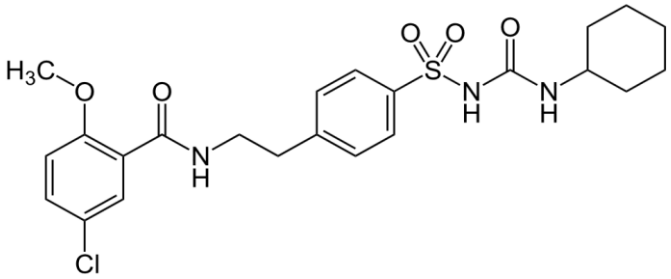
1.1 Introduction

Glibenclamide is one of the most commonly applied second generation sulfonyl urea's known as an oral antidiabatic agent; chemically it is; 1-{4{2-5-chloro-2-methoxy benzamido} ethyl benezene-sulfonyl}-3-cyclo hexyl urea}.



Chemical Structure of Glibenclamide

Physicaly; glibenclamide is a white crystalline powder; insoluble in water; sparingly soluble in methylene chloride; slightly soluble in alcohol and it dissolve in dilute solution of alkali hydroxides. glibenclamide (International Nonproprietary Names "INN"), also known as glyburide (United States Adopted Names "USAN"), is an antidiabetic drug in a class of medications known as sulfonylureas, closely related to sulfa drugs. It was developed in 1966. It is sold in doses of 1.25 mg, 2.5 mg and 5 mg, under the trade names Diabeta, glynase and micronase in the United States, and Daonil, Semi-Daonil and Euglucon in the United Kingdom and Delmide in India. It is also sold in combination with metformin under the trade names Glucovance and Glibomet. It is used in the treatment of type II diabetes. As of 2007, it is one of only two oral antidiabetics in the World Health Organization Model List of Essential Medicines (the other being metformin). As of 2003, in the United States, it was the most popular sulfonylurea (WHO, 2007).

Additionally, recent research shows that glibenclamide improves outcome in animal stroke models by preventing brain swelling. A retrospective study showed that in type 2 diabetic patients already taking glyburide, there was improved NIH stroke scale scores on discharge compared to diabetic patients not taking glyburide. The drug works by inhibiting adenosine tri

phosphate (ATP)-sensitive potassium channels in pancreatic beta cells. This inhibition causes cell membrane depolarization opening voltage-dependent calcium channel. This results in an increase in intracellular calcium in the beta cell and subsequent stimulation of insulin release (Serrano *et al*., 2006).

1.2 Solubility

Glibenclamide has high permeability and poor water solubility. The poor water solubility is responsible for its poor dissolution rate, which ultimately leads to variable absorption of Glibenclamide. Practically insoluble in water, sparingly soluble in methylene chloride, slightly soluble in alcohol and in methanol. It dissolves in dilute solution of alkali hydroxides (Nawale *et al*., 2013).

1.3 Stability Study

Stability is defined as the capacity of a drug substance or drug product to remain within time. It is influenced by a variety of environmental factors such as temperature, humidity and light. Knowledge from stability studies enables understanding of the long-term effects of the environment on drugs. Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and excipients in the drug product. The results are applied in developing manufacturing processes and selecting proper packaging, storage conditions, product's shelf life and expiration dates. Because the distribution environment is highly variable, products must be distributed in a manner that ensures product quality will not be adversely affected. The effect of possible temperature and humidity fluctuations, outside of labelled storage conditions, during transportation of drug products can be evaluated on the basis of the stability analysis of the drug. Established specifications to maintain its identity, strength, quality, and purity throughout the re-test expiration dating periods (Pamuru *et al*., 2012).

Stability tests represent and indispensable part of the testing program for pharmaceutical or cosmetic products since the instability of the preparation modifies requisites, like: quality, efficacy and safety. Stability can be affected by environmental factors such as temperature, pH, light and air,

which can provoke severe damages on the constituents of the product (André *et al*., 2007).

1.3.1. Stability Protocols

Usually, the Normal Stability Test is conducted in an interval of 90 to 120 days, maintaining the test samples stored, at minimum, in three distinct storage conditions involving temperature, humidity and luminosity. A standard or reference sample should be stored at conditions which conserves its physical, physicochemical and chemical characteristics or a condition that worthless modification are known and expected. The packaging material must be the final one for the future market accessibility or a neutral glass and all the stability assays must be realized, at least, in replicates of two. The results obtained from the normal stability test, after interpretation and statistical treatment, may be extrapolated, suggesting valuable theoretical data about the shelf life of the product (André *et al*., 2007).

1.3.2. Stability Assessment

The stability of biopharmaceuticals is complex and needs to be assessed using a raft of techniques. These techniques must include stability indicating analytical methods to assure chemical and physical stability and also confirmation of biological activity using a suitable biological or biochemical assay.

It is generally not possible to obtain reference standards for biopharmaceuticals and so the un-manipulated or freshly manipulated licensed medicine is often used as a reference.

1.3.3. Temperature and humidity

Special transportation and climatic conditions outside the storage conditions recommended should be supported by additional data. For example, these data can be obtained from studies on one batch of the product conducted for up to 3 months at 50°C/ambient humidity to cover extremely hot and dry conditions and at 25°C/80% RH to cover extremely high humidity conditions. Stability testing at a storage condition of extremely high humidity is not considered necessary (Parkar *et al*., 2015).

1.3.4. Storage Conditions

Accelerated conditions are generally not appropriate for stability trials. Hence all stability trials must be real time, real condition studies.

Generally only two storage conditions are required.

a. Refrigerated in the absence of light (5°C ± 3°C)
b. Room temperature (25°C ± 2°C)

For a refrigerator stored product the in-use room temperature data can be obtained by storing for a period at room temperature at the end of the study, this would normally be limited to 24 hours or 48 hours. This will help to reduce the costs of a study compared to an independent room temperature study being carried out. If the product is likely to be exposed to light, then the effect of exposure to continuous fluorescent light at room temperature should also be assessed.

1.3.5 Concentrations

Ideally each drug should be studied at a low and high clinically significant concentration. In this way if the drug shows a consistent stability profile it should be possible to interpolate to concentrations between the two studied concentrations. Note that with biological molecules there is a tendency for lower concentrations to be less stable, this could be related to dilution of stabilizing excipients or adsorption onto containers and components.

1.3.6 Storage Period / Study Length

Due to inherent variability in these molecules, for reconstituted products it is always best to manufacture or procure products with a shelf life which is applicable to the data but is as short as practicable. A longer shelf life does not necessarily mean a better product. Because degradation can be complex and the implications of the degradants may not be well understood it is not possible to define a safe level of degradation to define the end of a study. It is normal to assign a short but practical study period of between 48 hours and three months. It is not possible to extrapolate a shelf life beyond the study period. Ideally the study should be extended to one time point beyond the proposed shelf life of the product.

1.3.7 Sampling Strategy

It is recommended that each study includes at least four sampling points in addition to the baseline (T=0) data. These will be spread over the study period. However, if looking to assign a shelf life of six months or more then the sampling frequency must be a minimum of monthly for the first three months and three monthly thereafter as specified in the ICH. Due to the complexity of the analytical methods a different strategy can be employed to that for standard stability studies, whereby, for suitable prospective stability studies, samples are prepared at intervals (using the same batch of raw material) and the testing of all samples then takes place on the same day. Note that freshly prepared samples for T=0 must be included.

1.3.8 Sample Numbers

Strongly recommended that the stability studies include three independent batches. As a minimum each independent 'batch' must be a separate container. The ideal is for three truly independent batches of starting material to be included in the study, as expected by ICH, however, due to the cost of the samples and the analysis this is generally not possible. Each of these batches should have a minimum of three replicates. For some assay types more replicates may be necessary.

1.3.9 pH

The degradation of most drugs is calculated by extremes of pH, e.g $\{H_3O^+\}$ and$[OH^-]$and many drug are most stable between pH4 and pH8, if the acid is weak ,the concentration will depend not only on the acid concentration but also on the value of K_a (Crockford and Samuel, 1964).

1.3.10 Testing Protocols

The minimum testing protocol should include:

a. Color, clarity and particulates
b. Chemical stability
c. Physical stability

d. Assessment of sub-visible particle levels, for example light obscuration particle counting, micro flow imaging or other particle size analysis
e. Biological activity (cell based or biochemical assay as applicable)
f. Assessment of degradation and the clinical impact of degradation products

Additional parameters which may need to be considered include:

a. Moisture loss (particularly for infusion bags – can be carried out by weight check after storing at ICH low humidity storage conditions)
b. Container leachable
c. Excipients concentration

1.4. Validation

The objective of any analytical measurement is to obtain consistent, reliable and accurate data. Validated analytical methods play a major role in achieving this goal. The results from method validation can be used to judge the quality, reliability and consistency of analytical results, which is an integral part of any good analytical practice. Validation of analytical methods is also required by most regulations and quality standards that impact laboratories (Ludwig Huber, 2007).

1.4.1. The purpose of an analytical method

An analytical method details the steps necessary to perform an analysis. This may include: preparation of samples, standards and reagents; use of the apparatus; generation of the calibration curve, use of the formulate for the calculation, etc. The objective of validation of an analytical method is to demonstrate that the method is suitable for the intended use. The use of analytical methods drug development and manufacturing provides information: potency‹ which can relate directly to the requirement of a known dose. Impurities, which can relate to the safety profile of the drug. Evaluation of key drug characteristics such as crystal form ‹drug release uniformity properties‹which can compromise bioavailability. Degradation products methods need to be stability indicating. The validation of analytical

methods effect key of manufacturing parameters, to ensure that the production of drug substance and drug products consistent. The validation which is performed on the methods which generate this data needs to demonstrate that they can do so reliably and consistently (Oona, 2009).

1.4.2. Strategy for the Validation of Methods

The validity of a specific method should be demonstrated in laboratory experiments using samples or standards that are similar to unknown samples analyzed routinely. The preparation and execution should follow a validation protocol, preferably written in a step-by-step instruction format. This proposed procedure assumes that the instrument has been selected and the method has been developed. It meets criteria such as ease of use; ability to be automated and to be controlled by computer systems; costs per analysis; sample throughput; turnaround time; and environmental, health and safety requirements. Possible steps for a complete method validation are listed below (Ludwig Huber, 2006).

1. Develop a validation protocol, an operating procedure or a validation master plan for the validation.
2. For a specific validation project define owners and responsibilities.
3. Develop a validation project plan.
4. Define the application, purpose and scope of the method.
5. Define the performance parameters and acceptance criteria.
6. Define validation experiments.
7. Verify relevant performance characteristics of equipment.
8. Qualify materials, e.g. standards and reagents for purity, accurate amounts and sufficient stability.
9. Perform pre-validation experiments.
10. Adjust method parameters and /or acceptance criteria if necessary.
11. Perform full internal (and external) validation experiments.
12. Develop SOPs for executing the method in the routine.
13. Define criteria for revalidation.
14. Define type and frequency of system suitability tests and/or analytical quality control (AQC) checks for the routine.
15. Document validation experiments and results in the validation report.

1.4.3. Types of Analytical Procedures to be validated

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

a. Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product. Although there are many other analytical procedures, such as dissolution testing for drug products or particle size determination for drug substance, these have not been addressed in the initial text on validation of analytical procedures. Validation of these additional analytical procedures are equally important to those listed herein and may be addressed in subsequent documents. A brief description of the types of tests considered in this document is provided below.

b. Identification tests are intended to ensure the identity of an analyze in sample. This is normally achieved by comparison of a property of the sample (e.g., spectrum, chromatographic behavior, chemical reactivity, etc) to that of a reference standard;

c. Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test;

d. Assay procedures are intended to measure the analyze present in a given sample. In the context of this document, the assay represents a quantitative measurement of the major component(s) in the drug substance.

For the drug product, similar validation characteristics also apply when assaying for the active or other selected component. The same validation characteristics may also apply to assays associated with other analytical procedures e.g., dissolution.

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below:

a. Accuracy
b. Precision
c. Repeatability
d. Intermediate precision
e. Specificity
f. Detection limit
g. Quantitation limit
h. Linearity
i. Range

Each of these validation characteristics is defined in the attached glossary. The table lists those validation characteristics regarded as the most important for the validation of different types of analytical procedures. This list should be considered typical for the analytical procedures cited but occasional exceptions should be dealt with on a case-by-case basis. It should be noted that robustness is not listed in the table but should be considered at an appropriate stage in the development of the analytical procedure. Furthermore revalidation may be necessary in a certain circumstances; example changes in the synthesis of the drug substance, composition of the finished product and analytical procedure. The degree of revalidation required depends on the nature of the changes. Certain other changes may require validation as well (ICH, 2005).

1.4.4. Parameters for Method Validation

The parameters for method validation have been defined in different working groups of national and international committees and are described in the literature. The parameters, as defined by the ICH and by other organizations and authors, are summarized in brief in the following paragraphs (ICH, 2005).

1.4.4.1. Analytical procedure

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus,

generation of the calibration curve, use of the formulae for the calculation, etc.

1.4.4.2. Specificity

Specificity is the ability to assess unequivocally the analyze in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

1.4.4.3. Purity Tests:

To ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyze, i.e. related substances test, heavy metals, residual solvents content, etc.

Assay (content or potency):

to provide an exact result which allows an accurate statement on the content or potency of the analyze in a sample.

1.4.4.4. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

1.4.4.5. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of

measurements.

1.4.4.6. Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision

1.4.4.7. Intermediate precision

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

1.4.4.8. Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

1.4.4.9. Detection limit

The detection limit of an individual analytical procedure is the lowest amount of analyze in a sample which can be detected but not necessarily quantities as an exact value.

1.4.4.10. Quanititation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

1.4.4.11. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

1.4.4.12. Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical

procedure has a suitable level of precision, accuracy and linearity.

1.4.4.13. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain un effected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

1.5. Validation protocol

Depending upon the culture of the company, a method validation protocol could be simple (listed below) or exhaustive (in addition to the listed items, each parameter to be validated is described in detail): How solutions are going to be made, the experimental design, how the calculations are going to be performed, any software to be utilized (e.g., Excel). If a full-length protocol is required within a particular company, then the writing of this protocol and approval of the protocol would need to be completed prior to the commencement of the validation work. Otherwise, there may be many deviations to the protocol which will be needed to be referenced in the final method validation report. Some companies also have templates for the validation reports, thereby allowing for facile population of the results. Once populated, the file is reviewed to determine if all validation parameters and acceptance criteria were met. If they were not met, a deviation is added and the proper justification must be given. If it is deemed that the justification is not appropriate, then an action plan for the specific figure of merit in question is determined (i.e., repeat analysis, change of the analytical procedure, and revalidation). Also, if the analytical method has not been approved at the time of writing the validation protocol yet, it is recommended to attach a final draft of the method to the protocol. Before starting the experimental work, the protocols must be written by a qualified person and approved by a quality assurance department. Some of the items that are necessary to be specified in the validation protocol are listed below: (Michael *et al*., 1997).

a. The analytical method for a given product or drug substance.
b. The test to be validated.
c. The test parameters for each test, including type and number of solutions and number of tests.

d. The acceptance criteria for each parameter based on an internal standard operational procedure (product or method-specific adaptations may be necessary and are acceptable, if justified).
e. List of batches of drug substance and/or drug products.
f. For a drug product the grade/quality of the excipients used in the formulation.
g. List of reference materials to be used in the validation experiments.
h. Information of the instruments and apparatus to be used.
i. Responsibilities (author, chemists, analytical research project leader, quality assurance, and etc.).

1.6. Glibenclamide

1.6.1. Diabetes mellitus

Diabetes mellitus is condition arising due to abnormal metabolism of carbohydrates, fats and proteins. It is characterized mainly by an unusually high sugar level in the blood and the presence of sugar in urine (glucosuria). The normal blood glucose level in human plasma is 70-90 mg per 100ml. Hyperglycemia is characterized by more than normal concentration of the blood sugar level falls below the normal range. In the absence of insulin a much higher concentration of blood glucose is required before it can cross the cell surface. Glucose is produced first from glycogen reserves in the liver and the resulting hyperglycemia can be regarded directed towards increasing the extracellular-intracellular glucose gradient and hence the passage of glucose into the cell. The compensatory value of this mechanism is limited, for as soon as the renal threshold is reached glucose overflows into the urine, preventing any further rise in blood sugar and leading to a depletion of the liver stores of glycogen. Insulin inhibits the formation of cyclic adenosine monophosphate and hence the breakdown of glycogen (Nolte *et al*., 2001).

Two types of diabetes mellitus do exist; type I diabetes mellitus which is insulin-dependent and is due to deficiency of insulin following autoimmune of pancreatic beta-cell. Patient with type 1 require administration of insulin. Type II diabetes mellitus is a non-insulin dependent and is due to reduced secretion of insulin or to peripheral resistance to the action of insulin. Although patients may be controlled on diet only, they may require

administration of oral antidiabetic drug or insulin to maintain controlled treatment of diabetes mellitus aimed at alleviating symptoms and minimizing the risk of long-term complication. The Objective of the therapy is to maintain glucose level as normal as possible throughout the day without producing hypoglycemia or severely restricting the patient life (Lebovitz *et al*.,1992).

1.6.2. Antidiabetic Agents

For the treatment of type II diabetes mellitus, oral hypoglycemic agents are generally used. These agents are categorized into salicylates, diguanides, biguanides, alph-glycosidase inhibitors, thiazolidinedioones and sulphonylurea. Among these agent, sulphonylureas are the most frequently utilized.

1.6.3. Sulphonylureas

Sulphonylurea antidiabetic agents are used to treat type II diabetes mellitus in which insufficient amount of insulin is being produced by the pancreas (Lebovitz *et al*., 1992). The agent work by two approaches one of them is to stimulate the pancreas to release more insulin into the blood stream. All of the body cells need insulin to help turn the food into energy. This is done by either utilizing sugar(or glucose) in the blood as a quick energy source or the sugar may be stored in the form of fat, sugar, and proteins for use later, such as for energy between meals. The other approach is that sulphonylurea helps insulin get into the cells where it can work properly to lower blood sugar and in this way, sulphonylurea will assist in lowering blood sugar and help restore the way the food is used to make energy (Nolte *et al*., 2001). The sulphonylurea are considered to be subdivided into two subcategories: the first generation agent, e.g. tolbutamide, chlorpropamide, tolazamide, acetohexamide, and the second generation agent, e.g. glyburide (glibenclamide), glipizide and gliclazide.

1.6.4. Glibenclamide Mechanism of Action

The principal action of the drug is on the beta cells of islets of langerhans where it stimulates insulin secretion and thus reducing plasma glucose concentration. The drug reduces the potassium channels causing

depolarization, calcium ion entry and hence insulin secretion. Moreover, glibenclamide reduces the serum glucagon concentration, which contributes to the hypoglycemic effect of the drug. Furthermore, the drug potentiates insulin action on target tissues, on the liver, muscles and adipose tissue by increasing insulin receptor number and by enhancing the post receptor complex enzyme reaction mediated by insulin. The principal result is decreased hepatic glucose output and increased glucose uptake in muscle (Lebovitz *et al*., 1992).

1.6.5. Glibenclamide pharmacokinetics

Following oral administration, the drug is absorbed rapidly from the gastrointestinal tract and transported in the blood as highly protein bound complex. Owing to its low aqueous solubility, the drug is characterized by dissolution-limited absorption and is metabolized in the liver into products with low hypoglycemic activity (Coppack *et al*., 1990).

1.7. Aim of the Study.

The purpose of stability testing is to provide evidence on how the quality of an active pharmaceutical ingredient or medicinal product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and to establish a re-test period for the active pharmaceutical ingredient or a shelf life for the medicinal product and recommended storage conditions. The purpose of the stability study in this thesis is to establish, based on testing a minimum of three batches of the GLB and evaluating the stability information (including, as appropriate, results of the physical, chemical, tests), a re-test period can be applicable to all future batches of the GLB manufactured under similar circumstances. The degree of variability of individual batches affects the confidence that a future production batch will remain within specification throughout the assigned re-test period.

In formulation development of any drug molecule, analysis is important. It becomes essential to develop a simple, sensitive, accurate, precise, reproducible method for the estimation of drug sample. The study described a methodology to develop a new method for quantitative determination of glibenclamide (GLB). The validated method developed for linearity,

accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) according to the guidelines of the International Conference on Harmonization (ICH).The main concern of the thesis is the development and validation of UV spectrometric method as per ICH guideline; for estimation and validation of Glibenclamide, It is simple, fast, accurate and cost efficient and reproducible spectrophotometric method. The proposed method can be successfully applied for Glibenclamide in routine analysis work.

The specific objectives of this study are as follows:

1. Stability studies should guarantee the maintenance of quality, safety, and efficacy throughout the shelf-life of a product. The effect on products of the extremely adverse climatic conditions existing throughout this study.
2. To evaluate the assay of the studied GLB samples, moreover the levels of degradation products and other appropriate attributes.
3. To make sure that the studied product retain to its original size, weight, roughness, colour, variation, craking under normal handling and storage conditions throughout its shelf life.
4. Provide specific guideline to checks for operation of total quality control system for glibenclamide (GLB).
5. Provide a suitable stability –indicating assays both in solution and solid phase in order to follow glibenclamide (GLB stability.
6. To develop and validate a simple and precise stability indicating UV spectroscopic method for the estimation of Glibenclamide in bulk and tablet dosage form.
7. To provide recommendation on how to submit analytical procedures and methods validation data to support the documentation of the identity, strength, quality, purity, and potency of drug substances and drug products, this will assemble information and present data to support analytical methodologies.

2. Materials and Methods

2.1.Chemicals .

Glibenclamide sample (from India) was a gift from Wafrapharma laboratories, Omdurman, standard: EP5, Batch no GLB/B016/12 , manufacturing date: March 2012 , expiry date :February 2017 characteristics :white crystalline powder , melting point 172 °c , sparingly soluble in methylene chloride and practically insoluble in water .

Glibenclamide standard was a gift from wafrapharma laboratories.

Ethanol MW 46.07g/mole absolute, density0.875 g/cm and boiling point 78 °c application ,QC, UV Carlo ERBA Spain .

Hydrochloric acid. MW 36.46 g/ml, Assay 35 -38% (36.5%), density 1.19 g/cm AlphaChemika. India

Methanol. HPLC grade, MW 32.04 g/m density0.791g/cm , boiling point 64.7 °c Assay 99.9%, Duksan Korea .

2.2. Instruments.

2.2.1. UV-Spectrophotometer1800,vision 32 software V123 , serial No.A11454835469/CD Shimadzu Corporation, Japan.

2.2.2. UV-Spectrophotometer1800, Code in 008 vision 32 software V123 , serial No.A11454804719/CD Shimadzu Corporation, Japan.

2.2.3. pH meter, power supply 12-20 D (6w- max) , serial No 46088, Portugal.

2.2.4. Disintegrationtype 2T822, serial No. 1216490708, Erweka , Germany.

2.2.5. temperature and humidity chamber. Model SW- 8111 H – 360 D. serial No. E 36342 JC 0001, Korea.

2.2.6. General equipment. Glassware of pyerx type(doarig company), magnetic stirrer(Shimadzu corporation, Japan) sensitive balance Shimadzu corporation, Japan) ultra sonic bath (young line company) , oven (Shimadzu corporation, Japan), furnace (Shimadzu corporation, Japan)

2.3. Stability Studies.

2.3.1. Identification tests.

Several Identification tests were carried out using samples of glibenclamide as a finish product, as follows:

2.3.1.1 Melting point.

The melting point were determined by the capillary method is the temperature at which the last solid particle of a compact column of a substance in a tube passes into the liquid phase.

2.3.1.2 Detection of lamda max.

50.0 mg of the sample was accurately weighted and dissolved in methanol, with the aid of ultrasound. diluted to 50.0 cm^3 with the same solvent. 10.0 cm^3 of the solution was transferred to 100 cm^3 volumetric flask . 1.0 $cm^3$of a 103 g/l of hydrochloric acid was added and the solution was completed to the mark with methanol. The absorbance was scanned between 230 nm and 350 nm to find lamda max.

2.3.2 Moisture content.

Loss on drying were determined by accurately weighting 1 gm of glibenclamide and the sample was transferred to Oven at 105 C° for 3 hours then cooled and weighted .

2.3.3 Sulphated ash.

Porcelain crucible was ignited firstly in the furnace at 625 C° for 30 min then it was coaled over silcagel desiccator. 1gm of the sample was accurately weighted and placed into crucible and ignited in the furnace at 625 C° for 30 min it was cooled in the desiccator and the weight was been recorded .

2.3.4 The effect of pH.

Series of buffer solutions were prepared (pH 1-13) as shown in table (2.1) . 100mg of the sample was accurately weighted and transferred to 100cm^3 volumetric flask, dissolved by each buffer. The solution was scanned by UV spectrophotometer (200-400 nm).

2.3.5 Effect of solvent.

50mg of glibenclamide was accurately weighted and transferred to 50 cm^3 volumetric flask and was dissolved and completed to mark by ; hexane, dimethylformamide, water, acetone, methanol water, cloroform and nitric acid separately, each solution was scanned by UV spectrophotometer (200-400 nm).

2.3.6 Effect of pH/temp.

50mg of glibenclamide was accurately weighted and transferred to 50 cm^3 volumetric flask then it was dissolved and completed to mark by ; 0.1M hydrochloric acid, water, 0.1M sodium hydroxide separately, scanned by UV spectrophotometer (200-400 nm) for each solvent in different temperature (RT, 50C°, 70C°).

2.3.7 Assay.

For standard solution :an accurate weight of 10mg of glibenclamide STD was dissolved in amount of methanol (0.1M HCl), the solution transferred to 100 ml volumetric flask and the volume was completed by the same solvent.

For assay solution: 10 tablet was weighted and grinded to be fine homogenous powder, an accurate weight equivalent to 10mg of glibenclamide was dissolved into amount of methanol(0.1M HCl) and sonicate for 5 min the solution was transferred to 100 ml volumetric flask the volume was completed with same solvent .

The absorbance for standard and assay solution was determined by using UV spectrophotometer.

2.4. UV. Spectrophotometric analytical method validation

2.4.1. Preparation of sample stock solution.

An accurate weight of 100mg of Glibenclamide was dissolved in amount of ethanol and (0.1M HCl) the solution was transferred to 100ml volumetric flask; volume was completed by same solvent. Solution was stirred for 5 min.

2.4.2. Preparation of standard solution.

An accurate weight of 10 mg of glibenclamide was dissolved in amount of ethanol (0.1M HCl), the solution was transferred to 100ml volumetric flask; the volume was completed by same solvent and stirred for 5 min.

2.4.3. Selection of wavelength and solvents.

An accurate weight of 10 mg of Glibenclamide was dissolved in each solvent methanol (0.1 M HCl) ethanol (0.1M HCl), water, the volume of each solvent was adjusted to 100 ml and the solution scanned in the UV spectrophotometer between (200-400 nm).

2.4.4. Linearity study.

Series of sample solution 0.05, 0.075, 0.1, 0.125, 0.15, 0.175 mg/ml was prepared respectively from stock solution (Conc=1mg/ml), the sample was read in UV at wavelength 300 nm.

2.4.5. Precision study.

10cm^3 of stock solution was transferred to 100m cm^3 volumetric flask, completed to mark with ethanol (0.1 M HCl). The absorbance of sample was read by UV at wavelength 300 nm. The method was repeated five times by different three analysts.

2.4.6. Repeatability study.

It was carried out by analyzing 0.1 mg/ml concentration of glibenclamide solution for six times .

2.4.7. Accuracy study.

The accuracy study was carried out by adding 0.02, 0.04, 0.06, 0.08, 0.1, mg/ml of standard at different level 40% 80% 120% 160% 200% to 0.05 mg/ml reanalyzed sample solution, the recover solution was read by UV at wavelength 300 nm.

2.4.8. Sensitivity.

The sensitivity of the proposed method was measured and carried out using the term limit of detection (LOD) and limit of quantification (LOQ) as follow:

LOD=3.3 (δ/ s)
LOQ=10 (δ/ s)
respectively, where δ is the standard deviation of blank and (s) is slope of calibration curve.
The LOD was calculated from linearity study curve. The slope and standard deviation was measured and applied in the role of LOD and LOQ as shown above. The LOD was found to be 0.005mg/ml and LOQ is found to be 0.0167 mg/ml

2.4.9. Range.

The range was carried out in low and high level of concentration to get RSD% ≤ 2% on concentration 0.03mg/ml and0.2 mg/ml respectively.

2.4.10. Application.

For standard solution :an accurate weight of 10mg of glibenclamide STD was dissolved in amount of ethanol (0.1 M HCl), the solution transferred to 100 ml volumetric flask and the volume was completed by same solvent .
For assay solution : 10 tablet of each product (company) was weighed and grinded to be fine homogenous powder. An accurate weight equivalent to 10 mg of glibenclamide was dissolved into amount of ethanol(0.1M HCl) and sonicate for 5 min, the solution was transferred to 100 ml volumetric flask the volume was completed with same solvent .
The absorbance for standard and assay solution was determined by used UV spectrophotometer.

Table 2.1 : preparation of buffer solutions pH(1-13)

PH	Salt solution /ml	Adjust solution /ml	Quantity of water/ml
1	125 of (0.2M KCl)	335 of (0.2M HCl)	40
2	125 of (0.2M KCl)	32.5 of (0.2M HCl)	342.5
3	250 of [$KC_6H_5(COO)_2$]	111.5 of (0.1M HCl)	138.5
4	250 of [$KC_6H_5(COO)_2$]	0.5 of (0.1M HCl)	249.5
5	250 of [$KC_6H_5(COO)_2$]	113 of (0.1 M NaOH)	137
6	250 of (0.1M [KH_2PO_4])	28 of (0.1 M NaOH)	222
7	250 of (0.1M [KH_2PO_4])	145.5 of (0.1 M NaOH)	104.5
8	250 of (0.1M [KH_2PO_4])	233.5 of (0.1 M NaOH)	16.5
9	200 of (0.1M [H_3BO_3])	48 of (0.1 M NaOH)	0
10	20 of (5.045M [NH_4Cl])	35 of 10M NH_3	45
11	250 of (0.05M [$NaHCO_3$])	113.8 of (0.1 M NaOH)	136.2
12	125 of (0.2M [KCl])	30 of (0.2 M NaOH)	45
13	125 of (0.2M [KCl])	330 of (0.2 M NaOH)	35

3.1. Result and discussion

The shelf-life of the product was predicted and conformed by following the stability study within climatic condition expected during trade storage , shipping , house store and use . The product release monograph does not include all stability test monograph only criteria that are potentially susceptible to change during storage and that may have impact on quality, safety or efficiency need to be evaluated. And the result shall be used to determine appropriate storage condition and expiration date.

The laboratory has the authority to verify the validity of analytical procedure , therefore documentation of successful completion of such studies is a basic requirement to determine whether procedure is suitable for its intended application(s).

Glibenclamide was subject through this work to intensive stability studies as a raw material, finish product. And a validation of analytical method was proposed as ICH guideline.

3.2 Identification test and stability studies of glibenclamide.

For each batch of product, appropriate laboratory determination of satisfactory conformance to final specification for the drug product is needed , including the identity and strength of each active ingredient, prior to release.

Glibenclamide identification test were carried out through this studies , including ; assay , melting point, the specific absorbance, loss and drying, sulphated ash , listed in table (3.1) .

Table (3.1): Identification test result

Test	Specification	Result
Melting point	169 °C -174 °C	172.5 °C
specific absorbance	61 to 65	63
Loss and drying	N.M.T 1%	0.58%
Sulphated ash	N.M.T 0.1%	0.08%
Assay	99%-101%	99.4%

The assay was carried out using UV spectrophotometry. The content of Glibenclamide was calculated as:

Content%= (Abs of sample*100)\ Abs of STD

An accelerated and ongoing study, (table (3.2),(3.3)) show result which prove the specifications and stability of glibenclamide sample , the result of such stability testing should be used in determining appropriate storage condition and expiration dates , and conform product shelf-life. Sample size and intervals based on statistical criteria for each attribute examined to ensure valid estimate of stability. Significant change is defined as 5% loss of potency. Dissolution failure using 12unit , failure in physical specification limit(hardness , color , ect). For less stable product, the storage condition may be reduced but the accelerated condition should still be at less 15 °C above those use long-term evaluation. The condition for storage of ongoing stability samples has not been universally agreed on (some company applied the 25°C RH 60%).

The stability test monograph need not include all of the criteria defined in the release monograph. Only those parameter that are potentially susceptible to change during storage and that may impact on quality, safety, or efficiency need to be evaluated.

Test frequency should be adequate to determinate any degradation and to provide enough data point for statistical evaluation for the scale-up batches. The first three commercial batches tests is expected initially ,at 3-month interval during first year, 6 month during second year. And yearly there after. Some companies do not evaluate beyond 36 –month different frequency may be more appropriate for ongoing stability evaluation.

Under accelerated conditions and the effect of stress condition such as temperature, humidity, light, acidity and oxidation can provide much useful information to formulator. The potential interactive effect of the bulk drug and anticipated dose from expedient may also be evaluated. It should also be noted that any ingredient which interfere with official assay automatically makes the tests non-complaint, regardless of whether an alternative assay has been developed.

The accelerated studies at elevated temperature on the dosage form should allow some extrapolation to provide a tentative shelf-life. The ICH guideline allow extrapolation of 6-month data under accelerated condition within 12-month data at 25°C \ 60% RH. To predict a shelf-life of up to 24 month. Which should rarely be extrapolated from accelerated data.

Showed the ongoing stability result for the tested drug during 18 month. The assay was performed with the validated- proposed analytical method (Table 3.2).

Table (3.2).Ongoing stability study for glibenclamide for 18 month

Batch No	GLB/B015/4/2012	EXP. Date	4/2014
specification	PB 2007	Report data	18 months
Temperature	30°C±2°C	RH	65%±5%

Test	Specification	Initial	Month				
			3	6	9	12	18
Friability	N.M.T 1%	0.41%	0.30%	0.2%	0.35%	0.17%	0.26%
Hardness	50-140 N	131.7 N	76 N	105 N	129 N	107.5 N	133.5 N
Water content%	N.M.T 4%	3.2%	3.5%	3.3%	3.6%	3.5%	3.4%
Assay	95%-105%	100%	99.4%	101.4%	98.7%	99.0%	99.2%

Table (3.3). Showed the effect of accelerated conditions in standard stability testing of glibenclamide which reveal slight variation in assay and specification when compared with the result of ongoing stability testing.

Table (3.3). Accelerated stability study for glibenclamide for 6 month.

Batch No	GLB/B015/4/2012	EXP. Date	4/2014
Specification	PB 2007	Report data	6 months
Temperature	40°C±2°C	RH	75%±5%

Test	Specification	Initial	Month					
			1	2	3	4	5	6
Friability	N.M.T 1%	0.59%	0.26%	0.64%	0.44%	0.3%	0.5%	0.5%
Disintegration time	N.M.T 15 min	4.40min	4.1 min	4.2 min	3.5 min	5.4 min	4.4 min	5.48
Hardness	50-140 N	66.3 N	64 N	90 N	120 N	77.9 N	99.1 N	107 N
Water content%	N.M.T 4%	3.0	3.2	3.5	3.7	3.73	3.81	3.9
Assay%	95%-105%	100.5	101.8	100.5	98.7	99.7%	100.3	99.8

3.3 Effect of solvent upon UV spectrum of glibenclamide

UV spectrophotometric analysis for glibenclamide with change of solvent, pH and pH/temp was carried out. The resulting solution were scanned in UV range (200nm-400nm) table (3.4), (3.5) and (3.6).

In solution , all participant in a chemical reaction are solvated, reactants, product and the transition state. The presence of solvent can speed up – or slow down a reaction , while solvent molecules help making up the structure of the product and determine their stabilities (Morrison and boyd, 1997).

The variation of absorption wavelength of glibenclamide in various solvents is due to the polarity sequence of these solvents and their possible interaction and stabilization of either the ground state or the excited state and these for affecting both $\Pi \longrightarrow \Pi^*$ and $n \longrightarrow \Pi^*$ transition energies and wavelength (Table 3.4).

Table (3.4) Effect of solvent upon UV spectrum.

No	Solvent	lamda max nm	Absorbance (A)
1	Nitric acid	300	2.768
2	Water	270	0.250
3	Hexane	360	0.031
4	Chloroform	299	0.543
5	Methanol	244	3.964
6	Acetone	325	0.012
7	dimethylformamide	308	3.995

It was clearly observed that the polarity of the solvent highly effect the λmax of glibenclamide as seen in the solvent shown in table (3.4). Glibenclamide containing partially polar group in his skeleton that will be effected by the polarity of solvent.

pH highly affect the UV spectra of the compounds which contain functional groups bearing lone pair of electrons or acidic hydrogen indirect attachment or relation with chromophores such as benzene (Table 3.5).

Table (3.5) Effect of pH upon UV spectrum

No	pH	lamda max nm	Absorbance (A)
1	1	341	0.009
2	2	342	0.002
3	3	289	0.922
4	4	293	0.526
5	5	292	1.825
6	6	341	0
7	7	299	0.06
8	8	300	0.038
9	9	300	0.125
10	10	300	0.186
11	11	300	0.537
12	12	300	0.528
13	13	300	0.529

Glibenclamide contain hydrolysable group and the effect of acidic, neutral and basic conditions with respect to temperature variation was observed (Table 3.6).

Table (3.6) Effect of pH/temp upon UV spectrum

Solvent									
	0.1M HCL			Water			0.1 NaOH		
	RT	50C°	70C°	RT	50C°	70C°	RT	50C°	70C°
ƛmax	246	246	202	201	201	201	234	253	232
ABS	0.114	0.737	0.306	3.499	3.506	3.630	3.974	3.956	3.952

3.4 Validation study

Validation is process used to confirm that analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability, and consistency of analytical results. It is an integral part of any good practice of analytical method need to be validated or revalidated before their introduction in to routine use:
Whenever the conditions change for which the method has been validated (e.g. an instrument with different characteristics or samples with a different matrix),and whenever the method is changed and the change is outside the original scope of the method. The key point is to develop methods for easy validation and revalidation. The validity of the performed method was demonstrated through laboratory experiments, using samples and standards similar to known samples and standards were UV spectrophotometrically analyzed.
This work had been established to meet criteria such as ease of use, ability to be automated. and controlled by computer system, costs per analysis, sample through-put, and turnaround time, beside the environmental, health and safety requirements. Obtained results from the performed method gave sufficient amount of satisfaction; especially to meet and address our laboratory's lack of instrumentation in Sudan and other similar countries.

Initial validation approaches were generally less rigorous and demanding than ones performed for standard reference material (SRM) development. The question arise here was how many samples should be analyzed in any validation approach 1,2,6,12,? general, the greater variety of samples validated and the wider concentration range are the better. Ideally, the method should be validated for the analyte using several different sample types, each of which is determined separately for statistical and validation purposes. There is no official guideline on the correct sequence of validation experiments and the optimal sequence may depend on the method itself. There is no single validation approach that must always be employed for a new method; the analyst's primary concern should be to select an approach that will prove to give true validation.

Acceptance of any new method by others in the field will depend on the specific validation approaches used. Throughout this work, the method's performance characteristics were based on the intended use of the method . Although it is not always necessary to validate all analytical parameters that are available for specific technique, for example, if the method is to be used for qualitative trace level analysis, there is no need to test and validate the method's limit of quantization, or the linearity, over the full dynamic range of the equipment. However, there was an obligation throughout this work to validate all available parameters in compliance with (ICH) guidelines. Ruggedness and robustness steps of (USP) guidelines, moreover, were also attempted to give sufficient amount of assurance. Initial parameters had been chosen according to the analyst's experience and best judgment, while the final parameters should be agreed between the analytical chemist who performed the validation, those who apply the validated method and those who used the data to be generated by it. To determine the requirements of the analytical method to be developed, their goals and defined analytical figures of merit should firstly be considered. The required detection limits, selectivity, linearity range, accuracy, and precision should be defined. The eventual use of the method, its adaptability to other uses, and any regulatory requirement that need to be met should secondly be considered in addition to other requirements including sample through put (time effort, money, and laboratories), analysis time, available instrumentation and cost per analysis. Finally, the method was developed as a regulatory laboratory performed

validated method. Evaluation and documentation were made to the steps of method validation which include precision, accuracy, linearity range, LOD, LOQ, ruggedness, and robustness (some of these steps such as linearity range, LOD, LOQ had already been evaluated as analytical figures of merit).

3.5. Analytical method validation

3.5.1 linearity study.

In all linear calibration models assume that the response is proportional to the analyte concentration. Most of chromatographic and spectroscopic methods use this approach. The main type of calculation adopted was the method of least square whereby the sums of the squares of deviation from the predict line where minimized. It was assumed that all the errors are contained in the response variable, Y, and the concentration variable ,X, is error free. The models variable where Y= bX and Y=bx+a, where b is the slope of the calibration line and a is the intercept. This value was the least square deviation of the true values. The linearity study that was obtaining in this work with solution prepared, the linear regression data for the calibration curves showed good linear relation over the concentration range 0.05-0.175 mg/ml in ethanol (0.1M HCl) for glibenclamide.

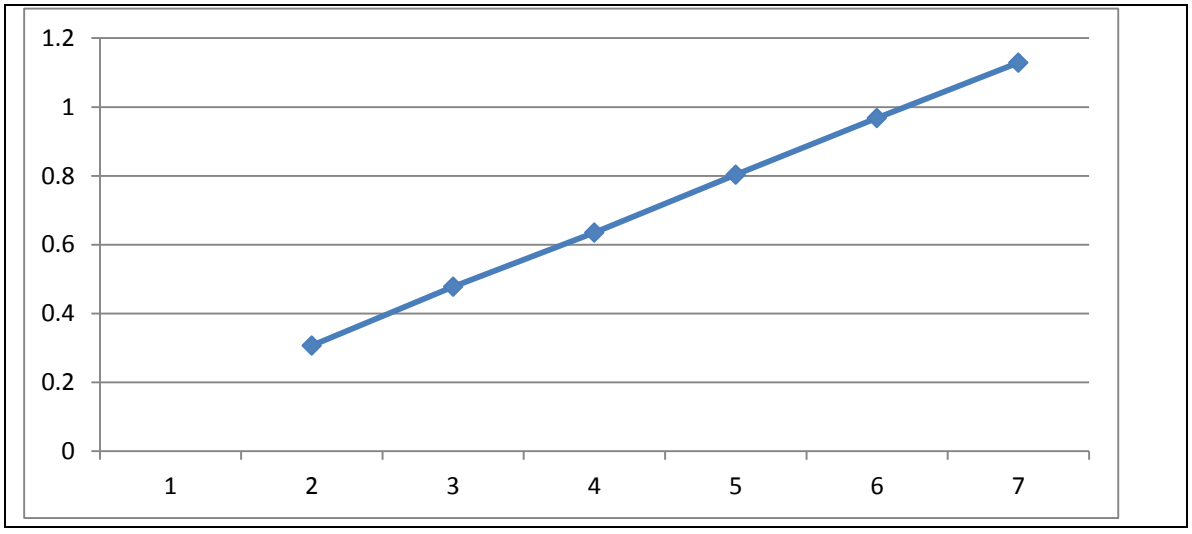
$$Y=0.1522X+0.0029$$

Table (3.7) linearity study of glibenclamide .

No	Conc mg/ml	Abs	R.S.D%
1	0.05	0.307	Zero
		0.307	
		0.307	
2	0.075	0.478	Zero
		0.478	
		0.478	
3	0.10	0.635	Zero
		0.635	
		0.635	
4	0.125	0.803	Zero
		0.803	
		0.803	
5	0.15	0.968	Zero
		0.968	
		0.968	
6	0.175	1.129	Zero
		1.129	
		1.129	

The ICH guidelines specify that minimum of five concentration level among with certain minimum specified ranges. From table (3.1)

Fig (1) show the linearity curve



3.5.2 Precision study.

Precision is the measure of the degree of repeatability of analytical method under normal operation and it is normally expressed as the percent relative standard deviation for statistically significant number of sample. These result show reproducibility of the assay. It's found less than 2% which indicate that method is precise. In this work reproducibility was measured by three different analyst for conc (0.1 mg/mL).

Table (3.8) Precision study for glibenclamide

NO	Conc mg/ml	Abs (analyst 1)	Abs (analyst 2)	Abs (analyst 3)
1	0.1	0.645	0.642	0.644
2	0.1	0.632	0.643	0.649
3	0.1	0649	0.645	0.648
4	0.1	0.642	0.648	0.650
5	0.1	0.643	0.641	0.645
6	Average	0.6422	0.6438	0.6472
7	SD	0.006301	0.002775	0.002588
8	RSD%	0.9812%	0.431%	0.400%

3.5.3 Accuracy.

Accuracy is the measure of exactness of an analytical methods or the closeness of agreement between the measured value and the value that is accepted either as conventional, true value or acceptance reference value. It's measured by assaying by spiking sample in blind study. It is not necessary to obtain 100% recovery as long as results are reproducible and known with high degree of certainty. More over developed method of quantization that will take percent recovery into account is one of the possible method of quantization which include standard addition, internal standard, and isotopic dilution, provides the most accurate and precise quantitative data.

The accuracy of the proposed method was determined throughout this work. To preanalyzed sample solution; a known amount of standard stock solution was added at different levels.

Result of recovery studies were reported in table (3.9). Reviewing the result accuracy can be reported as percent recovery by the assay of known added analyte in the sample. The percentage amount found was 100%(range 98.0%-102%) with RSD percentage less than 2%.

The recovery carried out through this work emphasized the validity of the proposed method and enhances its trueness.

Table (3.9) Result of recovery studies

Pre analyzed solution sample (mg/ml)	Amount added (mg/ml)	Amount recoverd	Abs	% amount recoverd	% RSD
0.05	0.02	0.07	0.452	100.3%	0.935%
	0.04	0.09	0.578	99.5%	
	0.06	0.11	0.717	101.5%	
	0.08	0.13	0.846	99.8%	
	0.1	0.15	0.967	99.1%	
Abs of pre analyzed solution 0.05mg/ml = 0.322					

3.5.4 Specificity and selectivity.

Term sensitivity is also used and defined as the changed of the measured signal as result of one unit change in the content of the analyte (calculated from the slope of the calibration line of the analyte) while the terms selectivity are often used interchange. The term specific refer to a method that produce a response for a single analyte only. While the term selective refers to method that provides response for a number of chemical entities that may or may not be distinguished from each other. The ICH divides the term specificity to two separated categories , identification and assay .

The sensitivity of the method was determined from the linearity equation. The LOQ and LOD for glibenclamide where found to be 0.0167 mg/ml and 0.005 mg/ ml respectively.

Table (3.10) sensitivity studies for glibenclamide

Conc mg /ml	Abs	LOD	LOQ
0.005 mg/ml	0.039	0.005	0.0167
	0.039		
	0.039		
	0.039		
	0.039		

3.5.5 Repeatability.

Repeatability should be obtained for short interval time or within same time for same method in the same laboratory by same operator and same equipment. The work done by analyzing the 0.1mg /ml for 6 time by preparing the solution was mention in chapter two. The range that was assumed is within 2% R.S.D and the percentage obtain is 0.458% R.S.D table (3.11).

Table (3.11) repeatability studies

Conc mg/ml	Abs	R.S.D%
0.1	0.654	0.458%
	0.659	
	0.66	
	0.655	
	0.655	
	0.661	

3.5.6 Linearity range.

Linearity range is the ability of the method to elicit test result that are directly proportional to analyte concentration within a given range. Linearity is generally report the variance of the slope. Of regression line. Range is the interval between the upper and lower level of analyte that have been demonstrated to be determined within precision, accuracy and linearity using the method. In this work the range is found to be from 0.03-0.2 mg/ml as in table (3.12) for low range and (3.13) for high range.

Table (3.12).Low range for linearity

Conc mg/ml	Abs
0.03	0.186
	0.188
	0.188
	0.184
	0.187
Average	0.187
RSD%	0.897%

Table(3.13). High range for linearity

Conc mg/ml	Abs
0.2	1.278
	1.28
	1.291
	1.276
	1.288
Average	1.283
RSD%	0.510%

3.5.7 Comparative study.

Four samples of glibenclamide manufactured by different companies were submitted for different physical test and assay determination. The method of the work was written in chapter two. Table (3.13) shows the absorbance of the samples the companies A<B<C are local company and the Company D is an international company.

Table (3.14) Comparative study for glibenclamide product from deferent company

Sample	Abs of sample	Abs of STD	Assay
Company A	0.658	0.665	98.9%
Company B	0.670		100.8%
Company C	0.680		102.3
Company D	0675		101.5

The result obtained from the same laboratory using same equipment within a short time interval to using the same methods .

3.6 Conclusion and recommendations.

The following points could be concluded and/or recommended for the validation for proposed UV spectrophotometric analytical method of glibenclamide and it is stability which had been predicted through this work of this thesis:

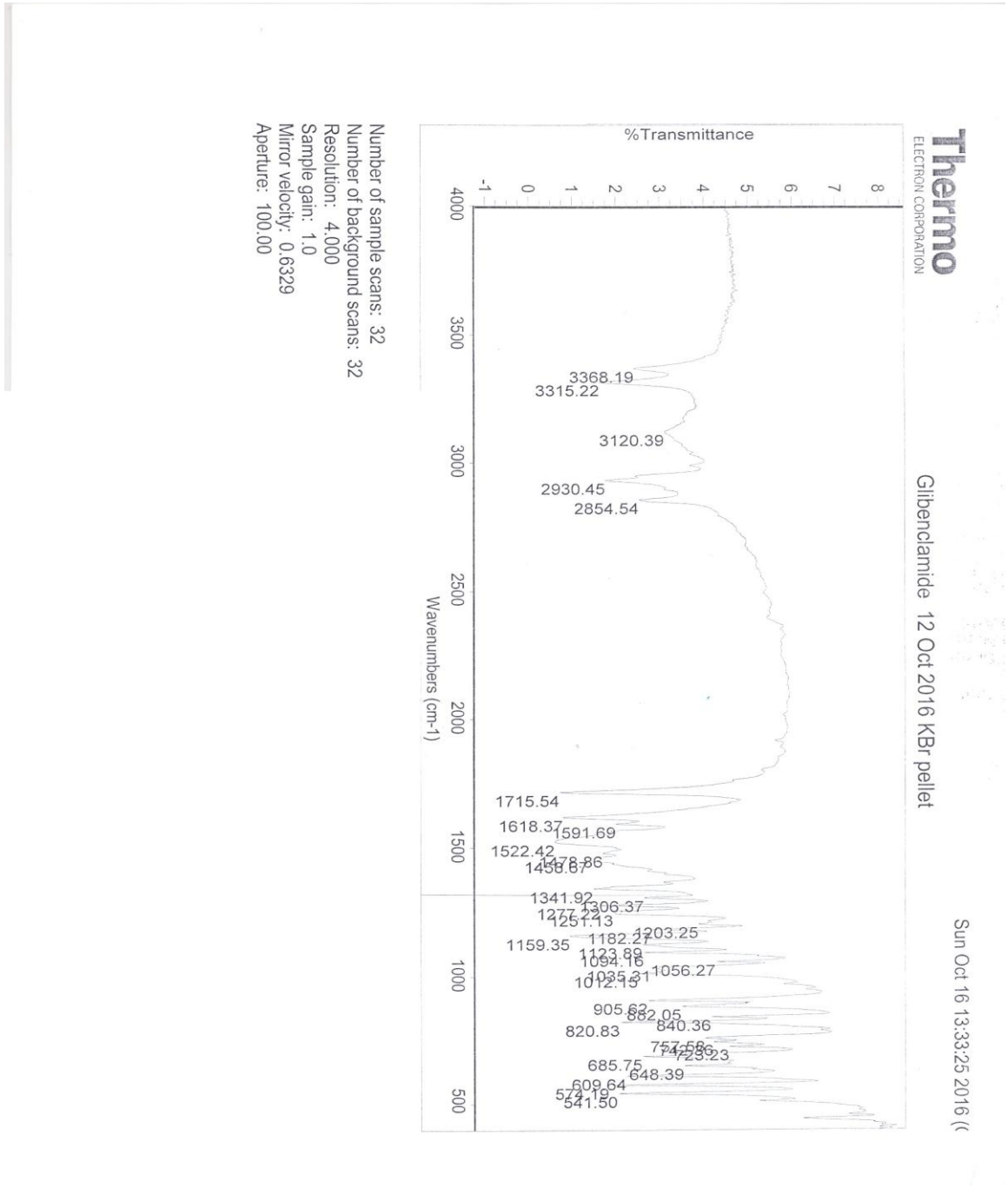
1. The concept of total quality control system is either limited in scope to the analytical method of assay or to the statistical techniques utilized in the work. Under these situations method development should investigate the stability of the analytes and standards. Throughout this work, system stability (investigated on sample solution) had been determined by replicate analysis of sample solution. System stability was considered appropriate , according to the values calculated on the assay result. Those obtained at different time intervals, did not exceed more than 2% of the corresponding value of the system. The work indicated that there is continuing need for reliable analytical system to comply with the national regulations as well as international requirements. For any method that will be used for routine analysis, quality control plan should be developed; this plan should ensure that, the method and equipment deliver consistently accurate results.
2. Regarding the stability studies of the glibenclamide there is no need to include all parameters in product release monograph but only parameters that are potentially susceptible to change during the storage and that may impact on the quality, efficiency and safety of the product. The test procedure for assessment of the quality control level of article are subject to various requirements especially those used for assessing the compliance of the pharmaceutical requirement which establish the specification. Throughout this work stability studies were intended to meet the proper standard of accuracy and reliability to guarantee pharmaceutical material and product. The status of the material and product were considered in relation to result of past and future quality tests. All parameters needed to test the quality of the product were considered in this work. It is recommended to rationalize the exclusion of the other parameters.

3. There should be written testing program designed to assess the stability characteristic of the drug product. To determine the appropriate storage conditions, and expiration data. The program must include the sample size and test intervals, storage conditions, specific test methods including testing in the same container –closure system and adequate number of batches to determine the expiration date. The scope of the method evaluation should be broad enough to include generation of information that is immediately usable for confirmation of the analyte in any sample. The preformed worked addressed the stability topic through compliance with the British pharmacopeia (2007), and ICH requirement stability data.
4. Stability storage conditions throughout this work involved long-term studies at 30±2C°. with 65 RH ±5C° accumulating for 18 months data before filing (ongoing study) or accelerated studies 45±2C° and 75 RH ±5C° accumulating for 6 months. The result obtained, as mention before, were in compliance with BP(2007), and ICH requirement and meet the specifications. Where a significant change (5% loss of potency) occurs during the 40C° accelerated study, an additional intermediate station should be used, such as 30±2C° with RH 60% ±5C°. Any degradants exceeding its specification limit, exceeding pH limit. For less stable products the storage condition may be reduced. But the accelerated condition should still be at least 15C° above those used for long term evaluation for product. Where water loss may be important it is more appropriate to replace the high RH condition are to be applied for the evaluation of bulk drugs 10-20% the same storages condition are to be applied for the evaluation of bulk drugs substance. The effect of long-term storage and freeze-thaw cycles can be investigated by analyzing a spiked sample immediately after preparation and on subsequent days of the anticipated storage period.
5. The present proposed validated UV spectrophotometric techniques is quite simple accurate, precise, reproducible and sensitive analytical procedure. The method has been developed for qualification of glibenclamide in raw material and tablet formulation. The validation procedure confirm that this is an appropriate method for their quantification in the plant material and formulation. It can also used in

routine quality control of this entire compound. The recently adopted increased requirements on internal and external laboratory quality control measures add an extra level of confidence to chemical measurements. This thesis agreed that the increase in such confidence permit alternative approaches to method validation to be considered. The thesis reviewed the procedure for assessing method validation criteria and noted the success of the test in inter laboratory method performance studies. The validity of an analytical procedure can be verified only by laboratories studies. Therefore the documentation of the successful completion of the such studies is basic requirement for determining whether a procedure is suitable for its intended use.

6. The suitability of an analysis method for its intended use is perquisite to obtaining accurate data; therefore, only validated methods should be used to acquire meaningful data. The initial analytical data of the proposed method that has been derived from the new method appears promising therefore it was important to evaluate its performance quantitatively. A greet care should be given to the method accessibility to generate data for the estimation of the proposed method. It is important that all the recommended available data on analytical performance characteristics are submitted to ICH or USP specification. And the submitted data is made available this will greatly help to minimize discrepancies in the mutual interpretation of data and the facilitated international harmonization.
7. The multi – laboratory model is the preferred option for validation of method of analysis. In case where less stringent model cannot be used, other validation protocols must be used. This can include a two laboratories or internal validation within a single laboratory. Comparison with a currently accepted analytical method(s) is yet another validation approach. This is usually done by two analyst using samples. This approach uses results from the currently accepted method as verification of the new method's results. The inter- laboratories collaborative studies perhaps are widely accepted procedure to validate any new analytical method. But it suffer from practical draw – backs, whereas, it is costly, time consuming (it can take years) difficult in coordinating, shipping samples, and so on. This approached is rarely employed when a method is being described for the first time in the literature.

Appendix No (1) shown the IR spectrum



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