THE EFFECT OF RIFAMPICIN ON THE PHARMACOKINETIC PARAMETERS OF NIFEDIPINE IN HEALTHY VOLUNTEERS

BY

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PHARMACEUTICAL ANALYSIS

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MARCH, 1995

DECLARATION

I hereby declare that this thesis has been written by me and it is a record of my research work carried out under the joint supervision of Dr. Abdullahi Mustapha and Dr. Ibrahim Abdu-Aguye. It has not been presented in any previous application for higher degree. The work of other investigators are acknowledged and referred to accordingly.

.....

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CERTIFICATION

This thesis entitled "THE EFFECT OF RIFAMPICIN ON THE PHARMACOKINETIC PARAMETERS OF NIFEDIPINE IN HEALTHY VOLUNTEERS" by NDANUSA BABANDAKO USMAN meets the regulations governing the awrad of the degree of MASTER OF SCIENCE (PHARMAEUTICAL ANALYSIS) OF AHMADU BELLO UNIVERSITY, and is approved for its contribution to Science, knowledge and literary presentation.

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DEDICATION

This work is dedicated to:

i My late Guardian..... Mallam Mohammed Ndanusa

ii My late Mother..... Malama Hawawu Nnaminin Usman

iii My late Father..... Alhaji Usman Ndakpako Manko

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ABSTRACT

Quality assessment of the three brands of nifedipine tablets/capsules were carried out. The identification tests, chemical assay and melting point method, determination showed that all the brands contained nifedipine as active ingredient. The results are in line with BP 1988 Addendun 1990 specifications for identification tests of nifedipine. Using U.V. spectroscopic methods, the U.V. spectra of all brands compared well with that of the reference with absorption maxima; at 206nm. 236nm and 344nm.

The estimated total content of active ingredient of the brands of nifedipine using HPLC and U.V.- assay methods gave results which complied with the BP 1988 specification for the drug content (98 - 102%).

The disintegration time for all the three brands of the nifedipine was less than 10 minutes. This result complied with BP 1988 specification for tab 1et/capsules disintegration time of not more than 15 minutes. The disssolution rate profiles of the three brands are similar. All the brands released more than 70% of their active ingredient in less than 30 minutes. These are within the limit specification by BP 1988 - of 45 minutes.

The effect of a single oral dose of rifampicin 1200mg on the pharmacokinetics parameters of nifedipine lOmg (Capsules) was studied in a cross-over design in six healthy human volunteers (Age 28 ± 6.3 years old and weight of 60.67 ± 3.50). The pharmacokinetic parameters; such as $ti_{a(jg}$, K_{abs} , t_{lag} , t_{raax} and Vd all showed no significant difference between the studies. Thus the

absorption kinetics related parameters are not significantly different between the studies. However, those kinetic parameters related with metabolism and excretion kinetics showed significant differences. AUC_{0-M} (relative bioavailability) was decreased by 62.2% (573.4 Vs 205.25 mg.hr/ml) $P < 0.0001$: $t_{½}$ el. was decreased by 67% (2.62 Vs 1.03 hr) $P < 0.0001$: k_e,. was increased by 157% (0.260 Vs 0.670 hr^{-1}) $P < 0.0001$; Cl_T was increased by 194% (17.33 Vs 50.17 ml/min.kg) $P < 0.0001$: C_{max} decreased by 67% (173.23 Vs 115.77 ng/ml) $P < 0.0001$.

This study showed that rifampicin increases the metabolism and elimination of nifedipine.

ABBREVIATIONS

B.P	=	British Pharmacopoeia
USP	=	United States Pharmacopoeia
HPLC	=	Hihg performance liquid chromatography
U.V.	=	Ultra Violet
PH	=	Hydrogen Ion Concentration
Hcl	=	Hydrochloric Acid
hr	=	Hour
ml	=	Millilitres
µl	=	Microlitres
mg	=	Milligramme
µg	=	Microgramme
ng	=	Nanogramme
gm	=	Gramme
Mw.	=	Molecular Weight
^0C	=	Degree centigrade (Celsius)
r.p.m.	=	Revolution per minute
log	=	Logarithm
In	=	Natural Logarithm
mm	=	Millimetres
GIT	=	Gastrointestinal tract.
Cl_T	=	Total body clearance
$t_{\frac{1}{2}}el$	=	Elimination Half life
$t_{\frac{1}{2}}abs$	=	Absorption Half life
K_{el}	=	Elimination rate Constant
$K_{abs.}$	=	Absorption rate constant

Vd = Apparent Volume of distribution

C_{max} = Maximum plasma concentration

t_{max} = Time to attain C_{max}

$AUC_{0-\infty}$ = Area under plasma - concentration Time curve

t_{lag} = Lag time.

nm = Nanometer

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CHAPTER ONE

1.0 *INTRODUCTION*:-

Drug action or effect can be regarded as ultimate consequences of physicochemical interactions between that drug and functionally important molecules in the living organisms. These important molecules are now recognised universally as receptors. Drug interaction is the modification of an effect of one drug by the presence of another, whether by direct or indirect means. Interactions may effect absorption, distribution, receptor action, metabolism or excretion. They may be beneficial or hazardous. Interaction may vary from person to person and from species to species. They may be of major clinical significance or no clinical significance at all (Reigelman et-al, 1970).

1.1 PHARMACOKINETIC STUDIES

The overall biological response of an organism to a drug depends on two major underlying phenomena. One is termed pharmacodynamics response, which is the action of the drug at specialized and specific site in the body to give pharmacological effect. The other is the action of the organism on the drug to impade its peneration or absorption, to dilute, distribute, transform and excrete the active principle(s), and thus diminish its availability at the site of action. The elucidation of the parameters which describe these procedures which modify the time course of the active drug in the body is a function of PHARMACOKINETICS.

Pharmacokinetics is concerned with obtaining the appropriate amount of the drug at receptor site for the appropraite length of time for that particular patient, thus obtaining maximum therapeutic benefit with minimum side effects.

For a drug to have effect, it must be absorbed into the body and must enter the organ or tissue where it exerts effect. For the effect to be terminated, the drug must be rendered in-active or be excreted from the body. This absorption, distribution elimination (metabolism and excretion) are the kinetic determinants of drug effect.

The absorption of the drug depends on the ability of the drug molecules to cross biological membranes which are chiefly lipoproteins. The transfer of drugs accross these membranes occurs mainly by passive diffusion, other methods are of transfer include - the active transport by specialized membranes (in guts and kidney), and the transfer of water soluble drugs (mol. wt. <100) through water filled pores. (Paxton, 1981).

The transfer of a drug from its absorption site to the blood, and the various steps involved in the distribution and elimination of the drug in the body are as follows:-

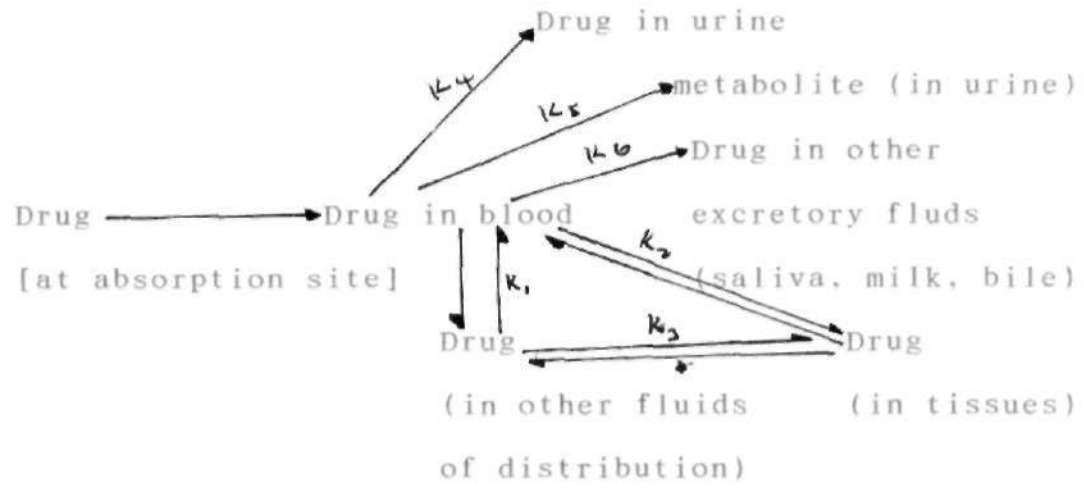


Figure 1.1:- Steps involved in the distribution and elimination of drugs in the body.

This figure 1.1 illustrates that drug distribution in the body *is reversible, while absorption and elimination phases are* irreversible. (Robert et-al 1975)

The extent and rate of absorption of drug from its absorption site is determined principally by the physico-chemical properties of the drug. Other factors are dissolution rate of the drug and ability of the drug to withstand the degrading effect of gastric enzymes and acids. (Paxton, 1981).

The primary standard of a pharmacokinetic study is based on the data obtained following intrevenois administration of drug in which the entire dose administered is placed directly into the blood stream; the pharmacokinetic parameters obtained are not *affected by all the factors that are potentially rate-limiting and* other metabolic factors associated with drug absorption following other routes of administration.

1.2 PRINCIPLES OF PHARMACOKINETICS

The basic approach used in pharmacokinetics is to fit experimental data on drug concentration in the plasma to mathematical equations. The complex flow of drugs and its metabolites through the body is simplified by considering the body to consist of a number of compartments. These are postulated to account for the experimental observations that drugs distribute into various fluids and tissues at different rates. These compartments have no anatomical boundaries, nor necessarily consist of one type of tissue. The number of compartments in any particular case will ultimately depend on the number of areas with different rate of drug penetration. Experimentally it is difficult to demontrate more than three compartments, and mostly only two can be differentiated.

Before any equation may be derived from these compartmental models, several assumptions have to be made. Firstly, drugs enter the system only via the central compartments, and are eliminated only from that compartment. Secondly reversible transfer occurs between central and peripheral compartments, and thirdly, the exit of drugs from compartments in the system is described by first-order kinetics. Under this last assumption the rate at which a drug is removed from a compartment is directly proportional to the drug concentration in it. Majority of the drugs enter the body via the blood stream, and are eliminated from the blood stream by the liver and kidney. Most of the drugs are eliminated by first-order kinetics. There are few exception which are primarily eliminated by

biotransformation, and if given in high enogh doses, result in saturation of the matabolic enzyme system. The elimination kinetics then become zero-order i.e. constant independant of drug concentration. (Paxton, 1981).

1.2.1 **Single compartiment model**

This is the simplest model. The whole body may be considered mathematically as a single compartment. In this mode it is assumed that exchange of drug between the plasma and tissues proceeds rapidly compared with the rate of elimination. Thus, there is instant distribution of the drug between plasma and other body tissues. Thus any change that occurs in the plasma drug concentration will be quantitatively reflected in the tissue drug levels. Assuming instantaneous distribution after intravenous (I.V.) injection of a drug into this model, the concentration (Co) in the plasma immediately after injection is equal to the dose (D) administered divided by the volume of this compartment (Vd_c). Vd_c - the apparent volume of distribution of the drug is not a true volume, but the volume into which all the drug in the body would appear to be distributed to achieve a concentration the same as that of plasma.

Also after instantaneous distribution, the concentration will fall according to first order kinetics, which means that a constant fraction of drug present is eliminated per unit time. Thus a plot of plasma drug concentration against time, with each unit representing the time for one half of the drug to be removed, results in exponential curve.

Reploting this curve with logarithms of concentration. results in a traight line.

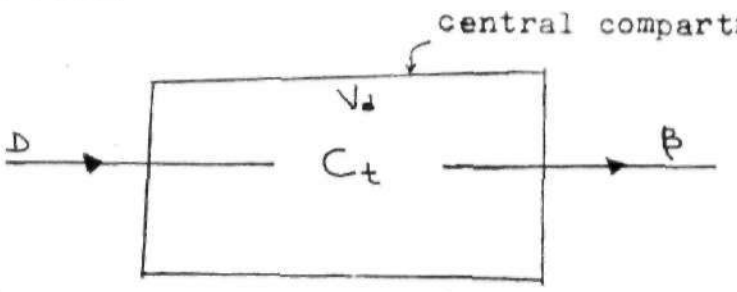


Fig. 1.2.1a. One compartment Model

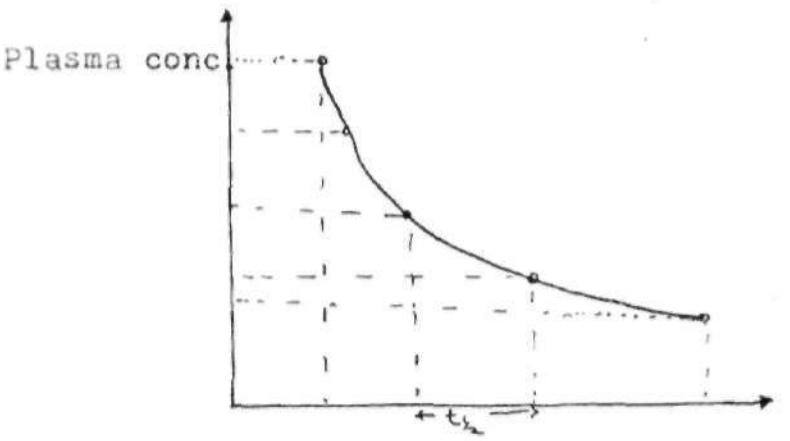


Fig. 1.2.1b. Plot of plasma conc. against time following rapid I.V. administration of a drug into single compartment.

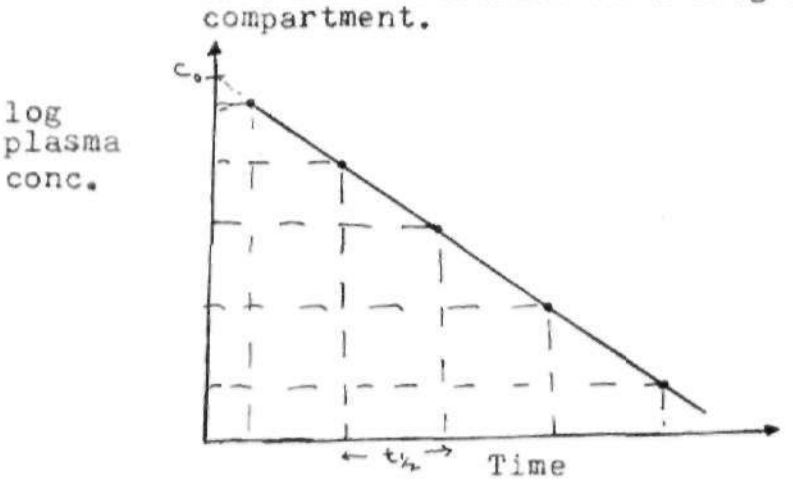


Fig. 1.2.1c. Plot of log plasma conc. against time.

The plot in figure 1.2.1 (c) can be extrapolated back to zero time to give theoretical initial concentration (Co).

The decline in drug concentration may be expressed *mathematically as:-*

$$\frac{dc}{dt} = -BC_t \quad \text{----> Equation 1}$$

Where : -

β ---> elimination rate constant

C_t ---> concentration

t ---> time

When equation 1 is intergrated -

$$C_t = C_0 \, e^{\beta} \quad \text{-----> Equation 2}$$

Logarithm

$$\text{In}c_t = \text{In}C_0 \, \beta t \quad \text{-----> Equation 3}$$

Logarithms to the base 10

$$\log c_t = \log C_0 - \frac{\beta t}{2.303} \quad \text{----> Equation 4}$$

1.2.2 Multi-compartment model

If the distribution of the drug is so slow that it cannot be disregarded, a model must then be considered which contain a central compartment and at least one peripheral compartment.

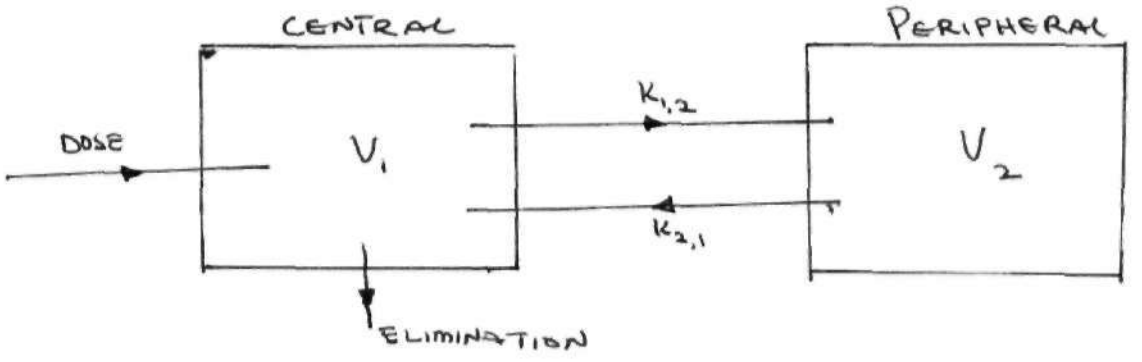


Fig 1.2.2.1- Two compartmental model

Figure:- 1.2.2.1 - Two compartment model kinetics. Although these compartments lack physical or anatomical reality, for many drugs the central compartment may correspond to the plasma volume together with extracellular fluid of highly perfused tissues such as heart, lungs, kidneys, liver and endocrine glands. Drugs distribute within few minutes between those tissues and equilibrium easily established. The peripheral compartment is formed by less perfused tissues such as muscle, skin or adipose tissues into which a drug may enter more slowly. The combined effect of two compartments give rise to a biphasic curve on I.V. injection with two distinct linear portions when drawn on a samilog ordinate scale (Fig 1.2.2.2 below). Although drug distribution to the peripheral compartment is slow, it is usually much faster than elimination. Thus the initial rapid fall in concentration known as alpha (&) phase or distribution phase mainly represents the relatively rapid process of drug distribution from central to peripheral compartment. Once the distribution is complete, the curve enters the relatively slow beta (β) or elimination phase during which the drug disappearance is determined mainly by irreversible elimination from central compartment.

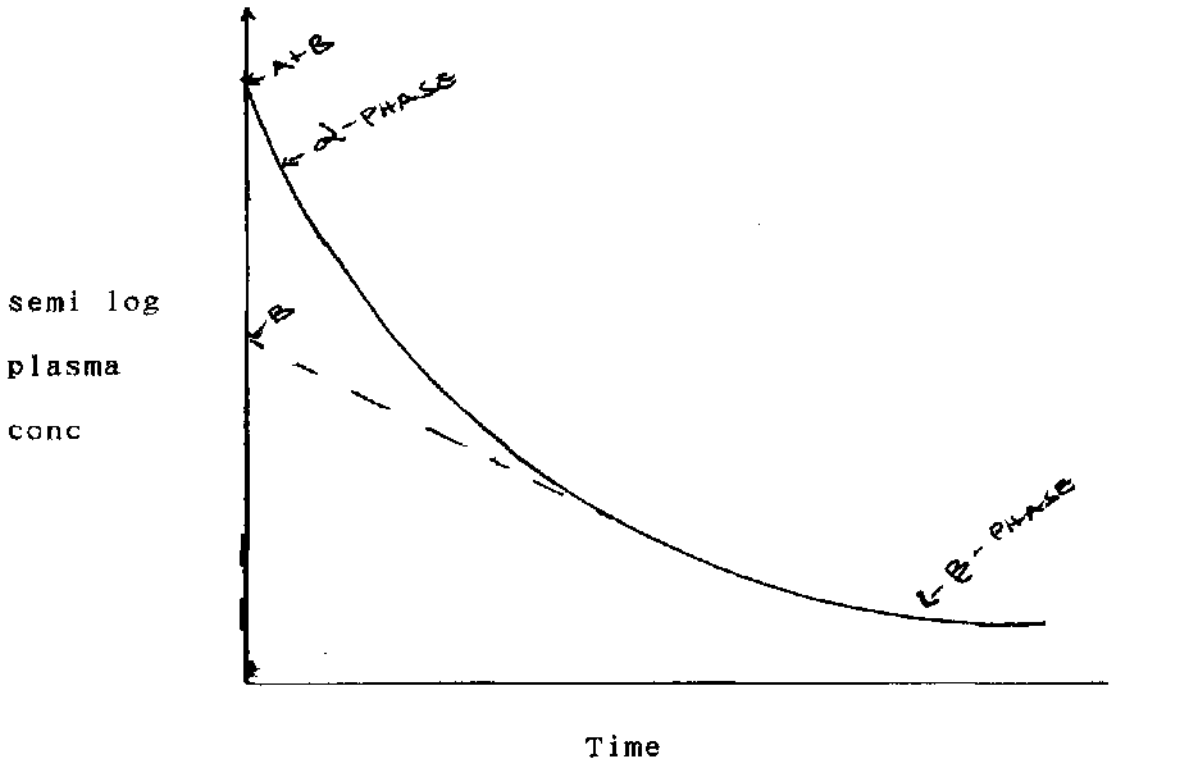


Figure 1.2.2.2:- Semi logarithmic plasma concentration time plot of a two compatment model.

The two compartment model may be expanded to contain additional compartment which can be described mathematically as the sum of as many individual exponential functions as there are relavant compartments. In reality, the available assay techniques in most cases allow the definition of a maximum of three compartments; with the two compartment model being the most popular.

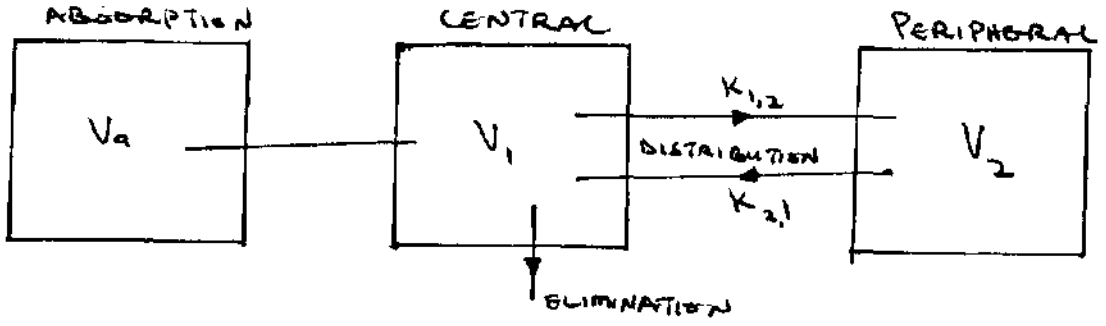


Figure 1.2.2.3 - Three compartment model.

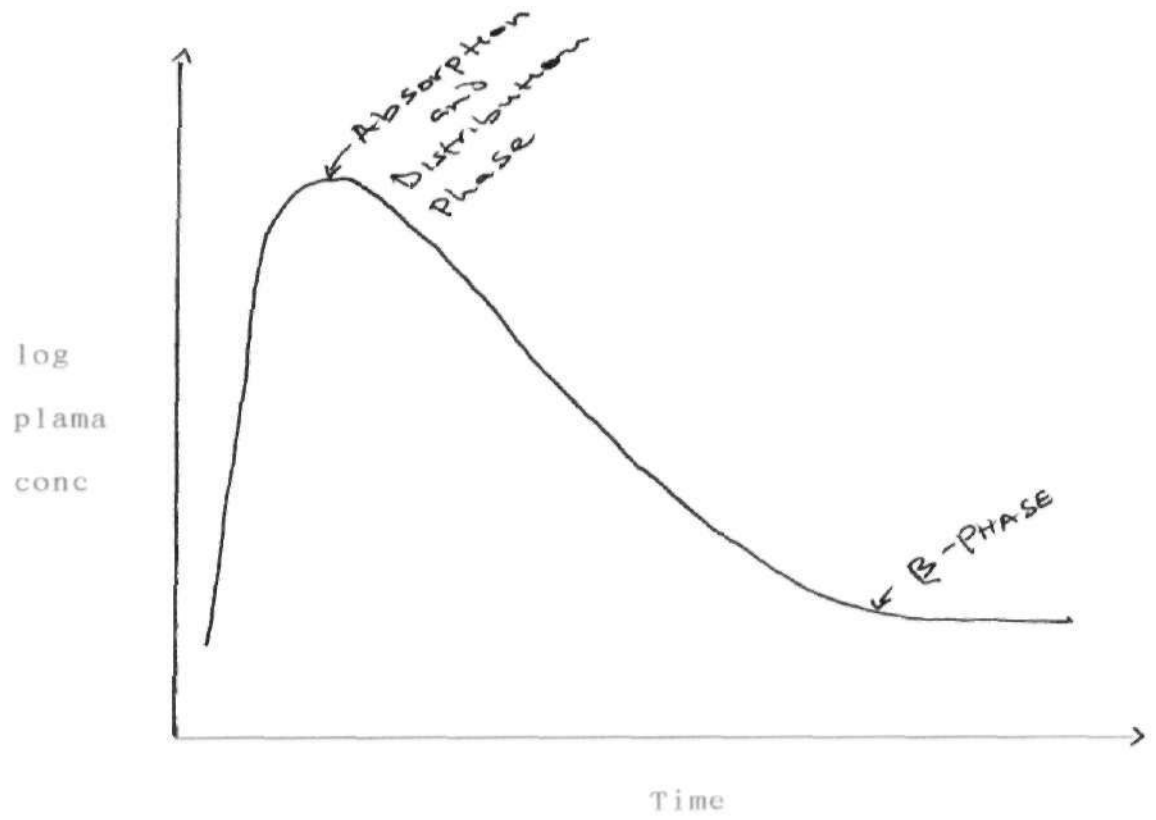


Figure 1.2.2.4 - A typical log concentration. time plot after a single oral dose is shown.

With an oral dose an additional absorption compartment is incorporated into the model to represent the volume (V_g), from which absorption occurs at a first-order rate (figure 1.2.2.2). In this model it is assumed that the entire drug is introduced rapidly into the absorption site, from which it is absorbed into the central compartment.

A typical equation for this type of plots with preliminary absorptive phase can be represented as the integral, $\int c.dt$, which represent area under curve, and which is a measure of the ammount of drug which is systemically available (bio-available); and is a major determinant of therepeutic efficacy (Paxton, 1981).

1.3 PHARMACOKINETIC PARAMETERS

Some of the most useful pharmacokinetic parameters obtained following drug administration are plasma half life, plasma clearance, apparent volume of distribution, Area Under Curve, bioavailability, rate constants for elimination, absorption constant.

1.3.1 ELIMINATION (PLASMA) HALF-LIFE:-

The elimination half-life ($t_{\frac{1}{2}}$) is the time taken for the half of the amount of drug present in the plasma to be eliminated, either by excretion or metabolism or both (Paxton, 1981). In practical terms it is the time taken for the plasma concentration to be reduced by fifty percent (50%) of its original value. (Paxton, 1981; Wagner, 1980).

It can be determined for both one and two compartment models from the terminal data of the log concentration - time plot. substituting for $t_{\frac{1}{2}}$ in equation - 3 results in:-

$$\ln x = \ln 2x - \beta t_{\frac{1}{2}}$$

$$\text{i.e. } \beta t_{\frac{1}{2}} = \ln 2$$

$$\text{But } \ln 2 = 0.693$$

$$\therefore \quad t_{\frac{1}{2}} = \frac{0.693}{\beta} \qquad \ldots\ldots \text{ Equation } 5$$

From the equation (5) it appears that a large value for β (Elimination rate constant) correspond to a short half life ($t_{\frac{1}{2}}$). However $t_{\frac{1}{2}}$ is a poor index of drug elimination as it depends not only on the clearance (elimination process), but also on the volume

through which the drug is distributed. This is supported by the fact that the half life is directly proportional to the apperent volume of distribution. (Paxton, 1981). Mathematically it is expressed as:-

$$t_{\frac{1}{2}} = \frac{0.693\ Vd}{Cls} \qquad \text{..... Equation (6)}$$

Although $t_{\frac{1}{2}}$ is a poor index of drug elimination processes and dosage requirements in individual patients, it is a useful parameter in the determination of suitable dosage intervals and the time required to attain the steady state during constant intravenous or chronic oral therepy.

1.3.2 PLASMA CLEARANCE:

Plasma or systemic clearance is defined as the volume of plasma from which a drug is totally and irreversibly removed per unit of time. It is a direct index of drug elimination. Total plasma clearance of a drug, which results from all processes of elimination (i.e. hepatic metabolism, renal and faecal excretion, exhalation by the lungs), can be expressed by two types of relationship.

Firstly, one can divide the absorbed dosed by area under curve, as the latter value basically represent the total bioavailable drug subsquently eliminated:-

$$Cls = \frac{FD}{AUC} \qquad \text{.....} \quad (7)$$

Cls ---> plasma clearance

FD ---> Absorbed dose

AUC ---> Area under curve (Bio availability)

Secondly, the product of the elimination constant and apparent volume of distribution equals the clearance.

$$Cls = \beta Vd \quad ..... \quad (8)$$

β - elimination rate constant.

The clearance has dimensions of volume per unit time, and express the efficiency with which a drug is eliminated and is made up of the sum of all clearances due to individual organs. (Paxton, 1981).

If only one organ is involved, for example the kidney, the plasma clearance is equal to renal clearance.

For any organ, the physiological determinants of a drug clearance are organ blood flow, the inherent ability of the organ to extract the drug from the blood and extent of binding of drug to plasma proteins (Paxton 1981).

For drugs that are eliminated by the liver (i.e. under goes hepatic clearance) - their clearance will depend on hepatic blood flow, or on the hepatic enzyme activity. For the drugs which liver has a metabolic capacity, their clearance is limited by hepatic blood flow. Such drugs are relatively insensitive to changes in matabolizing activity such as produced by hepatic enzyme induction. Thus changes which either increase or decrease hepatic blood flow increases or decreases hepatic clearance respectively. If the elimination is dependant only on enzyme activity there hepatic clearance will be effected by any change in enzyme activity, but

will not be effected by changes in hepatic blood flow. In practise drugs with high extraction ratio (i.e. >0.7) or hepatic clearance which is a large fraction of hepatic blood flow will experience flow limited clearance, whereas those with a small extraction ratio (<0.2) will be independant of hepatic blood flow, but on any changes in hepatic metabolizing enzyme activity and the binding properties of the drug (Paxton, 1981).

1.3.3 APPERENT VOLUME OF DISTRIBUTION:-

The term apparent volume of distribution is defined as that volume in which the amount of the drug in the body would need to be uniformly distributed to produce the observed plasma concentration. The concept of apparent volume of distribution is used to relate the plasma concentration of a drug to the dose administered (if no elimination has occurred), or to the amount of the drug in the body. It does not relate to a real physiological or anatomical space, but provide an estimate of the extent of drug's distribution through the body fluid compartment and its up take by tissues. A large apparent volume of distribution implies wide distrubution or extensive tissue up-take or both (Paxton, 1981). The apparent volume of distribution is inversely proportional to the blood level. Various ways of calculating apparent volume of distribution are known, but these depend on the pharmacokinetic profile of the drug (Gibaldi and Perrier, 1975). The simplest method after intravenous injection in a one conpartment model is by back extrapolation to find the initial concentration (Co) at zero time. More widely used, especially after oral doses on in

multicompartmental models, is the volume term derived from the area under curve which accounts for the observed plasma concentrations on the basis of the total amount of drug in the body at all times after distribution equillibrium was been attained:- (Paxton, 1981).

$$Vd_{(area)} = \frac{FD}{\beta\ AUC} \quad \text{..... Equation (9)}$$

1.3.4 AREA UNDER THE CURVE:-

Area under the curve is a measure of the amount of drug which is systemically available (bioavailable) and is a major determinant of therepeutic efficacy (Paxton, 1981). It is also the integral of drug blood level over time from zero to infinity. (Gibaldi and Pierrier, 1975). The area under the curve (AUC) can be evaluated after different routes of administration by several methods. Some of the common methods are planimeter methods, cut and weigh method (i.e. to cut out the area under entire curve on rectilinear graph paper and weigh on analytical balance); the other methods which are most commonly used are the trapezoidal rule method and computer application.

The trapezoidal rule is independent of any compartment model proposed.

The AUC is useful for calculating important pharmacokinetic parameters such as the total plasma clearance of the drug and apparent volume of distribution (Paxton, 1981).

The area under a plasma drug concentration versus time curve has the units of concentration time - (i.e. $\mu g.hr\ ml^{-1}$).

1.3.5 **BIO-AVAILABILITY**:-

Bio-availability is the term used to indicate measurement of the relative amount of an administered dose that reaches the systemic circulation. (Paxton, 1981).

Bio-availability is defined as the rate and extent to which active drug ingredient or therapeutic moiety from a drug product becomes available at the site of action which is normally estimated by its concentration in fluids, rate of excretion or acute pharmacological effect (skelly, 1976). According to this definition, the absorption of an intravenously administered drug is rapid and complete. However, for several reasons, most drugs are administered orally and absorption into the systemic circulation occurs in gastro-intestinal tract. Therefore, their rate and extent of absorption in individual's are usually not precisely known (Blanchard and Sawchuck, 1979). The absolute bioavailability of an oral dose (or by any other route) cannot be determined unless an intravenous study with same drug is performed. It is worthwhile noting here that low oral bioavailability may not necessarily indicate poor absorption. Loss of drug to various degrees may occur due to rapid uptake and metabolism in the liver, as the drug is being transfered by the portal system from its absorption site in the gastrointestinal tract to the general circulation. This so called "first pass elimination" during the first circulation through the organ is observed for a number of drugs in common use (Paxton, 1981).

The therapeutic action of a drug is dependant on an adequate

concentration of the drug reaching the site of action and this concentration is dependant on the passage of the drug across biological membranes.

If two dosage forms of the same drug make their active contents available to the general circulation at same extent and at the same rate, they are said to be demonstrating equal bio-availability; that is, they are bioequivalent (Abdul-monem and Henkel, 1978).

The extent of absorption of drug can be determined by two methods:

i) By determination of the total area under the plasma drug concentration versus time curve (AUC).

ii) By determination of the total amount of unchanged drug excreted in the urine.

In both cases, these results are taken in comparison with results obtained with a standard preparation. This standard may be an intravenous injection or an orally administered aqueous solution or another drug accepted as a standard.

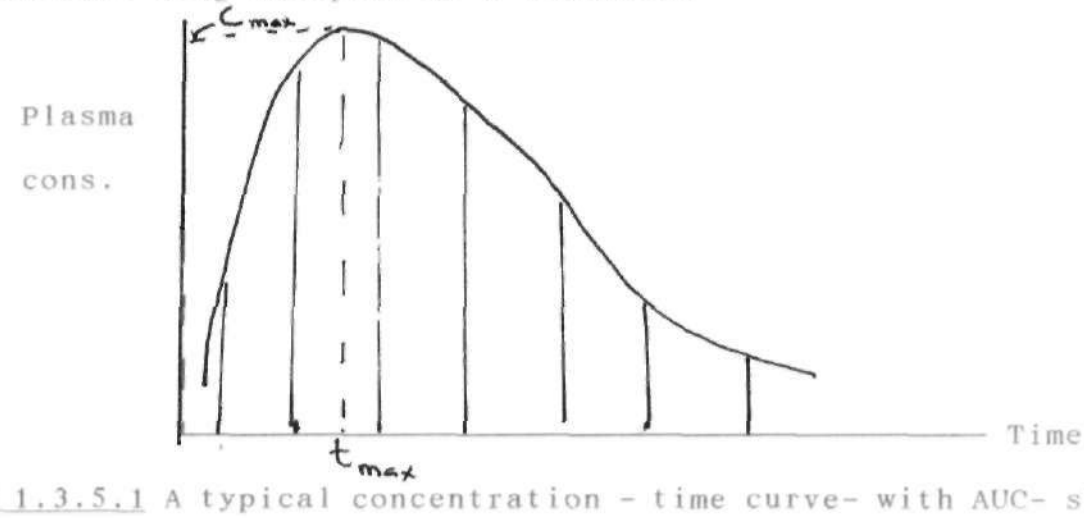


Fig 1.3.5.1 A typical concentration - time curve- with AUC- shaded.

where:- C_{max} ---> peak plasma concentration

t_{max} ---> time of peak plasma concentration.

The usual experiment does not yield plasma concentration data all the way to zero concentration. However when the experiment is carried out long enough to obtain post-absorptive data, then the area beyond the last concentration data point ($c^{'}$ at $t^{'}$) is readily estimated from the equation:-

$$AUC_{t^{'}-t^{'}} = C^{'}/\beta \qquad \text{..... Equation (10)}$$

This AUC must be added to the AUC calculated from time zero to time $t^{'}$ to obtain the total AUC.

$$\text{The total AUC} = \frac{FD}{\beta\ Vd} \qquad \text{..... Equation (11)}$$

F -------> fraction of the dose absorbed

$$F = \frac{\text{AUC (drug product)}}{\text{AUC (standard product)}} \qquad \text{..... Equation (12)}$$

-- Usually equal doses are administered.

The usual method for calculating the absolute bioavailability of an oral dose involves the comparison of the AUC after I.V. dose that for the same dose given orally (P.O.). The fraction absorbed

(f) is then equal to $\frac{\text{AUC (P.O.)}}{\text{AUC (I.V.)}}$ ..... Equation (13)

If the absorption is complete, the areas are equal and F = 1. However in most cases F < 1. The absorbed doses is then a product of fraction absorbed and administered dose (Gibaldi and Perreir, 1975; Blanchard and Sawchuk 1979).

1.3.5.1 FACTORS AFFECTING BIO-AVAILABILITY:-

The compressed tablet is the most commonly used oral dosage form, and is also most commonly involved in drug bioavailability and bioequivalence problems. This is not surprising owing to the reduction in drug surface area in the compressed formulation and the wide variety of drug forms, tabletting methodologies, excipients and coatings that are used.

Absorption may be affected not only by the different dosage forms, but also by similar dosage forms from different manufacturers and different batches of a dosage form from a single source. The most common conventional oral dosage forms, in increasing order of relative dissolution and absorption rates are in - sustained release prepration, coated tablets, tablets, capsules, suspensions and solution.

Dosage form related factors which can produce profound differences in bio-availability include formulation and manufacturing variables. Such as particle size, the physico-chemical properties of the drug, the type and quantity of excipients used (binders, lubricants, glidiants, diluents and disintegrants): the compaction force and other factors (Blanchard and Sawchuk, 1979).

Also various formulation and dosage form related factors which have been extensively discussed by Poole (1979), Wade (1976) and many other authors include particle size, crystal form, salt form, complex formation, PH of the drug and the absorbing medium and the solubility characteristics of the drug.

Poole (1979), showed that bioavailability of some drugs have been improved by particle size reduction.

The crystal form of a drug may change if the substance exhibits polymorphism. This is very common in organic compounds, some drugs may appear in non-crystalline form or amorphous forms. Usually amorphous compounds are always more soluble then the corresponding crystalline form and may exhibit different therapeutic properties (Poole, 1979).

Complex formation is a mechanism that can influence the biopharmaceutical properties of a drug. Drugs may interact reversibly to form complexes with substances occuring in the body, or with other drugs or with other pharmaceutical dosage forms. Such complexes may differs in its physico-chemical properties from the pure drug. This may alter the ability of the drug to permeate biological membrance leading to changes in bioavailability. Also such complexes are pharmacologically inactive since their dissolution prior to drug absorption usually fails to occur (Poole, 1979). Influence of food and specific dietary components on drug absorption have been extensively reviewed by Winstanley and Orme (1989); Hayes and Borzelleca (1985). The reviews reflect the complex and unpredictable nature of drug-food interactions, and the difficulty of establishing patterns which may be used as a guideline for optimum drug therapy in relation to food. The presence of food had been shown to decrease, delay, increase and OR not to affect drug absorption. The observed effects have been influenced by the type of drug and dosage form, the nature and size

of meal, fluid intake, the condition of the subject and the time sequence between eating and drug administration.

Although drug bioavailability studies are routinely carried out in a healthy human subjects, drugs are administered in practice to patients who may be suffering from variety of illnesses. Diseases affecting cardiovascular system, liver and gastro intestinal tract (GIT) may alter circulating drugs or metabolite levels after oral dosing. Surgical removal of part of the gastrointestinal tract reduces the absorption of most drugs, suggesting that patients who have undergone such proceedure may need to be monitored carefully when taking drugs whose absorption efficiency is critical (Bakare, M.T., 1992).

1.4 DRUG INTERACTION AND METABOLISM

Drugs and other foreign agents entering the body are excreted in an unchanged form via urine, bile and other body secretions only if relatively water soluble. Since a large number of drugs are lipid-soluble they would tend to accumulate in the organism and disrupt cellular processes if they are not converted to more water soluble product. This process whereby a drug or chemical compound introduced into the body is converted into a water-soluble compound to be excreted out of the body via the excretory organs especially kidney is referred to as metabolism. It is now generally accepted that the liver is the principal site of drug metabolizing activity (Testa and Jenner, 1976).

Drug metabolism reaction can be divided into two phases. Phase I (functionalization), and Phase II (conjugation) biotransformations.

In phase I, the compound is made more water soluble by addition of a polar functional group or deletation of a non-polar alkyl group by oxidation, reduction or hydrolysis. The metabolite is not always less active than the parent drug. Some metabolites have pharmacological actions as the parent drugs e.g. cyclosphosphamide. Phase I may lncrease drug toxicity as in acetaminophen or even carcinogenicity as in benzopyridine. The water solubility of these metabolites formed can be increased by conjugating enzymes (Phase II) that link the metabolites to endogenous compounds such as glucuronic acid, sulphate, acetate or glycine (Powell et al., 1983).

The liver is by far the most important and the most studied site of drug metabolism in the body. Disruption and separation of the liver cells components showed that the greatest drug metabolising activity is localized in the microsomes of the endoplasmic reticulum and cytosol. Although numerous enzymes are responsible for the metabolism of the drugs, quantitatively the most important is the microsomal enzyme oxidation system which is also referred to as mixed function oxidase system. The microsomal enzyme oxidation system is composed of two enzymes; cytochrome P-450 and nicotinamide-adenine dinucleotide phosphate (NADPH) for its actions.

Some drugs have the ability to induce or inhibit liver

microsomal enzymes, leading to faster or slower metabolism of the compounds (drugs) that are administered with them. This is a form of drug-drug interaction.

1.4.1 Characterization Cytochrome P-450 enzymes

From a functional view point the major components of the microsomal membrane are its enzyme system. The two microsomal electron transport chain systems have been described, namely, the NADPH-linked system with cytochrome P-450 as the terminal oxidase, and NADPH-linked system with cytochrome b_5 as the electron acceptor. The metabolic function of the NADPH-cytochrome P-450 system is to participate in the oxidation (hydroxylation) of a number of compounds such as steroids, fatty acids, drugs and carcinogens. On the other hand, the function of the NADPH-cytochrome b_5 chain is not fully understood, although it appears to play a role in fatty acid desaturation (Testa and Jenner, 1976).

Cytochrome P-450 gives a reduced carbon monoxide that had an u.v. absorption band at 450nm from which it derives its name (Klingenberg, 1958;). Cytochrome P-450 is a b-type haemoprotein (Omura and Sato, 1961). Researches by some workers have shown that multiple forms of P-450 are classified based on primary amino acid sequence alignment data (Sato et al., 1987; Nebert et al., 1989). In this system the P-450s are grouped into a gene super family, that is further subdivided into gene families and finally into gene sub-families. The limits of amino acid similarities that define families and subfamilies are some what arbitrary. To date, 13 gene families have been found. A protein in one gene family is ≤36%

similar to the P-450s in another family, and within a family a protein in a given subfamily is about 40 to 65% similar to a protein in another subfamily.

The primary hepatic drug metabolizing enzymes currently comprise of four gene families designeated I through IV. They are usually written as P-450I, P-450II, P-450III and P-450IV. The subfamilies are usually written by the side of the family number with capital letters, i.e. P-450 IA, P-450 IIA and so on. However some families are designated by trivial names taken from individual references.

Humans have at least three P-450III genes, P-450 NF designated as NF-25 (Beune et al., 1986), and P-450p (Molowa et al, 1986) and the third one is P-450 PCNI.

The P-450IV gene family is regarded as a clofibrate-inducible lauric acid w-hydroxylase; and was first purified from rats. This enzyme is very specific for fatty acid hydroxylations and has no detectable activity towards other substrates such as testerterone, benzyphetamine. It is also active in oxidation of archidonic acid (Bain et al., 1986).

The P-450NF (NF-25) - is designated as human cytochrome P-450 nifedipine oxidase (Baune et al., 1986). Also another P-450 designated as NF-10, is also referred to nifedipine oxidase related enzyme (Book et al., 1989).

A study by Bork et al., (1989) also established that the P-450 NF MRNA is about 8 fold more abundant than that of P-450p MRNA in several human liver samples.

The P-450 IA genes are ubiquitos in mammals, and for most part these enzymes have similar catalytic activities in several mammalian species. More interestingly, benzo(a)pyrene hydorxylase activity, which is usually associated with P-450IA$_1$ (Guengerich, 1988; Johnson et al., 1977; Ryan et al., 1982).

P-450 II gene family have been found to hydroxylate testesterone, and other steriods especially P-450 IIA (Nagata et al., 1987; Wood et al., 1985). These enzymes were isolated from rat liver. These enzymes were phenobarbital inducible. The are also said to have high catalytic activity for s-mephenytoin 4-hydroxylation (Gut et al, 1986), and hexobarbital 3^1-hydroxylation, and tolbutamide methyl hydroxylation (Ted et al., 1988).

The P-450 PC NI has also been shown to be nifedipine oxidase (Gonzalez et al., 1988). These P-450 IIIs (nifedipine oxidase) are responsible for the oxidation of the nifedipine to its nitropyridine analogue. The P-450 III - family have recently been investigated in rats and human and a high level similar to those in the liver are detected in the human intestine. Expression of P450 III members in the intestine may have profound clinical significance in drug metabolism and absorption (Watkins et al., 1987).

Other several groups of P-450 IIIs have been investigated in human. Interestingly a P-450 III enzyme has been expressed in foetal liver (Kitada et al., 1985).

Immunochemically detectable levels of one or more P-450 III

enzymes, varing from 5 to 10 fold, have been detected in human liver samples (Waxman et al., 1988:. The variability of P-450 III expression could have considerable clinical implications, since P-450 III metabolizes variety of drugs, including the calcium channel blocker nifedipine, (Guengerich et al., 1986; Gonzalez et al., 1988); the contraceptive agent 17α-ethonyl-estradiol, progesterone, *quinidine, midazolam, cyclosporine and erythromycin and* testerterone. Individual variability of hepatic P-450 III content could, therefore, have serious consequences in drug therapy.

Microsomal metabolizing enzymes activity in human is affected by genetics, age, hormones, diseases, nutrition, stress, temporal factors and exogenous chemicals, and so it is not surprising that the rate of elimination for a drug that is primarily metabolized by these enzymes is far less predictable than a drug that is eliminated inchanged in the urine (Park et al., 1981). In the same manner, one will expect the impact an enzyme inducer or inhibitor on drug metabolism to vary within and between patients due to these same modulators of metabolic activity. For instance the magnitude of metabolic inhibition from a drug might be far different in a well nourished, healthy patient than in a malnourished severely-ill patient (Park et-al 1981).

1.5 MICROSOMAL ENZYME INDUCTION

Enzyme induction can be defined as an adaptive increase in the number of molecules of a specific enzyme, secondary either to an increase in its rate of synthesis or to a decrease in its rate of

degredation (Breckenridge et al., 1987).

The activity of a hepatic microsomal enzymes such as the cytochrome P-450 mixed function oxidase system can be markedly increase upon exposure to a number of drugs, pesticides, polycyclic aromatic hydrocarbons, and environmental xenobiotics. The process by which the activity of these drug metabolizing enzymes are increased is referred to as enzyme induction (Testa et al., 1959). The overal effect of enzyme inductions is increase in the rate of metabolism and decrease in the duration of drug action.

Morphologically, the administration of enzyme inducers is accompanied by an increase in smooth endoplasmic reticulum. Within this membrances the monooxygenese system is arranged in such a way as to allow activation of molecular oxygen as well as reduction of cytochrome P-450. Enzyme induction is only demonstrable in-vivo and there is a delay before increase in enyzme activity can be observed. True enzyme induction is caused by an increase in cytochrome p.450 biosynthesis. The induced form of cytochrome P-450 has a substrate affinity similar to the uninduced form.

There are at least two mechanisms of induction within the liver cell, and probably within the other tissues as well. In the induction caused by phenobarbitone, increased in the concentration of isoenzymes of cytochrome P-450 and the corresponding MRNA are found in the cell. Phenoborbitone induces a 2kb MRNA which carried a code for translation of phenobarbitone induction. There is however, no specific receptor responsible for the effect of phenobarbitone. In contrast the second type of induction, caused

by polycyclic aromatic hydrocarbons, depends on presence of a genonic locus which makes certain animal species responsive to this type of induction. 3-methylcholanthrene is a typical of this class of inducers. They act via a specific receptor which combines with inducer, transfers it to the nucleus and intiate the induction process (Netter, 1987).

Some inducing agents may increase the rate of their own metabolism as well as those of other unrelated drugs and foreign compounds. For instance glutethimide, meprobamate, rifampicin, phenobarbitone. These agents are known as auto inducers. The obvious consequence of this interaction is a marked decrease in the biological activity of the agent involved. Some of the inducing agents may also enhance the metabolism of endogenous compounds such as steriodal hormones and billrubin (Williams, 1959). For instance phenobarbitone and rifampicin have been observed to increase metabolism of cortisol, testesterone, vitamin-D and bilirubin in man (Drayer, 1976). These inducers are referred to as cross-inducers and their interaction have significant effect on the biological activity of the affected drugs which may be beneficial or harmful. An example of significant drug interaction which is of relevance to therapy is the interaction between phenobarbital and warfarin (Testa, 1978). The enzyme induction action of phenobarbital leads to rapid metabolism to Warfarin leading to its decrease anticoagulant effect. Its therapy with oral conctraceptive has led to ineffectiveness of oral contraceptive in women (Kropp, 1974).

In addition to drugs, other chemicals such as polycyclic aromatic hydrocarbons and environmental pollutants such as pesticides may also induce certain oxidative path way thereby altering drug path ways (Nelson, 1977). Cigarette smoke contains minute amounts of polycylic aromatic hydrocarbons which are potent inducers of microsomal cytochrome P-450 enzymes. This type of induction is referred to as foreign induction. For instance theophylline is metabolized more rapidly in smokers than in non-smokers (Testa et al., 1978).

Variation in the extent of induction has been shown by some workers. Ohnhaus and Park (1979) have shown that the extent of enzyme induction is dose dependant in man using rifampicin as the inducing agent and measuring the antipyrine clearance. The same result was obtained by Breckenridge et al (1973) - using quinalbarbitone on six patients on long term warfarin therapy. The therapy leads to fall in steady state plasma warfarin concentration and further increase in quinalbarbitone resulted in a dose dependant increase in extent of induction.

Some times the inducing action of a drug is age dependant i.e. rifampicin increase antipyrine clearance in a group of young subjects, but it had no such effect in a group of elderly individuals (Twum-Barrima et al 1984).

Ohnhaus and Park (1979) - have also shown in their work that potency of induction of the enzyme vary from drug to drug. In the cause of their work they concluded that rifampicin's augmentation of the steroid excretion was more rapidly achieved than when

phenobarbitone or antipyrine are used as inducers. It seems that *different forms of cytochrome P-450 are being induced at varying* rates by various inducing agents.

Alcohols are knwon to be microsomal enzyme inducers if taken on chronic level (Qureshi et-al 1992).

Continued ingestion of such foods as charcoal broiled beef and brussell sprouts causes the rate of metabolism of various drugs to be increased in volunteers. Further alteration in the balance of protein and carbonhydrate in the diet determines the in-vivo rate of metabolism of drugs such as theophylline and antipyrine (Kappes et-al. 1978). The effects of enzyme induction depend not only on the inducing agents and substrate but also on variations in the patients.

More than 200 drugs and other chemicals have been discovered to induce hepatic microsomal enzymes in experimental animals, and in man. Enzyme inducing agents are structurally extremely diverse and there is no apparent structure activity relation. However most are lipid soluble at physiological PH.

1.6 MICROSOMAL ENZYME INHIBITION

Many drugs inhibit the metabolic detoxification of other drugs and thus cause increased elimination half-life of the drug and subsequently increase in the pharmacologic response. The metabolism of phenobarbital and meprobamate was inhibited by chlorcyclizine, glutethimide, phenagylcodol, imipramine and phenylisopropylhydrazine during the first 30 minutes after

administration to rats (Kato et-al, 1964).

Some compounds which inhibit the metabolism of a drug 1-6 hours after a single dose may stimulate the metabolism of the same drug 12-24 hours later or after chronic administration (Serrone and Fujimoto, 1962).

In humans, there are fewer reports of drug-drug interactions resulting from metabolic inhibition than induction which is in contrast to the in-vitro and in-vivo experience in mammals and insects where inhibition of Xenobiotics metabolism is well characterised (Park, 1982; Nelfer, 1980).

Clinical manifestations from inhibition of drug metabolism will usually result in an enhanced pharmacologic-toxic effect of the inhibited drug (i.e. bleeding from warfarin). However, metabolic inhibition can lead to decreased toxicity when the toxicity is due to a metabolite or can produce no change in drug activity when the metabolite has a similar pharmacologic profile to the parent drug. Sulphaphenazole given for 8 days (2gm) inhibited drug metabolizing enzyme activity on tolbuntamide and changed the later's plasma mean half-life from 4-hours (before giving inhibitor, sulphaphenazole) to 27.5 hours (after inhibitor). This lead to hypoglyceamic collapse of patients (in 2 cases).

1.6.1 **Mechanism for Inhibition of drug metabolism**

Mechanism for inhibition of drug metabolism have been reviewed (Testa et-al, 1981). The predominant types of inhibitions are competitive and noncompetitive. Competitive inhibition occurs when the inhibitor bounds to the enzyme at the same site as the

substrate and it is metabolized by the enzyme. For a significant change in drug clearance, the inhibitor drug concentration at the enzyme binding site is greater than the inhibitor concentration that doubles the Michelis-menten constant (Km) of the substrate drug, (Km is the substrate concentration that produces half maximal enzymatic metabolism rate). Non-competitive inhibition occurs when the inhibitor irreversibly binds to and inactivate the enzyme.

This may allow the substrate to bind to the enzyme as usual: however, the maximum velocity of the enzyme-substrate reaction (Vmax) is decreased. Inhibitors are usually lipophillic in nature. The nature of interaction may be complex in that there may be inhibition acutely followed by induction. The inhibition can also be stereospecific.

1.7 DRUG INTERACTION AND RENAL EXCRETION

Changes in renal function can modify a number of pharmacokinetic processes in the body and the thereby lead to unanticipated drug effects or drug interactions. A major drug interaction involving the kidney is that effect of one drug on the renal tubular secretion and subsequently effect on the excretion of another drug. In general, administration of an organic acid will slow the tubular secretion of another concurrently administered organic acid and the administration of a base will slow the tubular secretion of other bases (Reindenberg, 1973). Salicylates, for instance, were found to slow methotraxate excretion and phenylbutazone potentiates the hypoglycemic effect of acetohexamide

- i.e. the phenylbutzone slow down the excretion of hydroxyhexamide, an active metabolite of aceto hexamide (Leigler et al., 1969).

Interaction may also occur if a drug affects urine PH. This effect will be on elimination of a drug that is filtered at the glomerulus and is re-absorbed by non-Ionic diffusion back into the blood as the glomerular filtrate passes down the nephron (Woddel and Burtler, 1957).

1.8 COMPUTER IN PHARMACY

The demonstration and application of computer in Pharmacy have been well established (Schentang et al, 1985). This covers such areas as storing patients' records of drug reactions, history of allergies, polypharmacy and therapeutic failures and side effects of the drug used.

The role of microcomputers in the analytical laboratory is growing rapidly as applications of software becomes more generally available. The impact of micro-computers on the practices of analysis in pharmaceutical and biomedical areas is increasing rapidly. Of recent analytical chemists have began to use computers on some activities as storage and search of literature references and the fitting of non-linear analytical calibration curves. Also computers are now being utilized in the optimization of measurements and interpretation of results.

Many more possibilities for the applications arise when analytical instruments such as spectrophotometers and liquid

chromatograph are interfaced to micro-computers for on line data acquisition and. were appropriate instrument control (O'Haver. 1983). This can result in much greater flexibility in designing measurement protocols for research. method development and routine quality control.

One specific example relevant to pharmaceutical analysis and biomedical field is the simultaneous multicomponent quantitative analysis of a mixture of known constituents by multiwavelength UV-visible spectrophotometry. Using micro-computer controlled instrumentation based on conventional scanning instruments or based on the linear photodiode array. It is quite convenient to acquire a large number of absorbance values at very precise wavelenghs and to compute the concentrations of the constituents and their standard deviation matrix methods for which programmes have been published. This technique is one of the feature of commercially available. diode array multichannel spectrophotometer.

The use of microcomputers for on-line data aquisition and post-run manipulation in HPLC will continue to increase. especially for automated method development utilizing simplex procedures in the pharmaceutical and related industries. At present there are commercially available rapid scanning HPLC detectors. based on the linear photodiode array. and will utilize microcomputers for data acquisition. processing and presentation. This technology is apparent in pharmaceutical fields. In particular. the presentation of data as three dimensional 'spectrochromatograph' generated by microcomputer open new approaches for HPLC detection in

pharmaceutical and bio-analytical studies (O'Haver, 1983).

Microcomputer programmes have now been developed for elucidation of pharmacokinetic parameters after analytical studies.

CHAPTER TWO

GENERAL LITERATURE REVIEW

2.1 NIFEDIPINE

2.1.1 HISTORY AND CHEMISTRY

Dihydropyridine chemistry began in 1882 when Hantzsch (1882) first reported them as stable intermediates in the pyridine synthesis that bears his name. In the subsequent 50 years modifications of the original synthesis were developed and some reactions of dihydropyridines were studies. The recent interest in dihydropyridines can be traced to co-enzyme reduced nicotinamide adenine dinucleotide (NADH) and the unique ability this compund in biological systems to reduce unsaturated functional groups (Einer et al 1982 & Stout et al. 1982). In 1949 Phillips et al (1949) reported on the weak analgesic and curare-like activities of a few dihydropyridine derivatives. In search for orally active drugs for the treatment of coronary insufficiency. Bossert and co-workers, 1966 synthesised a compound designated Bay a 1040 which, following its introduction 1975 as Nifedipine (Adalat^{R}) has since become one of the major cardisvascular drugs (Bossert et al 1986). The cardiovascular activities of 4-aryl, 1,4-dihydropyridine, 3,5-dicarboxylic acid esters were discovered independently in the sixties in the laboratories of Bayer, Germany (Bossert et al 1971 & Vaters et al 1972) and Smith, Kline and French (Loev et al 1974).

Of 1,4-dihydropyridines, nifedipine is the most superior and the one that is most commonly used as cardiovascular drug.

Therapeutically nifedipine and other 1.4-dihydropyridines are grouped as calcium antagonists or calcium chanell blockers.

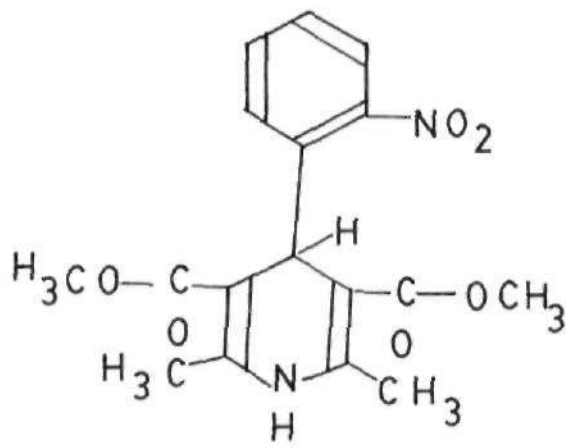


Fig. 2.1.1: Nifedipine Structural Formula

The nomenclature of Nifedipine -

(i) 1,4,-Dihydro- 2,6-dimethyl - 4-(2-nitrophenyl)-pyridine-3,5-dicarboxylic dimethyl ester.

(ii) Dimethyl 1,4-dihydro - 2,6-dimethyl - 4(o-nitro phyenyl) - 3,5-pyridin-dicarboxylate.

The molecular formula $C_{17}H_{18}N_2O_2$.

The molecular weight 346.34

The elemental composition:-

Carbon	-	58.95%
Hydrogen	-	5.24%
Nitrogen	-	8.09%
Oxygen	-	27.72%

Other chemical compounds in this class of 1,4-dihydropyridines include - Nimodipine, Nisoldipine, Nivaldipine, Bendipine, Manidipine, Nicardipine, Nitrendipine, Flordipine, Felodipine, Amlodipine, Isradipine, MPC-1304, and NG 818 (Anhoff and Persson, 1990).

The general structural formular for some of 1,4-dihydropyridines used medicinally is:

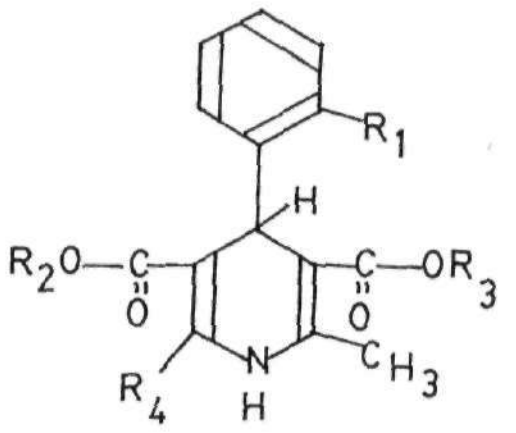


Fig 2.1.2: Structural formula of 1,4-dihydropyridines

Table 2.1.1 - below show some of the 1.4-dihydropyridines.

Table 2.1.I: 1,4-dihydropryridines.

Compound	M.Wt.	R1	R2	R3	R4	Other substituents
Nifedipine*	346	2-No_2	CH_3	CH_3	CH_3	
MPC 1304	388	2-No_2	CH_3	CH_2COCH_3	CH_3	
Nitrandipine*	360	3-No_2	CH_3	CH_2CH_3	CH_3	
Nimodipine	418	3-No_2	$CH(CH_3)_2$	$(CH_2)_2OCH_3$	CH_3	
Nisoldipine*	388	2-No_2	CH_3	$CH_3CH(CH_3)_2$	CH_3	
Nivaldipine	397	3-No_2	$CH(CH_3)_2$	CH_3	CN	
Nicardipine*	515	3-No_2	CH_3	$CH_2-N(CH_3)CH_2C_6H_5$		
Benidipine	505	3-No_2	CH_3	-Benzyl-3-piperidinyl	CH_3	
Manidipine	610	3-No_2	CH_3	2-(4-diphenyl-methyl-1-piperazinyl) ethyl	CH_3	
Falodipine*	370	2.3-cl	CH_3	CH_2CH_3	CH_3	
NB 818	457	2.3-cl	CH_3	$CH(CH_3)_2$	$CHOCONH_2$	
Amlodipine	419	2-cl	CH_3	CH_2CH_3	$CH_2O(CH_2)_2NH_2$	
Israldipine*	371	N_2O	CH_3	$CH(CH_3)_2$	CH_3	
Flordipine	500	2-cf_3	CH_2CH_3	CH_2CH_3	CH_3	1-(2-(4-morpholiny)-ethyl)

* - 1,4-dihydropyridines currrently used in the treatment of hyptertension.

2.1.2 DESCRIPTION AND PHYSICAL PROPERTIES

Nifedipine is a yellow, odourless, and tasteless crystalline powder, thermostable and non-hygroscopic.

Nifedipine is freely soluble at 20^{0}C in acetone (250 g/l), in methylene chloride (160g/L), in chloroform (140 g/L), solube in ethylacetate (50 g/L), slightly soluble in methanol (26 gm/l) and ethanol (17 g/L) and practically in soluble in water. Further the solubility at 37^0 in buffer solutions of different PH-values can be given as PH4, 0.0058g/L; PH7, 0.0056g/L; PH9, 0.0078g/L; PH13, 0.0060g/L (Syed Laik Ali, 1989).

2.1.2.1 Melting point:- Nifedipine has a melting point of about 171-175^{0}C.

2.1.2.2 Loss on drying:- Nifedipine loses not more than 0.5% of its weight when dried at 105^{0}C to constant weight.

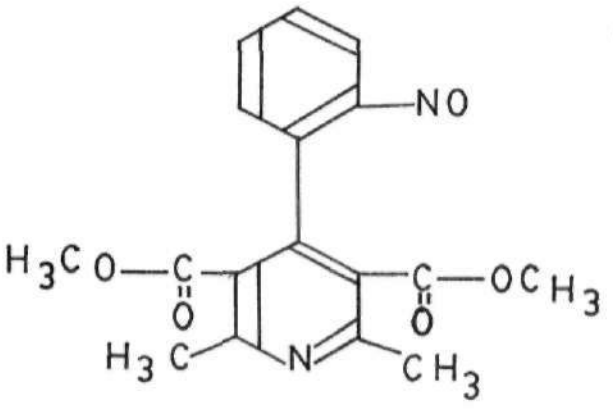
2.1.2.3 Sulphated Ash, Residue on Ignition:- Not more than 0.1%, an ignition temperature of 600^{0}C being used, and determined with 1-2gm. nifedipine powder (USPXX).

2.1.2.4 Dissociation constant:- The dissociation constant of calcium antagonist such as nifedipine were measured using a potentiometric microtitration technique. Nifedipine is not protonated and must exert its action via the unchanged form (Manhold, 1984). The PKa-value (acidic) is found to be greater than 13 [PKa > 13]. The PKa-value (Basic) is about -0.9.

Owing to the extreme low basicity of the dihydropyridine nitrogen it is not possible to obtain stable salts with acids.

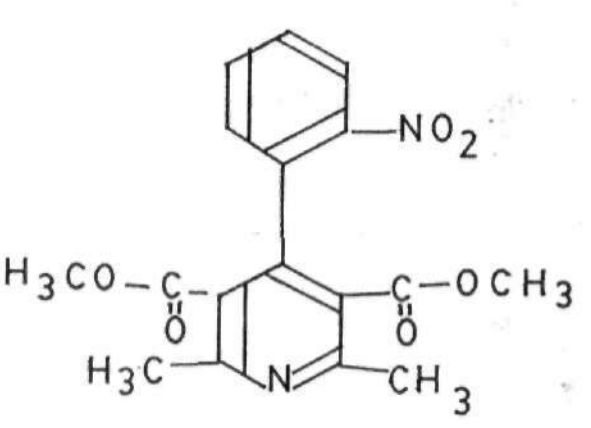
2.1.2.5 Light Sensitivity:- Nifedipine is sensitive to light in

solid form and extremely sensitive to light in dissolved state in solution. In the day light nifedipine decomposes to give 4(2-nitrosophenyl)-pyridine homologue (Fig. 2.1.3) and under u.v. light it decomposes to give oxidation product 4-(2-nitrophenyl)-pyridine homologue (Fig. 2.1.4) (Piergiorgio et al., 1981; Pietta et al., 1981).



4(2-nitrosophenyl)-pyridine

Fig. 2.1.3



4-(2-nitrophenyl)-pyridine

Fig 2.1.4

2.1.3 **ABSORPTION AND DISTRIBUTION:**

Nifedipine is absorbed over the whole length of gastrointestinal tract, close to 100% of an oral dose is absorbed largely in the small intestine (Raemseh and Sommer, 1983). However, there has been considerable variation among subjects in the manner and extent to which nifedipine is absorbed. Some investigators have indicated that nifedipine is up to 90% absorbed after single oral dose (Horster, 1975; Kleinbloesem et-al 1984), it has been theorized that substantial first pass metabolism is reponsible for the lower bioavailability of the drug (Kleinbloesem et-al 1984b). With the use of nifedipine - ^{14}C - labelled in man, some concentrations of nifedipine ^{14}C is detectable in the serum withim ten (10) minutes after sublingual and at most twenty minutes after oral administration. With both routes of administration, the maximum plasma (Serum) concentrations are attained in 30-120 minute after the administration (Horster et.al, 1975;Jokobson et-al, 1979; Raemsah and Sommer, 1983; Snadden et-al; 1984a).

The rate absorption from both oral and sublingual capsules varies widely among individuals. Several investigators have described 'fast' and 'slow' absorbers of nifedipine depending on the time required to reach maximum plasma concentrations which ranged from 30 to 90 minutes in 'fast' group and from 120 - 240 minutes in the 'slow group' (Forster et-al, 1983; Nakashima et-al, 1984, Snadden et-al 1984b).

When nifedipine was administered to rats in doses of 0.03-3 mg/kg intravenously or orally, the distribution pattern did not

vary significantly between the intravenous and oral treatments after 1 hour. While the liver, kidneys, serum and lungs contained higher concentration of nifedipine, lower concentrations were found in the brain, skeletal muscle and testes (Horster et-al, 1972).

In the blood of rats, nifedipine activity is mainly found in the serum, with concentrations 3 to 5 times higher than those in the erythrocytes (Horster et-al, 1972).

Rapid average distribution half lives of 0.09 and 0.17 hours have been seen after intravenous administration of nifedipine 1 and 4.5 mg respectively, in human volunteer (Kleinbloesen et-al 1984ab). The steady state volume of distribution of nifedipine in man after oral administration has been investigated and was found to be 1.32 L/kg (Forster et-al 1983). In a study by Kleinbloesem et-al (1984) - the steady state volume of distribution was noted to be 0.8 L/kg, whereas the volume of central or plasma compartment was only 0.25 L/kg. Since nifedipine is very highly bound to plasma protein, to the extent of 92 - 98% (Rosenkrenz et-al, 1974; Schlossman et-al, 1975) this would appear to indicate that nifedipine undergoes extensive tissue distribution in man. Schlossman et-al (1975) found that nifedipine protein binding increased from 92% - 98% as total nifedipine concentration decreased from 20 to 0.2 mg/l. Thus it is concentration dependant. It is the extensive plasma binding effect of nifedipine that is responsible for its long duration of action (Schlossman et-al 1975). The main metabolite of nifedipine is bound to plasma protein a lesser extent than the parent drug, 54% being bound to plasma

proteins (Scholossman et-al 1975).

Study of the pharmacokinetics of a single dose level of nifedipine capsules (10 mg thrice daily) for 7 days have shown that, this dose level of, there is no cummulative effect or other changes in the pharmacokinetic behavour (Raemsch and Sommer, 1983).

2.1.4 METABOLISM AND EXCRETION

Nifedipine undergoes extensive first pass hepatic metabolism in man following oral administration. (Forster et-al 1983; Waller et-al 1984).

This first pass metabolism results in 45 to 68% absolute bioavailability of drug (Sorkin et-al, 1985).

Radiolabelled nifedipine has been found to undergo hepatic oxidation to three pharmacologically in-active metabolites which are excreted in the urine (kroneberg and krebs, 1980). Two of these metabolites, an hydroxy carboxylic acid derivative and a mathoxy caboxylic acid derivative, contain 95% of the total detectable urinary radio activity.

Thus, this amount of nifedipine undergoes biotransformation: only traces of unchanged parent drug are excreated in the urine (Dockladalova et-al, 1982; Kroneberg, 1975; Raemsch and Sommer, 1983). In most cases after a single dose of nifedipine more of the hydroxy than methoxy carboxylic acid derivative is present in the plasma (Raemsch and Sommer, 1983).

However, Waller et-al (1984) - found that the nitropyridine analogue of nifedipine, which is also formed from nifedipine by photochemical degredation under ultraviolet light shows a marked

presence in the plasma after nifedipine is given orally, but is present only in trace amount after intravenous administration in humans. Horster et-al (1972) - reported that in the human body nifedipine is rapidly oxidised enzymatically to its nitropyridine metabolite. In the biotrasformation process the ester moiety of the side chain is hydrolysed and further oxidation occur in the methyl *side chain position*.

Diagramatic metabolic path way of nifedipine is presented below.

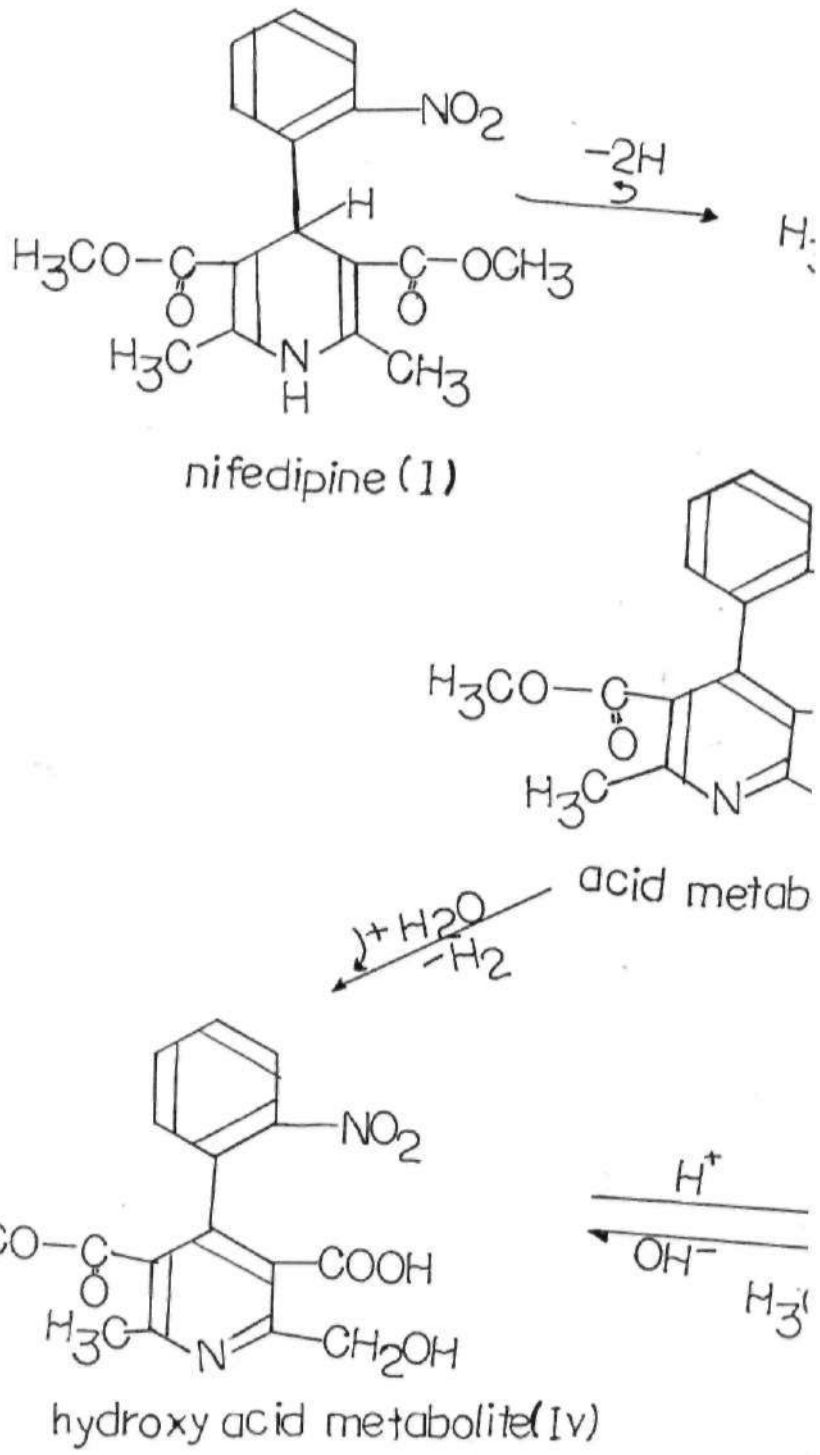


Fig. 2.4.1 Metabolism of nifedipine (R

Following the enteric absorption of nifedipine, some drug (30 to 40%, if 100% absorption is assume) is eliminated matabolically during the first pass through the liver and does not in enter systemic circulation (Raemsch and Sommer, 1983).

The elimination half life of nifedipine after admininstration of oral tablets has been found to vary between 6 and 11 hours, which is about 2 to 3 times longer than previously reported after the administration of oral capsules (Banzet et-al, 1983; Kleinbloesem et-al, 1984a; Forster et-al, 1983). The elimination half lives after intravenous and rectal administrations ranges from 1.3 to 2 hours (Hamman and Mc Allister, 1983; Forster et-al, 1983; Klembloesem et-al, 1984b).

After rectal and intravenous administration of radio activity lebeled nifedipine, 70 to 80 % of activity (in the form of highly water soluble metabolite) is eliminated in the urine and 90% of this ammount is eliminated after 24 hours (Horster et-al, 1975) Raemsch and Sommer, 1983), reminder is excreated in faeces also in metabolized form (Raemsch and Sommer, 1983). The unchanged nifedipine is excreted in the urine in trace ammount approximating 0.1% of the total dose Kleinbloesen et-al, 1984)

The total systemic clearance (intravenous) of nifedipine from plasma ranges from 27 to about 66 L/hour, while its intrinsic clearance (oral) ranges from 33 to 37 L/h (Banzet et-al 1983; Forster et-al, 1983). Kleinblosen et-al, (1984a) found the clearance of nifedipine after oral and intravenous adminsitration in volunteers to be 27 to 33 L/h and suggested that the rate of

nifedipine elimination depended on the drug metabolizing enzymes activity as well as on hepatic blood flow.

2.1.5. CLINICAL USES OF NIFEDIPINE

Nifedipine has been used to treat patients suffering from different forms of ischemic heart diseases, including those pre and post infarction, and in most instances (stable angina, variant angina, mixed angina, and unstable angina) with large degree of success. However, its efficacy compared with that of other drugs widely used to treat these diseases is not well documented.

In majority of chronic classic stable angina patients, chest pain and ST segment depression are reproducible at a given work load and this apprears to be directly related to inbalances between myocardial oxygen supply and demand (Glossman et-al 1985).

Until recently, nitrates and β-blocker have been the drugs principally chosen for the clinical management of stable angina, although the calcium antagonists, including nifedipine, seem to represent a major advance in these therapeutic area . Results reported from manufacturers (approximately 7000 case reports) indicates that nifedipine 30 to 60 mg daily significantly reduced the frequency of angina attack after a period of 2 to 3 weeks in about 80% of the patients treated (Muller et-al 1984).

In recent double blind placebo controlled studies nifedipine 30 to 60 mg/day for period of up to 6 weeks was found to be significantly more effective than placebo in of stable angina (Muller et-al 1984). The incidence of angina attacks and consumption of glyceryl trinitrate tablets were reduced

approximately 50 to 60% with nifedipine. It is suggested that in patients with chronic stable angina the vasodilator properties of nifedipine reduce after load while the heart rate remains relatively unchanged, and the drug confers clinical benefits on such patients by decreasing myocordial oxygen demand. Duhm et-al, (1972), in their works have shown evidence that indicates that nifedipine may augument myocardial oxygen supply by direct coronary vasodilation although this appears of lesser importance.

Nifedipine has been used also in treatment of variant (prinzmetal's) angina. The clinical efficacy of nifedipine for the treatment of prinzmetal's angina has been extensively documented (international Adalat(B) symposia 1st 2nd 3rd 4th and5th; 1975 - 1983). Nifedipine 40 to 160 mg/day significantly reduce the incidence of attack of angina in a clinical trial within 127 patients with severe variant angina. In fact 67% of the patients gets complete control of angina attacks (Antman et-al 1980). Also various centres reported by term up to 6 years clinical experience using nifedipine to treat patients with variant angina (Vincenneux et-al 1986).

Beneficial response ranged between 50 to 90% depending upon the criteria used to define a positive response.

In treatment of unstable angina, Muller et-al (1984) carried out a study which gave support to the use of nifedipine alone in the initial medical management of the disease also, they confirmed the added benefit of combining nifedipine and propranolol when monotherapy is not - effective. In a recent prospective randomised trial in 40 patients with unstable angina nifedipine 80 to

160mg/day is found to be effective in controlling episodes of angina. The drug stabilized the underlying unstable angina in 45% of the patients, and those who did not, a combination of nifedipine, and propranodol (180 to 240 mg/day) increase the response rate to approximatly 90% (Sorkins et-al 1985) - Nifedipine has also been used both prophylactically, in an attempt to attenuate threatened infarction, and therapeutically, to limit the extent of myocardial necrosis or to stabilize those patients with recurrent ischaemic pain immediately after infaraction (Muller et-al, 1984). Bossert et-al (1986) found nifedipine 30mg administered via nasogastric tube to be beneficial when used adjunctively with intravenous glyceryl trinitrate in preventing further re-occurances of coronary artery spasm in a patients with triple vessel coronary artery disease under going coronary artery by pass sugery.

Other works (Lederballe et-al, 1978: Muller et-al, 1984) showed that nifedipine administration suppresed spasm in majority of patients during coronary arteriography.

Nifedipine is also used in treatment of systemic hypertension. Single dose studies indicates that antihypertensive effects of nifedipine are primarily mediated through peripheral arterior dilation with an accompanying increase in sympathetic tone which results in parallel increases in heart rate (depending on the formulation administered), indices of left ventricular contractility, cardiac out-put and stroke work index. With longer time administration the increase in sympathetic tone seem to diminish as assessed by a reduction of the heart rate response and

lower circulation of nor-adrenatine concentration (Lederlle Perdersen et-al, 1978).

Clinical data are accumulating rapidly supporting the view that nifedipine may be useful in all grades of hypertension.

In the treatment of acute episodes of severe hypertension, and particularly in hypertensive crises, the efficacy and safety of nifedipine have been consistently reported. In single studies in patients with mild to severe hypertension, nifedipine 10 to 20 mg (orally or sublingually) has been shown to be a safe and effective drug for the rapid reduction of elevated blood pressure (Zylber-katz et-al, 1988).

Nifedipine did not produce a precipitous fall in blood pressure, and postural hypotension was rarely encountered (Laderbelle et-al, 1978). Also nifedipine 10 to 20 mg has been administered by various routes for the rapid reduction of very high blood pressure during hypertensive emergency. Most authors concluded that nifedipine is reasonably first line choice in this hypertensive emergency. Studies have aslo shown that nifedipine is effective in controlling hypertension in patient with acute renal failure requiring heamodialysis for survival (Glossman et-al, 1985; Achter et-al, 1987).

The results obtained from these renal patients are similar to those obtained in patients with essential hypertension.

There are also some data available, although very limited, resulting from clinical experience with nifedipine in patients with other diseases such as congestive heart failure, hypertrophic

cardiomyopathy, pulmonary hypertension and Roynaud Phenomenon.

In congestive heart failure, the potent vasodilatory properties of nifedipine seem to have offset its negative inotropic effects, thus resulting in an increase cardiac output (Gilman et-al, 1985). Many authors who have studied patients with pulmonary hypertension suggest that nifedipine should be considered as a potentially useful drug for initial treatment of this condition. Clinical trials with nifedipine in hypertrophic cardiomyopathy have shown that nifedipine can have both beneficial (Bossert et-al, 1986; Kroneberg et-al, 1975) and deleterious (Laderballe et-al, 1978) effects on the patients. Bossert et-al (1986), have reported that nifedipine 0.6 to 0.9 mg/kg/day produce immediate and sustained (up to 6 month) symptomatic improvement in neonates with hypertrophic cardiomyopathy.

Nifedipine 20mg three times daily for one week produced a great protection against Raynauds phenomenon by significantly reducing the number and severity of vasospastic attacks (Sorkins et-al, 1985).

2.1.6 PLACE OF NIFEDIPINE IN THERAPY

Clinical trials to date support the view that nifedipine should be considered a first line choice in patients with a predominant vasospastic components to their angina, i.e. variants or unstable disease, and in those patients whose angina is complicated by hypertension or congestive heart failure. There is also increasing evidence that nifedipine, used alone, may be a useful alternative in the clinical management of stable exertional

angina. In studies where nifedipine and a β-blocker have been used together, the conbination always produce more favourable result than monotherapy without any significant deterioration of left ventricular function. Nifedipine have also been used in treatment of various grades of hypertension. Many authors report that for those situations requiring acute reduction of blood ressure, sublingual nifedipine is the drug of choice because of its convenient route of administration, and its rapid lowering of elevated blood pressure without over correction or execessive adverse effects. Nifedipine has also been shown to be an extremely useful adjunct for controlling blood pressure in patients refrectory to conventional treatment regimens with combination of β-blocker, diuretics, and various vasodilators. To assist with compliance in long time management of hypertension, a sustained release formulation of nifedipine, allowing twice daily administration has been marketed, and this preparation appears to maintain 24 hour control of blood pressure as well as conventional capsule formulation of nifedipine .

Recently, nifedipine has been used in the management of Raynaud's disease and produced subjective improvement in the number, duration and severity of vasospastic attacks in more than 60% of the patients treated. Additionally, it was found to be helpful in healing of cutaneous ulceration that occured in some patients. It would seem to be first-line choice this setting as it is shown to be more effective than prozosin.

Clinical trials in other therapeutic areas such as congestive

heart failure, hypertrophic cardiomyopathy, and pulmonary hypertension have been encouraging but are limited in terms of the total number of patients studied, and more information is needed to clarify any role nifedipine may have in treating such patients.

Thus, nifedipine has shown itself to be a useful addition to the drugs available to the clinicians for the management of angina and hypertension, and will be of benefit to patients with Raynaud's and those with pulmonary hypertension.

2.1.7 MECHANISM OF ACTION

The degree of contraction and relaxation of arterial smooth muscle is controlled by changes of the calcium concentration in the cytosol. An increase in the intracellular concentration of calcium is detected by calmodulin (calcium - modulating protein). The calcium - calmodulin complex interacts with the myosin light chain kinase (MLCK) leading to phosphorylation of the myosin. Calcium - dependants myosin phosphorylation is the prerequisite for its interaction with action and for shortening or tension development. Simultaneously, the phosphorylated myosin act as an enzyme catalysing the hydrolysis of ATP to ADP, thus supplying energy for the mechanical movement of the myofilaments and for cellular contraction conversely, when the level of calcium is reduced, the MCLK is inactivated. Following the dissociation of calmodulin, the myosin is dephosphorylated to form a dormant state which causes the muscle to relax. Also energy supply through ATP hydrolysis ceases due to the decrease in cytosolic calcium concentration.

The calcium concentration is regulated by the activity of the

cell membrane and sarcoplasmic reticulum. In resting state the cell membrane has little permeability for calcium. thus maintaining large transmembrane concentration gradient. Membrane excitation by depolarization induces calcium influx through potential - sensitive channels (electro mechanical coupling). Agonist induced activation of the membrane receptors (Pharmaco mechanical coupling) leads to calcium influx through receptor operated channels (Bolton. 1979). In turn. relaxation is induced by active calcium pumping into extracellular space or the sarcoplasmic reticulum. Three distinct drug receptor sites of the calcium channel have been postulated by Glossman et-al. (1985): 1.4-dihydropyridine. verapamil. and diltiazam. receptor sites. Each exist in low and high affinity state. The high and low affinity states were proposed as equivalent to the non-conducting (resting or inactivated) or conducting (activated or open) states of the channels determined electrophysiologically in the intact cardiac membrane (Glossmann et-al. 1985).

It is believed that the hydrophobic 1.4-dihydropyridines (i.e. nifedipine) bind primarily to receptor sites associated with the in-activated calcium channel. and this binding presumably tends to keep the channel in the in-activated state. The calcium channel blockers relax arterial smooth muscle. but they have little effect on most venous beds and hence do not effect cardiac pre-load.

2.1.8 SIDE EFFECTS AND TOXICITY

The predominant side effect associated with nifedipine is execessive vasodilatation. which result commonly in peripheral

oedema and dizziness and less commonly headache, hypotension, flushing, nausea, vomiting and sedation. These side effects occur mainly in 20% of the patients on the drug (Lewis, 1983), and are usually benign and may abate with time or with adjustment of the dose. The character, severity and incidence of side effects is no greater during long term (greater than six months) treatment than during shorter courses or therapy (Terry 1982). Aggravation of myocardial ischemia has been reported, potentially due to excessive hypotension, and decrease coronary perfusion, selective coronary vasodilatation in non-ischemic regions of the myocardium, or an increase in oxygen demand due to execessive tarchycardia.

There are some reports (few) on poisoning with nifedipine. All were voluntary overdoses and doses up to 900mg were ingested (Hernington et-al, 1986; Gilman et-al 1985). The main symptoms of poisoning are throbbing headache, profound hypertension; cardiac rythm or arterioventricular node function and consciousness are not markedly impaired. The medical counter measures taken were mainly gastric larvage, activated charcoal, and close observation of the patient condition.

2.1.9 NIFEDIPINE DRUG INTERACTIONS

Nifedipine is widely believed to be metabolized by liver cytochrome p - 450, enzyme, (Schellens et-al, 1990; Waller et-al, 1984; Raemsch and Sommer, 1983). Thus all agents that can induce or inhibit liver cytochrome p - 450 production have the potential to alter the bioavalibility, clearance and elimination of nifedipine.

The pharmacokinetic interaction of alcohol and nifedipine

shows that acute dose of alcohol increases the bioavailability of nifedipine by 54%. However there is no significant difference between the treatments in the maximum plasma concentration (C_{max}), maximum plasma concentration time (t_{max}) or half life and there is decrease time for onset of increase heart rate (Qureshi et-al, 1992), also there is decrease in nifedipine plasma clearance by 49% (Boje et-al 1989).

Conflicting reports have been published concerning the effect of nifedipine on the steady state plasma concentration and elimination of digoxin. In acute trial nifedipine has been reported to elevate plasma concentration and decrease the renal clearance of digoxin in healthy subjects by 45 and 30% respectively, but this reduction in renal clearance was not of sufficient magintude to explain the observed increases in plasma concentration of digoxin (Berltz et-al, 1983). Other works by kirch et-al, (1986), shows that the increase in digoxin plasma concentration and its bioavailability (15%) is slight but significant. Also, they stated that this result is not dependant on the nifedipine dose administered. Since increase in digoxin plasma concentration is associated with increase glycoside effects, it was suggested that all patients receiving these drugs conbination should have their digoxin dose reduced by 40-50%, and their plasma concentration checked and be monitored for signs of digoxin to toxicity (Beltz et-al 1983.).

Conversely several single and multiple dose studies in both cardiac patients and healthy volunteers have indicated no

significant pharmacokinetic interaction between nifedipine and digoxin or digitoxin (Zylber - katz et-al, 1984; Kuhlman, 1985; Halawa et-al, 1990).

When both nifedipine and rifampicin were used together in essential hypertensive patients that developed peritoneal tuberculosis, both the maximum plasma concentration, and bioavailability of nifedipine were markedly decreased to about 40% of those patients without rifampicin. This finding suggests that rifampicin may increase the elimination of nifedipine presumably by induction of its hepatic metabolism. This interaction is significant and thus suggested that extra caution should be taken when nifedipine is prescribed along with rifampicin (Tada et-al, 1992).

Nifedipine when taken together with amoxycillin enhances the absorption kinetic and bioavailability of amoxycillin in human. Results in healthy volunteers shows that the uptake of amoxycillin is increased by 70% (significant) and also the bioavailability by 21.4% without modifying its distribution and elimination. This suggests that nifedipine could enhance amoxycillin intestinal uptake by stimulating its active transport. (Westphal et-al, 1990).

Workers have shown there is no significant effect on both pharmacokinetic and pharmacodynamic properties of nifedipine when it is taken together with cotrimoxazole (Edward et-al, 1990).
Some studies have shown that the combination of nifedipine and β-blockers is safe and may produce favourable clinical responses in patients with angina pectoris and hypertension. Some researchers

have reported that there is no significant pharmacokinetic interaction between nifedipine and propranolol, atenolol, nadolol, and metoprolol- (Kendall, et-al 1984: Wilkin et-al, 1985: Zylber katz et-al, 1988). Other workers find that there is significant pharmacokinetic interaction between nifedipine and propranolol. Nifedipine is said to increase the bio-availability, maximum plasma concentration (C_{max}) and reduces the time to achieve the max. Plasma concentration (t_{max}) of propranolol. They claimed that, that could be due to enhanced intestinal absorption and or reduced first pass effect, induced by nifedipine (Vinceneux et-al, 1986).

Quinide pre-treatment significantly inhibited the aromatization of nifeddipine to its major first pass pyridine metabolite and prolong elimination half life of nifedipine by about 40% (Schellens et-al, 1991). Case reports of decreased plasma quindine have been reported (Ahmed, 1984). There is no significant pharmacokinetic interaction between nifedipine and phenytoin and sparteins (Ferringer et-al, 1984: Green et-al, 1983: Shellens et-al, 1991). Also there is no significant interaction between antipyrine and nifedipine in the saliva pharmacokinetic parameter of the two when taken together (Ediki et-al, 1990). There is also no significant pharmacokinetic interaction between theophylline and nifedipine, though the clearance of theophylline is increased by 25% (Upton, 1991: Adebayo et-al, 1990: Christopher et-al, 1989 and Jackson et-al, 1986).

Cimetidine not raintidine produce significant changes in the pharmacokinetic of nifedipine at steady state. i.e. there is

increase in nifedipine bioavailability. Neither of them produce significant changes in the pharmacological response to nifedpine (Kirch et-al. 1985: Khan et-al. 1991).

Non-steroidal anti-inflamatory drugs (NSAID) have no significant pharmacokinetic and pharmacodynamic effect on the nifedipine when taken together (Salvetti et-all. 1990).

The citrus juice when taken together with nifedipine. increases the bioavailability of the latter to 108-169% to that of water (Barley et-al. 1991).

Low fat meal when taken with nifedpine reduces the rate of absorption of nifedipine and also decresed time for peak plalsma concentration. Though this is statistically insignificant. Also the total bioavailability of nifedipine is not significantly affected (Mustapha et-al. 1992).

2.1.10 NIFEDIPINE DOSAGE AND ADMINISTRATION

For the prophylatic management of the various forms of angina and for the treatment of hypertension, the recommended starting dose of nifedipine capsules is 10mg three (3) times daily during or after meals. The dosage should then be titrated to produce an optimal response; a 5mg capsule is available for this purpose. Typically the usual optimal dosage is between 30 and 90mg daily. This may be gradually increased to 120mg per day. For Raynauds' phenomenon initial dosage of 10mg three times daily, which is then increased gradually to 20mg three times daily.

A sustained release dosage formulation of nifedipine is now available, for the treatment of hypertension, - recommended dosage

of which is 20mg twice daily after food, increased if necessary to 40mg twice daily. Recently an intravenous dosage form of nifedipine was made available for use in cases where rapid action is required i.e. - in coronary vasospasm, hypertensive emergencies. It is present in ampoules of 1mg per 10ml. The usual dosage is 0.5 - 1.0mg per patient (0.007 to 0.015mg/kg weight) administered slowly (over about 3 minutes) at a constant rate. A total dose of 9 x 0.02mg/kg body weight over 7 hours should not be exceeded. There is also intracoronary injection of nifedipine 0.2mg/2ml ampoule. It is used to treat coronary vasospasm that occurs during diagnostic or therapentic intervations, e.g. in coronary artery bypass grafting.

Nifedipine may be used in combination with β-blockers and other antiphyertensive drugs; although the possibility of addictive effect should be borne in mind.

2.1.11 PROPRIETARY PREPARATIONS

i. Nifedipine 5mg tablets (non-propreitary).

ii. Adalat® (Bayer) 10mg and 5mg tablets and capsules.

iii. Adalat Retard® (Bayer) 20mg and 10mg tablets
These are sustained release dosage forms.

iv. Adalat®IC (Bayer) - coronary injection 100ug/ml.

v. Nifedipine Redard® - (Volpino Arg). - 20mg tablets
Procardia® (Pfizer USA) - 10mg and 5mg tablets.

vi) Nifecard Retard® - 20mg (sustained relear tablets).
(Lek. Yugoslavia).

2.2 RIFAMPICIN

2.2.1 HISTORY AND CHEMISTRY

The rifamycins originated as a group of anitbiotics produced by fermentation of Streptomyces mediaterranei. This Streptomyces was discovered in Northern Italy in the late 1950s and subsquently by a series of synthetic steps a great variety of other rifamycins were produced, some of which, including rifampicin, were well absorbed orally.

Rifampicin is a semi-synthetic derivative of rifamycin B. They are complex macrocyclic antibiotics (Burley, 1976).

It has the following chemical structure:-

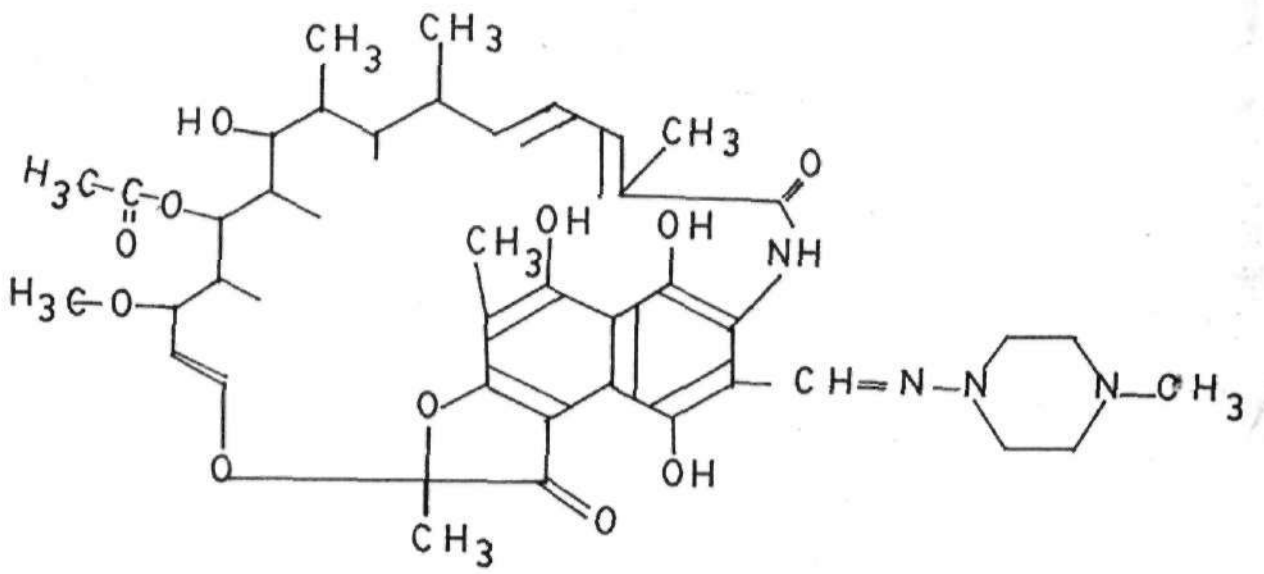


Figure 2.2.1:- Structural formula of Rifampicin.

The nomenclacture of rifampicin: O-5,6,9,19-pentahydroxy-23-methoxy-2,4,12,16,18,20,22-heptomethyl-8[N-(4-methyl-1-piperazinyl)formimidoyl]-2,7-(epoxypentadeca[1,11,13]-trienimino)-naphtho[2,1,6]furan-1,11(24)-dione-21-acetate (Martha, 1976).

The molecular formula:-

$C_{43}H_{58}N_4O_{12}$

The molecular Weight = 823.0

2.2.2 PHYSICAL PROPERTIES:-

Rifampicin is a tasteless brick red to reddish brown crystalline powder.

It is a zwitterion and is soluble in organic solvents and in water at low (acidic) PH.

Usually rifampicin is slightly soluble in water, ethanol, acetone and ether. It is freely soluble in chloroform; soluble in ethyl acetate and methanol. A 1% suspension in water has a PH of 4-6.5.

The dry powder is stable for more than 5 years at 25^0C. It losses 20% of its potency in aqueous solution in 10 hours at room temperature, but decomposition is diminished by addition of a reducing agent such as ascorbic acid (Wade et-al, 1982).

Ultra-violet spectroscopy of rifampicin suspension at PH 7.38 - shows absorption bands at maximum at 237nm, 254nm, 334nm, and 475nm. The maximum absorbance occurs at 334nm (Martha, 1976).

Melting point is 185^0 - 186^0C.

2.2.3 ABSORPTION AND DISTRIBUTION

Rifampicin is readily absorbed from gastro-intestinal tract and peak plasma concentration of 7-9ug/ml is achieved between 2-4 hours, after ingestion of 600mg; and 27ug/ml after ingestion a dose of 900mg (Radner, 1973). About 75 to 85% of rifampicin in the circulation is bound to plasma proteins (Vallner 1977).

Rifampicin is distributed throughout the body with ease, except the central nervous system. It is also present in effective concentration in many organs and body fluids, including the cerebrospinal fluid (CSF) (Sippel et-al, 1974).

Rifampicin also crosses the placenta and diffuses into breast milk and into CSF when the meninges are inflamed (Sippel et-al, 1974). The absorption of rifampicin is reduced or delayed when it is given with food. However, effective plasma concentration is still achieved when given with food. Also disease like coeliac disease delayed the absorption of rifampicin (Goodman and Gilman et-al, 1985).

The distribution of the rifampicin throughout the body and its presence in effective concentration in many organs and body fluids, may perhaps be best examplified by the fact that the drug may impart an orange-red colour to the urine, faeces, saliva, sputum, tears and sweat, the patients should be warned
(Goodman and Gilman, 1985).

2.2.4 METABOLISM AND EXCRETION

Following absorption in the gastrointestinal tract, rifampicin is rapidly eliminated in the bile, and enterohepatic circulation

ensures. During this time there is progressive de-acetylation of the drug, such that nearly all the antibiotic in the bile is in de-acetylated form after 6 hours. This metabolite retain essentially full antibacterial activity. The intestinal reabsorption is reduced by de-acetylation (as well as food), and metabolism thus facilitate elimination of the drug. The plasma half life of rifampicin varies from 1.5 - 5 hours and is increased in the presence of hepatic dysfunction (Goodman and Gilman, 1985).

Up to 30% of a dose of 900mg may be excreted in the urine and about half of this may be within 24 hours; and less than half of it may be unaltered rifampicin. A formyl rifampicin (metabolite) is formed in urine (Accocella, 1978).

There is a progressive shortening of the half-life of rifampicin by about 40% during the first 14 days of treatment, due to induction of hepatic microsomal enzymes with deacetylation of the drug. Thus rifampicin increases its own rate of metabolism (ie. its an 'autoinducer') - (Braimmer et-al, 1977).

2.2.5 CLINICAL USES OF RIFAMPICIN

Rifampicin in combination with isoniazid are the most effective drugs for the treatment of turberculosis. Rifampicin (like isoniazid) should not be used alone for this disease because of the rapidity with which resistance may develop. The usual dose is 600mg of rifampicin before food daily as a single dose.

Rifampicin is also used in the treatment of extrapulmonary lessions including tuberculosis, meningitis as well as elimination of menigococci from carriers. Usually in the dose of 600mg daily

for 4 days. Rifampicin is a drug of choice for chemoprophylaxis of meningococci disease and meiningitis due to H. Influenza in household contact of patients with such infections. The drug also show promise in the treatment of certain non-mycobacterial diseases. Combined with beta-lactam antibiotic or vancomycin, rifampicin may be useful for therapy of selected cases of staphylococcal endocarditis or osteomyelitis, especially those caused by penicillin 'tolerant' Staphylococci (Goodman and Gilman, 1985).

Rifampicin is also used in treatment of gonorrhoea caused by Neisseria gonorrhea. The usual dose is 1200mg daily as a single dose for one or two days (Reynolds, 1982).

In combination with other drugs like dapsone, rifampicin is used in treatment of leprosy. Usual dose is similar to that used in treatment of tuberclosis.

Also, rifampicin is used in treatment of brucellosis, leggionnaires, leishmaniessis and enteric infections. It is advisable that rifampicin should be reserved only for treatment of mycobacterial infections (Reynolds, 1982).

2.2.6 MECHANISM OF ACTION

Rifampicin inhibits DNA dependant RNA polymerase of mycobacteria and other microorganisms, leading to suppression of initiation of chain formation (not chain elongation) in RNA sysnthesis. More specifically, the β-sub unit of this complex enzyme is the site of action of this drug.

While rifampicin can inhibit RNA synthesis in mammalian

mitochondria, considerable higher concentrations of the drug are required for inhibition of the bacteria enzyme. Rifampicin is bactericidal for both intracellular and extracellular micro-organisms (Goodman and Gilman, 1985).

2.2.7 SIDE EFFECTS AND TOXICITY

Rifampicin does not cause untoward effects with great frequency. When given in usual doses, less than 4% of the patients with tuberculosis develop significant adverse reactions: the most common are rash (0.8%), fever (0.5%), and nausea and vomitting (1.5%) (Groset et-al, 1983).

The most notable problem is the development of jaundice. Sixteen deaths associated with this reaction have been recorded in 500,000 treated patients. The jaundice may be associated with concomitant use of other agents, including isoniazid, and fatalities have been reported in patients with pre-existing liver dis-orders or who have taken other potentially hepatotoxic agents. Hepititis from rifampicin rarely occurs in patients with normal liver function. However, chronic liver disease, alcoholism, and old age appear to increase the incidence of severe hepatic problems when rifampicin is given alone or concurrently with isoniazid (Gronhagen-Riska et-al, 1978).

Administration of on an intermittent schedule (less than twice weekly) and/or daily doses of 1200mg or greater is associated with frequent side effects, and the drug should not be used in this manner. A flulike syndrome with fever, chills, and myalgias develops in about 20% of the patients. The syndrome may also

include eosinophillia, interstitial nephritis, acute tubular necrosis, thrombocytopenia, heamolytic anemia and shock (Girling et-al, 1979).

The gastrointestinal disturbances produced by the rifampicin have occasionally required discontinuation of the drug. Various symptoms related to the nervous systems have been noted: ataxia, confusion, inability to concentrate, generalized numbness, pain in the extremities, muscular weakness, headache, dizziness, fatigue and drownsiness. Among the hypersensitivity reactions are fever, pruritus, uticaria, various types of skin erruptions, soreness of the mouth and tongue.

Immunoglobulin light-chain proteinuria have been noted by Graber et-al (1973) in about 85% of patients with tuberclosis treated with rifampicin. Renal failure has however, been associated with light chain protenuria. Rifampicin also causes immunosupression in animal models: this may be related to inhibition of protein synthesis by the cells involved in the immune process. There is no evidence that rifampicin induced immunosuppression causes deleterious effects in patients receiving influenza vaccine.

Potential tetragenicity of rifampicin is unknown it is best to avoid the use of it during pregnacy: as the drug is known to cross the placenta.

2.2.8 *RIFAMPICIN - DRUG INTERATION*

Since rifampicin is a potent inducer of hepatic microsomal enzymes (Ohnhaus et al, 1979), its administration results in a

decreased half-life for a number drugs, including prednisolone, digitoxin, quinidine, ketoconazole, propranolol, metoprolol, clofibrate, sulphonylureas, oral anitcoagulants (coumarin type): Rifampicin appears to enhance catabolism of a variety of steroids and oral contraceptives leading to decrease in their effectivness. Methadone metabolism is also increased, and precipitation of withdrawal syndromes have been reported.

Rifampicin may reduce biliary excretion of contrast media used for visualization of the gallbladder. These effects of rifampicin are noted to appear about 4 to 8 days after the administration of rifampicin is started and persist for 5 to 7 days after its is stopped (O'Reilly, 1975). Some workers have reported that the induction ability of the rifampicin is dose dependant and interindividual varibaility in response to it. Ohnhaus and Park (1979) - have demonstrated the dose dependancy and interindividual variability in hepatic microsomal enzyme induction using rifampicin as the inducing agent and measuring antipyrine clearance.

The table below gives a summary of rifampicin during interactions and this significant level of the interation.

Table 2.2.1: TABLE OF RIFAMPICIN DRUG - INTERACTIONS AND THEIR SIGNIFICANT LEVELS.

Drugs	Significance	Reference
I ANTI-COAGLANTS	There is diminished effect	
i Acenocumarol	Significant	O'Reilly, 1974; Michot et-al, 1970
ii Warfarin	Significant	O'Reily, 1974.
II ANTI-DIABETICS	There is decreased anti-diabetic activity.	
i Chlorpropamide	Significant	Self and Moris, 1980.
ii Tolbutamide	Significant	Syvalahti et-al, 1974
III CORTICOSTEROIDS	There is decreased response to corticosteroids.	
i Cortisone Acetate	Significant	Humfar, 1974; Edward, et-al, 1974
ii Fludrocortisol	Significant	Hunter, 1974; Edward, et-al;
iii Predmsolone	Significant	Hendrickses, 1979; Steenbergy et-al, 1977.
IV ORAL CONTRACEPTIVE	Decreased activity of oral contraceptives is usually noted. And it is significant.	Kropp's et-al 1974. Reimmer et-al,1971; Bolt et-al 1975
V Clofibrate	Decreased half-life Not Significant.	Houin et-al, 1978
VI Barbiturates	Significant decrease in half-life.	Zilly et-al, 1977.
VII Isoniazid	Not significant Interaction	Mouton et-al, 1979.

Drugs	Significance	Reference
VIII Quinidine	Significant decrease in its half-life	Ahmed et-al, 1979: Twum-Barima et-al 1980.
IX Probenecid	No significant interactions	Levi et-al, 1973: Fallon et-al, 1975.
X Dapsone	No significant interactions	Rusell et-al, 1975: Gelber et-al, 1977: Acocelle et-al,1980.
XI Antipyrine	Significant decrease in plasma half-life	Ohnhaus and Park, 1979.
XII Digoxin	Significant decrease in half-life	Peters et-al, 1974: Novi et-al, 1980.
XIII Nifedipine	Significant	Tada et-al, 1992.

2.2.9 PROPRIETARY PREPARATIONS:

i. Rifampicin capsules (usp) 300mg.

ii. Rifadin® (Murell, UK) - available as capsules of 150mg and 300mg. Also as syrup 100mg/5ml.

iii. Rimactane® (Ciba, UK) - as capsules 150mg and 300mg: syrup - 100mg/5ml.

iv. Rimactazid® (Ciba, UK) - 150mg rifampicin and 100mg Isoniazid - usually as tablets.

Rimactizid® 300mg - tablets containing 300mg rifampicin and and 150mg Isoniazid.

Several other proprietary preparations are also available.

2.3. CLINICAL SIGNIFICANCE OF DRUG INTERACTION

It is traditional practice to prescribe a large number of drugs, in the treatment of a patient with a given disease condition. In modern medicine this process is regarded as polypharmacy. One of these prescribed drugs may have effect on the pharacokinetic parameters of another, i.e. one drug alters the absorption, distribution, metabolism or excretion of another thus increasing or reducing the amount of the drug available to produce its pharmacological effects.

For instance, Cluff and Steward (1972) found that the average patient admitted to a medical ward of their hospital received 14 drugs. In the light of this and many other relevant informations, it seems reasonable to speculate that a very large number of patients may be at risk of having potentially harmful drug interactions and that an important problem in modern threapeutics might exist.

Examples are given of various drug interactions with clinical significant effects involving rifampicin. It is therefore, of clinical relevance for a careful monitoring to instituted when rifampicin and suspected drugs have to be concurrently administered.

2.4 OBJECTIVES AND SCOPE OF PRESENT STUDY

Nifedipine a dihydropyridine calcium channel blocker is well known to undergo first-pass effect if administered orally. And due to numerous reported cases of drug-drug interactions involving rifampicin (some clinically significant) it was pertinent to look

at a local possible experience using healthy volunteers.

In Nigeria like any other developing country, there are million cases of people suffering from angina disease, hypertension, staphylococcus endocarditis and meningicoccal diseases, and in this situation rifampicin is prescribed together with other anithypertensive drugs especially nifedipine. Therefore, there is need to carry out a well controlled study in healthy volunteers to investigate *rifampicin - nifedipine* interactions in this respect. The proposed study is therefore aimed at understanding the nature and extent of the possible interaction between the two drugs.

These two drugs are selected because they are the most commonly used here for the treatment of ailments highlighted above. Their used may not be far from their low incidence of side and toxic effects. Also the literature cited contained very scanty information on the potential interactions between the two drugs.

On the whole, the study is expected to address itself to the following objectives:-

i. To determine the pharmacokinetic profiles in man, of the nifedipine.

ii. To determine the effect of Rifampicin on the pharmacokinetic profiles of the nifedipine.

iii. To evaluate the effect of rifampicin on the bioavailability of nifedipine in man.

iv. The study also aims at quality control assessment of different brands of nifedipine tablets.

This study in general employs a sensitive, rapid and selective high performance liquid chromotographic (HPIC) method for estimating nifedipine in tablets and plasma samples.

GMBH 6056 Hensenstamm Kr. Offenback-Main).

- Kerry Ultrasonic Vibrator (Kerry Components, England).
- Quick fit centrifuge tubes 15ml.
- Quick fit conical flasks (250ml, 500ml, 1000ml).
- Technico Volumetric flasks (10ml, 25ml, 50ml, 100ml, 1000ml).
- 100µl Syringe (Hamilton Reno, Nevads).
- Technico pippettes (0.5ml, 1.0ml, 2.0ml, 5.0ml and 10ml).
- Technico burette (50ml).
- Oven - BS model OV 330 (Gallen kamp England).

3.1.2 REAGENTS

- Methanol (Rectapur®) - Analar grade (Prolabo - Group Rhone-Pouleric, Machenster M30 7RT); For May and Baker Nigeria PLC.
- Sodium Acetate - (Anhydrous) Analar grade (BDH chemicals, Poole, England).
- Dichloromethane - Analar grade (BDH chemicals, Poole, England).
- 4-Dimethylaminobenzaldehyde - (BDH chemicals, Poole, England).
- Acetic Acid Analar grade (BDH chemicals, Poole, England).

3.1.3 DRUGS

- Rifampicin capsules 300mg. Batch number 920937; manufactured September, 1992, Expiry date Sepember, 1995. Manufacturers - Midicalex, Millan, Italy.
- Nifedipine Powder (standard sample) Pat. No. 198208k. - Supplied by Bayer AG, Lever Kunsen, West Germany.
- Adalat® capsules 10mg, Batch no. 83226B manufatured by Bayer AG, Level Kunsen, West Germany. Manufacturing date; June, 1993. Expiry date; June, 1996.

- Nifecard® (Sustained Released Nifedine Tablets 20mg). Batch no. 0144. Manufactured by Lek Pharmaceuticals, Yugoslavia. manufactured date: March, 1993; expiry date, March, 1996.
- Adalat® Retard Tablets 20mg, Batch no. 025103A - Manufactured by Bayer AG, Lever Kunsen, West Germany, manufacturing date - October, 1993: expiry date October 1996.

3.1.4 SOLVENT SYSTEM

The solvent system used is a buffered system consisting of 0.01M sodium acetate buffer (pH4) mixed with methanol in the ratio of 45:55 respectively. The pH of the solvent system was adjusted to 5.2 using 0.01M sodium acetate. Before constituting the solvent system, the 0.01M sodium acetate buffer was filtered with Waters Millipore filter size 0.45µm; while the methanol was filtered with 0.5µm size waters millipore filter.

The solvent system was degassed by ultrasonication for 15 minutes.

3.2 METHODS

3.2.1 IN-VITRO STUDIES

3.2.1.1 u.v. Spectrophotometric methods of analysis and identification

20 tablets of each brand of nifedipine was weighed and average weight taken.

The tablets were then crushed with pestle and mortar into powdered form.

A 0.02gm equivalent of the powdered tablets was dissolved in 25ml of methanol in 50ml volumetric flask and made up to 50ml with

sufficient mathanol. The flask was wrapped with alluminium foil to prevent photodegradation of the drug and shaken on the flask shaker for 20 minutes. The solution was then filtered and 0.5ml of the filtrate was transfered into a 10ml wrapped volumetic flask and the volume made up to 10ml with 0.1M HCL and shaken for 5 minutes. The absorbance of the resulting solution was measured at the λ maximum 349nm using 0.1M HCL as blank (using 1cm silica cells). Also the peaks of maxima absorption were identified and the wavelength recorded.

The percentage contents of nifedipine was calculated using the relationship:-

$$\frac{A^{1\%,1cm} \text{ of drug}}{A^{1\%,1cm} \text{ of standard drug}} \times 100\%$$

Six replicate solutions were prepared from the same sample and the aborbance determined.

3.2.1.2 **Chemical Identification Test**

The identification test was carried out as described in BP 1988 addenddum 1990.

Procedure:-

Equivalent of 0.025gm of each brand of nifedipine tablets were weighed and 10ml mixture of 5 volumes of ethanol (96%), 3.5 volume of water and 1.5 volume of hydrochloric acid was added and dissolved with gentle heating. 0.5gm of zinc (granulated) was added and allowed to stand for 5 minutes with occasional swirrling and filtered. To the filtrate was added 5ml of a 1% w/v solution of sodium nitrates and allowed to stand for 2 minutes. Then 2ml of

a 5% w/v solution of ammonium sulphamate was added, and the mixture shaken vigorously with care and 2ml of 0.5% w/v solution of N-(1-naphthyl) ethylene diamine dihydrocholride was added. The colour that was formed was them noted.

3.2.1.3 Melting Point Determination.

The melting point was determined as described in BP 1988 addeddum 1990.

Procedure:-

A quantity of the powdered tablets of the three different brands equivalent to 0.02gm of nifedipine were extracted with four quantities each of 5ml of acetone and filtered. The filtrate was evaporated carefully to dryness on a water bath. A sufficient portion of the dried residue of different brands and standard nifedipine powder were placed in series of capillary tubes and packed well by tapping on a hard surface to form a tightly packed column of about 3mm in height. The capillary tubes were then introduced into the melting point apparatus and then switch on. The temperature at which the last particle disappeared was regarded as the melting point of the substance.

3.2.1.4 *Disintegration Test*

This was carried out as specified by BP 1988 using an Erweka disintegrating apparatus (see Appendix II).

Procedure:-

One tablet each was placed in the tubes of the basket, a disc was added to each tube. The assembly was suspended in a beaker containing distilled water maintained at 37±0.5^{0}C and the apparatus

was operated for fifteen minutes.

The mean of five replica determination was calculated for each brand of tablets.

3.2.1.5 Dissolution Test:

The rotary Basket method as described in the BP 1988 was used (see Appendix I).

Procedure:-

One tablet of nifedipine was placed in the basket, and this in turn was placed in the round bottom flask containing 1000ml of 0.1M HCL maintained at 37±0.5^{0}C. The stirrer was set at 100 revolutions per minute. Samples of 10ml of the dissolution medium were withdrawn at 5, 10, 15, 30, 45 and 60 minutes. These were at the same time being replaced by equal volume of 0.1M HCL previously maintained at 37±0.5^{0}C. The samples were withdrawn from a point midway between the basket wall and the side of the vessel to ensure that the normal pattern of flow of the dissolution medium was not disturbed. The samples were filtered using what man filtered paper and assayed by reference to a standard calibration curve. The absorbance of each sample was measured at a λ maximum of 349nm.

The dissolution was performed five times. The cummulative concentration at a given time was calculated using the formula of Wurster and Taylor (1965):

$$C_n = C_{n(measured)} + \frac{x}{1000} \sum_{s=1}^{n=1} (C_{n\ measured})$$

Where:-

$C_{n(measured)}$ ➡ the spectrophotometrically measured concentration.

C_n --------> *Concentration of the nth sampling* expected in the medium if the previous samples had not been removed.

x ---------> Volume of the sample removed at each time withdrawal was made

1000 -------=> Volume of the dissolution medium in millilitres.

3.2.1.6 <u>Calibration Curve - (u.v. spectroscopy)</u>

50mg of Nifedipine standard powder was accurately weighed, and dissolved and made up to mark with methanol in 100ml wrapped *volumetric flask. The solution thus contains 500µg/ml of* nifedipine. 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0ml of the standard solution above were transfered into series of wrapped 100ml volumetric flasks. Enough 0.1MHCL (methanolic) was added to made the volume of each flask to 100ml. Solutions of various *concentrations (5 - 30µg/ml) were obtained. The absorbances were* measured at λ max. 349nm in 1cm cell using 0.1MHcl as blank. The graph of the absorbance versus concentration was plotted.

3.2.1.7 <u>**Preparation of Standard Solutions**</u>:-

3.2.1.7.1 <u>Preparation of Internal Standard (4-Dimethylamino-*benzaldehyde)*</u>

10mg of the 4-Dimethylaminobenzaldehyde was weighed and dissolved in wrapped 100ml volumetric flask and the volume made up

to 100ml with methanol. The resulting solution was 0.1mg/ml. 1.0ml of the solution was further diluted with methanol in 100ml volumetric flask and the volume made up to 100ml with methanol. The resulting solution was 1µg/ml. The working concentrations of 500ng of 4-dimethylaminobenzaldehyde were prepared from the above solution (1µm/ml).

3.2.1.7.2 Preparation of 0.01M Acetate Buffer (pH 4.0)

0.01M Acetate buffer (pH 4.0) was prepared by dissolving 11.55ml of acetic acid in 1000ml of distilled water in a volumetric *flask. The resulting solution is referred to as solution A.* 16.4gm. of sodium acetate anhydrous was dissolved in to 1000ml of distilled water in 1000ml volumetric, and referred to as solution B. 41.0ml of solution A was added to 9.0ml solution B in a 100ml flask. The volume of the mixture was then made up to 100ml with distilled water. The resulting solution was referred to as the acetate buffer pH 4.0.

3.2.1.7.3 Preparation of Standard Nifedipine Solutions

10mg of nifedipine powder (standard) was weighed and dissolved in 40ml of methanol in a wrapped 100ml volumetric flask and sufficient methanol was added to make the volume up to 100ml. 1.0ml of the resulting solution was made up to 100ml with methanol in a 100ml volumetric flask. The resulting solution was 1µg/ml. From this the working solutions of 20-200ng were prepared by serial dilutions with methanol.

3.2.2 IN-VIVO STUDIES

3.2.2.1 Protocols of the study

Six healthy volunteers (aged between 28.5±6.3 years old and weight of 60.67±3.45kg) were involved in the study. The volunteers have normal laboratory values and are free from liver, kidney and respiratory diseases. They are also non-smoking and do not consume alcohol.

The study was divided into two phases:-

PHASE I:-

This phase is also referred to as pilot studies (OR control).

Each of the volunteers was administered with 10mg of nifedipine soft gelatin capsule (Adalat® capsule 10mg) after over night fasting using 200ml of water to swallow the capsules. The fasting was continued for another 5 hours after the commencement of the study.

PHASE II:-

This is referrred to as cross-over studies, usually 2 weeks wash out period after Phase I.

In this phase, 1200mg of rifampicin capsule was administered orally to the volunteers with 200ml of water. After 8 hours, 10mg of nifedipine capsule (Adalat® capsule) was administered orally with 200ml of water.

The volunteers usually fast over night and had a breakfast of one bottle of coke (coca-cola) with biscuits 5 hours after the administration of nifedipine capsules.

The blood sampling was done via a cubitally placed in dwelling

cannula. Blood samples (5ml) were withdrawn immediately prior to each dosing and at 0.5. 1.0. 1.5. 2.0. 3.0, 4.0. 6.0, 8.0 and 12.0 hours respectively. The samples were collected in heparinized tubes, centrifuged at lOOOgm for 15 minutes and the plasma harvested into Bijou bottles and kept at -10°C until analysis.

3.2.2.2 **Analysis**

3.2.2.2.1 Extract ion

The extraction method of Mustapha et-al (1992) was adopted.

The plasma (0.5ml) was placed in a 15ml centrifuge tubes: 0.5ml of methanol containing 500ng of 4-dimethyl aminobenzaldeh (internal standard) was added. The mixture was then deproteinized with 0.5ml of acetic acid and whirlmixed on an autovortex mixer for 15 seconds. Then 5ml of d i chloromethane was added to the mixture and whirlmixed again for 15 seconds and centrifuged at lOOOgm for 15 minutes. The aqueous layer was removed using appendof pipette and the organic layer was transfered to a clean 15ml centrifuge tube and the contents was evaporated to dryness under a stream of nitrogen at 40°C. The dry residue was reconstituted with lOOul of methanol and 15ul aliquot was injected into the HPLC machine.

3.2.2.2.2 Calibration Curve - (HPLC Method)

A fresh plasma was collected from the healthy volunteers who had not taken any drug for about three weeks.

0.5ml of the plasma was placed in a 15ml centrifuge tube and extracted as above (3.2.2.2.1). Two other samples were prepared as above; one containing 50ng of nifedipine standard powder and the

other containing 500ng of 4-dimethylaminobenzaldehyde and the extraction was repeated as was discribed above. The retention time of both the nifedipine and 4-dimethylaminobenzaldehyde was determined.

The plasma calibration curve was prepared by spiking the blank plasma sample (0.5ml) with 10, 20, 5o, 100 and 150ng of nifedipine and with 500ng of 4-dimethylaminobenzaldehyde as the internal standard. The samples were extracted as above.

The calibration curve was based on peak-height ratio between the nifedipine and internal standard. A plot of the peak height ratio against the standard concentration was constructed. A linear calibration curve was obtained and the co-efficient of linear regression of the slope was determined. The calibration curve was used to determine the concentration of nifedipine in individual plasma samples using microcomputer programme GRAPHAD[8].

The extraction procedure was repeated with 50ng/ml of nifedipine in methanol without plasma and the percentage extraction recoveries of nifedipine was calculated using the relationship:-

$$\frac{\text{Peak-Height ratio of plasma nifedipine}}{\text{Peak-Height ratio of nonplasma nifedipine}} \times 100$$

3.2.2.2.3 Conditions of Analysis of nifedipine using HPLC:-

The conditions of analysis of the nifedipine was adopted from the work of Mustapha et-al (1992).

- The solvent system consists of methanol: 0.01M sodium acetate buffer (pH 4.0) in the ratio of 55:45 respectively.
- pH of mobile phase:- 5.2 (adjusted with 0.01M sodium acetate).

- Flow Rate:- 2.0ml/min.
- Pressure:- 2000 psi
- Sensitivity:- 0.005 Aufs.
- Sample size:- 15µl.
- Chart speed:- 0.25cm/min.
- Filter:- 254nm.

3.2.2.2.4. Data Handling:

The values for the concentration of nifedipine in individual volunteers plasma samples were determined by the use of the computer programmes GRAPHAD® and STATIS 3®. Also the pharmacokinetic parameters of the nifedipine were determined using the same computer programmes.

CHAPTER FOUR

4.0. RESULTS AND DISCUSSIONS

4.1 IN-VITRO STUDIES

4.1.1 Identification Tests

The chemical identification test was carried out as specified in 3.2.1.2. An intense red colour which persisted for more than five minutes was produced for all the brands of the nifedipine tablets/capsules tested. This result is in agreement with BP 1988 Addendum 1990 specification.

Thus, all the three brands of the nifedipine contain the same active ingredient.

The absorption spectra of the three brands of nifedipine (MBY) preparations in 0.1HCL (methanolic) is shown in figure 4.1.1-abcd. The spectra gave three absorption maxima each at 205nm, 236nm, and 342nm. These absorption maxima compared with that obtained by Syed Laik Ali (1989).

The absorption maxima represent the nature of the chromophoric groups in the chemical structure of nifedipine i.e - the pyridine ring, aromatic ring, large alkyl substituent groups.

Melting point:- The melting points of the extracted nifedipine residue from the MBY - brands of nifedipine and the reference samples are 174^0, 174^0, 175^0 and 174^0C respectively. These values are within the acceptable limits for the melting point of nifedipine as stipulated in the BP 1988 Addendum, 1990 ($171-175^0$C).

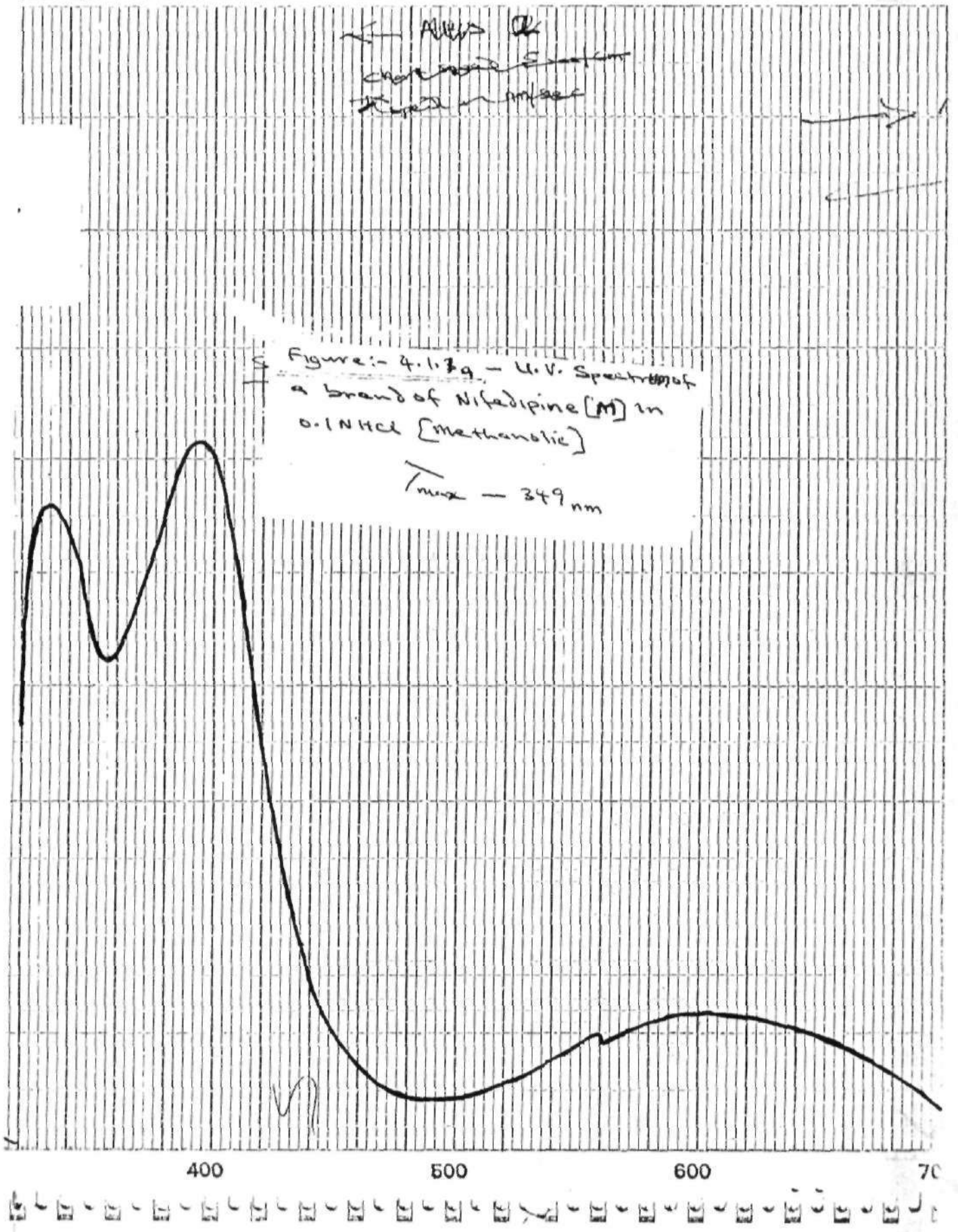
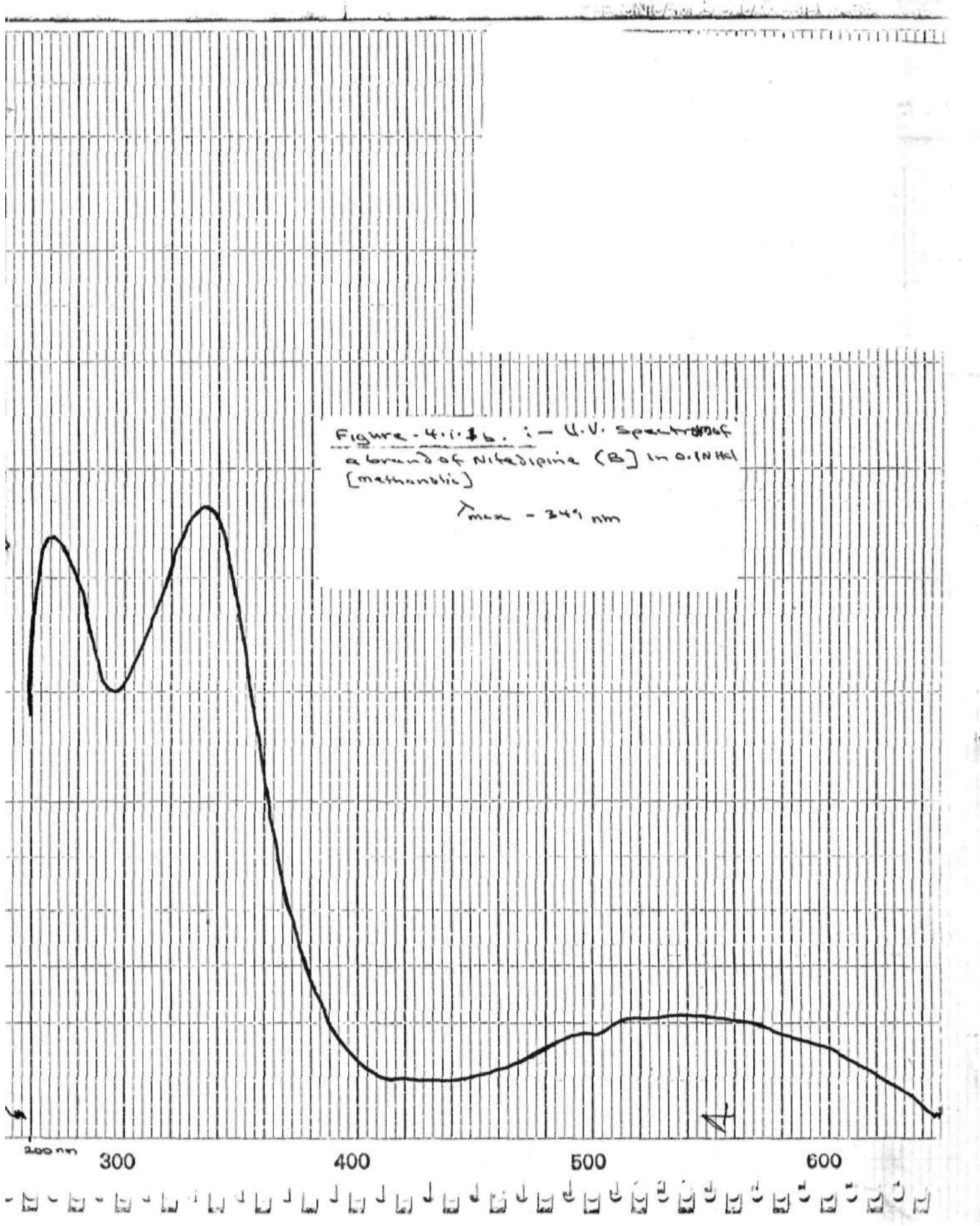


Figure:- 4.1.39 – U.V. Spectrum of a brand of Nifedipine [M] in 0.1N HCl [Methanolic]

λ_{max} – 349 nm


Figure - 4.1.16. :- U.V. Spectrum of
a brand of Nitedipine (B) in 0.1N HCl
[methanolic]
λmax - 341 nm
200 nm
300
400
500
600

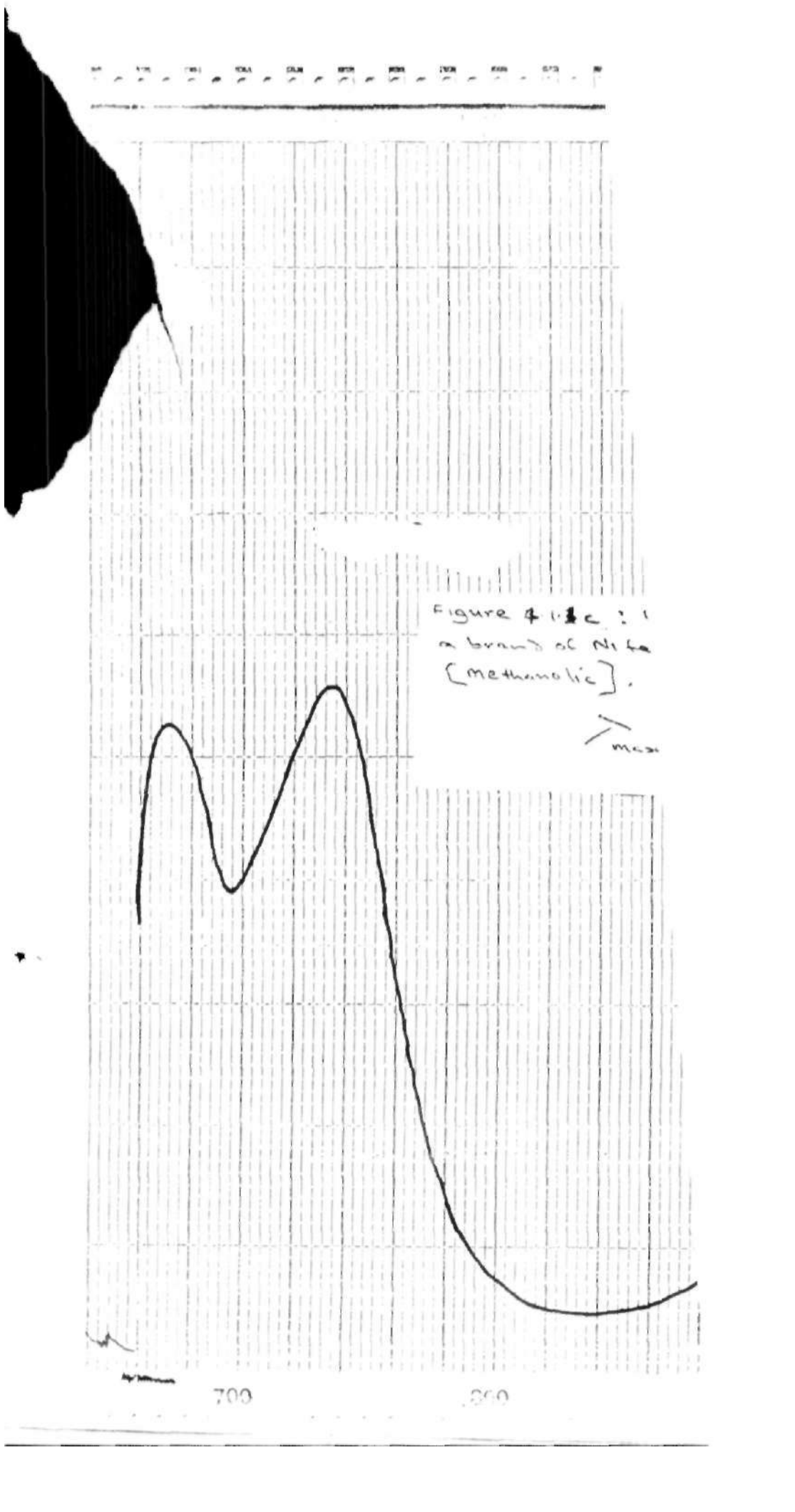


Figure 4.1.1c : A
a brand of Nike
[methanolic].

λ max

4.1.2 **Disintegration Test**

The disintegration profile obtained for the three brands of nifedipine (MBY) is presented in the Table 4.1.1 below. The result showed that the time taken for all the brands to disintegrate was less than 10 minutes. The BP 1988 stipulated time limit for tablet/capsule disintegration is 15 minutes. Hence all the brands passed the official requirement for tablet/capsule disintegration.

Disintegration test is not a measure of bioavailability of a drug. This is because the disintegrated particles have to be dissolved before absorption into the body.

Table 4.1.1: Disintegration times for the MBY - brands of Nifedipine tablets/capsules.

Tablet No.	Disintegration time (minutes)		
	M (Capsules)	B (Tablet)	Y (Tablet)
1	6.5	7.5	8.5
2	7.5	7.5	9.5
3	6.5	8.0	8.0
4	6.5	9.0	8.0
5	7.3	8.5	7.5
MEAN ± SEM	6.76 ± 0.15	8.10 ± 0.26	8.30 ± 0.30

SEM = Standard Error of the Mean.

4.1.3 Dissolution Rate Test:

The dissolution rate profiles of the three brands of nifedipine (MBY) in 0.1N Hcl are shown in the Table 4.1.2. Figure 4.1.2 shows graphical representation of the dissolution rate profiles. The plotted points represent average of six tablets. Complete release of the nifedipine (active ingredient) was obtained within 30 minutes for all the brands. B. P. 1988 requirements for the dissolution rate test is that not less than 70% of the stated amount of the active ingredient is in solution at the end of 45 minutes for any dosage form unless otherwise stated in the monograph. The result obtained complied with the stipulated official specification.

Table 4.1.2: In Vitro disssolution profile of three (MBY) brands of Nifedipine in 0.1N HCl (Methanolic) using BP 1988 Rotating Basket Method - At 100 rpm at 37 ± 0.5^{0}C.

Time [Mins]	CUMMULATIVE PERCENTAGE RELEASED ± SEM		
	M	B	Y
5	18.50 ± 0.40	10.80 ± 0.60	11.50 ± 0.70
15	68.60 ± 0.75	56.20 ± 0.80	58.64 ± 0.98
30	99.90 ± 0.20	99.60 ± 0.35	99.80 ± 0.26
45	99.70 ± 0.45	99.90 ± 0.28	99.90 ± 0.34
60	99.50 ± 0.30	99.60 ± 0.50	99.40 ± 0.83

n = 6
SEM = Standard Error of the Mean.

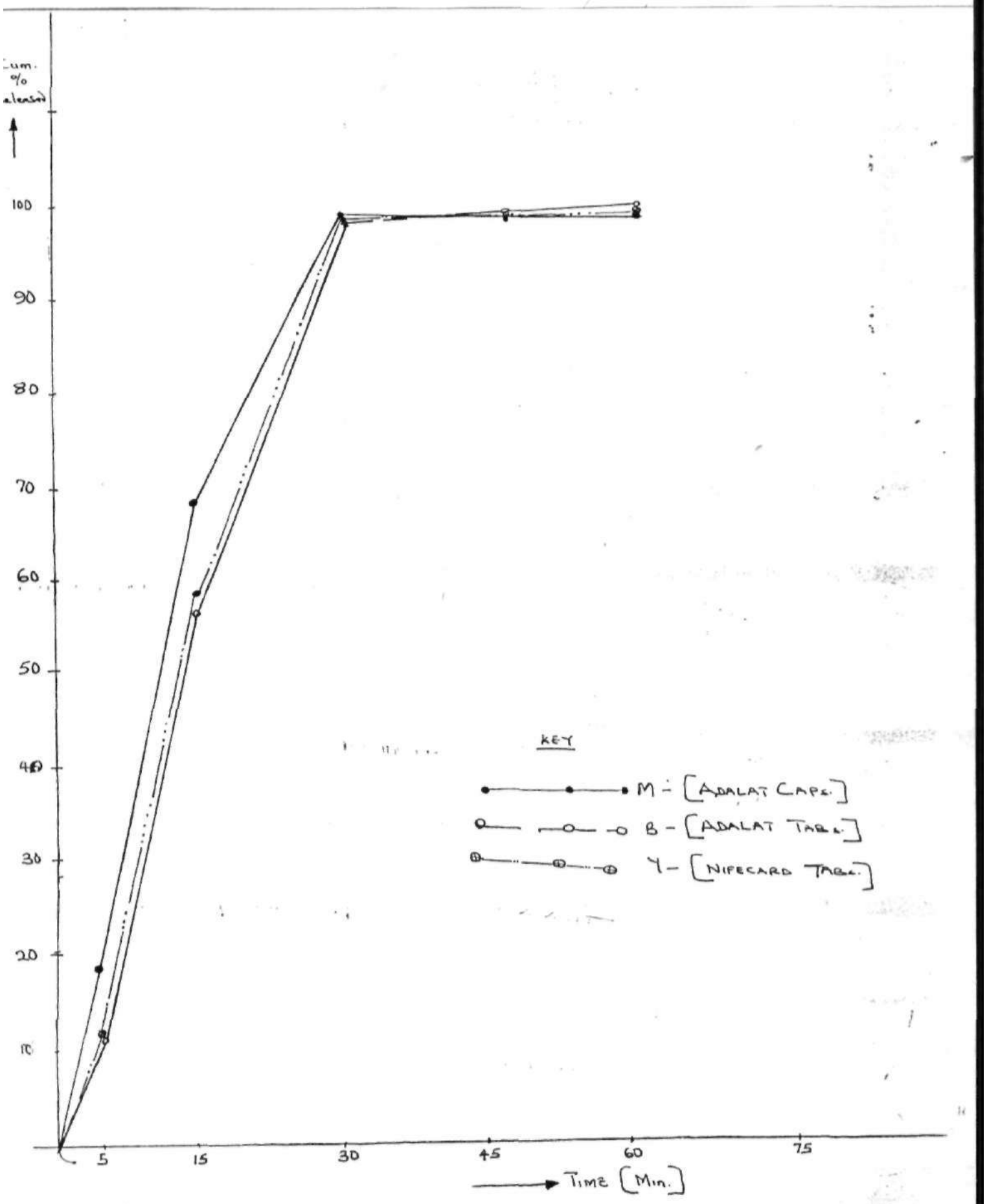


Fig. 4.1.2: Dissolution Rate Profile of three brands of nifedipine in 0.1NHCl (methanolic). Using BP 1988 Rotating Basket method.

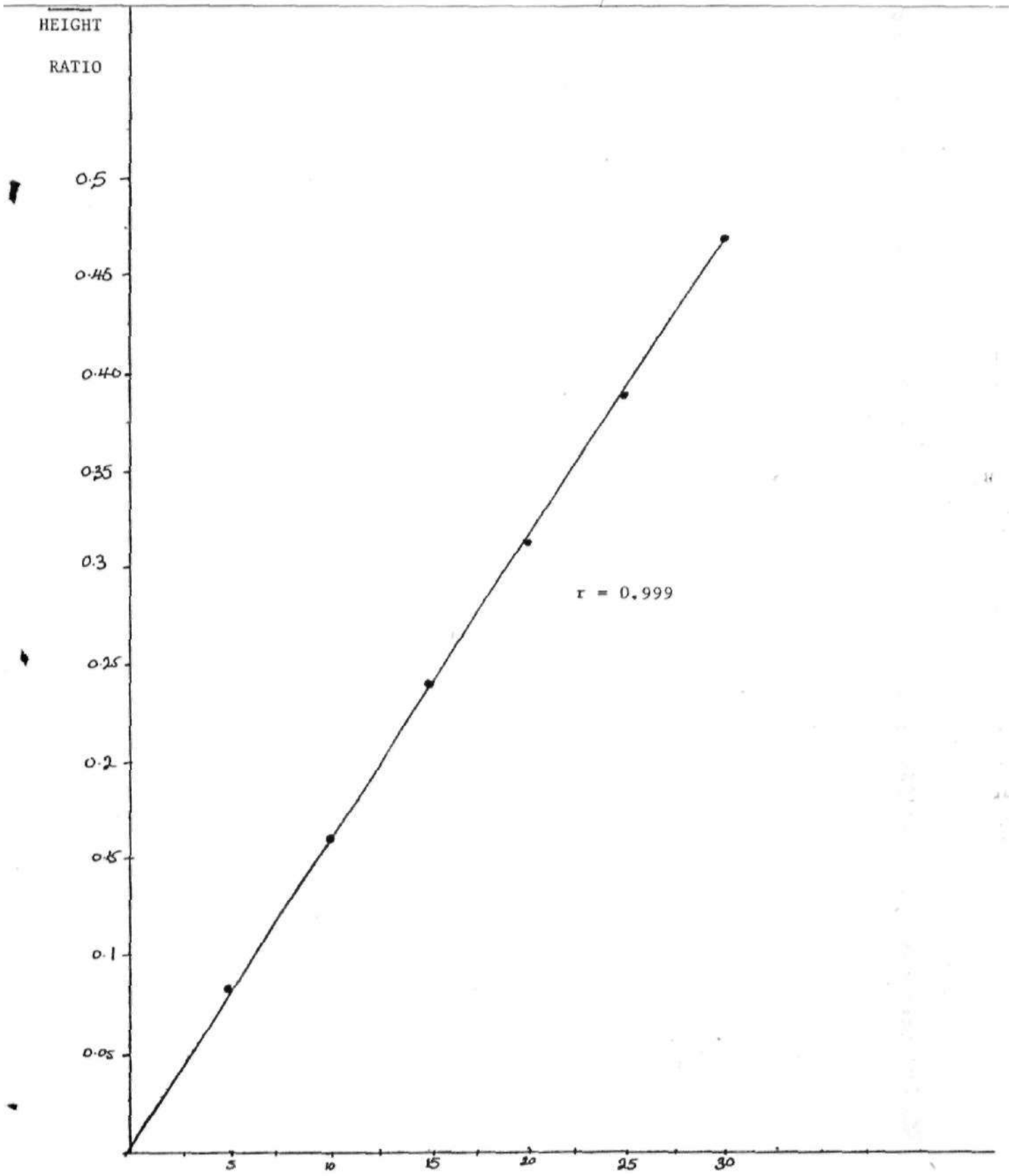


Figure: 4.1.3.: Calibration curve of Nifedipine (5 - 30 µg/ml) in 0.1 N HCl (Methanolic) - Using U.V. Spectrophotometer SP8 - 100.
(- max 349nm)

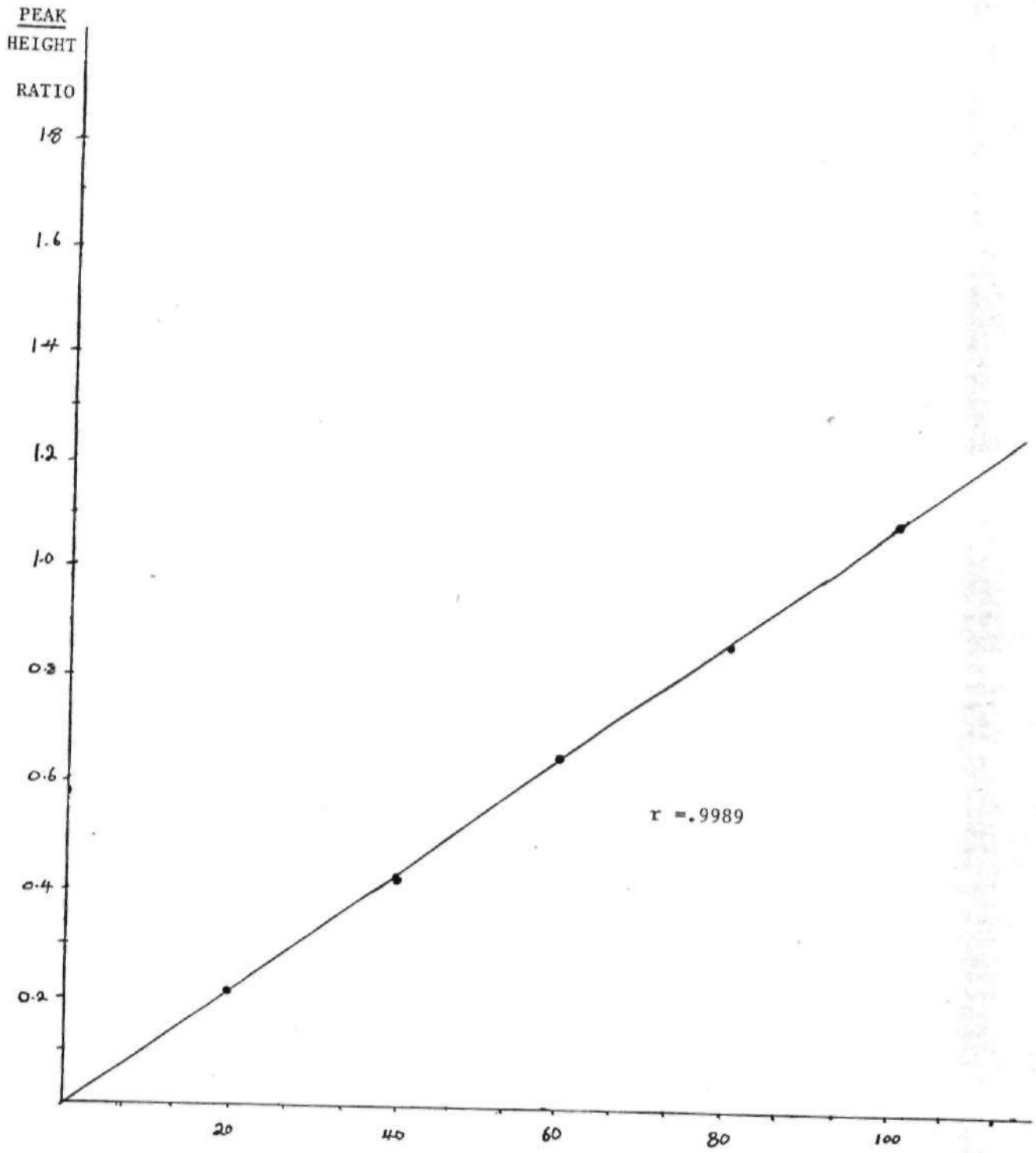


Figure: 4.1.4: Calibration curve for Nifedipine (20.0 - 100.0 µg/ml) Using HPLC.

4.1.4 Assay

Linear calibration curve (5-30µg/ml) with good correlation coefficient (r = 0.9990) of nifedipine in 0.1N HCl (methanolic) using u.v. spectrophotometer (λ_{max} 349nm) is shown in figure 4.1.3.

The quantitative determination of the three brands of the nifedipine using u.v. spectrophotometric methods as above gave the percentage contents of the brands of nifedipine as presented in table 4.1.3. The absorption spectra of the three brands are shown in figure 4.1.1 abcd.

Assay of these brands of nifedipine (MBY) was also conducted using HPLC technique (see chapter 3). With this technique, a linear calibration curve (20 - 100µg/ml) with a good correlation coefficient (0.9989) was obtained as shown in figure 4.1.4. The result is shown in table 4.1.3.

The assay result for the contents of the nifedipine from the two techniques i.e. the u.v. spectroscopy and HPLC are within the limits of 98.0 - 102.0% set by BP 1988 Addendum 1990 for drug contents.

The results indicate that the nifedipine brands tested are of the same chemical equivalence.

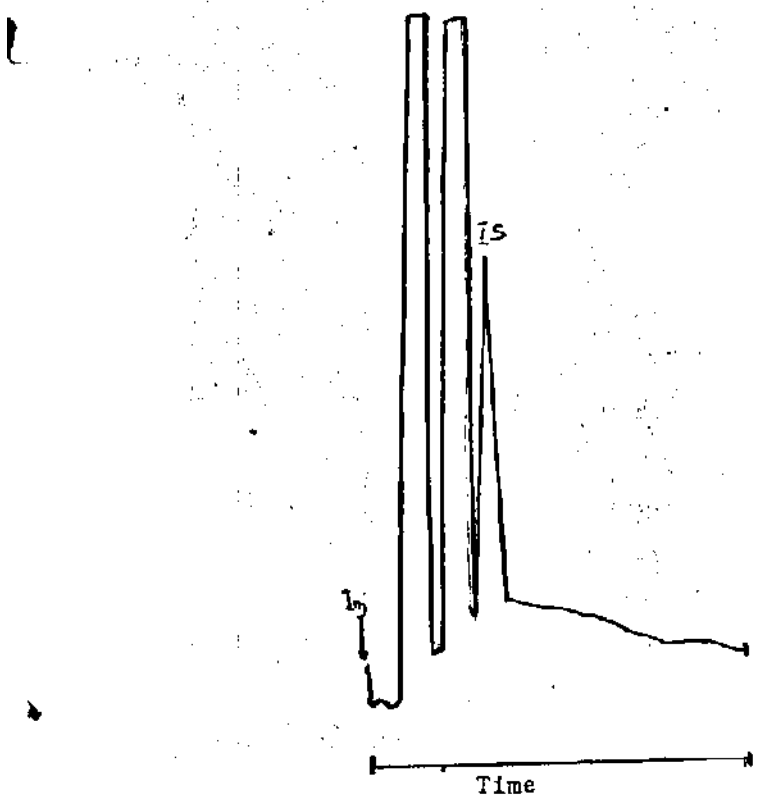


Figure: 4.2.1. HPLC - chromatogram of blank plasma with 4-Dimethy Amino benzaldehyde 500ng/ml [IS]

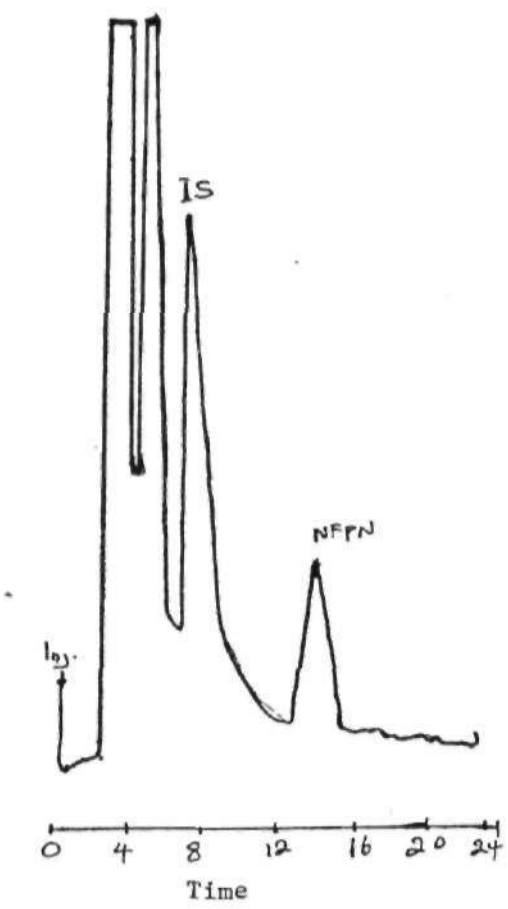


Figure: 4.2.2: HPLC - Chromatogram of plasma of volunteer I 1.5 hours post dosing with Adalat(R) capsule 10mg (Nifedipine)

Table 4.1.3 : Quantitative Determination of Nifedipine preparations using U.V. Spectrophotometric and HPLC methods of analysis.

Drug		Drug Content		Drug Content	
		U.V. Spectros. Method (mg)	% ± SEM	HPLC Method (mg)	% ± SEM
M	10mg Adalat capsule	10.01	100.1 ± 0.30	10.10	101.00 ± 0.55
B	20mg Adalat Tablet	19.98	99.9 ± 0.25	20.04	100.2 ± 0.15
Y	20mg nifecard Tablet	20.01	100.05 ± 0.80	20.02	100.1 ± 0.80

4.2 IN - VIVO STUDIES

4.2.1 Analysis

A reverse - phase HPLC method developed by Mustapha et-al (1992) was used to determine the nifedipine. This method has already been discussed in chapter 3.

The high performance liquid chromatogram of blank plasma and plasma of a voluteer I 90 min. post dosing with 10mg of adalat capsule are shown in figures 4.2.1 and 4.2.2 respectively.

The solvent system achieved a good resolution of nifedipine from its internal standard (4-Dimethylaminobenzaldehyde). The retention time of nifedipine and the internal standard were 14.2 and 7.6 minutes respectively. The capacity factors (k^1) were 4.07 and 1.7 respectively for nifedipine and internal standard. The resolution factor (d) was 2.4. Capacity factor should be between

1 and 10 (i.e. $k^1 \geq 1$). B.P. 1988 Addendum 1990 requires that the minimum standard resolution factor (d) should be 1.5 ($d \geq 1.5$). Thus it can be deduced that at the (d) value of 2.4, the nifedipine peak was well resolved from the internal standard peak.

The calibration curve of nifedipine in the plasma (fig. 4.2.3) gave a good correlation coefficient (r) of 0.997 which showed linearity at the relevant concentrations.

The result of the precision analysis of the method and percentage recovery are shown in tables 4.2.1 and 4.2.2 respectively.

These results compared well with those of obtained by Gulaid et-al (1978).
The limit of detection of this method was found to be 4.5ng/ml.

Table 4.2.1. Precision of Analytical Method

SAMPLE	CONC (ng/ml)	C.V. %	n
Within the run: Nifedipine	10	3.1	5
	40	3.6	5
	80	3.9	5
	120	4.0	5
	150	4.3	5
Between the run Nifedipine	10	3.4	5
	40	3.5	5
	80	3.3	5
	120	4.1	5
	150	4.0	5

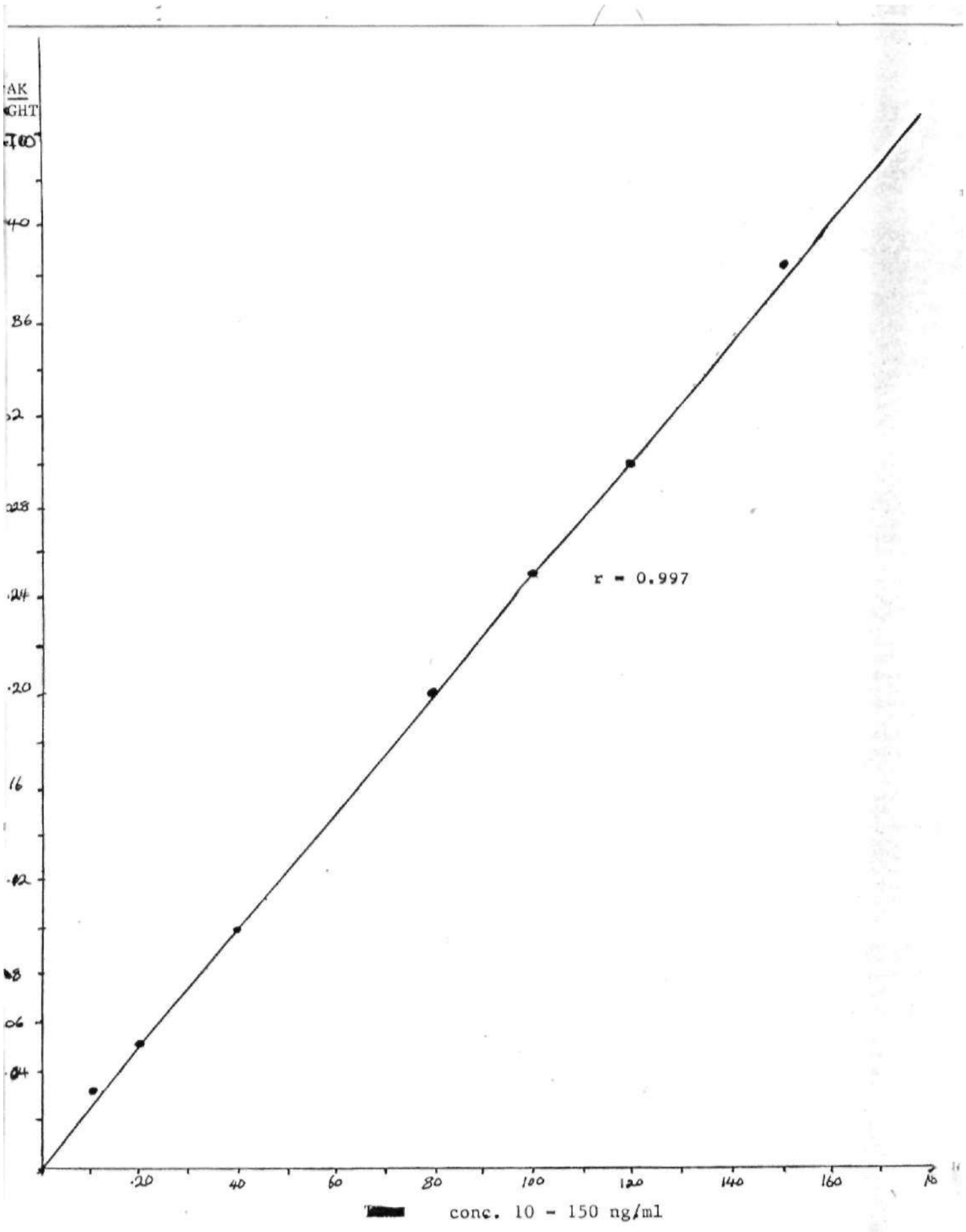


Figure: 4.2.3 - Calibration curve for Nifedipine in plasma sample (10-150ng/ml)

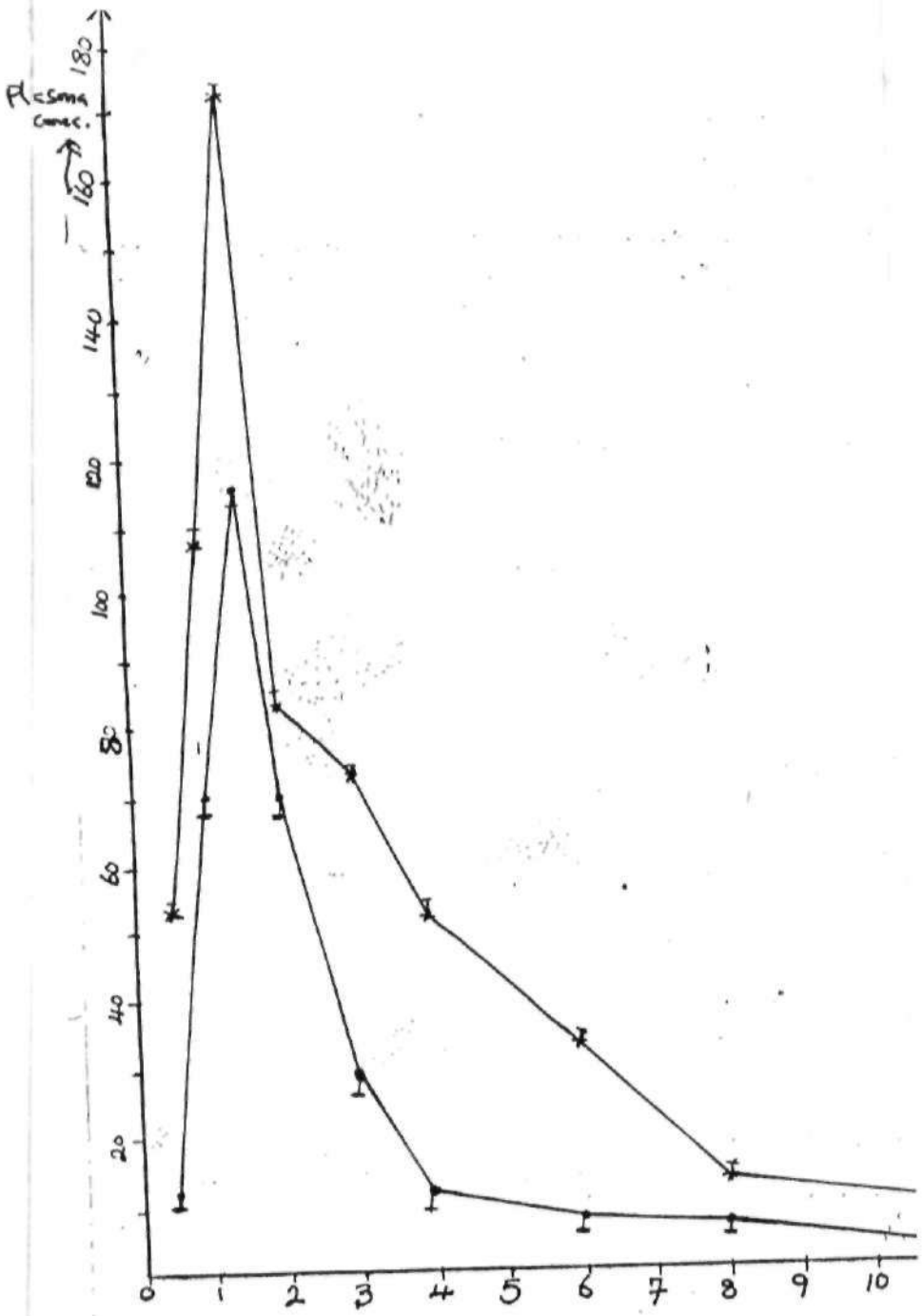


Figure: 4.2.4: Mean plasma concentratio
of Nifedipine capsules [
administration of Rifamp

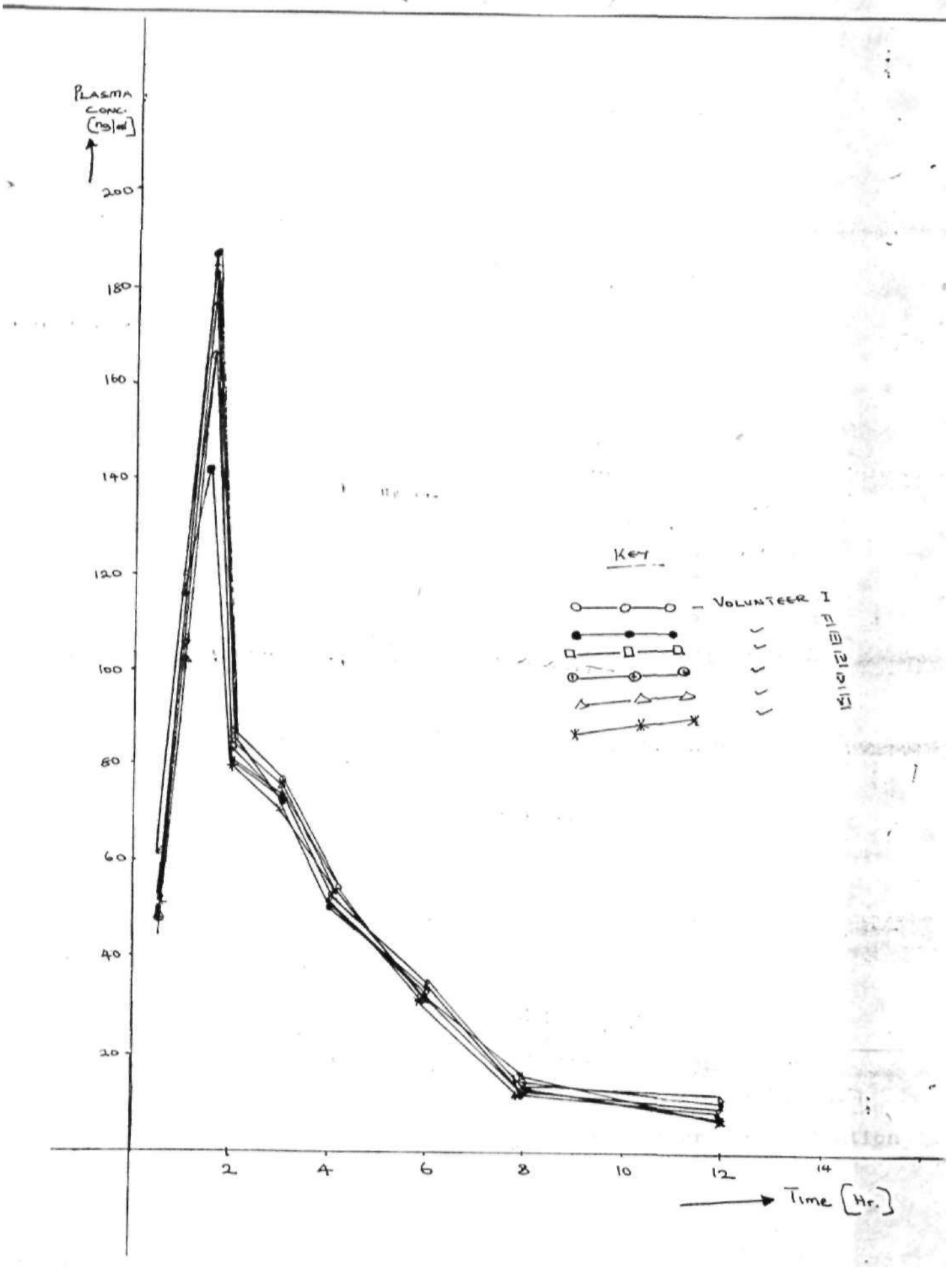


Fig. 4.2.5: Individual volunteers plasma-concentration

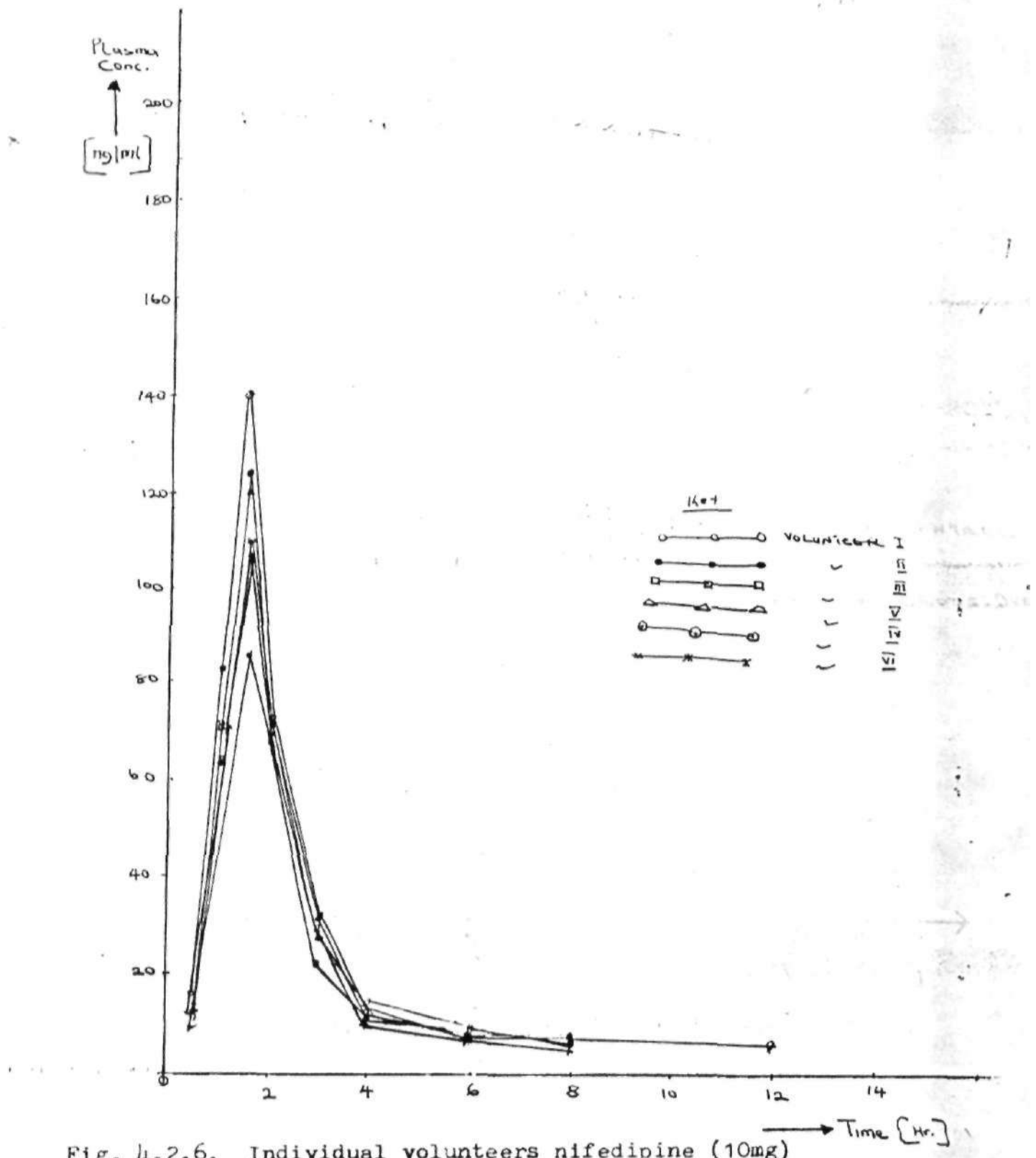


Fig. 4.2.6. Individual volunteers nifedipine (10mg) plasma conc. - time curve 8 hrs. after administration of rifampicin (1,200 mg).

Table 4.2.2 Percentage recovery of nifedipine from Biological metrix.

SAMPLE	CONC. (ng/ml)	MEAN RECOVERY % ± SEM	n
NIFEDIPINE	10	96.50 ± 0.92	5
	40	97.80 ± 1.60	5
	80	97.20 ± 2.93	5
	120	95.80 ± 3.10	5
	150	96.90 ± 1.86	5

SEM = Standard Error of the Mean.

Using the same method of extraction of nifedipine from biological metric, Mustapha et-al (1992) got percentage recovery of nifedipine of 98.1 ± 1.40 (sd).

4.2.2 **Pharmacokinetics**

The pharmacokinetics of nifedipine and its major metabolites have been extensively investigated (Waller et-al, 1984: Kleinblosem, 1984 a,b; Forster, 1975). Forster et-al, (1983) and Horster, (1975) have all shown that there are varying inter individual differences in the plasma level and pharmacokinetics of nifedipine, as shown in this study.

After the oral administration of 10mg nifedipine capsules, the mean maximum plasma concentration (C_{max}) was 173.23 ± 6.23ng/ml at time 1.5 hrs. Some workers like Jokobsen et-al (1979) reported 120ng/ml at time 1.0hr. Dokladalova et-al (1982) reported 150ng/ml at 1.0 hr.: Kleinbloesem reported a C_{max} of 117 ± 15ng/ml at time (t_{max}) of 1.4 ± 0.5 hrs. Achert et-al (1987) reported a C_{max}) of 120 ng/ml at t_{max} of 0.5 hr. after administration of 5mg capsules. Quereshi et-al (1992) reported 186 ± 126 ng/ml.

The elimination half life of the nifedipine observed from this

study was 2.62 ± 0.071 hr. This is against 3.1 ± 0.25 hr reported by Foster et-al (1983) and 1.9 ± 0.5 hr by Schellens et-al (1991).

The total body clearance (cl_T) in this study was found to be 17.33 ± 0.037 ml/min xkg. as against 62.0 ± 0.09 ml/hr/kg obtained by Foster et-al (1983). Kleinbloesem et-al (1984a) reported cl_T of 27L/hr. Quereshi et-al (1992) reported cl_T of 13.5 ± 3.2 ml/min-kg, while schellen et-al (1991) reported 1094 ± 555 ml/min. The volume of distribution was 66.2 ± 2.5 ml/kg as against 132ml/kg reported by Forster et-al (1983). The area under the plasma concentration-time curve ($AUC_{0-\infty}$) was found to be 573.4 ±14.29ng.hr/ml as against 469±140 reported by Waller et-al (1984) and 360 ± 100ng.hr/ml by Quereshi et-al (1992).

The lag time, the absorption rate constant and absorption half life were found to be 0.083±0.10hr: 1.687±0.084hr^{-1} and 0.410 hr respectively.

The elimination rate constant was 0.26±0.008hr^{-1}, as against 0.22±0.02hr^{-1} reported by Mustapha et-al (1992).

The oral administration of rifampicin capsule 1200mg eight hours before nifedipine in a cross-over fashion caused some changes in the mean pharmacokinetic parameters of nifedipine (Figure 4.2.4).

Table 4.2.3 to 4.2.7 shows plasma concentrations and pharmacokinetic parameters of oral nifedipine 10mg in individual volunteers before and after administration of rifampicin 1200mg orally.

After the oral administration rifampicin 1200mg in a cross-

over fashion, the mean maximum plasma concentration (C_{max}) of nifedipine was decreased to 115.77±16.20ng/ml from 173.23±6.23ng/ml, a decrease of about 67%, though the time to attain the C_{max} remained the same (1.5hrs). This change in C_{max} is statistically significant ($P < 0.0001$).

The total body clearance was increased from 17.33 ± 0.37 to 50.17 ± 3.88 ml/min.kg that is an increase of about 194%. This change is statistically significant ($P < 0.0001$). The area under plasma concentration time curve ($AUC_{0\infty}$) was decreased from 573.40 ± 14.29 to 205.25 ± 16.25 ng.hr/ml: that is a reduction of about 62.2%. The change is significant statistically ($P < 0.0001$). Tada et-al, (1992), in their work using tuberculosis patients already on rifampicin 600mg orally daily reported a reduction of $AUC_{(0-\infty)}$ of about 40%. This they said was statistically significant.

The elimination half-life was decreased from 2.62 ± 0.71 to 1.03 ± 0.023 hrs (67% reduction) while the elimination rate constant increased from 0.26 ± 0.008 to 0.67 ± 0.16 hr^{-1} (157.7% increase). These changes are statistically significant ($P < 0.0001$).

The volume of distribution was also increased by 13.75% - an increase from 66.20 ± 0.00 to 75.3 ± 2.5 ml/kg. The change is not significant ($P > 0.1$).

The lag time, absorption half life and absorption rate constants were increased from - 0.083 ± 0.10 to 0.243 ± 0.091 hr (193% increase): 0.41 ± 0.019 to 0.474 ± 0.089 hr (14.63% increase); and 1.687 ± 0.084 to 1.702 ± 25 hr^{-1} (1.2% increase)

respectively. The increments are statistically insignificant ($P > 0.1$, $P > 0.1$, and $P > 0.5$) respectively.

The pharmacokinetic parameters may be roughly divided into two groups.

i. Those parameters that are mainly related to the rate and extent of absorption i.e absorption rate constant, lagtime, absorption half life, maximum plasma concentration, time to attain maximum plasma concentration.

ii. Those parameters that mainly related to OR that are mainly indicators of metabolism and excretion, they include elimination half-life, elimination rate constant, total body clearance, area under plasma concentration - time curve and volume of distribution.

From the result obtained in this study, those changes due to the pharmacokinetic parameters that are mainly concerned with the absorption kinetic of nifedipine with the exception of C_{max} were statistically in-significant when nifedipine was administered orally 8 hours after the oral administration of 1200mg rifampicin. However, those pharmacokinetic parameters that are indicators of drug metabolism and excretion of nifedipine with the exception of volume of distribution were significantly changed.

From these results it could therefore be inferred that rifampicin increases the metabolism of nifedipine. This fact is supported by the reports of several researchers which reported that rifampicin is a powerful microsomal enzyme inducer (Ohnhaus et-al. 1979; Ohnhaus and Park. 1979; O'Relly. 1974; Kropps et-al. 1974 and Combalbert et-al 1989).

Waller et-al (1984) suggested that the high total body clearance of nifedipine of 11 L/hr was an indication that nifedipine elimination is dependant on hepatic blood flow and that nifedipine undergoes extensive first pass metabolism after oral administration. Similarly, Forster et-al (1983) in their work suggested that nifedipine had high hepatic extraction ratio because of high total body clearance (600ml/min.).

The decrease in C_{max} without a change in the t_{max}, and that the changes in the absorption rate constant, lagtime and absorption half-life were not statistically significant implied that the decrease in the C_{max} may be as a result of increase in nifedipine metabolism by rifampicin. Also the decrease in the elimination half-life implied that the conversion of nifedipine into its water soluble metabolite analogue was increased in the presence rifampicin. The increase in the elimination rate constant points to the fact that the rate of metabolism and excretion of nifedipine was increased by rifampicin.

The present study confirmed the report of other researchers mentioned above that rifampicin is a powerful liver microsomal enzyme inducer. Combalbert et-al. (1989); Guengrich et-al. (1986); Bork et-al. (1989); identified some liver cytochrome P-450 enzymes which belong to gene super family of cytochrome P-450 III (P - 450 $_{NF-25}$. $P\text{-}450_{NF-10}$; P - 450 $_{PCN1}$) as the main microsomal enzymes responsible for the oxidation of nifedipine to its nitropyridine analogue. They are referred to as nifedipine oxidase. These researchers mentioned above also indicated that these enzymes are

inducible at varying rates and extent by rifampicin and other inducers. This implied that metabolism of nifedipine mostly takes place in the liver.

Breckenridge, (1987) and Ohnhaus and Park, (1979) reported that there was usually a delay of about two to seven days before the enzyme induction activity can be observed from the start of the administration of the inducer. They used 600 mg rifampicin orally daily to measure plasma clearance of theophylline. They concluded that the effect of some inducers last up to fourteen days after their withdrawal.

From the present study, we observed that the inducing effect of rifampicin 1200mg orally, i.e. twice the dose administrated by Breckenridge, (1987) and Duhm and Poole (1979), Ohnhaus and Park, (1979) was evident within 8 hours of its administration. The reason for the quicker on set of induction of liver microsomal enzymes may be due to the rate and extent of enzyme induction which is dose dependant (Combalbert et-al, 1989). Ohnhaus and Park, (1979) - in their work showed that enzyme induction varies with individual (interindividual variability in response to various inducing agents) and also dose dependant in man using rifampicin as the inducing agent and measuring antipyrine clearance. Twum Barrima et-al (1984) - reported that the potency of induction of cytorome P-450's age dependant. They explained that rifampicin 600mg increased antipyrine clearance in a group of young subjects, but no such effect on a group of healthy old individuals. This reports suggestively supported our finding since the volunteers used were

young and are of less than 30 years of age.

Gonzelez et-al (1988) - have reported that the rate and extent of the induction of cytochrome P-450 IIIs varies from person to person (interindividual variation). They also reported that other P-450 IIIs that are not nifedipine oxidase can to some extent oxidise nifedipine.

Other factors such as genetics, stress, nutrition and hormone also affect the microsomal enzyme activities in human as well as the extent of induction (Park et-al. 1981). The alteration in the balance of protein and carbonhydrate in the diet determines the rate of liver metabolism of drugs such as theophylline and antipyrine (Kreppes et-al. 1978). They also reported that different forms of P-450 IIIs are induced at varying rate by an inducing agent.

Reduction of nifedipine C_{max} up to 67% observed after administration of rifampicin may also be attributed pre-hapatic metabolism of nifedipine in the small intestine. This is supported by the works of Watkins et-al (1987). They reported that inducible cytochrome P-450 III members have been investigated in the intestine of rats and man and a high level similar to those in liver were detected in the human intestine. Thus the induction of these intestinal P-450 IIIs will contribute significantly to the lowering of C_{max}, as the rate of metabolism of nifedipine will be increased.

The plasma concentration of nifedipine 10mg orally 8 hours after the administration of 1200mg rifampicin orally is reduced

significantly. At 6 hours after the administration nifedipine, i.e. 14 hours after the administration of rifampicin, the plasma concentration of nifedipine was 8.27 ± 0.23 ng/ml. This level is lower than the minimum effective plasma concentration (10ng/ml) of nifedipine. It could therefore, be inferred that clinically, rifampicin may cause severe lowering of plasma concentration of nifedipine when they are given together. This effect may necessitate either increase in frequency of the administration of nifedipine or increasing the dose to be administered.

Thus from the results obtained, it is advised that caution should be observed when the two drugs have to be given together.

CONCLUSION:

All the three brands of nifedipine (Adalat capsules 10mg, Adalat retard tablets 20mg, Nifecard retard tablets 20mg) passed the official tests for identification, disintegration, dissolution and assay according to BP 1988 Addendum, 1990.

The method of extraction and solvent system used in analysis (Mustapha et-al, 1992) - achieved a high recovery and high resolution of nifedipine from its internal standard (4-Dimethylamino benzaldehyde). The resolution factor is 2.4. The reproducibility of the procedure compares well with already published values (Gulaid et-al, 1978).

The mean pharmacokinetic parameters of nifedipine in the healthy volunteers vary in some cases when compared to those obtained by other researchers. This is supported by the work of Breimmer et-al (1989) - which reported that there were variabilities in nifedipine kinetics and metabolism in man.

Rifampicin 1200mg orally 8 hours before the administration of 10mg Nifedipine orally had been shown to change significantly the mean pharmacokinetic parameters concerned with metabolism and excretion of nifedipine and does not significantly change the pharmacokinetic parameters concerned with the absorption kinetics of nifedipine. It can therefore be concluded that rifampicin affects metabolism of nifedipine by inducing the cytochrome P-450 microsomal enzyme system responsible for the metabolism of nifedipine.

Clinically, caution should be exercised when patients on

nifedipine medication are to be given rifampicin and vice-versa. Also caution should be exercised in when rifampicin 1200mg is to be given as a chemoprophylaxis of meningococcal diseases and meningitis due to *Haemophilous Influenza* in patients already on nifedipine therapy.

TABLE 4.2.3 PLASMA CONCENTRATION OF NIFEDIPINE IN SIX HEALTHY *VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 10mg OF* NIFEDIPINE CAPSULES [CONTROL]

Time	Plasma concentration (ng/ml)						
	Volunteers						Mean ± SEM
[hrs]	I	II	III	IV	V	VI	
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0±0.0
0.5	52.30	50.00	52.80	62.00	48.40	50.60	52.76±2.24
1.0	106.60	116.10	120.20	12.00	106.20	96.40	107.85±3.29
1.5	182.70	142.70	166.00	184.80	186.80	176.40	173.23±6.23
2.0	86.00	79.80	83.10	84.00	86.80	79.80	83.25±1.11
3.0	72.00	73.00	72.50	74.80	76.80	71.00	73.35±0.79
4.0	52.80	51.00	50.60	53.00	52.90	52.10	52.1±0.39
6.0	32.00	31.70	33.00	34.00	32.20	33.80	33.00±0.35
8.0	14.00	11.60	13.20	13.50	12.80	13.40	13.10±0.31
12.0	9.00	8.30	8.00	9.60	7.90	9.10	8.70±0.25

TABLE 4.2.4 PLASMA CONCENTRATION OF NIFEDIPINE IN SIX HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 10mg NIFEDIPINE CAPSULES EIGHT HOURS AFTER ORAL ADMINISTRATION OF 1200mg OF RIFAMPICIN CAPSULES [CORSS-OVER].

Time	Plasma concentration (ng/ml)						
	Volunteers						Mean ± SEM
[hrs]	I	II	III	IV	V	VI	
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0±0.0
0.5	16.0	9.00	10.60	11.80	14.10	12.10	12.26±0.93
1.0	62.80	64.00	83/00	70.60	70.10	69.90	70.10±2.67
1.5	40.00	86.30	110.00	120.00	124.80	116.50	116.26±7.69
2.0	72.30	67.10	70.00	69.40	71.30	68.90	69.83±0.68
3.0	31.80	21.80	29.30	27.80	32.80	28.60	28.68±1.45
4.0	14.30	12.20	13.00	10.80	11.50	10.20	12.00±0.56
6.0	9.00	8.50	8.80	8.00	8.00	7.30	8.27±0.23
8.0	6.50	7.00	6.80	7.00	6.60	5.80	6.62±0.17
12.0	5.80	-	-	-	-	-	0.967±0.88

TABLE 4.2.5: PHARMACOKINETIC PARAMETERS AFTER 10mg NIFEDIPINE CAPSULES IN SIX HEALTHY VOLUNTEERS.

P-kinetic parameters [unit]		Volunteers I	II	III	IV	V	VI	Mean ± SEM
	Wt. [kg]	56.00	60.00	67.00	58.00	61.00	62.00	60.67±3.45
Kel [hr^{-1}]		0.250	0.295	0.275	0.249	0.264	0.279	0.260±0.008
$t_{\frac{1}{2}}$(el) [hr]		2.760	2.350	2.700	2.808	2.621	2.482	2.62±0.071
Ka [hr^{-1}]		1.500	1.870	1.500	1.500	1.920	1.830	1.687±0.084
$t_{\frac{1}{2}}$ [abs] [hr]		0.462	0.370	0.462	0.462	0.361	0.379	0.410±0.019
Vd[ml/kg]		67.00	66.00	67.00	66.00	65.00	66.00	66.20±2.50
$AUC_{(0-8)}$ [ng.hr/ml]		596.093	513.902	581.360	609.450	587.112	546.507	573.400 ± 14.29
C_{max} [ng/ml]		182.700	142.700	166.00	184.80	186.80	176.40	173.233 ± 6.23
T_{max} [hr]		1.5	1.5	1.5	1.5	1.5	1.5	1.5 ± 00
T_{lag}		0.09	0.10	0.066	0.040	0.100	0.100	0.083 ± 0.100
CL_T [ml/min.-kg]		17.00	19.00	17.00	16.00	17.00	18.00	17.33 ± 0.37

Table 4.2.6 PHARMACOKINETIC PARAMETERS OF 10MG NIFEDIPINE CAPSULES EIGHT HOURS AFTER THE ADMINISTRATION OF RIFAMPICIN CAPSULES 1200MG.

P-kinetics Parameters [UNITS]	VOLUNTEERS						
	I	II	III	IV	V	VI	mean±SEM
Wt. [kg]	56.00	60.00	67.00	58.00	61.00	62.00	60.67±3.45
K_{el} [hr^{-1}]	0.65	0.65	0.65	0.735	0.712	0.65	0.67±0.016
$t_{\frac{1}{2}}$ (el) [hr]	1.066	1.066	1.066	0.942	0.974	1.066	1.03±0.023
K_a [hr^{-1}]	2.030	2.260	2.200	0.934	0.909	1.940	1.702±0.250
$t_{\frac{1}{2}}$(abs) [hr]	0.341	0.315	0.315	0.742	0.767	0.357	0.474±0.089
Vd [ml/kg]	87.00	84.00	84.00	50.00	54.00	89.00	75.30±2.5
$AUC_{0\infty}$ [ng.hr/ml]	177.90	184.12	184.22	247.07	262.46	173.68	205.25±16.20
C_{max} [ng/ml]	140.00	86.30	110.00	120.00	124.80	116.50	115.77±7.69
T_{max} [hr]	1.5	1.5	1.5	1.5	1.5	1.5	1.5±0.00
T_{lag} [hr]	0.100	0.586	0.465	0.100	0.100	0.100	0.243±0.09
Cl_T [ml/ml.kg]	57.00	55.00	55.00	37.00	39.00	58.00	50.17±3.88

Table 4.2.7 MEAN EFFECT OF RIFAMPICIN 1200MG - ON THE PHARMACOKINETICS OF NIFEDIPINE 10MG CAPSULES ADMINISTERED EIGHT HOURS AFTER RIFAMPICIN.

Pharmacokinetics Parameters	n = 6 Mean ± SEM NFPN -ALONE	n=6 Mean ± SEM NFPN +RFP	% CHANGE	P-VALUE [t-test]
K_{el} [hr^{-1}]	0.260±0.008	0.67±0.16	+157.70	P < 0.0001
$t_{\frac{1}{2}}(el)$ [hr]	2.62±0.071	1.03±0.023	-67.00	P < 0.0001
K_a [hr^{-1}]	1.687±0.084	1.702±0.0250	+1.20	P > 0.5
$t_{\frac{1}{2}}(abs)$ [hr]	0.410±0.019	0.0474±0.089	+14.63	P > 0.1
Vd [ml/kg]	66.20±0.00	75.30±2.5	+13.75	P > 0.1
CL_T [ml/min.kg]	17.33±0.037	50.17±3.88	+194.00	P < 0.0001
$AUC(_{\infty})$ [ng.hr/ml]	573.40±14.29	205.25±16.20	-62.2	P < 0.0001
C_{max} [ng/ml]	173.23±6.23	115.77±7.69	-67.00	P < 0.0001
T_{max} [hr]	1.5±0.00	1.5±0.00	-	P > 0.5
T_{lag} [hr]	0.083±0.100	0.243±091	+193.00%	P > 0.1

NFPN = Nifedipine
RFP = Rifampicin
SEM = Standard Error of the Mean

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bottom of the vessel and the basket is maintained at 23 to 27mm during the test.

(c) A water bath that will maintain the dissolution medium at 36.5 to 37.5^{0}C.

APPENDIX II

DISINTEGRATION TEST FOR TABLETS AND CAPSULES APPARATUS.

The BP 1988 - disintegration apparatus for tablets and capsules consist of:-

(a) A rigid basket rack assembly supporting six cylinderical glass tubes 75.0 to 80.0mm long, 21.5mm in internal diameter and with a wall thickness of about 2mm.

(b) A cylinderical disc for each tube, each 20.55 to 20.88mm in diameter and 9.35 to 9.65mm thick, made of transperent plastic with a relative density of 1.18 to 1.20, pierced with five holes, each 2mm in diameter, one in the centre and the other four spaced equally on a circle of radius 6mm from the centre of the disc. Four equally spaced grooves are cut in the lateral surface of the disc in such a way that at the upper surface of the disc they are 9.5mm wide and 2.55mm and at the lower surface 1.6mm square.

(c) The tubes are held vertically by two superimposed transperent plastic plates 90mm in diameter and 6mm thick, perfirated six holes having the same diameter as the tubes. The holes are equidistant from the centre of the plate are equally spaced from one another. Attached to the under side of the lower plate is a woven gaze made from stainless steel wire 0.635mm in diameter and having nominal mesh aperatures of 2.00mm.

(d) The plates are held rigly in position and 77.5mm apart by vertical metal rods at the periphery and a metal rod is also fixed to the centre of the upper plate to enable the assembly to be attached to a mechanical device capable of raising and lowering it

smoothly through a distance of 50 to 60mm at a constant freqency of between 28 and 32 cyles per minute.

(e) The assembly is suspended in the liquid medium in a suitable vessel, preferably a 1000ml beaker. The volume of the liquid is such that when the assembly is in the highest position the wire mesh is at least 1.5mm below the surface of the liquid and when the assembly is in the lowest position the wire mesh is at least 25mm above the bottom of the beaker and the upper open ends of the tubes remain above the surface of the liquid.

(f) A suitable device that maintains the temperature of the liquid at 36^0 to 38^0c.

The design of the basket - rack assembly may be varied provided that the specifications for the glass tubes and wire mesh are maintained.