CHAPTER-1
BRIEF INTRODUCTION AND PHARMACOLOGICAL IMPORTANCE OF PIOGLITAZONE, RANOLAZINE, CLOPIDOGREL, ZAFIRLUKAST, TADALFIL AND SIGNIFICANCE OF IMPURITY PROFILE IN PROCESS DEVELOPMENT
1.1.1: Introduction and pharmacological importance of Pioglitazone HCl: An anti diabetic drug

1.1.1.1 History of Diabetes

Diabetes is one of the oldest ailments and was first documented in Ebers Papyrus, which was written in 1552 BC. Physicians from India were discovered this disease at same time and named as madhumeha or honey urine. Indian physicians were observed that ants and flies were attracted to urine of the people having diabetes and they have suggested that this is preliminary test for identification of diabetes. In 230 BC, the word “diabetes” was initially named by scientist from Greek, Aretaeus of Cappadocia and meaning of “diabetes” in Greek is “to pass through”.

Before 20th century, diabetes was treated by controlling the intake sugar content at minimal level, which was extended the life time of the diabetic patients for few years. In 1920, Dr. Frederick Banting discovered insulin, naturally-occurring hormone secreted by the pancreas for treating diabetes. In 1922, Leonard Thompson (14 years old boy) was successfully treated with insulin. Dr. Frederick Banting and Professor John Macleod jointly awarded Noble prize in physiology or medicine category in 1923 for discovery of insulin. November 14, Dr. Frederick Banting birth day was declared as world diabetes day.

As per world health organization (WHO), about 285 million people are having diabetes in worldwide and this number was expected to
indicated that India having highest number of diabetic patients (50.8 million) as compared with the other countries in the world\(^3\).

### 1.1.1.2 Classification of Diabetes

Diabetes was mainly classified into type-1 diabetes, type-2 diabetes and gestational diabetes. Type-1 diabetes resulted due to the body failure to produce insulin, can be treated by injecting the insulin and this is also called as insulin dependent diabetes. Type-2 diabetes resulted due to either pancreas does not produce enough insulin or body cannot use the insulin properly. Increasing blood sugar levels during the pregnancy time causes gestational diabetes and this can affect the both mother and baby.

**Figure 1.1:** Pancreas Insulin production for type-1 and type-2 diabetes

Type-1 diabetes also known as juvenile diabetes was currently preventable at the latent autoimmune stage, before it starts destroying beta cells. Symptoms for the type-1 diabetes are weight loss, frequent urination, feeling hungry, blurry eye sight and increased thirst. About 5-10% of the patients are having type-1 diabetes from all the diabetic
patients and this was often more in childrens having the age of less than 14 years. This can be treated by injecting insulin, transplantation of pancreas or transplantation of Islet cell.

Type-2 diabetes also calls as adult-onset diabetes or noninsulin dependent diabetes, can be differentiated from the type-1 diabetes using C-peptide assay test. It is more likely in people having age of more than 40 years, overweight or family members who having type-2 diabetes. The majority of the diabetes patients, about 90% have type-2 diabetes. Symptoms for type-2 diabetes are tiredness, increased hunger, increased thirst, frequent needs for urinate, dry skin or itchy skin, erectile dysfunction, blurred sight and slow healing of cuts or sores. Many of the type-2 diabetes patients have high level of triglycerides and low level of high-density lipoprotein cholesterol (HDL-C). As per the American diabetes association, limits of the cholesterol for diabetes patients, triglycerides are less than 150 mg/dL, bad cholesterol (LDL-C) is less than 100 mg/dL, good cholesterol (HDL-C) is greater than 40 mg/dL for men and 50 mg/dL for women.

1.1.1.3 Available drugs for treating Diabetes

Metformin is the widely prescribed an oral anti diabetic drug in the world for the treatment of type-2 diabetes, in particular patients having normal kidney function. Metformin was first reported in 1922, approved by health Canada and United States food and drugs administration (US FDA) in 1972 and 1994 respectively. If metformin
was not enough for treating type-2 diabetes, other class of medications available for treating type-2 diabetes is thiazolidinediones, DPP-IV inhibitors, sulfonylureas, alpha-glucosidase inhibitors, GLP-1 analogs and meglitinides alone or in combination with metformin.

![Chemical structures of glitazones](image)

**Figure 1.2:** Chemical structures of glitazones

Thiazolidinediones also known as glitazones are chemically 2, 4-thiazolidinediones derivatives that include pioglitazone, rosiglitazone,
troglitazone, netoglitazone, rivoglitazone, ciglitazone and balaglitazone used for treating diabetes.

1.1.1.4 Importance of Pioglitazone for treating diabetes

Among all the glitazones rosiglitazone and pioglitazone are the widely used drugs to manage type-2 diabetes. Rosiglitazone was approved by US FDA in May 25, 1999, later this was approved in Europe, Japan, New Zealand along with some other countries. Recent reports indicate that rosiglitazone associated with high level of cardiovascular risks such as heart attacks. In view of this, US FDA placed strict restrictions for use of the rosiglitazone in 2011 and this was withdrawn from Europe in 2010.

Pioglitazone was approved by US FDA in the form of hydrochloride salt in July 15, 1999 for treatment of type-2 diabetes. Latter on this was approved in about 45 countries including Europe, Canada, Japan and India. Pioglitazone HCl was marketed under the brand name of Actos® and it is available in 15 mg, 30 mg and 45 mg tablets. Pioglitazone is also available in combination with other anti diabetic agents such as metformin and glimepride. Worldwide sales of pioglitazone hydrochloride in year 2011 are about 5500 million USD (~INR 28,000 crores) with the consumption of 75,000 kgs. The major contribution is from USA (4000 million USD) and this was the one of the top 10 best selling drugs in USA.
1.1.1.5 Pioglitazone hydrochloride Mechanism of Action

ACTOS® is a thiazolidinedione that depends on the presence of insulin for its mechanism of action. ACTOS® decreases insulin resistance in the periphery and in the liver resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output. Pioglitazone is not an insulin secretagogue. Pioglitazone is an agonist for peroxisome proliferator-activated receptor-gamma (PPARγ). PPAR receptors are found in tissues important for insulin action such as adipose tissue, skeletal muscle, and liver. Activation of PPARγ nuclear receptors modulates the transcription of a number of insulin responsive genes involved in the control of glucose and lipid metabolism.

In animal models of diabetes, pioglitazone reduces the hyperglycemia, hyperinsulinemia, and hypertriglyceridemia characteristic of insulin-resistant states such as type 2 diabetes. The metabolic changes produced by pioglitazone result in increased responsiveness of insulin-dependent tissues and are observed in numerous animal models of insulin resistance. Because pioglitazone enhances the effects of circulating insulin (by decreasing insulin resistance), it does not lower blood glucose in animal models that lack endogenous insulin.

1.1.2: Introduction and pharmacological importance of Ranolazine: An anti angina drug

1.1.2.1: Introduction
Angina also known as Angina pectoris is indication for heart disease caused by lack of blood circulation to the heart. The most widespread reason for the angina is Atherosclerosis. In coronary heart disease patients, arteries become narrow and stiff when compared with the healthy heart arteries. These narrow and stiff arteries cause difficulties to reach oxygen rich blood for heart.

**Figure 1.3:** Oxygen rich blood flow in healthy heart and angina heart

### 1.1.2.2: Classification of Angina

Angina is divided into three categories (i) chronic angina or stable angina, (ii) unstable angina and (iii) variant angina. Chronic angina is often predictable when compared other type of anginas, can be occurred during physical exercise, emotional stress or mental strain, exposure to cold weather or extreme temperature, after taking excess meal. Clots in coronary artery lead to significant reduction of oxygen rich blood flow to the heart causes severe chest pain, known as unstable angina. This is not predictable and it can be occur at rest or in sleep and it needed immediate medical attention. About 17 million Americans are suffering
with coronary heart diseases and about 9 millions are suffering with chronic angina.

1.1.2.3 **Available drugs for treating Angina**

Medications those are available for treating Angina is nitroglycerin, Aspirin, heparins (dalteparin and heparin) Beta-blockers (carvedilol, nadolol, propranolol, metoprolol and atenolol) and calcium channel blockers (verapamil and amlodipine).

1.1.2.4 **Importance of Ranolazine for treating angina**

Ranolazine is the one of the medicament used to manage chronic angina, developed by Roche Bioscience (formerly Syntex) and marketed by CV Therapeutics. Ranolazine has anti-ischemic and antianginal effects that do not depend upon reductions in heart rate or blood pressure. For this reason, it is of particular use in individuals with angina who are nonresponsive to maximal tolerated doses of other anti-anginal medications. It is also used along with the other medications that are used for treating blood pressure and heart problems.

USFDA approved Ranolazine (brand name is Ranexa®) in January 27, 2006. Subsequently European medical agency (EMEA) has approved in July 09, 2008. Latter on it was approved in few other developing countries. Ranexa ® is available in market in the form of 500 mg and 1000 mg film coated tablet and the maximum daily dosage should be less than 2.0g. Over dosage of Ranexa ® lead to dizziness, nausea, and vomiting. Worldwide sales of Ranexa® by December 2011 is about 400
millions USD (~2000 crores) with the consumption of 1, 00, 678 kg. Major contribution is from USA i.e. about 300 millions USD.

1.1.2.5 Ranolazine Mechanism of Action

The mechanism of action of ranolazine’s antianginal effects has not been determined. Ranolazine has anti-ischemic and antianginal effects that do not depend upon reductions in heart rate or blood pressure. It does not affect the rate-pressure product, a measure of myocardial work, at maximal exercise. Ranolazine at therapeutic levels can inhibit the cardiac late sodium current (INa). However, the relationship of this inhibition to angina symptoms is uncertain. The QT prolongation effect of ranolazine on the surface electrocardiogram is the result of inhibition of IKr, which prolongs the ventricular action potential.

1.1.3: Introduction and pharmacological importance of Clopidogrel bisulfate: An anti platelet drug

1.1.3.1: Introduction

Platelets are the one of the key component in blood, produced by megakaryocyte (bone marrow cell) and these are small cell fragments having average life time of about 5-9 days. Platelets provide proteins and hormones that are essential for blood to coagulate. The average number of platelets present in healthy human blood is about 0.15-0.45 millions per millimeter of blood. Blood coagulation is an important phenomenon
that helps to prevent excessive blood loss when blood vessels are injured.

![Figure 1.4: Platelet clump](image)

Platelets and proteins in plasma can help to prevent the blood loss by forming a blood clot on the injured blood vessels. The blood clots are dissolved naturally after the injury has cured. Sometimes, the blood clots form on the inside of blood vessels without an injury and these clots will not dissolve naturally. Blood clots can occur in both the veins & arteries blood vessels. The blockage can develop larger and further limit the blood flow. Blood coagulation in the coronary arteries leads to heart attack and blockage in a cerebral artery may cause a stroke.

### 1.1.3.2 Available drugs for treating platelet aggregation

Different classes of drugs used as antiplatelet agents including irreversible cyclooxygenase inhibitors (Aspirin), adenosine diphosphate (ADP) receptor inhibitors (Clopidogrel, Prasugrel, Ticagrelor and Ticlopidine), phosphodiesterase inhibitors (Cilostazol), glycoprotein
IIB/IIIA inhibitors (Abciximab, Eptifibatide and Tirofiban) and adenosine reuptake inhibitors (Dipyridamole)

**Figure 1.5:** Antiplatelet drugs

### 1.1.3.3 Importance of Clopidogrel bisulfate for treating platelet aggregation

Though there are several antiplatelet drugs available in market, the widely used drug for management of blood clots in coronary artery disease, peripheral vascular disease, and cerebrovascular disease is clopidogrel bisulfate. Recent studies have shown that clopidogrel is more effective in blocking platelet aggregation than aspirin and ticlopidine even at much lower dosage. This is ranked as one of the top selling drugs (ranked as second top selling for few years) in the globe. Clopidogrel bisulfate was developed by French pharmaceutical company,
Sanofi-aventis and marketed by Sanofi-aventis and Bristol Mayers Squib (BMS). US FDA approved clopidogrel bisulfate in year 1997 in the form of oral tablets having dosage of 75 mg and 300 mg and it is marketing under the brand name of Plavix®. Thereafter, clopidogrel bisulfate was approved in more than 110 countries. The worldwide sales of clopidogrel bisulfate is about USD 10,535 millions (~58,000 crores) with the consumption of 517,267 Kg.

**1.1.3.4 Clopidogrel Mechanism of Action**

Clopidogrel is an inhibitor of platelet activation and aggregation through the irreversible binding of its active metabolite to the P2Y12 class of ADP receptors on platelets.

**1.1.4: Introduction and pharmacological importance of Zafirlukast: An anti Asthma drug**

**1.1.4.1: Introduction**

Asthma is a disease that causes swelling and narrowing the airways of the lungs. Airways are air carriers to and from lungs. Swollen and narrower airways affect the air flow to and from the lungs and this lead to tightness of chest, wheezing, shortness of breath and cough. These symptoms are often occurs in early morning and in night. About 300 million people are affecting with asthma throughout the world and approximately 2,50,000 deaths are caused by asthma globally in 2009.
1.1.4.2: Classification of Asthma

Asthma is categorized into allergic asthma, no allergic asthma, nocturnal asthma, asthma during the pregnancy and occupational asthma. Allergic asthma is most common asthma in both childrens and adults. Asthma is caused by genetic and environmental factors, it was not curable completely but this can be controlled with good medical care.

1.1.4.3 Available drugs for treating Asthma

Medications that are used for treating asthma are divided into two categories. First category medications such as short acting beta₂-adrenoceptor agonists (salbutamol) and anticholinergic (ipratropium bromide) used for quick-relief to treat acute symptoms. Second category medications such as glucocorticoids, long acting beta-adrenoceptor
agonists (LABA), leukotriene antagonists and mast cell stabilizers are used as long term control medications to prevent further exacerbation. The first category medications are fast acting medications whereas second category medications are long control medications. Leukotriene antagonists also known as leukast are the medicaments that are used to reduce leukotrienes, which are produced by several types of cells and causes inflammation in asthma and bronchitis. Leukotriene antagonists that are available in market are Montelukast, Zafirlukast and Pranlukast.

**Figure 1.7:** Structures of Montelukast, Zafirlukast and Pranlukast
1.1.4.4 Importance of Zafirlukast for treating Asthma

Zafirlukast is the first leukotriene compound approved for management of Asthma. US FDA approved zafirlukast in the form of 10 mg and 20 mg tablet with the brand name of Accolate®. Subsequently this was approved and launched by innovator in few other countries.

1.1.4.5 Zafirlukast Mechanism of Action

Zafirlukast is a selective and competitive receptor antagonist of leukotriene D4 and E4 (LTD4 and LTE4), components of slow-reacting substance of anaphylaxis (SRSA). Cysteinyl leukotriene production and receptor occupation have been correlated with the pathophysiology of asthma, including airway edema, smooth muscle constriction, and altered cellular activity associated with the inflammatory process, which contribute to the signs and symptoms of asthma. Patients with asthma were found in one study to be 25-100 times more sensitive to the bronchoconstricting activity of inhaled LTD4 than nonasthmatic subjects.

In vitro studies demonstrated that zafirlukast antagonized the contractile activity of three leukotrienes (LTC4, LTD4 and LTE4) in conducting airway smooth muscle from laboratory animals and humans. Zafirlukast prevented intradermal LTD4-induced increases in cutaneous vascular permeability and inhibited inhaled LTD4-induced influx of eosinophils into animal lungs. Inhalational challenge studies in sensitized sheep showed that zafirlukast suppressed the airway
responses to antigen; this included both the early- and late-phase response and the nonspecific hyperresponsiveness.

In humans, zafirlukast inhibited bronchoconstriction caused by several kinds of inhalational challenges. Pretreatment with single oral doses of zafirlukast inhibited the bronchoconstriction caused by sulfur dioxide and cold air in patients with asthma. Pretreatment with single doses of zafirlukast attenuated the early- and late-phase reaction caused by inhalation of various antigens such as grass, cat dander, ragweed, and mixed antigens in patients with asthma. Zafirlukast also attenuated the increase in bronchial hyperresponsiveness to inhaled histamine that followed inhaled allergen challenge.

1.1.5: Introduction and pharmacological importance of Tadalafil: PDE-5 inhibitor

1.1.5.1: Introduction

Phosphodiesterase type 5 (PDE5) is an enzyme present in corpus cavernosum, the spongy like erectile tissue of the penis. This enzyme is the target enzyme for management of pulmonary hypertension and erectile dysfunction. In normal conditions, sexual stimulation in the male arouses neurons in the corpus cavernosum to release nitric oxide that causes the formation of cyclic guanosine monophosphate (cGMP). The chemical compound, cGMP facilitates the relaxation of corpus cavernosal smooth muscle, resulting increased blood flow into the penis and produce erection. PDE-5 enzyme causes the degradation of cGMP
and this degradation prevented by inhibits the PDE5 enzyme. The male erectile dysfunction significantly increases with age. The large patient populations, about 30 million men in the United States have erectile dysfunction (ED).

Pulmonary hypertension is one of the rare disease causes due to the increase of blood pressure in pulmonary artery, which are the vessels that carrying blood from the right hand side of the heart to lungs. In pulmonary hypertension patients, walls of the pulmonary artery became stiff and this restrict to allow the more blood through arteries. Common symptoms are shortening of breath, non-productive cough, dizzy, tiredness.

**1.1.5.2 Available PDE-5 inhibitors and for treating pulmonary hypertension**

PDE5 inhibitors that are available in market for the treatment of ED is sildenafil, vardenafil, tadalafil. There are several drugs available in market for treatment of pulmonary hypertension including warfarin sodium, furosemide, spironalactone, nifedipine, diltiazem, ambrisentan, treprostinil sodium, sildenafil and tadalafil.
1.1.5.3 Importance of Tadalafil for treating ED and pulmonary hypertension

First approved pharmaceutical compound in this class is sildenafil. In 1998, US FDA approved Sildenafil and followed by vardenafil and tadalafil was approved. Clinically significant adverse effects have been noted when sildenafil was used for the treatment of ED.\textsuperscript{10-11} Tadalafil is found to better PDE-5 inhibitor when compared with sildenafil and vardenafil since tadalafil having longer duration action (more than 24 hr) by men with ED. So usage of tadalafil is safe for men with erectile dysfunction. In 2009, tadalafil was approved for treating pulmonary hypertension.

1.1.4.5 Tadalafil Mechanism of Action

Penile erection during sexual stimulation is caused by increased penile blood flow resulting from the relaxation of penile arteries and corpus cavernosal smooth muscle. This response is mediated by the release of nitric oxide (NO) from nerve terminals and endothelial cells,
which stimulates the synthesis of cGMP in smooth muscle cells. Cyclic GMP causes smooth muscle relaxation and increased blood flow into the corpus cavernosum. The inhibition of phosphodiesterase type 5 (PDE5) enhances erectile function by increasing the amount of cGMP. Tadalafil inhibits PDE5. Because sexual stimulation is required to initiate the local release of nitric oxide, the inhibition of PDE5 by tadalafil has no effect in the absence of sexual stimulation.

The effect of PDE5 inhibition on cGMP concentration in the corpus cavernosum and pulmonary arteries is also observed in the smooth muscle of the prostate, the bladder and their vascular supply. The mechanism for reducing BPH symptoms has not been established.

Studies in vitro have demonstrated that tadalafil is a selective inhibitor of PDE5. PDE5 is found in the smooth muscle of the corpus cavernosum, prostate, and bladder as well as in vascular and visceral smooth muscle, skeletal muscle, platelets, kidney, lung, cerebellum, and pancreas.

1.2 Significance of impurity profile in process development

Related substance also known as an impurity is undesired material, apart from API and ingredients, which forms either during the synthesis of API, formulation, or upon storage. The presence of undesired chemical compounds (impurities), even in small amount, may influence the efficacy, toxicological properties and safety of the pharmaceutical products. In view of this, Impurity profiling
(identification, synthesis, quantification, and control of impurities in the API and drug product) is now gaining critical attention from regulatory authorities.

Impurities can originate from several sources such as Crystallization-related impurities, Stereochemistry-related impurities, Residual solvents, Synthetic intermediates and by-products, Formulation-related impurities, Impurities arising during storage, Method related impurity, Mutual interaction amongst ingredients, Functional group-related typical degradation.

Various Pharmacopoeias, such as the British Pharmacopoeia (BP), United States Pharmacopoeia (USP), and Indian Pharmacopoeia (IP) have incorporated limits to certain levels of impurities present in the API’s or formulations. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)\(^\text{19}\) has also published guidelines for validation of methods for analyzing impurities in new drug substances. Mainly, the source of impurities will depend on synthetic route of an API, reaction conditions, and purity of the starting materials, reagents, solvents, excipients, packaging, and storage of the end product. The following are major categories of impurities as per ICH guidelines.

\textbf{1.2.1: organic impurities}

According to ICH guidelines, proposed limit for known and unknown organic impurities are fixed as below.
1. Limit for unknown impurity is 0.10% (or 1 mg per day intake, whichever is lower) and for known impurity is 0.15% when \( \leq 2 \text{ g/day} \) intake

2. Limit for known and unknown impurity is 0.05% whenever daily intake is more than 2 g.

**1.2.2: Residual solvents**

A number of solvents that are used for the synthesis of API and formulation of drug product and these are present in the final drug product. The content of these solvents, which are commonly called organic volatile impurities (OVI), is generally determined by the OVI methods specified in the compendia. These residual solvents are classified into Class 1, Class 2 and Class 3. Particularly, Class 1 solvents such as Benzene, Carbon tetrachloride should be avoided during the synthesis. The use of Class 2 solvents such as Acetonitrile, Chloroform, Dichloromethane and methanol are allowed to use with specific limits in the final drug substance or product. Class 3 solvents are low toxic potential solvents and the allowable upper limit of these solvents is 5000 ppm. To get the approval for drug compound from the regulatory authorities, it is essential to maintain the limit of solvents based on their classes.

**1.2.3: Impurities generated through Inorganic Substances**

Metals that were used as a catalyst during synthesis of a drug substance may be evaluated for their potential risk to human health.
There are mainly three categories of metals are described along with their maximum allowable limit in the drug substance or product. Metals such as Platinum, Palladium, Rhodium, Ruthenium and Osmium are belonging to Class 1A/1B and their allowable limit is not more than 10 ppm. The limit of Chromium, Molybdenum and Nickel which are belongs to Class 1C is up to 30 ppm. Copper and Magnesium are categorized as Class 2 metals and the limit would be 250 ppm. The third category limit is 1300 ppm for the metals like Iron and Zinc.

1.3 References

1. Leonid Poretsky, Principles of diabetes mellitus (2nd edition) 2009,


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