In-vitro preformulation studies for the development of a novel antidiabetic combination therapy involving linagliptin and dextromethorphan

A project submitted by

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Dedicated to my parents for their love, motivations and continuous support…

CERTIFICATION STATEMENT

This is to certify that this project titled “A systematic review and Preformulation studies, for the development of a novel antidiabetic combination therapy: Linagliptin & Dextromethorphan” submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.) from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Rubayat Islam Khan, Senior Lecturer, Department of Pharmacy, BRAC University and that appropriate credit is given where I have used the language, ideas or writings of another.

Signed,

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Countersigned by the supervisor

_____

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ABSTRACT

Hyperglycemia and decreased insulin secretion and its less sensitivity seems to be the primary defects associated with diabetes and available treatments focus on reducing those defects. Sulfonylureas and metformin were the first line treatment for type 2 diabetes mellitus despite knowing their progressive action towards beta cell damage and within 3 years of their use, 50% beta cells failure take place and patients require additional pharmacological agents to control type 2 diabetes. A new method of treatment is to target the incretin mimetic hormones, which are secreted by intestinal cells in response to food intake, provoking glucose-dependent insulin secretion from the pancreas. Gliptins are the agents, which improve beta cells health and suppress glucagon, resulting in improved post-prandial and fasting glucose by preventing the metabolism of incretin hormones by DPP-4 enzymes. Among gliptins, Linagliptin is considered as the most potent because of its improvements of HbA1c level as well as efficacy, safety and least incidence of hypoglycemia, which makes it a unique drug. In addition, recent study found that, NMDARs antagonist dextromethorphan increase the postprandial insulin secretion from beta cells by depolarizing the cell membranes of beta cells in the pancreas. The research suggests, gliptins in combination with dextromethorphan improves both postprandial and fasting glucose level of diabetes patients as well as keeps the HbA1c level below 7%, better than gliptins monotherapy. Therefore, the aim of this project is to do the preformulation studies of linagliptin and dextromethorphan as a novel combination therapy to treat type 2 diabetes mellitus. In this work, preformulation studies were done to ascertain the particle size distribution, angle of repose, compressibility index, bulk and tapped density, dissolution profile and interaction at dissolve state. The particle size distribution were below 250µ for both linagliptin and dextromethorphan, which indicates a good dissolution pattern. Angle of repose were 22.5^{o} and compressibility index were 18.75, while mixed linagliptin and dextromethorphan together ensures better flow ability as well as compression property. Moreover, the dissolution data suggest that, linagliptin get dissolved within 3 minutes whereas dextromethorphan takes 1.5 minutes to get dissolved in pH 6 phosphate buffer medium. On the other hand, both linagliptin and dextromethorphan did not demonstrate change in concentration in dissolve state with excipients in UV-spectrophotometer analysis, indicates no interaction among the ingredients. The methods used in the project were found to be accurate, liner and precise which put forward further work on this project for developing the novel combination therapy of linagliptin and dextromethorphan.

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List of Acronyms

µg = Microgram
Abs. = Absorbance
AMP = Adenosine Monophosphate
AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazole Propionic Acid
API = Active Pharmaceutical Ingredients
BP = British Pharmacopoeia
C_{max} = Maximum Plasma Concentration
CNS = Central Nervous System
CVD = Cardiovascular Disease
dL = deciliter
DN = Diabetic Nephropathy
DPP-4 = Dipeptidyl Peptidase 4
DR = Diabetic retinopathy
DXM = Dextromethorphan
FDA = Food and Drug Administration
FDC = Fixed Dosage Combination
GDIS = Glucose Dependent Insulin Secretion
GDM = Gestational Diabetes Mellitus
GIP = Glucose-dependent Insulinotropic Peptide
GIT = Gastrointestinal Tract
GLP-1 = *Glucagon-like Peptide-1*
GLUT = Glucose Transporter
Gm = Gram
HbA1c = Glycated Hemoglobin
HBr = Hydrogen Bromide
IDDM = Insulin Dependent Diabetes Mellitus
K+ channel = Potassium Channel
mg = Milligram
MI = Myocardial Infarction
mins = Minutes
mL = Milliliter
NIDDM = Non-Insulin Dependent Diabetes Mellitus
nM = Nano mole
nm = nanometer
NMDA = N-Methyl-D-aspartate
NMDAR = N-methyl-D-aspartate receptor
NPH = Neutral Protamine Hagedorn
OGTT = Oral Glucose Tolerance Test
SU = Sulfonylureas
T1DM = Type 1 Diabetes Mellitus
T2DM = Type 2 Diabetes Mellitus
USP = United States Pharmacopeia
UV = Ultraviolet
WHO = World Health Organization

Chapter 1

1. Introduction

1.1 Diabetes

Diabetes mellitus, a very conventional form of disease exist worldwide with 422 million people suffering from it and global prevalence among adults was 8.5% by the year 2017 (WHO, July 2017). Diabetes mellitus may be defined as per a prolonged metabolic syndrome characterized by hyperglycemia because of the inadequate metabolism of carbohydrate, fat and protein due to insufficient insulin production by pancreas or insulin insensitivity, which leads to blood glucose level greater than before (WHO, 1999). It was ranked as the 6th deadly disease in the year 2015, almost 1.6 million people directly died because of diabetes and according to WHO projects it will be the 7th leading death causing disease by the year 2030 (WHO, January, 2017, July 2017). Regrettably, diabetes is rising terrifyingly in Asian countries; among top 10 countries, 6 countries are from the region Asia and top 5 Asian courtiers are India, China, Pakistan, Japan and Indonesia. Bangladesh is ranked at 8th position among Asian countries but predicted to replace Japan by the year 2030 (Wenying, 2010). However 80% of the diabetes patients are found in lower and middle-income countries where Bangladesh alone contribute 11% of them (Mendenhall, Norris, Shidhaye, & Prabhakaran, 2014).

1.2 Signs and Symptoms of Diabetes mellitus

Diabetes a severe disorder, which at times go undetected since its cautionary signs are sometimes mixed up with the symptoms of other diseases and sometimes diabetes even does not demonstrate symptoms. Type 1 diabetes mellitus occurs swiftly maybe after some sort of illness while type 2 takes years to develop (Lipsky, Gee, Liu, & Nansel, 2016). With the rapid development of type 1 diabetes, it does not show any particular symptoms; moreover, sometimes symptoms are mistaken with flu until they demonstrate a serious condition called diabetic ketoacidosis (Francoeur, 2016). Indicators of diabetic ketoacidosis are sweet smelling breathe, confusion and Kussmaul breathing (Riaz, 16 April, 2009). On the other hand, some patients are diagnosed after extreme suffering; therefore, people should go for periodic diabetes testing if they have risk factors to avoid life-threatening conditions. Some possible indicators of diabetes mellitus are blurred vision, dizziness or fainting, fatigue, lethargy or drowsiness, mood swings or irritability. Moreover, polyphagia, polydipsia and polyuria are the very

conventional symptoms among diabetes patients as well as slow healing of sores and pain in feet, legs and hands. In addition to all these symptoms, infections and skin problems such as, itching, acanthosis nigricans dry skin along with numbness and trembling indicates the presence of diabetes (Castensoe-Seidenfaden et al., 2017; Francoeur, 2016; Lipsky et al., 2016).

1.3 Complications of diabetes

Diabetes is the disease, which affects all age group of people, starting from children to olds. It is itself a malady as well as creates complications in the body for instance, retinopathy, nephropathy, neuropathy, ischemic heart disease, peripheral vascular disease, and cerebrovascular disease (UKPDS, 1991). Complications associated with diabetes are broadly classified into two categories:

1. Microvascular, which is related with the small vessels like capillaries
2. Macrovascular, which is related with large vessels like arteries, veins

1.3.1 Microvascular Complications of Diabetes

A. Diabetic Retinopathy

Diabetic Retinopathy is about visual disability where peripheral retina or macula, sometimes both of them are affected which leads to vision disability and blindness of people who are affected with diabetes. Diabetic retinopathy can be proliferative or non-proliferative and at severe stage, abnormal growth of some vessels occurs which eventually leads to partial vision loss through vitreous hemorrhage or retinal detachment whereas, full vision loss may occur through retinal vessel leakage and macular edema (Solomon et al., 2017). Studies have found that patients with diabetes for more than 20 years are more likely suffer from diabetic retinopathy (Orchard et al., 1990). The higher the duration of diabetes, the more chances to get affected by diabetic retinopathy and the indicator is loss of pericytes, which are the contractile cells that surround the small vessels of endothelial cells (J. Kim, 2004). These pericytes provide protection against ROS damage, capillary tone and capillary maintenance; therefore, its loss due to diabetic retinopathy leads to abnormal capillary contractions (Hammes, 2005; Hammes et al., 2002). Other symptoms of diabetic retinopathy are thickening of capillary basement membrane, increased penetrability of endothelial cells, and development of micro aneurysms

(Carlson et al., 2003). Even at severe state hypoxia may occur due to blood flow obstruction to retina, adhesion of inflammatory cell to the retinal blood flow vessels and capillary blockage (Kohner, Patel, & Rassam, 1995).

B. Diabetic Neuropathy

Diabetic neuropathy perhaps well defined as the signs and symptoms of peripheral nerve dysfunctions in diabetes patients (Lewko et al., 2007). Almost 50% diabetes patients persist with peripheral neuropathy whereas patients also may suffer from autonomic neuropathy as well as abnormal heart rate (Cha et al., 2016; Dafaalla, Nimir, Mohammed, Ali, & Hussein, 2016). Peripheral neuropathy can be both monodiabetic neuropathy and polydiabetic neuropathy. It affects the lower-extremity sensation which may cause pain and in extreme level cause lower-extremity ulceration (commonly in foot) when impaired with peripheral vascular function (Adler, Boyko, Ahroni, & Smith, 1999; Chiles et al., 2014). Peripheral neuropathy is the impairment of peripheral nervous system due to chronic high sugar level in blood and around 60% to 70% diabetes patients develop this inevitable condition, which leads to loss of sensation, numbness, and pain (Cho et al., 2017). Axon thickening that ultimately lead to axonal loss, basement membrane thickening, decreased nerve perfusion, disturbed capillary blood flow to C fibers are the characteristics of this life-threatening condition along with hypoxia, loss of pericytes, microfilaments loss (Cho et al., 2017; Kote, Bhat, K, Ismail, & Gupta, 2013). Diabetes and high sugar level in blood is the principal reason for diabetic peripheral neuropathy; however, there are several risk factors as well, such as age, cardiovascular disease (CVD), dyslipidemia, hypertension, tobacco use (Santoro et al., 2006). In addition, severe ketoacidosis and microalbuminuria also stimulates diabetic peripheral neuropathy (Carratu et al., 2008). Then again, parasympathetic nervous system that control the involuntary activities in the body damaged because of high blood sugar level cause diabetic autonomic neuropathy which remains mostly undiagnosed and not as frequent as peripheral neuropathy (Schofield & Hendrickson, 2017). It can be recognized by observing the dysfunctions of some body system such as cardiovascular, gastrointestinal systems. Diabetic autonomic neuropathy mostly coexist with peripheral neuropathy and causes cardiovascular complications almost 20% diabetic population (Emanuele & Emanuele, 1997). Moreover, it causes abnormal heart rate, dizziness and fainting, ejaculation problem in man, heartburn, increased rate of morbidity and mortality. Orthostasis, tachycardia, urinary tract infection, vaginal dryness are also considered as characteristic symptoms of diabetic autonomic neuropathy (Chico, Tomas, & Novials, 2005; Zilliox et al., 2011). Diabetes is the foremost

reason behind this life-menacing syndrome accompanied by high blood cholesterol, hyperglycemia, and hypertension (Dafaalla et al., 2016; Emanuele & Emanuele, 1997; Zilliox et al., 2011). A family history of diabetes and kidney disease, age, alcoholism and smoking are also found to aggravate this frightening condition (Allawi, 2017).

C. Diabetic nephropathy

Diabetic nephropathy is a severe syndrome, which steers to renal failure and seen both in type 1 and in type two diabetes patients. The very first stage of this complication is microalbuminuria, which ultimately results in albuminuria that is increased albumin level in urine leads to renal failure and progresses till the end stage of renal disease (ESRD) (Fagerudd, Pettersson-Fernholm, Riska, Gronhagen-Riska, & Groop, 2000). Terrifyingly, around 25% of the diabetes patients are having diabetic nephropathy and the rate is increasing 2% to 3% per year (Adler et al., 2003). The physiognomies of diabetic nephropathy are confusion, fatigue, nausea, vomiting, loss of appetite, swelling of organs, and persistent itching in the company of hypertension, increased urination and proteinuria (Li, Zheng, Chen, & Zhao, 2017; Nishi et al., 2000). Elevated sugar level in blood is the main reason of diabetic nephropathy, which is intensified by alcoholism, smoking, high blood cholesterol, hyperglycemia as well as Hypertension (Dafaalla et al., 2016; Pedro, Ramon, Marc, Juan, & Isabel, 2010).

1.3.2 Macrovascular Complications of Diabetes

Complications, which are related with the large blood vessels, are the macrovascular complications and people suffering from diabetes are more probable to persist with macrovascular complications (Donahue & Orchard, 1992). It can affect any part of the body like heart, lung etc. The mechanism of macrovascular complication is atherosclerosis that narrows the arteries (Sharma, Farmer, & Garber, 2011). Macrovascular complication arise due to inflammation in the arteries, which eventually results in the formation of atherosclerotic lesion with fibrous cap and if accidentally the lesion is raptured it may cause acute vascular infraction (van Wijngaarden et al., 2017).

A. Cardiovascular disease

Diabetes is an immense risk factor for cardio-vascular diseases and people with diabetes are at risk, which is almost four times greater than normal people (Skrha, 2001). CVD is the death

reason of about 70% people having diabetes as well as increase the risk of myocardial infraction (Sharma et al., 2011). Diabetes patients have five times greater risk of having first time MI and 2 times greater risk for patients who already had MI (Efimov, Gordienko, Slavnov, Sviatelik, & Kaminskii, 1977). Though it was predicted that CVD and diabetes were primarily present in the western world but recent studies claim that these conditions are rapidly spreading in lower income countries as well and assume that around 75% to 80% diabetes patients will die from CVD worldwide (El-Lebedy et al., 2016). Obesity, dyslipidemia, hypertension, hyperglycemia these are the risk factors for CVD, in the meantime diabetes itself is an independent risk factor (Skrha, 2001). All together, these factors increase the risk for CVD many times and eventually causes inflammation in the vascular endothelium, which results in macrovasculopathy, and CVD in diabetes patients (Jawalekar, Karnik, & Bhutey, 2013).

B. Cerebrovascular diseases

When there is lack of blood supply in brain that leads to cerebrovascular diseases, which include stroke, brain hemorrhage etc. (Abe, 2012). Stroke is the 3rd leading cause of death in USA after CVD and cancer and patients with diabetes have 4 to 5 times greater risk of stroke (Choi, Yu, Yoon, Kim, & Jeon, 2016). Diabetes itself is an independent risk factor for cerebrovascular diseases. Diabetes also promote the sudden death rate due to stroke (Oliveira, Gorz, & Bittencourt, 1988). When patients have previous stroke history and diabetes at a time, there is a chance that, that patient will be neurologically defected because of disturbance of blood supply into the brain due to atherosclerosis, which affects the intracranial and extra cranial blood circulation to the brain and eventually leads to stroke (Asplund, Hagg, Lithner, Strand, & Wester, 1979). Hyperglycemia, hypertension, heart failure, and atrial fibrillation these are the risk factors for cerebrovascular disease (Abe, 2012). However, though these factors are controlled, but still diabetes itself is a big challenge towards stroke. Diabetes related complication, for example, hyperlipidemia, hyperglycemia, and hyperinsulinemia are also some risk factors for stroke though their relationship is not clearly understood. Moreover, Diabetic retinopathy, microalbuminuria, proteinuria, hyperuricemia these complications also worsen the situation for cerebrovascular diseases (Francoeur, 2016).

C. Peripheral artery disease

Peripheral artery disease is the obstruction of blood flow to the lower-extremity arteries of the body, which eventually leads to pain and claudication while working or doing exercise and ultimately leads to disability (Ali, Ahmed, Bhutto, Chaudhry, & Munir, 2012; Cheung, Lam,

& Cheung, 2016). This is a major complication among diabetes patients and almost 3.5 million people in USA are affected in this disease (Yap, Chuang, Chien, & Tai, 2014). People with diabetes are in 15 times greater risk to suffer from this disease, which may at severe condition lead to foot ulceration and lower-extremity amputation (Taniwaki et al., 2001). As the number of diabetes patients are increasing, patients of this disease are also increasing and physical exercise is a place of hope for these patients (Y. Zou et al., 2017). Hyperglycemia, duration of diabetes are the main factors of this disease, however other risk factors include hypertension, obesity, smoking, dyslipidemia, physical inactivity etc. (Thiruvoipati, Kielhorn, & Armstrong, 2015).

1.4 Types of Diabetes

Determination of diabetes type is very important for treatment. For instance, a gestational diabetes mellitus (GDM) patient may persist diabetes even after delivery, then it will be considered as type 2 diabetes mellitus and the patient has to be treated accordingly. If physicians continue the treatment of gestational diabetes mellitus (GDM), it will lead to a hazardous condition. It is be said that, determination of diabetes types of individual patient depends on the situation of time of diagnosis (Kerner, Bruckel, & German Diabetes, 2014). Types of diabetes include gestational diabetes, type 1 diabetes and type 2 diabetes.

1.4.1 Gestational diabetes

Gestational diabetes is sort of diabetes that arises during the pregnancy and automatically restores after delivery and occurs among 2% to 5% pregnant women around the 24^{th} week of pregnancy (Gabbe, 1986). Pancreas of some pregnant woman do not produce sufficient amount of insulin for metabolic function which leads to this gestational diabetes; in the meantime, insulin resistance and hyperinsulinemia is observed in case of some pregnant woman that results in hyperglycemia (Carpenter & Coustan, 1982; Kerner et al., 2014). It occurs during pregnancy and solves after delivery, where earlier type 1 or type 2 diabetes are not prerequisite (Homko, Sivan, & Reece, 2004). The condition is just glucose intolerance for pregnant women, which was not seen before. During pregnancy, insulin resistance can take place for various factors like growth hormone alteration, lactogen and insulinase secretion by placenta (Simmons, Devers, Wolmarans, & Johnson, 2009). In addition, numerous forms of risk factors are concomitant with developing gestational diabetes, such as glycosuria, history of

macrosomia, hypertension, obesity, polycystic ovarian syndrome (Simmons et al., 2009). Age, race, BMI, family history of diabetes and GDM in previous pregnancy also elevate the chances of gestational diabetes (Lin, Mu, & Hsu, 2015; Simmons et al., 2009). However, there is no significant signs and symptoms of gestational diabetes, which makes the situation worse for pregnant women to manage this syndrome. Therefore, glycemic management is the cornerstone for the management of GDM along the nutritional diet and regular exercise (Adamikova, 2001; Bloomgarden, Stell, & Jovanovic, 2010). Blood glucose should ne monitor 4 times a day and if it is found that blood glucose level is not under control with diet and exercise, in that case oral hypoglycemic and if needed, insulin can be used to manage gestational diabetes (Kelly, 2008).

1.4.2 Type 1 diabetes

Relatively rare form of diabetes, which is correlated with beta cell destruction and insulin dependency, is type 1 diabetes or IDDM. Type 1 diabetes are of two categories:

Immune-mediated type 1 diabetes

This form of diabetes are in existence among 5% to 10% patients, which is insulin dependent and occurs due to the destruction of beta cell due to the autoimmunity (S. Y. Kim et al., 2012). The beta cell of the pancreas are destroyed due to cellular-mediated autoimmune destruction because of the presence of islet cell autoantibodies, autoantibodies of insulin, autoantibodies of glutamic acid decarboxylase, autoantibodies of tyrosine phosphatase. These autoantibodies are present in 85% to 90% patients when fasting glucose is detected; however, beta cell destruction rate is not parallel for all, it varies individual to individuals (Singh et al., 2016). The process is speedy for some patients and gentle for others. Some patients are present with ketoacidosis whereas others are with excessive fasting glucose. However, some patients persist some beta cell to fight against ketoacidosis but eventually loses its secretion and become totally insulin dependent. Patients with type 1 diabetes are more likely to suffer from some autoimmune disorders like Graves' disease, Hashimoto's thyroiditis, Addison's disease, vitiligo, celiac sprue, autoimmune hepatitis, myasthenia gravis, and pernicious anemia (Reichard & Pihl, 1994).

Idiopathic diabetes

It is remarkably a rare case of diabetes, which does not have any known etiology. Patients of these types are used to struggle with permanent insulinopenia and are susceptible to ketoacidosis (Kaneko et al., 2017). This form of diabetes are inherent and require insulin replacement therapy.

Risk factors and treatment of type 1 diabetes

Type 1 diabetes, the lingering autoimmune syndrome is thought to be prompted genetically as well as by environmental factors though the environmental factors are not yet evidently identified (Borchers, Uibo, & Gershwin, 2010). The major genetic contribution is the HLA complex mostly HLA class II causes type 1 diabetes in the company of geographical location of the patient and family history of diabetes (Antonela et al., 2017; Sahakyan, Klein, Myers, Tsai, & Klein, 2010). The changing environmental patterns are altering the genetic expression of people, which stimulates expression of diabetes at early age; therefore, management of type 1 diabetes is becoming an onus to us. Regular intake of insulin, hypoglycemic agents and regular exercise with proper diet is the keystone to manage type 1 diabetes, besides alcoholism and smoking should be avoided (Khardori, Oct 05, 2017).

Table 1.1: Types of Insulin

Types of Insulin	Onset of action	Peak conc.	Duration
Rapid acting			
Lispro	15-30 min.	30-90 min	3-5 hours
Aspart	10-20 min.	40-50 min.	3-5 hours
Glulisine	20-30 min	30-90 min	1-2.5 hours
Short acting			
Regular insulin	30 min. -1 hour	2-5 hours	5-8 hours
Intermediate-Acting			
NPH (N)	1-2 hours	4-12 hours	18-24 hours
Long acting			
Insulin detemir	1-2 hours	6-8 hours	Up to 24 hours

(Holterhus et al., 2007; Munshi et al., 2016)

1.4.3 Type 2 diabetes

Type 2 diabetes is the most recognized form of diabetes existent among 90% to 95% diabetes patients. There was 330 million type 2 diabetes patients over in 2011 and the number going to increase to 469 million by the year 2030 (Chamnan P., 2011). It is ranked as the 6th top death causing disease with million peoples' death of type 2 diabetes by the year 2016 (WHO, January, 2017). It is called the non-insulin dependent diabetes mellitus since the beta cell of the pancreas cannot produce sufficient volume of insulin as body's demand that leads to type 2 diabetes (Daubresse, 2001). On the other hand, the body become insulin resistant since the body cannot properly react to insulin yet plentiful insulin is produced by pancreas (Wiltshire et al., 2001). Various factors intensify the occurrence of this chronic syndrome such as age, race, heart disease, high blood pressure, acanthosis nigricans, obesity, physical inactivity, polycystic ovary syndrome (Unnikrishnan, Shah, & Mohan, 2016; Zou, Ye, Zou, & Yu, 2017). Furthermore, low level of HDL, family history of diabetes and depression also found to pledge occurrence of type 2 diabetes (Sharma et al., 2011). Management of this life-threatening condition is to prevent or slow the occurrence of complications associated with diabetes mellitus. Managements for type 2 diabetes involves smoking cessation, avoid alcoholism, regular exercise, losing weight, consuming insulin if needed and regular intake of oral hypoglycemic agents (American Diabetes, 2017; Kahkoska, Mayer-Davis, Hood, Maahs, & Burger, 2017).

1.5 Oral hypoglycemic

Oral hypoglycemic are chemical agents, which are used to control the blood glucose level of diabetes patients. These drugs are also called anti-hyperglycemic agents that act by increasing insulin secretion, promoting organs sensitivity towards insulin and sometimes reducing the absorption of glucose from the gastrointestinal tract into the blood (Pagkalos, 2011). There are six types of pharmacologically active oral hypoglycemic agents are present in the market and these are used as single drug and sometimes as a combination therapy (Grujic, Perinovic, & Rizvanbegovic, 1976).

A. Biguanides

These agents are the insulin sensitizer, means upsurges the body's sensitivity towards insulin (Prejac, 1963). The most common biguanides is Metformin, which is used to improve the

insulin sensitivity, suppress the glucagon production in liver (Romero et al., 2017; Seliger et al., 2017). Metformin is used basically in case of obese patients to increase fatty acid oxidation, reduce glucose absorption rate from GIT as well as, increase glucose uptake by phosphorylating GLUT-enhancer factor (Igel et al., 2016; Jackson et al., 1987). Moreover, a research revealed that metformin actives AMP-activated protein kinase that promotes gluconeogenic genes in liver (Zhou et al., 2001). Metformin is used as monotherapy, but it can be used as a combination with sulfonylureas agents. However, if the patients has renal failure syndrome, metformin may lead to lactic acidosis (Fimognari, Corsonello, Pastorell, & Antonelli-Incalzi, 2006).

B. Meglitinides

Meglitinides are the insulin secretagogues agents that stimulates the pancreas to release insulin (Quillen, Kuritzky, & Samraj, 1999). Repaglinide, nateglinide and glibenclamide are the agents of this class and repaglinide is the most potent one among them with five times more potency than other meglitinides (Rizzo, Barbieri, Grella, Passariello, & Paolisso, 2005). Meglitinides are taken orally immediately before the meal to control the postprandial hyperglycemia. These drugs are rapid acting oral hypoglycemic agents that act on the ATP-dependent K-channel of the beta cell in the pancreas and stimulate the release of insulin (Engelen et al., 2011; Rudovich et al., 2010). Since these agents are short acting (4-6hr), therefore chance of hypoglycemia is less.

C. Sulfonylureas

SU agents once were the first line antidiabetic drugs, which cause endogenous insulin secretion (Kunte et al., 2007). They were the oldest antidiabetic agents but their use is now condensed as they have greater rate of hypoglycemic events (Confederat et al., 2015). Glyburide and glipizide are the drugs of this class. Hypoglycemia is the most common adverse effect of SU agents as a result long acting SU agents as a monotherapy is suggested to avoid whereas short acting agents can be used. However, the use of SU monotherapies are condensed now a day but are used as a combination therapy with insulin sensitizer agents. Long-term use of SU agents are prohibited since there will be a chance of beta cell failure (Sawada et al., 2008). Weight gain is predicted to be another side effect due to anabolic effect of increased insulin secretion.

D. Thiazolidinediones

These are insulin sensitizer drugs, which functions through gene regulation binding with PPARγ (peroxisomes proliferator-activated receptor gamma), a protein that regulates the transcription of genes that regulate the fat and glucose metabolism (Ciaraldi & Henry, 1997; Grossman & Lessem, 1997). Pioglitazone and rosiglitazone are the drugs of this class, among them rosiglitazone is restricted by FDA since it causes cardiovascular events (Roehr, 2010). Moreover, pioglitazone has also some side effects like weight gain, peripheral edema etc. Thiazolidinediones group of drug should be avoided in case of heart failure patients, though it can be used in patients who has renal impairment (Tominaga et al., 1993).

E. Incretin mimetic

The chemical agents that which mimic the function of incretin hormones in our body and helps in insulin secretion, are incretin mimetic, which are injectable drugs to control the postprandial blood glucose level of patients to whom other oral hypoglycemic agents are not working (Fabreegas, 2008). There are two incretin hormones present in our body, one is glucose-dependent insulinotropic polypeptide (GIP) and another one is glucagon-like peptide (GLP-1). Mainly GLP-1 analogs are the basement of incretin mimetic therapy with no threat of hypoglycemic events (Suarez et al., 2014). On the other hand, these agents are predicted to have some beneficial effects on sleep, central nervous system, liver, heart as well as inflammation. Exenatide and liraglutide are the example of two GLP-1 Analog drug (Lam & See, 2006). They are used as monotherapy in addition, sometimes with other oral hypoglycemic agents (Lopez Simarro, 2014).

F. Alpha-glucosidase inhibitors

Alpha-glucosidase inhibitors are the chemical agents that obstruct the functions alpha-glucosidase enzymes, e.g. Maltase enzyme, thus postpone the glucose absorption from gut (Kalra, 2014). Though there is no evidence of benefits are observed to prevent mortality, morbidity and all other complications, but its effect on HbAc1 is comparable with biguanides and thiazolidinedione and better than sulfonylureas to some extent (Bolen et al., 2007). Acarbose and vigliose are example of this group of agents where acarbose is the most used. Their mechanism of action is delaying glucose absorption from gut, however recent studies suggest that, they also have metabolic effect in colon for starch fermentation (Luo, Wang, Imoto, & Hiji, 2001). Alpha-glucosidase inhibitors have very rare case of side effects like

hepatic impairment moreover, it do not cause weight gain and hypoglycemia, even at overdose. Therefore, alpha-glucosidase inhibitors can be used as a first line drug to treat type 2 diabetes mellitus to control the postprandial blood glucose level along with diet and exercise.

G. Dipeptidyl peptidase-4 inhibitor

Dipeptidyl peptidase-4 is a ubiquitous enzyme that promptly inactive GLP-1and GIP hormones which are responsible for almost 50% to 70% insulin secretion (Scheen, 2012). DPP-4 inhibitors inhibit the DPP-4 enzyme, and increase the insulin secretion from pancreas and promotes the glycemic control in case of type 2 diabetes patients (Ahren, 2007). This class of drugs are a new invention. Some of DPP-4 inhibitors are sitagliptin, linagliptin, vildagliptin, alogliptin etc. (Barnett, 2006). Their effectiveness is comparable with the other hypoglycemic agents present in current times; even sometimes, these drugs are more effective with very little chances of hypoglycemia (Crepaldi et al., 2007). They are expensive medication compared with other diabetes therapies in the market, used as monotherapy and sometimes as ad-on.

On the other hand, NMDA receptor are known to be present in the central nervous system maintaining neurotransmission and control neuronal function. NMDARs are also present in the pancreas, but their function there was not clear in earlier times (Rodriguez-Diaz & Caicedo, 2013). Recent studies have found that NMDARs are actually present in beta cell in islets of Langerhans of pancreas and reduce the insulin secretion (Marquard et al., 2015). NMDA receptors bind with glutamate and reduces insulin secretion from pancreas. Therefore, inhibition of NMDARs will result in the increased insulin production. Studies found that, NMDA antagonist enhanced the glucose stimulated insulin secretion as well as beta cell survival. Furthermore, NMDA antagonists prolong the duration of beta cell in depolarized state, increased glucose tolerance level as well as stimulated the effect of exendin-4 (Marquard et al., 2015). Therefore, it is predicted that, NMDARs antagonist will show effectiveness against type 2 diabetes mellitus. Ketamine, dextromethorphan (DXM), phencyclidine (PCP), methoxetamine (MXE), and nitrous oxide (N2O) are some common NMDAR agonist present in market and dextromethorphan is one of the most common among them.

The rationale of this project is to formulate a noble antidiabetic combination therapy with dextromethorphan and linagliptin. Since it is reviewed earlier, what diabetes is and what is its severity, how it affects different parts of the body like brain, eyes, kidney, liver, limbs. Risk

factors of diabetes are also discussed, how they can be treated that points are also given. Classification of diabetes are also discussed in details along with their risk factors and treatment. However, one thing to notice is that, until now there is no cure for diabetes. It is a lifetime disease. We can control diabetes, our blood glucose level by following rules and regulations, but regrettably, we cannot get rid of this death causing disease yet. There are several types of hypoglycemic agents are there in market but most of them has side effects and adverse effects. For example, treatment of diabetes started with sulfonylureas but they are no longer used alone for their adverse effects. Consequently, new drugs are replacing old drugs. For their not only side effects or adverse effects, patients are also becoming resistant to those old drugs for using a very long time. The latest approach to treat diabetes is DDP-4 inhibitors class of drug. They considered as the most potent drug to treat diabetes until now compared with other marketed oral hypoglycemic agents. On the other hand, a recent study found dextromethorphan having antidiabetic property. As a result, the approach of this project is to use dextromethorphan as an ad-on drug with linagliptin for better glycemic control than linagliptin alone.

Chapter 2

2. Motivation

Diabetes is the one of the ancient diseases in the world, as well as its medication timeline. The risk of diabetes is increasing day by day and becoming deadly; as a result, better treatment is the demand of time for peoples' safety. Throughout its long medication time, new drugs came into market and many became outdated, for example, sulfonylureas are rarely used now a day as a single therapy since they cause beta cell failure, hypoglycemia, heart attack, weight gain and stroke, though this group was the earliest treatment for diabetes (Evans, Ogston, Emslie-Smith, & Morris, 2006). Biguanides class of drug metformin was used to be used most, but unfortunately, it is found to be ineffective for many patients along with side effects. Moreover, thiazolidinedione group also associated with cardiovascular risks. To fight against all the odds, new medications are emerging and Dipeptidyl peptidase (DPP)-4 inhibitors are the latest advancement in the treatment line of diabetes. DPP-4 enzymes inactive incretin hormones, which results in, delayed insulin secretion; therefore, DPP-4 inhibitors promote the insulin secretion eventually postprandial blood glucose control (Barnett, 2006). However, still DPP-4 inhibitors also have some side effects and ineffective in some cases, therefore, need of antidiabetic combination therapy arise.

2.1 Combination therapy

Addition of two or more active pharmaceutical ingredients (APIs) in a single dosage form is defined as combination therapy, which is also called fixed dose combination (FDC) (Hall, 1997). When single dosage form or monotherapies are ineffective, shows hazardous side effects, then necessity of combination therapy arise to avoid those unwanted circumstances. For example, paracetamol and caffeine are a combination drug to treat pain. Combination therapies were designed to target single disease but now a day the concept is changed and they are used to target multiple condition. These conditions are maybe related with one another and since these related conditions are similar for almost every patients; therefore, combination therapies are produced in mass scale in recent times (Bell, 2013). However, combination therapy is not a very new concept since they are found to be present in 60s and WHO included seven FDC medicine in their essential medicine list among 240 medicines in the year 1982 and 18 FDCs among 314 medicines in the year 2013 it their 14^{th} EML (Bell, 2013). As a result, it

is understood that, combination therapies are very common scenario and are approved by FDA. There are several advantages of combination therapies involving, better medication compliance, increased efficacy, increased synergistic effect in addition to less side effects. FDC reduces the dosage required per day and cost of the therapies (Hall, 1997). In spite of these advantages, FDCs are not beyond shortcomings like altered pharmacokinetics, increased cost, increased toxicity or adverse effects, difficulty to identify the cause of adverse reaction along with dose ratio inflexibility (Bell, 2013; Hall, 1997).

To overcome these issues regarding combination therapies, FDA policy states that two ingredients can be combined to form combination dosage form when two of them will have claimed therapeutic effect, two of them are compatible, their dosage form is safe and effective for patients, and has therapeutic effects that is claimed in the label (Crout, 1974). Combination therapies are sometimes considered as the standard treatment for various critical situation to reduce mortality and morbidity. Studies have found that combination therapies are beneficial in many chronic clinical disorders like cardiovascular diseases, pulmonary diseases, pain diseases, malignancies, rheumatoid arthritis, neurologic diseases, infectious diseases etc. (Bell, 2013). Chemotherapies now a day are also present in combination form. Combination therapy to treat diabetes mellitus is may be the most frequently used FDC available in market. Most frequent use of antidiabetics are maybe due to some benefits e.g., better glycemic control, cost reduction, less side effects, patients compliance moreover both insulin secretagogues and insulin sensitivity activity can be achieved by means of using combination antidiabetic medications (Schernthaner, 2010). Patients sometimes may require additional APIs to treat diabetes related complications. For instance, aspirin to prevent cardiovascular risks, ARBs to prevent renal disorders etc.

Table 2.1: some examples of currently available combination oral antidiabetic medicines in market

Combination Drugs	Available Doses
Saxagliptin + metformin (Pfutzner et al., 2011)	5 mg + 500 mg 2.5 mg + 1000 mg 5 mg + 1000 mg
Glipizide + metformin (METAGLIP, 2009)	2.5 m + 250 mg 2.5 mg + 500 mg 5 mg + 500 mg
Rosiglitazone + glimepiride (AVANDARYL, 2009)	4 mg + 1 mg 4 mg + 2 mg 8 mg + 2 mg
Vildagliptin + metformin (Rombopoulos, Hatzikou, Athanasiadis, & Elisaf, 2015; Ved & Shah, 2013)	50 mg + 500 mg 50 mg + 850 mg 50 mg + 1000 mg
Pioglitazone + metformin (Vanderpoel, Hussein, Watson-Heidari, & Perry, 2004)	30 mg + 50 mg
Pioglitazone + glimepiride (DUETACT, 2007)	30 mg + 2 mg 30 mg + 4 mg
Mitiglinide + metformin (Jung et al., 2012)	10 mg + 500 mg
Empagliflozin + linagliptin (Lewin et al., 2015)	10 mg + 5 mg 25 mg + 5 mg
Glyburide + metformin (Chien et al., 2007)	2.5 mg + 500 mg 5 mg + 500 mg
Glibenclamide + metformin (Gonzalez-Ortiz et al., 2009)	5 mg + 500 mg
Glimepiride + metformin (Gonzalez-Ortiz et al., 2009)	1 mg + 500 mg 2 mg + 500 mg
Rosiglitazone + metformin (Bailey et al., 2005)	4 mg + 2 g
Sitagliptin + metformin (Green & Feinglos, 2008)	100 mg + 1000 mg 100 mg + 2000 mg
Acarbose + metformin (Joshi et al., 2014)	50 g + 500 mg

2.2 A Novel Antidiabetic Combination

Sulfonylureas were the prior drug to treat diabetes in the beginning and was the most widespread treatment before 1995 worldwide but hypoglycemia was a severe risk in case of SU drugs since SU agents increase the insulin secretion (Sawada et al., 2008). Therefore, after 1995, biguanides agent metformin became popular but still it affects the beta cells and causes to beta cell failure (Igel et al., 2016). There were no drugs to improve the function and health of beta cell until the function of incretin hormones were discovered (Holst & Gromada, 2004). Failure of incretin hormones leads to beta cell failure, which can be prevented by DPP-4 enzyme inhibitors (Ahren, 2007).

Incretin hormones are responsible for maintaining the postprandial glucose hemostasis in the body. Glucagon like peptide GLP-1 and gastric insulotropic peptide GIP are the two major hormones present in the body that maintains the postprandial blood glucose level and insulin secretion. GIP is secreted from stomach and proximal small intestine with half-life of 5 minutes for diabetes patients whereas; GLP-1 is secreted from distal portion of the small intestine with half-life of 2 minutes (Yabe & Seino, 2011). Dipeptidyl peptidase DPP-4 enzymes rapidly degrade both of the hormones. Therefore, agents that will prevent the DPP-4 enzymes eventually will regulate the body's glucose and insulin level (Pratley & Salsali, 2007).

To get rid of all the complications mentioned, a new class of drug was the demand of time and GLIPTINs were discovered as the hope. Gliptins are the new class of antidiabetic drug present in market, which maintains the insulin secretion without affecting the health of beta cell in pancreas (Chahal & Chowdhury, 2007). During hyperglycemia 50% beta cell functions are lost within 3 years of treatment with other antidiabetic therapies; as a result, gliptins are the choice of drugs to treat type 2 diabetes mellitus (Scheen, 2015). Alogliptin, Linagliptin, Saxagliptin, Sitagliptin, Vildagliptin are the available gliptins in the market.

These gliptin family is used both as monotherapy and combination therapy. As a monotherapy, gliptins showed equivalent effectiveness and less side effects, sometimes better effect than current medications available in market (Sliva & Prazny, 2014). Gliptins reduce fasting glucose level around (10-35) mg/dl, postprandial glucose level around (20-60) mg/dl and glycated hemoglobin HbA1c level almost (0.4-1.2) % (Chahal & Chowdhury, 2007). A recent study suggest that, gliptins family when used as monotherapy, they keep the HbA1c level below 7%,

which is a desired goal for type 2 diabetes patients (Ahren & Schmitz, 2004). Gliptins are also used as combination therapy like used as ad-on agents with metformin, SU agents, thiazolidinedione, and even with insulin. Nevertheless, when gliptins are combined with other antidiabetic therapies, those agents cause side effects, which then affects the patients' health. Moreover, gliptins are expensive drug that leads to a higher price of combination therapy (Baptista, Teixeira, Romano, Carneiro, & Perelman, 2016).

A novel approach is made to combine gliptins with other agents than current antidiabetic therapies to overcome the side effects and reduce the price of medicines. NMDA receptors, which are present in the pancreas, control the insulin secretion through K+ channels. If, NMDARs can be blocked with antagonist in pancreas, insulin secretion will be promoted (Collison et al., 2016). A recent study has found that, most common antitussive agent dextromethorphan has NMDA antagonistic effects, which promotes the insulin secretion (Sargent, 2015). Therefore, a research team in Germany conducted a research to see the effect of dextromethorphan as ad-on agents with a gliptin, Sitagliptin. The result was promising and glycemic control was as more effective than sitagliptin monotherapy; moreover, side effects with dextromethorphan and sitagliptin were negligible compared with other available therapies (Marquard et al., 2016).

Sitagliptin is a non-peptidomimetic gliptin drug that lowers the blood glucose level significantly compared with other oral hypoglycemic. However, sitagliptin in combination with NMDAR antagonist dextromethorphan has shown better result and lowered the maximum blood glucose concentration in a clinical trial. Sitagliptin is recommended 100mg/day for type 2 diabetes patients, however in addition with dextromethorphan 60 mg maximum effectiveness found in a randomized, placebo-controlled, double-blinded, multiple crossover, and single-dose clinical trial.

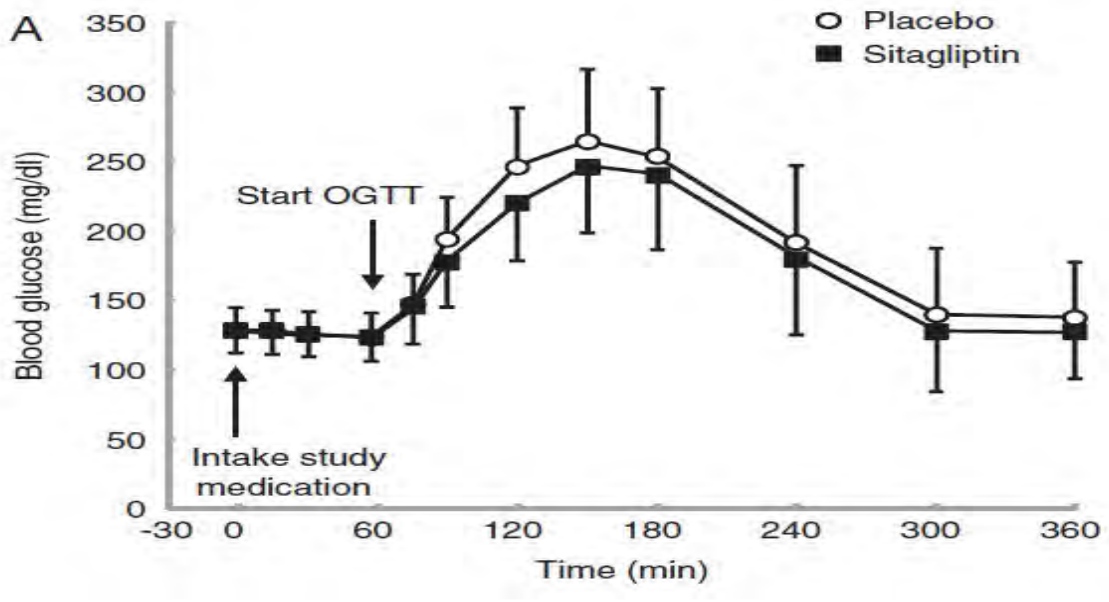


Fig 2.1: Comparison between placebo and sitagliptin (Marquard et al., 2016)

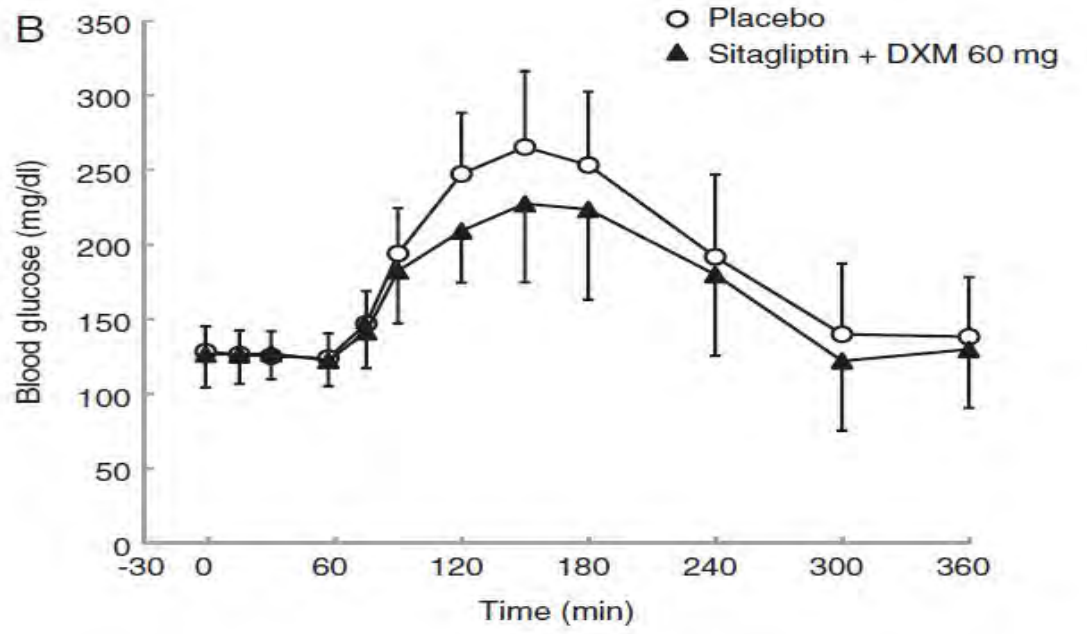


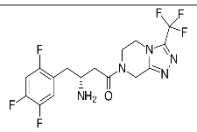
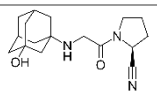
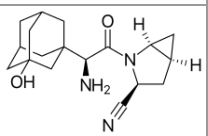
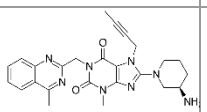
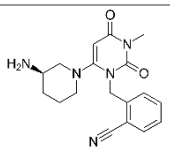
Fig 2.2: Comparison between placebo and sitagliptin + DXM (Marquard et al., 2016)

The study found that, adding of little amounts of dextromethorphan with sitagliptin increased the postprandial insulin level during OGTT test and reduced the blood glucose level without causing any event of hypoglycemia or hyperglycemia. Postprandial insulin secretion increased strongly during the first 30 minutes after oral administration of sitagliptin and dextromethorphan. The findings of the study highlighted that, addition of dextromethorphan

with sitagliptin increased insulin level by targeting pancreatic beta cell and NMDRAs, which functions by stimulating different glutamate-dependent molecular mechanisms. Therefore, the research suggest that, dextromethorphan as an ad-on with DPP-4 inhibitors based therapies can be the future of type 2 diabetes treatment.

Gliptin family show similar type of anti-hyperglycemic property, but they have various differences based on their pharmacokinetic, pharmacodynamics profile, adverse reactions, price etc.

Table 2.2: Comparison among sitagliptin, vildagliptin, saxagliptin, linagliptin and alogliptin

Criteria	**Sitagliptin**	**Vildaglipti n**	**Saxagliptin**	**Linagliptin**	**Alogliptin**
Chemical name & formula	(3R)-3-amino-1-[3-(trifluoromethyl) -6,8-dihydro-5H-[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one $C_{16}H_{15}F_6N_5O$	(2S)-1-[2-[(3-hydroxy-1-adamantyl) amino]acetic] pyrrolidine-2-carbonitrile $C_{17}H_{25}N_3O_2$	(1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2-azabicyclo [3.1.0]hexane-3-carbonitrile $C_{18}H_{25}N_3O_2$	8-[(3R)-3-aminopiperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]purine-2,6-dione $C_{25}H_{28}N_8O_2$	2-[[6-[(3R)-3-aminopiperidin -1-yl] -3-methyl-2,4-dioxopyrimidin-1-yl] methyl] benzonitrile $C_{18}H_{21}N_5O_2$
Structure					
Class	Non peptido-mimetic	Peptido-mimetic	Peptido-mimetic	Non peptido-mimetic	Non peptido-mimetic

Metabolism	Hepatic Rapid metabolism	Hepatic (hydrolysis)	Hepatic Rapid metabolism	Hepatic Minimal metabolism, 10%	Hepatic Limited metabolism
Elimination	Renal	Renal	Renal	Feces	Renal
Biological half life	8-14 hours	2-3 hours	2.5 hour for saxagliptin	24 hours	12-21 hours
Bioavailability	87%	85%	75%	30%	100%
Dosing	100mg/day	50mg twice/day	5mg/day	5mg/day	25mg/day
Drug interaction	NSAIDs Sulfa drugs	ACE inhibitors Sympathomimetic drugs	Nefazodone Quinolone antibiotics	Bexarotene Gatifloxacin	Alprazolam Acetylsalicylic acid
Side effects	Weight gain Headache Infection	Weight gain, Foot ulcers, Fluid retention	Angioedema Pancreatitis	Pancreatitis at overdose	Hypoglycemia Joint pain Sore throat
Molecular mass	407.314 g/mol	303.339 g/mol	315.41 g/mol	472.54 g/mol	339.39 g/mol
Protein binding	37%	9.3%	Negligible	75–99%	20%
Price	590 $/kg	2010 $/kg		2198 $/kg	3012 $/kg

(Deacon, 2011; Drugbank; Gupta & Kalra, 2011; Martin, Deacon, Gorrell, & Prins, 2011; Pubchem)

As per the research, all the gliptins can be used with dextromethorphan as combination therapy (Marquard et al., 2015). However, these gliptins have differences among themselves based on their efficacy, molecular characteristics, price, and pharmacokinetic profile. Linagliptin is the drug, which is considered as the most potent DPP-4 inhibitors until now which is structurally different from other DPP-4 inhibitors with a xanthine base in it, that increases its terminal half-life (Freeman, 2011). Considering the differences given in table 2, linagliptin is found to be the

most appropriate drug with less side effect and drug interaction; moreover, no dose adjustment is required for renal and hepatic impairment patients. Therefore, hypothesis of this project work is that, DPP-4 inhibitor linagliptin in combination with NMDARs antagonist dextromethorphan can be an innovative treatment to fight against type 2 diabetes.

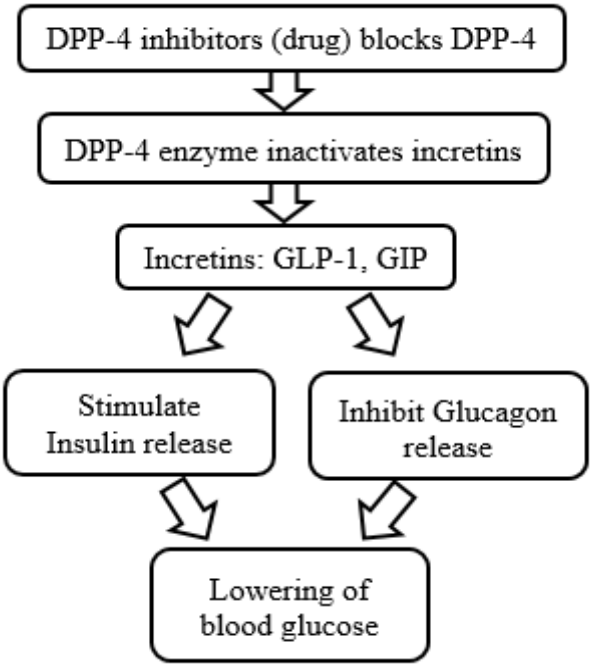
Chapter 3

3. Linagliptin

Linagliptin is a DPP-4 inhibitor; incretin analog which increases the level of incretin hormones GLP-1 and GIP by preventing degradation, which are responsible for the secretion of insulin from pancreas. DPP-4 inhibitors are the new class of antidiabetic drug, which are considered as more effective than other oral hypoglycemic agents. Among all DPP-4 inhibitors, linagliptin is the most potent drug manufactured yet (Gupta & Kalra, 2011). Until now, it is a patented compound and was approved by US FDA in the year 2011 for linagliptin 5mg once daily dose (FDA, 2011). It is an oral hypoglycemic agent that shows apparently no side effects or adverse effects; but in severe cases, it may cause pancreatitis, headache, sore etc. (Del Prato, Patel, Crowe, & von Eynatten, 2016). However, linagliptin monotherapy has negligible side effects but in combination with biguanides, it may lead to renal impairment (Ross et al., 2015). Linagliptin can be taken with food or without food (Gupta & Kalra, 2011). Its maximum bioavailability is 30% whereas excessive fatty food reduces its C_{max} by 15% (Retlich et al., 2010). Linagliptin helps to main the incretin level as normal throughout the day, nevertheless after meal is taken, it increase the level of incretin hormones, which eventually increase the insulin secretion and maintain the body insulin hemostasis (Del Prato et al., 2016). It is used to treat type 2 diabetes only, but not effective for treating type 1 diabetes.

3.1 Mechanism of action

Incretin hormones are responsible for retaining the blood glucose hemostasis in the body. Two major incretin hormones are GLP-1 glucagon-like polypeptide and GIP glucose-dependent insulinotropic polypeptide and both of the hormones enhance the biosynthesis of insulin and their release from pancreas; in addition, GLP-1 reduces the secretion of glucagon from alpha cells of pancreas, which results in decreased glucose output in liver (Yabe & Seino, 2011). DDP-4 is an enzyme that damage both the incretin hormones which results in increased blood glucose level (Ahren et al., 2004). Linagliptin is the drug that inhibits the DPP-4 enzymes and this activity ultimately results to the increased secretion of insulin from beta cell of pancreas and reduced secretion of glucagon, resulting in blood glucose hemostasis (Tradjenta, 2011).



3.2 Chemical structure

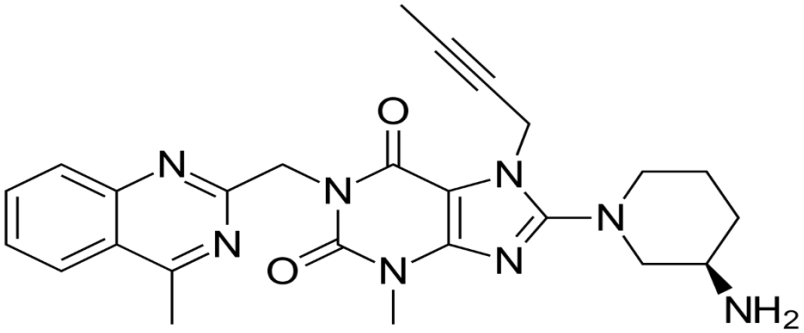


Fig 3.1: Chemical structure of linagliptin

Chemical structure of linagliptin is different from other DPP-4 inhibitors with the presence of a xanthine base (Deacon & Holst, 2010). The presence of xanthine base results in long terminal half-life (100 hours), whereas sitagliptin's and vildagliptin's terminal half-life is 2.5 hours and 12.4 hours respectively (FDA, 2011). The structure also makes it excellently selective for DPP-4 enzymes compared with DPP-8 (>40,000 fold) and DPP-9 (>10,000 fold) (Kalra, Unnikrishnan, Agrawal, & Singh, 2011). Moreover, the structure made linagliptin non-peptidomimetic class of drug (Gupta & Kalra, 2011).

3.3 Chemical properties

Table 3.1: Chemical properties of linagliptin

Criteria	Properties
Name	Linagliptin, trajenta
IUPAC name	8-[(3R)-3-aminopiperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]purine-2,6-dione
Molecular Formula	$C_{25}H_{28}N_8O_2$
Molecular weight	472.54 g/mol
Surface area	114 A^o
Charge	0
Color	Yellowish
Melting point	202 C^0
Water solubility	1mg/mL
LogP	2.85
Dissociation constant pKa	8.6

(Drugbank; Pubchem; Smelcerovic et al., 2015)

3.4 Pharmacodynamics profile

The *in vitro* potency of linagliptin was evaluated by Thomas and colleagues, the result found that linagliptin is more potent than other DPP-4 inhibitors approved by FDA. Research found half-inhibitory concentration (IC_{50}) of linagliptin was 1 nM, whereas sitagliptin's and saxagliptin's 19 nM and 50 nM respectively (Thomas et al., 2008). In one clinical trial with healthy male person, linagliptin revealed dose-dependent inhibition of blood glucose over 24 hours with a 5mg/day dose, 86% of the inhibition of the DPP-4 enzyme; whereas ≥80% enzyme inhibition results in maximum blood glucose reduction (Cox, Rowell, Corsino, & Green, 2010; Forst & Pfutzner, 2012; Huttner, Graefe-Mody, Withopf, Ring, & Dugi, 2008).

3.5 Pharmacokinetics profile

Table 3.2: Pharmacokinetics of Linagliptin

Criteria	Properties
Bioavailability	30%
C_{max}	1.5 hours
Protein binding	70%-80%
Half-life: therapeutic	12 hours, elimination: 131 hours
Metabolism	Hepatic (not widely metabolized)
Elimination	Hepatic 80% and renal 20%
Volume of distribution	1110 L
Clearance	70ml/min

(Blech, Ludwig-Schwellinger, Grafe-Mody, Withopf, & Wagner, 2010; Heise et al., 2009; Huttner et al., 2008; Retlich et al., 2010)

3.6 Efficacy

The efficacy of linagliptin was found very effective in two clinical trial conducted for 12 weeks and 24 weeks. The clinical trial found that, linagliptin was more effective in reducing HbA1c, glycated hemoglobin; placebo adjusted mean changes were -1.1%, -0.71%, -0.55% and -0.57% for HbA1c baseline ≥ 9%-8%. ≤ 9%- 7.5%, ≤8% and ≤7.5% and level of significance were P<0.000, P<0.0001, P<0.005 and P <0.0001 respectively (Del Prato et al., 2011). Moreover, linagliptin was found to be more successful controlling the fasting plasma glucose, the adjusted mean changes were found to be reduced -1.3 mmol/L. It also reduced postprandial glucose level 2 hours after meal, -3.2 mmol/L (Del Prato et al., 2011; Kawamori et al., 2012). Volunteers had taken 5mg linagliptin were found to have HbA1c level below 7% after 24 weeks nearly 25%; whereas with placebo control HbA1c with below 7% after 24 weeks only 11.6% (Del Prato et al., 2011). In another double-blinded study with 5mg linagliptin oral therapy found reduction of HbA1c level -0.4% with linagliptin whereas -0.1% with placebo and fasting plasma glucose reduction was 0.5mg/dL (FDA, 2011).

3.7 Adverse effects

Linagliptin is a DPP-4 inhibitor with less number of side effects. Less than 5% patients reported adverse effects against this new antidiabetic drug (FDA, 2011). However, hypoglycemia is the most common adverse reaction of linagliptin reported (Schernthaner et al., 2012). Hypoglycemia occurred only when used with sulfonylurea as a combination drug. Since linagliptin causes glucose dependent insulin secretion, as a result there is least chance of occurring hypoglycemia. Pancreatitis was another adverse effect reported against linagliptin medication (Schernthaner et al., 2012). Only 1 person among 538 patients face pancreatitis approximately. Moreover, other less frequent adverse reaction reported against linagliptin are diarrhea, back pain, headache, hypertension and infections such as nasopharyngitis, urinary tract infection, and upper respiratory tract infections (Gomis et al., 2012).

3.8 Drug interaction

When used with a strong CYP3A4 or P-glycoprotein inducer (rifampin), the efficacy of linagliptin is reduced (FDA, 2011). Sulfonylurea group of antidiabetic drugs need extra precaution when combined with linagliptin. Moreover, simvastatin, digoxin, glyburide, warfarin, metformin, and pioglitazone do not alter the efficacy of linagliptin when administered in parallel (Graefe-Mody et al., 2011; Moon et al., 2017).

To sum up, linagliptin is the new DPP-4 inhibitor with promising effectiveness and safety profile, which lowers HbA1c level almost 0.4% to 0.7%. In addition, dose adjustment is not required for renal impairment patients, no chance of weight gain and slightest chance of hypoglycemia makes linagliptin more potent than any other antidiabetic medication available in market, which has a very long half-life resulting in 24 hours DPP-4 inhibition.

Chapter 4

4. Dextromethorphan

Dextromethorphan is one of the most common antitussive drugs used worldwide, which belongs to morphine class with sedative and stimulant property at higher doses. However, its marketed forms are sometimes used for recreation that leads to misuse of this drug. Nevertheless, dextromethorphan is used not only for antitussive property but it has some other important pharmacological activity such as treating pain, epilepsy , neuroprotection for acute brain injury or mild stroke and neurodegenerative disorders (Kimiskidis et al., 1999; Taylor, Traynelis, Siffert, Pope, & Matsumoto, 2016). Combination of dextromethorphan with quinidine for treating pseudobulbar was approved by FDA in the year 2010 (Nguyen et al., 2016). A recent study has proved that dextromethorphan acts as a NMDA antagonists and promotes insulin secretion from pancreas (Sargent, 2015). Though dextromethorphan has various side effects nausea, dizziness, constipation, confusion, nervousness, euphoria and chance of dependency (Ziaee et al., 2005), but its controlled use with combination with other hypoglycemic agents may lead to a new stream to treat type 2 diabetes.

4.1 Mechanism of action

Glutamate is a neurotransmitter with glutamate receptors in the pancreatic islet cells and it is known that beta cell produce glutamate by glucose metabolism (Cabrera et al., 2008). Glutamate binds with receptors both intracellularly and extracellularly (Di Cairano et al., 2011). Extracellular binding involves binding with ionotropic and metabotropic receptors where ionotropic receptors are NMDARs, AMPARs and kinate receptors and metabotropic receptors are G-protein coupled receptors (Kalia, Kalia, & Salter, 2008). When glutamate binds with NMDARs in islet cell, it causes depolarization of membrane cells, opening of K+ channel and release of Mg+ which results in glucagon secretion from alpha cells with positive feedback mechanism and inhibition of insulin secretion (Cabrera et al., 2008); however NMDA receptors activation require binding with glutamate, glycine or D-serine. Insulin secretion from beta cell require closure of K+ channels and depolarization of membrane cells (Hatlapatka, Willenborg, & Rustenbeck, 2009). Therefore, NMDAR antagonist is found to be potential for the secretion of insulin. A NMDARs antagonist dextromethorphan binds noncompetitively with NMDA receptors in place of glycine and results in the closure of K+ channels what is required for insulin secretion from beta cell (Marquard et al., 2015).

4.2 Chemical properties

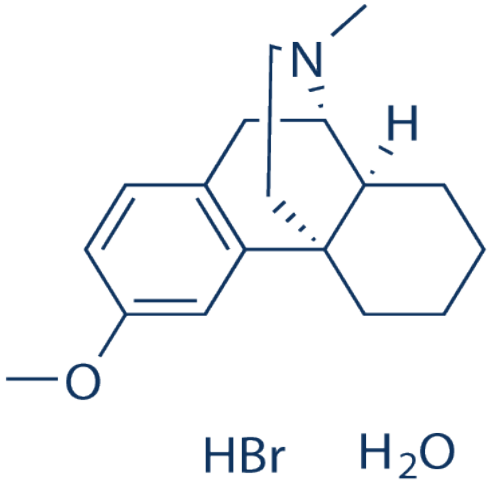


Fig 4.1: Chemical structure of dextromethorphan HBr

Table 4.1: Chemical properties of Dextromethorphan HBr

Criteria	Properties
Chemical name	Dextromethorphan HBr
Molecular formula	$C_{18}H_{28}BrNO_2$
Molecular weight	370.331 g/mol
Surface area	12.47 Aº
Charge	0
Water Solubility	1.5gm/100mL
LogP	3.75
pKa	9.89

(Drugbank; PubChem)

4.3 Pharmacokinetics

Table 4.2: Pharmacokinetics of dextromethorphan HBr

Criteria	Properties
Bioavailability	11%
C_{max}	1.6 to 1.7 hours
Half-life	2 to 4 hours
Metabolism	Hepatic
Elimination	Renal
Volume of distribution	No data available
Onset of action	30 minutes
Elimination half-life	4 hours

(G. L. Dickinson et al., 2007; Duedahl et al., 2005; Silvasti et al., 1987)

4.4 Therapeutic effect on type 2 diabetes

Dextromethorphan as a NMDARs antagonist promotes the insulin secretion from beta cells of pancreas though noncompetitive binding with glutamate receptors in the islet of Langerhans in pancreas. Moreover, clinical study found that, dextromethorphan protect pancreatic islets from cytokine induced cell death and promotes beta cells health (Marquard et al., 2015). Therefore, the study suggests that inhibition of pancreatic NMDARs can be an upright option to treat type 2 diabetes mellitus.

4.5 Drug interactions

Alcohols and CNS depressant drugs should not be taken with dextromethorphan as well as with serotonin reuptake inhibitors (e.g. Citalopram, Paroxetine) (Dy, Arcega, Ghali, & Wolfe, 2017; Schutz & Soyka, 2000). Moreover, concurrent use with monoamine oxidase inhibitors arise toxicity that in severe level results in death (Sinclair, 1973).

In spite of everything, considering all the data and effects of dextromethorphan, the hypothesis of this project is dextromethorphan can be used as an ad-on agent with linagliptin to treat type 2 diabetes to get better clinical effectiveness as well as safety. Moreover, addition of dextromethorphan will reduce the amount of linagliptin in the formulation that will result in cost reduction of the therapy.

Chapter 5

5. Materials and Methods

The very first step for developing any formulation is doing preformulation study to validate ingredients' physicochemical properties, which will enhance the rate of success of formulation. The purpose of doing preformulation study was to generating data, which will help in developing a stable, safe, bioavailable and acceptable formulation. The preformulation studies include physical appearance, particle size, bulk density and tapped density, angle of repose, dissolution profile and interaction.

5.1 Materials and equipment

Table 5.1: Materials used

Materials	Justification
Linagliptin	Active pharmaceutical ingredient
Dextromethorphan HBr	Active pharmaceutical ingredient
Monopotassium phosphate	Buffer preparation
Dipotassium phosphate	Buffer preparation
Mannitol	Diluent
Sodium starch glycollate	Disintegrating agent
Povidone K-30	Binder
Magnesium stearate	Lubricant

The materials used in this project were obtained from Eskayef Pharmaceuticals Limited, as gifts. Materials include linagliptin and dextromethorphan HBr where linagliptin is an antidiabetic drug and dextromethorphan HBr is antitussive agent. Moreover, Monopotassium phosphate (KH_2PO_4), Dipotassium phosphate (K_2HPO_4), mannitol, starch, povidone K-30 and magnesium stearate were used to check compatibility as excipients.

Table 5.2: Equipment used

Equipment name	Model
Electronic balance	OHAUS (pioneer) PA123
Electromagnetic sieve shaker	EMS-8
pH meter	HI 2211 pH/ORP meter
Dissolution apparatus	Logan instrumental UDT-804
UV spectrophotometer	Shimadzu, 1800 Hitachi, U-2910
Tap density tester	EDT-1020, Electro lab

5.2 Physical appearance analysis

Both linagliptin and dextromethorphan HBr were checked for their physical appearance. They were checked for their color visually, tested for their odor and taste. The results were found to be satisfactory and their physical properties were as same as specified in the standards.

5.3 Particle size analysis using sieve analyzer

Particle size is the analysis of characterizing particles distribution according to their diameter. Particle size analysis is of great importance in pharmaceutical industry since it affects physical stability, drug uniformity, release kinetics, dissolution, absorption, in vivo drug distribution as well as therapeutic action in the body (Khadka et al., 2014). Therefore, size distribution of both linagliptin and dextromethorphan were tested alone, as well as mixing them; by using Electromagnetic sieve shaker EMS-8.

A. Particle size distribution of Linagliptin

Particle size distribution was done by following USP General Test <786> Method I. 3 sieves were used to see the particle size distribution, and they were sieve of mesh 20, 30, 40 and 60. Accurately weighed 2gm linagliptin dry power was placed at the top sieve, which was a mesh 20 sieve and the lid was fixed at the top. Then the sieves were agitated for 5 minutes, sieves were removed and powders on the sieves were weighed carefully to determine distribution according to the size. In order to get a precise result, the process was done for 3 times.

B. Particle size distribution of Dextromethorphan HBr

Particle size distribution was done by following USP General Test <786> Method I. Accurately weighed 5gm dextromethorphan HBr solid powder was placed at the top sieve of mesh 20. Then the sieves were agitated for 5 minutes, sieves were removed and powders on the sieves were weighed carefully. The process was done for 3 times to ensure the precision of the test.

C. Particle size distribution of Linagliptin + Dextromethorphan HBr

Particle size distribution was done by following USP General Test <786> Method I. Accurately weighed 2gm linagliptin and 5gm dextromethorphan HBr was mixed uniformly using mortar pastle. Total 7gm of mixed powder was placed at the top sieve of mesh size 20 and agitated for 5 minutes. Afterward, sieves were removed carefully and powders left on the sieve was weighed. The process was done for three times to ensure precision.

5.4 Bulk density, Tapped density and Angle of repose

Bulk density is the ratio between mass of the powder and the untapped volume of that powder including the interparticulate void volume. Bulk density of any powder depends on powder's spatial arrangement on the powder bed. On the other hand, tapped density of powders are the ratio between the powder mass and the volume occupied after tapping. Tapped density is the increased bulk density. Angle of repose, tapped density and bulk density are important determinants of powder flow ability and compressibility, eventually the important determinants of the manufacturing of solid dosage forms (Sousa e Silva, Splendor, Goncalves, Costa, & Sousa Lobo, 2013).

A. Bulk density and Tapped density of Linagliptin

4gm of solid linagliptin powder was weighed accurately and poured into the graduated 100ml cylinder and the height of powder in the cylinder was measured to determine the bulk density of the power. Afterwards, the tapping machine was tuned for 250 taps per minutes and total 1250 taps carried out. Finally, the height of the powder in the cylinder measured to determine the tapped density of linagliptin.

B. Bulk density and Tapped density of Dextromethorphan HBr

15gm of dextromethorphan HBr powder was weighed accurately and poured into a 100ml graduated cylinder. The height of powder in the cylinder was measured to calculate the bulk density. Afterwards, the tapping machine was tuned for 250 taps per minutes and total 1000 taps carried out. Lastly, the height of the powder in the cylinder measured to determine the tapped density of linagliptin.

C. Bulk density and Tapped density of Linagliptin + Dextromethorphan HBr

5gm linagliptin and 15gm of dextromethorphan HBr were accurately weighed and poured into a 100ml graduated cylinder. The height of powder in the cylinder was measured to calculate the bulk density. Later, the tapping machine was tuned for 250 taps per minutes and total 1000 taps carried out. Last of all, the height of the powder in the cylinder measured to determine the tapped density of linagliptin.

D. Angle of repose Linagliptin

4gm of accurately linagliptin powder was poured onto a paper using a funnel and height (h) of the pile was calculated. A circle around the pile of linagliptin powder was drawn using a pencil and powders were removed. Afterwards, two diameter were drawn from two different sites, their average was taken and radius (r) was calculated. The same procedure was done for three times to ensure precision. Angle of repose was calculated using the equation

$$\tan \theta = \frac{h}{r}$$

E. Angle of repose Dextromethorphan HBr

12gm of accurately dextromethorphan HBr powder was poured onto a paper using a funnel and height (h) of the pile was calculated. A circle around the pile of linagliptin powder was drawn using a pencil and powders were removed. Afterwards, two different diameters were drawn from two different sites, their average was taken and radius (r) was calculated. The same procedure was done for three times to ensure precision. Angle of repose was calculated using the equation

$$\tan \theta = \frac{h}{r}$$

F. Angle of repose Linagliptin + Dextromethorphan HBr

2gm linagliptin and 8mg dextromethorphan HBr powder was properly mixed in a mortar pastel and poured onto a paper, using a funnel. Height of the pile was measured as well as a circle was dawn around the pile. Afterwards, two different diameters were drawn from two different sites and their average were taken to calculate the radius. The same procedure was done for three times to ensure precision. Angle of repose was calculated using the equation

$$\tan\theta = \frac{h}{r}$$

5.5 Dissolution profile

Dissolution is the process by which solute substances dissolve into solvent and goes into solution. In pharmaceutical industry, it may be defined as the amount of drug that goes into solution per unit time. Dissolution testing is one of the major quality control testing of solid dosage forms, which is sometimes considered as the determinant of bioavailability of drug substance (Anand, Yu, Conner, & Davit, 2011; P. A. Dickinson et al., 2008). Dissolution behavior of any drug substance directly affect the therapeutic actions. As a result, at times a relationship is established between *in vitro* dissolution and *in vivo* bioavailability, which is called *in vitro in vivo* correlation (IVIVC) (Takano, Kataoka, & Yamashita, 2012).

5.5.1 Linagliptin dissolution study

A. Preparation of phosphate buffer

1.3gm dipotassium phosphate and 1.02gm monopotassium phosphate was dissolved in 1.5L distilled water and the pH was maintained to 6.8 by using 1 M NaOH or 1 M HCl.

B. λ max determination

30µg/ml solution of linagliptin was analyzed in UV spectrophotometer for the range 200nm to 400nm. The solution gave maximum absorbance at which wavelength was used for the further analysis.

C. Preparation of calibration curve of linagliptin

The calibration curve of linagliptin was prepared by plotting absorbance against concentrations where the concentrations were 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml. Absorbance

was measure at 241nm wavelength, which is the standard for linagliptin and shows maximum absorbance. Afterwards, dissolution rate of linagliptin was calculated based on this standard curve.

a) Preparation of stock solution

10mg powder of linagliptin was taken into a 100ml volumetric flask and dissolved in 100ml water that makes the concentration 0.1mg/ml. This preparation was used as stock solution.

b) Preparation of 10µg/ml solution

1ml solution was taken from stock solution into a volumetric flask and 9ml buffer was added to make up the volume of 10ml. The final concentration was 10µg/ml.

c) Preparation of 20µg/ml solution

2ml solution was taken from stock solution into a volumetric flask and 8ml buffer was added to make up the volume of 10ml. The final concentration was 20µg/ml.

d) Preparation of 30µg/ml solution

3ml solution was taken from stock solution into a volumetric flask and 7ml buffer was added to make up the volume of 10ml. The final concentration was 30µg/ml.

e) Preparation of 40µg/ml solution

4ml solution was taken from stock solution into a volumetric flask and 6ml buffer was added to make up the volume of 10ml. The final concentration was 40µg/ml.

f) Preparation of 50µg/ml solution

5ml solution was taken from stock solution into a volumetric flask and 5ml buffer was added to make up the volume of 10ml. The final concentration was 50µg/ml.

D. Dissolution testing

The dissolution study was carried out in USP dissolution apparatus Logan instrumental UDT-804, which was a rotating paddle machine. 900ml buffer solution was taken in the vessel and temperature was set at 37.5 ^{o}C. After reaching the temperature, rotation was set at 75rpm and accurately weighed 5mg linagliptin powder was poured into the vessel and at the same time paddle rotation started. 10ml Sample solution was taken for first 10 minutes at one-minute

interval and from 10 minutes to 60 minutes at five minutes interval. Every time when sample solution was taken, fluid volume in the vessel was filled up with buffer solution. Then the absorbance for that solution was measured using UV-visible spectrophotometer at 280nm wavelength. The same procedure was done for 3 times to observe the precision of the testing procedure.

5.5.2 Dextromethorphan HBr dissolution study

A. Preparation of calibration curve of dextromethorphan HBr

The calibration curve of dextromethorphan HBr was prepared by plotting absorbance against concentrations where the concentrations were 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml. Absorbance was measured at 280nm wavelength, which is the standard for dextromethorphan HBr and shows maximum absorbance. Afterwards, dissolution rate of dextromethorphan HBr was calculated based on this standard curve.

B. Preparation of stock solution

10mg powder of dextromethorphan HBr was taken into a 100ml volumetric flask and dissolved in 100ml water that makes the concentration 0.1mg/ml. This preparation was used as stock solution.

a) Preparation of 10µg/ml solution

1ml solution was taken from stock solution into a volumetric flask and 9ml buffer was added to make up the volume of 10ml. The final concentration was 10µg/ml.

b) Preparation of 20µg/ml solution

2ml solution was taken from stock solution into a volumetric flask and 8ml buffer was added to make up the volume of 10ml. The final concentration was 20µg/ml.

c) Preparation of 30µg/ml solution

3ml solution was taken from stock solution into a volumetric flask and 7ml buffer was added to make up the volume of 10ml. The final concentration was 30µg/ml.

d) Preparation of 40µg/ml solution

4ml solution was taken from stock solution into a volumetric flask and 6ml buffer was added to make up the volume of 10ml. The final concentration was 40µg/ml.

e) Preparation of 50µg/ml solution

5ml solution was taken from stock solution into a volumetric flask and 5ml buffer was added to make up the volume of 10ml. The final concentration was 50µg/ml.

C. Dissolution testing

The dissolution study was carried out in USP dissolution apparatus Logan instrumental UDT-804, which was a rotating paddle machine. 900ml buffer solution was taken in the vessel and temperature was set at 37.5 ^{o}C. After reaching the temperature, rotation was set 75rpm and accurately weighed 60mg dextromethorphan HBr powder was poured into the vessel and at the same time paddle rotation started. 10ml sample solution was taken for first 10 minutes at one-minute interval and from 10 minutes to 60 minutes at five minutes interval. Every time when sample solution was taken, fluid volume in the vessel was filled up with buffer solution. Then the absorbance for that solution was measured using UV-visible spectrophotometer at 280nm wavelength. The same procedure was done for three times to observe the precision of the testing procedure.

5.5.3 Linagliptin + Dextromethorphan HBr dissolution testing

The dissolution study was carried out in USP dissolution apparatus Logan instrumental UDT-804, which was a rotating paddle machine. 900ml buffer solution was taken in the vessel and temperature was set at 37.5 ^{o}C. After reaching the temperature, rotation was set at 75rpm and accurately weighed 5mg linagliptin and 60mg dextromethorphan HBr powder was poured into the vessel and at the same time paddle rotation started. 10ml sample solution was taken for first 10 minutes at one-minute interval and from 10 minutes to 60 minutes at five minutes interval. Every time when sample solution was taken, fluid volume in the vessel was filled up with buffer solution. Then the absorbance for that solution was measured using UV-visible spectrophotometer at 280 nm and 241nm wavelength. The same procedure was done for three times to observe the precision of the testing procedure.

5.6 Ingredients interaction at dissolve state

Active pharmaceuticals are the therapeutic ingredients in a dosage form whereas excipients are used to manufacturing, drug administration and absorption. However, APIs or excipients used in a dosage form, more than one API can interact with each other as well as excipients and safety and quality of the product will be compromised. Interactions can be of two types, 1) physical 2) chemical. Interactions among APIs and excipients will change the property of the active ingredient as well as their concentration in the solution. UV-visible spectrophotometer can be used to measure the change in concentration, which will indicate if any interaction occurred, or not (Sirajuddin, Ali, & Badshah, 2013; Sultana, Arayne, & Shafi, 2007). Concentration of API is measured in the pure solution and then the concentration of API in measured in the solution mixed with other ingredients, change in the concentration will indicate if there any chemical changes occurred or not in the solution, which ultimately indicates the interactions (Jalali & Dorraji, 2012).

5.6.1 Linagliptin's interactions in solution

All the ingredients used in this project to test interaction with APIs are based on the current market preparation of linagliptin and dextromethorphan HBr tablets. The solutions were analyzed at 241nm wavelength.

A. Stock solution preparation

100mg linagliptin powder was taken in a 1L volumetric flask and mixed with 6.8 pH phosphate buffer; later on the volume was leveled 1L using buffer solution.

B. Linagliptin standard solution

10ml stock solution of linagliptin was mixed with 10ml buffer solution to make the concentration 0.05mg/ml.

C. Linagliptin + Dextromethorphan HBr

10ml solution of linagliptin was taken from stock solution and mixed with 10ml solution of dextromethorphan HBr of concentration 0.5mg/ml.

D. Linagliptin + Mannitol

10ml solution of linagliptin was taken from stock solution; 20mg mannitol and 10ml buffer solution was uniformly mixed with it.

E. Linagliptin + Sodium starch glycollate

10ml solution of linagliptin was taken from stock solution and mixed with 10ml solution of sodium starch glycollate of concentration 0.1mg/ml.

F. Linagliptin + Povidone K-30

10ml solution of linagliptin was taken from stock solution and mixed with 10ml solution of povidone K-30 of concentration 0.1mg/ml.

G. Linagliptin + Magnesium stearate

10ml solution of linagliptin was taken from stock solution and mixed with 10ml solution of magnesium stearate of concentration 0.01mg/ml.

5.6.2 Dextromethorphan HBr's interactions in solution

All the ingredients used in this project to test interaction with APIs are based on the current market preparation of linagliptin and dextromethorphan HBr tablets. The solutions were analyzed at 280nm wavelength.

A. Solution preparation

500mg dextromethorphan HBr powder was taken in a 1L volumetric flask and mixed with 6.8 pH phosphate buffer, later on the volume was leveled 1L using buffer solution.

B. Dextromethorphan HBr standard solution

10ml stock solution of dextromethorphan HBr was mixed with 10ml buffer solution to make up the final concentration 0.25mg/ml.

C. Dextromethorphan HBr + Linagliptin

10ml solution of dextromethorphan HBr was taken from stock solution and mixed with 10ml solution of linagliptin of concentration 0.1mg/ml.

D. Dextromethorphan HBr + Mannitol

10ml solution of dextromethorphan HBr was taken from stock solution; 20mg mannitol and 10ml buffer solution was uniformly mixed with it.

E. Dextromethorphan HBr + Sodium starch glycollate

10ml solution of dextromethorphan HBr was taken from stock solution and mixed with 10ml solution of sodium starch glycollate of concentration 0.1mg/ml.

F. Dextromethorphan HBr + Povidone K-30

10ml solution of dextromethorphan HBr was taken from stock solution and mixed with 10ml solution of povidone K-30 of concentration 0.1mg/ml.

G. Dextromethorphan HBr + Magnesium stearate

10ml solution of dextromethorphan HBr was taken from stock solution and mixed with 10ml solution of magnesium stearate of concentration 0.01mg/ml.

5.6.3 Linagliptin + Dextromethorphan HBr's interactions in solution

A. Linagliptin standard solution

10ml stock solution of linagliptin was mixed with 20ml buffer solution to make the final concentration 0.033mg/ml.

B. Dextromethorphan HBr standard solution

10ml stock solution of dextromethorphan HBr was mixed with 20ml buffer solution to make the final concentration 0.167mg/ml.

C. Linagliptin + Dextromethorphan HBr + Mannitol

10ml solution of linagliptin and 10ml solution of dextromethorphan HBr were taken from stock solutions; 10ml buffer solution and 20mg mannitol was uniformly mixed with it.

D. Linagliptin + Dextromethorphan HBr + Sodium starch glycollate

10ml solution of linagliptin and 10ml solution of dextromethorphan HBr were taken from stock solutions and mixed with 10ml solution of sodium starch glycollate of concentration 0.1mg/ml.

E. Linagliptin + Dextromethorphan HBr + Povidone K-30

10ml solution of linagliptin and 10ml solution of dextromethorphan HBr were taken from stock solutions and mixed with 10ml solution of povidone K-30 of concentration 0.1mg/ml.

F. Linagliptin + Dextromethorphan HBr + Magnesium stearate

10ml solution of linagliptin and 10ml solution of dextromethorphan HBr were taken from stock solutions and mixed with 10ml solution of magnesium stearate of concentration 0.01mg/ml.

5.6.4 All ingredients' interactions in solution

A. Linagliptin standard solution

10ml stock solution of linagliptin was mixed with 40ml buffer solution to make the final concentration 0.02mg/ml.

B. Dextromethorphan standard solution

10ml stock solution of dextromethorphan HBr was mixed with 40ml buffer solution to make the final concentration 0.1mg/ml.

C. Linagliptin + Dextromethorphan HBr + Mannitol + Sodium starch glycollate + Povidone K-30 + Magnesium stearate

10ml solution of linagliptin (0.1mg/ml), 10ml solution of dextromethorphan HBr (0.5mg/ml), 10ml solution of sodium starch glycollate (0.1mg/ml), 10ml solution of povidone K-30 (0.1mg/ml), 10ml solution of magnesium stearate (0.01mg/ml) were taken into a 100ml volumetric flask; afterwards, 20mg mannitol was added into the solution and properly mixed.

Chapter 6

6. Results

Data of all tests were taken more than one times to ensure the precision of the testing procedures and results were checked and verified.

6.1 Physical appearance

A. Linagliptin

Table 6.1.1: Physical appearance of linagliptin

Color	Slightly yellowish
Odor	Odorless
Taste	Tasteless
Appearance	Crystalline powder
Smell	No smell

B. Dextromethorphan HBr

Table 6.1.2: Physical appearance of Dextromethorphan HBr

Color	White
Odor	Odorless
Taste	Tasteless
Appearance	Crystalline powder
Smell	No smell

6.2 Particle size analysis

A. Particle size of linagliptin

Table 6.2.1: Particle size distribution of linagliptin

SL No.	Particle size (2gm powder)	Percentage
1	Mesh 20 = 0gm	0%
	Mesh 30 = 0gm	0%
	Mesh 40 = 0gm	0%
	Mesh 60 = 1.89gm	94%
2	Mesh 20 = 0gm	0%
	Mesh 30 = 0gm	0%
	Mesh 40 = 0gm	0%
	Mesh 60 = 1.92gm	96%
3	Mesh 20 = 0gm	0%
	Mesh 30 = 0gm	0%
	Mesh 40 = 0gm	0%
	Mesh 60 = 1.93gm	96.5%

B. Particle size of Dextromethorphan HBr

Table 6.2.2: Particle size distribution of dextromethorphan HBr

SL No.	Particle size (5gm powder)	Percentage
1	Mesh 20 = 0gm	0%
	Mesh 30 = 0gm	0%
	Mesh 40 = 0gm	0%
	Mesh 60 = 4.83gm	96.6%
2	Mesh 20 = 0gm	0%
	Mesh 30 = 0gm	0%
	Mesh 40 = 0gm	0%
	Mesh 60 = 4.92gm	98.4%

3	Mesh 20 = 0gm	0%
	Mesh 30 = 0gm	0%
	Mesh 40 = 0gm	0%
	Mesh 60 = 4.89gm	97.8%

C. Particle size of Linagliptin + dextromethorphan HBr

Table 6.2.3: Particle size distribution of linagliptin + dextromethorphan HBr

SL No.	Particle size (7gm powder)	Percentage
1	Mesh 20 = 0gm	0%
	Mesh 30 = 0gm	0%
	Mesh 40 = 0gm	0%
	Mesh 60 = 6.84gm	97.6%
2	Mesh 20 = 0gm	0%
	Mesh 30 = 0gm	0%
	Mesh 40 = 0gm	0%
	Mesh 60 = 6.81gm	97.2%
3	Mesh 20 = 0gm	0%
	Mesh 30 = 0gm	0%
	Mesh 40 = 0.61gm	8.7%
	Mesh 60 = 6.26gm	89.8%

6.3 Angle of repose, bulk density and tapped density, compressibility index, Hausner's ratio

A. Linagliptin

Table 6.3.1: Angle of repose, bulk density and tapped density, compressibility index, Hausner's ratio of linagliptin

SL No.	Angle of repose θ ($\tan\theta = \frac{h}{r}$)	Bulk density V_o (gm/cm3)	Tapped density V_f (gm/cm3)	Compressibility index (%) $\frac{100(Vo - Vf)}{Vo}$	Hausner's ratio V_f / V_o
1	26.38	0.62	0.76	22.58	1.23
2	28.5	0.59	0.71	20.33	1.20
3	25.73	0.58	0.72	28	1.24

B. Dextromethorphan HBr

Table 6.3.2: Angle of repose, bulk density and tapped density, compressibility index, Hausner's ratio of dextromethorphan HBr

SL No.	Angle of repose θ ($\tan\theta = \frac{h}{r}$)	Bulk density V_o (gm/cm3)	Tapped density V_f (gm/cm3)	Compressibility index (%) $\frac{100(Vo - Vf)}{Vo}$	Hausner's ratio V_f / V_o
1	15.37	1.21	1.33	10	1.1
2	18.52	1.18	1.29	9	1.09
3	17.4	1.19	1.28	7.5	1.075

C. Linagliptin + Dextromethorphan HBr

Table 6.3.3: Angle of repose, bulk density and tapped density, compressibility index, Hausner's ratio of linagliptin + dextromethorphan HBr

SL No.	Angle of repose θ ($\tan\theta = \frac{h}{r}$)	Bulk density V_o (gm/cm3)	Tapped density V_f (gm/cm3)	Compressibility index (%) $\frac{100(Vo - Vf)}{Vo}$	Hausner's ratio V_f / V_o
1	22.5	0.96	1.14	18.75	1.188
2	23.1	0.97	1.12	17.52	1.154
3	21.95	0.93	1.12	20.43	1.20

6.4 Dissolution profile

6.4.1 Linagliptin

A. λ max

Maximum absorbance was found at wavelength 241nm; shown in the figure. The wavelength was used for further analysis of linagliptin.

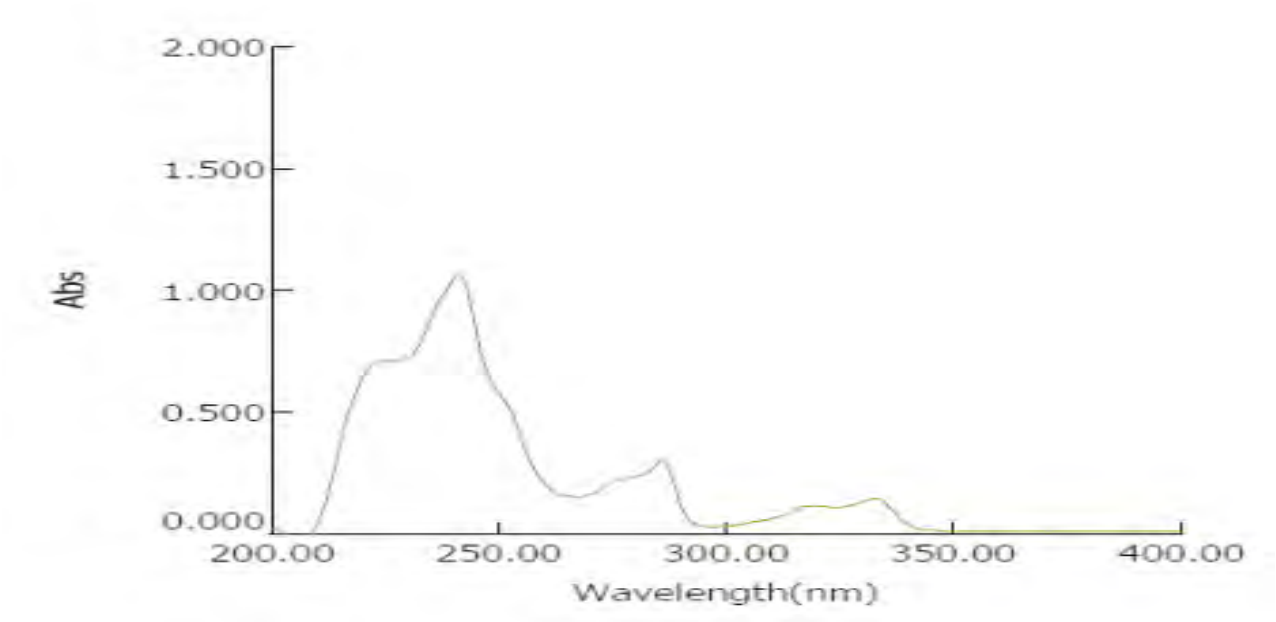


Fig 6.4.1.1: UV spectrum of linagliptin

B. Calibration curve

Table 6.4.1.1: Absorbance values of linagliptin at 241 nm

Sl no.	Concentration (µg/ml)	Absorbance
1	10	0.310
2	20	0.619
3	30	0.905
4	40	1.223
5	50	1.525

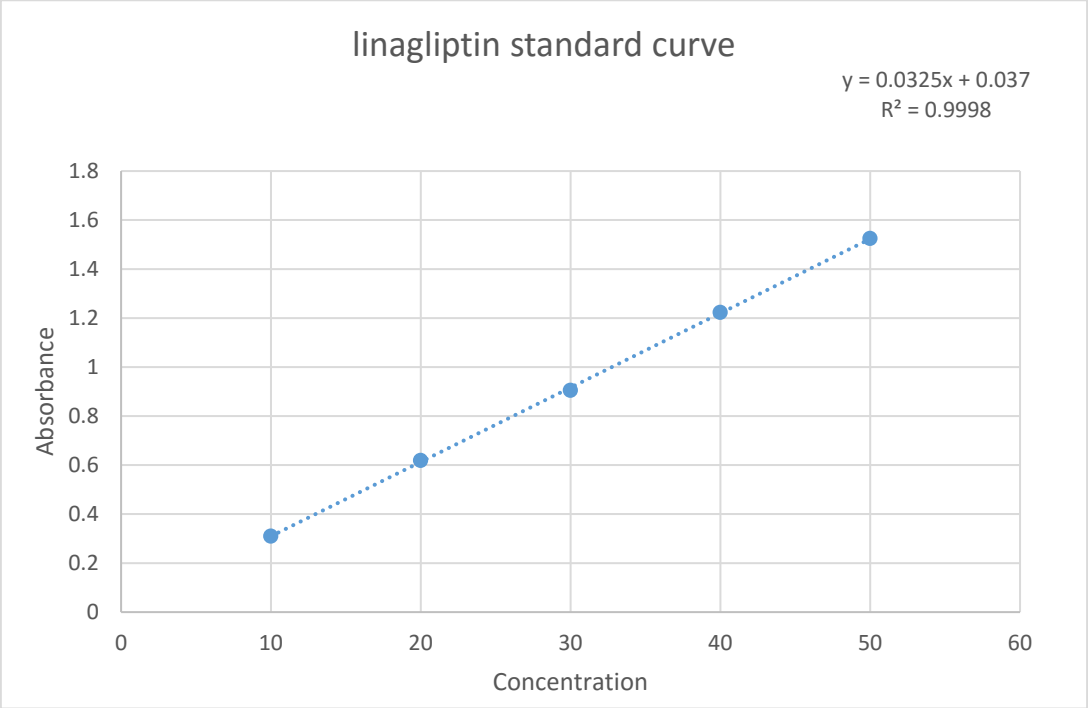


Fig 6.4.1.2: Standard curve of linagliptin

C. Dissolution data

The absorbance of sample solution was taken at 241nm wavelength and the concentration was calculated using the equation

$$Y = 0.0325x + 0.037$$

Table 6.4.1.2: Dissolution data of linagliptin

Sl no	Time (minutes)	Abs. 1	Conc. 1	Abs. 2	Conc. 2	Abs. 3	Conc. 3
1	1	0.230	5.938462	0.235	6.092308	0.233	6.030769
2	2	0.216	5.507692	0.217	5.538462	0.218	5.569231
3	3	0.179	4.369231	0.178	4.338462	0.185	4.553846
4	4	0.173	4.184615	0.176	4.276923	0.172	4.153846
5	5	0.171	4.123077	0.170	4.092308	0.170	4.092308
6	6	0.170	4.092308	0.172	4.153846	0.168	4.030769
7	7	0.167	4	0.167	4	0.165	3.938462
8	8	0.162	3.846154	0.166	3.969231	0.164	3.907692
9	9	0.160	3.784615	0.160	3.784615	0.159	3.753846
10	10	0.157	3.692308	0.156	3.661538	0.160	3.784615
11	15	0.154	3.6	0.155	3.630769	0.157	3.692308
12	20	0.148	3.415385	0.152	3.538462	0.153	3.569231
13	25	0.148	3.415385	0.148	3.415385	0.146	3.353846
14	30	0.146	3.353846	0.147	3.384615	0.145	3.323077
15	35	0.149	3.446154	0.145	3.323077	0.142	3.230769
16	40	0.139	3.138462	0.141	3.2	0.140	3.169231
17	45	0.139	3.138462	0.138	3.107692	0.137	3.076923
18	50	0.138	3.107692	0.138	3.107692	0.139	3.138462
19	55	0.136	3.046154	0.135	3.015385	0.134	2.984615
20	60	0.133	2.953846	0.132	2.923077	0.129	2.830769

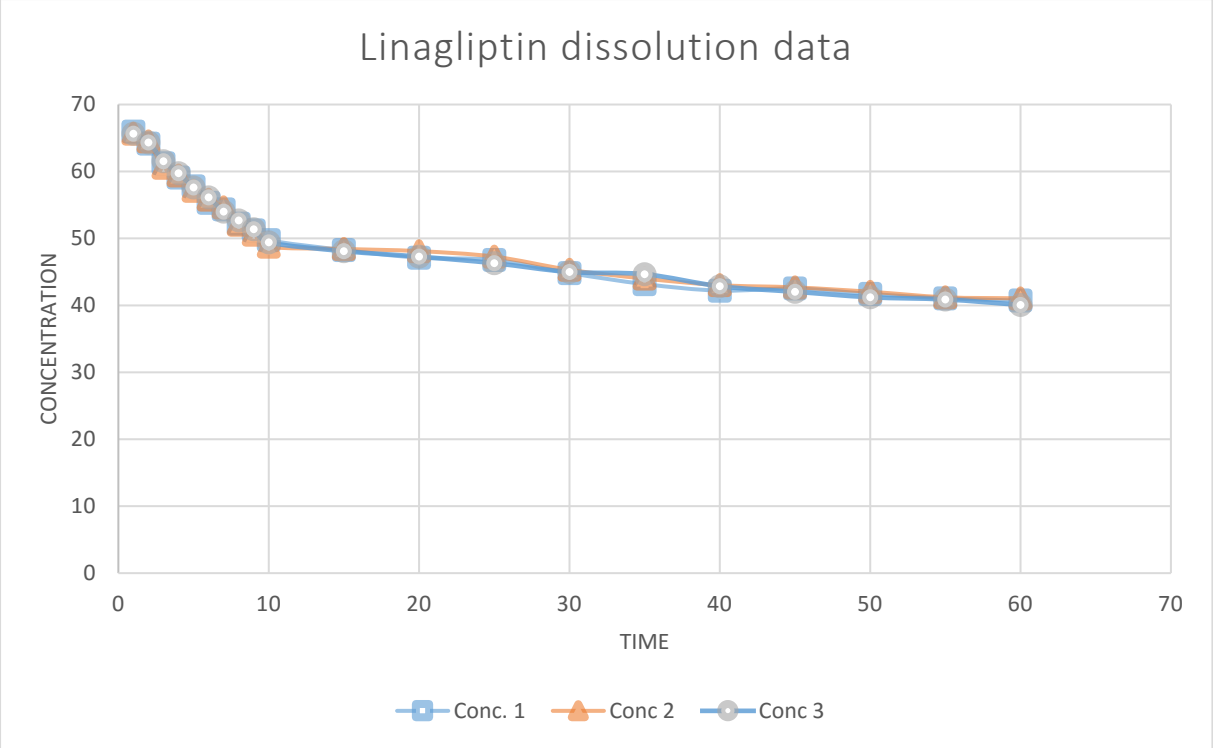


Fig 6.4.1.3: Dissolution pattern of linagliptin

6.4.2 Dextromethorphan HBr

A. Calibration curve

Absorbance of dextromethorphan HBr was measured at the wavelength 280nm which is USP standard for dextromethorphan HBr and this curve was used for further analysis of dextromethorphan HBr.

Table 6.4.2.1: Absorbance values of dextromethorphan HBr at 280 nm

Sl no.	Concentration (µg/ml)	Absorbance
1	10	0.105
2	20	0.171
3	30	0.230
4	40	0.291
5	50	0.352

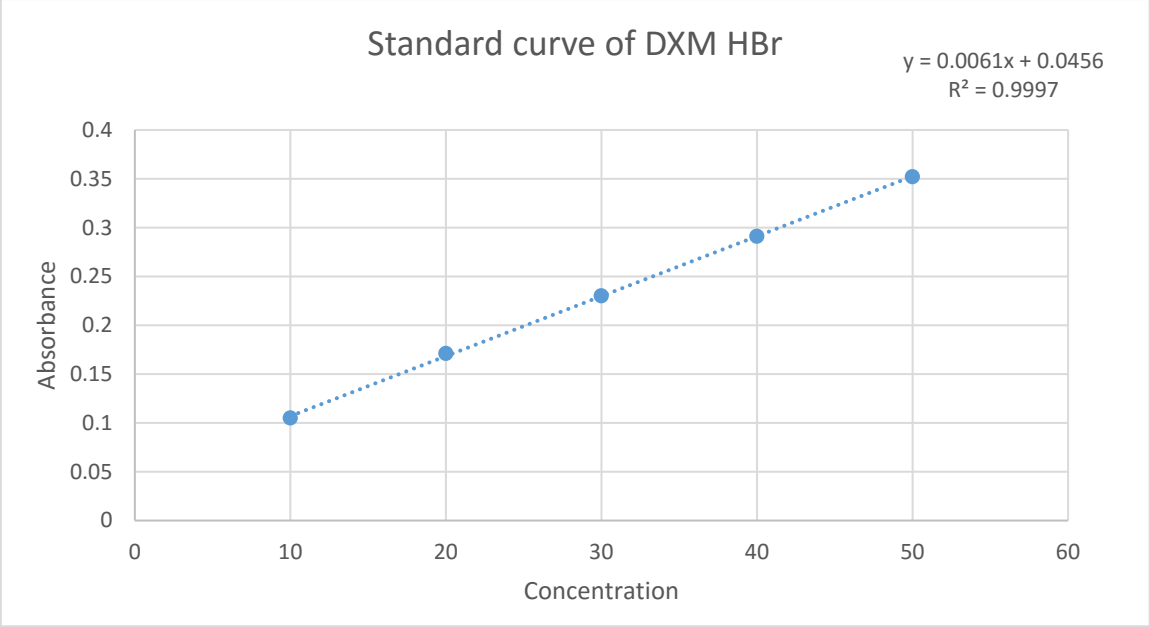


Fig 6.4.2.1: Standard curve of dextromethorphan HBr

B. Dissolution data

The absorbance of sample solution was taken at 280nm wavelength and the concentration was calculated using the equation

$$Y = 0.0061x + 0.0456$$

Table 6.4.2.2: Dissolution data of dextromethorphan HBr

Sl no	Time (minutes)	Abs. 1	Conc. 1	Abs. 2	Conc. 2	Abs. 3	Conc. 3
1	1	0.451	66.45902	0.452	66.57472	0.453	66.78689
2	2	0.432	63.34426	0.439	64.4918	0.434	63.67213
3	3	0.414	60.39344	0.416	60.72131	0.417	60.88525
4	4	0.401	58.2623	0.408	59.40984	0.402	58.42623
5	5	0.394	57.11475	0.399	57.93443	0.394	57.11475
6	6	0.388	56.13115	0.394	57.11475	0.386	55.80328
7	7	0.387	55.96721	0.387	55.96721	0.381	54.98361
8	8	0.364	52.19672	0.374	53.83607	0.368	52.85246
9	9	0.352	50.22951	0.366	52.52459	0.353	50.39344
10	10	0.346	49.2459	0.355	50.72131	0.348	49.57377

11	15	0.339	48.09836	0.348	49.57377	0.342	48.59016
12	20	0.331	46.78689	0.337	47.77049	0.334	47.27869
13	25	0.328	46.29508	0.326	45.96721	0.328	46.29508
14	30	0.318	44.65574	0.328	46.29508	0.329	46.45902
15	35	0.313	43.83607	0.316	44.32787	0.310	43.34426
16	40	0.309	43.18033	0.306	42.68852	0.302	42.03279
17	45	0.304	42.36066	0.305	42.52459	0.302	42.03279
18	50	0.311	43.5082	0.303	42.19672	0.298	41.37705
19	55	0.297	41.21311	0.300	41.70492	0.304	42.36066
20	60	0.295	40.88525	0.299	41.54098	0.292	40.39344

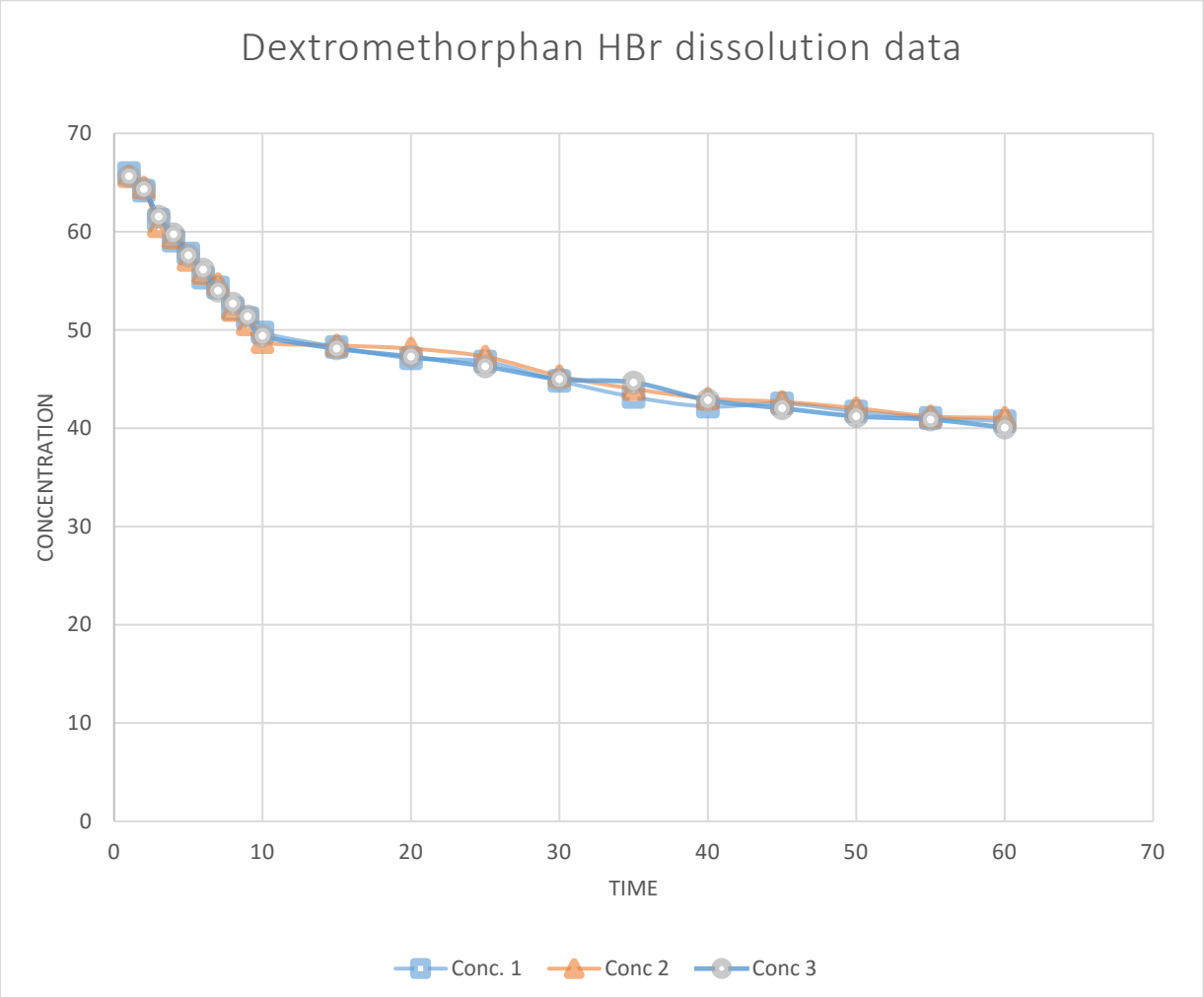


Fig 6.4.2.2: Dissolution pattern of dextromethorphan HBr

6.4.3 Linagliptin + Dextromethorphan HBr dissolution data

A. Linagliptin

Absorbance of linagliptin was measured at wavelength 241nm and concentration was calculated using the equation obtained from calibration curve

$$Y = 0.0325x + 0.037$$

Table 6.4.3.1: Dissolution data of linagliptin in linagliptin+ dextromethorphan HBr solution

Sl no	Time (minutes)	Abs. 1	Conc. 1	Abs. 2	Conc. 2	Abs. 3	Conc. 3
1	1	0.217	5.538462	0.214	5.446154	0.216	5.507692
2	2	0.210	5.323077	0.213	5.415385	0.209	5.292308
3	3	0.208	5.261538	0.209	5.292308	0.207	5.230769
4	4	0.201	5.046154	0.204	5.138462	0.203	5.107692
5	5	0.196	4.892308	0.200	5.015385	0.198	4.953846
6	6	0.193	4.8	0.197	4.923077	0.192	4.769231
7	7	0.189	4.676923	0.185	4.553846	0.190	4.707692
8	8	0.183	4.492308	0.185	4.553846	0.188	4.646154
9	9	0.174	4.215385	0.179	4.369231	0.176	4.276923
10	10	0.179	4.369231	0.175	4.246154	0.175	4.246154
11	15	0.172	4.153846	0.171	4.123077	0.173	4.184615
12	20	0.168	4.030769	0.166	3.969231	0.169	4.061538
13	25	0.165	3.938462	0.170	4.092308	0.168	4.030769
14	30	0.161	3.815385	0.162	3.846154	0.164	3.907692
15	35	0.158	3.723077	0.154	3.6	0.155	3.630769
16	40	0.147	3.384615	0.149	3.446154	0.149	3.446154
17	45	0.146	3.353846	0.143	3.261538	0.142	3.230769
18	50	0.138	3.107692	0.139	3.138462	0.134	2.984615
19	55	0.132	2.923077	0.131	2.892308	0.135	3.015385
20	60	0.131	2.892308	0.129	2.830769	0.132	2.923077

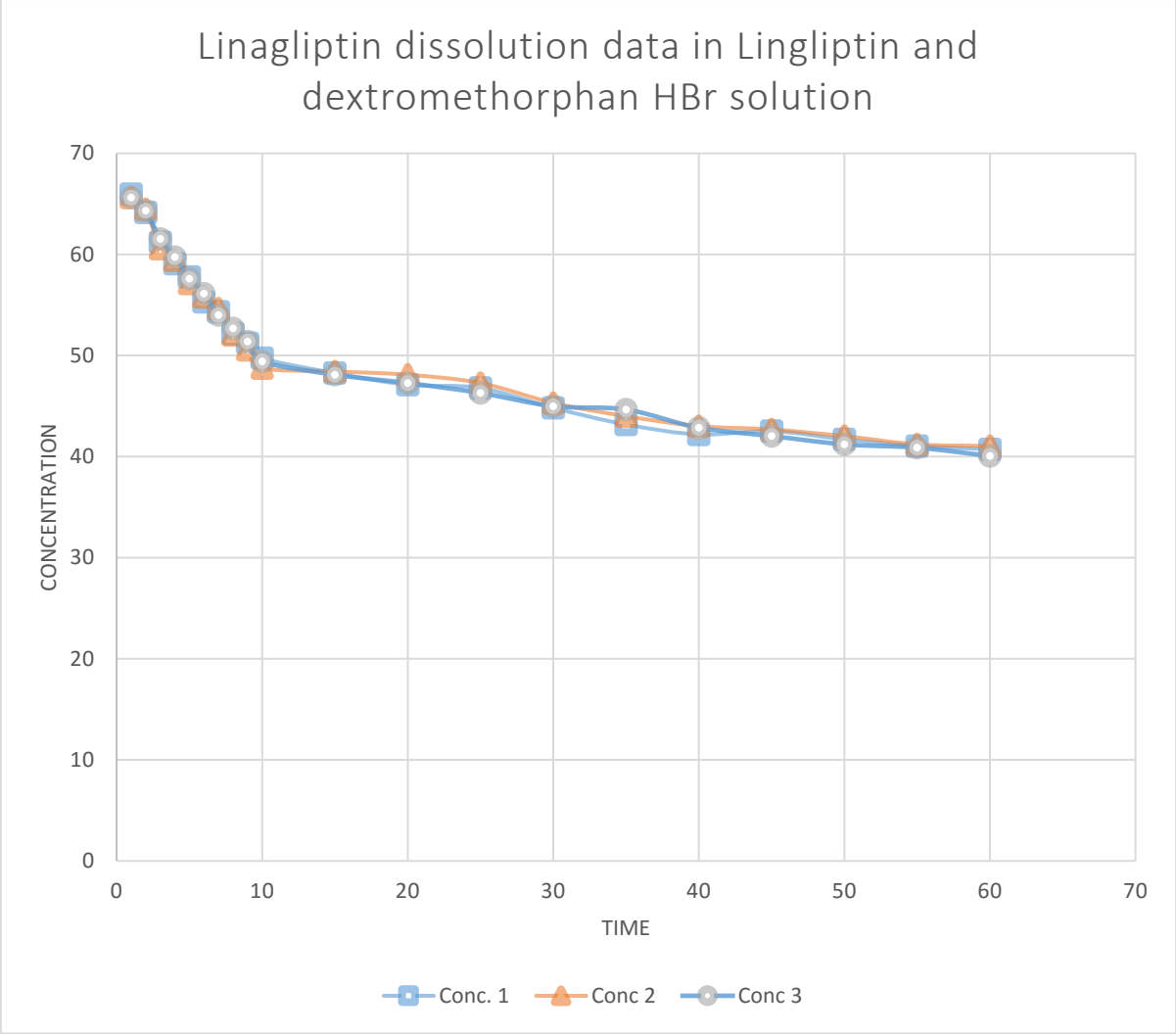


Fig 6.4.3.1: Dissolution pattern of linagliptin in linagliptin + dextromethorphan HBr solution

B. Dextromethorphan HBr

Absorbance of linagliptin was measured at wavelength 280nm and concentration was calculated using the equation obtained from calibration curve

$$Y = 0.0061x + 0.0456$$

Table 6.4.3.2: Dissolution data of dextromethorphan HBr in linagliptin+ dextromethorphan HBr solution

Sl no	Time (minutes)	Abs. 1	Conc. 1	Abs. 2	Conc. 2	Abs. 3	Conc. 3
1	1	0.448	65.96721	0.446	65.63934	0.446	65.63934

2	2	0.437	64.16393	0.439	64.4918	0.438	64.32787
3	3	0.419	61.21311	0.415	60.55738	0.421	61.54098
4	4	0.406	59.08197	0.408	59.40984	0.410	59.7377
5	5	0.398	57.77049	0.394	57.11475	0.397	57.60656
6	6	0.383	55.31148	0.386	55.80328	0.388	56.13115
7	7	0.377	54.32787	0.379	54.65574	0.375	54
8	8	0.364	52.19672	0.363	52.03279	0.367	52.68852
9	9	0.358	51.21311	0.354	50.55738	0.359	51.37705
10	10	0.349	49.7377	0.343	48.7541	0.347	49.40984
11	15	0.340	48.2623	0.341	48.42623	0.339	48.09836
12	20	0.333	47.11475	0.339	48.09836	0.334	47.27869
13	25	0.331	46.78689	0.334	47.27869	0.328	46.29508
14	30	0.319	44.81967	0.322	45.31148	0.320	44.98361
15	35	0.309	43.18033	0.314	44	0.318	44.65574
16	40	0.303	42.19672	0.308	43.01639	0.307	42.85246
17	45	0.305	42.52459	0.306	42.68852	0.302	42.03279
18	50	0.300	41.70492	0.302	42.03279	0.297	41.21311
19	55	0.296	41.04918	0.297	41.21311	0.295	40.88525
20	60	0.294	40.72131	0.296	41.04918	0.290	40.06557

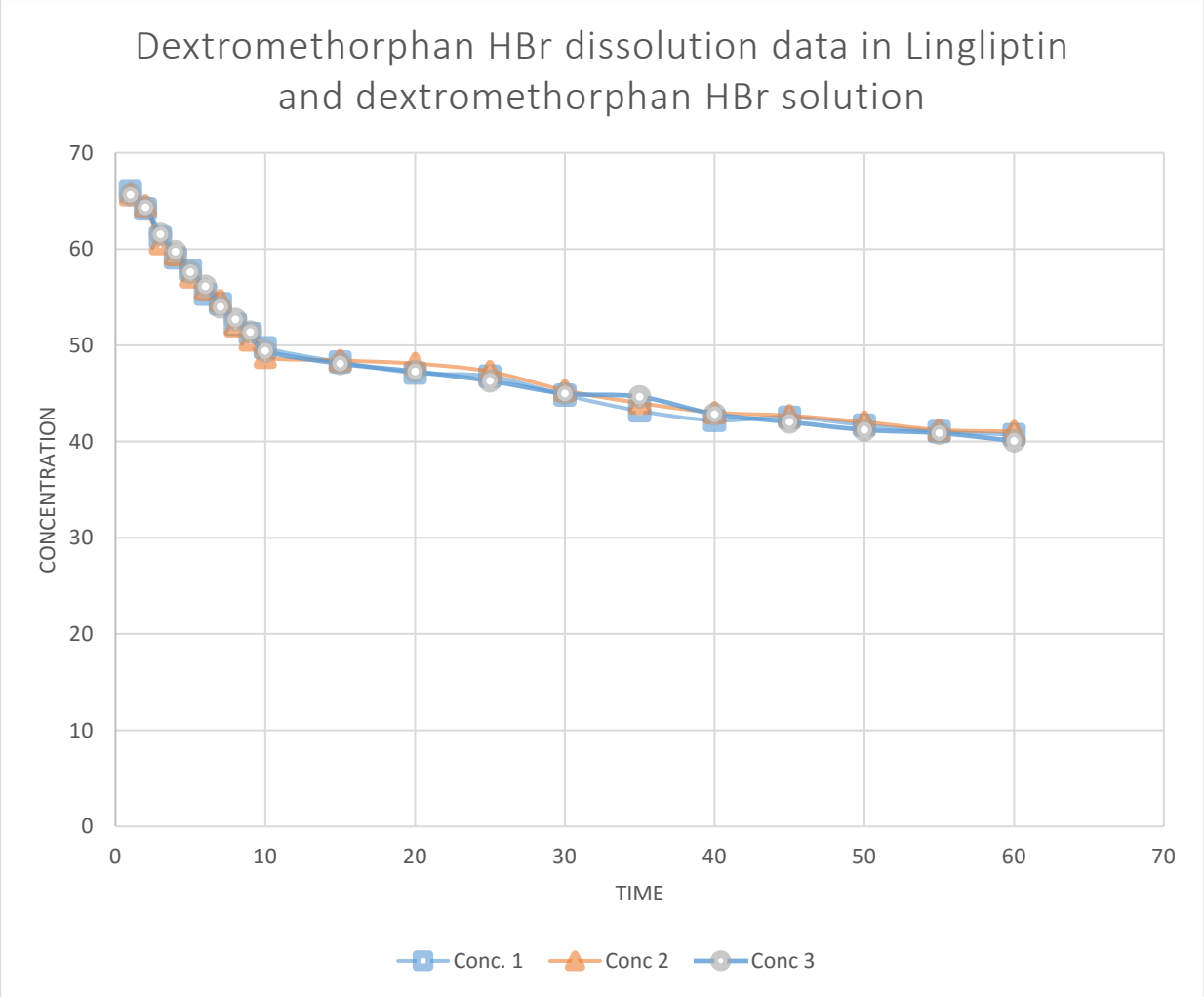


Fig 6.4.3.2: Dissolution pattern of dextromethorphan HBr in linagliptin + dextromethorphan HBr solution

6.5 Ingredients interaction at dissolve state

6.5.1 Linagliptin

The absorbance and concentration of sample solution in buffer medium was compared with the absorbance and concentration of pure linagliptin solution in buffer medium using the absorbance at 241nm wavelength and equation Y = 0.0325x + 0.037

A. Linagliptin + Dextromethorphan HBr

Table 6.5.1.1: Interaction between linagliptin and dextromethorphan HBr

SL no.	Linagliptin		Linagliptin + Dextromethorphan HBr	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	1.64	49.323	1.63	49.01538
2			1.63	49.01538
3			1.64	49.32308

B. Linagliptin + Mannitol

Table 6.5.1.2: Interaction between linagliptin and mannitol

SL no.	Linagliptin		Linagliptin + Mannitol	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	1.64	49.323	1.61	48.4
2			1.64	49.32308
3			1.62	48.70769

C. Linagliptin + Sodium starch glycollate

Table 6.5.1.3: Interaction among linagliptin and sodium starch glycollate

SL no.	Linagliptin		Linagliptin + sodium starch glycollate	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	1.64	49.323	1.60	48.09231
2			1.64	49.32308
3			1.64	49.32308

D. Linagliptin + Povidone k-30

Table 6.5.1.4: Interaction between linagliptin and povidone K-30

SL no.	Linagliptin		Linagliptin + sodium starch glycollate	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	1.64	49.323	1.62	48.70769
2			1.63	49.01538
3			1.66	49.93846

E. Linagliptin + Magnesium stearate

Table 6.5.1.5: Interaction between linagliptin and magnesium stearate

SL no.	Linagliptin		Linagliptin + Magnesium stearate	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	1.64	49.323	1.58	47.4769
2			1.61	48.4
3			1.62	48.70769

6.5.2 Dextromethorphan HBr

The absorbance and concentration of sample solution in buffer medium was compared with the absorbance and concentration of pure dextromethorphan HBr solution in buffer medium using the absorbance at 280nm wavelength and equation Y = 0.0061x + 0.0456

A. Dextromethorphan HBr + Linagliptin

Table 6.5.2.1: Interaction between dextromethorphan HBr and linagliptin

SL no.	Dextromethorphan HBr		Dextromethorphan HBr + Linagliptin	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	1.545	245.803	1.53	243.344
2			1.53	243.344
3			1.55	246.623

B. Dextromethorphan HBr + Mannitol

Table 6.5.2.2: Interaction between dextromethorphan HBr and mannitol

SL no.	Dextromethorphan HBr		Dextromethorphan HBr + Mannitol	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	1.545	245.803	1.54	244.984
2			1.53	243.344
3			1.548	246.295

C. Dextromethorphan HBr + Sodium starch glycollate

Table 6.5.2.3: Interaction between dextromethorphan HBr and sodium starch glycollate

SL no.	Dextromethorphan HBr		Dextromethorphan HBr + Sodium starch glycollate	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	1.545	245.803	1.52	241.705
2			1.53	243.344
3			1.52	241.705

D. Dextromethorphan HBr + Povidone K-30

Table 6.5.2.4: Interaction between dextromethorphan HBr and povidone K-30

SL no.	Dextromethorphan HBr		Dextromethorphan HBr + Povidone K-30	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	1.545	245.803	1.49	236.787
2			1.53	243.344
3			1.50	238.426

E. Dextromethorphan HBr + Magnesium stearate

Table 6.5.2.5: Interaction between dextromethorphan HBr and magnesium stearate

SL no.	Dextromethorphan HBr		Dextromethorphan HBr + Magnesium stearate	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	1.545	245.803	1.49	236.787
2			1.48	235.148
3			1.49.5	237.607

6.5.3 Linagliptin + Dextromethorphan HBr's interactions in solution

Concentration and absorbance of sample solution of linagliptin and dextromethorphan HBr mixed with other ingredient were compared with the pure absorbance and concentration of linagliptin and dextromethorphan HBr. The absorbance of linagliptin and dextromethorphan HBr were measured at wavelength 241nm and 280nm correspondingly.

A. Linagliptin + Dextromethorphan HBr + mannitol

a) Comparison with standard linagliptin

Table 6.5.3.1 A: Interaction among linagliptin, dextromethorphan HBr and mannitol compared with linagliptin

SL no.	Linagliptin		Linagliptin + Dextromethorphan HBr + Mannitol	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	1.1	32.7077	1.08	32.0923
2			1.09	32.4
3			1.09	32.4

b) Comparison with standard Dextromethorphan HBr

Table 6.5.3.1 B: Interaction among linagliptin, dextromethorphan HBr and mannitol compared with dextromethorphan HBr

SL no.	Dextromethorphan HBr		Linagliptin + Dextromethorphan HBr + Mannitol	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	1.05	164.6557	1.045	163.836
2			1.05	164.656
3			1.04	161.016

B. Linagliptin + Dextromethorphan HBr + Sodium starch glycollate

a) Comparison with standard linagliptin

Table 6.5.3.2 A: Interaction among linagliptin, dextromethorphan HBr and sodium starch glycollate compared with linagliptin

SL no.	Linagliptin		Linagliptin + Dextromethorphan HBr + Sodium starch glycollate	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	1.1	32.7077	1.08	32.0923
2			1.15	34.2462
3			1.06	31.4769

b) Comparison with standard Dextromethorphan HBr

Table 6.5.3.2 B: Interaction among linagliptin, dextromethorphan HBr and sodium starch glycollate compared with dextromethorphan HBr

SL no.	Dextromethorphan HBr		Linagliptin + Dextromethorphan HBr + Sodium starch glycollate	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	1.05	164.6557	1.042	163.344
2			1.035	162.197
3			1.038	162.689

C. Linagliptin + Dextromethorphan HBr + Povidone K-30

a) Comparison with standard linagliptin

Table 6.5.3.3 A: Interaction among linagliptin, dextromethorphan HBr and povidone K-30 compared with linagliptin

SL no.	Linagliptin		Linagliptin + Dextromethorphan HBr + Povidone K-30	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	1.1	32.7077	1.09	32.4
2			1.075	31.9385
3			1.083	32.1846

b) Comparison with standard Dextromethorphan HBr

Table 6.5.3.3 B: Interaction among linagliptin, dextromethorphan HBr and povidone K-30 compared with dextromethorphan HBr

SL no.	Dextromethorphan HBr		Linagliptin + Dextromethorphan HBr + Povidone K-30	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	1.05	164.6557	1.037	162.525
2			1.038	162.689
3			1.042	163.344

D. Linagliptin + Dextromethorphan HBr + Magnesium stearate

a) Comparison with standard linagliptin

Table 6.5.3.4 A: Interaction among linagliptin, dextromethorphan HBr and magnesium stearate compared with linagliptin

SL no.	Linagliptin		Linagliptin + Dextromethorphan HBr + magnesium stearate	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	1.1	32.7077	1.065	31.6308
2			1.07	31.7846
3			1.068	31.7231

b) Comparison with standard Dextromethorphan HBr

Table 6.5.3.4 B: Interaction among linagliptin, dextromethorphan HBr and magnesium stearate compared with dextromethorphan HBr

SL no.	Dextromethorphan HBr		Linagliptin + Dextromethorphan HBr + magnesium stearate	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	1.05	164.6557	1.036	162.361
2			1.043	163.508
3			1.042	163.344

6.5.4 All ingredients' interactions in solution

Concentration and absorbance of sample solution of linagliptin and dextromethorphan HBr mixed with other ingredients were compared with the absorbance and concentration of pure linagliptin and dextromethorphan HBr. The absorbance of linagliptin and dextromethorphan HBr were measured at wavelength 241nm and 280nm correspondingly.

a) Comparison with standard linagliptin

Table 6.5.4.1 A: Interaction among linagliptin, dextromethorphan HBr, mannitol, sodium starch glycollate, povidone K-30 and magnesium stearate compared with linagliptin

SL no.	Linagliptin		Linagliptin + Dextromethorphan HBr + Mannitol + Sodium starch glycollate + Povidone K-30 + Magnesium stearate	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	0.656	19.0462	0.638	18.4923
2			0.661	19.2
3			0.647	18.7692

b) Comparison with standard Dextromethorphan HBr

Table 6.5.4.1 B: Interaction among linagliptin, dextromethorphan HBr, mannitol, sodium starch glycollate, povidone K-30 and magnesium stearate compared with dextromethorphan HBr

SL no.	Dextromethorphan HBr		Linagliptin + Dextromethorphan HBr + Mannitol + Sodium starch glycollate + Povidone K-30 + Magnesium stearate	
	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)
1	0.638	97.1148	0.627	95.3115
2			0.621	94.3279
3			0.635	96.623

Chapter 7

7. Discussion

Preformulation studies are the very first step towards formulating a new formulation. It plays an important role to anticipate formulation related difficulties and determining the dosage form of any formulation. Moreover, critical drug information like solubility, flow ability, dissolution property and medium, interactions with excipients, preferences of excipients are also known from preformulation studies. In short, preformulation studies are the application of biopharmaceutical knowledge into the physicochemical factors of any drug substances. Microscopic methods and sieving are used to know the particle size and morphology of any drug substance, angle of repose determine the flow property and tapped density determine the compressibility of the drug substance. Moreover, dissolution studies provide critical information about the drug dissolution in vivo, whereas drug interaction can be determined by observing change in concentration using UV spectrophotometry. For the development of a noble antidiabetic formulation of linagliptin and dextromethorphan, a number of preformulation studies were done such as; morphological observations, particle size distribution, determination of angle of repose, bulk density tapped density, compressibility index, dissolution studies and drug interaction by observing change in concentrations.

First of all, morphological studies demonstrate that linagliptin powder was slightly yellow in color, odorless and crystalline powder. On the other hand, dextromethorphan HBr was white, crystalline powder with no odor. Particle size and size distribution is an important parameter for the formulation of any medicine. Particle size affects the solubility of the drug eventually absorption. The smaller the particle size, the more soluble it is (Khadka et al., 2014). In case of both linagliptin and dextromethorphan HBr, all the particles passed through the mesh 60, which means all the particles, were less than 250 micron in size. On the other hand, only 8% particles were found on mesh 40 while linagliptin and dextromethorphan HBr were mixed. This is maybe due to aggregation of two different compound or experimental error. However, particles of both linagliptin and dextromethorphan HBr were found to be very small which indicates their better solubility and absorption in the body. On the other hand, angle of repose is the interparticular friction among the particle that determines the flow property of any solid powder. Angle of repose value lower than 25 exhibits excellent flow property whereas more than 65 indicates very poor flow property (USP, 2017). Angle of repose values of linagliptin were 26.38, 28.5 and 25.73, which fall under the excellent flow property value. Besides,

dextromethorphan HBr had angle of repose value 17.4, 18.52 and 15.37 that also fall under excellent flow property value. Moreover, angle of repose values were found to be excellent when linagliptin and dextromethorphan HBr were mixed as well. Bulk density, tapped density, compressibility index and Hausner's ratio are the good determinants of flow property of any solid powder as well as the good determinants of compression property, moisture content, and cohesiveness of that powder for manufacturing process. According to USP Hausner's ratio below 1.11 indicates excellent compression and flow property, whereas, value above 1.6 indicates very poor property (USP, 2017). In case of linagliptin, the values were around 1.2, which indicates good compression property; on the other hand, dextromethorphan had excellent property with values around 1.08. In addition, mixing of linagliptin and dextromethorphan HBr had given a good Hausner's ratio value of flow property and compression of them. The values were checked for three times and they were found to be close. However, slight fluctuation in values maybe were due to error in operating procedure or experimental error.

Secondly, dissolution is the one of the most critical parameters for the formulation of any drug. The absorption of drug into the body depends on the dissolution or release of drug in the medium, which ultimately affects the therapeutic activity of the drug substance (Kobayashi, Sada, Sugawara, Iseki, & Miyazaki, 2001). The dissolution study data of linagliptin reveals that in buffer medium, linagliptin dissolve within 2 minutes and properly mixed with fluid. In the very first minute of dissolution study, data shows that, concertation of linagliptin were higher than expected concentration. This was due to the non-uniform distribution of linagliptin in the medium, however the expected maximum concentration was found at second minute of dissolution study, therefore it indicates that, linagliptin dissolves within 2 minutes at 6.8 pH. In addition, concentration were found to be decreased with time which was due to withdrawal of sample solution every minute and addition of buffer solution in the vessel. On the other hand, dextromethorphan HBr dissolved in the buffer medium within the first minute. However, 100% dextromethorphan was not found in the medium since dextromethorphan HBr is a salt of dextromethorphan and hydrogen bromide, but then again the wavelength 280nm is specific for dextromethorphan not for HBr, therefore 100% concentration of dextromethorphan HBr was not found. Concentration of dextromethorphan HBr also decreased with time likewise linagliptin due to withdrawal of sample and addition of buffer in the vessel. To sum up, both linagliptin and dextromethorphan HBr have excellent dissolution property; as a result, the absorption of drug in the body will depend on the disintegration of the formulated product.

Finally yet importantly, interactions of linagliptin and dextromethorphan HBr with excipients were checked based on observing the change of concentration using UV-spectrophotometry. Both linagliptin and dextromethorphan HBr did not show any kind of change in concentration when they were mixed, even when mixed with filler/diluent mannitol. There were slight change in concentration observed that was may be due to operational error. However, there was a bit change in concentration when linagliptin and dextromethorphan were mixed with binder povidone K-30 and lubricant magnesium stearate. The probable cause is use of higher amount of binder and lubricant since the higher amount of binder sticks the particle together and delay the dissolution; likewise, higher amount lubricants also delay the dissolution of solid particles. Now, comparing the concentrations of linagliptin and dextromethorphan HBr in sample solution with the standard concentrations of linagliptin and dextromethorphan HBr were found to be very close and satisfactory which indicates that no interaction took place among the compounds in the dissolved state. Therefore, the excipients might be considered for the formulation of oral dosage form of linagliptin and dextromethorphan HBr.

In conclusion, the preformulation studies data of both linagliptin and dextromethorphan HBr shown good flow property, good compression ability, excellent dissolution profile and no interaction with other ingredients, which would be used as excipients. Therefore, the preformulation studies indicate that, combination therapy of linagliptin and dextromethorphan HBr can be formulated with further work on this to improve the pharmacokinetics and pharmacodynamics properties.

Chapter 8

8. Conclusion

Type 2 diabetes mellitus is a multifactorial as well as multifunctional disease that remains undiscovered for a long time and affects various parts of the body, most importantly heart and blood vessels. Different therapies are currently available in market but not all the patients respond to the same medications and traditional antidiabetic medications achieve limited proper glycemic control, which are associated with weight gain and hypoglycemia. Incretin based therapies are the best substitutes of traditional antidiabetic therapies, which are well tolerated in addition to better glycemic control. Among incretin based therapies, gliptins has updated the concept of diabetes management by reducing HbA1c 0.5% to 2% with least side effects. Among all the gliptins, linagliptin is found to be one of the most effective treatments against type 2 diabetes with sustainable HbA1c reduction, least side effects, no weight gain, one dose per day and preserving the beta cells health. Moreover, linagliptin is found to be effective in wound healing and no dose adjustment is required for patients with renal impairment, which is one of the major advantage of linagliptin. However, the cost of linagliptin is a bit high. Therefore, linagliptin can be used in combination with dextromethorphan, which is cheaper in price but increase the insulin production by functioning as NMDARs antagonist in the islet cells of pancreas. To formulate the formulation of a novel antidiabetic combination therapy, the preformulation studies data suggest that both of linagliptin and dextromethorphan HBr had good solubility profile, no interactions occurred, and had good flow and compression property. As a result, findings of this project work recommend further work for the formulation of the combination therapy with linagliptin and dextromethorphan HBr to achieve better glycemic control and preserving beta cells health to fight against type 2 diabetes mellitus, the lethal disease.

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