# INFLUENCE OF METHYLDOPA, BENDROFLUAZIDE AND NIFEDIPINE ON THE PHARMACOKINETICS OF METFORMIN IN TYPE 2 DIABETIC PATIENTS WITH HYPERTENSION

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## DECLARATION

I declare that the work in this Dissertation entitled; INFLUENCE OF METHYLDOPA, BENDROFLUAZIDE AND NIFEDIPINE ON THE PHARMACOKINETICS OF METFORMIN IN TYPE 2 DIABETIC PATIENTS WITH HYPERTENSIONwas carried out by me in the Department of Pharmaceutical and Medicinal Chemistry under supervision of Prof. M.Garbaof Department of Pharmaceutical and Medicinal Chemistry, Dr.(Mrs).F.S. Bello of Department of Medicine, Ahmadu Bello University, Zaria and Prof. (Mrs).M.T. Odunola of Department of Pharmaceutical and Medicinal Chemistry, University of Ilorin. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for any degree or diploma at this or any other institution.

Sani Saidu Bello ____________________ __________________

Signature Date

## CERTIFICATION

This Thesis entitled; "INFLUENCE OF METHYLDOPA, BENDROFLUAZIDE AND NIFEDIPINE ON THE PHARMACOKINETICS OF METFORMIN IN TYPE 2 DIABETIC PATIENTS WITH HYPERTENSION" by SANI SA'IDU BELLO meets the regulations governing the award of degree of the Doctor of Philosophy of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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## ABSTRACT

Drug interaction may be the result of various processes, including alterations in the pharmacokinetics of the drug, such as in the absorption, distribution, metabolism and excretion (ADME) or combination of these effects or actions. This study investigated was the effect of methyldopa, bendrofluazide and nifedipine on the pharmacokinetics and pharmacodynamics of metformin in the treatment of type 2 diabetic patients with hypertension. Metformin was administered alone and then co-administered separately with either 250mg methyldopa, 5mg of bendrofluazideor 30mg of nifedipine in the patients. HPLC was used to analyse the blood samples of the subjects using Hypersil C18 column with acetonitrile, methanol and buffer(13:7:80) as mobile phase.The mean maximum absorptionconcentration ($C_{max}$) of metformin changedslightly from 1890.67±0.11ng/ml when administered alone to 1752.17±0.232ng/ml when administered with methyldopa,which is insignificant ($P > 0.05$) and decreased to 1865.67±0.002 when administered with bendrofluazide ($P < 0.05$) but increased significantly ($P < 0.05$) to 2357.16±0.10ng/ml when co-administered with nifedipine all at maximum absorption time ($T_{max}$,) of 3 hrs. AUC $_{0\text{-}8}$ also increased from 8882.10±0.21ng/ml/h alone to 8895.30 ± 0.22ng/ml/h, ($P > 0.05$) when interacted with methyldopa, 8985.85±0.04ng/ml/h when administered with bendroflazide($P > 0.05$) and to 10724.57±0.00 ng/ml/h when interacted with nifedipine($P > 0.05$). AUC $_{0\text{-}\infty}$ also increased in all the three cases but was statistically significant ($P < 0.05$) only when co-administered with nifedipine, from 12106.87±0.01ng/ml/h to 13238.58±0.01ng/ml/h($P < 0.05$). Similarly, mean blood sugar level of all the patients significantly ($P < 0.05$) increased postprandially,which signified hyperglycemic response. There was also a decrease in blood pressure level of the patients when Metformin was co-administered with methyldopa, bendrofluazide and nifedipine.The resultscompared favorably with the work of other researchers. Nifedipine enhanced metformin concentration significantly ($P < 0.05$) while methyldopa and bendrofluazide did not when co-administered separately. Therefore, co-administration of metformin with nifedipine should be closely monitored in diabetic patients taking the two drugs.

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| | |
|---|---|
| ABUTH | AHMADU BELLO UNIVERSITY TEACHING HOSPITAL |
| ADA | AMERICAN DIABETIC ASSOCIATION |
| ADME | ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION |
| AMP | ADENOSINE MONOPHOSPHATE |
| AMPK | ADENOSINE MONOPHOSPHATE-PROTEIN KINASE |
| AUC | AREA UNDER THE CURVE |
| BP | BRITISH PHARMARCOPEA |
| CAMP | CYCLIC ADENOSINE MONOPHOSPHATE |
| CDC | AMERICAN CENTRE FOR DISEASE CONTROL |
| CV | COEFFICIENT OF VARIATION |
| GH | GESTATIONAL HYPERTENSION |
| GIT | GASTROINTESTINAL TRACT |
| GLP-1 | GLUCAGON-LIKE PEPTIDE-1 |
| GLUT | GLUCOSE UPTAKE |
| HBP | HIGH BLOOD PRESSURE |
| HDP | HYPERTENSIVE DISORDER OF PREGNANCY |
| HDS | HYPERTENSION IN DIABETES STUDY |
| HOMO | HIGHEST OCCUPIED MOLECULAR ORBITAL |
| HPLC | HIGH PERFORMANCE LIQUID CHROMATOGRAPHY |
| LDL | LOW-DENSITY LIPOPROTEIN |
| LUMO | LOWEST UNOCCUPIED MOLECULAR ORBITAL |
| MODY | MATURITY ONSET DIABETES OF THE YOUNG |
| MAO | L-MONOAMINE OXIDASES |
| NIDDM | NON-INSULIN-DEPENDENT DIABETES MELLITUS |

| | |
|---|---|
| PDA | PHOTODIODE ARRAY |
| PIH | PREGNANCY-INDUCED HYPERTENSION |
| PKA | PROTEIN KINASE A |
| SHP | STRUCTURE OF HUMAN PROTEIN |
| TBC | TOTAL BODY CLEARANCE |
| VD | VOLUME OF DISTRIBUTION |
| W.H.O | WORLD HEALTH ORGANIZATION |

## LIST OF APPENDICES

# CHAPTER ONE

## 1.0 GENERAL INTRODUCTION

Diabetes mellitus is a disorder of metabolism,theprocess through which the body utilizes food that have been broken down by digestion. Most food is broken down to glucose, which the body can use for energy and growth. Glucose travels through the bloodstream to cells throughout the body.However, glucose cannot enter the cells without the help of a hormone called insulin. The later is produced by the pancreas, a large gland beneath the stomach. In people with diabetes mellitus, the body does not have enough insulin to move the glucose into the cell. This may be because the pancreas does not produce enough insulin or because the cells do not respond to insulin, even though, plenty is produced. Either way, glucose builds up in the blood and passes out of the body in urine without ever having being used as fuel. More than 200 million people worldwide have diabetes(International Diabetes Federation, 2006).

The early common symptom of diabetes is excessive drinking of water and frequent urination.People with hypertension associated with diabetes are usually treated with antihypertensive drugs,which result in the practice of polypharmacy.Thus, the need to research into the interaction, if any, of these drugs when co-administered is pertinent.

### 1.1 Diabetes Mellitus

Type 2 Diabetes mellitus (formerly called non-insulin-dependent diabetes mellitus (NIDDM), or adult-onset diabetes), is a disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency (Robbins and Cotran,2007). While it is often initially managed by increasing exercise and dietary modification, medications are typically needed as the disease progresses. There are an estimated 23.6 million people in the U.S. **(**7.8% of the population) with diabetes with 17.9 million being diagnosed (American Diabetes

Association, 2008) 90% of whom are type 2 (Inzucchi and Sherwin,2005). With prevalence rates doubling between 1990 and 2005, American Centre for Disease Control(CDC) has characterized the increase as an epidemic (Gerberding, 2007).

Traditionally considered a disease of adults, type 2 diabetes is increasingly diagnosed in children in parallel to rising obesity rates due to alterations in dietary patterns as well as in life styles during childhood(Vahidi*et al.*, 2010). Unlike type 1 diabetes, there is very little tendency toward ketoacidosis in type 2 diabetes, though it is not unknown. One effect that can occur is non-ketonic hyperglycemia which is also quite dangerous, though it must be treated very differently. Complex and multifunctional metabolic changes very often lead to damage and function impairment of many organs, most importantly the cardiovascular system in both types. This leads to substantially increased morbidity and mortality in both type 1 and type 2 patients, but the two have quite different origins and treatments despite the similarity in complications (Steinberger *et al*., 2001).

Insulin resistance means that body cells do not respond appropriately when insulin is present. Unlike type 1 diabetes mellitus, the insulin resistance is generally "post-receptor", meaning it is a problem with the cells that respond to insulin rather than a problem with production of insulin.Other important contributing factors are; an increase in hepatic glucose production (e.g. from glycogen to glucose conversion), especially at inappropriate times (typical cause is deranged insulin levels, as those levels control this function in liver cells), a decrease in insulin-mediated glucose transport in (primarily) muscle and adipose tissues (receptor and post-receptor defects)and an impaired beta-cell function—loss of early phase of insulin release in response to hyperglycemic stimuli(Shlomo*et al*., 2007).

Type 2 diabetes mellitus is more complex problem than type 1, but is sometimes easier to treat, especially in the early years when insulin is often still being produced internally. Type 2 may go unnoticed for years before diagnosis, since symptoms are typically milder (e.g no ketoacidosis, coma, etc) and can be sporadic. However, severe complications can result from improperly managed type 2 diabetes, including renal failure, erectile dysfunction, blindness, slow healing of wounds (including surgical incisions), and arterial disease, including coronary artery disease. The onset of type 2 has been most common in middle age and later life, although it is being more frequently seen in adolescents and young adults due to an increase in child obesity and inactivity. A type of diabetes called MODY is increasingly seen in adolescents, but this is classified as a diabetic due to a specific cause and not as type 2 diabetes.Type 2 diabetes mellitus is presently of unknown etiology (origin). Diabetes mellitus with a known etiology, such as secondary to other diseases, known gene defects, trauma or surgery, or the effects of drugs, is more appropriately called secondary diabetes mellitus or diabetes due to a specific cause. Examples include diabetes mellitus such as MODY or those caused by hemochromatosis, pancreatic insufficiencies, or certain types of medications (e.g. long-term steroid use)(Lippincott and Wilkins, 2007).

According to CDC, about 23.613 million people in the United States, or approximately 8% of the population, have diabetes. The total prevalence of diabetes increased 13.5% from 2005-2007 (Zimmet*et al*., 2001).In Nigeria,Studies conducted revealed the prevalence rate of less than 1 %for diabetes in Nigeria from 1960 to 2000with most patients having non-insulin dependent (type 2) diabetes (Ogbera and Ekpebeh, 2014).About 90–95% of all North American cases of diabetes are type 2 and about 20% of the population over the age of 65 has type 2 diabetes mellitus type 2. The fraction of type 2 diabetics in other parts of the world varies substantially, almost certainly for environmental and lifestyle reasons, though these are not known in detail. Diabetes affects over 150 million people worldwide and this number is expected to double by 2025 (Zimmet*et al.*, 2001).

Type 2 diabetes mellitus type 2 is often associated with obesity, hypertension, elevated cholesterol (combined hyperlipidemia) and with the condition often termed Metabolic syndrome (it is also known as Syndrome X, Reavan's syndrome, or CHAOS). It is also associated with acromegaly, Cushing's syndrome and a number of other endocrinological disorders. Additional factors found to increase risk of type 2 diabetes include aging (Jack *et al.,* 2004), high-fat diets (Lovejoy, 2002) and a less active lifestyle (Hu,2003).

The symptoms of diabetes include; chronic fatigue, general body weakness, malaise (feeling of unease),excessive urine production,excessive thirst, increased fluid intake,blurred vision (typically from lens shape alterations, due to osmotic effects, e.g., high blood glucose levels),unexplained weight loss,lethargy anditching of external genitalia**.** The World Health Organization definition of diabetes is for a single raised glucose reading with symptoms, otherwise raised values on two occasions, of either fasting plasma glucose ≥ 7.0 mmol/l (126 mg/dl) or with a glucose tolerance test, two hours after the oral glucose dose a plasma glucose ≥ 11.1 mmol/l (200 mg/dL)(WHO, 2007).Type 2 diabetes mellitus is a chronic, progressive disease that has no established cure but does have well-established treatments which can delay or prevent entirely the inevitable consequences of the condition. Often, the disease is viewed as progressive since poor management of blood sugar leads to a myriad of steadily worsening complications. However, if blood sugar is properly maintained, then the disease is effectively cured - that is, patients are at no heightened risk for neuropathy, blindness, or any other high blood sugar complication. There are two main goals of treatment; reduction of mortality and concomitant morbidity (from assorted diabetic complications)preservation of quality of life(ADA, 2015).

The first goal can be achieved through close glycemic control (i.e., to near 'normal' blood glucose levels); the reduction in severity of diabetic side effects has been very well demonstrated in several large clinical trials and is established beyond controversy. The second goal is often addressed (in developed countries) by support and care from teams of diabetes health workers (usually physician, nurse, dietitian or a certified diabetic educator). Type 2 is initially treated by adjustments in diet and exercise, and by weight loss, most especially in obese patients. The amount of weight loss which improves the clinical picture is sometimes modest (2–5 kg or 4.4-11 lb); this is almost certainly due to currently poorly understood aspects of fat tissue activity, for instance chemical signaling (especially in visceral fat tissue in and around abdominal organs). In many cases, such initial efforts can substantially restore insulin sensitivity. In some cases strict diet can adequately control the glycemic levels *(*Ripsin, *et al.*,2009).

### 1.1.1 Relationship between diabetes mellitus and hypertension

Diabetes and hypertension tend to occur together because they share certain physiological traits –- that is, the effects caused by each disease tend to make the other disease more likely to occur. In the case of diabetes and hypertension, these effects include: increased fluid volume -- diabetes increases the total amount of fluid in the body, which tends to raise blood pressure, increased arterial stiffness -- diabetes can decrease the ability of the blood vessels to stretch, increasing average blood pressure and impaired insulin handling -- changes in the way the body produces and handles insulin can directly cause increases in blood pressure. Though these common biological traits partially explain why diabetes and hypertension is such a common pair, in many cases, the two diseases are likely to occur together simply because they share a common set of risk factors. Some important shared risk factors are: Body Mass -- Being overweight significantly increases the risk of both diabetes and hypertension, Diet-- High fat diets rich in salt and processed sugars are known to contribute to the development of organ problems that can lead to both diabetes and hypertension and Activity Level -- A low level of physical activity makes insulin less effective (which can lead to diabetes) and can contribute to the development of stiff blood vessels, increasing the risk of hypertension. The preventive strategies for both high blood pressure and diabetes usually focus on these specific risk factors (HDS, 1993).

### 1.1.2 Prevalence of hypertension in people with diabetes mellitus

Data from one large, widely referenced study on type 1 diabetes showed that 5% of patients have high blood pressure within 10 years, 33% have high blood pressure within 20 years and 70% have high blood pressure by age 40. In studies of type 2 diabetes, data has shown that almost 75% of patients with kidney problems (a common complication) had high blood pressure. In those with type 2 diabetics but no kidney problems, the rate of high blood pressure was about 40%. Overall, when averaged across diabetes type 2 and age range, about 35% of all people with diabetes have high blood pressure (Sowers*et al.,* 2001).

## 1.2 Justification of the Study

One of the complications of diabetes mellitus is hypertension. The record of type 2 diabetes patients with hypertension attending diabetic clinic of Ahmadu Bello University Teaching hospital, Zaria (ABUTH) indicated 63% co-administration of metformin with antihypertensive drugs (methyldopa, bendrofluazide, nifedipine, etc) for a year review. Though the data is unpublished, it is not likely to be different from other Teaching Hospitals. This co-administration of drugs would likely leads to drug-drug interactions due to their metabolic link. These likely interactions of metformin with these antihypertensive drugs were not investigated. Therefore, the research is justified to investigate the clinical implications of the interactions.

## 1.3 Aim and Objective of the Research

The aim of the research is to determine the influence of co-administration of methyldopa, bendrofluazide or nifedipine on metformin pharmacokinetics and pharmacodynamics.

The aim will be accomplished through the following objectives:

1. To carry out Quality Control analysis of metformin, methyldopa, bendrofluazide and nifedipine
2. To determine concentration of metformin alone and when co-administered with methyldopa,bendrofluazide and nifedipine in plasma of the subjects using HPLC
3. To measure the blood sugar level of both the healthy and patients subjects
4. To determine the pharmacokinetic parameters of metformin

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

## 2.1 Different Methods of Pharmaceutical Analysis

### 2.1.1 Ultraviolet method of analysis

Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbital(Skoog, 2007). The more easily excited the electrons (i.e. lower energy gap between the HOMO and the LUMO), the longer the wavelength of light it can absorb. UV/Vis spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugatedorganic compounds, and biological macromolecules. UV Spectroscopic analysis is commonly carried out in solutions but solids and gases may also be studied. The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus for a fixed path length, UV/Vis spectroscopy can be used to determine the concentration of the sample in a solution (Skoog, *et al*., 2007).

The wavelengths of absorption peaks can be correlated with the types of bonds in a given molecule and are valuable in determining the functional groups within a molecule. The Woodward-Fieser rules, for instance, are a set of empirical observations used to predict $\lambda_{max}$, the wavelength of the most intense UV/Vis absorption, for conjugated organic compounds such as dienes and ketones. The spectrum alone is not, however, a specific test for any given sample. The nature of the solvent, the pH of the solution, temperature, high electrolyte concentrations, and the presence of interfering substances can influence the absorption spectrum. Experimental variations such as the slit width (effective bandwidth) of the spectrophotometer will also alter the spectrum. To apply UV/Vis spectroscopy to analysis, these variables must be controlled or accounted for in order to identify the substances present.The Beer-Lambert law has implicit assumptions that must be met experimentally for it to apply otherwise there is a possibility of deviations from the law to be observed.For instance, the chemical makeup and physical environment of the sample can alter its extinction coefficient. The chemical and physical conditions of a test sample therefore must match reference measurements for conclusions to be valid (MisraandDubinskii, 2002).

### 2.1.2 Fluorescence

Fluorescence spectroscopy,fluorometry or spectrofluorometry is a type of electromagnetic spectroscopy which analyzes fluorescence from a sample. It involves using a beam of light, usually ultraviolet light, that excites the electrons in molecules of certain compounds and causes them to emit light; typically, but not necessarily, visible light. A complementary technique is absorption spectroscopy. Devices that measure fluorescence are called fluorometers orfluorimeters. Molecules have various states referred to as energy levels. Fluorescence spectroscopy is primarily concerned with electronic and vibration states. Generally, the species being examined has a ground electronic state (a low energy state) of interest, and an excited electronic state of higher energy. Within each of these electronic states are various vibration states.In fluorescence spectroscopy, the species is first excited, by absorbing a photon, from its ground electronic state to one of the various vibration states in the excited electronic state. Collisions with other molecules cause the excited molecule to lose vibration energy until it reaches the lowest vibration state of the excited electronic state. The molecule then drops down to one of the various vibration levels of the ground electronic state again, emitting a photon in the process. As molecules may drop down into any of several vibration levels in the ground state, the emitted photons will have different energies, and thus frequencies. Therefore, by analyzing the different frequencies of light emitted in fluorescent spectroscopy, along with their relative intensities, the structure of the different vibration levels of molecules can be determined (James *et al.*, 2006).

Unlike in UV/visible spectroscopy, 'standard', device independent spectra are not easily attained. Several factors influence and distort the spectra, and corrections are necessary to attain 'true', i.e. machine-independent, spectra. Furthermore, no lamp has a constant intensity at all wavelengths. Additionally, the transmission efficiency of monochromators and filters must be taken into account. These may also change over time. The transmission efficiency of the monochromator also varies depending on wavelength. This is the reason that an optional reference detector should be placed after the excitation monochromator or filter. The percentage of the fluorescence picked up by the detector is also dependent upon the system. Furthermore, the detector quantum efficiency, that is, the percentage of photons detected, varies between different detectors, with wavelength and with time, as the detector inevitably deteriorates (Eisinger and Flores, 1979).

### 2.1.3 High performance liquid chromatography (HPLC)

High-performance liquid chromatography (formerly referred to as high-pressure liquid chromatography), HPLC, is a chromatographic technique 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 and a sample mixture through a column filled with a sorbent, leading to the separation of the sample components. The HPLC is considered an instrumental technique of analytical chemistry. In general, the method involves a liquid sample being passed over a solid adsorbent material packed into a column using a flow of liquid solvent. Each analyte in the sample interacts slightly differently with the adsorbent material, thus retarding the flow of the analytes. If the interaction is weak, the analytes flow off the column in a short amount of time, and if the interaction is strong, then the elution time is long. HPLC has been used in for medicine (e.g. detecting vitamin D levels in blood serum), legal (e.g. detecting performance enhancement drugs in urine), research (e.g. separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and manufacturing (e.g. during the production process of pharmaceutical and biological products) purposes (Lindsay andKealey, 1987).

The major components of HPLCas it relate to its application 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. Adigitalmicroprocessor and user software control the HPLC instrument and provide data analysis. Some models of mechanical pumps in HPLC instrument can mix multiple solvents together in ratios changing in time, generating a compositiongradient in the mobile phase. Various detectors are in common use, such as UV/Vis photodiode array (PDA) or based on mass. Most HPLC instruments also have a column oven that allows for adjusting the temperature the separation is performed at (Ahuja and Dong, 2005).

Sample mixture to be separated and analyzed is introduced, in a discrete small volume (typically micro liters), into the stream of mobile phase percolating through the column. The components of the sample move through the column at different velocities, which are function of specific physical interactions with the sorbent (also called stationary phase). The velocity of each component depends on its chemical nature, on the nature of the stationary phase (column) and on the composition of the mobile phase. The time at which a specific analyte elutes (emerges from the column) is called its retention time. The retention time measured under particular conditions is considered an identifying characteristic of a given analyte.Many different types of columns are available, filled with sorbents varying in particle size, and in the nature of their surface ("surface chemistry"). The use of smaller particle size packing materials requires the use of higher operational pressure and typically improves chromatographic resolution (i.e. the degree of separation between consecutive analytes emerging from the column). In terms of surface chemistry, sorbent particles may be hydrophobic or polar in nature.Common mobile phases used include any miscible combination of water with various organic solvents (the most common are acetonitrile and methanol). Some HPLC techniques use water-free mobile phases (Normal-phase chromatography). The aqueous component of the mobile phase may contain acids (such as formic, phosphoric or trifluoroacetic acid) or salts to assist in the separation of the sample components. The composition of the mobile phase may be kept constant (isocratic elution mode) or varied (gradient elution mode) during the chromatographic analysis. Isocratic elution is typically effective in the separation of sample components that are not very different in their affinity for the stationary phase. In gradient elution the composition of the mobile phase is varied typically from low to high eluting strength. The eluting strength of the mobile phase is reflected by analyte retention times with high eluting strength producing fast elution (short retention times). A typical gradient profile in reversed phase chromatography might start at 5% acetonitrile (in water or aqueous buffer) and progress linearly to 95% acetonitrile over 5–25 minutes. Periods of constant mobile phase composition may be part of any gradient profile, for example, the mobile phase composition may be kept constant at 5% acetonitrile for 1–3 min, followed by a linear change up to 95% acetonitrile.The chosen composition of the mobile phase (also called eluent) depends on the intensity of interactions between various sample components (analytes) and stationary phase (e.g. hydrophobic interactions in reversed-phase HPLC). Depending on their affinity for the stationary and mobile phases, analytes partition between the two during the separation process taking place in the column. This partitioning process is similar to that which occurs during a liquid–liquid extraction but is continuous, not step-wise. In this example, using a water/acetonitrile gradient, more hydrophobic components will elute (come off the column) late, once the mobile phase gets more concentrated in acetonitrile (i.e. in a mobile phase of higher eluting strength) (Ahuja and Dong, 2005).

The choice of mobile phase components, additives (such as salts or acids) and gradient conditions depends on the nature of the column and sample components. Often a series of trial runs is performed with the sample in order to find the HPLC method which gives adequate separation (Ahuja and Dong, 2005)

## 2.2 Pharmacokinetics Parameters

Some of the useful pharmacokinetic parameter obtained after drug administration include; Area under the curve (AUC), Volume of distribution (Vd), Elimination half-life ($t_{½}$ β), absorption half-life ($t_{½}$ α), systemic and total body clearance (TBC). Others are lag time, $C_{max}$ and $T_{max}$ (Paxton, 1981).

### 2.2.1 Volume of distribution

The volume of distribution Vd, also known as the apparent volume of distribution, is a calculated theoretical value. It is used clinically when trying to determine the loading dose necessary for a desired blood concentration of a drug, and is also used for estimating blood concentrations in the treatment of overdose. It can also be defined as volumes of plasma that would be necessary to account for the total amount of drug in the patient's body, if that drug were present throughout the body at the same concentration as found in the plasma. This value is usually further divided by the patient's body weight, and the result expressed in terms of liter per kilogram.

Volume of distribution corresponds to the ratio of an amount (A) of drug in the body at a given time ($A_t$) and plasma concentration (blood) at that time.

$$V_d = \frac{amount\ \ of\ drug\ \ in\ the\ body\ \ at\ time\ (A_t)}{Concentration\ \ \ at\ time\ \ (A_t)}$$

Thus volume does not necessary refer to an identifiable physiological volume, but merely to the blood volume that would be required to contain the entire drug in the body at the same concentration as in blood plasma (Shah, 1993). Volume of distribution ($V_d$) can exceed any volume in the body because the volume of distribution is the volume necessary to contain the amount of drug homogeneously at the concentration found in the blood plasma or plasma water (Wilkinson, 2001).

Drug with very high volumes of distribution have much higher concentration in intravascular tissue than in the vascular compartment. Similarly, drugs that are completely retained within the vascular distribution equal to the blood component in which they are distributed (Wilkinson and Rawlins, 1985).

### 2.2.2 Compartment models

Compartment models are often used to describe transport of material in chronological systems. Acompartment would contain a number of sub-compartments, each containing well mixed material (compartments exchange material with each other following certain rules). A compartment is an entity, which can be described by a definite volume and a concentration (of a drug contained in that volume) (Wilkinson and Rawlins, 1985).

The behavior of a drug in biological system can be described by a one-compartment model or two compartment model. Human body is a multi-compartment model as a whole considering drug concentration in different organelles, cells or tissues. Blood plasma and urine are the only two body fluids in a living body. Therefore, in pharmacokinetics, two compartment models are used for a lot of drugs; blood level versus time curves or urinary cumulative excretion verses time curve models. Two or more compartments can be linked together because a drug may move back from one compartment into another. The movement occurs at different rates (speeds).

Open one compartment model: this model results if the drug entering the body (inputs) does not instantly distributes (equilibrate) between the blood and other body fluids or tissues. In this model, the drug is not necessarily confined to the circulatory system. The drug may occupy the entire extracellular fluid, soft tissue or the entire body. However, distribution occurs instantly and is not pooled in a specific area.

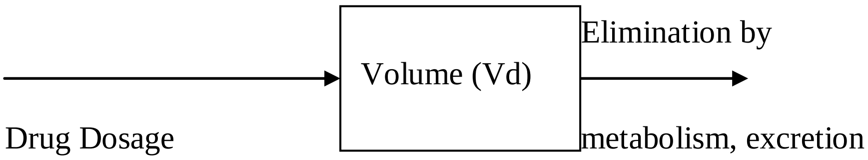


Figure 2.1: Schematic diagram of one compartment model

**Two compartment model;** Many drugs will exhibit multi-compartment pharmacokinetics (Rowland and Tozer, 1995) where more than one half-life term may apply to a single drug (Holford and Benet, 1995) and the "true" terminal half-life will be greater than that calculated from the equation $t½ = \frac{0.693\ Vd}{CL}$ (Wilkinson, 2001). In two compartment model, the central compartment composed of the blood and highly perfuse thin tissue (heart, lung, liver and brain), while the peripheral compartment composed of slowly perfuse tissue (muscle, skin, fat and bone).This model has had extensive use because it is the most tractable to parameter resolution (Gwilt, 1990); it also describes the appeal pharmacokinetics of memberous drugs and allows characterization of drug disposition in the absence of more specific information negating appropriate model.Primary parameters of two compartment model can be calculated from the same method of one compartment model provided that clearance occurs from the central compartment (Prescott, 1981).

### 2.2.3 Absorption plasma half-life

It is the time taken for the plasma concentration to reach half concentration of the drugs administered. Half-life is a parameter that changes as a function of both clearance and volume of distribution,clinically; it is given by the following relationship.

$$t_{½} = \frac{0.693\, Vd}{Cl}$$

Where: Vd = volume of distribution at steady state

Cl = clearance

This means that as the clearance decreases due to disease process, $t_{½}$would increase provided the disease does not change the volume of distribution.

In practical terms, half-life is the time taken for plasma concentration to reach half concentration of the drug administered (Prescott, 1981). Thus;

$$t_{½\ abs} = \frac{0.693}{Ka}$$

Where: Ka = absorption rate constant

For instance, the half-life of tolbutamide decreases in patients with acute viral hepatitis, this is the opposite of what one might expect (William, 2007).

### 2.2.4 Elimination (plasma) half-life

A drug half-life is the amount of time that it takes for half of the drug to be eliminated from the body. In a medical context, half-life may also describe the time it takes for the blood plasma concentration of a substance to have (plasma half-life) its steady state is a useful parameter in the determination of suitable dosage interval and the time required to attain steady state during intravenous or chronic oval therapy (Paxton 1981).

### 2.2.5 Area under the curve (AUC)

This is the integral of drug blood level over time from zero to infinity and is measure of thequantity of drugs absorbed (bioavailability) in the body. The various methods of calculating AUC include planimetry, cut and weight trapezoidal rule and computer application. Area under curve (AUC) can be calculated by

$$AUC = \frac{FxD}{Vd}$$

Where: F = Bioavailability

D= Dose

Vd = volume of distribution

## 2.3 Drug Interaction

A drug interaction is a situation in which a substance (usually drug) affects the activity of a drug when both are administered together. This action can be synergistic (when the drug effect is increased) or antagonistic (when the drug effect is decreased) or a new effect altogether. However, interactions may also exist between drugs and foods (drug-food interactions) as well as drugs and medical plant or herbs (drug-plant interaction) (*National Prescribing Service*, 2009).

If a patient is taking two drugs and one of them increases the effect of the other it is possible that an overdose may occur or interaction may increase the risk that side effects will occur. Similarly, if the action of a drug is reduced it may cease to have any therapeutic use because of under dosage (Baños*et al*. 2002).

Drug interaction may be the result of various processes, including alterations in the pharmacokinetics of the drug, such as in the absorption, distribution, metabolism and excretion (ADME) of a drug. Alternatively, drug interactions may be the result of the pharmacodynamic properties of the drug. Interaction may be due the combination of these effects or actions. In certain cases, the presence of a drug in an individual's blood may affect certain types of laboratory analysis (analytical interference). By studying the conditions that favor the appearance of interactions it would be possible to prevent them or at least diagnosed them in time. The factors or conditions that pre-dispose or favor the appearance of interactions include:

1. Old age: liver metabolism, kidney function, nerve transmission or functioning of bone marrow all decrease with age (Merle *et al.*,2005)

2. Polypharmacy: The more drugs a patient takes the more likely it will be that some of them will interact (García-Morillo, 2007)

3. Genetic factors: Genes synthesize enzymes that metabolize drugs. Some races have genotypic variation that could decrease or increase the activity of these enzymes. This is seen in genotype variation in the isozymes of cytochrome P450 (García-Morillo, 2007).

4. Hepatic/Renal diseases: The blood concentrations of drugs that are metabolized in the liver and or eliminated by kidneys may be altered if either these organs is not functioning correctly (García-Morillo, 2007).

5. Serious disease that could worsen if the dose of the medicine is reduced.
6. Drug dependent factors (Castells *et al.*,2007).

### 2.3.1 Mechanism of drug absorption interaction

Some possible mechanism of drug absorption interactions are due to pH effect on dissolution and ionization, changes in gastric emptying, GIT motility, formation of complex, interference with active transport and change in portal blood flow. For example, drugs that induce changes in pH of gastro intestinal fluids may have complex and unpredictable effects on the absorption of other drugs taken at the same time. According to the pH-partition theory, weak organic acids are largely absorbed from the stomach where as weak bases are absorbed best from the more alkaline contents of the upper small intestine. It was reported that the absorption of weak acid is reduced if they are given with alkali since less drug would be present in the un-ionized lipid soluble diffusible state.

Drugs may interact in the gastro-intestinal track to form complex, ion pairs and chelate which may be absorbed more rapidly or move slowly than the parent drugs. The absorption of tetracycline is inhibited by the formation of insoluble chelates with metals such as calcium and iron. Dicoumarol absorption is increased by the formation of a more soluble chelate with magnesium hydroxide. Similarly the absorption of a quaternary ammonium antiarrhythmic agent enhanced by ion-pair of drugs may also be reduced by adsorption into kaolin or charcoal. Changes in gastric emptying and gastro intestinal motility in man have significant effects on the rate of absorption of other drugs. Many other therapeutic agents that influence gastro intestinal motility and gastric emptying could cause absorption interaction through a similar mechanism (Tatro, 2004).

### 2.3.2 Drug distribution

Competition between different drugs for the same binding site on plasma protein (albumin) may have important consequences, if one of the drugs has high fractional binding at therapeutic plasma level. This action is more with highly bounded drugs then those which are less bounded. The affinity of plasma protein for one drug may be modified by the binding of another as in the case of aspirin and serum (Ahuja and Dong, 2005).

### 2.3.3 Drug absorption interaction

Some drugs, such as the prokinetic agents increase the speed with which a substance passes through the intestines. If a drug is present in the digestive tract absorption zone for less time, its blood concentration will decrease. The opposite will occur with drugs that decrease intestinal motility (Avdeef, 2003).Drugs can be present in either ionized or non-ionised form, depending on their pKa (pH at which the drug reaches equilibrium between its ionised and non-ionised form). The non-ionized forms of drugs are usually easier to be absorbed because they will not be repelled by the lipidonthelayer of the cell, most of them can be absorbed by passive diffusion, unless they are too big or too polarized (like glucose or vancomicyn), in which case they may have, or not, specific and non-specific transporters distributed on the entire intestine internal surface, that carries drugs inside the body. Obviously increasing the absorption of a drug will increase its bioavailability, so, changing the drugs between ionized or non-ionised stares. This can be useful or not for certain drugs. Drug solubility: The absorption of some drugs can be drastically reduced if they are administered together with food with a high fat content. This is the case for oral anticoagulants and avocado (Clark and Smith, 1986).

Formation of non-absorbable complexes**;** Chelation: The presence of di-or trivalent cations can cause the chelation of certain drugs, making them harder to absorb. This interaction frequently occurs between drugs such as tetracycline or the fluoroquinolones and dairy products (due to the presence of $Ca^{++}$) (Clark and Smith, 1986).

Binding with protein: Some drugs such assucralfate, bind to proteins, especially if they have a high bioavailability for this reason, its administration is contraindicated with food.

Finally, another possibility is that the drug is retained in the intestinal lumen forming large complexes that impede its absorption. This can occur with cholestyramine if it is associated with sulfamethoxazol, thyroxine, warfarin or digoxin (Clark and Smith, 1986).

Action of P-glycoprotein on the enterocytes appears to be one of the mechanisms promoted by the consumption of grapefruit juice in the case of an increase in bioavailability of various drugs, regardless of its demonstrated inhibitory activity on first pass metabolism (Clark and Smith, 1986).

### 2.3.4 Drug metabolism interactions

Many drug interactions are due to alterations in drug metabolism. Further, human drug-metabolizing enzymes are typically activated through the engagement of nuclear receptors. One notable system involved in metabolic drug interaction is the enzyme system comprising the cytochrome P450 oxidases (Danielson, 2002).

### 2.3.5 Drug-receptor interaction

The binding forces that hold a drug in combination with its receptor arise from the numerous bonds existing in the body system including, covalent bond, Vander Waals etc. Conformation changes in the drug-receptor combination also cause changes in the effect of drug. Variation in the drug molecule causes marked charges in its potency (Danielson, 2002).

### 2.3.6 Pharmacodynamic interactions

The change in an organism's response on administration of a drug is an important factor in pharmacodynamic interactions. These changes are extraordinarily difficult to classify given the wide variety of modes of action that exist and the fact that many drugs can cause their effect through a number of different mechanisms. This wide diversity also means that, in all but the most obvious cases, it is important to investigate and understand these mechanisms. The well-founded suspicion exists that there are more unknown interactions than known ones.

### 2.3.7 System/plasma clearance

Clearance may be defined with respect to blood, plasmadepending on the concentration measured (Wilkinson, 2001).Elimination of drug from the body may involve process of occupancy in the kidney, the lung, the liver and other organs (Wilkinson and Rawlins, 1985) and dividing the rate of elimination at each organ by the concentration of drug presented to it yield the corresponding clearance at that organ and when added together, these separate clearances equal total systemic clearance as;

Cl systemic = Cl renal + Cl liver + Cl other. For most drugs, clearance is constant over plasma or blood concentration range encountered in clinical settings, that is, elimination is not saturated. The rate of drug elimination is directly proportional to the concentration (Holford and Benet, 1995). Likewise, if clearance is first order, it can be measured by calculating the area under the curve (AUC) of the time concentration curve after a dose. Therefore, clearance is given by the equation; cl = $\frac{Dose}{AUC}$

The concept of clearance is very useful in clinical pharmacokinetics, because its value for a particular drug usually is constant over the range of concentrations encountered clinically (Paxton, 1981).Clinician usually wants to maintain steady concentrations of drug within a therapeutic window associated with therapeutic efficacy and a minimum toxicity (Wilkinson 2001). However, clearance does not indicate how much drug is being removed but rather the volume of biological fluid as blood or plasma from which drug would have to be completely removed to account or the elimination (Gibaldi and Prescott, 1983).

## 2.4 Drugs Used in the Study

The drugs used in the study are metformin tablets, methyldopa, bendrofluazide andnifedipine

### 2.4.1 Metformin 500 mg

One of the most widely used drugs for the treatment of type 2 diabetes is the biguanide, metformin. It works primarily by reducing liver release of blood glucose from glycogen stores and secondarily by provoking some increase in cellular uptake of glucose in body tissues (Baileyand Turner, 1996).

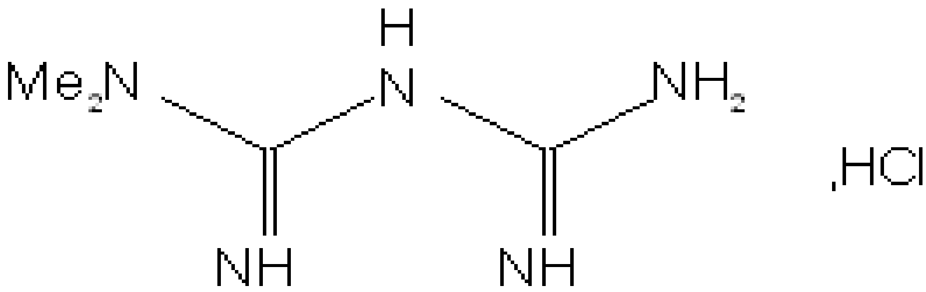


Figure 2.2: Structure of metformin

A systematic review of randomized controlled trials found that metformin and second-generation sulfonylureas are the preferred choices for most patients with type 2 diabetesmellitus, especially at early in the course of the disease (Bolen, 2007)

For patients who also have heart failure, metformin may be the best tolerated drug (Eurich*et al.*, 2007).

The variety of available drug agents and the clinical differences among type 2diabetic patientscan be confusing. At present, choice of drugs for type 2 diabetics is rarely straightforward and in most instances has elements of repeated trial and adjustment (Bailey and Turner, 1996)

#### *2.4.1.1 Therapeutic effect of metformin*

The main use of metformin is in the treatment of diabetes mellitus type 2, especially when this is accompanied by obesity and insulin resistance (Clinical Guidelines Task Force, 2005: National Collaborating Centre for Chronic Conditions, 2008 and American Diabetes Association, 2009). Metfomin is the only anti-diabetic drug that has been proven to protect against the cardiovascular complications of diabetes (International Diabetes Federation, 2005).

Unlike the other most-commonly prescribed class of oral diabetic drugs, the sulfonylureas, metformin (taken alone) does not induce hypoglycemia. Metformin also modestly reduces LDL and triglyceride levels (WHO,2010).

#### *2.4.1.2 Pharmacokinetics of metformin*

Metformin has an oral bioavailability of 50 – 60% under fasting conditions, and is absorbed slowly(Bristol-Myers,2008 and Garry *et al.*,2011). Peak plasma concentrations (C) are reached within one to three hours of taking immediate-release metformin and four to eight hours with extended-release formulations. The plasma protein binding of metformin is negligible, as reflected by its very high apparent volume of distribution (300-1000 L after a single does) and steady state is usually reached in one or two days (Bristol-Myers, 2008).

Metformin is not metabolized and it is cleared from the body by tubular secretion and excreted unchanged in the urine; metformin is undetectable in blood plasma after 24 hours of a single oral does(Robert *et al.,* 2003 and Bristol-Myers,2008). The average elimination half-life in plasma is 6.2 hours. Metformin is distributed to (and appears to accumulate in) red blood cells, with a much longer elimination half-life: 17.6 hours (reported as ranging from 18.5 to 31.5 hours in a single-does study of non-diabetic people (Bristol-Myers,2008).

*2.4.1.3 Mechanism of action of metformin*

Metformin decreases hyperglycemia primarily by suppressing glucose production by the liver (hepatic gluconeogenesis) (Shu*et al.,*2005). The "average" person with type 2 diabetes has three times the normal rate of gluconeogenesis; metformin treatment reduces this by over one third(Jayasagar*et al.*,2002).The molecular mechanism of metformin is incompletely understood: inhibition of the mitochondrial respiratory chain, activation of AMP-activated protein kinase (AMPK), inhibition of glucagon-induced elevation of cyclic adenosine monophosphate (cAMP) and consequent activation of protein kinase A (PKA), and an effect on gut microbiota have been proposed as potential mechanisms**(**Hundal*et al*., 2000 and Towler andHardie, 2007). A study in 2001 suggested that activation of AMPK, an enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats, **(** Zhou*et al.,*2001) was required for metformin's inhibitory effect on the production of glucose by liver cells (Kim *et al.,* 2008). Research published in 2008 further showed that activation of AMPK was required for an increase in the expression of SHP, which in turn inhibited the expression of the hepatic gluconeogenic genesPEPCK and Glc-6-Pase**(**Zhang *et al.,*2007). Metformin is frequently used in research along with AICAR as an AMPK agonist. More recent studies using mouse models in which the genes for AMPKα1 and α2 catalytic subunits (Prkaa1/2) or LKB1, an upstream kinase of AMPK, had been knocked out in hepatocytes have raised doubts over the obligatory role of AMPK, since the effect of metformin was not abolished by loss of AMPK function**(**Hundal*et al.,* 2000). The mechanism by which biguanides increase the activity of AMPK remains uncertain; however, research suggests that metformin increases the concentration of cytosolic AMP (as opposed to a change in total AMP or total AMP/ATP**(**Miller *et al.,*2013). Increased cellular AMP has also been proposed to explain the inhibition of glucagon-induced increase in AMP and activation of PKA (Hundal*et al.,* 2000).

Metformin and other biguanides may antagonize the action of glucagon, thus reducing fasting glucose levels (Collier *et al.,* 2006). Metformin also induces a profound shift in the faecal microbial community profile in diabetic mice and it has also been proposed that this may contribute to its mode of action possibly through an effect on Glucagon-like peptide-1(GLP-1) secretion (Towler and Hardie, 2007).

In addition to suppressing hepatic glucose production, metformin increases insulin sensitivity, enhances peripheral glucose uptake (by inducing the phosphorylation of GLUT4 enhancer factor), decreases insulin-induced suppression of fatty acid oxidation(Bailey and Turner,1996) and decreases absorption of glucose from the gastrointestinal tract. Increased peripheral utilization of glucose may be due to improved insulin binding to insulin receptors (Fantus and Brosseau, 1986). The increase in insulin binding after metformin treatment has also been demonstrated in patients with NIDDM (Musi*et al.*, 2002). AMPK probably also plays a role, as metformin administration increases AMPK activity in skeletal muscleAMPK is known to cause GLUT4 deployment to the plasma membrane, resulting in insulin-independent glucose uptake. Some metabolic actions of metformin do appear to occur by AMPK-independent mechanisms; a 2008 study found "the metabolic actions of metformin in the heart muscle can occur independent of changes in AMPK activity and may be mediated by p38 MAPK- and PKC-dependent mechanism(Saeedi *et al.,2008).*

### 2.4.2 Methyldopa

Methyldopa (L-α-Methyl-3,4-dihydroxypenylalanine) is an antihypertensive drug. Its use is now depreciated following introduction of alternative safer classes of agents. However, it continues to have a role in otherwise difficult to treat hypertension and gestational hypertension also knows as pregnancy-induced hypertension (PIH) (Reynolds, 1989).

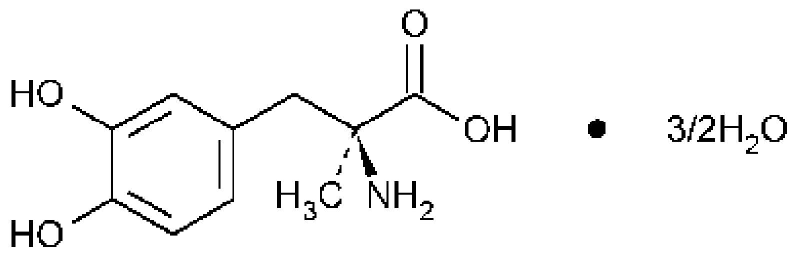

Figure2.3: Structure of Methyldopa

*2.4.2.1 Therapeutic effect of methyldopa*

Methyldopa is used in the clinical treatment of the following disorders:High blood pressure (HBP) or hypertension (HTN), Precelampsia and eclampsia or hypertensive disorder of pregnancy (HDP),Gestational hypertension (GH) or pregnancy – induced hypertension (PH) (American Medical Association, 1988).

*2.4.2.2 Side effects of methyldopa*

Methyldopa is capable of inducing a number of adverse, side effects, which range from mild to severe. Nevertheless, they are generally mild when the dose is less than 1 gram per day. Side effects may include: Nightmares,Variant, Wakefulness, Restlessness and Impairment of vision, Dizziness, lightheadedness, Dry mouth, Diarrhea and Migraine (Barnhart, 1987)

*2.4.2.3 Mechanism of action methyldopa*

Methyldopa has a dual mechanism of action (Winter, 2003). The first mechanism involved is a competitive inhibitor of the enzyme DOPA decarboxylase, also known as aromatic L-amino acid decarboxylase, which converts L-DOPA into dopamine. Dopamine is a precursor for norepinephrine (noradrenaline) and subsequentlyepinephrine (adrenaline). This inhibition results in reduced dopaminergic and adrenergic neurotransmission in theperipheral nervous system. This effect may lower blood pressure and cause central nervous system effects such asdepression, anxiety, apathy, anhedonia, and parkinsonism. Similarly, the second mechanism is convert methyldopa to α-methylnorepinephrine by dopamine beta-hydroxylase. Activation of these receptors in the brainstem appears to inhibit sympathetic nervous system output and lower blood pressure.

### 2.4.3 Bendrofluazide

It is chemically a 3-benzyl-3,4-dihydro-6-(trifluoromethyl)-2*H*-1,2,4-benzothiadiazine-7sulphonamide 1,1-dioxide with Molecular formula - $C_{15}H_{14}F_3N_3O_4S_2$ and Molecular weight 421.4.

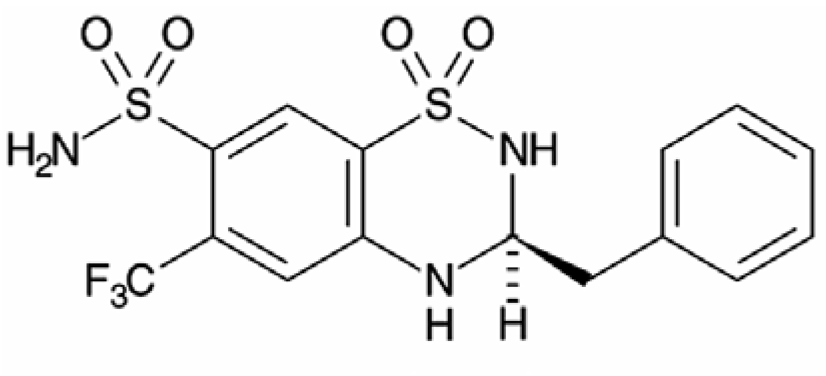


Figure 2.4: Structure of bendrofluazide

*2.4.3.1 Therapeutic effects of bendrofluazide*

It is use for the treatment of oedema and hypertension. Bendroflumethiazide tablets may alsobe used to suppress lactation.Bendroflumethiazide is a thiazide diuretic which reduces the absorption ofelectrolytes from the renal tubules, thereby increasing the excretion of sodium and chloride ions, and consequently of water. The excretion of other electrolytes, notablypotassium and magnesium, is also increased.The excretion of calcium is reduced. Thiazides also reduce carbonic anhydraseactivity so that bicarbonates excretion is increased, but this effect is generally smalland does not appreciably alter the acid base balance or the pH of the urine. Thiazidesalso have a hypotensive effect, due to a reduction in peripheral resistance and enhancethe effects of other antihypertensive agents**(**Brayfield, 2014).

*2.4.3.2 Adverse effects of bendrofluazide*

Bendrofluazide has as its adverse effects on the following systems;blood and lymphatic disorders, immune disorders, metabolism and nutrition disorders, cardiac and vascular disorders, respiratory, thoracic and intestinal disorders, gastrointestinal effects, skin and subcutaneous tissue disorderand reproductive disorders(Sydney *et al*., 1986).

*2.4.3.3 Pharmacokinetics of bendrofluazide*

Bendrofluazide is completely absorbed from the gastrointestinal tract and it isfairly extensively metabolized. About 30% is excreted unchanged in the urine. Theonset of diuretic action of the thiazides following oral administration occurs withintwo hours and the peak effect between three and six hours after administration. Theduration of the diuretic action of bendrofluazide is between 18 and 24 hours.The onset of the hypertensive action is generally three or four days (Beermann, *et al*., 1978).

*2.4.3.4 Mechanism of action of bendrofluazide*

As a diuretic, bendrofluazide inhibits active chloride re-absorption at the early distal tubule via the Na-Cl co-transporter, resulting in an increase in the excretion of sodium, chloride, and water. Thiazides like bendrofluazide also inhibit sodium ion transport across the renal tubular epithelium through binding to the thiazide sensitive sodium-chloride transporter. This results in an increase in potassium excretion via the sodium-potassium exchange mechanism. The antihypertensive mechanism of bendrofluazide is less well understood although it may be mediated through its action on carbonic anhydrases in the smooth muscle or through its action on the large-conductance calcium-activated potassium (KCa) channel, also found in the smooth muscle (Jättelä, 1979).

### 2.4.4 Nifedipine

Nifedipine is a yellow crystalline substance, practically insoluble in water but soluble in ethanol. Nifedipine has been formulated as both a long- and short-acting 1,4-dihydropyridine calcium channel blocker. It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. By inhibiting the influx of calcium in smooth muscle cells, nifedipine prevents calcium-dependent myocyte contraction and vasoconstriction. A second proposed mechanism for the drug's vasodilatory effects involves pH-dependent inhibition of calcium influx via inhibition of smooth muscle carbonic anhydrase (Haft and Litterer, 1984).

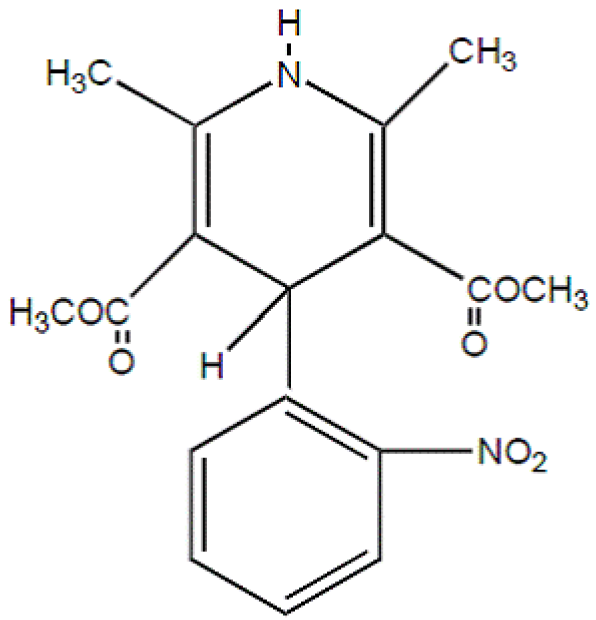


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Figure 2.5:Structure of nifedipine

*2.4.4.1 Therapeutic effects of nifedipine*

Nifedipine is use for the management of vasospastic angina, chronic stable angina, hypertension and Raynaud's phenomenon. It may also be used as a first line agent for left ventricular hypertrophy and isolated systolic hypertension (long-acting agents)(Varon and Marik, 2003).

*2.4.4.2 Adverse effect of nifedipine*

Nifedipine has the followings as its adverse effect when administered; dizziness, lightheadedness, giddiness, flushing, heat sensation, headache, weakness, nausea, heartburn, muscle cramps, tremor, peripheral edema, nervousness, mood changes, palpitation, dyspnea, cough, wheezing, nasal congestion and sore throat(Poole-Wilson et al., 2006).

*2.4.4.3 Pharmacokinetics of nifedipine*

Nifedipine is rapidly and fully absorbed following oral administration and extensively metabolized to highly water-soluble. Inactive metabolites accounting for 60 to 80% of the dose excreted in the urine. The remainder is excreted in the faeces in metabolized form, most likely as a result of biliary excretion. Hepatic metabolism is via cytochrome P450 system predominantly metabolized by CYP3A4, but also by CYP1A2 and CYP2A6 isozymes.It has a half-life of 2 hours and 92-98% protein binding(Chung*et. al.*, 1987)

*2.4.4.4 Mechanism of action of nifedipine*

The mechanism by which nifedipine reduces arterial blood pressure involves peripheral arterial vasodilatation and, consequently, a reduction in peripheral vascular resistance. The increased peripheral vascular resistance that is an underlying cause of hypertension, results from an increase in active tension in the vascular smooth muscle. Studies have demonstrated that the increase in active tension reflects an increase in cytosolic free calcium.Nifedipine is a peripheral arterial vasodilator which acts directly on vascular smooth muscle. The binding of nifedipine to voltage-dependent and possibly receptor-operated channels in vascular smooth muscle results in an inhibition of calcium influx through these channels. Stores of intracellular calcium in vascular smooth muscle are limited and thus dependent upon the influx of extracellular calcium for contraction to occur. The reduction in calcium influx by nifedipine causes arterial vasodilation and decreased peripheral vascular resistance which results in reduced arterial blood pressure(Pedersen *et. al.,* 1982)

## 2.5 Drug Interaction Studies

### 2.5.1 Metformin interaction studies

Metformin is often prescribed in combination with other drugs in treating Type 2 diabetes. Several brands are available as fixed-dose combinations, also with the purpose of reducing pill burden and making administration simpler and more convenient.

In the drugs list Review publication, (*RxList, 2010),* the followings were mentioned:

Furosemide– A single-dose, metformin-furosemide drug interaction study in healthy subjects demonstrated that pharmacokinetic parameters of both compounds were affected by co-administration. Furosemide increased the metformin plasma and blood Cmax by 22% and blood AUC by 15%, without any significant change in metformin renal clearance. On the other hand, Metformin reducedtheCmax and AUC of furosemide by 31% and 12% respectively, the half-life was decreased by 32%, without any significant change in furosemide renal clearance (*RxList*, 2010).

Nifedipine– A single-dose, metformin-nifedipine drug interaction study in normal healthy volunteers demonstrated that co-administration of nifedipine increased plasma metformin Cmax and AUC by 20% and 9%, respectively, and increased the amount excreted in the urine.

Tmaxand half-life were unaffected. Nifedipine appears to enhance the absorption of metformin. Metformin had minimal effects on nifedipine(*RxList,* 2010).

Cationic drugs– Cationic drugs (e.g., amiloride, digoxin, morphine, procainamide, quinidine, quinine, ranitidine, triamterene, trimethoprim, or vancomycin) that are eliminated by renal tubular secretion theoretically have the potential for interaction with metformin by competing for common renal tubular transport systems. Such interaction between metformin and oral cimetidine has been observed in normal healthy volunteers in both single- and multiple-dose, metformin-cimetidine drug interaction studies, with a 60% increase in peak plasma metformin and whole blood concentrations and a 40% increase in plasma and whole blood metformin AUC. There was no change in elimination half-life in the single-dose study. Metformin had no effect on cimetidine pharmacokinetics. Although such interactions remain theoretical (except for cimetidine), careful patient monitoring and dose adjustment of metformin and/or the interfering drug is recommended in patients who are taking cationic medications that are excreted via the proximal renal tubular secretory system (*RxList,* 2010).

Others– Certain drugs tend to produce hyperglycemia and may lead to loss of glycemic control. These drugs include the thiazides and other diuretics, corticosteroids, phenothiazines, thyroid products, estrogens, oral contraceptives, phenytoin, nicotinic acid, sympathomimetics, calcium channel blocking drugs, and isoniazid. When such drugs are administered to a patient receiving Metformin, the patient should be closely observed for loss of blood glucose control. When such drugs are withdrawn from a patient receiving metformin the patient should be observed closely for hypoglycemia (*RxList,* 2010).

Metformin is negligibly bound to plasma proteins and is, therefore, less likely to interact with highly protein-bound drugs such as salicylates, sulfonamides, chloramphenicol, and probenecid, as compared to the sulfonylureas, which are extensively bound to serum proteins (Bailey and Turner, 1996)

Glyburide—In a single-dose interaction study in type 2 diabetes patients, co administration of metformin and glyburide did not result in any changes in either metformin pharmacokinetics or pharmacodynamics. Decreases in glyburide AUC and $C_{max}$ were observed, but were highly variable. The single-dose nature of this study and the lack of correlation between glyburide blood levels and pharmacodynamic effects make the clinical significance of this interaction uncertain (*RxList*, 2010).

### 2.5.2 Methyldopa interaction studies

Some products that may interact with this drug include: iron products (such as ferrous sulfate, ferrous gluconate) and lithium. MAO inhibitors (isocarboxazid, linezolid, methylene blue, moclobemide, phenelzine, procarbazine, rasagiline, selegiline, tranylcypromine) should be avoided within 2 weeks before and during treatment with this medication. In some cases a serious (possibly fatal) drug interaction may occur.The drug react with other products that cause drowsiness including alcohol, antihistamines (such as cetirizine, diphenhydramine), drugs forsleep or anxiety (such as alprazolam, diazepam, zolpidem), muscle relaxants, and narcotic pain relievers (such as codeine), ibuprofen, naproxen may contain ingredients that could cause drowsiness or increase your blood pressure. This medication may interfere with certain laboratory tests (including urine catecholamine test), possibly causing false test results(Ellenhorn and Barceloux, 1988).

### 2.5.3 Nifedipineinteraction studies

NICE, (2006) in its report, explained the following interaction studies:

Beta-adrenergic blocking agents- Experience in over 1400 patients in a non-comparative clinical trial has shown that concomitant administration of nifedipine and beta-blocking agents is usually well tolerated, but there have been occasional literature reports suggesting that the combination may increase the likelihood of congestive heart failure, severe hypotension, or exacerbation of angina.

Long-acting nitrates:nifedipine may be safely co-administered with nitrates, but there have been no controlled studies to evaluate the antianginal effectiveness of this combination.

Digitalisshows isolated reports of patients with elevated digoxin levels, and since there is a possible interaction between digoxin and nifedipine, it is recommended that digoxin levels be monitored when initiating, adjusting, and discontinuing nifedipine to avoid possible over-or under-digitalization.

Quinidine: There have been rare reports of an interaction between quinidine and nifedipine (with a decreased plasma level of quinidine).

Coumarin anticoagulants: There have been rare reports of increasedprothrombin time in patients taking coumarin, anticoagulants, to which nifedipine was administered. However, the relationship to nifedipine therapy is uncertain.

Cimetidine: A study in six healthy volunteers has shown a significant increase in peak nifedipine plasma levels (80%) and area-under-the-curve (74%) after a one week course of cimetidine at 1000 mg per day and nifedipine at 40 mg per day.

Ranitidine produced smaller, non-significant increases. The effect may be mediated by the known inhibition of cimetidine on hepatic cytochrome P450, the enzyme system probably responsible for the first-pass metabolism of nifedipine.

Phenytoin is metabolized by CYP3A4. Co-administration of nifedipine with phenytoin, an inducer of CYP3A4, lowers the systemic exposure to nifedipine by approximately 70%. It is advisable to avoid co-administration of nifedipine with phenytoin or any known CYP3A4 inducer.

Grapefruit Juice;Co-administration of nifedipine with grapefruit juice resulted in approximately a doubling in nifedipine AUC and Cmax with no change in half-life. The increased plasma concentrations most likely result from inhibition of CYP 3A4-related first-pass metabolism. (Odou, *et al*., 2005)

### 2.5.4 Bendrofluazide interaction studies

Calcium;Thiazide diuretics decrease calcium loss in urine due to actions on the kidneys. (Threlkeld*et al*., 1993).

Folicacid;There was a study which showed that people taking diuretics for more than six months had dramatically lower blood levels of folic acid and higher levels of homocysteine compared with individuals not taking diureticshomocysteine, a toxic amino acid byproduct, has been associated with atherosclerosis(MorrowandGrimsley, 1999).

Magnesium and Potassium;Potassium-depleting diuretics, including thiazide diuretics, cause the body to lose potassium; they may also cause cellular magnesium depletion (Martin and Milligan, 1987), although this deficiency may not be reflected by a low blood level of magnesium (Kroenke*et al.,* 1987). Magnesium loss induced by potassium-depleting diuretics can cause additional potassium loss. Until more is known, it has been suggested that people taking potassium-depleting diuretics, including thiazide diuretics, should supplement both potassium and magnesium (Whang*et al.*, 1992).

People taking thiazide diuretics should be monitored by their prescribing doctor, who will prescribe potassium supplements if needed. Such supplementation is particularly critical before surgery in patients with a history of heart disease. In a preliminary study, people with low blood levels of potassium (in part related to diuretic use) had a higher incidence of serious problems resulting from surgery (including death) compared with those having normal potassium levels(Wahr*et al.*, 1999). A double-blind trial showed that thiazide diuretic use led to a reduction in blood levels of potassium in some participants. Those experiencing decreased potassium levels were also more likely to experience cardiovascular events, such as heart attacks, stroke, heart failure, aneurysm, and sudden cardiac death (Franse *et al.*, 2000). Fruit is high in potassium, and increasing fruit intake (especially bananas) is another way of supplementing potassium.Magnesium supplementation for people taking thiazide diuretics is typically 300–600 mg per day, though higher amounts (over 800 mg per day) have been reported in a controlled study to reduce side effects of thiazides (Ruml*et al.*, 1999). Combining supplementation of both potassium and magnesium has been reported to correct abnormally low blood levels of potassium and also to protect against excessive loss of magnesium(RumlandPak, 1999).

VitaminD:The reduction in urinary calcium loss resulting from treatment with thiazide diuretics is due primarily to changes in kidney function and may also be due, in part, to changes in vitamin D metabolism. However, there is no evidence to suggest that people taking diuretics have different requirements for vitamin D (Riis and Christiansen, 1985).

Interactions with Herbs; Herbs that have a diuretic effect should be avoided when taking diuretic medications, as they may enhance the effect of these drugs and lead to possible cardiovascular side effects. These herbsinclude dandelion, uvursi, juniper, buchu, cleavers,horsetail, and gravel root (Brinker, 1997).

Digitalis *(Digitalispurpurea)*;Digitalis refers to a family of plants commonly called foxglove, which contains digitalis glycosides, chemicals with actions and toxicities similar to the prescription drug digoxin. Thiazide diuretics can increase the risk of digitalis-induced heart disturbances (Threlkeld, 1993). Thiazide diuretics and digitalis-containing products should be used only under the direct supervision of a doctor trained in their use.

*Ginkgobiloba*;One case was reported in which ginkgo use was associated withhigh blood pressure in a person treated with a thiazide diuretic (Shaw, 1995).

Licorice *(Glycyrrhizaglabra)*; Licorice may increase the side effects of potassium-depleting diuretics, including thiazide diuretics (Shintani*et al.*, 1992).

# CHAPTER THREE

# 3.0 MATERIALS AND METHODS

## 3.1 MATERIALS

The brand, manufacturing date, batch number, expiry date and manufactures of the drugs administered to the patients and healthy subjects during the research are presented in table 3.1

### 3.1.1 Equipment

The followings equipment were used during the study:

Centrifuge: Heraeus (labafuge300) D-37520 ostencemated: 2003, Serial No.40267581,BN: 75003230, Auto-rorter mixer (Vorter, Stuart, England), Digital weighing balanceOHAUS model EP 64 BY Ohaus Corporation, Switzerland,5ml syringes, Refrigerator:Premier Thermacool, Nigeria, Hot Air oven: Big size one Gallenkamp, England, U.V.detector T80 + U.V/Vis spectrometer by PG instrument Ltd U.K, High Performance. Liquid Chromatography; Agilent Technologies, 1120LC series, USA,Disintegration-Rate Study: Eureka, England, Dissolution Rate Study: Eureka, England and PH Meter: HM 200 (Digital) England.

### 3.1.2 Reagents and standard drugs

The following standard solutions and reagents were used for the research:

Methanol: Sigma – Aldrich ≥ 99.9% U.K. HPLC Grade, Acetonitrile: Sigma – Aldrich ≥ 99.9%, U.K. HPLC Grade, Distilled water, Hydrochloric acid: May and Baker, England, Potassium dihydrogen phosphate (Buffer) by J.T. Baker 99.5% USA, Metformin HCl reference standard, Sulfadoxine: Internal standard source: Rambax pharmaceutical Ltd, Lagos.

*3.1.2.1 preparation of solutions*

**Metformin**: Stock solution of 3 µg/ml was prepared in distilled water by dissolving 5 mg of metformin sample in 5 ml of distilled water. 3 ml from this solution was diluted to 1000 ml mark. Serial dilutions were made from this resulting solution for concentrations ranging from 0.1- 3 µg/ml.

A solution of internal standard (sulfadoxine) was prepared by dissolving 5 mg in 5 ml of distilled water. Then 3 ml was pipetted out and diluted to 1000 ml. Serial dilutions of 0.1 µg- 3 µg/ml concentrations were prepared.

**Buffer solution:** 1.7g of potassiumdihydrogen phosphate was dissolved in 25 ml of distilled water to give the buffer concentration of 0.025 $MKH_2P0_4$ and acetic acid was used to adjust the pH to 5.6 – 5.8 range

Table 3.1 List of drugs used for the research

| Drug | Brand | Manufactured Date | Batch No | Expiry Date | Manufactured by |
|---|---|---|---|---|---|
| 1. Metformin | Diabetmin® 500 mg | June 2012 | Bco6597 | June 2015 | HovidBhd, 21 Malaysia |
| 2. Methyldopa | Dopatab® 250 mg | Aug 2012 | Bco8572 | Aug 2015 | HovidBhd, Malaysia |
| 3. Bendrofluazide | Bendro 5 mg | Nov 2011 | 411L | Nov 2014 | Ernest Chemist Ltd Accra Ghana |
| 4. Nifedipine | Nifecard® XL 30 mg | March 2012 | CK2723 | March 2015 | Lek Pharmaceutical and chemical Co. Solvenia |

## 3.2 METHODOLOGY

### 3.2.1 Quality control studies of metformin

*3.2.1.1 Identification test of metformin*

A quantity of the powdered tablets containing 20 mg of metformin hydrochloride was shaken with 20 ml of *absolute ethanol,* filtered and the filtrate was evaporated to dryness on a water bath. The residue was dried at $105^0$C for 1 hr.The color change was observed (B. P. 2002).

*3.2.1.2 Assay of metformin tablets*

Twenty (20) tablets wereweighed and powdered. A quantity of the powder (0.126 g)equivalent to 0.1g of metformin hydrochloride was dissolved in 70ml of distilled waterand shaken for 15 minutes and diluted to 100ml withdistilledwater. The mixture was filtered then 10ml was pipette from the filtrate and diluted to100ml with distilled water. Further 10ml was pipetted out from this second solution and made to 100ml with distilled water. Theabsorbance of the resultant solution was measured at the maximum at 232nm. But exactly 0.1 mg was used for the standard metformin powder.

The content of the $C_4H_{11}N_5HC1$ (metformin)was calculatedfrom the metformin tabletby taking 739 as the value of (A1% 1 cm) of metformin at 232 nm.

*3.2.1.3 Disintegration test of metformin*

Six(6) tablets of metformin were placed in six (6) tubes and a disk was added to each tube then the assembly was suspended in the beaker containing distilled water maintained at $37.5^0$C and apparatus was operated for 15 minutes(B.P 1998).

### 3.2.2 Quality control studies of methyldopa

*3.2.2.1 Identification test of methyldopa*

Standard powder of methyldopa (5mg) was dissolved in 1ml of water followed by 1ml of pyridine. Then 5ml of 4-nitrobenzoyl chloride was added and heated to boil. While shaking, 0.1 ml of sodium carbonate was also added (I.P 1979, Vol. 1). A quantity of powder tablets containing an equivalent weight of 5 mg of powdered tablets of methyldopa was used to repeat the test and color change was recorded

*3.2.2.2 Assay test of methyldopa tablets*

Twenty (20) tablets of methyldopa were weighed and powdered. A quantity of the powder(0.124 g) equivalent to 0.1g of anhydrous methyldopa was dissolved in sufficient 0.005Msulphuric acid to make 100ml and filtered. To 5ml of the filtrate, 2ml of iron (II) sulphate – citrate solution, 8 ml glycine buffer solution and sufficient water were added to produce 100ml. The absorbance of the solution was measured at the maximum wavelength of 545nm. The procedure was repeated with 5ml of 0.10% w/v solution of the methyldopa BPCRS in place of 5ml of the filtrate. The content of $C_{10}H_{13}NO_4$was calculated using the declared content of $C_{10}H_{13}NO_4$ in methyldopa Standard (B.P. 1998).

### 3.2.3 Quality control studies ofbendrofluazide

*3.3.1.1 Assay test of bendrofluazide*

Twenty (20) tablets were weighed and powdered.A quantityof the powder equivalent to 15mg of bendrofluazide was mixed with 50ml of 0.1 M of NaOH and shaken for 10 minutes. The mixture was then diluted to 100ml with 0.1M NaOH and filtered. 10ml of the filtrate was further diluted to 100 ml with water. The absorbance of the solution was measured at 275nm wavelength.The content of $C_{15}H_{14}F_3N_3O_4S_2$was calculated using 410 as (A1%,1cm) at maximum wavelength of

275nm (B.P. 1998). The test was repeated with accurately weight 15 mg of Standard powder of bendrofluazide.

### 3.2.4 Quality control studies ofnifedipine

*3.2.3.1 Identification test of nifedipine.*

A quantity of powderednifedipinetabletsequivalent to25mg of the sample was dissolved in 10ml of a mixture (5 ml of ethanol, 3.5 ml of water and 1.5 ml hydrochloric acid) followed by heating gently. 0.5g granulated zinc was also added and the mixture was allowed to stand for 5 minutes, swirling occasionally and then filtered.5ml of 1% w/v solution of sodium nitrite was added to the filtrate and allowed to stand for 2 minutes. To this, 5% w/v solution of ammonium sulphamate was added. The mixture was shaken vigorously with care and 2ml of a 0.5% w/v solution of N-(-1-naphthyl) ethylenediaminedihydrochloride was added (B.P 1998). A color change was observed and the test was repeated with standard powder of nifedipine.

*3.2.3 2 Assay test of nifedipine*

Ten (10) tablets of nifedipine (30 mg)were weighted and powdered. A quantity of powdered equivalent to0.13g of nifedipine was dissolved in a mixture of 25ml of 2-*methylpropan-2-ol* and 25 ml of 1M*perchoric acid.* The resultant solutionwas titrated with 0.1M*ammonium cerium (IV)sulphate* VS using 0.1ml of*ferroin* solution as indicator until the pink color was discharged.

The operation was repeated without the nifedipine sample. The difference between the titrations represents the amount of ammonium cerium (IV) sulphate required. Each ml of 0.1M*ammonium cerium IV) sulphate* VS is equivalent to 17.32mg of $C_{17}H_{18}N_2O_6$(B.P. 1998).

### 3.3 In-Vivo Studies

### 3.3.1 Study design

The study involved healthy subjects and type 2 diabetic patients who also have hypertension attending Clinics at ABUTH Zaria.Six healthy subjects and 18 patients participated in the study. The healthy subjects were screened to be free from both diabetes and hypertension. Their ages ranged between 28 and 45 years. They were also free from liver and kidneydiseases. The subjectsfasting blood sugar (FBS) and blood pressure (B.P) were takenbefore and after the study.

#### *3.3.1.1 Phase I*

Blood samples at fasting state were taken from both healthyhuman subjects and diabetic patients after overnight fasting. Thereafter,metformin tablet (1 g) was administered with 250ml of water to each subject. The healthy subjects were allowed to take food after 2 hours but patients after 30 minutes. A quantity (3ml) of blood sample was withdrawn at 0.0, 0.5, 1.5, 3, 4, 5, 6, 8, hours and transferred into anticoagulant bottles.

The blood sugar levels and blood pressure were determined for the subjects immediately after the sample collection. The collected samples were centrifuged and stored in a refrigerator at $-4^{0}C$.

#### *3.3.1.2 Phase II*

In this second phase of the study, which was conducted after 7 days washout period, the 18 patients were grouped into three(Group I, II and III) each containing six(6) patients. The administration of drugs was done as follows;

**Group I**: Receivedmetformin (1 g) and methyldopa (250mg) with 200ml water.

**Group II**: Received metformin (1 g) and nifedipine (30 mg) with 200ml of water.

**Group III**: Received metformin (1 g)and bendrofluazide(5mg)with 200ml of water.

After the administration of the drugs above, 3ml of blood samples were withdrawn at 0, 0.5. 1.5, 3,4,5,6 and 8 hours as in the phase I. The blood samples were tested for blood sugar level and metformin concentration determined. All the blood samples were centrifuged at 3000rpm for 15 min. The supernatant were collected as plasma into fresh sample bottles and stored at -4$^{o}$C.

## 3.4 Sample Analysis

### 3.4.1 Solvent system development

The solvent system used in this study was adopted from unpublished work in Pharmaceutical Chemistry Laboratory of University of Lagos and Central Research Laboratory of the same University. The method was modified by try and error of adjusting the ratios of the solvent combinationand validatedwith seven (7) point variation of solvent combinations. The solvent system with the best resolution was selected (13:80:7) for Acetonitrile: 25mM$KH_2P0_4$: Methanol.

After optimization of the solvent system, chromatograms of all the drugs were obtained. This was done by preparing 25 µg/ml of the drugs. Then 20µl of each standard solution was injected into the HPLC and allowed to run for 10 minutes after spiking the plasma with drug sample. The individual retention time were recorded. Then, a mixture of all the standards drugs was run for the same 10 minutes after spiking the mixture with drug free plasma. A chromatogram, showing individual peaks at various retention times was obtained (Appendix 12-16).

### 3.4.2 Extraction method

The method of Bhavesh*etal*., (2007) was adopted and modified for the extraction of metformin from samples.The method is; 100µl of metformin hydrochloride solution of appropriate concentration and 100µl of sulfadoxine solution (20µg $ml^{-1}$) were added to 900µl of drug free plasma contained in a clean 5 ml Ria Vial and were properly mixed. To this 50 µl of protein precipitating agent (perchloricacid: acetonitrile 50%v/v each) was added and was vortexed for 30seconds. After centrifugation at 3000 rpm for 10minutes, 700 µl of the supernatant was evaporated to dryness at $45^0$C. The residue wasreconstituted in 100 µl of mobile phase and 20 µl ofthis was injected on to the HPLC system. The modification was by air drying instead of drying over nitrogen gas.

The percentage of recovery was calculated byusing the following relationship:

$$PR = \frac{amount\ \ of\ metformiin\ \ recovered\ \ after\ \ plasma\ \ extraction}{amount\ \ in\ the\ standard\ \ preparation\ \ solution\ \ of\ metformin} \text{ x } 100$$

### 3.4.3 Calibration curve

Calibration curve based on peak-area ratio were prepared by spikingblank plasma in series of test tubes with standard solution of metformin to give concentration range 0.1 µg – 3 µg/ml. Thereafter,0.2 µg/ml of sulfadoxine as internal standard was added and each mixture ran in the HPLC machine. The respective peak area ratio for each mixture was plotted against the corresponding concentration.

### 3.4.4 HPLC chromatographic condition

| | | | |
|---|---|---|---|
| Mobile phase; | Acetonitrile : | 25mM$KH_2P0_4$: | Methanol |
| | 13 | 80 | 7 |

Column : ODS Hypersil –C8 4. 6 x 125mm, 5um

Detection wavelength: 238nm

Temperature: 30$^0$C

Flow rate: 1.00ml/min

Run time : 7 minute

Injection volume: 20 µl

pH : 5.8 (adjusted with acetic acid)

| | | |
|---|---|---|
| Chromatogram; | Metformin | sulfadoxine |
| Retention time(min): | 1.111 | 4.999 |

### 3.4.5 Validation of the modified method

*3.4.5.1 Precision*

Precision of the method was determined by selecting 0.2µg/ml, 0.5µg/ml and 1µg/ml concentrations from the prepared serial dilution and used to determine within-day and day-to-day variations. For within day variation, three concentrations were run 6 times in the morning and afternoon of same day. The same concentrations were run 6 times a day after to get the inter-day variations. The standard deviations of Peak Area Ratio obtained were calculated followed by coefficient of variation in percentage.

*3.4.5.2 Accuracy*

Two selected concentrations, 0.3 µg/ml and 0.5 µg/ml extracted six (6) times eachusing extraction method above. Percentage recovery and standard deviation were calculated. The results showed accuracy of the method.

### 3.4.6 Pharmacokinetic parameters calculation

The pharmacokinetic parameters were calculated from the concentrations derived from the corresponding peak area observed from the HPLC runs. Residual method and a software package, PKF Microsoft excel were used to compute the pharmacokinetic parameters. The results are as shown in tables 4.5, 4.6, 4.7 and 4.8

## CHAPTER FOUR

## 4.0 RESULTS

## 4.1 In-vitro Studies

### 4.1.1 Identification tests

*4.1.1.1 Metformin tablets*

When subjected to identification test using B. P 2002, an orange - red color was produced which darkens on standing for both tablet and standard samples

*4.1.1.2 Methyldopa tablet*

When subjected to identification test using B.P. 1998, an orange/amber color was produced, indicating the presence methyldopa in the tablet sample and the standard powder

*4.1.1.3 Nifedipine tablet*

When subjected to identification test using B.P. 1998, an intense red color developed which persisted for more than 5 minutes signifying the presence of nifedipine in the test sample.

### 4.1.2 Assay results of drugs used

The results for assay of the drugs used in this study are shown in table 4.1, from which it can be seen that the percentage content of active ingredient in all the drugs used for this study were within the official limits.

### 4.1.3 Disintegration test of metformin (B.P 2002)

The disintegration time (min) for all the six metformin tabletsin distilled water maintained at $37.5^{0}C \pm 0.5$was found to be between 2.9 and 3.0 min. This showed that they all passed the test according to the official book used.

**Table 4.1 Content (%) of active ingredient assayed in the drugs used**

| Drug | Content (%) of Drug assayed | Official range (%) |
|---|---|---|
| Metformin | 98.4 | 95 – 105 (B. P. 2002) |
| Methyldopa | 99.8 | 95 – 105 (B. P. 1998) |
| Bendrofluazide | 99.3 | 98 – 102 (B. P. 1998) |
| Nifedipine | 96.7 | 93.5 – 102 (B. P. 1998) |

## 4.2 Validation of Modified Method

### 4.2.1 Optimization of condition

The Optimization of solvent system was done by randomly selecting seven (7) different solvent combinations and run in the HPLC to deduce the appearance of peaks in the chromatograms. The solvent combination with the best peaks was adopted as presented on table 4. 2.

### 4.2.2 Precisionand accuracy

The precision of the modified method is presented in table 4.3 for both intra-day and inter-day analysis. Each concentration was run six (6) and their percentage coefficient of variation was calculated as in table 4. 3.The accuracy of the modified method is expressed as % recovery and presented in table 4.4

### 4.2.3 Calibration curve of metformin standard solution

The calibration curve obtained from the serial dilution concentrations range 0.1 µg - 3 µg/ml and the results of peak – area ratio recorded was plotted against the serial concentrations and a regression linear graph was obtained as shown in Figure 4.1. The linear correlation coefficient of the graph is 0.995.

Regression equation is $Y = 0.9484x - 64.6$

$Y$ = peak – area ratio

$X$ = concentration

Slope = 0.9484

Intercept = 64.6

**Table 4.2 Optimization of solvent system**

| Solvent system | | | pH | Flow rate | Retention time (min) | |
|---|---|---|---|---|---|---|
| $KH_2PO_4$ : ACN: Methanol | | | | 1ml/min | Metformin | Sulfadoxine |
| 60 | 20 | 20 | 5.7 | " | 2.03 | 4.93 |
| 65 | 25 | 10 | 5.6 | " | 1.94 | 5.08 |
| 65 | 28 | 7 | 5.6 | " | 1.07 | 4.70 |
| 75 | 20 | 5 | 5.6 | " | 1.09 | 4.84 |
| 80 | 15 | 5 | 5.8 | " | 1.09 | 4.88 |
| 80 | 13 | 7 | 5.7 | " | 1.11 | 5.00 |
| 70 | 20 | 10 | 5.8 | " | 2.16 | 4.95 |

*Note; ACN = Acetonitrile*

**Table 4. 3. Intra and inter-day assay variation of metformin**

| Sample | Concentration ng/ml | CV% | N |
|---|---|---|---|
| Intraday run | 200 | 3.4 ± 0.56 | 6 |
| (Metformin) | 500 | 1.8 ± 0.87 | 6 |
| | 1000 | 0.5 ± 0.64 | 6 |
| Inter-day run | 200 | 4.2 ±0.23 | 6 |
| (Metformin) | 500 | 3.5 ±0.41 | 6 |
| | 1000 | 1.2 ±0.04 | 6 |

CV =Coefficient of Variation, N= Number of samples

**Table 4.4: Percentage recovery of metformin**

| Sample | Concentration ng/ml | Recovery %±S.D | N |
| --- | --- | --- | --- |
| Metformin | 300 | 97.47±4.2 | 6 |
| | 500 | 97.58±6.7 | 6 |

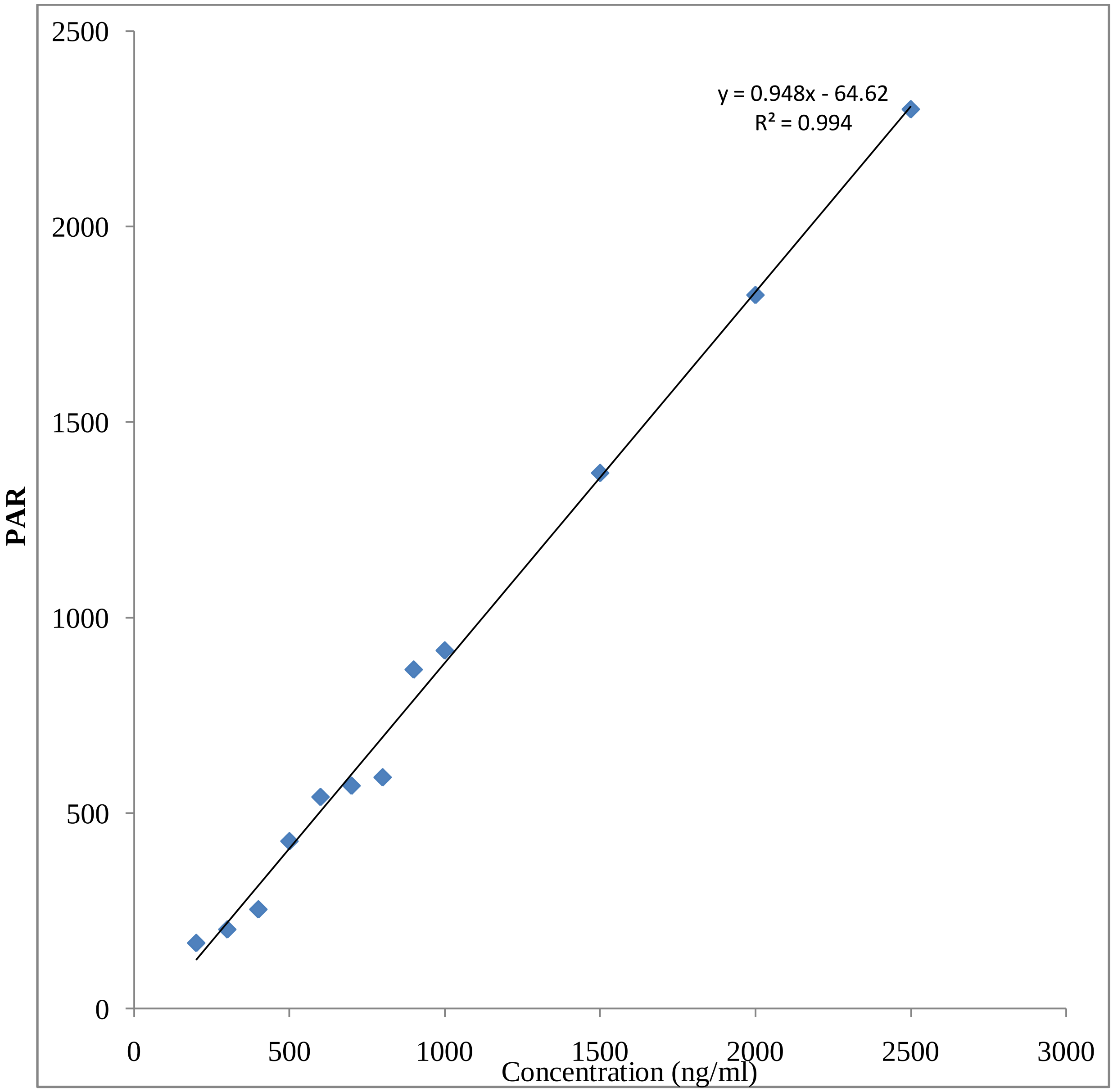
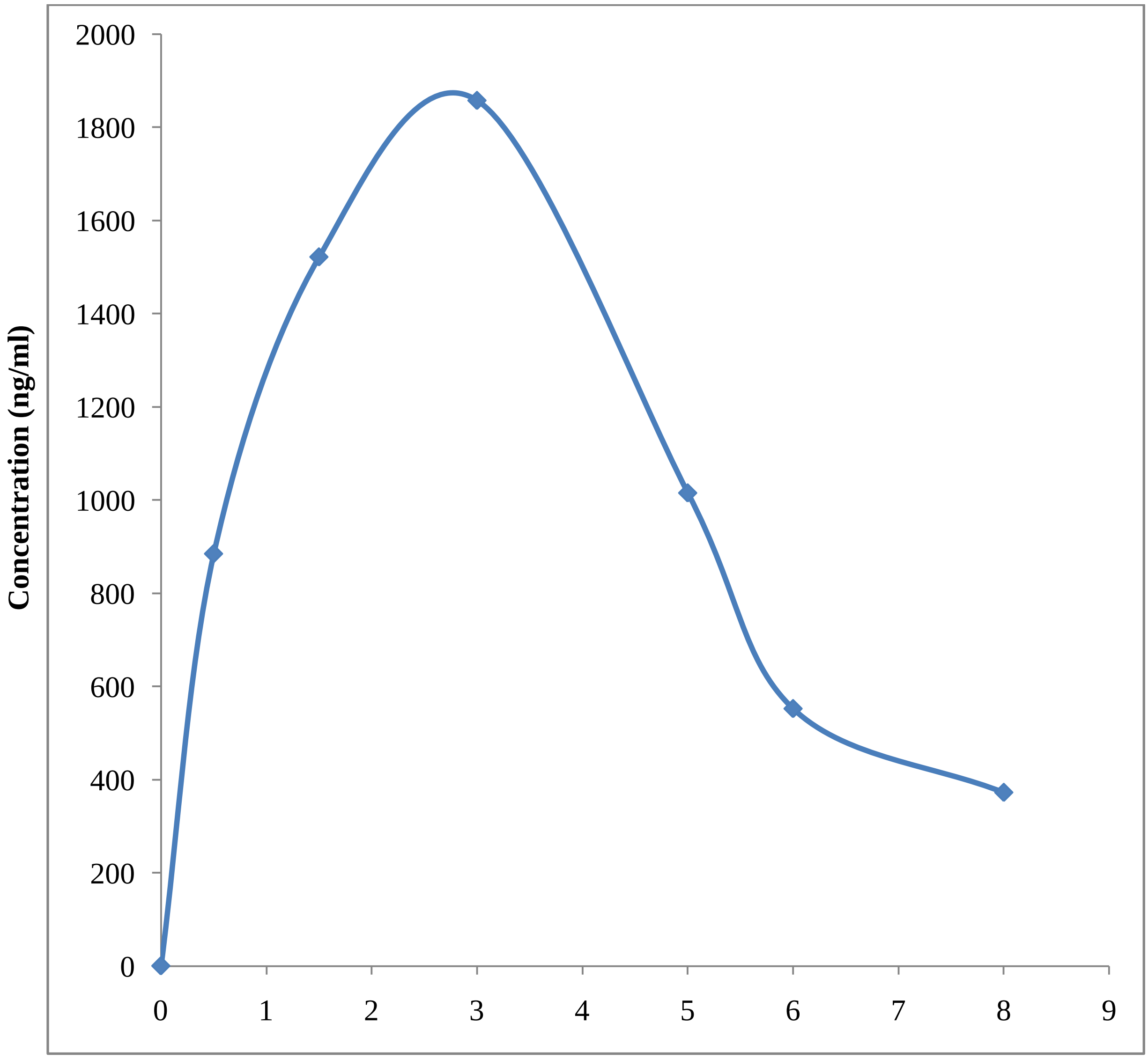



**Figure 4.1 Linear calibration curve of metformin**

## 4.3 In-vivo Studies

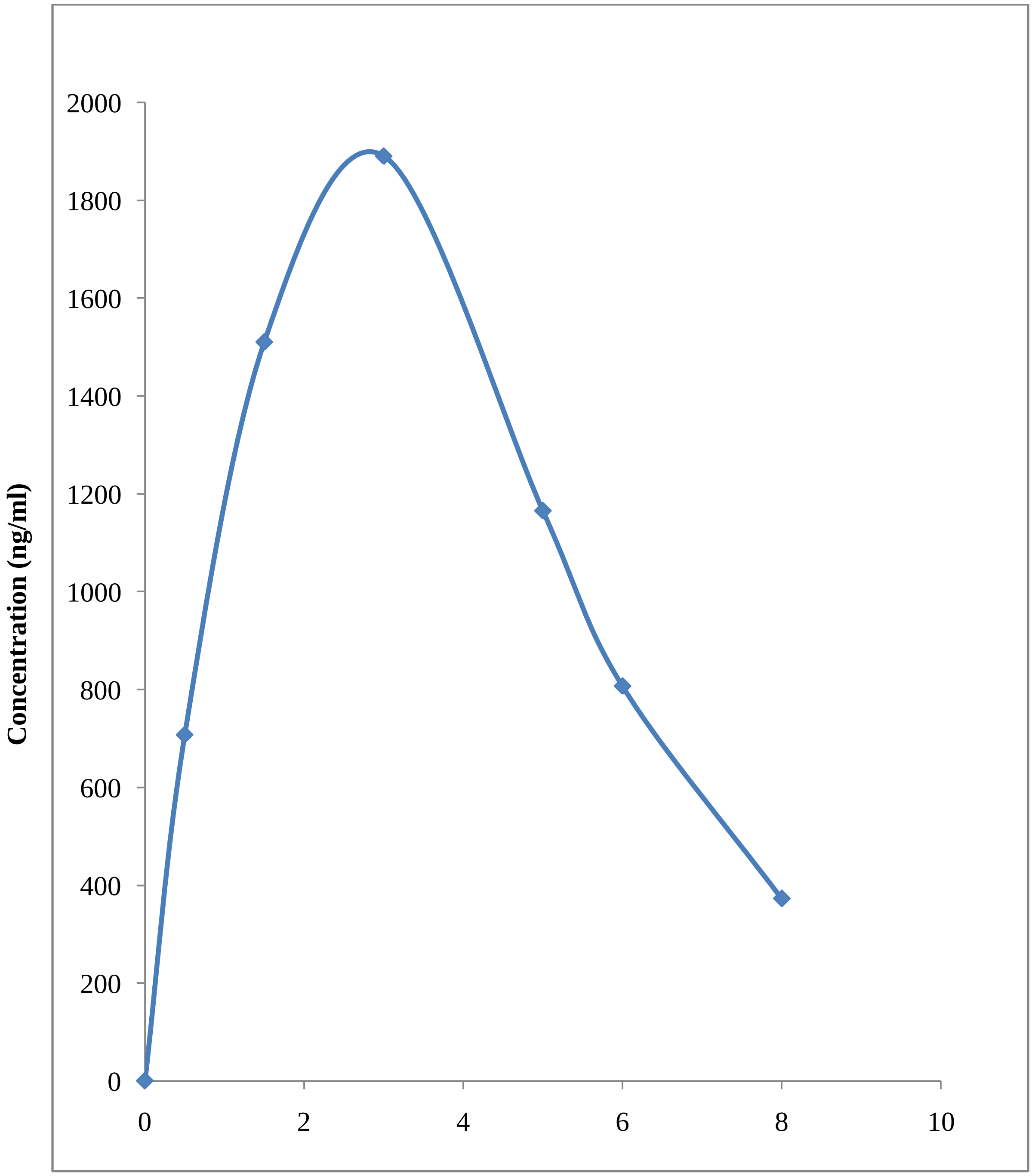
### 4.3.1 Metformin alone

The result for the administration of metformin (1 g) alone to healthy human subjects and in type 2 diabetic patients are shown in figures 4.2 and 4.3 respectively. The various concentrations detected were plotted against their corresponding sampling times. The $C_{max}$ and $T_{max}$for metformin in healthy human subjects were found to be 1857.67 ng/ml and 3 hrs respectively. In diabetic patients however, the $C_{max}$ and $T_{max}$ were 1890.67 ng/ml and 3 hrs respectively. Figure 4.4 shows a comparison of the pharmacokinetics profile of metformin in the two groups.




**Time (h) →**

**Figure4.2.Administration of metformin (1 g) tablets alone to healthyhuman subjects**




Time (h) →**Figure 4.3. Administration of metformin alone (1 g) in diabetic patients**

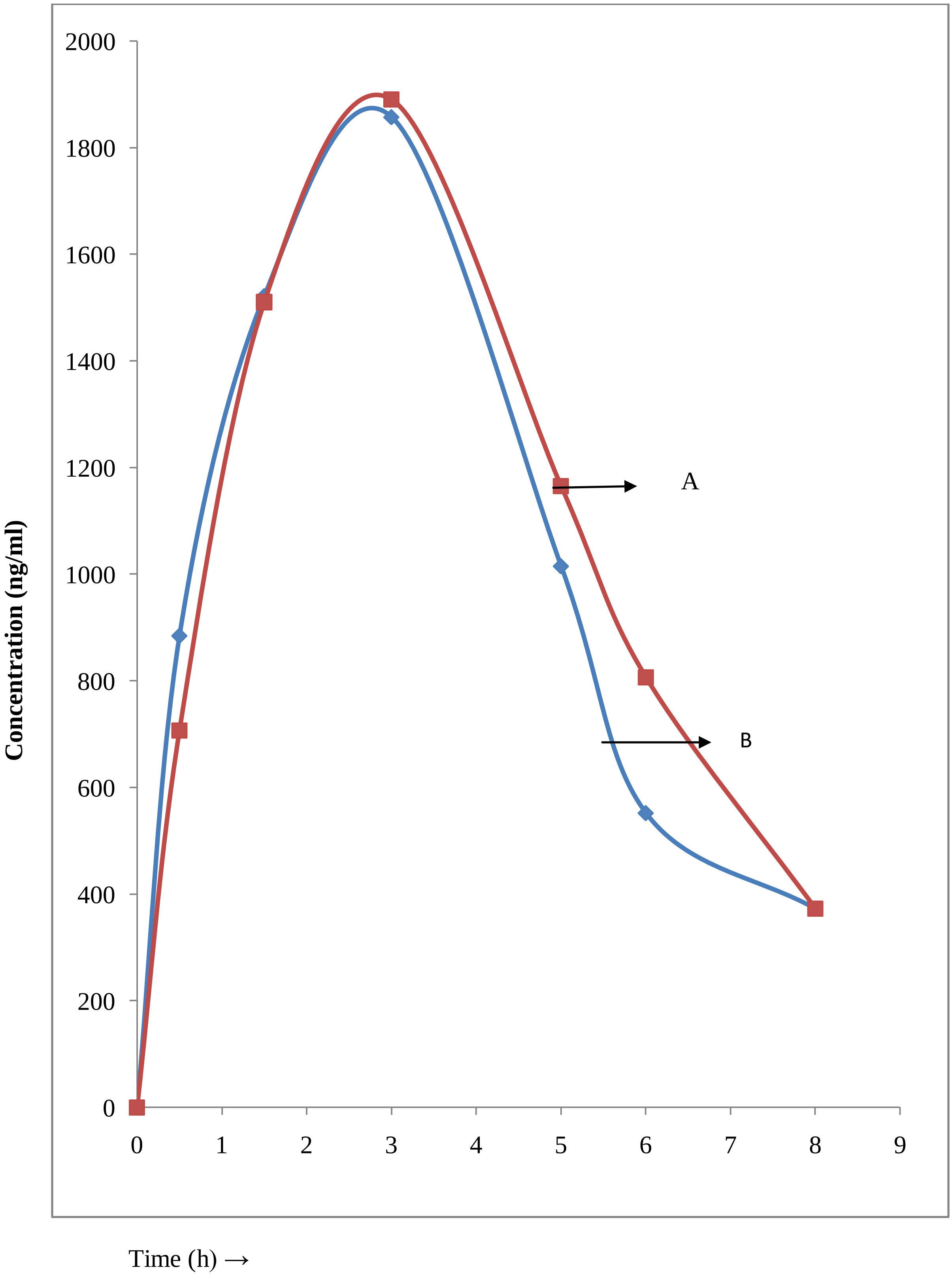
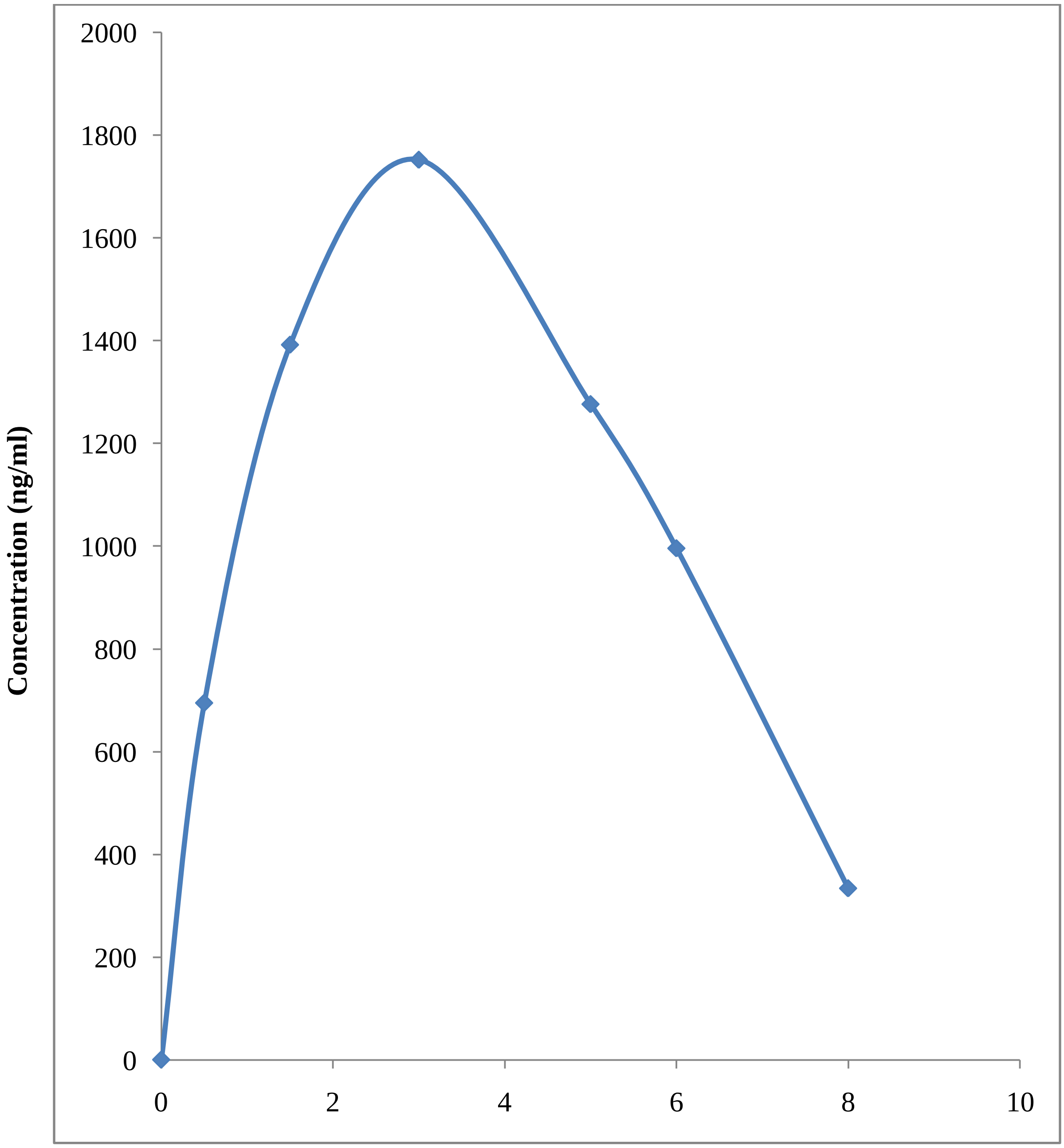



**Figure 4.4: Comparison of mean concentration of metformin in healthyhuman subjects (B) and Type 2 diabetic patientswith hypertension (A) after administration of metformin (1 g)**

### 4.3.2 Co-administration of metformin and methyldopa

The result of co-administration of metformin tablet (1 g) with methyldopa (250 mg) in type 2 diabetic patients and administration of metformin alone also in type 2 diabetic patientswere shown in figure 4.5 and 4.6. The various concentrations detected were plotted against their corresponding sampling times. The Cmax and Tmax of co-administered of metformin (1 g) with methyldopa (250 mg) were 1752.17 ng/ml and 3 hrs respectively. The Cmax has reduced from 1890.67 ng/ml, which is statistically insignificant ($P > 0.05$).




**Time (h) →**

**Figure 4.5: Co-administration of metformin (1 g) with methyldopa (250 mg)in type 2 diabetic patients**

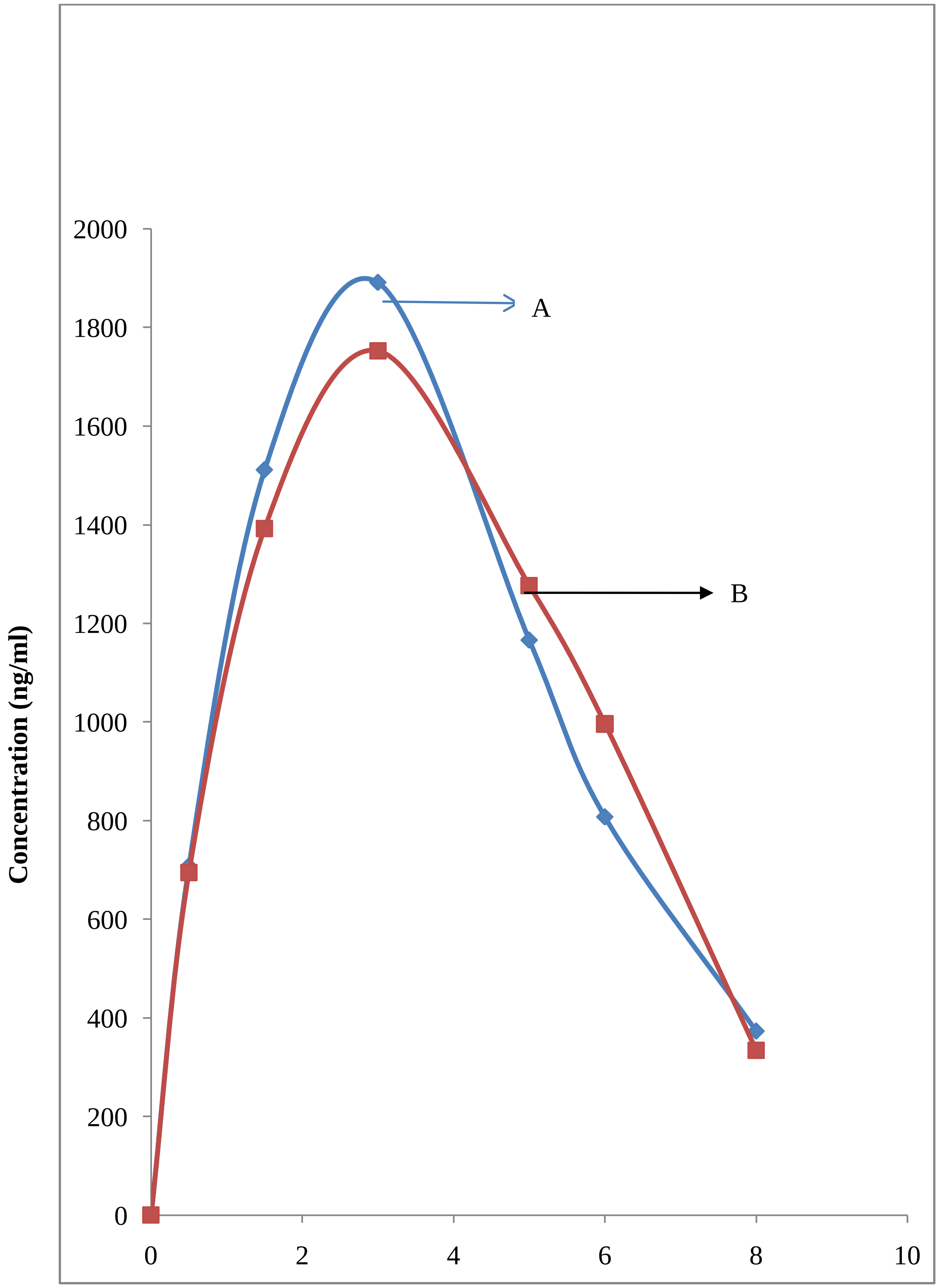


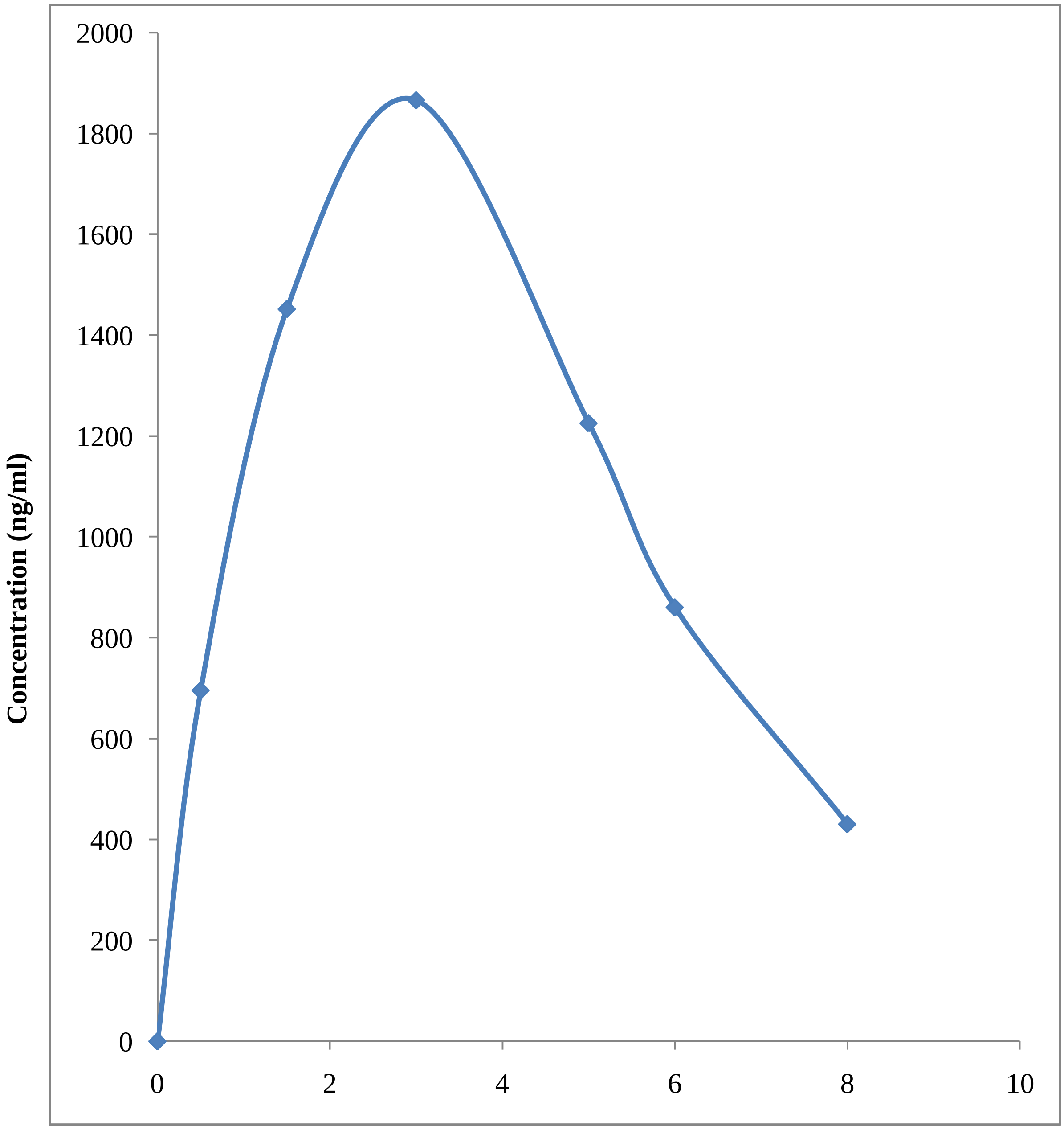

**Figure 4.6: Comparison of mean concentration of metformin following administration ofmetformin (1 g) alone (A) and co- administered with methyldopa (250 mg) in type 2 diabetic patients (B)**

**Time (h) →**

### 4.3.3 Co-administration of metformin and bendrofluazide

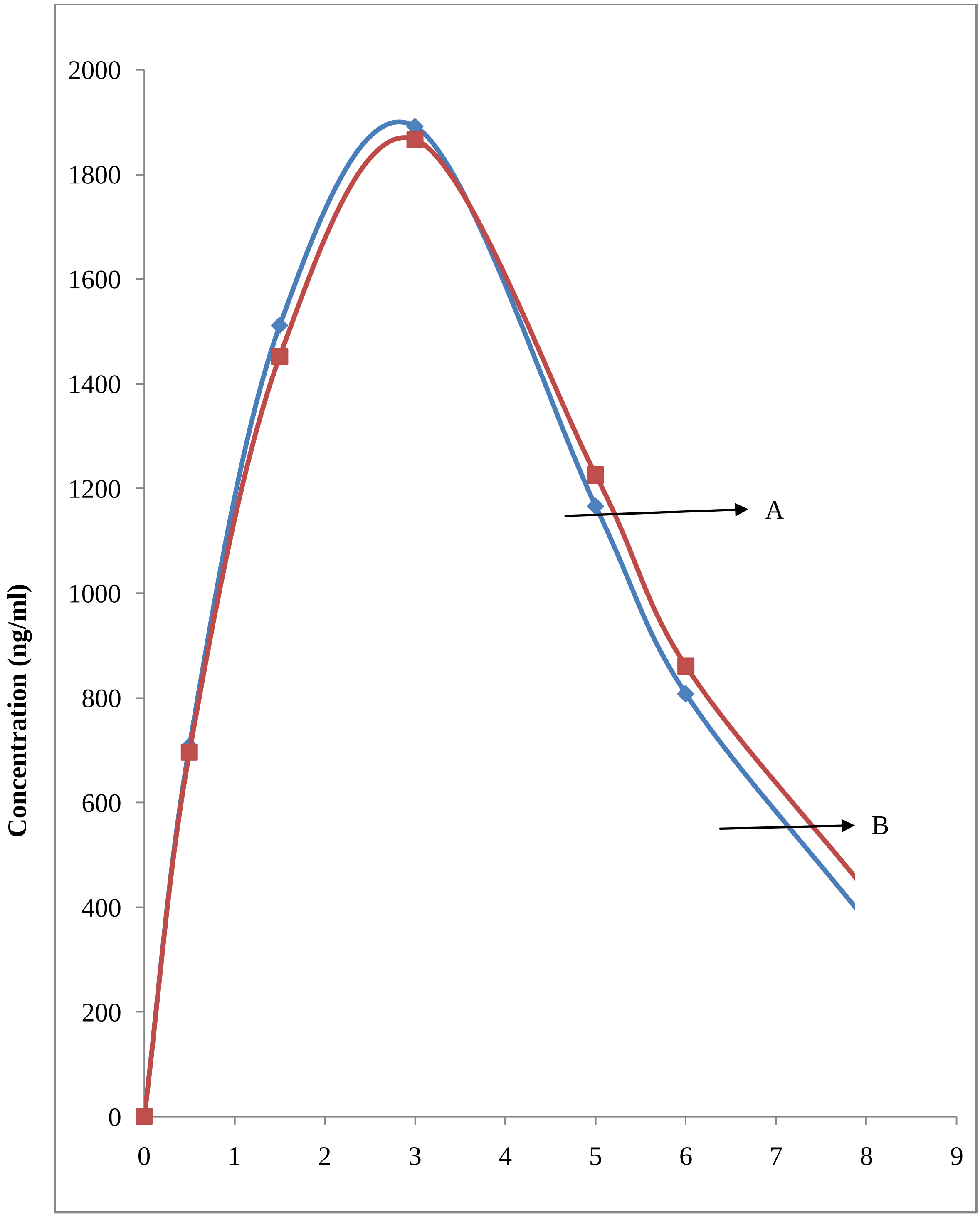
Results for the co-administration of metformin (1 g) with bendrofluazide (5 mg) in type 2 diabetic patients was shown in figure 4.7 and compared with that of administration of metformin (1 g) alone in the same type 2 diabetic patients in figure 4.8.

The Cmax and Tmax for co-administration of metformin (1 g) with bendrofluazide (5 mg) were 1865.67 ng/ml and 3 hrs respectively against 1890.67 ng/ml and 3 hrs for metformin (1 g) alone. The difference in Cmax is insignificant ($P > 0.05$).




Time (h) →

**Figure 4.7: Co-administered metformin (1 g) with bendrofluazide (5 mg) in type 2 diabetic patients**




**Time (h) →**

**Figure 4.8: Comparison of mean metformin concentration following administration of metformin (1 g) alone (A) and co-administered with bendrofuazide(5 mg) in type 2 diabetic patients (B)**

### 4.3.4 Co-administration of metformin and nifedipine

Results for the co-administration of metformin (1 g) with nifedipine (30 mg) in type 2 diabetic patients was shown in figure 4.9 and compared with that of administration of metformin (1 g) alone in the same type 2 diabetic patients in figure 4.10.

The Cmax and Tmax for co-administration of metformin (1 g) with nifedipine (30 mg) were 2357.16 ng/ml and 3 hrs respectively against 1890.67 ng/ml and 3 hrs for metformin (1 g) alone. The difference in Cmax is statistically significant ($P < 0.05$).

### 4.3.5 Metformin profile alone and when co-administered

The profiles of metformin (1 g) alone in healthy human subjects, in type 2 diabetic patients and when co-administered with methyldopa (250 mg), bendrofluazide (5 mg) and nifedipine (30 mg) were all compared in figure 4.11. The Cmax were 1857 ng/ml, 1890.67 ng/ml, 1752.17 ng/ml, 1865.67 ng/ml and 2357.16 ng/ml respectively. The Tmax remain the same at 3 hrs for all the drugs profile.

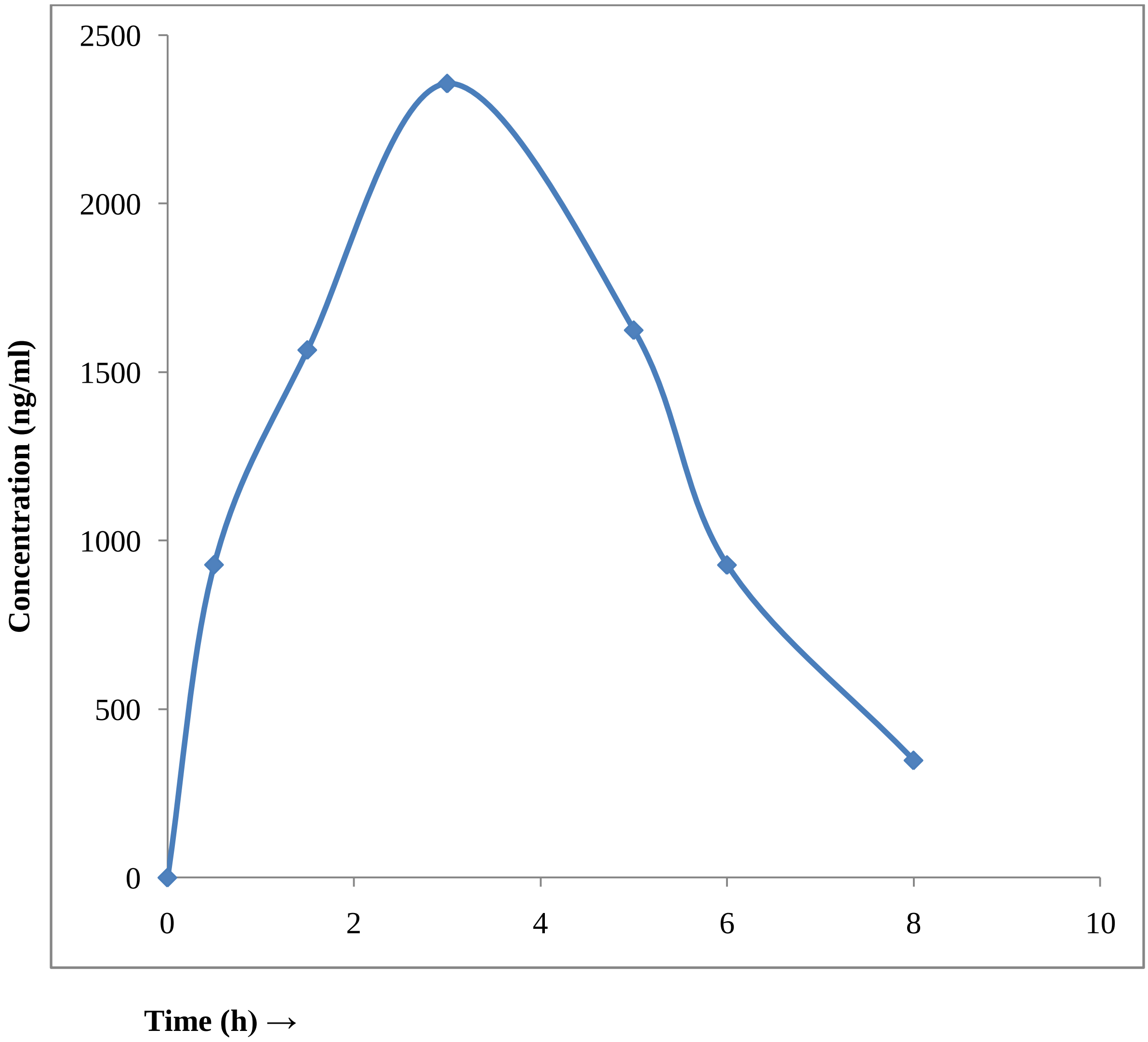



**Figure 4.9: Co-administration of metformin (1 g) with nifedipine (30 mg) in type 2 diabetic patients**

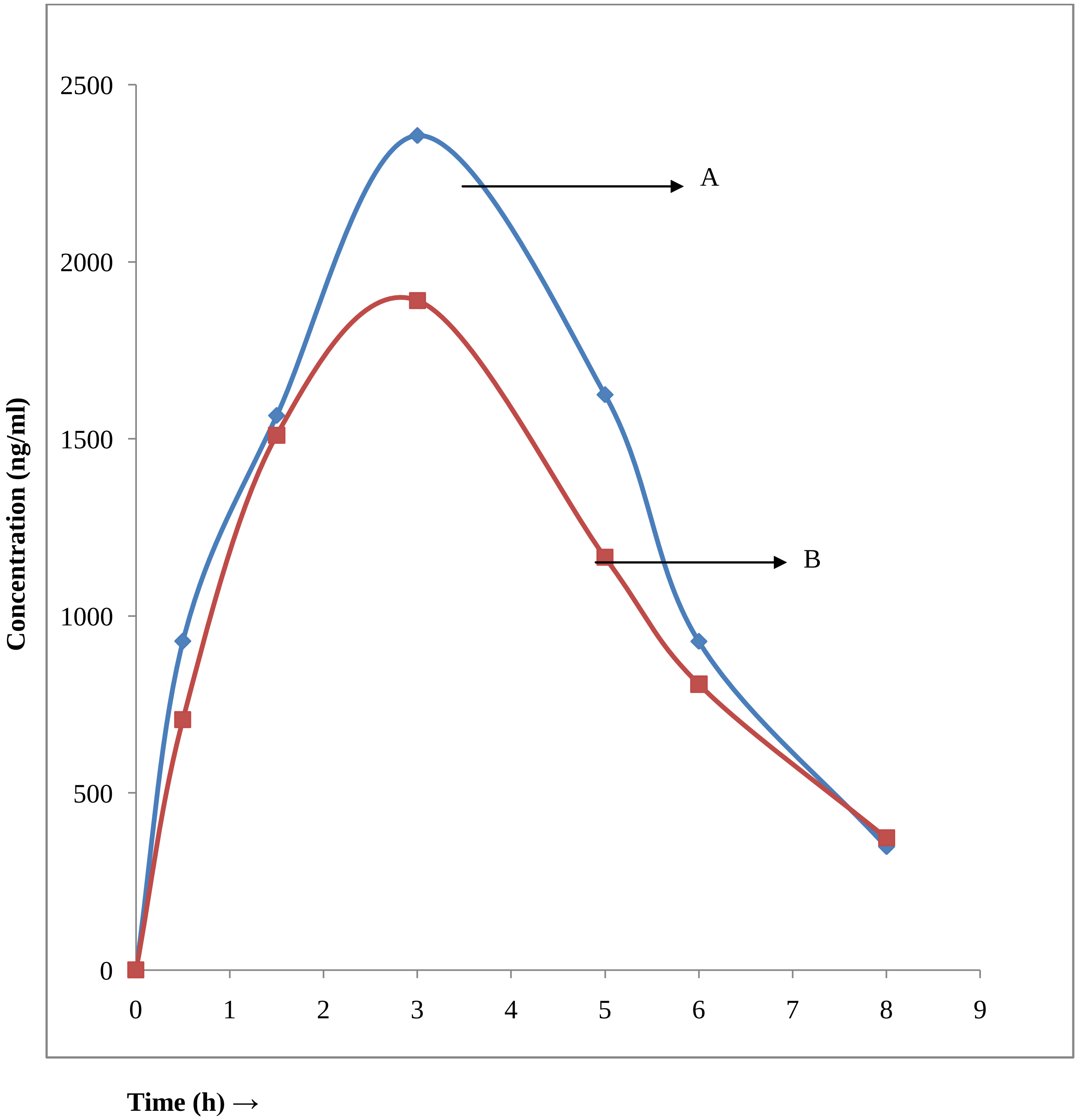
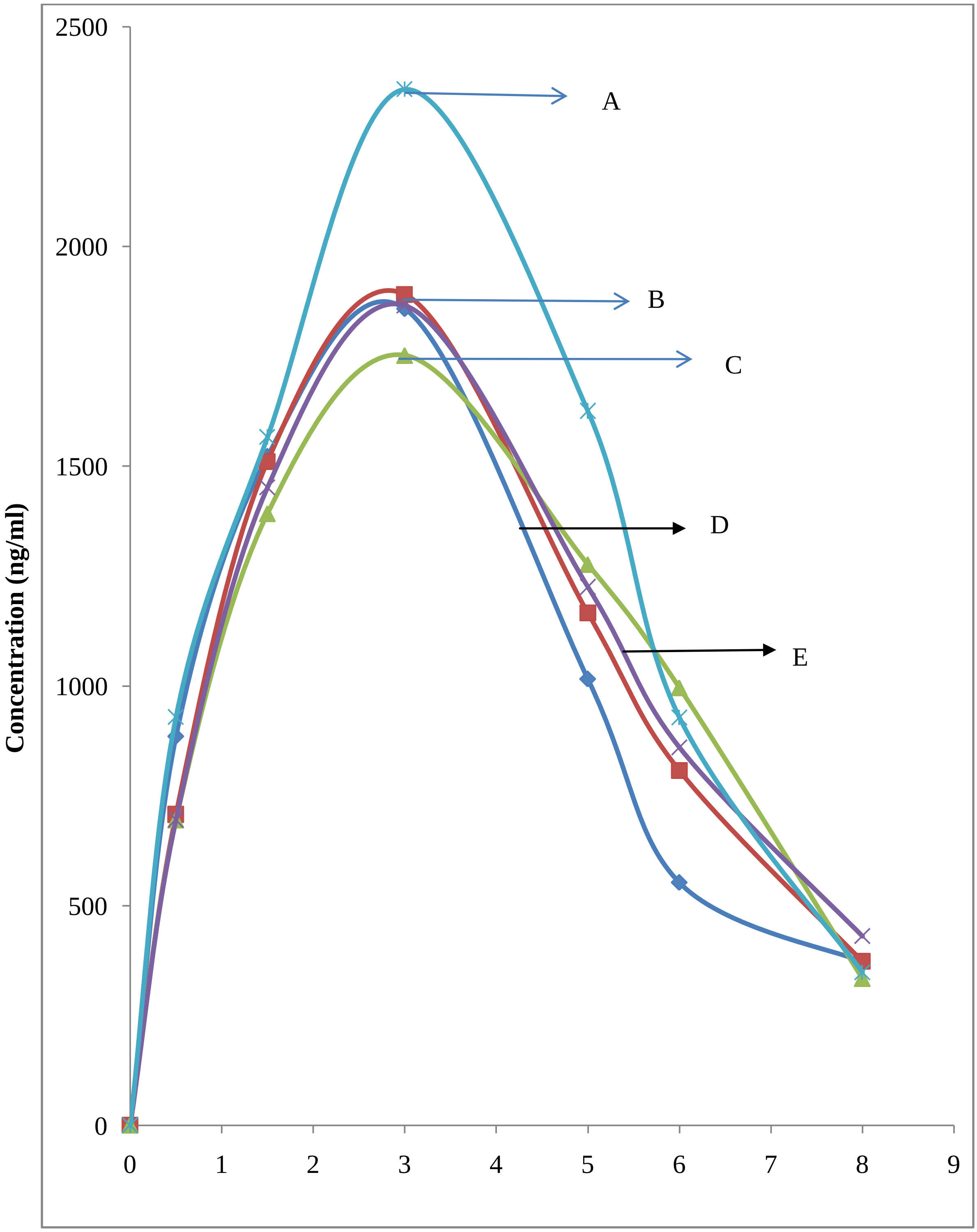



**Figure 4.10: Mean concentration of metformin alone (1 g)(B) and when co-administered with nifedipine (30 mg) (A) in type 2 diabetic patients**




**Time (h):**

**Figure 4.11: Mean metformin concentration alone (1 g) (B)withmethyldopa (250 mg) (C), bendrofluazide (5 mg) (E) andnifedipine (30 mg) (A) in type 2 diabetic patients and healthy human subjects (D).**

### 4.3.6 Pharmacokinetics of metformin (1 g) and when co-administered with the test drugs

The pharmacokinetics of metformin (1 g) alone in healthy human subjects and in type 2 diabetic patients was compared as shown in table 4.5. All the pharmacokinetics showed insignificant variation ($P > 0.05$). Likewise, table 4.6 showed variation of pharmacokinetics of metformin (1 g) alone in type 2 diabetic patients and when co-administered with methyldopa. The variation showed statistically insignificant ($P > 0.05$).

Table 4.7 showed insignificant ($P > 0.05$) variation of pharmacokinetics of metformin (1 g) alone in type 2 diabetic and when co-administered with bendrofluazide (5 mg). Similarly, pharmacokinetics of metformin (1 g) alone in type 2 diabetic patients was compared with its co-administration with nifedipine (30 mg) as shown in table 4.8 where variations in Cmax, AUCs were statistically significant ($P < 0.05$).

**Table. 4.5Comparison of pharmacokinetics of metformin(mean, n=6) alone in healthy human subjects and in type 2 diabetic patients.**

| Pharmacokinetic parameter | Healthy Subjects | Type II Diabetics | P-value |
|---|---|---|---|
| Lag time(h) | 0.1 ±0.000 | 0.12 ±001 | P>0.05 |
| t ½ abs (h) | 1.25 ±0.013 | 1.45 ±0.016 | P>0.05 |
| K abs (/h) | 0.554 ±0.002 | 0.478 ±.002 | P>0.05 |
| Cmax (ng/ml) | 1857.67 ±0.169 | 1890.67±0.107 | P>0.05 |
| $AUC_{0-8}$ (ng/ml/h) | 8318.89 ±.030 | 8882.10 ±0.205 | P>0.05 |
| $AUC_{0-\infty}$ (ng/ml/h) | 10688.05 ±0.120 | 12106.87 ±0.061 | P>0.05 |
| Vd (L) | 119.31±0.565 | 112.59 ±0.062 | P>0.05 |
| t ½ el (h) | 4.4 ±0.030 | 6.0 ±0.000 | P<0.05 |
| K el (/h) | 0.157 ±0.020 | 0.116 ±0.000 | P>0.05 |
| T max (h) | 3.0 ±0.000 | 3.0 ±0.000 | P>0.05 |
| CL(ml/h) | 760.0 ±0.200 | 970.7 ±0.330 | P>0.05 |

**Table 4.6 Comparison of pharmacokinetics of metformin (mean, n=6) alone and co-administered with methyldopa in type 2 diabetic patients.**

| Pharmacokinetic parameter | Met. Alone | Met.+ methyldopa | P-value |
|---|---|---|---|
| Lag time(h) | 0.12 ±001 | 0.12 ±0.001 | P>0.05 |
| t ½ abs (h) | 1.45 ±0.016 | 1.38 ±0.046 | P>0.05 |
| K abs (/h) | 0.478 ± 0.002 | 0.502 ±0.001 | P>0.05 |
| Cmax (ng/ml) | 1890.67±0.107 | 1752.17 ±0.232 | P>0.05 |
| $AUC_{0-8}$ (ng/ml/h) | 8882.10 ±0.205 | 8895.30 ±0.223 | P>0.05 |
| $AUC_{0-\infty}$ (ng/ml/h) | 12106.87 ±0.061 | 12061.80 ±0.043 | P>0.05 |
| Vd (L) | 112.59 ±0.062 | 112.50 ±0.061 | P>0.05 |
| t ½ el (h) | 6.0 ±0.000 | 6.58 ±0.014 | P>0.05 |
| K el (/h) | 0.116 ±0.000 | 0.105 ±0.020 | P>0.05 |
| T max (h) | 3.0 ±0.000 | 3.0 ±0.000 | P>0.05 |
| CL(ml/h) | 970.7 ±0.330 | 1022.7 ±0.316 | P>0.05 |

*Met. = Metformin*

**Table 4.7:Comparison of pharmacokinetics of metformin(mean, n=6) alone and when co-administered withbendrofluazide in type 2 diabetic patients**

| Pharmacokinetic parameter | Met. Alone | Met.+bendro. | P-value |
|---|---|---|---|
| Lag time(h) | 0.12 ±001 | 0.12 ±0.002 | P>0.05 |
| t ½ abs (h) | 1.45 ±0.016 | 1.23 ±0.052 | P>0.05 |
| K abs (/h) | 0.478 ± 0.002 | 0.563 ±0.067 | P>0.05 |
| Cmax (ng/ml) | 1890.67±0.107 | 1865.67 ±0.002 | P>0.05 |
| $AUC_{0\text{-}8}$ (ng/ml/h) | 8882.10 ±0.205 | 8985.83 ± 0.041 | P>0.05 |
| $AUC_{0\text{-}\infty}$ (ng/ml/h) | 12106.87 ±0.061 | 13626.77 ±0.006 | P>0.05 |
| Vd (L) | 112.59 ±0.062 | 111.29 ±0.120 | P>0.05 |
| t ½ el (h) | 6.0 ±0.000 | 7.48 ±0.038 | P>0.05 |
| K el (/h) | 0.116 ±0.000 | 0.093 ±0.073 | P>0.05 |
| T max (h) | 3.0 ±0.000 | 3.0 ±0.000 | P>0.05 |
| CL(ml/h) | 970.7 ±0.330 | 1200.7 ±0.01 | P>0.05 |

*Met + bendro = metformin co-administered with bendrofluazide*

**Table 4.8: Comparison of pharmacokinetics of metformin(mean, n=6) aloneand when co-administered withnifedipine in type 2 diabetic patients**

| Pharmacokinetic parameter | Met. Alone | Met.+nifedipine | P-value |
|---|---|---|---|
| Lag time(h) | 0.12 ±001 | 0.1 ±0.701 | P>0.05 |
| t ½ abs (h) | 1.45 ±0.016 | 1.12 ±0.031 | P>0.05 |
| K abs (/h) | 0.478 ± 0.002 | 0.619 ± 0.057 | P<0.05 |
| Cmax (ng/ml) | 1890.67±0.107 | 2357.16 ± 0.101 | P<0.05 |
| $AUC_{0-8}$ (ng/ml/h) | 8882.10 ±0.205 | 10724.57 ± 0.001 | P<0.05 |
| $AUC_{0-\infty}$ (ng/ml/h) | 12106.87 ±0.061 | 13238.58 ±0.011 | P<0.05 |
| Vd (L) | 112.59 ±0.062 | 93.24 ± 0.430 | P>0.05 |
| t ½ el (h) | 6.0 ±0.000 | 5.0± 0.310 | P>0.05 |
| K el (/h) | 0.116 ± 0.0 | 0.138 ± 0.003 | P>0.05 |
| T max (h) | 3.0 ±0.000 | 3.0 ± 0.000 | P>0.05 |
| CL(ml/h) | 970.7 ±0.330 | 675.65 ± 0.020 | P<0.05 |

*Met. = Metformin*

### 4.3.7 Effect of drugs administration on sugar level in type 2 diabetic patients

The results of blood sugar level of type 2 diabetic patients when metformin is administered alone and when it is co-administered with methyldopa, bendrofluazide and nifedipine were presented in tables 4.9, 4.10 and 4.11 respectively.

**Table 4.9:Comparison of mean sugar level in group treated with metformin alone and metformin when co-administered with methyldopa**

| Time (h) | Met.alone (mmol/l) (mean) | Met.+methyldopa(mmol /l)(mean) | P – value |
|---|---|---|---|
| 0 | 6.3 ± 0.02 | 8.0 ±0.67 | P<0.05 |
| 2 | 7.9 ± 0.73 | 13.1 ± 0.12 | P<0.05 |
| 3 | 6.4 ± 0.31 | 9.2 ± 0.22 | P<0.05 |
| 5 | 8.0 ± 0.43 | 9.2 ± 0.06 | P>0.05 |
| 8 | 8.0 ± 0.57 | 7.1 ± 0.38 | P>0.05 |

**Table 4.10:Comparison of mean sugar level in group treated with metformin alone and metformin when co-administeredwithbendrofluazide**

| Time (h) | Met. alone (mmol/l)(mean) | Met.+bendro (mmol/l) (mean) | P – value |
|---|---|---|---|
| 0 | 6.3 ± 0.02 | 6.4 ± 0.14 | P > 0.05 |
| 2 | 7.9 ± 0.73 | 10.8 ± 0.8 | P < 0.05 |
| 3 | 6.4 ± 0.31 | 7.9 ± 0.1 | P > 0.05 |
| 5 | 8.0 ± 0.43 | 4.0 ± 0.4 | P < 0.05 |
| 8 | 8.0 ± 0.57 | 2.7 ± 0.2 | P < 0.05 |

**Table 4.11:Comparison of mean sugar level in group treated with metformin alone in and metformin when co-administered withnifedipine**

| Time (h) | Met. alone (mmol/l) (mean) | Met.+ nifedipine (mmol/l) (mean) | P – value |
|---|---|---|---|
| 0 | 6.3 ± 0.02 | 3.8 ± 0.22 | P<0.05 |
| 2 | 7.9 ± 0.73 | 7.1 ± 0.19 | P>0.05 |
| 3 | 6.4 ± 0.31 | 5.5 ± 0.81 | P0.05 |
| 5 | 8.0 ± 0.43 | 4.3 ± 0.01 | P<0.05 |
| 8 | 8.0 ± 0.57 | 1.0 ± 0.67 | P<0.05 |

# CHAPTER FIVE

## 5.0 DISCUSSIONS

### 5.1 In-vitro Studies

The results of in-vitro studies have shown that all the drugs used in this study have passed Quality Control tests according to B.P. 1998 and 2002.

#### 5.1.1 Identification test of the drugs

The identification tests for all the drug tablets as well as the reference standards have shown positive results. This indicated that the drugs used contained the active ingredients. Therefore, the result of the analysis is not affected by fake drugs. Similarly, the positive results of reference standard clearly showed how that the test of identification is controlled

#### 5.1.2 Assay test of the drugs

The assay test result for the drugs used and the reference standard are within acceptable range of Official book. This confirmed that the drugs are of high quality standard and the assay method is précised. Therefore, the quality of the research was not affected by substandard drugs.

#### 5.1.3 Disintegration test of the drugs

Result of disintegration test of the drugs used also confirmed the quality of the drugs thereby, the outcome the research is assured.

## 5.2 In-vivo Studies

### 5.2.1 Pharmacokinetics of metformin in healthy human subjects and type 2 diabetic patients with hypertension

The results of the pharmacokinetic parameters of metformin (1 g) in healthy subjects and in type 2 diabetic patients showed that there were no significant changes when compared. This indicated thatdiseases did not affect the pharmacokinetics of metformin (1 g) in the population studied. Similarly, the pharmacokinetics of metformin (1 g) is unaffected by gene and environment.

Bhavesh*et al.* (2007) worked on healthy human subjects in India and their result showed that there were no significant changes in the pharmacokinetics of metformin (1 g) in the studied population when compared to the establishedpharmacokinetics of metformin (1 g).

There was however a significant difference in the elimination half- life $t½_{\beta}$ of metformin, which increased significantly from4.4 hrs to 6.0 hrs in type 2 diabetic patients with hypertension. The observation could be due to perfusion at absorption site which influences absorption of metforminas a result of the disease (Marchant, 1981).

### 5.2.2 Effects of methyldopa on pharmacokinetics of metformin

The changes observed in pharmacokinetics parameters were not statistically significant ($P > 0.05$). It was reported that pharmacokinetic drug interactions among medications used to treat diabetes are not very common because antidiabetic agents are generally not substrates, inducers, or inhibitors of the major CYP450 enzymes(Tatro, (2000);Hanstenand Horn, 2002). However,methyldopa would, in non-acid drugs, compete for the same pathway through the kidney (RxList, 2014).Methyldopa(oral) will increase the level or effect of metformin (oral) by basic (cationic) drug competition for renal tubular clearance (Chobanian, *et al.*, 2003).

However, co-administration of metformin (1 g) with cephelaxin (0.5 g) orally, increased Cmax and AUCs by 34 % and 24 % respectively and reduced clearance by 14 %. This is due to inhibition of renal tabular secretion of metformin which resulted in high cieculation of metformin concentration (Jayasagar, *et al.*, 2002)

The mean postprandial glucose level increased significantly at 2hrs (P <0.05) but reduced at 3hrs, 5hrs and 8hrs with administration of metformin alone and in combination with Methyldopa. The increment observed in glucose level could be due to increase in sugar level as a result of food taken.

There was direct relationship between metformin level alone, in combination with methyldopa and hypoglycemic response in the subjects investigated. .

### 5.2.3 Effects of bendrofluazide on pharmacokinetics of metformin.

The changes in pharmacokinetic parameters were not statistically significant when metformin was administered alone and co-administered with bendrofluazide. Though there was little variation, the changes were minor or insignificant. Because metformin affectsbendrofluazide by increasing sugar level thereby reducing the effect of antidiabetic drug (RxList, 2015).

Eileen *et al*. (2009) also reported insignificant changes in pharmacokinetics of metformin when co-administered with a thiazide,diuretics like bendrofluazide from healthy human subjects population work. This is in conformity with this work, though this work was in type 2 diabetic patients having hypertension because there was no variation in pharmacokinetics of metformin (1 g) as earlier stated.

Although, a clear theoretical mechanism is not obvious, metformin and the thiazides interact with different transporters, thiazides could affect transporter activity through altered ionic balance(Allhat, 2002).

### 5.2.4 Effects of nifedipine on pharmacokinetics of metformin.

$C_{max}$, AUCs and CL significantly increased (P<0.05) but the changes in other pharmacokinetic parameters are statistically insignificant when metformin (1 g) was co-administered with nifedipine (30 mg) in type 2 diabetic patient having hypertension. This could be due to competitive inhibition for hepatic metabolism of each drug.

RxList, (2010) reported increased in$C_{max}$ and AUC when metformin was co-administered with nifedipine in healthy human subjects.This was because nifedipine might have enhanced the absorption of metformin by enhancing stomach and intestinal absorption (RxList, 2014).

Another metformin – nifedipine drugs interaction studies conducted on healthy rats showed increase in Cmax and AUCs with Tmax unaffected. The changes in these pharmacokinetic parameters were explained in the study to be due to competitive inhibition for the metabolism of nifedipine and metformin via intestine CYPA31/2 in addition to competitive inhibition for hepatic metabolism of each drug (Choi and Lee, 2012).

It was also observed that blood sugar level dropped from 7.1mmol/L at 2hrs to 1.0mmol/L at 8hrs when metformin (1 g) was co-administered nifedipine (30 mg) in type 2 diabetic patient having hypertension in the population studied. This could likely, be due to an increased in intestinal absorption of glucose as a result of higher circulation of plasma metformin concentration (increased in $C_{max}$ and AUCs) (Howlett and Bailey, 1999).

Significant reduction ($P < 0.05$) of blood glucose levels were observed at 5 and 8hrs when metformin (1 g) was co-administered with nifedipine (30 mg) as compared with metformin (1 g) administered alone in type 2 diabetic patients. As $C_{max}$ and AUC increased the blood glucose level decreased. This could be due high circulation of concentration of metformin (Jayasagar, *et* *al.,* 2002). Therefore, there was direct relationship in the clinical effect observed and the increment in the serum levels of metformin when co-administered with nifedipine.

# CHAPTER SIX

## 6.0 CONCLUSION

The utility of the HPLC method for the determination of metformin concentration in blood plasma of healthy subjects was demonstrated and the results obtained compared favorably with those of other workers.

The results of metformin interaction with methyldopa and bendrofluazide showed insignificant interactions ($P>0.05$). However, nifedipine enhances the concentration of metformin.Therefore,there was a direct relationship between the hypoglycemic response of metformin at the absorptive phase when administered alone or with any of the drugs (methyldopa, bendrofluazide and nifedipine) investigated.

## 6.1 Recommendation

It is recommended that metformin can be co-administered with bendrofluazide and methyldopa in Type 2 diabetic patients with hypertension but patients who requirenifedipine should be closely monitored.

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**APPENDICES**

**APPENDIX I: Plasma concentration of metformin for individual healthy volunteers (ng/ml)**

| Time(h) | V1 | V2 | V3 | V4 | V5 | V6 | Mean ±SEM |
|---|---|---|---|---|---|---|---|
| 0.0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 ±0.00 |
| 0.5 | 884.87 | 890.92 | 868.68 | 970.89 | 985.10 | 710.92 | 884.67 ±0.35 |
| 1.5 | 1557.57 | 1517.17 | 1528.28 | 1653.53 | 1464.64 | 1412.12 | 1521.83±0.29 |
| 3.0 | 1972.72 | 1917.17 | 1928.28 | 1553.53 | 1964.64 | 1812.12 | 1857.67±0.27 |
| 5.0 | 1013.43 | 1020.20 | 1120.40 | 1011.31 | 1022.42 | 1015.15 | 1015.17±0.27 |
| 6.0 | 542.43 | 525.28 | 554.44 | 630.31 | 524.24 | 541.43 | 552.17±0.14 |
| 8.0 | 358.56 | 382.28 | 344.44 | 370.70 | 394.41 | 386.95 | 372.33±0.29 |

KEY :V1-V6 =Volunteer 1 – 6

**APPENDIX II: Plasma meanconcentrations of Metformin alone in Type 2 Diabetic patients (ng/ml)**

| Time(h) | P1 | P2 | P3 | P4 | P5 | P6 | Mean±SEM |
|---|---|---|---|---|---|---|---|
| 0.0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.0±0.00 |
| 0.5 | 622.22 | 723.23 | 741.41 | 711.11 | 723.23 | 724.24 | 707.33±0.39 |
| 1.5 | 1573.73 | 1453.53 | 1465.65 | 1539.39 | 1482.82 | 1552.52 | 1510.67±0.20 |
| 3.0 | 1825.25 | 1735.35 | 1765.65 | 1718.18 | 1862.62 | 1839.39 | 1890.67±0.22 |
| 5.0 | 1136.25 | 1158.30 | 1154.45 | 1174.25 | 1214.12 | 1157.56 | 1165.50±0.25 |
| 6.0 | 810.25 | 811.53 | 809.05 | 812.37 | 802.71 | 798.51 | 807.74±0.16 |
| 8.0 | 398.95 | 378.78 | 326.26 | 397.97 | 381.81 | 359.59 | 372.67±0.92 |

KEY:

P1-P6 = Patient 1-6

**APPENDIX III: Plasma meanconcentration of Metformin when co-administered with methyldopa in Type 2 patients (ng/ml)**

| Time(h) | MA1 | MA2 | MA3 | MA4 | MA5 | MA6 | Mean±SEM |
|---|---|---|---|---|---|---|---|
| 0.0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00±0.00 |
| 0.5 | 700.50 | 714.62 | 654.27 | 652.26 | 746.73 | 702.51 | 694.67±0.08 |
| 1.5 | 1398.32 | 1383.22 | 1377.18 | 1383.22 | 1381.21 | 1429.53 | 1392.28±0.05 |
| 3.0 | 1772.72 | 1732.32 | 1751.51 | 1760.60 | 1728.28 | 1770.70 | 1752.17±0.14 |
| 5.0 | 1282.82 | 1263.63 | 1258.58 | 1274.74 | 1271.71 | 1310.10 | 1276.33±0.08 |
| 6.0 | 998.98 | 976.76 | 1020.20 | 997.97 | 996.96 | 987.87 | 996.96±0.06 |
| 8.0 | 333.22 | 335.35 | 333.33 | 330.30 | 331.31 | 340.40 | 333.67±0.02 |

**APPENDIX IV: Plasma meanconcentration of Metformin when co-administered withbendrofluazide in Type 2 patients (ng/ml)**

| Time(h) | MB1 | MB2 | MB3 | MB4 | MB5 | MB6 | MEAN±SEM |
|---|---|---|---|---|---|---|---|
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00±0.00 |
| 0.5 | 705.35 | 699.33 | 690.30 | 693,31 | 720.40 | 666.22 | 695.52±0.02 |
| 1.5 | 1455.82 | 1455.82 | 1460.84 | 1430.72 | 1457.83 | 1452.81 | 1451.67±0.02 |
| 3.0 | 1863.63 | 1864.64 | 1873.73 | 1852.52 | 1874.74 | 1866.66 | 1865.33±0.03 |
| 5.0 | 1223.23 | 1226.26 | 1277.27 | 1224.24 | 1225.25 | 1227.27 | 1225.33±0.01 |
| 6.0 | 863.63 | 863.63 | 853.53 | 862.62 | 860.60 | 861.61 | 860.16±0.01 |
| 8.0 | 433.83 | 432.82 | 422.72 | 434.84 | 432.82 | 431.81 | 430.33±0.02 |

**APPENDIX V: Plasma meanconcentration of metformin when co-administered withnifedipine in Type 2 patients (ng/ml)**

| Time(h) | MN1 | MN2 | MN3 | MN4 | MN5 | MN6 | MEAN±SEM |
|---|---|---|---|---|---|---|---|
| 0.0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00±0.00 |
| 0.5 | 918.59 | 928.64 | 922.61 | 942.71 | 924.62 | 934.67 | 928.67±0.02 |
| 1.5 | 1567.45 | 1566.44 | 1552.35 | 1572.55 | 1555.48 | 1582.37 | 1566.16±0.03 |
| 3.0 | 2339.39 | 2374.74 | 2353.53 | 2361.61 | 2364.64 | 2348.48 | 2357.16±0.05 |
| 5.0 | 1631.31 | 1630.30 | 1622.22 | 1626.26 | 1618.18 | 1625.25 | 1625.25±0.02 |
| 6.0 | 920.20 | 924.42 | 927.27 | 933.33 | 924.24 | 926.26 | 926.28±0.02 |
| 8.0 | 351.51 | 349.29 | 345.45 | 347.47 | 346.46 | 350.50 | 348.48±0.01 |

**APPENDIX VI: Plasmamean concentration of metformin alone and in combination of other drugs**

| Time(h) | Metformin alone in healthy | Metformin alone in patients | Met. +methyldopat in patient | Met. + Bendro in patients | Met. + Nifedipine in patients |
|---|---|---|---|---|---|
| 0.0 | 0.00 ±0.00 | 0.0±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| 0.5 | 39.68 ±0.35 | 21.23±0.39 | 3.48±0.08 | 2.32±0.02 | 4.64±0.02 |
| 1.5 | 55.23 ±0.29 | 24.94±0.20 | 9.28±0.05 | 5.80±0.02 | 10.44±0.03 |
| 3.0 | 59.41±0.27 | 26.40±0.22 | 26.69±0.14 | 33.65±0.03 | 18.57±0.05 |
| 5.0 | 53.15±0.27 | 25.82±0.25 | 12.76±0.08 | 16.25±0.01 | 16.25±0.02 |
| 6.0 | 51.52±0.14 | 14.74±0.16 | 9.96±0.06 | 11.60±0.01 | 9.28±0.05 |
| 8.0 | 42.72±0.29 | 7.89±0.92 | 2.32±0.02 | 5.80±0.02 | 3.48±0.01 |

## APPENDIX VII: CALCULATION OF METFOMIN CONTENT

Weight of 20 tablets of 500mg metformin = 12.601g

Average weight $= \frac{12.601}{20} = 0.63g$

Therefore;

0.5g of metformin table = 0.63g

So,

0.1g of powder metformin $= \frac{0.1 \times 0.63}{0.5}$

$= 0.126g$

Weight of metformin powder used = 0.126g

Theoretical concentration of metformin solution prepared = 0.00126g/100ml

Actual concentration of prepared metformin solution $= \frac{\text{absorbance of the solution}}{(A_1\%, 1cm)}$

$= \frac{0.494}{739} = 0.00124g/100ml$

Therefore;

$\% \text{ content} = \frac{\text{Actual Concentration}}{\text{Theoretical Concentration}} \times 100$

$= \frac{0.00124}{0.00126} \times 100 = 98.4\%$

## APPENDIX VIII: CALCUALTION OF METHYLDOPA CONTENT

Weight of 250mg of 20 tablets = 7.075g

Average weight = $\frac{7.075}{20}$ = 0.35g

Therefore;

0.1g is equivalent to 0.142g of methyldopa powdered sample.

Methyldopa content was calculated by

$$\% \text{ content} = \frac{\text{absorbance of standard methyldopa}}{\text{Absorbance of sample prepared}} \times 100$$

$$= \frac{0.437}{0.438} \times 100 = 99.8$$

## APPENDIX IX: CALCULATION OF BENDROFLUAZIDE CONTENT

20 Tablets of 5mg tablet bendrofluazide

$$= 1.9826g$$

Average weight $= \frac{1.9826}{20} = 0.09913g$

Therefore

Weight of 0.015g of bendrofluazide

$$\frac{0.015 \times 0.09913}{0.005}$$

$$= 0.29739g$$

0.2939g was weighed and dissolved.

Theoretical concentration = 0.0015 g/100 ml

Actual concentration $= \frac{\text{absorbance of sample}}{(A_1\%, 1cm)}$

$$= \frac{0.609}{410} = 0.00149g/100ml$$

Therefore,

% contenct of Bendrofluazide in bendrofluzide tablet

$$= \frac{\text{Actual Concentration}}{\text{Theoretical Concentration}} \times 100$$

$$\frac{0.0015}{0.00149} \times 100 = 99.33\%$$

## APPENDIX X: CALCULATION OF NIFEDIPINE CONTENT

Weight of 10 tablets of 30mg nifedipine = 0.40415g

Average weight of nifedipine $= \frac{0.40415}{10} = 0.040415g$

0.13g of powdered tablet $= \frac{0.13 \times 0.040415g}{0.03}$

$= 1.75g$

1.75g was dissolved

Volume of sample titration = 120.7ml

Volume of blank titration = 16.2ml

Actual titre value = 120.7 – 16.2 = 104.5ml

1ml of ammonium cerium sulphate = 17.32mg nifedipine

Therefore;

104.5 x 17.32 = 1800.994mg

= 1.8099g of nifedipine

Percentage content of nifedipine $= \frac{\text{Actual weight}}{\text{Theoretical weight}} \times 100$

$= \frac{1.72}{1.810} \times 100$

$= 96.69\%$

## APPENDIX XI: PREPARATION OF IRON II SULPHATE-CITRATE SOLUTION

1g of sodium metabisulphate was dissolved in 200ml of distilled water followed by addition of 2 ml of 0.5MHCl, then 1.5g of Iron (II) Sulphate. 10g of sodium citrate was finally added.

## GLYCINE SOLUTION

42g of $NaHCO_3$ and 50g $KHCO_3$ were mixed with 180ml of distilled water followed by adding 15 ml 13.5M $NH_3$ with 180ml of distilled water. The solution was diluted to 500ml with distilled water. The solution was stirred until complete solution was formed.

## FERRION SOLUTION

1.76g of 1,10-phenanthroline HCl was dissolved in 70ml of distilled water than 0.7g of Iron (II) Sulphate was added. The solution was diluted

## APPENDIX XII:

)ata File C:\CHEM32\1\DATA\METFORMIN\METFORMIN 2014-12-18 11-03-57\009-0201.D
;ample Name: Mixed Std

==================================================================

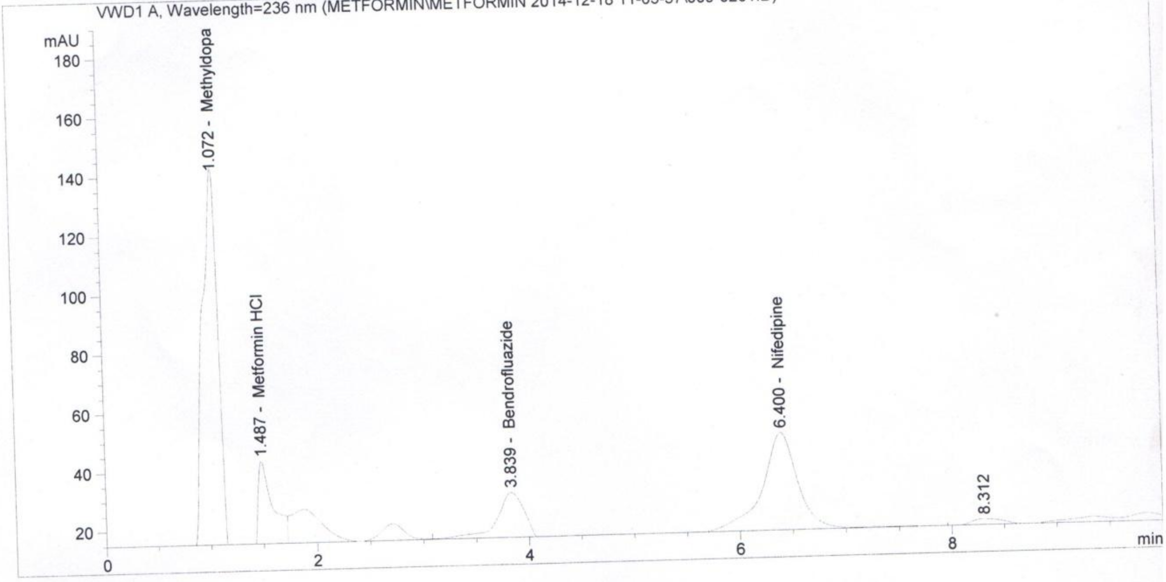
Acq. Operator : OLAJIDE M./BELLO S. Seq. Line : 2
Acq. Instrument : Instrument 1 Location : Vial 9
Injection Date : 12/18/2014 11:09:00 AM Inj : 1
Inj Volume : 20.000 µl

Acq. Method : C:\CHEM32\1\DATA\METFORMIN\METFORMIN 2014-12-18 11-03-57\METFORMIN.M
Last changed : 12/18/2014 11:11:03 AM by OLAJIDE M./BELLO S.
(modified after loading)

Analysis Method : C:\CHEM32\1\METHODS\METFORMIN.M
Last changed : 12/18/2014 11:23:12 AM by OLAJIDE M./BELLO S.
(modified after loading)

Sample Info : Mixed Std Metformin HCl; Methyldopa; Nifedipine & Bendr
ofluazide- 25Ug/mL
Column:ODS Hypersil C8 4.6 * 125mm, 5um
Mobile Phase: 25mMKH2PO4(pH10.63/ACETONITRILE:Methanol(
80:13:7)%
Wavelenght: 236nm
TEMP:30'C
Flow Rate: 1.000ml/min
Pharmaceutical Chemistry Laboratory
UNILAG

Additional Info : Peak(s) manually integrated




==================================================================

Area Percent Report

==================================================================

Sorted By : Signal
Calib. Data Modified : 12/18/2014 11:20:40 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Instrument 1 12/18/2014 11:23:16 AM OLAJIDE M./BELLO S. Page 1 of 2

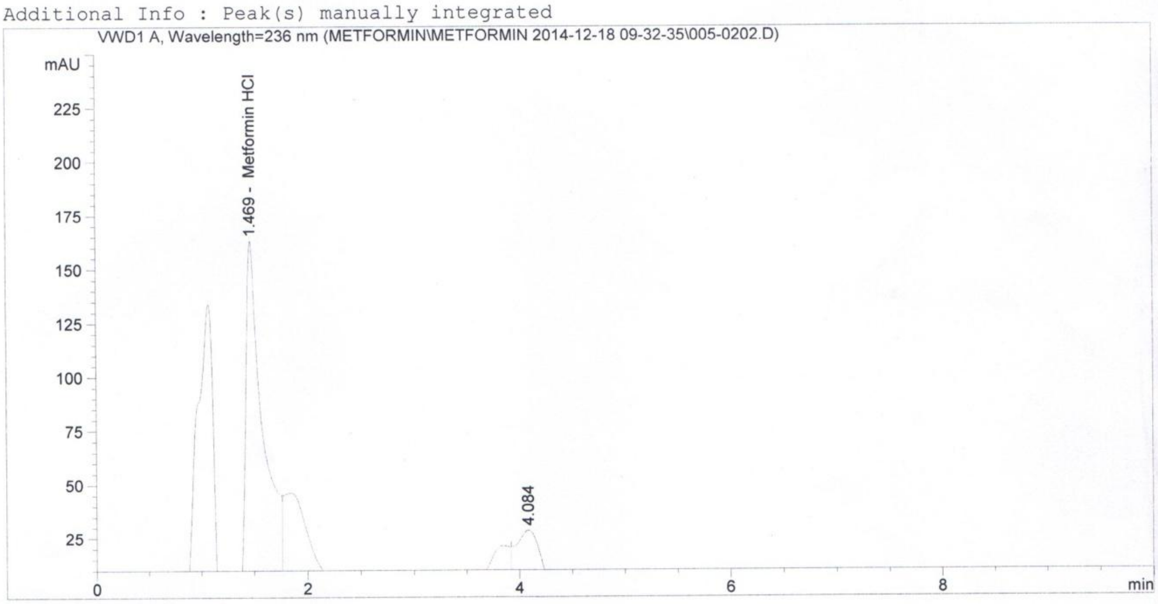
APPENDIX XIII

Data File C:\CHEM32\1\DATA\METFORMIN\METFORMIN 2014-12-18 09-32-35\005-0202.D
Sample Name: Std Metformin HCl

=====================================================================

```
Acq. Operator   : OLAJIDE M./BELLO S.            Seq. Line :   2
Acq. Instrument : Instrument 1                    Location : Vial 5
Injection Date  : 12/18/2014 9:45:15 AM                Inj :   2
                                                Inj Volume : 20.000 µl
Acq. Method     : C:\CHEM32\1\DATA\METFORMIN\METFORMIN 2014-12-18 09-32-35\METFORMIN.M
Last changed    : 12/18/2014 9:44:25 AM by OLAJIDE M./BELLO S.
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\METFORMIN.M
Last changed    : 12/18/2014 11:25:36 AM by OLAJIDE M./BELLO S.
                  (modified after loading)
Sample Info     : Std Metformin HCl - 25Ug/mL
                  Column:ODS Hypersil C8   4.6 * 125mm, 5um
                  Mobile Phase: 25mMKH2PO4(pH10.63/ACETONITRILE:Methanol(
                  80:13:7)%
                  Wavelenght: 236nm
                  TEMP:30'C
                  Flow Rate: 1.000ml/min
                  Pharmaceutical Chemistry Laboratory
                  UNILAG
```

Additional Info : Peak(s) manually integrated




=====================================================================

Area Percent Report

=====================================================================

```
Sorted By             :      Signal
Calib. Data Modified  :      12/18/2014 11:20:40 AM
Multiplier:                   :       1.0000
Dilution:                     :       1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Instrument 1 12/18/2014 11:25:45 AM OLAJIDE M./BELLO S.                    Page   1 of 2

APPENDIX XIV

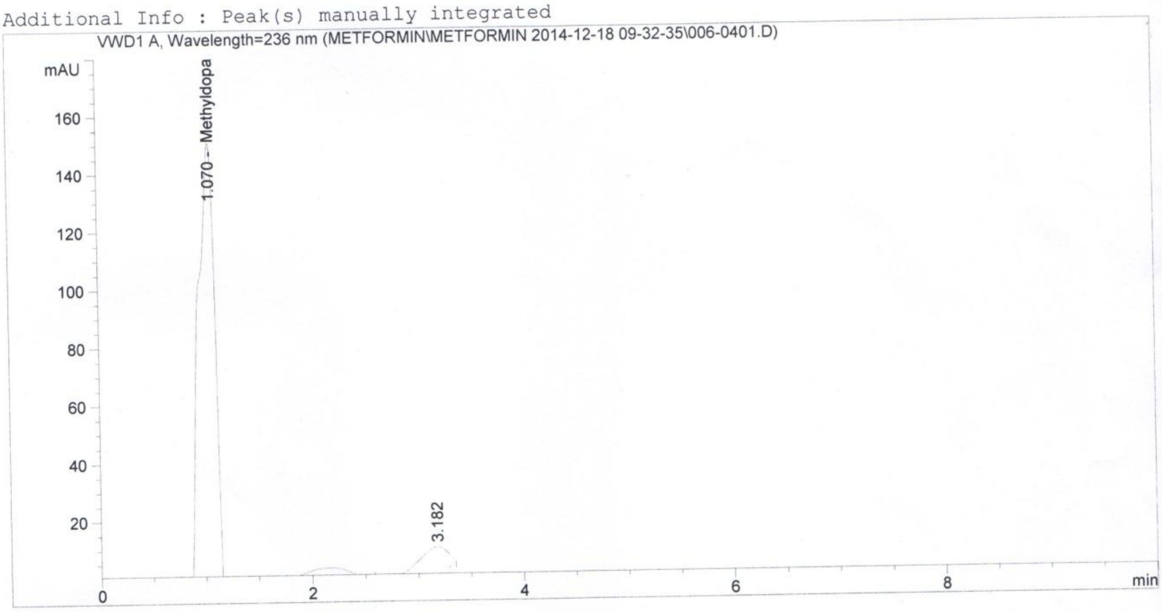
Data File C:\CHEM32\1\DATA\METFORMIN\METFORMIN 2014-12-18 09-32-35\006-0401.D
Sample Name: Std Methyldopa

=====================================================================

| | | | |
|---|---|---|---|
| Acq. Operator | : OLAJIDE M./BELLO S. | Seq. Line : | 4 |
| Acq. Instrument | : Instrument 1 | Location : | Vial 6 |
| Injection Date | : 12/18/2014 9:57:06 AM | Inj : | 1 |
| | | Inj Volume : | 20.000 µl |

Acq. Method : C:\CHEM32\1\DATA\METFORMIN\METFORMIN 2014-12-18 09-32-35\METFORMIN.M
Last changed : 12/18/2014 10:00:28 AM by OLAJIDE M./BELLO S.
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\METFORMIN.M
Last changed : 12/18/2014 11:28:00 AM by OLAJIDE M./BELLO S.
(modified after loading)
Sample Info : Std Methyldopa- 25Ug/mL
Column:ODS Hypersil C8 4.6 * 125mm, 5um
Mobile Phase: 25mMKH2PO4/ACETONITRILE:Methanol(80:13:7)
%
Wavelenght: 236nm
TEMP:30'C
Flow Rate: 1.000ml/min
Pharmaceutical Chemistry Laboratory
UNILAG

Additional Info : Peak(s) manually integrated




=====================================================================
Area Percent Report
=====================================================================

Sorted By : Signal
Calib. Data Modified : 12/18/2014 11:20:40 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Instrument 1 12/18/2014 11:28:04 AM OLAJIDE M./BELLO S. Page 1 of 2

APPENDIX XV

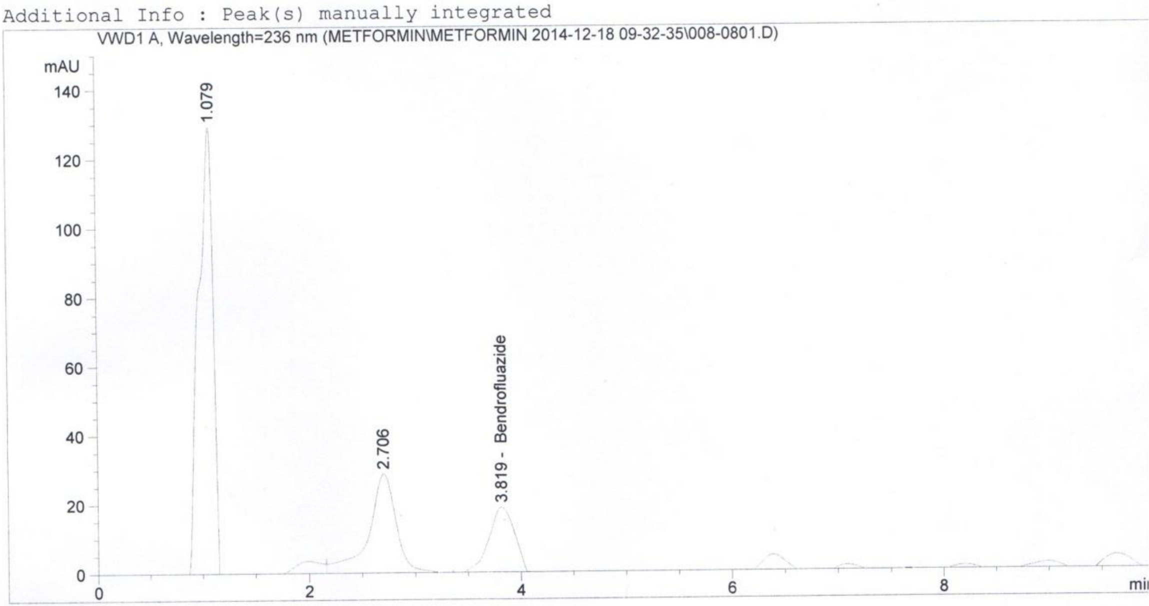
Data File C:\CHEM32\1\DATA\METFORMIN\METFORMIN 2014-12-18 09-32-35\008-0801.D
Sample Name: Std Bendrofluazide

=====================================================================

Acq. Operator : OLAJIDE M./BELLO S. Seq. Line : 8
Acq. Instrument : Instrument 1 Location : Vial 8
Injection Date : 12/18/2014 10:30:13 AM Inj : 1
Inj Volume : 20.000 µl

Acq. Method : C:\CHEM32\1\DATA\METFORMIN\METFORMIN 2014-12-18 09-32-35\METFORMIN.M
Last changed : 12/18/2014 10:29:49 AM by OLAJIDE M./BELLO S.
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\METFORMIN.M
Last changed : 12/18/2014 11:40:34 AM by OLAJIDE M./BELLO S.
(modified after loading)
Sample Info : Std Bendrofluazide - 25Ug/mL
Column:ODS Hypersil C8 4.6 * 125mm, 5um
Mobile Phase: 25mMKH2PO4(pH10.63/ACETONITRILE:Methanol(
80:13:7)%
Wavelenght: 236nm
TEMP:30'C
Flow Rate: 1.000ml/min
Pharmaceutical Chemistry Laboratory
UNILAG

Additional Info : Peak(s) manually integrated




=====================================================================
Area Percent Report
=====================================================================

Sorted By : Signal
Calib. Data Modified : 12/18/2014 11:40:12 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

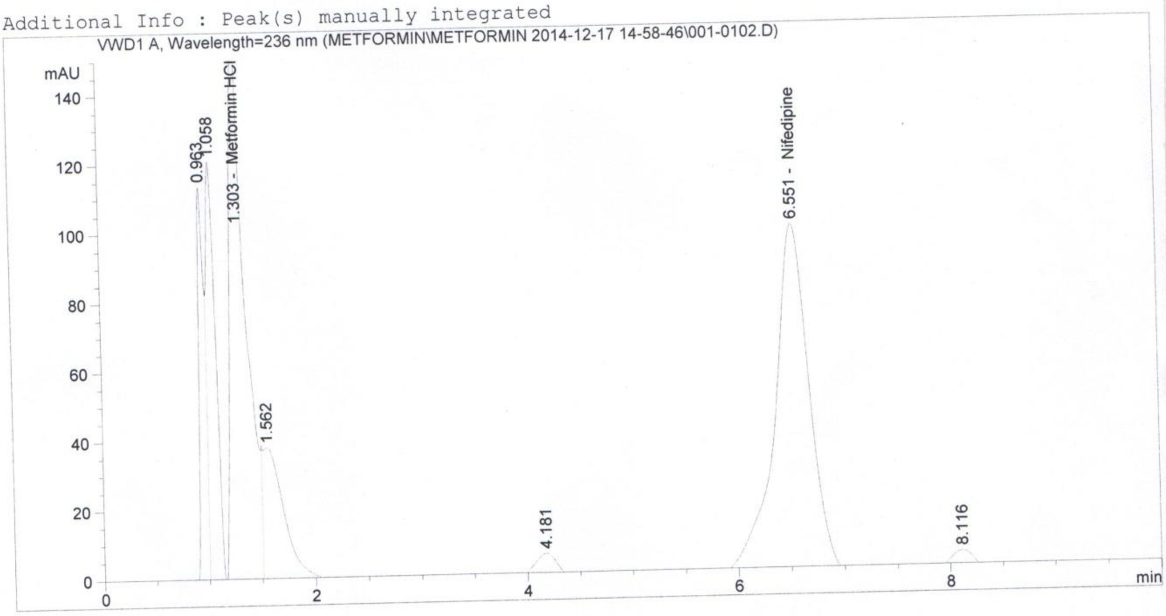
Instrument 1 12/18/2014 11:40:51 AM OLAJIDE M./BELLO S. Page 1 of 2

APPENDIX XVI

ata File C:\CHEM32\1\DATA\METFORMIN\METFORMIN 2014-12-17 14-58-46\001-0102.D
ample Name: Mixed Std

=====================================================================
Acq. Operator : OLAJIDE M./BELLO S. Seq. Line : 1
Acq. Instrument : Instrument 1 Location : Vial 1
Injection Date : 12/17/2014 2:59:43 PM Inj : 2
Inj Volume : 20.000 µl
Acq. Method : C:\CHEM32\1\DATA\METFORMIN\METFORMIN 2014-12-17 14-58-46\METFORMIN.M
Last changed : 12/17/2014 3:02:52 PM by OLAJIDE M./BELLO S.
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\METFORMIN.M
Last changed : 12/18/2014 11:43:39 AM by OLAJIDE M./BELLO S.
(modified after loading)
Sample Info : Std Metformin HCl & Nifedipine- 25Ug/mL-
Column:ODS Hypersil C8 4.6 * 125mm, 5um
Mobile Phase: 25mMKH2PO4 (pH 10.63)/ACETONITRILE:Methan
ol(70:30)%
Wavelenght: 236nm
TEMP:30'C
Flow Rate: 1.000ml/min
Pharmaceutical Chemistry Laboratory
UNILAG

Additional Info : Peak(s) manually integrated




=====================================================================
Area Percent Report
=====================================================================

Sorted By : Signal
Calib. Data Modified : 12/18/2014 11:43:40 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Instrument 1 12/18/2014 11:44:01 AM OLAJIDE M./BELLO S. Page 1 of 2

APPENDIX XVII

# POSTGRADUATE OFFICE
## FACULTY OF MEDICINE
## AHMADU BELLO UNIVERSITY TEACHING HOSPITAL
SHIKA - ZARIA, NIGERIA

P.M.B. 06 Samaru Zaria
Phone: 069 -551399
Fax: 069 - 555001
E-mail: abuthpg@yahoo.com

*Your Ref:*
ABUTH/PGO/COMM 9
*Our Ref:*

23rd November, 2010
*Date:*

Dr S S Bello
Dept of Pharmacentical Sciences
ABU
Zaria

Dear Dr Bello,

ETHICAL CERTIFICATE

Your research proposal titled **THE INFLUENCES OF ALDOMET, BENDROFLUAZIDE & NIFEDIPINE ON PHARMACCKINETICS AND PHARMACODYNAMIC OF METFORMIN IN HEALTHY AND DIABETIC PATIENS.** refers

This is to convey ethical committee's approval for commencement of the research.

You are required to keep this office informed of the progress being made on the research.

Yours sincerely,

DR. A J RANDAWA
Co-Ordinator Residency and ethics