Sudan University of Science
& Technology

Faculty of Science

Department of Scientific Laboratories
"Chemistry"

Graduation Project for a Bachelor Degree of Honor Title:

# The Effect of Heat and Direct Sun light

# (*Glibenclamide*)

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**September, 2014**

# الآيـــــــــــــة

بسم الله الرحمن الرحيم

(أَمَّنْ هُوَ قَانِتٌ آنَاءَ اللَّيْلِ سَاجِدًا وَقَائِمًا يَحْذَرُ الْآخِرَةَ وَيَرْجُو رَحْمَةَ رَبِّهِ ۗ قُلْ هَلْ يَسْتَوِي الَّذِينَ يَعْلَمُونَ وَالَّذِينَ لَا يَعْلَمُونَ ۗ إِنَّمَا يَتَذَكَّرُ أُولُو الْأَلْبَابِ )

صدق الله العظيم

سورة الزمر، الآيــة (٩)

# الإهـــــــداء

أمي الغالية هي شمعة تذوب لتنير دروب للآخرين هي زهر تذبل لتفوح برائحة الياسمين هي العطاء الذي يفيض بلاحدود هي رمز يجسد الكفاح والخلود، إلى الشمس التي حفت كل الأيام القاسية وتوسدت كل الهموم وتغطت بالمعاناة لتحميني من برد الظروف الصعبة ،حنيني الذي احن إليه وجنه السعادة في كلماتها وفرح للحزين إذا بكي، حبيبتي أمي أنـــا ...

أمــي الحنـون

الي الانسانية ورمز التقوى ،الطيبة المروءة الرجولة والكرم ..

من اوجدني في هذه الحياة سراج الطيبة الذي ينير دربي ويعطيني الاحساس بالامان

أبـي العـزيـز

إلى من قاسمتهم شهد الحياة وأزمة المحن القاسية... إلي تلك النسيمات العزبة حبات ذاك العقد الفريد...

أخــواتي

إلي اعز رفقه طابت الايام بوجودهم...

صديقاتي

# الشكر والعرفان

والشكر للدكتور / صلاح أحمد إبراهيم الذي قام بالإشراف علي هذا البحث باذلاً الجهد والوقت وكان لي خير ناصح ومشرف.

كما أتقدم بوافر الشكر والتقدير لكل الذين ساهموا معي في إخراج هذا البحث بصورة الجميلة .

# Abstract

The effect of heat and direct sun light of glibenclamide,by HPLC(high performance liquid chromatography) and UV(ultra-violet spectrophotometer).were put tablets in:

-Direct sun light.

-At 50C

Was reading at zero time and then after one day, after three days and after five days. After that calculated the assay.

From the result we found the glibenclamide is stable in direct sun ligt and heat.

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# Chapter One

# Introduction & Literature Review

# Introduction

## (1-1) Analytical chemistry:

### (1-1-1) Definition:

Analytical chemistry is te study of the chemical composition of natural and artificial materials Properties studied in anayltical chemistry include geometric features such as moleculer Morphologies and distriution of species ,as well features such as compostion and species Identity . Unlike the sub disciplines inorganic chemisrty and organic chemistry analytical Chemistry (like phyical chemistry ) is not restricted to any particular type of chemical compound or reaction .the contribution made by analyical chemistry have played critical rules in the sciences raning from deveolpment of concepets and theories (pure science)to a variety of practical applications, such as biomedical applications, environmental monitoring quality control of industrial manufacturin and forensic science (applied science).

### (1-1-2) Modern analytical chemistry:

Modern analytical chemistry is dominated by instrumental analysis . many analytical chemists focus on asingle type of instrument .Academics tend to either focus on new applications and discoveries or on new method of analysis .the discovery of chemical present in blood that increases the risk of cancer would be a discovery that and analytical chemist might be involved .An effort to develop A new method might involve the use of a tunable laser to increase the specificity and sensitivity of spectrometric method . many method , once developed ,are kept purposely static so that date can be compared over long periods of time this is particularly true in industrial quality assurance (QA) for ensic and forensic and environmental applications Analytical chemistry plays and increasingy important role and the phermaceutical industry where .asde QA, it is used in ddiscoverd of new drug candidats and clinical applicationts where un derstanding t he interiactions between the drug and the patient are critical.

### (1-1-3) Approaches:

Most modern analytical chemistry can be catrgorized in terms of the analytical target(problem or fied of application ).and the analytical methods used . the joural analytical chemisrty provids reviews of resent research in either analytical target or analytical method s , alternating by year .

1- By analytical target:

- Bio analytical chemistry .
- Material analysis.
- Envrionmental analysis.
- Forensics.

2- By Analytical Method :

- Spectroscopy.
- Mass spectrometry.
- Spectrophotometry and colorimetry
- Chromatography and electroporesis
- Crystallography
- Microscopy
- Electrochemistry.

## (1-2) Tablets:

### (1-2-1) DEFINITION:

Tablets are solid preparations each containing a single dose of one or more active substances. They are obtained by compressing uniform volumes of particles or by another suitable manufacturing technique, such as extrusion, moulding or freeze-drying (lyophilisation). Tablets are intended for oral administration. Some are swallowed whole, some after being chewed, some are dissolved or dispersed in

water before being administered and some are retained in the mouth where the active substance is liberated.

The particles consist of one or more active substances with or without excipients such as diluents, binders, disintegrating agents, glidants, lubricants, substances capable of modifying the behaviour of the preparation in the digestive tract, colouring matter authorised by the competent authority and flavouring substances.

Tablets are usually straight, circular solid cylinders, the end surfaces of which are flat or convex and the edges of which may be bevelled. They may have break-marks and may bear a symbol or other markings. Tablets may be coated.

Where applicable, containers for tablets comply with the requirements for materials used for the manufacture of containers (*3.1* and subsections) and containers (*3.2* and subsections).

Several categories of tablets for oral use may be distinguished:

- Uncoated tablets;
- Coated tablets;
- Effervescent tablets;
- Soluble tablets;
- Dispersible tablets;
- Orodispersible tablets;
- Gastro-resistant tablets;
- Modified-release tablets;
- Tablets for use in the mouth;
- Oral lyophilisates.

**(1-2-2) UNCOATED TABLETS:**

**DEFINITION:**

Uncoated tablets include single-layer tablets resulting from a single compression of particles and multi-layer tablets consisting of concentric or parallel layers obtained by successive compression of particles of different composition. The excipients used are not specifically intended to modify the release of the active substance in the digestive fluids.

Uncoated tablets conform to the general definition of tablets. A broken section, when examined under a lens, shows either a relatively uniform texture (single-layer tablets) or a stratified texture (multi-layer tablets) but no signs of coating.

**TESTS:**

**Disintegration :**

Uncoated tablets comply with the test. Use *water R* as the liquid. Add a disc to each tube. Operate the apparatus for 15 min, unless otherwise justified and authorised, and examine the state of the tablets. If the tablets fail to comply because of adherence to the discs, the results are invalid. Repeat the test on a further 6 tablets omitting the discs.

Chewable tablets are not required to comply with the test. [1]

**(1-3) High-performance liquid chromatography:**

(**HPLC**; formerly referred to as **high-pressure liquid chromatography**), is a technique in analytic chemistry used to separate the components in a mixture, to identify each component, and to quantify each component. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column.

HPLC has been used for medical ( detecting vitamin D levels in blood serum), legal ( detecting performance enhancement drugs in urine), research ( separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and manufacturing ( during the production process of pharmaceutical and biological products) purposess

Chromatography can be described as a mass transfer process involving adsorption. HPLC relies on pumps to pass a pressurized liquid and a sample mixture through a column filled with a sorbent, leading to the separation of the sample components. The active component of the column, the sorbent, is typically a granular material made of solid particles (. silica, polymers.), 2–50 micrometers in size. The

components of the sample mixture are separated from each other due to their different degrees of interaction with the sorbent particles. The pressurized liquid is typically a mixture of solvents ( water, acetonitrile and/or methanol) and is referred to as a "mobile phase". Its composition and temperature play a major role in the separation process by influencing the interactions taking place between sample components and sorbent. These interactions are physical in nature, such as hydrophobic (dispersive), dipole–dipole and ionic, most often a combination thereof.

HPLC is distinguished from traditional ("low pressure") liquid chromatography because operational pressures are significantly higher (50–350 bar), while ordinary liquid chromatography typically relies on the force of gravity to pass the mobile phase through the column. Due to the small sample amount separated in analytical HPLC, typical column dimensions are 2.1–4.6 mm diameter, and 30–250 mm length. Also HPLC columns are made with smaller sorbent particles (2–50 micrometer in average particle size). This gives HPLC superior resolving power when separating mixtures, which is why it is a popular chromatographic technique.

The schematic of an HPLC instrument typically includes a sampler, pumps, and a detector. The sampler brings the sample mixture into the mobile phase stream which carries it into the column. The pumps deliver the desired flow and composition of the mobile phase through the column. The detector generates a signal proportional to the amount of sample component emerging from the column, hence allowing for quantitative analysis of the sample components. A digital microprocessor and user software control the HPLC instrument and provide data analysis. Some models of mechanical pumps in a HPLC instrument can mix multiple solvents together in ratios changing in time, generating a composition gradient in the mobile phase. Various detectors are in common use, such as UV/Vis, photodiode array (PDA) or based on mass spectrometry. Most HPLC instruments also have a column oven that allows for adjusting the temperature the separation is performed at.(3)

**(1-4) Ultra violet Spectrophotometry (UV-Spectrophotometry) :**

In spectrophotometric analysis a source of radiation is used that extends into the ultraviolet region of the spectrum. From this, definite wavelengths of radiation are chosen possessing a bandwidth of less than 1nm. This process necessitates the use of a more complicated and consequently more expensive instrument. The instrument employed for this purpose is a spectrophotometer.[5]

An optical spectrometer is an instrument possessing an optical system which can produce dispersion of incident electromagnetic radiation, and which measurements can be made of the quantity of transmitted radiation at selected wavelength of the spectral range.

A photometer is device for measuring the intensity of transmitted radiation or a function of this quantity, When combined in the spectrophotometer and photometer are employed conjointly to produce a single corresponding to the difference between the transmitted radiation of reference material and that of a sample at selected wavelengths.[2]

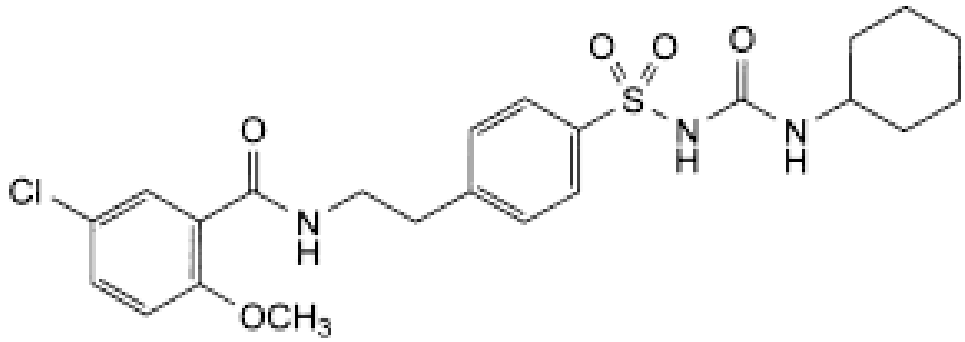
The chief advantage of colorimetric and spectrophotometric methods is that they provide a simple means for determination minute quantities of substances. The upper limit of colorimetric method is, in general, the determination of constituents which are present in quantities of less than 1 or 2 per cent. The sensitivity can, however, be improved if the technique of derivative spectrophotometry is employed. The development of expensive photoelectric colorimeters has placed this branch of instrumental chemical analysis within the of the means of even the smallest teaching institution.[2]

Analytical method that are based upon the absorption of electromagnetic radiation. Light consists of radiation to which the human eye is sensitive, waves of different wavelengths giving rise to light of different colors, while a mixture of light of these wavelengths constitutes white light. Covers the entire visible spectrum 400-760nm.[2]

The visual perception of color arises from the selective absorption of certain wavelengths of incident light by the colored object. The other wavelengths are either reflected or transmitted, according to the nature of the object, and are

perceived by the eye as the coloure of the object appears white, all wavelengths are reflected equally; if the object blue, the wavelengths that give the blue stimulus are reflected.[2]

***(1-5) Glibenclmide***




$C_{23}H_{28}ClN_3O_5S$

**(1-5-1) Definition:**

1-[[4-[2-[(5-Chloro-2-methoxybenzoyl)amino]ethyl]phenyl]sulfonyl]-3-cyclohexylurea.

**Content**

99.0 per cent to 101.0 per cent (dried substance).

**Characters:**

**Appearance**

White or almost white, crystalline powder.

**Solubility**

Practically insoluble in water, sparingly soluble in methylene chloride, slightly soluble in alcohol and in methanol.

Glibbenclamide tablets 5mg are an oral diabetes medication used to control blood sugar levels in people with mild to moderate type-2 diabetes mellitus (also called non insulin-dependent diabetes or maturity onset diabetes) who are unable to achieve adequate glycaemic control (normal blood sugar levels) with diet and exercise alone. glibenclamide tablets 5mg can be used alone as monotherapy, in

conjunction with diet and exercise, or in combination with other antihyperglycaemic medication (lowers blood sugar), like metformin, when a single drug does not provide adequate glycaemic control. If Glibenclamide tablets 5mg become less effective, they can be given together with insulin. Diabetes increases the risk of serious health complications, including diabetic retinopathy (damage to the retina in the eye with loss of vision), diabetic neuropathy (nerve damage) and diabetic nephropathy (kidney damage). Maintaining glycaemic control with Glibenclmide tablets 5mg reduces the risk of developing these vascular complications.

**(1-5-2) Mechanism of action:**

Glibenclamide tablets 5mg contain glibenclamide, an oral antihyperglycaemic medication belonging to the sulphonylurea group of drugs. Glibenclamide tablets 5mg controls blood glucose (sugar) primarily by acting directly on the beta cells, which are the insulin-producing cells of pancreatic islet tissue, to increase their sensitivity to glucose and to stimulate the cells to produce and release more insulin. Insulin is normally produced in response to food and increased blood glucose levels and it controls postprandial (after eating) blood glucose levels (glycaemic control). In diabetes, resistance to insulin results in loss of glycaemic control. Glibenclamide in Daonil tablets 5mg helps the body to produce sufficient insulin to maintain normal blood glucose levels after a meal as well as between meals and is effective for up to 24 hours. It is thought that as well as acting as an insulin secretagogue (stimulates insulin secretion) glibenclamide in Daonil tablets 5mg also acts on insulin-responsive cells in the liver, muscle and fat cells to increase the number of receptors, which means that insulin control of glucose production by the liver and uptake of insulin into peripheral tissues for energy and storage, is more efficient.

**(1-5-3) Contain:**

Daonil tablets 5mg contain the active ingredient glibenclamide, an oral antihyperglycaemic used to lower blood sugar levels. They also contain lactose, maize starch, talc, colloidal silica, magnesium stearate.

### (1-5-4) Side effects of Glibenclmide:

The most commonly reported side effects when taking Daonil tablets 5mg include: gastrointestinal complaints like nausea, diarrhoea, constipation and stomach or abdominal pain; allergic skin reactions like, redness (erythema), itching (pruritus), hives (urticaria); dizziness, drowsiness, headache, visual disturbances, confusion, malaise and tremor, which are usually transient and may be signs of hypoglycaemia. A more serious side effect of taking Daonil tablets 5mg is severe hypoglycaemia (very low blood glucose), as glibenclamide continues to work between meals to reduce blood sugar levels, so it is important to eat regularly; also certain other conditions like liver or kidney problems, or taking other medications like beta blockers, can make you more susceptible to hypoglycaemia. Other symptoms of hypoglycaemia include headache, hunger, restlessness, loss of consciousness, which can result in coma.

### (1-5-4-1) Hypoglycaemia (hypo):

Having a hypo is an unpleasant episode experienced by diabetics and refers to extreme hypoglycaemia or very low blood glucose. Glibenclamide in Daonil tablets 5mg continue to work between meals to reduce blood sugar levels and therefore if glucose levels fall too low, by missing a meal or waiting too long between eating, severe hypoglycaemia, which is very low blood glucose, can be the result. Symptoms of hypoglycaemia include headache, hunger, nausea, vomiting, restlessness, sensory disturbances, delirium, loss of consciousness, which can result in shallow respiration, bradycardia (slow heart beat) and coma.[1]

**(1-5-5) Drug interaction:**

- Protein bound drugs: because Glibenciamide Is highly protein bound it could be displaced from btnding sites by oral anticoagulants, hydantoins. salicylate and other nonstero4dal anti-inflammatory agents and sulfonamides.
- henylbutazone: may potentiate the hypoglycemic effects of Glibenclamide by decreasing the renal excretion of Ghbendamide.
- Thiazide diuremay exacerbate diabetes meUitus resulting n increased requirements of Ghbenciamide.
- A1coho Disutfirarn.like reactions have occurred very rarely. (3-adtenergic blocking agents: (3-adrenergic bloclcng agents may upair glucose tolerance increasing the frequency and seventy cf hypoglycemia. Other drugs Drugs that may enhance the hypoglycemic effect of Glibil
- Indude chioramphenicot, monoamine oxidase inhibitors and probenecid. are allergic to Glibenclamide or any ingredients in Daonil
- are pregnant or are breastfeeding
- have kidney, liver or thyroid disease
- have type 1 (insulin-dependent) diabetes or suffer from diabetic coma or diabetic ketoacidosis
- have G6PD deficiency as you may be at risk of haemolytic anaemia
- are malnourished or have adrenal or pituitary insufficiency, as this may increase risk of hypoglycaemia
- are taking medicines that interact with Daonil, including: the antifungals miconazole and fluconazole, non steroidal anti-inflammatory agents (NSAID) like phenylbutazone, salicylate analgesicis like aspirin, beta-blockers like propranolol for high blood pressure and certain heart conditions, clonidine for high blood pressure, ACE Inhibitors like benezapril for high blood pressure, cimetidine for peptic ulcers, monoamine oxidase inhibitors for depression, probenecid for gout, the anticoagulant warfarin,

the antibiotic chloramphenicol, sulphonamide antibiotics, corticosteroids for inflammation, bronchodilators like salbutamol, thiazides and other diuretics, thyroid hormones, oestrogens and progestogens for oral contraceptives and HRT, phenytoin for epilepsy, nicotinic acid for high cholesterol, calcium channel blocking drugs like verapamil for angina, isoniazid for tuberculosis[3]

- Drugs that affect Daonil and cause hyperglycaemia (high blood sugar) by reducing its glucose-lowering effect: Danazol for hormone treatment, the antipsychotic chlorpromazine, corticosteroids for inflammation, bronchodilators like salbutamol, thiazides and other diuretics, thyroid hormones, oestrogens and progestogens for oral contraceptives and HRT, phenytoin for epilepsy, nicotinic acid for high cholesterol, calcium channel blocking drugs like verapamil for angina, isoniazid for tuberculosis, clonidine for high blood pressure[3]

# Chapter Two
# The Objective

## (2-1) Objective:

Study the effect of heat and direct sun light on efficiency of glibenclamide (5mg)
.

# Chapter Three
# Materials and Methods

**(3-1) Material:**

- Glibenclamide Tablets
- Hydrochloric acid (1M)
- Methanol
- Aceto nitrile
- Potassium dihydrogen ortho phosphate(1.36%W\V)
- Ortho phosphoric acid ( ph:3)
- Methnolic HCL
- Lead standard solution (10ppm)
- Buffer solution PH 3.5
- Magnesum oxide
- Phenolphthalein solution
- Ammonia
- Sulfuric acid

**Equipments:**

- HPLC Instrument
- Ultra Violet Spectrophotometer (uv-1800series) (shimadzu)

**(3-2) Method:**

**(3-2-1) Method of HPLC**

**Mobile Phase :**

47 volumes of acetonitrile and 53 volumes of a 1.36% w/v solution of potassium dihydrogen orthophosphate previously adjusted to pH 3.0 with orthophosphoric acid

**Preparation of Sample:**

Weigh and powder 20 tablets. Carry out the method for liquid chromatography, Appendix III D, using the following solutions.

(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing 5 mg of Glibenclamide with a mixture of 2 mL of water and 20 mL of methanol until fully dispersed and filter through a 0.2-µm membrane filter (Anatop LC is suitable). (1)

**Preparation of Standard:**

(2) Dissolve 50 mg of glibenclamide BPCRS in 10 mL of methanol with the aid of ultrasound for 20 minutes, add sufficient methanol to produce 50 mL and dilute 1 volume of this solution to 4 volumes with methanol . To 20 mL of this solution add 2 mL of water and mix[1].

**(3-2-2) Method of heavy metals:**

**Test solution** In a silica crucible, mix thoroughly the prescribed quantity of the substance to be examined with 0.5 g of magnesium oxide R1. Ignite to dull redness until a homogeneous white or greyish-white mass is obtained. If after 30 min of ignition the mixture remains coloured, allow to cool, mix using a fine glass rod and repeat the ignition. If necessary repeat the operation. Heat at 800 °C for about 1 h. Take up the residue in 2 quantities, each of 5 mL, of a mixture of equal volumes of hydrochloric acid R1 and water . Add 0.1 mL of phenolphthalein solution and then concentrated ammonia until a pink colour is obtained. Cool, add glacial acetic acid until the solution is decolorised and add 0.5 mL in excess. Filter if necessary and wash the filter. Dilute to 20 mL with water .

**Reference solution (standard)** Prepare as described for the test solution using the prescribed volume of lead standard solution (10 ppm Pb) instead of the substance to be examined and drying in an oven at 100-105 °C. To 10 mL of the solution obtained add 2 mL of the test solution.

**Monitor solution** Prepare as described for the test solution, adding to the substance to be examined the volume of lead standard solution (10 ppm Pb)

prescribed for preparation of the reference solution and drying in an oven at 100-105 °C. To 10 mL of the solution obtained add 2 mL of the test solution.
**Blank solution** A mixture of 10 mL of water and 2 mL of the test solution.
To 12 mL of each solution, add 2 mL of buffer solution pH 3.5 . Mix and add to 1.2 mL of thioacetamide reagent . Mix immediately. Examine the solutions after 2 min. [1]

**(3-2-3) Method of sulphated ash:**

Ignite a suitable crucible (for example, silica, platinum, porcelain or quartz) at 600 ± 50 °C for 30 min, allow to cool in a desiccator over silica gel or other suitable desiccant and weigh. Place the prescribed amount of the substance to be examined in the crucible and weigh. Moisten the substance to be examined with a small amount of *sulfuric acid* (usually 1 mL) and heat gently at as low a temperature as practicable until the sample is thoroughly charred. After cooling, moisten the residue with a small amount of *sulfuric acid* (usually 1 mL), heat gently until white fumes are no longer evolved and ignite at 600 ± 50 °C until the residue is completely incinerated. Ensure that flames are not produced at any time during the procedure. Allow the crucible to cool in a desiccator over silica gel or other suitable desiccant, weigh it again and calculate the percentage of residue.

If the amount of the residue so obtained exceeds the prescribed limit, repeat the moistening with *sulfuric acid R* and ignition, as previously, for 30 min periods until 2 consecutive weighings do notdiffer by more than 0.5 mg or until the percentage of residue complies with the prescribed limit.

The amount of substance used for the test (usually 1-2 g) is chosen so that at the prescribed limit the mass of the residue (usually about 1 mg) can be measured with sufficient accuracy.[1]

**(3-2-4) Method of ultra violet:**

**Reagent:**
0.1M methanol -HCL(8.3ml Conc.HCL/ 1000ml

**Standard:**
Weight 10 mg of glienclamide powder dissovle in 100ml methanol HCL and stir for 15min.

**Sample:**

Weight and powder 20tablts .of glienclmide and take apowder equivalent dissolve in 0.1M methanolic HCL and complete volume to 100ml ,stir for 15min.take and incubate in water bath at 60 or 2min and filter, read abs at 300nm.

**Blank:**

0.1M methanolicHCL.

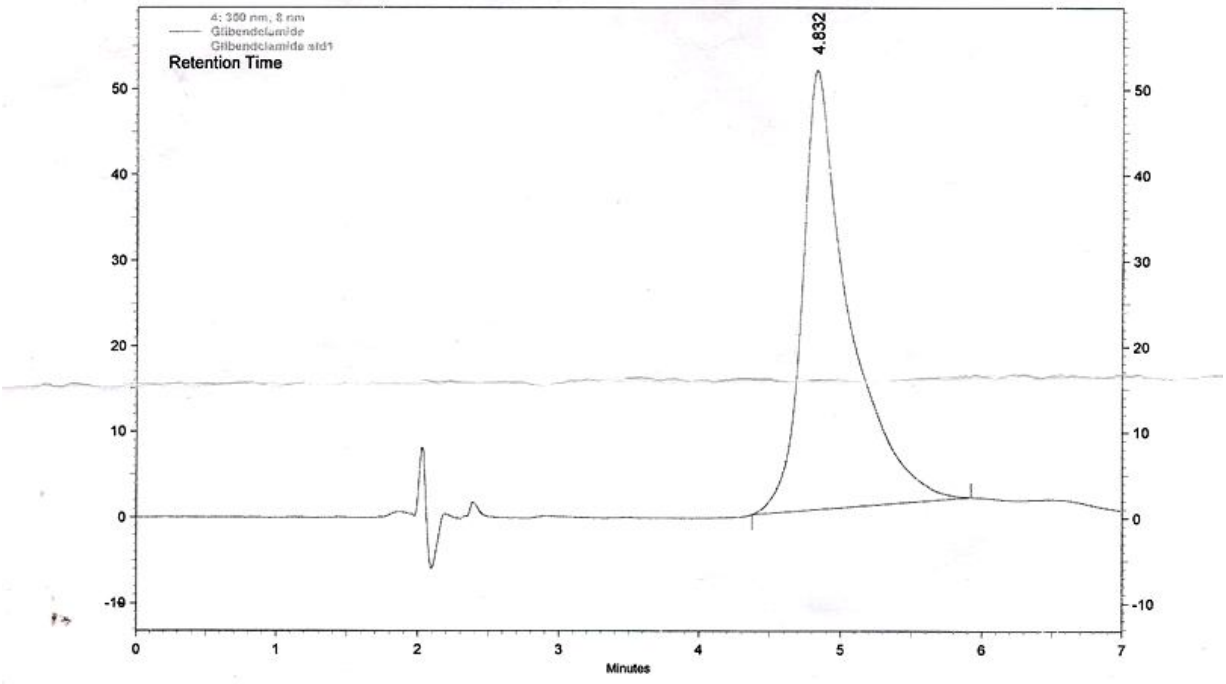
# Chapter Four
# The Result & Calculation

## (4-1) The Result:

## (4-1-1) HPLC Result:

**Shimadzu CLASS-VP V6.14 SP1**
***Area % Report*** **Page 1 of 2**

**Method Name:** **C:\CLASS-VP\Methods\Glibenclamide.met**
**Data Name:** **C:\CLASS-VP\Glibendclamide std1**
**User:** **System**
**Acquired:** **7/7/2014 2:30:12 PM**
**Printed:** **7/7/2014 2:43:40 PM**
**injection volume 20**




**4: 300 nm, 8 nm**

| Name | Retention Time | Area | Height |
|---|---|---|---|
| Glibendclamide | 4.832 | 1177091 | 51219 |

**Shimadzu CLASS-VP V6.14 SP1**
*Area % Report* **Page 1 of 2**

**Method Name:** **C:\CLASS-VP\Methods\Glibenclamide.met**
**Data Name:** **C:\CLASS-VP\Glibendclamide JON zero**
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**Acquired:** **7/7/2014 1:51:34 PM**
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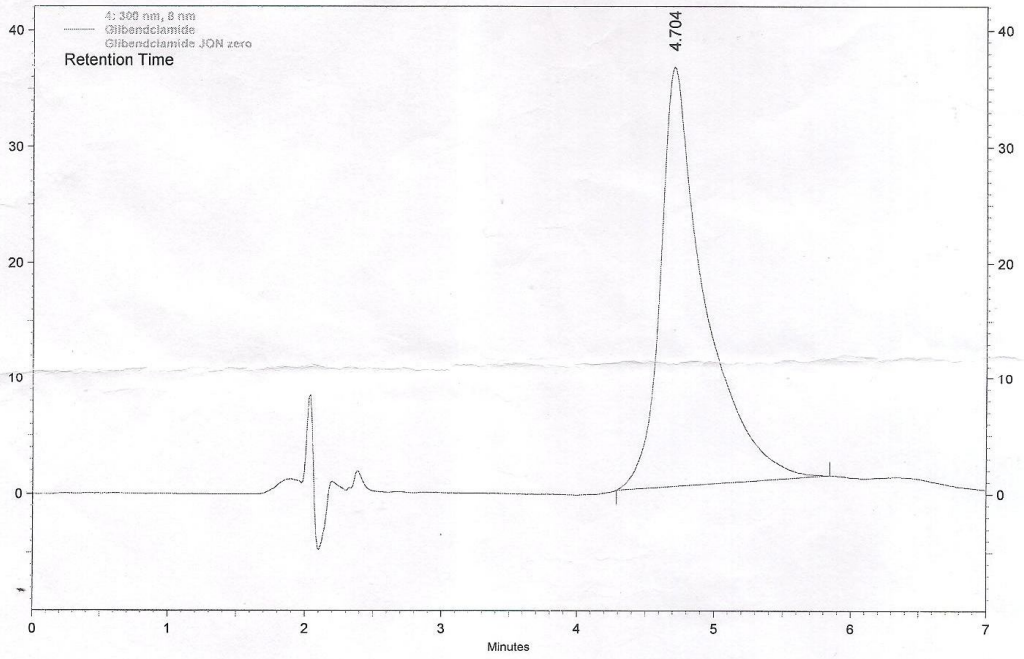
injection volume 20




**4: 300 nm, 8 nm**

| Name | Retention Time | Area | Height |
|---|---|---|---|
| Glibendclamide | 4.704 | 814910 | 36192 |

**Shimadzu CLASS-VP V6.14 SP1**
***Area % Report*** **Page 1 of 2**

**Method Name:** **C:\CLASS-VP\Methods\Glibenclamide.met**
**Data Name:** **C:\CLASS-VP\Glibendclamide JON sun**
**User:** **System**
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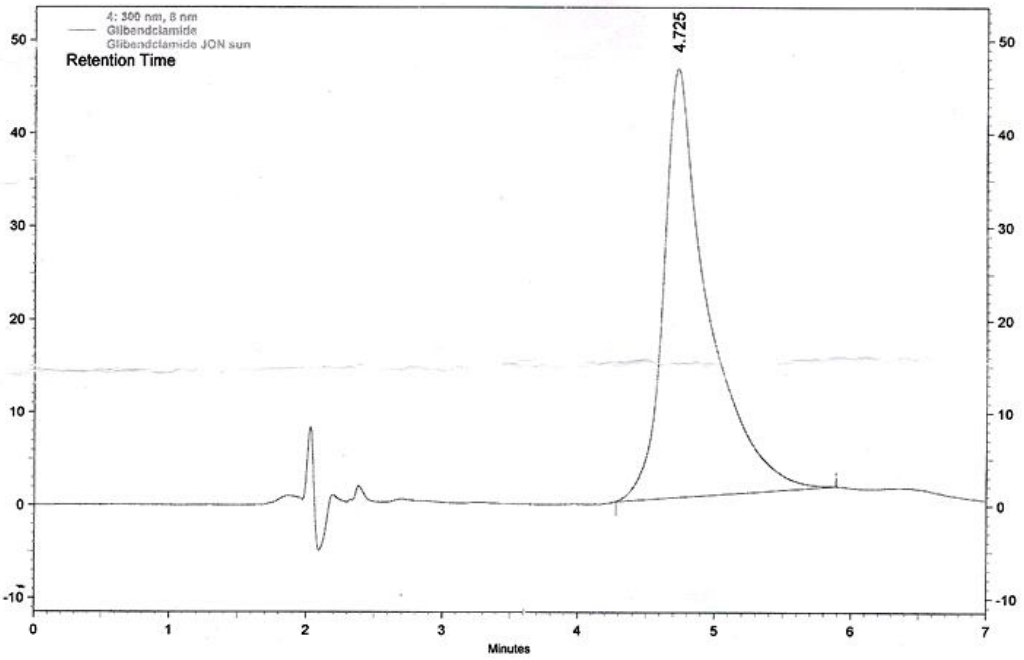
**Printed:** **7/7/2014 2:49:01 PM**
injection volume 20

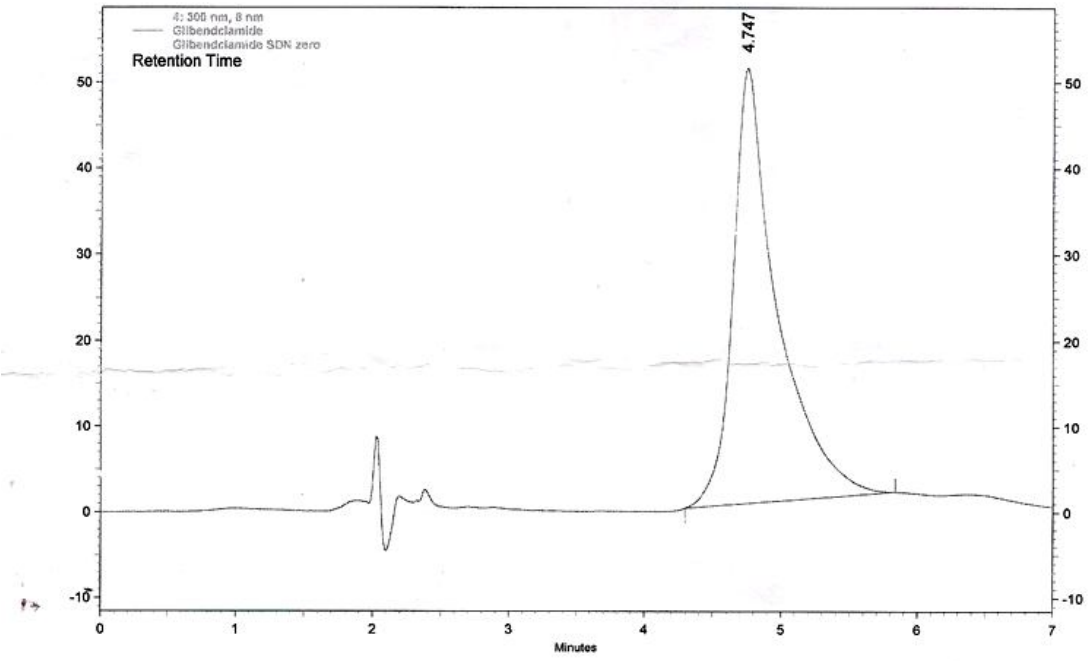



**4: 300 nm, 8 nm**

| Name | Retention Time | Area | Height |
|---|---|---|---|
| Glibendclamide | 4.725 | 1046596 | 46155 |

**Shimadzu CLASS-VP V6.14 SP1**
***Area % Report*** **Page 1 of 2**

**Method Name:** **C:\CLASS-VP\Methods\Glibenclamide.met**
**Data Name:** **C:\CLASS-VP\Glibendclamide SDN zero**
**User:** **System**
**Acquired:** **7/7/2014 2:09:13 PM**
**Printed:** **7/7/2014 2:50:23 PM**
injection volume 20

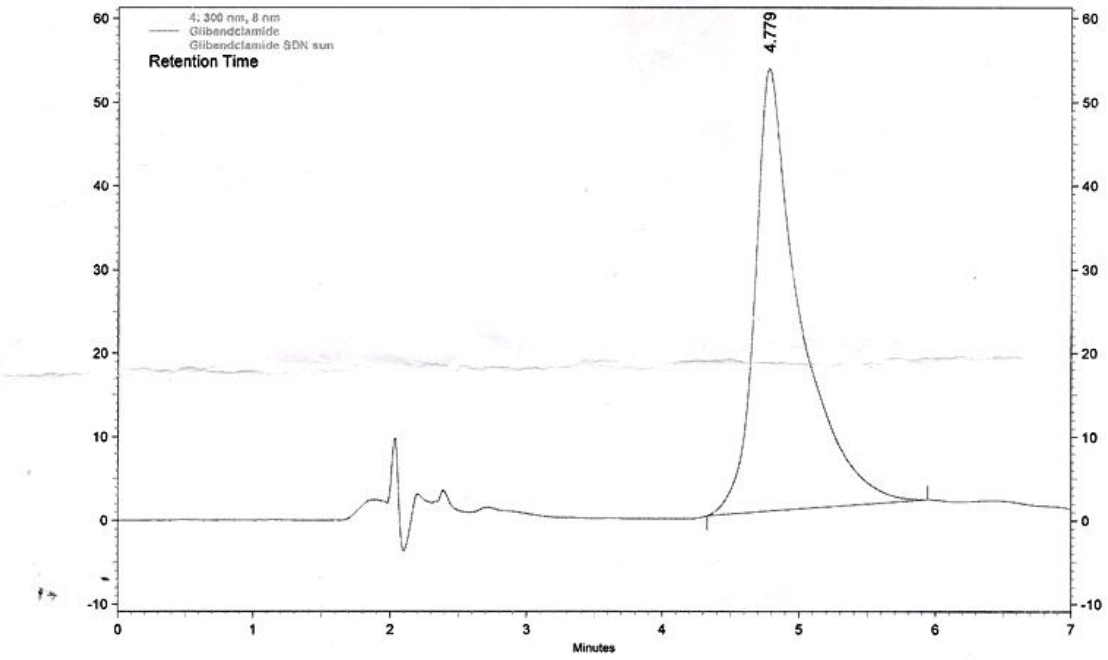



**4: 300 nm, 8 nm**

| Name | Retention Time | Area | Height |
|---|---|---|---|
| Glibendclamide | 4.747 | 1143626 | 50620 |

**Shimadzu CLASS-VP V6.14 SP1**
***Area % Report*** **Page 1 of 2**

**Method Name:** **C:\CLASS-VP\Methods\Glibenclamide.met**
**Data Name:** **C:\CLASS-VP\Glibendclamide SDN sun**
**User:** **System**
**Acquired:** **7/7/2014 2:17:56 PM**
**Printed:** **7/7/2014 2:51:48 PM**
injection volume 20




**4: 300 nm, 8 nm**

| Name | Retention Time | Area | Height |
|---|---|---|---|
| Glibendclamide | 4.779 | 1211302 | 52939 |

**(4-2) Calculation:**

Assay= read sample\read standerd *concentration of standar

Standard % = 1177091\1177091*100 = 100%

1- Sample-1(zero time) % = 814910\1177091*100 = 69.23%
2- Sample-1(sun)% = 1046596\1177091*100 = 88.9%
3- Sample-2(zero time) % = 1143626\1177091*100 = 97.16%
4- Sample-2(sun) % =1211302\1177091*100 =102.91%

- **Result of Heavy metal:**

| **Sample ID** | **Heavy metal** |
|---|---|
| Sample-1 | complies |
| Sample-2 | complies |

- **Result of sulphated ash:**

| **Sample ID** | **Ash (%)** |
|---|---|
| Sample-1 | 0.04 |
| Sample-2 | 0.051 |

- **Result of UV:**
- **At zero time:**

| | **Sample ID** | **WL300.0** |
|---|---|---|
| 1- | Standard | 0.633 |
| 2- | Sample-1 | 0.633 |
| 3- | Sample-2 | 0.632 |

**At 1 day sun light:**

| | **Sample ID** | **WL300.0** |
|---|---|---|
| 1- | Standard | 0.630 |
| 2- | Sample-1 | 0.630 |
| 3- | Sample-2 | 0.631 |

**At 3 day sun light:**

| | **SampleID** | **WL300.0** |
|---|---|---|
| 1- | Standard | 0.626 |
| 2- | Sample-1 | 0.626 |
| 3- | Sample-2 | 0.625 |

**At 5 day sun light:**

| | **SampleID** | **WL300.0** |
|---|---|---|
| 1- | Standard | 0.622 |
| 2- | Sample-1 | 0.622 |
| 3- | Sample-2 | 0.622 |

**At 1 day 50c:**

| | **SampleID** | **WL300.0** |
|---|---|---|
| 1- | Standard | 0.630 |
| 2- | Sample-1 | 0.630 |
| 3- | Sample-2 | 0.630 |

**At 3 days 50c:**

| | **SampleID** | **WL300.0** |
|---|---|---|
| 1- | Standard | 0.628 |
| 2- | Sample-1 | 0.628 |
| 3- | Sample-2 | 0.628 |

**At 5 days 50c:**

| | **SampleID** | **WL300.0** |
|---|---|---|
| 1- | Standard | 0.623 |
| 2- | Sample-1 | 0.623 |
| 3- | Sample-2 | 0.623 |

**Calculation:**

**At zero time:**

Sample-1= 0.633\0.633x100=100%

Sample-2 =0.632\0.633x100=99.8%

**Sun light:**

**At 1 day:**

Sample-1 =0.630\0.633x100=99.52%

Sample-2 = 0.631\0.633x100=99.68%

**At 3 days:**

Sample-1 =0.626\0.633x100 =98.89%

Sample-2 =0.625\0.633x100=98.73%

**At5days :**

Sample-1 =0.622\0.633x100=98.26%

Sample-2 =0.622\0.633x100=98.26%

**At 50 c:**

**1 day :**

Sample-1 =0.630\0.633x100=99.52%

Sample-2= 0.630\0.633x100=99.52%

**At 3 days:**

Sample-1=0.628\0.633x100=99.21%

Sample-2 =0.628\0.633x100=99.21%

**At 5 days:**

Sample-1=0.623\0.633x100=98.42%

Sample-2=0.623\0.633x100=98.42%

# Chapter Five
# Discussion & Conclusion

**(5-1) Discussion:**

- The percentage of zero time is 100% for sample-1 and 99.80% for sample-2
- The percentage of 1st day is 99.52%forsample-1 and 99.68%for sample - 2(sun) and 99.52% for sample-1 and sample-2(at 50c). and the percentage of 3rd day is 98.89% for sample-1 and 98.73% for sample-2(sun) and 99.21% for sample-1 and sample-2(at50c).and the percentage of 5th day is 98.26% for sample-1 and sample-2(sun) and 98.42% for sample-1 and sample-2(at50c).
- From the UV result the glibenclamide is became stable in direct sun light and 50c.

## (5-2) Conclusion:

Found through experience that glibenclmide is stable in direct sun light and temperature (50c).

**References:**

(1) British pharmacopeia 2013,.

(2) Vogels (analatical chemistry).

(3) At.wikibedia.org.