EFFECTS OF STORAGE CONDITIONS ON PHARMACOKINETICS OF PARACETAMOL TABLETS

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DEPARTMENT OF PHARMACOLOGY AND CLINICAL PHARMACY
FACULTY OF PHARMACEUTICAL SCIENCES
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DECLARATION

I hereby declare that the work reported in this thesis was carried out by me under the supervision of Professor Ibrahim Abdu - Aguye, Dean Faculty of Medicine and Professor Abdullahi Mustapha, Dean Student affairs. It has not been presented in any previous application for higher degree. The work of other investigators are acknowledge and referenced to accordingly.



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CERTIFICATION

This thesis entitled **EFFECTS OF STORAGE CONDITIONS ON PHARMACOKINETICS OF PARACETAMOL TABLETS** by Zezi Abdulkadir Umar, meets the regulations governing the award of the degree of master of science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

Dedicated to:

my father

UMAR ZEZI

and

my mother

HADIZA UMAR

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ABSTRACT

Twenty (20) Hospitals/Clinics, thirty (30) registered Pharmacy shops and one hundred and twenty five (125) Patent medicine stores were screened for their storage facilities in Zaria areas. Two of each of drug storage areas with sub optimum storage facilities (lack of functional ceiling fan and air condition, direct sunlight into premises, cracks in the ceilings and walls, improper ventilation and exposure to dusts) were then selected for the study. The average consumption rate of 1000 tablets per month was obtained in these areas screened. The paracetamol tablets used were stored for two months in these areas. Samples taken before exposure (controls) and after two months exposure (test samples) for *in vivo* studies. The brand of paracetamol used passed all quality control analyses.

Twelve (12) healthy young adult male volunteers with average age of 27.67 years and average weight of 66.67 kg took part in the study. Each volunteer was administered 1 g of paracetamol tablets orally and blood sampled at 0, 5, 10, 15, 20, 30, 60, 90, 120, 180, 240, 300 and 360 mins.

A reliable, rapid and simple, calorimetric method was used for plasma concentrations determination. Student t-test table was used to compare tests results to control for data analysis. P value less than 0.05 is significant.

Blood levels derived from tablets obtains from the storage areas fitted to first order kinetics. There is statistically significant differences in peak plasma concentrations ($P < 0.05$) in most areas studied compared to the controls. Time taken to attain peak plasma levels (t_{max}) remains the same in all the storage areas and is 0.33 hr (20 mins.) Areas under the curve from zero to infinity did not show any statistically significant differences ($P > 0.05$) in most areas studied. There was bioequivalent in all areas studied compared to controls, since the differences in their relative bioavailabilities satisfied the standard requirement of not more than 25%.

The sub optimum conditions have shown variable kinetics of other parameters such as volume of distribution, plasma clearance, absorption and elimination rate constants, lag time, elimination and absorption half lives. These differences could be due to individual variations as shown in other similar works. This study has therefore shown that these storage conditions have no significant effect on the pharmacokinetics of paracetamol tablets.

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ABBREVIATIONS

Abbreviation	Meaning
B.P	British Pharmacopoeia
°C	Degree centigrade (Celsius)
g	Gram
µg	Microgramme
mg	Milligramme
ml	Millilitre
min.	Minute
hr.	Hour
HCl	Hydrochloric acid
NaOH	Sodium hydroxide
$NaNO_2$	Sodium nitrate
G.L.C.	Gas Liquid Chromatography
H.P.L.C.	High Performance Liquid Chromatography
pH	Negative logarithm of hydrogen - ion concentration
U.V.	Ultraviolet
nm	Nanometer
kg	Kilogramme
L	Litre
%	Percentage
r.p.m	rotation per minute

CHAPTER ONE

1.0 INTRODUCTION

1.1 PHARMACOKINETICS

Pharmacokinetics is the study of the rate process associated with absorption, distribution, metabolism and excretion of a drug, i.e. quantitating drug and or metabolities levels in body fluids, tissues and excreta following administration until completely removed from the body (Kaplan and Jack, 1980). It deals with changes of drug concentration in the different body fluids and tissues in the dynamic system of liberation, absorption, distribution, body storage, binding, metabolism and excretion following drug administration. Therapeutically, clinical pharmacokinetics are useful in selecting and adjusting drug dosage schedules and to facilitate the interpretation of measured serum concentration of drugs.

Drugs once absorbed, disperse to various areas within the body including the target organs, the plasma and tissue binding sites. and the organs of elimination. The rate of distribution into a tissue depends on its vascular perfusion, the permeability of the tissue membrane to the drug, and the relative partitioning of drug between tissue and blood.

The main standard of a pharmacokinetic study is based on the data obtained following intravenous administration. This is because the entire dose administered is placed directly into the blood stream. The pharmacokinetic parameters obtained are therefore not affected by rate-limiting and metabolic factors which are potential possibilities with drug absorption following other routes.

Basically in Pharmacokinetics, the approach is to fit experimental data on drug concentrations in plasma to a mathematical equations that represent the flow of drugs and

their metabolites through discrete compartments of a model system. This results in a precise description of drug disposition which can have major clinical applications.

In order to derive mathematical equation for pharmacokinetic studies, there are several assumptions that have to be made. Firstly, drugs enter the system only via the central compartment and are eliminated only from that compartment. Secondly, reversible transfer occurs between central and peripheral compartments and thirdly the exit of drugs from all compartments in the model is described by first order kinetics (i.e. the rate at which a drug is removed from a compartment is directly proportional to the concentration in it). All three assumptions are relatively valid for most drugs. The majority of drugs enter the body via the blood stream and are eliminated from the blood stream by the liver or kidney. Similarly, most drugs are eliminated by first order kinetics.

1.1.1 Single - Compartment Model

The one-compartment model, the simplest model, depicts the body as a single homogenous in entity. This model is of use in pharmacokinetic analysis of blood, plasma or serum concentration, salivary and urinary excretion data for drugs which rapidly distributes between plasma and other body fluids and tissues upon administration into the systemic circulation. One-compartment model does assume that any changes that occur in the plasma quantitatively reflects changes occurring in tissue drug level. In one-compartment model, first-order kinetic of drug elimination occurs (i.e. the rate of elimination of drug from the body at any point in time is proportional to the amount of drug in the body at that time). The proportionality constant relating the rate and amount is

the first-order elimination rate constant which has units of reciprocal time (e.g. min^{-1}, hr^{-1}) (Gibaldi and Perrier, 1975).

Assuming instantaneous distribution after an intravenous (Iv) injection of a drug into this model (fig. 1.1.1), the concentration C_o in the plasma immediately after injection is equal to the dose D divided by the volume of the compartment Vd:

$$C_o = D/V_d$$

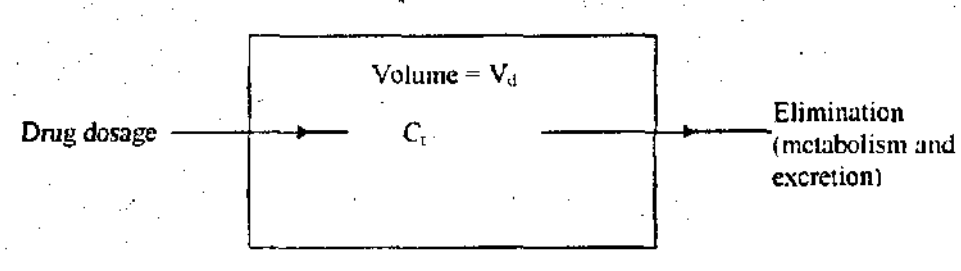


Fig. 1.1.1: Single Compartment model

After instantaneous distribution, the concentration will fall according to first-order kinetics which means a constant fraction of drug 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 eliminated ($t_{\frac{1}{2}}$), results in an exponential curve:

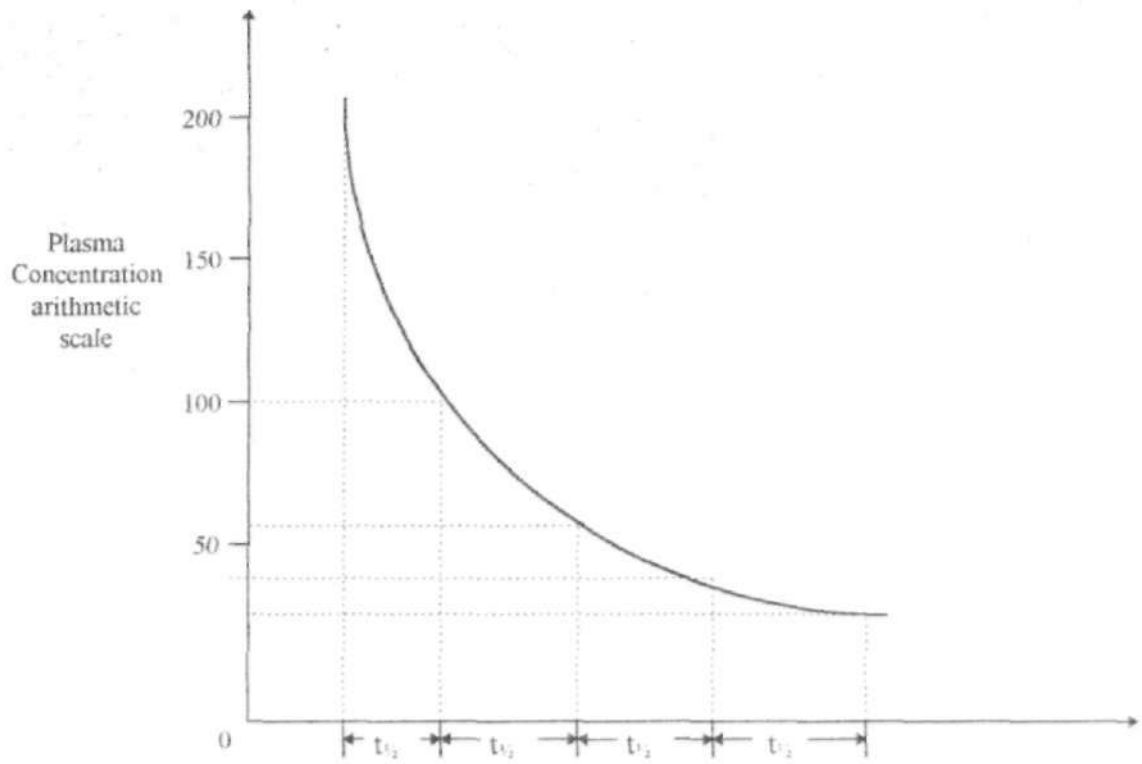


Fig. 1.1.2 exponential - decay graph for single compartment model.

Replotting this with concentration on a logarithmic scale results in a straight line :

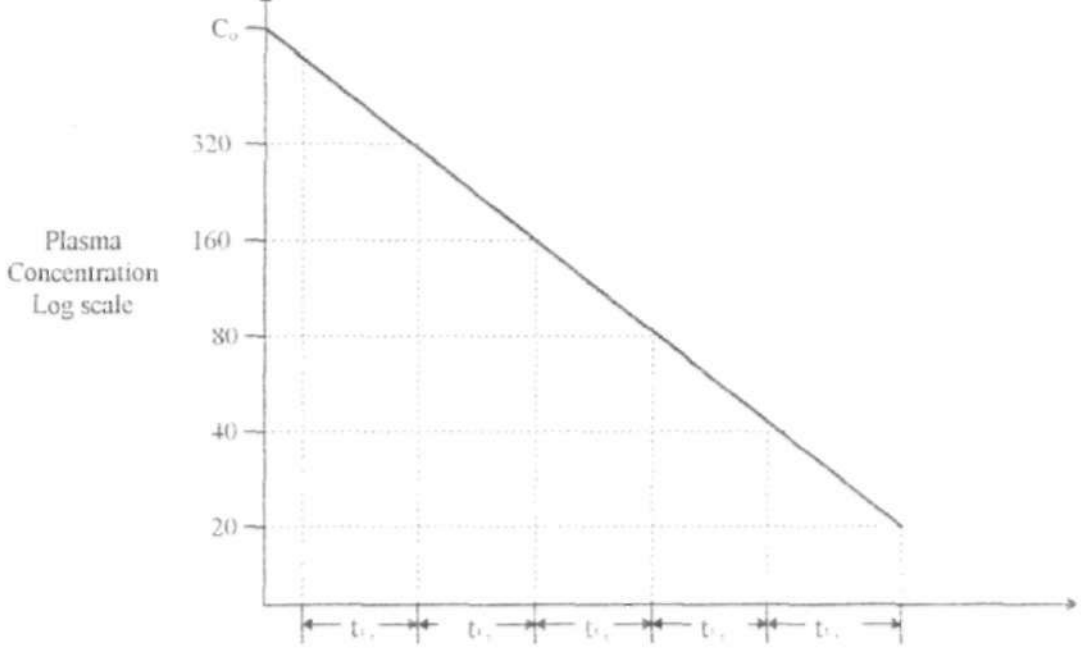


Fig. 1.1.3 Semi - log plot of concentration against time for single compartment model.

If the graph is extrapolated to a time - zero gives the theoretical initial concentration Co. By applying first order kinetics between drug concentration and time, the decline in drug concentration may be expressed mathematically as below

$$\frac{dC}{dt} = -\beta C_t$$

Where: C_t = Concentration

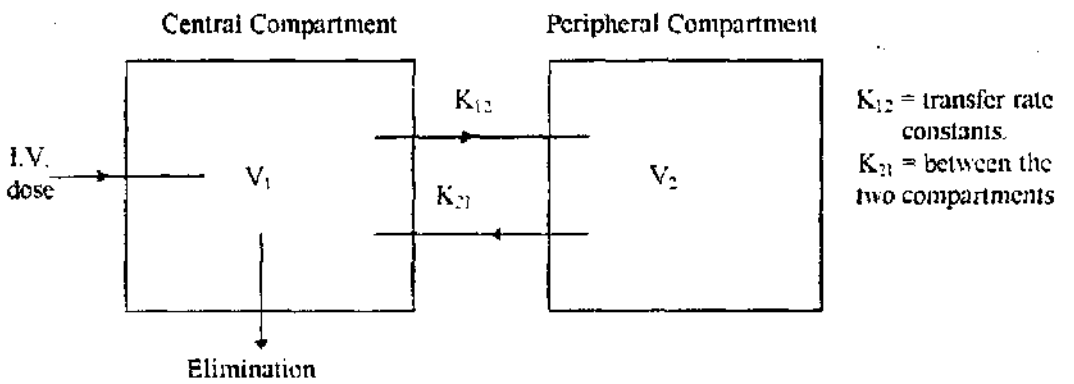
β = Rate Constant of elimination

Integration and conversion to logarithms to the base 10 gives:

$$\log C_t = \log C_o - \frac{\beta t}{2.303}$$

1.1.2 **Two Compartment Model.**

The concentration - time curve of a drug in plasma is often not a straight line on a semi logarithmic plot. If distribution of the drug is so slow that it cannot be disregarded, a model must then be considered which contains a central compartment and at least one other peripheral compartment.



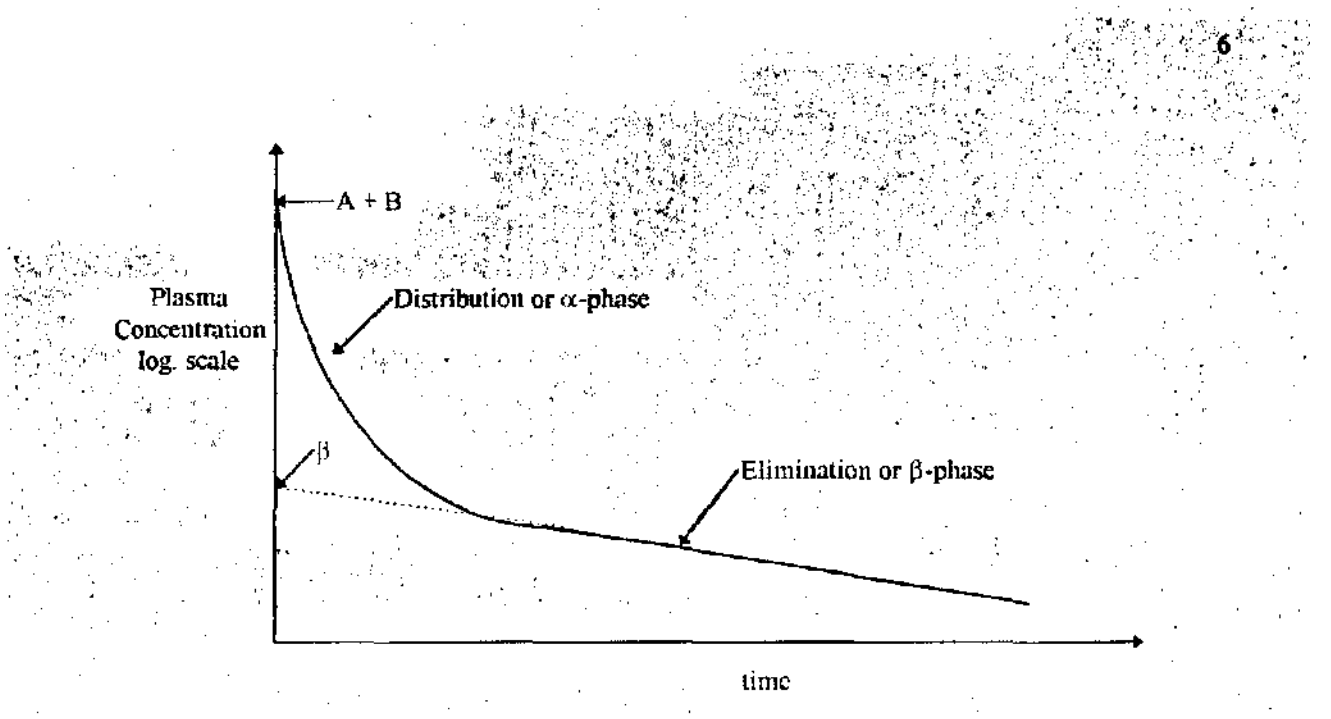


Fig. 1.1.4: Plasma log-concentration-time curve for a two-compartment model.

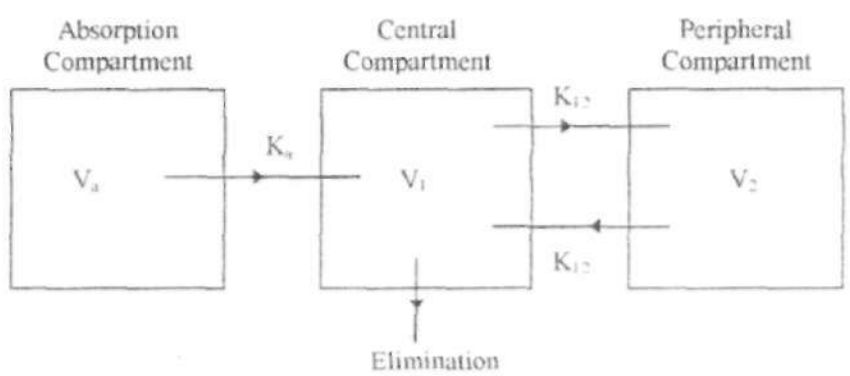
Even though these compartments lack physical or anatomical reality, for many drugs the central compartment represent the plasma or blood volume, together, with the extracellular fluid or highly perfused tissues such as heart, lungs, liver, kidneys and endocrine glands. Drugs distribute within a few minutes through this compartment and equilibrium between plasma and tissue is rapidly established. The peripheral compartment is then formed by less perfused tissues such as skin, muscle or adipose tissue in which drugs enter more slowly. The combined effect of two compartments give rise to bi-phasic curve on I.V. injection with two distinct linear portions when drawn on a semi-log scale (see fig. 1.1.4).

Although drug distribution is slow, it is usually much faster than elimination. Thus the initial rapid fall in concentration (α - distribution phase) mainly represents the relatively rapid process of drug distribution from central to peripheral compartment. Once distribution is complete, the curve enters the relatively slow β or elimination phase during

which drug disappearance is determined mainly by irreversible elimination from the central compartment.

1.1.3 Modification for Oral dosing

In considering oral dose, an additional absorption compartment is introduced into the model to represent the volume from which absorption occurs at a first order rate (see figure 1.1.5). In this case, it is assumed that the entire dose is rapidly introduced into the site of absorption, from which it is absorbed into the central compartment.



K_a = Absorption rate constant, K_{12} & K_{21} are Transfer rate constants between compartments

Fig. 1.1.5: Three compartment model.

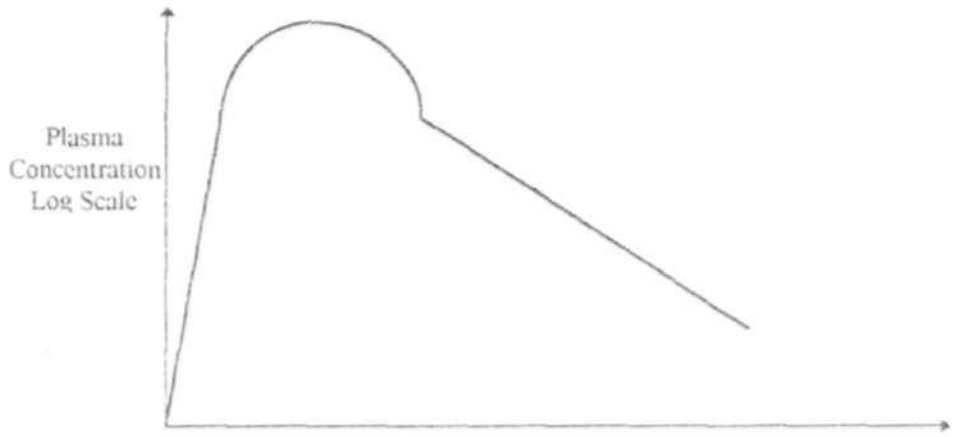


Fig. 1.1.6: A three-compartment model which includes a compartment for absorption. A typical log. Concentration-time curve, after a single oral dose.

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1.1.4 Area Under the Concentration - Time Curve (AUC)

The AUC, which is represented by the integral $\int_0^t C_p dt$ is a measure of the amount of drug that is available systematically (bioavailable) which is a measure of therapeutic efficacy. It is important for calculating such important pharmacokinetic parameters as relative oral bioavailability, Plasma clearance and apparent volume of distribution. It can be determined by the trapezoidal rule and is independent of any model used.

1.1.5 Plasma clearance (Cls)

Plasma or systematic 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 may be determined after oral administration from the relationship.

$$Cl_s = \frac{FD}{AUC}$$

where F = fraction absorbed

D = Dose

AUC = area under the concentration - time curve.

It may also be calculated from the relationship:

$Cl_s = \beta V_d$

where β = Elimination rate constant

V_d = Apparent Volume of distribution.

Clearance has the dimension of volume per unit time and is made up of the sum of all the clearances due to individual organs. If only one organ is involved for example the kidney, the plasma clearance is equal to renal clearance.

For any particular organ, the physiological determinant of drug clearance are organ blood flow, the inherent ability of the organ to extract the drug from the blood and the extent of binding of the drug to plasma proteins. Considering the liver, for example, hepatic clearance Cl_h is given by the relationship:

$$Cl_h = Q\left[\frac{f\,Cl'\,\text{int}}{Q + f\,Cl'\,\text{int}}\right]$$

where ; Q = hepatic blood flow

f = fraction of unbound (free) drug

and Cl' int = intrinsic free drug clearance.

The term in parenthes is referred to as the extraction ratio ER and can vary from zero to 1. If, a drug has very high intrinsic clearance, ER will tend towards I and the hepatic clearance would be equal to Q, the hepatic blood flow, and will show flow dependent kinetics. Thus, the hepatic clearance of a drug such a propranolol for which the liver has a high metabolic capacity, is limited by hepatic blood flow and is relatively insensitive to changes in metabolising activity. However, a reduction in the rate at which the drug is transported to the liver such as a decrease in blood flow would result in reduced hepatic clearance.

On the other extreme, when intrinsic hepatic clearance is very slow compared to blood flow, then ER is low and the equation for hepatic clearance reduces to:

$Cl_h = fCl'int$

and elimination is dependent on enzyme activity and drug binding. Thus for a drug such as theophylline for which there is insufficient enzyme activity present in the liver and which has low ER, its hepatic clearance will be affected by any change of enzyme activity (e.g. enzyme inhibition or induction) but will not be affected by changes in blood flow. The hepatic clearance will also be dependent upon the free-fraction of the drug in blood so that increase in binding reduce clearance, and vice versa. In contrast, altered plasma protein binding will have little effect on organ clearance of drugs with high ER as such drugs can be functionally stripped from their binding sites during passage through the liver.

1.1.6 **Apparent Volume of Distribution**

Apparent Volume of distribution V_d is defined as the volume in which the amount of drug in the body would need to be uniformly distribution to produce the observed plasma concentration. The knowledge of volume of distribution is used to relate the plasma concentration of a drug to the dose administered as the body is not homogenous, even if it can be treated as such in a mathematical model. Drug concentrations in the liver, kidney, muscle, fat and other tissues will therefore differ from one another as well as from the plasma concentration. The apparent volume of distribution does not relate to a real physiological or anatomical space, but provides an estimate of the extent of the drug's distribution through the body fluid compartments and its uptake by tissue. A large, value for V_d implies wide distribution or extensive tissue uptake or both. After oral administration, V_d can be determined from the relationship:

$$V_d = \frac{FD}{\beta AUC}$$

where F = fraction of dose absorbed

D = dose

β = elimination rate constant

AUC = area under concentration - time curve.

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1.1.7 **Elimination (Plasma) Half-life.**

The elimination half-life ($t_{\frac{1}{2}}$) is the time taken for half the amount of drug present to be eliminated either by excretion or metabolism or both. In other words, it is the time taken for the plasma concentration to be reduced by 50% of its original value, and can be determined for both one and two compartment models from the terminal data of the log concentration - time plot.

It may be also be determined from the equation (see page 5).

$$\log C_t = \log C_o - \frac{\beta t}{2.303}$$

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substituting for $t_{\frac{1}{2}}$ gives

$$\log x = \log 2x - \frac{\beta t}{2.303}$$

$$\text{i.e } t_{\frac{1}{2}} = \frac{0.693}{\beta}$$

From this equation, it shows that a large value for β corresponds to a short $t_{\frac{1}{2}}$ and is a reflection of the rate at which the drug leaves the body. However, $t_{\frac{1}{2}}$ is a poor index of drug elimination as it depends not only on the clearance but also on the volume

through which the drug is distributed (volume of distribution V_d). Indeed, if clearance is increased, one would expect a shorter half-life. Not so obvious is the concept that if clearance is kept constant but the volume into which the drug is distributed is increased, half life increases; this is supported the following mathematical relationship.

$$t_{\frac{1}{2}} = \frac{0.693}{Cl_s}$$

Although $t_{\frac{1}{2}}$ is 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 steady - state during intravenous or chronic oral therapy.

1.1.8 Non - Linear Pharmacokinetics (Fallibility of Assumptions).

It can be seen so far that all the systems operating on the drug obey first - order kinetic i.e. the drug has linear Pharmacokinetics characteristics. This implies that elimination half-life, clearance, volume of distribution and bioavailability are independent of dose or concentration of drug. It can be assumed that the Pharmacological effect is elicited by the drug administered. Even though the general concepts of Pharmacokinetics are applicable, these assumptions do not apply to all drugs. Therefore where these assumptions are not valid, significant clinical consequences accrue and modifications of the models are necessary.

Deviations from linearity are possible with respect to one or more processes in absorption, distribution, binding to biological materials in tissue, plasma protein binding and elimination of drug. (Ritschel, 1980). Deviations from linearity with respect to

absorption may be due to low solubility of the drug, low dissolution rate of the drug from the dosage form, changes in gastro-intestinal pH, and, in the case of active absorption processes, due to saturation of carriers.

Deviation from linearity with respect to tissue distribution may occur when tissue or active transport systems in the tissues become saturated. Deviation from linearity with respect to plasma protein binding can occur if increasing dose sizes approach saturation of binding sites or if only limited number of binding sites are available. Non-linearity with respect to excretion can be caused by changes of urinary pH for drugs that are week acids or week bases undergoing urinary recycling, by saturation of reabsorptive processes or by diurnal variation in the elimination of drugs.

1.19 **Dose - Dependent Kinetics**.

One of the commonest causes of non-linearity is the limited capacity of certain drug metabolising enzyme systems. Drug elimination is always linear below the maximal capacity of the drug metabolising enzymes. However, in some situations, drug concentrations are sufficiently high enough to cause saturation of the enzyme, system causing elimination to occur at a fixed rate (i.e. zero - order kinetics). In this situation, drug administration will depend on the magnitude of dose, and drug accumulation will not readily be predictable. Once saturation has been reached, the plasma concentration will increase disproportionately with subsequent dosing and the time required to reach the steady state will increasing half-life.

Example of a drug whose metabolism becomes saturated at clinically achievable concentrations is Phenytoin (Richens and Dunlop, 1975).

1.1.10 Active Metabolites

Some drugs are bio-transformed to a metabolite that has a Pharmacological action like that of the parent drug. With these drugs, the combined Phamacodynamic (and sometimes Pharmacokinetic) action may rise and fall in a complex way because of the different time courses, distribution, and rates of elimination of the active metabolite. For drugs with such properties e.g. the antiepileptic trimethadone, a study of pharmacokinetics alone would be of little value in predicting a therapeutics regimen (Harvey and Dean, 1985). Two or more active metabolites may greatly increase the complexity.

1.2 STORAGE AND STABILITY OF DISPENSED PRODUCTS

It could be said that no pharmaceutical product is stable indefinitely and certainly the majority of products are stable only for a limited time.

The instability of Pharmaceutical Products may be due to drug or excipient degradation. Drug instability may be enhanced by poor formulation, poor packing and poor storage conditions.

Rhodes (1984) has reported six possible results of pharmaceutical product instability.

1. Loss of active drug (e.g. acetylsalicylic acid hydrolysis, oxidation of adrenaline)
2. Loss of vehicle (e.g. evaporation of alcohol from alcoholic mixtures, evaporation of water from oil-in-water creams.
3. Loss of content uniformity (e.g. impaction of suspensions, creaming of emulsions).
4. Reduction in bioavailability (e.g. aging of tablets resulting in a change in dissolution profile).

5. Loss of pharmaceutical elegance (e.g. fading of coloured solutions and tablet).
6. Production of potentially toxic materials (e.g. breakdown products from drug degradation).

The above list is not meant to be fully comprehensive or exhaustive.

Generally degradation of pharmaceutical products could be classified as being due to chemical, physical and biological mechanisms. In some cases the degradation may be due to one or more of the mechanisms or what appears to be the result of one mechanism may be the result of another mechanism. For instance, a cracked emulsion (a physical effect) may be the result of microbial degradation of the emulsifier.

1.2.1 **Chemical Degradation**

Solvolysis

Solvolysis involves the degradation of the drug or excipients through reaction with the solvents present in the formulations. In most pharmaceutical products the solvent is water so that the degradation will be hydrolysis. However, in some pharmaceutical products the solvent may include such co-solvents as ethylalcohol, propylene glycol and glycerol together with water.

Most of the solvolysis reactions involved in the degradation of drugs and excipients involve 'labile' carbonyl compounds, such as esters, amides, lactones and lactams. Examples include esters such as acetylsalicylic acid, the local anesthetics, amides such as sulphonamides and chloramphenicol, lactones such as spironolactone and pilocarpine, and lactam such as the penicillins and cephalosporins.

Oxidation

Oxidation is a common cause of drug and excipient degradation. This method of degradation occurs in both water - and oil - soluble drugs, as well as fixed and volatile oils.

A substance is said to be oxidized if it gains electronegative atoms or radicals or loses electropositive atoms or radicals. Oxidation often involves the addition of oxygen or removal of hydrogen. There are two types of oxidation processes which affect pharmaceutical products:

1. Those involving atmospheric oxygen.
2. Those involving the reversible loss of electron.

The latter process can proceed under anaerobic conditions and is independent of atmospheric oxygen. Example of drugs undergoing such an oxidation process are riboflavine, ascorbic acid, adrenaline and ferrous salt. Oxidation is usually demonstrated in the latter stages by a change in colour e.g. adrenaline solution changes from a clear to a red - coloured solution.

Oxidation processes involving atmospheric oxygen, at ambient temperatures, are usually referred to as autoxidation. Autoxidation is usually a chain reaction involving the formation of free radicals in the initiation stage. In the propagation stage, the formation of peroxy radicals from oxygen and the free radicals produced in the initiation stage react with intact drug to produce hydroperoxides and more free molecular oxygen to form peroxy radicals and so on. Therefore in the propagation stage the products of the oxidation process are used to induce more oxidation, and so once the process has started

it becomes self propagating unit all the free drug has been used up and the termination stage reached. At this point the peroxy radicals and hydroperoxides combine to form inactive products.

Photolysis

Degradation of drugs or excipient molecule can be brought about by light, either room light or sunlight. Such reactions are termed photolysis and light - sensitive drugs are known as photolabile.

The shorter the wavelength of the light then the more potentially damaging is the light. Thus U.V. light is more harmful than red/orange light. However, in order to cause a photolytic reaction the energy from the light radiation must be absorbed by the molecules. If the energy absorbed is sufficient to achieve the activation energy, then degradation of the molecules is possible. In some cases, the molecules absorbing the light do not themselves take part in the reaction, but pass on their increased energy to other molecules, which then degrade. In such cases, the light energy absorbing drug or molecules is said to be a photosensitizer.

Although the energy obtained from light may be due to the initiator of a reaction, the reaction itself may be an oxidation process, polymerization or ring re- arrangement, etc. Also light energy may be converted to heat, photolysis may be accompanied by a thermal reaction. Alternatively, a photolytic reaction may produce a catalyst, which catalyses a thermal reaction. Once a thermal reaction is started it may proceed when the light source is removed.

Photolytic reactions include the decomposition of chlorpromazine hydrochlorides,

and other phenothiazines, the darkening of morphine and codeine, the fading of tartrazine dye and the initiation of many autoxidation process.

Polymerization

Polymerization involves the combination of two or more identical molecules to form a much larger and complex molecule. Degradation of Pharmaceutical products by polymerization is not very common and such reactions may occur after the initial degradation of the drug. Such an example of polymerization is the production of a straw - coloured solution after the autoclaving of dextrose injection. The coloured solution is attributed to the polymerization product of the initial major degradation product, 5-hydroxymethyl furfural.

Optical Isomerization

A change in the optical activity of a drug may result in a change in its biological activity. Racemization is the main type of optical isomerization which affects drug molecules and it occurs when the optically active form of the drug is converted into its enantiomorp,. Racemization continues until 50% of the original drug has been converted to its enantiomorph. In most cases the enantiomorph has less therapeutic effect than the original drug. An example of racemization is the conversion of (-) hyoscyamine into the racemic mixture of (+)- and (-)- hyoscyamine. Other examples include adrenaline, tetracycline and pilocarpine (Nunes & Brochmnn-Hausse, 1974).

Other methods of chemical degradation include hydration, dehydration decarboxylation, acylation and transesterification.

1.2.2 Physical Degradation

Polymorphism

Polymorphs are different crystal form of the same compound. Poylmorphs differ from one another in their crystal energies, the more energetic ones converting to the least energetic (or most stable) one. Different polymorphs of the same drug may exhibit different solubilities and melting point. These physicochemical properties have significant implications for pharmaceutical dosage forms. For example, theobroma oil used in the preparation of suppositories has two polymorphs. The β form has melting point at 37 °C, while the α form has a melting point at 30 °C. Conversion by exessive heating of β form to the α form makes theobroma oil unsuitable as a suppository base. Another well documented example is the conversion of cortisone acetate to a less soluble form when the drug is formulated in aqueous suspension. The less soluble polymorph forms an insoluble cake in the aqueous suspension (Macek, 1954).

This type of phenomena also occurs in ointments and creams when the presence of a less soluble drug gives a gritty feel to the product and poor release characteristics.

Vaporization

Many drugs and excipient may be lost from pharmaceutical products at ambient temperatures through vaporization. These drug and excipients posses a sufficiently high vapour pressure that they are volatile at room temperatures. Examples of such drugs are glyceryl trinitrate, alcohols, camphor, menthol, chloroform and volatile oils. These drugs may be lost from the product because of a loose - fitting closure.

Water loss

Evaporation of water from liquid preparation will cause concentration of the drug with the possibility of crystallization occurring if the solubility of the drug in the solvent is exceeded. Water loss from oil-in-water cream may result in a decrease in volume and a surface rubbery feel. Further evaporation of the water will cause the emulsion to crack. Some drugs are efflorescent, which means that they will lose water to the atmosphere resulting in a concentration of the drug and overall weight loss. Water loss to the atmosphere can be prevented by storing the pharmaceutical product in a well - closed container.

Absorption of water

Water will be absorbed from the atmosphere by some drugs and pharmaceutical products. For example, some drugs are deliquescent (calcium chloride and potassium citrate), whereas others are hygroscopic (glycerol and dry plant extracts). Effervescent powders and tablets will deteriorate if stored in a moist atmosphere.

1.2.3 **Factors Influencing degradation**.

Temperature

An increase in temperature generally increases the rate of reactions and thus the degradation of Pharmaceutical products is generally increased with an increased in temperature. The Arrhenius equation quantitatively describes the relationship between reaction (or degradation) and rate (k) to temperature (T).

where A = is the frequency factor, R is the gas constant, Ea is the activation energy and T is the temperature in degree absolute.

Because an increase in temperature results in increased rate of degradation, then one method of preventing or reducing degradation is to store the pharmaceutical product at low temperature. For example, insulin injections are normally required to be stored between 2 °C and 8 °C.

However, storage at low temperatures may not decrease the degradation rate as much as would be expected from the Arrhenius equation. For example, although the rate of oxidation is decreased at low temperature, the solubility of oxygen in solution is increased. This increase in solubility may promote the oxidation process, so that the overall result is usually a decrease in oxidation but not as much as would have been reported by Savello & Shangraw (1971) who showed an increased degradation rate of ampicillin solution on freezing.

A change of temperature, besides influencing chemical reaction rates, may also affect physical degradation rates. For example, an increase in temperature can cause creams and emulsions to become unfit for use and separate into two phases. Alternatively, freezing creams and emulsions will usually damage the emulsion structure due to the crystallization of water at 0 °C. On reheating to ambient temperature the emulsion does not reform.

A decrease in temperature may cause a drug to exceed its solubility and crystallize out from the solution. Sodium lactate infusion compound BP (1988) frequently exhibits crystallization at temperatures just below ambient.

An increase in temperature may result in an increased loss of volatile material or increased loss of water from Pharmaceutical products. Such product should be stored in a cool place.

Moisture (atmospheric)

Moisture or humidity can adversely affect the stability of dry drug formulations, such as tablets and capsules. For example, gelatin Capsules become soft and swell in the presence of moisture and the drug content of tablets may undergo surface hydrolysis (Cartensen & pothisiri, 1975). The presence of moisture may encourage the growth of micro organisms.

Additives

Buffer salts may be used to adjust and maintain the pH of a formulation. However, they may cause general acid-base catalysis and ionic effects (Aulton, 1988)

Surfactants may be added to formulation as solubilizers or emulsifiers. However, the presence of a surfactant may increase or decrease or leave unchanged the degradation of a drug or the excipients. Acceleration in the presence of surfactants acting as micellar catalysts is well documented, other references demonstrate the ability of surfactants to decrease the hydrolysis of poorly water - soluble drugs (Reigelman, 1960). The presence of surfactants in aqueous solution have been shown to protect some drugs from oxidation (Amin & Bryan, 1973). Some surfactants will trap preservative content of the aqueous phase, resulting in the possibility of microbial degradation of the product.

Complexing agents may be added to some drugs to produce a complex which is more stable than uncomplexed drug. Example of complexation include benzocaine and

procain complexed with caffeine to reduce hydrolysis (Higuchi & Lackman, 1955). More recently, Uekema *et al* (1983) have improved the thermal and photochemical stability of benzaldehyde in water by complexing the drug with α, β, and γ- cyclodextrins.

Antioxidants are added to drug formulations to prevent or reduce oxidation. They could be classified into antioxygens , reducing agents and chelating agents.

Antioxygens act by providing the necessary electrons and easily available hydrogen ions which are accepted by the free radicals and this stops the chain reaction in auto-oxidaion. Antioxygens act as chain breakers. Examples of antioxygens are the gallates, α-tocopherols and butylated hydroxyanisoles / hydroxytoluenes.

Reducing agents are substances which are preferably oxidized and offer protection to the drug until they themselves are used up or until all the oxygen in the container has been removed. Sodium metabisulphite is the most commonly used reducing agent. Other reducing agents include hypophosphorous, acid, dextrose, sulphurous acid and ascorbic acid.

Chelating agents act by complexing or chelating heavy metals present in solution which may be responsible for acting as catalysts in the oxidation process. Examples of chelating agents are sodium edetate and 2,3-dimer captopropane sodium in vitamin C injection.

1.2.4 **General Requirements for Drug Storage and Keeping.**

1. ***Floor space***

It should be wide enough.

Free from clutter which would interfere with traffic flow.

It should be free from dampness and leakages.

2. ***Cooling system***.

Functional fans

Functional Air conditions

Adequate ventilation.

3. ***Cold chain***.

Functional refrigerators

Functional freezers

Temperature charts.

4. ***Store condition***.

Roofs should be free from leaking, walls and floors should be free from cracks.

Lighting should be provided

Drugs should be free from direct sunlight

Drugs should be protected from direct wind and dusts.

Clean Shelves

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 GENERAL

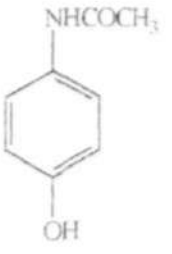
2.1.1 Names

Acetaminophen; paracetamol; *p*-acetamidophenol; N-acetyl-*p*-aminophenol.

2.1.2 History and chemistry.

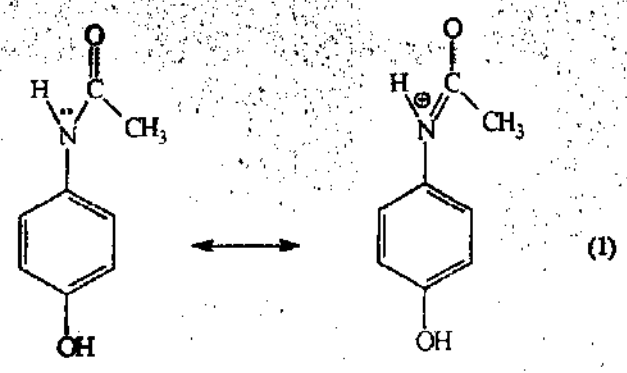
Acetanilide was introduced into medicine under the name of antifebrin by Cann and Hepp, who has accidentally discovered its antipyretic action in 1886. However, it proved to be toxic. Para-aminophenol which was taught to be the oxidized product of acetanilide in the body was tried in search of less toxic compound. P-aminophenol itself proved to be as toxic as acetanilide but toxicity was reduced if the hydroxyl or amino group received alkyl or acid substituents respectively. Acetophenetidine (Phenacetin) and N - acetyl-*p*-aminophenol (Paracetamol) were thus produced and found useful. Phenacetin is metabolised in the body to paracetamol (Crossland, 1980).

Structure.



$C_8H_9NO_2$; Mol. Wt. 151.16

Paracetamol has $NHCOCH_3$ side chain. The Lone pair of the NH_2 group enters into resonance with the carbonyl group, and consequently resonance with the ring is strongly diminished. This gives the drug a very weak basic character.



(I)

The amino benzene structure confers antipyretic activity to paracetamol. Introduction of other radicals into the hydroxyl group of para-aminophenol and into the free amino group of aniline reduces toxicity without loss of antipyretic action.

2.1.3 **Physical properties**.

White, odourless, crystals or crystalline powder with a bitter taste. Melting point 169° to 172 °C.

Solubility: One gram dissolves in about 70 ml of water at 25°, 20 ml of boiling water, 7 ml of alcohol, 13 ml of acetone, 50 ml of chloroform, 40 ml of glycerin, and 9 ml of propylene glycol. It is insoluble in benzene and ether, and soluble in solutions of alkali hydroxides. A saturated solution has a pH of about 6 (Martindale 26th ed.). PKa = 9.51) (Remington's Pharmaceutical sciences 15th ed.)

2.1.4 **Stability Summary**.

Acetaminophen is very stable in aqueous solution. Its pH - rate profile reveals specific acid and specific base catalysis with the maximum stability in the pH range 5 - 7 (Koshy *et al.* 1961)

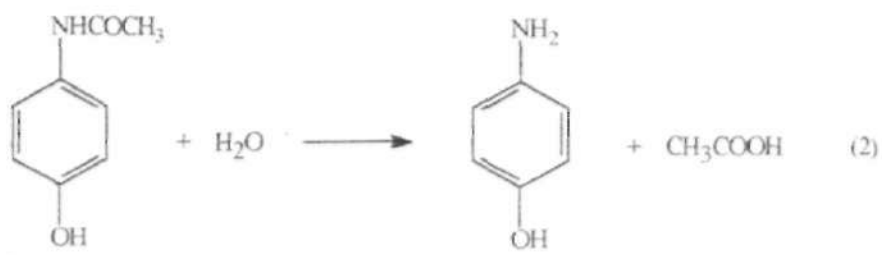
2.1.5 **Tablet Storage**

It should be protected from light (B.P. 1973)

2.2 **Drug Kinetics**

2.2.1 **Reactions and Rate Equation**.

The major route of degradation contributing to the instability of acetaminophen is its hydrolysis to yield p-aminophenol and acetic acid.



(2)

The stability of acetaminophen was studied by Koshy *et al* (1961). The hydrolysis is both acid - and base - catalyzed. Spontaneous hydrolysis was found to be negligible. The important reactions are, therefore,

$$HO-C_6H_4-NHCOCH_3+H \xrightarrow{K_{H^+}} HO-C_6H_4-NH_3^+ + CH_3COOH \quad (3)$$

$$HO-C_6H_4-NHCOCH_3+OH^- \xrightarrow{K_{OH^-}} HO-C_6H_4-NH_2+CH_3COO^- \quad (4)$$

the rate equation may be written as follows:

$$\text{rate:} = K_H + [H^+][APAP] + K_{OH^-}[OH^-][APAP] \quad (5)$$

where K_{H^+} and K_{OH^-} are specific acid and base second order rate constants, respectively, and APAP represents acetaminophen. At constant pH the reaction is pseudo first order, with the apparent first order rate constant K^1 being related to K_{H^+} and K_{OH^-} as shown below.

$$K^{1} = K_{H+}\left[H^{+}\right] + K_{OH-}\left[OH^{-}\right] \qquad (6)$$

2.2.2. **pH - Rate Profile**.

Figure 2.1.1 is the log K', pH plot for the hydrolysis of acetaminophen at 25° (Koshy *et al*, 1961). This pH - rate profile shows specific acid and specific base catalysis: It is accounted for by Eq.(6). The rate is insensitive to ionic strength. The maximum stability occurs at pH 6. At pH 6 and 25 °C, the rate constant is 1.005 x 10^{-9} s^{-1} corresponding to a half - life of 21.8 yr.

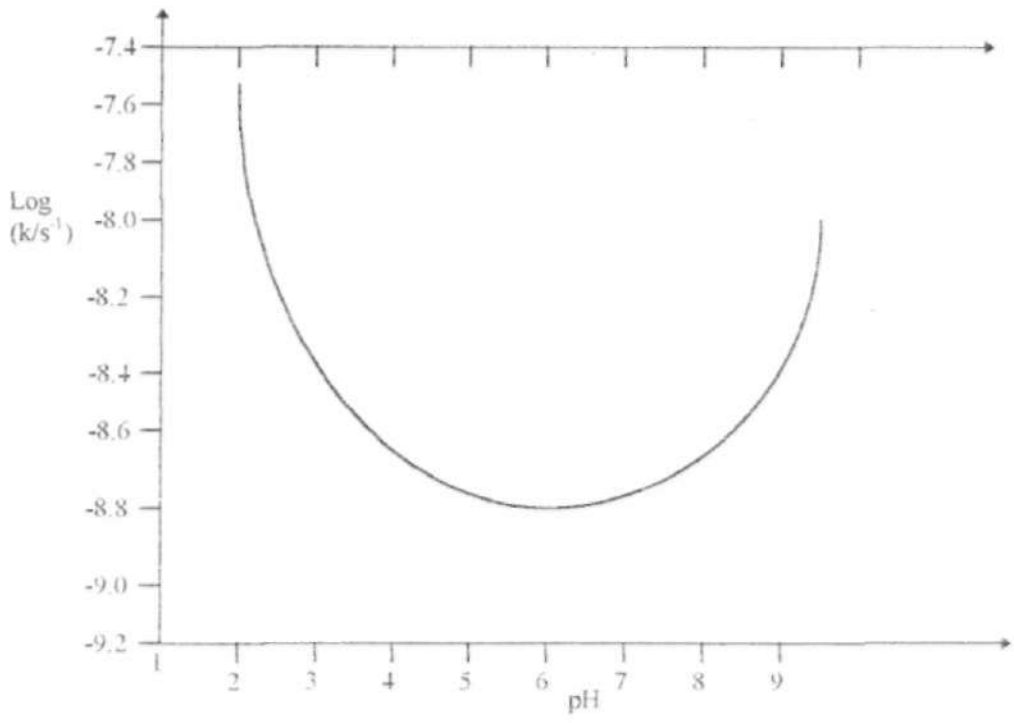


Fig. 2.1.1: Acetaminophen. pH-rate profile for hydrolysis of acetaminophen at 25 °C (rate constants in s^{-1})

2.2.3. **Activation Energy**.

Table 2.1.1 lists the activation energies and half-lives at different pH values. The activation energy, E_a, does not seem to be dependent on pH. A value of 17 kcal/mol was

reported by Zoglio *et al* (1968) and by Kay and Simon (1971), supporting those of Koshy *et al*.

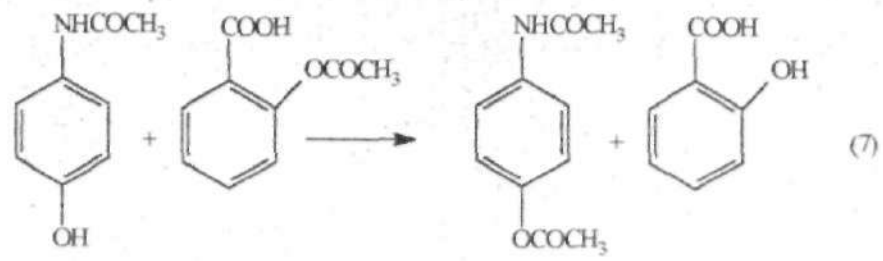
Table 2.1.1: Half-lives,$t_{½}$, and Activation Energies, E_a, for the Hydrolysis of Acetaminophen at different pH values (Koshy *et al*., 1961).

pH	$t_{½}$ (yr. at 25 °C)	E_a (kcal/mol)
2	0.8	16.7
3.	5.8	18.0
4.	15.4	
5.	19.8	
6.	21.8	17.4
7.	12.6	
8.	7.1	18.0
9	2.3	17.0

2.3 FORMULATIONS AND COMBINATIONS

2.3.1 Degradation Reactions

An aspirin - acetaminophen reaction was noticed by Koshy *et al* (1967) and Boggiano *et al* (1970), who reported that acetaminophen, reacts with aspirin giving salicylic acid and diacetyl-*p*-aminophenol ;



(7)

A linear relationship between the rate of formation of salicylic acid and that for diacetyl-*p*-aminophenol was reported by Koshy, *et al.*, (1967). Kalati on the other hand, observed no such reaction at 25 °C. The stability of such mixtures, however, decreased rapidly with the increase in moisture content, temperature, codeine phosphate, and magnesium stearate (Kalatzis, 1970). It is important to mention that caffeine made no contribution to the stability of aspirin - acetaminophen mixtures (Boggiano *et al*, 1970).

Acetaminophen was found to form 1:1 complexes with antipyrine (Dearden, 1972) and with caffeine and theophylline, but not with theobromide (Chowad, *et al*. 1972). This complexation increased the solubility of acetaminophen.

Acetaminophen formed complexes with polyethlene glycol 4000 (PEG 4000) and polyvinyl pyrolidones (PVP). This complexation increased the water solubility and dissolution rate of acetaminophen, the effect being greatest for the acetaminophen - PEG mixture in a rate of 1:2 w/w.

2.3.2 **Stabilization Methods**

In liquid preparations, the pH should be adjusted to about 6 for maximum stability. Acetaminophen tablets are stable. However, in combination with aspirin and Codeine phosphate, moisture and alkali stearates should be eliminated.

2.4 PHARMACOKINETICS AND METABOLISM

Paracetamol is rapidly and almost completely absorbed from the gastrointestinal tract. The concentration in plasma reaches a peak in about thirty minutes to one hour and the therapeutic half-life is approximately three hours (Swinyard, 1980). Therapeutic concentration is within the range of one to l0 μg/ml plasma (Horwitz and Jatlow, 1977, Prescott *et al.* 1971). It has been found that in rats paracetamol is absorbed from the stomach and all portions of the small intestine and relatively insensitive to changes in gastric emptying rates. (Weikel and Lish, 1969). Meal of high fat or protein content or a balance test - meal taken before administration of paracetamol did not delay significantly absorption whereas a high carbohydrate meal, reduced absorption by 60 - 76% in the first 1.5 hours compared with the fasting state (Jaffe *et al.*, 1971).

Studies conducted by McGilveray and Mattok (1972) on the effect of food and sleep on the absorption and excretion of paracetamol showed that concomitant administration of food produced little effect on the extent of its absorption. Less paracetamol is absorbed and excreted when the drug was taken before sleep than after morning ingestion. They are, however, not sure of the cause of the reduced absorption.

It has been reported by Chiou (1975) that food could significantly reduce the absorption rate and peak blood levels of paracetamol in humans; it had no significant effect on total bioavailability, as indicated by similar areas under blood concentration curves of paracetamol. He suggested that differences in the contribution of the first - pass effect for rectal and orally administered paracetamol would not be of clinical significance.

If paracetamol is metabolized appreciably during absorption, its systemic availability should be relatively increased by administering its precusor, acetophenetidine.

which has its phenolic group blocked and therefore protected from conjugation in the gut wall (Levy, 1971)

Paracetamol is relatively uniformly distributed throughout most body fluid (Flower *et al.*, 1985). Gwilt *et al* (1963) showed that paracetamol is distributed uniformly in all tissues with the exeption of fat in dogs.

The major metabolites of paracetamol are its sulphate and glucuronide conjugates, which are excreted into the urine (Andrews *et al.*, 1976). Further urinary metabolites include the corresponding 3-hydroxy and 3-methoxy conjugates (Andrews *et al* 1976) and 3-methylthio conjugates (Klutch *et al.* 1978).The C-3-cystein and C-3-mercapturate conjugates are found in the urine in rather small amounts after low doses of paracetamol, but are considerably increased after high, toxic doses (Andrews et al., 1976).

In normal subjects, less than 50% of paracetamol is usually excreted in the urine while the rest is eliminated largely as the glucuronide and sulphate conjugates (Cummings *et al.*, 1967; Levy and Regardh, 1971).

Studies have been conducted on the elimination of paracetamol and its metabolites. Results indicated that oral administration of 1g dose of paracetamol, the average recovery of drug in all forms was 92%, mostly as acetaminophen glucuronide and acetaminophen sulphate which are non toxic (Figure 2.1.2). Only 3.4% of the dose was recovered as free paracetamol (Levy and Yamada, 1971 and Miller and Fischer, 1974).

Paracetamol is the major metabolites of phenacetin and acetanlide (Batty and Robinson, 1977). Miller and Fischer (1974) concluded that rat is not an appropriate animal model for paracetamol metabolisms in humans. Paracetamol appears to be excreted in the urine primarily as acetaminophen sulphate when administered to rats in

doses comparable to human doses. In contrast, the glucuronide conjugate constitutes the largest fraction of the dose of paracetamol excreted in the urine by humans. However, children have less capacity for glucuronidation of the drug than do adults.

The ratio of glucuronide and sulphate metabolites varies widely between the different species; mice excrete more glucuronide than sulphate (Dybig, 1975). Administration of paracetamol with single and multiple doses of aspirin produce no effect on paracetamol metabolism in man (Amsel and Davison, 1972).

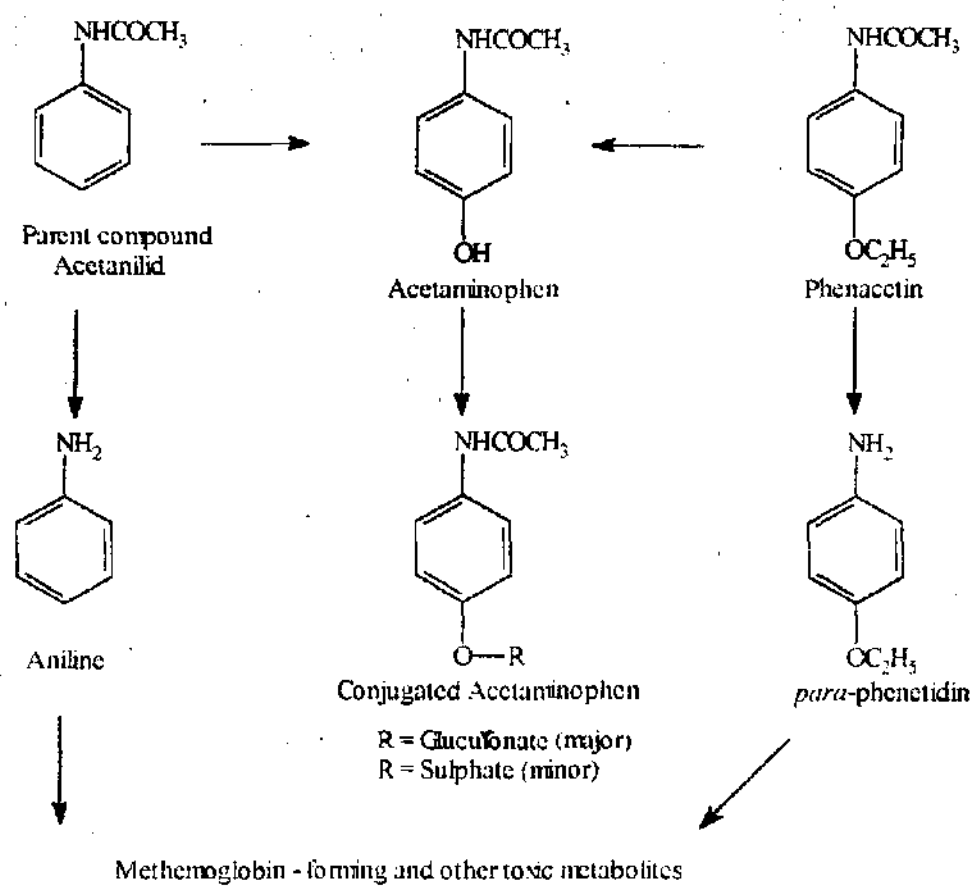


Fig.2.1.2: Structural formulas of major *para*-aminophenol derivatives and their interrelations (from Flower *et al.* 1985).

Excretion rate of the glucoronide and sulphate metabolites of paracetamol was reduced after administration of salicylamide, and paracetamol reduced the formation of the

sulphate metabolite of salicylamide (Levy and Yamada, 1971). Ascorbic acid caused a significant decrease in the fraction of paracetamol excreted as the sulphate and a significant increase in the fraction excreted as paracetamol and paracetamol glucuronide, but it had no effect on the recovery of total paracetamol i.e. the sum of paracetamol, its glucuronide and its sulphate (Houston and levy, 1976).

2.5 PHARMACOLOGICAL PROPERTIES.

Paracetamol is used mostly as mild analgesic and antipyretic (Plankogiannis and Saad, 1978; Chiou, 1975; Levy, 1971 and Swinyard, 1980). This property is used to relieve low intensity pain and reduce fever. Paracetanol is effective in treating pain arising from intergumental structures and malaise associated with viral infections, especially those components associated with headache and muscle aching (Bonica and Halpern, 1972). It is useful especially as an analgesic - anti pyretic in patients sensitive to aspirin. It has few side effects and is well tolerated by aspirin - sensitive patients.

Paracetamol may reduce the post traumatic inflammatory response and may be preferable to acetylsalicylic acid in bilateral oral surgery (Lokken and Skjelbred, 1980). Therapeutically paracetamol is not as effective as indomethacin and aspirin in the treatment of rheumatoid arthritis (Watson *et al*, 1975). In therapeutic doses paracetamol has no serious adverse reaction (Benson, 1983). The effective serum concentration of paracetamol for analgesia and antipyresis is 10 - 20 ug/ml and this is obtained usually after single doses of less than 2g is taken orally. Therapeutic concentration of 1 - 10 µg/ml plasma has also been reported (Horwitz and Jatlow, 1977; Prescolt *et al*, 1971).

2.5.1 Mechanism of Action.

Endogenous pyrogens derived from polymorphonuclear leucocytes may cause fever produced by bacterial endotoxins or viruses. Endogenous pyrogens act directly upon the thermoregulatory neurons in the hypothalamus to increase the 'set-point' temperature (Parkhouse, 1979). Paracetamol inhibits the enzyme prostaglandin synthetase in the brain. By preventing the biosynthesis of prostaglandin, the antipyretic analgesics could inhibit the generation of prostaglandin E type substances by pyrogen, a process necessary for an increase in the 'set-point' temperature (Flower and Vane, 1972). Paracetamol may be more effective against enzyme in the CNS than those in the periphery. This fact may partly account for its well documented ability to reduce fever and to induce analgesia, effects that involve actions on neuronal tissue (Flower *et al.*, 1985).

2.6 TOXICITY.

In conventional dose (325 to 1000 mg), paracetamol is well tolerated. Skin rash and other allergic reactions occur occasionally. The rash is usually erythematous or urticarial, but sometimes it is more serious and may be accompanied by fever and mucosal lesions (Flower *et al.*, 1985). Patients who show hypersensitivity reactions to the salicylates only rarely exhibit sensitivity to paracetamol and related drugs. In a few isolated cases, the use of paracetamol has been associated with neutropenia, thrombocytopenia and pancytopenia. When high doses are ingested paracetamol undergoes N-hydroxylation to form N-acetylbenzoquinoneimine, a highly reactive intermediate. This metabolite reacts with sulfohydryl groups in proteins and glutathione. (see figure 2.1.3).

When hepatic glutathione is depleted, after massive single doses, the excess reactive arylating intermediate covalently binds to vital hepatocellular macromolecules, leading to necrosis (Flower *et al*, 1985). The continuing problem of acute hepatic necrosis following paracetamol overdose has been reviewed (Barwick *et al.*, 1976; Fernandez and Brito, 1977; Boyer and Rouff, 1971; Byer and Traylor, 1982). Children have been considered to be relatively resistant to the hepatotoxic effect of paracetamol and severe liver damage was thought to be very uncommon. The possible reason is that its metabolism in children is different, with a greater proportion being converted to the sulphate conjugates.

Severe hepatic damage was likely if the patient had vomited within six hours of paracetamol overdose (Gazzard *et al.*, 1977). A toxic reaction of paracetamol on a patient's bone marrow has been reported by Mackinnoon and Menon (1974).

Intravenous N-acetylcysteine is now considered the treatment of choice for paracetanol poisoning. It is more effective than intravenous cysteamine or methionine in preventing liver damage. It also prevents renal failure and death when given within ten hours of ingestion (Maurer and Zeisler 1978; Jacobsen and Wiik-Larsen, 1981).

In studies conducted in patients with paracetamol overdose, it was revealed that severe hepatic lesions occurred when the plasma paracetamol concentrations, reached more than 30 μg per ml after four hours; none occurred in patients with concentrations of less than 120 μg, 50 μg per ml at 12 hours, but hepatic necrosis developed in 16 out of 18 with concentrations greater than 50μg/ml (Prescott *et al* 1971). Toxicity is associated with a prolongation of the normal plasma elimination half -life from 2 hr to over 4 hour to 10 hr and with the persistence of high paracetamol levels over several days (Boyer and Rouff,

1971, McJunkin *et al.*, 1976). Ambre and Alexander (1977) note that paracetanol plasma levels are more reliable index of the risk of toxic liver injury than in the total dose ingested.

Overdose of paracetamol is characterised by the following symptoms - vomiting, gastrointestinal haemorrhage, liver damage, cerebral oedema and renal tubular necrosis. Liver necrosis may be fatal several days after overdose.

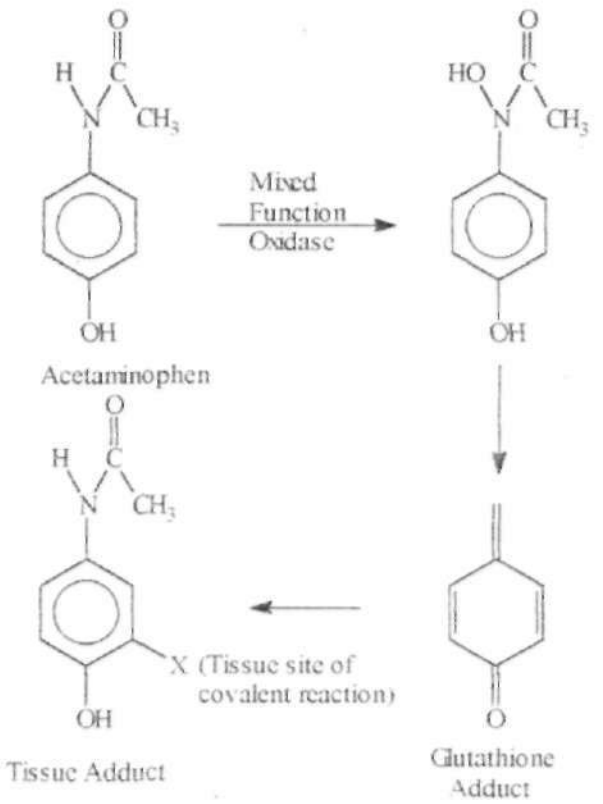


Fig. 2.1.3 Formation of Reactive Intermediates during metabolism of environmental substances and Drugs (from Benet and Sheiner, 1985).

2.7 DRUG INTERACTIONS

Phenytoin appears to enhance the hepatic metabolism of acetaminophen. Patients on combination anticonvulsant therapy that included phenytoin tended to have reduced acetaminophen bioavailability and half life (Perucca., 1971). Although little clinical

evidence is available, one might expect that phenytoin would tend to increase the hepatotoxicity of acetaminophen overdoses by increasing the production of toxic acetaminophen metabolites. Acetaminophen (1.5 g/day) did not significantly effect serum phenytoin concentrations in nine epileptic patients on chronic phenytoin therapy (Neuvonen, 1979). Until more is known about this interaction, it would be prudent to avoid large doses and or prolonged administration of acetaminophen in patients on Phenytoin.

A study of 21 subjects who were given various combinations of aspirin and acetaminophen demonstrated that acetaminophen increased blood concentration of unhydrolysed aspirin but not total salicylate. (Cotty, 1977). It was proposed that the increase in unhydrolysed aspirin would increase the therapeutic effect; however, it has also been suggested that combinations of aspirin with either acetaminophen or phenacetin may increase the likelihood of analgesic nephropathy (Wilson, 1982). Neither of these possibilities has been proven clinically. No special precautions appear necessary. However in the absence of a proven advantage of combined therapy over either agent alone, it may be better to use one agent or the other.

Acetaminophen probably reduces the sulfation of ethinyl estradiol in the gut mucosa, thus increasing the plasma concentration of the estrogen. Oral contraceptives appear to enhance the glucuronidation of acetaminophen. In six healthy women receiving oral contraceptives , the pharmacokinetics of ethinyl estradiol were studied with and without pre-treatment of a single 1 - g dose of acetaminophen (Rogers, 1987). Acetaminophen was associated with a modest increase in serum ethinyl estradiol concentrations. The clinical importance of such increase in estrogen levels is not

established, but it is reasonable to assume that periodic use of acetaminophen may result in fluctuating levels of ethenyl estradiol. Several studies have shown that women receiving oral contraceptives have considerably higher clearance of acetaminophen than those not receiving oral contraceptives (Miners, 1983; Ochs, 1984). The Magnitude of the reduction in plasma acetaminophen concentrations is probably sufficient to inhibit the effect of acetaminophen, in at least some patients. It does not appear necessary for women on oral contraceptives to avoid taking acetaminophen. Nevertheless one should consider the possibility that patients on the combination may develop increased ethinyl estradiol concentrations or decreased acetaminophen concentrations. If such changes in serum concentrations are suspected of causing problems, it would be prudent to use an alternative to acetaminophen.

It is proposed that enzyme induction from prolonged intake of large amounts of alcohol results in enhanced formation of hepatotoxic metabolites of acetaminophen under conditions of acetaminophen overdose. Data from acetaminophen over dose in chronic alcoholics indicate that they are more susceptible to acetaminophen - induced hepatotoxicity (McJunkin, 1976. Licht, 1980). These findings are consistent with the known ability of chronic alcoholism to result in enzyme induction and with the likelihood that enzyme induction results in accelerated formation of hepatoxic acetaminophen metabolites. Whether alcoholism could predispose to hepatoxicity in patients chronically receiving large therapeutic doses of acetaminophen is not established, but some of the reported cases have involved excessive therapeutic doses of acetaminophen rather than intentional overdoses (Barker, 1977, Licht, 1980). In contrast to chronic alcohol abuse, acute alcohol intoxication tends to inhibit hepatic microsomal drug metabolism and thus

might be expected to reduce the formation of toxic acetaminophen metabolites. Animal studies indicate that acute ethanol administration tends to protect against the hepatotoxicity of large acetaminophen does (Sato, 1981) and ethanol intoxication may have contributed to a favourable outcome in a patient who took 60g of acetaminophen (Lyons, 1977). Alcoholics may have lower bioavailability of acetaminophen, presumably due to increased first pass metabolism (Dietz, 1982). This may partly explain the tendency of alcoholics to take large quantities of acetaminophen for therapeutic purpose (Barker., 1977; McClain, 1980). No Special precautions appear necessary, but one should be aware that patients ingesting alcohol for prolonged periods may be more susceptible to hepatotoxicity from acetaminophen overdose.

In a study, acetaminophen (2.6g/ day for 2 weeks) given to patients on various oral anticoagulants resulted in a mean increase of 3.7 seconds in the prothrombin time compared with the effect of a placebo (Antlitz, 1968). In a similar study, acetaminophen (3 g/day for 2 weeks) had no effect on the prothrombin-time response to warfarin (Udall, 1970). Likewise, Antlitz (1969) found that two doses of 650 mg of acetaminophen had no effect on prothrombin-time response to oral anticoagulants. In another study, 10 of 20 patients on chronic oral anticoagulants were given acetaminophen (2.0 g day for 3 weeks), and the other 10 were given a placebo (Boeijinga, 1982). There was a moderate increase in the hypoprothrombinemic response in the patient given acetaminophen. However none of the patients was receiving warfarin, and it is not clear that the results can be applied to warfarin. Patients should be watched for evidence of enhanced hypoprothrombinemic response to oral anticoagulants when acetaminophen is used concurrently and the anticoagulant dosage reduced as needed.

After a prolonged chloramphenicol half-life observed in children concurrently taking acetaminophen therapy, six adults were given chloramphenicol (1.0g iv) followed by acetaminophen (100mg iv) 2 hours later (Buchanan (1979). The chloramphenicol half - life was 3.25 hours prior to the acetaminophen and increased to 18 hours following acetaminophen administration. In a subsequent study, chloramphenicol pharmacokinetics were not significantly different in 18 children who received concurrent acetaminophen and in eight children who did not (Kearns, 1985). In a study of five children receiving IV. chloramphenicol, acetaminophen (50mg kg/day for 48 - 72 hours) was associated with an increased in chloramphenical clearance (Spika, 1986). The reasons for the disparate results in these three studies probably result from differences in study design, number of subjects, assay techniques or route of acetaminophen administration. Until clinical evidence of excessive or insufficient chloramphenical effect associated with acetaminophen administration is reported, the clinical significance of this interaction will remain speculative.

Anticholinergics slow gastric emptying, thus reducing the rate of acetaminophen absorption in the intestine. Acetaminophen (1.5 g orally) was given to six patients with, without pretreatment with propantheline 30 mg IV (Nimmo, 1973). The rate of absorption of acetaminophen was considerably slow in the presence of propantheline than in its absence, but the extent of acetaminophen absorption did not appear to be affected. Oral administration of propantheline is likely to have a similar effect. Other agents with anticholinergic activity such as tricyclic antidepressants and antihistamines probably delay acetaminophen absorption. Because the onset of acetaminophen effect may be delayed in

patients receiving anticholinergics, excessive repeated doses of acetaminophen should not be given.

Barbiturates appear to enhance the metabolism of acetaminophen. The fate of acetaminophen (1.0 g orally and IV) was compared in six healthy subjects and six epileptic patients receiving chronics anticonvulsant therapy e.g. barbiturate (Perucca, 1979). The epileptic patients had a lower oral acetaminophen bioavailability and shorter serum half - life of acetaminophen following its iv administration. Although , clinical evidence is lacking, one might expect that patients taking enzyme inducing drugs would experience a reduced therapeutic response to acetaminophen. With acetaminophen overdoses, however, one would expect long-term phenobarbital therapy to increase (1) conjugation of acetaminophen to its non toxic glucuronide metabolite and (2) its oxidative biotransformation to hepatotoxic metabolites. The overall effect of these two opposing mechanisms is not predictable, but available evidence indicates that phenobarbital pretreatment tends to increase the hepatotoxicity of toxic acetaminophen doses. Phenobarbital has been shown to increase the hepatoxicity and nephrotoxicity of acetaminophen overdose in rats (Mclean, 1976), but evidence from humans is limited to isolated case reports (Wilson, 1978). Until more is known about this interaction, large doses of acetaminophen should be administered with caution to patient on barbiturate therapy.

Cholestyramine inhibits the gastrointestinal absorption of acetaminophen. In four healthy subjects concurrent administration of cholestyramine 12 g orally markedly reduced plasma levels of acetaminophen 2.0 g orally (Dordoni, 1973), the magnitude of the effect appears sufficient to reduce the therapeutic response to acetaminophen. It is likely that

colestipol (Colestid) would also reduce plasma acetaminophen levels, but clinical studies are lacking. Give acetaminophen I hour or more before or several hours after cholestyramine or colestipol to minimise the inhibition of acetaminophen absorption.

Acetaminophen (3.0 g/day for 5 days) some what reduced the 96-hour urinary excretion of diazepam and its metabolites in four subject following a single 10 - mg dose of diazepam (Mulley, 1978). The effect was greater in the two female subjects, but additional study is needed to confirm those results and to define the magnitude of the interaction.

It is proposed that metoclopramide increases the absorption rate of acetaminophen by stimulating gastric emptying (Nimmo, 1973). Although the absorption rate of acetaminophen has been shown to be increased by metoclopramide in healthy subjects, the amount of acetaminophen, absorbed did not seem to be affected. Theoretically, acetaminophen effect would occur somewhat sooner in those taking metoclopramide, but the difference in onset time probably would not be noticed by most patients. No special precautions are necessary during cotherapy with metoclopramide and acetaminophen.

2.8 ASSAY METHODS.

The best important requirements for a particular assay method are specificity, sensitivity and easy and quick to perform. The medium of analysis is also important.

There are various methods for quantitative determination of paracetamol as such in pharmaceutical preparations and in combination with other drugs Several methods involve colorimetric and require the hydrolysis of paracetamol to *p*-aminophenol. Brodie and Axelrod (1949) proposed the earliest colorimetric assay of paracetamol in plasma. After

acid hydrolysis the formed *p*-aminophenol is diazotized and coupled to alpha naphthol, and the azo dye is measured by spectrophotometry. Other procedures utilize the reaction of *p*-aminophenol with $NaNO_2$ - HCl to form the coloured 2 - nitro - *p* - aminophenol (chembers and Jones, 1976; Glynn and Kendal, 1975; Wiener, 1977) and with vanillin to a stable imine with an absorbance maximum at 395 nm (Plakogiannis and Saad, 1978). *P*-aminophenol can also be coupled with phenol in the presence of hypobromite to form an indophenol dye (Welch and Conney, 1965) which may be interfered with by ascorbic acid (Swale, 1977).

Gotelli *et al.* 1977 utilized reverse Phase High Performance Liquid Chromatography assay with UV detection at 254 nm requiring only 0.1 ml plasma with a sensitivity of 0.5 mg acetaminophen/ml and analysis time of 5 mins. The extraction technique is rapid and includes acetoacetanilide as the internal standard. Of 36 other drugs tested, only theophylline interfered with the determination of acetaminophen. The extraction solvent is ethyl acetate. Horwitz and Jatlow (1977), include an, ether extraction step and report no assay interference by theophylline; this may be due to the ether extraction step, rather than the ethyl acetate used by Gotelli *et al.*, (1977). The recovery of acetaminophen is 65% with ether and above 80% with ethyl acetate; however, theophylline might not be extractable into ether under the assay conditions of Horwitz and Jaflow (1977).

Evans and Harbison (1977) reported Gas Chromatography method using ether for extraction of plasma and urine samples at approximately neutral pH and Flame Ionization detection of acetaminophen. The gas chromatographic detection of acetaminophen in urine without derivatizaton allows a sensitivity of 2 µg/ml after fluorisil column

chromatography prepurification and ether extraction (Grove, 1971). However, underivatised acetaminophen leads to tailing GC peaks and nonlinear standard curves at low concentrations. The following compounds have been suggested as internal standards. N-propionyl - P - aminophenol (Dechtiaruk *et al.*, 1971), and *p*-bromoacetanilide (Evans and Harbison, 1977; Prescott, 1971) and *p*- chloroacetanilide (Prescott, 1971).

There are many recent methods of assay of acetaminophen. Peng *et al.*, (1994) described direct injection of urine and determination of acetaminophen by micellar liquid chromatography with a wall- jet cell carbon fibre microelectrodes. Urine was filtered and diluted tenfold with mobile phase. Portions (10 µl) were analysed by micellar LC on a column (25 cm x 4.0 mm i.d.) of Nucleosil C_{18} (5 µm) with a precolumn (5 cm x 4.6 mm i.d.) of silica gel (15 - 25 µm). The mobile phase (1 ml/min) contained 50 mM - SDS of pH 7.15, 20 mM - Na_2HPO_4, 3% propan-1-ol and 1 mM - EDTA. Acetaminophen was detected in a flow - through detector with a large volume wall - jet at a preconditioned carbon fibre microelectrode at +0.65 V vs. SCE. The peak due to paracetamol was well separated from those due to ascorbic acid, uric acid and 4-aminophenol. Calibration graphs were linear for 0.02 (detection limit) to 70 µg/ml of acetaminophen. At 10 µg/ml, the RSD (n = 20) was 3.1%. Recovery of 5 - 3000 ng of paracetamol added to three samples of urine averaged 95.7 - 98.3%. The presence of the surfactant micells both kept proteins in solution and enhanced the detector response.

Nese *et al.*, (1996) described simultaneous spectrophotometric determination of acetaminophen and caffeine in tablets by absorbance ratio technique. Powdered tablets equivalent to 94.4 mg paracetamol and 5.6 mg caffeine were sonicated for 20 mins with 80 ml water and the solution was diluted to 100 ml. The solution was filtered and a 10 ml

portion of the filtrate was diluted to 100 ml with water. The absorbance of the resulting solution was measured at 241.6, 259.7 and 272 nm and quantity of drugs were calculated from a given equation. Calibration graphs, were linear for 1 - 18 µg/ml paracetamol and 1 - 17 µg /ml caffeine.

Shirke, *et al.*, (1993) simultaneously estimated Ibuprofen and paracetamol in combined dosage formulation by high - performance thin-layer chromatography. Powdered tablets were dissolved in methanol and the solution was filtered. The filtrate was applied to silica gel GF 254 HPTLC plates which were developed with hexane:ethyl acetate :anhydrous acetic acid (18:5:2): densitometric scanning at 265 nm was used for quantitation. Calibration graphs were linear for 10 - 50 and 16-60 µg of paracetamol and Ibuprofen, respectively, for 325 mg of paracetamol , the recovery was 101.5% with a RSD of 1.8%. For 400 mg of Ibuprofen, the recovery was 99% with a RSD of 2.1%.

Dasgupta *et al.* (1993) described microwave-induced rapid hydrolysis of paracetamol and its conjugates in urine for emergency toxicological screen. Paracetamol was detected by a method involving hydrolysis, by boiling with HCl, to 4-aminophenol which is reacted with O-cresol and NH_4OH to form a distinctive indophenol blue colour, the absorbance of which is measured at 620 nm. The hydrolysis is still uncompleted after 10 min at 100 °C full hydrolysis requiring 20 min, so that hydrolysis is the time limiting step in the procedure. Paracetamol could be rapidly hydrolysed by microwave irradiation for 2 min at 120 W. Results agreed with those by conventional hydrolysis for 20 min.

Goicoechea, A.G. *et al.* (1995) described a validated high - performance liquid in the urine. Paracetamol and its glucuronide and sulphate metabolites chromatographic method for the determination of paracetamol and its major metabolites were diluted with

water and filtered to 0.45 µm. Analysis was carried out using a Waters Radical - PAK column (10 cm x 8 mm i.d.) of Resolve C_{18} silica (5 µm) with a similar Guard - PAK precolumn. Elution (2.5 ml/min) was with aqueous 0.1M-KH_2PO_4/methanol /acetic acid (95:4:1); detection was at 248 nm. Calibration graphs were linear from 5 - 100ug\ml for each analyte; RSD were from 0.4 - 2.4% (n-3). Detection limits were 2 ng/ml for paracetamol and 5 ng/ ml for glucuronide and sulphate.

Damian, P.C. *et al* (1995) gave account of rapid determination of paracetamol in blood serum samples by first derivative UV absorption spectroscopy. Serum (1 ml) was deprotonized with 2 ml TCA (240 g/L). Following centrifugation, 1 ml of the supernatant solution was treated with 0.2 ml 10M - NaOH - The UV absorption spectrum was recorded and the first derivative at 292 nm was calculated. Paracetamol was determined from a calibration graph of the first - derivative Vs., the original paracetamol concentration in the serum samples. The calibration graph was linear from 100 - 500 µg/ml of paracetamol. Other anti-inflammatory drugs except salicylate did not interfere. The RSD (n = 3) was 3.4 at the 40 µg/ml level. Recoveries ranged from 97.7 - 107%.

HPLC determination of paracetamol in serum with dialytic sample pretreatment was conducted by Gilar M. *et al* (1995). In this study, a centrifuged sample of serum was dialysed and trace enriched on an automated Gilson ASTED system - operated according to manufacture's instructions. Then the sample was analysed on a 4 µm Nova - pack C_{18} column (15 cm x 3.9 mm i.d.) and eluted (0.5 ml/min) with methanol/0.05% aqueous acetic acid (3:7) with detection at 254 nm. Calibration graphs were linear from 0.5 - 10 mg/1 paracetamol with 8-chlorotheophylline as internal standard; RDS were 1.3 - 3.4% (n = 10). Recoveries were 95.8 - 101.7%.

2.9 AIMS AND OBJECTIVES OF THE STUDY.

Paracetamol is used mostly as analgesic and antipyretic (Swinyard, 1980). It is well tolerated and lacks many of the side effects associated with use of Aspirin. It is obtained without prescription. Because of these advantages it is widely used in clinical practice alone and in combination with other drugs in treatment of some disease conditions. It has also earned a prominent place as a common, house hold analgesic. Consequently a large percentage of our population is exposed to the use of paracetamol.

There are very many places where drugs are kept. Commercially available paracetamol are stored in places which can be classified into three categories. These are (1) registered Pharmacy Shops (2), Hospitals/Clinics, and (3) Patent Medicine Stores. Some of these areas where drugs are kept lack standard storage facilities and the drugs kept may be subjected to harsh conditions. The aims and objective of this study is to investigate how storage conditions and drug handling in these three different areas affect the pharmacokinetic profile of paracetamol in normal healthy individuals. Paracetamol is chosen because of its wide usage and availability without prescription. The study will also include comparative study among these three different areas.

Results from this study could serve as a guide as to where best to obtain our drugs in case of significant differences using pharmacokinetic profiles of paracetamol tablets. This will reveal in what ways storage conditions could affect quality of drugs kept in these areas.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 DRUG

PARACETAMOL B.P. 500 mg tablet

Dependol ® Manufactured by PAAM PHARMACEUTICALS

(DELHI) LTD. 26, BHARGAVA LANE, NITYANAND

MRG, CIVIL LINES, DELHI, INDIA

MFG, CODE; UP/DRUGS/28 OF 1992 DISTRIBUTED BY:

BATCH NO: DPT 5 - 19 VITAL PHARMACEUTICALS

MFG DATE: JUNE, 1995 LS AND HEALTH CARE LTD., 79,

EXP. DATE: May, 2000 FORSYTHE STREET

LAGOS, NIGERIA.

3.2 EQUIPMENT

ERWEKA dissolution apparatus and accessories, Erweka appalebau G.M.B.H. 6056. Heusenstamm Kr. Offenbuch - main.

Analytical balance, type H35 AR Mettler Gallenkamp

Silica Cuvettes B.S. 3875. 310mm F.O.

Thermometer - 31/3 Quickfit

Centrifuge Machine Gallenkamp

Electric Stirrer.

M302 CAMSPEC Ultraviolet spectrophotometer.

CAMSPEC LTD. CAMBRIDGE CB24BG

UNITED KINGDOM.

3.3 **REAGENTS**

10% Trichloroacetic acid (BDH chemicals Ltd., Poole, England).

15% Sulphamic acid (BDH chemicals Ltd. Poole, England)

10% Sodium Nitrate (May and Baker Ltd., Dagen ham, England).

Sodium Hydroxide pellets (BDH Chemicals, Poole, England)

Hydrochloric acid (Analar grade BDH chemicals, poole, England).

Acetone - Analar grade (BDH chemicals Poole, England).

Potassium dichromate - BDH Chemicals Ltd., Poole, England.

Feric chloride - M & B Ltd., Dagenham England.

3.3.1 **Test for uniformity of weight (B.P. 1993 method)**

Twenty tablets were randomly selected and their average weight determined. The tables were then weighed individually and the percentage deviation from the mean weight determined.

3.3.2 **Identification Test (B.P. 1993 Method).**

Powdered tablets containing 0.5g of paracetamol was extracted with 20 ml of acetone, filtered and the filtrate evaporated to dryness and then dried at 105 °C. the residue obtained complies with the following test.

i) 0.1 g of residue when boiled with 1 ml of conc. HCl for 3 minutes, 10 ml of water added and cooled, no precipitate was produced. But when 0.05 ml of

0.0167M potassium dichromate was added a violet colour was produced slowly which did not turn red.

ii) The residue melt at 168 - 170 °C.

Pure standard paracetamol powder was tested concurrently with test samples and both gave similar result.

3.3.3 Chemical Assay (B.P. 1993 method).

Twenty tablets of paracetamol were accurately weighed and finely powdered. A quantity of the powder containing 0.15 g of paracetamol was added to 5ml of 0.1 m NaOH in a 200ml flat bottom flask diluted with 100 ml of water and shaken for 15 minutes. Sufficient water was added to produce 200ml. The solution was filtered. 10ml of the filtrate was diluted to 100 ml with water, 10 ml of the resulting solution was added to 10 ml of 0.1M NaOH, diluted to 100ml with water and the assorbance of the resulting solution measured at the maximum at 257nm. The percentage content of paracetamol was calculated taking 715 as the value of A (1%,1 cm) at the wavelength of 257 nm. The procedure was repeated five times.

3.3.4 Disintegration Test (B.P. 1993 method).

One tablet of paracetamol each was added to 6 tubes of the basket and a disk added to each tube. The assembly was suspended in the beaker containing 0.1M HCl maintained at 37 °C ± 0.5 °C and the apparatus operated. Disintegration is considered to be achieved when no residue remains. The tablets are considered to pass the test if all six have disintegrated before 15 minutes.

KAS

3.3.5 *In vitro* Dissolution Test (rotary basket method B.P. 1993)

One tablet of Paracetamol was placed in the basket and then placed in the flask containing 100ml of 0.1mHcl, warmed and maintained between 36.5 °C and 37.5 °C. The stirrer was set at 100 r.p.m and the apparatus operated for 45 minutes. Sample of the dissolution medium was then withdrawn and filtered. The filtrate was then assayed using UV. Spectrophotometer at wavelength of 257 nm. The procedure was repeated five times. The percentage released after 45 minutes was calculated by comparing the absorbance of the sample obtained with the absorbance given by a known concentration of paracetamol in 0.1M HCl at 257 nm.

B.P. (1993) specifies that for a tablet to pass the dissolution test, the amount of active ingredient released after 45 minutes is not less than 70%.

3.4 *IN VIVO* STUDY

3.4.1 Study Design

Twelve (12) healthy young adult male volunteers with average age of 27.67 years and average weight of 66.67 kg took part in the study. Prior to participation, they were all properly screened and certified healthy. No drugs including alcohol and cigarette, were allowed two weeks before their participation in the study. The first six volunteers received paracetamol tabs 1g each, from the first three different drug selling areas on different occasions. The second six volunteers were also given 1g paracetamol tabs each from the second three different drug selling areas on different occasions. These are registered Pharmacy Shops, Hospitals/Clinics and Patent Medicine Stores. The wash out period was two weeks between attendance by any individual for both controls and

samples. The paracetamol tablets were obtained directly from the distributor at the same time.

Prior to this study twenty (20) Hospitals/Clinics thirty (30) registered Pharmacy Shops and one hundred and twenty five (125) Patient Medicine Stores were screened for their storage facilities in Zaria areas. Two of each of drug storage areas with poorest storage facilities were then selected for the study. The average consumption rates in all these areas were also obtained (20 Hospitals/Clinics, 30 Pharmacy Shop and 125 Patient Medicine Stores). The average consumption rate of 1000 tablets per month was obtained.

Dependol ® brand of paracetamol manufactured by PAAM PHARMACEUTICALS (DELHI) INDIA was used. The product distributed by VITAL PHARMACEUTICALS LTD LAGOS.

The drugs were stored for two months to allow for drugs that are stocked in excess of normal consumption rate. Samples taken before exposure (controls) and after two months exposure (test samples). The sub optimum conditions used are lack of ceiling fan and air condition, direct sunlight into the premises, cracks in the ceilings and walls, improper ventilation and exposure to dusts.

3.4.2 **Test Protocol**.

The subjects fasted overnight before taking part the following morning. Prior to oral ingestion of 2 x 500 mg tablets of the paracetamol each volunteer attended the trial room and was allowed a 5 - minutes relaxation on a couch and made used to the room. Sampling took place at the clinical pharmacology trial room of Ahmadu Bello University Teaching Hospital Zaria. A size 20 g cannula was placed in an antecubital vein and 5 ml of blood taken (0 minutes).

3.4.3 Sampling Time .

After sampling of pre dose blood of 5 ml, 2 x 500 mg of paracetamol was then administered with 200 ml of water. Blood was then sampled at 5 minutes, 10, 15, 20, 30, 60, 90., 120, 180, 240, 300 and 360. All volunteers remained on the couch and no food or other drink was allowed during the 6 hours of the study. Blood was collected into plain glass tubes and allowed to clot. Serum was obtained by centrifuging for 15mins. Using pasteur pipettes, the serum was transferred into bijoux bottles, stoppered and stored in a deep freezer at -20 °C until the time of analysis.

3.4.4 Analysis

The method of Glynn and kendal (1975) which is specific for paracetamol without interference from the sulphate and glucuronide conjugates of paracetamol was used. 1ml of the sample was added to a 25 ml stoppered centrifuge tube containing 2 ml 10% trichloroacetic acid. After thoroughly mixed, the tube was centrifuged briefly and the claer supernatant decanted into a 25 ml test tube containing 1 ml 6N hydrochloric acid and 2 ml 10% sodium nitrate. After allowing the contents to stand for 2 minutes, 2 ml of 15% sulphamic acid was added carefully, followed by 5ml 10% NaOH. The ensuing yellow colour was measured at 430nm against a reagent blank of water. The absorbances of the control serum (obtained prior to administration of the tablets) were subtracted from the absorbances of the serum specimens obtained subsequently. The samples were assayed by reference to standard calibration curves.

3.4.5 Calibration Curves.

5mg of paracetamol powder was weighed and dissolved in distilled water transferred into a 100ml volumetric flask and made to volume with distilled water. A

standard paracetamol solution of 50 ug/ ml was obtained. 1.0, 2.0, 3.0, 4.0 and 6.0 ml of the standard solution were transferred into series of 10 ml volumetric flasks. 1 ml of blank serum was added to each flask and diluted to volume with distilled water. Solutions of various concentrations (5,10,15, 20 and 30 ug /ml) were therefore obtained.

All samples were treated as mentioned in the Colorimetric method assay procedure. The absorbances were measured at 430 nm in 1 cm cells against a reagent blank of water. Graphs of absorbance versus concentration were plotted.

3.5 DATA HANDLING AND STATISTIC.

The results of the various studies were carefully recorded. Graphs and tables were drawn were appropriate. The data were statistically analysed were appropriate. Student's paired t test was used for all the data. P. values less than 0.05 were considered to be statistically significant.

TABLE 3.1: THE AGES AND WEIGHTS OF TWELVE VOLUNTEERS THAT INGESTED IG OF PARACETAMOL EACH

VOLUNTEER	SEX	AGE(YRS)	WEIGHT (kg)
1	Male	28	68
2	"	30	72
3	"	25	66
4	"	26	69
5	"	31	71
6	"	29	69
7	"	27	67
8	"	32	67
9	"	23	61
10	"	26	64
11	"	30	66
12	"	25	60
Mean ± S.E..M.		27.67 ± 0.80	66.67 ± 1.05

S.E.M. standard Error of Mean.

CHAPTER FOUR

4.0 RESULTS

4.1 Test for uniformity of weight of paracetamol tablets

The result of test for uniformity of weight is shown in table 4.1

B.P. (1993) stated that not more than two of the individual weights (for tablets of average weight of 250 mg or more) deviates from the average weight by more than 5% and none deviates by more than twice that percentage.

The results show that the samples passed the test for uniformity of weight, since the deviations from the mean weight obtained are within the specified limit.

TABLE 4.1: Results of test for Uniformity of Weight for the Brand of Paracetamol used

TABLET NO.	WEIGHT OF TABLET (g)	% DEVIATION FROM THE MEAN
1	0.59340	0.0579
2	0.59245	0.2179
3	0.58999	0.6322
4	0.59360	0.0242
5	0.59310	0.1085
6	0.59200	0.2937
7	0.59430	0.0936
8	0.58900	0.7990
9	0.59550	0.2958
10	0.59995	1.0452
11	0.59270	0.1758
12	0.59240	0.2264
13	0.59424	0.0835
14	0.59337	0.0630
15	0.59200	0.2937
16	0.59444	0.1172
17	0.59479	0.1762
18	0.59812	0.7370
19	0.59361	0.0226
20	0.59592	0.3665

Total weight of 20 tablets = 11.87488

Average weight = $\frac{11.87488}{20}$ = 0.593744

4.2 IDENTIFICATION TEST

The samples comply with identification test for paracetamol tablets as contained in B.P. 1993 method.

4.3 CHEMICAL ASSAY

Table 4.2 shows the result of Chemical Assay of the brand of paracetamol used. The sample investigated has passed the test since the mean percentage content is 99.36 which complied with B. P. (1993) specification of drug content (95.00 - 105.00%).

TABLE 4.2: Results of the Chemical Assay for the Sample of Paracetamol Tablets.

Trail	Percentage content	Content per tablet (mg)	Remarks
1	98.53	492.65	Pass
2	99.00	495.00	Pass
3	101.20	506.00	Pass
4	99.32	496.50	Pass
5	98.84	494.20	Pass
Mean ± S.E.M.	99.38 ± 0.473	496.87 ± 2.366	Pass

S.E.M. Standard Error of mean

4.4 DISINTEGRATION TEST

The Disintegration time for the six tablets of paracetamol investigated is shown in table 4.3, All the tablets disintegrated before 15^{00} minutes which complied with B.P. (1993) specification.

TABLE 4.3: Results of Disintegration test for the six tablets of the Brand of Paracetamol investigated

Tablet No	Disintegration time (Minutes)	Remark
1	7.00	Pass
2	5.50	Pass
3.	6.00	Pass
4.	6.50	Pass
5.	5.00	Pass
6.	7.50	Pass
Mean ± S.E.M.	6.25 ± 0.382	Pass

S.E.M. = Standard error of mean

4.5 DISSOLUTION TEST

The mean percentage dissolution in 0.1M HCl after 45 minutes for the six tablets of paracetamol tried is shown in table 4.4. The mean percentage dissolution is shown to be 85.9 . This complied with B. P. (1993) specification which states that for a tablet to pass the dissolution test, the amount of the active ingredient released after 45 mins. is not less than 70%.

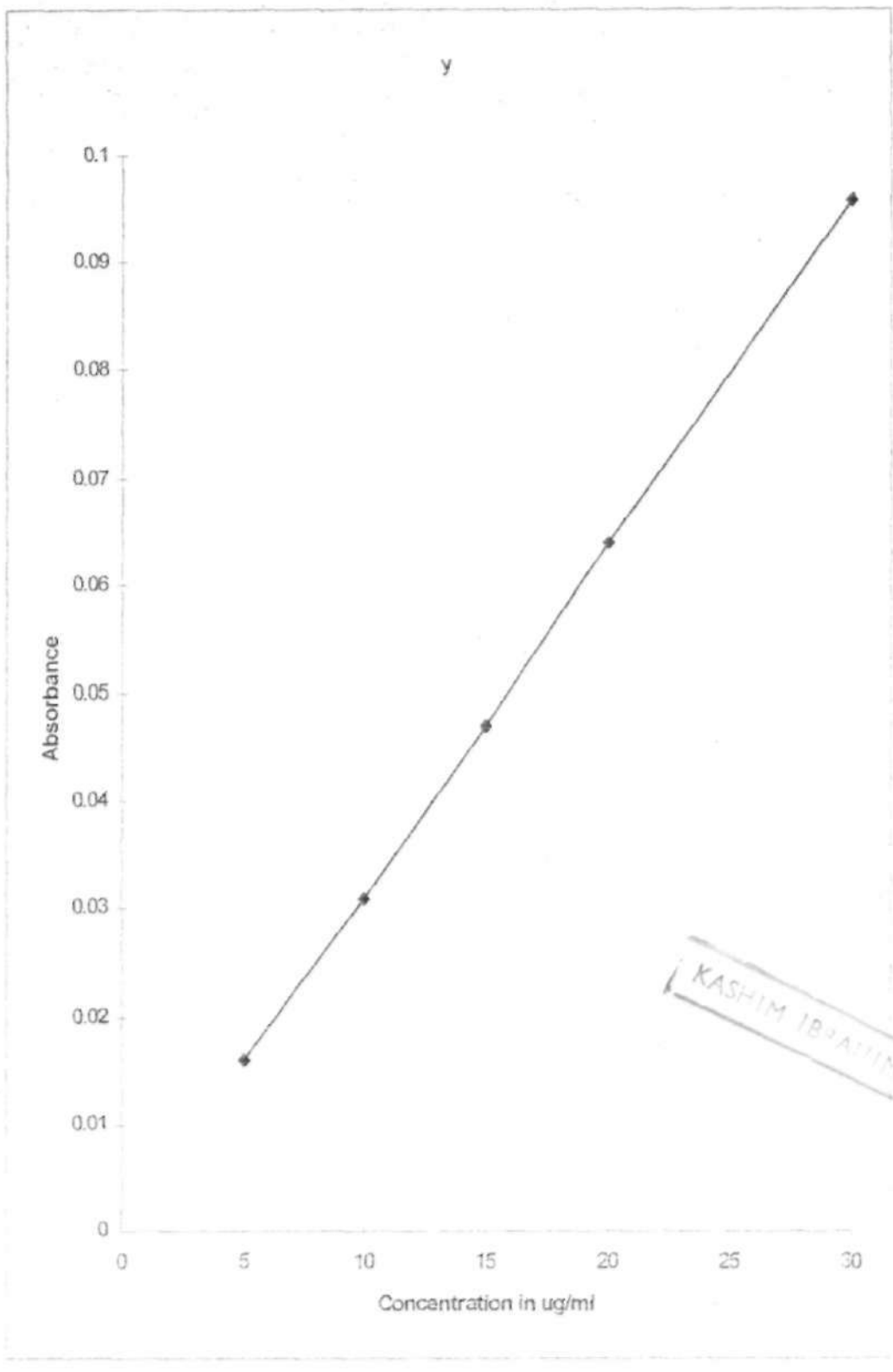
TABLE 4.4: *Results of Dissolution test for six tablets of Paracetamol investigated.*

Tablet No	Conc. mg/L dissolved	% dissolved after 45mins	Amount dissolved after 45 mins (mg)	Remarks
1	446.15	89.23	446.15	Pass
2	421.20	84.24	421.20	Pass
3.	430.05	86.01	430.05	Pass
4.	429.10	85.82	429.10	Pass
5.	436.25	87.25	436.25	Pass
6	415.30	83.06	415.30	Pass
Mean ± S.E.M		85.935 ± 0.890	429.675 ± 4.448	Pass

S.E.M. Standard error of Mean.

4.6 CALIBRATION CURVE

The calibration curve of paracetamol in serum using spectrophotometer is shown in figure 3. It is linear with correlation coefficient (r) of 1.0. Slope = 0.0032189.



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Fig. 3: Calibration curve for paracetamol in serum using spectrophotometer.

4.7 *IN VIVO* STUDIES

Tables 4.5 - 4.7 show the mean serum concentrations of paracetamol following administration of 1g to each volunteers for the areas studied. For Hospital 1 and 2, the mean serum paracetamol concentration ranged between 3.27 ± 0.26 to 7.38 ± 0.16 for the controls and between 3.07 ± 0.13 to 6.42 ± 0.15 for the tests. For pharmacy 1 and 2, the range is between 3.45 ± 0.23 to8.01 ± 0.13 for controls and 3.03 ± 0.15 to 7.52 ± 0.11 for the tests. For patent medicine store 1 and 2 the range is between 3.68 ± 0.11 to 7.25 ± 0.18 for controls and 2.50 ± 0.10 to 7.25 ± 0.18 for the tests.

The semilog mean serum concentration time graphs are shown in figures 2 - 7 for both control and test groups for the areas studied. All graphs show first order kinetics.

The Pharmacokinetic parameters were analysed from serum concentrations using computer programme SIMP.

The mean pharmacokinetic parameters are shown in table 4.9 - 4.11 for control and test groups for the areas studied. Using student t-test the values for tests are compared to control groups.

Table 4.12 shows the degree of significance of p-values for the pharmacokinetic parameters. P-values less than $P < 0.05$ is considered significant.

The P-values for volume of distribution (V_d) are significant in Hospital 1 and 2; Pharmacy 1 and patent medicine 1. But differences are not significant in pharmacy 2 and patent medicine 2.

For total plasma clearance (Cl), the differences are significant in all areas studied except patent medicine 2.

For maximum concentration (C_o), the differences are significant in all areas studied except in Hospital 1 and 2.

For elimination rate constant (k_e) the P-values are significant in all areas, except in pharmacy 2 and patent medicine 2.

The absorption rate constant (k_a), the P-values are less than 0.05 in all areas expect in Hospital 2 and patent medicine 2.

Lag time shows significant difference in all areas except in Pharmacy 2 and patent medicine 1.

Elimination half life ($t_{1/2}\beta$) is significantly affected in Hospital 1, Pharmacy 1 and patient medicine 2 but not in hospital 2, pharmacy 2 and patent medicine 2.

Absorption half life ($t_{1/2}\alpha$) is significantly affected in all areas except in Hospital 2 and patent medicine 2.

Area under the curve from time 0 - 6 hours (AuC_o-6) shows significant difference in Pharmacy 1, Pharmacy 2 and patent medicine store 1.

Area under the curve from 0 - infinity (AuC_o-α) is not significantly affected in all areas expect in Hospital 2 and Pharmacy 1.

Areas under concentration - time curve (AuC_o-α) were used to find relative bioavailability in the three different areas. Relative bioavailability is highest in Hospital 2 (101.61%). Table 4.8.

Table 4.5: Mean Serum Paracetamol concentrations in Healthy control and Test Groups Following Oral administration of the Tablet (1 g) for Hospitals 1 and 2

		MEAN CONCENTRATIONS μg ml ± SEM.			
TIME hrs	mins	HOSPITAL 1 Control	HOSPITAL 1 Test	HOSPITAL 2 Control	HOSPIT 2 Test
0.08	5	7.38 ± 0.16	6.38 ± 0.12	4.28 ± 0.78	6.42 ± 0.15
0.17	10	12.52 ± 0.12	11.4 ± 0.15	12.16 ± 0.22	12.16 ± 0.22
0.25	15	14.90 ± 0.11	13.3 ± 0.02	14.97 ± 0.08	14.94 ± 0.08
0.33	20	22.26 ± 0.14	20.6 ± 0.12	20.3 ± 0.11	20.47 ± 0.13
0.5	30	18.00 ± 0.18	16.9 ± 0.22	17.42 ± 0.23	17.40 ± 0.22
1	60	17.13 ± 0.19	16.33 ± 0.11	16.00 ± 0.17	15.73 ± 0.43
1.5	90	14.98 ± 0.10	13.0 ± 0.30	12.83 ± 0.09	12.83 ± 0.09
2	120	13.00 ± 0.16	11.73 ± 0.14	12.03 ± 0.11	12.67 ± 0.08
3	180	8.45 ± 0.19	8.5 ± 0.10	8.67 ± 0.13	8.67 ± 0.13
4	240	7.20 ± 0.15	6.48 ± 0.08	6.48 ± 0.08	6.43 ± 0.12
5	300	5.23 ± 0.17	4.42 ± 0.09	4.67 ± 0.34	5.0 ± 0.09
6	360	4.17 ± 0.08	3.07 ± 0.13	3.27 ± 0.26	3.43 ± 0.18

SEM = standard error of Mean.

TABLE 4.6: Mean Serum Paracetamol Concentration in Healthy Control and Test Groups Following Oral Administration of the Tablet (I g) for Pharmacy Shop I and 2

		MEAN COPNCENTRATION µg/ml ± SEM.			
TIME		PHARMACY SHOP I	PHARMACY SHOP I	PHARMACY SHOP 2	PHARMACY SHOP 2
hrs.	Mins.	CONTROL	TEST	CONTROL	TEST
0.08	5	7.13 ± 0.18	6.25 ± 0.09	8.01 ± 0.13	7.52 ± 0.11
0.17	10	12.3 ± 0.24	12.6 ± 0.05	12.73 ± 0.11	12.33 ± 0.11
0.25	15	14.88 ± 0.19	12.6 ± 0.13	15.6 ± 0.54	14.4 ± 0.10
0.33	20	21.47 ± 0.44	19.10 ± 0.13	22.63 ± 0.54	22.27 ± 0.11
0.5	30	17.47 ± 0.38	14.4 ± 0.11	19.03 ± 0.11	15.07 ± 0.11
1	60	16.40 ± 0.29	15.56 ± 0.11	17.33 ± 0.16	16.03 ± 0.11
1.5	90	14.95 ± 037	12.73 ± 0.11	15.83 ± 0.11	14.80 ± 0.13
2	120	12.13 ± 0.30	11.06 ± 0.21	14.53 ± 0.17	12.93 ± 0.18
3	180	8.7 ± 0.14	8.03 ± 0.12	9.70 ± 0.16	8.63 ± 0.15
4	240	6.81 ± 0.18	6.4 ± 0.06	7.9 ± 0.11	7.17 ± 0.17
5	300	4.59 ± 0.44	3.73 ± 0.14	6.53 ± 0.08	6.10 ± 0.10
6	360	3.45 ± 0.23	3.03 ± 0.15	5.53 ± 0.14	4.67 ± 0.13

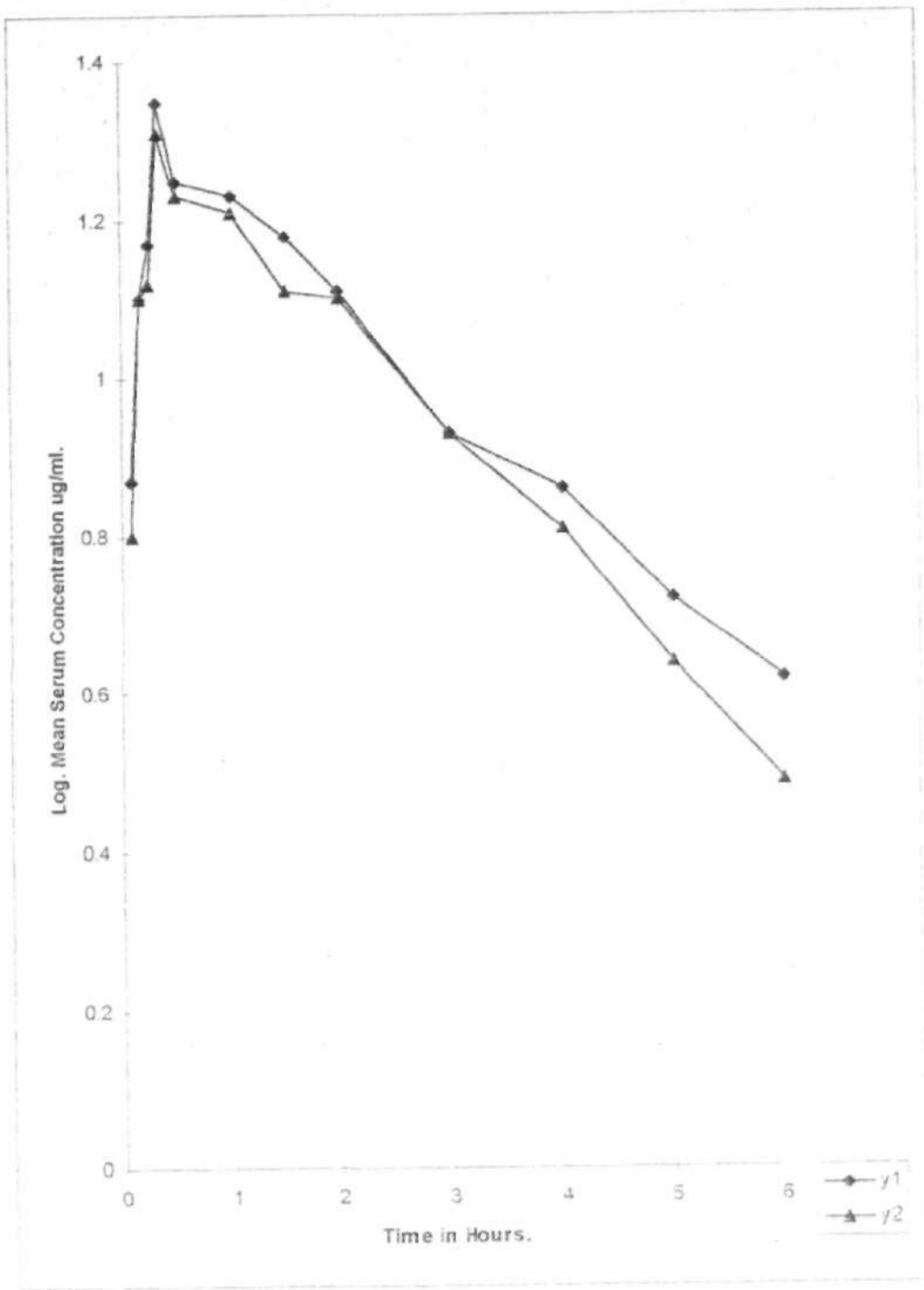
SEM = standard Error of Mean

TABLE 4.7: Mean Serum Paracetamol concentrations in Healthy control and Test Groups following Oral Administration of the Tablet (I g) for Patent Medicine Store I and 2.

		MEAN CONCENTRATION µg/ml ± SEM.			
TIME		PATENT MED. STORE I	PATENT MED. STORE I	PATENT MED. STORE 2	PATENT MED. STORE 2
hrs	mins	CONTROL	TEST	CONTROL	TEST
0.08	5	7.03 ± 0.15	5.41 ± 0.14	7.25 ± 0.18	7.25 ± 0.18
0.17	10	12.83 ± 0.09	9.46 ± 0.09	12.50 ± 0.13	12.53 ± 0.16
0.25	15	14.87 ± 0.06	12.18 ± 0.07	14.57 ± 0.20	14.48 ± 0.21
0.33	20	22.15 ± 0.15	18.82 ± 0.14	22.13 ± 0.12	22.20 ± 0.08
0.5	30	17.97 ± 0.09	15.27 ± 0.15	18.33 ± 0.11	18.23 ± 0.14
1	60	16.8 ± 0.15	13.5 ± 0.44	16.20 ± 0.07	16.20 ± 0.07
1.5	90	15.8 ± 0.13	11.9 ± 0.21	15.07 ± 0.15	15.10 ± 0.13
2	120	13.67 ± 0.26	10.88 ± 0.33	12.73 ± 0.11	12.8 ± 0.33
3	180	8.53 ± 0.08	6.05 ± 0.14	8.17 ± 0.09	8.08 ± 0.08
4	240	7.52 ± 0.11	8.33 ± 0.15	7.22 ± 0.20	7.15 ± 0.21
5	300	6.52 ± 0.13	4.67 ± 0.18	5.70 ± 0.11	5.7 ± 0.11
6	360	3.68 ± 0.11	2.50 ± 0.10	4.55 ± 0.20	4.37 ± 0.08

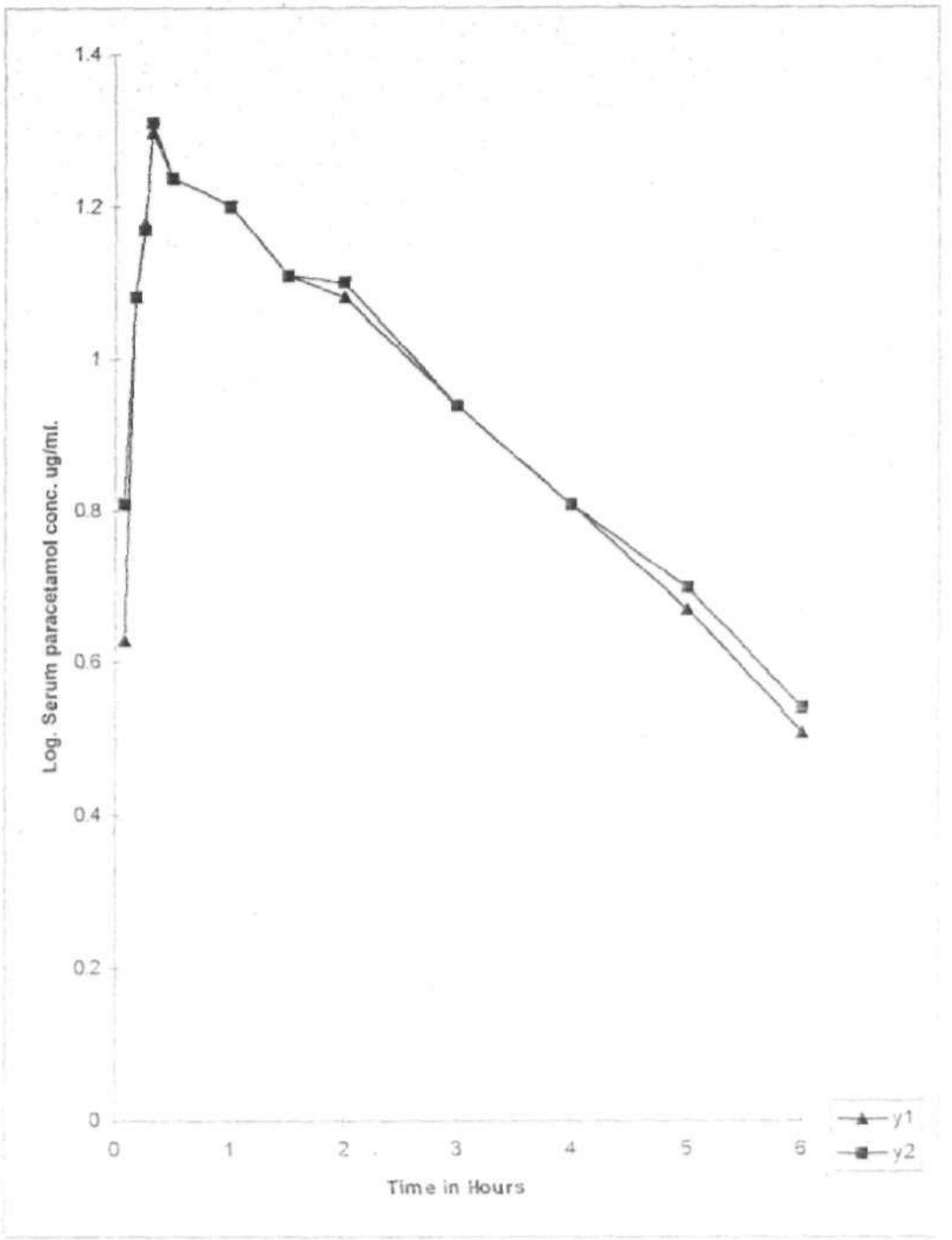
TABLE 4. 8: The Relative Bioavailability of Paracetamol kept in the three Different Drug keeping areas

Drug keeping area	AuCo-α µg /ml h	Relative bioavailability %
Hospital 2	67.2019	101.61
Hospital 1	64.3316	87.64
Pharmacy 1	61.5627	88.93
Pharmacy 2	76.2063	87.62
Patent Med Store 1	47.6366	76.46
Patent Med Store 2	74.3505	98.80



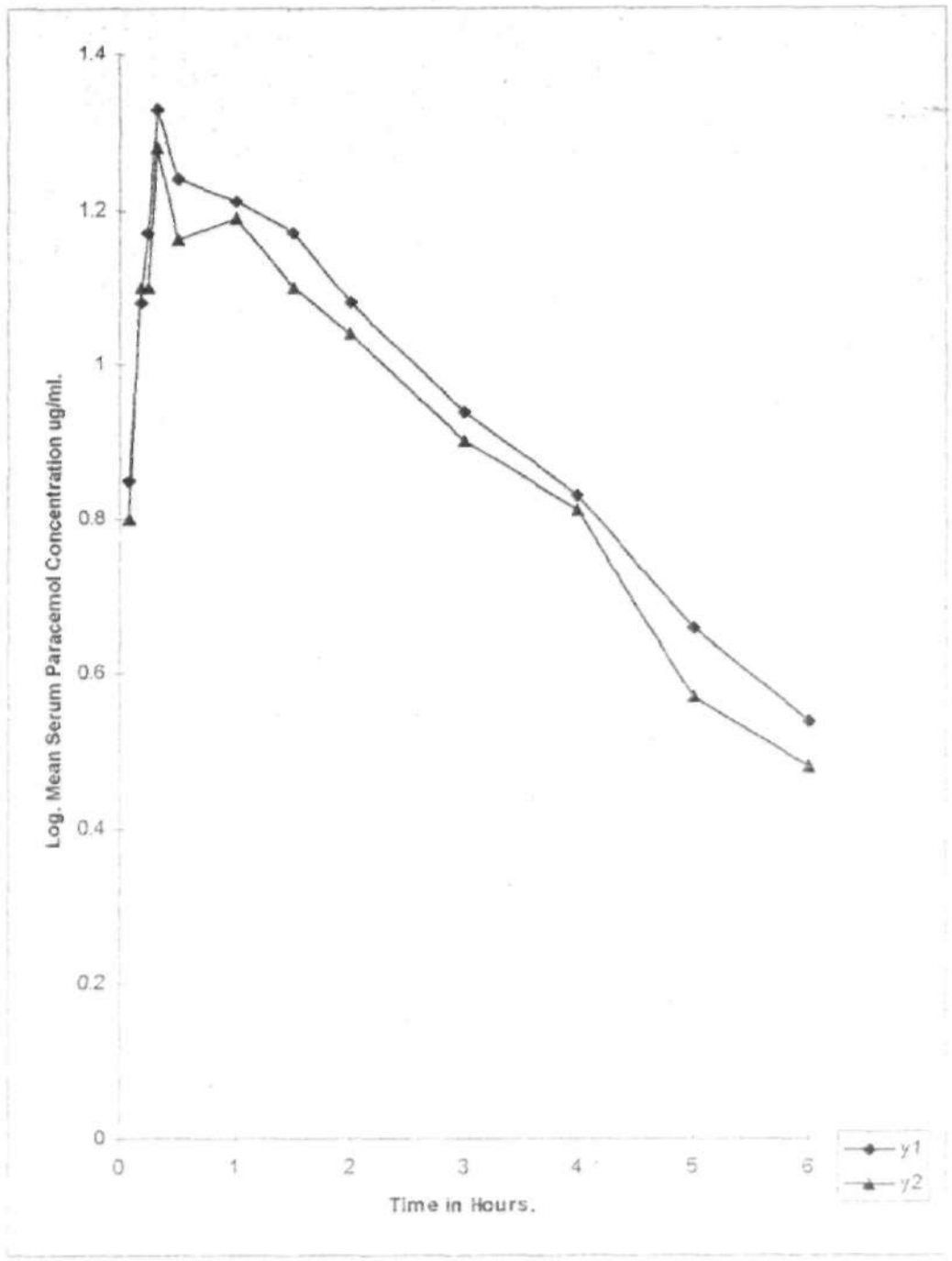
KEY: y1 = Control, y2 = Test.

Fig. 4: Semi-Log Mean Serum Paracetamol Concentraion-time Graph for Hospital I Control and Test after ingestion of IG.



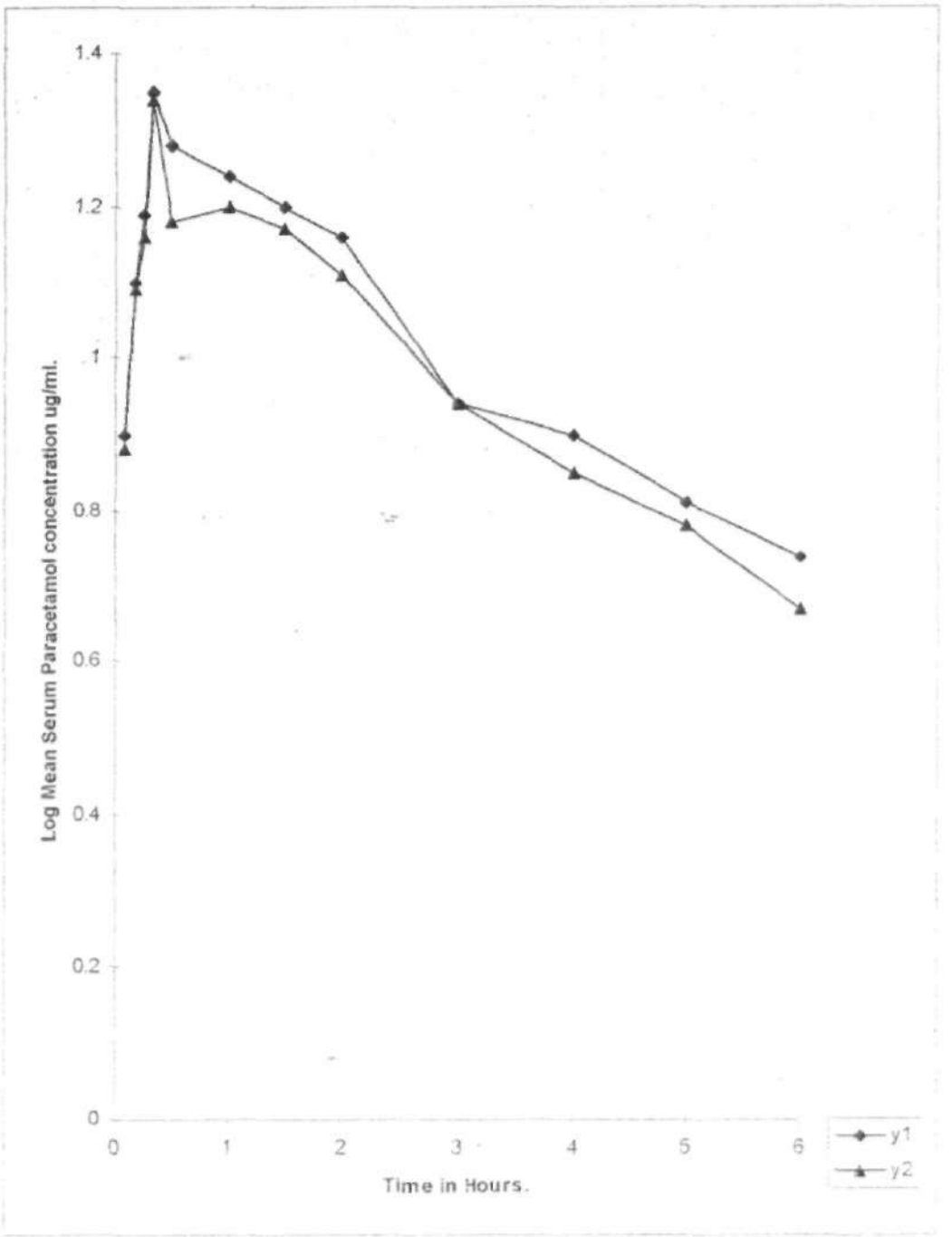
KEY: y1 = Control, y2 = Test.

Fig. 5: Semi-log Mean Serum Paracetamol Concentration -time Graph for Hospital 2 Control and Test After Ingestion of IG



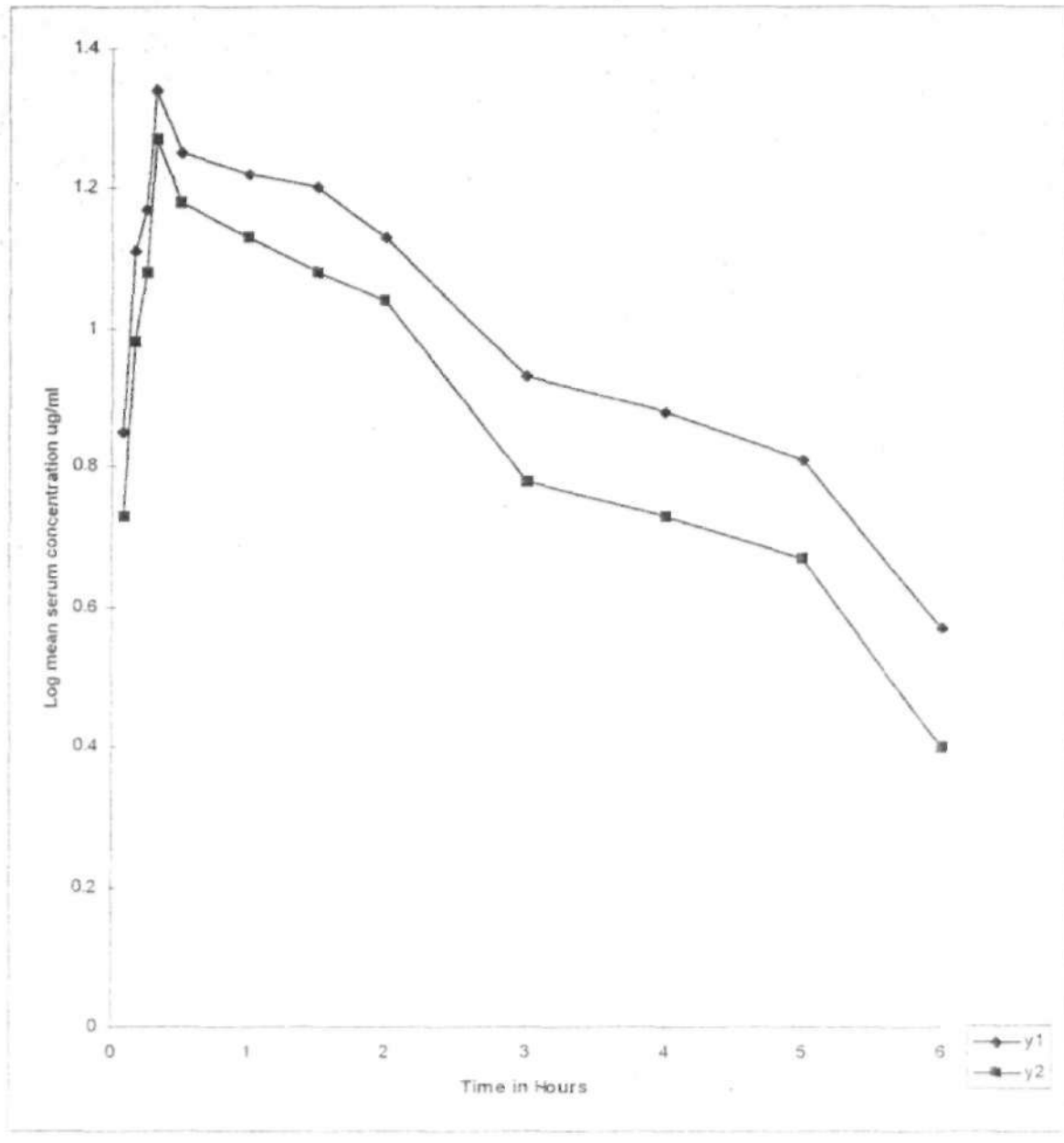
KEY: y1 = Control, y2 = Test

Fig. 6: Semi-Log Mean Serum Paracetamol Concentration-time Graph for Pharmacy Shop I Control and Test after Ingestion of IG.



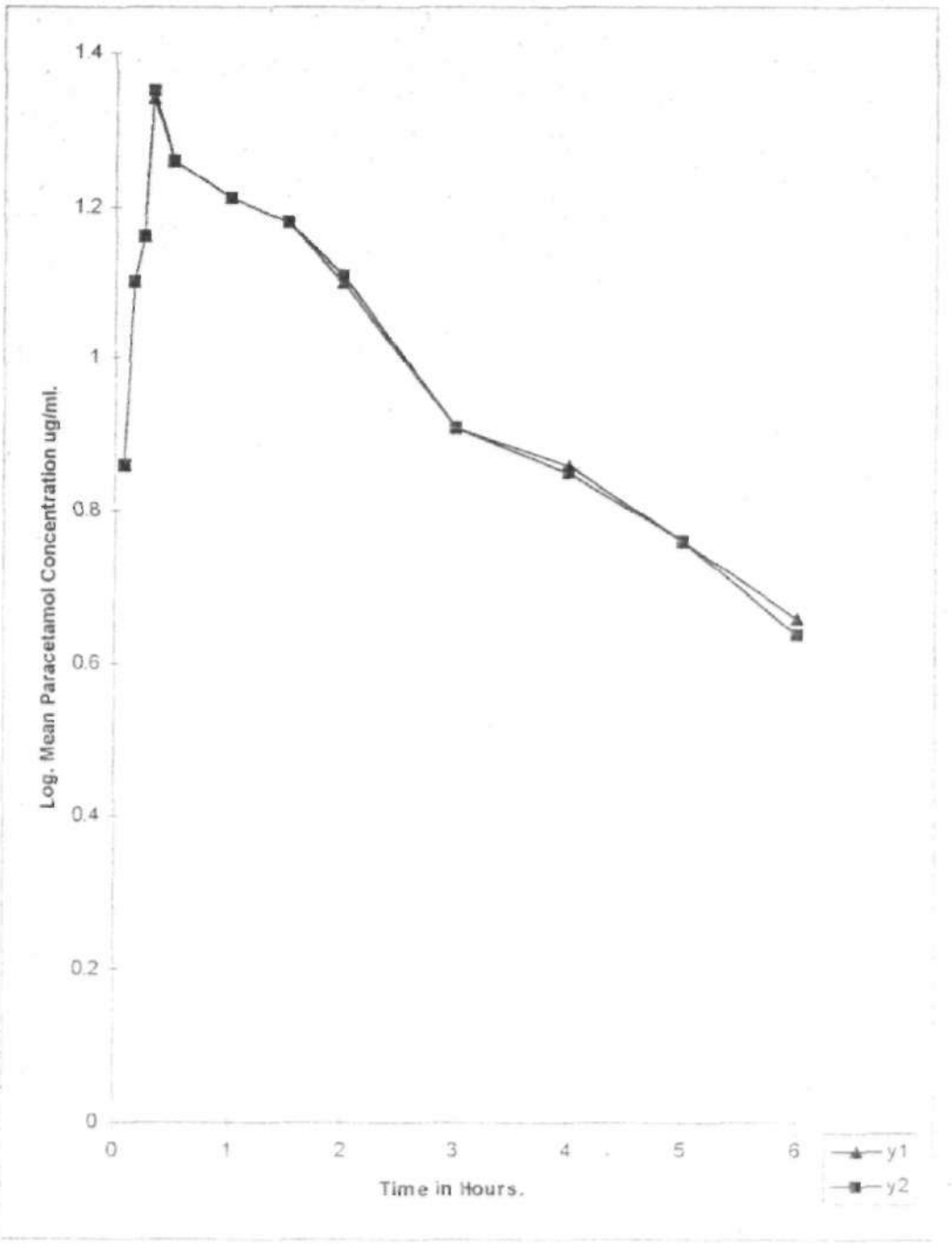
KEY: y1 = Control, y2 = Test

Fig. 7: Semi-Log Mean Serum Paracetamol Concentration-time Graph for Pharmacy Shop 2 Control and Test after Ingestion of IG.



KEY: y1 = Control, y2 = Test

Fig. 8. Semi-Log Mean Serum Paracetamol Concentration-time Graph for Patient Medicine 1 Control and Test after Ingestion of IG.



KEY: y1 = Control, y2 = Test

Fig. 9: Semi - log Mean Serum Paracetamol Concentration-time Graph for Patent Medicine Store 2 Control and Test after Ingestion of IG.

TABLE 4.9
MEAN PHARMACOKINETIC PARAMETERS FOR HOSPITAL 1 AND 2 CONTROL AND TEST GROUPS

Pharmacokinetic Parameters	HOSPITAL 1 CONTROL	HOSPITAL 1 TEST	HOSPITAL 2 CONTROL	HOSPITAL 2 TEST
Vd (ml)	43976.35 ± 554.3	47445.34 ± 290.7	48163.84 ± 486.6	48809.82 ± 824.0
Cl (ml/h)	13627.96 ± 83.4	15549.83 ± 129.7	15168.65 ± 394.6	14897.22 ± 224.4
Co (µg/ml)	23.70 ± 0.2	22.35 ± 0.3	21.70 ± 0.3	21.54 ± 0.3
Ke (µg/ml/h)	0.31 ± 0.0	0.33 ± 0.0	0.27 ± 0.0	0.31 ± 0.0
Ka (µg/ml/h)	6.18 ± 0.6	6.46 ± 0.3	8.18 ± 0.3	7.95 ± 0.2
Lag time (h)	0.03 ± 0.0	0.03 ± 0.0	0.05 ± 0.0	0.04 ± 0.0
t ½ β (h)	2.24 ± 0.0	2.12 ± 0.0	2.21 ± 0.1	2.25 ± 0.0
t ½ α (h)	0.12 ± 0.0	0.11 ± 0.0	0.09 ± 0.0	0.09 ± 0.0
AuCo-6 (µg/ml,h)	59.94 ± 0.0	54.95 ± 0.3	55.71 ± 0.5	56.03 ± 0.5
AuCo-α(µg/ml,h)	73.39 ± 0.4	64.33 ± 0.5	66.14 ± 1.7	67.20 ± 1.0
T_{max} (hr)	0.33	0.33	0.33	0.33

TABLE 4.10
MEAN PHARMACOKINETIC PARAMETERS FOR PHARMACY SHOP 1 AND 2 CONTROL AND TEST GROUPS

Pharmacokinetic Parameters	PHARMACY SHOP 1 CONTROL	PHARMACY SHOP 1 TEST	PHARMACY SHOP 2 CONTROL	PHARMACY SHOP 2 TEST
Vd (ml)	46173.23 ± 1077.4	49636.96 ± 325.2	42990.93 ± 413.0	45727.55 ± 306.4
Cl (ml/h)	14474.18 ± 287.5	16254.13 ± 184.5	11505.12 ± 120.1	13139.16 ± 212.0
Co (µg/ml)	22.96 ± 0.6	21.25 ± 0.2	23.57 ± 1.7	22.42 ± 0.1
Ke (µg/ml/h)	0.31 ± 0.0	0.33 ± 0.0	0.27 ± 0.0	0.29 ± 0.
Ka (µg/ml/h)	6.82 ± 0.2	6.47 ± 0.1	7.01 ± 0.2	7.49 ± 0.1
Lag time (h)	0.03 ± 0.0	0.03 ± 0.0	0.03 ± 0.0	0.03 ± 0.0
t ½ β (h)	2.21 ± 0.0	2.12 ± 0.0	2.59 ± 0.0	2.41 ± 0.00
t ½ α (h)	0.10 ± 0.0	0.11 ± 0.0	0.10 ± 0.0	0.09 ± 0.00
AuCo-6 (µg/ml,h)	58.14 ± 0.8	52.26 ± 0.4	66.27 ± 0.3	60 6 ± 0.70
AuCo-α(µg/ml,h)	69.22 ± 1.4	61.56 ± 0.7	86.97 ± 0.9	76.21 ± 1.2
T_{max} (hr)	0.33	0.33	0.33	0.33

TABLE 4.11
MEAN PHARMACOKINETIC PARAMETERS FOR PATENT MED STORE 1 AND 2 CONTROL AND TEST GROUPS

Pharmacokinetic Parameters	PATENT MED. STORE 1 CONTROL	PATENT MED. STORE 1 TEST	PATENT MED. STORE 2 CONTROL	PATENT MED. STORE 2 TEST
Vd (ml)	46235.35 ± 190.5	53291.28 ± 944.4	44640.68 ± 381.8	44957.76 ± 320.7
Cl (ml/h)	13321.16 ± 125.8	18222.95 ± 321.3	13292.96 ± 115.6	13453.02 ± 92.9
C_o (µg/ml)	23.13 ± 0.1	19.91 ± 0.5	22.81 ± 0.1	22.80 ± 0.1
K_e (µg/ml/h)	0.29 ± 0.0	0.34 ± 0.0	0.30 ± 0.0	0.30 ± 0.0
K_a (µg/ml/h)	7.04 ± 0.1	6.75 ± 0.2	7.18 ± 0.1	7.14 ± 0.1
Lag time (h)	0.031 ± 0.0	0.04 ± 0.0	0.17 ± 0.1	0.07 ± 0.0
$t_{½}\beta$ (h)	2.41 ± 0.02	2.03 ± 0.0	2.33 ± 0.0	2.32 ± 0.0
$t_{½}\alpha$ (h)	0.10 ± 0.0	0.10 ± 0.0	0.10 ± 0.0	0.10 ± 0.0
AuCo-α (µg/ml,h)	62.30 ± 0.4	47.64 ± 0.9	59.97 ± 0.3	59.75 ± 0.3
AuCo-α(µg/ml,h)	75.10 ± 0.71	54.96 ± 0.9	75.26 ± 0.7	74.35 ± 0.5
T_{max} (h)	0.33	0.33	0.33	0.33

TABLE 4.12
PHARMACOKINETICS OF THE TEST GROUPS AND THEIR 'P' VALUES IN RELATION TO 0.05 AS COMPARED TO THE CONTROL GROUPS.

Pharmacokinetic Parameters	HOSPITAL 1 P 0.05	HOSPITAL 2 P 0.05	PHARMACY SHOP 1 P 0.05	PHARMACY SHOP 2 P 0.05	PATENT MED. STORE 1 P 0.05	PATENT MED. STORE 2 P 0.05
Vd (ml)	47445.34 <	48809.82 <	49636.96 <	45727.55 >	53291.28 <	44957.76 >
Cl (ml/h)	15549.83 <	14897.22 <	16254.13 <	13139.16 <	18222.95 <	13453.02 >
T_{max}	0.33 >	0.33 >	0.33 >	0.33 >	0.33 >	0.33 >
C_o (µg/ml)	22.35 >	21.54 >	21.25 <	22.42 <	19.91 <	22.80 <
K_e (µg/ml/h)	0.33 <	0.31 <	0.33 <	0.29 >	0.34 <	0.30 >
K_a (µg/ml/h)	6.46 <	7.95 >	6.47 <	7.49 <	6.75 <	7.14 >
Lag time (h)	0.03 <	0.04 <	0.03 <	0.03 >	0.04 >	0.07 <
$t_{½}\ \beta$ (h)	2.12 <	2.25 >	2.12 <	2.41 >	2.03 <	2.32 >
$t_{½}\ \alpha$ (h)	0.12 <	0.09 >	0.11 <	0.09 <	0.10 <	0.10 >
AuCo-6 (µg/m,h)	54.95 >	56.03 >	52.26 <	60.63 <	47.64 <	59.75 >
AuCo-α (µg/ml,h)	64.33 >	67.20 <	61.56 <	76.21 >	54.96 >	74.35 >

CHAPTER FIVE

5.0 DISCUSSION

5.1 QUALITY CONTROL ANALYSIS

The brand of paracetamol used passed all the quality control analyses.

Results from test for uniformity of weight indicated not more than two of the individual weight (for tablets of average weight of 250mg or more) deviated from the average weight by more than 5% and none deviated by more than twice that percentage. This is within the specified limit (B.P.1993).

The brand has complied with identification and chemical assay tests. The mean percentage content is 99.21 which is within the official limit (95.00 - 105.00%)

All the tablet disintegrated before 15.0 minutes with mean disintegration time of 6.25mins.

The percentage dissolution of the tablets is 85.9 complying with official specification which states that for a tablet to pass the dissolution test, the amount of the active ingredient released after 45mins should not be less than 70%.

These various tests confirm that the brand used is of good quality.

5.2 *IN VIVO* STUDIES

The semi log mean serum concentration time graphs shown that results obtained from samples in all the areas studied fitted into first order kinetics. There is no difference as regards this characteristic in all the areas between the tests and controls. This means the rate of elimination of the drug from the body at anytime is proportional to the amount of

drug in the body at that time. The proportionality constant relating the rate and the amount is the first order elimination rate constant (Gibaldi and Perrier, 1975).

The Pharmacokinetics of paracetamol have been investigated extensively (Rawlings 1977; Clements, 1978, Bello 1990; and Thomas 1993).

They have all shown varying inter-individual differences in the plasma levels and Pharmacokinetics of paracetamol. All the pharmacokinetic parameters derived compared well with already established values.

From the values derived, there are significant differences in the maximum Plasma concentrations in the brand from the various areas studied compared to the controls ($P < 0.05$) except for Hospital 1 and 2. The maximum plasma concentrations range between 18.82 - 22-68ug ml (tables 4.9 - 4.11). These concentrations are reasonably higher than therapeutic concentration of 1 - 10 ug/ml reported by Horwitz and Jatlow, 1977. However, the time taken to attain maximum plasma levels (T_{max}) remains the same in the storage areas and is 0.33hr (20mins). Areas under the curve from zero to intinity have not shwon any statistically significant differences except in Hospital 2 and Pharmacy Shop I ($P < 0.05$) (Table 4.12). These three parameters affect the bioavailability of a particular drug. Peak plasma concentration (Co) and the time to attain peak plasma concentration (Tmax) determine rate of absorption while area under the curve, Auc determines extent of drug absorption. These two combine, will tell us the extent and rate of drug availability in systemic circulation. It could be said that in most storage areas studied except for Hospital 2 and Pharmacy, 1, the bioavailability is not greatly affected. This is based on the following reasons. One, the differences in plasma peak concentration range is small 18.82 - 22.63 ug/ml and since this is above therapeutic concentration the difference is negligible.

Secondly, the time to attain maximum plasma levels remains the same in all the areas. These therefore suggest rate of drug availability to be the same. Thirdly Areas under the curve to infinity have been shown not be statistically different except for Hospital 2 and Pharmacy shop 1. This means extent of drug availability in systemic circulation is similar.

The relative bioavailabilities (Table 4.8) determined showed bioequivalent in all the areas studied, since the difference in their relative bioavailabilities satisfied the standard requirement of not more than 25% (Wagner, 1975).

The lag time is significantly affected in most areas studied where the P-values are less than 0.05, except Pharmacy shop 2 and patent medicine 2. The range is between 0.0259 ± 0.00186 - 0.1730 ± 0.064(h) for controls and between 0.0263 ± 0.00176 - 0.0701 ± 0.038h for the tests. The statistical difference could be due to storage conditions. In those with higher lag time than the controls, it could be due to hardness of the tablets since some of these areas lack functional ceiling fan , air condition and the ventilation was generally poor. Also there were cases of these areas being exposed to sun rays. These factors could increase the tablets hardness caused by constant exposure as these tablets are dispensed. For those with reduced lag times there are cases of cracks in the ceilings, and zincs and walls which allow rain water into the premises. This could affect moisture contents making the drug dissolution faster when the tablets were administered reducing lag time. Individual variations are another possible reasons.

These facts could affect absorption rate constants, and absorption half Lives ($t_{½}$ α). The absorption half lives range between 0.0852h to 0.1195h for the controls and between 0.0875h - 0.1085h for the tests. The absorption rate constants range between

6.180 ug/ml/h - 8.184 ug/ml/h for controls and between 6.459 - 7.138 ug/ml/h for the test samples. Absorption half life, absorbtion rate constants and lag time affect onset of action. The on set is quick with reduced values and delayed with increased values.

Another factor that could affect paracetamol absorption is gastric emptying as may be due to individual variations. Gastric emptying is an important rate - limiting step in the absorption of paracetamol (Clements et al 1978). The degree of intersubject variability depend on the type of drug and may differ from group of subjects to another depending on age and other factors (Koup and Gabaldi, 1980).

For total body clearance (Cl), there are statistically significant differences in all storage areas studied except for patent medicine store 2. In these areas the test values are greater than control values meaning more volumes of plasma were cleared of the drug per unit time and reduced incidence of toxicity. Using plasma levels, Rawlings *et al*, 1977 reported total body clearance to be 23040ml h.It is not clear how the storage conditions could affect total body clearance but it is obvious that the physiological determinants of drug clearance are organ blood flow, the inherent ability of the organ to extract the drug from the blood and the extent of binding of the drugs to plasma proteins. These factors could vary from individuals to individuals.

The elimination half lives are generally lower than controls even though the low levels are not statistically significant in some area studied. This is in agreement with the definition of elimination half life, which is inversely proportional to clearance. The values compared with that obtained by Rawlings *et al*., 1977) of 2.4h.

Elimination rate constants follow the same pattern as all the test values are higher than the control values. This is expected since clearance is directly proportional to

elimination rate constants. All these parameters affect the dosing intervals.

Volume of distribution are generally higher in tests compared to controls. It is directly proportional to total body clearance and this has been shown in this study. The higher values are expected since areas under the curve are generally low compared to the control groups. This is because apparent volume of distribution is inversely proportional to Area under the curve.

5.3 CONCLUSION AND RECOMMENDATION.

The brand of paracetamol tablets used have passed the official tests for identification percentage content, weight variations, disintegration and dissolution tests according to B.P. 1993.

The results obtained from this study after storing the tablets for two months showed that samples from all the storage areas fitted into first order kinetics,.

The study revealed statistically significant differences in peak Plasma concentrations in most areas studied compared to the controls. These differences are not therapeutically significant, since the maximum concentrations in all areas are above therapeutic concentration. This shows that there are enough concentrations to elicit pharmacological actions. These differences could only be significant therapeutically in cases of drugs with low plasma concentrations as low as nanogram/ml and drugs with low therapeutic indices.

The results have also shown that the storage conditions have not affected bioavailability and bioequivalence.

The sub-optimum conditions have led to variable kinetics of other parameters such as volume of distribution, clearance, absorption and elimination rate constants, lag time, elimination and absorption half lives of paracetamol. These differences could also be due to individual variations as shown in other studies.

It is therefore recommended that such studies should be extended to other preparations such as liquid preparations and injectables. The study could be modified to include microbial identification and counts. This will improve the determination of suitability of pharmaceutical preparations as they are kept for patients to use.

The pharmaceutical inspectors should intensify effort in ensuring standard storage conditions and this should be taken as important as premises registration. This can effectively be done through the support of the government by post registration monitoring.

The high ratio of patent medicine stores to pharmaceutical chemists is of serious concern (as high as 40). The percentage of sub-optimum storage conditions is higher in these areas. Apart from this, they also serve as depot for expired, adulterated and fake drugs. No body knows the level of this. Government should intensity action to arrest the situation.

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APPENDICES

HOSPITAL I CONTROL GROUP (H I C)

Time mins.	Concentrations µg/ml						
	1	2	3	4	5	6	SD
5	7.6	7.20	6.80	7.60	7.90	7.20	0.39
10	12.40	12.20	12.80	12.40	13.00	12.40	0.30
15	14.80	15.00	15.20	14.40	15.00	15.00	0.28
20	22.40	22.10	22.40	21.80	22.80	22.10	0.34
30	18.40	17.80	18.60	18.00	17.80	17.40	0.44
60	17.60	17.20	16.40	16.80	17.60	17.20	0.47
90	14.81	15.20	14.70	14.80	15.20	15.20	0.24
120	12.40	13.00	12.80	13.40	13.00	13.40	0.38
180	8.40	8.20	9.20	8.80	7.90	8.20	0.47
240	7.20	7.20	6.80	7.60	6.80	7.60	0.36
300	4.80	5.40	5.70	5.00	4.80	5.70	0.42
360	4.40	4.10	3.90	4.10	4.10	4.40	0.20

1 --------- 6 ⇒ indicates number of Volunteer

HOSPITAL I TEST GROUP (H I T)

Time mins.	Concentrations µg/ml						
	1	2	3	4	5	6	SD
5	6.30	6.60	6.80	6.00	6.60	6.30	0.29
10	11.20	12.00	11.00	11.20	11.60	11.40	0.36
15	14.80	13.00	13.40	13.80	13.40	11.60	1.05
20	20.20	20.40	20.80	20.20	21.20	20.80	0.29
30	16.40	16.80	16.80	17.20	17.80	16.40	0.53
60	16.20	16.00	16.40	16.80	16.40	16.20	0.27
90	11.80	13.80	13.00	12.80	13.00	13.80	0.74
120	11.40	12.00	11.20	12.00	12.00	11.80	0.35
180	8.40	8.80	8.20	8.40	8.80	8.40	0.24
240	6.60	6.30	6.60	6.30	6.80	6.30	0.21
300	4.40	4.10	4.40	4.80	4.40	4.40	0.22
360	3.60	3.20	2.80	3.20	2.80	2.80	0.33

PHARMACY SHOP I CONTROL GROUP (PHIC)

Time mins.	Concentrations µg/ml						
	1	2	3	4	5	6	SD
5	6.60	6.60	7.60	7.20	7.60	7.20	0.45
10	11.80	11.20	12.00	12.20	12.00	13.00	0.59
15	14.80	14.00	15.20	14.90	15.20	15.20	0.47
20	20.00	20.20	22.10	21.90	22.10	22.50	1.08
30	16.20	16.40	18.00	18.40	18.00	17.80	0.93
60	16.00	15.20	16.40	16.80	16.80	17.20	0.72
90	13.40	14.80	15.20	14.90	15.20	16.20	0.91
120	11.80	12.20	13.00	11.40	11.40	13.00	0.73
180	8.20	8.40	8.80	8.80	8.80	9.20	0.33
240	6.30	6.60	6.80	7.60	6.80	6.80	0.43
300	5.00	5.40	6.00	5.70	5.40	3.00	1.07
360	3.20	3.60	3.20	4.20	3.90	2.60	0.57

PHARMACY SHOP I TEST GROUP (PHIT).

Time mins.	Concentrations µg/ml						
	1	2	3	4	5	6	SD
5	6.00	6.30	6.00	6.30	6.60	6.30	0.23
10	12.00	12.00	12.20	12.00	11.80	12.00	0.13
15	12.40	12.80	13.00	12.80	12.20	12.40	0.31
20	19.20	19.00	19.60	19.20	18.60	19.00	0.33
30	16.20	16.40	16.80	16.20	16.00	16.40	0.27
60	15.40	15.60	16.00	15.60	15.20	15.60	0.27
90	12.80	12.80	12.40	13.00	12.40	13.00	0.27
120	11.00	11.20	11.20	11.80	10.70	11.00	0.52
180	7.90	8.20	7.90	8.40	7.60	8.20	0.29
240	6.30	6.60	6.30	6.60	6.30	6.30	0.15
300	3.60	3.20	3.60	3.90	4.20	3.90	0.34
360	2.80	2.60	2.80	3.20	3.60	3.20	0.37

HOSPITAL II CONTROL GROUP (H II C).

Time mins.	Concentrations µg/ml						
	I	2	3	4	5	6	SD
5	3.0	6.30	6.80	6.60	3.00	6.80	1.9
10	11.20	12.40	12.80	12.40	12.20	12.00	0.54
15	14.90	15.20	14.80	15.20	14.90	14.80	0.19
20	20.20	20.40	20.20	20.80	20.20	20.00	0.28
30	16.80	17.20	17.80	17.80	18.10	16.80	0.56
60	16.20	16.40	16.20	16.00	16.00	15.20	0.42
90	13.00	12.80	13.00	13.00	12.40	12.80	0.23
120	12.00	12.00	12.20	12.40	12.00	11.60	0.26
180	9.20	8.40	8.80	8.80	8.40	8.40	0.33
240	6.30	6.60	6.80	6.30	6.30	6.60	0.21
300	4.80	5.00	5.40	3.00	4.80	5.00	0.84
360	3.90	3.90	3.60	2.20	2.80	3.20	0.67

HOSPITAL II TEST GROUP (H II T)

Time mins.	Concentrations µg/ml						
	1	2	3	4	5	6	SD
5	6.00	6.30	6.80	6.60	6.00	6.80	0.37
10	11.20	12.40	12.80	12.40	12.20	12.00	0.54
15	14.80	15.20	14.80	15.20	14.81	14.80	0.20
20	20.20	20.40	20.20	20.80	21.20	20.00	0.45
30	16.80	17.20	17.80	17.80	18.00	16.80	0.54
60	16.20	16.40	16.20	16.00	16.00	13.60	1.05
90	13.00	12.80	13.00	13.00	12.40	12.80	0.23
120	12.00	12.00	12.20	12.40	12.00	11.80	0.21
180	9.20	8.40	8.80	8.80	8.40	8.40	0.33
240	6.00	6.60	6.80	6.30	6.30	6.60	0.29
300	4.80	5.00	5.40	5.00	4.80	5.00	0.22
360	3.90	3.90	3.60	3.20	2.80	3.20	0.44

PHARMACY SHOP II CONTROL GROUP (PHIIC)

Time mins.	Concentrations µg/ml						
	1	2	3	4	5	6	SD
5	8.40	7.90	8.40	7.60	7.90	7.90	0.32
10	13.00	12.80	12.40	13.00	12.80	12.40	0.27
15	15.60	15.40	15.40	15.60	16.00	15.60	0.22
20	23.20	22.80	22.60	22.80	22.60	22.80	0.22
30	18.60	19.00	19.30	19.00	19.00	19.30	0.26
60	16.80	17.20	17.80	17.20	17.20	17.80	0.39
90	15.60	15.60	16.00	15.60	16.20	16.00	0.27
120	14.80	14.80	14.00	14.80	14.00	14.80	0.41
180	9.90	9.20	9.20	10.00	10.00	9.90	0.39
240	7.90	8.20	7.60	8.20	7.60	7.90	0.27
300	6.60	6.30	6.80	6.60	6.30	6.60	0.20
360	5.00	5.40	5.70	6.00	5.40	5.70	0.34

PHARMACY SHOP II TEST GROUP (PHIIT)

Time mins.	Concentrations µg/ml						
	I	2	3	4	5	6	SD
5	7.90	7.60	7.60	7.20	7.60	7.20	0.27
10	12.80	12.40	12.20	12.00	12.20	12.40	0.27
15	15.40	14.80	14.80	14.80	14.80	14.80	0.24
20	22.80	22.10	22.20	22.20	22.10	22.20	0.27
30	18.40	18.00	17.80	17.80	18.40	18.00	0.27
60	16.40	16.20	16.00	15.60	16.00	16.00	0.26
90	15.40	14.80	14.60	14.4.	14.80	14.80	0.33
120	13.40	13.00	12.80	12.20	12.80	13.40	0.45
180	9.20	8.80	8.40	8.20	8.40	8.80	0.37
240	7.60	7.20	6.80	6.60	7.20	7.60	0.41
300	6.30	6.00	6.30	5.70	6.00	6.30	0.24
360	4.80	4.80	4.50	4.10	4.50	4.10	0.31

PATENT MEDICINE II CONTROL GROUP (PMIIC)

Time mins.	Concentrations µg/ml						
	1	2	3	4	5	6	
5	7.20	6.80	7.60	7.20	7.90	6.80	0.44
10	12.20	12.40	12.80	13.00	12.40	12.20	0.33
15	15.40	14.40	14.00	14.40	14.80	14.40	0.48
20	22.20	21.80	22.60	23.20	21.80	22.20	0.30
30	18.00	18.40	18.60	18.60	18.40	18.00	0.27
60	16.40	16.20	16.00	16.40	16.00	16.20	0.18
90	15.40	15.40	14.80	14.60	14.80	15.40	0.37
120	12.80	13.00	12.40	12.80	13.00	12.40	0.27
180	8.40	8.20	7.90	8.40	8.20	7.90	0.22
240	7.90	7.20	6.60	6.80	7.20	7.60	0.48
300	6.00	5.40	5.70	6.00	5.40	5.70	0.27
360	4.50	4.20	5.40	4.50	4.20	4.50	0.50

PATENT MEDICINE II TEST GROUP (PMIIT)

Time mins.	Concentrations µg/ml						
	1	2	3	4	5	6	SD
5	7.20	7.90	6.80	6.80	7.60	7.20	0.44
10	12.00	12.80	12.20	12.40	12.80	13.00	0.39
15	15.40	14.70	14.00	14.40	14.00	14.40	0.52
20	22.10	21.80	22.20	21.80	22.10	22.20	0.19
30	18.00	18.00	17.80	18.40	18.60	18.60	0.34
60	16.40	16.00	16.20	16.20	16.00	16.40	0.18
90	15.40	14.80	15.40	15.40	14.80	14.80	0.33
120	12.80	13.40	12.40	13.00	12.40	12.80	0.8
180	8.20	7.90	7.90	8.20	7.90	8.40	0.21
240	7.90	6.80	7.60	7.20	6.60	6.80	0.51
300	5.40	6.00	5.70	5.40	5.70	6.00	0.27
360	4.50	4.10	4.50	4.10	4.50	4.50	0.21

PATENT MEDICINE I CONTROL GROUP (PM I C)

Time mins.	Concentrations µg/ml						
	1	2	3	4	5	6	SD
5	7.20	6.80	6.60	7.20	6.80	7.60	0.37
10	12.40	13.00	12.80	13.00	12.80	13.00	0.23
15	14.80	14.80	14.80	15.20	14.80	14.80	0.16
20	21.80	22.20	21.80	22.10	22.20	22.80	0.37
30	18.00	17.00	18.00	18.40	17.80	17.80	0.23
60	16.80	16.40	16.80	17.20	16.40	17.20	0.36
90	15.40	15.60	15.60	16.00	16.20	16.00	0.3
120	13.40	13.00	14.80	13.40	14.00	13.40	0.64
180	8.40	8.40	8.40	8.80	8.40	8.80	0.21
240	7.60	7.20	7.90	7.60	7.20	7.60	0.27
300	6.00	6.30	6.60	6.80	6.60	6.80	0.31
360	3.90	3.60	3.90	3.20	3.60	3.90	0.28

PATENT MEDICINE I TEST GROUP (PMIT)

Time mins.	Concentrations µg/ml						
	1	2	3	4	5	6	SD
5	5.70	5.40	5.70	5.00	5.70	5.00	0.34
10	9.50	9.80	9.50	9.20	9.50	9.20	0.23
15	12.50	12.20	12.00	12.20	12.20	12.00	0.18
20	19.30	19.00	18.60	19.00	18.60	18.40	0.34
30	15.60	15.40	15.60	15.40	14.80	14.80	0.37
60	15.40	14.00	12.80	13.40	13.00	12.40	1.08
90	12.50	12.20	12.00	12.00	11.80	11.00	0.51
120	12.00	11.00	10.70	11.20	10.90	9.50	0.81
180	6.30	6.60	6.00	5.70	6.00	5.70	0.35
240	5.70	5.40	5.70	5.00	5.40	4.70	0.37
300	4.80	5.00	5.00	4.10	5.00	4.10	0.45
360	2.20	2.60	2.80	2.60	2.20	2.60	0.24

HOSP 1 CONTROLS

PARAMETERS	Vol. 1	Vol. 2	Vol. 3	Vol. 4	Vol. 5	Vol. 6	Mean ± SEM
Vd (ml)	42320.29	44010.36	45006.82	44783.29	42329.55	45407.80	43976.35 ± 554.30
Cl (ml/h)	13649.85	13646.24	13647.60	13477.76	13973.94	13372.40	13627.96 ± 83.42
Co (µg/ml)	24.05	23.56	23.08	23.27	24.34	23.89	23.70 ± 0.20
Ke (µg/ml/h)	0.32	0.31	0.30	0.30	0.33	0.29	0.310 ± 0.01
Ka (µg/ml/h)	6.45	6.64	7.59	6.46	6.46	3.47	6.18 ± 0.57
Lag time (h)	0.03	0.03	0.04	0.02	0.02	-0.05	0.03 ± 0.00
t ½ β (h)	2.15	2.23	2.29	2.30	2.10	2.35	2.24 ± 0.04
t ½ α (h)	0.11	0.10	0.09	0.11	0.11	0.20	0.12 ± 0.02
AuCo-6 (µg/ml,h)	59.62	60.06	60.41	60.57	59.14	59.84	59.94 ± 0.01
AuCo-α (µg/ml,h)	73.26	73.28	73.27	74.20	71.56	74.78	73.39 ± 0.45
t_{max} (h)	0.33	0.33	0.33	0.33	0.33	0.33	0.33

HOSPITAL 1 TESTS

PARAMETERS	Vol. 1	Vol. 2	Vol. 3	Vol. 4	Vol. 5	Vol. 6	Mean ± SEM
Vd (ml)	48719.38	47450.89	47333.30	46749.68	46819.23	47599.54	47445.34 ± 290.72
Cl (ml/h)	15224.81	15363.19	16000.7	15326.08	15504.91	15879.27	15549.83 ± 129.66
Co (µg/ml)	21.05	22.36	22.52	22.50	22.88	22.77	22.35 ± 0.27
Ke (µg/ml/hr)	0.31	0.32	0.34	0.33	0.33	0.33	0.33 ± 0.00
Ka (µg/ml/hr)	7.73	6.25	6.23	6.47	6.48	5.53	6.46 ± 0.30
Lag time (h)	0.04	0.03	0.03	0.04	0.03	0.02	0.03 ± 0.00
t ½ β(h)	2.22	2.14	2.05	2.11	2.09	2.08	2.12 ± 0.02
t ½ α(h)	0.09	0.11	0.11	0.11	0.11	0.13	0.11 ± 0.00
AuCo-6 (µg/ml,h)	54.16	55.21	54.21	55.49	56.04	54.58	54.95 ± 0.31
AuCo-α (µg/ml,h)	65.68	65.09	62.50	65.25	64.50	62.98	64.33 ± 0.53
t_{max} (h)	0.33	0.33	0.33	0.33	0.33	0.33	0.33

HOSPITAL 2 CONTROLS

PARAMETERS	Vol. 1	Vol. 2	Vol. 3	Vol. 4	Vol. 5	Vol. 6	Mean ± SEM
Vd. (ml)	48350.08	47379.59	48041.79	46927.48	47917.74	50366.35	48163.84 ± 486.65
Cl (ml/h)	14422.76	14436.98	14361.84	16780.00	15733.87	15276.47	15168.65 ± 394.55
Co (µg/ml)	21.13	21.48	21.65	23.24	21.83	20.87	21.70 ± 0.34
Ke (µg/ml/hr)	0.30	0.30	0.30	0.36	0.33	0.30	0.27 ± 0.04
Ka (µg/ml/hr)	8..77	8.65	7.77	7.08	8.87	7.97	8.18 ± 0.29
Lag time (h)	0.07	0.04	0.03	0.04	0.07	0.03	0.05 ± 0.00
t ½ β(h)	2.32	2.27	2.32	1.94	2.11	2.28	2.21 ± 0.06
t ½ α(h)	0.08	0.08	0.09	0.10	0.08	0.09	0.09 ± 0.00
AuCo-6 (µg/ml,h)	56.26	56.47	57.59	54.00	55.03	54.91	55.71 ± 0.33
AuCo-α (µg/ml,h)	69.33	69.27	69.63	59.59	63.56	65.46	66.14 ± 1.66
t_{max} (h)	0.33	0.33	0.33	0.33	0.33	0.33	0.33

HOSPITAL 2 TESTS

PARAMETERS	Vol. 1	Vol. 2	Vol. 3	Vol. 4	Vol. 5	Vol. 6	Mean ± SEM
Vd. (ml)	47541.35	47516.03	48106.71	47282.81	47045.88	52366.16	48309.82 ± 823.98
Cl (ml/h)	14524.65	14448.03	14357.78	14994.21	15717.12	15341.53	14897.22 ± 224.40
Co (µg/ml)	21.55	21.49	21.63	22.17	22.34	20.05	21.54 ± 0.33
Ke (µg/ml/hr)	0.31	0.30	0.30	0.32	0.33	0.30	0.31 ± 0.00
Ka (µg/ml/hr)	7.38	8.19	7.75	7.70	7.81	8.86	7.95 ± 0.21
Lag time (h)	0.04	0.04	0.03	0.04	0.04	0.04	0.04 ± 0.00
t ½ β(h)	2.27	2.28	2.32	2.19	2.07	2.37	2.25 ± 0.04
t ½ α(h)	0.09	0.08	0.09	0.09	0.09	0.08	0.09 ± 0.00
AuCo-6 (µg/ml,h)	56.08	56.39	57.59	56.60	55.24	54.26	56.03 ± 0.47
AuCo-α (µg/ml,h)	68.85	69.21	69.65	66.69	63.62	65.18	67.20 ± 1.00
t_{max} (h)	0.33	0.33	0.33	0.33	0.33	0.33	0.33

PHARMACY 1 CONTROLS

PARAMETERS	Vol. 1	Vol. 2	Vol. 3	Vol. 4	Vol. 5	Vol. 6	Mean ± SEM
Vd. (ml)	49380.49	49434.60	45738.46	44901.28	44408.12	43176.51	46173.23 ± 1077.42
Cl (ml/h)	15299.48	14501.48	14205.71	13517.85	14051.54	15268.97	14474.18 ± 287.45
Co (µg/ml)	21.33	21.38	23.44	22.87	23.34	25.42	22.96 ± 0.62
Ke (µg/ml/hr)	0.31	0.29	0.31	0.30	0.32	0.35	0.31 ± 0.00
Ka (µg/ml/hr)	7.46	6.73	6.68	7.17	6.84	6.01	6.82 ± 0.20
Lag time (h)	0.03	0.03	0.03	0.03	0.03	0.03	0.03 ± 0.00
t ½ β(h)	2.24	2.36	2.23	2.30	2.19	1.96	2.21 ± 0.57
t ½ α(h)	0.09	0.10	0.10	0.10	0.10	0.11	0.10 ± 0.00
AuCo-6(µg/ml,h)	55.03	56.69	60.09	60.03	58.84	58.14	58.13 ± 0.81
AuCo-α(µg/ml,h)	65.36	68.96	70.39	73.98	71.17	65.49	69.22 ±1.37
t_{max} (h)	0.33	0.33	0.33	0.33	0.33	0.33	0.33

PHARMACY 1 TESTS

PARAMETERS	Vol. 1	Vol. 2	Vol. 3	Vol. 4	Vol. 5	Vol. 6	Mean ± SEM
Vd. (ml)	49998.11	49698.02	48433.16	50203.27	50533.52	48955.65	49636.96 ± 325.17
Cl (ml/h)	16656.44	16723.66	16559.87	15627.42	15952.13	16005.3	16254.13 ± 184.54
Co (µg/ml)	21.24	21.65	21.81	21.08	20.29	21.42	21.25 ± 0.22
Ke (µg/ml/hr)	0.33	0.34	0.34	0.31	0.32	0.33	0.33 ± 0.00
Ka (µg/ml/hr)	6.55	6.25	6.71	6.52	6.64	6.16	6.47 ± 0.09
Lag time (h)	0.03	0.03	0.03	0.03	0.02	0.02	0.03 ± 0.00
t ½ β(h)	2.08	2.06	2.03	2.23	2.20	2.12	2.12 ± 0.03
t ½ α(h)	0.11	0.11	0.10	0.11	0.10	0.11	0.11 ± 0.00
AuCo-6 (µg/ml,h)	51.63	52.07	52.20	53.71	51.28	52.69	52.26 ± 0.35
AuCo-α (µg/ml,h)	60.04	59.80	60.39	63.99	62.69	62.48	61.56 ± 0.70
t_{max} (h)	0.33	0.33	0.33	0.33	0.33	0.33	0.33

PHARMACY2 CONTROLS

PARAMETERS	Vol. 1	Vol. 2	Vol. 3	Vol. 4	Vol. 5	Vol. 6	Mean ± SEM
Vd. (ml)	44479.37	43221.74	41692.00	43525.55	42972.26	42054.95	42990.93 ± 413.03
Cl (ml/h)	11776.53	11657.68	11590.65	10998.16	11692.63	11315.05	11505.12 ± 120.08
Co (µg/ml)	23.22	23.48	24.06	23.04	23.58	24.06	23.57 ± 1.72
Ke (µg/ml/hr)	0.26	0.27	0.28	0.25	0.27	0.27	0.27 ± 0.00
Ka (µg/ml/hr)	7.29	7.04	6.40	7.53	7.16	6.64	7.01 ± 0.17
Lag time (h)	0.02	0.03	0.02	0.03	0.03	0.03	0.03 ± 0.00
t ½ β(h)	2.62	2.57	2.49	2.74	2.55	2.58	2.59 ± 0.03
t ½ α(h)	0.10	0.10	0.11	0.09	0.10	0.10	0.10 ± 0.00
AuCo-6 (µg/ml,h)	66.03	65.76	65.77	67.18	65.68	67.19	66.27 ± 0.29
AuCo-α (µg/ml,h)	84.91	85.78	86.28	90.92	85.52	88.38	86.97 ± 0.93
t_{max} (h)	0.33	0.33	0.33	0.33	0.33	0.33	0.33

PHARMACY 2 TESTS

PARAMETERS	Vol.. 1	Vol.. 2	Vol.. 3	Vol.. 4	Vol.. 5	Vol.. 6	Mean ± SEM
Vd. (ml)	44855.64	45331.19	46031.21	46078.90	45169.56	46898.80	45727.55 ± 306.38
Cl (ml/h)	12434.04	12827.97	13237.45	13999.23	13210.74	13125.55	13139.16 ± 212.03
Co (µg/ml)	22.81	22.38	22.14	22.27	22.63	22.28	22.41 ± 0.10
Ke (µg/ml/hr)	0.28	0.28	0.29	0.30	0.29	0.28	0.29 ± 0.00
Ka (µg/ml/hr)	7.55	7.35	7.69	7.57	7.19	7.59	7.49 ± 0.08
Lag time (h)	0.03	0.03	0.04	0.03	0.03	0.03	0.03 ± 0.00
t ½ β(h)	2.50	2.45	2.41	2.28	2.37	2.48	2.41 ± 0.03
t ½ α(h)	0.09	0.09	0.09	0.09	0.10	0.09	0.09 ± 0.00
AuCo-6 (µg/ml,h)	63.11	60.99	59.90	57.94	60.31	61.54	60.63 ± 0.71
AuCo-α (µg/ml,h)	80.42	77.95	75.54	71.43	75.70	76.19	76.21 ± 1.22
t_{max} (h)	0.33	0.33	0.33	0.33	0.33	0.33	0.33

PATENT MEDICINE 1 CONTROLS

PARAMETERS	Vol.. 1	Vol.. 2	Vol.. 3	Vol.. 4	Vol.. 5	Vol.. 6	Mean ± SEM
Vd. (ml)	45612.69	46653.99	46634.82	46076.63	46629.82	45804.17	46235.35 ± 190.55
Cl (ml/h)	13392.13	13695.48	12908.52	13534.50	13402.77	12993.57	13321.16 ± 125.82
Co (µg/ml)	23.12	22.75	22.94	23.67	23.04	23.24	23.13 ± 0.13
Ke (µg/ml/hr)	0.29	0.29	0.28	0.29	0.29	0.28	0.29 ± 0.00
Ka (µg/ml/hr)	6.79	7.52	6.87	6.88	7.15	7.02	7.04 ± 0.11
Lag time (h)	0.03	0.04	0.03	0.03	0.03	0.03	0.03 ± 0.00
t ½ β(h)	2.36	2.36	2.50	2.36	2.41	2.44	2.41 ± 0.02
t ½ α(h)	0.10	0.09	0.10	0.10	0.10	0.10	0.10 ± 0.00
AuCo-6 (µg/ml,h)	61.39	60.75	63.38	62.99	62.09	63.21	62.30 ± 0.44
AuCo-α (µg/ml,h)	74.67	73.02	77.47	73.89	74.61	76.96	75.10 ± 0.71
t_{max} (h)	0.33	0.33	0.33	0.33	0.33	0.33	0.33

PATENT MEDICINE 1 TESTS

PARAMETERS	Vol.. 1	Vol.. 2	Vol.. 3	Vol.. 4	Vol.. 5	Vol.. 6	Mean ± SEM
Vd. (ml)	49808.48	53014.55	54493.52	51590.40	56177.52	54663.21	53291.28 ± 944.41
Cl (ml/h)	17621.74	17518.92	17626.20	18542.71	18499.33	19528.82	18222.95 ± 321.31
Co (µg/ml)	21.91	19.99	19.25	20.33	19.18	18.82	19.91 ± 0.46
Ke (µg/ml/hr)	0.35	0.33	0.32	0.36	0.33	0.36	0.34 ± 0.00
Ka (µg/ml/hr)	5.64	6.79	7.02	6.61	7.00	7.42	6.75 ± 0.25
Lag time (h)	0.03	0.04	0.04	0.04	0.04	0.04	0.04 ± 0.00
t ½ β(h)	1.96	2.10	2.14	1.93	2.10	1.94	2.03 ± 0.04
t ½ α(h)	0.12	0.10	0.10	0.10	0.10	0.09	0.10 ± 0.00
AuCo-6(µg/ml,h)	50.53	49.21	48.08	46.70	47.38	43.93	47.64 ± 0.93
AuCo-α (µg/m,h)	56.75	57.08	56.73	53.93	54.06	51.21	54.96
t_{max} (h)	0.33	0.33	0.33	0.33	0.33	0.33	0.33

PATENT MEDICINE 2 CONTROLS

PARAMETERS	Vol.. 1	Vol.. 2	Vol.. 3	Vol.. 4	Vol.. 5	Vol.. 6	Mean ± SEM
Vd. (ml)	45460.59	44458.96	43002.64	44395.37	45537.09	44989.44	44640.68 ± 381.83
Cl (ml/h)	12950.86	13594.82	12974.74	13313.34	13591.87	13332.12	13292.96 ± 115.57
Co (µg/ml)	22.64	23.19	22.76	22.86	22.71	22.69	22.81 ± 0.08
Ke (µg/ml/hr)	0.28	0.31	0.30	0.30	0.30	0.30	0.30 ± 0.00
Ka (µg/ml/hr)	7.43	6.85	7.15	7.33	7.08	7.23	7.18 ± 0.08
Lag time (h)	0.03	0.03	0.03	0.03	0.02	0.04	0.17 ± 0.06
t ½ β(h)	2.43	2.27	2.30	2.31	2.32	2.34	2.33 ± 0.02
t ½ α(h)	0.09	0.10	0.10	0.09	0.10	0.10	0.10 ± 0.00
AuCo-6 (µg/ml,h)	61.42	59.82	59.18	60.11	59.50	59.82	59.97 ± 0.32
AuCo-α (µg/ml,h)	77.21	73.56	77.07	75.11	73.57	75.01	75.26 ± 0.66
t_{max} (h)	0.33	0.33	0.33	0.33	0.33	0.33	0.33

PATENT MEDICINE 2 TESTS

PARAMETERS	Vol. 1	Vol. 2	Vol. 3	Vol. 4	Vol. 5	Vol. 6	Mean ± SEM
Vd. (ml)	44818.28	45747.56	46070.92	44683.64	44116.45	44309.69	44957.75 ± 320.69
Cl (ml/h)	13175.80	13644.40	13272.73	13665.17	13662.4	13297.63	13453.02 ± 92.91
Co (µg/ml)	22.86	22.64	22.31	23.21	22.89	22.90	22.80 ± 0.00
Ke (µg/ml/hr)	0.29	0.30	0.29	0.31	0.31	0.30	0.30 ± 0.00
Ka (µg/ml/hr)	7.23	7.11	7.35	6.86	6.98	7.30	7.14 ± 0.08
Lag time (h)	0.03	0.02	0.04	0.03	0.26	0.03	0.07 ± 0.04
$t_{½}$ β(h)	2.36	2.32	2.41	2.27	2.24	2.31	2.32 ± 0.03
$t_{½}$ α(h)	0.10	0.10	0.09	0.10	0.10	0.09	0.10 ± 0.00
AuCo-6 (µg/ml,h)	60.59	59.54	59.72	59.77	58.66	60.21	59.75 ± 0.27
AuCo-α (µg/ml,h)	75.90	73.29	75.34	73.18	73.19	75.20	74.35 ± 0.51
t_{max} (h)	0.33	0.33	0.33	0.33	0.33	0.33	0.33

P. VALUES FOR PHARMACOKINETIC PARAMETERS IN THE DRUG KEEPING AREAS STUDIED.

Pharmacokinetic parameters	P.05 t= 2.228	HOSPITAL 1 t-values P-values	HOSP. 2 t-value P-value	Pharmacy 1 t-value P-value	Pharmacy 2 t-value P-value	Patent Med Store 1 t-value P-value	Patent med Store 2 t-value P-value
Vd.		3.64< 0.05	2.87 < 0.05	10.98 < 0.05	1.82 > 0.05	24.56 < 0.05	1.42 > 0.05
Cl		2.42 < 0.05	3.09 < 0.05	2.43 < 0.05	3.12 < 0.05	6.52 < 0.05	1.55 > 0.05
Co		1.89 > 0.05	1.08 > 0.05	7.95 < 0.05	2.77 < 0.05	12.99 < 0.05	2.23 < 0.05
Ke		2.32 < 0.05	61.6 6< 0.05	3.04 < 0.05	1.3 > 0.05	5.54 < 0.05	1.20 > 0.05
Ka		3.57 < 0.05	1.85 > 0.05	5.02 < 0.05	5.11 < 0.05	5.14 < 0.05	1.15 > 0.05
Lag time		3.12 < 0.05	20.79 < 0.05	506.38 < 0.05	1.77 > 0.05	2.13 > 0.05	2.77 < 0.05
$t_{½}\beta$		2.71 < 0.05	2.16 > 0.05	3.10 < 0.05	1.12 > 0.05	2.68 < 0.05	1.14 > 0.05
$t_{½}\alpha$		38.54 < 0.05	2.00 > 0.05	4.43 < 0.05	6.77 < 0.05	8.01 < 0.05	1.13 > 0.05
AuCo-6		2.05 > 0.05	1.27 > 0.05	5.33 < 0.05	5.78 < 0.05	4.48 < 0.05	1.41 > 0.05
AuCo-α		1.42 > 0.05	2.75 < 0.05	3.82 < 0.05	1.72 > 0.05	1.75 > 0.05	1.63 > 0.05