“Comparative Study of Different Brands of Linagliptin Tablet with Innovator drug”

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APPROVAL

This Project, **Comparative Study of Different Brands of Linagliptin Tablet with Innovator drug,** submitted by **Sabiha Kamal** to the Department of Pharmacy, Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy and approved as to its style and contents.

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Dedication

I would like to dedicate my work to my parents for their never ending support in my life.

Showkat Kamal

Halima Kamal

DECLARATION

I hereby declare that, this project report is done by me under the direct supervision of **Mrs. Sharifa Sultana**; Senior Lecturer**,** Department of Pharmacy, Daffodil International University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy. I am declaring that this Project is my original work. I also declare that neither this project nor any part thereof has been submitted elsewhere for the award of Bachelor or any degree.

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ABSTRACT

Linagliptin is a dipeptidyl peptidase 4 (DPP-4) inhibitors developed by Boehringer Ingelheim,which can be used to treat diabetes mellitus type-II. This study is done to compare the parameters (weight variation, hardness, disintegration, dissolution and assay) of film coated Linagliptin tablets. Different brands of Linagliptin tablets were collected from retail pharmacy of Bangladesh market for their evaluation test and compare with innovator drug (Trajenta). As it is an INN (International Nonproprietary Names) drug, specified method from ICH Guideline is followed for their evaluation test. The results of weight variation (±2), hardness (3.8-5.3kg/cm2), disintegration time (1.47 to 3.85 minutes), dissolution (97-106% in 0.1N HCl within 45 minutes) and assay (95-105%) tests of all marketed products comply with the Innovator drug and also with the specification. This study is done to view the scenario of the quality of different brands of Linagliptin tablets in Bangladesh market and to compare with innovator drug.

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CHAPTER ONE

INTRODUCTION

1.1: AN OVERVIEW

An estimated 371 million people worldwide have type 1 and type 2 diabetes. Type 2 diabetes is the most common type, accounting for an estimated 90% of all diabetes cases.Diabetes is a chronic disease that occurs when the body either does not properly produce, or use, the hormone insulin.

Rates of type-2 diabetes have increased markedly over the last 50 years. Type-2 diabetic patients require multiple therapies to effectively control hyperglycemia. Linagliptin is a new approved oral antidiabetic drug and it acts by inhibiting the enzyme dipeptidyl peptidase-4 (DPP-4). Linagliptin was approved by the US Food and Drug Administration on 2 May 2011 based on a large development program, including four pivotal trials in patients with type-2 diabetes (T2DM). The efficacy and safety of linagliptin has seen when used as mono therapy or in combination with other oral antidiabetic drugs. Linagliptin was associated with significant improvements in glycosylated hemoglobin, fasting plasma glucose and postprandial glucose, and more patients receiving linagliptin showed meaningful improvements and achieved targets for glycosylated hemoglobin. Linagliptin was well tolerated, with an adverse event profile similar to that of placebo, and low rates of hypoglycemic events. Taken together, the pivotal trials confirm linagliptin is effective and safe in patients with T2DM: the convenience of oral dosing with no requirement for dose adjustment in patients with renal or hepatic impairment make linagliptin a valuable option when considering therapies for patients with T2DM. Type 2 diabetes mellitus (T2DM) is a progressive disease, and it occurs with increasing prevalence in the elderly and those with other comorbidities. Blood glucose control presents a challenge that is magnified by these co-existing problems. To achieve glycemic targets, many patients need more than one antidiabetic drug, and additional medications are often required as glucose control deteriorates. Consequently, the development of new antidiabetic drugs that can help meet this challenge has been an area of intensive research.

The dipeptidyl peptidase-4 (DPP-4) inhibitors are one of the recently developed therapeutic classes for treatment of hyperglycemia in T2DM. The various agents in the class have differing chemical structures, but all act by inhibiting the DPP-4 enzyme, thus prolonging the life of incretin hormones, which in turn raise insulin levels and suppress glucagon secretion in a glucose-dependent manner

High-throughput screening using an assay to detect inhibition of DPP-4 led to the discovery of linagliptin, a xanthine-based molecule with a high selectivity for DPP-4. The pharmacokinetics and pharmacodynamics of linagliptin have been reviewed in detail elsewhere. Of note, unlike other DPP-4 inhibitors, which are predominantly excreted via the kidneys, linagliptin is mainly excreted unchanged via the enterohepatic system. Based on pharmacokinetic studies, no dose adjustment is needed for patients with renal or hepatic impairment.

1.2: TABLET

Tablets are solid preparations each containing a single dose of one or more active ingredients and obtained by compressing uniform volumes of particles. They are intended for oral administration. Some are swallowed whole, some are after being chewed, some are dissolved or dispersed in water before being administered and some are retained in the mouth where the active ingredients liberated. Tablets are usually circular solid cylinders, the end surfaces of which are flat or convex. These are the most widely used solid dosage form medicaments because they offer a number of advantages to the patient, prescriber, manufacturer to the patient, manufacture and the manufacturing pharmacist. Because of these advantages their popularity is continuously increasing day by day.

"Welcome" in Britain first use the term 'tablet' to describe the compressed dosage form.

Figure 1 : Different types of Tablet

1.2.1: CLASSIFICATION OF TABLETS

Mainly tablets are classified into two classes

A. Compressed tablets

B. Molded tablets

1.2.1.1: COMPRESSED TABLETS

The compressed tablets usually prepared on large scale production methods, whereas the molded tablets are prepared extemporaneously on small scale. This two main type of tablet are further classified as follows :

1. Chewable tablets
2. Sublingual tablets
3. Effervescent tablets
4. Soluble tablets
5. Dispersible tablets
6. Gastro-resistant tablets
7. Modified release tablets
8. Tablets for use in the mouth

9. Implants
10. Soluble tablets
11. Layered tablets

1.2.1.2: MOLDED TABLETS

1. Hypodermic tablets
2. Dispensing tablets

1.2.2: PROPERTIES OF A GOOD TABLET

- It should be accurate and uniform in weight.
- The size and shape should be reasonable for easy administration.
- The tablets should not be too hard to disintegrate in the stomach.
- There should not be any incompatibilities.
- They should be chemically and physically stable during storage.
- They should not break during transportation or crumble in the hands of the patient.
- They should be attractive in appearance.
- There should not be any manufacturing defects like cracking or chipping or discoloration.
- They should be easy and economical in production.
- After administration, it should disintegrate readily.

1.2.3: ADVANTAGES

- They are easy to swallow, carry.
- They are attractive in appearance
- Sugar coating can mask unpleasant taste
- They don't require any measurement of dose. The strip or blister packing has further facilitated the process of taking the dose by the patient. Moreover it providing a sealed covering which protects the tablets from atmospheric conditions likes as air, moisture and light etc
- Some of the tablets are provided into halves and quarters by drawing lines during manufacturing to facilitate breakage whenever a fractional dose is required.
- An accurate amount of medicament even if very small can be incorporated
- Tablets provide prolonged stability to medicament
- The incompatibilities of medicaments and their deterioration due to environmental factors are less in tablet form.

1.3: QUALITY AND ITS CRITERIA

Quality is an absolute necessity for medicines. The quality of drugs means quality of treatment that ensures the well being of the patients. According to the WHO (World Health Organization), the manufacturers must assume responsibility for the quality of the drugs he produces. A medicinal product must satisfy certain pharmacopoeial standards to claim it to be a quality drug. The principal criteria for a quality drug product are shown in the following figure :

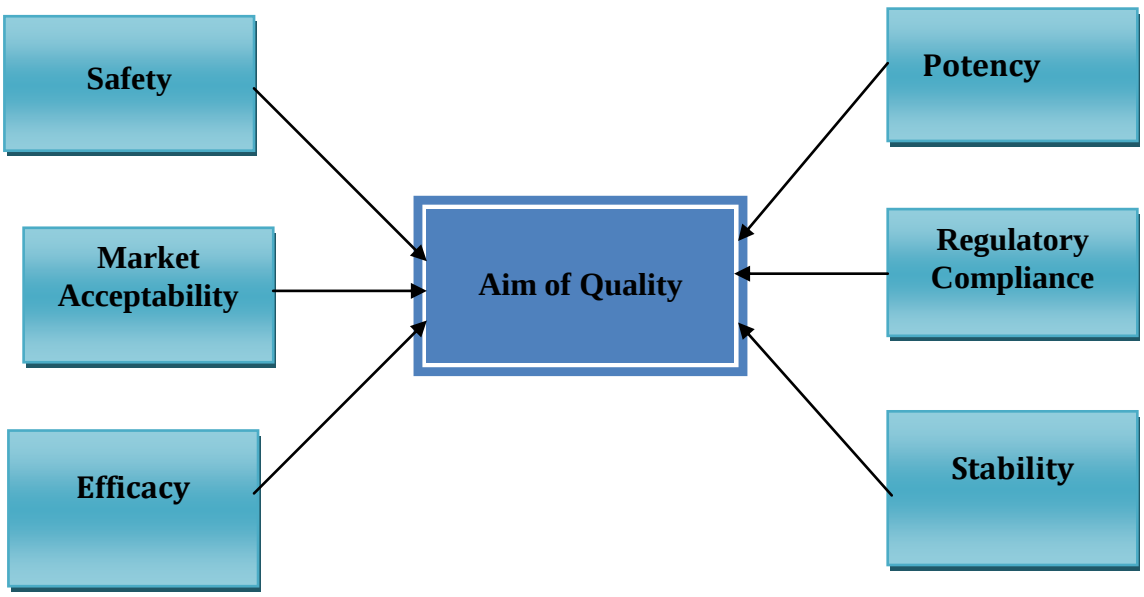

Fig.2: The aim of Quality.

SAFETY

Safety of medicine implies that the drug substance must meet certain safety requirements relating to its intended use. No drug, particularly prescription drugs, can be called absolutely safe. In the real life situation the drug-related risks (side effects) need to be compared with the risk associated with the benefit to the patient to evaluate the risk-benefit ratio. It is this ratio which one must use to judge the drug's therapeutic value.

Apart from the serious side-effect which is inherent in the drug itself, such as teratogenicity, a medicinal product can become unsafe due to many other factors such as cross-contamination, contamination with pathogenic organisms, very high or low potency, wrong labeling, inadequate packaging and storage conditions. So a careful and rational evaluation should be needed.

POTENCY

The product must contain adequate drug substance in its active form. Harmful degradation products must be absent or below defined limits.

EFFICACY

The effectiveness of a drug indicates its biological activity in animals or in human. The active substance should be adequately released from its dosage form.

STABILITY

Pharmaceutical preparations may exhibit chemical or physical instability. This may result in:

(a) Reduced activity of the drug.

(b) Formation of toxic degradation products and

(c) The drug may become inelegant and thus unacceptable.

The drug substance itself and its dosage form must be sufficiently stable to retain its minimum potency requirements satisfying the national or international pharmacopoeial monograph. In most western countries now-a-days ±5%, beyond the labeled potency is considered acceptable, unless the manufacturer has sound arguments for a greater variation. The finished product must be marketed in suitable packs to ensure its stability for use up to the expiry date when stored under specified condition.

ACCEPTABILITY

Acceptability refers to the consumer or market acceptability. This relates to the organoleptic properties such as its taste, odor, color, mode of use and qualities which are not directly noticeable to patients, e.g. too high a level of microbial contamination. A medicine should have pharmaceutical elegance for market acceptability.

REGULATORY COMPLIANCE

Each unit pack of the product must be clearly and correctly labeled. Moreover, the product must fulfill the regulatory requirements. Various information in support of the product such as potency claim, indications, side–effects, precautions, storage conditions, self-life, manufacturing date, batch number, instructions for use etc. must comply with the drug legislation.

1.4: EVALUATION OF TABLET

Tablets are evaluated according to their physical and chemical characteristics. To monitor tablet's quality, quantitative evaluations and assessments of chemical, physical and bioavailability properties must be made.

1.4.1: WEIGHT VARIATION TEST

In the process of compressing a tablet, of course there are problems; one of them is the weight variation. Usually, the range is still tolerable for large-sized tablets (diameter> 10 mm) was 3%, while for small tablet (diameter <7mm) is 5%. However, this specification ranges vary depending on the respective industry and the active ingredient of the drug. If the active ingredient is an extremely potent drug, in terms of the number of doses are very small (microgram scale) has a large effect, then the range specifications for tablet weight variation would be minimized.

Tablet weight variation in compressing process is not a trivial thing. Moreover, when affecting the uniformity of dosage units.

Tablet weight variation may be caused by:

- However, distribution at caused the vibration. So, small granule pushed, large granules will come out first, because there is a process of consolidation. Therefore, needs to be put a uniform granule size. So, before the compressing process begins, better evaluation the particle size distribution first.
- The flow of granules is not good / not free-flowing granules
- Particle distribution is not normal, because the specific gravity is different, so that the flow is bad.
- Keep the uniform of particle size distribution. Not too many fines and not too many granules. Granules with a large particle diameter which causes the resultant tablet has a variety of unsightly weight, while too fine granules which causes unsightly flow time.
- Lubricant or glidant less or not mixed evenly.

How to overcome the weight variation of tablets*:*

- **Evenly distribution of particle size**

If too many fines, then need to do are create a number of more granular. This case is commonly found on the direct compression process. This need not happen if are careful in choosing excipient for direct compression. The problem is excipient for direct compression is usually relatively more expensive.

If the active ingredient of the drug is stable to heat and humidity, then an easier way is to produce by wet granulation. Through granulation, drying and sifting, which formed granules can be more evenly. Critical points that need more attention is the moisture content and size of mesh used at the time of sifting.

- **Proper use of lubricant and glidant**

To solve tablet weight variation, excipient aerosil or colloidal silicon dioxide can be added. This excipient was added to the external phase. The amount used is usually 1-2% of the total weight of the tablet. Mixing for 10-15 minutes.

- **Specific gravity too different**

This case often occurs in the manufacture of tablets that contain more than one type of granules. Two or more active ingredients each made in separate granules (usually because of incompatible), then at the time of compression into one and coupled with the outer phase. Or two granules remain separate but when compression using two different hopper then compress into one tablet.

- **Proper tooling**

Proper tooling of the compression machine can solve the problem of weight variation. It means uniform size of each punch and diameter of the copression machine and as well as same speed in every time.

- **Optimum machine speed**

Optimum machine speed can control the weight variation of tablets because too much high or too much slow speed can varying the weight of tablets of different station.

Requirements:

Requirement is met if the weight variation of tablets is of no more than 10 tablets differs from average weight by more than percentage given below-

Table No. 1

Average weight of tablets	Percentage of difference
130mg or less	10%
130-324mg	7.5%
More than 324 mg	5%

- **ACCEPTED TABLET**

  Not more than two tablets are outside the percentage limit and no tablet differs by more than two times the percentage limit according to the above table.

- **SUSPECTED TABLET**

Not more than six tablets are outside the percentage limit and no tablet differs by more than two times the percentage limit according to the table.

- **REJECTED TABLETS**

  One tablet differs by more than two times the percentage limit according to the table.

  More than six tablets are outside the percentage limit.

Tablet weight variation in compressing process is not a trivial thing. Moreover, when affecting the uniformity of dosage units.

1.4.2: TABLET HARDNESS TEST

Tablet hardness testing is a laboratory technique used by the pharmaceutical industry to test the breaking point and structural integrity of a tablet "under conditions of storage, transportation, and handling before usage". The breaking point of a tablet is based on its shape. It is similar to friability testing, but they are not the same thing.

Tablet hardness testers first appeared in the 1930s. In the 1950s, the Strong-Cobb tester was introduced. It was patented by Robert Albrecht on July 21, 1953 and used an air pump. The tablet breaking force was based on arbitrary units referred to as Strong-Cobbs. The new one gave readings that were inconsistent to those given by the older testers. Later, electro-mechanical testing machines were introduced. They often include things like motor drives, and the ability to send measurements to a computer or printer.

There are 2 main processes to test tablet hardness: compression testing and 3 point bend testing. For compression testing, the analyst generally aligns the tablet in a repeatable way, and the tablet is squeezed by 2 jaws. The first machines continually applied force with a spring and screw thread until the tablet started to break. When the tablet fractured, the hardness was read with a sliding scale

1.4.3: DISINTEGRATION TIME TEST

Disintegration test is widely used in the pharmaceutical industry for evaluation of disintegration capability of formulations and quality control of different dosage forms. Disintegration tests are performed as per the pharmacopoeial standards. Disintegration is a measure of the quality of the oral dosage form like tablets and capsules. Each of the pharmacopoeia like the USP, BP, IP etc each have their own set of standards and specify disintegration tests of their own. USP, European pharmacopoeia and Japanese pharmacopoeia have been harmonized by the International conference on Harmonization (ICH) and are interchangeable.
The disintegration test is performed to find out the time it takes for a solid oral dosage form like a tablet or capsule to completely disintegrate. The time of disintegration is a measure of the quality. This is because,

for example, if the disintegration time is too high; it means that the tablet is too highly compressed or the capsule shell gelatin is not of pharmacopoeial quality or it may imply several other reasons. And also if the disintegration time is not uniform in a set of samples being analyzed, it indicates batch inconsistency and lack of batch uniformity.

DISINTEGRATION TEST METHOD

This test is provided to determine whether tablet disintegrate within the prescribed time when placed in a liquid medium under the experimental conditions presented below.

For the purposes of this test disintegration does not imply complete dissolution of the unit or even of its active constituent. Complete disintegration is defined as that state in which any residue of the unit, except fragments of insoluble coating or capsule shell, remaining on the screen of the test apparatus or adhering to the lower surface of the discs, if used, is a soft mass having no palpably firm core.

APPARATUS

Basket-rack assembly: The basket-rack assembly consists of six open-ended transparent tubes, each 75.0-80.0 mm long and having an internal diameter of 20.70-23.00 mm and a wall 1.0-2.8 mm thick; the tubes are held in a vertical position by two plates, each 88-92 mm in diameter and 5.00-8.50 mm in thickness, with six holes, each 22-26 mm in diameter, equidistant from the centre of the plate and equally spaced from one another. Attached to the lower surface of the lower plate is a woven stainless steel wire mesh, which has a plain square weave with 1.8-2.2 mm apertures and with a wire diameter of 0.570-0.660 mm. The parts of the apparatus are assembled and rigidly held by means of three bolts passing through the two plates. A suitable means is provided to suspend the basket-rack assembly from the raising and lowering device using a point on its axis. The design of the basket-rack assembly may be varied somewhat provided the specifications for the glass tubes and the screen mesh size are maintained.

Discs: The use of discs is permitted only where specified or allowed. Each tube is provided with a cylindrical disc 9.35-9.65 mm thick and 20.55-20.85 mm in diameter. The disc is made of a suitable, transparent plastic material having a specific gravity of 1.18-1.20. Five parallel 1.9-2.1 mm holes extend between the ends of the cylinder. One of the holes is centered on the cylindrical axis. The other holes are centered 5.8-6.2 mm from the axis on imaginary lines perpendicular to the axis and parallel to each other. Four identical trapezoidal-shaped planes are cut into the wall of the cylinder, nearly perpendicular to the ends of the cylinder. The trapezoidal shape is symmetrical; its parallel sides coincide with the ends of the cylinder and are parallel to an imaginary line connecting the centres of two adjacent holes 6 mm from the cylindrical axis. The parallel side of the trapezoid on the bottom of the cylinder has a length of 1.5-1.7 mm and its bottom edges lie at a depth of 1.50-1.80 mm from the cylinder's circumference. The parallel side of

the trapezoid on the top of the cylinder has a length of 9.2-9.6 mm and its centre lies at a depth of 2.5-2.7 mm from the cylinder's circumference. All surfaces of the disc are smooth. If the use of discs is specified, add a disc to each tube and operate the apparatus as directed under procedure.

The use of automatic detection employing modified discs is permitted where the use of discs is specified or allowed. Such discs must comply with the requirements of density and dimension given in this chapter.

PROCEDURE OF DISINTEGRATION FOR DIFFERENT TABLETS

The disintegration test for each dosage form is given in the pharmacopoeia. There are some general tests for typical types of dosage forms. However, the disintegration test prescribed in the individual monograph of a product is to be followed. If the monograph does not specify any specific test, the general test for the specific dosage form may be employed. Some of the types of dosage forms and their disintegration tests are:

Uncoated tablets: Tested using distilled water as medium at 37 ± 2 °C at 29-32 cycles per minute; test is completed after 15 minutes. It is acceptable when there is no palpable core at the end of the cycle (for at least 5 tablets or capsules) and if the mass does not stick to the immersion disc.

Coated tablets: The same test procedure is adapted but the time of operation is 30 minutes.

Enteric coated/ Gastric resistant tablets: The test is carried out first in distilled water (at room temperature for 5 min.; USP and no distilled water per BP and IP), then it is tested in 0.1 M HCL (upto 2 hours; BP) or Stimulated gastric fluid (1 hour; USP) followed by Phosphate buffer, pH 6.8 (1 hour; BP) or Stimulated intestinal fluid without enzymes (1 hour; USP).

Chewable tablets: exempted from disintegration test (BP and IP), 4 hours (USP).

These are a few examples for illustration. The disintegration tests for capsules, both hard and soft gelatin capsules are also performed in a similar manner. Also, the USP also provides disintegration tests for suppositories, peccaries etc.

FACTORS AFFECTING DISINTEGRATION: Several factors can significantly affect the disintegration time of tablets-

- **Disintegrants**: A good disintegrant will quickly break up a tablet into primary particles and ensures that the drug molecules are exposed for dissolution. Examples include corn and potato starches, sodium starch glycolate, cellulose derivatives such as sodium carboxymethyl cellulose, polyvinyl pyrollidone etc.

- **Manufacturing process**: The manufacturing processes have great influence on the disintegration behavior of tablets. The total amount of disintegrants is added in two portions. The major part is incorporated to the powders before granulation and the rest part is mixed with the dried granules along with lubricants. Disintegrants added in this manner serves two purposes those added after granulation breaks the tablet apart into granules and the portion added before granulation breaks the granules into fine particles.

- **Binders and lubricants**: The concentration of binder and lubricant used in the formulation has effect on disintegration time. At lower concentration of lubricant and binder the disintegration time is lower than that at higher concentration.

APPLICATIONS OF DISINTEGRATION TEST

- ✓ Disintegration test is a simple test which helps in the pre-formulation stage to the formulator.
- ✓ It helps in the optimization of manufacturing variables such as compressional force and dwell time.
- ✓ This test is also a simple in-process control tool to ensure uniformity from batch to batch and among different tablets.
- ✓ It is also an important test in the quality control of tablets and hard gelatin capsules.

1.4.4: DISSOLUTION TEST

In the pharmaceutical industry, drug **dissolution testing** is routinely used to provide critical in vitro drug release information for both quality control purposes, i.e. to assess batch-to-batch consistency of solid oral dosage forms such as tablets, and drug development, i.e., to predict in vivo drug release profiles.

In vitro drug dissolution data generated from dissolution testing experiments can be related to in vivo pharmacokinetic data by means of in vitro-in vivo correlations (IVIVC). A well established predictive IVIVC model can be very helpful for drug formulation design and post-approval manufacturing changes.

APPARATUS

All parts of the apparatus, including any metal that may come into contact with the sample to be tested or the dissolution medium, should be made from a chemically inert material and should not adsorb, react or interfere with the preparation or the dissolution medium. The dissolution assembly should be constructed in such a way that any vibration is reduced to a minimum. Use an apparatus that allows full visibility of all operations.

Paddle: The apparatus consists of a cylindrical vessel of suitable glass or other suitable transparent material with a hemispherical bottom and a nominal capacity of 1000 ml. The vessel is covered to prevent evaporation of the medium with a cover that has a central hole to accommodate the shaft of the stirrer and other holes for the thermometer and for devices for withdrawal of liquid. The stirrer consists of a vertical shaft with a blade at the lower end. The blade is constructed around the shaft so that it is flush with the bottom of the shaft. When placed inside the vessel, the shaft's axis is within 2mm of the axis of the vessel and the bottom of the blade is 25 ± 2mm from the inner bottom of the vessel. The upper part of the shaft is connected to a motor provided with a speed regulator so that smooth rotation of the stirrer can be maintained without any significant wobble. The apparatus is placed in a water-bath that maintains the dissolution medium in the vessel at 37 ± 0.5 °C.

Basket: The apparatus consists of the same apparatus as described for "Paddle", except that the paddle stirrer is replaced by a basket stirrer. The basket consists of two parts. The top part, with a vent, is attached to the shaft. It is fitted with three spring clips, or other suitable attachments, that allow removal of the lower part so that the preparation being examined can be placed in the basket. These three spring clips firmly hold the lower part of the basket concentric with the axis of the vessel during rotation. The lower detachable part of the basket is made of welded-seam cloth, with a wire thickness of 0.254 mm diameter and with 0.381 mm square openings, formed into a cylinder with a narrow rim of sheet metal around the top and the bottom. If the basket is to be used with acidic media, it may be plated with a 2.5-µm layer of gold. When placed inside the vessel, the distance between the inner bottom of the vessel and the basket is 25 ± 2mm.

FACTORS AFFECTING DISSOLUTION:

There are various factors that affect the dissolution property of drugs like-

Physicochemical factors of drug:

These include the size and shape of the drug particles.

- The surface area is directly related with the dissolution rate i.e., with the more surface area (decreased particle size) the dissolution rate will also be increased (e.g., in case of griseofulvin).
- The solid phase characteristics of drugs, such as amorphicity, crystallinity, state of hydration and polymorphic structure have shown to have a significant influence on the dissolution rate. For example, the amorphous form of novobiocin has a greater solubility and higher dissolution rate than the crystalline form.

Formulation factors:

To satisfy certain pharmaceutical functions, various adjuncts such as diluents, binders, disintegrates, granulating agents, lubricants, etc. are almost always used. They have very significant effects on

dissolution process e.g. usually hydrophilic lubricants like sodium lauryl sulfate increases the dissolution rate of the drug than the hydrophobic that of lubricants.

1.4.5: ASSAY / CONTENT UNIFORMITY TEST

Potency of tablet is expressed in term of grams, milligrams or micrograms (for some potent drugs) of drugs per tablet and is given as the label strength of the product.

Official compendia or other standards provide an acceptable potency range around the label potency. For highly potent, low dose drugs such as digitoxin, this range is usually not less than 90% and not more than 110% of the labeled amount. For most other larger dose drugs in tablet form the official potency range that is permitted is not less than 95% and not more than 105% of the labeled amount.

In general official potency analytical methods require that a composite sample of the tablets be taken, ground up, mixed and analyzed to produce an average potency value. In composite assays, individual discrepancies can be masked by use of the blended sample.

IMPORTANCE OF ASSAY / CONTENT UNIFORMITY TEST

- To provide same dose to the patient.
- To provide optimum Plasma concentration of the drugs.
- To provide excellent output of the drug by recovering the disease.

1.5: FACTORS/ SOURCE OF QUALITY VARIATION

Because of the increasing complexity of modern pharmaceutical manufacture arising from a variety of unique drugs and dosage forms, complex ethical, legal and economic responsibilities have been placed on those concerned with manufacture of modern pharmaceuticals. An awareness of these factors is the responsibility of all those involved in the development, manufacture, control and marketing of quality products. A systematic effective quality assurance program takes into consideration potential raw material, in- process checking, packaging material, and labeling and finished product variables. The major causes that lead to substandard drugs are given below:

(a) Addition of incorrect quantity of active ingredient or date expired sub-potent.
(b) Non-uniform distribution of active ingredients and
(c) Poor stability of active ingredients in the finished product materials.

1.6: INFORMATION ABOUT THE DRUG UNDER ANALYSIS

1.6.1: History, Discovery and Development of LINAGLIPTIN:

Type-2 diabetes is a metabolic disorder and it is characterized by high blood glucose level due to insulin resistance and relative insulin deficiency. The classic symptoms of type-2 diabetes are frequent urination (polyurea), increased thirst (polydipsia), increased hunger (Polyphagia) and weight loss. Rates of type-2 diabetes have increased markedly over the last 50 years. Recent estimates revealed that in the year 2007, 246 million people suffered from diabetes worldwide. In 2010, approximately 285 million people suffered from diabetes. This number is projected to rise to 366 million people affected in the year 2030. Type-2 diabetes caused various diseases such as heart disease, strokes, diabetic retinopathy where eyesight is affected, kidney failure.

DPP-4 inhibitors increased and prolong the effects of endogenous glucagon-like peptide-1(GLP-1) and glucose-dependent insulinotropic (GIP) by inhibiting the DPP-4 enzyme that rapidly degrades this incretin hormone. DPP-4 inhibitors prolonging GLP-1 half life and significantly reduce hemoglobin A1c (HBA1c), fasting plasma glucose (FPG) and postprandial blood glucose (PPG). GLP-1 is a gut-derived peptide and it is secreted from intestinal L-cells after meal. GLP-1 has various physiological functions such as

- Inhibition of glucagon release, gastric emptying and food intake
- Enhancement of β-cell growth and survival
- Potential of glucose simulated insulin secretion

GLP-1 and GIP-1 exert their effects by binding to their specific receptor, the GIP receptor (GIPR) and the GLP-1 receptor (GLP-1R) which belongs to the G-protein coupled receptor family. DPP-4 inhibitor treatment is associated with a low incidence of hypoglycaemia due to their glucose-dependent mode of action. DPP-4 inhibitors are body-weight neutral.

Linagliptin is a highly potent, selective, and long-acting dipeptidyl peptidase-4 (DPP-4) inhibitor of a novel chemotype that was attained upon structural optimization of a modest DPP-4 inhibitor discovered through high throughput screening (HTS). This chapter discusses high throughput screening (HTS) optimization; rationalization of DPP-4 inhibition potency by crystal structure analysis and studies of binding kinetics; basic physicochemical, pharmacological, and kinetic characteristics; and some preclinical studies. The X-ray crystal structure of compound I in complex with human DPP-4, makes it possible to highlight the main interactions of the inhibitor within the active site of the enzyme and to rationalize the observed structure-activity relationship (SAR). The development of linagliptin included a clinical pharmacology program encompassing several single- and multiple-dose randomized studies of the

absorption and disposition of linagliptin in healthy subjects and patients with type 2 diabetes mellitus (T2DM).

Linagliptin (BI-1356) was approved on 2 May 2011 by US FDA for treatment of Type-2 diabetes. Its trade names are Tradjenta and Trajenta. It is a DPP-4 inhibitor. It is being marketted by Boehringer Ingelheim and Lilly. Tradjenta is specifically indicated as an adjunct to diet and exercise to improve glycemic control in adults with type-2 diabetes mellitus. Linagliptin has been approved for monotherapy or in combination with other medications, in conjunction with exercise and dietary modification. It is administered orally in tablet form. The recommended dose is 5 mg once daily with or without food. High-throughput screening is a method for scientific experimentation is used in Linagliptin drug discovery to detect inhibition of DPP-4 enzyme in type-2 diabetes.

1.6.1.1: Clinical Studies :

Linagliptin has been studied as monotherapy and in combination with metformin, glimepiride, pioglitazone, and insulin.

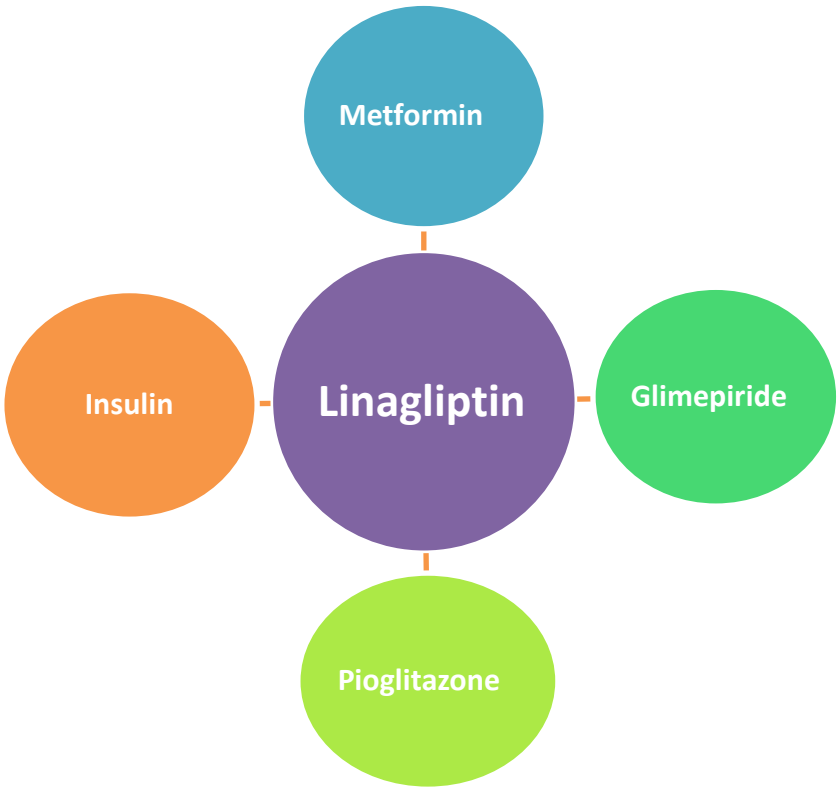

Figure 3 : Combination of Linagliptin with Metformin, Glimepiride, Insulin, Pioglitazone

A total of 3648 patients with type 2 diabetes were randomized and exposed to Linagliptin for at least 12 weeks in 10 double-blind, placebo-controlled clinical efficacy studies evaluating the effects of Linagliptin

on glycemic control. The overall ethnic/racial distribution in these studies was 69% White, 29% Asian, and 2.5% Black, and included 16% Hispanic/Latino patients. Fifty two percent of patients were male. Patients had an overall mean age of 57 years (range 20 to 91 years). In addition, an active (glimepiride)-controlled study of 104 weeks' duration was conducted in 1551 patients with type 2 diabetes who had inadequate glycemic control on metformin, and a placebo-controlled study of 52 weeks' duration was conducted in 133 patients with type 2 diabetes and severe chronic renal impairment (eGFR < 30 mL/min).

In patients with type 2 diabetes, treatment with Linagliptin produced clinically significant improvements in hemoglobin A1c (A1C), fasting plasma glucose (FPG), and 2-hour post-prandial glucose (PPG) compared with placebo.

Monotherapy:

A total of 730 patients with type 2 diabetes participated in 2 double-blind, placebo-controlled studies, one of 18 weeks' and another of 24 weeks' duration, to evaluate the efficacy and safety of Linagliptin monotherapy. In both monotherapy studies, patients currently on an antihyperglycemic agent discontinued the agent and underwent a diet, exercise, and drug washout period of about 6 weeks that included an open-label placebo run-in during the last 2 weeks. Patients with inadequate glycemic control (A1C 7% to 10%) after the washout period were randomized; patients not currently on antihyperglycemic agents (off therapy for at least 8 weeks) with inadequate glycemic control (A1C 7% to 10%) were randomized after completing the 2-week, open-label, placebo run-in period. In the 18-week study, only patients ineligible for metformin were recruited. In the 18-week study, 76 patients were randomized to placebo and 151 to TRADJENTA 5 mg; in the 24-week study, 167 patients were randomized to placebo and 336 to TRADJENTA 5 mg. Patients who failed to meet specific glycemic goals during the 18-week study received rescue therapy with pioglitazone and/or insulin; metformin rescue therapy was used in the 24-week trial.

Treatment with LINAGLIPTIN 5 mg daily provided statistically significant improvements in A1C, FPG, and 2-hour PPG compared with placebo (Table 4). In the 18week study, 12% of patients receiving TRADJENTA 5 mg and 18% who received placebo required rescue therapy. In the 24-week study, 10.2% of patients receiving LINAGLIPTIN 5 mg and 20.9% of patients receiving placebo required rescue therapy. The improvement in A1C compared with placebo was not affected by gender, age, race, prior antihyperglycemic therapy, baseline BMI, or a standard index of insulin resistance (HOMA-IR). As is typical for trials of agents to treat type 2 diabetes, the mean reduction in A1C with LINAGLIPTIN appears to be related to the degree of A1C elevation at baseline. In these 18 and 24 week studies, the changes from baseline in A1C were -0.4% and -0.4%, respectively, for those given LINAGLIPTIN, and 0.1% and 0.3%, respectively, for those given placebo. Change from baseline in body weight did not differ significantly between the groups.

Table 2 : Glycemic Parameters in Placebo-Controlled Monotherapy Studies of LINAGLIPTIN *

	18-WEEK STUDY		24-WEEK STUDY	
	TRADJENTA 5 MG	PLACEBO	TRADJENTA 5 MG	PLACEBO
A1C (%)				
Number of patients	n= 147	n= 73	n= 333	n= 163
Baseline (mean)	8.1	8.1	8.0	8.0
Change from baseline (adjusted mean***)	-0.4	0.1	-0.4	0.3
Difference from placebo (adjusted mean) (95% CI)	-0.6(-0.9, -0.3)	--	-0.7(-0.9, -0.5)	--
Patients [n (%)] achieving A1C < 7%**	32 (23.5)	8 (11.8)	77 (25)	17 (12)
FPG (mg/dL)				
Number of patients	n = 138	n = 66	n = 318	n = 149
Baseline (mean)	178	176	164	166
Change from baseline (adjusted mean***)	-13	7	-9	15
Difference from placebo (adjusted mean) (95% CI)	-21 (-31, -10)	--	-23 (-30, -16)	--
2-hour PPG (mg/dL)				
Number of patients	Data not available	Data not available	n= 67	n= 24
Baseline (mean)	--	--	258	244
Change from baseline (adjusted mean***)	--	--	-34	25
Difference from placebo (adjusted mean) (95% CI)	--	--	-58 (-82, -34)	--
* Full analysis population using last observation on study				

**18-week study: Placebo, n=68; TRADJENTA, n=136 24-week study: Placebo, n=147; TRADJENTA, n=306

***18-week study. HbA1c: ANCOVA model included treatment, reason for metformin intolerance and number of prior oral anti-diabetic medicine(s) (OADs) as class-effects, as well as baseline HbA1c as continuous covariates. FPG: ANCOVA model included treatment, reason for metformin intolerance and number of prior OADs as class-effects, as well as baseline HbA1c and baseline FPG as continuous covariates. 24-week study. HbA1c: ANCOVA model included treatment and number of prior OADs as class-effects, as well as baseline HbA1c as continuous covariates. FPG: ANCOVA model included treatment and number of prior OADs as class-effects, as well as baseline HbA1c and baseline FPG as continuous covariates. PPG: ANCOVA model included treatment and number of prior OADs as class-effects, as well as baseline HbA1c and baseline postprandial glucose after two hours as covariate.

Combination Therapy

Add-on Combination Therapy with Metformin:

A total of 701 patients with type 2 diabetes participated in a 24-week, randomized, double-blind, placebo-controlled study designed to assess the efficacy of Linagliptin in combination with metformin. Patients already on metformin (n = 491) at a dose of at least 1500 mg per day were randomized after completing a 2week, open-label, placebo run-in period. Patients on metformin and another antihyperglycemic agent (n = 207) were randomized after a run-in period of approximately 6 weeks on metformin (at a dose of at least 1500 mg per day) in monotherapy. Patients were randomized to the addition of either Linagliptin 5 mg or placebo, administered once daily. Patients who failed to meet specific glycemic goals during the studies were treated with glimepiride rescue.

In combination with metformin, Linagliptin provided statistically significant improvements in A1C, FPG, and 2-hour PPG compared with placebo (Table 5). Rescue glycemic therapy was used in 7.8% of patients treated with Linagliptin 5 mg and in 18.9% of patients treated with placebo. A similar decrease in body weight was observed for both treatment groups.

Table 3 : Glycemic Parameters in Placebo-Controlled Study for Linagliptin in Combination with Metformin*

	TRADJENTA 5 MG + METFORMIN	PLACEBO + METFORMIN
A1C (%)		

Number of patients	n = 513	n = 175
Baseline (mean)	8.1	8.0
Change from baseline (adjusted mean***)	-0.5	0.15
Difference from placebo + metformin (adjusted mean) (95% CI)	-0.6(-0.8, -0.5)	--
Patients [n (%)] achieving A1C < 7%**	127(26.2)	15(9.2)
FPG (mg/dL)		
Number of patients	n= 495	n= 159
Baseline (mean)	169	164
Change from baseline (adjusted mean***)	-11	11
Difference from placebo + metformin (adjusted mean) (95% CI)	-21(-27, -15)	--
2-hour PPG (mg/dL)		
Number of patients	n= 78	n= 21
Baseline (mean)	270	274
Change from baseline (adjusted mean***)	-49	18
Difference from placebo + metformin (adjusted mean) (95% CI)	-67(-95, -40)	--

* Full analysis population using last observation on study

**TRADJENTA 5 mg + Metformin, n=485; Placebo + Metformin, n=163

***HbA1c: ANCOVA model included treatment and number of prior oral OADs as class-effects, as well as baseline HbA1c as continuous covariates. FPG: ANCOVA model included treatment and number of prior OADs as class-effects, as well as baseline HbA1c and baseline FPG as continuous covariates. PPG: ANCOVA model included treatment and number of prior OADs as class-effects, as well as baseline HbA1c and baseline postprandial glucose after two hours as covariate.

Add-On Combination Therapy with Pioglitazone:

A total of 389 patients with type 2 diabetes participated in a 24-week, randomized, double-blind, placebo-controlled study designed to assess the efficacy of Linagliptin in combination with pioglitazone. Therapy was stopped in patients on oral antihyperglycemic therapy for a period of 6 weeks (4 weeks followed by a 2week, open-label, placebo run-in period). Drug-naïve patients entered directly into the 2-week placebo run-in period. After the run-in period, patients were randomized to receive either Linagliptin 5 mg or placebo, both in addition to pioglitazone 30 mg daily. Patients who failed to meet specific glycemic goals during the studies were treated with metformin rescue. Glycemic endpoints measured were A1C and FPG.

In initial combination with pioglitazone 30 mg, Linagliptin 5 mg provided statistically significant improvements in A1C and FPG compared to placebo with pioglitazone (Table 8). Rescue therapy was used in 7.9% of patients treated with Linagliptin 5 mg/pioglitazone 30 mg and 14.1% of patients treated with placebo/pioglitazone 30 mg. Patient weight increased in both groups during the study with an adjusted mean change from baseline of 2.3 kg and 1.2 kg in the Linagliptin 5 mg/pioglitazone 30 mg and placebo/pioglitazone 30 mg groups, respectively ($p = 0.0141$).

Add-On Combination Therapy with Insulin:

A total of 1261 patients with type 2 diabetes inadequately controlled on basal insulin alone or basal insulin in combination with oral drugs participated in a randomized, double-blind placebo-controlled trial designed to evaluate the efficacy of Linagliptin as add-on therapy to basal insulin over 24 weeks. Randomization was stratified by baseline HbA1c ($< 8.5\%$ vs $\geq 8.5\%$), renal function impairment status (based on baseline eGFR), and concomitant use of oral antidiabetic drugs (none, metformin only, pioglitazone only, metformin + pioglitazone). Patients with a baseline A1C of $> 7\%$ and $< 10\%$ were included in the study including 709 patients with renal impairment (eGFR < 90 mL/min), most of whom (n=575) were categorized as mild renal impairment (eGFR 60 to < 90 mL/min). Patients entered a 2 week placebo run-in period on basal insulin (e.g., insulin glargine, insulin detemir, or NPHinsulin) with or without metformin and/or pioglitazone background therapy. Following the run-in period, patients with inadequate glycemic control were randomized to the addition of either 5 mg of Linagliptin or placebo, administered once daily. Patients were maintained on a stable dose of insulin prior to enrollment, during the run-in period, and during the first 24 weeks of treatment. Patients who failed to meet specific glycemic goals during the double-blind treatment period were rescued by increasing background insulin dose.
Linagliptin used in combination with insulin (with or without metformin and/or pioglitazone), provided statistically significant improvements in A1C and FPG compared to placebo (Table 11) after 24 weeks of treatment. The mean total daily insulin dose at baseline was 42 units for patients treated with Linagliptin and 40 units for patients treated with placebo. Background baseline diabetes therapy included use of: insulin alone (16.1%), insulin combined with metformin only (75.5%), insulin combined with metformin and pioglitazone (7.4%), and insulin combined with pioglitazone only (1%). The mean change from baseline to Week 24 in the daily dose of insulin was +1.3 IU in the placebo group and +0.6 IU in the Linagliptin group. The mean change in body weight from baseline to Week 24 was similar in the two treatment groups. The rate of hypoglycemia, defined as all symptomatic or asymptomatic episodes with a

self measured blood glucose was also similar in both groups (21.4% Linagliptin; 22.9% placebo) in the first 24 weeks of the study.

1.6.1.2: Innovator: Tradjenta Approval History:

- FDA approved: Yes (First approved May 2nd, 2011)
- Brand name: Tradjenta
- Generic name: Linagliptin
- Company: Boehringer Ingelheim Pharmaceuticals Inc.
- Treatment for: Diabetes, Type 2

Tradjenta (linagliptin) is a dipeptidyl peptidase-4 (DPP-4) inhibitor indicated as adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus.

The U.S. Food and Drug Administration today approved Tradjenta (linagliptin) tablets, used with diet and exercise, to improve blood glucose control in adults with Type 2 diabetes.
People with Type 2 diabetes do not produce or respond normally to insulin, a hormone that regulates the amount of glucose in the blood. Over time, high blood glucose levels can increase the risk for serious complications, including heart disease, blindness, and nerve and kidney damage.

"This approval provides another treatment option for the millions of Americans with Type 2 diabetes," said Mary Parks, M.D., director of the Division of Metabolism and Endocrinology Products in the FDA's Center for Drug Evaluation and Research. "It is effective when used alone or when added to existing treatment regimens."

Type 2 diabetes is the most common form of the disease, affecting between 90 percent and 95 percent of the 24 million people in the United States with diabetes. Tradjenta increases the level of hormones that stimulate the release of insulin after a meal by blocking the enzyme dipeptidyl peptidase-4 or DPP-4, which leads to better blood glucose control.

Tradjenta was demonstrated to be safe and effective in eight double-blind, placebo-controlled clinical studies involving about 3,800 patients with Type 2 diabetes. The studies showed improvement in blood glucose control compared with placebo.

Tradjenta has been studied as a stand-alone therapy and in combination with other Type 2 diabetes therapies including metformin, glimepiride, and pioglitazone. Tradjenta has not been studied in

combination with insulin, and should not be used to treat people with Type 1 diabetes or in those who have increased ketones in their blood or urine (diabetic ketoacidosis).

Tradjenta will be dispensed with an FDA-approved Patient Package Insert that explains the drug's uses and risks. The most common side effects of Tradjenta are upper respiratory infection, stuffy or runny nose, sore throat, muscle pain, and headache.

FDA approves updated prescribing information for Tradjenta (linagliptin) tablets for add-on therapy to insulin in adults with type 2 diabetes:

RIDGEFIELD, Conn. and INDIANAPOLIS, August 17, 2012, 2012 /PRNewswire/ -- Boehringer Ingelheim Pharmaceuticals, Inc. and Eli Lilly and Company today announced the U.S. Food and Drug Administration (FDA) has approved a supplemental new drug application (sNDA) for Tradjenta (linagliptin) tablets for use as add-on therapy to insulin. Tradjenta is a prescription medication used along with diet and exercise to lower blood sugar in adults with type 2 diabetes, and can be used as monotherapy or in combination with other commonly prescribed medications for type 2 diabetes, such as metformin, sulfonylurea, pioglitazone or insulin. Tradjenta should not be used in patients with type 1 diabetes or for the treatment of diabetic ketoacidosis (increased ketones in the blood or urine).

The FDA's decision is based on data from a 52-week, phase 3 trial demonstrating the efficacy of Tradjenta in combination with insulin (with or without metformin and/or pioglitazone). The trial results showed adding Tradjenta to insulin produced better glucose control than insulin alone, with similar incidence of hypoglycemia (low blood sugar) in both treatment groups. Tradjenta belongs to a class of prescription medications called dipeptidyl peptidase-4 (DPP-4) inhibitors and is the first member of its class to be approved at one dosage strength (5 mg, once-daily).

Additionally, the FDA-approved label includes a clinical study in people with severe chronic renal impairment. Data from a 52-week, double-blind, randomized, placebo-controlled trial showed that use of Tradjenta 5 mg plus other glucose-lowering therapies in this patient population provided a statistically significant improvement in glycated hemoglobin (HbA1c or A1C) compared to placebo (placebo-adjusted reduction of 0.7 percent).

"Many people with type 2 diabetes taking insulin also require additional medication. With today's FDA decision, Tradjenta can be an effective add-on therapy with a demonstrated safety profile to help adult patients on insulin to improve their blood sugar control," said John Smith, M.D., Ph.D., senior vice president for clinical development and medical affairs, Boehringer Ingelheim Pharmaceuticals, Inc. "Tradjenta is the only once-daily, one-dose drug in its class without the need for dose adjustment regardless of declining renal function or hepatic impairment."

Tradjenta lowers blood sugar in a glucose-dependent manner by increasing incretin levels, which increase insulin levels after meals and throughout the day. Among many considerations when treating patients with type 2 diabetes, approximately 40 percent of individuals have some degree of renal impairment. With Tradjenta, no dose adjustment is required regardless of declining renal function or hepatic impairment.

The efficacy of Tradjenta as an add-on to basal insulin therapy was evaluated in a 52-week randomized, double-blind, placebo-controlled trial with the primary endpoint measured after 24 weeks. In this trial, a total of 1,261 patients with type 2 diabetes inadequately controlled on insulin glargine, insulin detemir, or NPH insulin were randomized to receive either Tradjenta 5 mg once daily or placebo. The trial enrolled patients with a baseline A1C of greater than or equal to 7 percent and less than or equal to 10 percent, and included 709 patients with renal impairment, most of whom were categorized as having mild renal impairment (estimated glomerular filtration rate [eGFR] 60 to <90 ml/min). A1C is measured in patients with diabetes to provide an index of blood glucose control for the previous two to three months. Patients were kept on a stable dose of insulin prior to and during the trial for the first 24 weeks. Additional background therapy combinations included basal insulin plus metformin (75.5%), basal insulin plus metformin and pioglitazone (7.4 %), and basal insulin plus pioglitazone (1%).

The primary endpoint of this trial was change in A1C after 24 weeks of treatment. At 24 weeks, Tradjenta plus basal insulin demonstrated a placebo-adjusted reduction in hemoglobin A1C of 0.65 percent from a baseline A1C of 8.3 percent. The mean change in basal insulin dose after 24 weeks was +0.6 IU/day for Tradjenta versus +1.3 IU/day for placebo. The differences in A1C seen between Tradjenta and placebo were comparable for patients with or without renal impairment, and regardless of the severity of impairment. Overall the mean change in body weight from baseline to week 24 was similar in both treatment groups. The rate of hypoglycemia also was similar in both groups (21.4%, Tradjenta and 22.9 percent, placebo) in the first 24 weeks of the study.The use of Tradjenta in combination with insulin in patients with severe renal impairment was associated with a higher rate of hypoglycemia.

The 52-week trial evaluated the efficacy and safety of Tradjenta in patients (n=133) who had both type 2 diabetes and severe chronic renal impairment, defined as eGFR of less than 30 ml/min. In addition to the study medication, patients also received background antihyperglycemic therapy, which included insulin or any combination with insulin; sulfonylurea or glinides as monotherapy; and pioglitazone or any other glucose lowering medications excluding any other DPP-4 inhibitors. For the initial 12 weeks of the study, doses of background antihyperglycemic were kept stable. During the subsequent 40-week period, the doses of background antihyperglycemic therapy could be adjusted if certain blood sugar targets were not met. At baseline, 62.5 percent of patients were receiving insulin alone as background diabetes therapy, and 12.5 percent were receiving sulfonylurea alone.

The primary endpoint of this study was the change from baseline in A1C after 12 weeks of treatment. After 12 weeks of treatment, Tradjenta 5 mg provided statistically significant improvements in A1C with an adjusted mean change of -0.6 percent, compared to placebo. Efficacy was maintained for 52 weeks with an adjusted mean change from baseline in A1C of -0.7 percent, compared to placebo.

Severe hypoglycemic events, defined as an event requiring the assistance of another person to actively administer carbohydrate, glucagon or other resuscitative actions, were reported in three (4.4 percent) Tradjenta treated patients and three (4.6 percent) placebo-treated patients.

Tradjenta is a prescription medicine that is used along with diet and exercise to lower blood sugar in adults with type 2 diabetes.

Tradjenta is not for people with type 1 diabetes or for people with diabetic ketoacidosis (increased ketones in the blood or urine).

Symptoms of a serious allergic reaction to Tradjenta are rash, raised red patches on your skin (hives), swelling of your face, lips, and throat that may cause difficulty breathing or swallowing. If you have any symptoms of a serious allergic reaction, stop taking Tradjenta and call your doctor right away.

Tell your doctor if you take other medicines that can lower your blood sugar, such as a sulfonylurea or insulin.

Tradjenta may cause serious side effects, including low blood sugar (hypoglycemia). If you take Tradjenta with another medicine that can cause low blood sugar, such as sulfonylurea or insulin, your risk of getting low blood sugar is higher. The dose of your sulfonylurea or insulin may need to be lowered while you take Tradjenta.

Signs and symptoms of low blood sugar may include headache, drowsiness, weakness, dizziness, confusion, irritability, hunger, fast heartbeat, sweating, or feeling jittery.

1.6.2: CHEMISTRY

LINAGLIPTIN tablets contain, as the active ingredient, an orally-active inhibitor of the dipeptidyl peptidase-4 (DPP-4) enzyme.

Linagliptin is described chemically as 1H-Purine-2,6-dione, 8-[(3R)-3-amino-1-piperidinyl]-7-(2-butyn-1-yl)-3,7-dihydro-3-methyl-1-[(4-methyl-2quinazolinyl)methyl]

Linagliptin

- Molecular Formula: $C_{25}H_{28}N_8O_2$
- Average mass: 472.542 Da
- Monoisotopic mass: 472.233521 Da
- MP: 202 degree C
- Density: 1.39

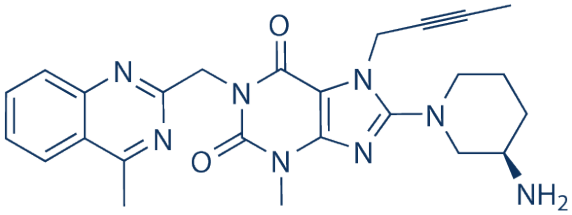

Figure 4 : Chemical Structure of Linagliptin

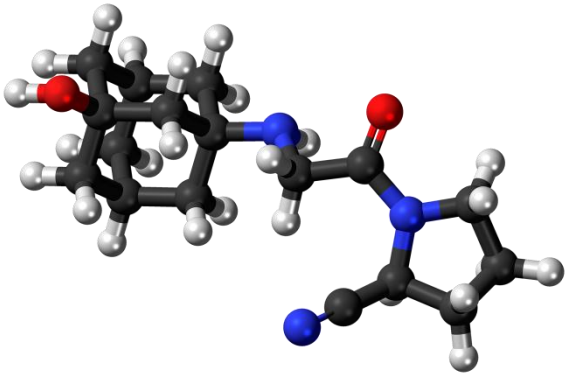

Figure 5 : 3D Structure of Linagliptin

Properties of Linagliptin:

- **Appearance:** Linagliptin is a white to yellowish, not or only slightly hygroscopic solid substance
- **Solubility:** It is very slightly soluble in water (0.9 mg/mL). Linagliptin is soluble in methanol (ca. 60 mg/mL), sparingly soluble in ethanol (ca. 10 mg/mL), very slightly soluble in isopropanol (< 1 mg/mL), and very slightly soluble in acetone (ca. 1 mg/mL).

- **API:** Each film-coated tablet of LINAGLIPTIN contains 5 mg of linagliptin free base.
- **Excipient:** and the following inactive ingredients: mannitol, pregelatinized starch, corn starch, copovidone, and magnesium stearate. In addition, the film coating contains the following inactive ingredients: hypromellose, titanium dioxide, talc, polyethylene glycol, and red ferric oxide

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1.6.3: PHARMACOLOGY

Mechanism Of Action:

Linagliptin is an inhibitor of DPP-4, an enzyme that degrades the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Thus, linagliptin increases the concentrations of active incretin hormones, stimulating the release of insulinin a glucose-dependent manner and decreasing the levels of glucagon in thecirculation. Both incretin hormones are involved in the physiological regulation of glucose homeostasis. Incretin hormones are secreted at a low basal level throughout the day and levels rise immediately after meal intake. GLP-1 and GIP increase insulin biosynthesis and secretion from pancreatic beta-cells in the presence of normal and elevated blood glucose levels. Furthermore, GLP-1 also reduces glucagon secretion from pancreatic alpha-cells, resulting in a reduction in hepatic glucose output.

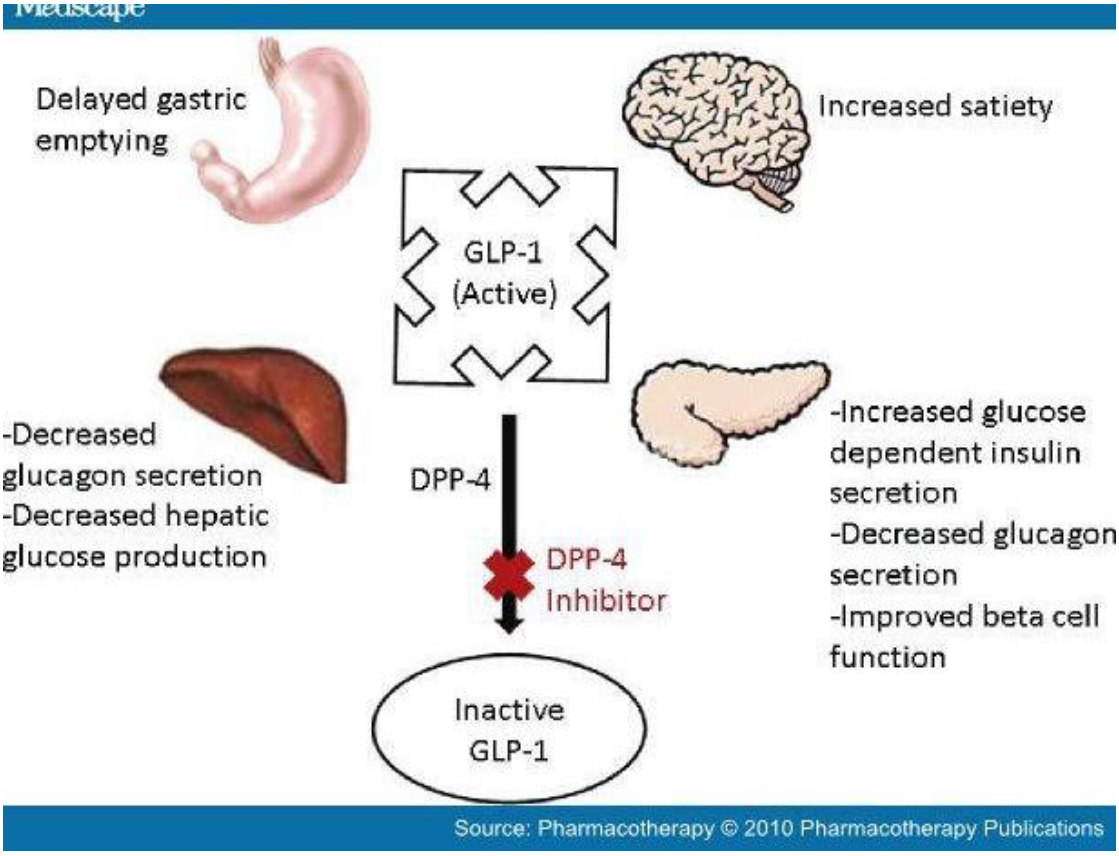

Figure 6: Mechanism of action of Linagliptin

1.6.3.1: PHARMACODYNAMICS

The treatment process of type-2 diabetes by linagliptin resulted in rapid, potent and long-lasting inhibition of plasma DPP-4 in clinical studies. Linagliptin does not bind to DPP-8 or DPP-9 activity in vitro at concentrations approximately therapeutic exposures. Linagliptin has the tendency to increase the concentration of incretin hormones and increases insulin secretion and lowers glucagon secretion [13]. As a result, glucose level is regulated in a controlled manner. Glucose excursion was measured by an OGTT, as the increment of the area under the plasma concentration-time curve (AUC) of glucose.

Linagliptin binds to DPP-4 in a reversible manner and thus increases the concentrations of incretin hormones. Linagliptin glucose dependently increases insulin secretion and lowers

glucagon secretion, thus resulting in better regulation of glucose homeostasis. Linagliptin binds selectively to DPP-4, and selectively inhibits DPP4 but not DPP-8 or DPP-9 activity *in vitro* at concentrations approximating therapeutic exposures.

Cardiac Electrophysiology

In a randomized, placebo-controlled, active-comparator, 4-way crossover study, 36 healthy subjects were administered a single oral dose of linagliptin 5 mg, linagliptin 100 mg (20 times the recommended dose), moxifloxacin, and placebo. No increase in QTc was observed with either the recommended dose of 5 mg or the 100-mg dose. At the 100-mg dose, peak linagliptin plasma concentrations were approximately 38-fold higher than the peak concentrations following a 5-mg dose.

1.6.3.2: PHARMACOKINETICS:

The pharmacokinetics of linagliptin has been characterized in healthy subjects and patients with type 2 diabetes. After oral administration of a single 5-mg dose to healthy subjects, peak plasma concentrations of linagliptin occurred at approximately 1.5 hours post dose (Tmax); the mean plasma area under the curve (AUC) was 139 nmol*h/L and maximum concentration (Cmax) was 8.9 nmol/L.

Plasma concentrations of linagliptin decline in at least a biphasic manner with a long terminal half-life (> 100 hours), related to the saturable binding of linagliptin to DPP-4. The prolonged elimination phase does not contribute to the accumulation of the drug. The effective half-life for accumulation of linagliptin, as determined from oral administration of multiple doses of linagliptin 5 mg, is approximately 12 hours. After once-daily dosing, steady-state plasma concentrations of linagliptin 5 mg are reached by the third dose, and Cmax and AUC increased by a factor of 1.3 at steady state compared with the first dose. The intra-subject and inter-subject coefficients of variation for linagliptin AUC were small (12.6% and 28.5%, respectively). Plasma AUC of linagliptin increased in a less than dose-proportional manner in the dose range of 1 to 10 mg. The pharmacokinetics of linagliptin is similar in healthy subjects and in patients with type 2 diabetes.

Absorption

The absolute bioavailability of Linagliptin is approximately 30%. High-fat meal reduced Cmax by 15% and increased AUC by 4%; this effect is not clinically relevant. TRADJENTA may be administered with or without food.

- Bioavailability: 30%
- Peak Plasma Time: 1.5 hr
- Peak Plasma Concentration: 8.9 nmol/L
- AUC: 139 nmol•h/L

Distribution

The mean apparent volume of distribution at steady state following a single intravenous dose of Linagliptin 5 mg to healthy subjects is approximately 1110 L, indicating that Linagliptin extensively distributes to the tissues. Plasma protein binding of Linagliptin is concentration-dependent, decreasing from about 99% at 1 nmol/L to 75%-89% at ≥ 30 nmol/L, reflecting saturation of binding to DPP-4 with increasing concentration of Linagliptin. At high concentrations, where DPP-4 is fully saturated, 70% to 80% of Linagliptin remains bound to plasma proteins and 20% to 30% is unbound in plasma. Plasma binding is not altered in patients with renal or hepatic impairment.

- Protein Bound: 75-99%; concentration dependent
- Vd: 1,110 L

Metabolism

Following oral administration, the majority (about 90%) of Linagliptin is excreted unchanged, indicating that metabolism represents a minor elimination pathway. A small fraction of absorbed Linagliptin is metabolized to a pharmacologically inactive metabolite, which shows a steady-state exposure of 13.3% relative to Linagliptin.

- Small fraction metabolized to inactive metabolite

Excretion

Following administration of an oral [^{14}C]-linagliptin dose to healthy subjects, approximately 85% of the administered radioactivity was eliminated via the enterohepatic system (80%) or urine (5%) within 4 days of dosing. Renal clearance at steady state was approximately 70 mL/min.

- Half-Life: 12 hr
- Terminal Half-Life: >100 hr
- Enterohepatic system (80%), urine (5%)
- Renal clearance: 70 mL/min

Specific Populations

Renal Impairment

An open-label pharmacokinetic study evaluated the pharmacokinetics of linagliptin 5 mg in male and female patients with varying degrees of chronic renal impairment. The study included 6 healthy subjects with normal renal function (creatinine clearance [CrCl] ≥ 80 mL/min), 6 patients with mild renal impairment (CrCl 50 to < 80 mL/min), 6 patients with moderate renal impairment (CrCl 30 to < 50 mL/min), 10 patients with type 2 diabetes mellitus and severe renal impairment (CrCl < 30 mL/min), and 11 patients with type 2 diabetes mellitus and normal renal function. Creatinine clearance was measured by 24-hour urinary creatinine clearance measurements or estimated from serum creatinine based on the Cockcroft-Gault formula.
Under steady-state conditions, linagliptin exposure in patients with mild renal impairment was comparable to healthy subjects.

In patients with moderate renal impairment under steady-state conditions, mean exposure of linagliptin increased ($AUC_{\tau,ss}$ by 71% and Cmax by 46%) compared with healthy subjects. This increase was not associated with a prolonged accumulation half-life, terminal half-life, or an

increased accumulation factor. Renal excretion of linagliptin was below 5% of the administered dose and was not affected by decreased renal function.

Patients with type 2 diabetes mellitus and severe renal impairment showed steady-state exposure approximately 40% higher than that of patients with type 2 diabetes mellitus and normal renal function (increase in AUCτ,ss by 42% and Cmax by 35%). For both type 2 diabetes mellitus groups, renal excretion was below 7% of the administered dose.

These findings were further supported by the results of population pharmacokinetic analyses.

Hepatic Impairment

In patients with mild hepatic impairment (Child-Pugh class A), steady-state exposure (AUCτ,ss) of linagliptin was approximately 25% lower and Cmax,ss was approximately 36% lower than in healthy subjects. In patients with moderate hepatic impairment (Child-Pugh class B), AUCss of linagliptin was about 14% lower and Cmax,ss was approximately 8% lower than in healthy subjects. Patients with severe hepatic impairment (Child-Pugh class C) had comparable exposure of linagliptin in terms of AUC0-24 and approximately 23% lower Cmax compared with healthy subjects. Reductions in the pharmacokinetic parameters seen in patients with hepatic impairment did not result in reductions in DPP-4 inhibition.

Body Mass Index (BMI)/Weight

No dose adjustment is necessary based on BMI/weight. BMI/weight had no clinically meaningful effect on the pharmacokinetics of linagliptin based on a population pharmacokinetic analysis.

Gender

No dose adjustment is necessary based on gender. Gender had no clinically meaningful effect on the pharmacokinetics of linagliptin based on a population pharmacokinetic analysis.

Geriatric

Age did not have a clinically meaningful impact on the pharmacokinetics of linagliptin based on a population pharmacokinetic analysis.

Pediatric

Studies characterizing the pharmacokinetics of linagliptin in pediatric patients have not yet been performed.

Race

No dose adjustment is necessary based on race. Race had no clinically meaningful effect on the pharmacokinetics of linagliptin based on available pharmacokinetic data, including subjects of White, Hispanic, Black, and Asian racial groups.

1.6.4: Physical Characteristics of Linagliptin Tablet

Dosage form

LINAGLIPTIN 5 mg tablets are light red, round, biconvex, bevel-edged, film-coated tablets with "D5" debossed on one side and the Boehringer Ingelheim logo debossed on the other side.

Indication

Monotherapy and Combination Therapy :

Linagliptin tablets are indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus

Important Limitations of Use:

Linagliptin should not be used in patients with type 1 diabetes or for the treatment of diabetic ketoacidosis, as it would not be effective in these settings.

Linagliptin has not been studied in patients with a history of pancreatitis. It is unknown whether patients with a history of pancreatitis are at an increased risk for the development of pancreatitis while using Linagliptin.

Dosage and administration

Recommended Dosing:

The recommended dose of Linagliptin is 5 mg once daily. Linagliptin tablets can be taken with or without food.

Concomitant Use With An Insulin Secretagogue (e.g., Sulfonylurea) Or With Insulin:

When LINAGLIPTIN is used in combination with an insulin secretagogue (e.g., sulfonylurea) or with insulin, a lower dose of the insulin secretagogue or insulin may be required to reduce the risk of hypoglycemia.

Side effects

- Anxiety
- Blurred vision
- Cold sweats
- Confusion
- Cool, pale skin
- Depression
- Dizziness
- Fast heartbeat
- Headache
- Increased hunger
- Nausea
- Nightmares
- Unusual tiredness or weakness

Rare:

- Bloating
- Constipation
- Darkened urine
- Fever
- Indigestion
- Loss of appetite
- Pains in the stomach, side, or abdomen, possibly radiating to the back
- Vomiting
- Yellow eyes or skin

Postmarketing Experience:

Additional adverse reactions have been identified during post-approval use of Linagliptin(TRADJENDA). Because these reactions are reported voluntarily from a population of uncertain size, it is generally not possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

- Acute pancreatitis, including fatal pancreatitis.
- Hypersensitivity reactions including anaphylaxis, angioedema, and exfoliative skin conditions.
- Rash.

Precautions:

Pancreatitis:

There have been postmarketing reports of acute pancreatitis, including fatal pancreatitis, in patients taking Linagliptin. Take careful notice of potential signs and symptoms of pancreatitis. If pancreatitis is suspected, promptly discontinue Linagliptin and initiate appropriate management. It is unknown whether patients with a history of pancreatitis are at increased risk for the development of pancreatitis while using Linagliptin.

Use with Medications Known to Cause Hypoglycemia:

Insulin secretagogues and insulin are known to cause hypoglycemia. The use of Tradjenta in combination with an insulin secretagogue (e.g., sulfonylurea) was associated with a higher rate of hypoglycemia compared with placebo in a clinical trial The use of Tradjenta in combination with insulin in subjects with severe renal impairment was associated with a higher rate of hypoglycaemia. Therefore, a lower dose of the insulin secretagogue or insulin may be required to reduce the risk of hypoglycemia when used in combination with Tradjenta.

Hypersensitivity Reactions:

There have been postmarketing reports of serious hypersensitivity reactions in patients treated with Linagliptin. These reactions include anaphylaxis, angioedema, and exfoliative skin

conditions. Onset of these reactions occurred within the first 3 months after initiation of treatment with Linagliptin, with some reports occurring after the first dose. If a serious hypersensitivity reaction is suspected, discontinue Linagliptin, assess for other potential causes for the event, and institute alternative treatment for diabetes.

Angioedema has also been reported with other dipeptidyl peptidase-4 (DPP-4) inhibitors. Use caution in a patient with a history of angioedema to another DPP-4 inhibitor because it is unknown whether such patients will be predisposed to angioedema with Linagliptin.

Macrovascular Outcomes:

There have been no clinical studies establishing conclusive evidence of macrovascular risk reduction with Tradjenta tablets or any other antidiabetic drug.

Contraindications:

Linagliptin is contraindicated in patients with a history of a hypersensitivity reaction to linagliptin, such as anaphylaxis, angioedema, exfoliative skin conditions, urticaria, or bronchial hyperreactivity.

Use in pregnancy and lactation:

There are no adequate and well-controlled studies in pregnant women. Linagliptin tablets should be used during pregnancy only if clearly needed.

Adverse Effects:

1-10%

- Nasopharyngitis (4.3%)
- Hyperlipidemia (2.8%; with pioglitazone)
- Cough (2.4%; with metformin and sulfonylurea)
- Hypertriglyceridemia (2.4%; with sulfonylurea)

- Weight gain (2.3%; with pioglitazone)

Hypoglycemia

- 7.6% overall incidence
- 22.9% incidence compared with placebo plus metformin and a sulfonylurea
- Incidence similar to placebo with monotherapy or combined with metformin or pioglitazone.

Postmarketing Reports

- Acute pancreatitis, including fatal pancreatitis
- Rash
- Hypersensitivity reactions including anaphylaxis, angioedema, and exfoliative skin conditions

Drug interaction:

Inducers of P-glycoprotein or CYP3A4 Enzymes

Rifampin decreased linagliptin exposure, suggesting that the efficacy of Tradjenta may be reduced when administered in combination with a strong P-gp or CYP3A4 inducer. Therefore, use of alternative treatments is strongly recommended when linagliptin is to be administered with a strong P-gp or CYP3A4 inducer

Overdose:

In the event of an overdose with Linagliptin, contact the Poison Control Center. Employ the usual supportive measures (e.g., remove unabsorbed material from the gastrointestinal tract, employ clinical monitoring, and institute supportive treatment) as dictated by the patient's clinical status. Removal of linagliptin by hemodialysis or peritoneal dialysis is unlikely.

During controlled clinical trials in healthy subjects, with single doses of up to 600 mg of Linagliptin (equivalent to 120 times the recommended daily dose) there were no dose-related clinical adverse drug reactions. There is no experience with doses above 600 mg in humans.

1.6.5: SOME MARKET PREPARATIONS OF LINAGLIPTIN

Table No. 4

Brand Name	Contains	Dosage Form	Manufacturer
Trajenta	Linagliptin 5mg	Tablet (Film-coated)	Boehringer Ingelheim Pharmaceuticals
Linita	Linagliptin 5mg	Tablet (Film-coated)	Square Pharmaceuticals Ltd.
Linatab	Linagliptin 5mg	Tablet (Film-coated)	Incepta Pharmaceuticals Ltd.
Lijenta 5	Linagliptin 5mg	Tablet (Film-coated)	Nipro JMI Pharma Ltd.

CHAPTER TWO

MATERIALS AND METHODS

2.1: MATERIALS

2.1.1: COLLECTION OF SAMPLES

There are few brands of Linagliptin (tablets) in Bangladesh. Samples were collected from retail medicine shop of different areas of Dhaka city. The samples were properly checked for their physical appearance, name of the manufacturer, batch number, manufacturing data, expiry date, manufacturing license number, D.A.R. number and maximum retail price at the time of purchase. No samples were bought and analyzed whom date of expiry had already been passed. Collected samples also covered small, medium and big companies. The samples were then coded with ethics for analysis.

2.1.2: COLLECTION OF STANDARD

The working standard of Linagliptin was obtained from Incepta Pharmaceutical Ltd. as a gift sample for research purpose. The purity of the reference standard was 99.911%.

2.1.3: CODING OF TABLETS

Linagliptin tablet from 4 different pharmaceutical companies were coded as

- Innovator
- L01
- L02
- L03

2.1.4: LABELING ON THE INNER CARTON OF THE COLLECTED SAMPLES

Each of the containers of tablets labeled with the following particulars:

(a) Brand name of the product
(b) Name of the manufacturer
(c) Composition of the product
(d) Batch number
(e) Manufacturing date
(f) Expiry date

(g) Manufacturing license number

(h) D.A.R. number

(i) Maximum retail price (M.R.P.)

2.1.5: REAGENTS

- Distilled water
- Standard Linagliptin
- 0.1N HCL
- Methanol

2.1.6: INSTRUMENTS

Table No. 5 : Instruments used in this study

Sl. No.	Instruments	Manufacturer
1	Electronic balance	Ohaus CP213, China
2	Hardness tester	Monsanto, India
3	Disintegration test apparatus	Aesico, India
4	Dissolution test apparatus	VERAGLE RC-8, China
5	T80+UV- visible spectrophotometer	PG instrumentation, England
6	Ultra sonicator bath	RICO, India

- **Samples**

For the analytical studies, the sample products of Linagliptin were collected from local market. The samples were properly checked for their batch number and expiry date. These are also of different strengths and dosage forms.

Table 6 : Samples designed in the study

SI No.	Sample Name	Company Name	Brand Name	Color	Shape	Size (Diameter) mm	Dosage Form
01.	Innovator	Boehringer Ingelheim Pharmaceuticals	Trajenta	Pink	Round	8mm	Tablet (Film-coated)
02.	L01	Square Pharmaceuticals Ltd.	Linita	Reddish	Round	6mm	Tablet (Film-coated)
03.	L02	Incepta Pharmaceuticals Ltd.	Linatab	Light Pink	Triangle Bisect line	9mm	Tablet (Film-coated)
04	L03	NIPRO JMI Pharma Limited	Lijenta 5	Light Green	Caplet NJP Embossed Bisect	9mm	Tablet (Film-coated)

2.2: METHODS

2.2.1: PHYSICAL ANALYSIS

2.2.1.1: WEIGHT VARIATION TEST

The weight variation is routinely measured to help ensured that a tablet contains proper amount of drug.

PROCEDURE

10 tablets were taken and weighed individually by an analytical balance. The average weight of the tablets was calculated. Then % of weight variation is calculated by using the following formula.

$$\% \text{ of weight variation} = \frac{\text{Individual weight} - \text{average weight}}{\text{Average weight}} \times 100$$

In this way the weight variation for 4 different brands of Linagliptin 5 Mg tablets were measured and the observed value for each sample was recorded.

Fig. 7: Analytical balance

2.2.1.2: HARDNESS TEST OF TABLETS

IMPORTANCE OF HARDNESS TEST

Hardness test of material is indicative of its strength. On the other hand, one can say it also indicates resistance power to damage its intactness. For tablet, it reflects the internal bonding strength of granules/powder which can able to hold composite structure under applied external force.

How hardness of oral formulation related to pharmacokinetics is shown by figure;

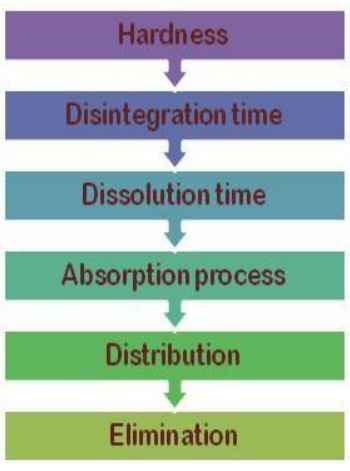

Fig. 8 & 9: How hardness of oral formulation related to pharmacokinetics & Hardness tester

2.2.1.3: DISINTEGRATION TIME TEST OF TABLETS

Disintegration time is the length of time required for causing disintegration of tablet. This test is important to evaluate a tablet since it directly influences the onset of action. This test not only evaluates the quality but also the bioavailability and effectiveness of tablets.

PROCEDURE

About 700ml 0.1N HCl was taken in 1000ml beaker and the beaker was placed into the device. One Linagliptin 5 Mg tablet was placed in each tube of basket rack and plastic disk is placed over each tablet and the basket rack is accurately positioned into the beaker. The temperature was maintained as 37±2 ℃. A motor driven device helps to move the basket up down through a distance of 5-6cm at a rate of 28-32 cycles per minutes. The time at which all the Linagliptin tablets passed through the sieve was the disintegration time was calculated. In this way disintegration time was determined for 4 different brands of Linagliptin tablets and the observed result for each sample was recorded.

Fig. 10: Disintegration apparatus used for disintegration time test

2.2.1.4: DISSOLUTION RATE TEST OF TABLETS

Dissolution is the property or tendency of a drug to undergo solution, which affects the rate of drug absorption.

MEDIUM: 0.1 N HCl

PROCEDURE

900 ml of 0.1N HCL solution was filled into 1000 ml beaker of dissolution apparatus. Each Linagliptin 5 Mg tablet of each brand was placed into each beaker. The test was repeated for 3 times for 3 sample of each brand. The dissolution medium was heated up to 37±o.5 ℃ by an auto heater and 50 R.P.M was adjusted. 5 ml of solution was withdrawn from beaker after 10minutes and fill with 5 ml 0.1N HCl. Then withdrawn solution was filtered through filter paper. The withdrawn solution of the sample absorbance was measured at 297 nm by using UV-visible spectrophotometer. Finally the percentage (%) release of Linagliptin 5 Mg tablet was determined.

In this way the dissolution rates of 4 different brands of Linagliptin 5 Mg tablet were determined and the observed value for each sample was recorded.

Fig. 11: Dissolution apparatus used for dissolution test

2.2.2: CHEMICAL ANALYSIS

2.2.2.1: PREPARATION OF STANDARD CURVE OF LINAGLIPTIN:

10 mg of Linagliptin 5 Mg was measured by the electronic balance (Ohaus) and placed in 100ml volumetric flask and dissolved by methanol. Then the concentration of solution was attained 100µg/ml by adding 0.1N HCl up to 100 ml. Then 1ml of solution was taken from the 100 ml of volumetric flask and phosphate buffer was added up to 10 ml and the concentration was 10µg/ml. Then a series of standard solution of standard Linagliptin 5 Mg e.g. 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml, 5µg/ml, 6µg/ml, 7µg/ml, 8µg/ml, 9µg/ml, 10µg/ml were taken for check absorbance at 297 nm against a blank for each solution by UV-spectrophotometer. The measured absorbance's were plotted against the respective concentration of the standard solutions which give a straight line.

Fig. 12: UV spectroscopy used for measuring the absorbance

Table No. 7: Absorbance of different concentration of standard *Linagliptin* solution measured at 297 nm.

Concentration (mcg /ml)	Absorbance	Average absorbance
00	00 00 00	00
01	0.079 0.081 0.083	0.081
02	0.166 0.170 0.179	0.171
03	0.207 0.209 0.217	0.211
04	0.258 0.256 0.261	0.258
05	0.302 0.297 0.299	0.299
06	0.349 0.357 0.359	0.355
07	0.403 0.405 0.408	0.404
08	0.454 0.449 0.451	0.451
09	0.497 0.499 0.491	0.495
10	0.51 0.55 0.53	0.53

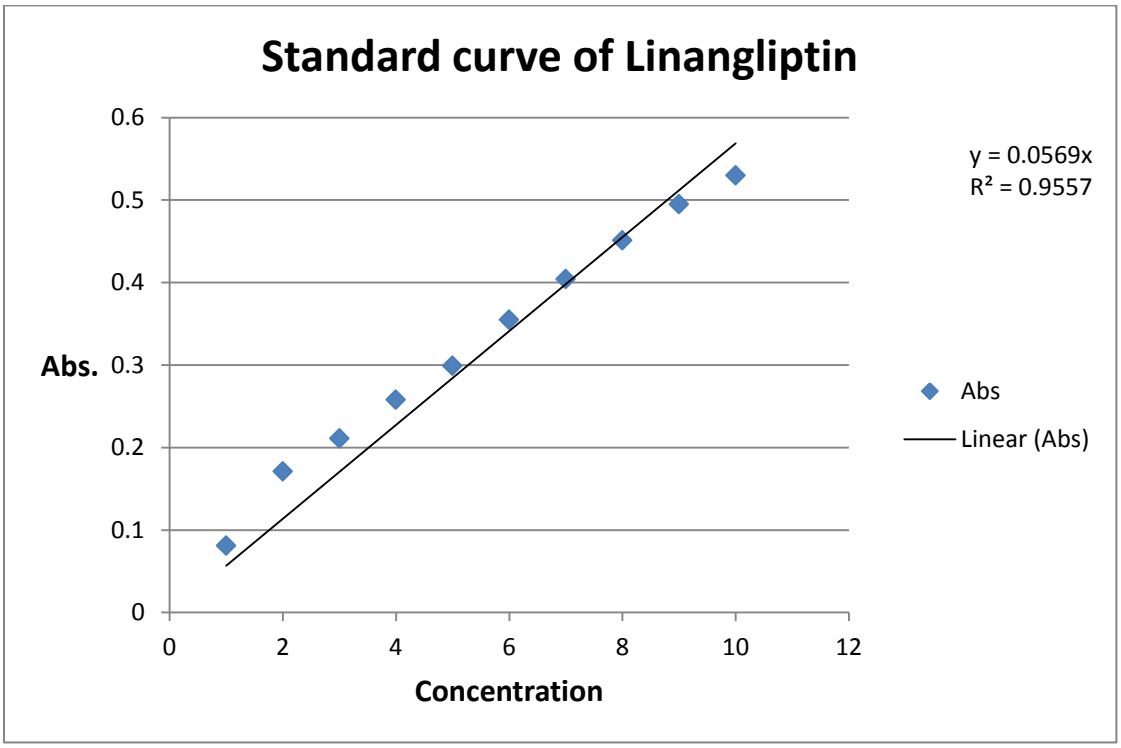

Fig. 13: Standard curve of Linagliptin

2.2.2.2: ASSAY / CONTENT UNIFORMITY / POTENCY TEST

PREPARATION OF STANDARD SOLUTION OF LINAGLIPTIN:

To prepare a standard solution, 10 mg of Linagliptin was measured by the electronic balance (Ohaus) and placed in 100 ml volumetric flask and dissolved by methanol. Then the concentration of solution was attained 100µg/ml by adding methanol to 100 ml. Then 1 ml of solution was taken and diluted to 10 ml with methanol and absorbance was measured at 295nm. To get more precise absorbance it was done at least two times.

PREPARATION OF ASSAY SOLUTION:

10 tablet of each brand of Linagliptin tablet was weighed and powdered. Equivalent weight of 10 mg of Linagliptin was weighed and dissolved by methanol and added methanol up to about 70

ml. Then the solution was sonicated about 15 minutes in the sonicator. After cooling the solution methanol was added into the volumetric flask up to 100 ml and the solution was filtered. 1 ml of sample was taken in a test tube and made the volume 10 ml with methanol. Absorbance was measured at 295 nm using T80+UV/Vis Spectrophotometer. This was done at least 3 times for each brand of Linagliptin tablet.

MEASUREMENT

The absorbance of both standard and sample were measured at 295 nm using T80+UV/Vis Spectrophotometer.

CALCULATION

Finally the assay was calculated by using the following equation.

Assay of sample = Abs. of Sam. × Wt. of Std. × Potency × Avg. Wt. of Sam. × DF / Abs. of Std. × Wt. of Sam.

Where,

- DF = Dilution Factor'
- Wt. of Sam. = Weight of sample;
- Wt. of Std. = Weight of sample;
- Abs. of Sam. = Absorbance of sample;
- Abs. of Std. = Absorbance of standard.
- Avg. Wt. of Sam. = Average weight of sample;

CHAPTER THREE

RESULTS & DISCUSSION

3.1: WEIGHT VARIATION

The weight variations of four brands of Linagliptin were determined and the observed results are shown in the following table.

USP specification of weight variation:

- ✓ ±10% for **130mg or less** average weight of tablet.
- ✓ ±7.5% for **more than 130mg** average weight of tablet.

Table No. 8: Weight Variation of Innovator

SI. No.	Individual weight(mg)	Average Weight(mg)	Weight variation %	Average Weight variation %	Specification
01	174	181.6	4.18	1.70	±7.5%
02	185		1.87		
03	182		0.22		
04	186		2.42		
05	179		1.43		
06	185		1.87		
07	177		2.53		
08	181		0.33		
08	185		1.87		
10	182		0.22		

Table No. 9: Weight variation of L 01

SI. No.	Individual weight(mg)	Average Weight(mg)	Weight variation %	Average Weight variation %	Specification
01	82	84.2	2.61	2.228	±10%
02	83		1.42		
03	83		1.42		
04	84		0.23		
05	83		1.42		
06	84		0.23		
07	87		3.32		
08	86		2.13		
09	81		3.80		
10	89		5.70		

Table No. 10: Weight variation of L02

SI. No.	Individual weight(mg)	Average Weight(mg)	Weight variation %	Average Weight variation %	Specification
01	192	192.1	0.05	1.19	±7.5%
02	193		0.46		
03	195		0.016		
04	189		0.016		
05	190		1.09		
06	193		0.47		
07	188		2.13		
08	195		1.50		
09	199		3.59		
10	187		2.65		

Table No. 11: Weight Variation of L03

SI. No.	Individual weight(mg)	Average Weight(mg)	Weight variation %	Average Weight variation %	Specification
01	108	103.1	4.75	3.996	±7.5%
02	98		4.94		
03	102		1.07		
04	102		1.07		
05	94		8.83		
06	107		3.78		
07	96		6.89		
08	105		1.84		
09	108		4.75		
10	101		2.04		

Result: From the above tables, it is appeared that all brands of Linagliptin tablets complied with the specification of weight variation of Innovator drug.

3.2: HARDNESS

The hardness of four brands of Linaglipti tablet was determined and the observed results are shown in the following table.

Table No. 12: Hardness of 4 brands of Linagliptin tablet

Sample Code	S1(kg/cm2)	S2(kg/cm2)	S3(kg/cm2)	S4(kg/cm2)	S5(kg/cm2)	Avg.(kg/cm2)
Innovator	4.5	4.9	4.2	4.5	4.5	4.51
L01	3.5	4.0	3.8	3.5	4.5	3.86
L02	5.0	5.5	5.7	5.0	5.6	5.36
L03	4.25	4.5	4.4	4.0	4.5	4.33

Result: From the above tables, it is appeared that all brands of Linagliptin tablets complied with the specification of hardness of Innovator drug.

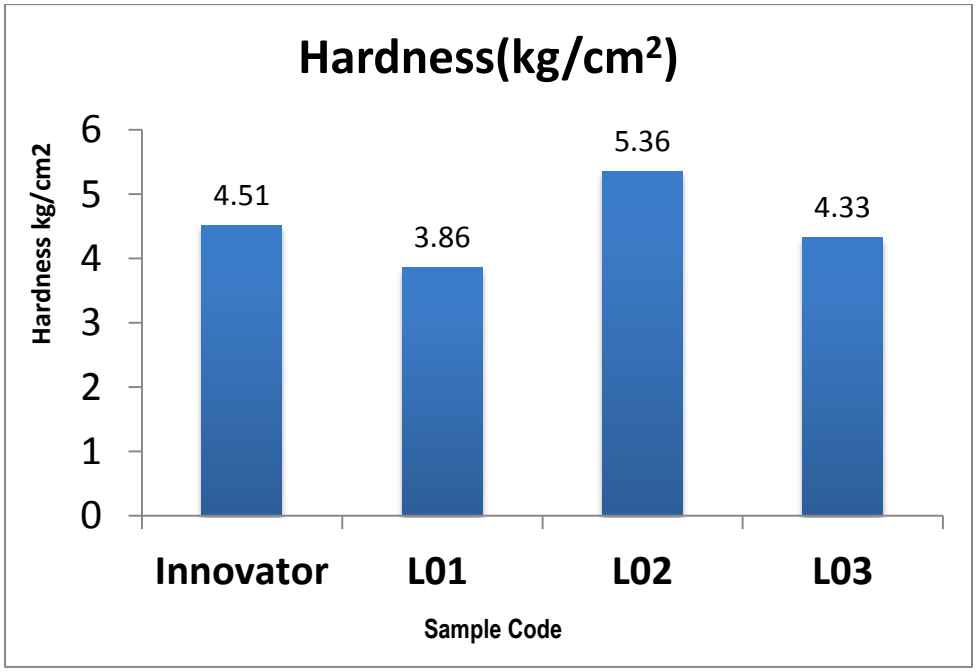

Figure14: Hardness (kg/cm^2) of four brands of Linagliptin tablet

3.3: DISINTEGRATION TIME

The disintegration time of four brands in dist. water of Linagliptin are shown in table. The specification of disintegration time is not more than 30 minutes.

Table No. 13: Disintegration time of four brands of Linagliptin tablet

Sample Code	T1(min.)	T2(min.)	T3(min.)	T4(min.)	T5(min.)	T6(min.)	Avg.(min)
Innovator	3.15	3.18	3.20	3.20	3.15	3.28	3.19
L01	3.29	3.57	3.43	3.39	3.56	4.00	3.54
L02	3.30	3.56	4.17	4.28	3.49	4.31	3.85
L03	1.34	1.48	1.54	1.38	1.57	1.49	1.47

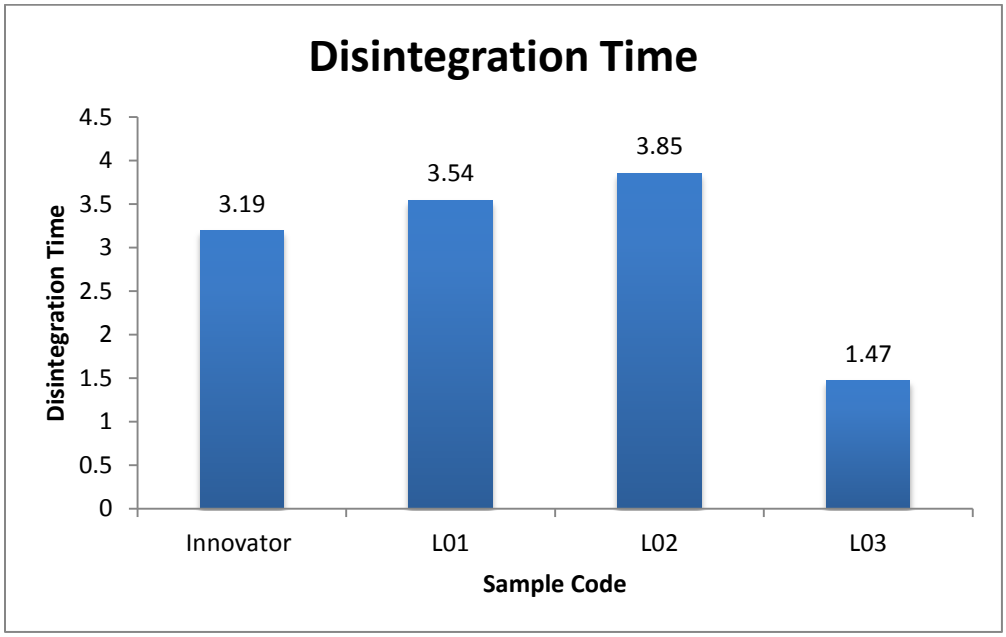

Fig. 15: Disintegration time of four brands of Linagliptin tablet in Distilled Water

It was seen from the result (table) that none of the marketed Linagliptin sample exceeded the specification and therefore it can be said that all the marketed sample complied with the Innovator drug for tablet disintegration time.

3.4: DISSOLUTION TEST

The dissolution rate of four brands of Linagliptin tablets was determined. The observed results were shown in table. The drug release % was plotted against the times, which give dissolution curve.

Specification: Not less than 75% in 0.1 N HCl of the labeled amount of Linagliptin to be dissolved in 45 minutes.

Table No. 14: Dissolution rate of Innovator

Dissolution in 0.1 N HCL

SI. No.	Time interval	drug release %				Average %	SD	RSD%
		Sample 1	Sample 2	Sample 3	Sample 4			
01	After 10 min	51.47	45.80	53.19	47.68	49.53	3.39	6.84
02	After 20 min	99.44	88.59	97.82	92.41	94.56	4.99	5.27
03	After 30 min	102.42	101.67	106.42	100.32	102.70	2.62	2.55
04	After 45 min	106.85	105.60	108.51	104.95	106.47	1.56	1.47

Table No. 15: Dissolution rate of L01

SI. No.	Time interval	Drug release %				Average %	SD	RSD%
		Sample 1	Sample 2	Sample 3	Sample 4			
01	After 10 min	78.50	82.14	76.48	69.32	76.61	5.39	7.0
02	After 20 min	97.32	93.88	99.69	100.74	97.90	3.04	3.12
03	After 30 min	101.39	99.97	102.53	110.66	106.88	4.79	4.62
04	After 45 min	98.57	95.88	99.87	98.32	97.66	2.61	1.69

Table No. 16: Dissolution rate of L02

Dissolution in 0.1 N HCL

SI. No.	Time interval	Drug release %				Average %	SD	RSD%
		Sample 1	Sample 2	Sample 3	Sample 4			
01	After 10 min	86.69	87.01	88.23	87.08	87.25	0.67	0.771
02	After 20 min	99.13	98.46	98.55	99.32	98.86	0.424	0.429
03	After 30 min	102.95	100.76	101.29	103.55	102.13	1.324	1.293
04	After 45 min	105.16	104.84	103.32	105.5	104.70	0.962	0.917

Table No. 17: Dissolution rate of L03

Dissolution in 0.1 N HCL

SI. No.	Time interval	drug release %				Average %	SD	RSD%
		Sample 1	Sample 2	Sample 3	Sample 4			
01	After 10 min	66.65	79.46	82.98	80.38	77.36	7.29	9.43
02	After 20 min	87.82	85.43	98.84	95.44	91.88	6.30	6.86
03	After 30 min	93.25	95.44	102.2	99.07	97.49	3.95	4.05
04	After 45 min	104.02	103.53	105.73	106.11	104.84	1.26	1.20

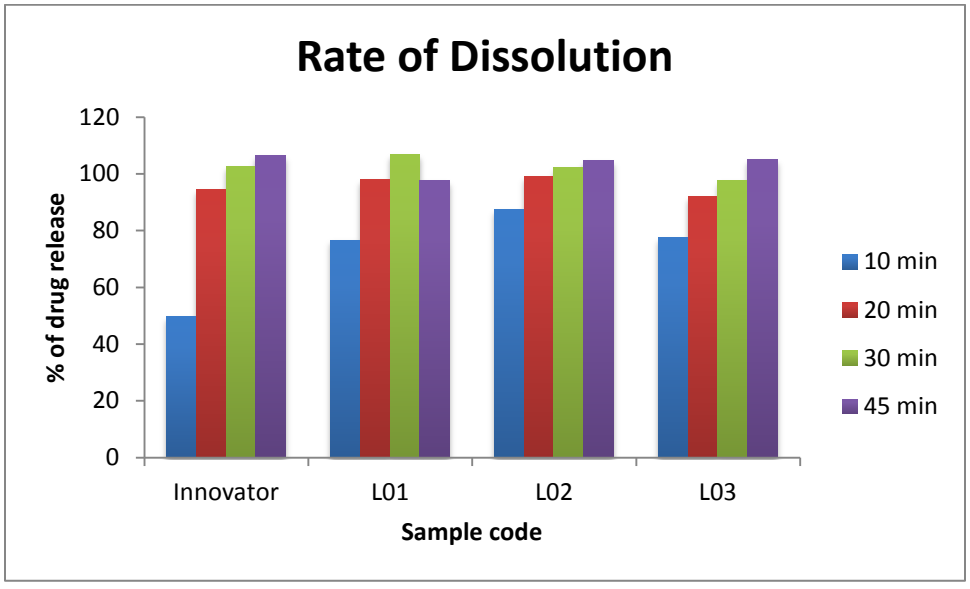

Fig. 16: Dissolution rate of four brands of Linagliptin tablet in 0.1N HCl

Result: All the brands meet the specification of the U.S.P standard as they release in cases of all brands more than 75% within 45 minutes in 0.1 N HCl dissolution medium.

3.5: ASSAY AND POTENCY TEST

Table No. 19: Potency of Innovator

SI. No.	Wt of sam(mg)	Abs of sam	Wt of Std (mg)	Abs of Std	Assay (mg)	Potency %	SD	RSD %
01	384.2	0.54	10	0.53	5.11	102.3	0.989	0.974
02	380.1	0.51	10		5.04	100.9		
Average Potency = 101.6%								

Result: All the tablets meet the specification of potency and the RSD value is lower than 2%.

Table No. 20: Potency of L01

SI. No.	Wt of sam(mg)	Abs of sam	Wt of Std (mg)	Abs of Std	Assay (mg)	Potency %	SD	RSD %
01	174.2	0.57	10	0.53	5.01	100.2	1.69	1.71
02	170.4	0.51	10		4.89	97.8		
Average Potency = 99.0%								

Result: All the tablets meet the specification of potency and the RSD value is lower than 2%.

Table No. 21: Potency of L02

SI. No.	Wt of sam(mg)	Abs of sam	Wt of Std (mg)	Abs of Std	Assay (mg)	Potency %	SD	RSD %
01	384.5	0.57	10	0.53	5.08	101.7	0.176	0.161
02	396.6	0.60	10		4.85	97.17		
Average Potency = 99.43%								

Result: All the tablets meet the specification of potency and the RSD value is lower than 2%.

Table No. 22: Potency of L03

SI. No.	Wt of sam(mg)	Abs of sam	Wt of Std (mg)	Abs of Std	Assay (mg)	Potency %	SD	RSD %
01	214.3	0.51	10	0.53	4.62	92.50	1.343	1.467
02	219.2	0.58	10		5.39	107.8		
Average Potency = 100.15 %								

Result: All the tablets meet the specification of potency and the RSD value is lower than 2%.

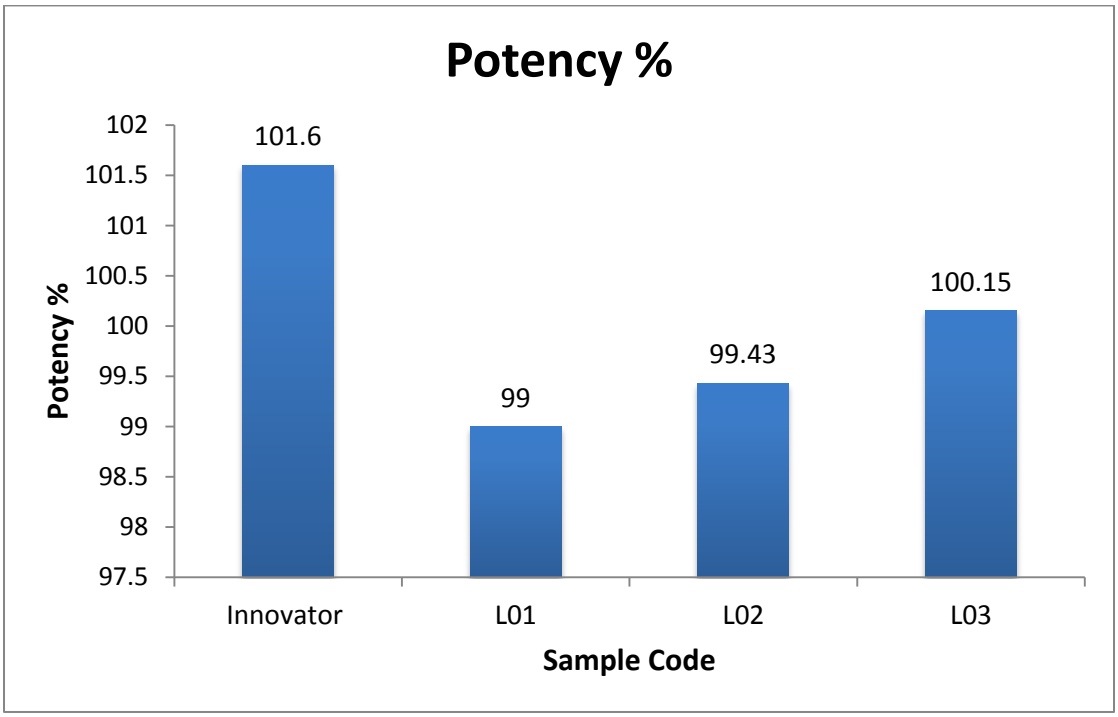

Fig. 17: Average potency of Innovator and three market preparations of Linagliptin tablet

CHAPTER FOUR

CONCLUSION

CONCLUSION

Linagliptin tablets have been analyzed to find their correct Quality status. For this purpose, the marketed sample of four brands of Linagliptin tablets was analyzed by using established methods from ICH Guidelines and apparatus. The result of weight variation, hardness, disintegration time, dissolution and assay potency tests of all marketed products comply with innovator drug. Weight variation is within the limit for all brands, hardness is within the limit for all brands, disintegration time is also within the official limit for all brands. Dissolution rate of each brands are also within the limit that all the drugs release more than 75% drug for all brands within 45 minutes in 0.1N HCl dissolution medium. All of the brands have proved that they have the potency which meets the specification. The present study, although performed on a limited scale yet on the basis of professional judgment the data reported in this study can help the Drug Control Authority to get an idea about the quality status of the marketed Linagliptin preparations in Bangladesh.

CHAPTER FIVE

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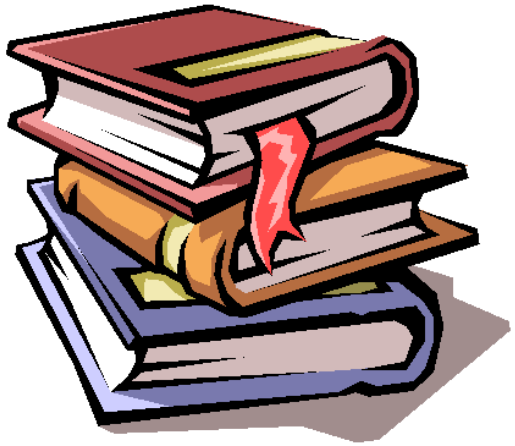

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