in the following sub-sections.

3.1. Data Analysis of Combination Preparation of Sitagliptin and Metformin

3.1.1. Average Weight-Weight Variation

Result of average weight-weight variation test for the combination formulation containing sitagliptin and metformin is shown in Table 9 and Figure 9.

Table 9: Average Weight-Weight Variation Test Result of Marketed Tablets Containing Sitagliptin in Combination with Metformin

Brand	IB	LC1	LC2	LC3	LC4
Weight (mg)	697.00	700.00	695.00	670.00	656.00
Tablet no. 1					
Tablet no. 2	697.00	703.00	729.00	661.00	653.00
Tablet no. 3	708.00	701.00	726.00	668.00	651.00
Tablet no. 4	698.00	704.00	720.00	662.00	655.00
Tablet no. 5	688.00	706.00	705.00	662.00	642.00
Tablet no. 6	693.00	707.00	712.00	660.00	646.00
Tablet no. 7	700.00	694.00	716.00	667.00	647.00
Tablet no. 8	701.00	710.00	700.00	660.00	650.00
Tablet no. 9	694.00	708.00	693.00	649.00	675.00
Tablet no. 10	698.00	703.00	710.00	655.00	659.00
Tablet no. 11	708.00	709.00	721.00	640.00	654.00
Tablet no. 12	686.00	700.00	705.00	670.00	651.00
Tablet no. 13	700.00	701.00	700.00	665.00	667.00
Tablet no. 14	698.00	690.00	702.00	660.00	640.00
Tablet no. 15	702.00	695.00	680.00	659.00	650.00

Table 9 (Continued)

Tablet no. 16	697.00	701.00	700.00	660.00	651.00
Tablet no. 17	695.00	702.00	687.00	641.00	652.00
Tablet no. 18	700.00	702.00	658.00	650.00	635.00
Tablet no. 19	691.00	699.00	705.00	669.00	655.00
Tablet no. 20	695.00	698.00	710.00	660.00	661.00
Average (mg)	697.00	701.65	703.70	659.40	652.50
Standard Deviation	5.53	5.06	16.49	8.67	8.95
RSD%	0.79	0.72	2.34	1.31	1.37

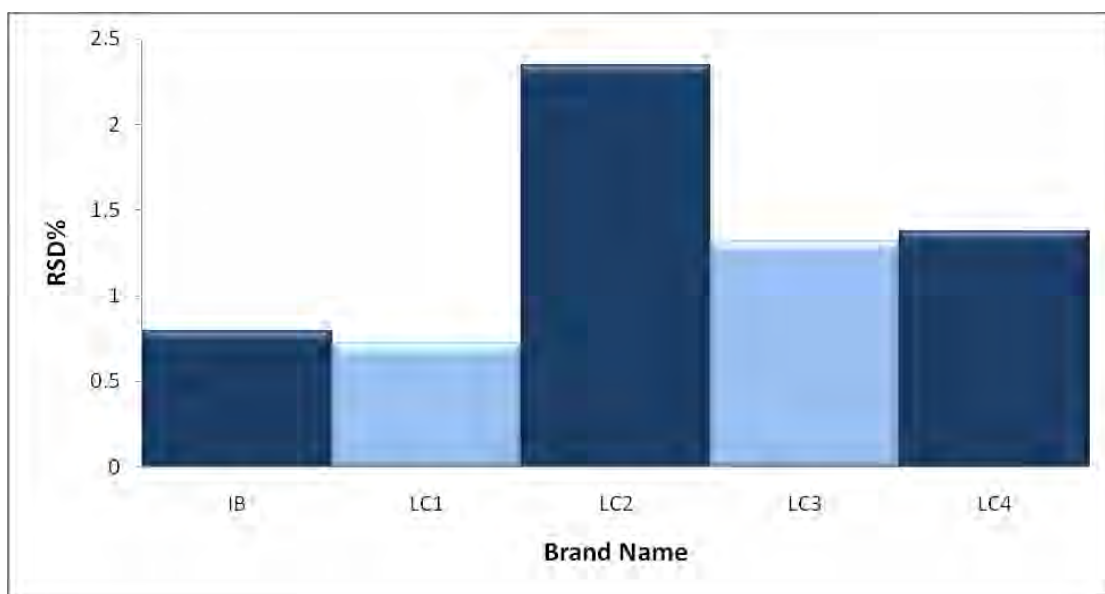


Figure 9: Average Weight-Weight Variation Test Result of Marketed Tablets Containing Sitagliptin in Combination with Metformin

Interpretation

From the result of experiment for average weight-weight variation test it has been observed that the relative standard deviations are less than 5% for all the marketed products which is within acceptable range according to USP29-NF24. However, the RSD of LC2 is very much higher than the innovator brand which may be due to the lack of uniformity in granule size during compression, poor flow ability of granules, improper distribution of particles, improper use of lubricant and glidant, etc.

3.1.2. Friability

Result of friability test for the combination formulation containing sitagliptin and metformin is shown in Table 10.

Table 10: Friability Test Result of Marketed Tablets Containing Sitagliptin in Combination with Metformin

Brand	Initial Weight, W_0 (mg)	Final Weight, W_f (mg)	$\frac{W_0 - W_f}{W_0} \times 100\%$
IB	6972.00	6972.00	0.00
LC1	7036.00	7036.00	0.00
LC2	7109.00	7109.00	0.00
LC3	6614.00	6602.00	0.18
LC4	6534.00	6534.00	0.00

Interpretation

From the result of experiment for friability test it has been observed that for most of the marketed products the amount of friability found is 0%. Only one has the friability of 0.18% which is very negligible.

3.1.3. Hardness

Result of hardness test for the combination formulation containing sitagliptin and metformin is shown in Table 11 and Figure 10.

Table 11: Hardness Test Result of Combination Preparation of Marketed Tablets Containing Sitagliptin in Combination with Metformin

Brand	IB	LC1	LC2	LC3	LC4
Hardness (kg)	20.58	17.91	13.71	19.25	13.38
Tablet no. 1					
Tablet no. 2	18.11	15.20	17.63	16.46	13.06
Tablet no. 3	18.52	15.77	21.31	20.34	12.82
Tablet no. 4	20.14	19.33	20.01	18.32	12.09
Tablet no. 5	22.28	19.81	17.55	17.59	12.21
Tablet no. 6	21.39	18.07	19.17	19.00	14.64
Tablet no. 7	21.87	18.36	13.10	17.31	11.36
Tablet no. 8	21.03	19.53	16.01	18.36	12.21
Tablet no. 9	21.51	19.61	20.01	20.42	12.01
Tablet no. 10	21.45	19.00	21.07	22.00	14.84
Average (kg)	20.68	18.26	17.96	18.91	12.86

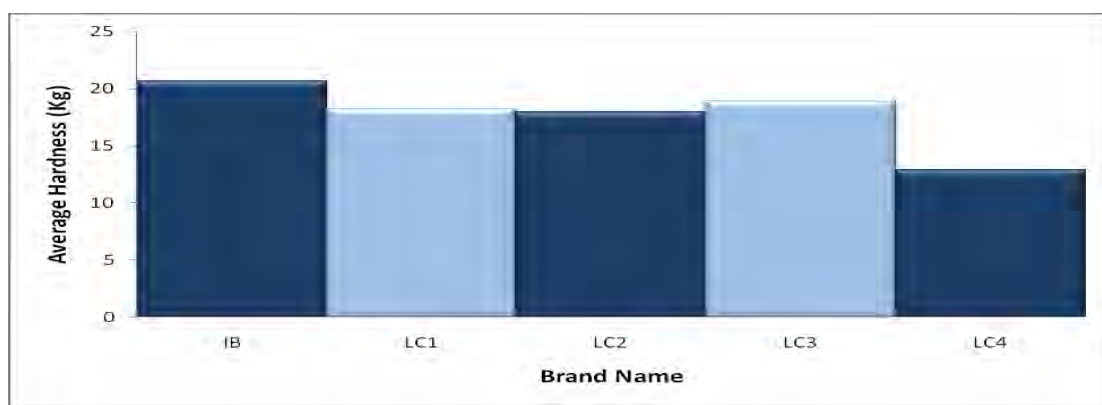


Figure 10: Hardness Test Result of Combination Preparation of Marketed Tablets Containing Sitagliptin in Combination with Metformin

Interpretation

Analysis of the result received from the above data, it can be said that most of the tablets were not so hard or too soft compared to innovator brand. Therefore, these tablets are considered as acceptable except LC4 from this evaluation test. The possible reason behind this may be the lack of proper binding agent during formulation.

3.1.4. Disintegration Test

Result of disintegration test for the combination formulation containing sitagliptin and metformin is shown in Table 12 and Figure 11.

Table 12: Disintegration Test Result of Marketed Tablets Containing Sitagliptin in Combination with Metformin

Brand	IB	LC1	LC2	LC3	LC4
Time (minute)	5.06	6.21	7.12	5.34	4.12
Tablet no. 1					
Tablet no. 2	5.08	6.25	7.18	5.43	4.2
Tablet no. 3	5.10	6.33	7.22	5.52	4.38
Tablet no. 4	5.18	6.56	7.36	5.58	4.5
Tablet no. 5	5.22	7.05	7.51	5.58	4.59
Tablet no. 6	5.25	7.15	7.59	6.18	5.08
Average (minute)	5.15	6.59	7.33	5.61	4.48

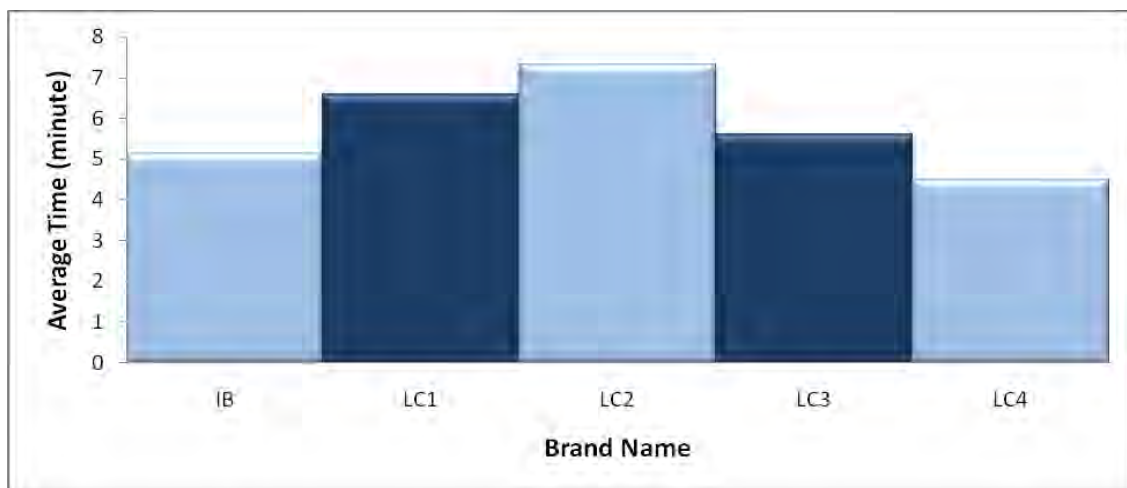


Figure 11: Disintegration Test Result of Marketed Tablets Containing Sitagliptin in Combination with Metformin

Interpretation

From the above result it has been seen that the total disintegration time for all the marketed products were not too much. So the onset time of these film coated tablets are acceptable. However, there was a difference between some of the brands which may be due to the binding agent used, type and amount of disintegrant, method of manufacture, etc.

3.2. Data Analysis of Single Sitagliptin Preparation

3.2.1. Average Weight-Weight Variation

Result of average weight-weight variation test for the formulation containing sitagliptin is shown in Table 13 and Figure 12.

Table 13: Average Weight-Weight Variation Test Result of Marketed Tablets Containing Sitagliptin

Brand	IB	LC1	LC2	LC3	LC4
Weight (mg)	205.00	201.00	190.00	204.00	156.00
Tablet no. 1					
Tablet no. 2	210.00	205.00	189.00	205.00	159.00
Tablet no. 3	209.00	208.00	191.00	204.00	160.00
Tablet no. 4	206.00	204.00	186.00	204.00	157.00
Tablet no. 5	211.00	203.00	187.00	207.00	159.00
Tablet no. 6	211.00	206.00	191.00	205.00	162.00
Tablet no. 7	207.00	204.00	192.00	208.00	161.00
Tablet no. 8	209.00	203.00	192.00	208.00	160.00
Tablet no. 9	205.00	200.00	192.00	213.00	164.00
Tablet no. 10	209.00	204.00	187.00	205.00	161.00
Tablet no. 11	206.00	202.00	191.00	205.00	161.00
Tablet no. 12	209.00	207.00	188.00	207.00	163.00
Tablet no. 13	211.00	211.00	186.00	208.00	157.00
Tablet no. 14	210.00	209.00	189.00	205.00	160.00
Tablet no. 15	209.00	204.00	193.00	206.00	158.00
Tablet no. 16	206.00	205.00	186.00	209.00	159.00
Tablet no. 17	207.00	208.00	192.00	203.00	160.00
Tablet no. 18	209.00	209.00	195.00	209.00	155.00
Tablet no. 19	207.00	207.00	188.00	205.00	158.00

Table 13 (Continued)

Tablet no. 20	208.00	204.00	189.00	205.00	157.00
Average (mg)	208.20	205.20	189.70	206.25	159.35
Standard Deviation	1.96	2.88	2.59	2.38	2.32
RSD%	0.94	1.40	1.36	1.15	1.45

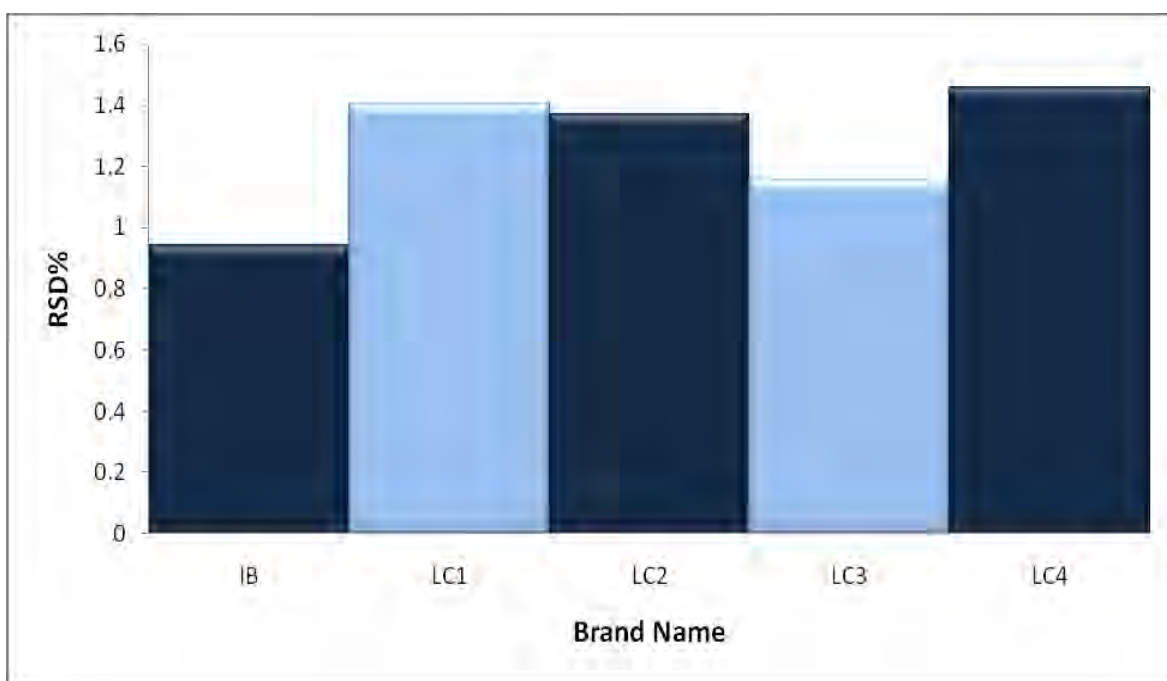


Figure 12: Average Weight-Weight Variation Test Result of Marketed Tablets Containing Sitagliptin

Interpretation

From the result of experiment for average weight-weight variation test it has been observed that the relative standard deviations are less than 7.5% for all the marketed products which is within the limit according to USP29-NF24. However, the RSD of LC1, LC2 and LC4 is higher than the innovator brand which may be due to the lack of uniformity in granule size during compression, poor flow ability of granules, improper distribution of particles, improper use of lubricant and glidant, etc.

3.2.2. Friability

Result of friability test for the formulation containing sitagliptin is shown in Table 14.

Table 14: Friability Test Result of Marketed Tablets Containing Sitagliptin

Brand Name	Initial Weight, W ₀ (mg)	Final Weight, W _f (mg)	$\frac{W_0 - W_f}{W_0} \times 100\%$
IB	2093	2093	0
LC1	2043	2043	0
LC2	1887	1887	0
LC3	2059	2059	0
LC4	1598	1598	0

Interpretation

From the result of experiment for friability test it has been observed that for all of the marketed products the amount of friability found is 0%.

3.2.3. Hardness

Result of hardness test for the formulation containing sitagliptin is shown in Table 15 and Figure 13.

Table 15: Hardness Test Result of Combination Preparation of Marketed Tablets Containing Sitagliptin

Brand	IB	LC1	LC2	LC3	LC4
Hardness (kg)	9.02	9.26	6.02	8.45	3.19
Tablet no. 1					
Tablet no. 2	9.58	7.56	6.39	9.54	1.82
Tablet no. 3	9.58	7.16	7.16	7.80	2.91
Tablet no. 4	9.54	6.31	6.31	9.06	2.35
Tablet no. 5	9.42	6.87	5.54	8.45	2.59
Tablet no. 6	9.78	6.06	5.82	8.45	2.79
Tablet no. 7	9.10	5.74	6.39	8.65	3.76
Tablet no. 8	8.85	6.95	5.42	9.06	2.91
Tablet no. 9	8.73	6.43	5.94	8.32	2.65
Tablet no. 10	9.45	5.74	6.06	8.62	2.42
Average (kg)	9.31	6.81	6.11	8.64	2.74

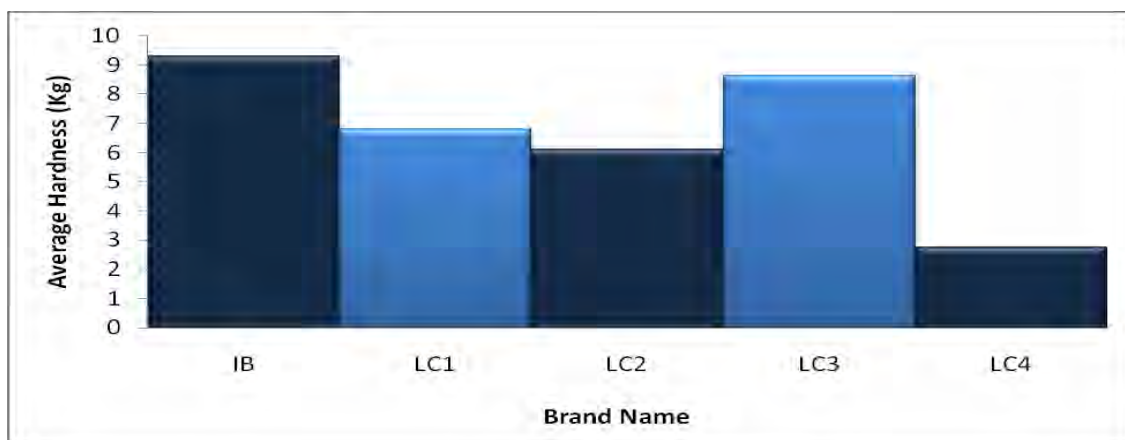


Figure 13: Hardness Test Result of Marketed Tablets Containing Sitagliptin

Interpretation

From this result we can conclude that one of the generics had a very low hardness compared to innovator brand while others passed the test. It may be due to the lack of proper binding agent during formulation.

3.2.4. Disintegration Test

Result of disintegration test for the formulation containing sitagliptin is shown in Table 16 and Figure 14.

Table 16: Disintegration Test Result of Marketed Tablets Containing Sitagliptin

Brand	IB	LC1	LC2	LC3	LC4
Time (minute)	0.30	0.08	3.20	0.15	0.52
Tablet no. 1					
Tablet no. 2	0.31	0.09	3.25	0.17	0.55
Tablet no. 3	0.31	0.10	3.36	0.18	1.00
Tablet no. 4	0.33	0.13	3.45	0.20	1.02
Tablet no. 5	0.34	0.15	4.10	0.22	1.05
Tablet no. 6	0.35	0.16	4.21	0.25	1.09
Average (minute)	0.32	0.12	3.59	0.19	0.87

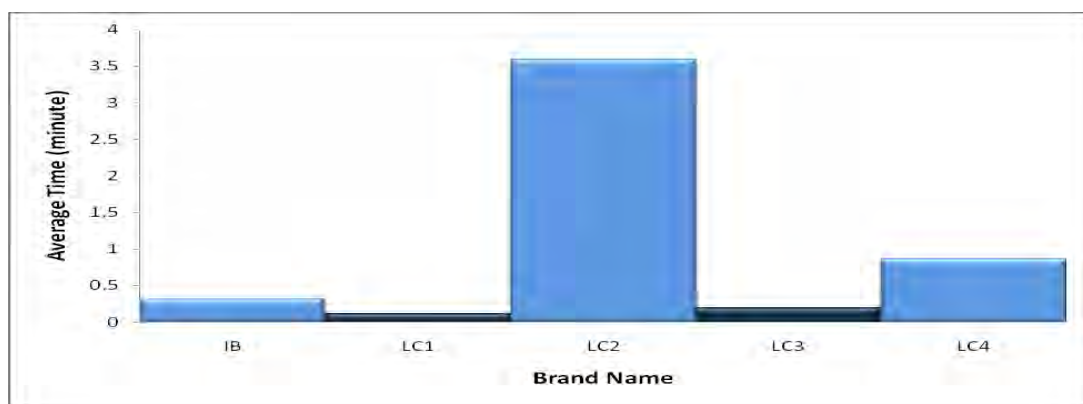


Figure 14: Disintegration Test Result of Marketed Tablets Containing Sitagliptin

Interpretation

From the above result it has been seen that the total disintegration time for all the marketed products were not too much. In fact very fast disintegration is found except generic. Therefore, compared to the innovator brand this generic did not pass the test while others are acceptable. It is may be due to the binding agent used, type and amount of disintegrant, method of manufacture, etc.

3.3. Estimation of Release Kinetics

3.3.1. System Suitability Test

After sufficient time had passed for HPLC system to achieve a stable baseline, system suitability was determined by injecting standard solution to the HPLC system. This suitability test was applied to the chromatograms of taken under optimum conditions to check various parameters such as column efficiency (theoretical plates), peak area, tailing factor, retention time, and resolution (Celebier et al., 2010).

Freshly prepared standard stock solution of sitagliptin alone and sitagliptin in combination with metformin were injected into the chromatographic system under the optimized chromatographic conditions (Sankar et al., 2013). The test is considered valid if the following considerations are met:

- The relative standard deviation for the peak area response for six replicate injections of standard preparation is not more than 2% (Qiu et al., 2009)
- Tailing factor for the peak should not be more than 2%.
- Theoretical plate for the peak obtained in the chromatogram of standard solution is not less than 3000.

After the test we obtained the following chromatograms shown in Figure 15 and 16, and the results are interpreted in Table 17 and 18.

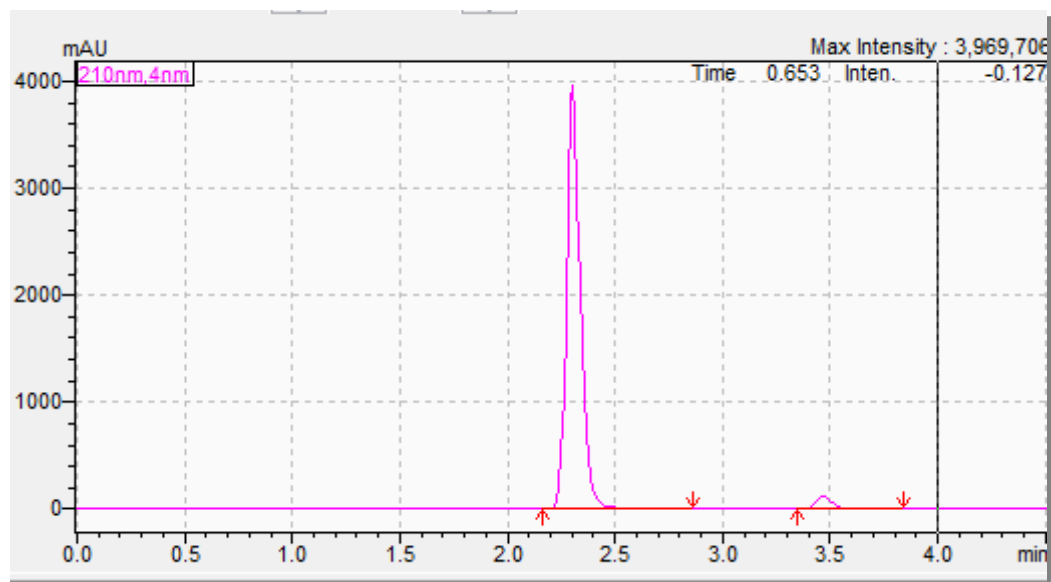


Figure 15: Chromatogram of Standard Sitagliptin and Metformin

Table 17: System Suitability Parameters of Standard Sitagliptin and Metformin

No.	Tailing factor	Theoretical plate	Peak area	Retention time
1	1.290	3072.000	3934890.000	2.292
2	1.292	3141.000	3938814.000	2.286
3	1.293	3051.000	3937037.000	2.290
4.	1.286	3120.000	3961279.000	2.284
5.	1.289	3153.000	3963069.000	2.295
6.	1.290	3102.000	3936220.000	2.286
Average	1.290	3106.500	3945218.167	2.288
STD	0.002	39.561	13207.463	0.004
RSD (%)	0.189	0.012	0.334	0.184

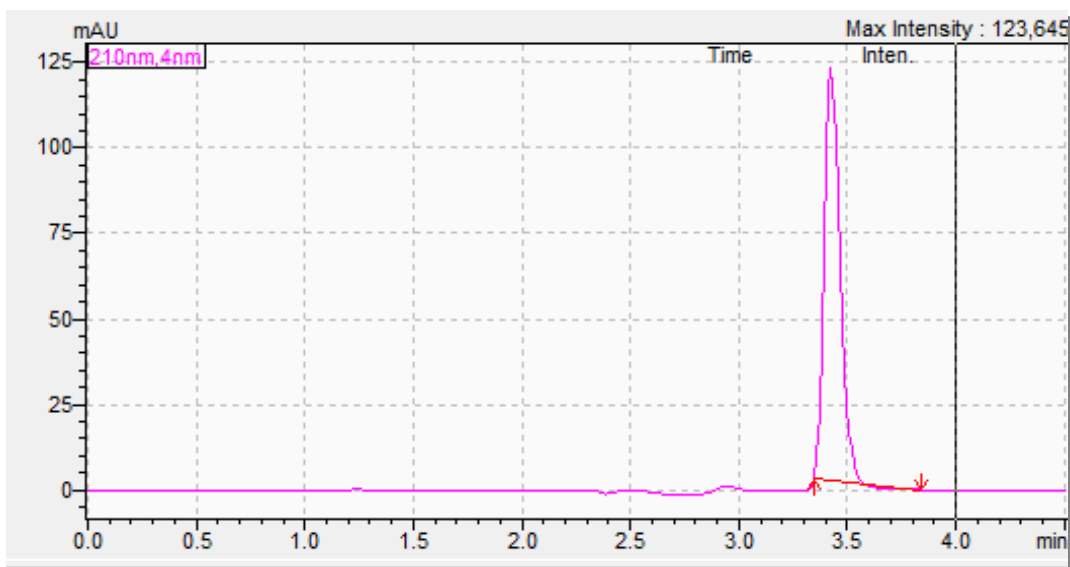


Figure 16: Chromatogram of Standard of Sitagliptin

Table 18: System Suitability Parameters of Standard Sitagliptin

No.	Tailing factor	Theoretical plate	Peak area	Retention time
1	1.292	8317.000	579943.000	3.420
2	1.306	8038.000	553714.000	3.424
3	1.291	8398.000	549199.000	3.417
4.	1.297	7570.000	577619.000	3.467
5.	1.294	7660.000	556273.000	3.459
6.	1.298	7560.000	565321.000	3.455
Average	1.296	7924.000	563678.166	3.440
STD	0.005	379.375	12848.018	0.022
RSD (%)	0.421	4.787	2.279	0.649

Interpretation

It is observed from the above tabulated data that the method complies with the system suitability parameters. Here, the relative standard deviation for the peak area response for six replicate injections of standard preparation of sitagliptin is approximately 2%. Hence, it can be concluded that the system suitability parameters meets the requirement of this method.

3.3.2. Estimation of Release Kinetics of Sitagliptin in Combination with Metformin

After injecting the sample containing sitagliptin in combination with metformin into the chromatographic system, following chromatograph was obtained (Figure 17) and the results are interpreted in Table 19 and Figure 18.

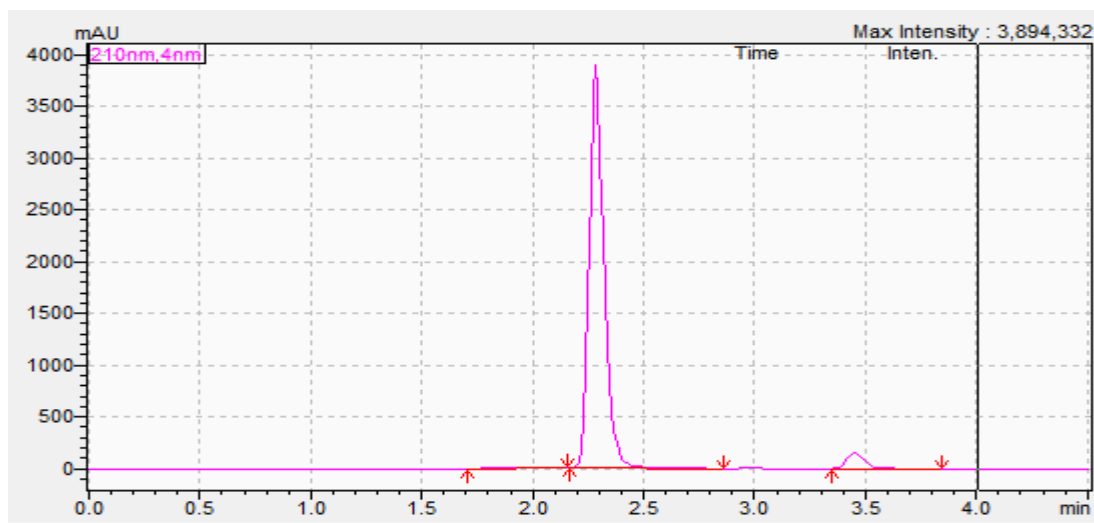


Figure 17: Chromatogram of Sample Sitagliptin and Metformin

Table 19: Dissolution Test Result of Marketed Tablets Containing Sitagliptin in Combination with Metformin

Brand	IB		LC1		LC2		LC3		LC4	
	A	B	A	B	A	B	A	B	A	B
Tablet no. 1	97.44	119.79	98.08	120.05	100.73	125.18	99.17	127.10	98.26	124.34
Tablet no. 2	97.43	119.68	98.02	119.56	100.33	123.63	99.16	126.28	98.18	123.23
Tablet no. 3	96.70	119.52	97.83	119.05	97.90	118.84	99.10	125.74	97.11	123.11
Tablet no. 4	96.65	119.35	97.75	118.95	97.83	117.95	95.72	124.26	97.09	123.06
Tablet no. 5	94.94	115.03	96.98	118.55	97.82	117.29	95.61	120.16	97.01	122.62
Tablet no. 6	94.78	114.69	96.95	118.40	97.45	117.16	95.44	120.01	96.99	122.32
Average	96.32	118.01	97.60	119.09	98.67	120.01	97.36	123.92	97.44	123.11

A= Dissolution% of Metformin, B= Dissolution% of Sitagliptin



Figure 18: Dissolution Test Result of Marketed Tablets Containing Sitagliptin in Combination with Metformin

Interpretation

In the above result all the generics compared to the innovator brand have shown a satisfactory amount of release of sitagliptin in binary mixture with metformin.

3.3.3. Estimation of Release Kinetics of Sitagliptin

After injecting the sample containing sitagliptin into the chromatographic system, following chromatograph was obtained (Figure 19) and the results are interpreted in Table 20 and Figure 20.

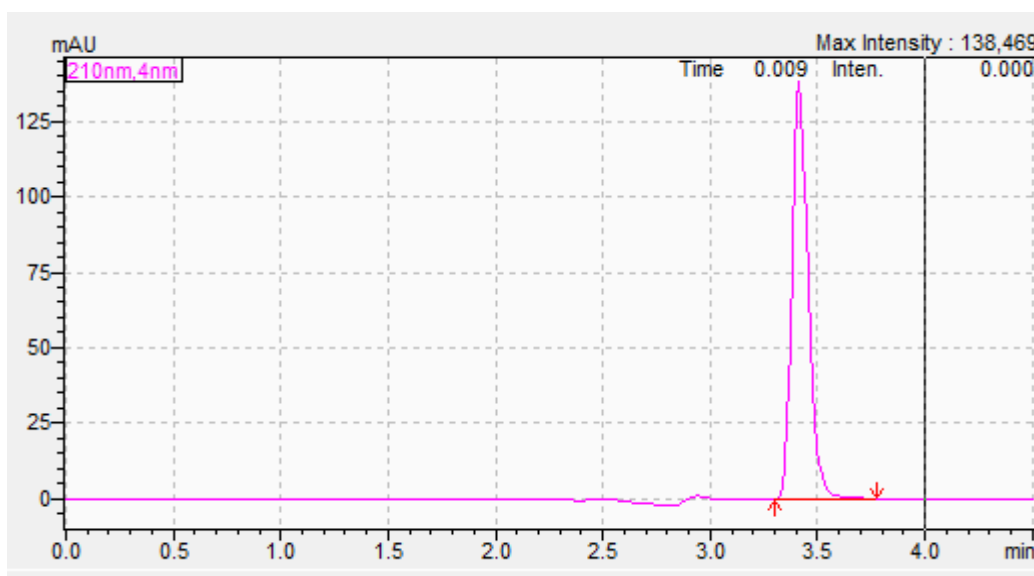


Figure 19: Chromatogram of Sample Sitagliptin

Table 20: Dissolution Test Result of Marketed Tablets Containing Sitagliptin

Brand	IB	LC1	LC2	LC3	LC4
Dissolution%	114.37	97.45	102.95	115.94	119.96
Tablet no. 1					
Tablet no. 2	114.09	96.91	102.73	115.53	119.64
Tablet no. 3	113.49	94.97	101.18	113.6	118.69
Tablet no. 4	113.25	94.47	101.08	113.23	118.63
Tablet no. 5	111.24	93.74	100.39	110.70	118.25
Tablet no. 6	110.22	93.42	100.34	109.86	117.98
Average	112.77	95.16	101.44	113.14	118.85

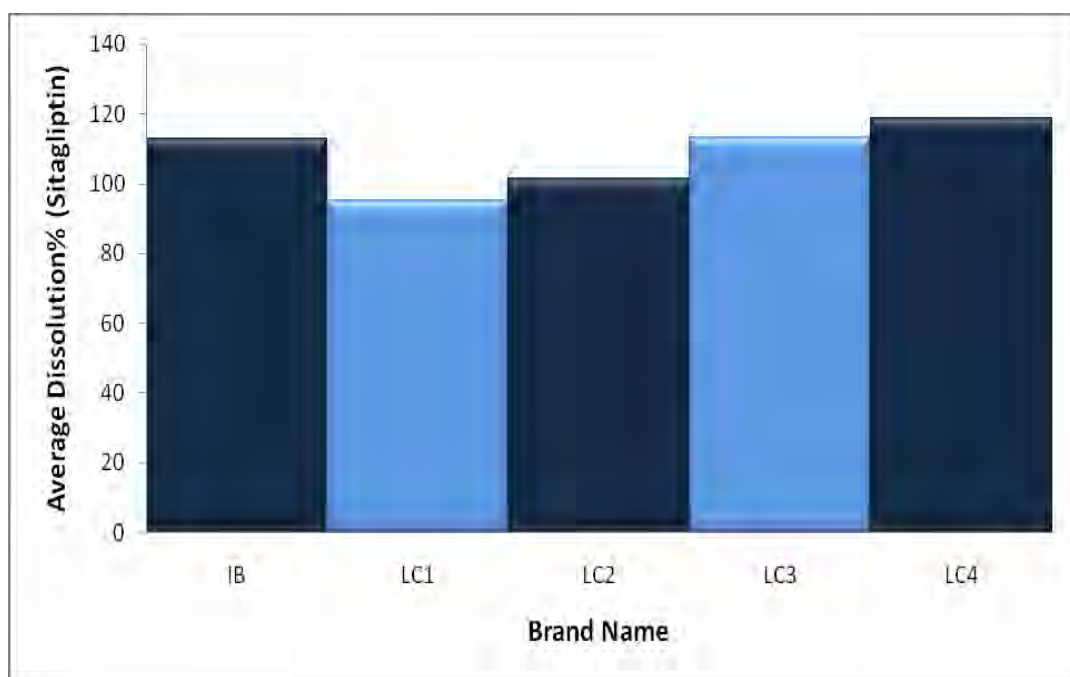


Figure 20: Dissolution Test Result of Marketed Tablets Containing Sitagliptin

Interpretation

In the above result most generics compared to the innovator brand have shown a satisfactory amount of release of sitagliptin except one which may be due to putting inappropriate amount of active ingredient during formulation.

Chapter 4

Discussion

Chapter 4

Discussion

The present work done on this combination preparation of sitagliptin and metformin, and sitagliptin alone in pharmaceutical tablet formulation encompasses a comparison of the local generic products with the innovator brand. Following the Good Quality Control Laboratory Practice (GQCLP), a comparative analytical evaluation between these formulations containing sitagliptin of several physicochemical parameters was performed to assure the quality of the generics. In average, weight variation test for both the combination preparation and single sitagliptin preparation though the generics passed the test according to USP, although some of them were found to be deviated from the innovator brand. Similarly, in both hardness tests and in vitro disintegration tests there were anomaly in the tablets of some generics. Therefore, compared to the innovator brand those particular generics did not pass those specific tests. These deviations may be the consequence of lack of uniformity in granule size, poor flow ability, inappropriate distribution of particles, improper use of binder, lubricant, disintegrant, etc. However, some of the generics were found to meet the criteria of these physicochemical parameters. In fact, better results have been observed in some cases which may be due to the development of new formulation by local companies. On the whole, all the generics have shown an acceptable result in friability test.

Most of the generics met the criteria of release kinetics compared to innovator brand for both combination preparation and single sitagliptin preparation. Only one generic has found with lower amount of release in sitagliptin single preparation compared to innovator brand. Here, the one important factor that was noticed was the release of sitagliptin in both single and combination preparation to be greater than 100%. Companies adding an excess amount of active ingredient in order to get better patient compliance may be a possible reason of this result.

During the study of release kinetics, several analytical methods were reviewed and several trials were undertaken on the chosen methods. The chosen optimized method was found to comprise of a simple, precise and accurate method by reverse phase high performance liquid chromatography for the estimation of sitagliptin. The method employed a RP-HPLC procedure with PDA detector consisting of Luna 5 μ C18 column (250 mm x 4.60 mm) inserting an injection volume of 10 μ L and eluted by the mobile phase of 0.02 M potassium dihydrogen phosphate (pH4) and acetonitrile in a ratio of 60:40 respectively, which is pumped at a flow rate of 1.0

mL/min, carried out the detection at a wavelength of 252 nm for the binary mixture and of 210 nm for the single sitagliptin. The peaks of both sitagliptin and metformin were found to be precise and well separated at approximate 3.45 and 2.28 minutes respectively. The chosen RP-HPLC method was found rapid, accurate, precise, specific, robust, economical and less time consuming. The values for system suitability parameter also showed feasibility of this selected method for routine pharmaceutical application. The changes in proportion of solvents were studied also in the initial phase of the study. Hence, the selected method was itself sophisticated enough to estimate simultaneously sitagliptin alone and in binary mixture with metformin in bulk and tablet dosage form.

Chapter 5

Concluding Remarks

Chapter 5

Concluding Remarks

Presently, it has become an urge to develop new strategies in the treatment of diabetes mellitus. Since diabetes is a lifelong condition, it cannot be cured. It can only be managed or controlled where the most effective treatment is based on the collaboration between patients and health professional. However, the life style of a patient is not persistent. So, with the modification of life style, the patient should be treated through newer strategies which can be combination therapy. This strategy has been already proved as convenient, economical with fewer side effects. This combination therapy also has synergistic effect with complementary mechanism of action since it contains more than one active pharmaceutical ingredient. Reducing the pill burden improves patient compliance and delays the need for insulin therapy. In our study, we estimated sitagliptin, a DPP-4 inhibitor, either alone or a binary mixture with metformin that is a biguanide derivative by using RP-HPLC method. Here, we collected almost all the available products from local market and have shown a comparison of the quality of marketed product between the local companies with the innovator brand. Though most of the local companies passed the test, some failed to meet the criteria of innovator brand. Therefore, physicians prescribing this drug must be aware of this. On the other hand, the method which we utilized for the assessment of sitagliptin was highly sophisticated giving an optimum, precise and accurate results rapidly. Therefore, it can be concluded that the present RP-HPLC method is appropriate and can be used for routine analysis in the quality control laboratories for any tablet dosage form containing sitagliptin. Furthermore, this method can be applied by changing various parameters or formulation of the dosage forms in future so that it can be determined that whether there is any interference of excipients on the rapidity and accuracy of the method. This will definitely be an actual robustness of this method.

Appendix

Appendix 1

Table 21: Name of the Marketed Product

Sitagliptin in combination with Metformin					
Brand Name	Amount of API	Manufacturer	Batch no.	Manufacturing Date	Expiry Date
Janumet® (Innovator Brand)	Sitagliptin 50 mg, Metformin 500 mg	Merck Sharp & Dohme, The Netherlands	L002021	September, 2014	September, 2016
Glipita®-M 50/500	Sitagliptin 50 mg, Metformin 500 mg	Beximco Pharmaceuticals Ltd., Bangladesh	SYD057	April, 2015	April, 2017
Sitomet® 50/500	Sitagliptin 50 mg, Metformin 500 mg	ACI Ltd., Bangladesh	EO56	February, 2015	February, 2017
Janmet 500	Sitagliptin 50 mg, Metformin 500 mg	The ACME Laboratories Ltd., Bangladesh	T2385004	February, 2015	February, 2017
Sliptin-M 500	Sitagliptin 50 mg, Metformin 500 mg	Drug International Ltd., Bangladesh	0215	January, 2015	January, 2017
Sitagliptin Alone					
Brand Name	Amount of API	Manufacturer	Batch no.	Manufacturing Date	Expiry Date
Januvia® (Innovator Brand)	Sitagliptin 50 mg	Merck Sharp & Dohme, Italy	15FC002A	July, 2014	June, 2016

Table 21 (Continued)

Glipita [®] 50	Sitagliptin 50 mg	Beximco Pharmaceuticals Ltd., Bangladesh	SYC060	March, 2015	March, 2017
Sitap [®] 50	Sitagliptin 50 mg	ACI Ltd., Bangladesh	EO75	February, 2015	February, 2017
Janvia 50	Sitagliptin 50 mg	The ACME Laboratories Ltd., Bangladesh	T2484055	December, 2014	December, 2016
Sliptin 50	Sitagliptin 50 mg	Drug International Ltd., Bangladesh	0515	May, 2015	May, 2017

Other marketed products are reported in Bangladesh National Formulary (BDNF) but were unavailable in the market at the time of study.

Table 22: List of Chemicals Used

Name	Manufacturer
Acetonitrile	Active Fine Chemicals Ltd, Bangladesh
Methanol	Active Fine Chemicals Ltd, Bangladesh
Potassium Dihydrogen Phosphate	Scarlab, Spain
Dipotassium Hydrogen Phosphate	Merck Specialties Private Ltd., India
Sodium Chloride Cryst. Pure	Merck Specialties Private Ltd., India
Sodium Hydroxide Pellets	Merck Specialties Private Ltd., India
Orthophosphoric Acid	ACI Labscan, RCI Labscan limited, Thailand.

Table 23: List of Apparatus Used

Name	Manufacturer	Model
Electronic Balance	Shimadzu, Japan	ATY-224
High Pressure Liquid Chromatography (HPLC)	Shimadzu, Japan	SPD-M20A Prominence
Friability Tester	Electrolab, India	EF-2
Disintegration Tester	Electrolab, India	ED-2L
Dissolution Tester	Logan Instruments Corp., USA	UDT-804
Hardness Tester	Electrolab, India	EH-01
pH meter	Mettler Toledo, North America	S220
Disposable Syringe	JMI Syringes & Medical Devices Ltd., Bangladesh	5 mL
Syringe Filter	Restek, USA	25 mm, 0.22 μm
Filter Paper	Whatman, UK	110 mm
Membrane Filter	Restek, USA	47 mm, 0.22 μm

Appendix 2

Sitagliptin

Sitagliptin phosphate is an orally active Dipeptidyl peptidase 4 inhibitor which is a monohydrate phosphate salt. Its chemical name is 7-[(3*R*)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-[3-(trifluoromethyl)-1,2,4-triazolo[4,3-*a*]pyrazine phosphate (1:1) monohydrate according to the IUPAC nomenclature and the chemical formula is $C_{16}H_{15}F_6N_5O$ (European Medicines Agency, 2008). Figure 21 shows the chemical structure of sitagliptin.

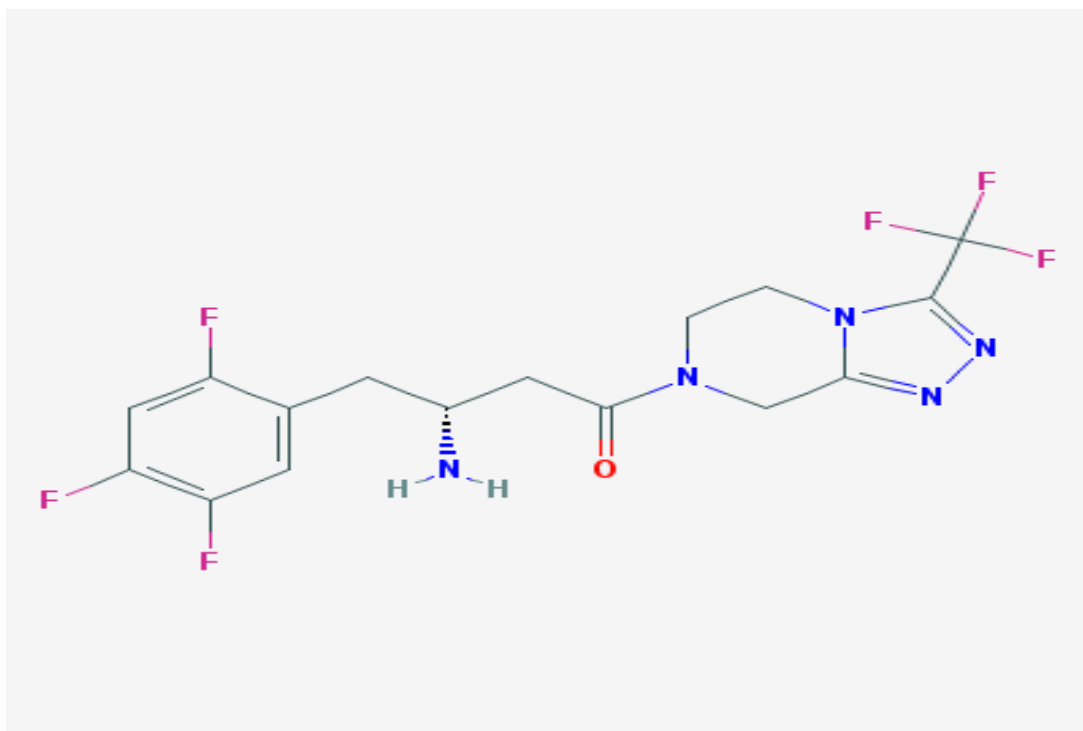


Figure 21: 2D Chemical Structure of Sitagliptin (Pubchem)

Sitagliptin is a white to off-white crystalline, non-aqueous powder that exhibits pH dependent aqueous solubility. It is soluble in water and *N,N*-dimethyl formamide, slightly soluble in methanol, very slightly soluble in ethanol, acetone and acetonitrile and insoluble in isopropanol and isopropyl acetate. The above-mentioned active substance contains a chiral centre and is used as a single enantiomer (*R*) (European Medicines Agency, 2008; Sankar et al., 2013).

It is stable if stored as directed condition and strong oxidizing agents must be avoided (Pubchem).

Toxicological Information (Pubchem)

Sitagliptin may be risk factor in the development of pancreatitis. Human toxicity includes an increased risk of heart failure among patients with type 2 diabetes who already have pre-existing heart failure. Thermal decomposition of sitagliptin phosphate may produce toxic gases such as carbon monoxide, carbon dioxide, and nitrogen oxides.

Metformin Hydrochloride

Metformin is a hypoglycemic agent belonging to the biguanide class of antidiabetics. The chemical name of its hydrochloride form is 1, 1-Dimethylbiguanide monohydrochloride according to the IUPAC nomenclature. The chemical formula of metformin is $C_4H_{11}N_5$ and the chemical structure is given in Figure 22.

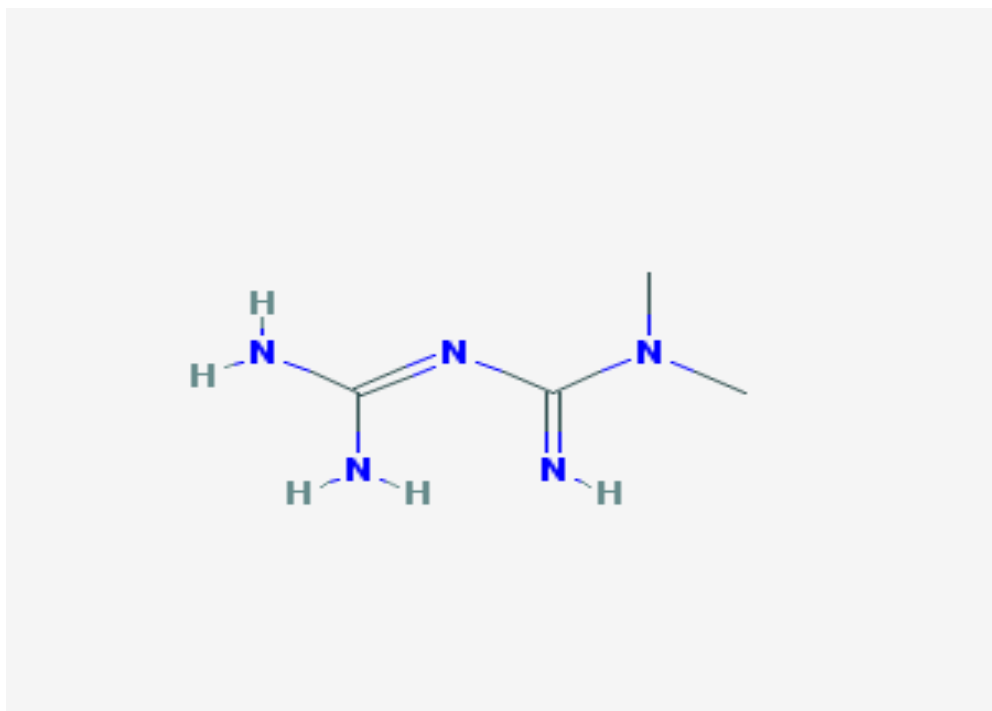


Figure 22: 2D Chemical Structure of Metformin (Pubchem)

It is a white to off-white crystalline, non-aqueous powder that is odourless and has a bitter taste. The compound is freely soluble in water, slightly soluble in ethanol and practically insoluble in chloroform, acetone, ether and in ethylene chloride. It has a specific crystalline form and has not demonstrated polymorphism or solvates. Particle size does not significantly influence dissolution of metformin hydrochloride, because it is freely soluble in water (European Medicines Agency, 2008; Sankar et al., 2013).

Toxicological Information

Lactic acidosis can occur because of the accumulation of metformin during treatment and the risk of metformin accumulation and lactic acidosis elevate with the degree of renal impairment (primary source). On the other hand, metformin is seen to be bound to plasma proteins negligibly (Pubchem).

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