Sudan Universityof ScienceandTechnology

College of Graduate Studies

Method Validation for Analysis of Drug Combination of Metformin Hydrochloride and Glebinclamide

التحقق من طريقة تحليلية لخليط عقار من الميتفورمين هيدروكلوريد و الجليبينكلاميد

A Thesis Submitted in Partial Fulfillment for the Requirements of the Degree of M.Sc. in Chemistry

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Dedication

To

My parents,

Wife,

Brothers,

And Sisters.

Acknowledgment

First of all mysincere thanks to Allah Almighty for helping and supporting me to complete this work.

It's an opportunity to offer great thanks to my guidance and supervisor Dr. Mohamed Sulieman Ali Altoum for his wonderful support.

The chance was extended to thanks wafrapharmastaff for open their laboratory to do practical part of the research.

A lot of thanks for our tutor and our teacher who was guide us to learn to know what's chemistry is, those who open our eyes to see , those people who work in chemical department staff .

Finally to my colleague, my parent, my friend and my wife for their unlimited greaten and wider support with no objection or angers.

Abstract

In this thesis the combination of metformin hydrochloride and glibenclamide drug used for diabetes mellitus type II, it was analyzed by developed method using simpleisocraticReverse phase high performance liquid chromatography (HPLC)with ultraviolet (UV)detector, mobile phase consist of acetonitrile and monobasic sodium orthophosphate (adjust to pH 2.5 by orthophosphoric acid) (50:50 V/V) with flow rate 1ml/minat 228nm.The drug combination wasdetermined according to the accepted worldwide verification standards.

The proposed method was investigated according toInternational Council for Harmonization (ICH) guidelines and the characters showed simple, reliable, fast, accurate, linear, precise and low cost with relative stander deviation RSD less than 2%.

The method was applied on finish product of different companies and the assay was determined.The results obtained agreed wellwith the pharmaceutical specification with good accuracy and it can be used in routine quality control analysis.

المستخلص

في هذه االدراسة يتم دراسة العقار المكون من الميتفورمين هيدروكلويد والجليبنكلاميد الذي يستخدم لمرضي السكري من النوع الثاني حيث يتم استخدام طريقة تحليل تستخدم فيها جهاز كروماتغرافيا السائل ذات الضغط العالي مع المكشافية الضوئيةباستخدام خليط من الأسيتونيتريل ومحلول منظم من فوسفات الصوديوم ثانئية الهيدروجين (الرقم الهيدروجيني 2.5 بواسطة حمض الفسفوريك)بنسبة (50:50) وبمعدل سريان للطور المتحرك 1مل/دقيقة بإستخدام طول موجي 228 لتحليل العقار وفقا لمواصفات تحقق عالمية.

وكان التحقيق في الطريقة المقترحة وفقا للمجلس الدولي للموائمة وأظهرت المتغيرات التي تم اختبارها بساطة وسرعة ودقة وقلة التكاليف وخطية مع معدل انحراف معياري أقل من 2٪.

تم إستخدام هذه الطريقة في تحليل منتجات نهائية من مختلف الشركات واعطت نتائج ضمن المواصفات الدستور البيرطاني للأدوية بمصداقية عالية.

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Chapter One

Introduction and Literature review

1. Introduction :

1.1 Metformin hydrochloride (MET)

The biguanide class of antidiabetic drugs, which also includes the withdrawn agents phenformin and buformin, originates from the French lilac or goat's rue (Galegaofficinalis), a plant used in folk medicine for several centuries.

Figure (1)Galegaofficinalis, a natural source of galegine

Metformin was first described in the scientific literature in 1922, by Emil Werner and James Bell, as a product in the synthesis of N,N-dimethylguanidine. In 1929, Slotta and Tschesche discovered its sugar-lowering action in rabbits, finding it the most potent biguanide analog they studied, (Delgado Carreño 2015).

Interest in metformin resumed at the end of the 1940s. In 1950, metformin, unlike some other similar compounds, was found not to decrease blood pressure and heart rate in animals. That year, Filipino physician Eusebio Y. Garcia used metformin (he named it Fluamine) to treat influenza; he noted the drug "lowered the blood sugar to minimum physiological limit" and was not toxic. Garcia believed metformin to have bacteriostatic, antiviral, antimalarial, antipyretic and analgesic actions. In a series of

articles in 1954, Polish pharmacologist Janusz Supniewski was unable to confirm most of these effects, including lowered blood sugar. Instead he observed antiviral effects in humans, (Janusz Supniewski 1950)

French diabetologist Jean Sterne studied the antihyperglycemic properties of galegine, an alkaloid isolated from Galegaofficinalis, which is related in structure to metformin and had seen brief use as an antidiabetic before the synthalins were developed. Sterne was the first to try metformin on humans for the treatment of diabetes; he coined the name "Glucophage" (glucose eater) for the drug and published his results in 1957, (Bailey2004).

Metformin became available in the British National Formulary in 1958. It was sold in the UK by a small Aron subsidiary called Rona.

Chemically is N, N-dimethyl imidodicarbonimidicdiamide hydrochloride

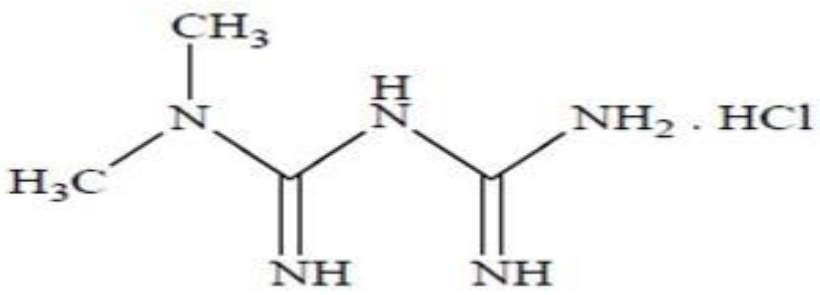


Figure (2) Chemical Structure of Metformin

Metformin, itself produces the antidiabetic effect, but its hydrochloride salt is more soluble in aqueous medium. It is the first line drug for the treatment of type II diabetes or non-insulin dependent diabetes mellitus (NIDDM), particularly in overweight and obese people and those with normal kidney function and evidence suggests it may be the best choice for people with heart failure. It is also used in the treatment of polycystic ovary syndrome.

Indeed, MET may produce a small decrease in body weight during the initial months of treatment. This cannot be attributed to a chronic decrease in food consumption or increased physical activity, suggesting that the

drug increases metabolic energy expenditure.

Physically, metformin hydrochloride is White crystals;freely soluble in water, slightly soluble in alcohol, practically insoluble in acetone and in methylene chloride,(Karim, et al 2012)

1.1.1 Mechanism of action

Metformin is an antihyperglycemic agent, which improves glucose tolerance in patients with type 2 diabetes, lowering both basal and postprandial plasma glucose. Its pharmacologic mechanisms of action are different from other classes of oral antihyperglycemic agents. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Unlike sulfonylureas, metformin does not produce hypoglycaemia in either patients with type 2 diabetes or normal subjects (except in special circumstances) and does not cause hyperinsulinemia. With metformin therapy, insulin secretion remains unchanged while fasting insulin levels and daylong plasma insulin response may actually decrease, (Dale, J. M. Ritter et al 2003).

1.1.2 Side effects

Gastrointestinal side effects such as abdominal discomfort, anorexia, bloating and diarrhoea are observed, depending on the dose.The reason for these effects is not known, but like a carbose metformin has been associated with decreased intestinal glucose absorption Hypoglycemia is not a side effect of metformin when used as monotherapy. Similarly, unlike some of the other oral hypoglycemic agents (OHAs), weight gain is not a side effect, and some patients experience weight loss. Lactic acidosis is rare but potentially fatal toxic effect. Metformin should not be given to patients with renal or hepatic disease, hypotoxic pulmonary disease heart failure or shock, (Dale, J. M. Ritter et al 2003).

1.2 Glibenclamide

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

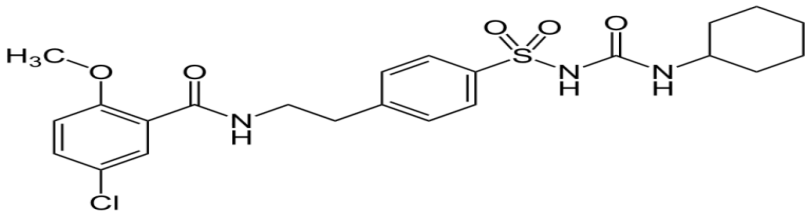


Figure (3) Chemical Structure of Glibenclamide

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 also known as glyburide, 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

triphosphate (ATP)-sensitive potassium channels in pancreaticbeta 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)).

1.2.1 Mechanism of action

The principal action of sulfonylureas is on Beta cells stimulating insulin secretion and thus reducing plasma glucose. High affinity receptors for sulfonylureas are present on the KATP channels in B cell plasma membranes, and the binding of various sulfonylureas parallels their potency in stimulating insulin release. The drug reduces the K^+ permeability of Beta cells by blocking KATP channels causing depolarization, calcium entry and insulin secretion, (Dale, J. M. Ritter et al 2003).

The acute administration of sulfonylureas to type 2 DM patient's increases insulin release from the pancreas. Sulfonylurea also may further increase insulin levels by reducing hepatic clearance of the hormone. In the initial months of sulfonylurea treatment, fasting plasma insulin levels and insulin responses to oral glucose challenges are increased. With chronic administration circulating insulin levels decline to those that existed before but despite this reduction insulin levels reduced plasma glucose levels are maintained. The explanation for this is not clear, but it may relate to reduced plasma glucose allowing circulating insulin to have more pronounced effects on its target tissues and to the fact that chronic hyperglycemia impairs insulin secretion glucose toxicity, (Dale, J. M. Ritter et al 2003).

1.2.2 Side effects

The main side effects of sulfonylureas are hypoglycemia and weight gain .Given that these drugs directly stimulate insulin secretion from pancreatic β cells irrespective of plasma glucose levels, the risk of hypoglycemia is associated with all sulfonylureas. Most episodes are mild and easily treated with glucose in the form of fruit juice, sweetened beverages or glucose tablets. However, prolonged and severe hypoglycemia can occur, especially in the setting of renal or hepatic impairment or in frail, elderly patients. Glipizide and Glimepiride are less associated with hypoglycemia than is glyburide.

Since these medications are metabolized in the liver; sulfonylureas are contraindicated in patients with moderate to severe liver dysfunction. The dose of glyburide should either be markedly reduced or avoided altogether in elderly patients and patients with moderate renal dysfunction. Dose adjustment is not required for glipizide or glimepiride in patients with moderate kidney dysfunction. However, there are insufficient data to support their use in those with end-stage renal disease, in which case insulin is the preferred option. The weight gain seen with sulfonylureas, which is typically 2–5 kg, is likely related to the increase in plasma insulin levels.

This may be discouraging in a population that is already prone to obesity and often struggling to lose weight. At the same time, metabolic control should not be compromised by withholding treatment in an attempt to avoid weight gain. Side effects of first-generation agents include skin rash, hyponatremia and alcohol-induced flushing and bone marrow damage, though very rare, can be severe during acute myocardial infarction, sulfonylureas are discontinued and type ıı diabetic patients are treated with insulin.

1.3 The uses of combination drug of metformin and glibenclamide:

Pharmaceutical formulation that contains two oral anti hyperglycemic drugs used in the management of type II diabetes, namely glyburide and metformin hydrochloride. Glucovance_ is available in three formulations including metformin:glyburide (500:5, 500:2.5 and 250:1.25 mg). It has been proven that intensive management of type II diabetes with glucovance improves glycaemic control and facilitates the attainment of glycaemic targets at lower doses of metformin or glibenclamide compared with the respective monotherapies.

1.4 previous studies :

There is many method was studied to investigate the assay analysis of metformin and glibenclamide :

Method developed to metformin (MET) and glibenclamide (GLB)) in Pharmaceutical dosage form by A high-performance liquid chromatographic, fist and second derivative spectrophotometric methods used for the simultaneous determination of MET and GLB. The first derivative amplitudes at 236 nm and 275.7 nm were selected for the assay of MET and GLB, respectively. Calibration curves were established at 5–120 μg/ml-1 for and 1–20 μg/ml-1, with limits of detection of 0.21μg/ml-1 and 0.29 μg/ml-1 and limits of quantification of 0.64μg/ml-1 and 0.89 μg/mL-1 for MET and GLB, respectively. The second derivative amplitudes at 244.6 nm and 229 nm were selected for the assay of MET and GLB, respectively. Calibration curves were established at 5–120 μg/ml-1 for and 1–20 μg/ml-1, with limits of detection of 0.46 μg/ml-1 and 0.30 μg/ml-1 and limits of quantification of 0.1.41μg/ml-1 and 0.91 μg/ml-1 for MET and GLB, respectively. In the HPLC method separation was performed by using C18 reversed phase column and a mobile phase of

acetonitrile: 0.05 M KH2PO4 (60:40v/v) adjusted by phosphoric acid to pH 3, at flow rate of 1 ml/min and the detection wavelength were 210 nm and 238 nm ,the retention time was found to be 3.145 and 7.792 min, linearity over the concentration ranges of 5–75 μm/ml-1 and 2-45 μg/ml-1, with limits of detection of 0.64 μm/l-1 and 0.02 μg/ml-1 and limits of quantification of 1.95 μg/l-1 and 0.07 μg/ml-1 for MET and GLB, respectively. The methods were also applied for the determination of MET and GLB in the presence of their degradation products formed under variety of stress conditions.

Simple spectrophotometric method for simultaneous estimation of Glibenclamide and Metformin HCl in combined dosage form. Using methanol as a solvent. The two wavelengths 229.5 nm and 237 nm were selected for estimation of Glibenclamide and Metformin HCl respectively.the results were validated as per ICH guidelines. The method can be employed for estimation of pharmaceutical formulations with no interference from any other excipient and diluents.

Analysis of combine of Metformin and Glibenclamide drug are prescribed for the treatment of patients with Diabetes. The RP-HPLC method for simultaneous estimation of Metformin and Glibenclamide in their combined tablet dosage form. The separation was achieved on a Oyster BDS RP- C18 Column (150mm x 4.6mm, 5 μm) column with an isocratic mixture of Methanol: Acetonitrile: Water in 30:60:10 (v/v), at a flow rate of 1.0 ml/min and UV detection at 228 nm. The retention time for Metformin and Glibenclamide were 3.17 and 8.10min respectively. Good linear relation was observed with in a concentration range of 2-4.5μg/ml for Glibenclamide and 200-450μg/ml for Metformin with high r2 value. The developed method was validated as per ICH guide lines. The

developed method was found to be precise, accurate and was used for the simultaneous estimation of Metformin and Glibenclamide in fixed dosage forms

The development and validation of RP-HPLC method for the analysis of Metformin. In this a simple RP-HPLC method was developed for the quantification of Metformin hydrochloride in raw materials and in pharmaceutical preparations. Analytical Reverse Phase Column C (18) was used and the mobile phase consisted of methanol-water (30/70v/v) the analytes were then determined by using UV detector. This method was validated according to ICH guidelines. The proposed method is rapid, accurate, economical and selective and it was used for the quantitative analysis of metformin in Neodipar tablets because of its sensitivity and reproducibility.

Literature shows that a RP-HPLC method was developed and validated for the simultaneous determination of Glipizide, Rosiglitazone, Pioglitazone, Glibenclamide and Glimepiride in Pharmaceutical dosage Forms and human plasma. Elution was performed using a mobile phase mixture of 0.05% triethylamine, acetonitrile and methanol in the ratio of 55:15:30 at a flow rate of 1 ml/min, RP column C (18) was used. The method was successful in detecting the drugs at a concentration of less than 0.1 μg ml-1. This method was validated according to ICH guidelines with suitable accuracy and precision and the results was found to be within acceptable range.

Spectrophotometric and HPLC determinations of anti-diabetic drugs, Rosiglitazone maleate and Metformin hydrochloride, in pure form and in pharmaceutical preparations. In this method he developed three Spectrometric method and one HPLC method for analysis of Anti-diabetic drugs. Two Spectrometric methods were based on the reaction of

rosiglitazone with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and bromocresol green(BCG), the third Spectrophotometric method consists of a zero-crossing first-derivative Spectrophotometric method for simultaneous analysis of RSG and metformin (MTF) in tablets. The fourth method is a rapid stability indicating HPLC method. The proposed method was successfully applied to the tablet analysis.

Method development and validation of simultaneous determination of pioglitazone and glimepiride in pharmaceutical dosage form by RP-HPLC was carried out by. Reddy B and team. Here the RP column used was a Phenomenex Luna C18 column. The mobile phase was acetonitrile with a flow rate of 1.5 ml/min. This method was validated according to ICH guidelines in terms of accuracy, precision, specificity, range, system suitability and robustness.

An accurate RP-HPLC method was developed for the simultaneous determination of glimipremide, rosiglitazone and pioglitazone hydrochloride. The column used Nucleodur C-18 and the mobile phase used was orthophosphoric acid and acetonitrile (80:20). Detection was done by using an UV detector. The proposed method was successfully validated and used for the the estimation of glimiprimide, rosiglitazone and pioglitazone hydrochloride.

A simple, precise and stability indicating RP-HPLC method for the quantitative analysis of glibenclamide present in the tablet formulation and bulk drug was developed. The column used was symmetry C-18 with the mobile phase and detection at 228nms. The proposed model provided linear response with acceptable range. The precision of the method was demonstrated using intra-day assay and RSD% values which were less than 1% in all instances.

RP-HPLC developed method for the determination of six anti diabetic drug products. The objective of this study was to develop a single RP-HPLC method for the simultaneous determination of six active ingredients including Metformin Hydrochloride, Pioglitazone, Glimepiride, Gliclazide, Glibenclamide and Glipizide in pharmaceutical products. The Column used was C-18 containing gradient mobile phase such as Sol A: Phosphate buffer and Sol B: Acetonitrile. This method was successfully validated in terms of specificity, precision, linearity, accuracy, ruggedness and robustness. This RP-HPLC method is simple and reproducible, with high resolution and has been successfully applied for the simultaneous determination of the six components (metformin hydrochloride, pioglitazone, glipizide, gliclazide, glibenclamide and glimepiride) in pharmaceutical drug products.

A method for the simultaneous estimation of Glibenclamide and Metformin HCl in Bulk and Tablets using UV – visible spectroscopy was developed and validated. This method reveals a spectrophotometric method for the simultaneous estimation of glibenclamide and metformin hydrochloride in combined dosage form using methanol as a solvent, the two wavelengths 229.5 nm and 237 nm were selected for estimation of glibenclamide and metformin HCl respectively. The method was validated successfully as per ICH guidelines, the method can be employed for estimation of pharmaceutical formulations with no interference from any other excipients and diluents.

developed and validated a stability indicating capillary electrophoresis method for the determination of Metformin hydrochloride in tablets. Citrate buffer was used as a background electrolyte with an applied voltage of 15 kv. The capillary used was of 68.5 cm length and detection was carried out at a wave length of 230 nm, the method was validated in

accordance with the ICH requirements, which involved accuracy, precision, linearity, selectivity and both limit of detection and limit of quantization.

1.5. Validation

Analytical methods can be considered as a complex and multi-step issue, ranging from sampling to generating the result. It is internationally recognized that the validation of methods is required to obtain high quality data. 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.

1.5.1. 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 formula for the calculation, etc. The objective of validation of an analytical method is to establish the performance characteristics of analytical applications through experimental tests, resulting in a suitable analytical method for its purpose and to demonstrate that the method is suitable for the intended use. The use of analytical methods development for drug 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 is 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, (Ludwig Huber 2007).

1.5.2. Strategy for the Validation of analytical 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.

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 or/and 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

1.5.3. 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. (Ludwig Huber 2007).

1.5.4 Analytical method validation characteristics:

Typical analytical performance characteristics that should be considered in the validation of the types of procedures described in this chapter are listed below. Each validation characteristic is defined to ensure consistency in usage of terminology and interpretation:

i. Precision
ii. Accuracy
iii. Repeatability
iv. Intermediate precision
v. Specificity
vi. Detection limit
vii. Quantitation limit
viii. Linearity
ix. Range

1.5.5 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).

1.5.5.1. 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 (ICH 2005).

1.5.5.2. 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). This definition has the following implications: Identification: to ensure the identity of an analyte, Purity Tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.

And assay (content or potency) to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample. ICH (2005).

1.5.5.3. Accuracy

Accuracy of an analytical procedure is defined as the closeness of agreement between the values that are accepted either as conventional true values or an accepted reference value and the value found. For drug substance, accuracy may be defined by the application of the analytical procedure to an analyte of known purity (e.g., a reference standard). For the drug product, accuracy will be determined by application of the analytical procedure to synthetic mixtures of the drug product components to which known amounts of analyte have been added within the range of the procedure.

Accuracy is usually reported as percent recovery by the assay (using the proposed analytical procedure) of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals. The range for the accuracy limit should be within the linear range.ICH (2005).

Typical accuracy of the recovery is expected to be about 98 – 102%

1.5.5.4. 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. (ICH (2005)).

1.5.5.5. Repeatability

Repeatability is a measure of the precision under the same operating conditions over a short interval of time, that is, under normal operating conditions of the analytical method with the same equipment. It is sometimes referred to as intra - assay precision. (ICH 2005).

1.5.5.6. Intermediate precision

Intermediate precision expresses the variation within the same laboratory. The extent to which intermediate precision needs to be established depends on the circumstances under which the procedure is intended to be used.

Typical parameters that are investigated include day - to - day variation, analyst variation, and equipment variation. (ICH 2005).

1.5.4.7. Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).ICH (2005).

1.5.5.8. 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.

There are several approaches to establish the Detection limit. Visual evaluation may be used for non-instrumental (e.g., solution color) and instrumental methods. In this case, the Detection limit is determined by the analysis of a series of samples with known concentrations and establishing the minimum level at which the analyte can be reliably detected.

Presentation of relevant chromatograms or other relevant data is sufficient for justification of the Detection limit.

For instrumental procedures that exhibit background noise, it is common to compare measured signals from samples with known low concentrations of analyte with those of the blank samples. The minimum concentration at which the analyte can reliably be detected is established using an acceptable signal - to - noise ratio of 2:1 or 3:1. Presentation of relevant chromatograms is sufficient for justification of the Detection limit.

Another approach estimates the Detection limit from the standard deviation of the response and the slope of the calibration curve. The standard deviation can be determined either from the standard deviation of multiple blank samples or from the standard deviation of the y intercepts of the regression lines done in the range of the Detection limit.

This estimate will need to be subsequently validated by the independent analysis of a suitable number of samples near or at the Detection limit:

$$DL = \frac{3\sigma}{S}$$

Where σ is the standard deviation of the response and S is the slope of the calibration curve. (ICH 2005).

1.5.5.9. Quantitation 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.

There are several approaches to establish quantitation limit the first approach is to evaluate it by visual evaluation and may be used for non-instrumental methods and instrumental methods. Quantitation limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantitated with acceptable accuracy and precision.

The second approach determines the signal - to - noise ratio by comparing measured signals from samples with known low concentrations of anlayte with those of blank samples. Quantitation limit is the minimum concentration at which the analyte can be reliably quantified at the signal - to - noise ratio of 10:1.

The third approach estimates quantitation limit by the equation

$$QL = \frac{10\ \sigma}{S}$$

Where σ is the standard deviation of the response and S is the slope of the calibration curve. (ICH 2005).

1.5.5.10. Linearity

Linearity of an analytical procedure is the ability (within a given range) to obtain test results of variable data (e.g. absorbance and area under the curve) which are directly proportional to the concentration (amount of analyte) in the sample. The data variables that can be used for quantitation of the analyte are the peak areas, peak heights, or the ratio of peak areas (heights) of analyte to the internal standard peak. Quantitation of the analyte depends on it obeying Beer's law for the spectroscopic method over a concentration range. Therefore, the working sample concentration and samples tested for accuracy should be in the linear range. There are two general approaches for determining the linearity of the method.

The first approach is to weigh different amounts of standard directly to prepare linearity solutions at different concentrations. However, it is not suitable to prepare solution at very low concentration, as the weighing error will be relatively high.

Another approach is to prepare a stock solution of high concentration. Linearity is then demonstrated directly by dilution of the standard stock solution. This is more popular and the recommended approach. Linearity is best evaluated by visual inspection of a plot of the signals as a function of analyte concentration. Subsequently, the variable data are generally used to calculate a regression line by the least - squares method. At least five concentration levels should be used. Under normal circumstances, linearity is acceptable with a coefficient of determination (r^2) of $\geq$ 0.995.ICH (2005).

1.5.5.11. 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. ICH (2005).

1.6. Validation protocol

Depending upon of 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.).

1.7 The Aim of the research:

- ❖ To investigate the new method using the ICH characters to validate the method.
- ❖ Uses the new isocratic method for simultaneous determination of glibenclamide and metformin hydrochloride.
- ❖ To determine the wave length that we can use to analysis the combine drug.
- ❖ Apply the method on the finish product and determine the assay of active ingredient.

Chapter Two

Materials and Methods

2. Materials and Methods

2.1. Chemicals.

- Glibenclamide sample (from India) was a gift from Wafrapharma laboratories, Omdurman, standard: Batch no 20150612 , manufacturing date: 12 June 2015 , expiry date :11 June 2018 characteristics :white crystalline powder , melting point 172 °c , sparingly soluble in methylene chloride and practically insoluble in water.
- Metformin sample (from India) was a gift from Wafrapharma laboratories, Omdurman, standard: Batch no 16070418, manufacturing date: July 2016 , expiry date : June 2021 characteristics :white crystalline powder , melting point 172 °c , slightly soluble in alcohol, practically insoluble in acetone and in methylene chloride .
- Acetonitrile HPLC grade MW 41.05/mole, density 0.786 g/cm and boiling point 82 °c application assay 99.9% made in Korea.
- Mono basic sodium orthophosphate dihydrate. MW 156.01 g/ml, central drug house (P) Ltd .New Delhi India
- Orthophosphoric acid MW 98/mole, density 1.71 g/cm and boiling point 158 °c application assay 85% made in Korea.

2.2. Instruments.

2.2.1. HPLC –young ling model YL 9100 HPLC vaccum degasser serial No D3042150624 AC 100-240 V made in Korea.

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. General equipment. Glassware of pyerxtype (Doarig Company), magnetic stirrer (Shimadzu Corporation, Japan) sensitive balance Shimadzu Corporation, Japan)

2.3 HPLC Analytical Method Validation

2.3.1. Preparation of diluent.

The diluent was acetonitrile: water (4:1 V/V) respectively.

2.3.2. Preparation of mobile phase.

An accurate weight of 1g of monobasic sodium orthophosphate was dissolved in amount of water and was transferred to 1000 ml volumetric flask then was completed to mark. The pH of solution was adjust to 2.5 by orthophospric acid. The buffer was mixed with equal amount acetonitrale the mobile phase is acetonitrile buffer (50:50 V/V).

2.3.3. Preparation of sample stock solution (1).

An accurate weight of 1250 mg of Metformin was dissolved in amount of diluent and solution was transferred into 100ml volumetric flask; volume was completed by same diluent. Solution was stirred for 10 min.

2.3.4. Preparation of sample stock solution (2).

An accurate weight of 50 mg of glibenclamide was dissolved in amount of diluent and solution was transferred into 100ml volumetric flask; volume was completed by same diluent. Solution was stirred for 10 min

2.3.5. Selection of wavelength

For determine the wavelength three solution was prepared:

Sample 1:

1ml of stock solution (1) was transferred into 100ml volumetric flask then was completed to the mark by diluent.

Sample 2:

1ml of stock solution (2) was transferred into 100ml volumetric flask then was completed to the mark by diluent.

Sample 3:

1ml of stock solution (1) and 1ml of stock solution (2) was transferred into 100ml volumetric flask then was completed to the mark by diluents

Then the sample was scanned in the UV spectrophotometer between (200-400 nm).

2.3.6. Chromatographic conditions.

The mixture of 0.1% w/w Sodium di hydrogen Phosphate Buffer, pH 2.5 (adjusted with H_3PO_4) and Acetonitrile (50:50 v/v) as mobile phase in an isocratic elution on a C18 column, the flow rate of 1.0 ml/min was set for elution and detection was carried out using UV-Visible detector set at 228 nm. All determinations were performed at a constant column temperature of 30ºC with a load of 20µl.

2.3.7. System suitability

For system suitability the solution of mixed metformin- glibenclamide 0.5-0.005 mg/ml respectively was prepared from stock solution and was injected for six time

2.3.8. Linearity study.

Series of mixed sample solution of 0.5-0.005, 1.5-0.015, 2.50.025, 3.5-0.035, 4.5-0.045, 5.5-0.055 mg/ml metformin-glibenclamide was prepared respectively from stock solution then 20 µl of the sample was injected into HPLC.

2.3.9. Intraday Precision study.

The mixture of metformin-glibenclamide 0.5-0.005mg/ml was injected different three day.

2.3.10. Repeatability study.

It was carried out by analyzing 2.5-0.025 mg/ml concentration of metformin-glibenclamide solution for six times.

2.3.11. Accuracy study.

The recover solution for accuracy study was carried out by adding 0. 4, 0. 8, 1.2, mg/ml of standard to 2 mg/ml reanalyzed sample solution and then the recover solution was injected on HPLC.

2.3.12. 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 fallow:

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

2.3.13. Range.

The range was carried out in low and high level of concentration to get RSD% $\leq$ 2% on concentration of metformin-glebinclamide 0.5-0.005 mg/ml and 5.50.055mg/ml respectively.

2.3.14. Application.

Standard solution: 2.5ml of stock solution (2) and 10 ml of stock solution (1) was transferred into 50 ml volumetric flask and completed to the mark with diluent.

Assay solution: 10 tablet of each product (company) was weighed and grinded to be fine homogenous powder then an accurate weight equivalent to 500-5 mg of metformin-glibenclamide was dissolved into amount of diluent and stirred for 5 min the solution was transferred into 100 ml

volumetric flask the volume was completed with same solvent and stirred for 30 min.

The standard solution and assay solution was injected in HPLC instrument.

Chapter Three

Result and discussion

3. Results and discussions:

3.1 Selecting wavelength for drug combination:

To select wave length for drug combination the samples were prepared as shown in chapter two and scanned in UV spectrophotometer from 200-400 nm, the spectrum that was obtain from scanned sample showed that metformin was absorbed at 228.5 nm and 242.5nm and glibenclamide absorbed at 228nm and 306nm. The spectrum of the combination of metformin and glibenclamide show that the mixed active ingredient absorbance in 228nm, 242.5nm and306nm therefore we 228nm was used for analysis the combination of drug.

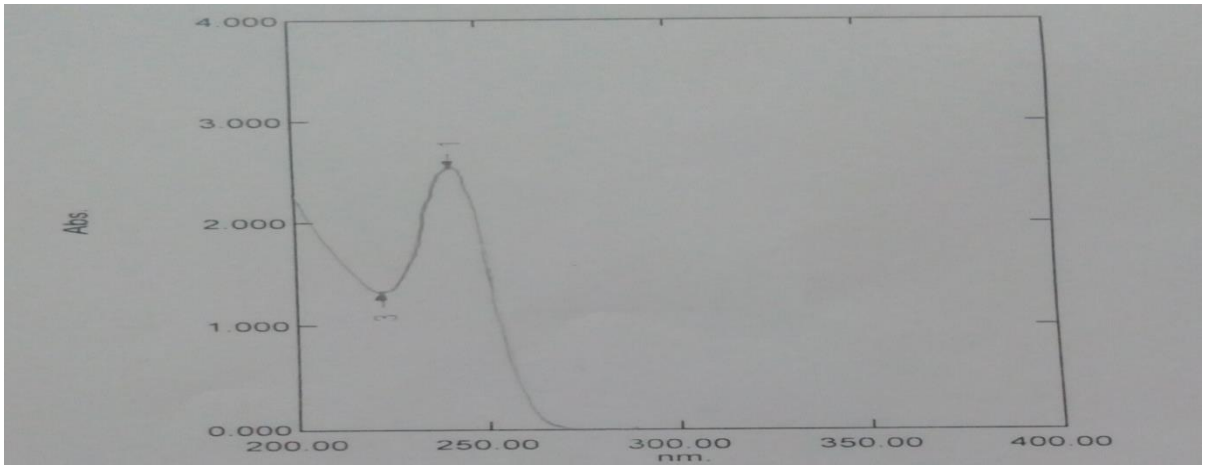


Figure (4) the UV scanned of metformin

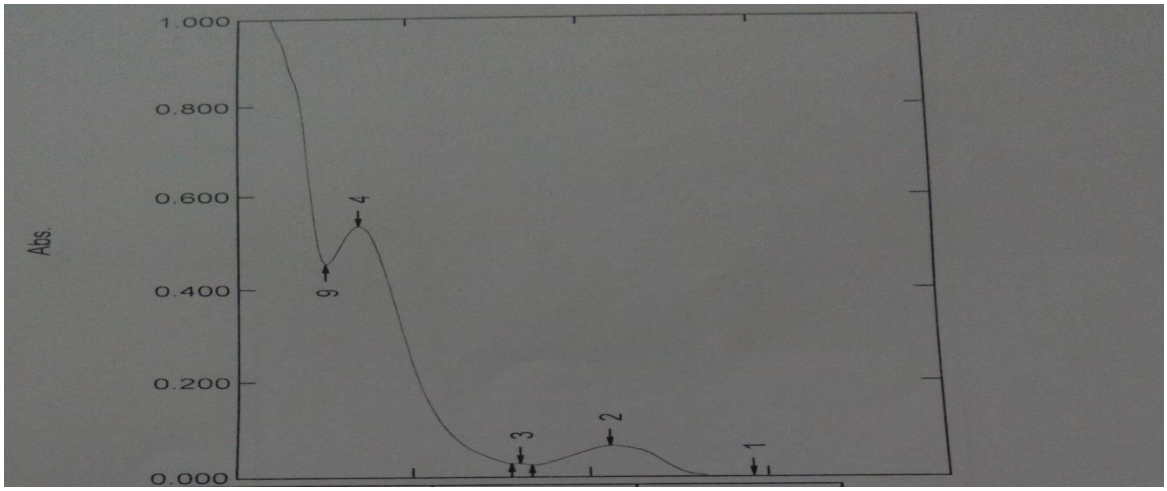


Figure (5) the UV scanned of glibenclamide

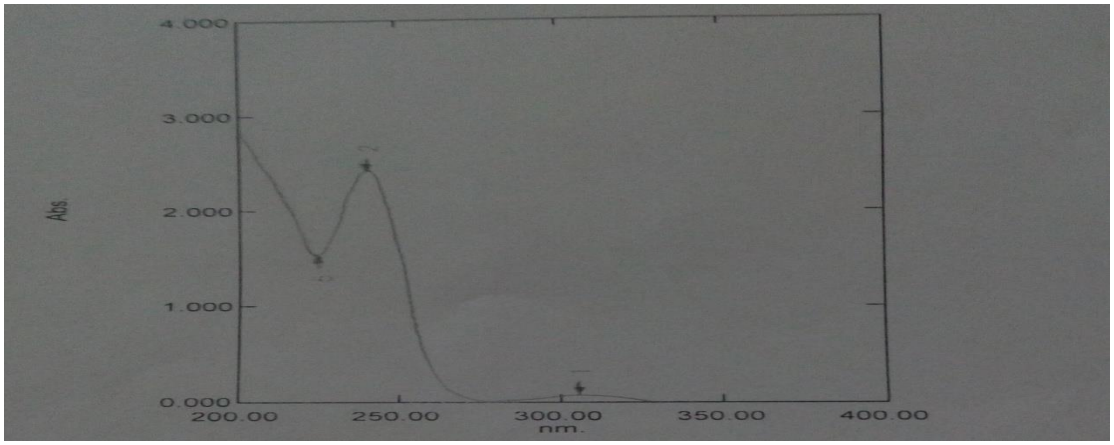
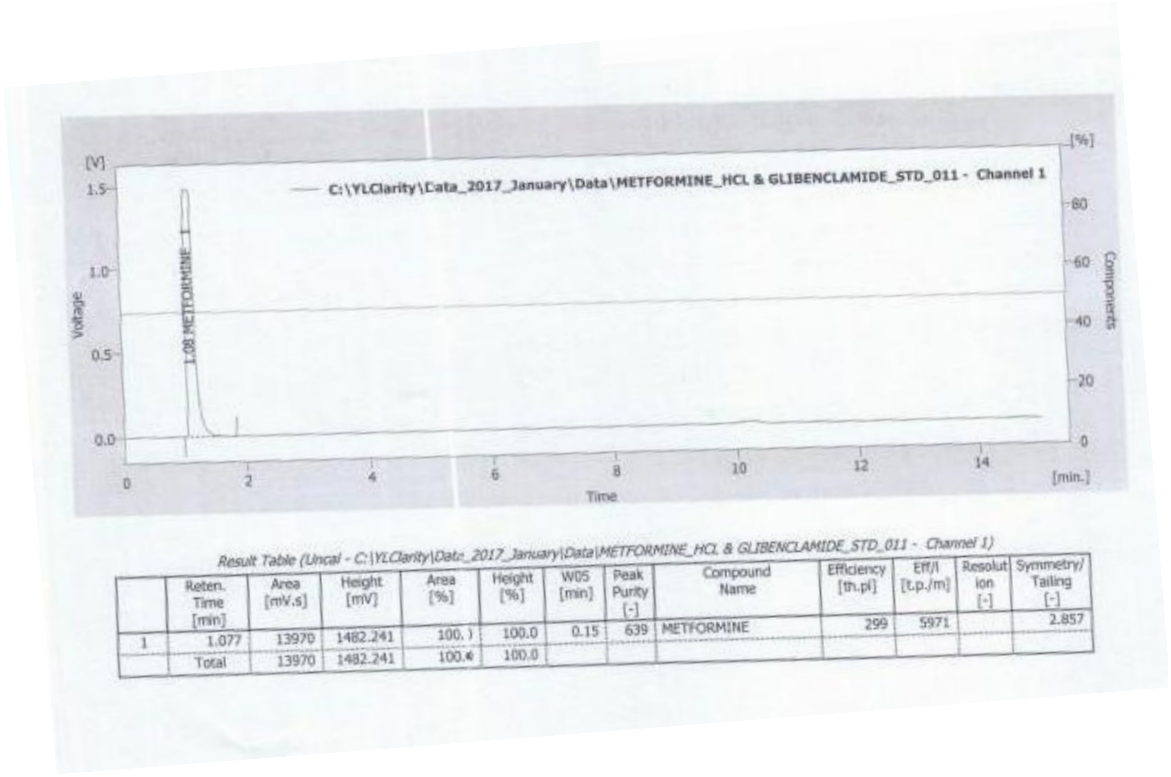


Figure (6) the UV scanned of metformin and glibenclamide

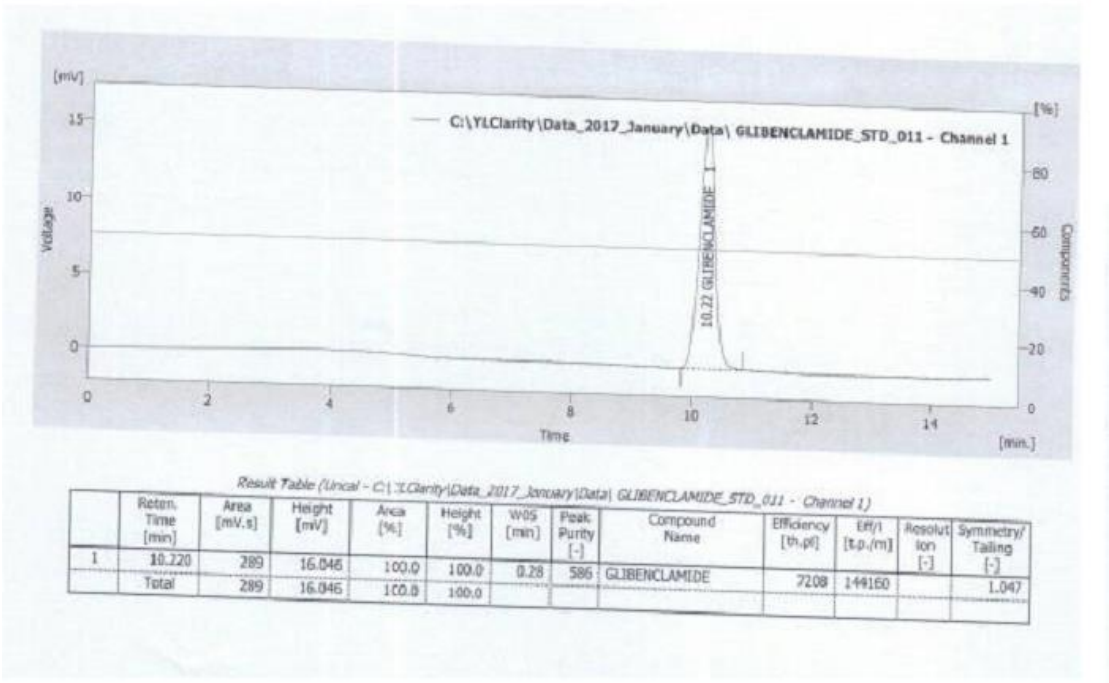
3.2. Retention time:

The retention time of the metformin and glebinclamide was determine by inject the 0.5mg/ml of metformin standard and 0.005mg/ml of glebinclamide standard and then inject the sample drug. The retention time that was obtain from the chromatograms were 1.08min and 10.2min and for sample were 1.09min and 10.22min for metformin and glebinclamide respectively.



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Compound Name	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]	Symmetry/ Tailing [-]
1	1.077	13970	1482.241	100.0	100.0	0.15	639	METFORMINE	299	5971		2.857
	Total	13970	1482.241	100.0	100.0							

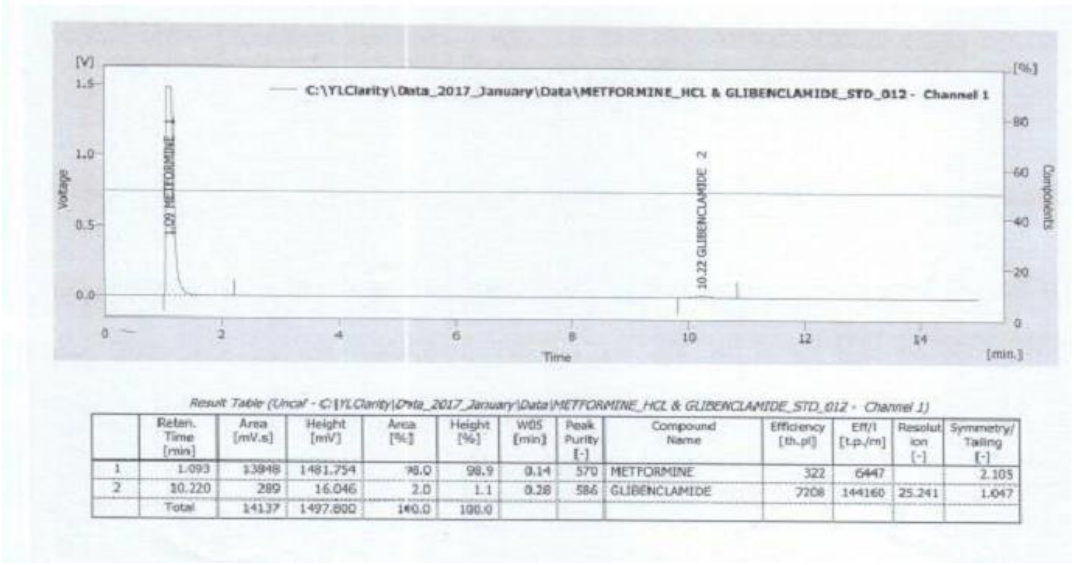
Figure (7) chromatogram of metformin standard



Result Table (Uncal - C:\YLClarity\Data_2017_January\Data\ GLIBENCLAMIDE_STD_011 - Channel 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Compound Name	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]	Symmetry/ Tailing [-]
1	10.220	289	16.046	100.0	100.0	0.28	586	GLIBENCLAMIDE	7208	144160		1.047
	Total	289	16.046	100.0	100.0							

Figure (8) chromatogram of glebinclamide standard



Result Table (Uncal - C:\YLClarity\Data_2017_January\Data\METFORMINE_HCL & GLIBENCLAMIDE_STD_012 - Channel 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Compound Name	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]	Symmetry/ Tailing [-]
1	1.093	13848	1481.754	98.0	98.9	0.14	570	METFORMINE	322	6447		2.105
2	10.220	289	16.046	2.0	1.1	0.28	586	GLIBENCLAMIDE	7208	144160	25.241	1.047
	Total	14137	1497.800	100.0	100.0							

Figure (9) chromatogram of metformin and glebinclamide sample

3.3. System suitability:

Table (3.1) below showed the results obtained for System suitability, the test is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. The System

suitability is assumed due to variability of the measurement itself is addressed in system precision, also termed instrument/injection precision, or injection repeatability. The maximum permitted relative standard deviation for replicate injections (at least five) of the prescribed reference solution does not exceed 2.0%.

Table (3.1) the system suitability

No	Metformin Conc. mg/ml	Area	Glibenclamide Conc. mg/ml	Area
1	0.5	14188	0.005	303
2	0.5	14178	0.005	301
3	0.5	14158	0.005	306
4	0.5	14120	0.005	304
5	0.5	14098	0.005	308
RSD%		0.27%		0.89%

3.4. Linearity study.

From table (3.2), fig (7) and fig (8) it's clear that the method was linear. In all linear calibration models 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, and the correlation coefficient (r^2) was 0.9993 and 0.9990 for metformin and glibenclamide respectively.

Table (3.2) linearity study of glibenclamide.

No	Metformin Conc. mg/ml	Area	glibenclamide Conc. mg/ml	Area
1	0.5	13927	0.005	313
2	1.5	18218	0.015	916
3	2.5	22590	0.025	1535
4	3.5	26247	0.035	2143
5	4.5	31053	0.045	2791
6	5.5	34541	0.055	3372

The ICH guidelines specify that minimum of five concentration level among certain minimum specified ranges. From table (3.2)

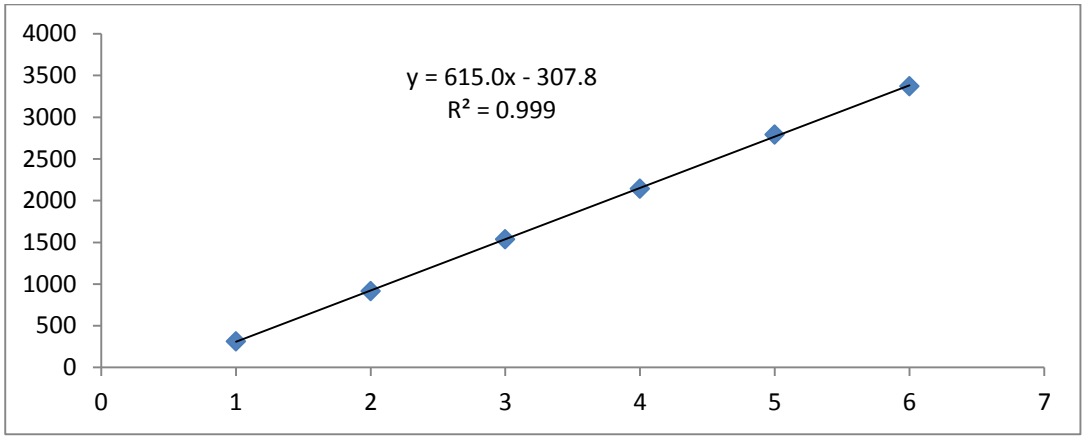


Fig (10) metformin linearity curve

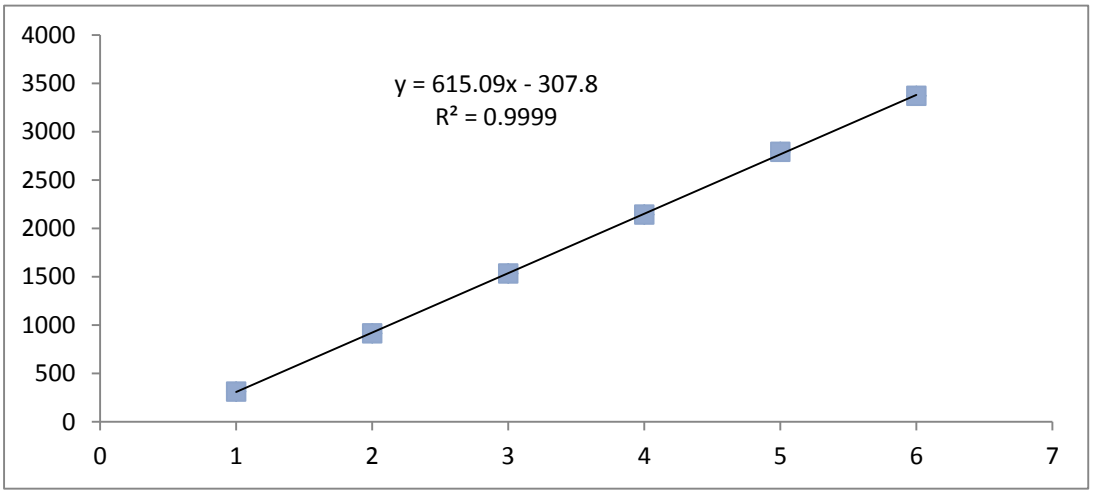


Fig (11) glibenclamide linearity curve

3.5. 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 showed in table (3.3) and table (3.4) intraday precision it's found less than 2% which indicate that method is precise.

Table (3.3) Precision study for metformin

NO	metformin Conc. mg/ml	Day (1)	Day (2)	Day (3)
1	0.5	14190	13782	14276
2	0.5	14187	13848	14269
3	0.5	14190	13831	14183
6	Average	14189	13820	14242
7	RSD%	0.012%	0.25%	0.36%

Table (3.4) Precision study for glibenclamide

NO	glibenclamide Conc. mg/ml	Day (1)	Day (2)	Day (3)
1	0.005	295	294	316
2	0.005	297	289	318
3	0.005	294	292	318
6	Average	295	291	317
8	RSD%	0.52%	0.86%	0.36%

3.6.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, provides the most accurate and precise quantitative data.

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

Result of recovery studies were reported in table (3.5). Reviewing the result accuracy can be reported as percent recovery by the assay of known added analyte in the sample. The range of the percentage 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 (3.5) Result of recovery studies metformin

Pre analyzed solution sample (mg/ml)	Amount added (mg/ml)	Amount recovered	Area	% amount recovered	% RSD
2mg/ml	0.4	2.4	12990	99.7	0.14%
	0.8	2.8	15202	100.1	
	1.2	3.2	17352	99.9	
Area of pre analyzed solution 10847					

Table (3.6) Result of recovery studies for glibenclamide

Pre analyzed solution sample (mg/ml)	Amount added (mg/ml)	Amount recovered	Area	% amount recovered	% RSD
0.02mg/ml	0.004	0.024	753	98.8	0.56%
	0.008	0.028	882	99.1	
	0.012	0.032	1015	99.9	
Area of pre analyzed solution 635					

3.7. Specificity and selectivity.

Term sensitivity is also used and defined as the change 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 metformin were found to be 0.06mg/ml and 0.0198 mg/ ml respectively and for glibenclamide were found to be 0.0019 mg/ml and 0.00064 mg/ ml respectively.

3.8. Repeatability.

Repeatability should be obtained for short interval time or within the same environment. The Repeatability was studied through out analyzing mixture solution contain 2.5 mg /ml and 0.025mg/ml of metformin and glebinclamiderespectively5 time. The R.S.D of Repeatability test should be not more than 2%.

Table (3.7) repeatability studies

No	Metformin Conc. mg/ml	Area	glibenclamide Conc. mg/ml	Area
1	2.5	23922	0.025	766
2	2.5	24229	0.025	771
3	2.5	23971	0.025	759
4	2.5	23796	0.025	767
5	2.5	23778	0.025	764
RSD%		0.76%		0.57%

3.9. Linearity range.

Linearity range is the ability of the method to elicit test result that is directly proportional to analyte concentration within a given range. 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. Table (3.8) showed low range for linearity and table (3.9) showed high range for linearity

Table (3.8) low range for linearity

No	Metformin Conc. mg/ml	Area	Glibenclamide Conc. mg/ml	Area
1	0.5	13680	0.005	303
2	0.5	13781	0.005	303
3	0.5	13798	0.005	300
4	0.5	13799	0.005	303

5	0.5	13792	0.005	302
RSD%		0.37%		0.43%

Table (3.9) high range for linearity

No	Metformin Conc. mg/ml	Area	Glibenclamideb Conc. mg/ml	Area
1	5.5	33522	0.055	3384
2	5.5	33543	0.055	3382
3	5.5	33552	0.055	3389
4	5.5	34141	0.055	3368
5	5.5	33826	0.055	3385
RSD%		0.80%		0.24%

3.10. Comparative study.

There are tow samples of metformin and glibenclamide manufactured by different companies in the market and it were submitted for different physical test and assay determination.

Table (3.10) Comparative study for metformin and glibenclamide product from different companies

Table (3.10) show the comparative study

Sample	Area of		area of STD		Assay%	
	metformin	glibenclamide	metformin	glibenclamide	metformin	glibenclamide
Company A	23225	1559	23623	1514	98.3	102.9
Company B	23113	1477			97.8	97.6

The result obtained in table (3.10) above was done in the same laboratory using same equipment within a short time interval to using the same methods.

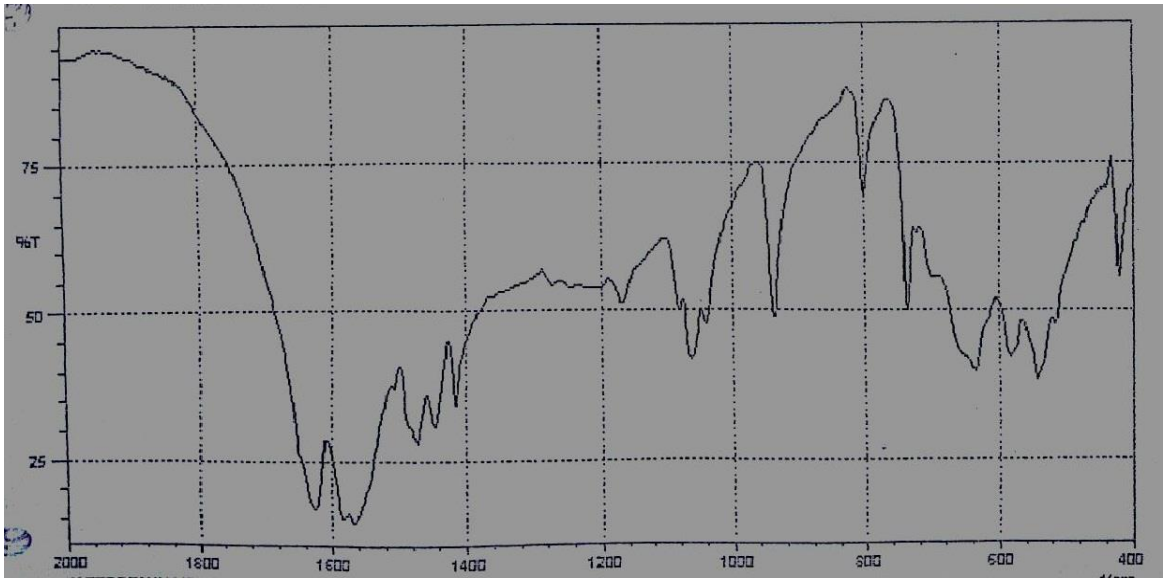


Figure (12) the IR spectrum for metformin

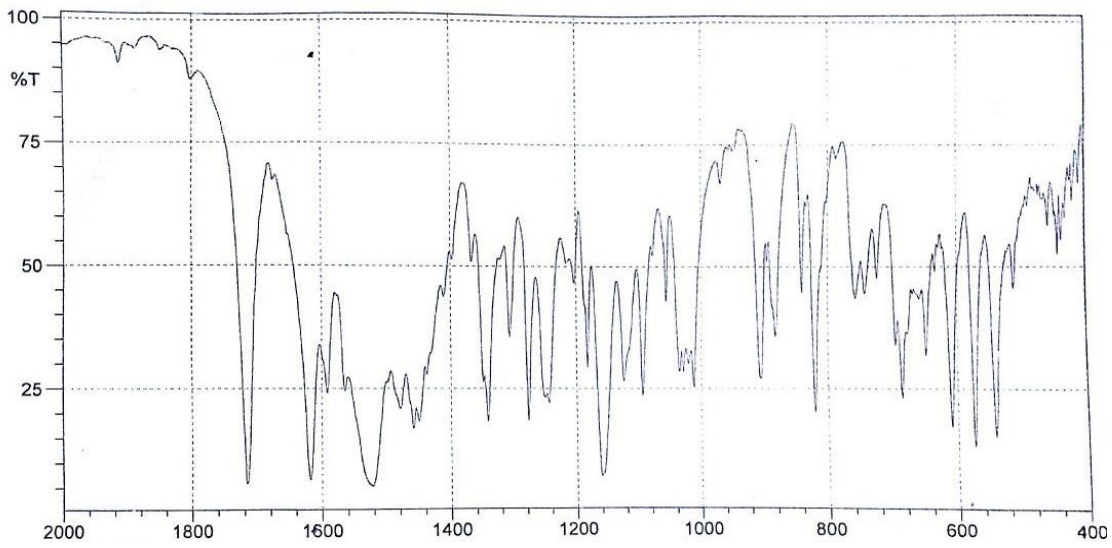


Figure (13) shown the IR spectrum for glibenclamide

Conclusion and recommendation.

According to the ICH guidance, the objective of method validation is to demonstrate that analytical procedures "are suitable for their intended purpose. "Therefore the method's purpose should be linked to the clinical studies and the pharmaceutical purpose of the product being studied.

The method should be continues to tracking the degradation, impurities and should be stability indicating and capable of measuring the effect of key manufacturing parameters to ensure consistency of the drug substance and drug product.

The result that was obtain from working agreed and approves that the method was simple, fast and reliable. The Performance of validation parameters linearity, Repeatability, Accuracy, Precision, repeatability, Specificity, range. The developed method offers several advantages in terms of simplicity in mobile phase, isocratic mode of elution and sample preparation steps and comparative short run time makes the method specific, repeatable and reliable for its intended use in simultaneous determination of Metformin HCl and Glibenclamide in tablet dosage form as well as in other formulations.

Establish the method for routine quality control laboratory analysis for simultaneous determination of drug combination metformin hydrochloride and glibenclamide.

Stability indicating of an analysis method for its intended use is perquisite to obtaining accurate data; therefore, 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.

Extend method in future work to consist the related substance of the drug combination and investigate all validation characters .

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